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African Journal of Agricultural Research

Table of Contents: Volume 11 Number 43, 27 October, 2016

ARTICLES

African Journal of Agricultural Research

Table of Contents: Volume 11 Number 43, 27 October, 2016

ARTICLES

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Full Length Research Paper

Yield and growth characteristics of Cowpea (Vigna unguiculata) as affected by prior heat stress and nutrient addition

Olusanya A. OLATUNJI[1,2]*, Gideon O. OKUNLOLA[3], Emmanuel T. KOMOLAFE [4], Akinjide M. AFOLABI[4], Akash TARIQ[1,2] and Ademayowa A. ODELEYE[4]

[1]Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization and Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, PR China.
[2]University of Chinese Academy of Sciences, Beijing, 100039, PR China.
[3]Department of Biological Sciences, Faculty of Basic and Applied Sciences, Osun State University, Osogbo, Nigeria.
[4]Department of Botany, Obafemi Awolowo University, Ile Ife, Nigeria.

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In the tropics, high temperatures and nutrient inaccessibility are often the most limiting factors affecting plant growth and final crop yield. However, it remain incomprehensible the actual impact of simultaneous interaction of prior heat stress and nutrient addition on growth and yield of cowpea plant, knowing that cowpea plant is an important grain legumes for over 200 million people in dry savanna of tropical Africa. In this study, the effect of interaction between prior heat stress and nutrient addition on growth and yield of cowpea plant was carried out in a randomized pot experiment at Obafemi Awolowo University, Ile-Ife, Nigeria. Cowpea seedlings were exposed to prior heat stress of 40°C a week after germination and different level of nutrient addition. The result obtained from the study showed that there was no significant ($p<0.05$) difference in the interactive effect of nutrient addition and prior heat stress on the shoot height of cowpea in most of the treatments compared to control, except in NNH_3 (200 ml of the nutrient solution on daily basis and 3 h of prior heat stress) and N_5H_6 (200 ml of nutrient in every five days and six hours of prior heat stress). The shoot height of N_5H_6 was significantly higher (41.17) than NNH_3 (20.26 cm). Cowpea plant subjected to only water with no prior heat stress had the highest leaf dry weight (2.44 g), while the cowpea plant under the interactive effect of nutrient supplied at every five days and six hours of prior heat stress had the highest shoot dry weight (2.63 g). The feedback of cowpea plant to interactive effect of prior heat stress and nutrient addition indicated that Longer period of prior heat stress had better advantages on growth parameter cowpea plant. However, neither prior heat stress nor nutrients addition had significant benefit on the leaf yield of cowpea, the interaction of the two significantly benefit shoot biomass and pod number of Cowpea plant.

Key words: Cowpea, growth, nutrient. heat, nodule, stress, treatment, yield.

INTRODUCTION

The frequency of extreme stress event on plant growth, development and yield differ among varieties of plant species, depending on the prevailing optimal environments as well as the susceptibility capacity of individual

species to a particular stress type. Commonly, plants are considered to be under stress when they experience a relatively severe scarcity of an essential element or an excess of a potentially toxic or damaging substance (Okunlola and Adelusi, 2012). Nevertheless, the ability of individual plant species to tolerate multiple stresses through morphological adjustments is a key feature that regulates species survival and colonization, and hence the ecological breadth of the species (Bazzaz, 1996; Sultan et al., 1998). Temperature is one of the major prevailing factors affecting the life of all living organisms. At the cellular level, heat stress beyond a threshold level plays a critical role in affecting the constancy of various proteins, fluidity of membrane lipids and metabolic imbalance (McClung and Davis, 2010; Wahid, 2007). Heat as a complex function of intensity, duration and rate of increase in temperature can cause declines in photosynthetic efficiency, leaf senescence, shoot and root growth inhibition, impairment of pollen and anther development, and subsequently reduction in yields (Karim et al., 1999; Sato et al., 2006; Wahid, 2007; Zhang et al., 2005). Heat stress due to high temperatures is a crucial threat to crop production worldwide (Hall, 2001) as it causes reduction in yield and dry matter production in many crops (Giaveno and Ferrero, 2003).

Around the world, nutrient addition is considered one of the major factor, to linearly increase crop production. However, sub-optimal availability of essential nutrients or toxicity of nutrient or non-nutrient minerals may pose primary restraint to plant growth and development over the majority of the earth's land surface (Okunlola et al., 2015). For instance, nitrogen being the most important macronutrient for plant due to it essential component in plant cell compounds such as chlorophyll and proteins (Srivastava and Singh, 1999), which are closely associated with leaf colour, crop growth status and yield (Fageria et al., 2011), constitutes only 2 to 4% of plant dry matter. Thus, it deficiency or supererogatory can affect leaf growth, leaf area, leaf duration, the photosynthetic rate per leaf area, and the size of the vegetative storage organs (Abdelhalim et al., 2016), thereby affecting crop yields negatively.

Despite the vast array of research on the effect of stress on crop growth, development and yields, the interactive effect of different type of stress on food crop are still rare. Particularly, the interactive effect of prior heat stress and nutrient addition on growth, development and yield of Cowpea plant (*Vigna unguiculata* (L.) Walp) has not been well researched. Thus, it is important to understand the interactive effect of prior heat and nutrient stress on the overall productivity of cowpea, being an economically viable food crops. Cowpea plant (*V. unguiculata* (L.) Walp.), is an annual bushy herb plant with erect, creeping or climbing stems depending on the varieties, (Ferreira, 2004; Shao et al., 2008). *V. unguiculata* is an important economic seed legumes in many developing regions due to its high protein content, resistance to drought, adaptability to different types of soil and intercropping systems (Shao et al., 2008). It is also desirable in that it has little dependence on chemical fertilizer for optimum yield if successfully nodulated with appropriate Rhizobium (Abayomi et al., 2008). Several studies such as Sunita and Uma (1993), Sharma and Gill (1994), Shinozaki and Yamaguchi-Shinozaki (2000) have reported the effect of different single stress factors on growth and yield of cowpea. However, the response of cowpea growth, development and yield to interactive effect of multiple stress factors has not been adequately established, especially in the savannah ecological zone which is the principal area of cultivation.

Therefore, in order to close this gap in knowledge, the present study evaluate the magnitude of effect due to simultaneous interaction of prior heat and nutrient stress on growth, development and yield of cowpea. We theorized that (i) the interaction of moderate or no prior heat stress and nutrient addition will significantly improve the overall productivities of cowpea plant, this was with the aim that optimal availability of essential nutrients will negate the effect of prior heat stress, (ii) at higher prior heat stress, the overall productivities of cowpea plant will significantly decline.

MATERIALS AND METHODS

Experimental design

A randomized pot experiment was conducted at the premises of Department of Botany, Obafemi Awolowo University, Ile-Ife. Topsoil was collected from a re-growth forest behind the Biological garden of Obafemi Awolowo University, Ile Ife, Nigeria. The soil samples were thoroughly mixed to obtain homogeneity and later sieved to remove the particles other than soil. Three wooden pots of approximately 30 cm x 30 cm were filled with the soil (Okunlola and Adelusi, 2013) and seeds of *V. unguiculata* were sown at a depth of about 1cm. The three wooden pots were divided into three regimes of (i) no heat stress (H_O), (ii) 3 h prior heat stress (H_3) and (iii) 6 h prior heat stress (H_6). The seedlings were allowed to germinate in the open environment for a period of one week and, then subjected to prior heat stress using Gallenkamp illuminated cooled incubator at 40°C (Okunlola and Adelusi, 2013). The first regime was incubated for three hours while the second regime was incubated for six hours. At the end of the incubation period, all the three wooden pots were transferred into the screen house. Thirty-six plastic pots were filled with the topsoil, with holes at the base of each plastic pot to ensure drainage of excess water. In order to further unravel the possible interaction between prior heat and nutrient addition, the experiment was further categorized into twelve (12) treatments based on the combined factors of the duration of nutrient supplied; no heat stress and prior heat stress (Table 1).The

*Corresponding author. E-mail: olusanya084@mails.ucas.ac.cn, olusanya084@yahoo.ca.

Table 1. Experimental treatment (prior heat stress× nutrient addition).

Prior heat stress	Nutrient addition			
	N_0	NN	N_3	N_5
H_O	$N_0H_0^{3*}$(control)	NNH_0^{3*}	$N_3H_0^{3*}$	$N_5H_0^{3*}$
H_3	$N_0H_3^{3*}$	NNH_3^{3*}	$N_3H_3^{3*}$	$N_5H_3^{3*}$
H_6	$N_0H_6^{3*}$	NNH_6^{3*}	$N_3H_6^{3*}$	$N_5H_6^{3*}$

3* - Three replicate per treatment, N_0H_0 – (control): only water with no prior heat stress, NNH_0 – daily nutrient supply (everyday) with no prior heat stress, N_3H_0 – 200 ml of nutrient solution every three day with no heat stress, N_5H_0 – 200 ml of nutrient solution in every five days and no prior heat stress, N_3H_3 – 200 ml of nutrient solution every three day and three hours of prior heat stress, N_0H_3 – three hours of prior heat stress with no nutrient supply, N_0H_6- six hour of prior heat stress with no nutrient supply, NNH_3 - 200 ml of the nutrient solution on daily basis and 3 h of prior heat stress, N_5H_3 - 200 ml of nutrient in every five days and three hours of prior heat stress, NNH_6 - 200 ml of the nutrient solution on daily basis and six hours of prior heat stress, N_3H_6 – 200 ml of nutrient in every three days and six hours of prior heat stress, N_5H_6 – 200 ml of nutrient in every five days and six hours of prior heat stress.

nutrient solution was prepared in accordance with the modified long Ashton formula (Hewitt, 1952).

Determination of growth and yield parameters

During the growing period, measurement of some growth parameter such as leaf area, {leaf length and leaf breath}, leaf number and shoot height were carried out up to the period of harvesting. Leaf area was calculated using the formula of Osei-Yeboah et al. (1983); A= LxWx2.325. In order to determine the yield response of *V. unguiculata* to treatments, each of the plant was carefully harvested. The dry weight of leaves, stems, roots and pods were determined after oven dried at 80°C. The pod number and root nodules were also estimated.

Statistical analysis

The result obtained from the study was subjected to both inferential and descriptive statistical analysis The effect of different treatments on each growth parameter were tested using Analysis of variance (ANOVA), least significant different (LSD) was used to evaluate the difference between separated means under each treatment. Single linkage cluster analysis was also performed on the selected yield parameter in other to show the likely similarity among the treatments. All statistical analysis were performed using SAS (SAS system for window 8) and PAST 3.01 software.

RESULTS

Growth and developmental characteristics of *Vigna unguiculata* under interactive effect of prior heat stress and nutrient addition

Evaluating the interactive effect of nutrient addition and prior heat stress on shoot height, leaf area and number of leaves of *V. unguiculata* during growing period, our result revealed that there was a progressive increase in the number of leaves, leaf area and shoot height from the first week (week 1) of the nutrient treatment to week 8 (Figures 1, 2 and 3). In addition, the result of the ANOVA revealed that there was no significant difference in the interactive effect of nutrition addition and prior heat stress on the shoot height of *V. unguiculata* in most of the treatments compared to control, except in NNH_3 and N_5H_6 which are significantly different from each other and also from the control (Table 2). The shoot height recorded when 200 ml of the nutrient solution was applied on daily basis to 3 h prior heat stress (NNH_3) *V. unguiculata* plant was significant difference ($P<0.05$) from *V. unguiculata* subjected to 200 ml of nutrient in every five days and six hours of prior heat stress. Particularly, the shoot height of N_5H_6 was significantly higher (41.17) than NNH_3 (20.26). Similar to the observed trend in shoot height, the leaf area of *V. unguiculata* when subjected to daily nutrient supply (everyday) with no prior heat stress (NNH_0) and when subjected to 200 ml of nutrient in every five days and six hours of prior heat stress (N_5H_6) treatments was significantly higher ($P<0.05$) than NNH_3. The average leaf area recorded during the study period range from 21.27 in NNH_3 treatment to 31.63 in N_5H_6 treatment. Furthermore, there was no significant different in the number of leaf recorded among the treatments. However, there was a decreased in the total number of leaf at NNH_3 (14.40) and N_3H_3 (15.7) treatments (Table 2).

Yield characteristic of *Vigna unguiculata* under the interactive influence of prior heat stress and nutrient addition

At harvest, there was no significant interaction of prior heat stress and nutrient addition majority on the yield parameter evaluated (Table 3), except in few treatments. The total pod number recorded among treatments were not significantly different from each other. Nevertheless, the pod number recorded when 200 ml of nutrient solution interact on daily basis with 3 hours of prior heat stress ($NNH_{3)}$ (1.40) was significantly ($p<0.05$) different from the total pod number recorded at N_5H_0, N_0H_0 and NNH_0 (3.75, 4.75 and 3.62) treatments respectively (Table 3). *V. unguiculata* subjected to three hours of prior heat stress with no nutrient supply (N_0H_3) had significantly ($p<0.05$) higher number of nodules than the number of

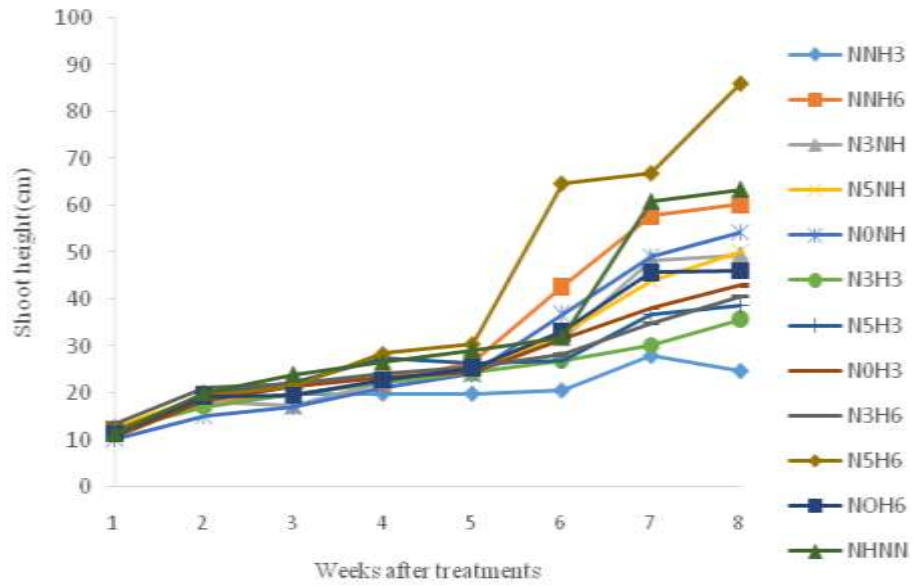

Figure 1. Weekly response of shoot height to prior heat stress and nutrient addition.

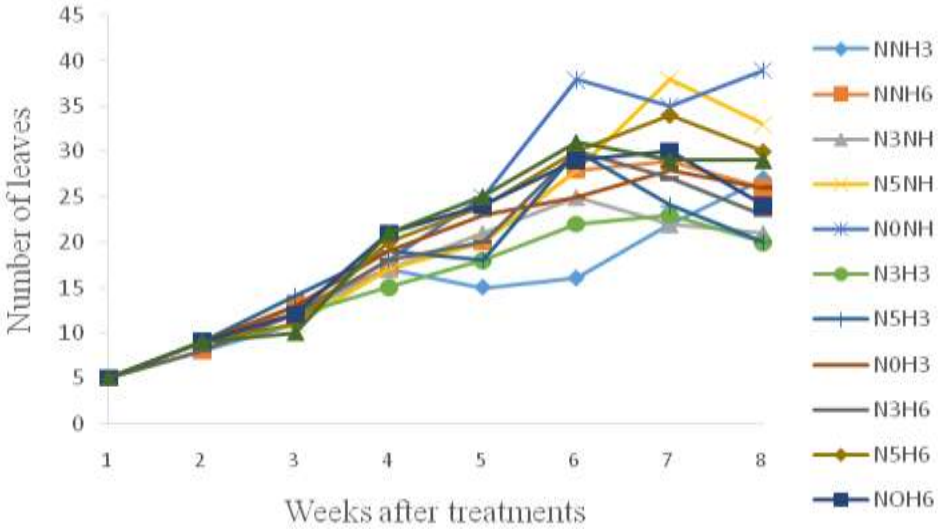

Figure 2. Weekly responses of number of leaves to nutrient addition and prior heat stress during growing period.

nodules recorded among other treatments. Although there was no significant ($p<0.05$) different in the total leaf biomass (leaf dry weight) and shoot biomass (shoot dry weight) among the treatments, however, *V. unguiculata* subjected to only water with no prior heat stress had the highest leaf dry weight (2.44), while the *V. unguiculata* under the interactive effect of 200 ml of nutrient supplied in every five days to six hours of prior heat stress plant had the highest shoot dry weight (2.63). Furthermore, *V. unguiculata* under the interactive effect of 200 ml of nutrient solution supplied every three day with no heat stress ($N_3H_{0)}$ and 200 ml of nutrient solution supplied every three day with three hours of prior heat stress recorded the highest seed biomass (Table 3). In addition, the result of this study further showed that cowpea plant under NNH_6 treatment had higher pod number (3.28) compared to cowpea plant under NNH_3 (1.40) treatment. In contrary, N_5H_3 had higher number of nodules (3.42) compared to N_5H_6 (2.25). The result of the cluster analysis further revealed that *V. unguiculata* under the interactive influence 200ml of nutrient solution supplied in every five days with no prior heat stress N_5H_0 and those

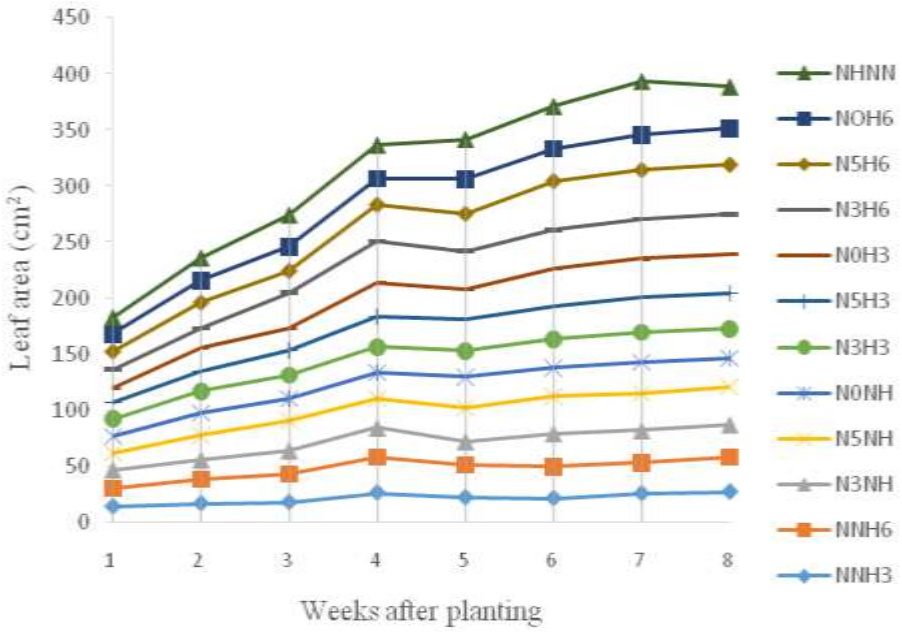

Figure 3. Weekly responses of leaf area to nutrient addition and prior heat stress during growing period.

Table 2. Effect of prior heat stress and nutrient addition on the growth parameters during the developmental stage.

Treatment	NNH_3	N_3H_3	N_0H_3	N_3H_6	N_5H_3	N_0H_6	N_3H_0	N_0H_0	N_5H_0	NNH_6	NNH0	N_5H_6
Shoot height (cm)	20.26^{c}	23.6^{bc}	25.64^{bc}	26.23^{bc}	26.80^{bc}	27.87^{bc}	27.90^{bc}	28.43^{b}	28.61^{bc}	32.09^{bc}	33.47^{ab}	41.17^{a}
Leaf area (cm^2)	21.27^{d}	22.2^{bd}	26.65^{bcd}	29.95^{ac}	26.48^{bcd}	26.15^{bcd}	23.47^{bd}	23.16^{bd}	27.44^{abc}	26.12^{abc}	31.58^{a}	31.63^{a}
No. of leaf	14.40^{b}	15.7^{b}	19.29^{ab}	17.29^{ab}	16.75^{ab}	19.33^{ab}	16.75^{ab}	22.87^{a}	20.45^{ab}	18.45^{ab}	20.04^{ab}	20.41^{ab}

^{abcd} Mean within the same row and with different letter are significantly different @ $P \leq 0.05$.

supplied with only water and also with no prior heat stress (N_0H_0) are more related to each other, but distantly related to the other treatments and cluster together with similarity index of above 90% (Figure 4).

DISCUSSION

The extent to which stress affect plant is a complex process. While high light intensity may induce severe photo-oxidative damage to chloroplasts, and consequently cause decrease in the yield capacity of plants, the mineral nutritional status of plants may significantly affects their ability to acclimatize to adverse environmental conditions (Okunlola et al., 2015; Okunlola and

Table 3. Yield characteristic of *Vigna unguiculata* under the influence of prior heat stress and nutrient addition.

Treatment	Fresh weight				Dry weight			
	NOP	NOD	WP (g)	DS (g)	DW (g)	DL (g)	DR (g)	DSH (g)
NNH_3	1.40^{b}	56.87^{abc}	31.16^{a}	2.37^{a}	5.61^{a}	2.21^{a}	1.59^{ab}	1.71^{a}
NNH_6	3.28^{ab}	53.50^{abc}	20.04^{a}	3.57^{a}	4.67^{a}	1.83^{a}	1.55^{ab}	1.28^{a}
N_3H_0	3.33^{ab}	62.66±^{abc}	21.76^{a}	5.62^{a}	4.35^{a}	1.53^{a}	1.32^{ab}	1.49^{a}
N_5H_0	3.75^{a}	38.88^{ab}	28.62^{a}	3.64^{a}	6.19^{a}	2.19^{a}	1.92^{ab}	2.07^{a}
N_0H_0	4.12^{a}	35.00^{a}	26.56^{a}	3.17^{b}	5.40^{a}	2.44^{a}	0.98^{b}	1.96^{a}
N_3H_3	2.77^{ab}	46.11^{ab}	22.14^{a}	4.34^{a}	4.41^{a}	1.53^{a}	1.52^{ab}	1.32^{a}
N_5 H_3	3.42^{ab}	59.77^{abc}	22.53^{a}	3.50^{b}	4.99^{a}	1.54^{a}	1.76^{ab}	1.67^{a}
N_0H_3	3.50^{ab}	85.11^{c}	26.51^{a}	3.42^{b}	5.33^{a}	1.82^{a}	1.39^{ab}	2.12^{a}
N_3 H_6	3.00^{ab}	44.00^{ab}	26.05^{a}	3.85^{b}	5.27^{a}	2.05^{a}	1.69^{ab}	1.52^{a}
N_5 H_6	2.25^{ab}	56.87^{abc}	30.83^{a}	3.92^{b}	6.81^{a}	2.12^{a}	2.04^{ab}	2.63^{a}
N_0 H_6	2.60^{ab}	74.14^{bc}	23.48^{a}	2.08^{a}	5.75^{a}	2.10^{a}	1.67^{b}	2.01^{a}
NNH_0	3.62^{a}	45.77^{ab}	26.18^{a}	4.97^{a}	6.26^{a}	2.22^{a}	2.13^{a}	1.86^{a}

^{abcd} Mean within the same column and with the same letter are not significantly different 0.05. NOP: Number of pod; NOD: number of nodles; DS: Dry seeds; WP: Whole plant fresh weight; DW: whole plant dry weight; DL: Dry leaf; DR: dry root and DSH: dry shoot.

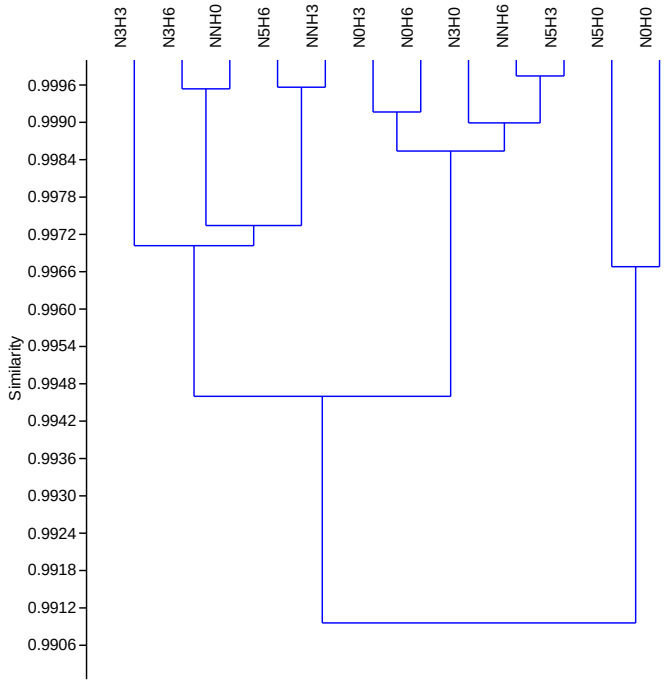

Figure 4. Cluster analysis of the relationship among the various treatments based on the observed yield parameters.

Adelusi, 2014). In the present study, the combine effect of different hours of prior heat stress and different nutrient addition level have dissimilar effect on the overall growth and yield characteristic of cowpea plant. While some treatment do not significantly affect the growth and yield parameters of cowpea, other posed significant ($p<0.05$) effects. For instance, when 200 ml of the nutrient solution supplied on daily basis interacted with 3 h of prior heat stress, the shoot height and leaf area was significantly reduced. However, when 200 ml of nutrient supplied every five days interacted with six hours of prior heat stress, shoot height, leaf area and number of leaves significantly increased. Since the prior heat stress used in the present study is below the temperature at which plant

tissues is expected to damaged (46.1°C), thus, the present observation suggested that longer period of prior heat stress have better advantages on cowpea plant, even when nutrient was not supplied or inadequately supplied. According to Thompson et al. (1988), medium irradiance and high nutrient levels will result into optimal leaf expansion, Chlorophyll content and Photosynthesis. Furthermore, the observed higher growth parameter when nutrient was supplied on daily basis with no prior heat stress agreed with our hypothesis that the interaction of moderate or no prior heat stress and nutrient addition will significantly improve the overall productivities of cowpea plant. The present findings suggested the ability of cowpea plant to ameliorate the equivocal effect of prior heat stress when interacted with nutrient addition. This agreed with the finding of Okunlola and Adelusi (2014) that plants grown at high nutrient availability generally produce larger leaves and greater allocation to phosynthetic protein than those at low nutrient availability.

According to Jurik (1991), variation in temperature will greatly affects plant growth and development, therefore, the low leaf area observed when nutrient solution supplied on daily basis interacted with 3 h of prior heat stress and when nutrient solution supplied every three days interacted with 3 h of prior heat stress maybe attributed to the combine effect of nutrient and prior heat stress which may impede the production of leaf. This agreed with Azcon-Bieto and Osmond (1983) that nutrient stress affects the development of mesophyll cell which make up the bulk of the internal leaf tissue and also affect leaf development. Furthermore, the high pod number observed when nutrient was supplied to cowpea plant without prior heat stress compared to cowpea plant under prior heat stress may be attributed to absence of heat stress in those treatments. According to Ghosh et al. (2000) and Sato (2006) subjecting plant to either moderate or intense heat stress will resulted into severe yield loss and subsequently extended into total crop failure. Reproductive development of many crop species is damaged by heat such that they produce no flowers or if they produce flowers they may set no fruit or seeds (Hall, 1993).

Crop sensitivity to heat stress hinge on the length of anthesis and also on the duration of heat stress (Hatfield and Prueger, 2015). The present findings revealed that longer duration of prior heat stress when interacted with daily addition of nutrient solution resulted in higher pod number in cowpea plants. However, interaction of longer prior heat stress (six hours) with nutrient addition at every five days interval resulted into decreased in number of nodules compared to three hours of prior heat stress. This suggested that duration of prior heat stress may impact the fertility of cowpea flower and subsequently impacting the pod yield of cowpea plant. Generally, both prior heat and nutrient addition significantly increased number of nodules in cowpea plant compared to when neither prior heat stress nor nutrient was applied. This finding corroborated the work of Simoes-Araujo et al. (2008) that in the tropical area, cowpea nodules are very resistant to heat and high temperature stress and, thus enhanced the number of nodules produced. Abdel-Wahaab et al. (1994) and Norman et al. (1995) reported that nutrients addition, particularly phosphorus will stimulate root and plant growth, initiate nodules formation and also influences efficiency of rhizobium-legumes symbiosis, consequently optimizing the biological nitrogen fixation systems of legumes.

The significant of parameters like shoot, plant height, leave biomass and total plant biomass as measure of yield had been suggested in several study (Hatfield and Prueger, 2015), therefore they may be used as a measure of yield response of cowpea to prior heat stress and nutrient addition. Although there was no significant ($p<0.05$) different in the total leaf biomass (leaf dry weight) among the treatments, however, the leaf biomass of *V. unguiculata* subjected to only water addition with no prior heat stress and nutrient addition had the highest leaf dry weight. This suggested that neither prior heat stress nor nutrients addition had significant benefit on the leaf yield of cowpea plant. furthermore, the shoot biomass of *V. unguiculata* under the interactive effect of nutrient supplied at every five days and six hours of prior heat stress was highest compared to other treatment. In general, among the different yield parameter studied leaf biomass had the highest dry weight in all the treatment. This may be attributed to food production capacity of the leaf, since the leaf is the site for food manufacture through photosynthesis.

Conclusion

The interactive effect of prior heat stress and nutrient addition on cowpea plant was quite universal. Longer period of prior heat stress have better advantages on growth parameter of cowpea plant, even when nutrient was not or inadequately supplied compared to shorter or no prior heat stress. It can further be concluded that while neither prior heat stress nor nutrients addition had significant benefit on the leaf yield of cowpea plant, the interaction of the two significantly benefit shoot biomass and pod number of cowpea plant. However, interaction of longer prior heat stress (six hours) with inadequate nutrient addition decreased number of nodules compared to shorter hours of prior heat stress. To obtain comprehensive overview of growth and yield response of cowpea plant, we suggested further studies to elucidate the physiological basis for growth and yield responses in cowpea plant under the interactive effect of prior heat stress and nutrients addition. We further suggested that the potential adaption mechanism underline the response of cowpea plant to interactive effect of different stress factor should be determined.

Conflict of Interests

The authors have not declared any conflict of interests.

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Chemical composition and antioxidant capacity in edible biomass of tagasaste (*Chamaecytisus proliferus* var. *palmensis*)

Leonardo Sulas[1]*, Simone Canu[2], Antonio Melchiorre Carroni[3], Giovanni Antonio Re[1], Mauro Salis[3] and Giovanna Piluzza[1]

[1]National Research Council (CNR), Institute for the Animal Production System in Mediterranean Environment (ISPAAM), Sassari, Italy.
[2]National Research Council (CNR), Institute of Biomolecular Chemistry (ICB) Sassari, Italy.
[3]Council for Agricultural Research and Economics (CREA), Research Unit on Mediterranean Agropastoral Systems, Sanluri, Italy.

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Tagasaste (*Chamaecytisus proliferus* (L. fit.) Link var. *palmensis* (Christ) Hansen and Sunding is a perennial fodder shrub naturalized in a range of environments for its valuable forage traits. This research was aimed at evaluating the seasonal variation in chemical composition, bioactive compounds and antioxidant properties in edible biomass components (leaves, twigs, flowers, and pods) of tagasaste grown in a Mediterranean environment of Sardinia (Italy). Season influenced the composition of edible biomass and chemical composition and antioxidant capacity of leaves and twigs. On average, crude protein concentration was 200, 97 and 66 g kg^{-1} dry matter, in leaves, twigs and mature pods, respectively. Neutral and acid detergent fibre were high in twigs. In late summer, antioxidant capacity of leaves reached 44 mmol/100 g dry weight of Trolox equivalent and was correlated with the levels of total phenolics and flavonoids. Research highlights that the edible biomass of tagasaste is a high quality feed source available during the most critical forage shortage for rainfed farming systems of Mediterranean climatic areas.

Key words: Bioactive compounds, forage quality, plant parts, polyphenols, tree lucerne.

INTRODUCTION

Browse forages and multipurpose trees are widely used in Southern Europe, Eastern Mediterranean region and African savannas, to alleviate feed shortages and supply vital nutrients for growth and reproduction for ruminants, during long and dry periods (Assefa et al., 2008; Franzel et al., 2008; Papanastasis et al., 2008; Kökten et al.,

*Corresponding author. E-mail: l.sulas@cspm.ss.cnr.it.

2012; Fomum et al., 2015). The indigenous *Chamaecytisus* from the Canary Islands comprises a taxonomic complex (Francisco-Ortega et al., 1992); the form that is endemic to La Palma is commonly known as "tagasaste" (*Chamaecytisus proliferus* (L. fit.) Link var. *palmensis* (Christ) Hansen and Sunding), which is cultivated as a fodder tree and has achieved agricultural importance around the world, particularly in areas of Australia and New Zealand (Chinea et al., 2013). Tagasaste has become naturalized in Java, the Hawaiian Islands, California, Chile, North Africa, Ethiopia, Kenya, Tanzania and South Africa (Francisco-Ortega et al., 1991). Since 1879, it has been grown as a drought-resistant fodder plant in Australia (Dann and Trimmer, 1989).

It has been reported that this fodder tree also appears to be well suited to the drained sandy soils in both the winter and summer rainfall regions of South Africa (Meissner, 1997; Lindeque and Rethman, 1998). According to Assefa (1998), tagasaste could be the best browse tree for the highlands of East Africa. A marked feature of this shrub species is its ability to retain evergreen leaves and hence maintain a relatively high nutritive value during the dry season (González-Rodrıguez et al., 2005). Edible biomass of tagasaste is represented by leaves and other parts such as twigs (that is, fine stems) and pods (Becholie et al., 2005). Based on its favourable chemical composition, tagasaste may have a role as a high quality feed source and/or supplement for ruminants, which could be used to correct the critical seasonal shortage of forage caused by prolonged dry periods and harsh environmental conditions (Varvikko and Khalili, 1993; Assefa, 1998; Kumara Mahipala et al., 2009a; Kitaw et al., 2012).

Increasing attention is being paid to forage secondary compounds such as polyphenols and condensed tannins, which can reduce intestinal infections in grazing animals (Marley et al., 2003; Piluzza et al., 2013). Polyphenols are among the most significant compounds related to the antioxidant properties of plant materials. This information could be very helpful in establishing relationships such as between forage polyphenol contents and their feeding values for ruminants. Unfortunately, there is limited knowledge regarding the antioxidant capacity of tagasaste biomass in spite of its potential.

In the last decade, tagasaste germplasm has been introduced to Sardinia, Italy, where a private breeder has also started a selection program.

As different plant organs constitute the edible biomass of tagasaste, a study of their relative contribution and chemical composition is necessary in view of the local exploitation of this valuable leguminous shrub. We hypothesized that growth seasonal conditions affect biomass components ratios and chemical composition of plant organs in tagasaste. Therefore, our specific objectives were to investigate the relative contribution of leaves, twigs, flowers and pods to the total edible biomass of tagasaste and their seasonal chemical composition, bioactive compounds content (total polyphenols, non-tannic phenolics, tannic phenolics, flavonoids, condensed tannins) and antioxidant capacity.

MATERIALS AND METHODS

The field experiments were conducted during 2013 to 2014 in Southern Sardinia (39° 31' N, 8° 51' E, Italy), where the climate is Mediterranean with mild winter. The area has a long-term average annual rainfall of 446 mm received mainly in the autumn and winter months, and a mean annual air temperature of 17.6°C. The total rainfall from September 2013 to August 2014 did not substantially differ from climatic data. The soil at the experimental site, classified as *Typic Fluvaquents*, is sandy-clay-loam, with pH 7.8, sufficient average nitrogen content of 1.1‰ and phosphorous 16.2 ppm.

Plant material, sampling and measurements, chemical composition

Tagasaste used in the study was grown on experimental plots of the Council for Agricultural Research and Economics (Sanluri, Italy). Plots size consisted in tagasaste plants (three year old) each spaced 2.5 m between rows and 2 m apart within rows, under a randomised block design with three replicates. The trees had never been pruned, with the exception of eliminating the branches on the basal part of the plant (up to 50 cm in height). No mechanical intervention on the soil, fertilisation, herbicide application or irrigation was applied. At bi-monthly intervals, starting from November 2013 and ending in September 2014, three undisturbed plants per plot were pruned at a cut height of 50 cm. Sampling months corresponded to late autumn (November), winter (January), early spring (March), late spring (May), summer (July) and late summer (September), respectively. Plant aerial biomass was separated into branches, lignified stems and edible biomass. Edible biomass was then subdivided in leaflets (thereinafter referred as leaves), young twigs (thin stems < 3 mm of diameter), flowers, green pods, and mature (that is, brown-dry) pods. Tagasaste leaves, twigs and reproductive organs were immediately weighted to determine fresh weight and the contributions of each plant component. Phytomass sub-samples were oven dried at 65°C for 48 h, then ground to 1 mm screen to be analysed for quality traits. Total N was determined using the Kjeldahl method and crude protein (CP) was calculated by multiplying the N content by 6.25. Neutral and acid detergent fibres (NDF and ADF) and acid detergent lignin (ADL) were determined by using the procedure of Van Soest et al. (1991) and ether extract (EE) using Soxhlet extraction.

Total digestible nutrients (TDN), digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV) and net energy for lactation (NE_l) of edible biomass were estimated according to the following equations adapted from Lithourgidis et al. (2006) and Sadeghpour et al. (2014):

$TDN = (-1.291 \times ADF) + 101.35$
$DMI = 120 / \%NDF$ dry matter basis
$DDM = 88.9 - (0.779 \times \%ADF$, dry matter basis)
$RFV = \%DDM \times \%DMI \times 0.775$
$NE_l = (1.044 - (0.0119 \times \%ADF) \times 2.205$

Antioxidant capacity and bioactive compounds

Harvested subsamples of edible biomass components were kept on

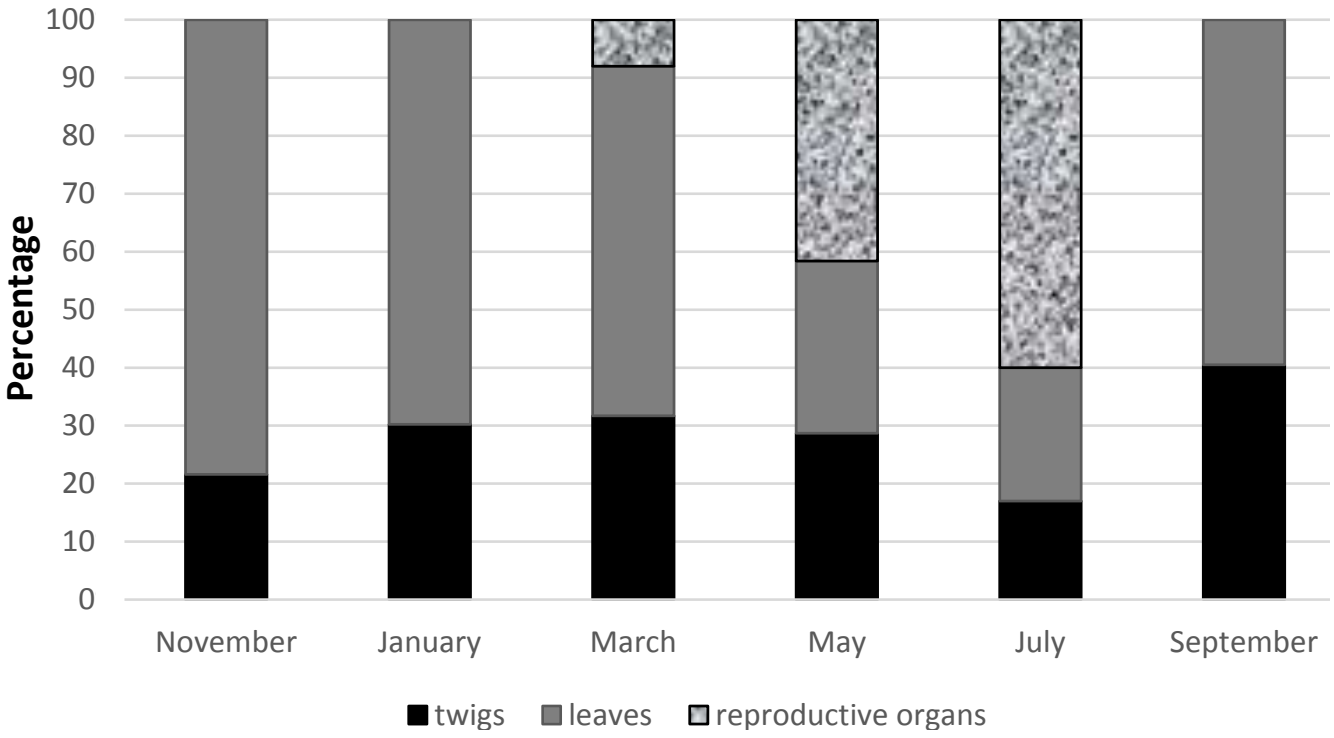

Figure 1. Contribution of twigs, leaves and reproductive organs to the edible biomass of tagasaste.

ice, freeze dried and ground to a fine powder for chemical analysis. The powdered material was then used for extract preparation following Piluzza et al. (2014). Antioxidant capacity was determined by means of the improved ABTS ((2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt)) and by DPPH (1,1-diphenyl-2-picrylhydrazyl) (Surveswaran et al., 2007; Piluzza and Bullitta, 2011) assays. Trolox, a water-soluble analogue of vitamin E was used as the reference standard. The results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC), as mmol Trolox equivalents per 100 g dry weight of plant material (mmol TEAC/100 g DW).

Total phenolics (TotP), non-tannic phenolics (NTP) and tannic phenolics (TP) were determined using the Folin Ciocalteau colorimetric assay according to procedures previously described by Sulas et al. (2016). Results were expressed as g gallic acid equivalent (GAE) kg^{-1} dry weight of plant material (g GAE kg^{-1} DW).

Total flavonoids (TotF) were quantified by colorimetric assay using Aluminium trichloride, following procedures previously reported (Piluzza and Bullitta, 2011). Catechin was used as a standard and the flavonoid content was expressed as g catechin equivalent kg^{-1} dry weight of plant material (g CE kg^{-1} DW).

For each bi-monthly sampling, three subsamples of each available plant organ were analysed and all the assays were performed in triplicate.

Statistical analysis

The data were subjected to analysis of variance, using Statgraphics Centurion XVI version (StatPoint Technologies Inc., 2009), considering month of sampling as independent variable and forage quality parameters, antioxidant capacity and bioactive compounds as dependent variables. Differences between means were assessed by the Fisher's least significant difference (LSD) procedure for means separation. Coefficients of determination (R^2) were calculated using Microsoft Excel 2000.

RESULTS

Edible biomass and its chemical composition

The contribution of leaves to the total edible biomass exceeded 60% from September to March, representing the most important component, whereas the contribution of twigs ranged from 17 to about 40% (Figure 1). Flowering started in March (early spring) and mature pods represented about 60% of edible biomass in July, making them a relevant component in summer.

The time of sampling significantly affected chemical composition of leaves and twigs (Tables 1 and 2). Dry matter content in leaves ranged from 268 (January) to about 400 g kg^{-1} fresh sample in July and September (Table 1). Leave dry matter content recorded in January, March and May was significantly lower than in the remaining months. Crude protein concentration peaked in January (254 g kg^{-1} DM), followed by March and significantly decreased in May and July to about 170 g kg^{-1} DM, with intermediate values in September. Except for January, when NDF reached the highest value (522.3 g kg^{-1} DM), the remaining NDF values did not significantly differ. ADF showed a quite similar trend. ADL content ranged from 70 (May) to 149 (January) g kg^{-1} DM, the

Table 1. Bi-monthly trend of chemical composition (g kg^{-1} DM) in tagasaste leaves: Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), ether extract (EE) and ash.

Cutting	DM	CP	NDF	ADF	ADL	EE	Ash
	(g kg^{-1} DM)						
Nov	362.9^{a}	191.0^{cd}	370.6^{b}	193.9^{c}	86.8^{b}	30.7^{cd}	70.1^{b}
Jan	267.7^{b}	254.7^{a}	522.3^{a}	296.9^{a}	149.6^{a}	35.2^{b}	72.4^{b}
Mar	287.5^{b}	229.5^{b}	387.0^{b}	203.0^{bc}	100.6^{b}	34.8^{bc}	57.8^{c}
May	304.8^{b}	173.6^{d}	396.1^{b}	219.9^{bc}	69.9^{b}	41.7^{a}	81.9^{a}
Jul	392.9^{a}	172.0^{d}	390.4^{b}	231.5^{b}	110.3^{ab}	38.7^{ab}	62.0^{c}
Sep	395.6^{a}	195.2^{c}	338.0^{b}	209.4^{bc}	74.8^{b}	29.3^{d}	44.9^{d}

Values with different letters in a column are significantly different at $p \leq 0.05$ (Fisher's test).

Table 2. Bi-monthly trend of chemical composition (g kg^{-1} DM) in tagasaste twigs: Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), ether extracts (EE) and ash.

Cutting	DM	CP	NDF	ADF	ADL	EE	Ash
	(g kg^{-1} DM)						
Nov	394.7^{d}	96.9^{a}	623.8^{b}	409.6^{c}	122.7^{b}	34.4^{a}	54.2^{a}
Jan	288.3^{f}	112.1^{a}	675.2^{a}	463.2^{ab}	151.9^{a}	22.1^{d}	38.0^{cd}
Mar	325.8^{e}	97.8^{a}	630.9^{b}	448.9^{b}	101.3^{c}	13.5^{e}	46.3^{b}
May	425.0^{c}	72.3^{b}	664.5^{a}	465.5^{ab}	126.0^{b}	22.9^{cd}	37.1^{cd}
Jul	485.6^{a}	99.9^{a}	630.1^{b}	481.3^{a}	126.3^{b}	26.1^{bc}	33.6^{d}
Sep	445.5^{b}	100.8^{a}	680.2^{a}	485.2^{a}	126.1^{b}	39.8^{b}	38.6^{c}

Values with different letters in a column are significantly different at $p \leq 0.05$ (Fisher's test).

Table 3. Total digestible nutrients (TDN), digestible dry matter (DDM), digestible dry matter intake (DMI), relative feed value (RFV) and net energy for lactation (NE_l) of tagasaste leaves.

Cutting	TDN (g kg^{-1} DM)	DDM (g kg^{-1} DM)	DMI (g kg^{-1} of body weight)	RFV (%)	NE_l (Mcal kg^{-1})
Nov	763^{a}	738^{a}	32	185^{a}	1.793^{a}
Jan	630^{c}	658^{c}	23	117^{b}	1.523^{c}
Mar	751^{ab}	731^{ab}	31	182^{a}	1.769^{ab}
May	730^{ab}	718^{ab}	30	171^{a}	1.725^{ab}
Jul	715^{b}	709^{b}	31	174^{a}	1.695^{b}
Sep	743^{ab}	726^{ab}	35	200^{a}	1.753^{ab}

Values with different letters in a column are significantly different at $p \leq 0.05$ (Fisher's test).

latter significantly higher than the remaining values, except for July. Ash content ranged from 45 to 82 g kg^{-1} DM.

In twigs, dry matter content significantly increased from January to July, reaching 486 g kg^{-1} fresh sample (Table 2). Crude protein concentration ranged from 72 to 112 g kg^{-1} DM and the value recorded in May was significantly lower than the remaining. Significant but slight variations were found in the values of NDF. ADF values also showed significant but limited variations. ADL content ranged from 101 (March) to 152 (January) g kg^{-1} DM. Ether extract varied from 14 to 34 g kg^{-1} DM and ash content from 34 to 54 g kg^{-1} DM. Overall, chemical composition of tagasaste twigs showed little variation across the year.

The chemical composition of flowers was similar to that of leaves at the same cutting time (March); the CP concentration of green pods reached 126 g kg^{-1} DM, whereas it decreased to 66.6 g kg^{-1} DM in mature pods. The values of NDF of both green and mature pods were within the range recorded for twigs.

In leaves, the lowest values of TDN, DDM, DMI, and NE_l were recorded in January (Table 3). TDN ranged

Table 4. Total digestible nutrients (TDN), digestible dry matter (DDM), digestible dry matter intake (DMI), relative feed value (RFV) and net energy for lactation (NE_l) of tagasaste twigs.

Cutting	TDN (g kg^{-1} DM)	DDM (g kg^{-1} DM)	DMI (g kg^{-1} of body weight)	RFV (%)	NE_l (Mcal kg^{-1})
Nov	485^{a}	570^{a}	19^{a}	85^{a}	1.227^{a}
Jan	416^{bc}	528^{bc}	18^{b}	73^{cd}	1.087^{bc}
Mar	434^{b}	539^{b}	19^{a}	80^{ab}	1.124^{b}
May	412^{bc}	526^{bc}	18^{b}	74^{cd}	1.080^{bc}
Jul	392^{c}	514^{c}	19^{a}	76^{bc}	1.039^{c}
Sep	387^{c}	511^{c}	18^{b}	70^{d}	1.028^{c}

Values with different letters in a column are significantly different at $p \leq 0.05$ (Fisher's test).

Table 5. Bi-monthly trend of trolox equivalent antioxidant capacity (TEAC) by ABTS and DPPH methods, total phenolics (TotP), non-tannic phenolics (NTP), tannic phenolics (TP), total flavonoids (TotF) of tagasaste leaves.

Cutting	TEAC(mmol/100 g DW)		TotP	NTP	TP	TotF
	ABTS	DPPH	(g GAE kg^{-1} DW)	(g GAE kg^{-1} DW)	(g GAE kg^{-1} DW)	(g CE kg^{-1} DW)
Nov	14.4^{b}	22.2^{b}	76.8^{b}	65.4^{b}	11.3^{b}	43.5^{b}
Jan	8.6^{c}	9.5^{d}	48.5^{d}	45.2^{d}	3.3^{c}	21.3^{d}
Mar	11.5^{bc}	10.2^{d}	65.6^{c}	54.1^{c}	11.5^{b}	36.6^{bc}
May	7.0^{c}	7.1^{d}	49.7^{d}	40.3^{d}	9.4^{bc}	20.5^{d}
Jul	9.1^{bc}	15.2^{c}	75.4^{b}	56.4^{c}	19.6^{a}	30.2^{c}
Sep	34.2^{a}	44.3^{a}	112.1^{a}	95.6^{a}	16.5^{ab}	67.7^{a}

Values with different letters in a column are significantly different at $p \leq 0.05$ (Fisher's test).

Table 6. Bi-monthly trend of trolox equivalent antioxidant capacity (TEAC) by ABTS and DPPH methods, total phenolics (TotP), non-tannic phenolics (NTP), tannic phenolics (TP), total flavonoids (TotF) of tagasaste twigs.

Cutting	TEAC (mmol/100 g DW)		TotP	NTP	TP	TotF
	ABTS	DPPH	(g GAE kg^{-1} DW)	(g GAE kg^{-1} DW)	(g GAE kg^{-1} DW)	(g CE kg^{-1} DW)
Nov	5.0^{b}	3.5^{bc}	23.0^{ab}	17.3^{bcd}	5.8^{a}	5.7^{d}
Jan	3.9^{bc}	3.2^{c}	18.2^{cd}	16.4^{cd}	1.8^{b}	7.7^{c}
Mar	2.8^{c}	2.7^{c}	16.6^{d}	14.0^{d}	2.6^{b}	5.4^{d}
May	3.9^{bc}	2.6^{c}	21.2^{bc}	18.4^{abc}	2.8^{b}	9.0^{b}
Jul	4.8^{b}	4.4^{b}	23.3^{ab}	21.4^{a}	1.9^{b}	7.7^{bc}
Sep	6.9^{a}	5.9^{a}	25.8^{a}	21.0^{ab}	4.8^{a}	11.3^{a}

Values with different letters in a column are significantly different at $p \leq 0.05$ (Fisher's test).

from 630 (January) to 763 (November) g kg^{-1} DM and variations were related to the ADF concentration. DDM ranged from 738 (November) to 658 (January). DMI, which is negatively correlated with NDF, ranged from 23 to 35. Except in the January sampling, RFV exceeded 151 value, which is indicative of a prime forage, and a similar trend was recorded for NE_l. In twigs, the most favourable values of TDN, DDM, RFV and NE_l were recorded in November. The value of RFV was on average 80 (Table 4).

Trolox equivalent antioxidant capacity (TEAC) and phenolic contents

The content of phenolics and the antioxidant activities detected by means of the two *in vitro* assays (ABTS, DPPH) on the tagasaste leaves and twigs for each cutting date are shown in Table 5 and 6. Statistically significant differences among sampling months where found for total antioxidant capacity, TotP, NTP, TotF. In leaves, both ABTS and DPPH assays exhibited wide

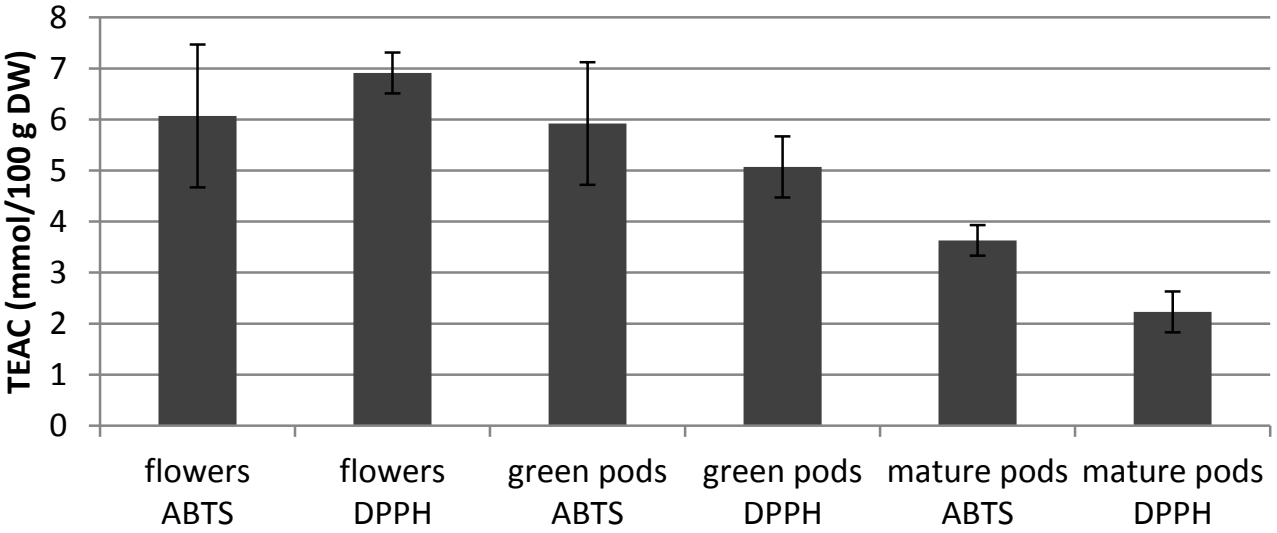

Figure 2. Antioxidant capacity in flowers and pods of tagasaste (Vertical bars indicate standard deviations of means).

seasonal variations of antioxidant capacities from May to September (Table 5). The highest antioxidant capacities were observed in late summer, and the values (34.2 and 44.3 mmol TEAC/100 g DW, respectively) were five to six-fold higher than those recorded in late spring. Total phenolics (TotP) ranged from 48.5 (January) to 112.1 (September) g GAE kg^{-1} DW. High TEAC values corresponded to high TotP contents, and low TEAC values to lower TotP contents. The seasonal trend also showed differences for the contents of Non-tannic phenolics (NTP) from 40.3 (May) to 95.6 (September) g GAE kg^{-1} DW, Tannic phenolics (TP) from 3.3 (January) to 19.6 g GAE kg^{-1} DW (July) in leaves. Total flavonoids (TotF) of leaves ranged from 20.5 (May) to 67.7 (September) g CE kg^{-1} DW.

In twigs, both ABTS and DPPH assays exhibited wide seasonal variations of antioxidant capacities from March/May to September, when again the highest values (6.9 and 5.9 mmol TEAC/100 g DW, respectively) were recorded (Table 6). However, they were about twice as high as the early spring values. Total phenolics (TotP) ranged from 16.6 (March) to 25.8 (September) g GAE kg^{-1} DW, whereas the contents of NTP from 14.0 to 21.1 g GAE kg^{-1} DW at the same cutting times. The values of TP varied from 1.8 (January) to 5.8 g GAE kg^{-1} DW (November) and TotF from 5.4 (May) to 11.3 (September) g CE kg^{-1} DW.

The antioxidant capacity of flowers and green and mature pods of tagasaste is shown in Figure 2. Both ABTS and DPPH assays evidenced high TEAC values in flowers and green pods compared to mature pods. The concentrations of TotP, NTP and TP in flowers were high (Figure 3).

The correlations between the antioxidant activity and TotP, NTP, TP and TotF (Table 7) were highly significant for the different months, except for TP in January, May and September (with ABTS), and in September (with DPPH), when no significant correlations were found.

DISCUSSION

The six consecutive samplings, carried out at bi-monthly intervals across a complete productive year, indicated quantitative and qualitative variations occurred in the edible biomass of tagasaste. In particular, leaves resulted the predominant component of the edible biomass during the vegetative growth, whereas the relative contribution of pods in summer was important. Season affected the chemical composition of edible biomass and the lowest CP values of leaves were recorded in May (late spring) and July (summer). However, the above-mentioned CP values, coupled with moderate levels of NDF, indicated a high quality feed source, which is also available during the most critical forage shortage for Mediterranean farming systems.

Overall, results indicate that quality of tagasaste remained high during all the year. Regarding seasonal chemical composition of tagasaste, our data if expressed as averaged means of leaves and twigs are quite similar to those reported from South Africa, Canary Islands, Ethiopia, Australia and New Zealand (Borens and Poppi, 1990; Lindeque and Rethman, 1998; Ventura et al., 2002; Becholie et al., 2005; Assefa et al., 2012). However, only few authors have reported a separate analysis for each component of edible biomass, as in this study. In addition, less pronounced changes in phenological stages (that is, no pod production) were noticed under different environmental conditions (Assefa et al., 2012) and different methodological approaches were used sometimes, by including stems up to 6 mm diameter in the edible biomass. As regard Mediterranean conditions

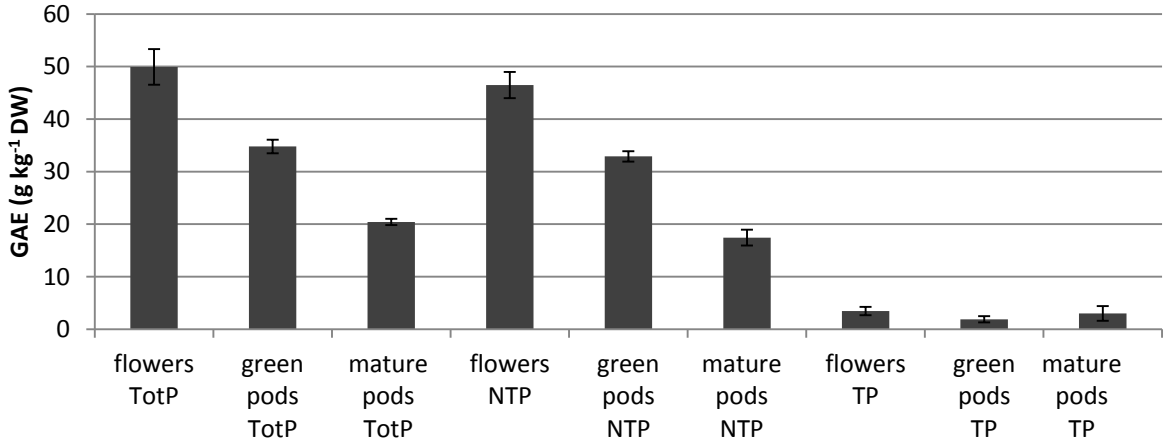

Figure 3. Polyphenolic contents in flowers and pods of tagasaste (Vertical bars indicate standard deviations of means).

Table 7. Correlations (R^2) established between total phenolics (TotP), non-tannic phenolics (NTP), tannic phenolics (TP), flavonoids (TotF) and antioxidant capacity (ABTS, DPPH) in tagasaste.

Parameter	ABTS						DPPH					
	Nov	Jan	Mar	May	Jul	Sep	Nov	Jan	Mar	May	Jul	Sep
TotP	0.918**	0.855**	0.784**	0.746**	0.892**	0.987**	0.991**	0.880**	0.836**	0.886**	0.940**	0.971**
NTP	0.902**	0.855**	0.697**	0.809**	0.886**	0.961**	0.989**	0.860**	0.793**	0.889**	0.925**	0.995**
TP	0.914**	0.451ns	0.789**	0.271ns	0.869**	0.634ns	0.868**	0.710*	0.634*	0.445*	0.934**	0.489ns
TotF	0.919**	0.849**	0.917**	0.830**	0.810**	0.956**	0.981**	0.914**	0.766**	0.804**	0.849**	0.984**

** Significance level at P ≤0.001, * Significance level at P ≤0.05.

of Sardinia, crude protein level of standing hay (that is, ungrazed dry residuals) decreased to 4% in summer, with relevant reduction in nutritive value (Sulas et al., 1995). It is worth nothing that the lowest CP values recorded in tagasaste leaves and twigs during summer were 17.2 and 7.2%, respectively. Moreover, only in January, the RFV of tagasaste leaves was less than 151 value, which is indicative of a prime forage. In addition, our study evidenced that tagasaste pods represent a complement instead of an alternative to green leaves and twigs on offer. In contrast to herbaceous plants, browse has the advantage of maintaining a sufficient nutritional level of protein, digestible fibre and minerals during critical periods of the year (Decandia et al., 2008). Another weak point linked with annual forage crops management deals with the possible delay in their establishment and subsequent first utilization by ruminants (Ligios et al., 2000) that are both affected by the occurrence of late summer rains. Based on the obtained results, tagasaste can be regarded as a strategic and inexpensive green fodder and protein source for ruminants.

Our study has indicated variations regarding phenolics and antioxidant capacity in edible biomass and sampling times (Tables 5 and 6). The highest antioxidant capacity of leaves was recorded in late summer and it was 2 to 5 times higher than in the other sampling times. The highest antioxidant capacity found in tagasaste leaves was twice as high as in leaves of forage chicory (Piluzza et al., 2014), but similar to values found in leaves of *Mirtus communis* L. populations (Melito et al., 2016). On the other hand, leaves of other Mediterranean perennial shrubs, such as *Cistus creticus* L., and *Pistacia lentiscus* L., showed higher values of antioxidant capacity (Piluzza and Bullitta, 2011), but the latter with known limitations in terms of intake and digestibility (Decandia et al., 2000). As late summer is a very critical period for animal feeding reared in rainfed Mediterranean farming systems, the above mentioned peaks of antioxidant capacity from tagasaste represent a valuable trait of this species.

Ver Elst and Pieterse (2006) studied the biomass composition as a factor influencing the possible utilization as mulches of eight legume species, including tagasaste. They evaluated parameters such as lignin, polyphenols, cellulose and hemicellulose contents; the content of polyphenols was 3.47% and similar levels were also indicated by Ventura et al. (2012). Our results showed a higher content in leaves during the different cutting times. Kumara Mahipala et al. (2009b) found a content of total

polyphenols of 44 g kg^{-1} and total tannin of 8.9 g kg^{-1} in tagasaste grown in Western Australia. The present study reported similar results for total polyphenols in the cutting of January (48.5) and May (49.7) in leaves; for tannic phenolics similar results were at the May cutting (9.4) in the leaves.

The levels of TotP, NTP and TP in tagasaste leaves were 3 to 6 times higher than values found in a range of Mediterranean annual grass, legume and herb species such as *Lolium rigidum* Gaud., *Medicago polymorpha* L., *Medicago arabica* L., *Plantago lanceolata* L., *Trifolium cherleri* L. and *T. resupinatum* L. (Cabiddu et al., 2013). The levels of TotP, NTP found in Mediterranean populations of forage chicory (Piluzza et al., 2014) were half than those reported for tagasaste. Karimi et al. (2013) reported TotP and TotF contents of 37 and 12.6 mg g^{-1} DM, respectively in leaves of *Medicago sativa* L., investigated as medicinal plant; these values were markedly lower than our results, but they were obtained with a methanol extraction.

Overall, our findings agree with previous studies that document the relationship between antioxidant activity and total phenolic compounds in different plants (Salama et al., 2012; Hariprasanna et al., 2015; Sulas et al., 2016). To the best of our knowledge, this study provides new insights into quality and antioxidant activities in all edible biomass organs of tagasaste. The results represent basic information not available so far, and could be useful for a proper exploitation of tagasaste, for complementing forage resources already available in the local context.

Conclusions

Season affected composition of edible biomass, chemical composition and antioxidant capacity of leaves and twigs. The highest antioxidant capacities of both leaves and twigs were detected in late summer. At each sampling, antioxidant capacity was correlated with TotP, NTP and TotF. Results highlight that tagasaste has remarkable potential for improving animal nutrition and welfare and the overall high quality of its fodder may be exploited for compensating typical seasonal feed shortages associated to drought seasons. Such results encourage further investigations dealing with the chemical characterization of its phenolic compounds for contributing to a full valorisation of this leguminous shrub as source of natural antioxidants. Additional research is required to set up a proper management of the tagasaste crop, taking into account the available forage resources and chains in each context.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Trichoderma-bean interaction: Defense enzymes activity and endophytism

Omari Dangelo Forlin Dildey*, Laline Broetto, Bruna Broti Rissato, Edilaine Della Valentina Gonçalves-Trevisoli, Sidiane Coltro-Roncato, Emanuele Guandalin Dal'Maso, Cristiane Claudia Meinerz, Nicanor Pilarski Henkemeier, José Renato Stangarlin, Odair José Kuhn and Tulya Fernanda Barrientos Webler

State University of West Paraná - UNIOESTE, Marechal Cândido Rondon, Paraná, Brazil.

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Biotic inducing agents such as Trichoderma spp. are an alternative for the induction of resistance by activating defense mechanisms of plants. This work aimed to study the interaction between bean and Trichoderma spp. evaluating the effects of the induction of resistance in bean through the defense enzymes activity and ability to endofitism. The experimental was design in randomized blocks, with four replications, being 21 isolates of Trichoderma spp. and control. The isolates were inoculated at planting, with adding 0.8 ml of the suspension 1×10^8 conidia/ml in groove, in sterilized soil. After 30 days, leaf and roots samples were taken to determine the enzyme activity. To verify the endophytic colonization capacity of isolated, bean root fragments were sanitized and placed in Petri dishes containing PDA medium. Data were submitted to analyzes of variance and compared by Scott-Knott test ($p<0.05$). The enzymatic activity of peroxidase, polyphenoloxidase and phenylalanine ammonia-lyase was not significant, but the β-1-3-glucanase activity in leaf tissue was suppressed by isolates TI1, TM1, TLB15 (*Trichoderma virens*), TI2, TLB3, TLB4, TLB12, TOD1, TOD3 (*Trichoderma harzianum*), TLB6 (*Trichoderma asperellun*), TLB14, TLB17 (*Trichoderma koningiopsis*) and TOD2B (*Trichoderma longibrachiatum*). The isolates TM4, TLB9, TLB15 (*T. virens*), TI2, TI4, TLB2, TOD1 (*T. harzianum*) and TLB17 (*T. koningiopsis*), were able to colonize endophytically the bean roots. Trichoderma has endophytic capacity and interfere in β-1-3-glucanase activity.

Key words: Induced resistance, *Phaseolus vulgaris* L., Trichoderma spp.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is exposed to factors that causes reduction on productivity and final quality of grain. Brazil is one of the major producer and consumer of this legume, with national production of 3,185,400,000 tons in the 2014/1015 harvest (Conab, 2015). However, there are several phytosanitary

*Corresponding author. E-mail: omaridildey@hotmail.com. Tel: +55(45) 32847878. Fax: +55(45) 32847879.

Table 1. Name, specie of the isolated used on trials.

Isolated	Specie
TI1	*Trichoderma virens*
TI2	*Trichoderma harzianum*
TI3	*Trichoderma virens*
TI4	*Trichoderma harzianum*
TM1	*Trichoderma virens*
TM2	*Trichoderma virens*
TM3	*Trichoderma virens*
TM4	*Trichoderma virens*
TLB2	*Trichoderma harzianum*
TLB3	*Trichoderma harzianum*
TLB4	*Trichoderma harzianum*
TLB6	*Trichoderma asperellum*
TLB9	*Trichoderma virens*
TLB12	*Trichoderma harzianum*
TLB14	*Trichoderma koningiopsis*
TLB15	*Trichoderma virens*
TLB17	*Trichoderma koningiopsis*
TOD1	*Trichoderma harzianum*
TOD2A	*Trichoderma longibrachiatum*
TOD2B	*Trichoderma longibrachiatum*
TOD3	*Trichoderma harzianum*

problems, that have great destructive potential, causing enormous losses in production of the country.

The Trichoderma is a fungus naturally found in different types of soils, living saprophyte and parasitizing other fungi, behaving as an antagonist to various phytopathogens, being the most searched fungus nowadays, once they are able to act as biological control agents, growth promoters and plant disease resistance inducers (Louzada et al., 2009).

Biological control is considered to be one of the method that may occur simultaneously throughout the antagonist life cycle, whose capacity can induce the synthesis of substances with antagonistic action by producing secondary metabolites which inhibit or stimulate the development of a microorganism or plant. In addition to biological control, there may be resistance induction, in which the plant actives defense mechanisms, signaled by elicitors of biotic and abiotic origin (Papavizas, 1985; Ridout et al., 1988).

The interaction between plant and fungus may have endophytic relations, establishing symbiotic relations still not well understood, but of great importance for plants (Azevedo, 1998). In interactions, the microorganisms produce or induce the production of secondary metabolites, which can provide many benefits, such as inducting resistance by the activation of defense mechanisms present at low levels in plant (Hammerschmidt and Dann, 1997; Schwan-Estrada et al., 2000).

Thus, the research aimed to study the interaction of different isolates of Trichoderma spp. in bean plants, by the effect on enzymes activity related to plant defense and endophytic capacity.

MATERIALS AND METHODS

The experiment was conducted in laboratory and greenhouse. Isolates of Trichoderma spp., from native forest soils and of agricultural property in system of organic production, were isolated by the bait method (Ethur et al., 2005) and identified in species level by the sequencing region method internal transcriberd spacer (ITS) and part of gene elongation fator (EF) gene (Table 1).

To prepare the Trichoderma spp. inoculum, potato dextrose agar (PDA) discs having mycelium of each isolates, were transferred to erlenmeyers containing rice (20 g of rice to 25 ml of water and autoclaved for 20 min at 120°C), then, were incubated at 25 ± 2°C at photoperiod of 12 h, according to Silva et al. (2011) with adaptations.

After seven days, conidia suspension was prepared with 50 mL of sterile and distilled water, added to the Erlenmeyer to make rice washing. The suspension was filtered in gauze and the concentration was adjusted to 1×10^6 conidia mL^{-1}, with the aid of a Neubauer chamber.

The experiment was conducted in plastic pots for up to three liters, which contained soil, sand and organic compound in a proportion of 3: 2: 1, autoclaved for 25 min at 120°C, repeating the operation in 24 h.

Seed were treated by the suspension of Trichoderma spp. isolates at planting, by application of 0.8 ml of the adjusted suspension (Broetto, 2013). In each pot were seeded two bean seeds of the cultivar IAPAR 81 (Iapar, 2015).

The experimental design was in randomized blocks, being 22 treatments with four replications (21 Trichoderma spp isolates and a control (without Trichoderma spp. inoculation)), deployed in 88 experimental plots.

For carrying out the biochemical analysis, 30 days after the emergence of bean the samples were collected samples from leaf and root tissue, wich were weighed and packed in aluminum envelopes, frozen and kept in a freezer at -20°C until the moment of analysis.

Samples from leaves and roots (0.5 g) were macerated mechanically in 4 mL of sodium acetate buffer 100 mM (pH 5.0), being added 0.04 g of polyvinylpyrrolidone. The mixture was centrifuged at 20.000 g for 25 minutes at 4°C. The supernate was taken as the enzyme extract and frozen at -20°C for determination of peroxidase (POD), polyphenoloxidase (PPO), phenylalanine ammonia-lyase (PAL) and β-1,3-glucanase (β-GAZE) activities (Lusso and Pascholati, 1999).

The POD activity was determined by direct spectrophotometric method, by the measurement of the conversion of guaiacol in tetraguaiacol to 30ºC, with spectrophotometric reading at 470 nm, for a period of 2 minutes, with optical density recorded every 15 s (Hammerschmidt et al., 1982). The reaction mixture contained 100 µL of the enzyme preparation and 900 µL of solution (12.5 mL of guaiacol and 310 µL of hydrogen peroxide in 87.5 ml of sodium phosphate buffer 0.01 M (pH 6.8)). The activity was expressed by the variation between the values in the linear increase range (E.U. (enzyme unit) min^{-1} g^{-1} fresh weight).

The PPO activity was determined using the methodology of Duangmal and Apenten (1999), adapted by Kuhn et al. (2010). The assay measured the catechol oxidation converted into quinone, reaction measured by the enzyme PPO. The substrate was composed by catechol in a concentration of 0.002 M, dissolved in sodium phosphate buffer 0.01 M (pH 6.8). The reaction was developed by mixing 900 µL of substrate and 100 µL of enzymatic

preparation, with readings in spectrophotometer at 420 nm, for a a period of one minute, with optical density recorded every 15 s. The activity was determined by the variation between the values in the linear increase range E.U. (enzyme unit) min^{-1} g^{-1} fresh weight.

The PAL activity was determined by quantitation of trans-cinnamic acid released from the substrate phenylalanine; the methodology is described by Umesha (2006), in which 50 µL of the enzyme preparation were added to 450 µL of Tris-HCl 0.025 M buffer (pH 8,8) and 500 µL of a solution L-phenylalanine 0.05 M, incubated at 40°C for 2 h. The reaction was stopped by adding 60 µL of HCl 5 M, with read in a spectrophotometer at 290 nm. The PAL activity constitutes of the difference between the absorbance of the mixture and of the control (100 µL of protein extract and 900 µL of Tris-HCl 0.025 M buffer (pH 8.8)), which was guinea fowl in the standard curve to trans-cinnamic acid (y = 0.0095x + 0.0255, where y is the absorbance at 290 nm and x is the concentration of trans-cinnamic acid (µg)), expressed in µg trans-cinnamic acid h^{-1} g^{-1} fresh weight.

The β-GAZE activity was determined by colorimetric measurement of reducing sugars released from the laminarin (Vogelsang and Barz, 1993). The reaction involves 100 µL of the enzyme preparation, 50 µL of sodium phosphate buffer 0.01 M (pH 6.0) and 150 µL of laminarininarina (2 mg mL^{-1}), which was developed at 40 ° C for one hour. In control, laminarin was added after the incubation. The sugars formed were quantified by Lever Method (1972), which consists of extracting an aliquot part of 30 µL of the previous solution and adding to this 1.5 mL of hydrazine solution of the p-hydroxybenzoic acid 0.5% (PAHBAH). The mixture was maintained at 100°C for 10 min, cooled on ice for 10 min, and the absorbance determined at 410 nm. The β-1,3-glucanase activity was the difference between the absorbance of the mixture containing the sample and control, which was painted in standard for glucose curve (y = 0.002x + 0.0046, where y is the absorbance and x the concentration of reducing sugars (µg)) and expressed as glucose equivalent μg^{-1} mL^{-1} h^{-1} g^{-1} fresh weight.

After collecting the roots samples for biochemical analysis, a portion of 10 cm of root from each plant was washed to evaluate the ability of Trichoderma spp. isolates, about its endophytic colonization capacity. This was performed by the indirect method of isolation, using root fragments of 1.5 cm, superficially disinfected with 70% alcohol for one minute, immersed in 3% sodium hypochlorite for one minute and washed three times in distilled autoclaved water. Then, three fragments were transferred into Petri plate, containing PDA médium, in three repetitions. The petri dishes were incubated in BOD at 25 ± 2 ° C with a photoperiod of 12 hours. The evaluation was made by viewing the Trichoderma spp. growth, from the root.

Once tabulated, the data were submitted to analysis of variance, and the averages compared by Scott-Knott, at 5% probability, with the assistance of statistical software version 5.3 Sisvar (Ferreira, 2010).

RESULTS AND DISCUSSION

Despite the importance of Trichoderma sp. and research for resistance induction, just a little is understood about the relationship of microorganism-plant interaction of this fungus in the bean, by the fact of the paucity of studies involving the mechanisms by which Trichoderma spp. interacts, activating the biochemical defense systems of bean plants. Several hypotheses are presented, but few of them have been cogent, which are evaluated by chemical and cytological searches from plant tissues presenting these fungal agents (Hanson and Howell, 2004).

According to Table 2, the results of the enzymatic activity of POD, PPO and PAL in the leaves and roots showed no statistical difference between isolated of Trichoderma spp., but, the β-GAZE was reduced depending on the isolated, presenting statistical difference in bean leaves.

The POD activity in bean, even with no statistical difference between the isolated, can observe a tendency of suppression by the isolated TM1, TM4, TLB15 (*T. virens*), TOD2A (T. longibrachiatum) and TOD3 (*T. harzianum*) on the expression of this enzyme activity in leaf tissue of bean, as compared to control.

Similarly, the isolated TI1, TLB15 (*T. virens*), TOD2B (T. longibrachiatum) and TLB6 (T. asperellum), suppressed the enzymatic activity in root tissue. However, the isolated TM3 (*T. virens*) and TI2, TLB4 (*T. harzianum*), showed a tendency to increase the POD enzyme in bean plants, compared to the control.

Similar results for POD were obtained by Silva et al. (2011), that observed the effect of 60 isolated of Trichoderma spp. on growth promoting and systemic resistance induction to anthracnose in cucumber plants. They found no significant difference regarding the activation of the PPO enzyme in the plant between the 7^{th} and 14^{th} day after treated with Trichoderma spp., as compared to control.

However, Yedidia et al. (1999), working with hydroponic cucumber plants inoculated with Trichoderma, observed an increase in the POD enzyme after the time interval until seven days. However there was no increase in activity at 7 and 14 days after treatment, while the untreated plants showed a gradual increase over time, it was observed increase in plant growth, being confirmed, for the first time, the direct impact of this fungus in the interaction plant-fungus.

According to Yedidia et al. (1999), the increase in POD enzyme in leaves observed in their study, may be dued to either a systemic defense response, as the presence of Trichoderma in the rhizosphere, probably decrease of the enzymatic activity could be temporarily related to the root colonization by different isolated of Trichoderma spp.

A similar result was obtained by Dildey et al. (2013), wich have observed no POD and PPO activity induction in bean plants against the pathogen *Macrophomina* phaseolina, in inoculation of seeds with different isolates of *T. harzianum*. However, even it does not demonstrate significant results for enzymeshere, there was a tendency of such, on one of the different isolated of Trichoderma compared with the treatent control. From the results, the POD activity is not necessarily involved in this process, but further defense mechanisms such as chitinases, β-GAZE among others, may be acting in the induction of resistance of bean plants.

For the PPO activity, the results of this research showed no statistical difference, however, the isolated TI1, TI3, TLB15, TM4 (*T. virens*), TOD2A, TOD2B (*T.*

Table 2. Peroxidase activity (POD - unit of enzyme $^{-1}$ min^{-1} g^{-1} fresh tissue), polyphenoloxidase (PPO - unit of enzyme $^{-1}$ min^{-1} g^{-1} fresh tissue), phenylalanine ammonia-lyase (PAL - µg^{-1} trans-cinnamic acid h^{-1} g^{-1} fresh tissue) and β-1,3 glucanase (β-GAZE - µg^{-1} h^{-1} g^{-1} fresh tissue) in roots and leaves of bean in the treating seed with *Trichoderma* spp. isolated, 30 days after germination.

Peroxidase activity								
Isolated	**Leaf**				**Root**			
	POD	**PPO**	**PAL**	**β-GAZE**	**POD**	**PPO**	**PAL**	**β-GAZE**
TI1	1066.22[a]	438.72[a]	31.48[a]	869.70[a]	776.85[a]	655.15[a]	51.54[a]	1876.52[a]
TI2	973.85[a]	593.90[a]	81.43[a]	1318.42[a]	1120.42[a]	566.82[a]	44.53[a]	1678.07[a]
TI3	779.27[a]	506.65[a]	21.25[a]	1546.45b	924.02[a]	848.20[a]	58.63[a]	1592.40[a]
TI4	850.67[a]	516.97[a]	30.03[a]	1633.8b	864.85[a]	569.87[a]	65.83[a]	1630.20[a]
TM1	747.05[a]	629.37[a]	43.11[a]	1234.85[a]	879.32[a]	643.27[a]	60.32[a]	1640.30[a]
TM2	827.52[a]	601.92[a]	97.78[a]	1527.75b	1018.20[a]	852.25[a]	42.87[a]	1883.07[a]
TM3	1065.47[a]	581.02[a]	162.43[a]	1873.90b	1226.87[a]	741.62[a]	50.75[a]	1864.10[a]
TM4	689.47[a]	537.27[a]	36.40[a]	1698.47b	819.25[a]	500.25[a]	39.15[a]	1356.00[a]
TLB2	911.25[a]	512.05[a]	94.99[a]	2064.77b	1080.15[a]	655.22[a]	49.71[a]	1602.47[a]
TLB3	1069.52[a]	601.85[a]	71.69[a]	1302.17[a]	1040.37[a]	566.27[a]	110.46[a]	1833.70[a]
TLB4	1045.30[a]	590.30[a]	38.95[a]	1415.10[a]	1121.90[a]	775.07[a]	48.95[a]	1704.45[a]
TLB6	1025.45[a]	598.65[a]	57.22[a]	1395.10[a]	746.45[a]	715.12[a]	67.54[a]	1907.62[a]
TLB9	973.52[a]	662.97[a]	64.39[a]	2045.00b	922.75[a]	673.80[a]	92.21[a]	2050.42[a]
TLB12	944.85[a]	549.72[a]	32.47[a]	1033.17[a]	878.35[a]	671.85[a]	71.14[a]	2184.27[a]
TLB14	911.07[a]	583.15[a]	48.25[a]	1322.77[a]	988.47[a]	605.30[a]	38.46[a]	1791.10[a]
TLB15	800.97[a]	418.45[a]	71.75[a]	1308.60[a]	641.70[a]	546.20[a]	58.21[a]	1803.82[a]
TLB17	1049.35[a]	613.80[a]	70.71[a]	1263.40[a]	854.45[a]	700.82[a]	54.02[a]	2118.07[a]
TOD1	1043.97[a]	581.95[a]	100.60[a]	1366.50[a]	982.92[a]	522.27[a]	56.49[a]	1771.67[a]
TOD2A	922.87[a]	439.45[a]	41.02[a]	1831.80b	933.87[a]	595.92[a]	70.58[a]	1960.02[a]
TOD2B	733.80[a]	427.17[a]	17.91[a]	1187.20[a]	714.12[a]	632.15[a]	71.45[a]	2093.87[a]
TOD3	827.20[a]	519.42[a]	77.40[a]	1126.05[a]	828.82[a]	553.22[a]	66.46[a]	2252.90[a]
Controle	1014.50[a]	638.12[a]	111.30[a]	1747.75b	890.17[a]	644.25[a]	64.07[a]	2195.00[a]
(CV%)	30.50	32.80	125.40	32.90	37.70	30.50	47.18	26.80
DMS	747.52	481.48	212.34	1274.71	921.97	523.91	75.90	1319.19

TI1, TI3, TM1, TM2, TM3, TM4, TLB9 and TLB15 (*T. virens*); TI2, TI4, TLB2, TLB3, TLB4, TLB12, TOD1, TOD3 (*T. harzianum*); TLB6 (*T. asperellum*); TLB14, TLB17 (*T. koningiopsis*); TOD2A, TOD2B (*T. longibrachiatum*).* Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% significanceControl: soil without *Trichoderma* spp. Transformed data by √x+1.0.

longibrachiatum) and TI4, TLB2 (*T. harzianum*), had a suppression tendency of PPO enzymatic activity, compared with the control, for the leaf tissue.

For the PPO activity in the root tissues of bean plants, they have shown an increasing trend for the isolated TI3, TM2, TM3 (*T. virens*) and TLB4 (*T. harzianum*), however it can be seen that the isolated TLB15, TM4 (*T. virens*) and TI2, TI4, TLB3, TOD1, TOD3 (*T. harzianum*), suppressed this enzyme activity.

Pereira et al. (2008), working with chitosan extracted from Trichoderma spp. and Rhizopus sp., obtained a protective effect on cocoa plants, by noting that the application of biotic inducers are able to promote increased in POD and PPO enzymes activity, in addition to reducing Verticillium-wilt.

According to Pinol and Palazón (1996), in some cases, the polyphenols metabolism may act as antioxidant, leading to the inactivation of fenolases enzyme production. In another aspect, the biotic biosynthesis of secondary metabolites is attached the plant genetic constitution determining the activation of genes that synthesize the enzymes.

The PAL activity, showed no significant difference in bean plants. But, for leaf tissue, it can be noticed a sharp increase in the enzymatic activity of isolated TM3 (*T. virens*) compared to the control. On the other hand, all isolated present a suppression tendency for this enzyme, being this tred more evident in isolated TI1, TI3, TM4 (*T. virens*), TI4, TLB4, TLB12 (*T. harzianum*) and TOD2B (T. longibrachiatum), with no statistical difference

In expression of this enzyme in the root system, it is observed a increase tendency for isolated TLB3 (*T. harzianum*) and TLB9 (*T. virens*), however, the isolated TI1, TM2, TM3, TM4 (*T. virens*), TI2, TLB2, TLB4 (*T. harzianum*), TLB6 (T. asperellum) and TLB14 (T. koningiopsis), showed suppression in PAL enzymatic activity and no statistical difference.

Karthikeyan et al. (2006), evaluated the induction of

Table 3. Presence (+) and absence (-) of endophytic colonization in bean roots by *Trichoderma* spp. isolated, 30 days after germination.

Colonization in bean roots		
Isolated	**Specie**	**Colonization**
TI1	*T. virens*	-
TI2	*T. harzianum*	+
TI3	*T. virens*	-
TI4	*T. harzianum*	+
TM1	*T. virens*	-
TM2	*T. virens*	-
TM3	*T. virens*	-
TM4	*T. virens*	+
TLB2	*T. harzianum*	+
TLB3	*T. harzianum*	-
TLB4	*T. harzianum*	-
TLB6	*T. asperellum*	-
TLB9	*T. virens*	+
TLB12	*T. harzianum*	-
TLB14	*T. koningiopsis*	-
TLB15	*T. virens*	+
TLB17	*T. koningiopsis*	+
TOD1	*T. harzianum*	+
TOD2A	*T. longibrachiatum*	-
TOD2B	*T. longibrachiatum*	-
TOD3	*T. harzianum*	-
Control*		-

*Soil without *Trichoderma* spp.

phenol compounds, the defense enzymes involved in phenylpropanoid pathway and the protein-PR accumulation against Ganoderma in reply to the application of Pseudomonas fluorescens, *T. viride* and *T. harzianum* combined with chitin. The authors observed a high increase in PAL activity in all treated palms following application of P. fluorescens, *T. viride* and chitin. The highest level was reached 3 days after treatment and, then, declined slowly.

According to De Meyer et al. (1999) the rhizosphere colonization by P. aeruginosa, produced PAL activity in bean roots and, consequently, increased the salicylic acid level in the leaves.

As stated by Campos et al. (2003), a complex response wich involves production of phytoalexins and isoflavonoids may make a different role in the related response for the enzyme PAL induction. In addition of there being a complex response, involving phytoalexins and isoflavonoids production, it may play a different role in the response related to induction of that enzyme.

On the other hand, Nicholson and Hammerschmidt (1992), reported that the increase in PAL activity might be directly involved with a biochemical marker for induced resistance, once this enzyme is considered essential for the phenols synthesis, being important for resistance, enabling occurrence of a specific interaction between plant and inducer microorganism.

For β-GAZE activity, there was significant difference for the results of leaf tissue treated with the isolated TI1, TM1, TLB15 (*T. virens*), TI2, TLB3, TLB4, TLB12, TOD1 and TOD3 (*T. harzianum*). The isolated TLB6 (T. asperellun), TLB14, TLB17 (T. koningiopsis), TOD2B (T. longibrachiatum) suppressed the this enzyme activity. In tissue root system there was no difference, but was observed a more evident suppression trend in the isolated TM4 (*T. virens*).

According to Saksirirat et al. (2009), by working with fifteen isolated of Trichoderma spp. to control Xanthomonas campestres pv. vesicatoria there was a decrease of 69,32% of the symptoms, showed that four isolated of species *T. harzianum* e T. asperellum, induced activity of β-GAZE and chitinase in the leaves, showing that these species are promising for resistance induction in tomato plants. The same autors, They report that these enzymes are known to play an important role in the degradation of fungus cell wall. Besides that, are related to pathogenesis-related proteins (PR proteins) in several plants, including beans (Van Loon and Van Stien, 1999).

As stated by Salzman et al. (1998), these induced proteins are not always present during the infection process, they can be found in healthy tissue of certain plant part, as leaves or roots, or at specific developmental stages.

According to Akrami et al. (2011), several factors may interfere in the efficiency of biotic inducing agents among them the abiotics, mainly temperature, humidity and soil pH, wich are considered crucial on inducer efficiency, ensuring a successful performance of Trichoderma spp. isolated.

Among the 21 isolates of Trichoderma spp. evaluated for the colonization of bean plants roots, it was verified that 8 isolated feature the endophytism capacity in roots (Table 3), The results show that these isolates can be considered endophytic microorganisms

These data confirm those of Benítez et al. (2004), wich found that isolated of Trichoderma spp. are able of colonizing roots, producing secondary compounds that confer stimulate to plant growth by, as well as, induce resistance, by activation of plant defense mechanism. The same has been verified by Pedro (2012), that working with Trichoderma spp. isolated, in growth stimulating and anthracnose resistance induction in bean plants, showed that seven isolated of Trichoderma spp. evaluated were able to colonize the seedlings roots, being possible to check their growth on petri dishes, from the roots.

The gender Trichoderma may be associated with the roots of several plants of several species. Its penetration and colonization, associated with its antibiosis capacity, enhances an hydrolytic enzymatic arsenal, degrader of

the plant cell wall. This input on epidermal layers of the roots confers a higher growth, improving nutrient absorption, and, thereby, increasing plant productivity (Harman et al., 2004).

According to the results of the present work, the reduction of enzymatic activity verified in bean plants inoculated with Trichoderma spp., is not very well clarified, it is believed that the Trichoderma spp., stimulates the plant to the equilibrium of its enzymatic arsenal, for the sake of its own benefit, so that, the plant will not endanger the symbiotic relationship between its interaction with the Trichoderma spp., because in case of the plant be stimulated to produce defense enzymes, Trichoderma, probably, would be hampered by the degradation of its structures and its cell death would be occurred.

Lucon (2009), verify that, in the absence of phytopathogens, the action of the mechanisms may be related to the production of hormones or growing factors, increase in the availability and absorption of nutrients or efficiency in the use of some nutrients by the plant.

These same Trichoderma spp. isolated, tested in this study, were used for Broetto (2013), which verified the effectiveness of the isolated TLB2, TLB3, TLB12 and TLB17 in bean against M. phaseolina. Also, stood out isolated TI1, TM3, TLB3, TLB12 and TLB17 as good growth promoters of the bean plants, whereas, the isolated TLB12 presented highlighted grain yield.

Several studies have been conducted to extend the knowledge of the action mechanisms by the biocontrol in diverse cultures. It is believed that, to the extent that the potential of these mechanisms will getting to know, in interactions with plants and microorganisms, lead to promising expectations for use in agriculture, as well as contribute towards the environment. preservation.

Conclusion

Isolated of Trichoderma TI1, TM1, TLB15 (*T. virens*), TI2, TLB3, TLB4, TLB12, TOD1, TOD3 (*T. harzianum*), TLB6 (*T. asperellun*), TLB14, TLB17 (*T. koningiopsis*) and TOD2B (*T. longibrachiatum*), suppressed the β-1,3-glucanase activity in leaf tissue. Neither of the isolated interfered in the activity of peroxidase, polyphenoloxidase and phenylalanine-ammonia-lyase. Isolated of *Trichoderma* spp., TM4, TLB9, TLB15 (*T. virens*), TI2, TI4, TLB2, TOD1 (*T. harzianum*) and TLB17 (*T. koningiopsis*), colonized the bean root in endophytic form.

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Development of an automated system of aeration for grain storage

Arlindo Modesto Antunes*, Ivano Alessandro Devilla, Antônio Cruvinel Borges Neto, Beethoven Gabriel Xavier Alves, Guiliano Rangel Alves and Mateus Morais Santos

Department of Agricultural Engineering, State University of Goiás, 75132-400, Anápolis – GO, Brasil.

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Stored grain should be preserved as much as possible during storage process, because of the occurrence of chemical, biochemical, physical and microbiological changes. The grain storage process consists of storing this material for a later use after a certain period. This study aims at to develop a prototype for grain aeration; to develop a software to control aeration strategies; and to evaluate the development of strategies for controlling the aeration of stored corn. The metallic silo is built in a cylindrical shape (0.9 m diameter and 3 m high), with a fully perforated bottom, which was adapted to an aeration system (plenum, axial fan, with three-phase motor of 1.47 kW and PVC pipe Ø 0.10 m). The silo was externally isolated using glass wool (0.06 m) thick, and the airflow controlled and determined in 0.1 m^3 min^{-1} t^{-1}, where t is ton of grain. To work with data relating to the experiment, it was necessary to use sensors, positioned at the center of the silo (spaced 0.5 m), from the plenum. The sensors were connected to an Arduino board, in order to register the temperature of grain mass. After the installation of the sensors in the silo, this was loaded and the surface of the grain mass was leveled. Control strategies were implemented: Continuous aeration; control of dry bulb temperature; control via timer and temperature control via temperature difference between the grain and the ambient air. Operating results obtained with the software developed, were effective for control of grain aeration processes stored in the prototype. In conclusion, the system for automated aeration of stored grain was effective in the storage of grain and the control strategy, aeration of grain via the temperature difference between the grains and the environment and night aeration were the best for the aeration process in region of Anápolis - GO.

Key words: Storage, grain cooling, prototype, Arduino.

INTRODUCTION

The grain storage date centuries, with biblical references when Joseph advised Pharaoh to store all grain produced in Egypt in times of plenty, to ensure the supply of these grains in the Egyptian cities in extreme drought time. The

*Corresponding author. E-mail: arlindo.modesto1@hotmail.com

grain storage is then used to store the product for consumption after a certain time period (Araújo et al., 2012).

It is known that after harvesting the grains pass through a series of processes until it reaches the final consumer. Some of these operations include: Receiving, cleaning, drying and storage, handling, among others. According to Baal (2014), these operations are called pre-processing of grain, and the places where they are held are called grain packing houses. Usually grain processing sites are also composed of storage structures, so these sites can be called units processing and grain storage.

Stored grain should be preserved to the maximum during storage, due to the occurrence of chemical, biochemical, physical and microbiological changes. The speed and intensity of these processes depend on the intrinsic quality of the grain, the pre-processing operations, the storage system used and the prevailing environmental factors during the storage period (Alencar, 2006).

The main influential factor in any process of storage, is the grain water content. Deterioration reactions occur at high humidity levels, especially enzymatic hydrolysis and oxidation of lipids, however, these reactions occur less frequently with low water content. Because of this, the water content of the grains should be controlled in storage (Furquim et al., 2014).

Moisture migration varies with respect to the season. During periods of winter and autumn, the beans located near the silo walls and on top of the grain mass are cooled more easily than those located at the bottom of the silo. After some time due to the mass of the temperature gradient grains, convective currents are generated. That is, the air cold and dense intergranular located near the bin wall is pulled down, flowing through the center of the silo and driving up the warm, less dense air initially locating in this region. Already in the spring and summer, the temperature of the grain near the walls of the silo and increase grain located in the center of the facility remain cold. In these periods the convective currents change their direction. The cold, denser air, located in the center of the silo, flows down, resulting in a movement of the convective currents from the silo center towards their side (Muir and Jayas, 2014; Vasconcellos, 2012)

Currently, aeration is the most widespread control method employed in the preservation of stored grain. Aeration is the forced passage of the ambient air through the grain mass in such a way as to modify the microclimate intergranular voids, creating unfavorable conditions for the development of organisms that influence the preservation of the quality of grain. However, if the aeration is not well managed it can cause loss of heating, fermentation and excessive loss of water content, and it is highly dependent on local weather conditions. Thus, the efficiency of an aeration system is centered on obtaining a uniform air flow in all regions of the silo. Another objective of aeration is to prevent migration and condensation of moisture that occurs whenever there is a heating at some point of the grain mass (Luiz, 2012).

The management of aeration should be implemented from the comprehensive studies on the devices to be used. The management of aeration is directly related to a control strategy, as is the drive fans based on temperature and humidity of grain and air (Lopes, 2006; Lopes et al., 2008).

Faced with the need to control and optimize the process of aeration of stored grain, activities are being developed, aiming at automating the data acquisition system and consequent reduction in the cost of the grain storage process (Kaliyan et al., 2007; Lawrence and Maier, 2011; Rigo et al., 2012).

Finally, this study is aimed at developing a computer program developed in PHP platform for control of aeration processes, build a prototype for aeration of stored grain, evaluate strategies for aeration control corn grain stored and collect cooling data and water content of corn grain stored in metal silos with different strategies of aeration being used.

MATERIALS AND METHODS

This work was developed in Drying Laboratory and Plant Products Storage, Campus of Exact Sciences and Technology, Henry Santilo, the State University of Goiás in Anápolis - Goiás. The laboratory is located in the Brazilian Central Plateau,with latitude 16°19' 36'' S and longitude: 48° 57' 10'' and altitude 1.017 m, with tropical weather and temperatures ranging from 18 to 36ºC (IMB, 2012).

In the experiments we used corn kernels (Zeamays L.), with initial moisture content of 12.6% (B.U.), acquired by the State University of Goiás in local trade. The treatment plant of corn kernels was conducted with the use of phosphine, before starting the experiment to control pests.

We used a cylindrical metal silo (0.9 m diameter and 3 m high), made of smooth material, capable of storing 1500 kg of corn grain with specific weight of 750 kg m^{-3}.

The plenum is constructed using construction materials like brick, cement and sand. Its construction is designed to enable air to enter the silo, its dimensions were (0.30 × 1.2 × 1.2 m).

When the silo Plenum, an aeration system was adapted, it consisted of radial centrifugal fan blades, with three-phase motor 1cv, consisting of sheet metal and dimensioned to provide the air flow of 6 m^3 min^{-1}. The same was placed in a device to restrict the entry of air, hence achieving the aeration air flow 0.10 m^3 min^{-1} t^{-1}.

For determining the air flow, we used the methodology described by Delmée (1982), which was used in the Pitot tube for determining the air flow in pipes. Control of fan air flow, was given by a diaphragm positioned in the suction inlet (Figure 1).

In order to achieve the temperature of the grain after the drying process, around 30°C, a device was developed for heating of stored grains (Lawrence and Maier, 2011).

The device consisted of a metal box with dimensions of 0.50 × 0.30 × 0.35 m (Figure 2). Two finned resistors (type U) with dimensions 0.45 × 0.06 m and 1 kW were installed in the metal box so that there were no losses in the water content of the grains to the external environment. A closed system was developed with the objective of recirculated air aeration and consequent gradual

Figure 1. Diaphragm coupled to the fan for restricting the flow of air.

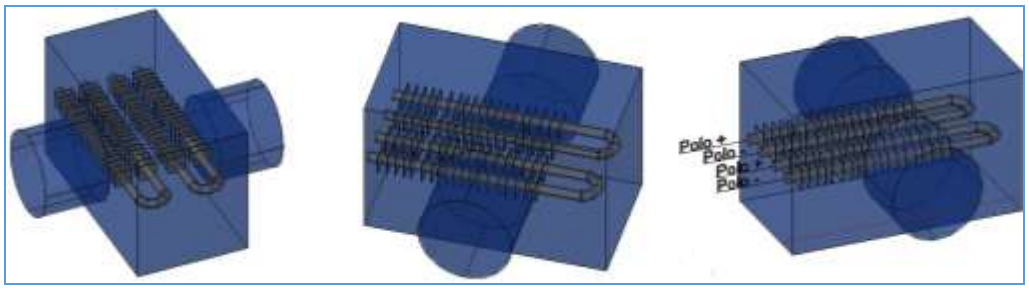

Figure 2. Device used to heat the grain mass.

increase in temperature (Lawrence and Maier, 2011). Control of the aeration system was designed by SHT 75 sensors (temperature and relative humidity) spaced 0.5 m in the grain mass with microcontroll Arduino Nano boards, relays, contacts and thermal relay, microcomputer and ventilator.

The main structure for the fan drive is guided in the components of the Arduino Nano board and Arduino 1.0.1 system, installed in the microcomputer, which controls the temperature sensors and humidity.

From the drive and return of the information from the sensors, which occurs every minute, the system turns on the fan through the relay board, which meets the criteria set out in topic 3.6 Validation of the computer program.

The Figure 3 shows the operating steps of the control system and data acquisition used to evaluate the different strategies of grain aeration process.

Reviews of developed software were performed according to the following aeration system control strategies: Continuous aeration, aeratoron 24 h, ambient air temperature control (T_{air} < 22°C ± 0.9), control via timer (aerator on from 21:00 pm to 10:00 am) and temperature control via temperature difference between the grain and the ambient air (T_{DIF} = 3°C).

RESULTS AND DISCUSSION

The storage capacity of the built silo was 1500 kg of corn grains (Zeamays L). Thus, this work was stored in 30 bags of 50 kg of maize, which were discharged vertically into the silo. The upper part of the silo has been accessed with the help of a metal ladder.

The results of the determination of the fan air flow, the pitot tube method are shown in Table 2. The average flow provided by the fan was [0.00152 ± 0.00158] $m^3 s^{-1}$. At the center pipe of 0.2 m the collected mean values were [0.00198 ± 0.0001] $m^3 s^{-1}$, as desired values (Silva et al., 2000).

The pitot tube was placed at various depths within the tube (PVC) of 0.2 m, which is being shown in Table 1 in reading position. As Macintyre (1990), the readings were spaced at 0.02 m.

The device comprises two finned resistors (type U) of 1 kW work properly when the resistors are arranged in series and connected to a cable 2.5 mm in diameter. The heating system resulted in temperatures up to 40°C, and monitored by digital thermo-hygrometer Cycloar arranged after the air outlet of the heater device.

In Table 2, to start the process, the average initial temperatures were not fixed, only the final average temperature, 30.0 ± 2.0. The various heights of the silo

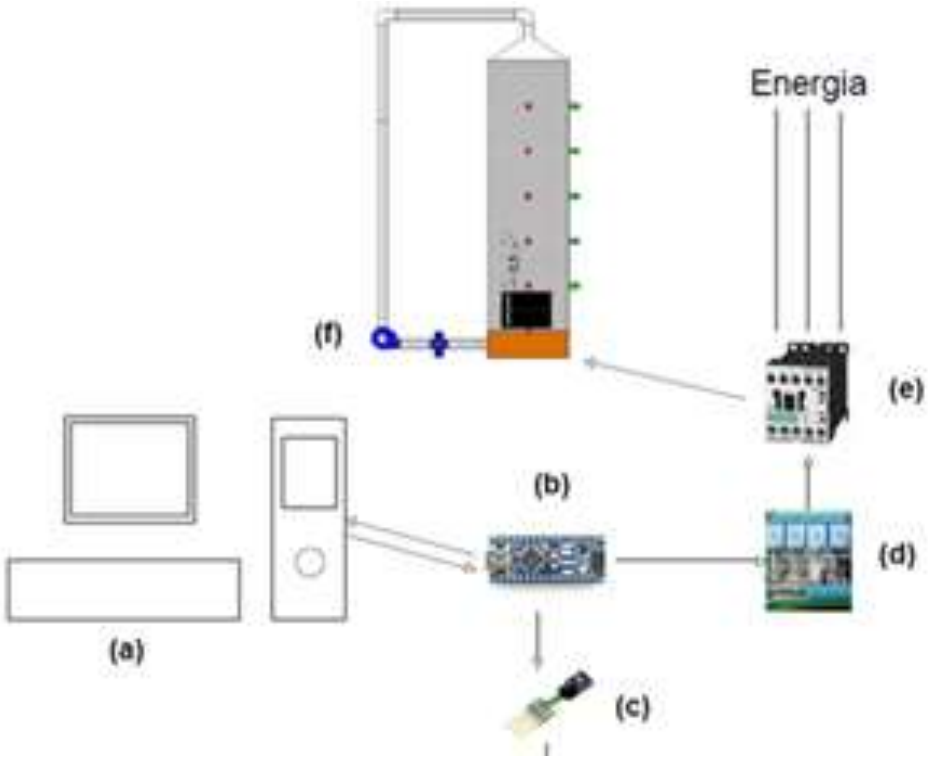

Figure 3. Control system diagram and acquisition aeration data follows the flow (a) Microcomputer (b) nano Arduino, (c) sensors, (d) relay board, (e) Contactor (f) of the fan motor.

Table 1. Means and standard deviation of speed, gauge air flow and height in different positions of the pitot tube.

Readings	Reading position (m)	Height gage (Pa)	Speed (m s^{-1})	Flow rate (LPM)	Flow rate (m^3s^{-1})
Top edge	0.02	0.980 ± 0.0	0.04 ±0.0	83.366±0.0	0.0014 ±0.0
	0.04	1.883 ± 0.107	0.06±0.002	115.477 ± 3.314	0.0019 ±0.0001
	0.06	1.687 ± 0.407	0.06±0.007	108.570±14.411	0.0018 ±0.0002
	0.08	1.765 ± 0.0	0.06±0.0	111.847 ±0.0	0.0019 ±0.0001
	0.10	1.295 ± 0.429	0.05±0.008	94.758±15.599	0.0016 ±0.0006
Center	0.12	0.981 ± 0.0001	0.04±0.0001	83.366 ±0.0001	0.0014 ±0.0
	0.14	0.981 ±0.0	0.04±0.0	83.365 ±0.0001	0.0014 ±0.0
	0.16	0.981 ±0.0	0.04±0.0	83.365 ±0.0001	0.0014 ±0.0
Lower border	0.18	0.666 ±0.161	0.04±0.004	68.318±8.553	0.0011 ±0.0001
	0.2	0.883 ±0.098	0.04±0.002	78.989 ±4.400	0.0013 ±0.0001
	Average	1.210±0.42	0.049±0.008	91.377±0.0002	0.00152±15.80

Table 2. Initial average temperature (TMI), final average temperature (TMF) and heating time for each airing strategy.

Strategy	TMI (°C)	TMF (°C)	Time (h)
a	28.5±1.22	30.98±1.33	2.5
b	25.5±0.62	33.6±0.65	8.0
c	27.2±0.35	31.2±1.41	5.2
d	27.3±0.82	31.5±1.01	6.0

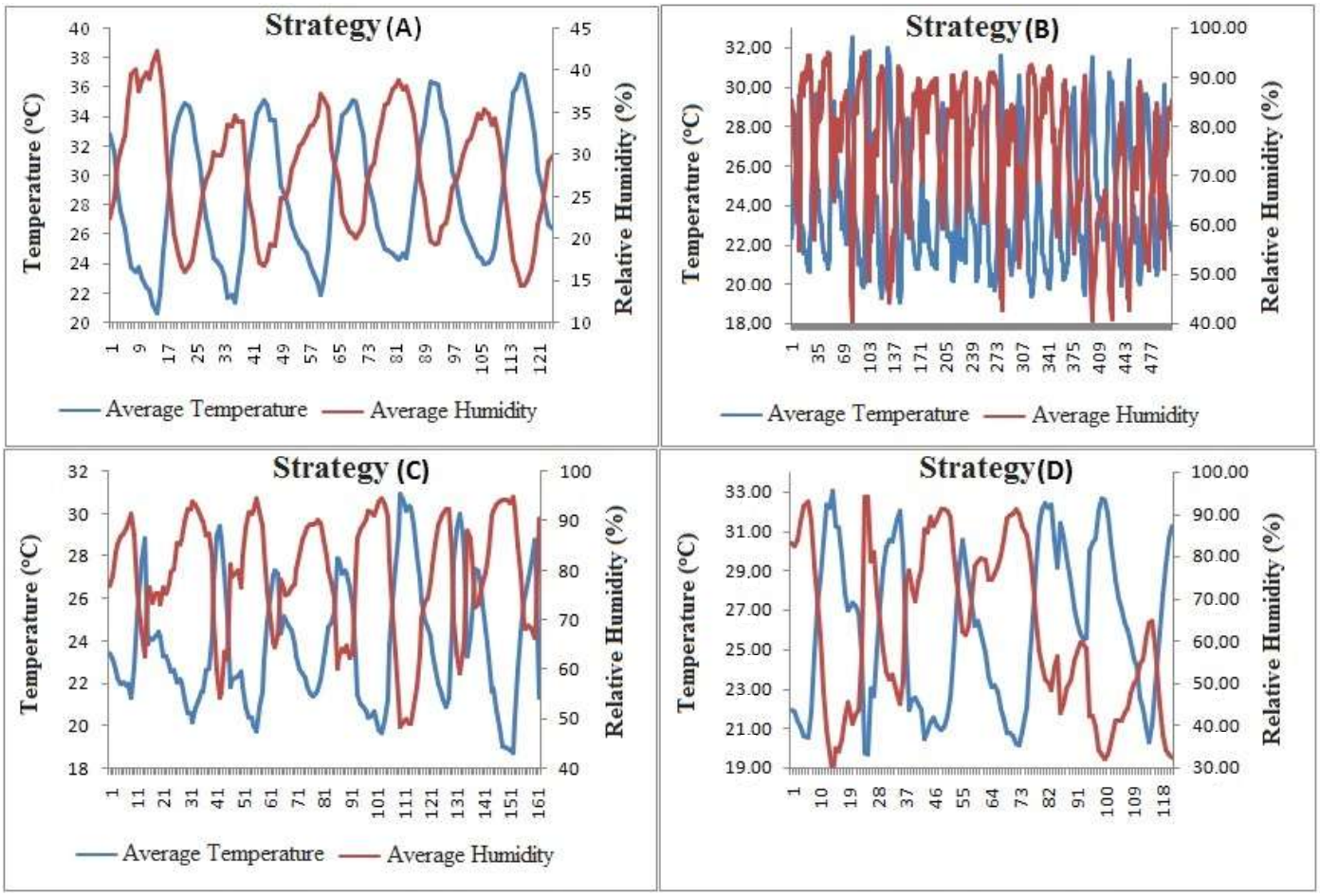

Figure 4. Averages of the ambient temperature and relative humidity during aeration strategies, b, c and d, in hours.

led different times to achieve TMF.

The heating process of the grain mass was faster in continuous ventilation strategy (a), since the initial temperature of the grain mass was 28.5°C. The total elapsed time for heating and air recirculation was 2.5 h to warm 2.4°C.

Heating the grain mass in strategy b the ambient air temperature control (T_{ar} < 22 ± 0.9°C) was conducted for a prolonged period of time due to the outside ambient temperature of 23°C. To complete the heating process, it was necessary to have 8 h to warm 8°C, since the initial average temperature of the grain mass was 25.5 ± 0.62°C.

The uniform heating of the grain mass was possible because of the prototype recirculation developed for the experiment. Figure 3 shows that the PVC pipe was connected to the fan and the top of the silo. The ventilation air outlet and the heater was placed immediately after a tubing connected to the plenum, allowing the recirculation of air.

As for the controller, it was observed that the use of Arduino was adequate for the process, since during the period of aeration of the mass of grains, the microcontroller remained in perfect operation, controlling the cooling of the grain mass, as strategy pre established. Other authors such as Cavalcante et al. (2011), Evans et al. (2013), Dilly and Mendes (2015), also cite the Arduino as functional for the acquisition of temperature and humidity data.

The strategies, during storage, environmental data and storage in silo prototype were collected to evaluate the aeration process. It was observed that the four aeration strategies, regardless of the storage time, there was cooling the grain mass, considering that all the simulations started from temperatures in the grain mass in the range of 30 to 32°C. Thus, the study corroborates other authors who also found the same cooling trend of the grain mass (Rigo et al., 2012; Lawrence and Maier, 2011).

Figure 4 shows the environmental meteorological data collected during testing of control strategies studied. Note that, the city of Anapolis - GO, with tropical climate, has striking feature, as it relates temperature and relative humidity. At night-time, it was possible to note an ambient

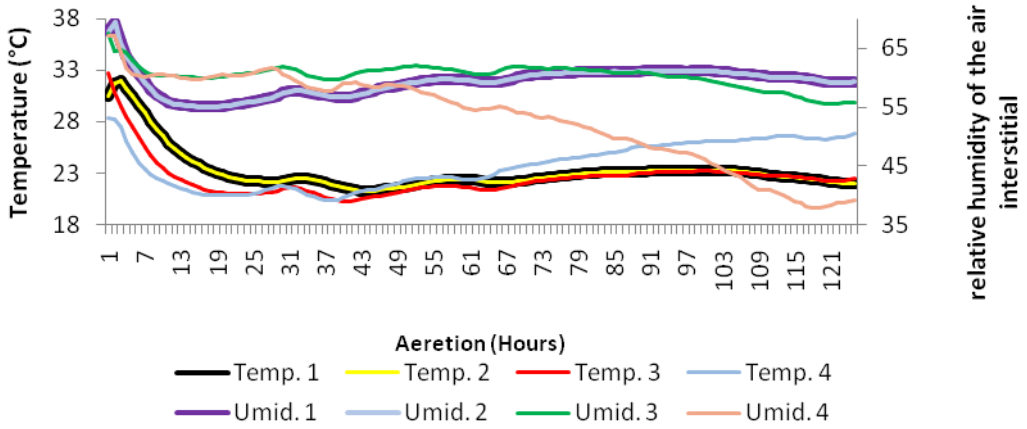

Figure 5. Means of temperature and relative humidity of the air interstitial grain silo heights in four (1, 2, 3 and 4) during continuous aeration control strategy.

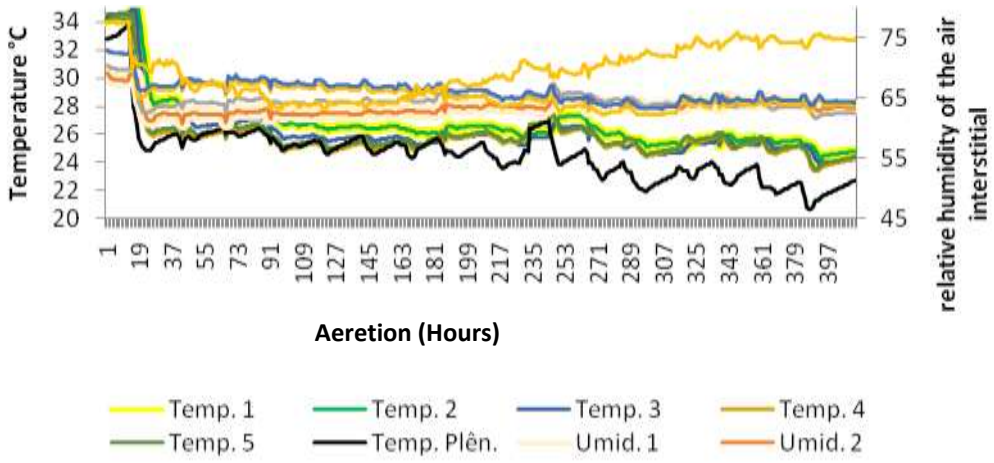

Figure 61. Medium temperature and relative humidity of interstitial air grain silo in six heights (1, 2, 3, 4, and 5 Plen) for ambient air temperature control strategy.

temperature reduction, combined with an increase in relative humidity. This characteristic shows the need for an efficient control of the aeration process of the stored grains (Lopes et al., 2015).

Control strategy, continuous aeration (a), ambient temperature ranged from 20.3 to 39.0°C and relative humidity of 12.74 to 43.7%, a fact which differed from other strategies, where their averages ranged from 18 to 32°C and relative humidity 30 to 95%, which can be explained by the beginning of the rainy season in the region.

Regarding the temperature in the grain mass, it was noted that at the beginning of the period in which the system has been in operation there occurred sudden changes in temperatures that were observed in the silo due to internal gradients, the temperature difference between the inside and the outside of the silo and a probable insect action. These temperature variations in the grain mass were observed in all strategies (Figures 5 to 8).

In the process of continuous aeration, aeration was done by a period of six days, reaching an average temperature of the grain mass is 23.3 ± 0.54°C. Temperatures lower than this could not be achieved, given the fact that ambient temperatures during the aeration process continued with a minimum of 20.5°C and maximum 39°C.

In continuous aeration strategy (a) and the room air temperature control (b) the mass of the cooling process grains can be seen intensely from 24 to 48 h of aeration. Devilla et al. (2004) and Nascimento and Queiroz (2011) also found this feature in the study of aeration of corn grain stored.

As in Figures 5 to 8, it is possible to note that in all tests the aeration mass of the cooling direction is vertically upward in the silo way, corroborating Lawrence and

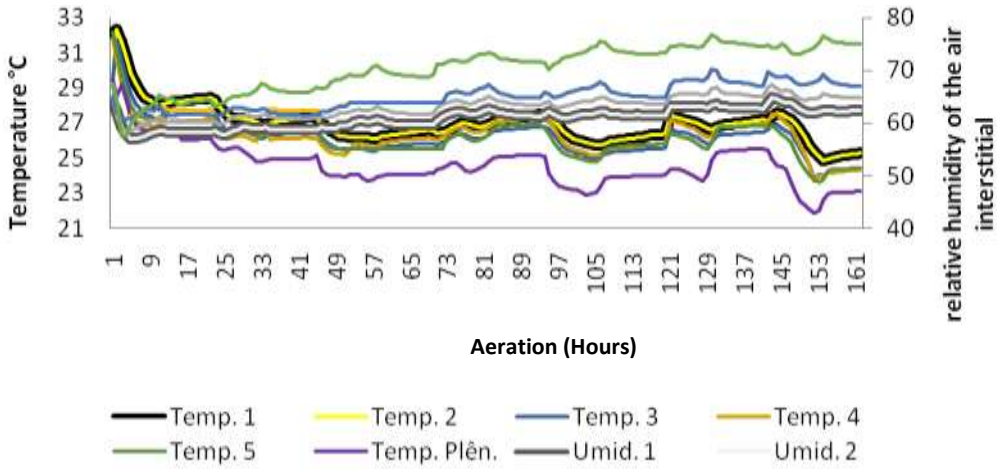

Figure 7. Means of temperature and relative humidity of the air interstitial grain silo in six times (1, 2, 3, 4, and 5 pollen) over night aeration control strategy.

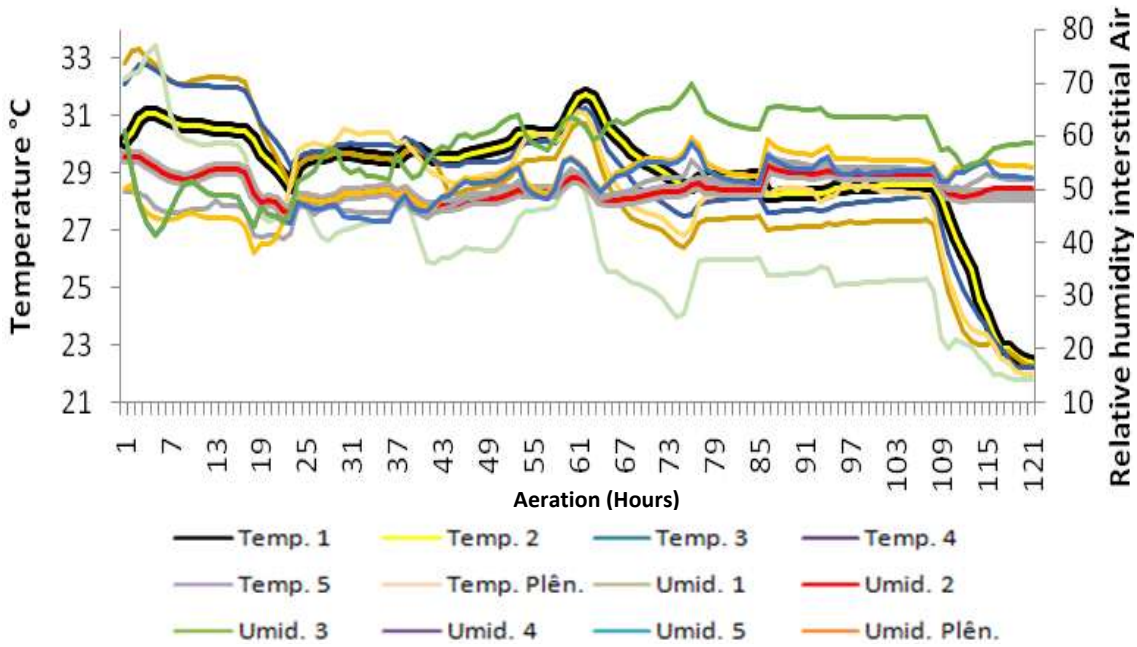

Figure 8. Means of temperature and relative humidity of the air interstitial grain silo in six times (1, 2, 3, 4, and 5 pollen). For temperature control strategy via the temperature difference between the grain and the ambient air.

Maier (2011) who also observed this fact when studying fifteen grain aeration strategies. Thus, it appears that the front of the cooling mass of the grain silo base to 1.5 m in height, making it slower subsequent to this point.

In Figure 7, it was possible to assess that when controlled aeration via timer, at night, 22 to 10 h the next day, there is a greater control of the temperature gradient of the grain mass, lengthening this way, the period of aeration is nine days. This was also by lifting by Lopes et al. (2010) when studying the effects of different control strategies in the aeration environment.

It was found that during the aeration process for all simulations, the heights (1 and 2) within the silo, showed similar temperature and relative humidity during the process. It is known that the deepest parts of the silo tend to warm very slowly, because of its low thermal conductivity (Elias, 2002; Santos, 2002; Quirino et al.,2013), however, it is known that for the upper parts of the silo is higher the influence of environment in storage.

Figure 8 relates to aeration control strategy via the temperature difference between the grain and the environment. This can be seen that in six days the grains

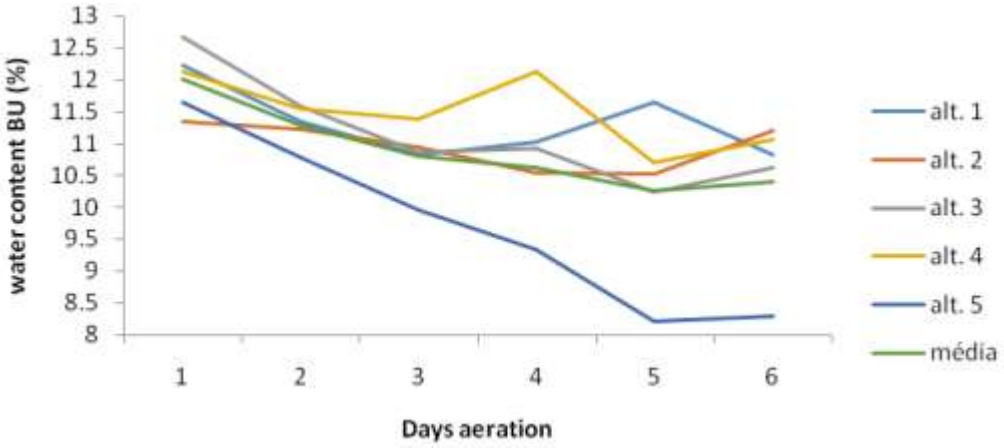

Figura 9. Grain water content em % B.U. related to six times and days in the silo aeration during continuous aeration control strategy.

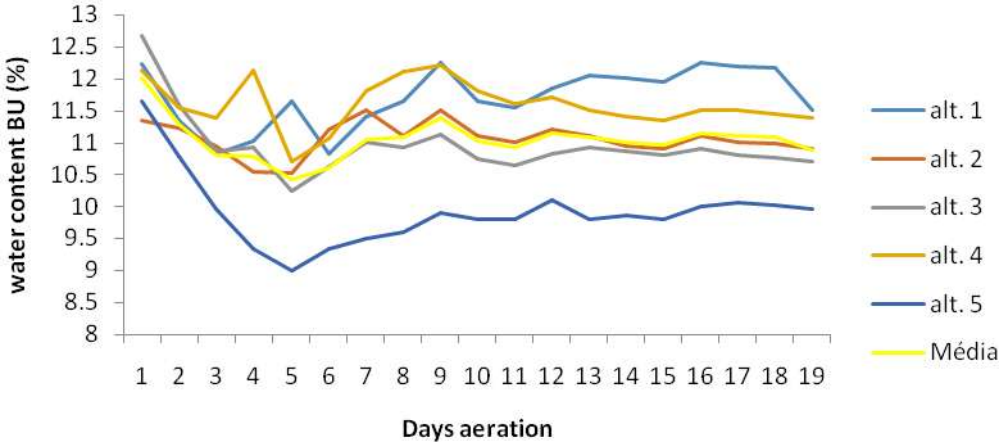

Figura 10. Water content of the grains in percentage, related to six times in the silo and days of aeration for ambient air temperature control strategy.

are cooled, which may be due to the long periods of low ambient temperature and the control strategy has been shown effective (Lopes et al., 2010).

In Figures 9 to 12 the variation of the grain water content in "% B.U." in six different heights in the storer prototype grain during test period are shown.

Based on Figure 9, continuous aeration, it is observed that there is super drying the lower profile of the grain mass, near the base of the silo, however, during the aeration process, the beans lost on average 1.6% B.U. finalizing the process with (10.39 ± 1.2)% B.U.

Note that if the top of the silo heights 1 and 2, the climatic conditions are fully exposed, win or lose water due to direct contact with the environment.

It was noted that the aeration night was the best preserved in the average grain moisture content, ranging 11.2 ± 0.64 to 11.4 ± 0.33% B.U. the end of the aeration process. Justified by environmental conditions (low night time temperatures combined with relative humidity between 50 and 65%).

In control via temperature difference between the beans and the ambient air the aeration process began with an average water content of [10.55 ± 0.8]% B.U, and was completed with 11.22 ± 1.0% B.U. Already airing via the external environment temperature control, allowed an average variation in grain moisture content of 12 ± 0.51 to 10.88 ± 0.62% B.U.

Through the strategies for, climate control of air (b), night aeration (c), and control via the temperature difference between the grains and the environment (d), it was possible to reach temperatures significantly affect the proliferation of insect pests in grain mass stored (Table 3).

It is known that temperatures between 27 and 34°C are permissible proliferation of most insect pests of stored grains in tropical and subtropical regions (ARO, 2014).

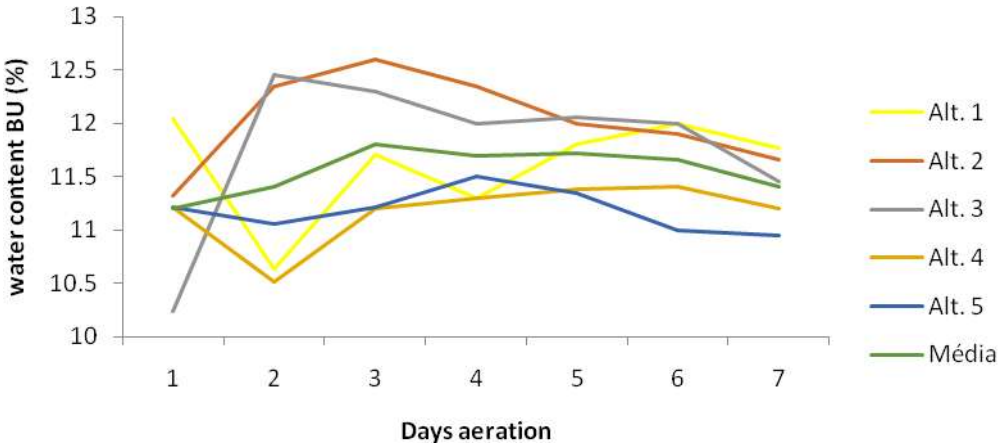

Figura 11. Grain water content in percentage, related to six times and days in the silo aeration, aeration during night time control strategy.

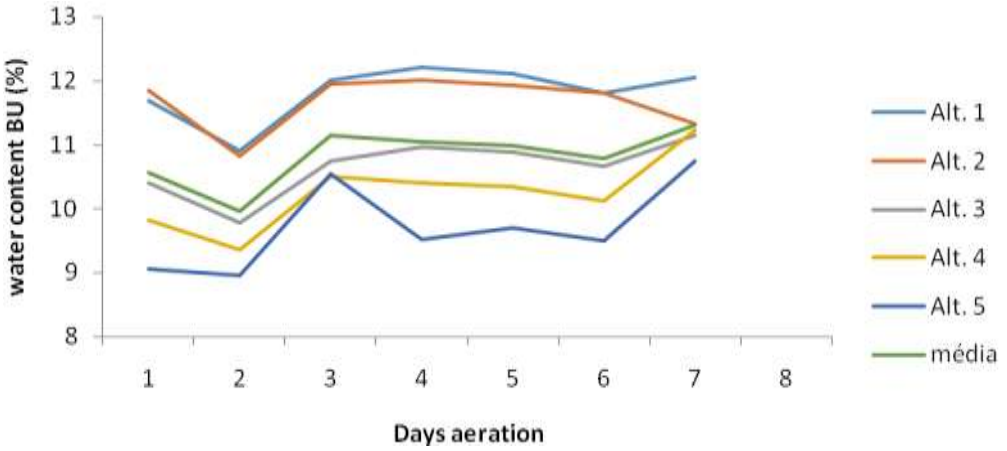

Figura 12. Grain water content in percentage, related to six times and days in the silo aeration for temperature control strategy via the temperature difference between the grain and the ambient air.

Table 3. Means and standard deviation of temperature in the strategies a, b, c and d related heights in silo.

Strategy	Height in silo						Mean
	1	2	3	4	5	Alt. Plen.	
a	23.6±1.99	23.6±1.99	23.3±2.17	22.5±1.60	-	-	23.3±0.50
b	24.0±1.91	23.8±1.92	23.9±1.77	23.3±1.87	23.5±1.76	20.6±2.21	23.2±0.85
c	24.8±1.06	24.8±1.06	23.2±0.95	23.8±1.06	23.5±1.05	21.9±1.94	23.7±0.85
d	22.4±1.51	22.4±1.51	22.2±1.43	22.3±2.12	22.0±1.98	21.8±2.31	22.2±0.95

However, the growth is suppressed by such temperature of 17 to 22°C (Garcia et al., 2000; Navarro and Noyes, 2010). Thus, it was found that the strategy d, temperature control between the grains, and environment <3 degrees Celsius was the best strategy, then the strategy B, temperature control air.

Figure 13 shows the period of operation of the aeration blower, wherein the aeration strategy d, controlling the aeration means of temperature difference between the grain and the ambient air was more economical in terms of consumption of electric power, and seen that for the mass of cooling grain using the "d" strategy was

Table 3. Means and standard deviation of temperature in the strategies a, b, c and d related heights in silo.

Strategy	Height in silo						Mean
	1	2	3	4	5	Alt. Plen.	
a	23.6±1.99	23.6±1.99	23.3±2.17	22.5±1.60	-	-	23.3±0.50
b	24.0±1.91	23.8±1.92	23.9±1.77	23.3±1.87	23.5±1.76	20.6±2.21	23.2±0.85
c	24.8±1.06	24.8±1.06	23.2±0.95	23.8±1.06	23.5±1.05	21.9±1.94	23.7±0.85
d	22.4±1.51	22.4±1.51	22.2±1.43	22.3±2.12	22.0±1.98	21.8±2.31	22.2±0.95

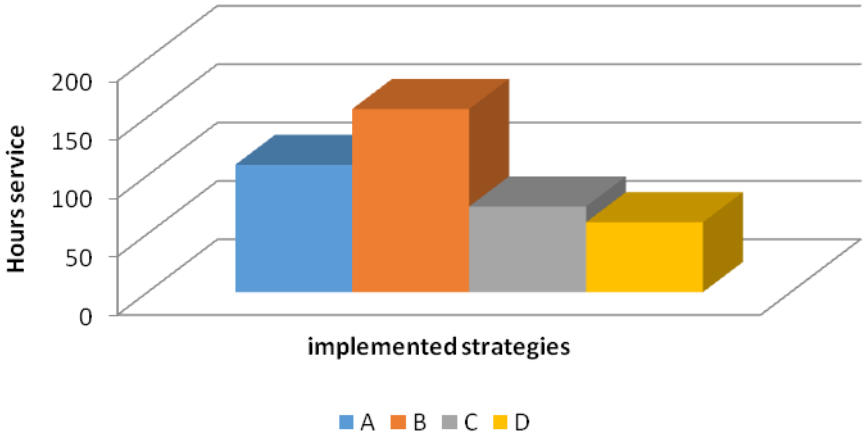

Figura 13. Number of hours with the fan on in the different evaluated aeration strategies.

completed with 60 h of ventilation. Then, the night aeration strategy was also economical, spending 73 h to cool the grain mass. Similar results were found by Lawrence and Maier (2011), when evaluated several controls for aeration of stored grains and concluded that flow rates of 0.1 m^3min^{-1} t^{-1} combined with temperature control strategies culminate in lower power consumption.

Conclusion

According to the results obtained and the conditions in which it was developed this work, it can be concluded that:

1. Operational results obtained with the software developed, were effective for control of grain aeration processes stored in the prototype.
2. The prototype for storage and control of aeration of stored grain was efficient in grain storage control process.
3. The control strategies, aeration of grain via the temperature difference between the grains and environment (TDIF = 3°C) and night aeration (21 to 10 h next day), were effective for the aeration process in Annapolis- GO.
4. Temperature and humidity data related to corn grain storage were collected and proved the efficiency of computational and structural system.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of *Allium sativum* (garlic) extract on the growth and nodulation of cowpea *(Vigna unguiculata)* and groundnut *(Arachis hypogea)*

Martina T. V. Adeleke

Rivers State University of Science and Technology, Port-Harcourt, Rivers State, Nigeria.

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It has been demonstrated that symbiotic N_2 fixation enhances soil fertility and productivity as well as increases carbon sequestration and nutrient conservation. Green house experiments were conducted to ascertain the effect of garlic extract on the nodulation of cowpea and groundnut plants. Different concentrations of the extract (20, 40, 60 and 80%), were applied to the plant at 2 weeks after planting. Both legumes were adversely affected by the garlic extract, but the effect was more pronounced on the groundnut plants. Before application of the garlic extract to the soil, the soil pH was 6.5, phosphorus 8.3 mol/kg, calcium 2.51 mol/kg, potassium 0.45 mol/kg, and nitrogen 0.295 mol/kg, and these make the soil fertile enough for nodulation. However, after garlic extract application, there was reduced nodulation in the roots and a marked drop in plant height, leaf area and root development in comparison to the control. This adverse effect was more with increasing concentration of the extract. It is recommended, that land for cowpea or groundnut product remains free of garlic and members of the families Allium and Cyperus plants. They should not be left in the soil or turned over in it, during land clearing and should be grown in inter-crop. This is because their exudates during decomposition or growth could have inhibitory effects on the growth and nodulation of legumes.

Keywords: Garlic extract, nodulation, legumes, nitrogen fixation.

INTRODUCTION

Legumes (Fabaceae) constitute the third largest family of flowering plants comprising more than 650 genera and 18000 species (Dadi and Bekele, 2006). Economically, legumes represent the second most important family of crop plants after Poaceae (grass family), accounting for approximately 27% of the world's crop production (Dadi and Bekele, 2006). The Leguminosae family has enormous diversity as it include every morphophylogical types of plants- viz. herbs, shrubs, small and big tree, climbers, creepers, lianes, annual, perennial etc. One of the most important attributes of legumes is their unique capacity for symbiotic nitrogen fixation, underlying their importance as a source of nitrogen in both natural and agricultural ecosystems.

Accordingly the family is subdivided into three subfamilies, Mimosoideae, Caesalpinioideae, and

E-mail: mtadeleke@yahoo.co.uk.

Papilionoideae. Papilionoideae subfamily contains nearly all economically important crop legumes, including soybean (*Glycine max*), peanut (*Arachis hypogaea*), mungbean (*Vigna radiata*), chickpea (*Cicer arietinum* L.*)*, lentil (*Lens culinaris)*, common bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), and alfalfa (*Medicago sativa*). Humans have used legumes in agriculture for thousands of years either as a food crop for humans and livestock, or as a rotational crop to supply N to non-legume crops. The shift from biological to industrial sources of N fertilizer in agricultural systems did not occur until the 20th century (Smil, 2001; Crews and Peoples, 2004). Symbiotic N_2 fixation enhances soil fertility and productivity as well as increases carbon sequestration and nutrient conservation (Cromwell and Winpenny, 1993).

A biochemical dialogue is initiated between the host plant and the rhizobia, causing nodulation to occur (Lum and Hirsch, 2003). A variety of molecular and genetic mechanisms have evolved between the legume and the rhizobia to regulate the nodulation and N2 fixation processes (Lum and Hirsch, 2003). Nod genes direct the various stages of nodulation; the initial interaction between the host plant and free-living rhizobia is the release of a variety of chemicals by the root cells into the soil. Some of these encourage the growth of the bacterial population in the area around the roots (the rhizosphere).

Reactions between certain compounds in the bacterial cell wall and the root surface are responsible for the rhizobia recognizing their correct host plant and attaching to the root hairs. Flavonoids secreted by the root cells activate the nod genes in the bacteria which then induce nodule formation. The whole nodulation process is regulated by highly complex chemical communications between the plant and the bacteria, once bound to the root hair the bacteria excrete nod factors (Madigan et al., 2000). These stimulate the hair to curl. Rhizobia then invade the root through the hair tip where they induce the formation of an infection thread; this thread is constructed by the root cells and not the bacteria and is formed only in response to infection.

Apart from the obvious potential for increased agricultural productivity, a more comprehensive understanding of nodulation will enhance our knowledge of plant development and plant–microorganism interactions. For example, the development of root nodules has parallels with lateral root formation (Krusell et al., 2002). Therefore, nodulation research will help provide insight on this general developmental process in plants. In addition, some of the steps in nodule formation are common to, and likely derived from the pathway leading to symbiosis between mycorrhiza and their respective plant hosts (Endre et al., 2002) similarly, *Meloidogyne incognita*, is proposed to infect susceptible plants via a process that shares similarities with *Rhizobium* infection (Weerasinghe et al., 2005).

Allelopathy is the suppression of growth of one plant species by another due to the release of toxic substances (Portales-Reyes et al., 2015; Cipollini et al., 2012). One of the studied aspects of allelopathy is the role of allelopathy in agriculture. Recent researches have focused on the effect of weeds on crop, crops on weeds and crops on crops. This research furthers the possibility of using allelochemicals as growth regulators and natural herbicides, to promote sustainable agriculture. For example the effect of yellow nutsedge (*Cyperus esculentus*) on cowpea has also been studied. When yellow nutsedge wad intercropped with cowpea, the yellow nutsedge root exudation inhibited the cowpea growth (Kadioglu and Yanar, 2005). Cocks comb (*Celosia argentea* L.) is one of the most famous allelopathic plants. It has effect on the seed germination, seeding growth and flowering of legumes (Kadioglu and Yanar, 2005).

Garlic (*Allium sativum L)*. Is a member of the Alliaceae family, has been widely recognized as a valuable spice and a popular remedy for various ailments and physiological disorders. The name garlic may have originated from the Celtic word 'all' meaning pungent. As one of the earliest cultivated plants, garlic is mentioned in the Bible and in the literature of Ancient Israel (The Talmud), Egypt (Codex Ebers) and India (Vedas and Purans, Charak Sanghita). As and Purans, garlic is reported to have medicinal as well as nutritive value in food items. It is a remarkable plant, which has multiple beneficial effects such as antimicrobial, antithrombotic, hypolipidemic, antiarthritic, hypoglycemic and antitumor activity. Allicin has antibacterial and antioxidant activity (Karin et al., 2008). This study therefore seeks to find out the effect of having garlic exudates around the roots of some legumes namely, cowpea and groundnut.

Intercropping of garlic with other crops could be advantageous in overcoming continuous cropping problems but could also harmful due to the allochemicals released into the environment which are considered to alter a variety of physiological and biochemical processes. There are increasing evidences that the allelochemicals have significant effect on cell division, ion and water uptake, phytohormone metabolism, respiration, photosynthesis, enzyme function, as well as gene expression (Sadaqa et al., 2016; Portales-Reyes, 2015; Ian et al., 2014; Hale and Kalisz, 2012; Singh and Thapar 2003). This study was conducted to ascertain the effect of garlic extracts on the growth and nodulation of the legumes - cowpea and groundnut.

MATERIALS AND METHODS

Plant materials

Green house experiment were conducted at the Department of Applied and Environmental Biology, Rivers State University of Science of Technology, Port Harcourt, Nigeria to determine the effect of garlic extracts on the nodulation of legumes (cowpea and groundnut). The groundnut and cowpea seeds were also collected from the mile 3 market in Port Harcourt Rivers State. Fresh garlic

Table 1. Plant height of groundnut plants following treatment with varying concentrations of garlic extract after 7 to 31 days.

Treatment applied	Plant height (cm)						
	DAP 7	DAP 9	DAP 11	DAP 13	DAP 27	DAP 29	DAP 31
Control	5	10	15	20	40	60	80
20% conc Trmt 1	5	12	17	22	46	50	60
40% conc Trmt 2	5	10	15	22	30	35	35
60% conc Trmt 3	4	8	13	18	26	30	35
80% conc Trmt 4	7	10	15	20	25	25	30

Table 2. Leaf area of cowpea plants following treatment with varying concentrations of garlic extract after 7 -31 days.

Treatment applied	Leaf area (cm^2)						
	DAP 7	DAP 9	DAP 11	DAP 13	DAP 27	DAP 29	DAP 31
Control	30	30	30	30	90	90	90
20% conc Trmt 1	30	36	36	42	60	66	72
40% conc Trmt 2	30	30	36	36	48	54	60
60% conc Trmt 3	30	30	36	48	48	60	60
80% conc Trmt 4	30	30	48	54	60	60	60

bulbs (2 kg) purchased from mile 3 market in Port Harcourt were thoroughly washed to remove soil and other debris. About (1 kg) of Garlic bulbs were sliced into small pieces and put in a clean blender with 500 ml of water and blended into a fine paste. About (1 L) of crude liquid extract was obtained by filtering the garlic paste through a piece of sterile muslin cloth. The extract was collected into a sterile bottle and kept in a refrigerator (at 4°) until used. The same thing was done with the second set of (1 kg) garlic bulbs.

Greenhouse experiment

About 3 kg capacity polyethylene bags were filled with 2 kg of air dried loamy soil. The soil used for the planting of the crop and for the analysis was collected from the university school research farm. Three replications were made for each treatment, for both cowpea and groundnut, and the control. So a total of 30 bags were half filled with soil, 15 bags for cowpea and 15 bags for groundnut. Cowpea and groundnut seeds were sown 2 cm deep in each bag. The pots were watered after every 2 days with 100 ml distilled water till after two weeks of germination and were kept weed-free by hand weeding. The garlic extract was retrieved from the refrigerator, allowed to warm up to room temperature and administered to the plants. About 100 ml of the extract solution of was added at the dilution of 20, 40, 60 and 80% treatment for each treatment the three replica respectively while distilled water was used on the control. Germination counts were made and measurements were taken of plant height and leaf area before and after treatment application. All the chemicals used in the experiment were of the analytical grade and were obtained from the department and from the soil science department in the faculty of agriculture.

RESULTS

Effect of garlic extract on cowpea nodulation and growth

After the application of the treatment (garlic extract) it was observed that the cowpea plant with the 20, 40, 60 and 80% concentration showed adverse response to all treatments after 2 days of application by wilting but recovered after 5 days (Tables 1 and 2). The data of higher the concentration of garlic extracts having an effect on the growth and leaf area of the plant is represented graphically in Figures 1 and 2. The plants with the highest concentration of garlic (80%) had the slowest growth rate followed by that of the 60 and 40% concentration. Pictures of reduced growth and nodulation are shown in Figures 3 and 4.

Effect of garlic extract on groundnut nodulation and growth

Similar to cowpea, groundnut plants treated with 20, 40, 60 and 80% garlic extracts showed wilting three days after application but recovered seven days after (Tables 3 and 4; Figures 5 and 6). The plants with the highest concentration (80%) all died, the plant with the 60 and 40% concentration recovered after seven days but showed very slow growth while the plants with the lowest concentration (20%) fully recovered but did not compare favorably with the control plants.

Tables 3 and 4, and Figures 7 to 11, showed that plants with the lowest concentration (20%) grew well but not as the control; while the plants with the highest concentration (80%) of the treatment were most affected that they all died.

A graph showing the relationship between the plant height and the date of planting before and after treatment application. This shows that the higher the treatment concentration the slower the growth of the plant.

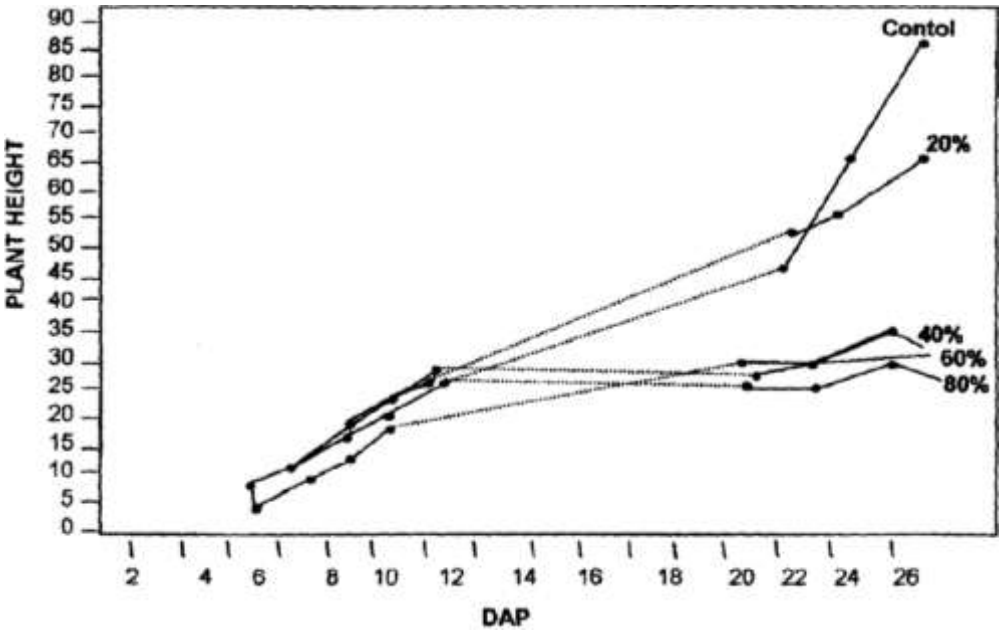

Figure 1. Relationship between plant height of cowpea days after planting (DAP) following treatment with different levels of garlic extract.

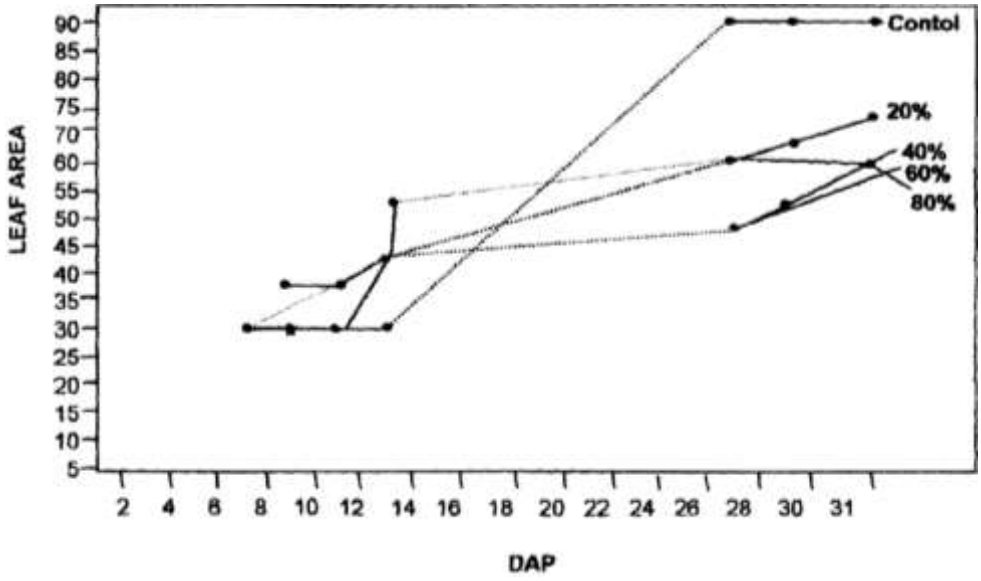

Figure 2. Relationship between leaf area of cowpea days after planting (DAP) following treatment with different levels of garlic extract.

DISCUSSION

The results of this research show that the plants treated with the lower concentration of garlic extract compared favorably with the ones in the control, while those given higher concentrations decreased in leaf area and plant height. The data shows that the effect of the garlic extract on cowpea and groundnut nodulation and growth is significant, with the effect being more pronounced on groundnut compared to cowpea. The degree of inhibition increased as the concentration of garlic extract increased for both groundnut and cowpea. A similar observation was made by Laosinwattana et al. (2009) in their study of the effect of garlic on the growth of pepper.

Figure 3. Cowpea plants before treatment with garlic extract.

Figure 4. Cowpea plants after treatment with garlic extracts at 20, 40, 60 and 80% concentration.

Table 3. Plant height of groundnut plants following treatment with varying concentrations of garlic extract after 7 to 31 days after planting (DAP).

Treatment applied	Plant height (cm)						
	DAP 7	DAP 9	DAP 11	DAP 13	DAP 27	DAP 29	DAP 31
Control	10	12	17	22	30	35	38
20% conc Trmt 1	5	10	15	20	24	26	28
40% conc Trmt 2	7	10	15	20	21	22	24
60% conc Trmt 3	6	8	13	22	22	X	23
80% conc Trmt 4	4	8	15	20	X	X	X

Table 4. Plant area of groundnut plants following treatment with varying concentrations of garlic extract after 7 -31 days after planting (DAP).

Treatment applied	Plant area (cm^2)						
	DAP 7	DAP 9	DAP 11	DAP 13	DAP 27	DAP 29	DAP 31
Control	29	29	31	32	40	60	65
20% conc Trmt 1	20	28	28	28	32	36	40
40% conc Trmt 2	24	24	28	30	27	30	30
60% conc Trmt 3	21	28	28	30	28	X	X
80% conc Trmt 4	28	28	28	30	X	X	X

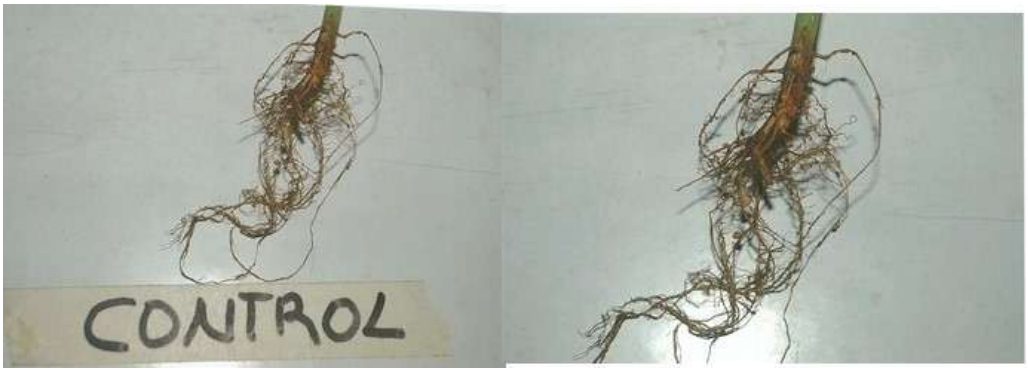

Figure 5. Roots of cowpea plants without treatment with garlic extract showing nodulation.

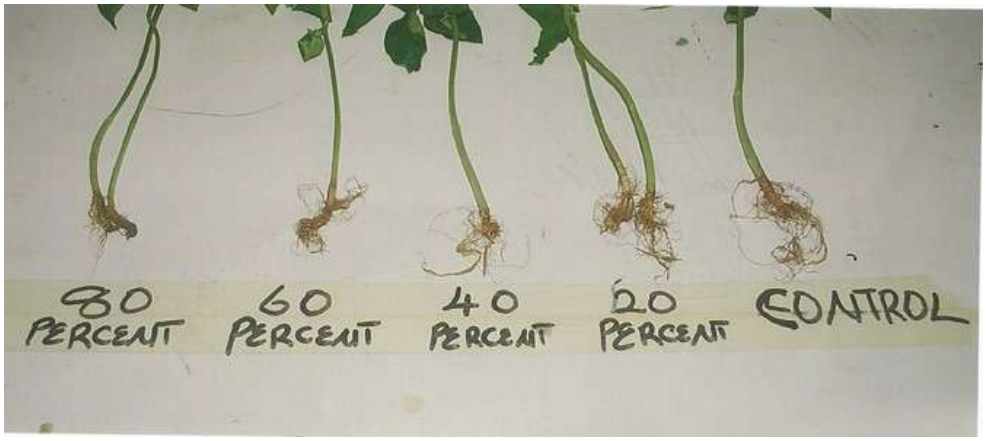

Figure 6. Roots of cowpea plants after treatment with 20, 40, 60 and 80% of garlic extract.

In addition, allelopathic effects of weed extract on crops have been observed earlier, for example the effect of yellow nutsedge (*Cyperus esculentus*) on cowpea has also been studied (Kadioglu and Yanar, 2005). When intercropped with cowpea, the yellow nutsedge root exudate inhibited the growth of the cowpea plants (Kadioglu and Yanar, 2005). Interaction effect indicated that higher concentrations produced lower vigour index. The increased inhibitory effect at higher concentration of weed extract may be due to increase in the concentration of allelo chemicals like phenolic acids namely, P-hydroxy benzoic acid, p-coumaric acid, caffeic acid, o-coumaric acid and ferulic acid (Portales-Reyes, 2015). Similar results were noticed by Singh and Thapar (2003).

It was also observed that all concentrations of garlic extracts in this study markedly did not promote root development of both cowpea and groundnut plants which suggests that root development is sensitive to allelopathic

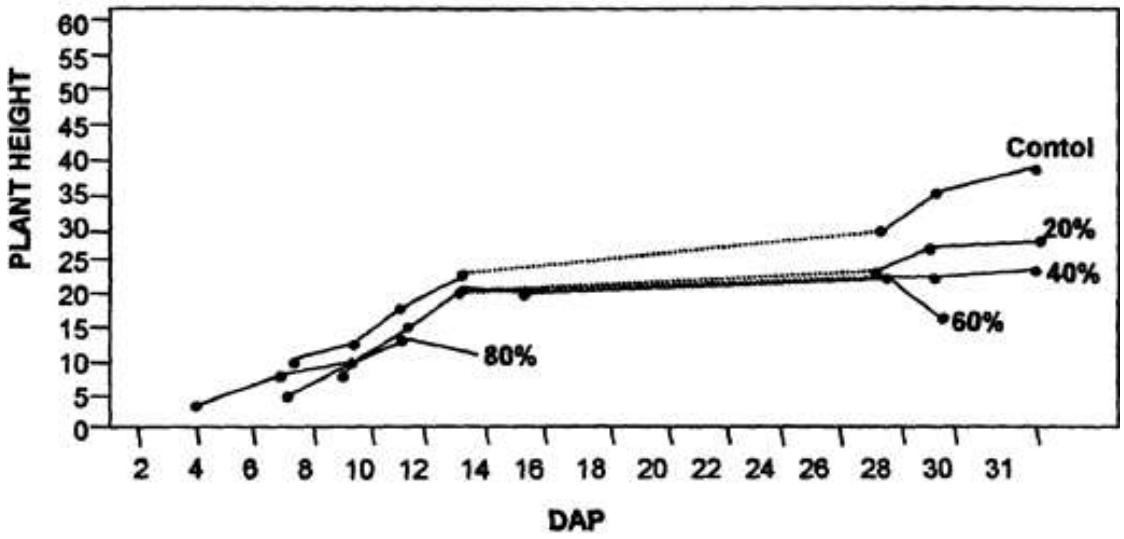

Figure 7. Relationship between plant height of groundnuts days after planting (DAP) following treatment with different levels of garlic extract.

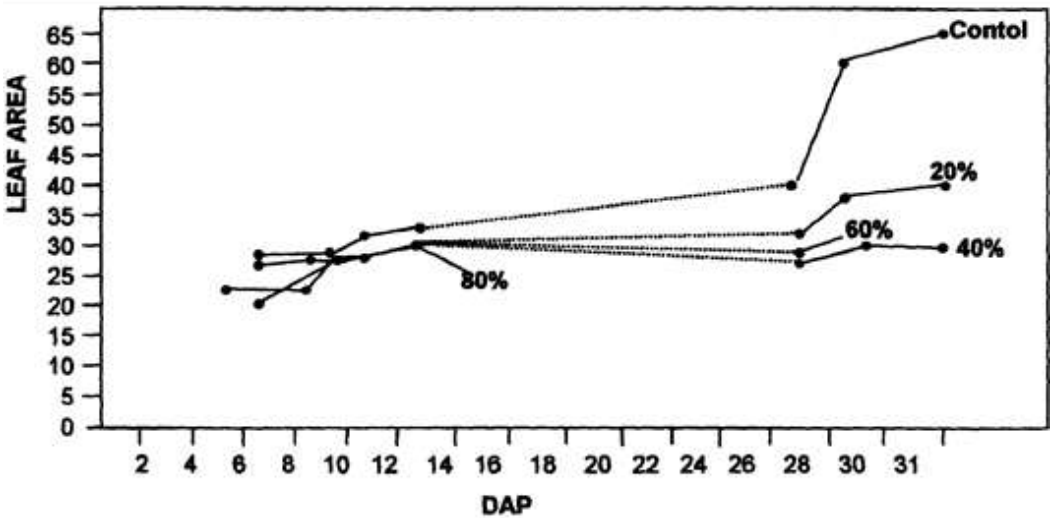

Figure 8. Relationship between leaf area of groundnuts days after planting (DAP) following treatment with different levels of garlic extract.

inhibition of extracts (Plate 4 and 7). This observation agrees with some of the earlier studies which indicate that water extracts of allelopathic plants causes more pronounced effects on root development (Batish et al., 2006). This could be due to the fact that roots are first to come in contact with the allelochemicals in the environment (Turk and Tawaha, 2002).

Root nodulation was observed only in the control. None of the treatment concentrations showed root nodulation and this may have been due to the fact that root development is adversely affected by garlic extract treatment. Moreover, the extent of nodulation and amount of nitrogen fixed by legumes is largely related to the plant size and root mass (Tlusty et al., 2004). The chemical constituent of garlic extract shows that it has antimicrobial properties (Allicin), and this could have further contributed to the hindering root nodulation in the legumes (Karin et al., 2008) since nodulation is a symbiotic relationship between Rhizobium and root. Legumes will not grow well or fix nitrogen in acidic soil with pH above 6.5. These findings provide evidence that the garlic extract contains some allelochemicals and causes allelopathy through releasing the allelochemicals to environment. So, it is certainly inferred that garlic extract may have allelopathic

Figure 9. Groundnut plants before treatment with garlic extract.

Figure 10. Groundnut plants after treatment with garlic extracts at 20, 40, 60 and 80% concentration.

effect on a great majority of legume plants from this present study.

The result of the experiment reveals that the garlic extract has a negative effect on both cowpea and groundnut plants at all concentrations; the effect increasing with concentration. The effect was however more pronounced in groundnut than in cowpea. Leaf area, plant height and root development were all inhibited, hence, there was no nodulation observed. It is suggested therefore, while weeding or clearing a piece of land for cowpea or groundnut product remains of garlic and members of the families Allium and Cyperus plants

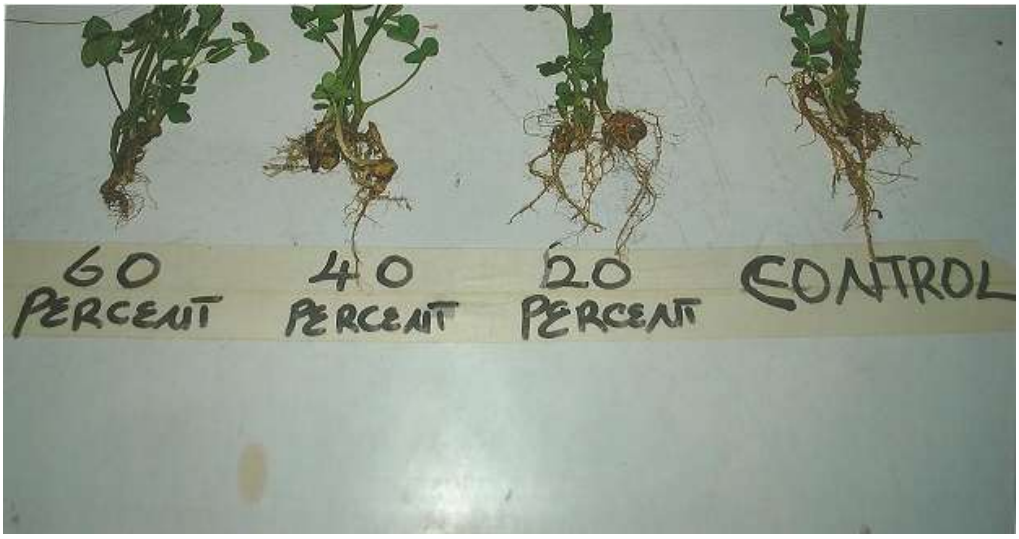

Figure 11. Roots of groundnut plants after treatment with 20, 40, 60 and 80% of garlic extract.

should not be left in the soil or turned over in it. This is because their exudates during decomposition could have inhibitory effects on the growth and nodulation of legumes. Alternatively, the land should be tilled to remove its rhizomes and root remains, and left for a while for proper decomposition of any remnants and for sufficient rains to wash away the exudates before sowing on it. Completely avoiding land that has been previously used for members of these families, for legume production would be best, where possible.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Agronomic performance of local and introduced plantains, dessert, cooking and beer bananas (*Musa* spp.) across different altitude and soil conditions in eastern Democratic Republic of Congo

M. Kamira[1], J. Ntamwira[1], C. Sivirihauma[2], W. Ocimati[3]*, P. van Asten[4], L. Vutseme[2] and G. Blomme[5]

[1]Bioversity International, Bukavu, South Kivu, DR Congo.
[2]Bioversity International, Butembo, North Kivu, DR Congo.
[3]Bioversity International, Kampala, Uganda.
[4]IITA, Kampala, Uganda.
[5]Bioversity International, Addis Ababa, P. O. Box 5689, Addis Ababa, Ethiopia.

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Agronomic performances of local and exotic *Musa* species were evaluated across seven agro-ecologies in Eastern Democratic Republic of Congo. Generally, all the cultivars performed well. Mean bunch yields varied between 11 and 42 kg across different use groups and agro-ecologies. Introduced cultivars had higher or comparable yields to the local cultivars across agro-ecologies. The exotic beer cultivar 'NARITA 27' generally outperformed other beer types. The green cooking exotic types 'NARITA 4' and 'NARITA 2' produced bunches of up to 37 and 39 kg, respectively at altitudes of 1066 and 1111 m, though having smaller bunches of 21 to 25 kg, at 900 and 1707 m in comparison to the local type 'Barhabeshya' (30 to 37 kg). Yields were mainly influenced by soil factors and altitude. Yields generally increased with increase in OM, N, P, K, Ca, and pH. Altitude had a non-linear relationship with the time from planting to flowering, with the time from planting to flowering declining at higher altitudes. In contrast, the fruit filling phase increased linearly with altitude. Bunch weights of most cultivars declined with increasing altitude; particularly, when N, K, P and OM concentrations were low; possibly because most assimilates go towards sucker development at the high altitudes. For example, bunch weights of 'Barhabeshya', 'Mbwazirume' and 'Nshika' strongly declined (R^2 = -0.56-0.99) with increasing altitude. 'Ndundu', 'FHIA21' and 'Gros Michel' thrived well at high altitude sites. Such variations in cultivar adaptability plus cultivar attributes, e.g. height can be exploited by selectively promoting cultivars in specific agro-ecologies/niches.

Key word: Agro-ecologies, cultivar, fruit filling phase, Kivu provinces, plantain cultivar, yield.

INTRODUCTION

Plantain (*Musa* AAB), as well as other cooking banana (*Musa* ABB), East Africa highland banana (*Musa* AAA-EA) and dessert banana (*Musa* AAA, AA), constitute the sixth most important global food commodity (FAOSTAT, 2015). They are an excellent food source and in some countries of the world (e.g. in parts of East and Central

Africa) they are the principal components of the diet. The all year round fruiting habit of banana puts the crop in a superior position in bridging the 'hunger gap' between annual crop harvests (INIBAP, 1996); therefore, contributing significantly to food and income security of people engaged in its production and trade, particularly, in developing countries. Moreover, banana is considered an important food, because of its chemical composition and high content of vitamins and minerals, particularly potassium (Silva et al., 2002). The pseudostems and leaves are commonly used as mulch in plantations, livestock feed and as wrapping material (Karamura, 1993). Dried leaf bases are extensively used as roofing material for houses, to weave ropes for tethering goats and sheep, and for mattress making (Karamura, 1993; Kamira et al., 2015). In Eastern and Central Africa, banana is commonly grown by small-scale farmers (Bagamba et al., 1999) whose farm size ranges from 0.5 to 1.5 ha. In East and Central Africa banana is mainly cultivated in association with other crops (Nyabyenda, 2006) and its importance is exemplified by the large proportion of land allocated to the crop (Bagamba et al., 1999).

A wide variety of *Musa* genome groups are cultivated worldwide (Pollefeys et al., 2004). This diversity manifests itself in the cultivars that are grown, the way they are prepared, eaten and marketed, and in the systems in which they are produced. Relatively few banana cultivars have moved from the center of origin in Southeast Asia, with the result that the diversity of these plants declines from Asia to Africa to America (Simmonds, 1995). The Great Lakes region covering parts of Uganda, Rwanda, Burundi, Tanzania, Kenya and Democratic Republic of Congo (DR Congo) is regarded as a secondary centre of diversity for bananas and plantains (Simmonds, 1966). This region is also the largest producer and consumer of bananas in Africa (Smales, 2006), with annual per capita consumption reaching 250 kg, the highest in the world (FAO, 1985).

The three major genomic groups under cultivation worldwide are AAA, AAB, and ABB (Simmonds, 1995). Most of the commercial cultivars are triploids and belong to the AAA dessert group. The cultivars grown vary with altitude. For instance, at lower elevations in the Eastern DR Congo and Congo basin, below 1,200 m above sea level (masl), AAB plantains are mainly cultivated (Dheda et al., 2011; Ocimati et al., 2013). In contrast, in the mid to high altitude regions of Eastern DR Congo (1,200 to 2,000 masl) the East African highland banana types (AAA-EA) predominate (Ocimati et al., 2013).

However, above 2,000 masl, the majority of *Musa* cultivars do not perform well due to the low temperature (Turner et al., 2007; Sikyolo et al., 2013).

Banana production in Central Africa is mainly hampered by pests, including nematodes and weevils, as well as diseases (e.g. *Xanthomonas* wilt of banana [XW], banana bunchy top disease and *Fusarium* wilt), lack of resistant cultivars, poor soil fertility, and plantation management (Gold et al., 1994; INIBAP, 2003; Speijer et al., 1999; van Asten et al., 2011).

All the important nematode species that feed on bananas are root parasites which cause lesions, thereby reducing water and nutrient uptake to the upper parts of the plant and also paving the way for other pathogenic micro-organisms (Coyne et al., 2003) to infect plants. Nematodes are found on all varieties of bananas but variation in susceptibility has been observed (Gowen, 1995; Speijer, 1996; Speijer et al., 1999; Kamira et al., 2013). Banana weevils are especially important at the low altitude areas. Weevil larvae primarily destroy the rhizome tissue by tunneling through it as they feed (Gold et al., 2001). Severe weevil damage in the corm leads to a reduction in plant growth and bunch size, and to possible corm snapping due to wind or the weight of the plant (Gold et al., 1994). The weevil has been implicated in the decline and disappearance of highland banana from traditional growing zones in East Africa (Gold et al., 1999).

Banana *Xanthomonas* wilt is a devastating disease caused by the bacterium *Xanthomonas campestris* pv. *musacearum*. It was first officially reported in 1968 in Ethiopia (Yirgou and Bradbury, 1974), where it remained confined until 2001 when the disease appeared in both Central Uganda and the North Kivu province of the DR Congo and all banana cultivars planted in these areas are susceptible to XW (Tushemereirwe et al., 2004; Ndungo et al., 2006). Black leaf streak (BLS), the most important foliar disease in banana, caused by an airborne fungus called *Mycosphaerella fijiensis* Morelet, reduces functional leaf area and thus can cause yield losses of 30 to 50% (Mobambo et al., 1996; Ploetz, 2004). The majority of exotic bananas, together with all the East African highland bananas, are susceptible to BLS.

One pillar of the Consortium for Improving Agriculture-based Livelihoods in Central Africa (CIALCA)'s intervention strategy in banana-based systems is the introduction of new high yielding, pest and disease resistant *Musa* cultivars that are highly acceptable to consumers (CIALCA, 2008). Increasing the diversity of *Musa* germplasm forms an integral part of technology packages intended to overcome a number of biotic and abiotic challenges that hamper banana production in central Africa, a region where few exotic *Musa* cultivars have been introduced over the past decades.

*Corresponding author. E-mail: w.ocimati@cgiar.org.

In this study, the agronomic performance of introduced (exotic landraces and hybrids) plantain, green cooking, dessert and beer cultivars was evaluated against plantains, green cooking and beer landrace cultivars in three sites across South Kivu and four sites across North Kivu in the eastern DR Congo. The introduced cultivars were selected for a range of attributes that include one or more of the following: tolerance to pests and diseases, large bunch size, short to intermediate growth cycle, and ability to serve multiple purposes. Of particular interest was the association between agronomic features of cultivars with the altitude and soil conditions.

MATERIALS AND METHODS

Musa germplasm experiments were established in 2007 in different agro-ecological zones with contrasting altitude, soil fertility and rainfall across the Kivu provinces of eastern DR Congo (Tables 1 and 2). These included four sites in North Kivu (Butembo, Maboya, Mavivi and Mutwanga) and three sites in South Kivu (Kamanyola, INERA-Mulungu and Mushweshwe).

Exotic plantain cultivars ('FHIA21', 'Obubit', 'T6'), green cooking ('NARITA 2' (previously 'NSH20'), 'NARITA 4' (previously 'NSH22'), 'Mpologoma'), dessert ('FHIA03'), and a beer cultivar (NARITA 27 (previously 'NSH42')) were planted and evaluated against the local plantain ('Musheba'), local green cooking ('Barhabeshya', 'Matooke', 'Mukingiro', 'Vulambya', 'Mbwazirume'), local beer ('Ndundu', 'Nshika') and dessert ('Gros Michel', 'Dwarf Cavendish', 'Giant Cavendish') cultivars (Table 3). The term 'exotic' is used in this text to refer to plantain and banana hybrids and landraces introduced from regions other than the study locations. The cultivars differ in their growth characteristics, tolerance or resistance to the various *Musa* pests and diseases (Table 3). The exotic materials from the Bioversity International Transit Centre (ITC) in Leuven, Belgium were multiplied at the Agrobiotech tissue culture lab in Bujumbura, Burundi, while the IITA/NARO hybrids were multiplied at the Phytolabu lab in Bujumbura.

Sword suckers of the local cultivars that served as checks were obtained from the Institut National pour l'Étude et la Recherche Agronomiques (INERA) Mulungu, South Kivu *Musa* germplasm collection (Table 3), while the highland cooking banana 'Mplologoma' was obtained from Rwanda. Sucker planting material was pared and a 30 cm section of the pseudostem was left on the corm.

Suckers were planted with a small portion (15 cm) of the pseudostem sticking out of the ground. Hardening of tissue culture (TC) plantlets was done at the INERA Mulungu research station in South Kivu, DR Congo. Three month old TC-derived plantlets (on average 30 cm high) were transported to the various sites in South and North Kivu for subsequent planting.

Fifteen plants of each cultivar (in three replicates of five plants) were planted at each experimental site. Plants were spaced at 3 × 2 m, providing a density of 1,667 plants/ha. The size of the planting hole was 60 × 60 × 60 cm and 10 kg of decomposed cow manure was applied in each planting hole at planting. Weeding was carried out at three-monthly intervals, while de-suckering and de-leafing of dead leaves was practiced on a case-by-case basis. Three plants were kept per mat (that is, parent, first ratoon and second ratoon). Mulching was carried out at the beginning of each dry season. Where necessary, forked wooden sticks were used to support mature plants with heavy bunches to prevent toppling. The geographical coordinates of each experimental site were recorded using a GARMIN Global Positioning System (GPS) unit (Table 1). In addition, composite soil samples (0 to 30 cm soil layer) were collected at each location to determine soil physical and chemical characteristics (Table 2). Rainfall data (Table 1) were derived from radar images and were provided by the International Center for Tropical Agriculture's Climate Change, Agriculture, Food Security programme.

Banana growth and yield data were collected and averaged over three cropping cycles (that is,plant crop cycle, 1st ratoon and 2nd ratoon). Data collected during each cycle comprised plant performance indicators at flowering (plant height, pseudostem circumference at soil level and at one metre above soil level and number of functional leaves) and at harvest (bunch weight, number of fruits per bunch, average number of fruits per hand, number of hands per bunch, average fruit length of the second lowest hand) and number of days from planting to flowering and from planting to harvest. Plant height was measured from soil level to the point where the leaf petioles of the youngest two leaves intersect, while the total number of functional leaves was determined by counting all the existing green leaves on a plant. The functional leaves had at least 50% green leaf lamina surface area. Mature bunches were harvested when the fingers of the second lowest hand had attained a round shape (Nguthi et al., 1999). Bunch weight was measured with a spring balance. Average annual production was calculated using the formula described by Gaidashova et al. (2008) as:

$$\text{Average annual production} = \text{Bunch weight/number of days to harvest} \times 365 \times \text{plant density ha}^{-1}$$

where 365 is the number of days in a year.

Statistical analysis was carried out using Statistics Analysis System (SAS) (SAS Institute Inc., 2008). The General Linear Model procedure was used to analyze the data and the Tukey's student range test was used for multiple comparisons. Averages for various growth and yield traits were computed across the three cropping cycles. Soil, weather and plant growth and yield attributes across the cultivars were subjected to a principal component analysis (PCA) using GenStat statistical software (GenStat, 2008) to determine the most important variables that influenced bunch yield (kg), time to flowering (days), time from flowering to harvest (days) and production (t ha^{-1} $year^{-1}$). To further our understanding of the effect of altitude, association plots between altitude and bunch weight, time from planting to flowering, time from flowering to harvest, and total production were developed.

RESULTS

Yield across banana use groups and sites was affected by a combination of factors that included soil characteristics, altitude and plant attributes (Tables 1 and 4, Figures 1 to 5).

Significant variations ($P < 0.05$) in agronomic performance within plantain cultivars (AAB and AAAB genomes), green cooking (AAA-EA), dessert (AAA and AABB) and beer (AAA-EA and other AAA) banana cultivars was observed within and across sites for plant height, pseudostem girth, number of leaves, crop cycle duration and all yield parameters (Tables 5 to 9).

The effect of altitude, soil and plant characteristics on plant performance and yield

A principle components analysis to determine the contribution of altitude, soil and plant characteristics on

Table 1. Site location, altitude and annual rainfall.

Region	Site	Latitude	Longitude	Altitude (masl)*	Annual rainfall (mm)
South Kivu	Kamanyola	S 2.72	E 29.01000	900	1293
	Mulungu	S 2.3345	E 28.79197	1707	1043
	Mushweshwe	S 2.31161	E 28.89186	1528	1043
North Kivu	Butembo	N 0.11786	E 29.25869	1815	1058
	Maboya	N 0.25019	E 29.32986	1412	1275
	Mavivi	N 0.56903	E 29.47894	1066	1389
	Mutwanga	N 0.32942	E 29.73389	1111	1004

*masl: Metres above sea level.

Table 2. Soil characteristics of the sites.

Region	Site	Om (%)	pH	N (%)	P (mg/kg)	Ca (cmolc/kg)	Mg (cmolc/kg)	K (cmolc/kg)	Soil texture
South Kivu	Kamanyola	5.3	6.3	0.24	3.4	7.37	1.63	0.24	Sandy clay
	Mulungu	7.2	6.2	0.32	118.9	6.16	1.08	0.83	Heavy clay
	Mushweshwe	4.8	5.5	0.22	12.4	1.40	0.42	0.46	
North Kivu	Butembo	4.8	5.9	0.25	28.1	2.72	0.75	0.49	Sandy clay
	Maboya	6.0	5.0	0.30	6.1	0.81	0.18	0.09	Clay
	Mavivi	3.6	6.5	0.20	4.4	3.98	0.51	0.16	Sandy loam
	Mutwanga	5.0	6.0	0.26	30.0	3.90	1.05	0.26	-

Om: Organic matter.

plant performance and yield revealed that soil characteristics had the highest contribution to the variation in banana yields (Table 4) across sites. The first, second and third PCs had eigenvalues > 1 and contributed 35, 28 and 18%, respectively to the total variation in the data set while combined together they accounted for 81% of the total variation. PC 1 was mainly influenced by organic matter and soil macro-nutrient content, that is, phosphorus, potassium and nitrogen levels. PC 2 was mainly influenced by soil pH, calcium and magnesium. PC 3 was explained by the yield attribute, the number of hands on a bunch and the growth characteristics (that is, plant girth and height) of the banana cultivars. Altitude (PC 2) contrasted with soil fertility content, pH and the banana growth and yield attributes (Table 4).

The principal component plot for bunch weight separated each location, with Mulungu showing the strongest positive association with K, N, OM and P concentrations in the soil and bunch weight. In soils with lower concentrations of K, N, P and OM, the association between bunch weight and Ca, Mg and pH became more apparent. Altitude had a negative association with bunch weight, particularly when N, K, P and OM concentrations are low. Within each site there is a slope of the plots towards the upper left section of the graph (Figure 1). This may reflect the consistent effect of soil fertility across the range of genotypes at each site.

The principal component plot for time from planting to bunch emergence divides the sites into five groups. Mulungu has a high association between time from planting to bunch emergence and soil N, P, K, and OM concentrations. Mavivi falls low on the PC1 axis with expected low concentrations of N, P, K and OM, but with large plants, and these features are associated with an increased time from planting to bunch emergence and low suckering. Maboya had a strong association with soil pH, Ca and Mg. The fourth group of sites straddles the PC1 axis close to the origin, but is spread along the PC2 axis. Here at moderate fertility the stronger associations between plant size (girth and height) and time from planting to bunch emergence appear.

Altitude did not significantly influence the time from planting to bunch emergence, while it influenced bunch weight and production (Figures 2 and 3). Since higher altitudes are associated with cooler temperatures that slow the development of bananas, one would expect an association between altitude and time from planting to

Table 3. Cultivar use, genome group, type, origin and presence across experimental sites in South and North Kivu, Eastern DR Congo.

Use	Cultivar (genome group)	Type	Origin of planting material	Cultivar characteristics and tolerance/ resistance to pests and diseases	South Kivu			North Kivu			
					A	B	C	D	E	F	G
Plantain	FHIA21 (AAAB)	Hybrid (French type plantain)	FHIA and ITC*, Leuven, Belgium	Resistant to Fusarium (Foc) Race 1 and 2[1, 2], black leaf streak (BLS)[1,2]; susceptible to nematodes[2]; large bunch size [2]; moderately resistant to BBTD[3].	x	x	x	x		x	x
	Musheba (AAB)	Landrace	Local	Susceptible to banana weevils [4, 5]	x	x	x	x	x	x	x
	Obubit Ntang (AAB)	Landrace	West Africa (ITC0519)	Large bunch size; resistant to Foc Race 4 and weevils[6]	x	x		x	x	x	x
	T6 (AAAA)	61-88 Highgate hybrid	Jamaica (ITC1247)	Multiple uses[2]; medium to high bunch yield, intermediate stature, resistant to BLS, Foc Race 1 and 2; highly resistant to yellow sigatoka; susceptible to Foc Race 4 [7].	x	x	x	x	x	x	x
Cooking	Barhabeshya (AAA-EA)	Landrace	Local	Susceptible to banana weevils, nematodes and BLS[5, 8], resistant to Foc (all Races) [6,8]	x	x	x	x	x	x	x
	NARITA 2 (AAA-EA)	Hybrid (secondary triploid)	IITA/NARO, Uganda	Good resistance to BLS [2, 9]	x	x	x	x	x	x	x
	NARITA 4 (AAA-EA)	Hybrid (secondary triploid)	IITA/NARO, Uganda	Good resistance to BLS[2, 9]	x	x	x	x	x		x
	Matoke (AAA-EA)	Landrace	Local	Susceptible to banana weevils [4 5,8], BLS and nematodes [5,8], resistant to Foc (all Races)[6, 8]				x	x	x	x
	Mukingiro (AAA-EA)	Landrace	Local	Susceptible to banana weevils, BLS and nematodes, resistant to Foc (all Races) [5,6 8]				x	x	x	x
	Mbwazirume (AAA-EA)	Landrace	INERA germplasm collection	Very palatable; resistant to Foc (all Races)[6, 8]; susceptible to banana weevils [4,5,8], BLS and nematodes [8]	x	x	x				
	Mpologoma (AAA-EA)	Landrace	Rwanda+	Large bunch size and very palatable[4]; susceptible to banana weevils [5, 8]; BLS and nematodes; resistant to Foc (all Races) [6,8]	x	x					

Table 3. Contd. Cultivar use, genome group, type, origin and presence across experimental sites in South and North Kivu, Eastern DR Congo.

Dessert	Giant Cavendish (AAA)	Landrace	Burundi (TC lab, Agrobiotech)	Moderately resistant to BBTD but susceptible to sigatoka diseases, and Foc Race 4[3]	x	x	x	x	x	x	x
	Dwarf Cavendish (AAA)	Landrace	Burundi (TC lab, Agrobiotech)	Short stature, moderately resistant to BBTD, susceptible to BLS and Foc Race 4[3]	x	x	x	x	x	x	x
	Gros Michel (AAA)	Landrace	Local	Very palatable; moderately resistant to BBTD and nematodes, susceptible to Foc Race 1[3]	x	x	x				
	FHIA03 (AABB)	Hybrid	FHIA and ITC, Leuven, Belgium	Short production cycle, large bunch size [2,7]; susceptible but tolerant to Foc Race 4[2,3,10,11]; resistant to BLS[3,11,12], partially resistant to nematodes[3,11]]	x	x	x	x	x	x	x
Beer	NARITA 27 (AAA-EA)	Hybrid (secondary triploid)	IITA/NARO, Uganda	Resistant to BLS[2, 9]	x	x	x	x	x	x	x
	Yangambi Km5 (AAA)	Landrace	Local	Moderately resistant to BBTD, highly resistant to Foc, BLS and sigatoka diseases and burrowing nematode, R. similis[3]				x	x	x	x
	Ndundu (AAA-EA)	Landrace	Local	Susceptible to banana weevils, BLS and nematodes [5,8]; resistant to Foc (all Races)[5,6,8]	x	x	x				
	Nshikazi (AAA-EA)	Landrace	Local	Susceptible to banana weevils, BLS and nematodes [5,8]; resistant to Foc (all Races)[5 6,8]	x	x	x				

'X' denotes included at the experimental site; '*ITC': Bioversity International Transit Centre, K.U. Leuven, Belgium; #: A, B, C, D, E, F and G, respectively denote Mulungu, Mushweshwe, Kamanyola, Maboya, Mavivi, Mutwanga and Butembo; +: Suckers were used for planting materials obtained locally and from Rwanda. Citations: 1 = Castillo (2002); 2 = Vezina and Van den Bergh (2016); 8 = Vezina (2016); 3 = dela Cruz et al. (2008); 4 = Ocan et al. (2008); 5 = Gold et al. (1999); 6 = Molina et al. (2010); 7 = Daniells (2000); 9 = Tushemereirwe et al. (2015); 10 = Ndungo (1997); 11= FHIA factsheet; 12= Krauss et al. (1999).

bunch emergence to be present in the PCA. A plot of the time from planting to bunch emergence against altitude (using data from Tables 5, 7, 8 and 9) resulted in variable data and the linear regression gave an R^2 of 0.045, indicating no association. However, when a polynomial line was fitted to the data the R^2 increased to 0.29 (Figure 4).

To further evaluate the association between altitude and development, altitude was plotted against the time from bunch emergence to harvest. As expected, and in line with for example Sikyolo et al. (2013), an increased fruit filling phase was observed with increasing altitude (Figure 5), with an R^2 of 0.30 for a polynomial function. The fruit filling phase is likely to be largely free of the effect of early or late suckering that is likely to influence the data on planting to bunch emergence.

Performance of exotic and local plantain cultivars

The cultivar 'T6' had the highest mean plant height (316 cm) and girth (75 cm), while 'FHIA21' had the smallest plant height (287 cm) and girth (69 cm) across the sites. Altitude did not have a consistent effect on plant height or girth. In general, the cultivar 'Musheba' had the highest mean number of functional leaves (8.8 leaves), while the

Table 4. Principle component (PC) scores for eleven variables that influenced the yield of 19 banana cultivars in multi-locational trials across 7 different sites in eastern Democratic Republic of Congo during 2007-2011.

Variable	Latent vectors (Loadings)			
	1	2	3	4
Altitude	0.24595	**-0.36691**	0.34367	-0.00412
Calcium (Ca)	0.28463	**0.42949**	-0.23350	-0.03423
Magnesium (Mg)	0.24033	**0.40609**	-0.28481	0.04469
Potassium (K)	**0.44659**	-0.05409	0.22688	0.03434
Nitrogen (N)	**0.36761**	-0.27999	-0.22269	-0.13740
Organic matter (OM)	**0.41995**	-0.21947	-0.24051	-0.00239
Phosphorus (P)	**0.48573**	-0.06250	0.08200	-0.13824
pH	0.15297	**0.50640**	0.07320	-0.14293
Plant height at flowering	0.12770	0.09087	**0.49777**	0.56764
Girth at flowering	0.10888	0.32841	**0.35650**	0.14440
Number of hands per bunch	-0.04800	0.10037	**0.44765**	-0.77081
Latent roots	3.845	3.061	1.976	0.853
Variation (%)	35	28	18	8

In bold are the scores that contribute most to the variation within the PC.

least were noted in 'Obubit' (Table 5). The highest number of functional leaves was recorded at Kamanyola, that has lowest altitude (900 masl), although no consistent trend was observed with increasing altitude. Generally the cultivars took longer to mature at the high altitude sites. For example the fastest developing plantain cultivar was 'T6' recorded at the low altitude site at Mavivi (1066 masl; flowering at 373 days and harvested at 502 days) followed by 'Musheba' at Kamanyola (900 masl; 546 days to harvest). 'Obubit' and 'FHIA21', respectively had the longest time to flowering at the high and mid altitude sites at Mulungu (1707 masl; 580 days) and Mushweshwe (1528 m; 579 days). The mid altitude sites of Maboya (1412 masl) and Mushweshwe, characterized by high erosion and poorer soils (Table 2), generally recorded long crop cycles. For example, 'T6' and 'FHIA21' took the longest time from planting to harvest at Maboya (727days) and Mushweshwe (741days), respectively (Table 5).

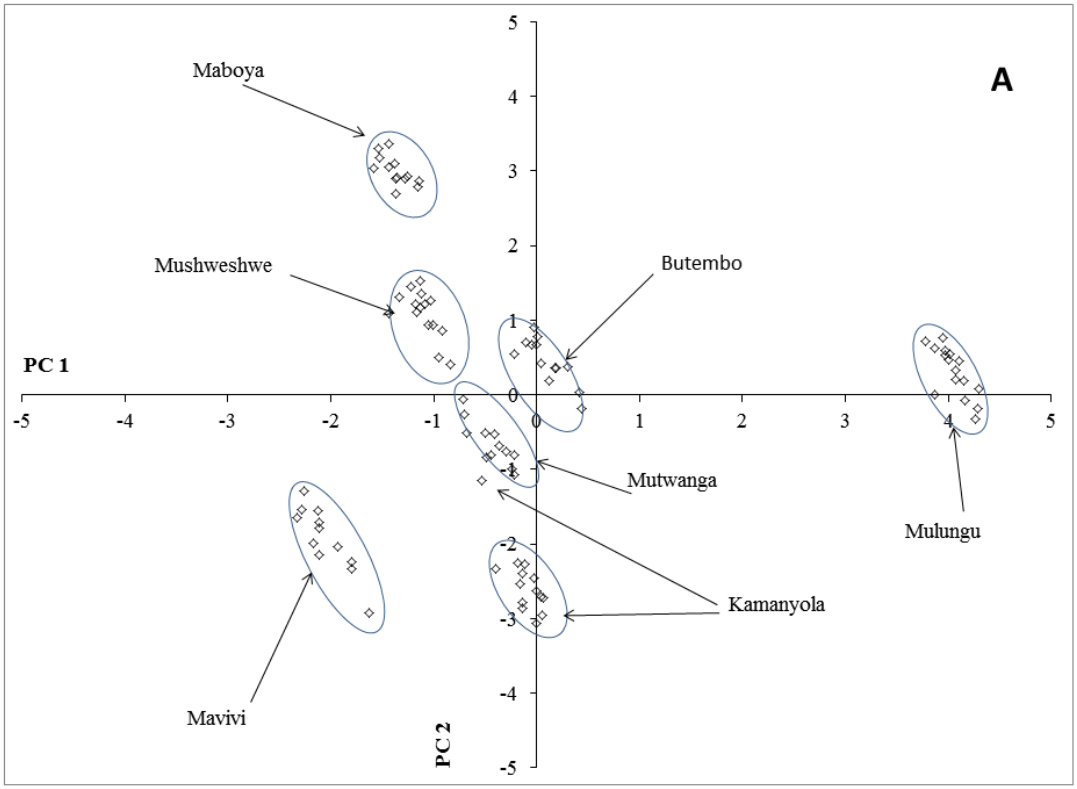
'Musheba' (36.6 kg), 'T6' (34.3 kg) and 'Obubit' (33.2 kg) had the heaviest bunch weights at the low altitude Mavivi (1066 masl) site, followed by 'Musheba' (32.1 kg) at the low altitude Mutwanga (1111 masl) site (Table 5). The bunch weights of the local cultivar 'Musheba' and the exotic cultivars 'T6' and 'Obubit' at these low altitudes did not differ significantly at $P<0.05$. These cultivars generally outperformed 'FHIA21' at low altitudes (Mutwanga, Mavivi and Kamanyola), whereas 'FHIA21' outperformed them at the mid to high altitude sites at Butembo (1815 masl), Maboya, Mulungu and Mushweshwe (Table 5). Similar trends to those in the bunch weight were observed in the other bunch attributes, that is, number of hands per bunch, number of fingers/fruits per bunch and finger length (Table 5). Across all the plantain cultivars, except 'FHIA 21' for which altitude was positively correlated with plant height (R^2 = 0.35) and bunch weight (R^2= 0.74), low correlations (-0.01 to 0.29) were observed between altitude and all growth and yield attributes (Table 6).

Performance of local/regional and hybrid green cooking banana cultivars

Among the green cooking cultivars, 'Barhabeshya' (359 cm) recorded the highest plant height, though 'Mukingiro' (415 cm) and 'NARITA 2' (408 cm) had above average heights at Butembo (1815 masl) and Mavivi (1066 m), respectively. 'Matooke' (231 cm) was the shortest cultivar (Table 7). Plant height generally increased with increasing altitude across all the cultivars evaluated except for 'Mpologoma' (Table 6). However, significant increases in plant height were only observed in 'Barhabeshya' (R^2=0.96) and 'Mbwazirume' (R^2=0.67). The largest mean pseudostem girth at soil level was recorded for 'Barhabeshya' (83 cm) followed by 'Mbwazirume' (82 cm) and the least in 'Matooke' (71 cm) (Table 7). Except for 'Mbwazirume' (R^2=0.86), plant girth generally increased with altitude (Table 6).

On average, the introduced cultivar 'Mpologoma' (11.3 leaves) had the h ighest mean number of functional leaves at flowering followed by 'Barhabeshya' (11.0 leaves) and the least in'Mukingiro' (6.4 leaves).

Mushweshwe (mid altitude) followed by Kamanyola (low) generally had the highest number of leaves (Table 7). Apart from 'Matooke' whose leaves increased with altitude (R^2=0.42) and 'NARITA 4' whose number of leaves profoundly declined with altitude (R^2=-0.33), leaves in other cultivars were not significantly impacted



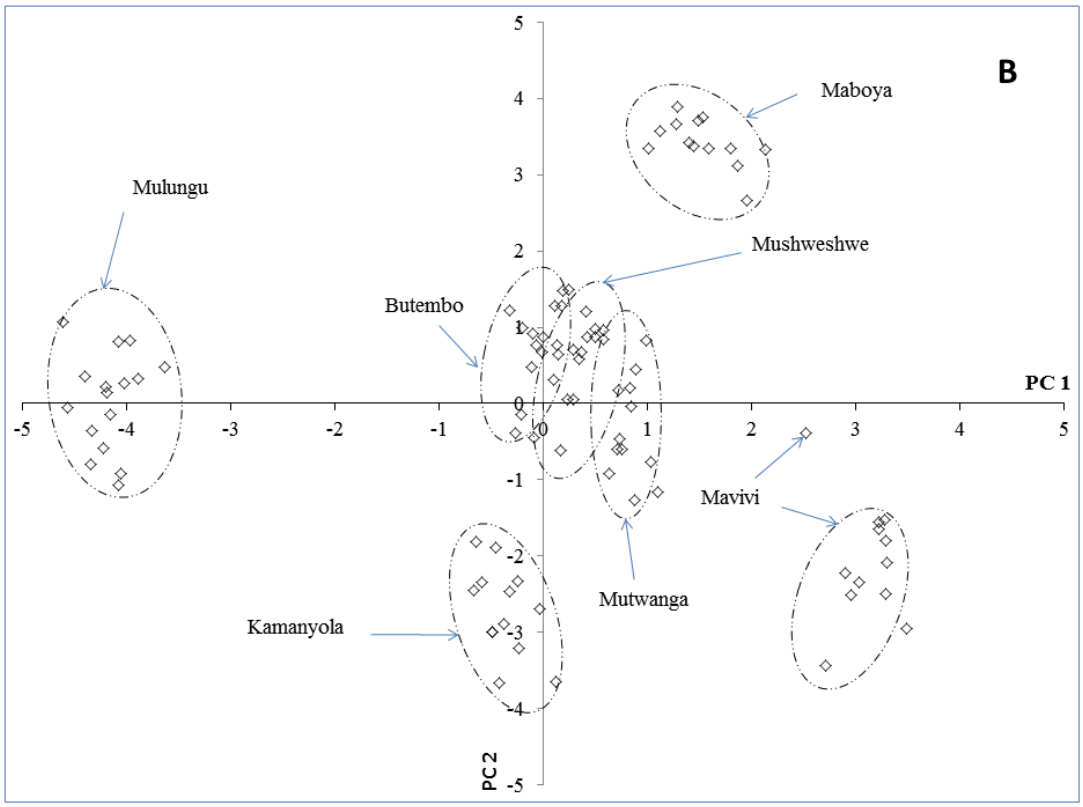


Figure 1. Plot of principal component 1 and 2 for banana yields (A) and time from planting to flowering (B) for 19 different banana cultivars, across 7 different sites in eastern Democratic Republic of Congo.

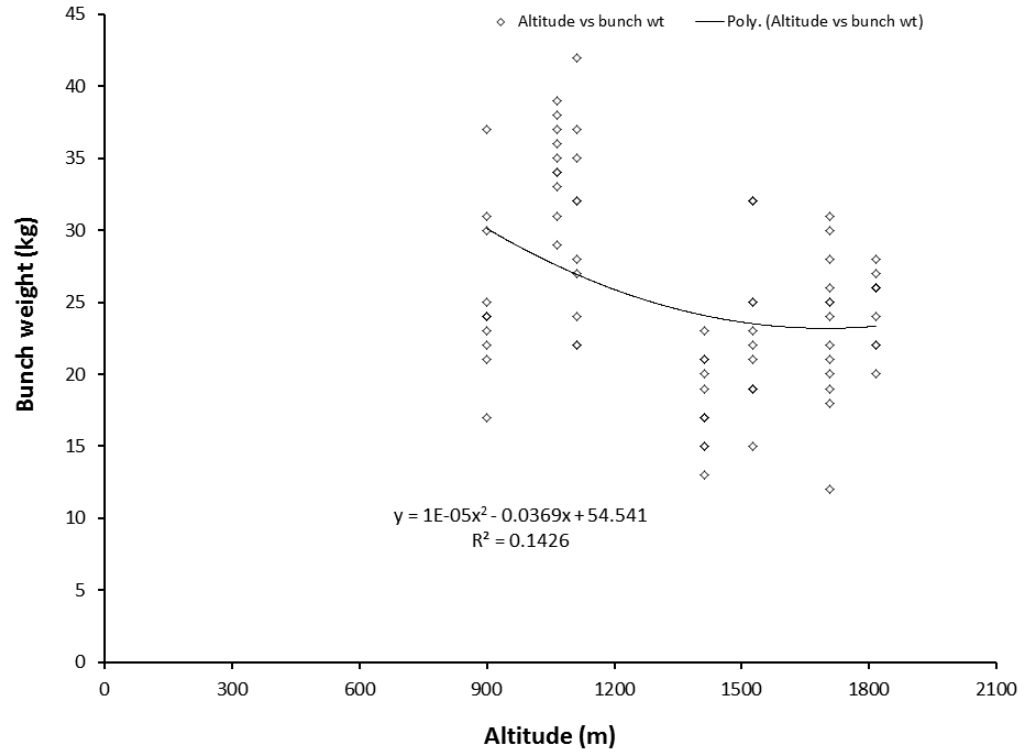


Figure 2. Variation in bunch weight (kg) with altitude across 19 cultivars, three crop production cycles and 7 experimental sites in eastern Democratic Republic of Congo.

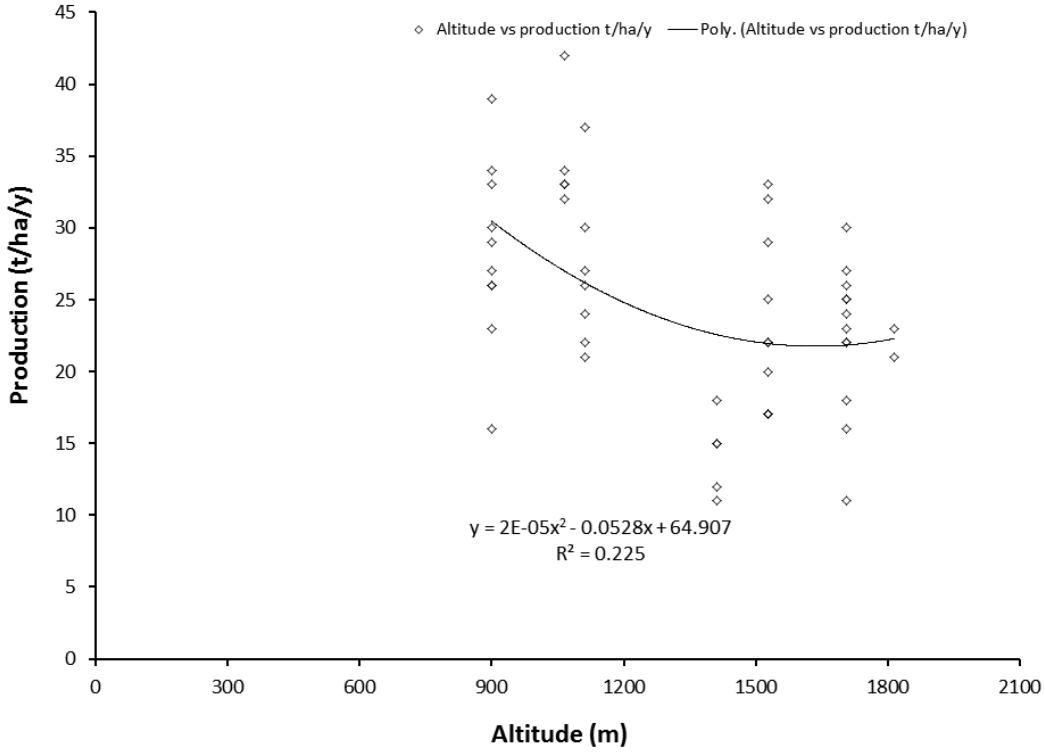


Figure 3. Variation in production (t/ha/y) with altitude across 19 cultivars, three crop production cycles and 7 experimental sites in eastern Democratic Republic of Congo.

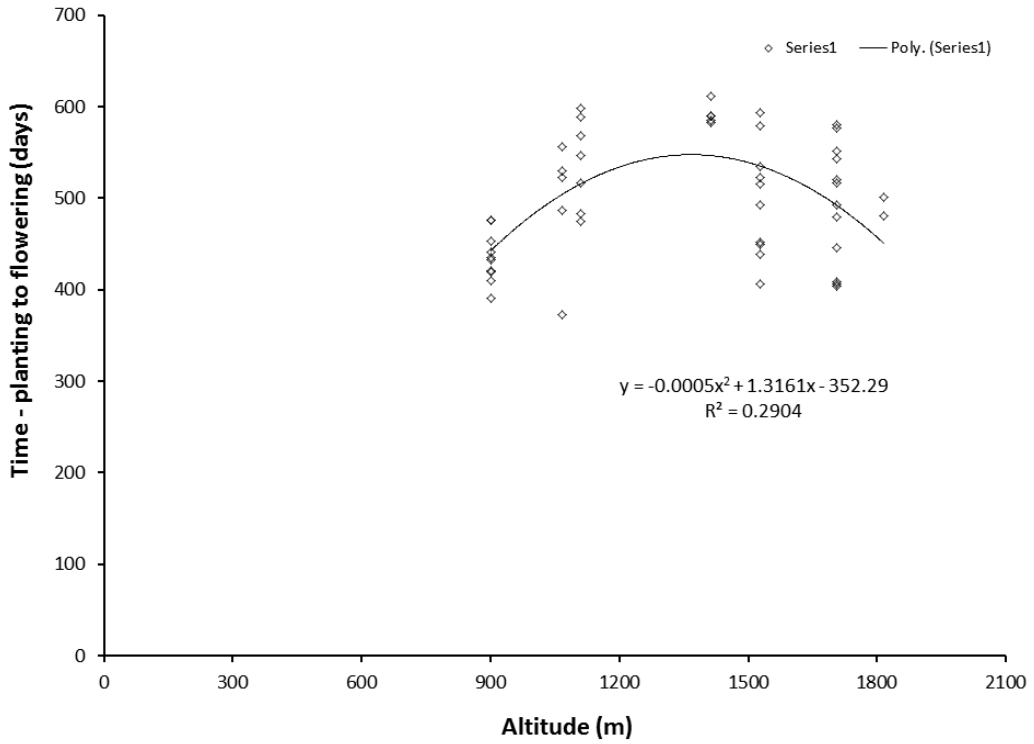


Figure 4. Variation in time from planting to flowering (days) with altitude across 19 cultivars, three crop production cycles and 7 experimental sites in eastern Democratic Republic of Congo.

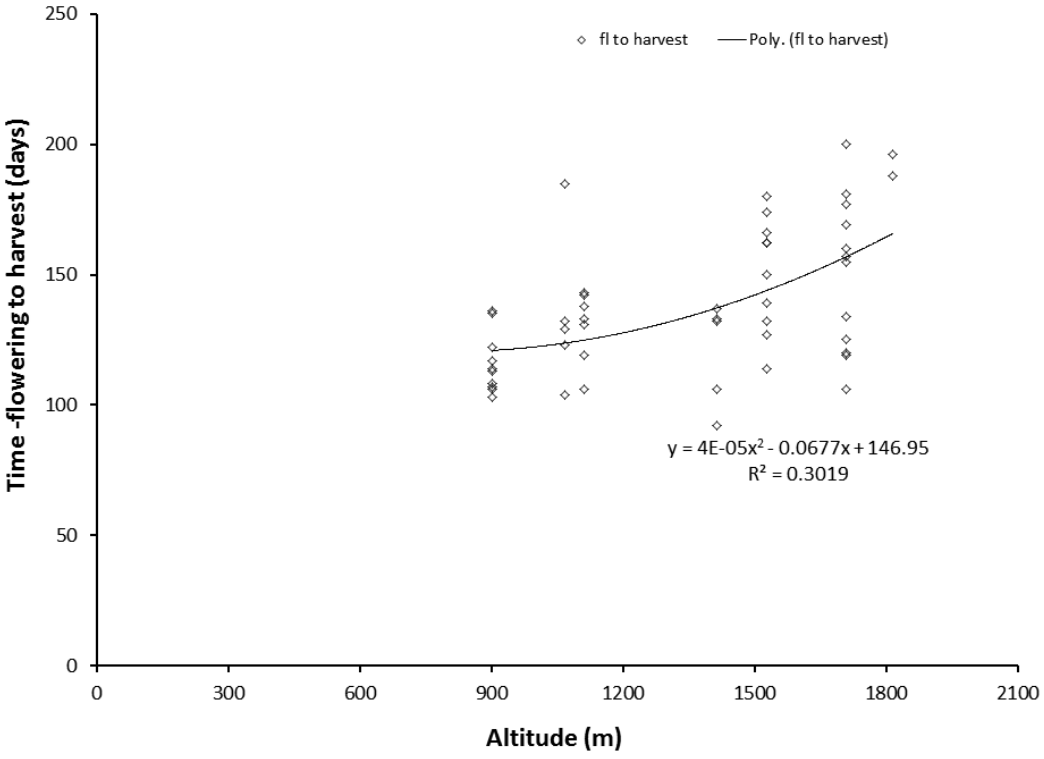


Figure 5. Variation in time from planting to flowering (days) with altitude across 19 cultivars, three crop production cycles and 7 experimental sites in eastern Democratic Republic of Congo.

Table 5. Plant performance traits of plantain (*Musa* AAB genome group) cultivars at flowering and harvesting stages, averaged per site. Means followed by the same letter within a column are not significantly different at P<0.05.

Genotype	Site (Altitude)	Plant performance traits at flowering			Crop cycle duration (days)		Plant performance traits at harvest				
		Height (cm)	Girth at base (cm)	Functional leaves at bunch emergence	Time to flowering	Time to harvest	N° hands	N° fingers	Finger length (cm)	Bunch weight (kg)	Production (Tonnes.ha^{-1} per year)
FHIA21 (AAAB)	Kamanyola (900 m)	279^{fg}	74^{efg}	10^{cb}	476^{ebdc}	611^{bdc}	7^{ij}	83^{kj}	19^{m}	17^{hi}	16^{o}
	Mutwanga (1111 m)	230^{i}	60^{mkl}	6^{i}	–	–	9^{gefcd}	136^{fcebd}	24^{fe}	22^{fcegd}	–
	Maboya (1412 m)	238^{hi}	54^{m}	8^{gfh}	–	–	9^{gefcd}	136^{cebd}	27^{ba}	23^{fcebd}	–
	Mushweshwe (1527 m)	304^{fe}	66^{jik}	9^{cd}	579^{a}	741^{a}	8^{gefijh}	111^{fiegjh}	19^{mlk}	21^{fhegd}	17^{m}
	Mulungu (1707 m)	318^{dec}	75^{efd}	7.6^{gf}	479^{ebdc}	648^{bdac}	7^{ijh}	149^{cbd}	21^{ijk}	25^{cebd}	23^{e}
	Butembo (1815 m)	301^{fe}	87b^{a}	8.7^{ed}	–	–	10a	158^{b}	29^{a}	27^{b}	–
Mean	-	278.1	69.4	8.2	529.3	694.4	8.5	128.9	23.2	22.5	18.9
Musheba (AAB)	Kamanyola (900 m)	339^{bdac}	82^{bcd}	13^{a}	432^{edf}	546de	8^{gijh}	–	21^{iljk}	24^{cebd}	27^{d}
	Mavivi (1066 m)	306^{fe}	79^{ecd}	7^{gih}	–	–	10^{a}	200^{a}	25^{fecd}	37^{a}	–
	Mutwanga (1111 m)	314^{dec}	75^{ef}	9e^{d}	–	–	9^{bcd}	136^{fcebd}	25^{fecd}	32^{a}	–
	Maboya (1412 m)	228^{i}	58^{ml}	6^{ih}	583^{a}	689^{ba}	7^{ij}	126^{fcegd}	18^{m}	17^{hgi}	15^{q}
	Mushweshwe (1527 m)	330^{bdec}	65^{jikl}	11^{b}	439^{edfc}	571^{dec}	7^{ij}	77^{k}	23^{fgh}	19^{fhgi}	20^{j}
	Mulungu (1707 m)	352^{ba}	76^{efd}	9^{ed}	406^{ef}	606^{bdc}	8^{gijh}	92^{ikjh}	22^{ijh}	22^{fcegd}	22^{g}
	Butembo (1815 m)	324^{bdec}	71^{ihfg}	8^{gf}	–	–	10^{ba}	153^{cb}	25^{fed}	26^{cb}	–
Mean	-	313.1	72.1	8.8	464.8	603.3	8.6	130.6	22.7	25.3	21
Obubit (AAB)	Mavivi (1066 m)	303^{fe}	75^{ef}	7^{gfh}	530^{bac}	634^{bdac}	9^{ecd}	146^{cbd}	26^{becd}	33^{a}	32^{b}
	Mutwanga (1111 m)	319^{dec}	70^{ihfg}	7^{gfh}	517^{bdac}	655^{bac}	9^{efcd}	123^{fcegdh}	26^{becd}	22^{fcegd}	21^{i}
	Maboya (1412 m)	252^{hgi}	62^{jkl}	7^{gih}	–	–	8^{gfijh}	109^{fiegjh}	25^{fe}	21^{fheg}	–
	Mushweshwe (1528 m)	344^{bac}	67^{jihk}	11^{b}	593^{a}	707^{ba}	8^{gefcdh}	93^{ikjh}	22^{igh}	19^{fhegi}	17^{n}
	Mulungu (1707 m)	300^{fe}	74^{ehfg}	7^{gfh}	580^{a}	700^{ba}	8^{gijh}	98^{ikgjh}	20m^{ljk}	18^{hgi}	16^{p}
	Butembo (1815 m)	310de	71^{ihfg}	8^{gf}	501^{ebdac}	697^{ba}	9^{ecd}	138^{cebd}	25^{fed}	26^{cbd}	23^{f}
Mean	-	304.7	69.6	7.8	544.2	679	8.5	117.8	23.7	23.3	21.5
T6 (AAB)	Kamanyola (900 m)	277^{fg}	85^{bc}	9^{ed}	–	–	7^{j}	–	19^{ml}	23^{fcebd}	–
	Mavivi (1066 m)	362^{a}	93^{a}	8^{ef}	373^{f}	502^{e}	9^{bc}	151^{cb}	26^{bcd}	34^{a}	42^{a}
	Mutwanga (1111 m)	341^{bdac}	80^{ecd}	8^{gf}	483^{ebdc}	616^{bdc}	8^{gefidh}	131^{fcebd}	27^{bc}	28^{b}	27^{c}
	Maboya (1412 m)	261^{hg}	65^{jikl}	7^{gih}	590^{a}	727^{a}	9^{gefcd}	117^{fiegdh}	24^{feg}	21^{fhegd}	18^{l}
	Mushweshwe (1528 m)	343^{bac}	70^{ihfg}	11^{b}	–	–	8^{gefcd}	89^{ikj}	21^{ij}	15^{i}	–
	Mulungu (1707 m)	319d^{ec}	68^{jihfg}	8^{gf}	543^{ba}	677^{bac}	8^{gijh}	105^{fikgjh}	19^{mlk}	20^{fheg}	18^{k}
	Butembo (1815 m)	313d^{ec}	67^{jihkg}	8^{gf}	480^{ebdc}	668^{bac}	9^{ecd}	134^{fcebd}	24^{feg}	24^{fcebd}	21^{h}
Mean	-	316.4	75.4	8.1	493.7	638.1	8.4	121.3	22.9	23.6	25.2

Table 6. Correlation coefficient between altitude and different banana growth and yield attributes.

Cultivar group	Cultivar	Plant Height	Number of functional leaves at flowering	Time to harvest	Bunch weight
Beer	'YangambiKm5'	0.02	0.11	0.83	0.12
	'Ndundu'	0.92	0.98	0.48	0.89
	NARITA 27	0.17	- 0.01	0.18	- 0.01
	'Nshika'	0.71	- 0.26	0.98	- 0.56
Plantain	FHIA21	0.35	- 0.01	- 0.01	0.74
	'Obubit'	0.01	0.03	0.02	- 0.22
	'Musheba'	0.01	- 0.12	0.02	- 0.21
	T6	0.01	- 0.01	0.01	- 0.29
Cooking	'Barhabeshya'	0.96	- 0.12	0.38	- 0.99
	'Mukingiro'	0.34	0.01	1.00	- 0.43
	'Matooke'	0.32	0.42	1.00	- 0.04
	NARITA 2	0.05	0.06	0.15	- 0.15
	NARITA 4	0.18	- 0.33	0.35	- 0.38
	'Mbwazirume'	- 0.12	- 0.12	- 0.04	- 0.74
	'Mpologoma'	- 0.33	-	-	-
Dessert	'Giant Cavendish'	0.25	0.06	0.39	- 0.32
	'Gros Michel'	0.95	- 0.07	0.99	0.88
	'Dwarf Cavendish'	0.13	0.09	0.43	- 0.16
	FHIA03	0.43	- 0.01	0.38	- 0.21

'-' Denotes negative correlation coefficient (R^2).

impacted by the changes in altitude (Table 6). Generally the time to harvest increased with altitude except for 'Mpologoma' (Tables 6 and 7). The lowest time to maturity was generally recorded at the lowest altitude site in Kamanyola (900 m) for the cooking cultivars. 'Mpologoma', an introduced cultivar, took the shortest time to reach shooting and harvesting at Mushweshwe (406 and 545 days) and Mulungu (410 and 528 days). In contrast, the longest time to maturity was recorded in 'NARITA 4' (716 days) at Mushweshwe (Table 7). 'NARITA 4' took generally longer to mature at the mid to high altitudes compared with the low altitudes (Table 7).

The local cultivar 'Barhabeshya' at 33 kg bunch weight had the heaviest bunch. Generally bunch weight, number of hands and fingers per bunch, and finger length were higher at the low altitude sites (Table 7) and declined with increasing altitude. The bunch weights of 'Barhabeshya' (R^2= 0.99) and 'Mbwazirume' (R^2= 0.74) were most strongly correlated with altitude while 'Matooke' (R^2= 0.09) and 'NARITA 4' (R^2= 0.15) showed no linear association (Table 6).

The heaviest bunches averaging 39 kg were recorded in 'NARITA 4' at Mavivi. Similarly 'NARITA

Table 7. Plant performance traits of cooking (*Musa* AAA-EA genome group) cultivars at flowering and harvesting stages, averaged per site. Means followed by the same letter within a column are not significantly different at P<0.05.

Genotype	Site (Altitude)	Plant performance traits at flowering			Crop cycle (days)		Plant performance traits at harvest				
		Height (cm)	Girth at base (cm)	Functional leaves	Time to flowering	Time to harvest	N° hands	N° fingers	Finger length	Bunch weight (Kg)	Production (tons ha^{-1} per year)
Barhabeshya (AAA-EA)	Kamanyola (900 m)	290^{kgijh}	83^{bc}	11^{bc}	453^{ih}	589^{gf}	10^{cb}	–	20^{hfg}	37b^{a}	39^{a}
	Mushweshwe (1528 m)	393^{ba}	78^{dce}	12^{ba}	452^{h}	579^{hgf}	9^{ghikj}	129^{hi}	22^{bcd}	32^{edc}	33^{e}
	Mulungu (1707 m)	394^{ba}	90^{a}	10^{ed}	577^{bdac}	702^{ba}	9^{ghfde}	164de	21^{efd}	30^{ed}	26^{i}
Means	-	359	83	11	494	623	10	146.8	21	33	33
Matooke (AAA-EA)	Mavivi (1066 m)	238^{lnm}	76^{gdfe}	6^{mnl}	–	–	10^{gcfde}	152^{hfgde}	22^{bcd}	34^{bdc}	–
	Mutwanga (1111 m)	215^{nm}	66^{ij}	7^{jkl}	546^{dec}	677^{bac}	9^{ghfie}	193^{cb}	23^{bc}	24^{jgifh}	22^{p}
	Maboya (1412 m)	196^{n}	61^{j}	6^{mnl}	611^{a}	703^{ba}	8^{ikj}	141^{hfg}	18^{hij}	17^{m}	15^{r}
	Butembo (1815 m)	273^{klij}	83^{bc}	8^{jki}	–	–	9^{ghfie}	155^{fgde}	21^{ecd}	28^{ef}	–
Means	-	231	71	7	578	690	9	160	21	26	18
Mbwazirume (AAA-EA)	Kamanyola (900 m)	306^{fgijh}	90^{a}	10^{ef}	476^{hg}	584^{hgf}	8^{ikj}	–	17^{l}	25^{gfh}	26^{h}
	Mushweshwe (1528 m)	348^{fbdec}	77^{gdfce}	11^{bc}	–	–	9^{ghfikj}	134^{hgi}	18^{kij}	23^{jgikh}	–
	Mulungu (1707 m)	331^{fgdeh}	79^{dc}	8^{jhi}	551^{bdec}	657^{bdc}	8^{hikj}	139^{hfg}	17^{kl}	24^{jgih}	22^{q}
Means	-	328	82	10	513	620	9	136	17	24	–
Mpologoma (AAA-EA)	Mushweshwe (1528 m)	304^{fgijh}	72^{gfh}	12^{a}	406^{j}	545h^{i}	8^{ikj}	118^{ji}	18^{kjl}	22^{jgikh}	25^{l}
	Mulungu (1707 m)	296^{gijh}	76^{gdfe}	10^{ecd}	409^{j}	528^{i}	8^{lk}	141^{hfg}	17^{kl}	21^{jlikh}	25^{m}
Means	-	300	74	11	408	536	8	130	17	22	25
Mukingiro (AAA-EA)	Mavivi (1066 m)	314^{fgieh}	77^{gdfce}	6^{n}	522^{fe}	645^{edc}	10^{cfde}	207^{b}	24^{ba}	35^{bac}	33^{d}
	Mutwanga (1111 m)	337^{fgdec}	83^{bc}	8^{jki}	589^{bac}	695^{ba}	9^{ghfiej}	193^{cb}	22^{ecd}	35^{bc}	30^{g}
	Maboya (1412 m)	263k^{lijm}	68^{ih}	6^{mn}	–	–	7^{m}	103^{j}	19^{hig}	19l^{km}	–
	Butembo (1815 m)	415^{a}	88^{ba}	7m^{lk}	–	–	9^{ghfie}	149^{hfge}	21^{ecd}	26^{gf}	–
Means	-	332	79	6	556	670	9	163	22	29	32
NARITA 2 (AAA-EA)	Kamanyola (900 m)	267^{klij}	72^{gfhe}	8^{jhi}	441^{ihj}	548^{hgi}	8^{ml}	–	11^{n}	21^{jlik}	23^{n}
	Mavivi (1066 m)	408^{a}	79^{dc}	7^{jkl}	486^{fhg}	671^{bac}	11^{b}	202^{b}	23^{bc}	36^{ba}	33^{f}
	Mutwanga (1111 m)	386^{bac}	79^{dc}	8^{ghi}	474^{hg}	616^{edf}	10^{cb}	212^{b}	25^{a}	37^{ba}	37^{b}
	Maboya (1412 m)	291^{kgijh}	65^{ij}	6^{mnl}	–	–	9^{ikj}	141^{hfg}	19^{hig}	17^{lm}	–
	Mushweshwe (1528 m)	357^{bdec}	68^{ih}	11^{bcd}	534de	696^{ba}	10^{cfde}	164de	19^{hifg}	25^{gfh}	22^{o}

Table 7. Contd.

	Mulungu (1707 m)	358^{bdec}	79^{dc}	9^{gf}	446^{ihj}	606^{ef}	10^{cfde}	158^{fde}	17^{kjl}	25^{gigh}	25^{k}
	Butembo (1815 m)	376^{bdac}	77^{dfce}	7^{jk}	–	–	10^{cd}	168de	20^{efg}	22^{jgikh}	–
Means	-	349	74	8	476	627	10	174	19	26	28
	Kamanyola (900 m)	258^{kljm}	79^{dc}	11^{bc}	421^{ij}	534^{i}	8.3^{lkj}	–	14^{m}	22^{jgikh}	26^{j}
	Mavivi (1066 m)	331^{fgdeh}	78^{dc}	8^{jki}	556^{bdec}	688^{bac}	12^{a}	234^{a}	23^{bc}	39^{a}	34^{c}
NARITA 4 (AAA-EA)	Maboya (1412 m)	241^{klnm}	62^{j}	7^{jkl}	–	–	9^{ghikj}	173^{cd}	20^{hfg}	20^{jlkm}	–
	Mulungu (1707 m)	284^{klijh}	71^{gh}	9^{gh}	516^{feg}	673^{bac}	9^{ghikj}	98^{j}	13^{n}	12^{n}	11^{s}
	Butembo (1815 m)	375^{bdac}	78^{dc}	7^{jk}	–	–	10^{cde}	171^{cde}	19^{hifg}	20^{jlkm}	–
Means	-	298	74	8	498	631	10	169	18	23	24

4' bore the highest mean number of hands per bunch (12.3) and fruits per bunch (234), and had longer fruits (22.6 cm) at this site (Table 7). High bunch weights were also obtained from 'NARITA 2' at both Mutwanga (37.3 kg) and Mavivi (35.8 kg).

At Kamanyola (900 m), the lowest altitude site, 'Barhabeshya' equally had a good bunch weight of 37.2 kg and number of hands (10.3) per bunch. 'Matooke' and 'NARITA 2' had the poorest performance at Maboya, yielding mean bunch weights of 17.3 and 17.0 kg, respectively (Table 7).

Performance of local and hybrid dessert banana cultivars

'Gros Michel' (374 cm), followed by 'Giant Cavendish' (349 cm) were the tallest, with the shortest plants (193 cm) observed in the 'Dwarf Cavendish' cultivar (Table 8). Plant height declined with increasing altitude across all cultivars. 'FHIA03' had the largest mean girth (90 cm) at soil level compared with other dessert banana types (Table 8). Girth declined with altitude only in FHIA03.The number of functional leaves at flowering varied between 11.8 leaves in 'Gros Michel'and 8.5 in 'Giant Cavendish'. There was no significant correlation between the number of functional leaves and altitude in 'Giant Cavendish', 'Dwarf Cavendish', 'FHIA03' or 'Gros Michel'.

Crop cycle duration generally increased with altitude across the cultivars (R^2 = 0.38 to 0.99) (Table 6). For example, the shortest times to flowering of 390, 410 and 435 days were recorded for 'Gros Michel', 'Giant Cavendish' and 'Dwarf Cavendish', respectively at the lowest altitude site of Kamanyola (900 m). 'FHIA03' was the best performing cultivar in terms of time to flowering and maturity at the higher altitude sites (Table 8). 'FHIA03' (30 kg) and 'Gros Michel' (29 kg), respectively had the heaviest bunch weights, with the lightest in 'Giant Cavendish'. The heaviest bunches were recorded at the low altitude sites of Mavivi, Kamanyola and Mutwanga. Except in 'Gros Michel', where bunch weight significantly (R^2=0.88) increased with altitude, bunch weight in the other three cultivars showed no significant correlation with altitude (Tables 6).

Performance characteristics of beer cultivars across sites

'Ndundu' was generally the most robust cultivar in the vegetative stage. It had the greatest plant height (341 cm), girth (81 cm) and number of functional leaves at flowering (11), while 'Yangambi Km5' and 'NARITA 27' were the least vigorous (Table 9). Plant height significantly increased with increasing altitude in 'Ndundu' (R^2= 0.7) and 'Nshika' (R^2= 0.9) while not significantly (R^2= 0.01 to 0.2) for 'Yangambi Km5'and 'NARITA27' (Table 6). The pseudostem girth in 'Nshika' significantly (R^2= 0.64) declined with the altitude whereas the other cultivars were not affected. The number of functional leaves in 'Yangambi Km5' was not correlated with altitude.

The banana beer cultivar 'Ndundu' (AAA-EA) took the shortest time to flower (417 days) and to

Table 8. Plant performance traits of dessert cultivars (*Musa* AAA genome group) at flowering and harvesting stages, averaged per sites. Means followed by the same letter within a column are not significantly different at P<0.05.

Genotype	Site (Altitude)	Plant performance traits at flowering			Crop cycle (days)		Plant performance traits at harvest				
		Height (cm)	Girth at base (cm)	Functional leaves	Time to flowering	Time to harvest	N° hands	N° fingers	Finger length	Bunch weight	Production (tons ha^{-1} per year)
Giant Cavendish (AAA)	Kamanyola (900 m)	269^{f}	77^{hji}	10^{ed}	410^{g}	513^{c}	8^{ih}	–	19^{g}	24^{ih}	29^{g}
	Mavivi (1066 m)	402^{a}	90^{c}	6^{i}	–	–	10^{bdac}	217^{a}	24^{ba}	29^{dfe}	–
	Mutwanga (1111 m)	345^{d}	76^{jki}	8^{gh}	598^{a}	741^{a}	9^{f}	149^{gdfce}	24^{a}	32^{dc}	26^{i}
	Maboya (1412 m)	304^{e}	69^{lm}	6^{i}	585^{bac}	717^{a}	7^{ij}	112^{gfh}	19^{g}	13^{l}	11^{o}
	Mushweshwe (1528 m)	354^{dc}	71^{lk}	11^{c}	515^{edc}	681^{a}	7^{ihj}	108^{gh}	17^{hji}	19^{k}	17^{m}
	Mulungu (1707 m)	391^{ba}	86^{dfce}	10^{ed}	–	–	8^{gh}	119^{gfhe}	18^{hg}	19^{jk}	–
	Butembo (1815 m)	381^{b}	81^{hfg}	8^{gh}	–	–	9^{gf}	141^{gdfce}	21de	22^{ji}	–
Mean	-	349	79	9	527	663	8	141	20	23	21
Dwarf Cavendish (AAA)	Kamanyola (900 m)	169^{j}	77^{hji}	11^{c}	435^{fg}	557^{cb}	9^{gf}	136^{gdfce}	19^{g}	31^{dc}	33^{b}
	Mavivi (1066 m)	213^{h}	79^{hgi}	6^{i}	–	–	10^{ebdac}	209^{ba}	23^{bc}	31^{dc}	–
	Mutwanga (1111 m)	191^{i}	74^{ljk}	8^{gh}	568^{bac}	687^{a}	10^{ed}	168^{c}	22^{dc}	27^{gfe}	24^{j}
	Maboya (1412 m)	158^{j}	66^{m}	7^{i}	590^{ba}	723^{a}	9^{f}	149^{dfce}	17^{hi}	15^{l}	12^{n}
	Mushweshwe (1528 m)	192^{i}	77^{hji}	13^{b}	523^{bdc}	703^{a}	10^{ebdc}	175^{bc}	17^{ji}	25^{gh}	22^{l}
	Mulungu (1707 m)	237^{g}	88^{dc}	12^{c}	492^{fed}	669^{a}	11^{a}	174^{bc}	16^{j}	26^{gfh}	24^{k}
	Butembo (1815 m)	194^{i}	80^{hgi}	9^{ef}	–	–	10^{bac}	214^{a}	21^{fe}	26^{gfh}	–
Mean	-	193	77	9	522	668	10	175	19	26	23
FHIA03 (AABB)	Kamanyola (900 m)	252^{gf}	96^{b}	14^{a}	419^{g}	536^{cb}	8^{gf}	112^{gfh}	20^{f}	30^{dce}	34^{a}
	Mavivi (1066 m)	357^{dc}	113^{a}	9^{gf}	–	–	10^{e}	177^{bc}	23^{bc}	38^{b}	–
	Mutwanga (1111 m)	265^{f}	79^{hjgi}	8^{gh}	–	–	10^{ebdac}	162^{dc}	24^{ba}	42^{a}	–
	Maboya (1412 m)	270^{f}	74^{ljk}	7^{h}	–	–	7^{j}	94^{h}	18^{hg}	15^{l}	–
	Mushweshwe (1528 m)	347^{d}	89^{dc}	14^{a}	449^{feg}	599^{b}	10^{ed}	143^{gdfce}	18^{hgi}	32^{c}	32^{c}
	Mulungu (1707 m)	347^{d}	83^{fge}	11^{d}	404^{g}	559^{cb}	8^{gf}	127^{gdfhe}	18^{hgi}	28^{gfe}	30^{d}
	Butembo (1815 m)	373^{bc}	98^{b}	10^{e}	–	–	9^{f}	161^{dc}	21^{fe}	26^{gfh}	–
Mean	-	316	90	10	424	565	9	139	20	30	32
Gros Michel (AAA)	Kamanyola (900 m)	316^{e}	84d^{fge}	12^{c}	390^{g}	496^{c}	8^{ih}	125^{gdfhe}	21de	24^{ih}	30^{e}
	Mushweshwe (1528 m)	405^{a}	88^{dce}	14^{ba}	493^{fed}	667^{a}	10^{edc}	138^{gdfce}	22^{dc}	32^{c}	29^{f}
	Mulungu (1707 m)	403^{a}	90^{c}	10^{ed}	520^{bedc}	701^{a}	11^{ba}	157^{dce}	19^{g}	31^{dc}	27^{h}
Mean	-	374	87	12	468	622	9	140	21	29	28

Table 9. Plant performance traits of beer (*Musa* AAA-EA genome group) cultivars at flowering and harvesting stages, averaged per site. Means followed by the same letter within a column are not significantly different at P<0.05.

Genotype	Site (Altitude)	Plant performance traits at flowering			Crop cycle (days)		Plant performance traits at harvest				
		Height (cm)	Girth at base (cm)	Functional leaves	Time to flowering	Time to harvest	N° hands	N° fingers	Finger length	Bunch weight (Kg)	Production (tons ha^{-1} per year)
Yangambi Km5 (AAA)	Mavivi (1066 m)	293^{dfe}	66^{fg}	5^{j}	502^{fdec}	613^{bdc}	9^{fed}	136^{gh}	16^{feg}	18^{gf}	18^{n}
	Mutwanga (1111 m)	305^{dce}	68^{fg}	8^{edf}	419^{hig}	579^{ed}	10^{ced}	238^{ba}	15^{fg}	26^{dce}	27^{f}
	Maboya (1412 m)	261^{g}	65^{g}	6^{hi}	485^{fdeg}	627^{bdc}	9^{feg}	174^{dfe}	17^{fe}	13^{h}	12^{p}
	Butembo (1815 m)	311^{dc}	68^{fg}	7^{egf}	540^{bdac}	671^{ba}	11^{b}	182^{dce}	19^{c}	28^{dc}	25^{i}
Mean	-	293	67	7	487	623	10	182	17	21	21
Ndundu (AAA-EA)	Kamanyola (900 m)	306^{dce}	78^{dc}	11^{bc}	380^{i}	480^{f}	7^{h}	–	18de	21^{f}	27^{g}
	Mushweshwe (1528 m)	362^{a}	77^{dc}	12^{a}	470^{feg}	593^{edc}	9^{fg}	157^{gfe}	17^{e}	26de	26^{h}
	Mulungu (1707 m)	357^{a}	87^{a}	10^{c}	402^{hi}	530^{ef}	10^{ced}	169^{gfe}	15^{g}	31^{dc}	35^{b}
Mean	-	342	81	11	417	535	9	133	17	26	30
NARITA 27 (AAA-EA)	Kamanyola (900 m)	226^{h}	44^{h}	8^{ed}	436^{fhig}	534^{ef}	6^{i}	–	17^{e}	15^{gh}	17^{o}
	Mavivi (1066 m)	314^{dc}	85^{ba}	7^{hgf}	471^{feg}	610^{bdc}	12^{a}	263^{a}	23^{a}	47^{a}	46^{a}
	Mutwanga (1111 m)	304^{dce}	75de	7^{egf}	512^{bdec}	661^{bac}	10^{cb}	213^{bc}	24^{a}	36^{b}	33^{d}
	Maboya (1412 m)	269^{gf}	68^{fg}	6^{hgi}	–	–	10^{cbd}	212^{bc}	22^{b}	27^{dce}	–
	Mushweshwe (1528 m)	282^{gfe}	67^{fg}	10^{c}	566^{bac}	721^{a}	10^{ced}	181^{dce}	19^{c}	28^{dc}	24^{k}
	Mulungu (1707 m)	288^{dfe}	66^{fg}	7^{hgf}	573^{ba}	700^{a}	10^{cd}	167^{gfe}	19^{dc}	27^{dc}	24^{j}
	Butembo (1815 m)	312^{dc}	71^{fe}	6^{ji}	438^{fhig}	577^{ed}	10^{cbd}	204^{dc}	21^{b}	28^{dc}	29^{e}
Mean	-	271	68	7	499	634	10	207	21	30	29
Nshika (AAA-EA)	Kamanyola (900 m)	292^{dfe}	81^{bc}	11^{bac}	452^{fheg}	556^{ed}	10^{cd}	–	16^{feg}	31^{c}	34^{c}
	Mushweshwe (1528 m)	341^{ba}	71^{fe}	12^{ba}	558^{bac}	704^{a}	8^{g}	121^{h}	16^{feg}	22^{fe}	19^{m}
	Mulungu (1707 m)	323^{bc}	75de	9^{d}	583^{a}	714^{a}	9^{fg}	141^{gfh}	16^{feg}	27^{dce}	23^{l}
Mean	-	319	76	10	531	658	9	131	17	27	25

reach harvest (535 days), while 'Nshika', with 531 and 653 days to flowering and harvest respectively, took the longest time (Table 9). The time to harvest generally increased with increasing altitude across the cultivars. 'Nshika' (R^2= 0.98) followed by 'Yangambi Km 5' (R^2= 0.83) were more sensitive to the changes in altitude while 'NARITA 27' (R^2= 0.18) was least sensitive (Table 6). 'NARITA 27' yielded the heaviest bunches (30 kg), and greatest number of hands and fingers and the longest finger length. In contrast, the lightest bunch weight was recorded

in ‘Yangambi Km5’ (21 kg). It was noticed that bunch weight significantly increased with increasing altitude in ‘Ndundu’ (R2= 0.89), while it declined for ‘Nshika’ (R2= 0.56). Yields in other cultivars, ‘Yangambi Km5’ (R2= 0.12) and ‘NHS42’ (R2 = 0.01) (Table 6) were not significantly associated with altitude.

DISCUSSION

The different banana cultivars (exotic and local, Table 3) generally responded differently across the three South Kivu sites and four North Kivu sites in eastern DR Congo. The PC results suggest that both soil factors, especially the level of soil pH, OM, N, P, K, Ca and Mg in the soil and altitude influenced bunch attributes and thus yield of the cultivars. Yield generally increased with increase in OM, N, P, K, Ca and pH. High OM, N, P, K and Ca have been reported to be vital for the growth of the banana plant. A high Ca and OM is reported to improve the availability of P that is easily fixed in the soil under low pH conditions (Pessarakli, 1999). It is thus not surprising that increasing pH was also observed to improve bunch yields. In addition to making P available to the banana plant, higher pH also improves the growth of banana roots, thus improving nutrient and water uptake by plants (Pessarakli, 1999). Altitude was observed to have a negative association with bunch weight, particularly when N, K, P and OM concentrations are low. This could be attributed to the fact that most assimilates go towards sucker development at the high altitudes (Sikyolo et al., 2013; Turner et al., 2016).

The relationship between altitude and time from planting to flowering had a non-linear relationship, with the time from planting to flowering observed to decline at high altitudes. It is understood that PCA uses linear regressions and the curvilinear response may appear as misleading. The planting to bunch emergence data are the means for three crop cycles and will be influenced by the effect of altitude on the time when the ratoon crop began to grow. High altitudes cause bananas to produce suckers at an earlier stage of development compared with plants grown at lower elevations (Sikyolo et al., 2013; Turner et al., 2016). Thus the time from planting to bunch emergence (flowering) when averaged over a number of crop cycles is likely to be different from the data obtained for that cultivar and location for the plant crop. The plant crop data may more accurately show a relationship between altitude and time from planting to bunch emergence than data combined for three crop cycles. For example, time from planting to flowering has been shown to increase with altitude in the plant crop (Sikyolo et al., 2013). This is further strengthened by the fact that the fruit filling phase increases linearly with altitude, that is, is largely free of the effect of early or late suckering that is likely to influence the data on planting to bunch emergence. The performance of the plantain cultivars was influenced by both the altitude and the prevailing soil conditions. Plantains have been reported to grow best at lower elevations (<1200 masl) (Sebasigari, 1985). However, a few plantain cultivars are also found at higher elevations in eastern DR Congo.

Vitousek et al. (1994) reported increased rates of soil mineralization along a decreasing elevation gradient which was most strongly associated with altitude, influencing banana production. Despite the ability of plantains to grow in a wide range of soils, optimum nitrogen, potassium and phosphorus are needed to satisfy plant requirements for profitable production (Zake et al., 2000). The introduced plantain cultivars ‘T6’ and ‘Obubit’ had good yields comparable to ‘Musheba’, the local check, at the low altitude sites in this study. These cultivars in addition to having heavier bunches, had shorter production cycles. A shorter crop cycle gives a higher annual yield (Gaidashova et al., 2008) and this is one of the traits desired by farmers. In contrast, ‘FHIA21’ was better adapted to the mid and high altitude sites, outperforming the local check ‘Musheba’ and the other introduced plantain cultivars. Plantain cultivars ‘Obubit’ and ‘T6’ can therefore be promoted with a good level of acceptance in the low altitude areas whereas ‘FHIA21’ can be promoted at the high altitude sites among the communities in eastern DR Congo. ‘FHIA 21’, ‘Obubit’ and ‘Musheba’ also yielded acceptable bunch sizes of above 25 kg at Butembo (1815 masl); and 28 kg at Mutwanga (1049 masl) (Tables 1 and 4). These mean bunch weight are slightly higher than plantain yields of 24 kg per bunch previously reported in Mutwanga (Ndungo Vigheri, personal communication, 2011). Butembo and Mutwanga have a favourable microclimate with excellent volcanic-derived soils with high potassium concentration (Table 2). Optimum potassium is especially vital for vigorous growth of banana as it increases uptake of other essential elements such as nitrogen and phosphorus (Twyford and Walmsley, 1973; Bolanos et al., 2003).

All the green cooking cultivars examined were outperformed by ‘Barhabeshya’ the local check. Other cooking cultivars with relatively good yield were the local cultivars ‘Mukingiro’ and ‘Matooke’ and the introduced cultivar ‘NARITA 2’. Yield of the cooking cultivars was influenced by the interaction between the soils and altitude. For example, a high correlation was observed between altitude and bunch weight in ‘Barhabesha’ and ‘Mbwazirume’ (Table 6). ‘NARITA 4’ only performed well at the low altitude site of Mavivi that has good soil physical characteristics and abundant and well distributed rainfall.

The performance of the dessert cultivars was influenced by changes in altitude, soil conditions and genotype. For example, the number of functional leaves increased with increasing altitude in the ‘Cavendish’ types, while it declined in ‘Gros Michel’ and ‘FHIA03’. These cultivars generally performed poorly at Maboya, with characteristic poor soils. This is consistent with

Bolanos et al. (2003) who observed that sufficient soil nutrients are essential in obtaining higher total fruit weight and consequently heavy bunches. The introduced hybrid 'FHIA03' significantly outperformed the other cultivars in bunch weight. Dela Cruz et al. (2008) described 'FHIA03' as a cultivar that can be grown in diverse soil types, tolerant to prolonged drought, and grows well between 0 and 1,500 m. The intermediate height of 'FHIA03' plants and robust pseudostem are desirable traits in the hilly areas where plants are prone to toppling or pseudostem breakage due to wind. Bunch weights in 'Gros Michel' increased with altitude, suggesting that it could establish well at these high altitudes with reduced pest and disease problems.

The beer cultivar 'Ndundu' was more vigorous, despite the introduced cultivar 'NARITA 27' having the best bunch yields and an intermediate crop cycle length. 'NARITA 27' also had several hands and long fingers, in addition to not being significantly impacted by the changes in altitude. These attributes make the cultivar ideal for all the evaluated altitudes. 'Nshika' was the best performing local cultivar for the low altitude sites. It had yields matching that of 'NARITA 27' at the low altitude sites. 'Nshika' has been reported as a productive banana cultivar (Dowiya et al., 2009) occupying over 68% of the landscape (Ocimati et al., 2013) across South Kivu. However, the yield of 'Nshika' was observed to decline with increasing altitude, suggesting it is better adapted to the low altitude sites. 'Ndundu' was more adapted to the high altitude sites (with yields matching that of 'NARITA 27' and 'Nshika' at low altitudes). This beer cultivar could thus be selectively promoted at the high altitude sites. Furthermore, 'Nshika' and 'Ndundu' benefit from their shorter production cycles.

CONCLUSION AND RECOMMENDATIONS

The results of this study indicate that the tested cultivars are all excellent in terms of yield, even though altitude and soils differently influenced their performance. For instance, at the Mavivi site, all plantains yielded bunch weights ranging from 33.2 kg ('Obubit') to 36.6 kg ('Musheba'). Beer cultivar 'Nshika' is more adapted to low altitudes while 'Ndundu' is adapted to the high altitude sites. In Mutwanga, all dessert cultivars yielded heavy bunches averaging between 27.4 kg ('Dwarf Cavendish') to 41.9 kg ('FHIA03'). 'Musheba' (plantain), 'Barhabesha' (green cooking), 'FHIA03' (dessert) and 'NARITA 27' (beer) were not significantly impacted by altitude. These variations in cultivar adaptability can be exploited by selectively promoting cultivars in specific agro-ecologies/ niches (as influenced by altitude and soils) to which they are more adapted.

Cultivar attributes such as height, pseudostem girth and leaf production can also be exploited. For example, 'FHIA21' (plantain), 'Matooke' (cooking), 'Dwarf Cavendish' (dessert) and 'NARITA 27' (beer) have short heights, a desirable trait for easy harvesting and areas prone to strong winds. 'Musheba' (plantain), 'Mpologoma' (cooking), 'FHIA03' (dessert) and 'Ndundu' (beer) have the shortest cropping cycles - an important criteria forfarmers in selecting cultivars to grow. Furthermore, in comparison with other cultivars, it was noticed that plantains yielded relatively heavy bunches averaging 17.4 kg to 23.4 kg in Maboya, despite this being the site with the lowest soil fertility.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Germination capacity of some halophytic plants species under increasing salinity

Hichem Hajlaoui[1*†], Samira Maatallah[1†], Nawel Nasri[2] and Hafedh Nasr[3]

[1]Regional Center for Agricultural Research in Sidi Bouzid - Tunisia.
[2]Faculty of Sciences of Tunis, University Campus, 2092 Tunis, Tunisia.
[3]National Research Institute of Rural Engineering, Water and Forests – Tunisia.

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In Tunisia, more particularly in semiarid and arid areas, the drought accentuated by the surface evaporation of water lead to gradual increase in salinity of the soil which a major hurdal in development of vegetation. In these highly salted ecosystems, some plants are growing naturally; however, various species show different tolerance levels to salinity during their development. Seed germination is the stage which is most susceptible to this abiotic constraint. The aim of this study is to investigate the influence of increasing NaCl concentrations from 0 to 200 mM on the germination behavior of some halophytic plants species (*Acacia cyanophylla, Acacia ampliceps, Medicago arborea, Hedysarum carnosum, Casuarina glauca and Ceratonia siliqua*) whose seeds, were collected from Tunisian stands. The germination is evaluated through the daily and the cumulated rates of germination, the corrected seed germination rate and the recovery rate. The effect of salt stress revealed that the elevation of NaCl concentration induces a reduction of germination capacity as good as germination speed. In this setting, 200 mM concentrations of NaCl constitute a physiological limit of germination for all studied species. However, the interspecific variability is relatively important. According to the principal component analysis (PCA), seeds of *C. glauca* were the most salt tolerant, followed by *M. arborea*. Seeds of *A. ampliceps* were the least salt tolerant. The increase of the recovery rate with high salinity supposed that the latter has an osmotic reversible effect and not a toxic irreversible one on the studied species seeds.

Key words: Halophytes, salt stress, germination, Tunisia.

INTRODUCTION

Under natural conditions, plants are subjected to several types of environmental stress (biotic, abiotic). The impacts on crop productivity may be determined by one and/or the interaction of these components (Dong et al.,

*Corresponding author. E-mail: hajlaoui2001@yahoo.fr.

†These two authors contributed equally to this work.

2015). Salinity in the soil is one of the non-living environmental stresses that affect crop production especially in arid and semiarid regions. Plants can develop strategies to grow in saline environments, but after successful seed germination, which is a key stage for plant establishment (Sattar et al., 2010). Salt stress is one of the most serious environmental factors limiting the germination of seeds and the growth of plants due to affecting the water and mineral status of the seeds and/or plants that leads to osmotic stress and an excess of sodium ions. Increase in salinity levels causes a reduction in seed germination percentage and delay germination process initiation, while salt stress can also cause complete inhibition of the germination process (Pujol et al., 2000).

The response of seeds germination to salinity has been reported to be more complex than plant growth because it depends on the availability of stored compounds. Several studies on the effect salinity on seeds germination focused on halophytes and non-halophytes (Joshi et al., 2005; Abari et al., 2011; Zhanwu et al., 2011). They responded to salt stress in similar way that achieves germination (Khajeh-Hosseini et al., 2003; El-Keblawy and Al-Rawai, 2005).

The inhibitory effect of salt stress on seeds germination is due to an osmotic effect and ion toxicity (Bajji et al., 2002; Tobe et al., 2004; Hajlaoui et al., 2007). Therefore, seeds may be generally less salt tolerant than mature plants (Dodd and Donovan, 1999). However, seeds of some halophytic plants may be more salt-tolerant than growing plants (Ungar, 1996). Bakhshi et al. (2013) demonstrated that germination factor such as germination rate and percentage was decreased with increasing NaCl concentration in some halophytic plants species.

Halophytes are potentially useful for ecological applications, such as landscaping, or rehabilitation of damaged ecosystems. They may also present economical interest as food, forage, or for production of metabolites. Laboratory investigations indicate that halophytes have adapted to saline habitats by their ability to adjust osmotically to increasing salinity levels (Gul et al., 2013).

Tunisia has a large diversity of halophytic plant species, found in different types of climax vegetation- in naturally occurring sabkhas (salt-flats) as well as saline soils and inland and littoral salt marshes. However, only limited published information is available on the status, biology and potential use of these plants.

In this study, we seek to provide detailed information on the germination responses of six halophytic plants species (*Acacia cyanophylla* Lindl., *Acacia ampliceps* Maslin, *Medicago arborea* L. *Casuarina glauca* Sieber ex. Spreng., *Ceratonia siliqua* L. and *Hedysarum carnosum* Desf.).

Therefore, the aim of this study is to evaluate and compare their germination performance under increasing levels of salinity, based on different germination parameters.

MATERIALS AND METHODS

Seed source

Seeds of *Acacia cyanophylla, Acacia ampliceps, Medicago arborea, Hedysarum carnosum, Casuarina glauca* and *Ceratonia siliqua* are collected from Tunisian stands. After harvest, seeds are immediately stored at 5 °C.".

Germination conditions

For each species seeds selected for uniformity (on the basis of colour and size), were surface sterilized with 0.1% $HgCl_2$ for 5 min followed by thorough washing under tap water. All instruments for seed germination process were autoclaved at 121°C temperature on 15 PSI pressure for 20 min.

One hundred seeds of each species were placed in Petri dishes containing two layers of filter paper continuously moistened with distilled water or NaCl solutions (50, 100 and 200 mM). The Petri dishes were incubated at 25°C, in darkness. The treatments were replicated five times.

Seeds of *A. cyanophylla*, *A. ampliceps* and *C. siliqua* were previously immersed for one hour in boiled water prior to germination at all treatments. This pre-treatment is currently used to alleviate the mechanical restriction of the germination in softening the cover layers of the embryo. A seed was considered germinated when the tip of the radical had grown free of the seed coat (Wiese and Binning, 1987). Germinated seeds are counted daily until no more occurred for a period of three consecutive days.

To study the recovery of seed germination from NaCl treatments, the ungerminated seeds are immersed in distilled water for 1 h. Then, they are placed on filter paper in Petri dishes, moistened with distilled water and germinated at 25°C.

Parameters studied

At the end of experiment, the following germination parameters were recorded:

(i) Final germination percentage:

FGP = (number of germinated seeds/number of total seeds) × 100.

(ii) Germination rate index (GRI) was calculated following Esechie (1994):

GRI = [(G1/1) + (G2/2) + (Gx/X)]

Where, G = germination on each alternate day after placement , 1, 2, x = corresponding day of germination.

(iii) Corrected germination rate index (CGRI) was defined following Smith and Dobrenz (1987):

CGRI (%) = (A/B) * 100

Where: A: Number of seeds germinated in a given concentration of salt for 17 days, B: Number of seeds germinated in distilled water (control) for 17 days.

(iv) Recovery Rate (RR) of germination was calculated as indicated by Gulzar and Khan (2001):

RR(%) = [(a-b)/(c-b)] * 100.

Where: a: Number of seeds germinated after being transferred

to distilled water, b: Number of seeds germinated in NaCl solution and c: Total number of seeds" describing the coefficient (c) of the formula of "Recovery Rate (RR) of germination.

Statistical analyses

One-way ANOVA is carried out to test for differences between species at a single salinity level and for differences between the effects of the different levels of salinity on a single species. When appropriate, differences between means are compared using Newman-Keuls test of Statistica version 5.5. $P<0.05$ is used to define statistical significance. Two-way ANOVA was used to test the effects of the main factors (species and salt stress) and their interactions on the different study parameters. Principal component analysis (PCA) was used to evaluate differences in salt tolerance between the study species. SPSS 13.0 for Windows was used to perform PCA. The PCA is an exploratory tool, useful for reducing the number of variables in a given data matrix by collapsing the dimensionality of the data. This was accomplished by projecting the original data onto new axes, or principal components (PCs). These PCs were ranked according to the amount of the variance of the original sample that was accounted for by each PC. PC 1 accounted for most of the variance, PC 2 accounted for the next greatest amount of variance and so on. The PCA was used to observe relationships between samples by plotting the scores of their PCs in two or more dimensions. Since the first two PCs accounted for the greatest amount of variance in the samples, they were typically the scores that were plotted (Schultz and Ducklow, 2000).

RESULTS AND DISCUSSION

Cumulative seed germination

The evolution of germination percentage of seeds of six halophytic plants species under control and saline condition (50, 100 and 200 mM NaCl) was investigated. Significant difference was observed between treatments and all six halophytic plants species. For all studied species, NaCl treatments reduced the final germination percentages and retarded germination process (Figure 1). At 0 mM NaCl, seed germination was delayed by 1 day for *Acacia cyanophylla*, *Acacia ampliceps*, *Medicago arborea* and *Hedysarum carnosum*, by 3 days for *Ceratonia siliqua* and by 4 days for *Casuarina glauca*. At 50 mM NaCl, the germination delay does not change significantly. At 100 mM NaCl, *C. siliqua* shows a significant increase in the lag time (11 days). At 200 mM NaCl, all the studied species shows an increase in lag time. It was noted that 12 days lag time for *A.* cyanophylla, 9 days for C. siliqua, 5 days for M. arborea and C. glauca, 4 days for H. carnosum and 2 days for A. ampliceps (Figure 1). Also, the speed of germination is slower in saline solutions than in distilled water, where optimum germination percentage was reached after 15 days. The effect of salt stress on the delay in germination was reported for others halophytic plants species like *Atriplex lentiformis,* where the speed of germination is slower in salinity treatments than in control (Mahmood, 1995). The delay in germination was also noted for *Hedysarum scoparium* (Tobe et al., 2001) and *Casuarina glauca* (Liu et al., 2006). Some reports interpret the effects of salinity on the germination of seeds of different plant species in terms of osmotic potential, while others explain the reduction in germination as toxic effects of ionic elements (Poljakoff-Mayber et al., 1994). Indeed, the high intracellular concentrations of both Na^+ and Cl^- can inhibit the metabolism of dividing and expanding cells (Neumann, 1997), retarding germination and even leading to seed death (Zhang et al., 2010).

The highest germination percentage, in control samples and 200 mM, was observed in *C. glauca.* In five other species, the germination percentage was reduced significantly in comparison with control values since the low dose of NaCl (50 mM). At higher NaCl concentrations (200 mM NaCl), germination was virtually suppressed in *Acacia cyanophylla* (Figure 1). These results are in agreement with other studies, salinity decreased the germination of *Prosopis juliflora* (El-Keblawy and Al-Rawai, 2005), *Atriplex halimus* (Abbad et al., 2004), *Atriplex lentiformis* (Mahmood, 1995), *Atriplex griffithii* (Khan and Rizvi, 1994; Khan and Ungar, 1997a), *Casuarina equisetifolia*, *Medicago ruthenica* (Guan et al., 2009), *Halocnemum strobilaceum* (Qu et al., 2008) and *Hedysarum scoparium* (Tobe et al., 2000).

Seed germination rate

For all the species studied, the germination rate index (GRI) was decreased significantly ($P<0.05$) with increasing salinity (Table 1).

The highest values of germination rate were recorded on distilled water (control). They were statistically similar for all species, except *C. glauca* which showed the highest value of germination rate index. It was fairly around 95.33%. At 50 mM NaCl, the germination rate index was decreased significantly and more extensively in *A. ampliceps, A. cyanophyllda,* and *H. carnosum* compared to *M. arborea*, *C. glauca* and *C. siliqua* for which the germination rate index was respectively around 68, 58.66 and 72.66% while, for the first three species, it was only about 46, 30.66 and 45.33% respectively. At 100 mM NaCl, *M. arborea* and *C. glauca* showed the higher rates of germination than others species. They have respectively 70 and 58.66% of their seeds germinated. At 200 mM NaCl, only *C. glauca* has the highest final germination rate (54.66%) compared to other species. Moreover, under this condition (200 mM NaCl) the germination of *A. cyanophylla* appears to be hampered and she had the lowest final germination rate which did not exceed 1.33%. This is in agreement with those obtained by Debez et al. (2004) on *Cakile maritima,* Yildirim et al. (2011) *on Physalis* and Dallali et al. (2012) on two *Hedysarum* species.

Most of these studies suggested that seed germination of halophytic plants usually occurs when soil salinity levels are low and soil moisture is relatively

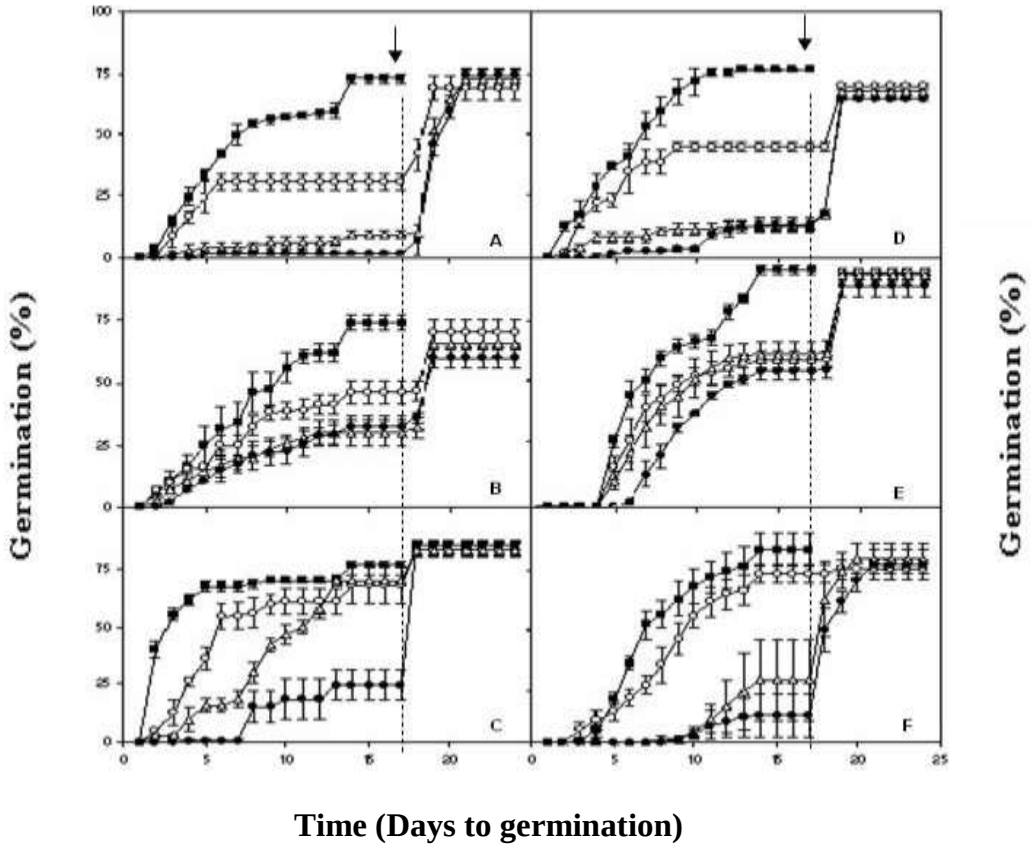

Figure 1. Effects of salinity treatments (■: 0 mM NaCl, ○: 50 mM NaCl, ∆: 100 mM NaCl, ●: 200 mM NaCl) on the germination kinetics of . *A. Cyanophylla (A)*, *A. ampliceps* (B), *M. Arborea (*C),: *H. carnosum* (D),: *C. Glauca (*E),: *C. siliqua* (F). The arrow and the discontinuous vertical line indicate the recovery date.

Table 1. Effects of salinity treatments (0, 50, 100 and 200 mM NaCl) on the germination rate index (%) of *A. Ampliceps, A. cyanophylla, C. glauca, C. siliqua, H. Carnosum* and *M. Arborea.*

Plant species	NaCl (mM)			
	0	50	100	200
A. ampliceps	74.00 (3.05)^{Ab}	46.00 (4.61)^{Bbc}	30.00 (5.03)^{Bb}	32.66 (4.37)^{Bb}
A. cyanophylla	73.33 (1.76)^{Ab}	30.66 (3.33)^{Bc}	9.33 (1.76)^{Cb}	1.33 (1.33)^{Dd}
C. glauca	95.33 (1.76)^{Aa}	58.66 (3.52)^{Bab}	58.66 (5.33)^{Ba}	54.66 (3.33)^{Ba}
C. siliqua	82.66 (7.51)^{Ab}	72.66 (4.06)^{Aa}	26.66 (17.6)^{Bb}	11.33 (9.33)^{Bcd}
H. carnosum	76.66 (0.66)^{Ab}	45.33 (1.76)^{Bbc}	12.66 (2.66)^{Cb}	13.33 (2.66)^{Ccd}
M. arborea	76.66 (1.33)^{Ab}	68.00 (8.08)^{Aa}	70.00 (2.30)^{Aa}	24.66 (6.36)^{Bbc}

Values between brackets are SE. Different upper case letters in a row indicate significant differences between salinity treatments for single species, whereas different lower case letters in a column indicate significant differences between species at each salinity level, based on the Newman-Keuls test at P<0.05.

high. Optimum germination of halophyte seeds is often obtained under freshwater and inhibited by increasing salinity concentrations (Gul et al., 2013), but the ability to germinate at higher salinities is varied with species (Table 1).

At these higher salinities, germination rate was decreased rapidly, which may be a result of osmotic stress or reduced enzyme activities, etc. Salt has been linked with the reduction of α-amylase activity in the seeds (Almansouri et al., 2001). Amylase is an important catalyst in the breakdown of starch reserves, which provides sugars for embryo growth and development.

Table 2. Variation of the corrected seed germination rate (%) of *A. Ampliceps, A. cyanophylla, C. glauca, C. siliqua, H. Carnosum* and *M. Arborea* submitted to different NaCl concentrations (50, 100 and 200 mM NaCl).

Plant species	NaCl (mM)			
	0	50	100	200
A. ampliceps	100.00 (0.00)^{A}	62.26 (6.43)^{Bb}	40.99 (8.07)^{Bbc}	43.92 (4.87)^{Bab}
A. cyanophylla	100.00 (0.00)^{A}	41.91 (4.86)^{Bb}	12.80 (2.61)^{Cc}	1.75 (1.75)^{Dd}
C. glauca	100.00 (0.00)^{A}	61.65 (4.45)^{Bb}	64.57 (6.84)^{Bb}	57.47 (4.31)^{Ba}
C. siliqua	100.00 (0.00)^{A}	88.25 (7.42)^{Aa}	30.35 (18.06)^{Bc}	15.77 (13.54)^{Bcd}
H. carnosum	100.00 (0.00)^{A}	59.10 (1.94)^{Bb}	16.46 (3.30)^{Cc}	17.45 (3.59)^{Ccd}
M. arborea	100.00 (0.00)^{A}	89.07 (12.04)^{Aa}	91.45 (4.52)^{Aa}	32.31 (8.46)^{Bbc}

Values between brackets are SE. Different upper case letters in a row indicate significant differences between salinity treatments for single species, whereas different lower case letters in a column indicate significant differences between species at each salinity level, based on the Newman-Keuls test at P<0.05.

Corrected seed germination rate

Similarly to the final seed germination rate, the corrected seed germination rate was reduced significantly (P<0.05) with increasing salinity for all the studied species (Table 2). At 50 mM NaCl, *M. arborea* and *C. siliqua* showed the highest percentages of corrected germination which reached respectively 89.07 and 88.25% At 100 mM NaCl, *M. arborea* has always preserved the higher corrected germination rate (about 91.45%) compared to others species whereas for *C. Siliqua,* the corrected seed germination rate dropped significantly to 30.35%. At the level of 200 mM NaCl, *A. ampliceps* and *C. glauca* illustrated the highest corrected germination rate with an average of about 43.92 and 57.47% respectively. Dealing with all treatments of salinity, *A. cyanophylla* presented more reduction in germination in response to salt stress than the other species (Table 2). Indeed for this last, the corrected seed germination rate was around 41.91, 12.80 and 1.75% respectively at treatments of 50, 100 and 200 mM NaCl.

Like the final germination rate, the corrected germination rate shows that the increase in salinity progressively inhibited germination. In agreement with previous results concerning halophytic species seeds, the imposition of salt stress resulted in decreases of both percentage and germination rates (Lachiheb et al., 2004; Sekmen et al., 2012). According to Prado et al. (2000), the decrease in germination may be ascribed to an apparent osmotic 'dormancy' developed under saline stress conditions, which may represent an adaptive strategy to prevent germination under stressful environment.

Correlation between the corrected germination rate and the lag time

The relationship between the corrected germination rate and the lag time is depicted in Figure 2. The results indicated that these parameters are relatively dependent. Indeed, the corrected germination rate decreased and lags time was increased with increasing NaCl concentration. For the two salinity treatments 50 and 100 mM NaCl, the relation between the corrected germination rate and the lag time is not strong. In fact, the correlation coefficient (R^2) values were respectively about 0.0481 and 0.0574, whereas, for 200 mM NaCl, this relation becomes relatively strong (R^2=0.5003) (Figure 2). Seeds of *A. cyanophylla*, *A. ampliceps*, *M. arborea* and *H. carnosum* have trivial primary dormancy as reflected by 1-day lag time at control treatment, whereas those of *C. siliqua* and of *C. glauca* showed significant primary dormancy. This last can be related to various inhibitors (Baskin and Baskin, 1998). The secondary dormancy, as indicated by germination delay under salinity treatments, strongly increased at 100 mM NaCl and was rather amplified at 200 mM NaCl. At this highest level of salt stress the relationship between the corrected germination rate and the lag time was linear, or close to being linear, which suggests a simple physical cause, and may be indicative that osmotic limitation is important in determining the germination rate. So what, delaying the germination of seeds and the increase of the lag time germination of studied species with increasing salt concentration is explained by the time required for the seed to establish mechanisms allowing it to adjust its internal osmotic pressure.

Recovery rate of seed germination

Transfer experiments of seeds to control medium after pretreatment with NaCl were conducted to clarify the mode of action of salt stress on germination. Our results (Table 3) showed that the recovery responses vary from one species to the other and against the level of salinity they are exposed. Indeed, the recovery rate of seeds of A. cyanophylla, H. carnosum and C. siliqua was increased significantly (P<0.05) with increasing salinity. This increase remains more pronounced specially in seeds of C. siliqua where the recovery rate of

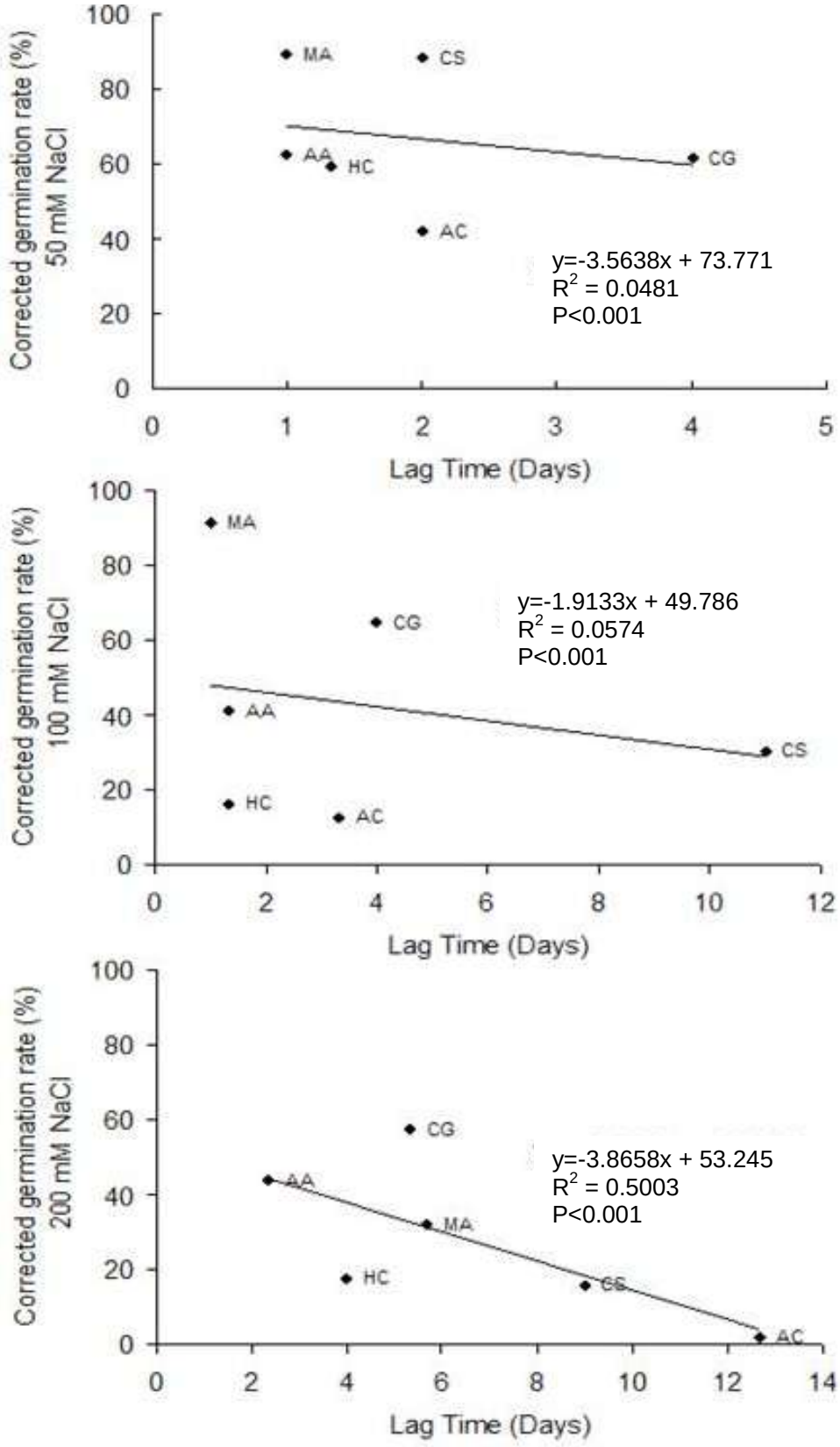

Figure 2. Relation between the corrected germination rate and the lag time of germination of A*. cyanophylla* (AC), *A. ampliceps* (AA), *M. arborea* (MA), *H. carnosum* (HC), *C. glauca* (CG) and *C. siliqua* (CS) at 50, 100, and 200 mM NaCl.

Table 3. Effects of salinity treatments (50, 100 and 200 mM NaCl) on the Recovery rate of germination (%) of *A. Ampliceps, A. cyanophylla, C. glauca, C. siliqua, H. Carnosum* and *M. Arborea.*

Plant species	NaCl (mM)		
	50	100	200
A. ampliceps	46.24 (5.65)^{Ab}	50.00 (3.36)^{Aa}	40.71 (2.29)^{Ab}
A. cyanophylla	56.19 (4.74)^{Bb}	69.77 (2.38)^{Aa}	74.30 (1.86)^{Aa}
C. glauca	86.48 (4.37)^{Aa}	74.45 (8.30)^{Aa}	75.76 (7.56)^{Aa}
C. siliqua	7.67 (1.21)^{Bc}	71.16 (5.67)^{Aa}	71.56 (10.67)^{Aa}
H. carnosum	45.09 (1.69)^{Bb}	62.47 (3.23)^{Aa}	59.17 (1.08)^{Aa}
M. arborea	43.54 (10.72)^{Ab}	42.25 (14.40)^{Aa}	80.60 (1.12)^{Aa}

Values between brackets are SE. Different upper case letters in a row indicate significant differences between salinity treatments for single species, whereas different lower case letters in a column indicate significant differences between species at each salinity level, based on the Newman-Keuls test at P<0.05.

germination raised from 7.67% at 50 mM NaCl to 71.16 and 71.56% respectively at 100 and 200 mM NaCl. Moreover we noticed also that the recovery rate does not differ significantly between *A. ampliceps*, *M. arborea* and *C. glauca* among the NaCl treatments exceptionally at 200 mM, where *A. ampliceps* presented the lowest recovery rate with an average of about 40.71% (Table 3). At 50 mM NaCl, *C. glauca* has the highest recovery rate, whereas *C. siliqua* has the lowest value. All the other species have intermediate rates. At 100 mM NaCl, no significant difference is noted between studied species for the recovery rate.

The increase of the recovery rate with increasing salinity seems to be a general rule. In this setting, Khan and Ungar (1997b) reported that halophyte seeds have typicallythe ability to maintain seed viability for extended periods of time during exposure to hyper-saline conditions and then to commence germination when salinity stress is reduced or removed. Most halophytes show an important recovery of germination when stress conditions are alleviated (Khan and Ungar, 1997b; Pujol et al., 2000; Gulzar and Khan, 2001; Qu et al., 2008). This may have ecological significance within highly saline environments, reflecting a physiological response that is understrong selection pressure during the evolution of these species. Moreover, seed survival under hypersaline conditions rather than germinability has been used as criteria for salt tolerance. Survival skills of halophyte seeds which was observed in *C. Glauca* and *M. arborea* under hypersaline conditions gives a selective advantage to halophytes and distinguishes them from most of glycophytes (Sekmen et al., 2012).

Previous studies that reported that a recovery of germination upon transfer of seeds from NaCl solution to pure water generally attribute the initial repression of germination and its subsequent recovery to osmotic factors, without exploring alternative hypotheses, such as the export of sodium from the seeds upon transfer to pure water (Debez et al., 2004). Likewise, it is unclear whether the high sodium concentrations seen here in non-germinating seeds are physiologically relevant (that is, a causal factor), or simply a result of the longer duration that non-germinating seeds spent in their respective solutions (Zhang et al., 2010).

Our data confirm the assumption that salinity usually impairs germination by preventing imbibition (Keiffer and Ungar, 1995). Nevertheless, at higher salinity (200 mM NaCl) toxicity phenomena due to the accumulation of Na^+ and Cl^- ions, have also appeared, as evidenced by the decline in germination recovery, compared to the control, especially for *A. ampliceps* seeds.

Discrimination of interspecific variability and interactions species x salt stress

The analysis of variance examining the germination rate index, corrected germination and recovery rates after imposing salt stress are presented in Table 4. Results of this analysis showed that the effect of the salinity level on germination indices is more significant than that of the plant species. The interaction between the two factors (salinity and species) is also significant, but at lower extent. However, the interaction affects the recovery rate rather than the two other rates.

The data processing by PCA is presented in Figure 3. The first two axes of the PCA represented 80.65% of the total variability. The first principal component (PCA 1) expressed 57.06%, while the second axis (PCA 2) accounts for 23.59% of the total variability. PCA 1 seemed to separate the scores of the control treatment from that of the other NaCl treatments. PCA 2 seemed to separate the scores of the best salt tolerant species than that of the worst.

Overall and from the positive side to the negative side of PCA 1, the germination performance was decreased with increasing salinity. Seeds germinated in 0 mM NaCl (control) recorded the greatest contribution to the PCA 1 and thus, they perform better than the other treatments. The graphical representation of the dispersion, of studied

Table 4. Results of analysis of variance examining final germination, corrected germination and recovery rates after imposing salt stress.

Source of variation	d.f.	MS	*F*-value	*P*-value
Final germination rate				
Species	5	2387.02	24.72	< 0.001
Salt stress	3	11076.81	114.72	< 0.001
Species × Salt stress	15	489.75	5.07	< 0.001
Corrected germination rate				
Species	5	2468.69	18.30	< 0.001
Salt stress	3	17770.39	131.74	< 0.001
Species × Salt stress	15	850.18	6.30	< 0.001
Recovery rate of germination				
Species	5	994.59	11.18	< 0.001
Salt stress	3	8873.61	99.78	< 0.001
Species × Salt stress	15	693.59	7.80	< 0.001

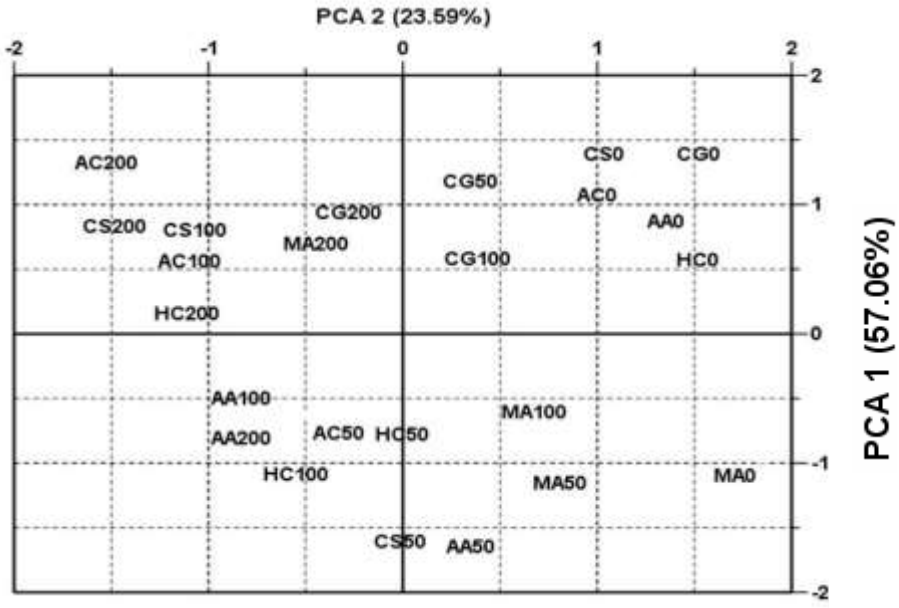

Figure 3. Results of Principal Component Analysis. Scores are the combinations of species [**AC**: *A. cyanophylla*, **AA**: *A. ampliceps*, **MA**: *M. arborea*, **HC**: *H. carnosum*, **CG**: *C. glauca*, **CS**: *C. siliqua*] and NaCl concentrations [**0**: 0 mM NaCl (control), **50**: 50 mM NaCl, **100**: 100 mM NaCl, **200**: 200 mM NaCl.] and study parameters.

species, in the diagram generated by the first two axes of PCA (1-2) showed the existence of a large interspecific variability. Globally, seeds of *C. glauca* showed to be the most salt tolerant. However, *A. cyanophylla* was the most sensible to NaCl treatment. *A. ampliceps* was the least performant species. For more details and depending on the PCA analyses, it can be make a classification that takes into consideration the germination behaviour of studied species at the level of each salinity treatment. At 50 mM NaCl, the salt tolerance of the study species' seeds can be classified as follows: *C. glauca* > *M. arborea* > *A. ampliceps* > *H. carnosum* > *C. siliqua* > *A. cyanophylla*. At 100 mM NaCl, the salt tolerance of the study species' seeds can be classified as follows: *C. glauca* > *M. arborea* > *C. siliqua* > *A. cyanophylla* > *H. carnosum* > *A. ampliceps*. Finally, at 200 mM NaCl, the salt tolerance of the study species' seeds can be classified as follows: *C. glauca* > *M. arborea* > *H. carnosum* > *C. siliqua* > *A. cyanophylla* > *A. ampliceps*. Thus, seeds of *C. glauca* were the most salt tolerant, followed by *M. arborea*. Seeds of *A. ampliceps* were the least salt tolerant.

The considerable interspecific variability in germination behaviour of halophytic plants species with increasing

salinity level was reported by Keiffer and Ungar (1995); Lachiheb et al. (2004) and Gul et al. (2013). For example, Keiffer and Ungar (1995) exposed seeds of five halophytes (*Atriplex prostrata, Hordeum jubatum, Salicornia europaea, Spergularia marina* and *Suaeda calceoliformis*) to an extended period of salinity treatments. They used Woodell (1985) classification system and placed *Atriplex prostrata* seeds in the Type 1, *Hordeum jubatum* and *Spergularia marina* in the Type 2, and *Salicornia europaea* and *Suaeda calceoliformis* in the Type 3 category.

Conclusion

In conclusion, for the majority of the studied halophytic plants species, NaCl treatments reduced the final percentages of germination. There is no significant difference between the final germination rates after recovery and those previously germinating in distilled water (control), suggesting that seed germination ability completely recovered at all salinity levels. The secondary dormancy, as indicated by germination delay under salinity treatments, strongly increased at 100 mM NaCl and was rather amplified at 200 mM NaCl. Our results suggested that salt tolerance of studied species is dose-dependent. Dealing with the imposition of increased salt stress, the interspecific variability includes a much greater capacity of *C. glauca* and *M. arborea* to tolerate high salinity environments. For some species, such as *A. Cyanophylla* and *A. ampliceps,* although they are halophytic. Our study proves that these species are relatively less tolerant to salt stress in germination phase than other wild plants in Tunisia. These results suggested that it is difficult to link the salinity tolerance during germination to the ecology of the species or its tolerance to adult plant stage. In Tunisia, where most sites were affected by salinity, selective breeding based on germination salinity tolerance appears to be feasible based on the germination variation found between halophytes species and/or cultivars. In this setting, the two selected species such as *C. glauca* and *M. arborea* may play an important role for the rehabilitation and recovery of saline soils and non-conventional water resources.

Conflict of interests

The authors have not declared any conflict of interests.

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Seeding density of *Brachiaria ruziziensis* intercropped with grain sorghum and effects on soybean in succession

Larissa Pacheco Borges[1], Alessandro Guerra da Silva[2]*, Maria Mirmes Paiva Goulart[1], Itamar Rosa Teixeira[3], Gustavo André Simon[2] and Kátia Aparecida de Pinho Costa[1]

[1]Instituto Federal Goiano, Rio Verde, Goiás, Brazil.
[2]Universidade de Rio Verde, Rio Verde, Goiás, Brazil.
[3]Universidade Estadual de Goiás, Anápolis, Goiás, Brazil.

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Sorghum is an alternative crop to produce grains in the off-season in Brazilian Cerrado and the intercropping with *Brachiaria* species enable to produce dry matter on the soil surface for a longer time. However, there is limited information on *Brachiaria ruziziensis* seed density to be applied to intercropping with sorghum without causing decreases in sorghum grain yield. The objective of the study was to evaluate the seeding density of *B. ruziziensis* in different intercropped systems with grain sorghum grown after soybean harvest, in off-season cultivation, to produce grains and the effects of straw on the agronomic performance of the soybean crop in succession to intercropping. This study was conducted in the field during the 2014 off-season in Rio Verde, Goiás. The experimental design was complete randomized blocks in a 3×5 factorial arranged with four replications corresponded to intercropping on rows, inter-rows and broadcast sowing with the densities of 2, 4, 6, 8 and 10 viable pure seeds m^{-2} of *B. ruziziensis*. Monocultures of sorghum and these forage crop were also evaluated. The results demonstrated the absence of effects of intercropping systems and seeding density on the yield of sorghum grains and on the cultivation of soybean in succession. The intercropping was effective in producing straw on soil surface to Cerrado no-tillage system.

Key words: *Brachiaria* species, *Glycine max,* straw, off-season crop, *Sorghum bicolor.*

INTRODUCTION

Currently, the diversification and integrated systems of activities on farms has become an essential tool for the stability of agribusiness. Modern agriculture has prioritized agricultural practices that intensify the land use, inputs and technological knowledge aiming increased profitability and competitiveness (Bonaudo et al., 2014; Lemaire et al., 2014).

In this context, the grain crops intercroppings with forages species allow to produce grains and straw on the soil surface or the formation of biomass for the cattle grazing (Morais et al., 2014). Thus, the intercropping of annual crops with tropical grasses, used in integrated

*Corresponding author. E-mail: silvaag@yahoo.com.br.

crop-livestock systems, has been increasingly adopted by farmers in the Brazillian Cerrado region (Oliveira et al., 2015; Freitas et al., 2016).

However, the great difficulty of this region is keeping the straw on the soil surface in the off-season (Kliemann et al., 2006; Borghi et al., 2013; Ensinas et al., 2016). The weather conditions are a great obstacle to this system, in which is characterized by dry winter, high temperatures throughout the year and a prolonged dry season, making it difficult to keep the straw on the soil surface (Silva et al., 2015). In this context, off-season crops are essential for the implementation and feasibility of the system as they provide ground cover for a longer period (Horvathy Neto et al., 2012).

In the Cerrado region, specifically in the Brazillian Midwest, the sorghum crop became increasingly relevant to grain production. It is a crop grown after the harvest of soybean, in off-season cultivation in the no-tillage under conditions lower precipitation. The increasing of the sorghum area in that region is due to great demand of agricultural industries installed in the region and to nutritional value similar to corn. In addition, it has a lower production cost in relation to corn and excellent adaptation to different environments (Baumhardt et al., 2005; Kouressy et al., 2008), especially during the off-season, in which there are water deficits coinciding with the reproductive development of the crop. Forage grasses, such as *Brachiaria* species, are alternatives to rotation, succession or intercropping systems in the Cerrado. It can provide an excellent vegetable cover (Lima et al., 2014), contributing to increases in the levels of organic matter in the soil, beyond to make the crop rotation (Loss et al., 2013). The *Brachiaria* spp. also has an abundant root system, contributing to water infiltration, aggregation and aeration in the soil (Kluthcouski et al., 2004; Silva et al., 2015).

The intercropping of sorghum with *Brachiaria* spp., in the off-season cultivation, is a promising technique because it allows the production of grains and forages (Ribeiro et al., 2015). In the Cerrado, the success of such intercropping systems is because the straw produced provides a favorable environment for the recovery or conservation of soil properties (Entz et al., 2002; Franzluebbers, 2007) to promote an improvement of physical, chemical and biological conditions and to contribute to the production and development of plants (Maughan et al., 2009; Bell et al., 2014). Besides, the intercropping enables the consolidation of no-tillage system in the Cerrado, resulting in positive effects on crops, such as soybean grown in succession (Silva et al., 2015). However, there is a need for more information about *Brachiaria* spp. implementation recommendations, such as intercropping systems and seeding density in order to increase dry matter production in the off-season.

Thus, the objective of this study was to evaluate the effects of grain sorghum intercropped with *Brachiaria ruziziensis* that provides higher sorghum grain yield and dry mass of both cultures, in different forage seeding densities on the row, inter-row and broadcast intercropping systems, besides the evaluation of cultural performance soybean in succession.

MATERIALS AND METHODS

Location and experiment characterization

The experiments were conducted in the field (17°47'22.3" S, 50°57'40.1" W; altitude: 737 m) in the agricultural area of the city of Rio Verde-Goiás, Brazil. The soil of the experimental area was classified as a dystrophic Red Latosol (Santos et al., 2013). The preceding summer crop was soybean grown under no-tillage system. Before starting the experiment, the soil had the following chemical and physical characteristics: pH in $CaCl_2$: 4.6; Ca, Mg, K, Al, H+Al, and cation exchange capacity: 1.55, 1.01, 0.16, 0.25, 5.7 and 8.40 in $cmol_c\ dm^{-3}$, respectively; P: 7.70 mg dm^{-3}; base and aluminum saturations: 32.25 and 8.45%, respectively; organic matter: 25.52 g dm^{-3}; and clay, silt and sand: 540, 170 and 290 g kg^{-1}, respectively. The average air temperature and rainfall during the conduction of the experiment are as shown in Figure 1.

Experiment design

The experimental design was randomized blocks in a 3×5 factorial with four repetitions, with three *B. ruziziensis* and grain sorghum intercropping systems (row, inter-row and broadcast sowing) with five seeding density (2, 4, 6, 8 and 10 viable pure seeds m^{-2}), beyond the corresponding additional treatments to grain sorghum monocultures (0 density of viable pure seeds m^{-2}) and the five *B. ruziziensis* seeding densities. It was the grain sorghum hybrid BRS 330 (medium cycle, semi-open panicles and red grains without tannin). The *B. ruziziensis* was chosen because it has decumbent culms, short racemes, intense hairiness and good production of biomass for soil covering. The plots consisted of seven sorghum planting rows 5.0 m long and spaced 0.50 m apart. The useful area was obtained disregarding the two side rows and eliminating 0.50 m at each end.

Experiment installation and conduction

After the soybean harvest and one week before the implementation of sorghum intercropped with *B. ruziziensis*, the desiccation of weeds was performed using the equivalent to 1,440 g a.e. ha^{-1} of glyphosate and 0.5 L ha^{-1} of 2,4 D with a spray volume of 150 L ha^{-1}. One day before sowing, the sorghum sowing furrows were marked using a seeder with a row spacing of 0.50 m. In this operation, the application of 333 kg ha^{-1} of 08-20-18 fertilizer was performed. Both sorghum and *B. ruziziensis* were manually sown on March 11, 2014. The forage crop was sowed in the row intercropping system at a 10 cm depth together with the fertilizer. Then, seeds were covered with 8 cm of soil. The sorghum was sown soon after and then covered by 2 cm of soil. In the inter-row intercropping, the furrows for the sowing of *B. ruziziensis* were opened in inter-rows of sorghum at a 10 cm depth. *B. ruziziensis* was seeded by throwing in the broadcast sowing system. Then, the monocultures of sorghum and *B. ruziziensis* were sown at 2 and 10 cm of depth, respectively. To define the amount of viable pure *B. ruziziensis* seeds, a germination/emergence test was performed in a sand bed to obtain the amount of viable pure seeds per m^2.

At 23 days after the emergency (DAE), the coverage at the side of the sorghum sowing line with nitrogen was performed using 550 kg ha^{-1} as ammonium sulfate. On the same day, 0.40 L ha^{-1} of

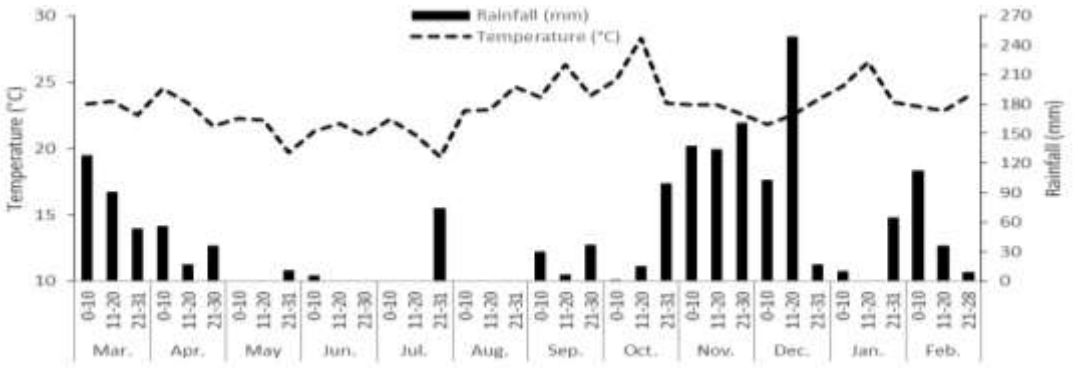

Figure 1. Variation of average air temperature and rainfall every ten days from March 2014 to February 2015, Rio Verde/GO, Brazil (source: Weather Station of the University of Rio Verde, Rio Verde).

bifenthrin + carbosulfan was spray mechanically. At 45 DAE, 0.20 L ha^{-1} of chlorantraniliprole was used for the control of *Spodoptera frugiperda*. In both applications, a spray volume of 200 L ha^{-1} was used. The harvest of sorghum was performed at 119 DAE (July 8, 2014).

Characteristics evaluated on *B. ruziziensis* and grain sorghum crops

Grain yield (harvest of panicles, with subsequent threshing and weighing of grains; moisture was corrected to 13%), thousand grain weight (weight of a thousand grains chosen randomly in the yield sample; moisture was corrected to 13%), plant height (measured from the base of the plant to the tip of the panicles in five randomly selected plants) and final population (counting the total number of plants harvested) were evaluated in the useful area of the plot.

After the sorghum harvest, a cut was made at 30 cm height to standardize plants. The *B. ruziziensis* plants remained for 124 days in the field together with sorghum stubble. On that date, the plant height was determined (measured from the base of the plant to the tip of last fully expanded leaf in the useful area of the plots in five plants chosen randomly). At the same time, the dry matter yield of each crop and their sum were evaluated for *B. ruziziensis* and grain sorghum to quantify the production of straw. In this evaluation, the dry matter of sorghum and *B. ruziziensis* plant were measured in 1 m^2 using a 1.0 × 1.0 m iron square. The cut in plant was made on the soil level. The samples were placed separately in paper bags and taken to dry in an oven at 65°C to determine dry weight. Then, sorghum, *B. ruziziensis* and total dry matter yield were calculated in kg ha^{-1}.

Upon cutting of biomass, the percentage of soil cover was quantified by evaluation at two points chosen randomly in the useful area of the plot. A 0.50 × 0.50 m iron square, containing a line with ten equidistant points, was used. The determination of the coverage percentage of the soil surface was calculated when these points coincided with the presence of vegetable cover of the sorghum and *B. ruziziensis*.

Soybean crop

After the harvest of *B. ruziziensis* and grain sorghum crop biomass, soybean was seeded in order to assess the performance of the crop in succession to intercropping. The desiccation of sorghum and *B. ruziziensis* biomass was mechanically performed 124 days after harvesting the sorghum using an equivalent to 1,920 g a.e. ha^{-1} of glyphosate and 0.50 L ha^{-1} of 2,4 D with a spray volume of 150 L ha^{-1}.

Six days after the desiccation of biomass (November 15, 2014), the soybean crop was implemented in the seeding furrows with a seven-line seeder and 408 kg ha^{-1} of 08-20-18 fertilizer. The variety NS 7490 RR was used (early circle, indeterminate growth and tolerant to the herbicide glyphosate).

Glyphosate (960 g ha^{-1}) + [bifenthrin + zeta-cypermethrin] (0.25 L ha^{-1}) and [carbendazim + kresoxim-methyl + tebuconazole] (1.0 L ha^{-1}) were used at 20 DAE to control weeds, pests and diseases. The two latter products were also used at 27 DAE. For the prevention of diseases and pests, the following pesticides were also used: [carbendazim + cresoxim-methyl + tebuconazole] (1.0 L ha^{-1}) and [bifenthrin + carbosulfan] (0.6 L ha^{-1}) at 52 DAE, and [carbendazim + cresoxim-methyl + tebuconazole] (1.0 L ha^{-1}) and acephate (1.00 kg ha^{-1}) at 86 DAE. All applications were performed using a mechanical sprayer with spray volume of 150 L ha^{-1}.

Characteristics evaluated on soybean crop in succession to intercropping

On soybean crop, the following was analyzed in the useful area of the plot: grain yield (plants harvest, with subsequent threshing and weighing of grains; moisture was corrected to 13%), thousand grain weight (weight of thousand grains; moisture was corrected to 13%), initial population (counting the number of plants) at the fully developed two trifoliate stage (stage V_2), final population (counting number of plants at harvest), initial plant, in stage V_2, and final plant height (measured from the soil surface to insertion on second trifoliate and on last node, respectively, in five randomly selected plants at harvest), height of insertion of the first pod (measured from the soil surface to the insertion of the first pod at harvest), number of pods on the secondary and main stems with one, two, three and four grains; number of secondary stems (counting the number of secondary stems in five randomly selected plants) and total number of pods (counting the number of pods in a same sample with five plants). The evaluations of the number of pods and secondary stems were performed at the harvest of soybean.

Firstly, an individual analysis of variance was performed and then an analysis of the data obtained in intercropping was performed combined with data from the monoculture. To compare the mean values of the intercropping, the Tukey test at 5% probability was performed to compare intercropping systems means. The values means of seeding densities were compared by regression analysis when significance was found on variance analyses. Among the means obtained in intercropping with the monoculture, a comparison by contrast using the Dunnett test at 5% probability was performed.

Table 1. Significance of the variables grain yield (GY), 1000-grain weight (1000-GW), plant height (SPH), plant population (POP) and dry matter yield (SDMY) of grain sorghum crop, and plant height (BPH), dry mass yield (BDMY) of *B. ruziziensis*, total dry mass yield (TDMY) and soil cover (SC) of sorghum intercropped with *B. ruziziensis* in the densities of 2, 4, 6, 8 and 10 viable pure seeds m^{-2} in the 2014 off-season, Rio Verde-GO, Brazil.

Sources of variation	FD	GY	1000-GW	SPH	POP	SDMY
Intercropping systems (IS)	2	ns	ns	ns	**	ns
Seeding density (SD)	4	ns	ns	ns	ns	ns
IS × SD	8	ns	ns	ns	ns	ns
Intercropping × Monoculture	1	ns	ns	ns	ns	**
CV (%)	-	20.5	10.7	4.8	12.9	32.7
Sources of variation	**FD**	**BPH**	**BDMY**	**FD**	**TDMY**	**SC**
Intercropping systems (IS)	2	*	ns	2	ns	*
Seeding density (SD)	4	ns	ns	4	ns	ns
IS × SD	8	ns	ns	8	ns	ns
Monoculture	4	ns	ns	5	ns	**
Intercropping × Monoculture	1	**	**	1	ns	**
CV (%)	-	10.3	23.3	-	14.2	8.7

**, * and ns: significant at 1 and 5% and not significant, respectively, by F test; CV: Variation coefficient; FD: free degree.

RESULTS AND DISCUSSION

Grain sorghum crop

The *B. ruziziensis* crop in the intercropping did not interfere on development and yield of the sorghum. This is evidenced by the lack of significant between intercropping systems and monoculture for the grain yield, thousand grain weight and plant height of the sorghum crop. However, the sorghum dry matter yield showed significant interaction, so *B. ruziziensis* intercropped with sorghum during off-season interfered in this trait of sorghum (Table 1). Besides, the intercropping systems influenced the sorghum plant population.

The *B. ruziziensis* and grain sorghum intercropping, under off-season conditions did not cause significant competition for water, light, nutrients and physical space. Thus, it was observed mean values similar for sorghum grain yield, thousand grain weight and plant height of sorghum crop in sorghum-based intercropping system and monoculture (Table 2).

It is noteworthy that no herbicide was applied to the intercropping to suppress the growth of *B. ruziziensis* plants, as the Cerrado region grower do in the maize crop. This is due the lack of selective grass herbicides for a post-emergence application in the sorghum crop. Moreover, it is important to note that *B. ruziziensis* has a prostrated initial growth and a slower establishment. This may have allowed sorghum plants to develop faster, without interference at the early development stage. Although, the research results are limited with regard to sorghum yield during off-season, the obtained values of grain yield allow to infer the technical feasibility of the *B. ruziziensis* and grain sorghum intercropping in succession to soybeans in the Brazilian Midwest, as evidenced by Silva et al. (2015).

The decrease in rainfall during the development of the species, associated with a slow initial growth of *B. ruziziensis*, caused the grass not to interfere with the height of sorghum plants (Table 2), corroborating with studies on sorghum intercropped with *Brachiaria* spp. (Horvathy Neto et al., 2012; Silva et al., 2015).

The analysis of plant population at the sorghum harvest is essential to establish possible effects of suppressive *B. ruziziensis* plants on the grain sorghum crop. In this respect, no significant differences were observed for plant populations between the intercropping and the monoculture (Table 2). However, differences in plant populations were observed among the intercropping systems, with lower values in the broadcast seeding. However, this did not result in differences in sorghum grain yield, as discussed earlier.

B. ruziziensis crop

Intercropping with sorghum interfered in the plant height and dry mass yield of *B. ruziziensis* relative to values of forage monoculture ($P \leq 0.05$) (Table 1). In the row and inter-row intercropping systems, the sorghum shading, causing etiolation, resulted in higher *B. ruziziensis* height, unlike the broadcast intercropping (Table 3). Consequently, most of the intercropping systems showed results lower for dry matter yield of *B. ruziziensis* in the intercropping.

After the harvest of sorghum grains, because of the low regrowth of sorghum plants during off-season, the lack of rain in the Cerrado (Figure 1) and the presence of *B. ruziziensis* plants at the vegetative stage causing suppression of sorghum plants in intercropping. Thus, the

Table 2. Mean values for grain yield (GY), 1000-grain weight (1000-GW), plant height (SPH), plant population (POP) and dry matter yield (SDMY) of grain sorghum crop intercropped with *B. ruziziensis* in the densities of 2, 4, 6, 8 and 10 viable pure seeds m^{-2} in the 2014 off-season, Rio Verde-GO, Brazil.

Intercropping systems	Seeding density (viable pure seeds m^{-2})					Mean values
	2	4	6	8	10	
GY (kg ha^{-1})						
Row	2,663[a]	2,050[a]	2,295[a]	2,336[a]	2,383[a]	2,345[a]
Inter-row	2,285[a]	2,295[a]	2,428[a]	2,089[a]	2,087[a]	2,237[a]
Broadcast	2,048[a]	2,151[a]	2,303[a]	2,345[a]	2,282[a]	2,226[a]
Mean values	2,332	2,165	2,342	2,257	2,251	2,269
Monocultures			2,028			
1000-GW (g)						
Row	12.56[a]	13.04[a]	12.29[a]	12.56[a]	12.54[a]	12.60[a]
Inter-row	12.48[a]	12.27[a]	12.22[a]	11.47[a]	11.83[a]	12.05[a]
Broadcast	11.02[a]	11.91[a]	12.06[a]	12.65[a]	11.78[a]	11.89[a]
Mean values	12.02a	12.41	12.19	12.23	12.05	12.18
Monocultures			13.09			
SPH (cm)						
Row	129[a]	132[a]	129[a]	130[a]	133[a]	131[a]
Inter-row	130[a]	132[a]	132[a]	131[a]	132[a]	131[a]
Broadcast	129[a]	129[a]	128[a]	130[a]	128[a]	129[a]
Mean values	129	131	130	130	131	130
Monocultures			126			
POP (plants ha^{-1})						
Row	145,778[a]	139,000[a]	138,333[ab]	141,667[a]	148,000[a]	142,556[a]
Inter-row	139,667[a]	150,000[a]	168,999[a]	147,333[a]	164,444[a]	154,089[a]
Broadcast	133,000[a]	141,000[a]	117,999[b]	142,667[a]	108,000[b]	128,533[b]
Mean values	139,481	143,333	141,777	143,888	140,148	141,170
Monocultures			131,333			
SDMY (kg ha^{-1})						
Row	1,108[a*1]	1,143[a*1]	1,126[a*1]	1,332[a*1]	1,110[a*1]	1,164[a]
Inter-row	1,214[a*1]	1,456[a]	1,256[a*1]	1,080[a*1]	1,312[a*1]	1,263[a]
Broadcast	1,251[a*1]	1,288[a*1]	1,342[a*1]	1,127[a*1]	2,362[a]	1,474[a]
Mean values	1,191	1,296	1,241	1,180	1,595	1,300
Monocultures			2,365			

Means followed by the same lowercase letter in columns do not differ by Tukey test at 5% probability. *[1]: Means differ significantly from the sorghum monoculture by Dunnett test at 5% probability.

most intercropping treatments showed lower mean values of dry mass of *B. ruziziensis* that the monoculture these forage crop (Table 3).

Dry matter production in the intercropping

The intercropping systems and monocultures showed similar mean values for total dry mass yield without decreasing the grain yield in the sorghum-based intercropping systems (Tables 1 and 3). However, the intercropping systems showed significant difference for the soil cover (SC). In this trait, the row intercropping system showed higher mean values than broadcast sowing intercropping system (Table 3). Also, the grain sorghum monoculture showed lower soil cover compared to *B. ruziziensis*.

The intercropping and monoculture interaction showed significant difference for the soil cover (Table 1). The row intercropping system and *B. ruziziensis* monoculture

Table 3. Mean values for plant height (BPH) and dry matter yield (BDMY) of *B. ruziziensis*, total dry mass yield (TDMY) and soil cover (SC) of the grain sorghum intercropping with *B. ruziziensis* in the densities of 2, 4, 6, 8 and 10 viable pure seeds m^{-2} in the 2014 off-season, Rio Verde-GO, Brazil.

Intercropping systems	Seeding density (viable pure seeds m^{-2})					Mean values
	2	4	6	8	10	
BPH (m)						
Row	1.06^{a*1}	1.12^{a*1}	1.15^{a*1}	1.04^{a*1}	1.18^{a*1}	1.11^{a}
Inter-row	1.07^{a*1}	1.08^{a*1}	1.10^{a*1}	1.07^{a*1}	1.12^{a*1}	1.09^{a}
Broadcast	0.89^{a*1}	0.97^{a}	0.96^{a}	1.04^{a*1}	1.02^{a}	0.98^{b}
Mean values	1.01	1.06	1.07	1.05	1.11	1.06
Monocultures	0.64^{A}	0.76^{A}	0.77^{A}	0.74^{A}	0.85^{A}	-
BDMY (kg ha^{-1})						
Row	2,693^{a}	2,337^{a}	2,330^{a*1}	2,435^{a}	2,261^{a*1}	2,411^{a}
Inter-row	2,148^{a*1}	2,572^{a}	2,171^{a*1}	2,459^{a}	2,372^{a*1}	2,345^{a}
Broadcast	1,582^{a*1}	1,925^{a*1}	1,990^{a*1}	2,234^{a}	2,344^{a*1}	2,015^{a}
Mean values	2,141	2,278	2,163	2,376	2,326	2,257
Monocultures	3,945	3,543	3,725	3,423	4,014	-
TDMY (kg ha^{-1})						
Row	3,801^{a}	3,480^{a}	3,456^{a}	3,767^{a}	3,371^{a}	3,575^{a}
Inter-row	3,363^{a}	4,028^{a}	3,427^{a}	3,539^{a}	3,684^{a}	3,608^{a}
Broadcast	2,833^{a}	3,213^{a}	3,332^{a}	3,361^{a}	4,706^{a}	3,489^{a}
Mean values	3,332	3574	3,405	3,556	3,920	Sorgo
Monocultures	3,945^{A}	3,543^{A}	3,725^{A}	3,412^{A}	4,014^{A}	2,566^{A}
SC (%)						
Row	80.0^{a}	81.2^{a}	80.0^{a}	88.7^{a}	82.5^{a}	82.5^{a}
Inter-row	72.5^{a*1}	77.5^{a*1}	80.0^{a}	82.5 ^{a}	82.5^{a}	79.0^{ab}
Broadcast	75.0^{a*1}	73.7^{a*1}	73.7^{a*1}	72.5^{a*1}	77.5^{a*1}	74.5^{b}
Mean values	75.8	77.5	77.9	81.2	80.8	Sorgo
Monocultures	96.2^{A}	97.5^{A}	97.5^{A}	100.0^{A}	100.0^{A}	78.75^{B}

Means followed by the same lowercase letter in lines and upper case letter in columns do not differ by Tukey test at 5% probability. $^{*1;2}$: Means differ significantly from the *B. ruziziensis* and sorghum monocultures, respectively, by Dunnett test at 5% probability.

showed similar mean values for the soil cover in the five different seeding density (Table 3). The contrast was observed to broadcast sowing intercropping (lower values; P≤0.05). The similar mean values to the first system could be due the fact that the forage crop in the intercropping was benefited by fertilization; it could favor the vegetative growth and consequently the covering of the soil surface.

However, the inter-row intercropping system showed lower mean values (P≤0.05) for the soil cover than *B. ruziziensis* monoculture only for the seeding density 2 and 4 seeds m^{-2}. In the higher seeding density (6, 8, and 10 seeds m^{-2}), those treatments did not show significant difference relative to monoculture. Thus, the *B. ruziziensis* growth, after the sorghum crop, when grown in row and inter-row intercropping in succession to soybeans crop, enables to increase the biomass production in intercropping the Cerrado soil, as observed in other researches (Horvath Neto, 2012; Silva et al., 2015).

It is worth noting that, during the off-season in the Brazillian midwest region, due the low rainfall (Figure 1), the pastures for cattle grazing are debilitated. The production of biomass during this period using inter-cropping systems, like *B. ruziziensis* and grain sorghum intercropping, will enable the production of forage. If the stocking rate is respected and high levels of animal unit are not adopted, the biomasses of *B. ruziziensis* and sorghum can be used as grazing. Otherwise, this biomass may be desiccated in advanced time, since this forage species is easily desiccated compared other *Brachiaria* spp. for the implementation of soybean crop (Ceccon and Concenço, 2014).

In addition, the *B. ruziziensis* and grain sorghum intercropping systems and sorghum monoculture showed similar mean values for grain yield, as discussed previously. The row and inter-row (6, 8, and 10 seeds m^{-2}) intercropping systems and *B. ruziziensis* monoculture showed similar mean values for the soil cover, proving

Table 4. Significance of the variables grain yield (GY), 1000-grain weight (1000-GW), initial (IPOP) and final plant populations (FPOP), initial (IPH) and final plant heights (FPH), first pod insertion heights (FPIH), number of pods on the main stem with one (NPMS1G), two (NPMS2G), three (NPMS3G) and four grains (NPMS4G) and in secondary stems with one (NPSS1G), two (NPSS2G) three (NPSS3G) and four grains (NPSS4G), number of secondary stems (NSS) and number of pods total per plant (NPT) for soybean grown in succession to grain sorghum intercropped with *B. ruziziensis* in the densities of 2, 4, 6, 8 and 10 viable pure seeds m^{-2} in the 2014 off-season, Rio Verde-GO, Brazil.

Sources of variation	FD	GY	1000-GW	IPOP	FPOP	IPH	FPH	FPIH	NPMS 1G
Intercropping systems (IS)	2	ns	*	ns	ns	ns	ns	ns	ns
Seeding Density (SD)	4	ns	ns	ns	**	ns	ns	ns	ns
IS × SD	8	ns	ns	ns	*	ns	ns	ns	ns
Monoculture	4	ns	ns	ns	ns	ns	ns	ns	ns
Intercropping × Monoculture	1	ns	ns	ns	*	ns	ns	*	ns
CV (%)		11.35	9.46	8.81	5.59	11.14	7.58	8.58	69.5

Sources of variation	FD	NPMS 2G	NPMS 3G	NPMS 4G	NPSS 1G	NPSS 2G	NPSS 3G	NPSS 4G	NSS	NPT
Intercropping systems (IS)	2	ns	ns	ns	ns	ns	ns	ns	*	ns
Seeding Density (SD)	4	ns	ns	ns	ns	ns	ns	ns	ns	ns
IS × SD	8	ns	ns	ns	ns	ns	ns	ns	ns	ns
Monoculture	4	ns	ns	ns	ns	ns	ns	ns	ns	ns
Intercropping × Monoculture	1	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)		55.22	23.87	46.67	117.69	51.17	64.49	125.18	42.16	23.1

**, * and ns: significant at 1% and 5% and not significant, respectively, by F test; CV: Variation coefficient; FD: free degree.

the importance of forage species in the intercropping to benefit the soil conservation in the no-tillage system (Kluthcouski et al., 2004).

Soybean crop

Once collected, the *B. ruziziensis* and sorghum biomass, desiccation was done for both species and six day later, the soybean crop was seeded. The results revealed that the intercropping did not affect (P≥0.05) the grain yield, initial population, initial and final plant heights and yield components (number of pods on the main and secondary stems, regardless of the number of grains) (Table 4). However, the broadcast sowing intercropping provided a greater thousand grain weight in relation to the inter-row intercropping (Table 5). Even so, this difference did not contribute to the increase in the grain yields of soybean crop.

The grains yield of soybean was not influenced by preceding crop [*B. ruziziensis* and grain sorghum intercropping systems and monocultures (grain sorghum or *B. ruziziensis*)], type of intercropping system, nor sowing density of *B. ruziziensis* (Table 5). Some case registers an increase in the yield of the culture successor to the intercropping system (Morais et al., 2014). The benefits of forage grass intercropping have been observed on the soil structure by using plants that have a large and aggressive root system with different geometries and soil spaces exploited, besides providing a greater vegetation cover (Kluthcouski et al., 2004).

The fact that total dry matter yield and soil cover values in intercropping is not differentiate from the monoculture of sorghum which might have led to the absence of response in the soybean yield. It is important to note that from mid-December until the end of January, there was a drastic reduction in rainfall in the region, with dry periods in the first twenty days of January (Figure 1). During this period, the soybean was at the reproductive stage, namely pod formation and early grain filling, which require more water. Thus, the higher percentage of soil cover provided by the straw favors the retention of moisture in the soil during the development of the summer crop, especially under drought conditions (Yin et al., 2016).

It was expected that the straw on the soil surface might influence the early development of soybeans, but it did not. Perhaps this is justified by the lack of differences in total dry matter yield, as discussed earlier. Consequently, the initial plant heights were not affected by the implementation systems and the seeding density of *B. ruziziensis* (Tables 1 and 5). Similarly, there were no significant differences from the results obtained in relation to the monoculture (Table 5). The same effect was observed for the insertion height of the first pod and plant height (Table 7). Although a linear increase in the final population of soybean was observed with the increase in the seeding density of *B. ruziziensis* in row intercropping, there were no increases in the yield of soybeans (Table 6).

The analysis of the other yield components (number of pods in the main and secondary stems, regardless of the

Table 5. Average values for grain yield (GY), 1000-grain weight (1000-GW), initial (IPOP) and final populations (FPOP) and initial plant height (IPH) of soybean crop in succession to grain sorghum intercropped with *B. ruziziensis* in the densities of 2, 4, 6, 8 and 10 viable pure seeds m^{-2} in the 2014 off-season, Rio Verde-GO, Brazil.

Intercropping systems	Seeding density (viable pure seeds m^{-2})					Mean values
	2	4	6	8	10	
GY (kg ha^{-1})						
Row	2,928^{a}	2,488^{a}	2,703^{a}	2,802^{a}	3,047^{a}	2,793^{a}
Inter-row	2,744^{a}	2,815^{a}	2,366^{a}	2,712^{a}	2,881^{a}	2,703^{a}
Broadcast	2,633^{a}	2,548^{a}	2,773^{a}	2,579^{a}	2,542^{a}	2,615^{a}
Mean values	2,768	2,617	2,614	2,698	2,823	Sorgo
Monocultures	2,813^{A}	2,601^{A}	2,966^{A}	2,633^{A}	2,614^{A}	2,482^{A}
1000-GW (g)						
Row	114^{a}	112^{a}	115^{a}	99^{a}	113^{a}	111^{ab}
Inter-row	116^{a}	99^{a}	110^{a}	106^{a}	99^{a}	106^{b}
Broadcast	117^{a}	113^{a}	125^{a}	115^{a}	109^{a}	116^{a}
Mean values	116	108	117	107	107	Sorgo
Monocultures	114^{A}	111^{A}	115^{A}	108^{A}	120^{A}	107^{A}
IPOP (plants ha^{-1})						
Row	424,375^{a}	448,125^{a}	480,832^{a}	436,665^{a}	448,125^{a}	447,624^{a}
Inter-row	470,625^{a}	495,625^{a}	447,500^{a}	463,125^{a}	458,750^{a}	467,125^{a}
Broadcast	432,500^{a}	431,250^{a}	455,000^{a}	429,375^{a}	444,375^{a}	438,500^{a}
Mean values	442,500	458,333	461,110	443055	450,416	Sorgo
Monocultures	425,000^{A}	416,875^{A}	446,250^{A}	405,000^{A}	438,750^{A}	437,708^{A}
FPOP (plants ha^{-1})						
Row	359,166^{a}*^{1}	395,625^{a}	435,625^{a}*^{2}	391,875^{a}	405,000 ^{a}	397,458^{a}
Inter-row	403,750^{a}	396,250^{a}	415,625^{a}	430,625^{a}*^{1}	420,000 ^{a}	413,250^{a}
Broadcast	379,582^{a}	403,760^{a}	394,166^{a}	396,875^{a}	389,375^{a}	392,749^{a}
Mean values	380,833	398,542	415,139	406,458	404,792	Sorgo
Monocultures	420,000^{A}	368,125^{A}	393,750^{A}	381,875^{A}	393,125^{A}	386,458^{A}
IPH (cm)						
Row	17.15^{a}	16.30^{a}	15.30^{a}	16.42^{a}	16.12^{a}	16.26^{a}
Inter-row	16.52^{a}	17.37^{a}	15.15^{a}	15.67^{a}	14.60^{a}	15.86^{a}
Broadcast	15.85^{a}	16.80^{a}	15.22^{a}	16.02^{a}	14.90^{a}	15.26^{a}
Mean values	16.51	16.82	15.22	16.04	15.21	Sorgo
Monocultures	17.32^{A}	17.27^{A}	17.05^{A}	18.40^{A}	16.85^{A}	15.65^{A}

Means followed by the same lowercase letter in lines and upper case letter in columns do not differ by Tukey test at 5% probability. *$^{1; 2}$: Means differ significantly from the *B. ruziziensis* and sorghum monocultures, respectively, by Dunnett test at 5% probability.

Table 6. Models, coefficient of determination (R^2) and significance (P value) of regressions adjusted for the characteristic final plant population (FPOP) of soybeans crop grown in succession to sorghum intercropped with *B. ruziziensis* in the densities of 2, 4, 6, 8 and 10 viable pure seeds m^{-2} in the 2014 off-season, Rio Verde-GO, Brazil.

Intercropping systems	Models	R^2	P (value) (%)
FPOP			
Row	Y = 376.408,76 + 3669,63x	29,15	9
Inter-row	Unadjusted	-	-
Broadcast	Unadjusted	-	-
Mean values	Y = 385.479,40 + 2644,85x	58,53	5

Table 7. Average values for final plant height (FPH), insertion height of the first pod (FPIH), number of pods on the main stem with one (NPMS1G), two (NPMS2G), three (NPMS3G) and four grains (NPMS4G) of soybean crop grown in succession to sorghum intercropped with *B. ruziziensis* in the densities of 2, 4, 6, 8 and 10 viable pure seeds m^{-2} in the 2014 off-season, Rio Verde-GO, Brazil.

Intercropping systems	Seeding density (viable pure seeds m^{-2})					Mean values
	2	4	6	8	10	
FPH (cm)						
Row	62.90^{a}	58.77^{a}	62.52^{a}	62.77^{a}	62.77^{a}	61.95^{a}
Inter-row	59.02^{a}	62.87^{a}	61.47^{a}	62.07^{a}	64.27^{a}	61.94^{a}
Broadcast	57.15^{a}	63.62^{a}	64.62^{a}	59.15^{a}*^{1}	56.27^{a}*^{1}	60.16^{a}
Mean values	59.69	61.75	62.87	61.33	61.10	Sorgo
Monocultures	64.40^{A}	66.27^{A}	69.90^{A}	69.22^{A}	68.75^{A}	62.27^{A}
FPIH (cm)						
Row	16.27^{a}	14.90^{a}	16.52^{a}	16.42^{a}	16.20^{a}	16.06^{a}
Inter-row	16.45^{a}	16.40^{a}	16.15^{a}	15.62^{a}*^{1}	15.85^{a}	16.09^{a}
Broadcast	16.80^{a}	16.47^{a}	17.50^{a}	16.32^{a}	15.10^{a}	16.44^{a}
Mean values	16.51	15.92	16.72	16.12	15.72	Sorgo
Monocultures	16.85^{A}	16.72^{A}	16.87^{A}	18.57^{A}	17.67^{A}	16.87^{A}
NPMS1G						
Row	1.10^{a}	1.00^{a}	1.00^{a}	1.65^{a}	0.90^{a}	1.13^{a}
Inter-row	1.25^{a}	1.03^{a}	1.05^{a}	0.70^{a}	1.00^{a}	1.01^{a}
Broadcast	0.60^{a}	1.20^{a}	1.35^{a}	1.02^{a}	0.70^{a}	0.97^{a}
Mean values	0.98	1.08	1.17	1.12	0.87	Sorgo
Monocultures	0.65^{A}	1.30^{A}	1.22^{A}	0.90^{A}	1.15^{A}	1.12^{A}
NPMS2G						
Row	4.45^{a}	3.55^{a}	2.60^{a}	7.50^{a}	4.05^{a}	4.43^{a}
Inter-row	3.55^{a}	3.73^{a}	3.35^{a}	3.35^{a}	3.65^{a}	3.53^{a}
Broadcast	4.15^{a}	4.50^{a}	3.51^{a}	3.67^{a}	3.00^{a}	3.77^{a}
Mean values	4.05	3.93	3.15	4.84	3.56	Sorgo
Monocultures	3.15^{A}	4.90^{A}	3.51^{A}	4.20^{A}	4.05^{A}	4.25^{A}
NPMS3G						
Row	10.60^{a}	9.95^{a}	10.45^{a}	8.90^{a}	10.30^{a}	10.04^{a}
Inter-row	7.20^{a}	9.76^{a}	10.70^{a}	9.70^{a}	11.10^{a}	9.69^{a}
Broadcast	10.43^{a}	10.05^{a}	12.00^{a}	8.27^{a}	9.25^{a}	10.00^{a}
Mean values	9.41	9.92	11.05	8.96	10.22	Sorgo
Monocultures	9.50^{A}	10.70^{A}	10.97^{A}	9.10^{A}	9.85^{A}	11.22^{A}
NPMS4G						
Row	1.55^{a}	1.50^{a}	2.15^{a}	2.40^{a}	2.05^{a}	1.93^{a}
Inter-row	1.55^{a}	1.11^{a}	2.05^{a}	1.50^{a}	1.85^{a}	1.61^{a}
Broadcast	2.05^{a}	2.35^{a}	2.18^{a}	2.55^{a}	2.20^{a}	2.27^{a}
Mean values	1.72	1.65	2.13	2.15	2.03	Sorgo
Monocultures	2.40^{A}	2.65^{A}	2.48^{A}	1.95^{A}	2.55^{A}	2.07^{A}

Means followed by the same lowercase letter in lines and upper case letter in columns do not differ by Tukey test at 5% probability.
*$^{1; 2}$: Means differ significantly from the *B. ruziziensis* and sorghum monocultures, respectively, by Dunnett test at 5% probability.

number of grains and the number of pods) were not influenced by the intercropping systems and the seeding density of *B. ruziziensis* (Tables 7 and 8). Differences were only observed for the number of secondary stems, with a higher value in broadcast sowing intercropping in relation to row intercropping (Table 8). This variation may be attributed to the phenotypic plasticity of the soybean crop in function of the population of plants used.

Table 8. Average values for number of pods on the secondary stem with one (NPSS1G), two (NPSS2G), three (NPSS3G) and four grains (NPSS4G) and number of secondary stems (NSS) and pods total per plant (NPT) of soybean crop grown in succession to sorghum intercropped with *B. ruziziensis* in the densities of 2, 4, 6, 8 and 10 viable pure seeds m^{-2} in the 2014 off-season, Rio Verde-GO, Brazil.

Intercropping systems	Seeding density (viable pure seeds m^{-2})					Mean values
	2	4	6	8	10	
NPSS1G						
Row	0.30^{a}	0.60^{a}	0.40^{a}	1.65^{a}	0.85^{a}	0.76^{a}
Inter-row	0.50^{a}	0.45^{a}	0.30^{a}	0.30^{a}	0.55^{a}	0.42^{a}
Broadcast	0.53^{a}	0.40^{a}	0.62^{a}	0.52^{a}	0.50^{a}	0.52^{a}
Mean values	0.44	0.48	0.44	0.82	0.63	Sorgo
Monocultures	0.25^{A}	0.35^{A}	0.51^{A}	0.65^{A}	0.95^{A}	0.57^{A}
NPSS2G						
Row	2.50^{a}	2.78^{a}	2.00^{a}	0.95^{a}	2.25^{a}	2.09^{a}
Inter-row	2.25^{a}	1.56^{a}	1.45^{a}	1.55^{a}	2.15^{a}	1.79^{a}
Broadcast	2.07^{a}	2.35^{a}	2.11^{a}	1.40^{a}	2.07^{a}	2.00^{a}
Mean values	2.27	2.23	1.85	1.30	2.15	Sorgo
Monocultures	1.65^{A}	1.60^{A}	1.61^{A}	2.30^{A}	2.25 ^{A}	2.50^{A}
NPSS3G						
Row	2.70^{a}	5.00^{a}	4.85^{a}	2.05^{a}	3.90^{a}	3.70^{a}
Inter-row	2.70^{a}	1.81^{a}	3.15^{a}	2.45^{a}	3.85^{a}	2.79^{a}
Broadcast	3.58^{a}	4.65^{a}	3.77^{a}	1.92^{a}	3.35^{a}	3.45^{a}
Mean values	2.99	3.82	3.92	2.14	3.70	Sorgo
Monocultures	3.85^{A}	2.70^{A}	3.37^{A}	2.90^{A}	4.70^{A}	3.67^{A}
NPSS4G						
Row	0.20^{a}	0.50^{a}	0.80^{a}	0.15^{a}	0.55^{a}	0.80^{a}
Inter-row	0.10^{a}	0.36^{a}	0.55^{a}	0.65^{a}	0.50^{a}	0.43^{a}
Broadcast	0.66^{a}	1.65^{a}	0.83^{a}	0.55^{a}	0.30^{a}	0.44^{a}
Mean values	0.32	0.83	0.72	0.45	0.45	Sorgo
Monocultures	0.40^{A}	0.50^{A}	0.91^{A}	0.30^{A}	0.60^{A}	0.47^{A}
NSS						
Row	2.10^{a}	2.80^{a}	2.85^{a}	2.05^{a}	2.30^{a}	2.42^{a}b
Inter-row	1.80^{a}	1.48^{a}	2.00^{a}	1.55^{a}	2.20^{a}	1.80b
Broadcast	2.96^{a}	2.85^{a}	2.87^{a}	2.22^{a}	2.32^{a}	2.64^{a}
Mean values	2.28	2.37	2.57	1.94	2.27	Sorgo
Monoculture	2.30^{A}	2.15^{A}	2.13^{A}	1.70^{A}	3.25^{A}	2.82^{A}
NPT						
Row	23.40^{a}	24.88^{a}	24.35^{a}	25.25^{a}	24.85^{a}	24.54^{a}
Inter-row	19.10^{a}	19.83^{a}	22.60^{a}	20.20	24.65^{a}	21.27^{a}
Broadcast	24.10^{a}	27.10^{a}	26.40^{a}	19.92^{a}	21.37	23.79^{a}
Mean values	22.20	23.95	24.45	21.79	23.62	Sorgo
Monocultures	21.85^{A}	24.70^{A}	24.61^{A}	22.30^{A}	26.10^{A}	25.87^{A}

Means followed by the same lowercase letter in lines and upper case letter in columns do not differ by Tukey test at 5% probability. $^{*1; 2}$: Means differ significantly from the *B. ruziziensis* and sorghum monocultures, respectively, by Dunnett test at 5% probability.

However, the variation in the number of pods did not influence the soybean yield.

Therefore, the *B. ruziziensis* seeding did not density not affected the sorghum grain and dry matter yields in the

off-season. This suggests that, under off-season conditions in the Brazillian Cerrado, it is possible to increase the seeding density these forage specie to increase the dry matter production on intercropping, without, however, causing decrease in the sorghum grain yield and of the soybean crop grown in succession. By conducting other field experiments under conditions similar, it is believed to be feasible to increase the *B. ruziziensis* seeding density above of 10 viable pure seeds m^{-2} in the intercropping in off-season cultivation.

The increase in biomass production on the soil surface would help to minimize the risk of crop losses caused by dry and temperature high periods, which are common in the Brazillian midwest region. In addition, the highest amount of biomass produced in intercropping will contribute to the maintenance of the no-tillage system. Therefore, sorghum intercropped with *B. ruziziensis* on rows, inter-rows and broadcast sowing are viable cultivation techniques for a no-tillage system aiming at the production of grains and dry matter (straw) in the Cerrado region.

Conclusions

Intercropping systems and the seeding density of *B. ruziziensis* until 10 viable pure seeds m^{-2} did not affect the sorghum grains yield in the off-season cultivation, as soybean crop grown in succession.

The *B. ruziziensis* and grain sorghum intercropping was effective in producing straw on soil surface to Cerrado no-tillage system.

Conflict of Interests

The authors have not declared any conflict of interests.

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Selection of $S_{0:2}$ maize progenies using a mixed-model approach

Leandro Lopes Cancellier[1]*, Luiz Paulo Miranda Pires[1], Luiz Antonio Yanes Bernardo Júnior[2], Ewerton Lelys Resende[1], Eduardo Lopes Cancellier[3] and Renzo Garcia Von Pinho[1]

[1]Department of Agriculture, Federal University of Lavras, Lavras, MG, CP 3037, Brazil.
[2]Department of Biology, Federal University of Lavras, Lavras, MG, CP 3037, Brazil.
[3]Department of Soil Science, Federal University of Lavras, Lavras, MG, CP 3037, Brazil.

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In breeding programs, obtaining breeding lines was important, and first selfing selection generations is common. In addition, an analytic approach through mixed models can lead to more success in genotype selection because it lends flexibility in analysis of unbalanced data and provides more precise genotypic values in regard to progenies evaluated. The goal of this study was to make early selection (in the S_2 generation) of progenies evaluated in top crosses, using a mixed-model approach. Five hundred S_2 progenies were plant derived from three populations using selection intensity (40%), which were crossbreeding with three testers. The hybrids obtained, together with control treatments, were set up in five experiments in Brazil: three in Minas Gerais, one in Santa Catarina, and one in Paraná, which evaluated grain yield. The REML method was used for calculation of variance components, and means were predicted through BLUP. The BLUPs of general combining ability (GCA) and specific combining ability (SCA) were also predicted, and the Spearman correlation coefficients among BLUPs were estimated. The dominance effects had a bigger influence on yield expression, as seen from wider amplitude in SCA values. There was an 86% coincidence considering strategy in which selection index was carried out within three populations, in relation to selection by the overall value of GCA. Considering superior hybrids, the progenies of population C exceeded the quantity of hybrids expected by 24.6%, whereas there was a reduction of 30.8% for A and 20% for B. The hybrids that exhibited the highest BLUP means were derived from crosses between progenies of population C together with the tester LE84. The low correlation among testers, both for SCA and for BLUPs, indicates that there is progeny per tester interaction.

Key words: *Zea mays*, combining ability, mixed models, development of breeding lines.

INTRODUCTION

Grain yield increase in the maize crop is directly related to the use of superior hybrids, associated with biotechnological events that contribute to taking better advantage of the genetic potential of these hybrids. Troyer (2006) reports the importance of the development of modern single hybrids that assisted in increasing

*Corresponding author. E-mail: leandrocancellier@gmail.com. Tel: (054)81063319.

maize production in the United States as of 1960. Thus, the exploitation of heterosis makes the use of hybrids in the production system viable.

Currently, there are a large number of high-yielding hybrids available to producers. Therefore, replacing current hybrids with even better ones is one of the big challenges facing breeders (Bison et al., 2003). Since this is an extremely important matter for the seed sector, breeding companies must adopt efficient breeding strategies to obtain new hybrids. In addition to its consequences for increasing crop yield, releasing new cultivars directly affects company profitability.

The probability of obtaining superior hybrids is greater when evaluating a larger number of lines and experimental hybrids. The use of adequate methodologies for identification of pairs of lines that can provide better hybrid combinations improves the efficiency of breeding programs (Iemma, 2003). In this regard, one strategy is evaluation of the combining ability between parents.

The selection of lines with greater values of general combining ability (GCA) increases the probability of obtaining high-yielding hybrids. This occurs due to greater contributions of favorable alleles in the lines. In addition, the identification of hybrids with higher values of specific combining ability (SCA) indicates the possibility of effective use of heterosis between lines (Costa et al., 2013).

One of the main steps in breeding programs is obtaining lines. In this step, early selection of these genotypes in the first generations of self-pollination is common, based on their combining ability with an elite tester line whose performance is already known by the breeder (Guimarães et al., 2012). This substantially reduces the number of lines before reaching homozygosity (Fuzatto, 2003), as well as the cost of carrying out the breeding program.

Success in early selection based mainly on GCA is due to good stability obtained over the self-pollination generations (VALÉRIO et al., 2009). In addition, this method allows greater variation to be obtained between progenies than within them, which shows the individuality of lines even in generations with a lower degree of inbreeding. A study conducted by Bernardo (1991) shows the high genetic correlation between topcrosses of S_2 progenies with S_6 topcrosses. This shows that it is possible to discard progenies in an efficient manner while lines are being obtained, keeping only the most promising for formation of hybrids.

In breeding programs, it is common to carry out large scale trials with many environments. Naturally, this imposes greater difficulties in controlling experimental balance, whether through the loss of plots or through the difficulty of obtaining seeds from all the crosses. Thus, data treatment that does not consider this imbalance will add error to the genotypic values and will lead to reduction in genetic gain.

Analysis through mixed models is a tool that can increase success in early selection and efficiency in breeding programs. This type of analysis, especially BLUP, generates more accurate estimates of the genotypic value of the individual (Arnhold et al., 2009; Mendes, 2011), provides great flexibility of analyses, and allows the breeder to better deal with the imbalance problem, which provides more adequate comparisons between genotypes with different numbers of observations.

Under conditions of experimental imbalance, genotypes can undergo a big change in ordering of the estimated phenotypic values in relation to the genotypic values predicted through BLUP. This leads to a gain in obtaining genotypic values. Thus, BLUP has come to be considered the most accurate method for analysis in these conditions, which justifies its use in this study, in which the quantity of hybrid seeds derived from the topcrosses was not sufficient for their evaluation in all the environments, or in all the genetic combinations (Santos et al., 2016).

In light of the foregoing, this study was carried out with the aim of selecting, in an early inbred generation ($S_{0:2}$), progenies evaluated in topcrosses, using the mixed model method.

MATERIALS AND METHODS

The experiments were conducted from November 2011 to May 2013. In the 2011/2012 crop season, the field was set up for obtaining top cross hybrids. We used 500 $S_{0:2}$ progenies derived from three distinct populations; $S_{0:2}$ progenies are derived from the second generation of selfed plants from F_2 populations coming from commercial hybrids commonly used in southern Brazil, composed of 175 progenies of population A, derived from the three-way hybrid Garra (Syngenta); 88 progenies of population B, derived from the single-cross hybrid AS 1532 (Agroeste); and 237 progenies of population C, derived from the single-cross hybrid 30B39 (DuPont Pioneer).

Phenotypic selection was carried out near flowering time and pre-harvest, adopting the criteria of most vigorous plants, lowest occurrence of leaf diseases, upright leaves, and uniformity of plants within the progeny, among others. Considering a selection intensity of 40%, the selected progenies were crossed with three testers, the elite line LE84, the experimental single-cross hybrid HS2532, and the commercial single-cross hybrid GNZ2004. The LE84 line was selected as an elite line from the maize breeding program and characterized as having high general combining ability. The hybrid GNZ2004 was used due to recommendation for use in same growing regions as A, B, and C hybrids. The experimental hybrid HS2532 was chosen as a good parental line and for allowing identification of high yielding three-way hybrids.

Of the 200 progenies selected, 189 were obtained with sufficient seed quantity to carry out at least one experiment, thus forming 457 top cross hybrids.

In 2012/2013 crop season, experiments were carried out at five locations, three at Crop and Livestock Scientific and Technological Development Center of UFLA (Universidade Federal de Lavras, Lavras, MG, Brazil), at different sowing times: on 09/11/2012 with 144 hybrids tested, on 16/11/2012 with 81 hybrids tested, and on 23/11/2012 with 289 hybrids. Another experiment was conducted at Guarapuava, PR, with sowing on 17/10/2012 with 316 hybrids, and at São Miguel d'Oeste, SC, sown on 07/11/2012 with 348 hybrids.

Table 1. Description of the crosses made from the progenies of the three populations and number of testers obtained in the crosses.

Population	Number of testers			No. of progenies		Possible crosses	Crosses made	
	1	2	3	No.	%	No.	No.	%
A	2	39	17	58	30.7	174	131	75.3
B	0	12	20	32	16.9	96	84	87.5
C	12	44	43	99	52.4	297	229	77.1
Total	14	95	80	189	100.0	567	444	78.3
Total (%)	7.4	50.3	42.3	100.0	-	-	-	-

Table 2. Description of the number of crossbreeding obtained for progenies combination and testers.

Population	Tester			Total hybrids	
	84	2004	2532	No.	%
A	19	57	55	131	29.5
B	20	32	32	84	18.9
C	55	88	86	229	51.6
Total	94	177	173	444	-
Total (%)	21.2	39.9	39.0	-	-

The hybrids were randomly distributed in the experiments so as not to confuse environmental effects with the effects of population or testers. Thus, 13.8% of the hybrids (63) were tested in only one experiment, 16.6% (76) were tested in only two experiments, 68.5% (312) in three experiments, 0.87% (4) in four experiments, and only 0.43% (2) were tested in five experiments, thus totaling the 457 hybrids evaluated.

Among 457 hybrids evaluated, 444 are top cross hybrids, resulting from crosses of progenies from the three populations with testers. However, for some progenies, not enough seeds were obtained in crosses with three testers, and 7.4% of the progenies were tested with only one tester, 50.3% were tested with only two testers, and 42.3% were tested with three testers (Table 1).

The number of crossbreeding from each population combination and testers can be seen in Table 2. In addition, 13 control treatments were included, consisting of 12 commercial hybrids and 1 experimental hybrid.

A simple square lattice experimental design was used in the trials set up in Lavras, MG; the first was 12 × 12, the second 9 × 9, and the third 17 × 17. In the trials set up in PR and SC, randomized blocks were used, with two replications. The experimental plots in Lavras, MG, consisted of two 5-meter rows at a spacing of 0.55 m; and in the trails of PR and SC, of four 5-meter rows at a spacing of 0.5 m.

Grain yield was evaluated as weight of grains obtained in plot, and data were extrapolated to Mg ha^{-1}, with moisture corrected to 13%.

Data analysis was carried out through the mixed model approach, using Restricted Maximum Likelihood (REML) method for genotypic and residual variance components calculation. From fitting the model through iterative process, prediction of variance components of each random vector was obtained, as well as solution of fixed-effects vector.

Initially, BLUP mean values (u+g) of hybrids were obtained through combined analysis of data using the following model:

$$y = X\beta + Z_1 g + Z_2 w + \varepsilon$$

in which y is phenotypic observations vector; β is fixed effects (locations, blocks, and overall mean) vector; g is genotypic effects vector, assumed as random; w is effects of genotype vector per environment interaction, assumed as random; and X, Z_1, and Z_2 represent incidence matrix of said effects.

The estimates of the BLUPs of general combining ability and specific combining ability were carried out with the assistance of SAS software, using proc mixed. In this study, we chose to use with all genotypes within a single group model, according to Balestre et al. (2010). According to these authors, model that considers the genotypes in a single group provides more accurate estimates of GCA and SCA than model that considers two distinct groups, even with kinship information.

Thus, the following model was considered:

$$y = X\beta + Z_1 g + Z_2 s + Z_3(ga) + Z_4(sa) + \varepsilon$$

in which y is observations vector; β is parameters of fixed effects (overall mean, locations, and blocks within locations) vector; g and s are general combining ability and specific combining ability respectively (assumed as random) vectors; ga and sa are interactions between general combining ability and specific combining ability with the environment, respectively (assumed as random) vectors; and ε is residues vectors. X, $Z_1, Z_2, Z_3,$ and Z_4 represent the incidence matrices of β, g, s, ga, and sa effects, respectively.

In abid to analyze best manner of selecting progenies, a comparison was made between two different ranking strategies. In the first, a selection intensity of 30% within each population was considered. In second strategy, same selection intensity was adopted. However, the populations that gave rise to progenies selected were disconsidered.

The Spearman ranking correlations were estimated between BLUP means (u+g), BLUE means, and SCA. Analysis was carried

Table 3. Estimate of the variance components obtained by REML/BLUP for general combining ability (GCA) and specific combining ability (SCA), interaction with the environment, and residual variance of S_2 progenies of maize crossed with three testers.

Parameter	Variance
GCA	0.03846*
SCA	0.1599**
GCA × Environment	0.1352**
SCA × Environment	0.1992**
Residual Variance	1.4522**

*, **; Significant at the level of <0.05 and <0.01, respectively.

out using proc corr of SAS software.

RESULTS AND DISCUSSION

Among parameters useful for selection of genotypes, combining ability stands out through ease of interpretation. In this study, the estimate of variance of SCA was 4.15 times greater than that of GCA (Table 3).

The variance components of models can express genetic variability of genotypes, which allows inferences to be made regarding the type of gene action predominant for each one characteristics. Such a result indicates that in set of progenies under study, dominance effects have a bigger influence on expression of grain yield.

The bigger contribution of dominant effects toward an increase in grain yield is reported in various studies (Balestre et al., 2010; Bordallo et al., 2005; Guedes et al., 2011; Viana and Matta, 2003; Vivek et al., 2010; Werle et al., 2014).

In contrast, Englesong et al. (2011), Oliveira et al. (2011), Paterniani et al. (2006), and Rovaris et al. (2014) studies higher estimate of GCA in relation to SCA indicated that additive gene action contributes more toward an increase in yield. Such divergent results can be explained by the simple fact that combining ability of a genotype is relative to other genotypes being tested; thus, a determined genotype can exhibit high or low GCA, depending on genotypes that are being tested. Oliveira et al. (2011) cited that the type of allelic interaction that is predominant depends on population involved in diallel; thus, the result is specific for genotypes evaluated. Even so, it is possible to verify the predominance of additive genetic effects and of dominance simultaneously in same breeding population. Guimarães et al. (2007), seeking to evaluate maize single-cross hybrids in regard to yield from diallel crosses between divergent lines, found significant contributions of GCA and SCA, thus showing the importance of aforementioned effects.

Expression of the predominant allelic interaction also depends on the breeding intensity to which the genotypes under testing have been subjected. According to Paterniani et al. (2006), lines that have been extensively bred in distinct environments tend to have a bigger contribution from additive effects on manifestation of yield. This effect is in agreement with this study, in which progenies with a low degree of inbreeding were used that were target of phenotypic mass selection. Thus, predominance of the dominance effects on the study of genetic control of the trait in question is possible.

The magnitude of the BLUPs of the GCA for all the progenies of population A ranged from -0.1622 to 0.1780 ton ha^{-1}. The magnitude of the BLUPs of the GCA for all the progenies of population B ranged from -0.1711 to 0.1399 Mg ha^{-1}, and variation for all the progenies of population C was from -0.1182 to 0.2001 Mg ha^{-1}. This scenario of low variation, considering the yield potential of the crop, is a direct reflection of the low estimate of the variance component related to GCA in comparison to SCA.

Oliveira et al. (2011) observed that low variance estimates of GCA were also reflected in low amplitude in the estimates of GCA of the progenies, just as observed in this study. Balestre et al. (2010) found that the variances of the BLUP values of the combining abilities (GCA and SCA), estimated from 90 $S_{0:2}$ progenies genotyped with 25 microsatellite markers, were low, corroborating the results obtained in this study. These same authors discuss importance of use of progenies as a viable alternative in synthesis of high production hybrids.

In Table 4, ranking of the 57 best progenies based on GCA can be observed, considering best progenies within each population. Considering 58 progenies of population A, 17 progenies with highest GCA were selected, with variation in values from 0.01248 to 0.1780 Mg ha^{-1}. In the case of 32 progenies of population B, the 10 progenies with highest GCA were considered, with values ranging from 0.00936 to 0.1399 Mg ha^{-1}. As for 99 progenies of population C, 30 were selected, exhibiting GCA values from 0.03899 to 0.2001 Mg ha^{-1}.

Considering selection of the progenies with highest values of GCA, among 58 progenies of population A, 13 were selected with GCA values from 0.0414 to 0.1780 Mg ha^{-1} (Table 5). In 32 progenies cases of population B, six progenies with highest GCA were considered with values from 0.0447 to 0.1399 Mg ha^{-1}. Furthermore, among 99 progenies of population C, 38 were selected, with variation from 0.0319 to 0.2001 Mg ha^{-1}.

There was coincidence of 49 progenies (86%) considering the strategy in which the selection index was carried out within each one of three populations in relation to selection by overall value of GCA, disregarding pedigree of the progenies. This indicates that there was superiority in progenies production of one population in relation to others. The superiority of population C in generating progenies of higher general combining ability

Table 4. BLUPs of general combining ability (GCA) (Mg ha^{-1}) of 57 of 189 maize progenies, considering selection of 30% for each population.

Progeny	GCA
Population A	
A153	0.17800
A23	0.11130
A178	0.10750
A131	0.07876
A71	0.07082
A98	0.06748
A119	0.06604
A176	0.06505
A3	0.05605
A9	0.05232
A100	0.04970
A86	0.04582
A16	0.04140
A151	0.01940
A41	0.01685
A26	0.01364
A114	0.01248
Population B	
B31	0.13990
B13	0.08256
B60	0.06669
B30	0.05645
B48	0.04476
B17	0.04437
B2	0.02853
B57	0.02482
B38	0.02066
B47	0.00936
Population C	
C134	0.20010
C122	0.18460
C259	0.17430
C143	0.16290
C223	0.14400
C141	0.14390
C193	0.11570
C103	0.10630
C113	0.10090
C100	0.10080
C110	0.09285
C40	0.09006
C251	0.08866
C180	0.08752
C192	0.08436
C236	0.08352
C77	0.08290
C124	0.07777
C67	0.07770
C234	0.07515
C247	0.07308
C24	0.07240
C188	0.06477
C109	0.06433
C190	0.05731
C106	0.05552
C250	0.05211
C147	0.05201
C135	0.04811
C18	0.04432

Table 4. Contd.

*In reference to 30% of total of 189 progenies with highest BLUPs of GCA per population.

can be observed, considering that in the classification by overall GCA (Table 5), 8 progenies of population C were included, together with fact that there was superiority of 3.24% in GCA mean values upon using second selection strategy that is, disregarding progenies origins.

The estimates of GCA, in their magnitude, generally occur for genotypes with highest and lowest frequencies of favorable alleles in relation to mean value of genotypes tested. Thus, it is understood that purpose of selection of progenies based on highest estimates of GCA is to increase frequency of favorable alleles in following generations. Thus, it can be inferred that population C has higher frequency of favorable alleles, reflected in superiority in progenies production. The importance of high frequency of favorable alleles as a strategy for analysis of the potential of a population for extracting lines has already been discussed in literature. Bison et al. (2003), evaluating progenies derived from two different commercial hybrids, describes in regard to the estimate of m+a that there is a close relationship to frequency of favorable alleles in a population. It follows that the highest estimates of GCA can be derived from the most promising populations.

Associating progenies with high GCA values with hybrids resulting from these progenies that also have high values of SCA should be prioritized in selection because they associate a high mean and wider genetic variance. There are thus greater chances that in future generations these progenies will be lines with high potential for generating high production hybrids.

The higher expression of the dominance effects reflected in wider variance of SCA (Table 3) can also be seen by wider amplitude in the SCA values in relation to amplitude in GCA values (Table 5).

Table 6 shows 133 hybrids among 444 (corresponding to a selection intensity of 30%) with highest values of

Table 5. BLUPs of general combining ability (GCA) (Mg ha^{-1}) of 57 of 189 maize progenies, considering selection of 30% of progenies with higher GCAs, regardless of population (Pop).

Pop	Progeny	GCA
C	C134	0.2001
C	C122	0.1846
A	A153	0.1780
C	C259	0.1743
C	C143	0.1629
C	C223	0.1440
C	C141	0.1439
B	B31	0.1399
C	C193	0.1157
A	A23	0.1113
A	A178	0.1075
C	C103	0.1063
C	C113	0.1009
C	C100	0.1008
C	C110	0.0929
C	C40	0.0901
C	C251	0.0887
C	C180	0.0875
C	C192	0.0844
C	C236	0.0835
C	C77	0.0829
B	B13	0.0826
A	A131	0.0788
C	C124	0.0778
C	C67	0.0777
C	C234	0.0752
C	C247	0.0731
C	C24	0.0724
A	A71	0.0708
A	A98	0.0675
B	B60	0.0667
A	A119	0.0660
A	A176	0.0651
C	C188	0.0648
C	C109	0.0643
C	C190	0.0573
B	B30	0.0565
A	A3	0.0561
C	C106	0.0555
A	A9	0.0523
C	C250	0.0521
C	C147	0.0520
A	A100	0.0497
C	C135	0.0481
A	A86	0.0458
B	B48	0.0448
B	B17	0.0444
C	C18	0.0443
C	C235	0.0433
A	A16	0.0414
C	C98	0.0395
C	C49	0.0390
C	C61	0.0383
C	C138	0.0363
C	C94	0.0328
C	C93	0.0325
C	C96	0.0319

Table 5. Contd.

* In reference to 30% of total of 189 progenies with highest BLUPs of GCA regardless of population.

SCA. There was variation in SCA values of hybrids derived from progenies of population A from -0.5251 to 0.5234 Mg ha^{-1}, and among superior ones, the lowest SCA value was 0.1010 Mg ha^{-1}. For population B, variation was from -0.4687 to 0.6916 Mg ha^{-1}, with lowest value within superior hybrids of 0.0980. Variation of population C was from -0.4921 to 0.6398 Mg ha^{-1}, and lowest value among superior hybrids was 0.1008.

To assist in recognition of best hybrid combination, the SCA is of fundamental importance and should be interpreted as deviation of a crossbreeding in relation to what would be expected based on GCA of its parents (Valente, 2010).

Expressive contributions of SCA have already been observed not only for grain yield (Balestre et al., 2010; Bordallo et al., 2005; Guedes et al., 2011; Vivek et al., 2010) but also for other traits of economic importance. Vivek et al. (2009), by means of a diallel among 12 elite lines and analysis using method 4 of Griffing (1956) found significant effects for SCA both for grain yield and for a complex of diseases that affect maize yield, such as common rust and *Phaeosphaeria* leaf spot, among others.

Considering the number of hybrids for each population, 27 hybrids derived from progenies of population A are on list of superior hybrids. For population B, 20 hybrids are among superior ones, and for population C, 86 hybrids.

The amplitude of variation for SCA of hybrids for populations A, B, and C was 1.0485, 1.1603, and 1.1319, respectively. It can be observed that, in regard to SCA, the variation in population C is intermediate; nevertheless, it was the population that generated the highest estimates of additive effects, providing the inference in regard to highest proportion of favorable alleles accumulated up to this generation of inbreeding.

Taking the proportion of hybrids tested into account, 39 hybrids would be expected for population A, 25 for B, and 69 for C (Table 7).

Thus, it can be seen that the progenies of population C have an ability for generating hybrids with mean production superior to hybrids derived from progenies of populations A and B because, among superior hybrids,

Table 6. BLUPs of specific combining ability (SCA) of 133 maize hybrids from different populations.

Hybrid	SCA	Hybrid	SCA	Hybrid	SCA	Hybrid	SCA
Population A		**Population B**		**Population C**		**Population C**	
A100 X 2532	0.5234	B31 X 2532	0.6916	C122 X 84	0.6398	C95 X 2532	0.1858
A153 X 2004	0.5087	B30 X 2004	0.4455	C143 X 84	0.5602	C186 X 2004	0.1857
A3 X 2004	0.3659	B60 X 2532	0.3685	C134 X 84	0.5173	C102 X 2004	0.1849
A119 X 2532	0.3251	B17 X 2004	0.2927	C259 X 84	0.5069	C181 X 2532	0.1821
A71 X 2004	0.3202	B48 X 2004	0.2815	C178 X 84	0.4807	C135 X 2532	0.1819
A98 X 84	0.3182	B85 X 2532	0.2687	C191 X 84	0.4441	C28 X 2004	0.1817
A178 X 2532	0.2782	B7 X 2532	0.2294	C192 X 84	0.4419	C235 X 84	0.1800
A42 X 2532	0.2618	B78 X 2532	0.2255	C223 X 84	0.4394	C134 X 2532	0.1791
A9 X 2004	0.2608	B13 X 2004	0.2128	C113 X 84	0.4279	C110 X 84	0.1765
A8 X 2532	0.2594	B57 X 2532	0.1933	C259 X 2004	0.4250	C110 X 2532	0.1719
A23 X 2532	0.2406	B47 X 2004	0.1807	C100 X 2004	0.4199	C96 X 2532	0.1704
A133 X 2532	0.2357	B90 X 2004	0.1547	C174 X 2004	0.3877	C247 X 2532	0.1698
A153 X 2532	0.2313	B2 X 2532	0.1515	C188 X 2004	0.3776	C67 X 2532	0.1665
A131 X 2532	0.2265	B23 X 2532	0.1463	C77 X 2004	0.3418	C75 X 2004	0.1653
A23 X 2004	0.2220	B34 X 2004	0.1352	C180 X 2532	0.3162	C85 X 2532	0.1634
A37 X 84	0.2125	B13 X 2532	0.1304	C141 X 2004	0.3128	C61 X 2004	0.1629
A178 X 2004	0.1686	B36 X 2532	0.1141	C234 X 84	0.3124	C109 X 2004	0.1605
A58 X 2532	0.1597	B47 X 2532	0.1076	C251 X 2004	0.3113	C103 X 2004	0.1589
A176 X 2004	0.1524	B17 X 2532	0.0989	C24 X 2004	0.3010	C124 X 84	0.1575
A86 X 2532	0.1433	B30 X 84	0.0980	C45 X 2532	0.2933	C15 X 2532	0.1566
A114 X 2004	0.1350			C141 X 2532	0.2856	C67 X 2004	0.1566
A151 X 2004	0.1335			C180 X 84	0.2810	C68 X 2532	0.1558
A16 X 2532	0.1214			C194 X 2532	0.2782	C65 X 2004	0.1549
A176 X 2532	0.1180			C40 X 2004	0.2733	C138 X 84	0.1543
A109 X 2004	0.1094			C143 X 2532	0.2621	C97 X 2532	0.1530
A87 X 2004	0.1011			C80 X 2004	0.2611	C247 X 84	0.1458
A131 X 2004	0.1010			C103 X 84	0.2517	C147 X 2532	0.1447
				C94 X 2004	0.2495	C131 X 2004	0.1407
				C193 X 2532	0.2482	C134 X 2004	0.1357
				C126 X 84	0.2432	C124 X 2532	0.1347
				C236 X 2004	0.2430	C151 X 2004	0.1328
				C93 X 2532	0.2422	C98 X 2532	0.1293
				C113 X 2004	0.2343	C18 X 2532	0.1265
				C190 X 84	0.2294	C182 X 2532	0.1245
				C144 X 2532	0.2293	C190 X 2532	0.1151
				C151 X 2532	0.2287	C223 X 2004	0.1141
				C49 X 2004	0.2269	C78 X 2004	0.1100
				C193 X 84	0.2175	C87 X 2004	0.1080
				C250 X 84	0.2110	C109 X 2532	0.1069
				C112 X 2004	0.2106	C155 X 84	0.1051
				C179 X 84	0.2040	C236 X 84	0.1043
				C146 X 2532	0.1974	C40 X 2532	0.1011
				C106 X 84	0.1956	C22 X 2004	0.1008
Mean		Population A	0.2309	Population B	0.2263	Population C	0.2344

*In reference to 30% of total of 444 hybrids with highest SCA values.

progenies of population C exceeded number of hybrids expected by 24.6%, whereas A and B had a reduction of 30.8 and 20%, respectively.

From breakdown tester and population combinations, it can be observed that progenies of population C with tester LE84 resulted in an increase of 56.3% in number of

Table 7. Breakdown in relation to population of origin of progenies and testers of hybrids with highest values of specific combining ability.

Pop.	Tester									Total		
	LE84			GNZ2004			HS2532					
	N_e	N_s	%	N_e	N_s	%	N_e	N_s	%	N_e	N_s	%
A	6	2	33.3	17	12	70.6	16	13	81.3	39	27	69.2
B	6	1	16.7	10	7	70.0	9	12	133.3	25	20	80.0
C	16	25	156.3	26	31	119.2	27	30	111.1	69	86	124.6
Total	28	28	100	53	50	94.3	52	55	105.8	133	133	100.0

N_e, Number of hybrids expected based on number of hybrids tested; N_s Number of superior hybrids.

hybrids among superior ones (Table 7). The only combination that generated results above that expected, except for those of population C, was combination of progenies of population B with HS2532 tester, leading to an increase of 33.3% in number of hybrids among superior ones. Included in this combination was hybrid that has highest value of SCA, B31 X 2532, with 0.6916 Mg ha^{-1}.

The ability to generate hybrids with superior production is connected with medium/high frequency of favorable alleles of parental lines, just as gene complementarity of parents, often expressed by high estimates of specific combining ability. Thus, progenies complementary of population C with LE84 tester can be explained by high proportion of contrasting loci between tester and population, which can, moreover, belong to distinct heterotic groups.

The high complementarity of population C with LE84 tester can furthermore be observed by SCA values, in which nine hybrids with higher SCA values are part of this combination. This scenario indicates that there is potential for obtaining high production single-cross hybrids after obtaining inbred lines derived from this population and crossed with this tester.

According to Bordallo et al. (2005), most favorable hybrid is that with highest estimate of specific combining ability, in which one of parents has higher general combining ability. Thus, we have 24 progenies example A100, A153, A3, A119, A71, A98, B31, B30, B60, C122, C143, C134, C259, C192, C223, C113, C100, C188, C77, C180, C141, C234, C251, and C24, in which hybrids resulting from these progenies have SCA values above 0.3000 (Table 6) and have high GCA values, above 0.0497 (Table 4).

To obtain inter population hybrids, populations derived from hybrids should be given priority in choice of population to be used for extracting lines. In these cases, hybrids should have high SCA estimates (Balestre et al., 2008).

There was wide variation in the estimate of SCA as a function of tester; such a situation can be important if one of the testers is of interest for obtaining hybrids in crosses with some lines that may come to be obtained from these populations. In fact, this is main argument upon using an inbred line as a tester in which result can be guide of high potential crossbreeding the future; preferentially, this tester will be from a distinct heterotic group (Elias et al., 2000).

The SCA is a very useful parameter in population choice for extraction of lines and obtaining inter population hybrids. Balestre et al. (2008), evaluating potential extraction of lines from ten commercial hybrids using mean components and mixed models with incorporation of multiallelic molecular markers, found a significant variation in SCA estimates, showing influence of non-additive effects in hybrid combinations.

Thus, considering that 40.2% of population C progenies are present among 33% of hybrids with higher SCA values (Table 6), and considering only crossbreeding with LE84 tester, these progenies of population C are present in more than half of superior hybrids. It can be inferred that greatest probability of obtaining a good hybrid will be using lines derived from population C in crossbreeding with LE84 tester. Such a situation reflects higher allelic complementarity in these crossbreeding's. Considering that LE84 tester is an elite line, the chance of obtaining good hybrids after obtaining inbred lines from population C increases.

Fritsche-Neto et al. (2010), evaluating efficiency of REML/BLUP approach in prediction of genotypic values and in unbalanced experiments in maize crop, simulated various conditions of imbalance in number of environments, as well as in number of hybrids within environments, finding good efficiency when imbalance is up to 20% of hybrids within environments and 23% of environments. Balestre et al. (2010) also evaluated the efficiency of BLUP with the incorporation of kinship information for prediction of the production of untested maize hybrids, observing significant estimates of correlation between observed and predicted genotypic values, which ranged from 0.55 to 0.7, depending on degree of imbalance.

In general, hybrids that had higher BLUPs were derived from crossbreeding between progenies of population C with LE84 tester (Table 8).

Of 133 genotypes selected, 93 were derived from

Table 8. BLUPs (u+g) of yield (Mg ha^{-1}) of 133 maize hybrids derived from progenies of three populations and crossed with three testers and 13 controls.

Hybrid	BLUP (u+g)	Hybrid	BLUP (u+g)	Hybrid	BLUP (u+g)	Hybrid	BLUP (u+g)
C122 X 84	12.4	C179 X 84	11.6	C151 X 2532	11.4	A131 X 2532	11.3
C143 X 84	12.3	C49 X 2004	11.6	B48 X 2004	11.4	C204 X 84	11.3
B31 X 2532	12.3	C80 X 2004	11.5	C93 X 2532	11.4	C124 X 2532	11.3
C134 X 84	12.2	C45 X 2532	11.5	A178 X 2532	11.4	C95 X 2532	11.3
C259 X 84	12.1	A23 X 2004	11.5	A176 X 2004	11.4	C22 X 2004	11.3
A153 X 2004	12.1	C106 X 84	11.5	C181 X 2532	11.4	C151 X 2004	11.3
C192 X 84	12.0	C250 X 84	11.5	C110 X 84	11.4	B47 X 2532	11.3
C113 X 84	12.0	C235 X 84	11.5	B57 X 2532	11.4	C127 X 84	11.3
C178 X 84	12.0	C112 X 2004	11.5	C224 X 84	11.4	C158 X 2004	11.3
C223 X 84	12.0	C94 X 2004	11.5	C223 X 2004	11.4	C190 X 2532	11.3
C191 X 84	11.9	C236 X 2004	11.5	C85 X 2532	11.4	C96 X 2532	11.3
C259 X 2004	11.9	C134 X 2532	11.5	C122 X 2004	11.4	B38 X 84	11.3
C100 X 2004	11.8	C134 X 2004	11.5	A42 X 2532	11.4	B23 X 2532	11.3
C77 X 2004	11.8	A178 X 2004	11.5	C110 X 2532	11.4	C182 X 2532	11.3
C234 X 84	11.8	A37 X 84	11.5	B13 X 2004	11.4	C155 X 84	11.3
C188 X 2004	11.8	B85 X 2532	11.5	C236 X 84	11.4	C109 X 2532	11.3
C141 X 2004	11.8	A9 X 2004	11.5	B90 X 2532	11.4	C110 X 2004	11.3
B60 X 2532	11.7	C138 X 84	11.5	B30 X 84	11.4	C98 X 2532	11.3
C251 X 2004	11.7	C67 X 2004	11.5	C147 X 2532	11.4	B47 X 2004	11.3
A100 X 2532	11.7	C109 X 2004	11.5	C131 X 2004	11.4	A87 X 2004	11.3
C180 X 84	11.7	C103 X 2004	11.5	A151 X 2004	11.4	C78 X 2004	11.3
C103 X 84	11.7	B17 X 2004	11.5	A131 X 2004	11.4	C97 X 2532	11.3
C40 X 2004	11.7	A119 X 2532	11.5	B13 X 2532	11.4	P30F53H	12.6
B30 X 2004	11.7	A23 X 2532	11.5	C28 X 2004	11.4	AS1581	12.3
C180 X 2532	11.7	C146 X 2532	11.5	C135 X 2532	11.4	GNZ9626	12.1
C174 X 2004	11.7	C61 X 2004	11.5	C15 X 2532	11.4	AG8088	11.9
C190 X 84	11.6	C126 X 84	11.5	C68 X 2532	11.4	AG8041	11.8
C24 X 2004	11.6	C144 X 2532	11.5	B7 X 2532	11.4	DKB240PRO2	11.5
A3 X 2004	11.6	C124 X 84	11.5	C131 X 84	11.4	AG1051	11.4
C193 X 84	11.6	C65 X 2004	11.5	C251 X 84	11.4	AG4051	11.4
C143 X 2532	11.6	C67 X 2532	11.5	B78 X 2532	11.4	AG8061	11.3
C141 X 2532	11.6	C194 X 2532	11.5	A114 X 2004	11.4	LG6304YG	11.0
A98 X 84	11.6	C247 X 2532	11.4	C87 X 2004	11.4	GNZ2004	10.8
C247 X 84	11.6	C102 X 2004	11.4	B2 X 2532	11.4	HS2532	10.1
C113 X 2004	11.6	C75 X 2004	11.4	C40 X 2532	11.4	BRAS3010	9.8
C193 X 2532	11.6	A153 X 2532	11.4	B17 X 2532	11.3		
A71 X 2004	11.6	A8 X 2532	11.4	C186 X 2004	11.3		

*In reference to 30% of total of 457 hybrids with highest yields.

population C, equivalent to 69.69% of total, regardless of tester used. Another important observation can be made upon comparing BLUPs of hybrids with hybrids used as controls. The amplitude of variation of BLUPs of controls was 2.81, whereas top crossbreeding hybrids exhibited lower amplitude of variation, namely, 1.1.

Considering BLUPs of hybrids, a large number of top crosses that have mean values above some commercial hybrids are observed, thus showing that in addition to populations resulting in progenies with high variability, they also allow high mean values to be obtained; it should be noted that this is considered to be ideal situation for selecting progenies and obtaining lines (Alves, 2006; Ferreira et al., 2009).

To obtain genetic gains in future generations, ensuring lines with good combining ability associated with ability to generate high yielding hybrids, progenies that have high GCA and that also contribute to obtaining hybrids with high SCA should be selected in early generations. In addition to good combining ability, they should exhibit

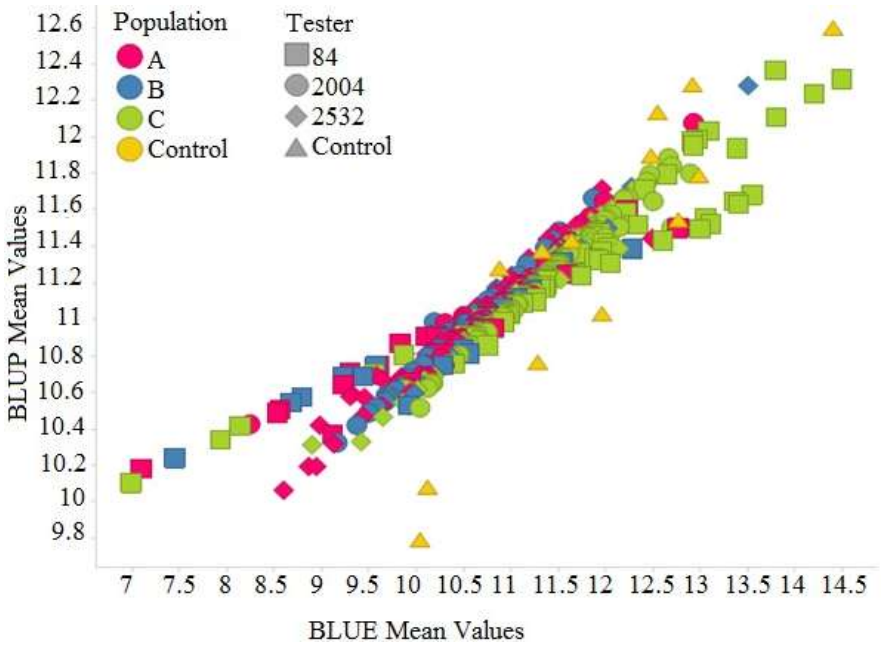

Figure 1. Dispersion between BLUP mean values and BLUE mean values of 457 maize hybrids. Points in orange represent hybrids coming from population A; points in blue, green, and yellow represent populations B, C, and controls, respectively. Points in square format represent hybrids with tester LE84; circle, diamond, and triangle represent the tester GNZ2004, the tester HS2532, and controls, respectively.

Table 9. Estimates of Spearman correlation between SCA, BLUP mean values, and BLUE mean values in 444 maize hybrids.

Source	r
BLUP mean x SCA	0.95**
BLUP mean x BLUE mean	0.97**
BLUE mean x SCA	0.90**
Tester GNZ2004 BLUP mean x SCA	0.95**
Tester HS2532 BLUP mean x SCA	0.96**
Tester LE84 BLUP mean x SCA	0.97**

** Significant at 1% probability by t test.

high mean values. In this study, progenies with highest values of GCA (Table 5) that contributed to formation of hybrids with high SCA values (Table 6) and that obtained high yields (Table 8) should be selected to advance generation and obtain lines.

Except for progenies A23, A86, and C18, all progenies selected based on GCA (Table 5), also formed hybrids with high SCA values associated with high grain yields. It is thus considered that even with the greater influence of dominance effects on yield expression, additive effects should not be disregarded.

Correlation coefficients of high magnitude were manifested among SCA, BLUP, and BLUE, as well as for correlation between BLUP and SCA within each tester (Table 9). It can be inferred that, regardless of the tester, using BLUP mean values or SCA, ranking of hybrids will not change very much. Therefore, one of these parameters alone can be adopted for use in progeny selection process. Due to considerable flexibility of analysis, mixed model approach (BLUP) can be an advantageous option, and problem of imbalance can be dealt with in a better way.

The high correlation between predicted BLUP means and estimated BLUE means can be observed in Figure 1. A similar result was found by Iemma (2003), comparing methodology of mixed models with fixed model in diallel analysis, obtaining correlation of 0.99 between BLUP and BLUE, attributing this to fact that BLUP considers matrix identity in structure of variances and covariances, and thus there are not big differences in methodology. Consequently, it can be inferred that for grain yield, classification of the hybrids undergoes little change when mixed model and fixed model methodologies were considered. Similar results were obtained by Iemma (2003).

The greater correlation of SCA with BLUP mean values (Table 10) in comparison to BLUE mean values were also observed in this case (Figure 2). It can be seen that the points are distributed in a more clustered manner around the mean value. This greater correlation between SCA and BLUP can be attributed to shrinkage method effect, which approximates observations to overall mean, more intensely penalizing hybrids with a smaller number

Table 10. Estimate of Spearman correlation between testers for specific combining ability (SCA) and BLUP mean in 444 maize hybrids.

Correlations between testers	BLUP mean	SCA
	r	r
GNZ2004 x HS2532	0.13	-0.15
GNZ2004 x LE84	0.36**	0.23
LE84 x HS2532	-0.03	-0.26

** Significant at 0.01 probability by the t test.

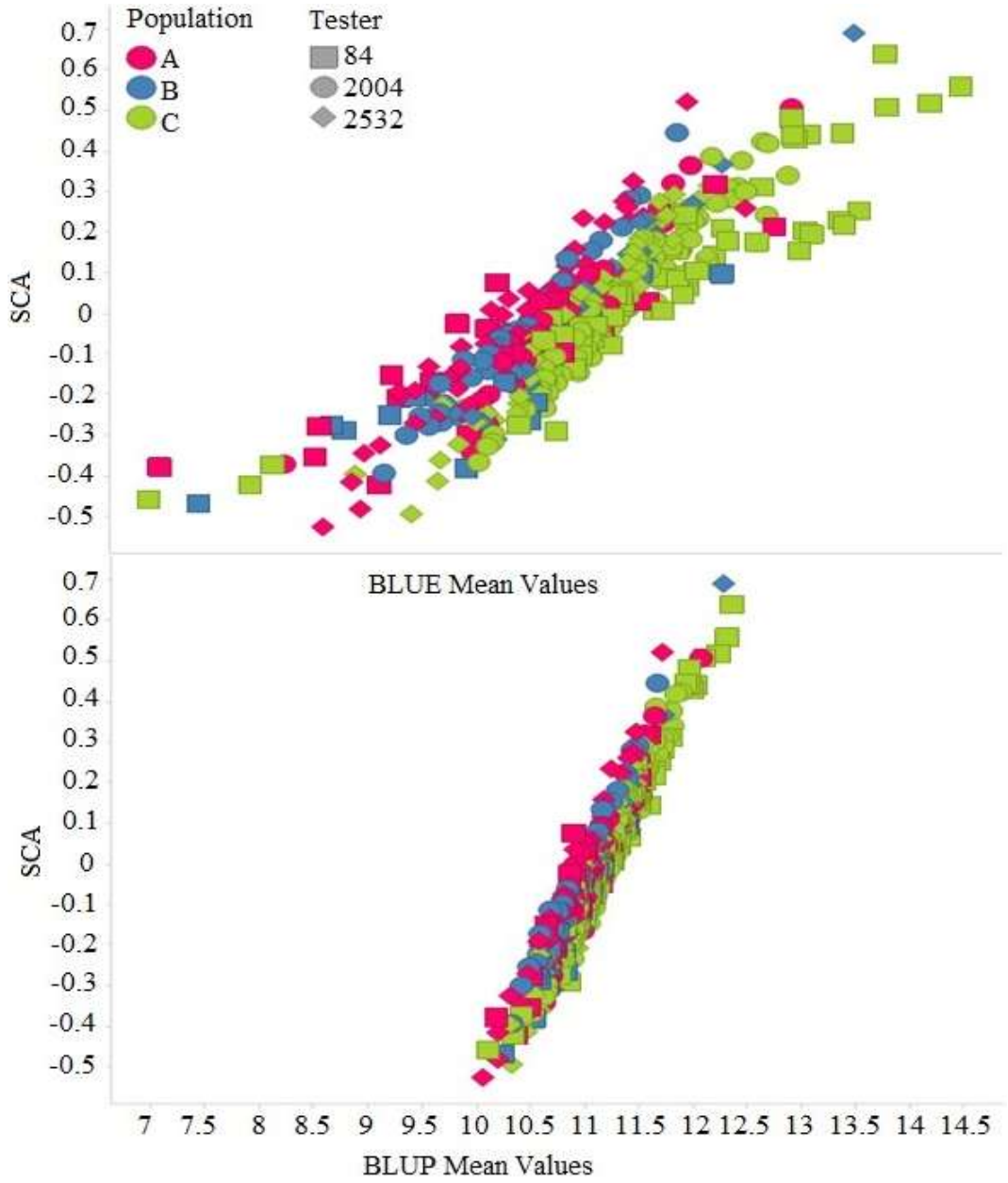

Figure 2. B and C populations, respectively. Points in square form represent hybrids with LE84 tester; circle and diamond represent GNZ2004 and HS2532 testers, respectively.

of plots and in environments with greater lack of experimental uniformity.

The high magnitude of correlation coefficient between SCA and BLUP mean was also observed by Balestre et al. (2008), which was attributed to fact that yield is main parameter used in these estimates. These authors furthermore report that SCA and heterosis are highly correlated with yield, and that these correlations are useful in choice of population used for extraction of lines.

Low correlation was detected between testers considering both BLUP means and SCA. Thus, production of progenies is totally dependent on tester, indicating that allelic frequency of tester is an important factor and must be considered in the progeny test. Elias et al. (2000) also reported that there is low correlation in the production and classification of top cross hybrids when different testers are used, attributing this difference to the genetic structures in populations, just as in testers.

When commercial hybrids or elite hybrids from breeding programs are used as testers, it is expected that they have high frequency of favorable alleles for diverse genes. Thus, there may be confounding effects of dominant favorable alleles in tester (Elias et al., 2000). This situation also contributes to low correlation between testers, as well as to results that do not represent real merit of progenies, but rather specific production of progeny per tester combination (Barreto et al., 2012). Even if testers with high frequency of favorable alleles are not considered ideal from conceptual point of view, results may be highly valuable if objective is to obtain three-way hybrids.

Low correlations between testers also have implications for intensity of selection because correlations between the combining abilities of progenies with diverse types of testers are normally too low to have some predictive value and, therefore, high selection intensity can only be applied to a specific tester because probability of selecting lines with high combining ability for distinct testers is very low.

Barreto et al. (2012) further state that unrelated testers used, on a strict genetic basis, as in case of single-cross hybrids, is justified because it allows expression of greater genetic variance in relation to evaluation *per se* of progenies, observing five times greater variance.

Thus, it can be concluded that progenies A100, A119, A131, A153, A176, A178, A23, A3, A71, A9, A98, B13, B17, B30, B31, B48, B60, C100, C103, C106, C109, C110, C113, C122, C124, C134, C135, C138, C141, C143, C147, C180, C188, C190, C192, C193, C223, C234, C235, C236, C24, C247, C250, C251, C259, C40, C49, C61, C67, C77, C93, C94, C96, and C98 were selected based on high production through GCA, SCA, and BLUP and are able to be selected to generation advance and obtain lines of high value.

The hybrids that showed greater yield potential were derived from crossbreeding between progenies of population C with 84 tester.

There was greater influence of dominance effects on yield expression, but additive effects should not be disregarded. There was high correlation between SCA and mean BLUP, allowing that one alone can be used in selection of progenies.

The low correlation between testers both for SCA and for mean BLUP indicates that there is progeny per tester interaction.

Conflict of Interests

The authors have not declared any conflict of interests.

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Seeding rate and physiological quality of dual purpose wheat seeds

Vinícius Jardel Szareski[1]*, Ivan Ricardo Carvalho[2], Maicon Nardino[3], Gustavo Henrique Demari[1], Alan Junior de Pelegrin[2], Mauricio Ferrari[2], Diego Nicolau Follmann[4], Tiago Corazza da Rosa[2], Etiane Skrebsky Quadros[5], Tiago Pedó[1], Paulo Dejalma Zimmer[1], Velci Queiróz de Souza[5] and Tiago Zanatta Aumonde[1]

[1]Department of Plant Science, Agronomy School Eliseu Maciel, Federal University of Pelotas (UFPel), Campus Capão do Leão, Rio Grande do Sul, Brazil.
[2]Departament of Plant Genomics and Breeding Center, Federal University of Pelotas (UFPel), Campus Capão do Leão, Rio Grande do Sul, Brazil.
[3]Departament of Mathematics and Statistics, Federal University of Pelotas (UFPel), Campus Capão do Leão, Rio Grande do Sul, Brazil.
[4]Federal University of Santa Maria (UFSM), Santa Maria, Rio Grande do Sul, Brazil.
[5]Federal University of Pampa (UNIPAMPA), Dom Pedrito, Rio Grande do Sul, Brazil.

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Wheat dual purpose (*Triticum aestivum* L.) has elevated biomass production, high tillering potential, long vegetative stage, short reproductive period. Therefore, this study aim to reveal the seeding rates effect on physiological attributes of wheat dual purpose seeds grown in three agricultural crops, and identify a population management strategy and check the response stability of the traits in southern Brazil. The tests were carried out during 2012, 2013 and 2014 agricultural crops. The experimental design was randomized blocks arranged in factorial scheme, being: three agricultural crops x two wheat genotypes dual purpose (BRS Tarumã and BRS Umbu) x five seeding rates (75, 150, 225, 300, 375 seeds m^{-2}), arranged in three repetitions. The variance analysis revealed significant interaction to p>0.05 of probability for agricultural crops x dual purpose wheat genotypes x seeding rates for the characters first germination counting (FGC), germinated seeds percentage (GP), seedlings dry mass (SDM), and field emergence (FE). The genotype BRS Tarumã with increases of seeding rates, reduced the magnitude of germination and first germination counting in all agricultural crops. For wheat dual purpose seedling dry mass and field emergence decreased with seeding rates increment. Management strategies to improve the physiological quality of seeds wheat with dual purpose are specific to agricultural crops, providing applications in scientific research and as agricultural.

Key words: *Triticum aestivum* L., growing plants, field crops, crop science.

INTRODUCTION

Wheat dual purpose (*Triticum aestivum* L.) belongs to the family Poaceae, characterized by providing forage during

the vegetative stages and by the grain yield, being considered of dual aptitude (Martin et al., 2010). Wheat dual purpose has elevated biomass production, high tillering potential, long vegetative stage and short reproductive period (Carvalho et al., 2016). This species presents tolerance to trampling, quick field establishment, grain yield, high protein values and good digestibility, allowing the crop-livestock integration and maximizing physical space on the farm (Martin et al., 2010; Martin et al., 2013). Its use occurs, in most cases, in the form of direct grazing, hay production (Fontaneli, 2007; Carvalho et al., 2015).

Wheat genotypes growth and development may be influenced by genetic and edaphoclimatic factors, genotype x environment interaction, management techniques (Zagonel et al., 2002). Among the influenced characters, it is highlighted the number of tillers per plant, ears per unit of area, plants grains mass and number (Martin et al., 2013). Among the management techniques that most influence these characters, seeding rate deserves attention (Valério et al., 2013). Seeding rate is characterized as determining for wheat crop, and can provide balance in the dynamics between characters of agronomic interest and grain yield (Valério et al., 2013; Ozturk et al., 2006; Benin et al., 2012). The seeding rate used for certain genotype is crucial for the magnitude of total tillers, viable tillers per plant, intraspecific and interspecific competition with weeds (Valério et al., 2013; Tavares et al., 2014).

The effects attributed to different wheat seeding rates may be defined as a change in the dynamics of plants competition, alteration in the photosynthetically active area, plants lodging, increase in incidence pests, unfavorable microclimate for plants growth, development, and increased competition for nutrients, that can reduce physiological seed quality (Carvalho et al., 2016). Low plant densities may reveal increased number of tillers and ears per plant, changing the direction of assimilates, their remobilization to seeds (Fioreze and Rodrigues, 2014). In contrast, plants density increase may result in a larger number of ears per unit of area, and grain yield (Da Silveira et al., 2010).

Seed quality is determined by elevated genetic, physical integrity, sanity and differential physiological performance (Barbieri et al., 2013). Low quality seeds may reduce seedling emergence speed, resulting in lower growth of the aerial part, and root system, thus compromising growth and crop development (Scheeren et al., 2010; Peske et al., 2012). Therefore, this study aim to reveal the seeding rates effect on physiological attributes of wheat dual purpose seeds grown in three agricultural crops, and identify a population management strategy and check the response stability of the traits in southern Brazil.

MATERIALS AND METHODS

The experiment was conducted in 2012, 2013 and 2014 agricultural crops, in the Federal University of Santa Maria, in Frederico Westphalen southern Brazil, the geographic coordinates of 27°23'26''S, 53°25'43''O, and altitude of 461 m. The soil is classified as ferric aluminicoxisol (Embrapa, 2006), and the climate, according to Köppen's characterization, as *Cfa* subtropical. The experimental design was randomized blocks arranged in factorial scheme, being: three agricultural crops x two wheat genotypes dual purpose (BRS Tarumã and BRS Umbu) x five seeding rates (75, 150, 225, 300, 375 seeds m^{-2}), arranged in three repetitions. The experimental units were composed by 12 lines with two meters length, spaced 0.17 m. System direct seeding was used in all agricultural crops, with NPK base fertilization of 250 kg ha^{-1} (10-20-20), and 90 kg ha^{-1} of nitrogen (N) in top-dressing at full tillering stage, in urea form (45% of N). The harvest was manually done, being considered the plants present in the six central lines of each experimental unit, disregarding 0.5 meters of each extremity aiming to minimize the border effects (Martin et al., 2010). Posteriorly, the seeds were accommodated in a chamber with constant temperature of 25°C during 15 days in order to homogenize the humidity, which is determined by the official oven method, at 105°C for 24 h, occurring the standardization at 13% of humidity (Brasil, 2009).

The physiological germination tests, first counting and dry mass were carried out with four sub-samples of 100 seeds for each experimental unit. The seeds were accommodated in rolls formed by three germination paper sheets of the *germitest* type, moistened with distilled water in a proportion of 2.5 times the mass of the dry paper. The rolls were wrapped in polyethylene envelopes aiming to keep the humidity, and were subsequently allocated in a B.O.D at 20°C (Brasil, 2009).

Germination (G) was evaluated eight days after sowing, and the data was measured by the percentage of normal seedlings (Brasil, 2009). Collectively, four days after sowing, it was evaluated the first germination counting (FGC), measured by the percentage of normal seedlings (Brasil, 2009). Determination of seedlings dry mass (SDM) occurred from ten normal seedlings at eight days after sowing. For this, the seedlings were dried in an oven with forced ventilation at temperature of 70°C until constant weight, and the results are expressed in grams (g). For field emergence (FE) evaluation, it was proceeded the sowing of four hundred seeds disposed in four repetitions, with one hundred seeds for each experimental unit. The number of emerged seedlings counting was performed on the twentieth-first day after sowing, and the results expressed as a percentage.

The data was submitted the normality test of Shapiro and Wilk (1965), and homogeneity variance by Bartlett (Steel et al., 1996). It was found interaction for agricultural crops x two wheat genotypes dual purpose x seeding rates, at 5% of probability. The characters that revealed significance were deployed to simple effects for qualitative factors. The quantitative factor was subjected to linear regression analysis, identifying the most significant polynomial degree, and the results were expressed in graphs. The analyses were performed using statistic software Genes (Cruz, 2013).

*Corresponding author. E-mail: viniciusszareaski@gmail.com.

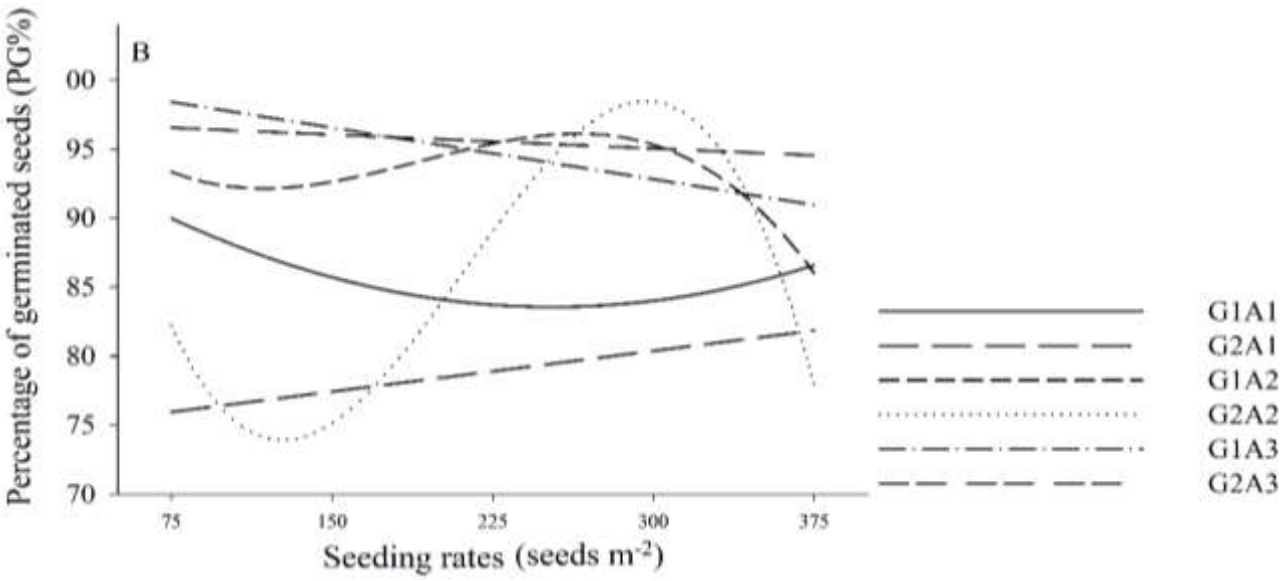

Figure 1. Regression equations for the character germinated seeds percentage (GP). Graph B. G1Y1 genotype BRS Tarumã agriculture crop 2011($y=96.55-0.10x+0.002x^2$ $R^2=0.30$); G2Y1 genotype BRS Umbu agriculture crop 2011($y=74.47+0.01x$ $R^2=0.09$); G1Y2 genotype BRS Tarumã agriculture crop 2012 ($y=103,86-0.23x+0.00x^2-0.00x^3$ $R^2=0.73$); G2Y2 genotype BRS Umbu agriculture crop 2012 ($y=248.66-3.95x+0.02x^2-0.00x^3+0.00x^4$ $R^2=0.92$); G1Y3 genotype BRS Tarumã agriculture crop 2013 ($y= 91.26+0.07-0.0002$ $R^2= 0.54$); G2Y3 genotype BRS Umbu agriculture crop 2013 ($y= 97.03-0.006x$ $R^2=0.04$).

RESULTS AND DISCUSSION

Analysis of variance for traits

Analysis of variance revealed significant interaction at p>0.05 probability for agricultural crops x two wheat genotypes dual purpose x seeding rates for the characters first germination counting (FGC), germinated seeds percentage (GP), seedlings dry mass (SDM), and field emergence (FE). The coefficients of variation obtained for the measured characters ranged from 4.53 to 16.53%, indicating reliable inferences about the results and good experimental precision (Pimentel Gomes, 2000).

Seed quality

Germination is crucial for commercializing seeds, which must provide minimum percentage of 80% (Brasil, 2003). Among seeding rates for germinated seeds percentage (GP) (Figure 1), it is observed that the genotype BRS Tarumã in agricultural crops 2011 (G1Y1) and 2013 (G1Y3), as well as the genotype BRS Umbu, in the agricultural crops of 2013 (G2Y3), reduced germination with the increase of seeding rates. However, the genotype BRS Umbu, in 2011 (G2Y1) revealed germination increase with the elevation of seeding rates. The genotype BRS Tarumã in 2012 (G1Y2), revealed germination increment when cultivated in the density of 225 seeds per square meter. It is observed differential behavior of genotypes at different sowing densities. The quality of the wheat seeds produced is closely related to plant density, where random plants reductions adversely affect seeds physiological quality (Barbieri et al., 2013).

It was verified superior germination performance for the genotype BRS Tarumã in 2011 and 2012 agricultural crops. However, in 2013, the genotypes behaved similarly, where the genotype BRS Umbu revealed superiority at density of 375 seeds per square meter. Between agricultural crops, the genotype BRS Tarumã revealed superiority in 2012 and 2013, while the genotype BRS Umbu presented higher percentages in 2013 (Table 2). Germination is evaluated under ideal conditions; however, it cannot be regarded as absolute because their results should be related to complementary vigor testing in order to better represent the seedlings performance in the field (Douradinho et al., 2015). The first germination counting consists of a vigor indicative test, the ability of quickly germinate relates to the vigor and can be an indicative of higher physiological quality (Marcos Filho, 2015).

Among seeding rates, it is observed for the character first germination counting (FGC), that the genotype BRS Tarumã in 2012 (G1Y2) and 2013 (G1Y3) agricultural crops (Figure 2), and BRS Umbu in 2013 (G2Y3) revealed an increase for first germination counting at density of 300 seeds per square meter, while the genotype BRS Tarumã in 2011 (G1Y1) demonstrated reduction of values in response to seeding rates increment. The genotype BRS Umbu in 2012 (G2Y1) incremented the character FGC. The results evidence

Table 1. Interaction for agriculture crops x two wheat genotypes dual purpose x seeding rates, for the character first germination counting (FGC).

Seeding rates seeds (m^2)	2011		2012		2013	
	Agricultural crops / Dual purpose wheat genotypes					
	BRS Tarumã	BRS Umbu	BRS Tarumã	BRS Umbu	BRS Tarumã	BRS Umbu
75	$89.2^{A\alpha}$	$71.7^{B\gamma}$	$94.5^{A\alpha}$	$82.5^{B\beta}$	$97.5^{A\alpha}$	$97.5^{A\alpha}$
150	$78.2^{A\beta}$	$85.7^{A\beta}$	$92.2^{A\alpha}$	$82.4^{A\alpha}$	$96.2^{A\alpha}$	$97.5^{A\alpha}$
225	$74.5^{B\beta}$	$86.5^{A\beta}$	$89.2^{A\alpha}$	$83.9^{A\beta}$	$97.2^{A\alpha}$	$97.2^{A\alpha}$
300	$81.2^{A\beta}$	$79.0^{A\beta}$	$94.9^{A\alpha}$	$94.9^{A\alpha}$	$98.2^{A\alpha}$	$85.9^{B\beta}$
375	$74.2^{B\beta}$	$83.7^{A\beta}$	$85.5^{A\alpha}$	$78.2^{A\beta}$	$88.9^{A\alpha}$	$93.2^{A\alpha}$
CV (%)	6.26					

*****Means followed by the same uppercase letter in the line, for genotypes, and the same Greek letter for agricultural years, did not statistically differ by Tukey test at 5% of probability.

Table 2. Interaction for agriculture crops x two wheat genotypes dual purpose x seeding rates, for the character germinated seeds percentage (GP).

Seedingrates seeds (m^2)	2011		2012		2013	
	Agricultural crops / Dual purpose wheat genotypes					
	BRS Tarumã	BRS Umbu	BRS Tarumã	BRS Umbu	BRS Tarumã	BRS Umbu
75	$91.0^{A\alpha}$	$71.7^{B\gamma}$	$92.9^{A\alpha}$	$82.5^{B\beta}$	$96.2^{A\alpha}$	$96.9^{A\alpha}$
150	$83.5^{A\beta}$	$80.5^{A\beta}$	$92.2^{A\alpha}$	$70.6^{B\gamma}$	$95.9^{A\alpha}$	$94.5^{A\alpha}$
225	$84.2^{A\beta}$	$83.0^{A\beta}$	$94.9^{A\alpha}$	$93.2^{A\alpha}$	$96.2^{A\alpha}$	$96.5^{A\alpha}$
300	$85.5^{A\beta}$	$79.7^{B\beta}$	$94.9^{A\alpha}$	$94.9^{A\alpha}$	$95.9^{A\alpha}$	$91.5^{A\alpha}$
375	$85.7^{A\alpha}$	$79.5^{B\beta}$	$85.5^{A\alpha}$	$78.2^{B\beta}$	$86.9^{B\alpha}$	$95.9^{A\alpha}$
CV (%)	4.53					

*****Means followed by the same uppercase letter in the line, for genotypes, and the same Greek letter for agricultural years, did not statistically differ by Tukey test at 5% of probability.

that the vigor attributed to wheat dual purpose seeds are linked with intrinsic characteristics of the genotype, and also with seeding rates and agricultural crops. Marcos Filho (2015), the evaluation of vigor has many implications in the seed sector as a basic monitoring of physiological potential during different stages of seed production, being an apparatus for strategic decisions regarding the selection of seeds with high physiological potential, aiming to meet the consumer's demand.

Vigor assigned seeds

For the character first germination counting (FGC) in 2011 agricultural crops, the genotype BRS Tarumã was inferior at densities of 275 and 375 seeds per square meter. In the agricultural crops of 2012, the genotype BRS Umbu reached lower values for FGC at density of 75 seeds per square meter. In 2013, the genotype BRS Umbu was inferior at density of 300 seeds per square meter (Table 1). Among agricultural crops, it is observed that the genotype BRS Tarumã was superior in 2012 and 2013. For the genotype BRS Umbu the superiority occurred only in 2013. The highest values of first germination counting report to the greater seed vigor and are indicative of higher capability of available reserves utilization, metabolic reactivation and the cellular structure of the seed, resulting in the formation of a seedling with proportional, well defined root and aerial structures (Bewley et al., 2013).

Among seeding rates for the character seedling dry mass (SDM), both genotypes in the agricultural crops of 2011 (G1Y1 and G2Y2) revealed no significant response for densities (Figure 3). The genotype BRS Umbu in 2012 (G2Y2), and the genotype BRS Tarumã in 2013(G1Y3) revealed reduction of seedlings dry mass with the increase of seeding rates. On the other hand, the genotype BRS Umbu in 2013 (G2Y3), demonstrated dry mass increment with seeding rates increase. In the agricultural crops of 2011, seedlings dry mass (SDM) did not differ for both genotypes. In 2012 the genotype BRS Umbu presented the highest magnitudes, however, it revealed superiority only at density of 150 seeds per square meter. In 2013 the genotype BRS Umbu revealed superiority for seedlings dry mass (Table 3). Between agricultural crops, it was observed superiority for the genotype BRS Tarumã in 2012, and the genotype BRS Umbu in 2013 (Table 3).

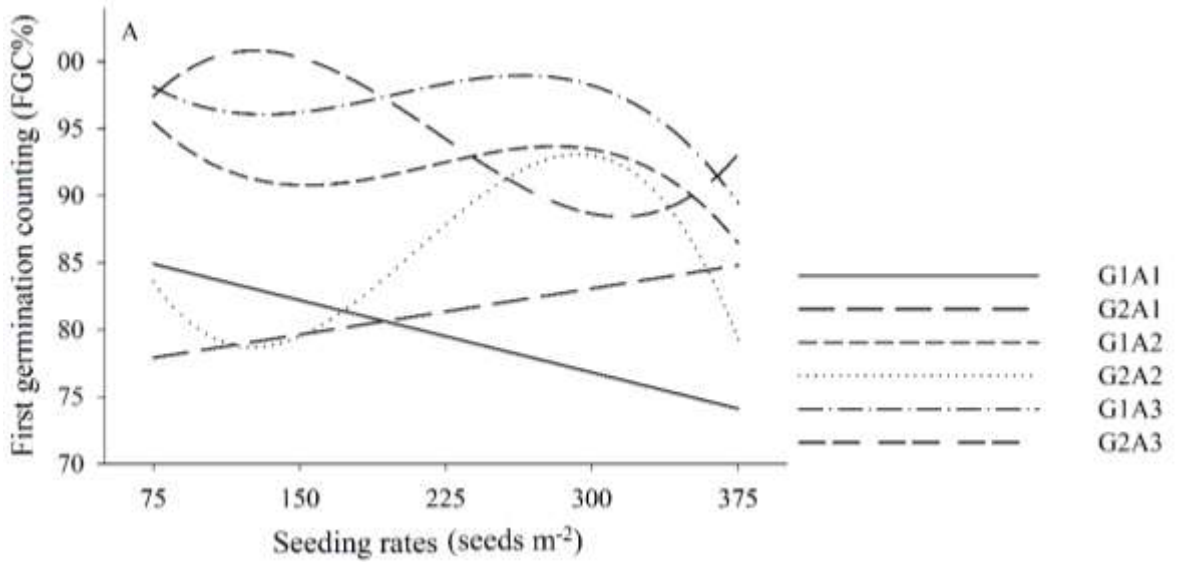

Figure 2. Regression equations for the character first germination counting (FGC). Graph A: G1Y1 genotype BRS Tarumã agriculture crop 2011($y=87.6-0.03x$ $R^2=0.19$); G2Y1 genotype BRS Umbu agriculture crop 2011($y=76.17+0.02x$ $R^2=0.08$); G1Y2 genotype BRS Tarumã agriculture crop 2012 ($y=54.33+1.12x-0.01x^2+0.00x^3-0.00x^4$ $R^2=0.84$); G2Y2 genotype BRS Umbu agriculture crop 2012 ($y=30.33+1.44-0.01x^2+0.00x^3-0.004x^4$ $R^2=0.84$); G1Y3 genotype BRS Tarumã agriculture crop 2013 ($y= 110.40-0.26x+0.01x^2-0.00x^3$ $R^2=0.86$); G2Y3 genotype BRS Umbu agriculture crop 2013 ($y= 148.66-1.37x+0.01x^2-0.01x-0.00x^4$ $R^2= 0.73$).

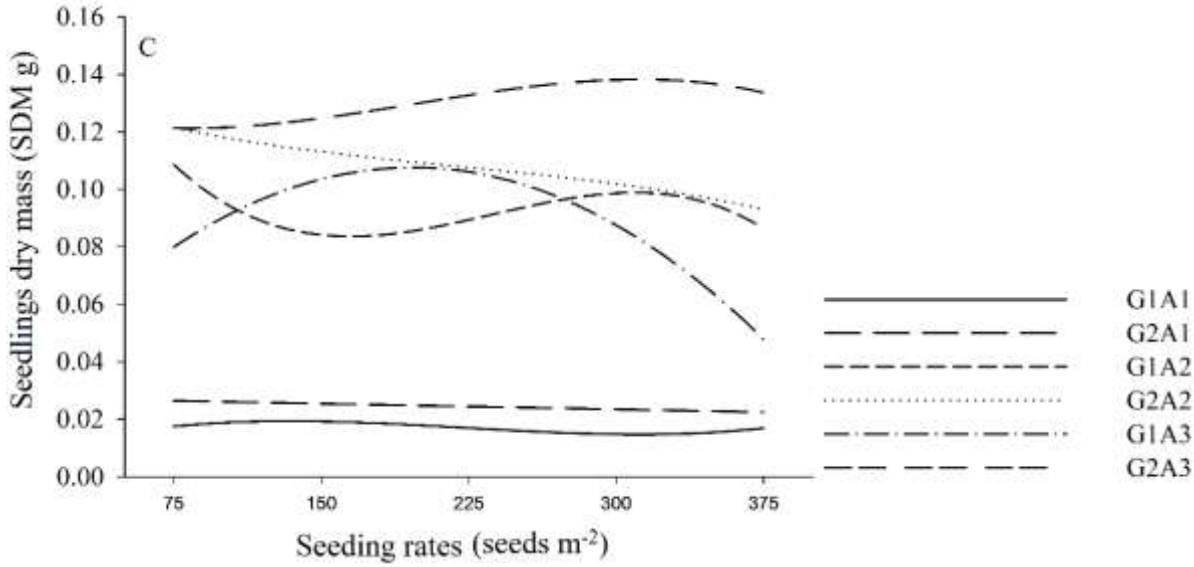

Figure 3. Regression equations for the character seedling dry mass (SDM). Graph C: G1Y1 genotype BRS Tarumã agriculture crop 2011($y=0.007+0.0002x-0.000x^2+0.00x^3$ $R^2=0.47$); G2Y1 genotype BRS Umbu agriculture crop 2011($y=0.02-0.00$ $R^2=0.29$); G1Y2 genotype BRS Tarumã agriculture crop 2012 ($y=0.18-0.001x+0.00x^2-0.00x^3$ $R^2= 0.73$); G2Y2 genotype BRS Umbu agriculture crop 2012 ($y= -0.24+0.009x-0.00x^2+0.00x^3-0.004x^4$ $R^2=0.84$); G1Y3 genotype BRS Tarumã agriculture crop 2013 ($y= 0.03+0.0007-0.00x^2$ $R^2=0.45$); G2Y3 genotype BRS Umbu agriculture crop 2013 ($y= 0.11+0.00005x$ $R^2=0.11$).

The character seedlings dry mass is also considered a vigor attribute because it constitutes a form of evaluation that relates more to seeds physiological performance, and plants in the field, depending on edaphoclimatic growing conditions, proper genotype positioning in micro regions, fertilization, harvest close to physiological maturity, and appropriate management techniques (Peske et al., 2012). The allocation of dry matter in seeds

Table 3. Interaction for agriculture crops x two wheat genotypes dual purpose x seeding rates, for the character seedlings dry mass (SDM).

Seeding rates seeds (m^2)	2011		2012		2013	
	Agricultural crops / Dual purpose wheat genotypes					
	BRS Tarumã	BRS Umbu	BRS Tarumã	BRS Umbu	BRS Tarumã	BRS Umbu
75	$0.017^{A\gamma}$	$0.027^{A\beta}$	$0.107^{A\alpha}$	$0.118^{A\alpha}$	$0.086^{B\beta}$	$0.121^{A\alpha}$
150	$0.018^{A\beta}$	$0.025^{A\beta}$	$0.084^{B\alpha}$	$0.124^{A\alpha}$	$0.093^{B\alpha}$	$0.122^{A\alpha}$
225	$0.018^{A\beta}$	$0.023^{A\gamma}$	$0.088^{A\alpha}$	$0.089^{A\beta}$	$0.094^{B\alpha}$	$0.135^{A\alpha}$
300	$0.014^{A\beta}$	$0.024^{A\gamma}$	$0.099^{A\alpha}$	$0.113^{A\beta}$	$0.111^{B\alpha}$	$0.136^{A\alpha}$
375	$0.016^{A\gamma}$	$0.022^{A\gamma}$	$0.085^{A\alpha}$	$0.089^{A\beta}$	$0.037^{B\beta}$	$0.133^{A\alpha}$
CV (%)	16.53					

*****Means followed by the same uppercase letter in the line, for genotypes, and the same Greek letter for agricultural years, did not statistically differ by Tukey test at 5% of probability.

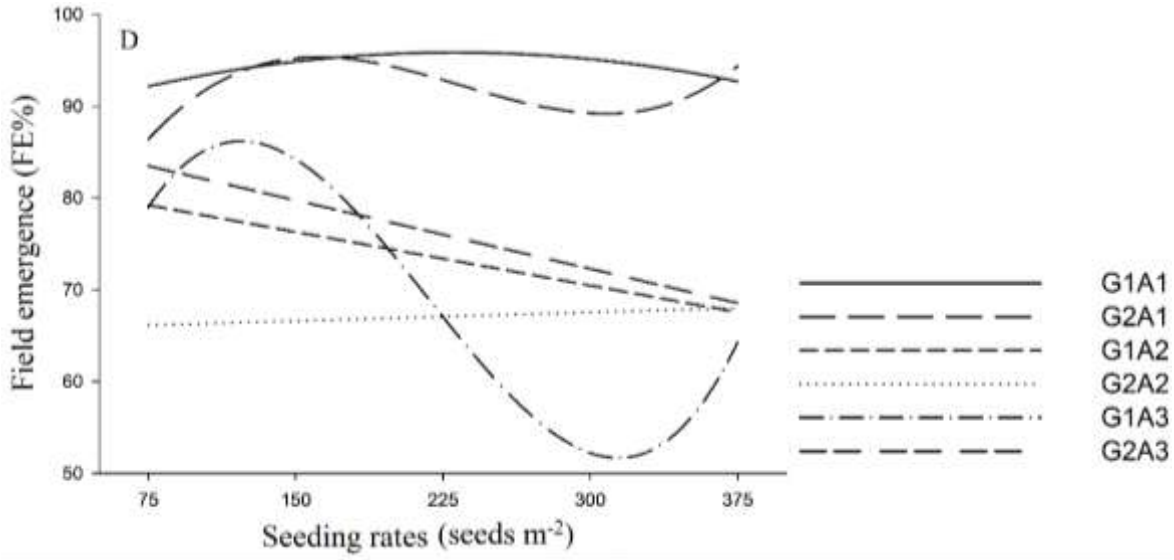

Figure 4. Regression equations for the character field emergence (FE). Graph D: G1Y1 genotype BRS Tarumã agriculture crop 2011($y= 87.73+0.07x-0.801x^2$ $R^2=0.27$); G2Y1 genotype BRS Umbu agriculture crop 2011($y=56.26+0.58x-0.002x^2+0.00x^3$ $R^2=0.45$); G1Y2 genotype BRS Tarumã agriculture crop 2012 ($y=82.11-0.03x$ $R^2=0.12$); G2Y2 genotype BRS Umbu agriculture crop 2012 ($y=65.66+0.006x$ $R^2=0.004$); G1Y3 genotype BRS Tarumã agriculture crop 2013 ($y=326.66-6.41x+0.05x^2-0.00x^3+0.00x^4$ $R^2=0.86$); G2Y3 genotype BRS Umbu agriculture crop 2013 ($y= 87.20-0.04x$ $R^2=0.16$).

has close relationship with the area of leaves available for photosynthetic process, which may be benefited by the crop population density. Plants with proper spacing tend to make better use of mineral resources, water and light, producing more assimilated (Lopes and Lima, 2015). In appropriate environmental and management conditions, the assimilates partitioning is sequential throughout the development, being the seeds favored in bigger or smaller intensity, depending on the level of competition between structures (Pedó et al., 2016).

Among seeding rates for the character field emergence (FE), the genotype BRS Tarumã in 2012 and 2013 (G1Y1 and G1Y3) agricultural crops, and the genotype BRS Umbu in 2013 (G2Y3), revealed reduction of vigor with seeding rates increment (Figure 4). On the other hand, the genotype BRS Umbu in 2012 (G2Y2) revealed seedlings emergence increment with density increases. The genotype BRS Tarumã in 2011 (G1Y1), and the genotype BRS Umbu in 2012 (G2Y2) presented higher magnitudes for seedlings emergence at density of 225 seeds per square meter. The character field emergence, in 2011 agricultural crops evidenced no difference between genotypes, in 2012 superiority was revealed for the genotype BRS Tarumã. In 2013, the genotypes behaved similarly, and BRS Umbu was superior only at density of 300 seeds per square meter (Table 4).

Between agricultural crops it is observed that BRS Tarumã genotype was superior in 2011. In 2012 and

Table 4. Interaction for agriculture crops x two wheat genotypes dual purpose x seeding rates, for the character field emergence (FE).

Seeding rates seeds (m^2)	2011		2012		2013	
	Agricultural crops / Dual purpose wheat genotypes					
	BRS Tarumã	BRS Umbu	BRS Tarumã	BRS Umbu	BRS Tarumã	BRS Umbu
75	$92.0^{A\alpha}$	$86.0^{A\alpha}$	$84.0^{A\alpha}$	$68.6^{B\beta}$	$81.3^{A\alpha}$	$81.3^{A\alpha\beta}$
150	$95.3^{A\alpha}$	$96.6^{A\alpha}$	$72.0^{A\beta}$	$70.6^{A\beta}$	$74.6^{A\beta}$	$78.6^{A\beta}$
225	$95.3^{A\alpha}$	$90.6^{A\alpha}$	$68.6^{A\beta}$	$59.3^{A\gamma}$	$81.3^{A\beta}$	$77.3^{A\beta}$
300	$95.3^{A\alpha}$	$90.6^{A\alpha}$	$76.8^{A\beta}$	$60.6^{B\beta}$	$42.6^{B\gamma}$	$81.3^{A\alpha}$
375	$92.6^{A\alpha}$	$94.0^{A\alpha}$	$68.6^{A\beta}$	$76.0^{A\beta}$	$66.6^{A\beta}$	$61.3^{A\gamma}$
CV (%)	9.83					

*****Means followed by the same uppercase letter in the line, for genotypes, and the same Greek letter for agricultural years, did not statistically differ by Tukey test at 5% of probability.

2013 agricultural crops, no difference was found for this character. The same behavior was observed for BRS Umbu genotype (Table 4).

Guedes et al. (2015), the character field emergence enables to evaluate the real physiological potential of seeds, because, when sown in non-controlled environmental conditions, seeds are subjected to a range of adverse factors, which could compromise the plants stand. Moreover, it should be noted that field emergence relates to the ability of metabolizing reserves, membrane reorganization and carbon allocation in seedlings. The main factors that determine seed vigor are determined in the field of production during the mother plant or seed development (Bewley et al., 2013). Therefore, plants grown in more appropriate densities and management tend to produce seeds with greater vigor and lower mass allocation.

Conclusion

The genotype BRS Tarumã with increases of seeding rates, reduced the magnitude of germination and first germination counting in all agricultural crops.

For wheat dual purpose seedling dry mass and field emergence decreased with seeding rates increment. Management strategies to improve the physiological quality of seeds wheat with dual purpose are specific to agricultural crops, providing applications in scientific research and as agricultural.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Agronomic performance, production components and agricultural productivity of maize (*Zea mays* L.) cultivars

Cicero Teixeira Silva Costa[1], Iêdo Teodoro[2], Samuel Silva[3], Fernando Nobre Cunha[1]*, Marconi Batista Teixeira[1], Frederico Antonio Loureiro Soares[1], Wilker Alves Morais[1], Nelmício Furtado da Silva[1], Flávio Henrique Ferreira Gomes[1] and Lays Borges dos Santos Cabral[1]

[1]Federal Institute of Goiás (Instituto Federal Goiano – IF Goiano), Campus Rio Verde, Goiás GO, Brazil.
[2]Federal University of Alagoas (UFAL) Rio Largo, Alagoas, Brazil.
[3]Federal Institute of Alagoas (IFAL) Campus Piranhas, Alagoas, Brazil.

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The rain fed crop is highly dependent on environmental conditions, and study aims to evaluate the agronomic performance, yield components and agricultural productivity of six maize cultivars in the region of the coastal tablelands of Alagoas State, Brazil. The experimental design was randomized complete block, with five replications and six treatments. Six maize cultivars, three varieties (Jaboatão, Batité and AL Bandeirante) and three hybrids (AG 7098, AG 7088 and AG 8677) were evaluated from June 8 to October 14, 2015. The experimental area of 2000 m^2 (0.2 ha) was divided into six cultivation rows of seven meters linear portions; spaced 0.8 m between rows and 0.25 m between plants, totaling 50.000 plants ha^{-1}. During the experiment, the following were evaluated: plant height, leaf area index (LAI), culm diameter and at the end of the experiment, the production components, agricultural productivity, water balance and the grains water content were determined. The highest grain yield was obtained by hybrid AG 8677 (4.2 t ha^{-1}) followed by the AG 7098 (3.6 t ha^{-1}), with an average of 3.0 t ha^{-1} for all cultivars. Variety AL Bandeirante achieved a productivity similar to that of hybrid AG 7088. The lowest productivities were verified by varieties Jaboatão (2.0 t ha^{-1}) and Batité (2.2 t ha^{-1}). However, these yields were statistically similar to that of variety AL Bandeirante. The maximum water excess during cultivation of maize was 504 mm. An increased productivity was shown by hybrid AG 8677, and the smallest of the range was the variety Jaboatão. The average productivity of the hybrids was 50% higher than that of the varieties.

Key words: Water balance, grain yield, water deficit.

INTRODUCTION

Maize (*Zea mays* L.) is consider as an important stable crop for agriculture and for the world economy; it is the most productive grain crop throughout the world with production corresponding to 38.1% of all grains, followed

*Corresponding author. E-mail: fernandonobrecunha@hotmail.com

by wheat with 29.1% and rice with 20.8% (Conab, 2016). Maize has been used worldwide for food, feed and production of biofuel among other uses. Although Brazilian agronomic technologies have evolved, productivity is still considered very low in some regions, especially in Alagoas, State, Brazil.

The average maize yield in Brazil is 5.3 with 2.0 t ha^{-1} in Northeastern and 1.0 t ha^{-1} in Alagoas (Conab, 2016). However, the agricultural potential of maize is much above this and among the factors responsible for the low productivity, is the lack of tolerant cultivars moisture variations of soil (water excess and deficit), caused by poor distribution of rainfall in the region of the coastal tablelands of Alagoas State, Brazil.

Globally, 15% of arable land is irrigated and currently accounts for 42% of all crop production; 7100 km^3 of water are consumed annually to produce food (Sumberg, 2012; Rockstrom et al., 2012).

In the forest region of Alagoas State, Brazil, the annual rainfall varies between 1.500 and 2.000 mm, despite being considered superior to the water requirements of maize, there is also water shortage due to poor distribution of rainfall in time, resulting in the occurrence of dry spells (Souza et al., 2004). Depending on the intensity and the phenological stage of the crop, the water deficit reduces the growth rate and yield of agricultural crops, especially in the critical period (pre-flowering to early grain filling). When soils have a small water storage capacity, it may not be possible to overcome impacts of the variability of rainfall as referred for maize (Popova et al., 2014).

This sensitivity can be observed in the physiological processes related to the formation of the zygote and early grain filling due to high perspiration, which occurs due to the higher leaf area index (Bergamaschi et al., 2004). Therefore, there is a need for resistant cultivars for adverse environmental conditions, preferably in rainfed crops, which reduces the risks caused by poor distribution of rainfall.

High soil water deficits resulted in severe decreases in yield and net income, however, in regions where water scarcity exists, irrigation managers and farmers should adopt the deficit irrigation approach to achieve economically sustainable crop production (Karasu et al., 2015).

Potential yield is relevant to benchmark crops where irrigation, the amount and distribution of rainfall, or a combination of irrigation and rainfall ensure that water deficits do not constrain yield (Sadras et al., 2014).

Due to yield instability of main crops associated with drought, the use of technologies such as irrigation and precision agriculture (PA) has been recently adopted in large scale (Vian et al., 2016).

The advantages of the use of irrigation in agriculture are associated directly to the correct management in order to increase productivity and reduce production costs (Pereira et al., 2015).

The effect of the deficit and/or excess of water on the growth and yield of maize depend on the development stage, the cultivar, duration and intensity of these factors (Machado, 2016). Therefore, the water balance has been used as a tool to determine the periods in which the plant is subjected to adverse water conditions. Another tool used to know the plant development standards is the analysis of crop growth, which differs at its various stages of plant development. Therefore, determining the growth rate for maize cultivation is of fundamental importance to adjust the critical stage of crop to periods of better water distribution.

Whereas the rainfed crop is highly dependent on environmental conditions, this study aims to evaluate the agronomic performance, yield components and agricultural productivity of six maize cultivars in the region of the coastal tablelands of Alagoas State, Brazil.

MATERIALS AND METHODS

The present study was conducted at the Agricultural Sciences Center of the Federal University of Alagoas, Rio Largo - AL, (09°28.02 "S, 35° 49 43" W; 127m), region of the coastal tablelands of Alagoas State, Brazil. The soil of the area is classified as cohesive argisolic Yellow Latosol texture medium/clayey, flat topography with slopes less than 2% (Carvalho, 2003). The authors evaluated six maize cultivars, three varieties (Jaboatão, Batité and AL Bandeirante) and three hybrids (AG 7098, AG 7088 and AG 8677), from June 8 to October 14, 2015.

The experimental design was randomized complete block, with five replications and six treatments. The experimental area was 2000 m^2 (0.2 ha) divided into six cultivation rows, seven meters linear portions; spaced 0.8 m between rows and 0.25 m between plants, totaling 50.000 plants ha^{-1}.

Soil preparation was done by a disc plows and two graders, both 25 cm depth. The depth of fertilization was determined based on chemical analysis of soil (Table 1), and to obtain a productivity of 10 t ha^{-1} grain. To this was applied 217 kg N ha^{-1}, 96 kg P_2O_5 ha^{-1} and 188 kg K_2O ha^{-1} (Coelho, 2008), which was used as source of urea, superphosphate and chloride potassium, respectively.

During the experiment, the following were evaluated: plant height, leaf area index (LAI), culm diameter and at the end of the experiment, the production components, agricultural productivity, water balance and the grains water content were determined.

The canopy height was measured from the root collar to the highest point of the plant (vegetative canopy) in 10 plants in each plot marked. The LAI was calculated by Equation 1 (Watson, 1947; Marafon, 2012):

$$LAI = \frac{LA \times NP}{10.000} \quad (1)$$

Where: LA- is the leaf area (m^2); NP- is the number of plants per hectare which is 10000 to one hectare area in m^2.

Leaf area was calculated every two weeks by ten plants in each plot sampling, using Equation 2 (Hermann and Câmara, 1999):

$$LA = L \times W \times 0.75 \times (N+2) \quad (2)$$

Where: L- is the length of the leaf "+3" (m); W- is the width of the leaf "+3" (m); 0.75 is a shape coefficient. This coefficient was determined by areas by Martins (2002) integration method, correction of shape maize leaves; N is the number of

Table 1. Chemical characteristics of the soil of the experimental area.

Layer[1]	pH	P	K	Ca	Mg	Al	V	M	OM
m	in H_20	mg dm^{-3}	---------- $cmol_c$ dm^{-3} ----------				---------- % ----------		
0.0-0.2	5.80	47.00	0.11	4.00	1.40	0.05	47.70	1.20	2.24

[1]OM = Organic matter; V = base saturation; m = aluminum saturation.

photosynthetically active leaves.

For the determination of diameter of culm, measurements were made at a time corresponding to 1/3 from the base with graduated calipers. For the determination of the production and agricultural productivity, four core lines of five meters long (16 m^2) in each plot were harvested. All the ears of maize with at least a grain formed were then counted. Of total maize harvested in each plot, were randomly selected fifteen ears for the determination of the following components: length, diameter, number of grains per line, lines per spike, grains per spike, mass of grains per spike and cobs.

The length of spike was measured with a tape and the diameter with calibre. The number of grains per line and lines per spike were obtained by counting the grains in each line and the number of lines, and the number of grains of cob from the multiplication of both. The mass of grains per spike, the 1.000 grains and spike were determined by weighing the grain and mass of cob. Then the rest of the material was threshed and weighed to determine the agricultural productivity. All variables related to mass of grain were adjusted to 13% water content (Brazil, 2009).

The water balance was carried out daily until the stage of physiological maturity of maize, considering that soil has a 40 mm water storage capacity. Thus, it was possible to monitor the water availability in the soil in ten days scale by Thornthwaite and Mather (1955) method, according to Pereira et al. (2002). Meteorological data were provided by the agrometeorology and solar radiation laboratory (ASRAL), which maintains a weather station next to the experimental area. The data were submitted to analysis of variance by F test and means were compared by Tukey test at 5% probability.

RESULTS AND DISCUSSION

Pluvial precipitation during the conduct of research was 831.3 mm, considered superior to the water requirements of maize. However, there was similarity in the distribution of rainfall during the crop cycle. In general, there was excess water until the ninetieth day after planting (DAP), and a small deficit in some growth stages (Figure 1). For Bergamaschi et al. (2006), the water need by the maize varies between 200-400 mm, and distributed during cultivation.

In the water balance of the maize cultivation, it is observed that there was excess respectively and water deficit of 503 and 111 mm, for cultivars AL Bandeirante and AG 8677 (Figure 1A and B), both reached physiological maturity with 112 DAP. A water deficit of 5 mm occurred between the 1st and 2nd ten-day period, another 13 mm between the 4th and 8th ten-day period and a third of 93 mm between the 9th ten-day periods to physiological maturity. During cultivation, in the variety Jaboatão, there was an excess of 506 and 145 mm water deficit (Figure 1B). The largest deficit during the cultivation of the variety Jaboatão was 128 mm, and took place between the 9th and 13th ten-day period, while the lowest occurred during the 1st and 2nd ten-day period (4 mm) and between 4th and 8th ten-day period (13 mm). Among the three varieties studied, it was found that during cultivation, Batité was the lowest excess (500 mm) and higher hydric deficit (151 mm). This deficit was distributed at three different times: 5 mm between the 1st and 2nd ten-day period, 13 mm between the 4th and 8th ten-day period and the largest deficit (133 mm) between the 9th and 13th ten-day period (Figure 1C).

The knowledge of the process that constitute the soil water balance equation or simply the soil water balance components in field cropping is important, for instance, to correctly detect water deficits periods during the crops cycle, to indicate the need for irrigation and to present nutrient losses by leaching (Moreira, 2012).

During the cultivation, the hybrid AG 7098 was the largest excess (504 mm) and the lowest water deficit (101 mm). There was a deficit of 4 mm between the 1st and 2nd ten-day period, 13 mm between the 4th and 8th ten-day period and 84 mm between the 9th and 12th ten-day period (Figure 1E). Regarding the hybrid AG 7088, there was an excess of water deficit and 501 and 145 mm, respectively. The distribution of the deficit was also very similar to the other cultivars. Between the 1st and 2nd ten-day period, the deficit was 5 mm, between the 4th and 8th ten-day period was 13 mm and between 9th and 12th ten-day period water deficit was 127 mm.

Although, planting have occurred on the same date, the physiological maturity occurred at different times, which was influenced by heat accumulation and efficiency in water use among cultivars. So, there were differences regarding water distribution throughout the crop. As shown in Figure 1, the largest deficit water was 13 mm, not interfering with the development of crop because it mostly occurred outside the critical period of the crop (Figure 1).

Souza et al. (2015) reported that in the stages of flowering and seed filling and most radiation interception, the ETc reached daily average values equal to 5.3 mm day^{-1}, characterizing the period of highest water demand of corn. On the other hand, the excess water occurring between the stages of emergency and grain doughy (Figure 1) may have leached the available nutrients in the soil solution; which reduces crop growth and physiological processes associated with formation of the zygote in maize plants.

The varietal characteristics and environmental factors determine the vegetative growth of crops. During the plant

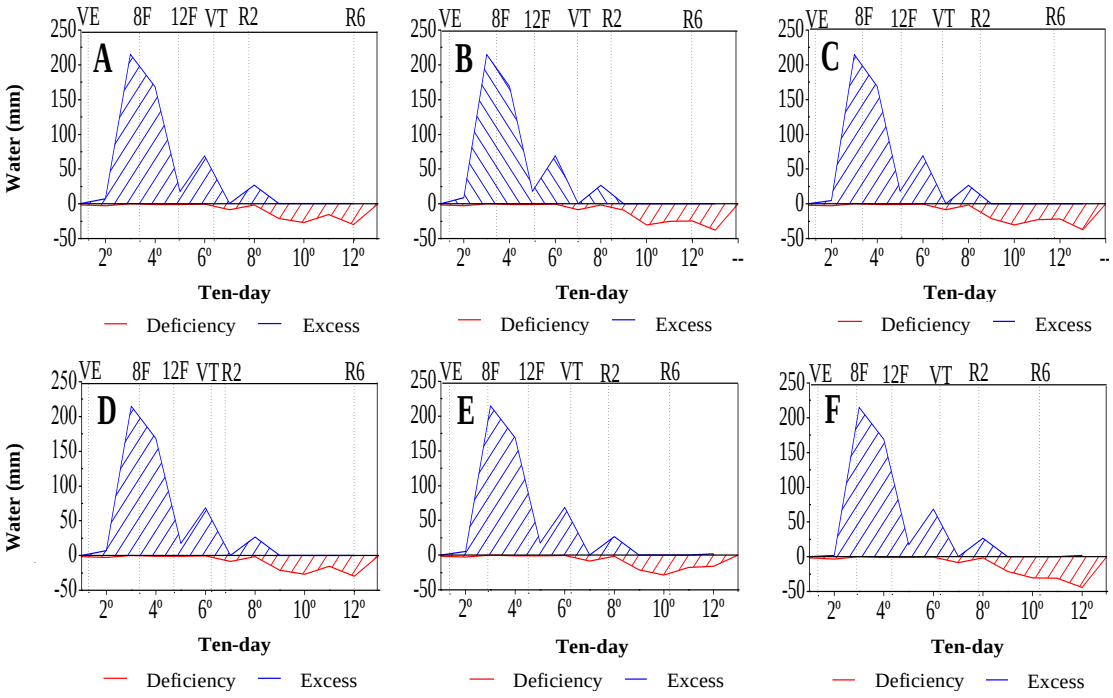

Figure 1. Deficit and excess water six maize cultivars: AL Bandeirante (A), Jaboatão (B), Batité (C), AG8677 (D), AG7098 (E) and AG7088 (F), in the region Rio Largo – Alagoas State, Brazil, in rainfed crop, from June 8 to October 14, 2015.

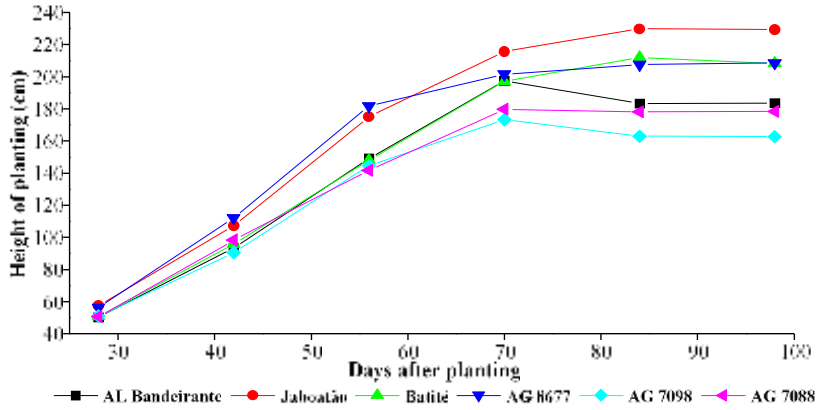

Figure 2. Height of the canopy of six maize cultivars, depending on the DAP, in the region Rio Largo, Alagoas State, Brazil, in rainfed crop, from June 8 to October 14, 2015.

height maize cycle characterized by presenting two growth stages (Figure 2). In the first phase growth, it was approximately linear and occurred between emergency and the start of flowering (at tasseling stage); and in the second phase, there was occurrence of stability in plant height in the period between the stages of flowering and grain doughy/hard (Figure 2). 70 and 98 DAP to Jaboatão variety had the highest and the hybrid AG 7098 the lowest. After the first growth phase (70 DAP), this height was 215.5 cm and the second stage (98 DAP) to 229.2 cm Jaboatão, while the hybrid AG 7098 obtained height of 173.2 cm to 70 DAP and 162.5 cm to 98 DAP. The average growth rate of the variety Jaboatão was 3.0 cm day^{-1} in the first growth phase and 0.5 cm day^{-1} in the second phase; while, the hybrid AG 7098 grew to 2.5 cm^{-1} in the first stage and 0.2 cm^{-1} in the second stage.

The average daily growth rate of varieties during the first phase was 2.9 and 0.1 cm in the second. The hybrids had

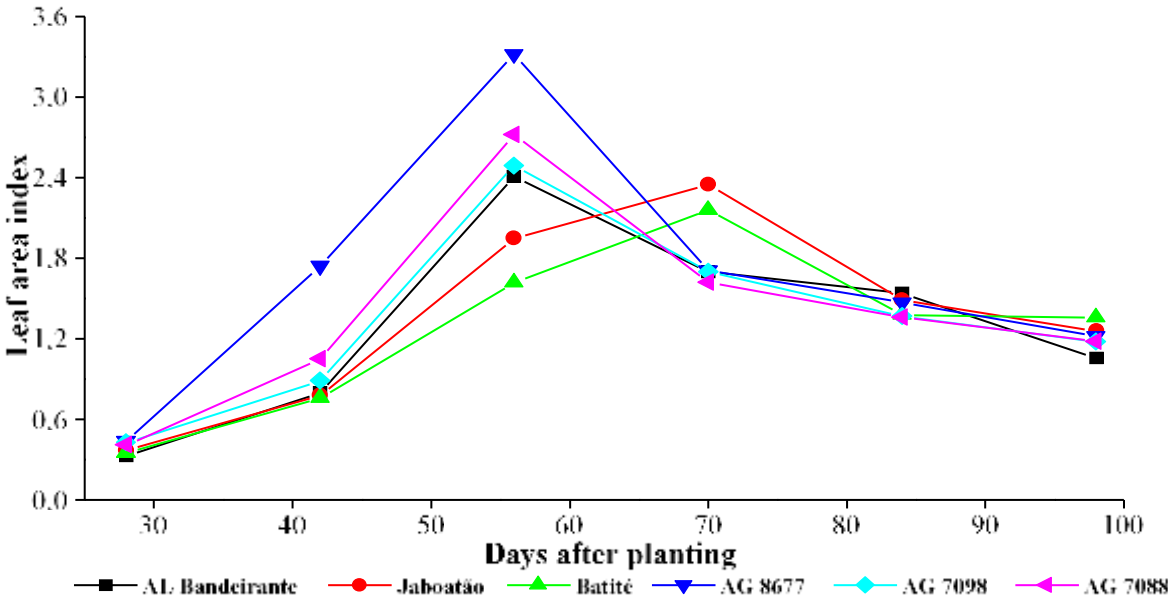

Figure 3. Leaf area index (LAI) of six maize cultivars, depending on the DAP in the region Rio Largo – Alagoas State, Brazil, in rainfed crop, from June 8 to October 14, 2015.

an average growth of 2.6 cm day^{-1} on the first stage and 0.06 cm day^{-1} on the second growth phase. The results showed that under the same environmental conditions, varieties were more efficient as compared to the growth rate. The reduction in the growth rate of the culture from 70 DAP, where the second growth stage took place because the photoassimilates are directed to spikes production and accumulation of starch in the grains.

The results of this study corroborate those obtained by Alvarez et al. (2006), who found that the height of the maize at 100 DAP was 197.3 cm; 193.7 and 203.5 cm for Pioneer 30F35 cultivars, and 2B7070 2B688, respectively. Gilo et al. (2011) observed similar results, working with six maize cultivars in the region of Aquidauana, MS State, Brazil, where daily growth rate of 2.6 cm and an average height of 183.8 cm was observed at 70 DAP.

The leaf area index (LAI) is responsible for interception of solar radiation in agriculture, and represents the leaf area of the plants on the ground coverage area. The results demonstrate that the cultivars were divided into two groups with the same behavior, and three stages of development throughout the growing cycle (Figure 3). Silva et al. (2015) verified that the AG30A91 genotype had a higher leaf area index, reflecting higher estimates of potential productivity.

The first group formed by the cultivars AG 8677, AG 7088, AG 7098 and AL Bandeirante showed a maximum LAI 2.7 at 56 DAP. At this point, the AG 8677 cultivar presented IAF 3.3 and variety AL Bandeirante 2.4; followed by a reduction of 48.5% for AG 8677 cultivar and 71.0% for the variety AL Bandeirante between 56 and 70 DAP (IAF second stage). In the third phase of development, which took place between 70 and 98 DAP, the IAF belonging to the first group of cultivars reduced by an average of 38.2% for the second phase, where aLAI average of 1.2 was found.

The second group also showed similar behavior with the first, but at different times. The maximum LAI was 70 DAP with values ranging from 2.3 for the variety Jaboatão to 2.16 for Batité, checking the end of the first phase of development, which ended after 70 DAP. The second stage of development occurred between 70 and 84 DAP, with an average reduction on the order of 36.2 and 8.4% in the third stage of development occurred between 84 and 98 DAP (Figure 3). At 98 DAP variety, Batité showed higher LAI (1.2), while the lowest AL Bandeirante (1.1).

The maximum LAI occurred at 56 DAP averaged 2.8 for hybrid and 2.0 for the varieties, while the minimum obtained at 98 DAP was 1.2 for both cultivars. Regarding the LAI hybrids, they were higher by 0.8 varieties, encouraging greater uptake of solar radiation by the canopy of plants.

After the maximum LAI, with the assessments carried out at 56 and 70 DAP, this index began to decrease (Figure 3) having commenced stability at different times; 70 DAP for cultivars AG 8677, AG 7098, AG 7088 and AL Bandeirante and 84 DAP for Jaboatão and Batité varieties. The reduction in LAI is a function of leaf senescence, physiological process that occurs in the maize crop at the end of the vegetative phase, at tasseling stage. At this time, there is a reduction of crop growth (Figure 2) and the leaf emission, since the produced assimilates is directed to the formation of spikes. This same behavior regarding the LAI was also verified by

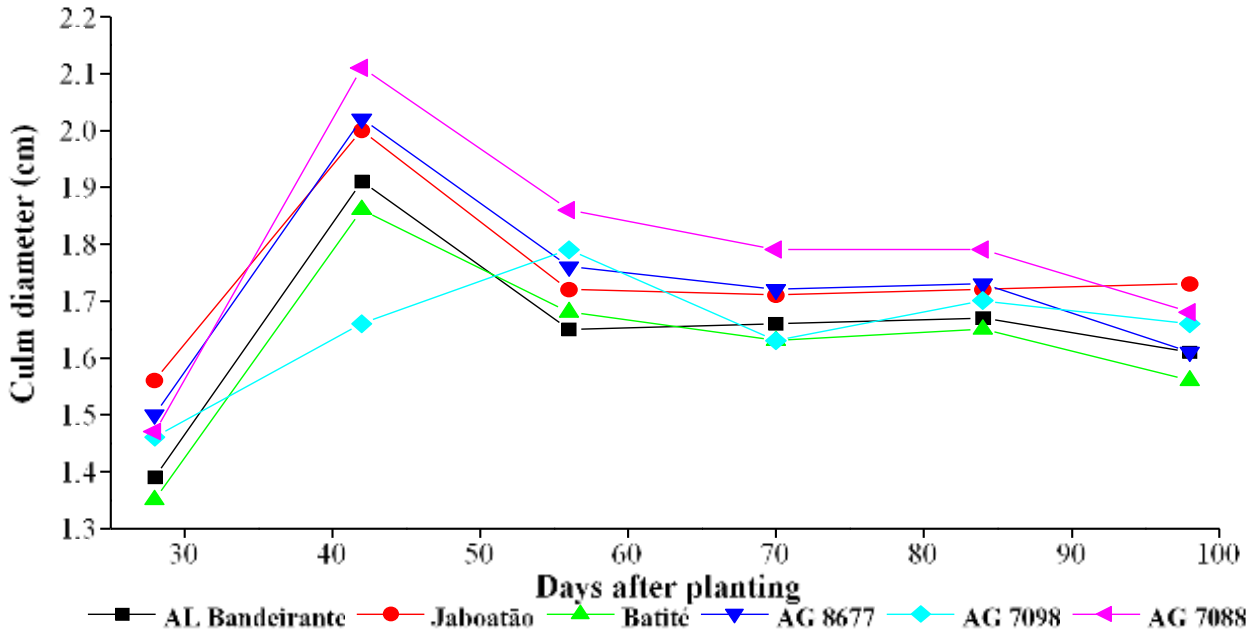

Figure 4. Behavior of diameter of culm of six maize cultivars, depending on the DAP in the region Rio Largo – Alagoas State, Brazil, in rainfed crop, from June 8 to October 14, 2015.

Mergener (2007) in the region of Campos Novos – SC State, Brazil, and Veloso et al. (2009) in the region of Piracicaba – SP State, Brazil.

In this study, there was variation in LAI of cultivars in all evaluation times, which shows that this index is also strongly influenced by genetic characteristics of cultivars. Although, below ideal, the greater IAF until the beginning of flowering (56 DAP) was verified by hybrid AG 8677.

The maximum diameter of culm occurred at 42 DAP for most cultivars, averaging 1.9 cm, except the hybrid AG 7098, which had a different behavior than the other. For this hybrid, the maximum diameter (1.8 cm) occurred at 56 DAP, the second largest diameter at this time of evaluation. However, after 42 DAP, the larger diameter (2.1 cm) was observed in the hybrid AG 7088 and the lowest (1.7 cm), the AG 7098, then there was a reduction in the diameter of the culm in the last evaluation (Figure 4).

At 98 DAP, mean diameter was 1.6 cm, and the maximum and minimum values observed were 1.7 cm for variety Jaboatão and 1.5 cm for Batité. Both hybrid varieties presented as a maximum diameter of 1.9 cm and 1.6 min at 42 and 98 DAP, respectively. The hybrid AG 7088 showed the largest diameter of culm in four evaluation times, lower than the hybrid AG 8677 to 28 DAP and variety Jaboatão to 98 DAP (Figure 4). This same behavior was also observed by Gilo et al. (2011), where the authors observed a maximum diameter of 1.9 cm at 45 min and 100 DAP to 1.5 cm.

Among the variables studied in growth analysis, the diameter of culm is less varied, since this variable depends on the genetic characteristics of the plant, population density, used spacing, the leaf area and environmental conditions throughout the crop cultivation.

The maize cultivars had no significant effect by the F test at 1% probability for the length of ear (LE), mass of cob and the beans water content (Table 2). On the other hand, it is observed that there were significant differences among cultivars for other production components. Cultivar AG 7088 showed a higher number of rows per ear of maize (17), followed by cultivar AG 7098 (15.6). The AG 8677 cultivars and AL Bandeirante were similar and lower than the AG 7088 and AG 7098. The cultivars Jaboatão and Batité varieties were similar and lower than others (Table 2).

The average results obtained in this study approached those obtained by Meira et al. (2009) and Nascimento et al. (2011), who found 15 and 13.6 rows of grains per ear of maize, respectively. They also reported that the number of rows of grains per ear of maize is highly dependent on the genetic characteristics of cultivars.

The larger numbers of grains per row were obtained by AG 7088 and AG 8677 cultivars, both were similar (Table 2). Cultivars AL Bandeirante, Batité and AG 7098 also had no significant effect on the row for grains number, and thus both were similar to cultivars with higher values (AG 7088 and AG 8677) and to cultivar Jaboatão that presented fewer numbers of grains per row (19). The number of grains per row is directly related to the thickness of grain and the length maize ear. The results obtained in this study were lower than the average observed by Meira et al. (2009), who reported that the

Table 2. Analysis of variance of yield components of six rainfed maize cultivars in the region Rio Largo – Alagoas State, Brazil, in rainfed crop, from June 8 to October 14, 2015.

Cultivars[1]	LE	NRE	NGR	Mass of cob	Water content
				------- g ------	------ % -----
Jaboatão	11.68^{a}	10.28^{a}	19.02^{a}	18.78^{a}	18.59^{a}
AL Bandeirante	11.58^{a}	14.18^{b}	23.32^{ab}	19.00^{a}	13.33^{a}
Batité	11.98^{a}	10.96^{a}	21.14^{ab}	16.09^{a}	14.73^{a}
AG 7088	10.68^{a}	17.00^{d}	23.89^{b}	17.71^{a}	17.07^{a}
AG 7098	11.99^{a}	15.58^{c}	23.59^{ab}	19.40^{a}	14.57^{a}
AG 8677	12.20^{a}	14.16^{b}	25.60^{b}	23.09^{a}	22.11^{a}
Means	11.83	13.69	22.76	19.01	16.73
CV%	8.74	2.83	6.90	12.89	7.07

[1]Means followed by the same letter in the column do not differ by Tukey test at 5% probability. LE: length of ear, NRE: number of rows per ear and NGR: number of grains per row.

Table 3. Analysis of variance of yield components and agricultural productivity of six rainfed maize cultivars in the region Rio Largo – Alagoas State, Brazil, in rainfed crop, from June 8 to October 14, 2015.

Cultivars[1]	DS	NGE	MGE	M1.000G	Productivity
	--- cm --		---------- g ----------		---- t ha^{-1} ----
Jaboatão	4.10^{b}	198.65^{a}	68.10^{ab}	355.26^{c}	2.08^{a}
AL Bandeirante	4.15^{b}	330.29^{b}	79.34^{ab}	260.47^{ab}	2.97^{ab}
Batité	3.66^{a}	232.00^{a}	60.60^{a}	279.37^{ab}	2.21^{a}
AG 7088	4.26^{bc}	406.91^{c}	91.04^{bc}	230.28^{a}	3.63^{bc}
AG 7098	4.32^{bc}	367.74^{bc}	89.77^{bc}	258.71^{ab}	3.11^{b}
AG 8677	4.51^{c}	360.94^{bc}	113.17^{c}	297.73b	4.20^{c}
Means	4.17	316.09	83.67	280.31	3.04
CV%	3.10	10.59	4.29	9.54	4.97

[1]Means followed by the same letter in the column do not differ by Tukey test at 5% probability. DE: Diameter of ear, NGE: number of grains per ear, MGE: mass of grains per ear; M1.000G: mass of 1.000 grains.

average length of ear was 18.4 cm.

Table 3 shows that there was a significant effect on the components of production and agricultural productivity. The largest diameter was obtained by the hybrid AG 8677, which did not differ statistically from the AG 7088 and AG 7098 hybrids, which were similar. The Jaboatão and AL Bandeirante varieties were similar and higher than the Batité variety that showed the smallest diameter of spike (3.7 cm). These results are close to those obtained by Born et al. (2011) in Botucatu – SP State, Brazil.

The hybrid AG 7088 had the highest number of grains per ear (406.91 grains), but did not differ from hybrid AG 7098 and AG 8677, which were similar and did not differ from variety of AL Bandeirante. The Jaboatão and Batité varieties did not differ statistically, but were lower than the range of AL Bandeirante (Table 3). On average, cultivars had 316.0 grains per ear of maize. These results were 40% lower than the results obtained by Gonçalves Junior et al. (2008).

The largest mass of grains per ear was observed in the hybrid AG 8677 (113.17 g), however, this did not differ statistically from the hybrid AG 7088 and AG 7098; both were similar (Table 3). The Jaboatão and AL Bandeirante varieties also did not differ among themselves, and were similar to the AG 7088 and AG hybrid 7098. The lower mass of grains per ear was observed in the range Batité (60g), similar to the mass of varieties Jaboatão and AL Bandeirante. Even under irrigation, corn yield showed high spatial variability (Vian et al., 2016).

The largest mass of 1000 grains was obtained by the variety Jaboatão (355.2 g). Varieties AL Bandeirante, Batité and hybrid AG 7098 were not statistically different from each other, but were similar to the AG 8677 and AG

7088 hybrids with a mass of 1000 grains 297.7 and 230.3 g, respectively (Table 3). The average mass of 1,000 grains obtained in this study was higher than the results found by Nascimento et al. (2011) and lower than that obtained by Meira et al. (2009).

The largest grain yield was obtained with the hybrid AG 8677 (4.2 t ha^{-1}) followed by the AG 7098 (3.6 t ha^{-1}), with an average of 3.0 t ha^{-1} for all cultivars. Variety AL Bandeirante achieved productivity similar to hybrid AG 7088. The lower productivities were verified by Jaboatão varieties (2.0 t ha^{-1}) and Batité (2.2 t ha^{-1}); however, these yields were statistically similar to variety AL Bandeirante. France et al. (2011) reported agricultural income in the order of 3, 6 and 9 t ha^{-1} using different doses of nitrogen (N) when the leaves ranged from 1.3, 2.2 to 2.6% (N), respectively. However, the agricultural productivity of the maize crop is directly related to several factors, among them are: the genetic potential of the cultivar, the water and nutrient availability, weather conditions, etc.

In this study, the water surplus of approximately 504 mm occurred between planting and on ninety days (90 DAP), may have resulted in a reduction in agricultural productivity due to the reduction of oxygen near the root system of the plants due to flooding of the experimental area, and consequently the leaching of nutrients from the soil solution. Taking into account the agricultural productivity, hybrid maize is more tolerant to water as compared to other varieties studied.

Conclusion

The maximum water excess during cultivation of maize was 504 mm. The mass of ear is influenced by the number of grains per row and the number of rows per ear of maize. The largest mass of 1,000 grains was obtained by variety Jaboatão and maximum grains per ear by hybrid AG 7088. There was increased productivity in the hybrid AG 8677, and the smallest in Jaboatão.

The average productivity of hybrid was 50% higher than the varieties. The agricultural yield of maize crop is directly related to LAI.

Conflict of interest

The authors did not declare any conflict of interest.

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Development and production of chia (*Salvia hispanica* L.) in different space arrangements

Thiago Furlan de Freitas[1], Bruna Broti Rissato[2*], Alexandre Luis Müller[1], Dyogo Bortot Brustolin[1], Sidiane Coltro-Roncato[2], Omari Dangelo Forlin Dildey[2], Guilherme Peixoto de Freitas[3] and Heloisa Ferro Constâncio Mendonça-Müller[2]

[1]Pontifical Catholic University of Paraná, PUCPR, Toledo, Paraná, Brazil.
[2]State University of West Paraná, UNIOESTE, Marechal Cândido Rondon, Paraná, Brazil.
[3]Federal University of Paraná, UFPR, Palotina, Paraná, Brazil.

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The development of management methods appropriate for the culture of chia (*Salvia hispanica* L.) is essential to improve productivity. This research aimed to evaluate and quantify the development and productivity of chia plants in different row spacing and planting density in Western region of Paraná. As the treatments used were four row spacing (0.15, 0.30, 0.45 and 0.60 m) in three plant densities (200,000, 400,000 and 600,000 plants ha^{-1}), totaling 12 treatments. The experiment was conducted in a randomized block design with four blocks, in which the vegetative growth and the productivity were evaluated. The results were submitted to the Tukey test (p> 0.05). The number of ears per plant was higher in the population of 40 plants m^2 with row spacing of 30 and 45 cm, and in the population of 60 plants m^2, with the row spacing of 15 and 45 cm. The number of spikelets per spike was superior for the population of 40 and 60 plants m^{-2} when grown in the row spacing of 15 and 45 cm. The population of 60 plants m^2 with the row spacing of 15 cm, stood out for thousand grain weight and productivity. Development of variables of chia showed no significant difference.

Key words: Chia, plant population, productivity, row spacing, *Salvia hispanica* L., vegetative growth.

INTRODUCTION

Chia (*Salvia hispanica* L.) is an oleaginous, annual and summery plant, belonging to the Lamiaceae family, native to southern Mexico and northern Guatemala (Ayerza and Coates, 2009). It is a staple food of the Central America civilizations in pre-Columbian period, along with corn, beans and amaranth (Fernandez et al., 2006). Chia is a plant characterized by low water consumption and well adapted to arid and semiarid regions (Ayerza, 1995).

In the last few years, the plant seeds have attracted importance for human nutrition and health due to its high fatty acid content of α-linolenic and also due to the beneficial health effects by the consumption of ω-3 fatty

*Corresponding author. E-mail. brunarissato@hotmail.com.

acids they contain, in addition to protein, glutamic acid, arginine, lipids, fibers, and, thereby, gaining space in the Brazilian agriculture (Ayerza, 2011). In this context, therefore, chia is a profitability alternative to farmers and industry.

The flour, a byproduct of oil extraction, can be used in human and animal nutrition and is high in fiber and constituents with antioxidant activity (Ayerza and Coates, 1996; Olivos-Lugo et al., 2010), which gives it an emulsifier character, intensifying the feeling of satiety when eating the grain (Antruejo et al., 2011).

The chia oil has superior quality than other oils such as soybean oil (*Glycine max*), sunflower oil (*Helianthus annuus* L.), rapeseed oil (*Brassica napus* L.) and olive oil (*Olea europaea* L.) (Ayerza, 2013), it concentrates higher percentage of fatty α-linolenic acid, reaching 68% (Ayerza, 2011). Given the above, even if it is not cultivated on a large scale today, chia deserves to a great attention due to the universal applicability of its products and derivatives.

With regards to the cultivation, the species adapts to different locations in Brazil, provided the conditions of temperature, altitude and precipitation fall within the requirements of culture (Migliavacca et al., 2014). Pozo (2010) mentioned that the ideal conditions for the culture development are: temperature between 14 and 20°C, no frosts, good insolation and pluviometric incidence of 250 to 300mm. In general, chia find better development conditions when seeded in October and November (Migliavacca et al., 2014), coinciding with the soybean crop, which reduces the interest of its production by farmers.

According to Migliavacca et al. (2014), that sowed chia in March to April, after the first harvest of grains, and leading the same until the end of August, they noticed that the culture appears as a highly profitable option in the crop rotation system, with great accumulation of vegetable raw materials on the ground after the removal of crop seeds, promoting the formation of straw which acts as vegetation cover for the soil.

In order to better manage soil and climatic conditions with the crop needs, studies have been performed in order to adjust row spacing and density of plants and to obtain, therefore, an increase in productivity. Some studies have shown that the best row spacing range for the culture would be between 30 and 50 cm, with an amount of five kilograms of seed per hectare (Ayerza and Coates, 2005; Kentucky, 2012).

In a study by Ayerza (1995), it was confirmed that the productivity of chia, as well as many cultures, is dependent on the local climate, and especially, the planting date. However, in the literature, information on cultivation and management pre and post-harvest of chia are still insufficient, so that research involving culture aimed mostly at the nutritional composition of the seeds, as well as the benefits it provides to the animal and human health.

Since the culture is highly dependent on the environment to express its maximum agronomic potential, additional studies are needed to determine the factors that really affect the chia yield. Thus, the most appropriate geographic regions for the production of chia may be identified. In this context, there is a need for further study on the mentioned subject, since each location can have different characteristics for the development of plants, and consequently for final production.

Therefore, the present study aims to quantify the growth and productivity of chia under different row spacings and populations of plants, the development of management methods appropriate for the culture of *Salvia hispanica* L., as well as show appropriate management techniques for the culture established in Brazil.

MATERIALS AND METHODS

The experiment was conducted in the municipality of Toledo - PR, with the following coordinates: altitude of 570 m, with latitude 24° 43'13 "S and longitude 53° 46'45" W. The climate is characterized by the Koppen method as subtropical CFA (subtropical, humid climate with hot summers and with four distinct seasons). The soil of the experimental local is classified as a Distroferric Red Latosol, characterized by a good natural fertility and clay texture.

After collection, chemical analysis and soil correction, they marked 36 experimental parcels in experimental design of randomized blocks, each one with 5 m in length and 2.55 m wide, totaling 25.5 m^2. The effective surface of the plot was of 7.2 m^2 (4 x 1.8 m), being the center lines used for evaluation of the productivity and the laterals used for culture growth assessments. Fifteen days after the soil correction, the culture was seeded by tillage system, being at that time, the experiment was implanted in a factorial 3 x 4, being the first factor, the plant population (200,000, 400,000 and 600,000 plants ha^{-1}) and the second factor, the row spacing (0.15, 0.30, 0.45 and 0.60 m).

The monitoring of pests, diseases and weed plants was performed every two days from the date of emergence of the crop, with the need for manual weeding once a week to keep the area free of weed plants. From the 28th day after emergence (DAE) and every 28 days, three plants were collected for each experimental unit for analysis of the variables: leaf area and dry weight of plants.

Leaf area (cm^2)

The leaves were collected and scanned on a graph paper and, then, digitized and casted into the QUANT program (Vale et al., 2001), such that the area sum of all the leaves of each plant represented the leaf area per plant.

Dry mass (g)

The plants were collected without roots and separated into stems, stalk, leaves and reproductive structures. After that, they were kept in a air forced circulation stove at 65°C until they reach a constant weight in order that, then, they were measured dry mass of leaves; dry mass of stem and stalk; dry mass reproductive structures; and total dry weight of shoot.

To determine the productivity of the culture, 10 plants were

collected per plot and the number of floral branches, number of grains per floral branch and weight of 1000 grains (g) were calculated. The productivity (kg ha^{-1}) was estimated manually, harvesting the useful area of the plot. The value was obtained after the humidity correction to 13%, on a wet basis. From data of dry mass and of leaf area, leaf area index (LAI), specific leaf area (SLA), leaf area ratio (LAR), net assimilation rate (NAR) and relative growth rate (TCR) were calculated according to the equations proposed by Benincasa (2003).

After tabulating, the data were submitted to analysis of variance ($p= 0.05$) and were estimated by regression equations and compared by Tukey test ($p<0.05$) using the statistical program SISVAR.

RESULTS AND DISCUSSION

There was no interaction effect between population and row spacing of chia plants for the variables dry mass of stems, leaves and reproductive parts, leaf area, leaf area index, leaf area ratio, relative growth rate of plants and assimilation rate liquid of chia plants.

From the 28 days after emergence, there was a great dry matter accumulation for reproductive parts + leaf + stem, leaf + stem and stem. This accumulation was constant from 28 to 56 days after emergence for the variables leaf + stem and stem during which time the plants were in the vegetative phase and was stabilized from 56 days after emergence, when the plants went into the reproductive phase, which occurred only to increase reproductive parts + leaf + stem.

The leaf area of chia plants was increased to approximately 56 days after emergence, phase in which the plants are in full vegetative development. After the vegetative phase, the plant set its size and then passes to the reproductive stage, in which the leaf area growth was not present, and the the plant started the development of reproductive parts. The same was observed for the variable leaf area index (m m^2) of chia plants, which was increased until the 56 days after emergence and stabilized after that period.

The leaf area ratio was maximum at the 28 days after emergence, decreasing in the reproductive stage. Such fact shows the source-drain relation between leaves and fruits (Taiz and Zeiger, 2004). The relative growth rates and net assimilation of chia plants were growing from 28 to 56 days after emergence, a considerably high reduction after that time until the end of the cycle, indicating that the initial period of development is the limiting factor for the species production, whereas in this phase, the plants make their full photosynthetic role, in order to accumulate photosynthates to the next stage.

For number of ears per plant (Table 1), there was no row spacing effect for the population of 20 plants m^2. For the population of 40 plants m^2, the 30 and 45 cm row spacings stood out, while for the population of 60 plants m^2, the row spacings of 15 and 45 cm showed the best results.

For the row spacing of 15 cm, the population of 60 plants m^2 had the highest number of ears per plant. For the row spacing of 30 cm, the higher average was presented by the population of 40 plants m^2. For the row spacing of 45 cm, populations of 40 to 60 plants m^2 showed no significant difference, being greater than population of 20 plants m^2. There was no effect for plant population spacing of 60 cm.

There was an increase of number of spikelets per spike (Table 2) for the population of 20 plants m^2 grown on rows spaced in 15, 30 and 60 cm among them; for the population of 40 plants m^2 when cultivated in row spacings of 15 and 45 cm; and for the population of 60 plants m^2 when cultivated in the spacings of 15, 45 and 60 cm among them.

For row spacing of 15 cm and population of 40 plants m^2, there was no beneficial effect on the studied variable. The same was concluded for row spacing of 30 cm and populations of 20 and 40 plants m^2; row spacing of 45 cm and population of 40 plants m^2; and spacing of 60 cm and population of 20 and 60 plants m^2 between rows. The number of grains per spikelets of chia plants was not affected by population (Table 3). However, there was influence of the row spacings, so that culture better responded to the spacings of 15 and 45 cm between rows.

For thousand grain weight (Table 4), there was no effect of the row spacings on the population of 40 plants m^2. In the population of 20 plants m^2, the 30 cm row spacing was best adapted, whereas for population of 60 plants m^2, row spacing of 15 cm, showed the best result. There was no interaction between the row spacings of 45 and 60 cm and the three studied populations, and, in these cases, all possible combinations showed beneficial effect on the variable in question. The thousand grain weight average was very similar to other works developed with the culture of chia. Singh and Goswami (1996) and Ista (2003) obtained an average of 1.31 g of thousand grain weight. In a study developed by Ixtaina et al. (2008), the average for the variable in question was of 1.32 g, whereas for Guiotto et al. (2011), the average was of 1.35 g.

Regarding the productivity of chia (Table 5), there was no difference between the row spacings tested for the population of 20 plants m^2. For the population of 40 plants m^2, the productivity was higher in the row spacings of 30 and 45 cm, while in the population of 60 plants m^2, the highest values were obtained in the spacings of 15, 45 and 60 cm between rows. There was no interaction between population and row spacing for variables dry mass of vegetative and reproductive parts. At 56 days of the emergency, the complete plant vegetative growth results in the reproductive phase.

The population of 60 plants m^2 was best adapted to the row spacings tested, except for the row spacing of 30 cm, for which the population of 40 plants m^2 was better. In general, the population of 60 plants m^2 was the one which had the highest productivity, in particular, when

Table 1. Number of ears per plant of chia conducted in different space arrangements.

Row spacing (cm)	Plant population (m²)			Average
	20	40	60	
15	8^{aB}	7b^{C}	11^{aA}	9
30	8^{aB}	15^{aA}	8^{bB}	10
45	10^{aB}	13^{aA}	12^{aA}	12
60	11^{aA}	8^{bA}	8^{bA}	9
Average	9	11	10	10

Averages followed by same letter, lowercase for the column and uppercase for the row, do not differ significantly at 5% in the Tukey test.

Table 2. Number of spikelets per spike of chia conducted in different space arrangements.

Row spacing (cm)	Plant Population (m²)			Average
	20	40	60	
15	51^{aB}	63^{aA}	47^{abB}	54
30	56^{aA}	47^{bAB}	39^{bB}	47
45	36^{bC}	52^{abA}	45^{abB}	44
60	58^{aA}	48^{bB}	57^{aA}	54
Average	50	52	47	50

Averages followed by same letter, lowercase for column and uppercase for row, do not differ significantly at 5% in the Tukey test.

Table 3. Number of grains per spikelet of chia plant conducted in different space arrangements.

Row spacing (cm)	Number of grains per spikelet
15	3.3^{a}
30	2.9^{c}
45	3.2^{ab}
60	3.1^{bc}
Average	3.1

Averages followed by same letter in column do not differ significantly at 5% in the Tukey test.

Table 4. Mass of a thousand seeds of chia (g) conducted in different space arrangements.

Row spacing (cm)	Plant population (m²)			Average
	20	40	60	
15	1.3^{bB}	1.4^{aB}	1.6^{aA}	1.4
30	1.8^{aA}	1.4^{aB}	1.3^{dC}	1.5
45	1.4^{bA}	1.4^{aA}	1.5^{bA}	1.4
60	1.4^{bA}	1.4^{aA}	1.4^{cA}	1.4
Average	1.5	1.4	1.5	1.4

Averages followed by same letter, lowercase for column and uppercase for row, do not differ significantly at 5% in the Tukey test.

Table 5. Productivity of chia (kg ha^{-1}) conducted in different space arrangements.

Row spacing (cm)	Plant population (m²)			Average
	20	40	60	
15	489^{aB}	770^{bB}	1446^{aA}	902
30	577^{aC}	1025^{aA}	732^{bB}	778
45	491^{aC}	1083^{aB}	1321^{aA}	965
60	584^{aB}	690^{bB}	1083a^{bA}	786
Average	536	892	1146	858

Averages followed by same letter, lowercase for column and uppercase for row, do not differ significantly at 5% in the Tukey test.

subjected to a spacing of 15 cm between rows. The lowest productivity was obtained by the population of 20 plants m^{2} at a spacing of 45 cm between rows, so that the difference between these arrangements was of 955 kg ha^{-1}.

This fact emphasizes the importance of the culture studied, particularly for cultivation in different spatial arrangements, and it is able to express its maximum physiological potential and, therefore, its maximum productive capacity.

The average productivity gained was of 858 kg ha^{-1}, higher than the Brazilian productivity, which is usually 500

to 600 kg ha^{-1} of seeds. However, Ayerza and Coates (2005) reported on results exceeding 1266, 2031 and 2120 kg ha^{-1} for experimental parcels seeded in Argentina, Venezuela and Colombia, respectively. These values can only be obtained if the climate and soil of the region are appropriate for culture, favoring it for the fertility of the soil, temperature and luminosity.

Therefore, the chia may be adopted as an alternative to traditional crop, in order to diversify and stabilize the local agricultural economy, as it grows well in the region and has potential for expansion in each harvest, due to its industrial, medicinal and food applications.

Conclusions

There was no significant difference for chia plants development variables when they were cultivated in different spatial arrangements. There was a significant difference for chia plants production variables, and higher grain yield was obtained by the population of 60 plants m^2 in a spacing of 15 cm between rows.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Evaluation of solvent retention capacity of wheat (*Triticum aestivum* L.) flour depending on genotype and different timing of nitrogenous fertilizer application

Alan Junior de Pelegrin[1]*, Ivan Ricardo Carvalho[1], Mauricio Ferrari[1], Maicon Nardino[2], Vinícius Jardel Szareski[3], Daniela Meira[2], Cleiton Antônio Wartha[4], Diego Nicolau Follmann[4], Carla Maria Garlet de Pelegrin[5], Luiz Carlos Gutkoski[6], Antonio Costa de Oliveira[1], Luciano Carlos da Maia[1] and Velci Queiróz de Souza[7]

[1]Plant Genomics and Breeding Center, Federal University of Pelotas, Capão do Leão, Rio Grande do Sul, 96010-165, Brazil.
[2]Departament of Agronomic and Environmental Sciences, Federal University of Santa Maria, campus Frederico Westphalen, Rio Grande do Sul, 98400-000, Brazil.
[3]Departament of Plant Sciences, Federal University of Pelotas, Capão do Leão, Rio Grande do Sul, 96010-165, Brazil.
[4]Departament of Plant Sciences, Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, 97105-900, Brazil.
[5]Departament of Biology, Federal University of Fronteira Sul, Cerro Largo, Rio Grande do Sul, 97900-000, Brazil.
[6]Center for Food Research − Cereal Lab, University of Passo Fundo, Passo Fundo, Rio Grande do Sul, 99052-900, Brazil.
[7]Federal University of Pampa, Dom Pedrito, Rio Grande do Sul, 96450-000, Brazil.

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Wheat is one of the most important cereal crops in the world. The objectives of this study were: (i) to evaluate the solvent retention capacity of wheat flour from three genotypes when nitrogenous fertilizer is applied at different stages of crop growth; and (ii) to evaluate the possible existence of correlations between different solvents when nitrogenous fertilizer is applied at different stages of crop growth. The experiment was conducted from June to November 2013 in Plant Breeding Laboratory and Plant Production, Federal University of Santa Maria, the experimental area is located at Frederico Westphalen, southern Brazil. The experiment consisted of 12 treatment combinations (3 genotypes and application of nitrogenous fertilizer at 4 crop growth stages) of a 3x4 factorial (genotype x nitrogenous fertilizer application) arranged in a randomized complete block design with three replications. The genotypes Fundacep 52, TBIO Mestre and TBIO Itaipu were arranged with the tested nitrogenous fertilizer applications (I = no nitrogen, II = tillering, III = tillering and flowering and IV = tillering, booting and flowering). Greater levels of solvent retention capacity were observed in TBIO Mestre, suggesting that the genotype has good baking quality. The amount of glutenin was not affected by the stage of nitrogenous fertilization application. The water retention capacity of the flour has a strong and positive correlation with the retention capacity of the sodium carbonate and sucrose, along with the associations between the solvents sodium carbonate and sucrose.

Key words: Agriculture science, plant management, plant science, quality grains, *Triticum aestivum* L.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important economy cereal crop in the Brazilian. In Brazil, wheat is widely used as a raw material for the production of bread, biscuits and pasta. The country imports wheat grain from other countries because domestic production does not meet the demand for wheat grain, both in quantity and quality (Carneiro et al., 2005). The quality evaluation of wheat flour is an essential tool for pursuing optimum nitrogen fertilizer application regime and improved wheat genotypes in order to achieve superior quality. Due to the great variability among Brazilian wheat genotypes, the wheat flour quality cannot be assessed without defining its final product use (Gutkoski et al., 2007).

Wheat flour is composed mainly by starch (70 to 75%), water (12 to 14%), proteins (8 to 16%), non-starch polysaccharides (2 to 3%), lipids (2%) and minerals (1%) in average (Scheuer et al., 2011). Moreover, the qualities, quantities and differences in the several flour constituents vary from genotypes (Morita et al., 2002). Final quality of products derived from wheat is a factor directly related to technological characteristics of the wheat grain. The characteristics are influenced by genetic factors and its interaction with biotic factors and abiotic (Mandarino, 1993). The genotypes have differences in grain composition and processing quality, and a cultivar that is suitable for preparing food, may be inadequate for the preparation of other (Miranda et al., 2011).

The application of nitrogenous fertilizer is essential for the expression of technological wheat quality. As reported by Almeida et al. (2013), nitrogen is a constituent of gliadin and glutenin proteins that are, responsible for the functional characteristics of wheat flour. The amount of nitrogen fertilizer applied varies depending on the content of organic matter in the soil, the previous crop and the expected yield of grain. The management of nitrogen throughout the culture is given in two stages: at sowing and the remainder applied to cover. For the use of higher doses of nitrogen in coverage can adopt the installment in two applications, which were, at the start of tillering and the rest at the beginning of stretching. Nitrogen application coverage can still be performed after wheat booting, in order to increase grain protein, improving their technological characteristics (Cunha and Caierão, 2014).

The evaluation method of wheat flour quality by solvent retention capacity (SRC) is commonly used in breeding programs. This is because sampling is carried out using small quantities of flour (Pallarés et al., 2007). This method is successfully used by many researchers in the world, to assess the quality of the flour of different genotypes and managements to increase the quality of wheat by-products (Pasha et al., 2009; Duyvejonck et al., 2012; Kaur et al., 2014; Gusmán et al., 2015), however, in Brazil, this method is not commonly used.

The SRC pattern of the four nitrogen application times presents a profile of the analyzed flour quality, making it possible to predict the end use quality (AACC, 2000). Therefore, this method may be promising as an indirect measure of wheat flour quality in genotypes and segregating populations. The study of techniques and management strategies to improve the quality of wheat grain is a key aspect in the search for greater competitiveness of the wheat produced in Brazil, reducing dependency on raw materials imported from other countries. Thus, the objectives of this study were: (i) to evaluate the solvent retention capacity of wheat flour from three genotypes when nitrogenous fertilizer is applied at different stages of crop growth; and (ii) to evaluate the possible existence of correlations between different solvents when nitrogenous fertilizer is applied at different stages of crop growth.

MATERIALS AND METHODS

The experiment was conducted from June to November 2013 in Plant Breeding Laboratory and Plant Production, Federal University of Santa Maria, in the experimental area located at Frederico Westphalen, RS, southern Brazil, at altitude of 460 m above sea level, latitude 27°23'48.17"S and longitude 53°25'34.82"W, in the state of Rio Grande do Sul, southern Brazil. The area has distrophic red Oxisol soil (Embrapa, 2006), sub-tropical humid climate with balanced rain system and the dry season is not defined. The area receives an average annual rainfall ranging from 1,250.0 to 2,000.0 mm (Sema, 2005).

The experiment consisted of 12 treatment combinations (3 genotypes and application of nitrogenous fertilizer at 4 crop growth stages) of a 3x4 factorial arranged in a randomized complete block design with three replications. The genotypes were: Fundacep 52, TBIO Mestre and TBIO Itaipu, which are widely grown cultivars in the country, and the treatments for the nitrogenous fertilizer application were as follows; I = no nitrogen (without the application of N in coverage), II = tillering (application of 115 kg ha^{-1} N at tillering stage), III = tillering and flowering (application of 57.5 kg kg ha^{-1} N in the phase of tillering and 57.5 kg ha^{-1} N at flowering stage) and IV = tillering, booting, and flowering (application of 38.3 kg ha^{-1} N at tillering, 38.3 kg ha^{-1} N at booting and 38.3 kg ha^{-1} N at flowering).

The genotypes were seed on the 10th of July 2013 using a tractor seeder in 7.14 m^2 plots measuring 12 rows by 3.5 m long, spaced 0.17 m apart. Grain yield was harvested from the eight center, discarding 0.5 m from both ends (length wise).The fertilization was carried out following the soil analysis before the implementation of the experiment, where basal fertilizer was applied at 200 kg ha^{-1} (08N:24P:12K) formulation. On the surface

*Corresponding author. E-mail: pelegrinagro@gmail.com.

Table 1. Summary of the analysis of variance for three wheat genotypes and four nitrogenous fertilizer.

Factor of variation	Means Square to SRC			
	Water	Na_2CO_3	Sucrose	$C_3H_6O_3$
Genotype	27.63*	176.41*	361.65*	2321.78*
Nitrogenous fertilizer	1.36^{ns}	20.34*	82.97*	639.61*
Genotype*nitrogenous fertilizer	2.23^{ns}	0.86^{ns}	64.02^{ns}	89.06^{ns}
Block	0.005	7.35	14.36	91.51
Residue	1.67	1.96	30.44	110.32
C.V. (%)	1.97	1.67	5.68	9.24

* Significant at 5% by F test; ^{ns} Not significant.

was applied 115 kg ha^{-1} urea (45% of nitrogen) for all trials. It is applied to phenological stages related to the tested nitrogen managements. The average plant population was 310 plants m^{-2}. Cultural treatments were performed according to the technical recommendations for the wheat crop (RCBPTT, 2011). Regarding pests and diseases control, pesticide and fungicide applications were done accordingly and where necessary.

The characters were measured at Laboratory of Cereals of Food Research Center of the University of Passo Fundo, RS. The SRC method expresses the solvent weight retained by flour after centrifuging and it is expressed as a percentage based on 14% of moisture determined according to the Equation 1.

$$\text{SRC}\ (\%) = \left(\left(\frac{\text{Gel mass}}{\text{Flour mass}}\right) \times \left(\frac{86}{\text{100-Moisture flour}}\right) \text{-1}\right) \times 100 \quad (1)$$

Four solvents were used independently in SRC method in order to obtain different values for SRC: 50% sucrose, 5% sodium carbonate, 5% lactic acid and water. The solvent retention capacity was determined according to the method No. 56-11 of AACC (2000), using 5 g of flour for each solvent. The procedure consisted of weighing 5 g of flour for each test tube and adding 25 ml of the solvent. The tubes were then vigorously agitated with the support of magnetic stirrer for 40 s at 5 min intervals until completion of the 20 min cycle. Subsequently, the samples were centrifuged at 1000 × g for 15 min. The results were calculated according to Equation 1.

The data was subjected to analysis of variance by F test. Simple effects were dismembered when the genotype x nitrogenous application interactions were observed. Moreover, the variables that showed no significant interactions were dismembered into principal effects. Mean separation was done using Duncan's multiple range test at 5% of probability. Analyses were performed with the Genes software (Cruz, 2013). The correlations were classified as described by Carvalho et al. (2004), where values equal to 0.00 correspond to null correlation; 0.00 to ≤ 0.30 corresponds to weak correlation; 0.30 to ≤ 0.60 corresponds to medium correlation; 0.60 to ≤ 0.90 corresponds to strong correlation; 0.90 < 1.00 corresponds to very strong correlation and 1.00 corresponds to perfect correlation.

RESULTS AND DISCUSSION

Analysis of variance for traits

The analysis of variance by F test revealed no significance (≤ 0.05) for the genotype wheat x nitrogenous fertilizer application interaction for the traits water retention capacity, sodium carbonate retention capacity, sucrose retention capacity, and lactic acid retention capacity, implying that the response pattern obtained by genotypes depending on nitrogenous fertilizer strategies in surface fertilization was similar (Silva, 2007). For traits, water retention capacity was observed to have significant differences for the factor genotypes, with no significant effect factor on nitrogen application, showing more effect of the genetic makeup of each genotype than the nitrogenous fertilizer for this trait. For characters, sodium carbonate retention capacity, sucrose retention capacity and lactic acid retention capacity significant differences were found between the main effects for both factors of variation, genotypes and nitrogen fertilization, inferring that for these characters, evidenced variation was due to the genetic differences of genotypes and adopted nitrogenous fertilizer. The coefficients of variation for the traits varied from 1.67 to 9.24% (Tables 1 and 2). According to the classification by Pimentel-Gomes (2000), coefficients of variation (CV) may be considered low (<10%) with high accuracy, medium (10 to 20%) with good accuracy, high (20 to 30%) with low accuracy and very high (> 30%) with low accuracy. Thus, CV obtained in this study show reliable results and adequate experimental accuracy.

Complementary analyzed

In the comparison of genotype effects, TBIO Mestre genotype presented greater magnitudes of retention capacity of the solvents (water, sodium carbonate and sucrose) as compared to the other studied genotypes (Table 2). This result demonstrates that this genotype revealed better flour quality indices for baking. The TBIO Mestre and TBIO Itaipu genotypes expressed greater lactic acid absorption than the Fundacep 52 genotype. The lactic acid retention capacity is indicative of forming ability of glutenin net and the strength of the gluten flour (Gaines, 2000). The glutenin are protein fraction of gluten, which most influences the quality of bread for flour (Brunori et al., 1989); therefore, as important as the quantity, the quality of proteins is critical for determining the technological wheat quality. Bushuk (1985) shows

Table 2. Results of solvent retention capacity (SRC) water, sodium carbonate (Na_2CO_3), sucrose and lactic acid ($C_3H_6O_3$) for genotype factor and nitrogenous fertilizer application.

Genotype	SRC (%)			
	Water	Na_2CO_3	Sucrose	$C_3H_6O_3$
Fundacep 52	66.02[b]	82.59[b]	94.90[b]	96.05[b]
Tbio Mestre	68.47[a]	88.22[a]	103.86[a]	125.33[a]
Tbio Itaipu	65.15[b]	79.58[c]	91.32[b]	120.94[a]
Nitrogenous fertilizer				
I	67.10[a]	86.08[a]	100.81[a]	101.83[b]
II	66.77[a]	82.11[bc]	93.35[b]	126.24[a]
III	66.59[a]	83.48[b]	95.18[ab]	119.40[a]
IV	65.84[a]	81.73[c]	96.49[ab]	114.83[a]

*Means followed by the same letter (a) in the column do not differ statistically with Duncan (p≤0.05).

that the quality of protein is a genotypic characteristics inherent in each genotype, corroborating the results obtained in this study. The selection of wheat genotypes seeking better baking quality should consider protein quality as one of the genotypes selection criteria (Wrigley, 1994).

According to Gaines (2000), flour with SRC of water ≤ 57%, SRC of sodium carbonate ≤ 72%, SRC of sucrose ≤ 96% and SRC of lactic acid ≥ 100% is suitable for "sponge and dough" systems, that is, in the production of panettone, sweet bread and bread. Based on this classification, the TBIO Mestre was the only genotype that presented flour with the desired SRC profile for a good performance in "sponge and dough" systems. However, according to the criteria used by Pike and Mac Ritchie (2004), TBIO Mestre and TBIO Itaipu genotypes are demonstrated as sources of good quality flour for baking because they presented a SRC of lactic acid above 100%. No significant differences were found for water absorption by wheat flour depending on nitrogenous fertilizer applications (Table 2), implying that the applications studied provided no change in the total quantity of wheat flour constituents (AACC, 2000). Nevertheless, this effect does not mean that the quantities of grain components did not have varied individually. Thus, this parameter should be analyzed together with the SRC in other solvents in order to predict objectively the flour quality.

The sodium carbonate absorption estimates the level of damaged starch in the flour, indirectly indicating its hardness. Moreover, sucrose absorption is associated with the pentosan quantity present in the flour (AACC, 2000). The nitrogenous fertilizer application I exhibited greater absorption capacity of sodium carbonate and sucrose solution, with 86.08 and 100.81%, respectively. This result indicates that when nitrogen was not applied to cover, there was great damage to starch levels and pentosans present in the flour. The pentosans are components of plant cell, being present in the aleurone layer and in the bran layers, especially in wheat grains. During the milling of wheat grains, parts of cells (pentosans) can stay in the flour and change its composition and quality. Along with damaged starch, which also comes from the grinding, the pentosans absorb 100% of water. Therefore, the presence of pentosans and damaged starch increase the water absorption of the flour, being a desirable feature for bread production (Kweon et al., 2011).

Nitrogenous fertilizer applications II, III, and IV demonstrated greater lactic acid absorption, while the same did not differ among themselves. According to AACC (2000), the absorption of lactic acid is associated with glutenin and it can be used as a gluten indicator. The glutenins are reserve proteins, which together with gliadins form the gluten, being responsible for the functional properties of wheat flour. This result is supported by findings of Boehm et al. (2004). These authors emphasized that the increase in grain protein content is due to the use of nitrogenous fertilizer. Similarly, Pelegrin et al. (2016), emphasized that the technological quality is influenced by management and nitrogen sources as well as the genotypic attributes of used cultivars. This fact is justified because nitrogen is the basic component of proteins and amino acids (Malavolta, 1981). So, despite the fact that Bushuk (1985) stated that protein quality is a genotypic trait, nitrogen supplies of this study favored the expression of this trait by genotypes.

Pearson linear correlation between traits

The *Pearson* linear correlation analysis identifies the existing linear dependence between two traits, showing the magnitude of their positive or negative interrelations, ranging from -1.0 to 1.0, the closer to 1 the value, the stronger the degree of association between these two traits and the closer to zero, the smaller the linear dependence between them (Carvalho et al., 2004). The SRC of water demonstrated a strong positive correlation with the SRC of sodium carbonate and sucrose, obtaining coefficients of 0.76 and 0.63, respectively (Table 3). These results corroborate those of Antunes (2014), which also found a positive correlation between SRC of water and SRC of sodium carbonate, when two types of flour were studied in a physicochemical level. Similarly, Duyvejonch et al. (2012), Colombo et al. (2008) and Gaines (2000) observed a strong correlation in the values of SRC of water, SRC of sodium carbonate and SRC of sucrose.

The SRC of sodium carbonate presented a strong positive correlation with the SRC of sucrose. Moreover, Costa (2013) found the same correlation studying the industrial quality of wheat lines. Also, Barrera et al. (2007) evaluated two wheat genotypes and one triticale genotype in different milling conditions. These authors

Table 3. Pearson correlation coefficients between the retention capacity (SRC) of the flour of the solvents water, sodium carbonate (Na_2CO_3), sucrose and lactic acid ($C_3H_6O_3$).

	Water	Na_2CO_3	Sucrose	$C_3H_6O_3$
Water	-			
Na_2CO_3	0.766*	-		
Sucrose	0.631*	0.693*	-	
$C_3H_6O_3$	-0.234	-0.049	0.113	-

*Linear correlation coefficient of Pearson (N = 24) significant at p≤0.05.

found positive correlation between the sucrose absorption capacity and sodium carbonate absorption capacity of the flour. Significant correlations of SRC of lactic acid with other solvents tested under the conditions of this research were not found. This result is similar to that found by Barrera et al. (2007), where there was no correlation between the SRC of lactic acid with the SRC of sodium carbonate and SRC of sucrose. Although, they found a strong correlation between the SRC of lactic acid and SRC of water.

Conclusions

Greater levels of solvent retention capacity were observed in TBIO Mestre, suggesting that the genotype has good baking quality. The application of nitrogen in coverage increases the retention capacity of lactic acid; however, the stage that is applied throughout the wheat crop cycle does not change this feature, under the conditions of this study.

The water retention capacity of the flour has a strong and positive correlation with the retention capacity of the sodium carbonate and sucrose, together with the associations between the solvents sodium carbonate and sucrose.

Conflict of Interests

The authors have not declared any conflict of interests.

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Effect of hydro-priming duration on germination and early seedling growth of rapeseed under salinity stress

Seyed Nader Mosavian[1]* and Mahjoobeh Esmaeilzade-Moridani[2]

[1]Department of Agricultural sciences, Payame Noor University, Tehran, Iran.
[2]Faculty of Agriculture, Guilan University, Guilan, Iran.

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A laboratory experiment was carried out to assess the possibility of decreasing the negative effects of salinity on seed germination and early seedling growth of canola by hydro-priming of seeds. A 7×5 factorial experiment based on completely randomized design was employed to compare the treatments. Seed priming comprised 0.0, 4.0, 8.0, 12.0, 16.0, 18.0 and 24.0 h seeds soaking in distilled water, and salinity stress was simulated by the solutions of 0.0, 25.0, 50.0, 75.0 and 100.0 mM NaCl. Germination percentage and uniformity, germination speed and seedling dry weight were measured as germination performance indicators. Results showed that all traits were significantly affected by salinity stress and seed priming. Germination uniformity and seedling dry weight were affected by the interaction of salinity × seed priming. Germination percentage and speed were slightly affected by the interaction of salinity × seed priming. However, it was not significant. Increasing in salinity stress from control to the highest level (125 mM NaCl) resulted in 26% reduction of germination percentage of rapeseed. However, germination speed was a trait showing higher sensitivity to the salinity stress. The difference between control treatment and the highest salinity stress in terms of germination speed was 45%. Hydro-priming improved germination percentage and speed of rapeseed seeds. The highest germination percentage and speed was observed in the treatment of 24 h which was not significantly different with 20 h hydro-priming. Germination percentage and speed of rapeseed improved 25 and 30 by hydro-priming of seeds, respectively. Generally, the length of hydro-priming solutions improved the performance of seeds and seedlings of rapeseed. Higher levels of hydro priming duration reduced negative effects of sever salinity stress, where there was no significant difference between two last salinity levels in terms of salinity.

Key words: Germination, rapeseed, salinity, seedling dry weight.

INTRODUCTION

Good characteristics such as suitable placement in crop rotation, desirable quality, high value of oil (40 to 45%) and protein (39%) that has changed rapeseed (*Brassica napus* L.) to an important crop (Ghassemi-Golezani et al., 2010). Since rapeseed do not produce tiller, its grain yield is considerably depended on plant density affecting

*Corresponding author. E-mail: nader_mosavian@yahoo.com.

by germination and seedling establishment. Rapid and uniform field emergence is an essential prerequisite to reach the yield potential, quality and ultimately profit in annual crops (Parera and Cantliffe, 1994). Out of many constraints regarding low production of oilseeds, seed quality is the prime importance. Oilseeds are deteriorated more rapidly during storage, which reduces the quality of seeds (Afzal et al., 2004). Furthermore, their germination and seedling establishment is adversely affected by suboptimal environmental conditions such as salinity stress.

Soil salinity, a common problem in rainfed areas of south-west Iran (Ghassemi-Golezani et al., 2008), is a major obstacle which may decrease the germination of seeds in two ways: by lowering external osmotic potential and by the toxic effects of Na^+ and Cl^- ions on the seeds (Khajeh-Hosseini et al., 2003). Salinity may individually or in combination with drought, adversely affect germination and seedling establishment (Cheng and Bradford, 1999). The sensitivity of rapeseed to salinity, especially during germination and early growth of seedling has been reported (Afzal et al., 2004). Therefore, if the stress effect can be alleviated at the germination stage, the chances for attaining good crop establishment would be increased.

Seed priming is a pre-sowing technique for the improvement of germination and seedling growth, and establishment of many crops. The beneficial effects of priming have been shown for many field crops including chickpea (Kaur et al., 2005) and lentil (Ghassemi-Golezani et al., 2008). Hydro-priming generally enhances seed germination and seedling emergence under both saline and non-saline conditions, although there are exceptions. Hydropriming is a low cost method with advantageous effects on many field crops, such as maize, rice, chickpea, soybean and sunflower (Ashraf and Foolad, 2005; Kaya et al., 2006). Eskandari and Alizadeh-Amraie (2014) reported that lentil germination performance was improved by hydro-priming of seeds under salinity stress which was related to faster germination.

Since seed priming has proved to be a successful strategy for reducing the adverse effects of adverse environmental conditions such as salinity stress and, thus, improvement of the germination and emergence of plants (Kaya et al., 2006), the current experiment was aimed to assess the potential of hydro-priming for overcoming salt stress, an effective factor for germination failure of rapeseed, on seed germination and primary seedling growth of rapeseed.

MATERIALS AND METHODS

This study was carried out at the seed laboratory of the Ramin University of Agriculture and Natural Resources, Iran. A 7×5 factorial experiment based on completely randomize design was employed to assess the effect of hydro-priming duration on seed germination and early seedling growth of rapeseed under salinity stress. Hydro-priming durations included 0 (unprimed or control), 4, 8, 12, 16, 20 and 24 h seed soaking in distilled water (all hydro-printed seeds soaked in distilled water).

After priming, seeds were backed to their initial humidity. Salinity treatment comprised five levels of 0.0, 25.0, 50.0, 75.0, and 100 mM Nacl started from the beginning of the germination process. Four replicates of 50 hydro-primed seeds were germinated between double layered rolled germination papers under different salinity levels. The rolled paper with seeds was put into plastic bags to avoid moisture loss. Seeds were allowed to germinate in the dark for 14 days. The seeds and seedlings kept in the salinity treatments until the germination rates were determined. Germination was considered to have occurred when the radicles were 2 mm long. During germination evaluation, seedlings with short, thick and spiraled hypocotyls and stunted primary roots were considered abnormal (ISTA, 2003) and were not included in the count for seedling growth assessment. Germination speed (GS) was calculated according to Ellis and Roberts (1980):

$$GS = [\Sigma n / \Sigma(Dn)]$$

Where n is the number of seeds germinated on day D, D is the number of days counted from the beginning of the test. At the end of germination process, germination uniformity needed time for germination of a seed) and seedlings dry weight of rapeseed under different salinity stress were measured by oven-drying of seedlings.

Analysis of variance of the data and mean comparison were carried out using MSTATC statistical software and Duncan's multiple range test, respectively.

RESULTS AND DISCUSSION

Analysis of variance of laboratory data showed that all traits were significantly affected by salinity stress and seed priming (Table 1). Germination uniformity and seedling dry weight were affected by the interaction of salinity × seed priming. However, germination percentage and speed were not significantly affected by the interaction of salinity × seed priming (Table 1).

The results of the experiment indicated that germination percentage of rapeseed was only significantly affected by the last two levels of salinity (100 and 125 mM) (Table 2). Increasing in salinity stress from control to the highest level (125mM NaCl) resulted in 26 percent reduction of germination percentage of rapeseed. However, germination speed was a rapeseed germination trait showing higher sensitivity to the salinity stress in which the second salinity stress led to significant reduction of germination speed (Table 2). The difference between control treatment and the highest salinity stress in terms of germination speed was 45 percent. In other words, the salinity level of 125 mM NaCl induced 45% reduction of germination speed.

The results revealed that hydro-priming improved germination percentage and speed of rapeseed seeds. The highest germination percentage and speed was observed in the treatment of 24 h which was not significantly different with 20 h hydro-priming. Germination percentage and speed of rapeseed improved 25 and 30 by hydro-priming of seeds, respectively (Table 2). Ghana and Schillinger (2003)

Table 1. ANOVA analysis of salinity, priming and their interaction on maximum of germination (Gmax), Germination speed, germination uniformity (GU) and seedling weight.

S.O.V	df	Means of square			
		GP	GS	GU	DM
Salinity	5	1670.832**	0.00051883**	219.7454**	0.00006558**
Priming	6	1080.508**	0.00006721**	810.9681**	0.00017981**
Salinity× priming	30	149.4095	0.00000724	297.3317**	0.00003142**
Error	84	129.0159	0.00000735	49.29836	0.00001003
CV (%)	-	14.97	11.68	12.91	27.22

** Significant at P<0.01; ns: not significant; GP: germination percentage, GS: germination speed, GU: germination uniformity, DM: seedling dry weight.

Table 2. Effect of salinity and hydro-priming duration germination percentage and speed of rapeseed.

Treatment		Germination percentage	Germination speed
Salinity (mM)	0.0	89.8^{a}	0.31^{a}
	25.0	81.6^{a}	0.25^{b}
	50.0	81.8^{a}	0.23^{b}
	75.0	80.0^{a}	0.23^{b}
	100.0	72.0^{b}	0.19^{c}
	125.0	59.0^{c}	0.17^{c}
	Mean	75.7	0.23
Hydro-priming duration (h)	0.0	66.4^{c}	0.20^{c}
	4.0	69.0^{bc}	0.20^{c}
	8.0	70.0^{b}	0.20^{c}
	12.0	73.8^{b}	0.23^{bc}
	16.0	75.5^{b}	0.24^{ab}
	20.0	81.5^{a}	0.25^{a}
	24.0	82.0^{a}	0.26^{a}
	Mean	75.98	0.23

Difference letters indicating significant difference at P≤0.01.

observed that germination and seedling growth of winter wheat improved by hydro-priming of seeds compatible with the finding of the current research. Mohammad and Shahza (2005) recorded higher root growth of rice with hydro-priming of seeds.

Germination uniformity (h) of rapeseed decreased with hydro priming of seeds (Figure 1). In the highest level of salinity stress, the hydro priming treatment had the lowest effect. However, in other levels of salinity stress (25, 50, 75 and 100 mM) hydro priming clearly improved germination uniformity of rapeseed. It can be concluded that hydro priming decreased the negative effects of salinity on germination uniformity of rapeseed (Figure1).

Seedling dry weight of rapeseed decreased with increasing salinity stress. However, hydro-priming of seeds improved seedling growth under salinity conditions. Under the treatments of 100 and 125 mM NaCl, hydro-priming inhibited negative effects of salt stress on seedling growth of rapeseed (Figure 2), in which no seedling dry weight reduction was observed in the 125 mM treatment.

In general, higher hydro-priming durations improved seedling dry weight of rapeseed under higher salinity stress, emphasizing the positive effects of hydro-priming on seedling growth performance under salt stress conditions. It has been reported that germination is a process occurring through cell elongation, while root growth occurs through cell division process. Thus, in germination process, salinity affects cell division more than cell elongation (Khajeh-Hosseini et al., 2002) suggesting that higher seedling growth under salinity stress induced by priming treatment is more important than germination. This results are compatible with the findings of the current research, where seedling dry

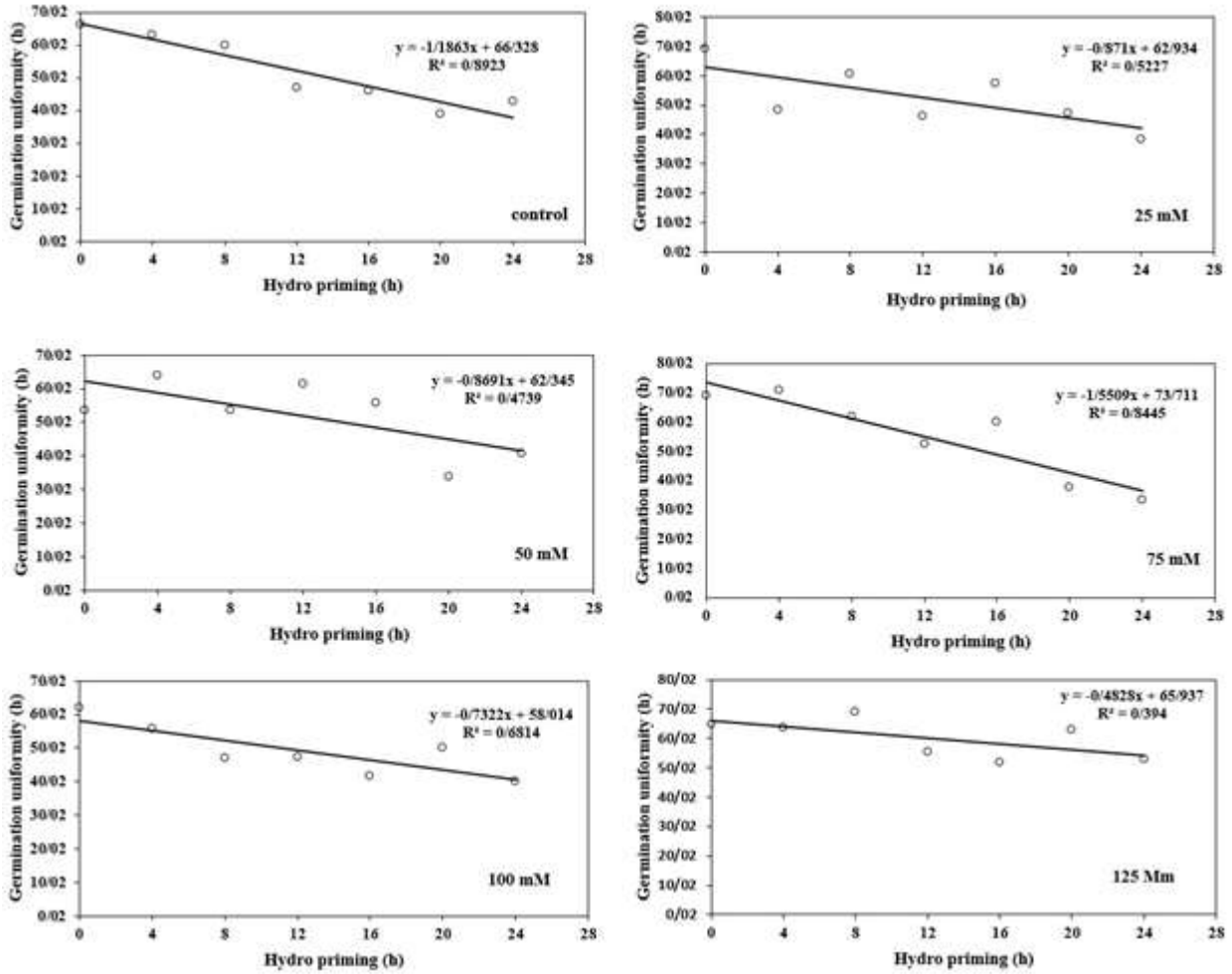

Figure 1. Effect of hydro-priming duration on germination uniformity of rapeseed under different salinity level.

weight produced by unprimed seeds decreased with increasing salinity stress (Figure 2).

However, hydro-priming inhibits negative effects of salinity stress, where there was no significant difference between seedling dry weight of 24 h hydro-primed seeds under 25 mM and 125 mM salinity stress (Figure 2). This results are in line with the findings of Salehi et al. (2010) who reported that priming improved seedling growth of rapeseed under salinity stress.

A pre-sowing treatment involving hydration of seeds in priming solutions improved the performance of seeds and seedlings of rapeseed. This improvement was reflected in higher germination and seedling dry weights. Hydropriming improved seedling vigor of rapeseed as indicated by seed germination percentage and speed and seedling dry weight (Table 2 and Figure 2). The earlier germination might be attributed to increased metabolic activities in the hydroprimed seeds (Soon et al., 2000; Basra et al., 2002). It is obvious that higher rate of seed germination can lead to the production of large and uniform seedlings.

According to McDonald (2000), primed seeds can rapidly imbibe and revive the seed metabolism, enhancing germination rate and uniformity. In many crops, seed germination and early seedling growth are the most sensitive stages to water limitation induced by salinity stress. Water deficit and salt stress may delay the onset and reduce the rate and uniformity of germination, leading to poor crop performance and yield (Kaya et al., 2006). Therefore, the beneficial effects of priming may be more evident under unfavorable rather than favorable conditions (Bradford, 1995) which was clearly showed in Figure 2 where seedling dry weight of rapeseed improved under higher levels of salinity stress compared with lower salt stress.

However, in some cases, longer hydro-priming decreased germination performance of rapeseed which is compatible with the findings of Omidi et al. (2005) who reported that priming is a technique improving germination performance of rapeseed, but higher levels

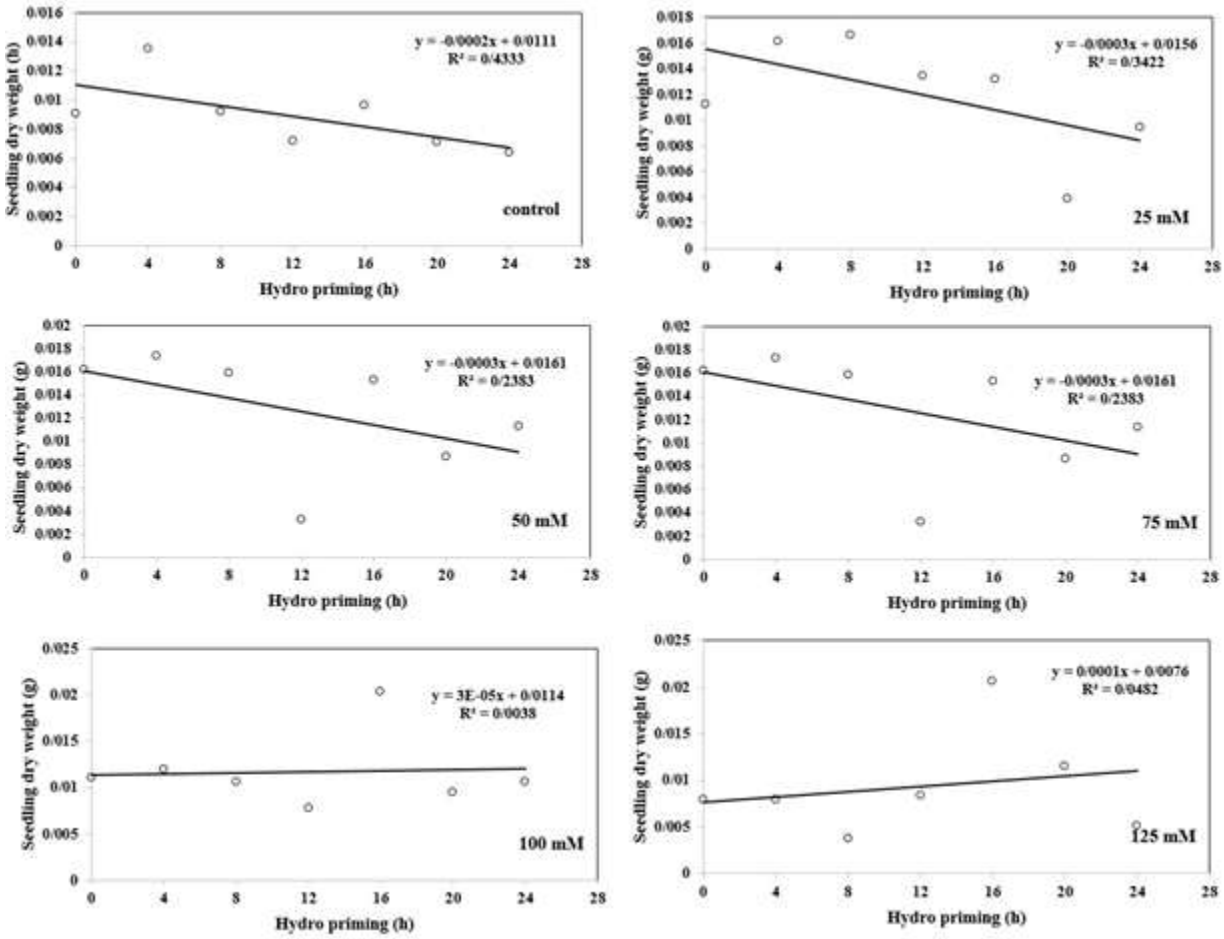

Figure 2. Effect of hydro-priming duration on seedling dry weight of rapeseed under different salinity level.

of priming may decrease seedling growth of rapeseed, confirming the findings of figure 2. It has been reported that high rate of water uptake in primed seeds accelerated their germination and enhanced germination percentage (Kaya et al., 2006).

Furthermore, during priming, the embryo expands and compresses the endosperm. The compression force of the embryo and hydrolytic activities on the endosperm cell walls may deform the tissues that have lost their flexibility upon dehydration, producing free space and facilitating root protrusion after rehydration. In this research, the fastest rate and percentage of germination was obtained by soaking seeds in water (hydro priming) compared with un-primed seeds, probably due to faster water uptake and earlier initiation of metabolism processes, which determine radicle protrusion.

Conclusion

Germination and subsequent seedling growth of rapeseed can be inhibited by adverse conditions such as salinity. Hydro-priming is a helpful method for reducing the risk of poor germination and stand establishment of rapeseed under salinity stress. The finding of the research revealed that with the simple and useful technique of hydro-priming enhanced germination and seedling growth of rapeseed will be attained which has positive effect on field performance of rapeseed under salinity stress.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Efficient control of conidium germination, mycelial growth and early blight in tomato *in vitro* with essential oils under farm conditions

C. L. M. Abreu[1,2], C. I. M. A. Feres[2,4,6]*, E. L. Furtado[2], L. C. Ming[2], M. O. M. Marques[3] , R. S. Acáciod, A. Riffel[5], H. F. Goulart[4], A. E. G. Santana[4], F. S. Bernardes[4] and F. L. A. Câmara[2]

[1]Empresa Mato-grossense de Pesquisa, Assistência e Extensão Rural do Mato Grosso - EMPAER-MT, Rua 02, s/n, Edifício Ceres, 3˚ andar, CPA, 78.050-250, Cuiabá – Mato Grosso, Brazil.
[2]Universidade Estadual Paulista, Faculdade de Ciências Agronômicas, Depto. de Produção Vegetal, Fazenda Experimental Lageado, Rua José Barbosa de Barros, 1780, C. Postal 237, 18610-307, Botucatu – São Paulo, Brazil.
[3]Instituto Agronômico, Centro de Pesquisa e Desenvolvimento de Recursos Genéticos Vegetais, Seção de Fitoquímica, C. Postal 28, 13001-970, Campinas - São Paulo, Brazil.
[4]Instituto de Química e Biotecnologia, Universidade Federal de Alagoas, 57092-970 Maceió, Alagoas, Brasil.
[5]Empresa Brasileira de Pesquisa Agropecuaria (Embrapa)- Tabuleiros Costeiros - UEP Rio Largo/AL, BR - 104 Norte, km 85, s/n - Zona Rural - Rio Largo/AL CECA - UFAL (Centro de Ciências Agrárias da UFAL) - Campus Delza Gitaí, Cep: 57.100-000, Brazil.
[6]Faculdades Integradas do Norte de Minas, Campus JK, Avenida Osmani Barbosa, nº 11111, Bairro JK, Montes Claros, Minas Gerais, Brazil.

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The aim of this work was to find a natural product and efficient dose to control early blight in tomato crop in agro-ecological or conventional agriculture system. The treatments were essential oils from *Cymbopogon citratus, Eucalyptus citriodora, Piper hispidinervum, Cymbopogon martini, Rosmarinus officinalis, Syzygium aromaticum, Cinnamomum zeylanicum, Mentha piperita, Citrus sinensis var. dulcis*, and *Melaleuca alternifolia* used against *Alternaria solani* in *Solanum lycopersicum* under greenhouse conditions. *In vitro*, mycelial growth and conidia germination were completely inhibited by *C. zeylanicum*, *C. martini, C. citratus*, and *S. aromaticum* oils of 750 µL L^{-1}, *E. citriodora* and *M. alternifolia* at 2000 µL L^{-1}, and *Mentha piperita* in 5000 µL L^{-1}. In tomato, plants under greenhouse conditions, early blight in leaf was inhibited by *C. zeylanicum*, *C. martini*, *C. citratus*, and *S. aromaticum* at 750 µL L^{-1}, and *E. citriodora* oil of 5000 µL L^{-1}. The best results for MIC 50 and 90 with minor dose to control was essential oil from *C. citratus* at 341.32 and 1.822.10 µL L^{-1} rich in geranial (46.91%), neral (34.34%), and geranyl acetate (6.30%). Therefore, it is possible to use this essential oils or manufacture a new and efficient product to control *Alternaria solani* in tomato plants.

Key words: Agroecology, *Alternaria solani*, *Cimbopogon* sp., Eucalyptus sp., Neral, *Solanum lychopersicon*.

INTRODUCTION

Plant diseases are caused by fungi, nematodes, bacteria, and viruses, among which fungi are the main pathogens, causing great yield losses in numerous important crops (Huang et al., 2010). Natural sources from plants play a significant role in the prevention and control of these diseases. In addition, products of higher plants may lead

to the discovery of the source of antimicrobial agents with possible novel mechanisms of action (Hada, 2014). Tomato (*Solanun lycopersicon* Mill.) is one of the most economically important vegetables in the world (Kurozawa and Pavan, 2011, 2005; Filgueira, 2000). It is the host for a wide spectrum of pests and diseases and thus requires specific ecological, nutritional and phytosanitary conditions in cropping and/or field (Leite et al., 2003). At post harvesting conditions, it is a highly perishable vegetable with a short shelf-life and high susceptibility to fungal disease during prolonged storage (Ibrahim, 2014).

Early blight, which is caused by *Alternaria solani* (Ell. and Martin) Jones *and* Grout, is one of the most important diseases affecting tomato plants, since it causes direct (fruits) and indirect (branches and leaves) damages (Yanar, 2011; Leite et al., 2003). Fungicides including mancozeb, chlorothalonil, thiophanate-methyl and copper are used to control this disease.

Losses of up to 75% of agrochemicals during application have been recorded for tomato crops. Nowadays, producers use 45 agrochemical applications during the cycle of this species. The indiscriminate use of agrochemicals in tomato plants, associated with the lack of knowledge by producers, results in the development and intensification of occupational diseases and environmental contamination besides exposure of consumers to such risk (Araújo at al., 2000; Kishore and Pande, 2007; Fawzi et al., 2009).

Natural products have great potential for the management of pests and diseases, among other utilizations from extracts and essential oils (Zanella, 2015). Historically, the origin of pyrethroids and carbamates is an example of the potential of natural products for phytosanitary uses.

In this context, essential oils from plants have great potential due to their intrinsic characteristics such as high bioactivity, great molecule diversity and volatility, and brief environmental persistence (Knaak and Fiuza, 2010; Lee et al., 2008). The mechanism of action of essential oils in live cells is related to cell membrane permeability alteration, enzyme synthesis inhibition and/or inactivation (Souza et al., 2005). Cruz et al. (2015) had significant results in essential oils from *Plectranthus amboinicus* leaves against *Fusarium solani* at 10 µL. In *Pyricularia grisea, Cymbopogon winterianus* has total mycelia growth inhibition with 10 µL (Perini et al., 2013).

According to Soylu et al. (2010) and Copping and Duke (2007), based on the activity and mechanism of action concerning natural products, they may be directly applied on plants for protection and have potential applicability in the establishment of programs for the development of new products as well as synthesis and/or semi-synthesis processes. Also, Pawar and Thaker (2007) studied the antifungal effect of essential oils from 75 plant species against *Alternaria porri* and *Fusarium oxysporum* f.sp. *ciceris*. Yanar et al. (2011) studied the antifungal effect of essential oils from 27 plant extracts, and Sallam and Kamal (2012) studied six plants. These authors emphasized the potentiality and importance of researching these compounds as an alternative for synthetic phytosanitary products, considering their economic and environmental viability.

Several authors have reported the efficiency and wide spectrum of action of essential oils against insects, fungi and pathogenic and phytopathogenic bacteria, both *in vitro* and *in vivo* (Paes, 2012,). Thus, the present work aimed at advancing applied research by evaluating, through preventive applications, the effect of different doses of essential oils from 10 plant species on conidium germination and mycelial growth *in vitro* as well as their controlling action, *in vivo*, on the severity of early blight caused by *A. solani* in the leaves of *S. lycopersicum* Mill. cv. Sta. Clara cultivated under greenhouse conditions.

MATERIALS AND METHODS

Since the present paper is composed of three different linked works, the result was adopted as eliminatory parameters of treatment efficiency for the subsequent studies.

The employed essential oils are of commercial origin and obtained through hydrodistillation. In all experiments, essential oils were homogenized with a 1:1 mixture of water and Tween 80 detergent. The used concentrations were calculated based on the essential oil.

The essential oils analysis

Analysis of essential oil components was analyzed through Gas Chromatography, ubMass Molecular Biology and Phytochemistry, Agronomical Institute of Campinas, São Paulo State, Brazil.

A 2 mg oil sample was diluted in 1 ml ethyl acetate (HPLC grade), from which a 1 µL aliquot was injected. The analysis was carried out in a GC/MS - Shimadzu/QP-5000, equipped with DB-5 column (30 m × 0.25 mm × 0.25 µm) electron ionization (70 eV) and mass scan range from 30 to 300 Da. Helium was used as carrier gas at 1.0 ml/min flow, and injector at 240˚C. The following program was used: 50˚C (5') to 160˚C, 3˚C per minute, and 160 to 220˚C, 10˚C per minute. Using a split ratio of 35, the temperatures of the ion source and GC-MS interface were 200 and 230°C, respectively. Compounds were identified by comparing their mass spectra with the GC/MS spectral library. Kovats retention index was calculated and determined by comparing the data with those found in literature (Adams, 1995).

Effect of essential oils at different doses on *A. solani* mycelial growth *in vitro* (Experiment 1)

Mycelia were obtained in the Mycology and Forest Phytopathology Laboratory, Department of Plant Production, College of Agronomical Sciences, São Paulo State University-UNESP,

*Corresponding author. E-mail: c.iezid@gmail.com.

Botucatu, São Paulo State, Brazil.

The experiment was carried out in Petri plates. The plates with each treatment were organized in a completely randomized design of 10 × 6 factorial arrangement, with five replicates. The treatments were essential oils from the following species: *Cymbopogon citratus* (lemon grass), *Eucalyptus citriodora* (lemon eucalyptus), *Piper hispidinervum* (long pepper), *Cymbopogon martini* (palmarosa), *Rosmarinus officinalis* (rosemary), *Syzygium aromaticum* (clove), *Cinnamomum zeylanicum* (cinnamon), *Mentha piperita* (peppermint), *Citrus sinensis* var. *dulcis* (orange), and *Melaleuca alternifolia* (tea tree), at 5 different concentrations (0, 250, 500, 750, 1000 and 5000 µL L^{-1}), except for *M. alternifolia*, *M. piperita* and *Eucaliptus. citriodora* oils, which were used at 0, 1000, 2000, 3000, 4000 and 5000 µL L^{-1}. After autoclaving, treatments were added to PDA culture medium. Plates were inoculated with 0.5 cm diameter disks and kept in BOD chamber at 25ºC in the dark.

The colony diameter (mm) was daily evaluated until the eighth day. The mean of the ratio between measures in two perpendicular directions considered the growth value. The growth rate over time was assessed through parallelism test among doses of each oil, in which angular coefficients and constants from the growth linear regression equations were calculated. The results were plotted and the fungi static effect was analyzed through intercept (angular coefficient from the progress equation by each oil and dose), in which, the lower value, the smaller will be the angular coefficient of the disease symptom progression rate, mycelial growth and conidia germination at each treatment. Thus, each angular coefficient from the linear regression analysis contrasted the Scott-Knott method ($p \leq 0.0001$).

To obtain the maximum inhibitory concentrations (50 and 90%), differential and integral calculus was applied. Regression statistical test was performed using "F" test.

Effect of essential oils at different doses on *A. solani* conidium germination *in vitro* (Experiment 2)

Conidia were obtained at Laboratory of Mycology and Forest Phytopathology, College of Agronomic Sciences, UNESP, Botucatu, São Paulo State, Brazil.

The experiment was carried out in 2% Agar medium in water. The design is completely randomized in 5 × 5 factorial arrangement, with three replicates. Treatments consisted of five essential oils selected among those from Experiment 1 (*C. citratus*, *C. martini*, *S. aromaticum*, *C. zeylanicum*, and *E. citriodora*). Each oil was tested at five concentrations (0, 250, 500, 750, and 1000 µL L^{-1}). The culture medium containing the treatments was applied onto glass slides placed in Petri plates and inoculated with 10 µL of a suspension containing 10^3 µL^{-1} *A. solani* conidia. The plates were kept in a Biological organism development chamber (BOD) for 24 h at 25^{o}C in the absence of light.

Conidium germination was evaluated with the aid of an optical microscope. The conidium that presented germ tube extension higher than the analyzed conidium length was considered germinated (Neely, 1978), and the germination percentage was calculated. Due to the pattern of conidium germination in response to treatments, only analysis of variance and mean contrasts were done through the Scott-Knott method ($p \leq 0.0001$), to the detriment of regression analysis.

Effect of essential oils at different doses on the control of early blight caused by *A. solani* in the leaves of *S. lycopersicon* Mill. cv. Sta. Clara under greenhouse cropping (Experiment 3)

S. lycopersicon Mill. cv. Santa Clara seedlings (30 days old) was transplanted to 13-L pots. The substrate consisted of a mixture of earth, sand and commercial organic substrate (1:1:1), in addition to 10 kg fertilizer formula 4-14-08 per m^3 of the mixture. Plants were kept for 57 days after transplanting (DAT) in a high tunnel under greenhouse condition; they were covered with 150 µ "Agrofilme" and subjected to daily irrigation through micro-aspersion (5 mm flow). The experimental design was in randomized blocks in 5 × 4 factorial arrangement, with 6 replicates; 5 essential oils (*C. citratus, E. citriodora*, *C. martini*, *S. aromaticum*, and *C. zeylanicum*) were used at 4 concentrations (0, 500, 750, and 1000 µL L^{-1}), except for *E. citriodora* (0, 750, 1000 and 5000 µL L^{-1}).

The pathogen inoculation consisted of the application of a 10^4 *A. solani* conidia/mL suspension twice on consecutive days after the first application of treatments, which was repeated at 3-day intervals until 57 DAT. It was done with the aid of a handheld backpack sprayer and plastic blanket for isolation of plots. At 57 DAT, the symptom severity was evaluated in the leaf tissue. The evaluator received a visual accuracy training with the software DISTRAN (public domain) of diagrammatic scale of damaged leaf area symptoms (Grade 0 = 0; 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; and 4 = 76-100% injured leaf area) (Azevedo, 1997). Nine leaves from basal, middle and apical part of the canopy in ten plants were evaluated; from each replicate we can calculate the index control of relative efficiency (EFCR). The EFCR was calculated in relation to each standard treatment per treatments and replications (Ming et al., 2012)

Data were subjected to regression analysis ($p \leq 0.0001$) with square root equations and Minimal Inhibitory Concentration (MIC) of 50 and 90% of symptoms of plant disease was determined. We do this study only in greenhouse field experiment with better oils after *in vitro* conidium and mycelial experiments.

RESULTS AND DISCUSSION

Chemical composition of the essential oils and their potential use

The results of GC/MS analysis from the essential oils tested and the activity reported on literature for each one and/or the most important compound detected are presented. The majority of antimicrobial compounds found in essential oils are terpenoids and phenylpropenes with the most active being phenols, although some aldehydes and non-phenolic substances also present promising antimicrobial activity. The target site and mode of action of most essential oil components still under is not well understood, especially in yeast (Hyldgaard, 2012).

The GC/MS results from the essential oils used in this work indicated that: The *C. citratus* (lemon grass) essential oil has the major constituents of geranial (46.91%), neral (34.34%), geranyl acetate (6.30%), camphene (1.02%), 6-methyl-5-hepten-2-one (1.28%), linalool (0.82%), citronellal (0.25%), isomenthol (1.81%), nerol (0.45%), geraniol (3.52%), and α-*trans*-bergamotene (0.85%).

C. martini (palmarosa) essential oil had major component of geraniol (86.98%), followed by geranyl acetate (9.03%), *trans*-ocimene (1.45%), *trans*-caryophyllene (0.63%), and geranial (0.41%). Geranial and neral aldehyde mixture constitutes citral, a potent bioactive substance against phytopathogens (Glamočlija et al., 2011).

E. citriodora (eucalyptus) essential oil main compound was citronellal (74.74%), followed by citronellol (6.26%), isopulegol (5.18%), iso-isopulegol (4.33%), geraniol (2.96%), α-pinene (0.45%), β-pinene (0.68%), limonene (0.33%), 1,8-cineole (1.51%), citronellyl acetate (1.24%), geranyl acetate (0.28%), and *trans*-caryophyllene (0.72%).

Both *S. aromaticum* (clove) and *C. zeylanicum* (cinnamon) had the major compound, eugenol: it is a major constituent in clove essential oil, and its antimicrobial activity is linked to its ability to permeabilize the cell membrane and interact with proteins; a phenylpropanoid of high antibiotic efficiency, specially against fungi; it can be used in crop phytopathogen control, as well as in food conservation. The antifungal mode of action of eugenol needs further investigation, but it is known to depend on cell proliferation. Eugenol treatment altered cell membrane and cell wall structures of proliferating *S. cerevisiae* cells resulting in the release of cellular content (Hyldgaard et al., 2012; Faria et al., 2006; Souza et al., 2005).

S. aromaticum had higher eugenol content (82.55%), relative to that of *C. zeylanicum* (73.45%). It had less diversity of substances - *trans*-caryophyllene (11.98%), eugenyl acetate (3.13%), α-humulene (1.74), and α-copaene (0.59%) compared with those present in *C. zeylanicum* essential oil: *Cis*-caryophyllene (4.67%), linalool (3.58%), benzyl benzoate (2.92%), *cis*-cinnamyl acetate (2.11%), safrole (1.25%), α-pinene (1.24%), α-phellandrene (1.32%), ortho-cymene (1.11%), β-phellandrene (1.10%), camphene (0.40%), β-pinene (0.47%), α-terpineol (0.36%), *trans*-cinnamaldehyde (0.77%), and α-copaene (0.75%). However, Pawar *and* Thaker (2007) attributed the antifungal activity of *C. zeylanicum* essential oil to cinnamaldehydes.

M. alternifolia (tea tree) had 4-terpineol (46.74%) and gamma-terpinene (18.16%) as the main components; it also presented α-terpinene (7.5%), 1,8-cineole (5.25%), ortho-cymene (4.38%), α-terpineol (3.43%), terpinolene (2.84%), α-pinene (2.32%, limonene (1.69%), α-selinene (1.27%), aromadendrene (1.00%), gamma-cadinene (0.99%), myrcene (0.82%), β-pinene (0.80%), sabinene (0.41%), *trans*-caryophyllene (0.37%), and delta-3-carene (0.34%).

P. hispidinervum (long pepper) presented Safrole with major constituent (93.85%) in the essential oil; it had α -pinene (0.35%), myrcene (0.18%), delta-3-carene (0.41%), ortho-cymene (0.14%), limonene (0.21%), *cis*-ocimene (0.35%), *trans*-ocimene (0.88%), terpinolene (2.37%), *trans*-caryophyllene (0.30%), bicyclogermacrene (0.62%), and pentadecane (0.25%). In this oil, the major compound, Safrole detected has high relevance to industrial and agriculture chemical use. It has approximately 90 to 94% of the essential oil compound produced by the plant; it is applied as synergistic element in the composition of insecticides and herbicides (Maia et al., 1987).

For *R. officinalis* (rosemary), the essential oil is mainly composed of 1,8-cineole (25.51%), α -pinene (22.23%), camphor (18.64%), camphene (10.63%), limonene (5.51%), β -pinene (4.76%), borneol (2.48%), myrcene (2.39%), 3-octanone (0.44%), ortho-cymene (1.87%), bornyl acetate (1.80%), α -terpineol (1.40%), *trans*-caryophyllene (1.15%), and linalool (0.92%). Rosemary oil, until, has no agriculture use. *M. piperita* (peppermint) essential oil main constituent was menthol (45.18%), followed by menthone (22.54%), 1,8-cineole (7.03%), meta-cresol acetate (6.39%), menthyl acetate (5.97%), neo-menthol (2.88%), limonene (1.79%), pulegone (1.55%), *trans*-caryophyllene (1.21%), β-pinene (1.12%), non-identified compounds (0.72%), gamma-terpinene (0.70%), α-pinene (0.60%), gamma-muurolene (0.59%), isomenthol (0.47%), sabinene (0.38%), 3-octanol (0.25%), ortho-cymene (0.25%), *trans*- β -ocimene (0.25%), and myrcene (0.12%).

For *Citrus sinensis* var. *dulcis* (orange) essential oil used in this work is limonene (96.86%); it also presents small quantities of myrcene (2.27%) and α-pinene (0.52%).

Considering the effect attributed to the essential oil major compound, this relation is almost direct when the oil has little diversity of molecules. It is important to point out the major compounds of essential oils, since they may be responsible, at least in part, for the oil antifungal activity (Salamci et al., 2007). However, is not true for all the essential oils and/ or microorganisms.

For essential oils with great diversity of molecules that is well distributed, the synergistic effect hypothesis is probably the most suitable. In some cases, large proportions of oxygenated monoterpenes may guarantee the essential oil antifungal activity (Santana, 2015).

The mechanism of action of essential oils in live cells is related to cell membrane permeability alteration, and enzyme synthesis inhibition and/or inactivation (Souza et al., According to the parallelism test for the growth curves of the analyzed doses of essential oils, *R. officinalis, C. sinensis* v. *dulcis*, and *P. hispidinervum* did not significantly reduce mycelial growth rate, presenting thus the highest angular coefficients (Table 1).

However, it can be seen that there are oils at different concentrations where the growth remains stagnant when the intercept remains at the abscissas (Table 1 and Figure 1). In this case, there is only fungistatic action and not fungicide effect. Aside oil type or dose, regular application is needed. However, such characteristics concerning monoterpenes constitute a dubious quality for the applicability of essential oils in crop protection, since they are highly volatile and instable. Commercial applications of essential oils would benefit from deeper insight into the mode of action behind individual compounds, as this could facilitate the exploitation of, e.g., synergistic combinations with more powerful antimicrobial properties (Hyldgaard, 2012).

The antifungal activity of a given essential oil may be

Table 1. Effect of different essential oils and doses on *A. solani* mycelial growth rate *in vitro*, according to the parallelism test of angular coefficients from linear regression equations for mycelial growth in colony diameter (mm) over eight days.

Oils	Doses (*μ*L L^{-1})					Mean R^2
	0	250	500	750	1000	
	Angular coefficients (α)					
R. officinalis	10.47^{Aa}	9.29^{Aa}	9.63^{Aa}	9.59^{Aa}	10.02^{Aa}	0.94
S. aromaticum	10.47^{Aa}	7.46^{Cb}	6.37^{Cc}	0.00^{Dd}	0.00^{Dd}	0.96
C. citratus	10.47^{Aa}	8.44^{Bb}	6.30^{Cc}	0.00^{Dd}	0.00^{Dd}	0.94
C. martini	10.47^{Aa}	8.86^{Bb}	6.00^{Cc}	0.00^{Dd}	0.00^{Dd}	0.96
C. zeylanicum	10.47^{Aa}	7.36^{Cb}	2.91^{Dc}	0.00^{Dd}	0.00^{Dd}	0.94
C. sinensis v. dulcis	10.47^{Aa}	9.91^{Aa}	8.77^{Bb}	9.97^{Aa}	9.73^{Aa}	0.96
P. hispidinervum	10.47^{Aa}	8.63^{B}c	8.42^{B}c	9.39^{Ab}	8.18^{B}c	0.97

	0	1000	2000	3000	4000	5000	
	Angular coefficients (α)						
E. citriodora	10.47^{Aa}	9.34^{Ab}	0.00^{Bc}	0.00^{Bc}	0.00^{Bc}	0.00^{Ac}	0.95
M. piperita	10.47^{Aa}	6.01^{Bb}	4.09^{Ac}	2.96^{Ad}	2.47^{Ad}	0.00^{Ae}	0.95
M. alternifolia	10.47^{Aa}	6.43^{Bb}	0.00^{Bc}	0.00^{Bc}	0.00^{Bc}	0.00^{Ac}	0.94

Means followed by the same uppercase letters in columns and lowercase letters in lines do not differ by the Scott-Knott test ($p \leq 0.0001$).

isolated effects or synergic action by two or more compounds (Cruz et al., 2015). However, increasing amounts of evidence indicate that the inherent activity of essential oils may not rely exclusively on the ratio in which the main active constituents are present, but also interactions between these and minor constituents in the oils. Various synergistic antimicrobial activities have been reported for constituents or fractions of essential oils when tested in binary or ternary combinations (García-García et al., 2011). However, if we can use this technology at this moment, there is need to think about environment and economic sustainability questions under agroecology system prerogatives. In this case, the direct use of the essential oil on the cropping process to control insects and pathogenic microorganisms is possible. To the resources, free input given by farmers is possible in planting economic or alimentary plants with phitossanitary plants for essential oil production or other environmental services.

Effect of essential oils at different doses on *A. solani* mycelial growth *in vitro* (Experiment 1)

A. solani mycelial growth was differently influenced, when the studied essential oils and their concentrations was compared (Table 1 and Figure 1).

Action of the mycelial growth control can be possible because it has a high variation on the essential oils compounds. There are two hypotheses for the complex action: First, synergic action among the compounds against *A. solani*; second, horizontal action with multiples attack points in the membranes and biochemical process in the mycelial tissue.

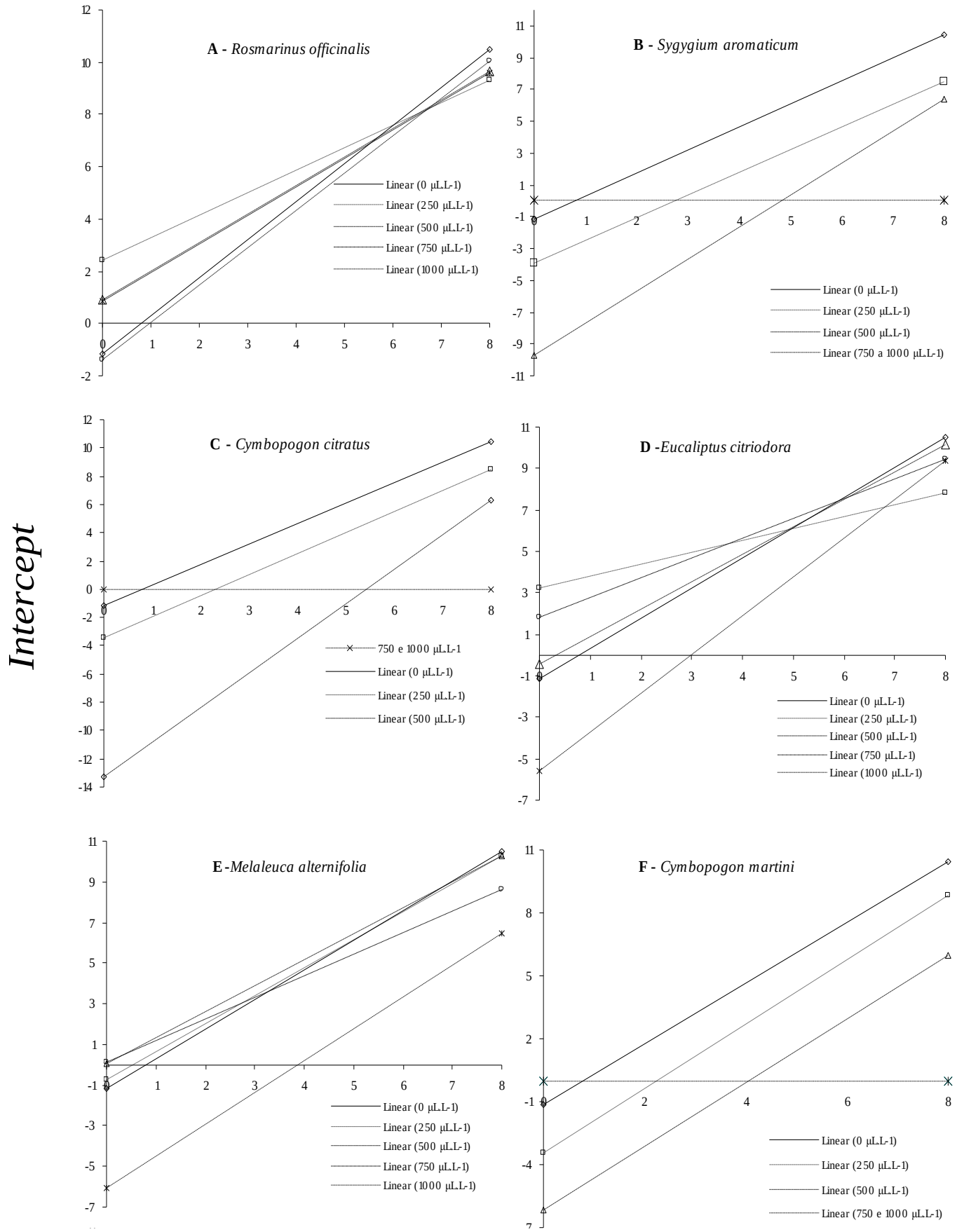
In addition, Daferera et al. (2003) reported that *R. officinalis* oil was inefficient against *B. cinerea* and *F. solani* mycelial growth, corroborating the present results.

As such, essential oils did not show any action against *A. solani* under the conditions of the present work. They were eliminated at this stage. The opposite was observed for *E. citriodora, M. alternifolia, M. piperita, S. aromaticum, C. citratus, C. martini,* and *C. zeylanicum* oils, which not only reduced the colony growth rate but also completely inhibited it at the highest doses (Table 1).

For the remaining oils, the fungistatic effect for five days on colony stagnation was confirmed, and mycelial growth was completely inhibited at doses higher than 750 and 1000 μL L^{-1} (Figure 1).

Similar to the results obtained in the present work, several authors have reported the fungicide and fungistatic effects of different doses of *S. aromaticum, C. zeylanicum, C. citratus, C. martini, E. citriodora, M. alternifolia* and *M. piperita* essential oils against *in vitro* pathogens development: *Aspergillus parasiticus, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Rhizopus sp, Penicillium sp, Europium repens, Rhizoctonia solani, Rhizoctonia bataticola, Fusarium moniliforme, F. oxysporum*, *F. solani, Bipolaris sorokiniana, Botrytis cinerea, Helminthosporium oryzae, Alternaria* sp., *Alternaria porri, Myrothecium verrucaria,* and *Curvalaria lunata* (Pawar and Thaker, 2007; Negrelle and Gomes, 2007; Faria et al., 2006; Fonseca et al., 2006).

Besides, Faria et al. (2006) reported *Alternaria* sp. was susceptible to the essential oil of *Ocimum gratissimum* chemotype-eugenol as well as to isolated eugenol. Pawar


Intercept
A - Rosmarinus officinalis
Linear (0 µL.L-1)
Linear (250 µL.L-1)
Linear (500 µL.L-1)
Linear (750 µL.L-1)
Linear (1000 µL.L-1)
B - Sygygium aromaticum
Linear (0 µL.L-1)
Linear (250 µL.L-1)
Linear (500 µL.L-1)
Linear (750 a 1000 µL.L-1)
C - Cymbopogon citratus
750 e 1000 µL.L-1
Linear (0 µL.L-1)
Linear (250 µL.L-1)
Linear (500 µL.L-1)
D -Eucaliptus citriodora
Linear (0 µL.L-1)
Linear (250 µL.L-1)
Linear (500 µL.L-1)
Linear (750 µL.L-1)
Linear (1000 µL.L-1)
E -Melaleuca alternifolia
Linear (0 µL.L-1)
Linear (250 µL.L-1)
Linear (500 µL.L-1)
Linear (750 µL.L-1)
Linear (1000 µL.L-1)
F - Cymbopogon martini
Linear (0 µL.L-1)
Linear (250 µL.L-1)
Linear (500 µL.L-1)
Linear (750 e 1000 µL.L-1)

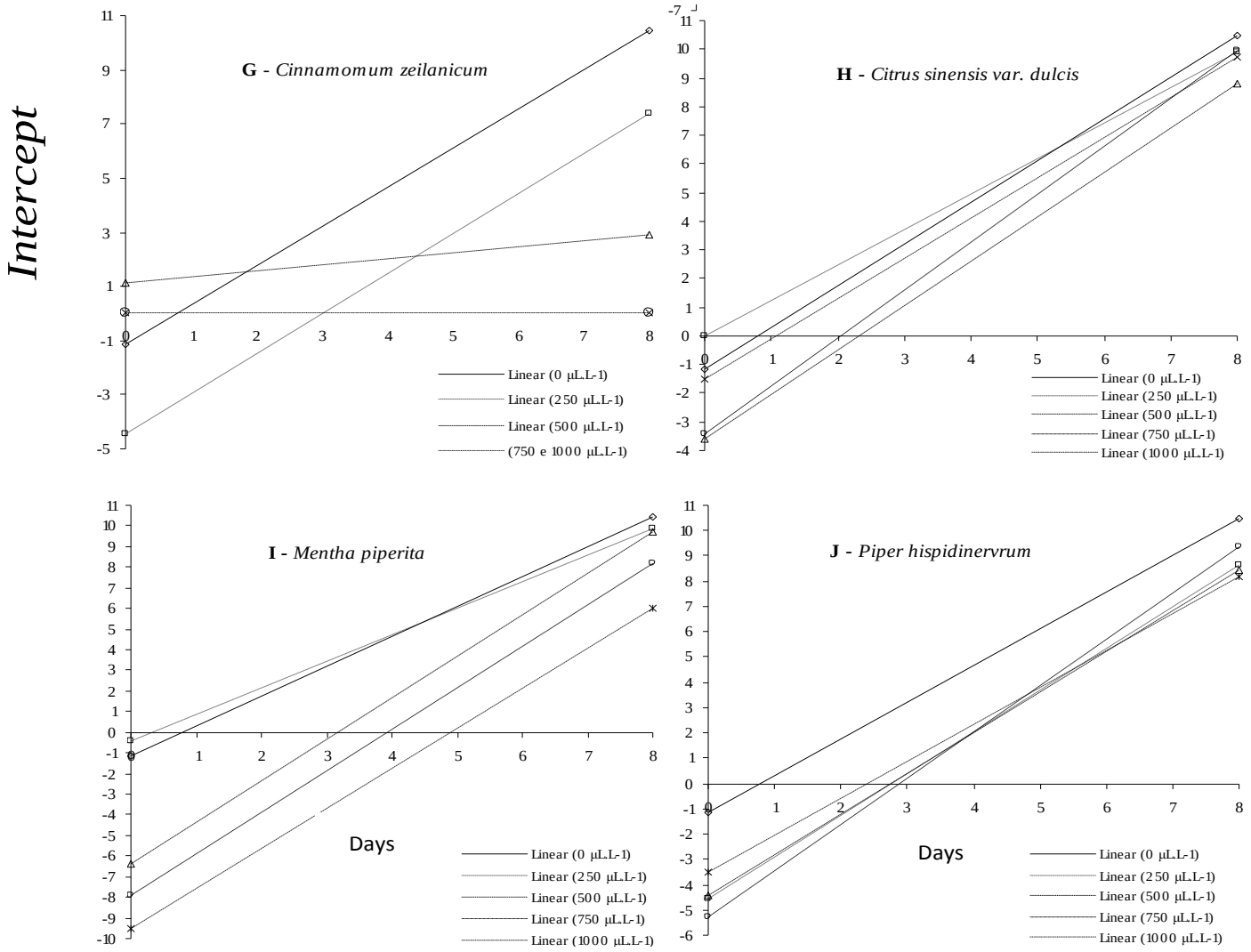


Figure 1. Fungistatic effect of essential oils and their concentrations on *A. solani* mycelium growth *in vitro*, on the axis "x" days for return to growth and "Y" axis angular growth rates. Intercept analysis between angular coefficients and constants of linear equations on mycelia growth.

and Thaker (2007) also observed that essential oils of other plant species rich in eugenol were efficient against *Alternaria porri.* Salgado et al. (2003) studied the bioactivity of several *Eucalyptus* sp. essential oils; *E. urophylla* oil was the most efficient; *E. citriodora* oil was against *F. oxysporum* Schlecht., *Botrytis sorokiniana* Shoemaker, and *Botrytis cinerea* Pers, with dose-dependent inhibition point.

The antifungal effect observed for *S. aromaticum* and *C. zeylanicum* is probably due to eugenol, the main compound in both species: 82.55 and 73.45%, respectively, of their composition. In addition, there are reports of the antifungal effect of isolated eugenol against *A. solani* (Faria et al., 2006), *Fusarium* spp*., Rhizopus* spp., and others (Souza et al., 2005).

Campaniello et al*.* (2010) found that eugenol (from 100 to 150 µg mL^{-1}) is an effective antifungal compound against *Aspergillus*, *Penicillium*, *Emericella* and *Fusarium* spp., suggesting that this activity could be attributed, in part, to the presence of a phenolic group.

C. citratus oil antifungal effect may be related to its major compounds, neral (34.34%) and geranial (46.91%), or to their associate action with the remaining oil components. This hypothesis is confirmed by Negrelle *and* Gomes (2007), who reported that the mixture of these aldehydes constitutes citral, to which important antifungal activity is attributed. Souza et al. (2005) also detected potent antifungal activity by citral against some phytopathogens like *Fusarium* spp*.* and *Rhizopus* spp. The activity of *E. citriodora* oil was probably due to its main compound, citronellal (74%). This hypothesis supports the results obtained by Salamci et al. (2007),

Table 2. Effect of different essential oils and their doses on *A. solani* conidium germination percentage *in vitro.*

Oils	Doses *(µL L $^{-1}$)*					
	0	250	500	750	1000	5000
	Germinated conidia (%)					
S. aromaticum	100.0^{Aa}	100.0^{Aa}	100.0^{Aa}	0.0^{Ab}	0.0^{Ab}	0.0^{Ab}
C. citratus	100.0^{Aa}	100.0^{Aa}	100.0^{Aa}	0.0^{Ab}	0.0^{Ab}	0.0^{Ab}
C. martini	100.0^{Aa}	100.0^{Aa}	100.0^{Aa}	0.0^{Ab}	0.0^{Ab}	0.0^{Ab}
C. zeylanicum	100.0^{Aa}	100.0^{Aa}	100.0^{Aa}	0.0^{Ab}	0.0^{Ab}	0.0^{Ab}
E. citriodora	100.0^{Aa}	100.0^{Aa}	100.0^{Aa}	100.0^{Aa}	100.0^{Aa}	0.0^{Ab}

Means followed by the same uppercase letters in columns and lowercase letters in lines do not differ by the Scott-Knott test ($p \leq 0.0001$).

who stated that the antifungal activity of essential oils is generally attributed to their major compound. It must be considered, however, that in certain cases the antifungal action, as well as its intensity, may be a result of the synergistic effect among the oil constituents. For example, neral and/or geranial activities are potentiated when they are associated with myrcene (Onawunmi et al., 1984).

Effect of essential oils at different doses on *A. solani* conidium germination *in vitro* (Experiment 2)

The effects observed for conidium germination was similar to those observed for *A. solani* mycelial growth (Table 2).

Corroborating the present results, Caccioni and Guizzardi (1994) also detected an inhibitory effect by the same concentrations of *C. zeylanicum* and *Cymbopogon* sp essential oils on *Monilinia laxa, Mucor piriformis* and *Rhizopus stolonifer* mycelial growth and spore germination. *S. aromaticum*, *C. citratus, C. martini*, and *C. zeylanicum* essential oils similarly inhibited conidium germination at 750 µL L $^{-1}$, whereas inhibition by *E. citriodora* oil occurred only at 5000 µL L^{-1} (Table 2).

Mishra and Dubey (1994) observed conidia germination and mycelial growth inhibition in *F. moniliforme, A. flavus* and *A. fumigatus* and other 47 species under *C. citratus* essential oil. On the other hand, these authors explain the importance of the cropping seasons of *C. citratus* on the essential oil compounds and their effect on fungi control.

Effect of essential oils at different doses on the control of early blight caused by *A. solani* in the leaves of *S. lycopersicum (L. esculentum)* Mill. cv. Sta. Clara cultivated under greenhouse conditions (Experiment 3)

Supporting the results obtained in the *in vitro* experiments for mycelial growth (Table 1 and Figure 1) and conidium germination (Table 2), *in vivo* studies indicated a significant reduction in early blight severity in the leaves of *S. lycopersicun* cv. Sta. Clara cultivated under field greenhouse conditions (Figures 2 and 3). However, we can identify the significant dose effect difference by the MIC 50, 90% and Ymax estimated by biological and mathematical model to reduce symptoms by each oil (Table 3).

C. zeylanicum essential oil reached its maximum response (78, 2%) lower than the remaining oils, indicating that even a ten-fold increase in its dose is inefficient (Figure 2, Table 3).

Only *E. citriodora* essential oil at 4.802,64 µL L^{-1} and *C. citratus* oil at 3.172,80 µL L^{-1} was 100% efficient in controlling early blight severity, relative to the remaining species. *S. aromaticum* and *C. martini* oils at 2.998,39 µL L^{-1} had 92% and 2898.642 µL L^{-1} 90% control, respectively (Figures 2 and 3; Table 3).

The chemical compounds observed at this treatment was geranial (46.91%), neral (34.34%), geranyl acetate (6.30%), camphene (1.02%), 6-methyl-5-hepten-2-one (1.28%), linalool (0.82%), citronellal (0.25%), isomenthol (1.81%), nerol (0.45%), geraniol (3.52%), and α-*trans*-bergamotene (0.85%) (Figure 4).

Nashwa et al. (2012) found that different concentrations of six plant extracts, *O. basilicum*, *A. indica*, *E. chamadulonsis*, *D. stramonium*, *N. oleander*, and *A. sativum,* significantly reduced the early blight disease. The best results for MIC 50 and 90 with minor dose necessary to control was essential oil from *C. citratus* at 341, 32 and 1.822,10 µL L^{-1} respectively (Table 3).

These results are corroborated by Glamočlija et al. (2011) and Geromini et al. (2015) on the effectiveness of Geranial and Neral aldehyde mixture that constitutes citral, a potent bioactive substance that fights against phytopathogens, especially fungi and post-harvest and storage diseases.

Farming under agroecological system is possible with the use of these essential oils. If the farmer has this species in their land, they can proceed to the oil

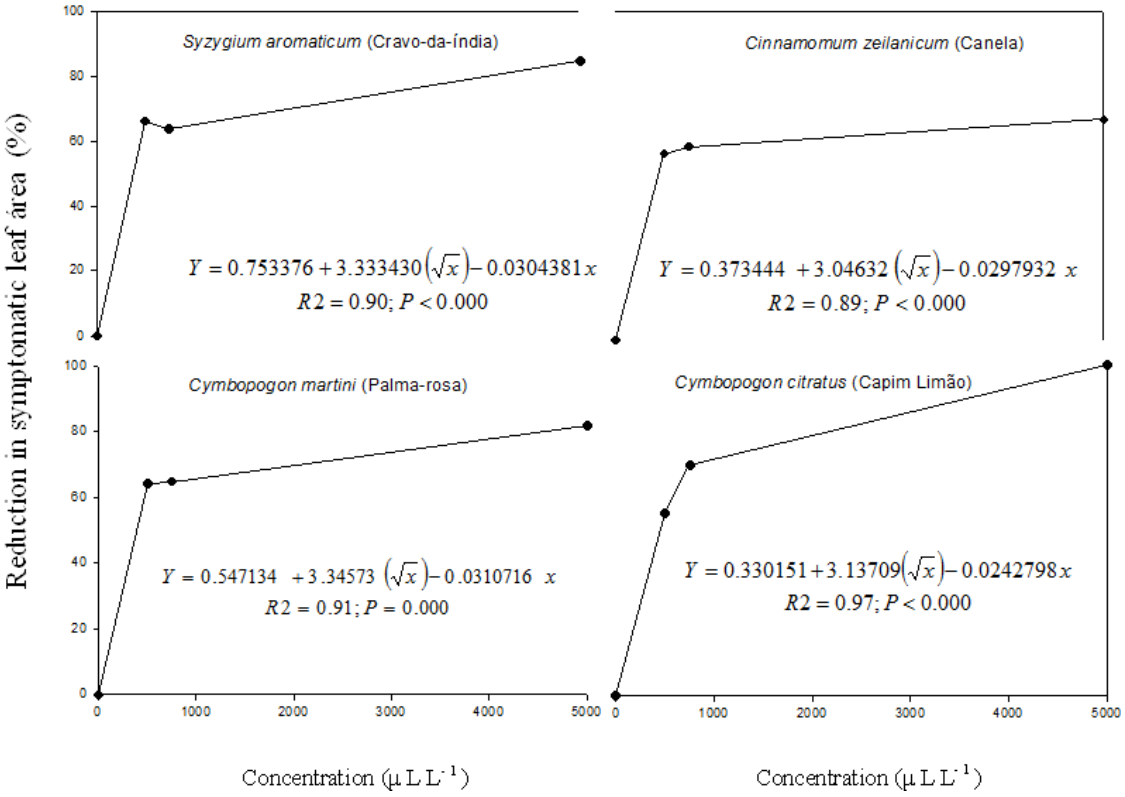


Figure 2. Efficiency of essential oils in controlling *A. solani* symptom severity in *S. lycopersicum (L. esculentum)* leaf area at 57 days after transplant under greenhouse conditions. Tested concentrations: 0, 500, 750 and 5000 µL L^{-1}. Equations tested by "F" method ($p \leq 0.0001$).

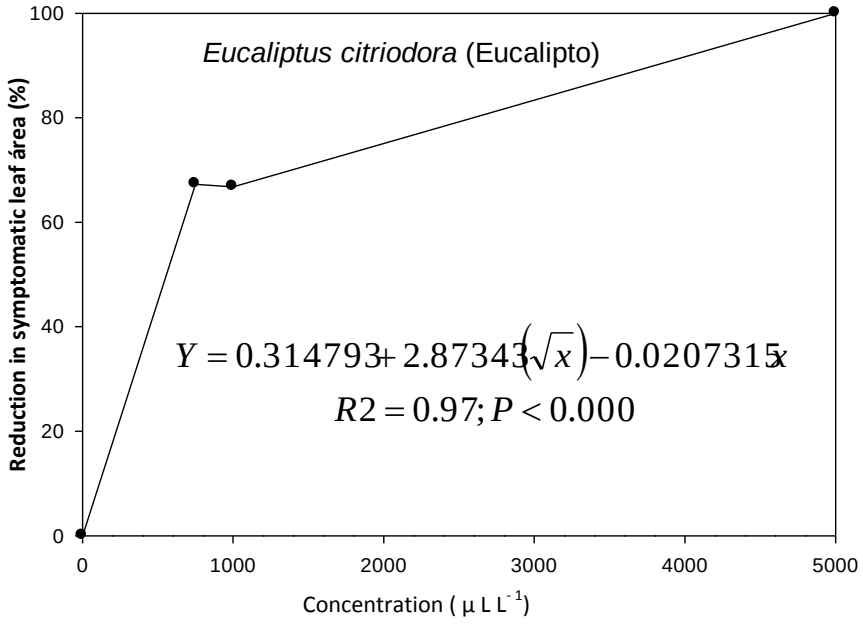


Figure 3. Efficiency of *E. citriodora* essential oil in controlling *A. solani* symptom severity in *S. lycopersicum (L. esculentum)* leaf area at 57 days after transplant under greenhouse conditions. Tested concentrations: 0, 750, 1000, and 5000 µL L^{-1}. Equations tested by "F" method ($p \leq 0.0001$).

Table 3. Inhibitory concentration (MIC 50, 90 and maximum effect) of different essential oils and their on *A. solani* symptoms reduction in tomato plants at experimental under field greenhouse conditions from response models at Figures 2 and 3.

Oils	Inhibitory concentration per Reduction in symptomatic leaf area (%)			
	MIC 50 (µL L $^{-1}$)	MIC 90 (µL L $^{-1}$)	MIC. Max. (µL L $^{-1}$)	Ŷ Max. (%)
S. aromaticum	309.86	2.172.86	2.998.39	92
C. citratus	341.32	1.822.10	3.172.80	100
C. martini	312.78	2.440.25	2.898.64	90
C. zeylanicum	413.50	*ni	2.613.71	78
E. citriodora	410.05	2.253.39	4.802.64	100

*ni = oil is not efficient at this level; Equations tested by "F" method ($p \leq 0.0001$).

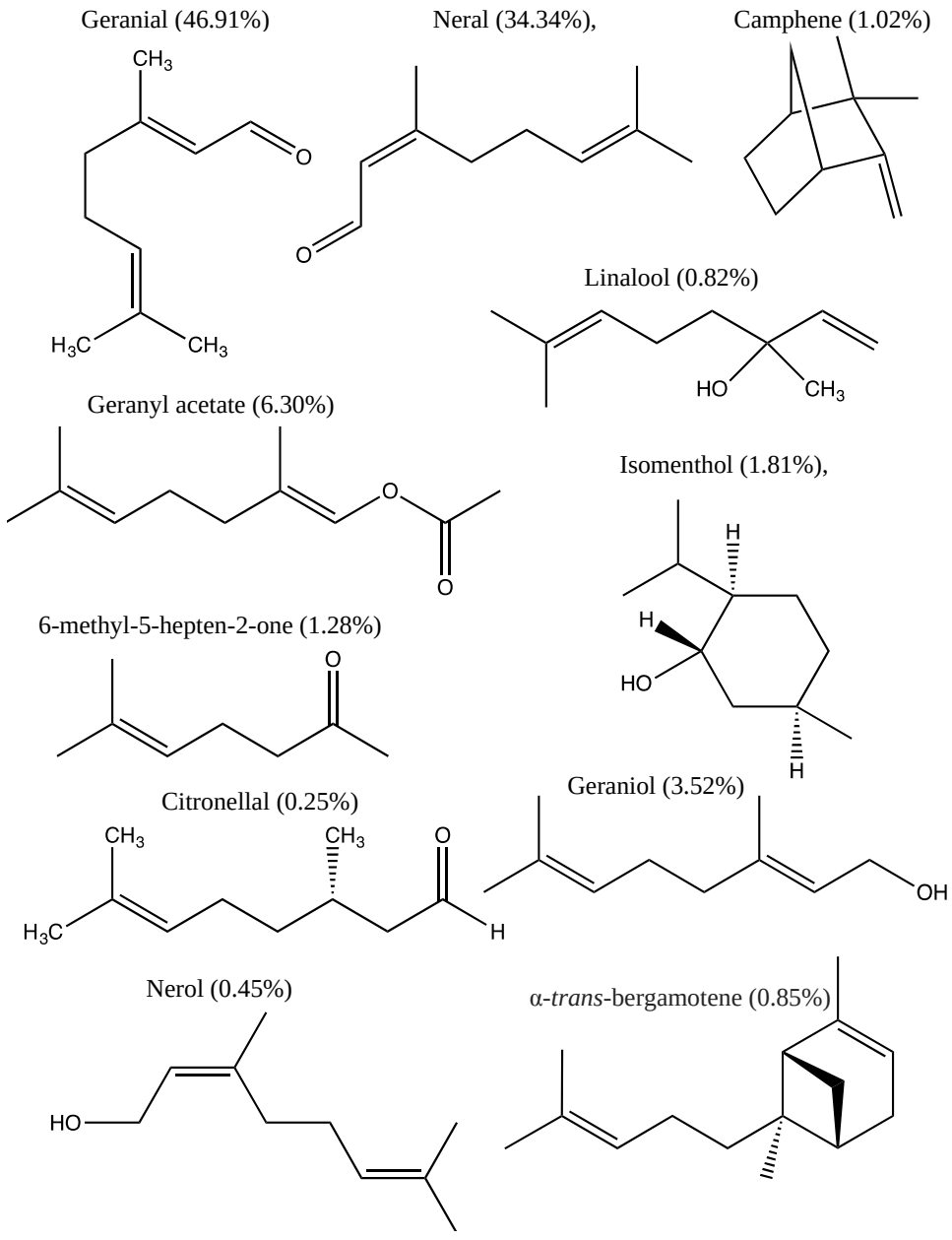


Figure 4. Chemical structure by compounds detected on *C. citratus* oil used on this experiment. GC/MS and Chemdraw Prime 15.0.

extraction by using steam distillation, and MIC 90 with a spreader-sticker. However, two applications are needed per week. Moreover, it is now also possible to develop a new product based on natural compound with more studies around formulation and chemical stability on field.

Conclusion

According to the results obtained in the present experiments, technological studies are recommended for the development of new phytosanitary products based on essential oils of C. citratus, and E. citriodora and possibly S. aromaticum and C. martini for the control of A. solani in tomato crop.

Conflict of Interests

The authors have not declared any conflict of interests.

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Comparison of mapping soybean areas in Brazil through perceptron neural networks and vegetation indices

Carlos Antonio da Silva Junior[1]*, Marcos Rafael Nanni[2], Paulo Eduardo Teodoro[3], Guilherme Fernando Capristo Silva[2], Mendelson Guerreiro de Lima[1] and Marta Eri[4]

[1]Geotechnology Applied in Agriculture and Forest (GAAF), State University of Mato Grosso (UNEMAT), Alta Floresta, Mato Grosso, Brazil.
[2]Federal University of Viçosa (UFV), Viçosa, Minas Gerais, Brazil.
[3]Department of Agronomy (DAG), State University of Maringá (UEM), Maringá, Paraná, Brazil.
[4]University of East Anglia (UEA), Norwich, United Kingdom.

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This study aimed to develop and evaluate the Artificial Neural Networks (ANNs) settings to differentiate and estimate areas of soybean by employing the vegetation index with and without time series. Study area comprises the state of Paraná, South Brazil. The images used to process the ANN were Normalized Difference Vegetation Index (NDVI), Perpendicular Vegetation Index (PVI) and Enhanced Vegetation Index (EVI) indices, including Julian day 017 and Crop Enhanced Index (CEI), which were derived from time series MOD13Q1 product from MODIS sensor Terra satellite. The samples were demarcated into polygon soybean, non-soybean and others (mainly streams). ANN architecture was performed by the module classification employing Multi-layer Perceptron (MLP) artificial neural networks trained by using back propagation algorithm. CEI as a vegetation index with timed series discrimination of soybean areas, pixels with higher than 0.28 rates, proved to be equivalent to ANN to separate soybean areas. Kappa parameter of 0.40 and 0.34 for CEI index and ANN, respectively, it was found in mapped areas. The major and unique contribution of the current study for remote sensing in agriculture was to show that vegetation indices coupled with artificial neural network techniques may improve the results of crop mapping, especially in soybean areas.

Key words: Back propagation neural network, remote sensing, perpendicular vegetation index (PVI), enhanced vegetation index (EVI), crop enhancement index (CEI), time-series.

INTRODUCTION

It is important and necessary to monitor crops and other agricultural activities in the country, mainly because some cultivated areas are carried out by government agencies in subjective forms. In recent decades the emergence of

*Corresponding author: E-mail: carlosjr@unemat.br.

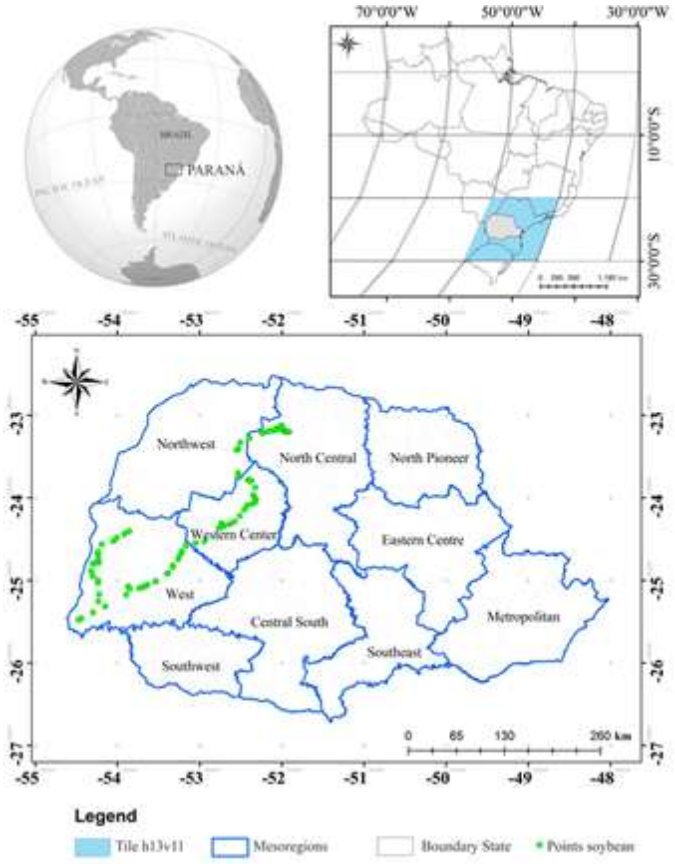

Figure 1. Study area corresponding to the state of Paraná, Brazil.

remote sensing, particularly the orbital level, made it possible to monitor and track the spatial and temporal evolution changes in the Earth's surface and began objective studied (especially with the use of vegetation indices). This was done with expectations on the remote sensing, and to meet the new methodological challenges for interpretation of low spatial resolution images. Also, in Brazil, there is no mapping for the soybean crop, only presentation of subjective numbers of agricultural areas.

Monitoring the Earth's surface and vegetation dynamics by remote sensing techniques is highly important for agriculture. In fact, crop scientists have employed these techniques mainly to assess plant biophysical parameters (Bsaibes et al., 2009; Duveiller et al., 2011) and acreage estimates (Pan et al., 2012).

The systematic mapping of agricultural crops in Brazil is a challenge, mainly due to the large size of the country and wide variety of crops. The systematization of soybean crop is highly relevant to the country due to its economic importance.

Artificial neural networks (ANNs) are computational techniques that use mathematical models to assess several issues by simulating the neuron organization of the human brain. They are commonly referred to as artificial intelligence (AI) and their principle factor is based on taking decisions through learning techniques and acquiring knowledge experience indicated by a user, that is, learning, making mistakes and going on to new discoveries. As the nervous system is composed of several neurons, ANNs is also formed by units of small modules which simulate the neuron mechanism (Haykin, 2008).

Weights in ANNs depend on real inputs and on the internal state of the network so that they may produce models for learning patterns. This allows the use of algorithms in economic and statistical analysis. Since multilayer network is one of the most used, it will be applied in the current study for forecasting exertion.

Artificial neural networks classifiers have been successfully employed to solve several issues in agriculture, such as soybean flowering and physiological maturity (Elizondo et al., 1994), soybean prediction yield (Kaul et al., 2005), maize grain quality (Liao et al., 1993), classification of grains as seeds (Luo et al., 1999), and maize yield (Liu et al., 2001).

This study was carried out aiming to develop and evaluate ANNs settings to differentiate and estimate areas of soybean through a vegetation index, with and without time series.

MATERIALS AND METHODS

Study area

The study area comprises the state of Paraná, southern Brazil, between 22º29' and 26°43' S and between 48º20' and 54°38' W (Figure 1). The average altitude ranges between the highest, above 600 m (52% of the state´s landmass) and the lowest, below 300 m (3% of the state´s landmass). According to *Köppen* classification, the climate is characterized by three predominant types: Cfa (subtropical with well distributed rainfall during the year and hot summers); Cfb (subtropical with well distributed rainfall during the year and mild summers); Cwa (subtropical with hot summers and dry winters).

An early aspect that should be taken into account in the remote-mapping of soybean is its phenology as a progressive process. Therefore, the analysis of soybean cultivation involves the use of a time series of satellite images. To cope with this situation, the acquisition of images has been selected to cover the study area in different periods of the crop and to highlight the soybean temporal dynamics, while allowing a better distinction from other land cover classes.

Data and equations of vegetation indices

Values of Enhanced Vegetation Index, Equation 1 (EVI), MOD13Q1 product, tile h13v11, collection 5.0, composite by MODIS images of 16 days (Huete et al., 1997) from Terra satellite, with spatial resolution of 250 m, downloaded from USGS LP-DAAC, it were used. One of its most important features is displaying the same temporal resolution almost every day and producing images with 12-bit quantization in 36 spectral bands.

$$EVI = g.\frac{\rho_{NIR} - \rho_B}{\rho_{NIR} + (c_1.\rho_R) - (c_2.\rho_B) + l} \quad (1)$$

Table 1. Dates of MODIS images for the time series used from the soil preparation to the final stage of soybean.

Julian day	Date	Year
161	6/10	2010
177	6/26	2010
193	7/12	2010
209	7/28	2010
225	8/13	2010
321	11/17	2010
337	12/3	2010
353	12/19	2010
001	1/1	2011
017	1/17	2011
033	2/2	2011
049	2/18	2011

Wherein, ρ_{NIR}, ρ_R and ρ_B - are red and blue, respectively, in the spectral reflectance near-infrared,; g - the gain factor (2.5); c_1 and c_2 - are the coefficients of correction for the atmospheric effects to red (6) blue (7.5), respectively; l - is the correction factor for soil interference.

EVI is an index designed to mitigate the effects of soil (adjusted by SAVI - Soil-adjusted Vegetation Index) and atmosphere (adjusted by ARVI - Atmospherically Resistant Vegetation Index) in vegetation mapping. Besides EVI, the study used subsequent analyses in images contained in MOD13Q1 product: Blue, red, near-infrared and Normalized Difference Vegetation Index - NDVI (Equation 2):

$$NDVI = \frac{\rho_{NIR} - \rho_R}{\rho_{NIR} + \rho_R} \quad (2)$$

Where ρ_{NIR} and ρ_R are respectively the spectral reflectance in near-infrared and red.

All images, originally obtained in Hierarchical Data Format (HDF) and sinusoidal projection format; it was processed according to the development of automated batch routines. Consequently, data have been redesigned to coordinate Lat./Long., DATUM WGS-84 (World Geodetic System 1984) and automatically converted to GeoTIFF format. The corresponding time series dates used are shown in Table 1.

To calculate the Crop Enhancement Index (CEI), developed by Rizzi et al. (2009), the maximum and minimum EVI planting culture and vegetative growth were employed, respectively, and the corresponding dates in Table 1 were used.

The preference of these time intervals was based on the agricultural calendar of soybean in the state of Paraná, featuring top soil tillage (liming and fertilization), harvest and desiccation (late March) and the beginning of sowing (between October and November). CEI index was calculated from these values by Equation 3:

$$CEI = g * \frac{(MaxEVI + S) - (MinEVI + S)}{(MaxEVI + S) + (MinEVI + S)} \quad (3)$$

Where: MaxEVI = maximum EVI at maximum development of the crop; MinEVI = minimum value of EVI observed in pre-planting and/or emergency; S = coefficient of enhancement (10^2); g = gain factor (10^2).

The value of CEI index (Rizzi et al., 2009) varies between [-1] and [+1], which allows scanning large positive differences respectively between maximum and minimum EVI observed along the soybean crop period. High CEI values indicate, as reflectance pixel, a high probability of soybean. The index is treated individually with time series and exclusive use for pattern recognition of soybean. However, it was used in the ANNs preparing to verify their integrity in soybean contribution patterns. ANNs is therefore composed of indexes with and without time series.

Perpendicular Vegetation Index (PVI) was calculated (Equation 4) for MODIS image 017 Julian day to mitigate reflectance of soil. In fact, soil is present in soybean early stages, causing a spectral mixture. For PVI execution, regressions of soil line were performed by spectral bands of red and near-infrared, following Nanni and Demattê (2006).

$$PVI = \frac{\rho_{NIR} - a\rho_R - b}{\sqrt{1 + a^2}} \quad (4)$$

Where *a* and *b* are the slope and intercept of the soil line, respectively, which feature band ρ_{NIR} as the dependent variable.

The images used for ANNs processing were NDVI, PVI and EVI indices, both Julian day 017 and CEI index, derived from time series MOD13Q1 product from MODIS sensor Terra satellite. Julian day 017 was used because on that day the soybean fields of Paraná are at their maximum vegetative vigor, thus we could obtain their maximum reflectance.

Artificial Neural Networks (ANNs)

ANNs training comprised the methodology processes described by Andrade et al. (2013). The definition of samples and their spectral signatures, ANNs architecture and training definition, network application and the achievement of the classified map were thus performed. Samples were then defined: Soybean, non-soybean and other land uses. IDRISI Selva platform merely recognized two classes and presented an error in the final classification map.

All ANNs process was performed in IDRISI Selva platform

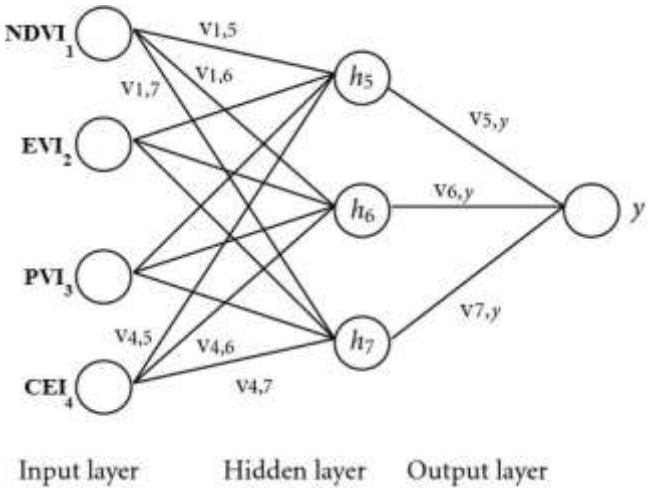

Figure 2. Structure of three-layer MLP neural networks with 4 input nodes, 3 hidden nodes, and 1 output node. Each hidden layer is directly connected to each component of the input layer and to each components of the output layer.

developed by the Graduate School of Geography, Clark University. The platform provides resources for environmental management and natural resources by employing time series or changes, decision analysis by multiple criteria and uncertain analysis. Three classifiers were implemented for the ANN module: Multi-layer perceptron (MLP), Self-organizing map (SOM) and Fuzzy ARTMAP.

The use of artificial neural networks in agriculture human vision for trial training assumes an important role. Visual selection in the form of polygons in creating swatch processes is an example of such judgments. Samples of training polygons are important because it has strong links with the final yield, that is, it works in the perception of identifying plots of agricultural areas, reflectance, and texture, among others.

Between 50 and 65 polygons of various sizes were defined and selected in this segment to provide better details of the areas where soybean was being cultivated. Samples in soybean polygons, non-soybean polygons and other uses (mainly watercourses) were defined for the process. Spectral thresholds of each image were then defined by the Makesig module, which consists of extracting spectral information from each image based on selected samples. With regards to the ANNs architecture, the classification module by artificial neural networks, type Multi-Layer Perceptron (MLP), it was performed. Output layer elements were defined by the number of classes to be demarcated in the image. In this case, they comprised three nodes with identified values in the making of the samples and processed by Makesig module. Certain tests were deployed for layer quantities, number of neurons per layer and activation function so that the best configuration of the architecture for soybean crop mapping could be identified.

Artificial neural networks were trained by employing backpropagation algorithm (Haykin, 2008). Backpropagation refers to the method of calculating the sum gradient according to the function of the quadratic error related to weights for a feed-forward network. The latter is a simple but effective application to the chain rule of elementary calculus. It is therefore assumed that there are n classes, m neurons in the hidden layer and one neuron in the output layer, as shown in Figure 2.

Backpropagation learning algorithm is essentially a optimization method able to find the weight coefficients and thresholds for the neural networks and the given training set. The network should be constituted by neurons behavior described by Equation 5.

$$y = S\left(\sum_{i=1}^{N} w_i x_i + \Theta\right) \quad (5)$$

Where *y* is the non-linear output function, S is defined by Equation 6.

$$S(\varphi) = \frac{1}{1+e^{-\gamma\varphi}} \quad (6)$$

Where γ determines the curve steepness in the origin of coordinates. Input and output values are assumed to be in the range [0, 1]. In the following formulas, the parameter *o* denotes the output layer, *h* the hidden layer, and *i*, *j* the indexes. Index *i* catalogs output neurons and the index *j* their inputs. This means *i*-th neuron output of the hidden layer and means the weight connecting *i*-th neuron of the output layer and *j*-th neuron of the previous hidden layer.

Heuristic method was employed for this step by varying the training configuration of the following parameters: Learning rate, momentum rate, number of training samples (pixels) and repetitions. When the network is trained, the distinction of classes is undertaken and identified as soybean and non-soybean to generate the final classified map.

The performance of artificial neural networks settings were evaluated with Root Mean Square Error (RMSE), prediction accuracy and Standard Error of Prediction (SEP). Furthermore, the coefficient of correlation (r) between the predicted and outputs, coupled with the slope and intercept of the linear regression model, it was also employed. RMSE was calculated by Equation 7.

$$RMSE = \sqrt{MSE} = \sqrt{\frac{SSE}{n-p}} \quad (7)$$

Where *n* is the number of observations; *p* is the number of parameters to be estimated; *SSE* and *MSE* are the sum of quadratic errors and mean square error, respectively.

Neurons between adjacent layers that define the neural networks structure (Figure 2) are not completely bonded because a specific entry (or all of them) affects each output.

For example, the first exit, that is h5 (V5, y), for the proposed neural networks is a function of four inputs, as shown in Equation 5. A hyperbolic sigmoid tangent transfer function with Equation 8 is applied to the hidden and output layers.

$$f(x) = \frac{2}{1+e^{-2x}}1 \quad (8)$$

Experimental protocol, statistical analysis and field samples

Having the images processed and applying the appropriate classifiers, thematic maps of soybean areas were generated. The complete process of placements and their respective processes are shown in Figure 3.

The evaluation of quality thematic maps, vegetation index (CEI) and artificial neural networks (ANNs) was achieved by using a completely independent set of sample points. This approach was adopted since all rating processes could be evaluated by such method.

A GPS device, Trimble GeoExplorer 2008 Series, it was used for an independent samples set. The state of Paraná was covered and coordinates containing soybean crops, totaling 172 sites, detailed

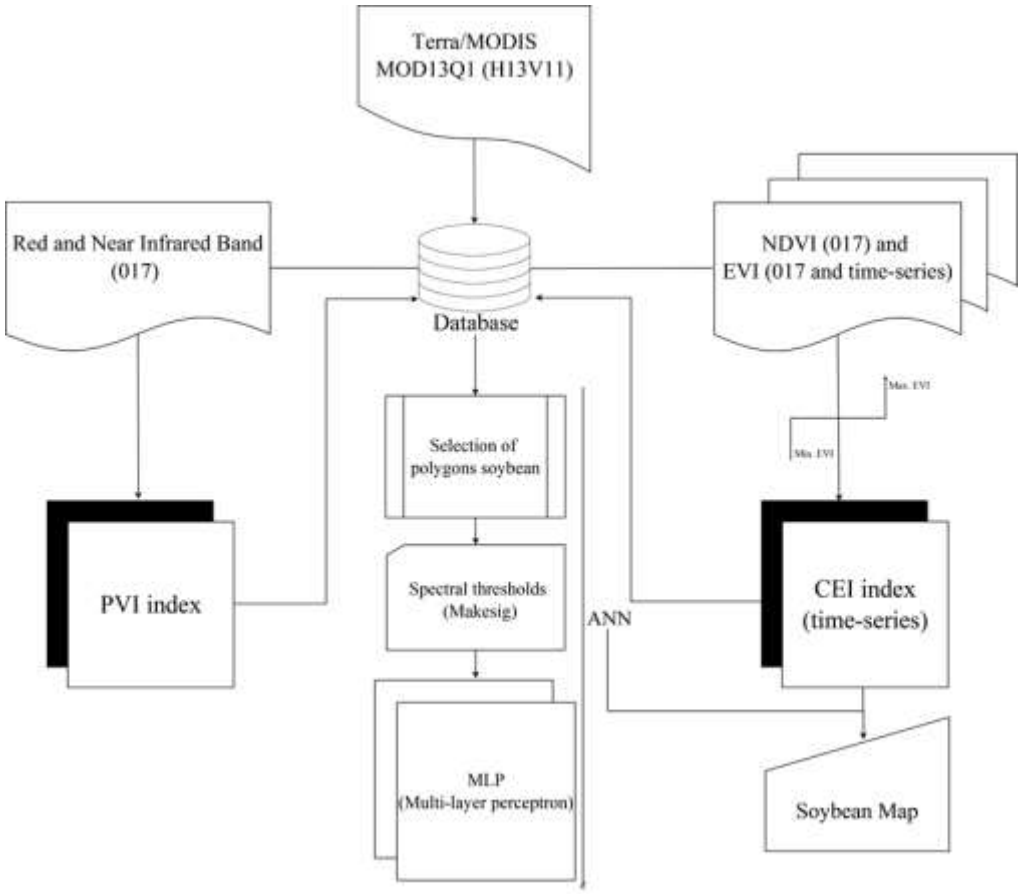

Figure 3. Flowchart of the methodology used to generate the thematic maps.

by green points in Figure 1. Non-soybean sites were collected by a qualified expert through a visual interpretation of a time series from MODIS images (Freitas et al., 2011). The 346 points distributed throughout the area were generated randomly and independently. The size of this set of samples may be considered satisfactory according to Congalton and Green (2009).

The quality of the classification was assessed quantitatively by the coefficients of Overall Accuracy (OA) and Kappa index (κ) (Equations 9 and 10, respectively), both derived from the confusion matrix (Congalton and Green, 2009). Further, errors and accuracy from the point of view of producers and users were taken (Antunes et al., 2012; Johann et al., 2012). These measurements provided a better assessment of the final classification of soybean areas.

$$OA = \left(\frac{\sum_{i=1}^{k} x_{ii}}{N} \right) \quad (9)$$

$$\kappa = \frac{N\sum_{i=1}^{k} x_{ii} - \sum_{i=1}^{k} (x_{i+}.x_{+i})}{N^2 - \sum_{i=1}^{k} (x_{i+}.x_{+i})} \quad (10)$$

Where: κ = estimated Kappa; k = number of rows; x_{ii} = number of observations in row i and column i; $\sum_{i=1}^{k} x_{ii}$ = the sum of the matrix elements on its main diagonal; x_{i+} = total sum of observations for lines; x_{+i} = total sum of observations for columns ; N = total number of observations.

With the given rates and under the hypothesis of equality between two ratios arising accuracy of different classifications (κ1 = κ2), the statistical test was performed by Equations 11 to 13.

$$Z = \frac{(\hat{\kappa}_1 - \hat{\kappa}_2) - (\kappa_1 - \kappa_2)}{\sqrt{\sigma^2(\hat{\kappa}_1) + \sigma^2(\hat{\kappa}_2)}} \sim N(0,1) \quad (11)$$

$$Z = \frac{\hat{\kappa} - \kappa}{\sqrt{\sigma^2(\hat{\kappa})}} \sim N(0,1) \quad (12)$$

$$\sigma^2(\hat{\kappa}) = \frac{1}{n}\left[\frac{\theta_1(1-\theta_1)}{(1-\theta_2)^2} + \frac{2(1-\theta_1)(2\theta_1\theta_2 - \theta_3)}{(1-\theta_2)^3} + \frac{(1-\theta_1)^2(\theta_4 - 4\theta)_2^2}{(1-\theta_2)^4} \right] \quad (13)$$

Table 2. Confusion matrix of classifications for different methods to estimate soybean area and their respective rates of κ and OA parameters.

		Reference			
		CEI			
		Soybean	**Non-Soybean**	∑	
Classification	Soybean	75	07	82	κ = 0.40
	Non-Soybean	97	167	264	OA = 0.70
	∑	172	174	346	
		ANNs			
Classification	Soybean	67	08	75	κ = 0.34
	Non-Soybean	105	166	271	OA = 0.67
	∑	172	174	346	
				Z = 0.88	p-value = 0.1891^{ns}

Where:

$$\theta_1 = \frac{\sum_{k=1}^{c} x_{kk}}{n}; \theta_2 = \frac{\sum_{k=1}^{c} x_{k+} x_{+k}}{n^2}; \theta_3 = \frac{\sum_{k=1}^{c} x_{kk}(x_{k+} + x_{+k})}{n^2}; \theta_4 = \frac{\sum_{i=1}^{c} \sum_{j=1}^{c} x_{ij}(x_{i+} + x_{+j})}{n^3}.$$

Finally, the estimated soybean area was compared with that provided by the Automatic Recovery System (SIDRA) of the Brazilian Institute of Geography and Statistics (IBGE), a parameter to check the mapping evaluation. This step could only be performed for the 2010/2011 harvest, since the agency has yet not published the soybean-sown areas for each municipality.

RESULTS AND DISCUSSION

When analyzing the data obtained by the methods used in the estimates of soybean areas (CEI and ANN), we found that classifications were equal to Z test reference for the Kappa (κ) and Overall Accuracy (OA) parameters (Table 2). However, the classification by artificial neural networks (ANN) had a lower κ rate, probably caused by a user on neurons training, that is, in the selected polygons. The κ rate shows the conformity of the classification obtained.

Kappa index has advantages over the Overall Accuracy parameter because it incorporates all the elements of the error matrix, objects classified correctly or not. Moreover, κ assesses thematic accuracy since it is more sensitive to changes caused by accuracy mistakes from users and producers and evaluates the spatial coincidence between two situations. Also, regarding the OA, accuracy score stretched to 70%. According to Foody (2002), an accuracy score index over 85% is desirable. Score rates for OA were lower than those considered desirable for CEI index and ANN.

CEI index was actually expected to be equal by Z test with regard to ANNs result because the index was part of the MLP´s input layer.

The κ, evaluating the agreement or disagreement between classifications, ranged between 0.34 (ANN) and 0.40 (CEI) (Table 2) and is of a reasonable quality (κ> 0.21 and 0.81) according to the classification described by Landis and Koch (1977). Therefore, thematic maps, generated for the soybean crops, mainly when CEI is employed, approached field reality. Rates were even more significant when compared with studies that used the same sensor, albeit with other methodologies. For instance, Lamparelli et al. (2008) obtained Kappa parameter between 0.60 and 0.80 when they estimated soybean crops with MODIS data.

Therefore, classifiers evaluation showed that those based on pixel by pixel structure, for example, ANNs which requires sample acquisition by the user, it have more refined and highly accurate results. Further, combination with CEI improved results since it took into account pixel values in the classification. In other words, assigned class depends on the observed value of the pixel.

The error from producer's and user's point of view was used to measure the accuracy of each category (soybean and non-soybean) (Table 3) through the analysis of errors inclusion and omission in ratings (Antunes et al., 2012). When producer's and user's data errors (inclusion and omission, respectively) were analyzed, it was underpinned that the lowest value for the inclusion error class for soybeans was obtained by CEI index, with a 0.56 error, whereas the error of omission was 0.08 for the same class. This sums up to the fact that 56% of areas classified as 0.92% soybean crop area actually belonged to that class (Table 3). Rudorff et al. (2007), estimating soybean with MODIS in the state of Rio Grande do Sul (Brazil), obtained 76.17% parameters from Overall Accuracy and 0.503 for Kappa. Pax-Lenney and Woodcock (1997) considered the above a good performance. Thus, producer's error occurs when an object is included in the class to which it does not belong and user's error occurs when an object is deleted from the class it belongs to (Johann et al., 2012).

On the other hand, regarding to the non-soybean class (Table 3), the value obtained in the producer's error by CEI and ANN, 0.04 and 0.05 respectively, should be

Table 3. Producer's and user's accuracy point of view with regard to matrix errors for soybean and non-soybean class constructed from images.

Analysis method		Producer's Accuracy	User's Accuracy
Soybean	CEI	0.44	0.91
	ANN	0.39	0.89
Non-Soybean	CEI	0.96	0.63
	ANN	0.95	0.61

Table 4. Settings used to perform tests for artificial neural networks for the discrimination of soybean areas.

Settings	Pixel	Hidden layers	Neurons		Rate of learning	Momentum	Iterations	RMS		Accuracy (%)
			1º Layer	2º Layer				Train.	Test	
1	1515	1	6	-	0.01	0.55	10000	0.2896	0.2903	83.32
2	**1473**	**1**	**7**	**-**	**0.01**	**0.50**	**10000**	**0.2796**	**0.2832**	**85.01**
3	1457	1	8	-	0.01	0.55	10000	0.3008	0.2991	82.16
4	1518	2	10	6	0.01	0.50	10000	0.2602	0.2738	83.23
5	1463	2	6	5	0.01	0.55	10000	0.2664	0.2651	85.52

enhanced. The same may be said with regard to user's accuracy since the value reached 0.39, with the consequent underestimation of soybean ratings and an increase in non-soybean estimates.

Similar results for both ratings were probably due to the fact that, different from other classifiers, techniques took into account other attributes rather than the mere spectral response of each pixel. Different attributes are taken into account within the space in which targets are inserted from the sampling process. However, in the non-soybean ratings, a small difference was detected between CEI´s and ANN´s errors since the number of spectral mixing was somewhat generalized for the classifiers.

Determining the number of neurons is the most fundamental problem in neural networks learning, since there is no general rule in the literature that accurately pinpoints the precise ANNs number of neurons to solve a given problem (Hippert and Taylor, 2010). In addition, there are practically no scientific researchers employing ANNs for soybean area studies. Table 4 shows the settings used for testing in an attempt to get a network that would best classify images and highlight the architecture with the best results. ANNs implementation featured a processing time ranging between 10 and 15 hours.

Setting 2, an artificial neural network with 7 neurons in a single hidden layer, it was the architecture with the best performance. Other defined parameters were a set of 1473 pixels by category for the test and training set; A 0.01 learning rate; a 0.5 momentum factor to enhance network convergence. The limit of 10,000 iterations or 0.0001 root mean square error (RMSE) of 0.0001 was established as ANNs training stopping criterion. The ceasing of ANNs training occurred by the number of iterations of the backpropagation algorithm, with the rate of 0.2796 for RMSE for data training and 0.2832 for the test data.

Although setting 2 achieved 85.01 precision rates, or rather, the highest setting chosen, the architecture failed to perform satisfactorily the distinction between soybean and non-soybean class (that is other land uses) when analyzing the final result of the classification. This fact may be due to the great number of layers, more than it was necessary, which caused difficulties in network converging.

Table 4 shows that the neural networks had a better performance with one hidden layer, with the variation in the number of neurons in layer and in the pixel set. Intermediate layers presuppose error estimates which, if in great amounts, lead to network convergence problems. Results obtained in greater areas of soybean revealed that an increase in the number of pixels of the training and test set, the network produced better results with a number of neurons of the first layer, varying between 6 and 10. In smaller areas, the network with the best results also had a small number of neurons in the intermediate layer. The greater the number of neurons in the layer, the greater the complexity of the network and the greater its range in terms of possible solutions (Hippert and Taylor, 2010).

Kappa and Overall Accuracy rates obtained by ANNs classification may have been impaired by environmental factors that affect the image's spectral pattern, such as variations in the phenological stage, vegetative vigor, plant spacing and nearby crops. In other words, due to mean spatial resolution of MODIS/Terra images (that is,

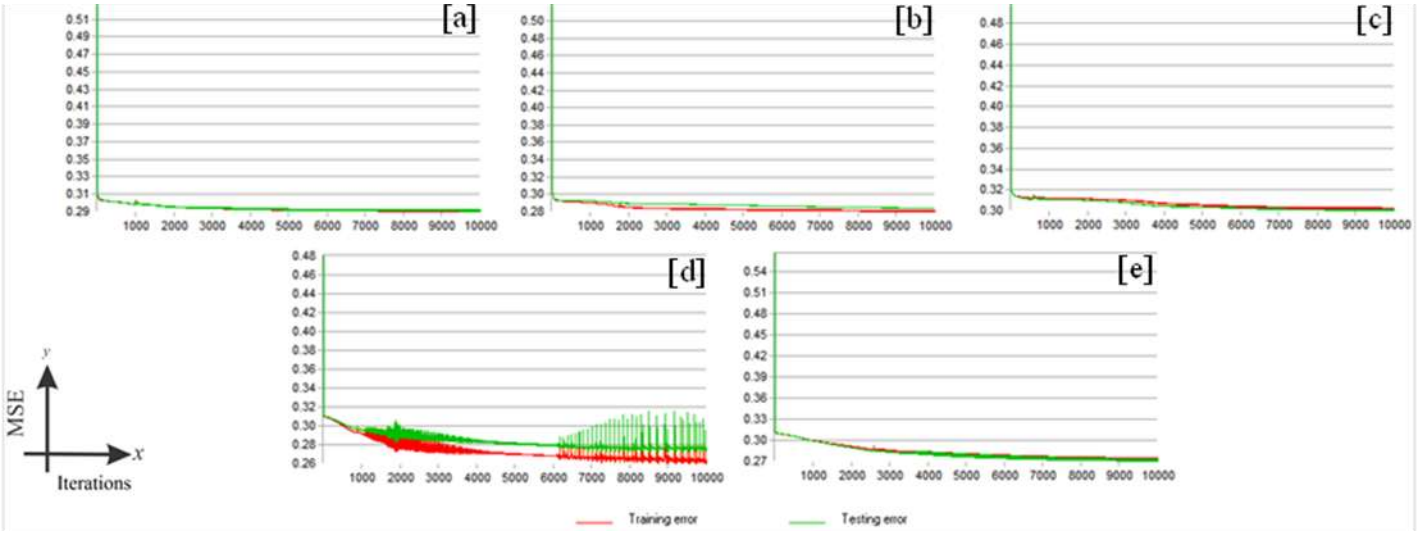

Figure 4. Training and test error for the respective configurations used in artificial neural networks.

Table 5. Estimated soybean areas (hectares) in mesoregions of the state of Paraná, Brazil.

Mesoregion	CEI	ANN
Northwest	118,060.84	91,782.00
Western center	274,906.94	319,579.23
North central	369,490.52	475,709.53
North pioneer	109,441.89	116,620.91
Eastern centre	104,039.44	160,931.76
West	505,982.16	379,364.49
Southwest	33,220.42	32,063.27
Central south	132,288.35	104,406.60
Southeast	40,302.95	52,039.74
Metropolitan	19,823.58	11,914.92
Total	1,707,557.08	1,744,412.45

250 m).

Figure 4 illustrates the training progress of an MLP in training and test error. It shows the number of learned patterns versus formation of iterations. The total number of learned patterns was 10,000. In the case of setting 2 learning (Table 4), the parameters were much more conservative as to the formation of training and error tests (Figure 4b). In the case of less than 10,000 times of training iteration, the RMSE for test data was reduced to less than 0.50 and stabilized at this level (Table 4 and Figure 4).

Quantified results in hectares for soybean crop were generated for each municipality under analysis. Table 5 limits itself to the mesoregions in the state of Paraná; the limitations of current paper make it unfeasible to list the great number of municipalities (399 in total). When compared with official Brazilian data (SIDRA/IBGE), the two techniques (ANNs and CEI) had a performance than lower expected (Figure 8). In the Z test analysis, all techniques used showed that values did not present a 95% probability statistical significance and equality when compared to κ parameters generated by the ratings. This result demonstrates that Table 5 data underestimated the true ground conditions.

Soybean areas estimated in Table 5 showed similarity between CEI and ANN classifications but varied on the underestimation of indexes predicted by official data (SIDRA/IBGE). The same occurred with estimates studies on soybean areas in the state of Maranhão, Brazil, where Silva Junior et al. (2014), using the CEI index, obtained underestimates when comparing government data. Convergence of official Brazilian data with those estimated in current study had been expected (Figure 7) since current analysis has been based on objective methods (statistical) with the use of satellite images, with a spatial resolution of 250 m, contrary to those by

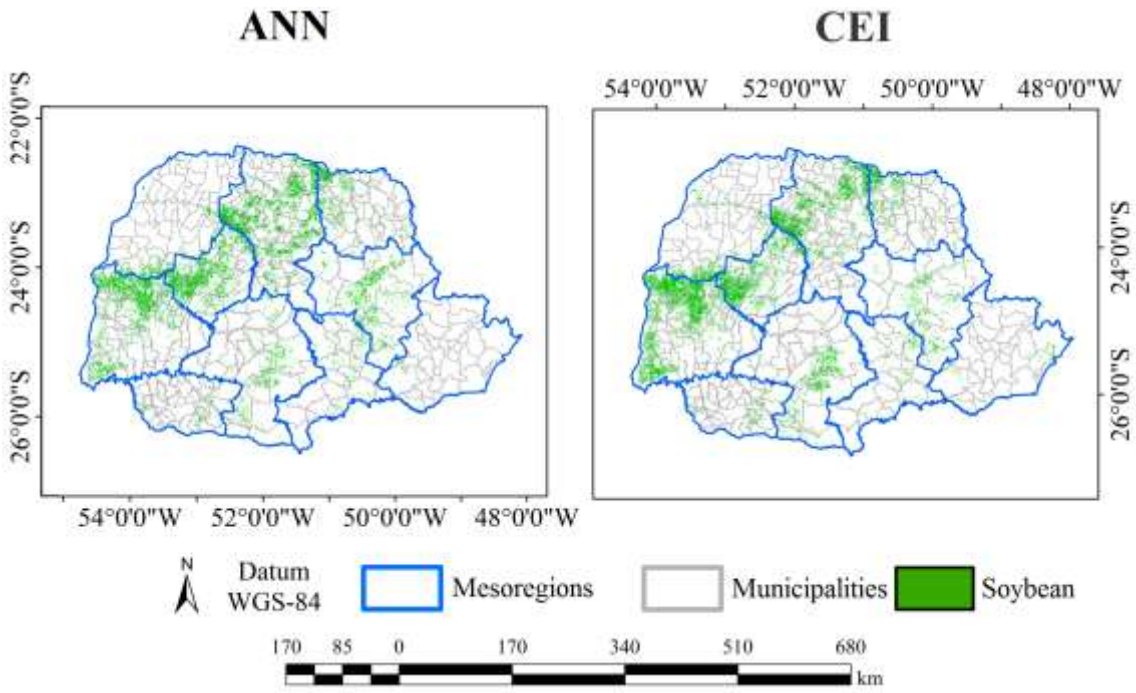

Figure 5. Spatial distribution of areas cultivated with soybean crop according to their respective classification techniques for the state of Paraná, Brazil.

SIDRA/IBGE, which have been obtained subjectively by interviews with producers, meetings with technicians to check sampled planting intentions and amount of inputs sold for the harvest.

However, objective methods also bring uncertainties. They are only confirmed by reliable reality-collected data. Overestimation or underestimation with regard to official data has been proven in several scientific studies by remote sensing techniques in the estimation of agricultural areas (Xiao et al., 2002; Peng and Gitelson, 2012; Wu and Li, 2012). Thus, it may be perceived that the reliability of a given mapping obtained by satellite images is greater when it comes to higher spatial resolution, and thus with increasing details. However, reported results have a great potential for estimating agricultural areas cultivated with soybean, especially in very vast areas. This is a highly positive fact since MODIS sensor data images are not only free (http://modis.gsfc.nasa.gov/), but feature an area of 2,330 km as width, circular orbit, near-polar, helio-synchronous, with a temporal resolution of 1 to 2 days, so that the probability of receiving images free of clouds is increased and favors the mapping of summer crops.

The relationship of artificial neural networks in agriculture, mainly soybean crop, and the mapping of agricultural areas are still scarce, although their use by several researchers can be highlighted. Andrade et al. (2013) identified coffee areas in the state of Minas Gerais, Brazil, where TM/Landsat-5 images were used with ANN-type Multi-layer Perceptron (MLP). The same authors found the method satisfactory with automatic mapping of ANNs. The evaluation of the efficiency of artificial neural networks for classifying land use can be confirmed in a study by Chagas et al. (2009) on levels of pasture degradation obtained by remote sensing imagery.

Figure 5 shows the map of soybean plantations classified by ANN and CEI. There is a similarity between the estimated spatial distribution of soybean areas in CEI and ANN classification. Since classifiers provided underestimation (Table 5), they presented more intensely the colored areas as potential soybean plantation areas. The methodological techniques demonstrated and evaluated relevant potential use for soybean mapping. It may thus supplement state and federal agencies in official surveys of the crop, detailing data for each municipality and furnishing agricultural statistics with even greater rapidity.

In addition to quantitative analysis, a map of disagreements was also produced (Figure 6). It is a thematic map containing pixels which ANNs classifiers labeled the soybean class differently from that by CEI index. The map of disagreement submitted a total of 147.42 pixels, representing 36,855.37 ha, with pixels featuring divergent classification.

Although the Perpendicular Vegetation Index (PVI) lies at ANNs entrance, with decreasing soil effect in its calculation, a spectral mixture of pixels may still be perceived. Figure 6 shows intense divergences in the southern region of the state and in the coastal areas due to the inherent spectral mixture of these pixels and to the

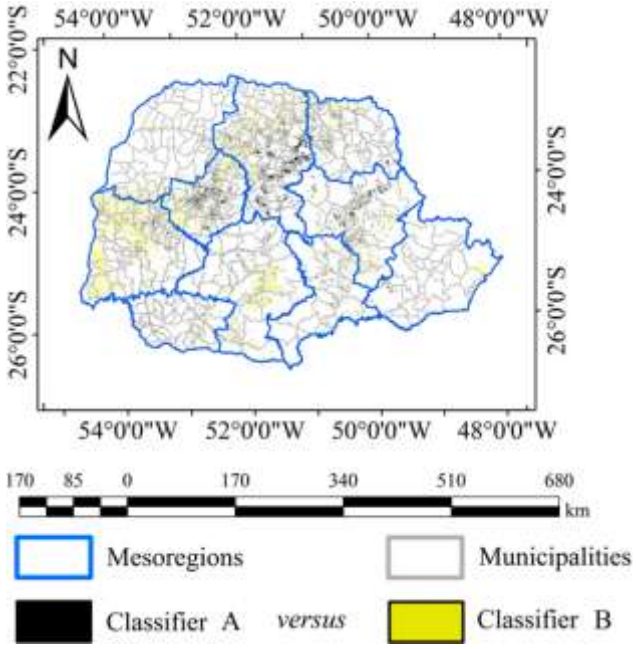

Figure 6. Map of disagreements between CEI map (yellow) and ANNs (black) index.

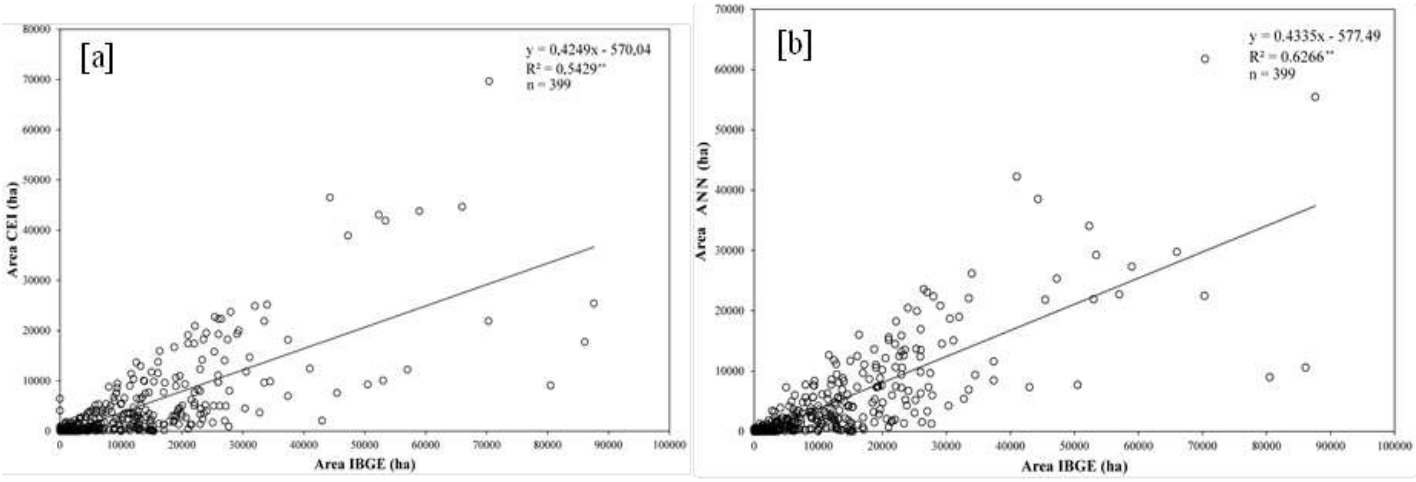

Figure 7. Relationship between soybean planted areas (ha) obtained with MODIS applied CEI index and ANN and the official data (IBGE) in 399 municipalities evaluated in the 2010/2011 crop harvest.

reflection of sand that makes the CEI assigned as soybean for the high reflectance pixels.

The mapping behavior of soybean areas of the state of Paraná (Brazil) conducted by CEI index and ANN is shown in Figure 6. Similarity between the areas mapped by two methods and their distribution throughout the state may be perceived. CEI map (yellow) reported up to 69,669.82 ha in a single municipality, whereas ANNs (black) features a maximum area of 61,000.00 ha of soybean plantations. The advantage of using ANNs with input PVI for the elimination of soil (soil line (Nanni and Demattê, 2006)) in its index should be enhanced. In fact, the coast areas (metropolitan mesoregion) are totally devoid of any soybean plantations. Such confusion occurred because of CEI index and its use of EVI in its calculation.

Figure 7 shows municipal areas obtained by CEI and ANNs (Figure 7ª and b, respectively) and their relationship with the data obtained by Brazilian official agencies (Brazilian Institute of Geography and Statistics - IBGE). Although ANNs showed inferiority in Kappa values (Table 2) when compared to CEI index, the rates in

hectares per municipality had a higher consistency with the data from official organ (R^2 = 0.6266).

Conclusions

This study describes the mapping of soybean areas with artificial neural networks with the implementation of vegetation indices. ANNs provides a new setting method with the aid of an experienced analyst to automate the mapping and the discrimination of soybean plantations in Brazil. The number of input variables of the network is not limited to training, just by changing ANNs configuration. We investigated four widely used vegetation indices, including NDVI, EVI, PVI and CEI, in the soybean area estimation study. The Crop Enhancement Index (CEI) is a technique of vegetation index with time series for discrimination of soybean areas (pixels with values greater than 0.28), it showed equality with ANNs performance in distinguishing the soybean areas. The criterion for ANNs setting featuring superiority in soybean mapping extended the limit of 10,000 iterations or the root mean square (RMS) of 0.0001 to stop the number of iterations of the backpropagation training algorithm with RMS 0.2796 for the training data and 0.2832 for test data. This current study approves MODIS images for the analysis of soybean areas in Brazil since the difficulty is to obtain pictures totally free of clouds. The major and unique contribution of this study for remote sensing in agriculture was to show that vegetation indices combined with the techniques of artificial neural networks could improve the results of crop mapping, especially regarding the soybean plantations. Also, we verified the effectiveness of mapping as a source of information for government agency for real statistical knowledge of the agricultural areas.

Conflict of Interests

The authors have not declared any conflict of interests.

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