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Review

A review of geographic information systems and digital imaging in plant pathology application

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The increasing rate of disease incidence resulting from devastating effects of plant pathogens, limits crop productivity globally, thus affecting food security. The current global population growth with many mouths to feed is dependent on vibrant agricultural productivity. The effects of globalization, climate change, evolution of pathogens and vectors to mention a few, have combined to increase spread of invasive plant pathogens. Consequently, early detection of pathogens, accurate diagnosis and assessment and surveillance are imperative to predicting disease outbreaks and ample time to develop and apply appropriate mitigation measures for crop protection and enhanced productivity. Diagnosis is the process to determine cause of disease, while detection deals with knowing pathogen. Both disease diagnosis and pathogen detection are central to protecting crops and natural plant systems, as well as crucial prelude to undertaking prevention and management measures. Visual assessments of disease in plants populations are unreliable and subjective, arising from human limitations. Failure in pathogen detection and disease diagnosis lead directly to inadequate disease control and reductions in crop production and quality. Geographic Information Systems (GIS) and Digital Imaging (DI) have been applied in plant pathology to improve speed and accuracy of disease assessment, diagnostics and pathogen detection. These new technologies have assisted in collection and analysis of field data in ways that were not possible before advent of computer and thus, minimize human errors. This paper presents a brief review on application of both emerging technologies in plant disease diagnosis and detection.

Key words: Geographic information systems, imaging, plant disease, detection, diagnosis.

INTRODUCTION

Plant disease remains a major threat to global agriculture, accounting for about 10% reduction in crop yield (Strange and Scott, 2005). Broadly speaking,

disease is defined as anything that adversely affects plant health. A conservative definition usually includes a persistent irritation, resulting in plant damage. In present

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context, it includes only those (living) things that replicate themselves and spread to adjacent plants to cause abnormalities. This includes such biological organisms as nematodes, fungi, bacteria, and viruses, which are sometimes referred to as pathogenic microorganisms. Their presence remains a challenge to plant health across the globe, especially resource-poor farmers in developing countries, whose limited resources are unable to deal with disease outbreaks (Mutka and Bart, 2015). Successful control of most plant diseases depends on early detection and accurate diagnosis. The terms diagnosis and detection were often used interchangeably. Diagnosis is the process of determining cause of a problem (disease) through careful examination; whereas detection is to find out pathogen responsible for disease (Kumar and Sreenivasulu, 2009). Diagnosis is as much an art as it is a science. The science deals with technology applied to detect pathogens; while art involves synthesis of information obtained from history case, symptoms and results of laboratory tests to determine pathogen that causes disease (Kumar et al., 2009). Diagnosis can be a long or short process, depending on diagnostician's expertise and nature of problem. Once cause, is known an appropriate control strategy can be developed. Thus, inaccurate identification of disease and disease-causing agent, affect disease control measures and can lead to a waste of time and money and further plant losses. Therefore, proper disease diagnosis and early detection are vital in disease management to prevent the establishment and dispersal of pests and pathogens after introduction and to minimize subsequent impact (Cook and Madden, 2002; Myerson and Reaser, 2002).

PLANT DISEASE DIAGNOSIS METHODS

Visual assessment

Traditionally, plant disease diagnosis normally relies on symptoms recognition through visual observation and rating based on these symptoms and signs. Symptoms are physical characteristics of disease expressed by plant and include wilt, galls, cankers, rots, necrosis, chlorosis, and general decline; while signs are physical evidence of pathogen causing disease. Signs can include fungal fruiting bodies such as mushrooms or pycnidia, mycelia, bacterial slime, presence of nematodes or insects, or insect holes presence. Literature is replete with different assessment methods in plant disease diagnosis, including Large (1966), James (1971) Cobb (1892), Horsfall and Barratt (1945), Chester (1950). In addition, Stover (1983), Gauhl et al. (1993) and Nwauzoma et al (2008) have given further insight on diagnosis of Sigatoka leaf spot diseases of banana and other leaf spot diseases using visual assessment. Disease diagnosis based on symptoms is unreliable because different pathogens may

cause similar symptoms as was Banana streak virus (BSV) case and Cucumber mosaic virus (CMV), resulting in considerable confusion (Wardlaw, 1961; Stover, 1972) and this is common with most foliar pathogens. Moreover, visual assessment depends on subjectivity of raters and often lack accuracy, reproducibility and traceability. Barbedo (2013), citing Bock et al. (2010) listed some shortcomings associated with visual assessment of disease symptoms:

1. Raters may get tired and lose concentration, thus decreasing their accuracy.
2. There can be substantial inter- and intra-rater variability (subjectivity).
3. There is a need to develop standard area diagrams to aide assessment.
4. Training may need to repeat to maintain quality. Raters are expensive.
5. Visual rating can be destructive if samples collected in field for assessment was later taken to laboratory.
6. Raters are prone to various illusions (for example, lesion number/size and area infected).

Microscopy

Usually, a plant pathology diagnostician relies on a combination of gross symptomatology and microscopic images to make a disease diagnosis. Yield losses from foliar diseases can reach as high as 58% when infections in field occur early and environmental conditions favor disease spread and development (Berry et al., 2000). Early detection and diagnosis of disease with timely applications of foliar fungicides are necessary to avoid devastating losses. Rapid diagnosis of plant pathogens is also critical because some fungicides cannot be applied after a certain stage in plant's maturity. In addition, some genetic leaf abnormalities like lesion mimics, leaf speckling, heat stripe, genetic stripe, among others, can be similar to symptoms produced by plant pathogens. Other reasons why it is important to assess disease early and accurately are to predict yield loss, monitor and forecast epidemics, to assess crop germplasm for disease resistance, and for understanding fundamental biological processes including coevolution (Bock et al., 2010). An improper diagnosis of a genetic abnormality or a plant pathogen can be very costly (Berry et al., 2000). Microscopy involves isolation and growing a pure culture of the pathogen in a suitable medium and observing for diagnostic features under the microscope.

Serology

As alternatives and over years, serological and immunological methods like Enzyme-linked Immunosorbent Assay (EIA), Immunoelectron microscopy

(IEM) and Polymerase Chain Reaction (PCR) have been in use at one time or other. In the last three decades, specifically PCR, enhance technique process is widely applied in plant disease diagnosis and phenotyping (Nutter et al., 2006; Jackson et al., 2007; Hilber and Scheupp, 1992). PCR offers several advantages compared to traditional methods of diagnosis: Organisms need not be cultured prior to their detection by PCR; the technique possesses exquisite sensitivity, with theoretical potential to detect a single target molecule in a complex mixture without using radioactive probes; and it is rapid and versatile. Hence, the use of PCR to diagnose plant diseases and other applications in plant pathology has remained unsurpassed.

Modern technologies

As impressive as these methods may be, there is a direct contact between diagnostician and plant or plant parts and this may introduce assessment errors. The use of Geographic Information Systems (GIS) and Digital Imaging (DI) in plant diseases diagnosis falls under broad definition of "remote sensing" (Bock et al., 2010), which implies obtaining information about an object without having direct physical contact with it (de Jong et al., 2006). Remote sensing of disease is a passive process, rather than an active method that would generate imaging radiation. Both GIS and DI provide systems and methods that allow diagnostic technician to diagnose diseases and other plant anomalies without physically handling plant tissue, which limits errors.

The technologically advanced application using remote sensing techniques to detect and measure plant diseases started with aerial photography (Neblette, 1927; Taubenhuis et al., 1929) and has been applied to various pathos systems (Colwell, 1956; Brenchley, 1964; Wallen and Jackson, 1971; Jackson et al., 1978; Lillesand et al., 1981; Gerten and Weise, 1984; Edwards et al., 1985; Lee, 1989). It had been applied as an accurate and reliable method to detect plant diseases, facilitated by highly sophisticated and innovative methods of data analysis that lead to new insights derived from sensor data for complex plant-pathogen systems (Mahlein, 2016). Indeed, automated image analysis-based phenotyping provides a powerful alternative to visual assessments. Indeed, automation eventually provides a calibrated image analysis, thereby eliminating any subjectivity of raters and ensuring reproducibility (Rousseau et al., 2013).

Geographical information systems

GIS is a computer system that assembles and stores, manipulates, and displays geographically referenced information. In addition, it analysis and provides an electronic representation of information called spatial

data from different sources including natural earth and other manufactured features. GIS references these real-world spatial data elements to a coordinate system, which later can be separated into different layers. GIS system stores each category of information in a separate "layer" for ease of maintenance, analysis, and visualization. For example, layers can represent terrain characteristics, census data, demographics information, environmental and ecological data, roads, land use, river drainage and flood plains, and rare wildlife habitats. The power of GIS lies in its ability to analyze relationship between features and their associated data (Samson, 1995). Different applications create and use different layers. GIS can also store attribute data, which is descriptive information of map features.

This attribute information placed in a database separate from graphics data, but linked to them. GIS allows examination of both spatial and attribute data at same time. In addition, GIS allows users to search attribute data and relate it to spatial data. Thus, it combines geographic and other types of data to generate maps and reports, enabling users to collect, manage, and interpret location-based information in a planned and systematic way. The sources of such data include satellite imagery, aerial photos, maps, ground surveys, and global positioning systems (GPS).

Application in plant pathology

GIS can be applied in different disciplines including veterinary activities, where it is used to understand dynamics and spreading pattern of a disease and quick response in the case of disease emergency (Jebara, 2007). In area of plant disease and pest management, Thomas et al. (2002) applied GPS technology to study outbreak of six insect pests and 12 diseases and risk map in various crops from six different states in United States of America and make management decisions. GIS is also important in spatial analysis of plant disease epidemics (Nutter et al., 1995; Orum et al., 1997); mapping distribution of diseases or specific genotypes of plant pathogens, plant disease epidemiology and management (Nelson et al., 1999). Jaime-Garcia et al. (2001) analyzed genetic structure of *Phytophthora infestans* that causes late blight disease in a mixed potato and tomato production area in Mexico.

Jaime-Garcia and Cotty (2006), studied spatial relationship texture, crop rotation and *Aspergillus* sp. community structure in soil. Sanyong and Amarakul (2001), applied GIS to study distribution of three tree species in different parts of Thailand and lastly, Azahar et al. (2011) and Taliei et al. (2013) compared effect of plant density on distribution pattern of diseases in Malaysia. Therefore, importance of GIS in plant disease detection and diagnosis has wide application in precision agriculture.

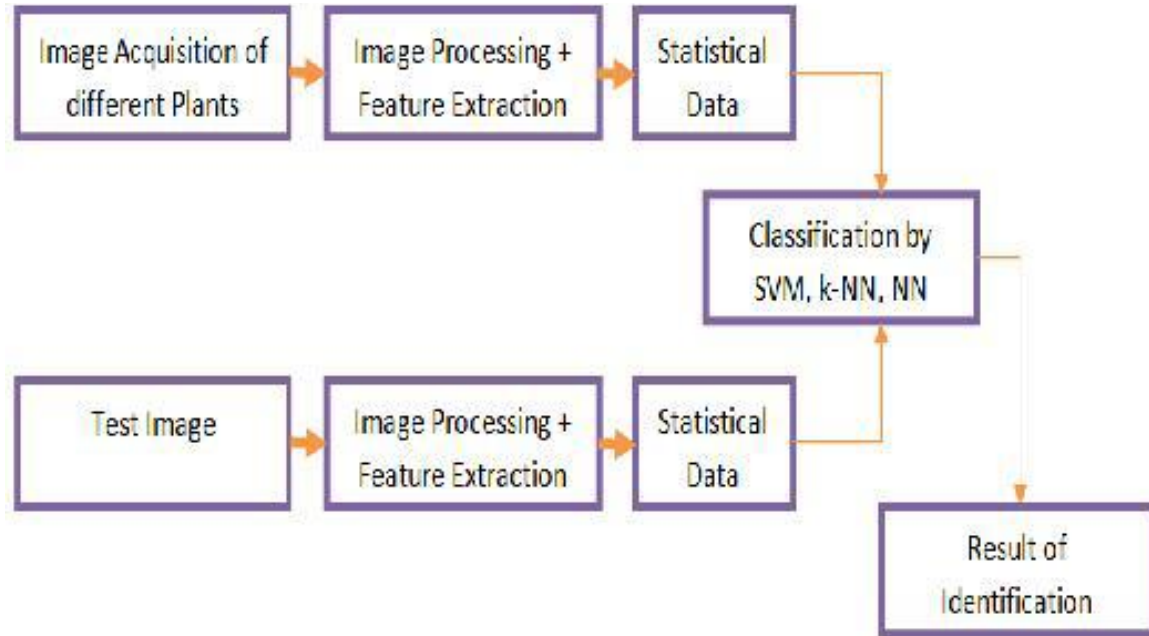


Figure 1. A flow chart showing GIS application in plant pathology (Priya, 2015). SVM, Support Vector Machine; k-NN, Known Nearest Neighbor; NN, Neural Network.

Digital imaging (DI)

Digital imaging is an art as well as science. It is the art of making images (pictures) like photographs and printed text or artwork using digital camera or image machine. Digital cameras are easy to handle and are a simple source of RGB (red, green, and blue) digital images for disease detection, identification and quantification. RGB colour images with red, green and blue channels are used to detect biotic stress in plants (Bock et al., 2010). Each image comprises of a certain amount of pixels, which are then mapped unto a grid and stored in a sequence by a computer. In all classes of digital imaging, information are converted by image sensors into digital signals processed by computer and outputted as a visible light image.

The steps in digital imaging are: Image acquisition, image pre-processing, features extraction and neural network based classification (Priya, 2015). A flow diagram for identification of plant diseases using image processing techniques is as follows (Figure 1).

Techniques of digital imaging

Digital imaging processing techniques include detection, quantification, and classifying plant diseases. Quantification estimates severity of a particular disease, either estimating leaves area affected by disease or how deep infection rooted is on the affected plant or part, which can be estimated by means of colour and texture

features.

Most quantification algorithms include a segmentation step to isolate symptoms, from which features can be extracted and properly processed in order to provide an estimate of disease severity. It is worth noting that problem of determining disease severity by analysing and measuring its symptoms is difficult even if performed manually by one or more specialists, which have to pair diagnosis guidelines with symptoms as accurately as possible.

As a result, manual measurements will always contain some degree of subjectivity, which in turn means that references used to validate automatic methods are always correct (Barbedo, 2013). It is important to take this to consideration when assessing the performance of those methods. Classification is an extension of detection methods, but instead of detecting only one specific disease amidst different conditions and symptoms, it identifies and labels whichever pathogen is affecting plant. Classification method includes a segmentation step, which is normally followed by extraction of a number of features that will feed some kind of classifier (Barbedo, 2013).

Types of digital imaging sensors

Multi and hyperspectral reflectance sensors: These sensors are grouped on the basis of spectral resolution of their spatial scale and type of detector (whether the sensors are imaging or non-imaging systems). This

notwithstanding, they assess spectral information of objects at broadband wavelengths. Multispectral imaging sensors provide data in the R, G and B wavebands, including infrared band. Modern hyperspectral sensors increase the complexity of data measured up to 350 to 2,500 nm (Steiner et al., 2008). While non-imaging sensors average the spectral information over a certain area, hyperspectral data can detect huge matrices along spatial x- and y- axes. The strong, spatial resolution influence pathogen interactions (Mahlein et al., 2012b, West et al., 2003). Sensors with approximately 1 m spatial resolutions are usually unsuitable to detect single symptoms on diseased plant parts.

Thermal sensors: Infrared thermography (IRT) correlates plant temperature with plant water status, micro climate in crop stands and changes in transpiration due to early infections by plant pathogens (Jones et al., 2002; Lenthe et al., 2007; Oerke et al., 2006). Emitted infrared radiation in the thermal infrared region from 8 to 12 μm can be detected by thermographic and infrared cameras and is illustrated in false colour images, where each image pixel contains the temperature value of the measured object. In pathosystem for apple and *Venturia inaequalis*, thermography visualized the spatial colonization of apple tissues by the pathogen above visible symptoms, where hyphae and conidia were only seen through microscopy (Oerke et al., 2011). Gomez (2014) monitored the infection and spread of *Peronospora sparsa* on different Rosa cultivars using IRT. IRT effectively analysis the heterogeneity between and within leaves, mean temperature difference within single leaves, plants and crop stands which are important indicators for appearance of disease symptoms.

Fluorescence imaging sensors: Chlorophyll fluorescence imaging assesses photosynthetic electron transfer and differences in plants photosynthetic activities (Bauriegel et al., 2014). It has been used to study differences in the photosynthetic activity due to biotic and abiotic stresses leaves (Bürling et al., 2011, Scholes and Rolfe, 2009).

A combination of fluorescence imaging with image analysis techniques is useful for discrimination and quantification of fungal infections (Konanz et al., 2014). Research has been directed at extracting fluorescence parameters from sun-induced reflectance in the field, which would have potential for plant disease assessment at canopy or field level (Rossini et al., 2014). A variety of sensors methods have been used for different plant pathogen systems (Table 1) (Mahlein, 2016).

Digital imaging in plant pathology application

A pioneering study to demonstrate capacity of remote sensing and image analysis in plant pathology was

Nilsson (1980, 1995). Previous reviews on use of images from digital cameras in agriculture and other plant sciences include Nutter (1990), Price and Osborne (1990) and Nilsson (1995). Specifically, Bock (2010) gave a comprehensive review on use of image analysis to assess disease severity. As an important tool in plant pathology, Digital Imaging is used to assess disease severity (Bock 2010), diagnose plant diseases and other disorders, to quantify host resistance and plant disease classification (Newton, 1989; Holmes et al., 2000; Mahlein, 2016). Detection can be partial or real time; in partial classification, a disease was identified amongst other several possible maladies. In partial classification, putative regions are classified as being disease result of interest or not, instead of applying a complete classification into any possible disease. Abdullah et al. (2007) applied this method, using neural networks to discriminate between *Corynespora* pathogen from other pathogens that affect rubber tree (*Hevea brasiliensis*) leaves. Real time monitoring continuously monitors crops and raises an alarm as soon as disease of interest was detected in any plants. For instance, Sena et al. (2003) discriminated between maize plants affected by fall armyworm from healthy ones.

Mahlein (2016) reported that image-based diagnosis of plant diseases has been refined for many crop systems over many years. The studies further showed that image-based phenotyping produced more accurate and precise results than visual assessments of disease in different pathogens. The use of automated, high-throughput digital imaging in plant disease phenotyping allows for collection of data at numerous time points, produce images from which quantitative phenotype data can be derived and improve reproducibility of experiments. Many different phenotype measurements can be obtained from image data.

For studies on plant growth and development, these measurements may be plant height or biomass. For studies of plant disease or other stresses, percent leaf area covered with symptoms or changes in photosynthetic responses can be derived from images. Tucker and Chakraborty (1997) used image analysis to count lesion number and measure severity of leaf blight (*Alternaria helianthi*) of sunflower (*Helianthus annuus*) and oat leaf rust (*Puccinia coronata* f.sp. *avenae*) on oats (*Avena sativa*). Newton (1989), used image analysis to measure sporulation area of powdery mildew (*Erysiphe graminis*) on barley leaves of various cultivars. Although this parameter showed significant positive correlation with most components of partial resistance, but image analysis was unable to discern colony size component reduced which could be performed through visual rating. However, Todd and Kommendahl (1994) found image analysis more discerning than raters at differentiating germplasm reaction of corn to *Fusarium* spp. causing stalk rot, and for differentiating among *Fusarium* spp., although no actual values were used in the study. Image

Table 1. Plant pathosystems and diseases assessed by different optical sensors.

Type of sensor	Crop species	Name of disease and pathogen	References
Red, Green, Blue (RGB) Colour Imaging	Cotton	Bacterial angular (<i>Xanthomonas campestris</i>), <i>Ascochyta</i> blight (<i>Ascochyta gossypii</i>)	Camargo and Smith (2009)
	Sugar beet	<i>Cercospora</i> leaf spot (<i>Cercospora beticola</i>); Leaf spot (<i>Ramularia beticola</i>), Phoma leaf spot (<i>Phomabetae</i>), bacterial leaf spot (<i>Pseudomonas syringae</i> , pv. <i>Aptata</i>)	Neumann et al. (2014)
	Grape fruit	Citrus canker (<i>Xanthomonas axonopodis</i>)	Bock et al. (2008)
	Tobacco	Anthracoze (<i>Colletotrichum destructivum</i>)	Wijekoon et al. (2008)
	Apple	Apple Scab (<i>Venturia inaequalis</i>)	Wijekoon et al. (2008)
	Canadian goldenrod	Rust (<i>Coleosporium asterum</i>)	Wijekoon et al. (2008)
Thermal sensors	Sugar beet	<i>Cercospora</i> leaf spot (<i>C. beticola</i>)	Chaerle et al. (2004)
	Cucumber	Downy mildew (<i>Pseudoperonospora cubensis</i>); Powdery mildew (<i>Podosphaera xanthii</i>)	Berdugo et al. (2014); Oerke et al. (2011)
	Apple	Apple Scab (<i>V. inaequalis</i>)	Okerke et al. (2011)
	Rose	Downy mildew (<i>Peronospora sparsa</i>)	Gomez (2014)
Flourescence imaging	Wheat	Leaf rust (<i>Puccinia triticina</i>); Powdery mildew (<i>Blumeria gramineas</i> f.sp. <i>tritici</i>)	Burling et al. (2011)
	Sugar beet	<i>Cercospora</i> leaf spot (<i>C. beticola</i>)	Chaerle et al. (2004), Konanz et al. (2014)
	Bean	Common bacterial blight (<i>Xanthomonas fuscans</i> sub. sp. <i>fuscans</i>)	Rousseau et al. (2013)
	Lettuce	Downy mildew (<i>Bremia lactucaae</i>)	Buriegel et al. (2014), Brabandt et al. (2014)
Spectral sensors	Barley	Net blotch (<i>Pyrenophora teres</i>), Brown rust (<i>Puccinia hordei</i>), Powdery mildew (<i>Blumeria graminis hordei</i>)	Kuska et al. (2015), Wahabzada et al. (2015a)
	Wheat	Head blight (<i>Fusarium graminearum</i>); Yellow rust (<i>Pucinia striiformis</i> f.sp. <i>tritici</i>)	Bauriegel et al. (2014), Bravo et al. (2003), Huang et al. (2007), Moshou et al. (2004)
	Sugar beet	<i>Cercospora</i> leaf spot (<i>C. beticola</i>), sugar beet rust (<i>U. betae</i>), Powdery mildew (<i>Erysiphe betae</i>), Root rot (<i>Rhizoctonia solani</i>), <i>Rhizotonia</i> (Beet necrotic yellow vein virus)	Bergstrasser et al. (2015), Hillnhutter et al. (2011), Mahlein et al. (2010 2012 2013), Rumpf et al. (2010), Steddom et al. (2003 2005)
	Tomato	Late blight (<i>Phytophthora infestans</i>)	Wang et al. (2008)
	Apple	Apple scab (<i>V. inaequalis</i>)	Delalieux et al.(2007)
	Tulip	Tulip breaking virus (TBV)	Polder et al. (2014)
Sugarcane	Orange rust (<i>Puccinia kuehnii</i>)	Apan et al. (2004)	

Source: Mahlein (2016).

analysis was as good as, and complimentary to visual assessment comparing resistance of potatoes to late blight based on internal images of symptoms in tuber (Niemira et al., 1999).

Studies with a variety of pathogens show that image-based diagnosis produces a more accurate and precise results than can be obtained with visual assessments of disease and allows for exploration of more dimensions of disease phenotypes. Stewart and McDonald (2014) used automated image analysis of infected wheat leaves to

analyse disease symptoms of septoria wheat blotch caused by *Zymoseptoria tritici*. This fungus is characterised by chlorosis, necrotic lesions and fruiting bodies (pycnidia). Typical visual disease assessment relied on estimates of percentage leaf area covered by pycnidia lesions. Pycnidia are small and accurate estimates of pycnidia cover are difficult to make especially when they are numerous. Image analysis made it possible to quantify pycnidial size and density along with other traits, which would not have been

possible with visual assessment alone. Olmstead et al. (2001) found that image analysis of powdery mildew (*Podosphaera clandestine*) on sweet cherry was inferior to rater estimates when compared to actual values. Despite these studies, most recent works suggest that image analysis most often provides a more accurate and precise, but generally more time-consuming way of rating disease. Image analysis has now been widely tested and explored as a tool in plant pathology, and for applicability in sectors within discipline having various research goals. Thus, image-based phenotyping can greatly enhance data available for characterizing plant disease (Mukta and Bart, 2015). Again, Bock et al. (2008) examined citrus canker disease symptoms on grape fruit leaves caused by bacterium *Xanthomonas axonopodis* P.v. *citri* using digital imaging. Studies on bacterial blight caused by *Xanthomonas* spp. on two different genotypes of bean found that image analysis enhanced ability to distinguish between genotypes with different levels of disease severity (Xie et al., 2012). Furthermore, all measurements of disease were reproducible between different disease susceptibility levels on different genotypes. Hence, image-based phenotyping offers potential to improve reproducibility and sensitivity of disease quantification.

Some advanced system with potential applications in field are imaging platform for detection of tulip breaking virus (TBV) infected tulip bulbs (Polder et al., 2014) or a prototype of a hyperspectral imaging platform for yellow rust detection (*Puccinia striiformis*) in wheat (Bravo et al., 2003). Polder et al. (2014) developed a robot with multispectral cameras and online machine vision analysis pipeline. This work was the result of limited technical experts for rating tulip bulbs. They were able to adjust and optimize this system to attain a level of accuracy equivalent to that obtained by experienced rating experts. Bravo et al. (2003) detected and classified yellow rust diseased patches in wheat fields with a success rate of 96% under ambient light conditions using hyperspectral imaging. The result was very encouraging, leading to development of cost effective optical sensor platform for early and accurate detection of plant diseases in different crops. Considering plant disease occurrence depends on specific environmental factors and often exhibit a heterogeneous distribution in fields, optical techniques such as RGB (Red, Green, Blue) imaging, multi and hyperspectral sensors, thermography or chlorophyll fluorescence that use digital imaging are useful in identifying primary diseases foci and areas differing in disease severity in the fields (Franke and Menz, 2007).

Conclusion

This article has reviewed useful additions on GIS and DI applications in plant disease diagnosis, detection and measurement of disease severity. Although previously known methods such as PCR, ELISA, and visual

assessment are already available and widely used for plant disease detection, but not without obvious setbacks. On other hand, GIS and digital imaging are particularly useful in diagnosing and identifying recurring patterns of plant disease as well as other problems such as insect and weed infestations without direct contact with plant or plant parts. A highly interdisciplinary approach with a close link to practical agriculture could lead to powerful solutions for diagnosis and disease detection with high accuracy and sensitivity that will improve plant health management. For best results successful applications of GIS and digital imaging in plant disease diagnosis requires teamwork, involving an experienced field diagnostician and an experienced computer user with some background in statistics. Incorporating traditional epidemiological statistical techniques into a GIS interface allows researchers to gain a greater insight into the spatial aspect of disease spread. In addition, availability of software capable of producing attractive maps provides an opportunity to communicate in visually form, plant disease situation to a wider audience.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Irrigation productivity and water-use efficiency in papaya crop under semi-arid conditions

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Papaya (*Carica papaya* L.) has a great economic importance in tropical and subtropical countries, Brazil being one of the largest producers of papaya in the world. This crop requires a considerable amount of water during its cycle, making proper irrigation management essential for optimal water use. Improving water-use efficiency can increase levels of agricultural production as well as the efficient use of water resources in semi-arid regions. The aim of this work was to evaluate productivity and water-use efficiency of irrigation in papaya. The research was carried out in the Curupati irrigation Perimeter, located in the semi-arid region of Brazil. The volume of water applied to the crop was quantified by calculating the number of operating hours of the pump unit supplying the irrigated lot, determining the flow rate of the emitters used in the irrigation system, and evaluating the soil moisture profile. The total volume of water applied was 2,663,296.20 m³, for a yield that ranged from 80 to 106 t ha⁻¹. For water-use efficiency, it was found that for each kilogram of papaya produced, 1,042 m³ of water were consumed, giving a productivity of 0.95 kg m³. The water-use efficiency was affected by the different types of soil in the irrigation perimeter.

Key words: Water-use efficiency, irrigation management, irrigated agriculture, production, *Carica papaya* L.

INTRODUCTION

Brazil is the world's third largest producer of fruit, ranking second in the production of papaya (*Carica papaya* L.) with around 12.5% of world production (FAO, 2015).

Irrigated agriculture is a substantial activity today, a result of the continuous increase in the demand for food due to the growth in population. The activity emerged in the Northeast of Brazil following a significant growth in the market, and has resulted in greater production and higher incomes for the sector, especially fruit farming, which has assumed a prominent place in this scenario

(Lopes et al., 2011).

In this context, the fruit farming is of great importance for the semi-arid region of northeastern Brazil, since the adoption of irrigation technology in the cultivation of papaya has resulted in increased production. However, the water use efficiency (WUE) is related to irrigation management. In this sense, in the region under study, the producers establish arbitrary periods of irrigation, generally over-irrigating, for fear that the crop may suffer from water stress and affect production. Thus,

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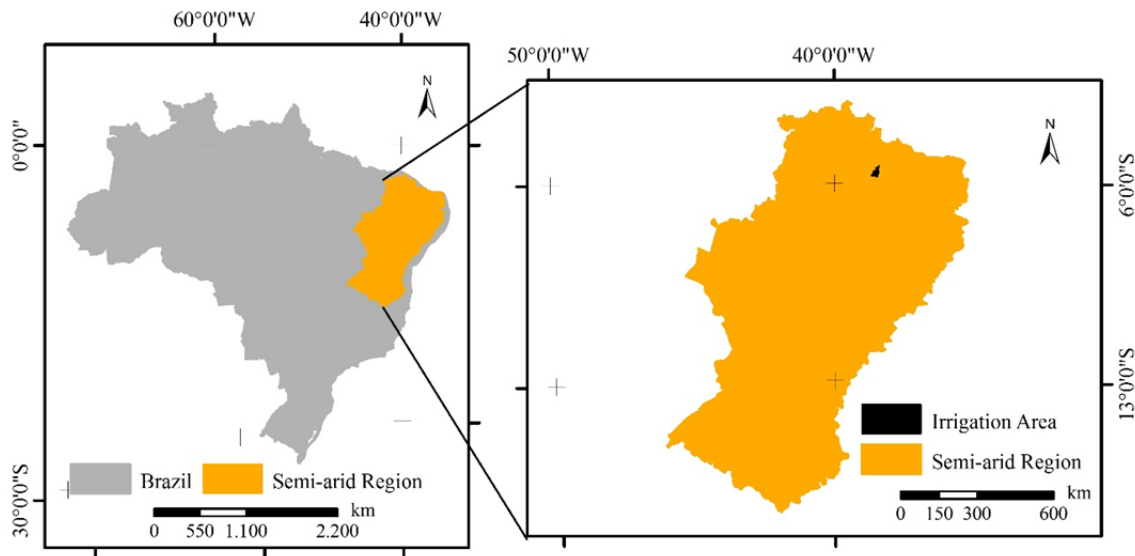


Figure 1. Location of the semi-arid region of Brazil and the Curupatiirrigation Perimeter.

occasioning a decrease in the WUE, which brings as consequence, application of excess water, soil nutrient profile washing and increase in energy consumption.

Water use efficiency (WUE) is one of the parameters used to calculate the relationship between the productivity obtained with a crop for a specific volume of applied water (Loomis, 1983). According to Melo et al. (2010), WUE increases when there is a reduction in the depth of water applied, with no reduction in production, essential in arid and semi-arid regions, due to problems of water shortage. Lima et al. (2010), point out that with an increase of only 1% in WUE in the semi-arid region of the Northeast of Brazil, it is estimated that there would be a saving of 165,000 L of water per irrigated hectare per year.

Second Guoju et al. (2016), improving water-use efficiency was a key factor in the continual increase in crop productivity in arid and semi-arid regions of northwestern China. For Gomes and Testezlaf (2007) the WUE reduces water loss and supplies the water required by plants for their development; also, irrigation management is important in obtaining high yields, in addition to the preservation of the environment.

Several researchers have sought to identify the interference that occurs and that impairs the efficient application of water, in order to maximise water-use efficiency (Souza et al., 2005; Medeiros, 2003; Peixoto et al., 2005; Carvalho et al., 2006). Such interference stems from the level of education of the producers and the quality of the irrigation water, to management techniques in the field.

It is worth pointing out that, although there are studies into sustainable water management, such reports are scarce, especially under arid and semi-arid conditions, requiring further investigation in order to ensure the best

economic, social and environmental use of the resource. With this in mind, the aim of this work was to evaluate productivity and water-use efficiency in irrigated papaya in the semi-arid region of Brazil.

MATERIALS AND METHODS

Location of the study area

The trial was carried out in an irrigated area located in the semi-arid region of Brazil (Figure 1). Under study was the Curupatiirrigation Perimeter, located in the town of Jaguaribara, in the State of Ceará, Brazil.

Edaphoclimatic characterisation

The climate according to the Köppen classification is type BSw'h', hot semi-arid with average monthly temperatures greater than 18°C. The average annual rainfall in the region is 810 mm, with 80% of the total rainfall occurring from January to April.

The predominant soils are Neosols, Luvisols and Argisols, with the predominant native vegetation in the region being deciduous thorn forest, dense shrub-like caatinga, open shrub-like caatinga, and mixed dicot-palm forest (IPECE- 2007). Other climatic characteristics of the region under study can be found in Table 1, including average values for the 2000 to 2015 historical series.

Description of the Curupatiirrigation Perimeter

The irrigation district comprises an area of 189 ha, divided into lots of 1.5 ha, benefitting 144 producers. Of these, 63 produce irrigated papaya through a system of drip irrigation, making up an area of 94.5 ha. The remaining 94.5 ha is cultivated with guava irrigated by a micro-sprinkler system, of benefit to 81 producers.

The water in the perimeter comes from the Castanhão reservoir, pumped by a 500 hp floating motor pump set with a flow rate of 0.33 m³s⁻¹. This was used to supply the distribution channel. The

Table 1. Climatic characteristics.

Parameter	Value	Unit
Average annual insolation	3,096	h year ⁻¹
Average annual potential evaporation	1,830	Mm year ⁻¹
Maximum average annual temperature	32.00	°C
Average annual temperature	27.50	°C
Minimum average annual temperature	26.00	°C
Average annual relative humidity	67.95	%
Average annual wind speed	3.80	m s ⁻¹

Source: INMET (2016).

water was pumped from the channel to the irrigated lots by a further set of four 75 hp motor pumps.

For all of the lots of papaya producers utilized the irrigation drip system located, in which The water was carried to the lots by 75 and 50 mm diameter PVC tubing. The papaya crop was planted in rows spaced 4 m apart with 1.8 m between plants, being that the drip emitters were spaced 0.4 m apart, with an equivalent flow rate of 2.0 L h⁻¹.

For better efficiency in applying the water to the soil, hourly irrigation pulses were adopted, giving a total of four pulses for each 0.75 ha sector day⁻¹. The rainfall volume during the crop cycle was also calculated, as the motor pump sets were turned off during the rainy season.

First, questionnaires were carried out to the papaya producers in the irrigated perimeter to obtain information on the irrigation system, the agricultural practices being used, the power installed and the general characteristics of the adopted production system, and to monitor the technical team in the irrigation perimeter.

For the sample calculation, we used a probabilistic technique, in which all elements of the population have equal probability, different from zero, being selected for the sample. estimated the sample size by applying the method proposed by Fonseca and Martins (1996):

$$n = \frac{Z^2 * p * q * N}{d^2 * (N-1) + Z^2 * p * q} \quad (1)$$

Where, N: the total population of 63 families; d: the sampling error definite at 10%; Z: standard deviation of 1.96 which corresponds to a confidence level of 90%; p and q: are the percentage of positive sample elements and unfavorable (50%) to the searched attribute.

Soil analysis

To characterise the textural and chemical class of the soil in the irrigated area, samples were collected from the 0-30, 30-60, 60-90 and 90-120 cm layers. The samples were analysed at the Soil and Water Laboratory of EmbrapaAgroindústria Tropical, in Fortaleza, Ceará.

To determine the moisture content of the soil profile in the irrigated area, further samples were taken from the 0-30, 30-60, 60-90 and 90-120 cm layers, and then analysed by the Laboratory of Hydrology of the Department of Agricultural Engineering at the Federal University of Ceará, Pici Campus. Samples were collected only a soil sample for each lot, corresponding to 18 samples, according to determination of the number of lots of papaya producers to be interviewed.

Productivity of the papaya

Analysis of average crop yield was based on the ratio of papaya

produced to the area under cultivation, as per Equation 2.

$$P_{av} = \frac{p}{x} \quad (2)$$

Where, P_{av} is average productivity, in kg ha⁻¹; p is papaya produced in kg; x is area under cultivation, in ha.

Water-use efficiency

The water-use efficiency (WUE) was obtained from the ratio between crop production and the volume of water applied, as per Loomis (1983).

$$WUE = \frac{p}{v} \quad (3)$$

Where, WUE is water-use efficiency in kg m⁻³; p is crop production, in kg; v is volume of water applied in m³.

Statistical analysis

Descriptive statistics were used to analyse the following parameters: Mean value, standard deviation and coefficient of variation. The data were analysed using the SPSS v 16 software. The Excel software was used for graphing and regression analysis of the correlations between the different parameters being evaluated.

RESULTS AND DISCUSSION

Producer profile

Interviews were conducted randomly in the Curupatiirrigation Perimeter, where 18 producers were interviewed, of which 67% reported that they had had no previous experience with irrigated agriculture. Due to the lack of knowledge of this technique of irrigation management, water-use efficiency was low.

According to Lopes et al. (2011), when conducting interviews with lot owners in the Lower Acaraúirrigation Perimeter, they found that 77.78% of the producers had had no experience with irrigated agriculture before arriving in the area. They concluded that existing irrigation management in the perimeter was inadequate, and that producers could not determine when or how

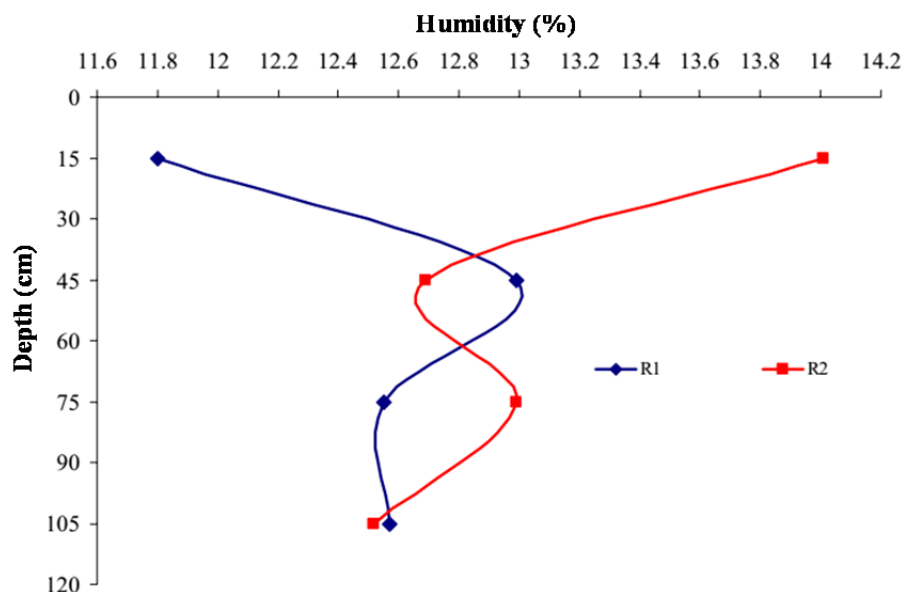


Figure 2. Soil moisture profile in the Curupatiirrigation Perimeter.

much to irrigate.

Thus a lack of knowledge or experience with the techniques of irrigation increases the likelihood of producer failure since, as per Branco (2003), Vanzela et al. (2003) and Andrade et al. (2009), the success of irrigated agriculture bears a close relation to the level of education and knowledge of the technique being practiced.

For Lacerda and Oliveira (2007) the low level of education explains the poor effectiveness of public policies which aim to promote development, since the low level of schooling acts to limit access to information, communications, human and social capital, and especially the adoption of technology.

Technical assistance in the CurupatiIrrigation Perimeter was provided by the Secretary for Agriculture of the State of Ceará, currently known as the Secretary for Agrarian Development (SDA), and by the Company for Technical Assistance and Rural Extension for the State of Ceará (EMATERCE), both with offices in the City of Jaguaribara. The permanent presence of two agricultural technicians in the area offered the same technical guidance to all the producers, who therefore received the same orientation and adopted the same irrigation management.

Irrigation management

As producers in the perimeter had no experiments with irrigation technique, was determined the soil moisture content to a depth of 120 cm, and thus identified the existence of water losses by deep percolation, in which were carried out two samples in two periods identified with R1 and R2 in four depths.

Figure 2 shows that in the beginning of the period of irrigation (R1) and in the period of greatest demand for water by the crop (R2) content of the soil showed differences in the first layer (0-30 cm), due the biggest difference of soil matric potential of in this layer, because the soil is dry at the start of irrigation, being that with the exception of the first layer (0-30 cm), the values for soil moisture were similar.

A water loss through deep percolation was observed too; thereby decreasing water-use efficiency, as calculation of the irrigation depth had been carried out to a depth of 80 cm. Coelho et al. (2005), studying the root systems of papaya crops under different irrigation systems in the Northeast of the country, concluded that 80% of the root system of papaya irrigated by surface drip occurred at a depth of 45 cm, with at least 60% of the roots being concentrated at 25 cm, reaching a maximum depth of 75 cm.

It was thus found that the soil moisture content from 90 to 120 cm is compatible with the other profiles studied, characterising the excessive use of water and fertiliser in a root zone not available to the root system of the papaya.

Lopes et al. (2009), when estimating the water requirement of the coconut (*Cocos nucifera*) in the Lower AcaraúIrrigation Perimeter, found that due to not employing any method of determining soil moisture, the producer usually over-irrigated, for fear that the crop might suffer from water stress and affect production.

The excessive application of water to a layer of soil not exploited by the roots, results in an increase in the volume of water used and power consumed. In addition, the soil profile is washed of nutrients in the root zone of the crop, thus affecting development and productivity, as

Table 2. Management adopted and productivity of the papaya in the Curupatiirrigation Perimeter.

Producer	Crop	Area (ha)	Irrigation System	Productivity (t ha ⁻¹)
01	Papaya	1.5	Drip	93
02	Papaya	1.5	Drip	106
03	Papaya	1.5	Drip	93
04	Papaya	1.5	Drip	93
05	Papaya	1.5	Drip	86
06	Papaya	1.5	Drip	93
07	Papaya	1.5	Drip	93
08	Papaya	1.5	Drip	80
09	Papaya	1.5	Drip	93
10	Papaya	1.5	Drip	93
11	Papaya	1.5	Drip	83
12	Papaya	1.5	Drip	100
13	Papaya	1.5	Drip	100
14	Papaya	1.5	Drip	100
15	Papaya	1.5	Drip	100
16	Papaya	1.5	Drip	100
17	Papaya	1.5	Drip	93
18	Papaya	1.5	Drip	93

well as decreasing water-use efficiency.

Another major problem is the risk of salinisation at deeper layers due to leaching. According to Chaves et al. (2006) and (2009) studying the risks of soil degradation and the dynamics of soil salinity in the Araras Norte Irrigation Perimeter in Ceará, they found increases in soil salinity at the deeper layers, expressing the occurrence of the leaching process as a result of excessive irrigation depths.

Productivity of the papaya

Based on the matrix formed from the questionnaires given to the 18 producers in the Irrigation Perimeter, a profile of the producers was prepared in relation to the irrigation management adopted, and a survey of the productivity in each of the lots (Table 2). Where in the management adopted in relation the time of irrigation, fertilization, phytosanitary treatment were the same for all lots.

It can be seen from Table 2 that the productivity varied from 80 to 106 t ha⁻¹, with an average yield of 96.78 t ha⁻¹ and a standard deviation of 6.40 t ha⁻¹. It was found that productivity for 77.78% (14 producers) was around the average (96.78±6.40), 5.56% (1 producer) had significantly higher productivity than the average, and 16.68% (3 producers) had a productivity lower than or equal to 90 t ha⁻¹, less than the average. The mean values found in the 18 lots of the Irrigation Perimeter exceeded the national average productivity of 60 t ha⁻¹ for the variety of the Formosa group grown in the area.

According to Tolk et al. (2016), differences in the productivity ranking of the soils of one region compared with those from other regions emphasize the effects of interaction between the environment and the soil on crop production, as does the structural class of the soil in which the crops are grown.

The significant increase in papaya production in the Curupati Irrigation Perimeter, compared to the national average, can be attributed to irrigation technology, as well as the climatic conditions of the region, which favour cultivation of the fruit. According to Lima et al. (2015), proper irrigation is essential to maintain or increase production in cultivated areas, where there is greater competition for water resources requiring an increase in water-use efficiency and a reduction in environmental impact.

For Santos et al. (2008), studying the effect of different irrigation depths on a papaya crop, there was a linear increase in crop productivity for the increasing volume of water applied. However, it should be investigated whether this increase in productivity justifies the large amount of water used, that is, water-use efficiency, and the relationship between production and the volume of water used, should be considered.

Water-use efficiency

To determine water-use efficiency as well as the volume of water used in the production of one kilogram of papaya, both the water applied by the irrigation system and rainwater throughout the crop cycle were considered,

Table 3. Volume of water applied in the Curupatiirrigation Perimeter.

Year	Volume of rain water (m ³)	Volume of irrigation water (m ³)	Total (m ³)
2006	-	165,628.80	165,628.80
2007	152,793.00	1,058,184.00	1,210,977.00
2008	260,712.00	1,025,978.40	1,286,690.40
	Total volume applied		2,663,296.20

Table 4. Water-use efficiency for each lot in the Curupatiirrigation Perimeter.

Producer	Production (kg)	Volume of water applied (m ³)	WUE (m ³ kg ⁻¹)
01	139,500	147,960.9	1.06
02	159,000	147,960.9	0.93
03	139,500	147,960.9	1.06
04	139,500	147,960.9	1.06
05	129,000	147,960.9	1.15
06	139,500	147,960.9	1.06
07	139,500	147,960.9	1.06
08	120,000	147,960.9	1.23
09	139,500	147,960.9	1.06
10	139,500	147,960.9	1.06
11	124,500	147,960.9	1.19
12	150,000	147,960.9	0.99
13	150,000	147,960.9	0.99
14	150,000	147,960.9	0.99
15	150,000	147,960.9	0.99
16	150,000	147,960.9	0.99
17	139,500	147,960.9	1.06
18	139,500	147,960.9	1.06

as shown in Table 3.

Based on the total volume of water applied, it was possible to calculate the water requirement for each kg of papaya produced, as shown in Table 4, in which total volume of water applied in each lot was the same for all, once the irrigation management adopted was the same, namely irrigation time was the same in all lots.

The average value for WUE in the Curupatiirrigation Perimeter was 1.05 m³ kg⁻¹, that is, for each 1.05 m³ of water applied, 1 kg of papaya was produced. The standard deviation was 0.08 m³ kg⁻¹, the coefficient of variation was 7.17%, and the minimum and maximum values for WUE were 0.93 and 1.23 m³ kg⁻¹, respectively.

The greatest production was shown in lot 02, with a consequent greater efficiency in the use of water, 0.93 m³ kg⁻¹, due to the irrigation management being suitable for the type of soil in the lot; unlike lot 08, which had the lowest production and consequently low water-use efficiency, 1.23 m³ kg⁻¹.

This variation in water use efficiency is attributed to the types of soil in the Irrigation Perimeter. In lot 02 the soil is a Luvisol; these are soils of high natural fertility, endowed

with clays with a high capacity for holding water and nutrients, and are commonly found in the Northeast of Brazil, where they are distributed mainly in the semi-arid region. However, in lot 08 the soil type is a Neosol; these are little-evolved soils, of sandy sediments with low water retention capacity, contributing significantly to the reduction in productivity, and consequently to the low water-use efficiency.

According to Gomes et al. (2008), the ratio of macro- to micro-pores in Neosols is large, given the high degree of rounding of the quartz grains that make up these soils and that favours significant vertical percolation of the water, reflected in high hydraulic conductivity. This, coupled with the low clay and low organic matter content, contributes to poor particle cohesion, almost total lack of aggregation, and an intense leaching process. As a result, the soils are also ecologically fragile, due to their low capacity for water and nutrient retention for plants (Zuo et al., 2008).

In relation to Luvisols, Maia Filho et al. (2013), in their study on the effect of manure on water consumption and sunflower production in two types of soil, found that

plants grown in Luvisols showed better growth characteristics, with the soil type significantly affecting both the water consumption and water-use efficiency of the sunflower crop.

In this regard, it is worth noting that the soil type has a significant influence on water-use efficiency. Furthermore, irrigation management cannot be the same for all lots in the area, due to the variability of the soil. According to Tenhunen et al. (2002), soil moisture conditions significantly influence water-use efficiency in arid and semi-arid regions.

Therefore, even with the application of an excessive irrigation depth in relation to the projected irrigation depth, which will be different for each type of soil, the volume of water per kilogram of papaya produced was lower than values found by Souza et al. (2006), when studying irrigation efficiency for different types of soil texture in Irrigation Perimeters of Ceará. Those authors obtained results that displayed a range of values from 0.18 to 0.5 kg m⁻³, showing that for a sandy-loam texture, only 0.18 kg of unhusked rice were produced for each 1.0 m³ of water applied. Whereas, for a silty-clay texture, 0.5 kg of unhusked rice were produced for each 1.0 m³ of water applied.

The values found for WUE can therefore be considered satisfactory, since irrigated agriculture in dry regions requires large quantities of water, whereas to produce one kilogram of grain in humid regions, less than 0.5 m³ of water is needed; in arid regions this volume varies from 1.5 to 2.5 m³ (Andrade and D'Almeida, 2006).

As per Tari (2016), who found values for water-use efficiency from 1.02 to 1.30 kg m⁻³, in a study of the effects of different irrigation strategies on productivity, quality and water-use efficiency in a wheat crop under semi-arid conditions.

For Guoju et al. (2016), improving water-use efficiency is a key factor for the continual increase in crop productivity in arid and semi-arid regions. However, it is also important to point out the possible decrease in production due to increases in the WUE, since the improvement in yield under conditions of limited water with greater water-use efficiency is substantial, a reduction in water consumption would increase the WUE, but possibly reduce production (Blum, 2005, 2009).

In this context, the WUE is an important parameter in identifying constraints on improving the efficient use of water, as well as being a tool for increasing crop production and economising water, in addition to preserving the environment. It should be considered an important attribute in helping farmers increase their income through productivity and water savings, especially in areas deprived of water (Liu et al., 2013).

Conclusions

The low level of education associated with lack of knowledge of irrigation technique favors the decrease of

WUE. In particular, the WUE was of 1.05 m³ to produce 1 kg of papaya, expressing a productivity of 0.95 kg m⁻³. The management of irrigation similar, adopted for the different types soils of Irrigated area affect significantly the water-use efficiency, as per observed in the variation of values of WUE from 0.93 to 1.23 m³ kg⁻¹. Therefore, emphasize the level of instruction as irrigation strategy to improve WUE and productivity, in view of aspects observed regarding the level of knowledge of farmers with the technique of irrigation, notably it becomes necessary to an instruction process of farmers with the purpose of capacitate them to the domain of technological degree that irrigation requires.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Multivariate analysis of the genetic divergence among populations of ornamental pepper (*Capsicum annuum* L.)

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The genus *Capsicum* presents a wide genetic variability. The most common way to determine this variability has been based upon morphological descriptors. We studied the genetic divergence among populations of ornamental pepper (*Capsicum annuum* L.) using two different multivariate techniques: Cluster analysis and canonical discriminant variables. The analyses enabled us to determine the morphological descriptors that contributed most to the genetic divergence. The study was carried out in a greenhouse in the Northeastern Brazil, in two years: 2013 and 2014. The experimental design was the completely randomized design, considering two crossed factors: Population and years. Thirteen populations of ornamental pepper were evaluated based on sixteen plant descriptors, six flower descriptors and ten fruit descriptors; eight F₃ populations, resulting from crossing the accessions 134 (P-9) and 77.1 (P-10), and five additional control populations: P-9, P-10, P-11, P-12 and P-13. There was an agreement between the two multivariate techniques in terms of distance between populations. Fruit descriptors contributed most to the genetic divergence, separating the populations used as control (P-11, P-12 and P-13) from the others. This separation is due to the uniformity of these populations in terms of fruit size and weight.

Key words: Canonical variables, morphoagronomic descriptors, genetic resources, genetic variability.

INTRODUCTION

Pepper belongs to the genus *Capsicum* and features wide morphological variability present in plants, flowers and fruits. This variability in fruit is portrayed by the differences in colors, shapes, sizes and flavors. The variability present in plant architecture gives the pepper

high potential for use as ornamental plants because this sector of the market prefers short plants with colorful and erect fruits, as well as plants resistant to diseases, pests and abiotic stress (Carvalho and Bianchetti, 2007; Rêgo et al., 2009).

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Despite this potential, the *Capsicum* improvement in Brazil is still not compatible with the relevance of pepper in the production chain (Reifschneider, 2000). However, this scenario is changing, considering the concern of breeders to develop cultivars with main focus on the fruit characteristics such as size, shape, capsaicin content, color, firmness, vitamin content and uniformity (Luz, 2007).

Thus, the study of genetic diversity is critical to the understanding of the genetic variability in populations or genotypes kept in Active Germplasm Banks (AGB). As it is known, in general, the morphologically far are the parents to be used in breeding programs the greater the heterotic effect (Gonçalves et al., 2008; Rêgo et al., 2011b; Sudré et al., 2005). However, some factors, such as lack of documentation, description and evaluation of collections of genetic materials, may hinder the use of AGB, which limits the action of breeders (Gepts, 2006).

The use of morphological descriptors has been a common way to qualitatively and quantitatively characterize the variability in AGBs. According to Sudré et al. (2010), characterization of domesticated *Capsicum* species is of great interest, particularly for the AGB, as the wide variability in species is not yet fully known and exploited. Gonçalves et al. (2008) emphasize the importance of characterization of AGBs, as it makes the variability between populations or accession available to researchers, which allows the selection of superior genotypes and enables the increase of frequency of favorable alleles. These genotypes can also be used in hybrid combinations of high heterosis for future use in selecting segregating generations (Rêgo et al., 2011a, 2012b).

To determine the genetic distance between individuals, a group of individuals or populations, biometric models are used. In general, these models are based on multivariate techniques which allow a combining multiple information from a set of characteristics. Several multivariate methods can be used on studies of genetic diversity, such as cluster analysis, principal components and canonical discriminant variables. Considerations of using these methods include what is the most appropriate to get a desired accuracy, interpretation of results and how the data were obtained (Cruz and Carneiro, 2006).

We aimed to study the genetic divergence among F_3 populations of ornamental pepper through multivariate methods, as well as to determine the morphoagronomic descriptors that contributed most to the genetic divergence.

MATERIALS AND METHOD

The study was conducted in a greenhouse in the Northeastern Brazil. Thirteen populations of ornamental pepper belonging to the Active Germplasm Bank (AGB) of the Laboratory of Plant Biotechnology of the Federal University of Paraíba were used. These populations consisted of eight F_3 populations: F21 (P-1), F24 (P-2), F25 (P-3), F27 (P-4), F29 (P-5), F210 (P-6), F211 (P-7) and

F231 (P-8), originated from crossing the accessions 134 (P-9) and 77.2 (P-10), and five populations used as additional control, accessions 134 (P-9), 77.2 (P-10), 10.1 (P-11), 10.2 (P-12) and 10.3 (P-13).

The seeds were sown in polystyrene trays with 200 cells filled in with commercial substrate Plantmax HT®. Thirty-five days after sowing, when the seedlings had three true leaves they were transplanted to plastic pots with volume capacity of 800 mL containing the same commercial substrate. The plants were watered daily, on alternate days with nutrient solution consisting of the following composition (g/1000 L): 1000 g of calcium nitrate; 1250 g of potassium nitrate; 250 g of MKP; 500 g of magnesium sulfate; 1.5 g of boric acid; 25 g of quelatec AZ; 25 g of ultraferro; 110 g of potassium chloride and 150 g of potassium sulphate. Phytosanitary treatment was carried out when necessary, throughout the growing cycle, in order to minimize the damage caused by pests and diseases.

The experimental design was completely randomized design. The experimental unit consisted of one plant per pot. Fifty plants of each of the eight F_3 populations were assessed, as well as ten plants of the additional controls.

The morphoagronomic characterization was performed in accordance with the recommendations of the *Capsicum* descriptors proposed by IPGRI (1995). Sixteen morphological descriptors (6 of vegetatives and 10 of reproductives) were used: PH (plant height), CD (canopy diameter), FFH (first fork height), SD (stem diameter), LL (leaf length), LW (leaf width), FL (fruit length), FLD (fruit largest diameter), FSD (fruit smallest diameter), PL (peduncle length), PT (pericarp thickness), PCL (placenta length), the ratio FL/FLD, FW (fruit weight), DMC (dry matter content), and NSF (the number of seeds per fruit), in two years: 2013 and 2014.

The data were subjected to multivariate analysis of variance according to a two-way MANOVA model with the factors years and populations. The effect of the interaction between these factors was analyzed. To quantify the relative contribution of the descriptors to the genetic divergence we used the criterion of Singh (1981). The canonical discriminant variables were then constructed, whose average scores for each combination of factors were presented in two-dimensional plane through the biplot technique (Gabriel, 1971). From the loadings of the canonical variables, we evaluated the importance of each characteristic related to plant and fruit on the genetic divergence among populations. Furthermore, a cluster analysis was also performed via Ward algorithm, based on the squared generalized Mahalanobis distance. All analyzes were performed using R version 3.2.1 software (R Core Team, 2015).

RESULTS AND DISCUSSION

The result of the multivariate analysis of variance shows that there is interaction between the two factors ($p < 0.01$), indicating that the populations of ornamental pepper used in this experiment responded differently in each year.

Based on Singh (1981) criterion (Table 1), it can be seen that the descriptor that contributed most to the genetic divergence was FW, with 44.7 and 38.8% for the first and second year, respectively. This slight decrease in contribution is probably due to the different accumulations of fresh fruit weight. The higher contribution in the first year was also high for the FL (15.1%) and low (7.8%) in the second year. The fact that FL is a descriptor closely related to the production (FW) or is a production component explains the changing in the contribution of FL to discriminate the populations, and will affect the

Table 1. Relative contribution of morphological descriptors with the calculation of Mahalanobis distances, according to Singh's criterion, for two years of evaluation of 13 populations of ornamental pepper (*C. annuum* L.).

Descriptor	2013	2014
	Relative participation (%)	
FW	44.7	38.8
CD	2.7	11.0
FSD	9.5	9.6
FL	15.1	7.8
FLD	1.7	7.0
LW	4.6	5.7
NSF	4.9	5.0
PT	5.7	0.0
LL	5.2	3.5
Other descriptors	5.9	11.6

FW, fruit weight; CD, canopy diameter; FSD, fruit smallest diameter; FL, fruit length; FLD, fruit largest diameter; LW, leaf width; NSF, the number of seeds per fruit; PT, pericarp thickness; LL, leaf length.

contribution of the FW (Table 1). This confirms the results obtained from the scores associated with the canonical variables, in which the contribution of the descriptors of fruit was decisive for determining the distance among populations. Sudré et al. (2006) reported that the most important descriptors were the length and the diameter of the fruit, and this is probably related to the number of species studied, since it is expected more differences in fruit morphology as the number of species increases.

One can also highlight the participation of the variables FL (15.1%, year 1) and (7.8%, year 2) and FSD (9.5%, year 1) and (9.6% year 2). This decrease on FL is due to differences in the growth rate and greater uniformity of fruits observed in the second year, as well as the fact that populations used as control (P-11, P-12 and P-13) have heavier fruits, thus contributing to a smaller distance among these populations and the greater distance from them to other populations. This could be explained by the fact that the diversity contributed by canopy diameter (CD) was low (2.7) in the first year and high (11.0) in the second year. The lower the use of assimilate for the vegetative growth (CD) might contribute to the higher the use for reproductive growth (FL and FW) and vice versa (Table 1).

The CD showed no effective contribution to the divergence among the populations, in both years. However, in the second year, it reached 11%. Probably, this major contribution is due to the fact that some populations naturally present different rates of development of plant canopy. According to Barbosa et al. (2002), Stommel and Bosland (2006), Rêgo et al. (2009) and Barroso et al. (2012), plants with compact size, lower height and smaller canopy diameter are of interest for selection of pepper plants with ornamental use purpose.

In Figures 1A and B, through the scores associated with the first two canonical variables (Can1 and Can2), the populations seem dispersed, although some form

coherent clusters. The variability retained at the first canonical variable (Can1) was 58.4%, in the first year. The variables that contributed most to this canonical variable, in descending order of importance, were: FW, FLD, FL, FSD, PCL, LL and LW.

The canonical discriminant analysis showed that, in general, the variables associated with fruit (FW, FLD, FSD, FL and PCL) contributed most to the distance between populations, both in the first (Figure 1A) and in second (Figure 1B) year. These results coincide with those obtained by Hand et al. (2011), who reported that the two variables that contributed most to the divergence were variables of fruit.

It is noteworthy to highlight the behavior of populations P-11, P-12 and P-13 (Figure 1). They presented fruits significantly greater than the others. The aforementioned fruit characteristics were also responsible for the proximity between the populations (P-8 and P-5) (P-2 and P-1) (P-3 and P-7), (P-7 and P-2), (P-4 and P-2), (P-4 and P-1) and (P-6 and P-3), which are thus clustered for presenting, in general, smaller fruits.

In the first year, the variability retained by the second canonical variable (Can2) was 18.2%, mainly due to the contribution of CD, FFH, PH, PT, SD, CD and LW (Figure 1A). Silva Neto et al. (2014), working with *C. annuum*, reported that the characteristics that contributed most to the diversity were: stem diameter, canopy diameter and the first fork height. It is also observed that the populations P-4, P-1, P-2, P-7, P-3 and P-11 showed lower values for variables related to the size of plants, while populations P-5 and P-8 showed the highest values. According to Rêgo et al. (2011a), variables related to growth habit and harmony between plant canopy and vase size are crucial for the potential of pepper as ornamental plant. Thus, populations presenting lower contribution to that characteristic can be selected for cultivation in vase, whereas when they have higher

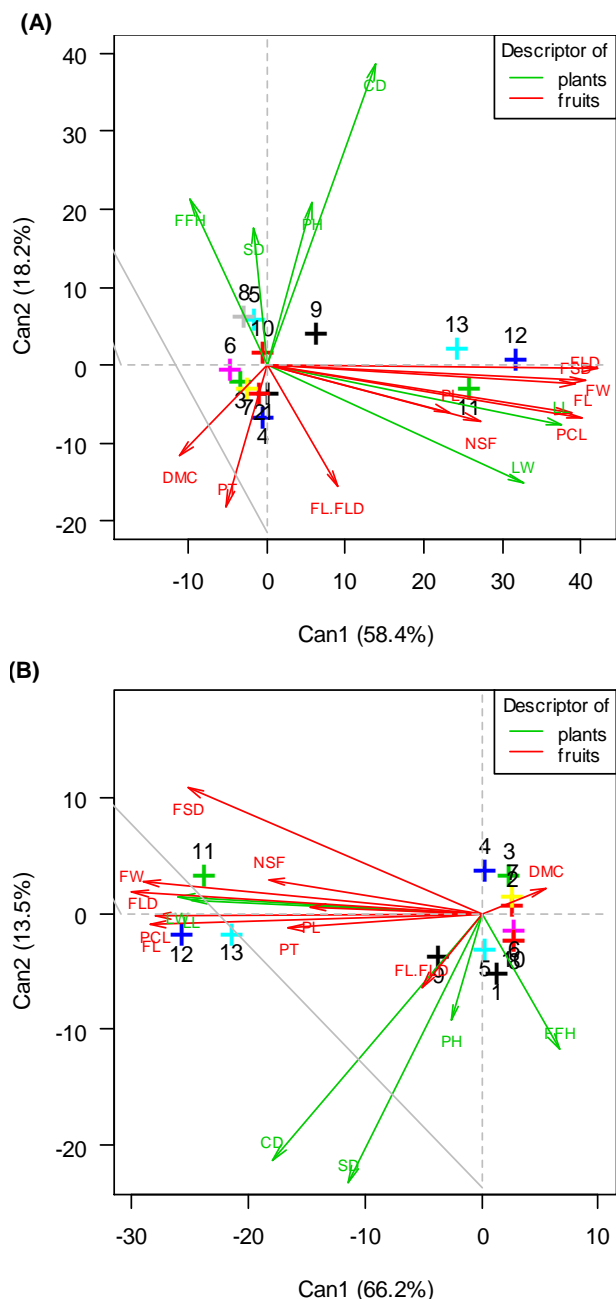


Figure 1. Scattering of scores of the first two canonical variables (Can1 and Can2), obtained from morphoagronomic descriptors of 13 populations of ornamental pepper (*C. annuum* L.), in 2013 (A) and 2014 (B). PH, plant height; CD, canopy diameter; FFH, first fork height; SD, stem diameter; LL, leaf length; LW, leaf width; FL, fruit length; FLD, fruit largest diameter; FSD, fruit smallest diameter; PL, peduncle length; PT, pericarp thickness; PCL, placenta length; the ratio FL/FLD; dimensionless; FW, fruit weight; DMC, dry matter content; and NSF, the number of seeds per fruit.

values, should be discarded.

The first two canonical variables retained together 76.6% of the variability between populations (Figure 1A)

and, then, it was possible to identify two clusters of F_3 populations: Cluster 1: P-1, P-2, P-3 and P-7; Cluster 2: P-8 and P-5. The distance between these two clusters and the populations P-11, P-12 and P-13 is evident. According to Cruz and Regazzi (2004), the formation of clusters with intracluster homogeneity and heterogeneity intercluster is the starting point for a more thorough assessment thereof, for use in future breeding programs.

In the second year of evaluation (Figure 1B), the variability retained in Can1 increased to 66.2%, and the descriptors that contributed to the distancing of the populations were: FW, FLD, FL, PCL, LW and FSD. In relation to Can2, which retained 13.5% of the total variability, the variables that contributed most were: SD, CD, FFH, PH, FSD and FL/FLD (Figure 1B). It highlights the phenotypic proximity between P-2 and P-7, also found in the first year, and the proximity of P-6, P-8 and P-10. This last cluster was formed probably due to the higher contribution of the characteristics relating to the size of the plant, presented by the populations P-6 and P-10, higher than that observed in plants of P-8, in the second year.

The first two canonical variables retained together 79.7% of the variability among populations (Figure 1B). The relationship between the characteristics studied and the evaluation period (first and second year) decisively influenced the behavior of the populations, because it is observed that in the first year, the P-1 population had one of the lowest values for the size of plant characteristics while in the second year, showed relatively larger plants. Figure 1A can be seen that the P-5 and P-8 populations showed a similar behavior to that seen in the first year of assessment. Nevertheless, in the second year, both had increased relative to the size characteristics. It is worth noting the behavior exhibited by the population P-2, P-3, P-4, P-7, P-9, P-12 and P-13, which did not change when evaluated in the first and second years. Probably this fact is related with genetic factor, since there were no drastic changes in environmental issues.

In Figure 2A and B we present the dendrograms obtained with Ward's algorithm, for the first and second year, respectively. In both years, only two clusters of populations were identified: (i) Populations from P-1 to P-10, and (ii) P-11, P-12 and P-13. These clustering were also identified by the canonical variables. Therefore, the canonical discriminant variables were more effective than the cluster analysis via Ward algorithm based on Mahalanobis distance. For instance, it can be seen through the canonical scores that P-6 diverges from P-9 (Figure 1A). This result was not verified using the cluster analysis (Figure 2A).

According to Bosland (1993), low-sized plants that produce small fruits, are considered promising for ornamental pepper agribusiness. One can also point out that plants with low-size and small canopy diameter are more harmonics. Furthermore, it is important to consider the relationship between plant architecture and vase size.

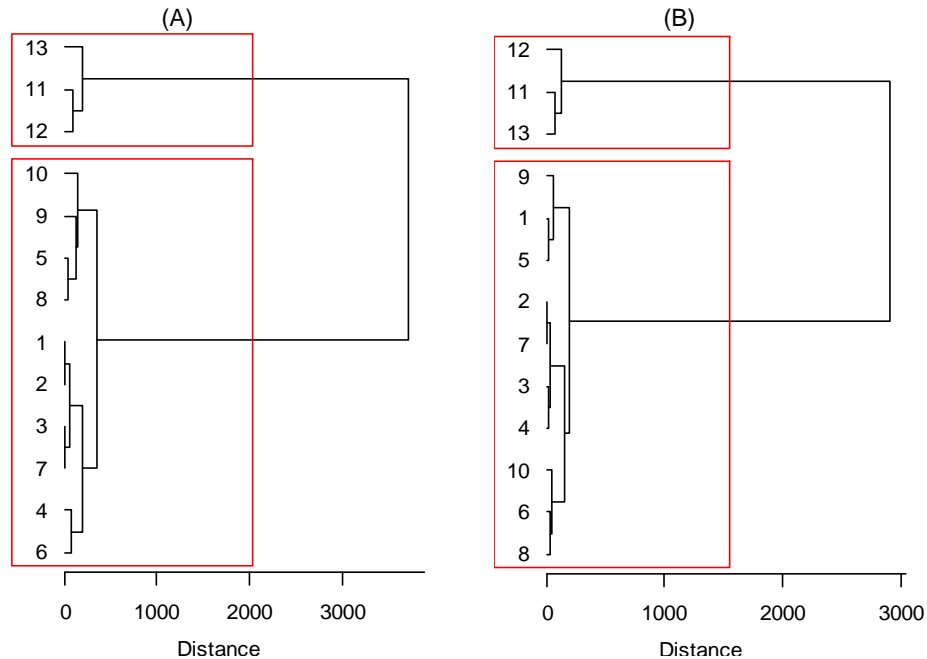


Figure 2. Dendrogram obtained using Ward's algorithm based on Mahalanobis distance from morphoagronomic data of 13 populations of ornamental pepper (*C. annuum* L.), in 2013 (A) and 2014 (B).

The plant height and canopy diameter should be 1.5 to 2 times larger than the size of the vase (Barbosa et al., 2002; Barroso et al., 2012).

Conclusions

It can be inferred that the populations are divergent, enabling the formation of different clusters, with the multivariate techniques - canonical discriminant variables and cluster analysis - presenting considerable agreement on the composition of the main clusters and on the contribution of the descriptors. Nonetheless, the canonical discriminant variables were more effective than the cluster analysis via Ward algorithm based on Mahalanobis distance. Fruit weight, fruit smallest diameter, fruit length and fruit largest diameter explained most of the variation among the populations, in the two years of evaluation. In breeding programs aimed at obtaining ornamental sized pepper, one should give importance to populations P-2, P-3, P-4 and P-7, with small values for plant and fruit size, that is, it is recommended to carry out selection within these populations, in order to continue the ornamental pepper breeding program.

Abbreviation

PH, plant height; **CD**, canopy diameter; **FFH**, first fork height; **SD**, stem diameter; **LL**, leaf length; **LW**, leaf

width; **FL**, fruit length; **FLD**, fruit largest diameter; **FSD**, fruit smallest diameter; **PL**, peduncle length; **PT**, pericarp thickness; **PCL**, placenta length; **FL/FLD**, the ratio between fruit length and fruit largest diameter; **FW**, fruit weight; **DMC**, dry matter content; **NSF**, the number of seeds per fruit; **MANOVA**, multivariate analysis of variance; **Can1**, first canonical variable; **Can2**, Second canonical variable.

Conflict of Interests

The authors have not declared any conflict of interest.

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Full Length Research Paper

Operating and energetic performance of the assembly tractor-scarifier in different liquid ballasting and working depths

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The working depth and ballasting are factors that can influence directly the operational and energy performance of a mechanized set. The objective of this study was to evaluate the operational and energetic performance of the set tractor-scarifier, working at three depths under two ballasting conditions. The study was conducted in the experimental area of mechanization of the Department of Agricultural Engineering at the Federal University of Ceará in Fortaleza. The experimental design was of randomized blocks in a factorial scheme 2 x 3, with four replications, with two liquid ballasting (0 and 75% water) and three working depths (0.15; 0.30 and 0.40 m). The parameters evaluated were the soil water content, periodic and specific consumption of fuel, the overall work rate, slipping of the front and rear wheels of the tractor, travel speed, specific operational resistance, mobilized and lifting area, blistering, strength and power in the drawbar. The ballasting with 75% of water associated with a lower depth provided greater operational field capacity, lower demand for strength and power in the drawbar with lower fuel consumption by area.

Key words: Soil tillage, tyre, consumption of fuel.

INTRODUCTION

Soil compaction and formation of the densified layer can be considered one of the main limiting factors of productivity. According to Fernandes et al. (2012) it is a process that can occur artificially by constant moving

machines that compress the soil surface or naturally by rainfall and long dry spells.

Scarification or subsoiling are recommended techniques for soil unpacking, revolving hardened layers

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in the subsurface or in greater depth, however, the soil decompression process or disruption of dense layers is an operation with high energy demand and low operating performance, requiring studies on the subject.

According to Russini (2012), assessing the energy and operational performance of mechanized sets, is a complex task because of the many variables that must be analyzed within a fairly wide area of influence. In this context, the working depth and ballast to be used are factors which may directly influence the operational performance and energy assembly.

Cortez et al. (2011) reports that some implements have better operational capacity than others. Thus, Fernandes and Gamero (2010) by studying the operational performance in reduced tillage and conventional technique found that the theoretical field capacity light grid was 1.28 ha h^{-1} , while for the scarifier was 0.80 ha h^{-1} , in the speeds 5.01 and 2.87 km h^{-1} , respectively.

Compagnon et al. (2013) on evaluating the performance of the tractor-scarifier assembly in two different depths, concluded that the greater the depth of the scarifier work, the greater the increase in consumption and operating time, fuel tensile strength, power, and slipping of the bar and front wheeled of the tractor.

Lopes et al. (2005) evaluated the tractor performance depending on the type of ballast, tires and working speed and concluded that the combination of ballast condition and the range selected for output variables in the bar and effective field capacity, allowed the tractor to work more efficiently at the speed 4.57 km h^{-1} in the tillage operation with the scarifier.

Carvalho Filho et al. (2007) evaluated the mobilization of a red Latosol, and found that the scarifier provided less tillage compared to moldboard plow. However, Mazurana et al. (2011) observed that the mobilization promoted by scarification reduces bulk density, mechanical resistance to penetration and increases water infiltration.

Therefore, the aim of the present study was to evaluate the operational and energetic performance ripper tractor set, working at three depths under two conditions of liquid tractor ballasting.

MATERIALS AND METHODS

The study was conducted in the experimental area in the Department of Agricultural Engineering of the Federal University of Ceará, located in the geographical coordinates $03^{\circ}44' \text{ S}$ latitude and $38^{\circ}34' \text{ W}$ longitude, with an average altitude of 26 m. According to Koeppen's classification (1948), the region is defined as Aw^{\prime} , that indicates rainy tropical climate. The soil of the area was classified with a Red-yellow Argisol, with sandy frank textural class, with approximately 82.90% sand, 10.60% clay and 6.40% silt, following the methodology of (EMBRAPA, 1997).

The experimental design was of randomized blocks in a factorial scheme 2×3 , with four replications, being two liquid ballasting (0 to 75%) of the front and rear tires and three depths of scarification (0.15, 0.30 and 0.40 m), totalling 24 experimental units, 3.5 m wide and 15 m long.

In the scarification operation Marchesan scarifier was used, AST / MATIC 450 model, it was configured with five rods spaced 0.4 m, 0.08 m narrow tip, harrowing roller, automatic disarming security system with total mass of 1560 kg. The work depth control was carried out through the scarifier tires, with the help of rings stuck to the hydraulic cylinder.

The scarifier was pulled by the tractor BM120 4 x 2 TDA (front wheel assist), of 88, 26 kW (120 hp (cv)) in the engine in the rotation of 2000 rpm, with front-wheel drive on, equipped with bias tires, front axle with tires 14.9-24 R1 and rear axle with 18.4-34 R1 tires, with inflation pressure of 12 and 16 psi (82.8 and 110.4 kPa), respectively according to manufacturer's recommendation. The power-to-weight ratio according to liquid ballasting treatment was of 52 and 58 kg hp^{-1} respectively.

Through Equation 1, the slippage was determined by counting the number of turns from the tractor wheeling in experimental part pulling the implement (with load) and the implement in transport mode (without load).

$$PR = \left[\frac{n^1 - n^0}{n^1} \right] \times 100 \quad (1)$$

In which:

PR = Slippage of the tractor wheels (%);

n^0 = Number of turns from the wheels without load;

n^1 = Number of turns from the wheels with load.

The overall work rate was obtained according to the working width of the implement, travelling speed and efficiency of the operation. The travelling speed was determined by dividing the length of the portion by the time reckoned by digital timer, triggered on and off according to the passage of the front wheels of the tractor laterally to the stakes that bordered the parcels.

For the acquisition of fuel consumption data and time of each route, an electronic system with pulse counters for obtaining readings from the flowmeters and a stopwatch to measure the tractor time in each parcel were used.

In order to measure the fuel consumption, two flow meters were used, both of the brand "Flowmate" Oval Model M-III and LSF 41 with a precision of 0.01 ml installed in series at the entrance and return of the injection pump, thus the volume of fuel consumed by tractor along the way in ml can be obtained, it is possible by means of Equation 2, to determine consumption in L h^{-1} .

$$C_H = \left(\frac{q}{t} \right) \times 3.6 \quad (2)$$

In which:

C_H = Hourly fuel consumption (L h^{-1});

q = Volume consumed in the parcel (ml);

t = Time to go through the parcel (s);

3.6 = Unit conversion factor.

Subsequently to the obtainment of hourly fuel consumption (L h^{-1}), the consumption was calculated in L ha^{-1} (Equation 3)

$$C_A = \frac{C_H}{CC_e} \quad (3)$$

In which:

C_A = Fuel consumption by area, L ha^{-1} ;

C_H = Hourly fuel consumption, L h^{-1} ;

CC_e = Effective field capacity (ha h^{-1}).

The specific fuel consumption was determined by means of

Equation 4.

$$C_E = \frac{C_H \times d}{P} \quad (4)$$

In which:

CE = specific fuel consumption (kg kWh⁻¹);

CH = Hourly fuel consumption (L h⁻¹);

d = density of the fuel (kg L⁻¹);

P = power in the bar (kW)

To determine the power requirement in drawbar, a load cell of the HBM brand was used. To collect the load cell data, the data acquisition system from HBM model Quantum XMX804A was used with ability to monitor and record information at a frequency of 19.200 Hz. With the values obtained, the average power on the draw bar was determined by Equation 5. The average power in the drawbar was calculated based on the average tractive force and the actual travelling speed of the set.

$$F = \left(\frac{\sum F_i}{\sum n} \right) \times 0.0098 \quad (5)$$

In which:

F = Average power on the draw bar, kN;

Fi = instant traction force, kgf;

n = Number of recorded data;

0.0098 = Adequacy factor.

The mobilized area corresponds to the area between the natural soil profile and bottom profile of the furrow left by the implement, in order to determine it a wood profilometer of 3 m wide and 1 m in height with a vertical base for fixing millimetered paper was used, for that, a survey of the natural surface profile, background and ground elevation was conducted. According to the theory of differential and integral calculus after the construction of curves delimiting the natural soil profile and the bottom soil profile, we obtain the upper and lower amounts, for performing estimation of the area. Thus, lower amounts were used, with the construction of vertical rectangles with 0.005 respectively m width and height not exceeding the established lines. The mobilized area (Equation 6) is the sum of these partial areas.

$$MSA = \sum_{i=1}^n 0.5h_n \quad (6)$$

In which:

h_n – rectangle height of order n.

The operating specific resistance was obtained from Equation. 7, taking into account the average tensile strength and ground area mobilized.

$$SOR = \frac{F_m}{AMS} \quad (7)$$

In which:

SOR - specific operational resistance, kN m⁻²;

F_m - mean traction force, kN; and

MSA - mobilized soil area, m².

Finally the decision was for the rectangle area formula to determine each partial area, following Thomas methodology et al. (2012). By using Equation 6, soil blistering was determined.

$$E_m = \frac{AE}{AM} \times 100 \quad (8)$$

Where:

E = Blistering (%)

AE = Elevation Area (m²)

AM = Mobilized area (m²)

RESULTS AND DISCUSSION

The data was submitted to normality test using the coefficients of symmetry and kurtosis according to Mesquita et al. (2003). After checking the normality of the data, variance analysis was carried out and when significant, the Tukey test was applied at 5% probability for weighted average.

In Figure 1, the symmetry coefficients (A) and kurtosis (B) can be found for the studied parameters. It can be observed that all the values obtained for the symmetry and kurtosis coefficients are within the range -2 to 2.

Values of symmetry and kurtosis coefficients within the range of -2 and 2 indicate that the data follow a normal distribution, since according to (Montgomery, 2004) the coefficients of symmetry and kurtosis with values less than 2 and greater than -2, represent small deviation from the normal distribution, the hypothesis of data normality can be considered, a necessary condition to carry out a variance analysis and obtain results safely.

In Table 1, for the hourly fuel consumption, according to variance analysis, it can be observed that there was no significant difference between the averages (p < 0.05) of the assessed ballasting and depth factors.

These results contrast with those observed by Monteiro et al. (2013) who found significant differences in fuel consumption as a function of liquid ballast in the tires. However, these authors worked in clay texture soil, a condition that requires greater force from the tractor to pull the implements and consequently higher energy demand, yet this study was developed in sandy texture soil which may have contributed to this result.

The specific consumption for different working depths presented significant difference between the average, the lowest value being in the depth of 0.40 m, a result that can be associated with greater demand for power by the implement working in greater depth P1 – 15.69; P2 – 23.96 e P3 – 25.99 kW respectively, because the specific fuel consumption is obtained as a function time and power consumption.

Similar result was found by Palma et al. (2010), that in assessing the fuel consumption of a Valtra BL 88 4X2 tractor with front-wheel assisted drive (TDA) pulling a precision fertilizer-seeder, with chisel plow at different depths (100, 150, 200 and 250 mm), biggest consumptions was observed in the smallest depth.

The specific consumption for different ballasts was not significant. These results disagree with those observed by Lopes et al. (2005), who evaluating the performance of an agricultural tractor 4 x 2 TDA, of 89 kW (121 cv (hp)) maximum engine power, pulling a drag scarifier combined with a harrowing roller and cutting wheels with seven angled straight rods and ferrules without wing with 7 cm wide, the lowest specific consumption found with

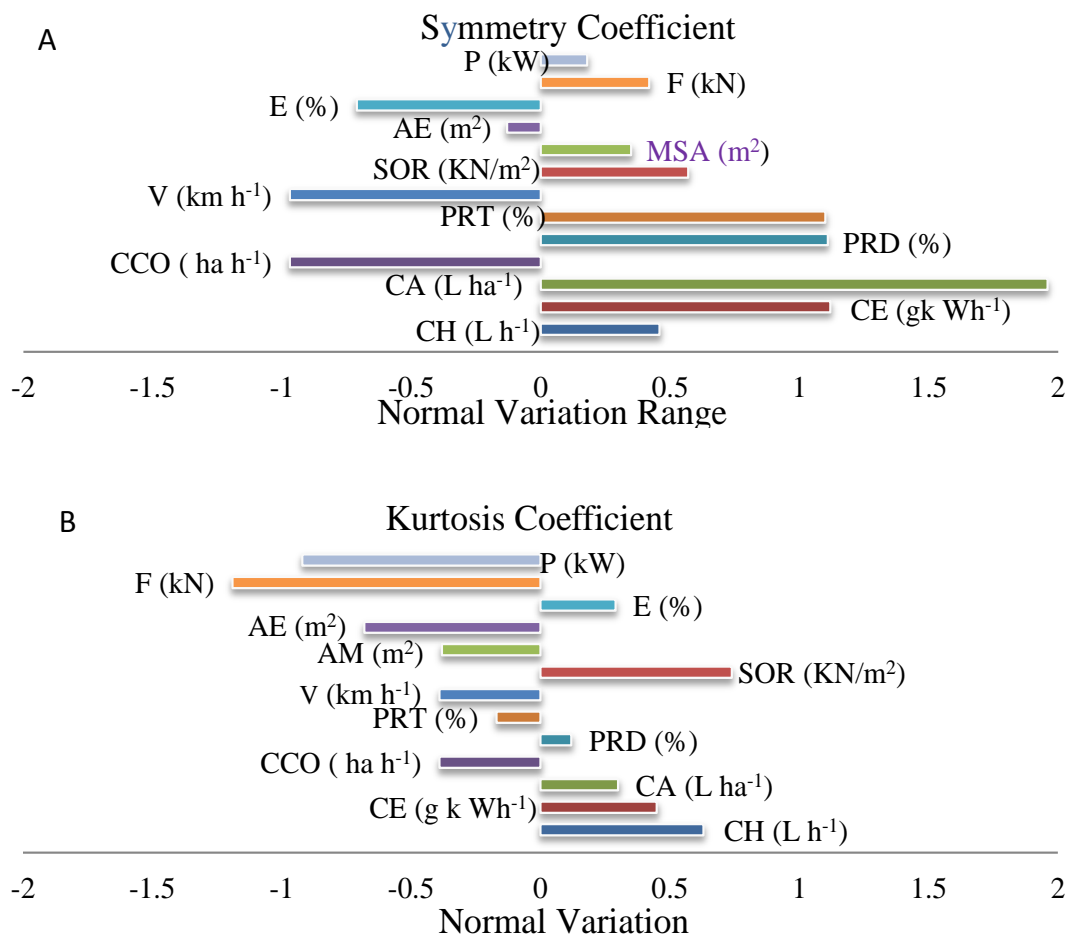


Figure 1. (A) Symmetry coefficient and (B) Kurtosis for all of the assessed parameters. CH- Fuel consumption in L.h⁻¹; CE- Specific fuel consumption; CA- Fuel consumption in L.ha⁻¹; CCO- The overall work rate; PRD- Slippage of the front axles; PRT- Slippage of the rear axles; V- Traveling speed; SOR- Specific operational resistance; MSA- mobilized area; AE- elevation area; E- blistering; F- Strength on the drawbar; P- Power on the drawbar.

ballasting being 75% of water in the tire. For consumption per area the result of variance analysis was significant for the working depth factor, being greater in depth of 0.40 m result that may be associated with greater ground area mobilized by scarifier rods and also by higher tensile force required by the equipment to overcome the resistance offered by the soil.

Compagnon et al. (2013), assessing the energy and operational performance of a tractor of Valtra brand, BM 125i model, 4 x 2 TDA, pulling the Marchesan scarifier, AST / MATIC 450 model, with a total mass of 1400 kg in red clayey textured eutroferric Oxisol, they also found that the greater the working depth the greater the fuel consumption in L ha⁻¹.

For the overall work rate it can be observed that there was a significant interaction between the analyzed variables, the consequences of the interaction are shown in Figure 2.

It can be observed in the unfolding of the ballasting in

the depths (C) that the only one that differed significantly was L1, corresponding to 0% of liquid ballast, with the lowest value in the depth P3 (0.40 m) a result that may be associated with higher slippage of the tractor in that same treatment that contributed to reduce the speed and consequently lower the overall work rate. Lopes et al. (2005) evaluating the performance of a tractor on red eutroferric Oxisol also found that the field capacity was lower when he worked without liquid ballast in the tire.

According to the values presented for the unfolding of the depths within the ballasting (D), it is observed that only the depth P3 (0.40 m) shows significant difference with lower values in the evaluated ballasting L1 and L2 (0 and 75% water), a result that can be associated with the fact that greater working depth mobilize greater ground area P1 – 0.26; P2 – 0.32 and P3- 0.46 m² respectively, contributing for speed reduction, directly affecting the overall work rate. Similar results were obtained by Compagnon et al. (2013) in the depths of 0.20 and 0.30 m.

Table 1. Average values for fuel consumption per hour (CH), Specific consumption (CE), Consumption per area (CA) and the overall work rate (CCO).

Sources of variation		CH (L h ⁻¹)	CE (kg k ⁻¹ Wh ⁻¹)	CA (L ha ⁻¹)	CCO (ha h ⁻¹)
Ballasting (L)	L1	9.85	0.41	4.47	0.59
	L2	9.67	0.34	4.18	0.65
Depth (P)	P1	9.55	0.51 ^a	3.00 ^b	0.71
	P2	9.69	0.34 ^b	4.93 ^a	0.69
	P3	10.04	0.32 ^b	5.04 ^a	0.47
F Value	L	0.04 ^{NS}	3.81 ^{NS}	0.57 ^{NS}	28.32 ^{**}
	P	0.11 ^{NS}	4.65 [*]	12.51 ^{**}	191.0 ^{**}
	L*P	3.55 ^{NS}	3.51 ^{NS}	2.99 ^{NS}	5.0 [*]
DMS	L	1.78	0.11	0.79	0.02
	P	2.65	0.17	1.19	0.03
CV (%)		21.29	32.74	21.17	4.40

Averages followed by the same letter or no letter in the columns do not differ from each other by Tukey test at 5% probability. * - significant ($p < 0.05$); ^{NS} - non significant ($p > 0.05$). L- Ballasting. P- Depth. L1- Ballasting 1 (0% water); L2- ballasting 2 (75% water); P1- depth 1 (0, 15 m); P2- depth 2; (0, 30 m) P3- depth 3 (0, 40 m). DMS- minimum significant difference. CV- variation coefficient.

In Table 2 it can be observed that for the variable specific resistance there was no significant difference for the analyzed factors, demonstrating that the soil used to perform the scarification process has no resistance to shearing, as it's a soil with sandy loam texture class.

Similar results were found by Sasaki et al. (2005), working with single-stem subsoiler attached to the hydraulic system in three points of the tractor, with depth control by mounting clip, found that the requirements of structure and soil texture is closely related to the dynamic resistance because soils with high sand contents give lower hardness due to their mineralogy.

For the slippage of the front and rear axles of the tractor and travelling speed variables it can be observed that there was a significant interaction, the developments are shown in Figures 3, 4 and 5. It can be observed for the slippage of the front wheels of the tractor in the unfolding of the ballasting at all depths (E), the only one that differed was L1, corresponding to 0% of liquid ballast, with biggest value in depth 0.40 m.

A result that can be associated with low weight to power of the tractor ratio, not being suitable for working at this depth, it requires the addition of weight, because the slipping values are above the index envisioned by the ASAE (2003), to firm ground, which is 8 to 10%.

Monteiro et al. (2011) while evaluating the performance of an agricultural tractor equipped with radial and diagonal tires with three levels of liquid ballast on solid ground condition, obtained different results with slippage below that recommended with liquid ballast 0%, however, the tractor used by him was not pulling a scarifier at

different depths.

In the deployment of depths in each ballast is observed that the slippage increases with increasing depth and the highest value was found working in P3 (0.40 m) with liquid ballast of 0% in the tires, indicating that the tractor is with little ballast to overcome the soil resistance at greater depths.

For the depth of 0.15 m with slippage of 5.78 and 6.92% associated with ballastings of 0 and 75% water, show the absence of need for ballasting, because these amounts are below the index envisioned by ASAE (2003) to firm ground, which is 8-10%, evincing that the tractor was with ballast above the recommended to work with scarifier, with possible ballast removal.

In the unfolding of ballasting inside the depths to the slipping of the rear wheel (G), it is observed that the only one that was significantly different was 0% of water in the depth of 0.40 m, with higher slippage and finding values above those recommended by ASAE (2003), indicating that the tractor is with inadequate ballasting for the operation. Similar results were verified by (Gamero, 2008) evaluating the operating performance of a shank subsoiler with lateral curvature ("Paraplow"), finding greater slippage in the depth of 0.35 m.

In the unfolding of depths within the ballastings (H) the depth P3 (0.40 m) in all ballastings provided greater slippage, with greater value for L1 (0% of liquid ballast in the tire). This result may be associated with greater demand for strength and power by the scarifier for it is working in greater depth, associated with a lower load on the tractor which provides a lower weight-power ratio,

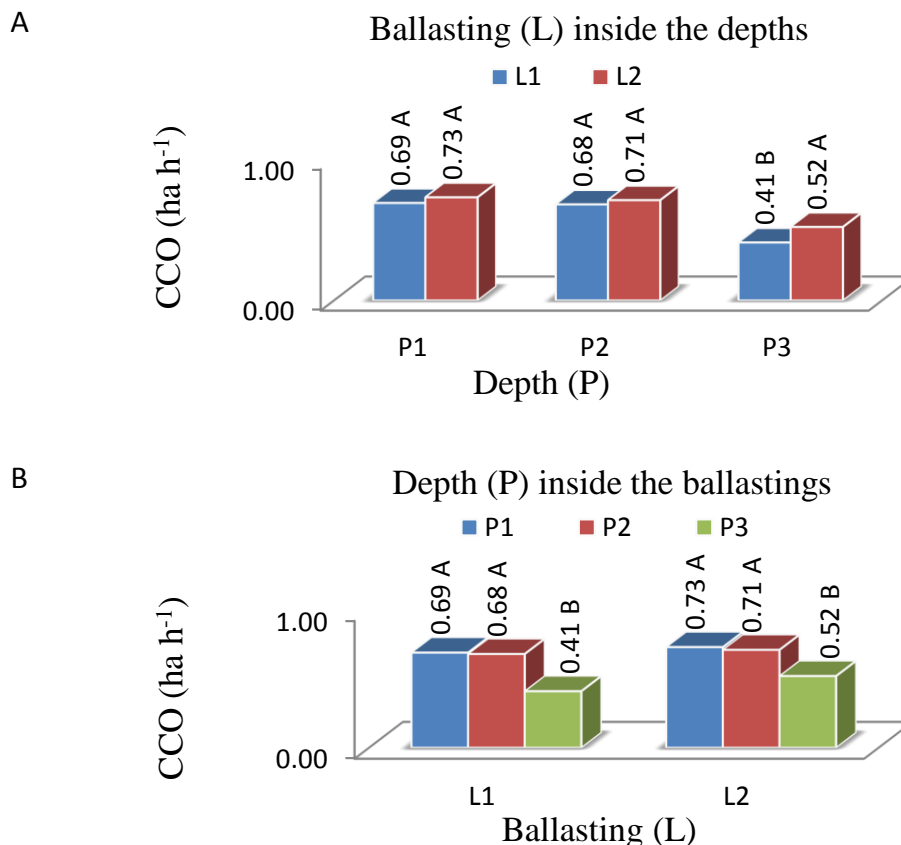


Figure 2. Ramifications of the interaction between factors a) working depth and b) ballasting for the overall work rate variable. Averages followed by capital letters in the columns do not differ by Tukey test 5% probability.

Table 2. Average values for specific operational resistance (ROS), slippage of the front axles (PRD), and rear (PRT) from the tractor and travelling speed (V).

Variation sources		ROS (kN m ⁻²)	PRD (%)	PRT (%)	V (km h ⁻¹)
Ballasting (L)	L1	603.38	20.36	20.07	3.99
	L2	582.03	14.44	13.93	4.39
Depth (P)	P1	551.99	6.34	4.65	4.78
	P2	586.84	11.53	10.11	4.64
	P3	639.28	34.33	36.23	3.15
F Value	L	0.11 ^{NS}	19.46*	37.23*	28.32**
	P	0.63 ^{NS}	163.96*	374.90*	191.0**
	L*P	1.45 ^{NS}	11.57*	40.59*	5.06*
DMS	L	134.24	2.81	2.11	0.15
	P	199.83	4.19	3.14	0.23
CV (%)		26.42	18.89	14.50	4.40

Averages followed by the same letter or no letter in the columns do not differ by Tukey test at 5% probability. * - Significant ($p < 0.05$); ^{NS} - non significant ($p > 0.05$). L1- ballasting 1 (0% water); L2- ballasting 2 (75% water); P1- depth1 (0.15 m); P2- depth 2; (0.30 m) P3- depth 3 (0.40 m). CV- variation coefficient. DMS- minimum significant difference.

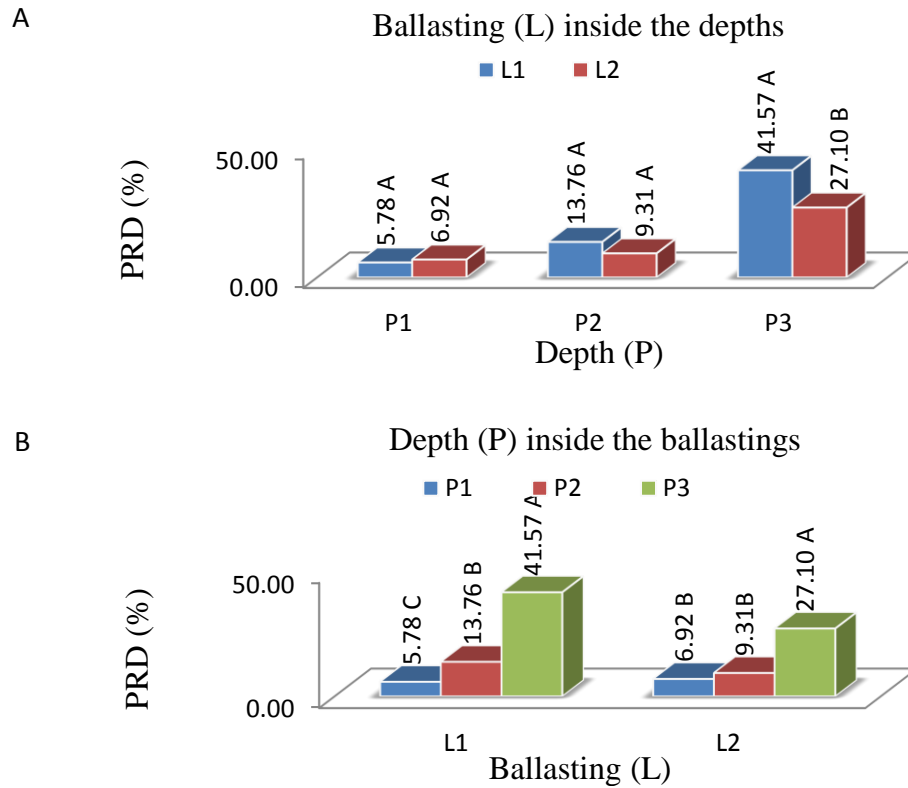


Figure 3. Consequences of the interaction between a) ballasting and b) rod depth factors for speed variable. Averages followed by capital letters in the columns do not differ by Tukey test at 5% probability.

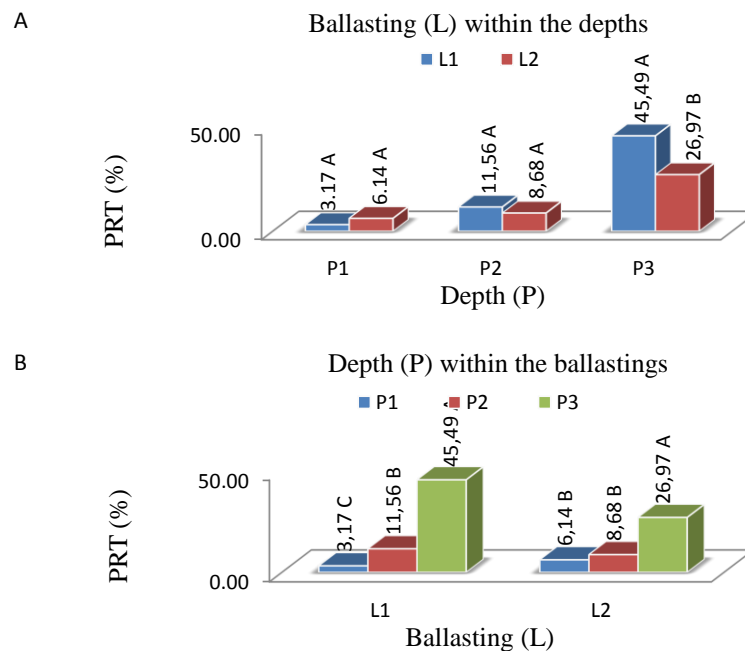


Figure 4. Consequences of the interaction between A) working depth and B) ballasting for slippage of the rear wheels of the tractor factors. Averages followed by capital letters in the columns do not differ by Tukey test at 5% probability.

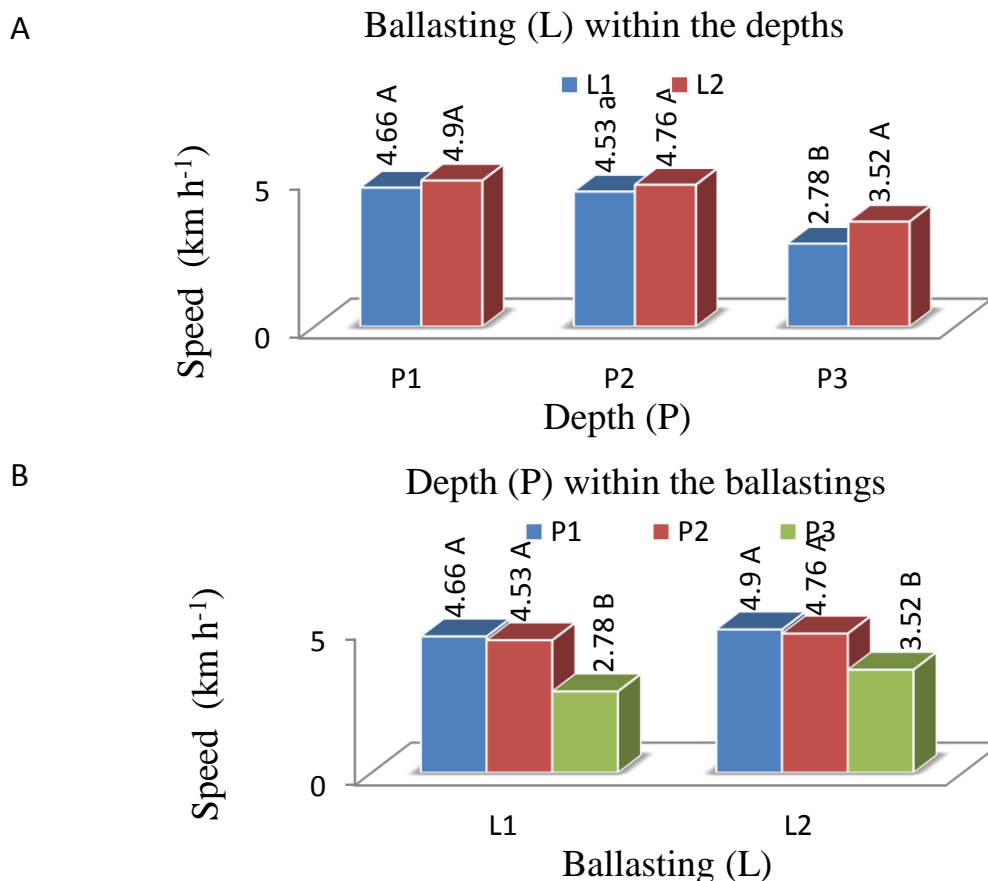


Figure 5. Developments of significant interaction between a) ballasting and b) rod depth factors for speed variable. Averages followed by capital letters in the columns do not differ by Tukey test at 5% probability.

thus contributing to increased slippage with values above those recommended by the ASAE (2003), indicating that the tractor is with inadequate ballast for the operation.

In the unfolding of ballasting within the depths (I) for traveling speed variable, it's noticeable that there was a significant difference only in P3, that working with L2 (75% of liquid ballasting in the tire) provided greater travelling speed, this result may be associated with the fact that bigger cargo provide greater contact area of the wheels with the ground, possibly increasing the traction coefficient, reducing slippage and favoring greater speed. Differently, in the unfolding of depths within the ballasts (J) it was observed that there were significant differences in the greatest depth (P3) within the two ballasts, resulting in lower travelling speeds values, this result might be associated with an increased slippage of wheel sets and the bigger force demand to pull the implement. Gamero (2008) when working with different depth and marches scheduling also observed lower speed in the greatest depth pulling a subsoiler.

In Table 3, it can be observed that for mobilized area, there is no significant difference ($p > 0.05$) between

averages for the ballasts used, a result which may be attributed to minimum weight transfer from the tractor to the scarifier attached to the draw-bar, whereby the addition of liquid ballast to the tractor wheels interferes little with the penetration of the rods into the ground.

For the depth of the stems, the result was significant with highest average value of 0.46 m² in the working depth 0.40 m, attributable to adjustment of greater depth, mobilizing greater amount of soil. The values obtained for mobilized ground area were close to the values found by Santos et al. (2014), evaluating soil tillage, water infiltration speed and soil coverage rate in the emerald grass under mechanized handlings with scarifier.

The elevation area presented significant result ($p < 0.05$) difference between the averages for the two factors, ballasting and depth. The L2 (75% water) was the one that provided greater area of elevation, a result that may be related to higher speed developed in the same treatment. The depth with greater elevation area was 0.40 m with an average value of 0.08 m², which may be associated with a greater soil area mobilized.

For soil blistering there was significant results only for

Table 3. Average values for mobilized soil area (MSA), elevation area (AE), soil blistering (E), strength (F) and power (P) according to two liquid ballast and three depths of the chisel plow.

Variation causes		MSA (m ²)	AE (m ²)	E (%)	F (kN)	P (kw)
Ballasting (L)	L1	0.33	0.04 ^b	15.84 ^b	19.10	19.97
	L2	0.34	0.07 ^a	22.36 ^a	20.64	23.79
Depth (P)	P1	0.23 ^c	0.04 ^b	19.92	11.80	15.69
	P2	0.32 ^b	0.06 ^{ab}	19.09	18.57	23.96
	P3	0.46 ^a	0.08 ^a	18.29	29.25	25.99
F Values	L	0.38 ^{NS}	8.23 [*]	4.69 [*]	3.48 ^{NS}	13.37 ^{**}
	P	35.04 ^{**}	5.54 [*]	0.09 ^{NS}	151.20 ^{**}	36.45 ^{**}
	L*P	0.32 ^{NS}	0.09 ^{NS}	0.21 ^{NS}	7.0 ^{**}	12.00 ^{**}
DMS	L	0.04	0.01	6.39	17.34	22.22
	P	0.07	0.02	9.55	25.82	33.18
CV (%)		16.60	35.84	38.53	9.62	11.68

Averages followed by the same letter or no letter in the columns do not differ by Tukey test at 5% probability. * - Significant ($p < 0.05$); ^{NS} - non significant ($p > 0.05$). L1- ballasting 1 (0% water); L2- ballasting 2 (75% water); P1- depth 1 (0.15 m); P2- depth 2; (0.30 m) P3- depth 3 (0.40 m). DMS- minimum significant difference; CV- variation coefficient.

ballasting with the highest average value of 22.36 m² to L2. Result that may be related to the higher speed developed in the ballasting of 75% of water. Rosa et al. (2011) when evaluating the effect of compaction and deformation under the action of the subsoiler tip, found no difference in elevation area at depths of 0.23 and 0.15 m, however, they found greater blistering in the depth of 0.15 m, associating this result to the occurrence of compacted layers.

For the power on the draw bar, it can be observed that there was a significant interaction between the variables analyzed, and the unfolding of the interaction are shown in Figure 6. Verifying ballasting at each depth (K), the greatest power with difference between the averages was observed in the depth of 0.40 m in ballast L2 (75% water). A result that can be attributed to larger contact area between tire and soil due to its deformation when adding liquid ballast, higher power to weight ratio, associated with greater rupture resistance of soil structures on account of greater depth. Monteiro et al. (2013) obtained a similar result, in accomplishing the energetic evaluation of a 4 x 2 TDA tractor in the light of ballasting with water, as a result, higher power values were found when increasing the ballasting.

Checking the behavior of power with respect to depth for each evaluated ballast (L) with 0% water (L1) highest power values were obtained in the greatest depths (P2 and P3). In the ballast with 75% water was found highest power value at greater depth, corresponding to 31.34 kW. The largest power values observed at greater depths may be associated with greater demand for rupturing the

soil structure at greater depths. Lopes et al. (2005) when evaluating the tractor performance according to the type of ballast, tires and working speed, in the soil preparation operation with scarifier also revealed higher power values when using greater liquid ballasting.

To force values in the drawbar Figure 7, relating ballasting into the depths (M), it can be seen that only the depth of 0.40 m, the average differentiated from each other, with higher value in the ballasting 75% of Water. Result that can be attributed to larger contact area between tire and soil due to its deformation when adding liquid ballast, higher power to weight ratio with the ballast of 75% water, associated with increased resistance to rupture of the soil structures due to the higher depth.

Verifying the strength in relation to the depths for each ballast (N), with 0% water (L1) higher strength values were obtained in the greatest depth (P3). In the ballast with 75% water, the highest force value was found in the greatest depth, corresponding to 3.02 kN. The highest force values observed in the greatest depth may be associated with greater demand to rupture the soil structures for working at greater depths. Rosa et al. (2011) when evaluating the effect of compaction and deformation under the action of the subsoiler tip, also found greater demand for power in the greater depths.

Conclusion

The ballasting with 75% of water associated with a smaller depth provides greater operational field capacity,

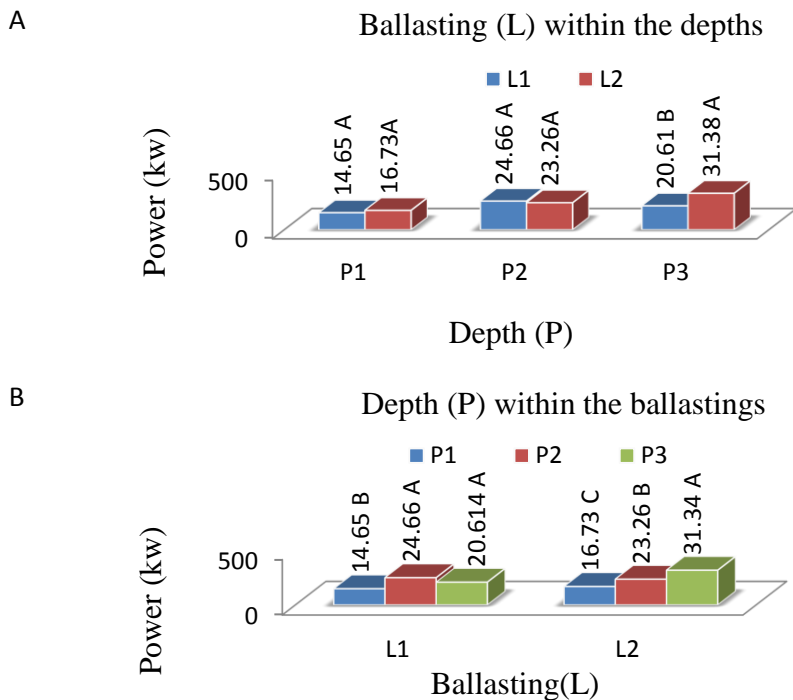


Figure 6. Graphical representation of the consequences of significant interaction between the factors, ballasting and depth rod for variable power. Averages followed by capital letters in the columns do not differ by Tukey test at 5% probability.

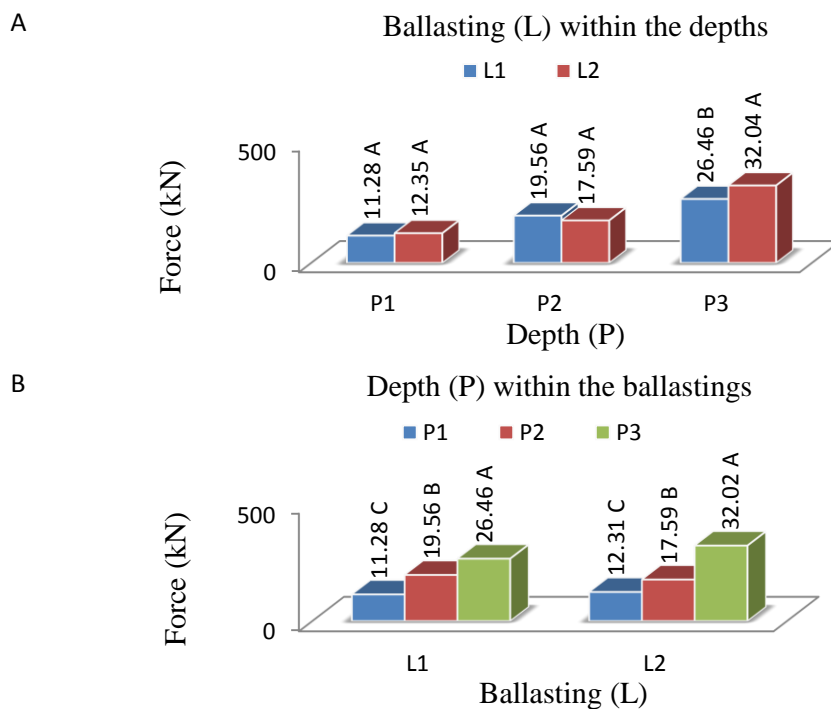


Figure 7. Graphical representation of the consequences of the significant interaction between ballasting and stem depth factors to the force variable. Averages followed by capital letters in the columns do not differ by Tukey test at 5% probability.

lower demand for force and power in the drawbar with lower fuel consumption per area.

Most ripper working depth increases fuel consumption by area, slipping, speed, ground area mobilized, strength and power in the drawbar.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Exploitation of variability for salinity tolerance in maize hybrids (*Zea mays* L.) at early growth stage

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Salinity is extremely serious problem that has a drastic effect on maize crop, environment and causes economic losses of country. An advance technique to overcome salinity is to develop salt tolerant genotypes which require screening of huge germplasm to start a breeding program. Therefore, present study was undertaken to screen out 25 maize hybrids of different origin for salinity tolerance at seedling stage under three levels of salt stress 250 and 300 mM NaCl including one control. The existence of variation for tolerance to enhanced NaCl salinity levels at seedling stage in maize proved that hybrids had differing ability to grow under saline environment and potential variability within specie. Almost all the twenty five maize hybrids behaved varyingly in response to different salinity levels. However, the maize hybrids H₆, H₁₃, H₂₁, H₂₃ and H₂₄ expressed better performance under salt stress in terms of all six characters and proved to be as highly tolerant while H₂₂, H₁₇, H₂₀, H₁₈, H₄, H₉, and H₈ were identified as moderately tolerant. Hybrids H₁₄, H₅, H₁₁ and H₃, H₁₂, H₂, were expressed as most sensitive to salinity suggesting that screening is an effective tool to exploit genetic variation among maize hybrids and salt tolerance in maize can be enhanced through selection and breeding procedure.

Key words: Salinity, hybrids, maize, variation.

INTRODUCTION

Salinity is one of the most important abiotic factor limiting plant growth and productivity. In fact, it is the accumulation of water soluble salts in the growth medium to such an extent that has a drastic effect on crops, surrounding atmosphere and makes losses economically to the country (Rengasamy, 2006). Salinity in the soil is mainly result of reduced rainfall and high evapotranspiration. It not only adversely affects crop

lifecycle but also ground water that becomes brackish (Rhoades and Loveday, 1990; Evans, 1998). Worldwidely, about one quarter of irrigated land (60 million hectares) is salt affected that extremely damaging to crop plants (Lewis, 2002). High concentrations of soluble salts in the root medium results in reduced flowering and yield (Gill, 1979), reduction in photosynthesis (Yeo, 1998), plant nutrient uptake

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(Grattan and Grieve, 1999), plant growth rates and causes physiological drought (Mahajan and Tuteja, 2005). Salinity reduces fresh and dry weights of maize shoot (Raptan et al., 2001). According to Rabie (2005) who also found that salinity decreases the growth of mungbean. Similarly, Ghoulam et al. (2002) stated that salinity affects all growth parameters such as leaves fresh and dry weights.

Maize is one of the major food grain crops in Pakistan and occupies a significant position in agricultural economy of the country. It is third most important cereal crop in the world after wheat and rice but its production is affected negatively owing to high salt concentrations (Ashraf and McNeilly, 1989).

Salinity is tolerated by several crops including maize to a threshold level and above that yield reduces (Khan et al., 2006). To overcome salinity, plant breeders have been adopted many strategies, among them the most important one is exploitation of genetic variability in germplasm for identification of salinity tolerant genotypes pertaining yield potential even in presence of salinity in the soil (Ashraf et al., 2006).

To start a breeding programme, screening of huge germplasm is first and extremely important step in evolving high yielding and salt tolerant maize genotypes. This approach requires complete understanding about mechanism of plant response to different salinity levels at various plant growth stages as reported in several crops such as sorghum (Azhar and Khan, 1997), rice (Shannon et al., 1998), cotton (Azhar and Ahmad, 2000), wheat (Ali et al., 2002; Khan et al., 2003b), maize (Khan et al., 2003a), soybean (Kamal et al., 2003). Rao and McNeilly (1999) studied the genetic components of variation for salt tolerance in maize and concluded that salinity tolerance is high heritable and governing by additive and non additive genetic effects. Similarly, Akram et al. (2010) studied the screening of salt tolerance in maize (*Zea mays* L.) hybrids at an early seedling stage and found that overall salt tolerance performance was best at all salinity levels.

To use variation that already exists in plant material is crucial to develop salt tolerant genotypes within short time span (Flowers and Yeo, 1995). But, heterogeneity of soil physico-chemical properties and rainfall fluctuations are two main factors which cause difficulties for screening of salt tolerant genotypes in maize crop under field conditions. However, maize is basically cross pollinated crop which is highly polymorphic in nature that is why its cultivated species have great genetic variation; hence salinity tolerance exists in it (Paterniani, 1990). Therefore, present study was planned to screen out 25 maize hybrids for salinity tolerance under different levels of salinity stress.

MATERIALS AND METHODS

The present study on salinity tolerance in maize hybrids (Table 1)

was carried out in the Department of Plant Breeding and Genetics, University College of Agriculture, Bahauddin Zakariya University, Multan during 2009-10. Three levels of NaCl salinity including a control were developed. Three seeds of each hybrid were sown in plastic bags filled with 0.5 kg of soil under proper moisture conditions. The pH, EC and saturation percentage of the soil medium was 7.6, 0.79 and 25.6, respectively. Twenty five hybrids were randomized in three replications following Two-Factor Factorial Completely Randomized Design. The desired levels of salinity were developed using anhydrous NaCl. Two desired levels of 250 and 300 mM NaCl were applied following three steps, that is, after five days of germination first dose of 250 and 300 mM NaCl was applied. After 4 days of first application, second dose of 250 and 300 mM NaCl was applied. After three days of second application, third dose of 250 and 300 mM NaCl was applied to growing seedlings. To measure the salt tolerance, the data on root length, shoot length, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight were recorded after 16 days of sowing from three seedlings of each hybrid grown under each treatment.

To measure the significance, the data of 25 hybrids were subjected to analysis of variance (Steel et al., 1997). The means of treatments were separated by LSD (Least Significant Difference) test at 5% level of significance to establish difference between the genotypes, salinity levels and their interaction.

RESULTS

Tukey's LSD at 5% separated hybrids, salinity levels and their interaction into several groups, with no significant difference between the means within the groups, while great significant difference was found among groups. Results showed that hybrids, salinity levels and their interaction had a significant effect on root length, shoot length, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight. According to mean values of the data, hybrid H₂₁ gained maximum root length 40.56 cm followed by H₂₂, H₁₇ obtaining 37.92 and 36.22 cm, respectively as compared to the others. However, minimum root lengths 26.21, 27.21, 27.18 and 26.99 cm were recorded by hybrids H₂₄, H₁₄, H₁₃ and H₁₁ (Table 2). Similarly, hybrids H₂₁, H₂₃ and H₁₃ obtained highest shoot lengths 31.63, 31.22 and 30.44 cm followed by H₂₀, H₁₈, H₄, H₆ and H₉ obtaining 27.18, 27.02, 26.89, 26.75 and 26.53 cm, respectively (Table 5). Whereas, hybrid H₂₄ showed minimum shoot length 19.77 cm. Among the salinity levels, highest level S₂ (300 mM NaCl) produced minimum root and shoot lengths which were 57.20 and 66.21% less than that of the control (0 NaCl), respectively. Highest root and shoot lengths 54.64 and 51.36 cm were recorded by H₁₅ and H₂₁ hybrids, respectively at 0 NaCl level. Whereas, H₁₁ and H₃ hybrids produced minimum root and shoot lengths 13.96 and 7.84 cm, respectively at 300 mM NaCl salinity level.

Data regarding mean root and shoot fresh weights of twenty five maize hybrids at different levels of salinity are presented in (Tables 3 and 6). Hybrids H₆ and H₂₄ recorded maximum root and shoot fresh weights 3.73 and 3.9 g followed by H₈ and H₂₃ those gained 3.59 and 3.69 g, respectively. Whereas, minimum root and shoot fresh weights 2.84 and 2.01 g were observed by hybrids

Table 1. Name of maize CIMMYT Hybrids, Tropical and High Land Yellow.

Hybrids	Parentage
H ₁	(cl02725/clrcy015)//cl02450
H ₂	(clrcy034/clg2502)//cl02450
H ₃	(clrcy038/clg2502)//cl02450
H ₄	(clrcy040/clg2502)//cl02450
H ₅	(clrcy040/clrcy038)//cl02450
H ₆	(clrcy040/clrcy039)//cl02450
H ₇	(clrcy040/cl02450)//cml451
H ₈	(clrcy044/clg2502)//cl02450
H ₉	(clrcy044/clrcy038)//cl02450
H ₁₀	(clrcy044/clrcy039)//cl02450
H ₁₁	(clrcy044/clrcy040)//cl02450
H ₁₂	cml451*c102450
H ₁₃	(clqg2508/clqrcyq47)//cml165
H ₁₄	(clqrcyq49/clrcyq47)//cml165
H ₁₅	(clqrcyq49/clrcyq66)//cml165
H ₁₆	(clqrcyq49/clrcyq67)//cml165
H ₁₇	(clqrcyq59//((cml165*c102450)-b*c102450)-62-1-2*c102450)-28-1-b-))//cml161
H ₁₈	(clqrcyq59/cml165)//cml161
H ₁₉	(clqrcyq60/clqrcyq62)//cml161
H ₂₀	(clqrcyq60/clqrcyq63)//cml161
H ₂₁	(cml462/cml461)//cml245*p86c4f13-3-1-2-1-3-1-1-1)b-4tl-2-1-b-b-b
H ₂₂	(cml462/cml461)//cml245*p86c4f13-3-1-2-1-3-1-1-1)b-4tl-2-1-b-b-b
H ₂₃	(cml462/cml461)//p88c5f6-6-1-2-1-2-1-b*s.morado tardio tl93a-5-b-1tl-1-1-1-b)-b-22tl-1-1b
H ₂₄	(cml462/cml461)//p88c5f6-6-1-2-1-2-1-b*s.morado tardio tl93a-5-b-1tl-1-1-1-b)-b-22tl-1-1b
H ₂₅	(cml460/cml461)//p86asdpc1f90-1-2-1-b-1-b-4-b-b-b-b-b-b-b

H₂₄ and H₁₂, respectively. Fresh root and shoot biomass was also affected by salinity. Maximum root fresh weight 4.73 g was recorded in control but minimum 1.92 g in S₂ when salt was applied at the rate of 300 mM. Same pattern was studied for shoot fresh weight. Hybrids H₁₂ and H₁₉ obtained maximum root and shoot fresh weights 5.53 and 5.53 g at control followed 5.40 and 5.52 g by H₆ and H₂₁, respectively.

Different maize hybrids had significant effect on the root and shoot dry weights (Tables 4 and 7). Maize hybrid H₆ recorded maximum root dry weight 0.198 g followed by H₈ that gained 0.193 g whereas, minimum 0.151 g was observed by hybrid H₂₄. Hybrid H₂₄ recorded maximum shoot dry weight 0.19 g followed by H₂₃ that gained 0.18 g, respectively whereas, minimum 0.10 g was observed by hybrid H₁₂. Salinity also had significant effect on root and shoot dry weights. Maximum root and shoot dry weights 0.252 and 0.20 g were recorded in control but minimum 0.102 and 0.08 g, respectively were observed in S₂ when salt was applied at the rate of 300 mM. The interactive effect of both sources of variation (hybrids and salinity levels) had a significant effect on the root and shoot dry weight. Hybrid H₁₂ recorded maximum root dry weight 0.293 g at control followed by H₆ that obtained 0.286 g. Minimum root dry weight 0.213 g was recorded

by hybrids H₅ and H₂₄. Hybrids H₁₉ and H₂₁ recorded maximum shoot dry weight 0.28 g followed by H₁₈ that obtained 0.26 g. Minimum shoot dry weight 0.11 g was observed by H₁₁, H₂, H₄ at control.

DISCUSSION

Salinity tolerance is entirely important for whole plant life cycle from germination till harvesting for production of seed in grain producing crops like maize. It has been revealed from a lot of studies that in crop plants salinity tolerance at early seedling stage also reflects great tolerance at adult stage (Akram et al., 2010). In accordance with that, the present investigation expressed the existence of variation for tolerance to enhanced NaCl salinity levels at seedling stage in maize. Our findings were supported by several scientists like, Ashraf et al. (1986) investigated that variation exists in several forage grass species, Ashraf and McNeilly (1990) exploited variation for improved salinity tolerance in maize, Al-Khatib et al. (1993) studied salinity tolerance in lucerne, Kebebew and McNeilly (1994) studied variation for salinity tolerance in pearl millet, Maiti et al. (1996) found that in maize, variation for salinity tolerance that is

Table 2. Effect of salinity and maize hybrids on root length (cm).

Hybrids	S ₀ (Control)	S ₁ (250 mM)	S ₂ (300 mM)	Means
H ₁	48.45 ^{cd}	29.46 ^{p-t}	20.75 ^{z-d}	32.89 ^{h-k}
H ₂	41.50 ^{ij}	34.19 ^{mn}	24.70 ^{w-y}	33.46 ^{g-j}
H ₃	52.18 ^{ab}	27.95 ^{r-u}	14.66 ^{fg}	31.59 ^{k-m}
H ₄	47.30 ^{d-f}	30.76 ^{o-q}	22.75 ^{w-a}	33.60 ^{f-j}
H ₅	42.03 ^{ij}	34.08 ^{mn}	29.06 ^{p-t}	35.06 ^{c-g}
H ₆	52.24 ^{ab}	31.54 ^{n-p}	22.15 ^{y-a}	35.31 ^{c-e}
H ₇	43.85 ^{g-i}	36.78 ^{k-m}	24.87 ^{v-y}	35.16 ^{c-f}
H ₈	47.54 ^{d-f}	30.26 ^{o-s}	18.47 ^{b-d}	32.09 ^{j-m}
H ₉	45.65 ^{e-g}	31.47 ^{n-p}	16.30 ^{d-g}	31.14 ^{lm}
H ₁₀	49.26 ^{cd}	29.52 ^{p-t}	18.18 ^{b-e}	31.32 ^{i-m}
H ₁₁	41.76 ^{ij}	25.24 ^{q-x}	13.96 ^g	26.99 ⁿ
H ₁₂	50.55 ^{bc}	36.55 ^{lm}	22.76 ^{x-a}	36.62 ^{bc}
H ₁₃	42.26 ^{h-i}	24.72 ^{w-y}	14.56 ^g	27.18 ⁿ
H ₁₄	37.82 ^{kl}	28.35 ^{q-t}	15.46 ^{e-g}	27.21 ⁿ
H ₁₅	54.64 ^a	34.13 ^{mn}	18.32 ^{b-d}	35.69 ^{cd}
H ₁₆	46.83 ^{d-f}	36.11 ^{lm}	20.34 ^{a-c}	34.43 ^{b-h}
H ₁₇	52.15 ^{ab}	32.75 ^{no}	23.76 ^{xy}	36.22 ^c
H ₁₈	42.06 ^{ij}	31.52 ^{n-p}	24.38 ^{w-y}	32.65 ^{i-l}
H ₁₉	47.85 ^{c-e}	30.50 ^{o-r}	13.98 ^g	30.77 ^m
H ₂₀	44.92 ^{f-h}	39.38 ^{jk}	17.41 ^{d-f}	33.90 ^{e-i}
H ₂₁	52.97 ^{ab}	41.10 ^{ij}	27.60 ^{s-v}	40.56 ^a
H ₂₂	52.06 ^{ab}	38.30 ^{kl}	23.41 ^{x-z}	37.92 ^b
H ₂₃	46.93 ^{d-f}	28.84 ^{p-t}	20.45 ^{a-c}	32.07 ^{j-m}
H ₂₄	37.59 ^{kl}	26.90 ^{t-w}	14.13 ^g	26.21 ⁿ
H ₂₅	48.54 ^{cd}	30.97 ^{o-q}	17.85 ^{c-e}	32.45 ^{i-l}
Mean	46.76 ^a	32.05 ^b	20.01 ^c	

Table 3. Effect of salinity and maize hybrids on root fresh weight (g).

Hybrids	S ₀ (Control)	S ₁ (250mM)	S ₂ (300mM)	Means
H ₁	4.39 ^{l-k}	2.93 ^{t-x}	2.07 ^{y-d}	3.13 ^{i-k}
H ₂	5.12 ^{b-e}	3.41 ^{m-r}	1.99 ^{y-e}	3.51 ^{b-d}
H ₃	4.55 ^{g-k}	3.03 ^{s-w}	2.21 ^{yz}	3.26 ^{f-j}
H ₄	4.76 ^{f-h}	2.17 ^{q-u}	2.12 ^{y-b}	2.25 ^{d-h}
H ₅	4.01 ^l	2.67 ^x	1.82 ^{b-h}	2.84 ^m
H ₆	5.40 ^{ab}	3.60 ^{mn}	2.12 ^{yz}	3.73 ^a
H ₇	4.90 ^{d-f}	3.27 ^{o-s}	1.99 ^{y-e}	3.39 ^{c-g}
H ₈	5.14 ^{b-e}	3.42 ^{m-r}	2.21 ^{yz}	3.59 ^{ab}
H ₉	4.65 ^{f-j}	3.10 ^{r-v}	2.32 ^y	3.35 ^{d-h}
H ₁₀	5.15 ^{b-e}	3.44 ^{m-q}	1.69 ^{e-i}	3.43 ^{b-f}
H ₁₁	4.30 ^{kl}	2.87 ^{u-x}	2.18 ^{y-a}	3.12 ^{i-k}
H ₁₂	5.53 ^a	3.68 ^m	1.51 ^{hi}	3.57 ^{a-c}
H ₁₃	5.25 ^{a-c}	3.50 ^{m-p}	1.83 ^{b-h}	3.53 ^{b-d}
H ₁₄	4.77 ^g	3.18 ^{p-u}	1.67 ^{f-i}	3.21 ^{g-k}
H ₁₅	4.68 ^{f-i}	3.11 ^{r-v}	1.77 ^{d-i}	3.19 ^{h-k}
H ₁₆	4.94 ^{c-f}	3.33 ^{n-s}	1.80 ^{c-i}	3.36 ^{d-h}
H ₁₇	4.34 ^{jk}	3.23 ^{o-t}	1.88 ^{a-g}	3.15 ^{i-k}
H ₁₈	4.44 ^{h-k}	3.23 ^{o-t}	2.10 ^{y-c}	3.26 ^{f-j}
H ₁₉	4.31 ^{kl}	3.22 ^{o-t}	1.73 ^{e-i}	3.09 ^{j-l}
H ₂₀	5.21 ^{a-d}	3.20 ^{p-t}	1.96 ^{z-f}	3.46 ^{b-e}

Table 3. Contd.

H ₂₁	4.84 ^{e-g}	3.54 ^{m-o}	1.49 ⁱ	3.29 ^{e-i}
H ₂₂	4.56 ^{g-k}	2.73 ^{wx}	1.82 ^{b-i}	3.04 ^{kl}
H ₂₃	4.76 ^{f-h}	3.17 ^{q-u}	2.13 ^{y-b}	3.35 ^{d-h}
H ₂₄	4.01 ^l	2.67 ^x	1.82 ^{b-h}	2.84 ^m
H ₂₅	4.31 ^{kl}	2.82 ^{v-x}	1.63 ^{g-i}	2.92 ^{lm}
Mean	4.73 ^a	3.1 ^{ab}	1.92 ^c	

Table 4. Effect of salinity and maize hybrids on root dry weight (g).

Hybrids	S ₀ (Control)	S ₁ (250mM)	S ₂ (300mM)	Means
H ₁	0.233 ^{ij}	0.153 ^{f-e}	0.113 ^{w-z}	0.166 ^{h-j}
H ₂	0.270 ^{b-f}	0.183 ^{m-p}	0.106 ^{x-a}	0.186 ^{b-d}
H ₃	0.243 ^{h-j}	0.160 ^{q-u}	0.120 ^{wx}	0.174 ^{f-h}
H ₄	0.253 ^{f-h}	0.170 ^{p-r}	0.116 ^{w-y}	0.180 ^{d-f}
H ₅	0.213 ^{kl}	0.143 ^{uv}	0.096 ^{z-c}	0.151 ^l
H ₆	0.286 ^{ab}	0.193 ^{mn}	0.116 ^{w-y}	0.198 ^a
H ₇	0.263 ^{c-g}	0.173 ^{o-q}	0.106 ^{x-a}	0.181 ^{d-f}
H ₈	0.276 ^{a-d}	0.183 ^{m-p}	0.120 ^{wx}	0.193 ^{ab}
H ₉	0.250 ^{g-i}	0.163 ^{q-t}	0.126 ^{vw}	0.180 ^{d-f}
H ₁₀	0.273 ^{b-e}	0.183 ^{m-p}	0.090 ^{a-c}	0.182 ^{c-f}
H ₁₁	0.230 ^{jk}	0.153 ^{r-u}	0.116 ^{w-y}	0.166 ^{h-j}
H ₁₂	0.293 ^a	0.200 ^{lm}	0.080 ^c	0.191 ^{a-c}
H ₁₃	0.280 ^{a-c}	0.183 ^{m-p}	0.096 ^{z-c}	0.186 ^{b-d}
H ₁₄	0.253 ^{f-h}	0.166 ^{p-s}	0.086 ^{bc}	0.168 ^{g-j}
H ₁₅	0.250 ^{g-i}	0.163 ^{q-t}	0.093 ^{a-c}	0.168 ^{g-j}
H ₁₆	0.260 ^{d-h}	0.173 ^{n-q}	0.096 ^{z-c}	0.177 ^{d-g}
H ₁₇	0.233 ^{ij}	0.170 ^{p-r}	0.100 ^{y-b}	0.167 ^{h-j}
H ₁₈	0.233 ^{ij}	0.170 ^{p-r}	0.116 ^{w-y}	0.173 ^{f-i}
H ₁₉	0.230 ^{jk}	0.170 ^{p-r}	0.093 ^{a-c}	0.164 ^{i-k}
H ₂₀	0.280 ^{a-c}	0.170 ^{p-r}	0.103 ^{w-b}	0.180 ^{b-e}
H ₂₁	0.256 ^{e-h}	0.190 ^{m-o}	0.080 ^c	0.175 ^{e-h}
H ₂₂	0.243 ^{h-j}	0.146 ^{tu}	0.096 ^{z-c}	0.162 ^{jk}
H ₂₃	0.253 ^{f-h}	0.170 ^{p-r}	0.116 ^{w-y}	0.180 ^{d-f}
H ₂₄	0.213 ^{kl}	0.143 ^{uv}	0.096 ^{z-c}	0.151 ^l
H ₂₅	0.230 ^{jk}	0.150 ^{s-u}	0.086 ^{bc}	0.155 ^{kl}
Mean	0.252 ^a	0.169 ^b	0.102 ^c	

expressed at early seedling stage produces high yields at maturity. Our findings expressed that screening at seedling stage for salinity tolerance in maize is a productive method, considering that variation at the seedling stage is controlled genetically.

A lot of morphological seedling traits in maize have been employed for identification of salinity tolerance. Among them, broadly used trait is the root length of seedlings grown in control and saline solutions; root length has been reduced rapidly when the seedlings is exposed to salinity (Rao and McNeilly, 1999; Khan and McNeilly, 2005). Accordingly, our results show that root

length was one of the morphological traits that suffered major losses as compared to the non-saline treatment throughout the experiment of screening (Table 2). This attitude was expectable, because root is the first important plant organ that has direct contact with the growth medium supplying all the necessary nutrients from soil to growing plant and is first to be affected, therefore roots provide the important information in context to salinity tolerance in crops like maize (Collado et al., 2010; Akram et al., 2010). Similarly, Cramer et al. (1988) and Ashraf et al. (2005) were of the opinion that root growth and development is extremely sensitive to high salinity

Table 5. Effect of salinity and maize hybrids on shoot length (cm).

Hybrids	S ₀ (Control)	S ₁ (250 mM)	S ₂ (300 mM)	Means
H ₁	39.84 ^{e-g}	26.67 ^{n-q}	8.71 ^{ij}	25.07 ^{c-g}
H ₂	32.76 ^{j-l}	22.40 ^{s-u}	11.87 ^{d-i}	22.34 ^{h-j}
H ₃	39.66 ^{e-g}	21.99 ^{s-v}	7.84 ⁱ	23.16 ^{g-i}
H ₄	41.3 ^{de}	23.40 ^{q-t}	15.73 ^{z-c}	26.89 ^{bc}
H ₅	38.32 ^{e-i}	19.77 ^{u-y}	9.30 ^{h-j}	22.46 ^{h-j}
H ₆	44.98 ^{bc}	21.87 ^{s-v}	13.40 ^{c-g}	26.75 ^{bc}
H ₇	35.57 ^{h-j}	17.43 ^{w-a}	14.04 ^{a-e}	22.34 ^{h-j}
H ₈	31.00 ^{lm}	22.58 ^{s-u}	11.58 ^{e-i}	21.72 ^{i-k}
H ₉	46.27 ^{bc}	23.39 ^{q-t}	9.94 ^{h-j}	26.53 ^{bc}
H ₁₀	32.06 ^{k-m}	29.01 ^{m-o}	14.39 ^{a-e}	25.15 ^{c-f}
H ₁₁	27.03 ^{n-p}	21.05 ^{t-v}	13.92 ^{b-e}	20.67 ^{ik}
H ₁₂	39.02 ^{e-g}	24.51 ^{p-s}	14.15 ^{a-e}	25.89 ^{b-e}
H ₁₃	44.57 ^{b-d}	27.14 ^{n-p}	19.60 ^{u-y}	30.44 ^a
H ₁₄	47.91 ^b	17.43 ^{w-a}	10.35 ^{f-j}	25.23 ^{b-e}
H ₁₅	36.74 ^{g-i}	22.93 ^{r-u}	10.06 ^{g-j}	23.24 ^{f-i}
H ₁₆	35.10 ^{i-k}	23.57 ^{q-t}	14.40 ^{a-e}	24.26 ^{d-h}
H ₁₇	38.73 ^{e-h}	24.62 ^{p-s}	15.03 ^{a-d}	26.13 ^{b-d}
H ₁₈	35.57 ^{h-j}	31.88 ^{k-m}	13.63 ^{c-f}	27.02 ^{bc}
H ₁₉	31.70 ^{k-m}	20.70 ^{t-w}	11.87 ^{d-i}	21.42 ^{i-k}
H ₂₀	43.93 ^{cd}	26.15 ^{o-r}	11.46 ^{e-i}	27.18 ^b
H ₂₁	51.36 ^a	26.27 ^{o-r}	17.26 ^{x-b}	31.63 ^a
H ₂₂	38.08 ^{f-i}	23.40 ^{q-t}	16.79 ^{y-c}	26.09 ^{b-d}
H ₂₃	45.92 ^{bc}	29.13 ^{m-o}	18.60 ^{v-z}	31.22 ^a
H ₂₄	29.83 ^{l-n}	17.31 ^{w-b}	12.17 ^{d-h}	19.77 ^k
H ₂₅	40.25 ^{ef}	20.53 ^{t-x}	11.23 ^{e-j}	24.00 ^{e-h}
Mean	38.71 ^a	23.40 ^b	13.08 ^c	

Table 6. Effect of salinity and maize hybrids on shoot fresh weight (g).

Hybrids	S ₀ (Control)	S ₁ (250 mM)	S ₂ (300 mM)	Means
H ₁	2.98 ^{n-q}	2.22 ^{t-c}	1.82 ^{a-j}	2.34 ^{k-m}
H ₂	2.32 ^{t-z}	2.89 ^{o-r}	1.89 ^{y-h}	2.37 ^{i-m}
H ₃	4.63 ^{ef}	3.32 ^{l-o}	1.54 ^{g-k}	3.16 ^{de}
H ₄	2.23 ^b	2.56 ^{q-w}	1.98 ^{x-g}	2.26 ^{l-n}
H ₅	2.64 ^{q-u}	2.15 ^{u-e}	1.84 ^{z-i}	2.21 ^{mn}
H ₆	4.62 ^{ef}	2.58 ^{q-v}	1.65 ^{f-j}	2.95 ^{e-g}
H ₇	4.47 ^{fg}	2.92 ^{n-r}	2.17 ^{u-d}	3.19 ^{de}
H ₈	4.44 ^{fg}	1.69 ^{d-j}	1.85 ^{y-i}	2.66 ^{hi}
H ₉	4.12 ^{gh}	2.29 ^{t-a}	1.77 ^{b-j}	3.73 ^{f-i}
H ₁₀	3.52 ^{j-m}	3.00 ^{n-q}	1.43 ^{h-k}	2.65 ^{h-j}
H ₁₁	2.33 ^{t-z}	3.33 ^{l-o}	1.78 ^{b-j}	2.48 ^{i-m}
H ₁₂	2.43 ^{t-x}	2.23 ^{t-b}	1.38 ^{i-k}	2.01 ⁿ
H ₁₃	4.42 ^{fg}	2.67 ^{p-t}	1.66 ^{e-j}	2.92 ^{e-h}
H ₁₄	5.06 ^{a-e}	2.06 ^{x-f}	1.68 ^{e-j}	2.93 ^{e-h}
H ₁₅	5.03 ^{b-e}	2.57 ^{q-v}	1.45 ^{h-k}	3.02 ^e
H ₁₆	3.87 ^{h-k}	2.09 ^{v-f}	1.74 ^{c-j}	2.56 ^{i-k}
H ₁₇	4.01 ^{g-j}	3.04 ^{m-q}	1.91 ^{y-h}	2.99 ^{ef}
H ₁₈	5.26 ^{a-c}	3.40 ^{k-n}	2.08 ^{w-f}	3.58 ^{bc}
H ₁₉	5.53 ^a	2.64 ^{q-u}	1.35 ^{jk}	3.17 ^{de}

Table 6. Contd.

H ₂₀	4.80 ^{c-f}	2.58 ^{q-v}	1.79 ^{b-j}	3.05 ^e
H ₂₁	5.52 ^{ab}	3.13 ^{l-p}	1.43 ^{h-k}	3.36 ^{cd}
H ₂₂	3.60 ^{i-l}	2.82 ^{p-s}	1.08 ^k	2.50 ^{i-l}
H ₂₃	4.34 ^{f-h}	4.67 ^{d-f}	2.06 ^{x-f}	3.69 ^{ab}
H ₂₄	5.15 ^{a-d}	4.80 ^{c-f}	1.74 ^{c-j}	3.9 ^a
H ₂₅	4.05 ^{g-i}	2.34 ^{s-y}	1.64 ^{f-j}	2.68 ^{g-i}
Mean	4.05 ^a	2.80 ^b	1.71 ^c	

Table 7. Effect of Salinity and maize hybrids on shoot dry weight (g).

Hybrids	S ₀ (Control)	S ₁ (250mM)	S ₂ (300mM)	Means
H ₁	0.16 ^{j-m}	0.11 ^{q-v}	0.09 ^{u-a}	0.12 ^{j-m}
H ₂	0.11 ^{q-u}	0.14 ^{l-p}	0.09 ^{u-a}	0.11 ^{k-m}
H ₃	0.23 ^{c-e}	0.16 ^{j-m}	0.08 ^{y-b}	0.16 ^{de}
H ₄	0.11 ^{q-v}	0.12 ^{p-s}	0.10 ^{t-y}	0.11 ^{l-n}
H ₅	0.13 ^{n-q}	0.11 ^{q-w}	0.09 ^{u-a}	0.11 ^{mn}
H ₆	0.23 ^{c-e}	0.13 ^{q-r}	0.08 ^{w-a}	0.15 ^{e-g}
H ₇	0.22 ^{d-f}	0.14 ^{l-p}	0.11 ^{q-w}	0.16 ^{de}
H ₈	0.22 ^{d-f}	0.08 ^{w-a}	0.09 ^{u-a}	0.13 ^{g-j}
H ₉	0.21 ^{e-g}	0.11 ^{q-u}	0.09 ^{v-a}	0.13 ^{f-i}
H ₁₀	0.17 ^{i-k}	0.15 ^{k-o}	0.07 ^{z-b}	0.13 ^{h-j}
H ₁₁	0.11 ^{q-v}	0.16 ^{j-m}	0.08 ^{w-a}	0.12 ^{j-m}
H ₁₂	0.12 ^{p-t}	0.11 ^{q-v}	0.07 ^{ab}	0.10 ⁿ
H ₁₃	0.22 ^{d-g}	0.13 ^{n-q}	0.08 ^{w-a}	0.14 ^{e-h}
H ₁₄	0.25 ^{a-c}	0.10 ^{s-y}	0.08 ^{x-a}	0.14 ^{e-h}
H ₁₅	0.25 ^{a-c}	0.12 ^{p-s}	0.07 ^{z-b}	0.15 ^{ef}
H ₁₆	0.19 ^{g-i}	0.10 ^{s-y}	0.09 ^{b-a}	0.13 ^{i-k}
H ₁₇	0.20 ^{f-h}	0.15 ^{k-o}	0.09 ^{u-z}	0.15 ^{ef}
H ₁₈	0.26 ^{ab}	0.17 ^{j-l}	0.10 ^{s-y}	0.17 ^{bc}
H ₁₉	0.28 ^a	0.13 ^{o-r}	0.07 ^{ab}	0.16 ^{de}
H ₂₀	0.24 ^{b-d}	0.13 ^{q-r}	0.09 ^{u-a}	0.15 ^{de}
H ₂₁	0.28 ^a	0.15 ^{k-n}	0.07 ^{z-b}	0.17 ^{cd}
H ₂₂	0.18 ^{h-j}	0.14 ^{m-p}	0.05 ^b	0.12 ^{i-l}
H ₂₃	0.22 ^{d-g}	0.23 ^{cd}	0.10 ^{r-x}	0.18 ^{ab}
H ₂₄	0.25 ^{a-c}	0.24 ^{b-d}	0.08 ^{x-a}	0.19 ^a
H ₂₅	0.20 ^{f-h}	0.11 ^{q-u}	0.08 ^{x-a}	0.13 ^{g-j}
Mean	0.20 ^a	0.14 ^b	0.08 ^c	

level in the soil that is reduced rapidly in size.

Measurement of shoot length under salt stress may be a more effective and useful parameter than root length to identify salinity tolerance (Eker et al., 2006). In accordance with that, our findings also showed an important reduction in shoot length of seedlings in salinity compared to the controls and this trait could be useful in screening salinity tolerance (Table 5). Our results were also supported by (Collado et al., 2010; Akram et al., 2010) that increasing the concentration of NaCl declines the root and shoot lengths of the maize hybrids.

Increasing salinity is accompanied by a significant reduction in shoot length, plant fresh and dry biomass in tomato Mohammad et al. (1998) and in maize Akram (2010). Accordingly, our studies revealed that salinity significantly reduced root and shoot fresh weights and dry weights (Tables 3, 4, 6 and 7). Similar findings were also reported in upland cotton (*Gossypium hirsutum* L.) by Hanif et al. (2008). This showed that the increasing levels of salinity hampered the root and shoot growth which ultimately resulted in reduced root and shoot fresh and dry weights among the 25 maize hybrids.

In the present experiment, almost all the twenty five maize hybrids behaved varying in response to different salinity levels. But the maize hybrids H₆, H₁₃, H₂₁, H₂₃ and H₂₄ expressed better performance in term of root and shoot lengths, fresh and dry weights and have proved as salt tolerant hybrids.

Conclusion

The results of our study concluded that screening is an effective tool to exploit genetic variation among maize hybrids. These variations can further be utilized in a breeding programme to develop high yielding salt tolerant genotypes of maize through selection and breeding procedures. Our findings will provide guidelines about selection of salt tolerant hybrids in maize and this information will be very necessary and relevant to plant breeders and physiologists who are indulged in improving salt tolerance of maize. This criterion is also applicable for other crops to develop high yielding salt tolerant varieties.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Consequence of different ripeners on germination and initial growth of morning glory

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At the application time of sugarcane ripeners, most times, morning glory plants, that have escaped, survived or emerged, are entwined on sugarcane stalks and these plants are at this time in different reproductive stages (and it becomes more difficult to control) causing a mechanical and crop yield damage. The main aim of this research was to evaluate sugarcane crop ripeners effect on the seeds germination, seedling development and the abscission of the reproductive structures of morning glory. The treatments consist of five ripeners: ethyl-trinexapac (300 ga.i.ha⁻¹), glyphosate (216 ga.i.ha⁻¹), fluazifop-p-butyl (75 ga.i.ha⁻¹), sulfometuron-methyl (15 ga.i.ha⁻¹), ethephon (480 ga.i.ha⁻¹) and one control plot (without ripener application). They were applied during the reproductive stages of morning glory. The experimental design used was completely randomized design with four replications and six treatments, to a total of 24 plots. After ripeners application, abscised reproductive structures were counted to determine the percentage of abscission. The seeds of each phenological stage were collected when it reached the point of physiological maturity and it was set to germinate in Petri dishes to evaluate the germination rate, germination mean time, germination velocity, initial growth and dry mass weight. Trinexapac-ethyl, glyphosate and fluazifop-P-butyl application when plants are with open flowers can reduce seed viability. Glyphosate showed the smallest length value and it can affect seedling growth. Glyphosate and ethephon caused the highest percentages of abscission of morning glory reproductive structures and they can contribute to reduce the seeds inflow to the soils.

Key words: Abscission, *Ipomoea hederifolia*, plant growth regulator, reproductive structure

INTRODUCTION

Sugarcane became one of the main crops of Brazilian economy (Mapa, 2016). In the last decades the system passed through major changes due the prohibition of burning the sugarcane areas before harvesting in São

Paulo state, mainly because of environmental and social impacts derived from the crop (Monqueiro et al., 2011). With the adoption of this practice, harvesting after burning is being substituted with raw sugarcane

Table 1. Phenological stages of *Ipomoea hederifolia* submitted to application of different ripeners in the flowering and fruiting period.

Phenophases	Stage
Beginning of flowering organs	1° - G51
Closed flowers	2° - G55
Open flowers	3° - G65
Fruits	4° - G75

harvesting, following progressive legal restrictions. As a consequence, the raw sugarcane system provided important modifications for weeds (Kuva et al., 2011). The remaining straw above the surface that originates from the harvesting process changes the light, temperature and humidity conditions, and these factors favor the development of weeds with voluble stems in those areas, such as the morning glory (Azania et al., 2011).

The morning glory develops at the same time with the biggest sugarcane growth phase, and adult specimens become intertwined in stalks and leaves, hindering the crop development, the agricultural practices and harvesting operations (Azania et al., 2002). Silva et al. (2009) verified that the presence of *Ipomoea hederifolia* during the entire crop cycle of RB855536 sugarcane reduces 46% of its productivity. The same authors also wrote that during harvesting, its fruits and seeds may be found attached to the mother-plant, thus allowing the dissemination of these seeds by the harvester machines to medium and long distances.

After the biggest sugarcane growth phase, the stabilization of its growth and the maturation starts. During this process, the morning glory plants show various flowering fluxes that, usually, coincide with the sugarcane ripener application. Most of these chemicals are used to provide a consistent ripening effect on sugarcane, to suppress flowering (which can reduce sugar content) and to enhance sucrose concentration (Dalley and Richard Jr., 2010). Among traditional products used as sugarcane ripeners that belong to growth inhibitors group or herbicide compounds group are glyphosate, sulfometuron-methyl, ethephon, trinexapac-ethyl, and fluzifop-P-butyl (Leite et al., 2008).

This way, it is expected that ripeners applied on these plants may reduce the fruit production and, consequently, seeds. This abortion induction and or seed reduction may happen depending on the reproductive stage of the plant. New alternatives which egress blockage of viable weed seeds on the ground, eliminating the production

and/or viability of these seeds still on the mother-plant as well as hinder the development of seedlings represent promising implements for weed management.

Given all that has been explained above, the aim of this work is to evaluate the usage of sugarcane ripeners on the seed germination, seedling development and reproductive structures abscission of *I. hederifolia*.

MATERIALS AND METHODS

The experiment was developed at Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal Campus, São Paulo State, located at 21°14'05" S, 48°17'09" W coordinate and 615 m altitude. *I. hederifolia* seeds were seeded on raised beds of 50 cm width and 50 cm length.

As soon as the species had reached the four stages (or phenophases) needed for the experiments, five ripeners were applied: ethyl-trinexapac (300 ga.i.ha⁻¹), glyphosate (216 ga.i.ha⁻¹), fluzifop-p-butyl (75 ga.i.ha⁻¹), sulfometuron-methyl (15 ga.i.ha⁻¹) and ethephon (480 ga.i.ha⁻¹) on the *I. hederifolia* plants, and a control plot was left without application.

On *I. hederifolia*, those stages were defined following Bleiholder et al. (1991), through the BBCH-Code scale, which enables one to identify weeds development stages (Table 1). Each stage was marked with different colored ribbon strip, to identify which flowering or fructification stage that branch was on the application day. The experimental design used was completely randomized design with four replications and six treatments, to a total of 24 plots. For each plot, 30 reproductive structures per stage were marked, to a total of 124 reproductive structures per plot.

Ripener application was performed with a CO₂ pressurized backpack sprayer equipped with a XR 110.02 nozzle. The equipment was regulated at 2.2 bar pressure applying 200 L.ha⁻¹ of herbicide mix.

After the ripener application, daily observations were carried out on the number of marked structures on which abscission was carried out in each phenophase during the period between ripener application and seed harvesting, to determine the percentage of structures that suffered abscission. After that, the fruits of each treatment, when they have reached maturation point, were harvested for their seeds (right before the dehiscence).

For the next part of the experiment, the seeds were tested through tetrazolium chloride test for each batch (Brasil, 2009). Seeds were then put to germinate on Petri dishes with filter paper as medium, embedded with 2.5 times the weight of paper on water and arranged on BOD (Biological Oxygen Demand) germination chambers at 27°C temperature. The experiment was conducted on completely randomized design, with four replications and 25 seeds per Petri dish. Daily observations were carried for 15 days after the first seed germinated (radical > 2 mm), counting the number of germinated seeds.

Thereafter, the seeds from each stage were evaluated by the germination test, through analyzing the germination rate, germination velocity index (Maguire, 1962), mean germination time (Labouriau, 1983), length and dry matter of seedlings aerial parts and roots.

The results were analyzed on completely random design on a 6x4 split-plot design (treatments x stage), being the whole-plot A

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Table 2. Interaction effect between treatments and reproductive stages on abscission percentage of reproductive stages of morning glory.

Treatments	Beginning of flowering organs	Closed flowers	Open flowers	Fruits	F
	Abscission (%)				
Ethephon	70.5 ^{Aa}	27.1 ^{Bbc}	21.5 ^{Bc}	30.7 ^{Aab}	139.33**
Fluazifop-P-butyl	10.5 ^{Bb}	5.0 ^{Cb}	8.2 ^{Cb}	25.3 ^{ABa}	22.49**
Glyphosate	64.5 ^{Aa}	42.3 ^{Ab}	34.0 ^{Ac}	11.5 ^{Cd}	134.47**
Sulfometuron-methyl	7.0 ^{Bb}	7.5 ^{Cb}	7.0 ^{Cb}	18.3 ^{BCa}	8.63**
Trinexapac-ethyl	11.5 ^{Bb}	7.7 ^{Cb}	10.2 ^{BCb}	25.7 ^{ABa}	18.44**
Control	7.5 ^{Bab}	10.0 ^{Ca}	4.0 ^{Cab}	2.5 ^{Db}	3.22*
F	244*	59.74**	34.91**	29.54**	

Means followed by different letters through Tukey test at 5% probability. Upper-case letters for treatments and lower-case letters for stages. ** Significant at 1% probability through F-test. * Significant at 5% probability through F-test. F (treatments x stages) = 51.95**; VC (%) plots = 21.02; VC (%) split-plots = 19.27.

and split-plot B. Results were submitted to analysis of variance and means compared through the Tukey test at 5% probability.

RESULTS AND DISCUSSION

Abscission of reproductive structures

Analyzing the treatments among each stage, all the applied products provoked increase on abscission percentage when compared to the control plot (Table 2).

The results obtained with glyphosate show a more harmful effect on the beginning of flowering structures, still closed flowers and open flowers, at 57, 32 and 30%, respectively, compared to the control plot. Viator et al (2003) showed increased number of cotton bolls abscised per plant with the increasing of glyphosate rates.

When the fruits were formed, the abscission due to glyphosate application is 9% higher. In this stage, the abscission was very low (35% lower than previous stages).

Ethephon application caused an increase of 63, 17, 28 and 28% on beginning of flowering structures, closed flowers, open flowers and fruits stages, when compared to the control plot. On the remaining treatments (trinexapac-ethyl, Fluazifop-P-butyl and sulfometuron-methyl), the results differ only on the fruit stages, on a 20% higher mean value. Hole and Hardwick (1978), Iglesias et al. (2006), Ninot and Romero (2012) reported that ethephon may promote the abscission of different plant organs such as leaves, flowers, and fruits.

Among the stages, it is possible to see the application on the beginning of flowering organs, closed and open flowers promoted the highest abscission rates when glyphosate and ethephon were applied. The application on the fruits, all treatments provided increase on the abscission rates.

The results found for glyphosate and its effects on the abscission rates in the reproductive stages may be

explained by the raise of ethylene and the consequential cellulase activity raise on the abscission zones, diminishing the resistance to abscission. That occurs because amino acid and ureid biosynthesis branches into tryptophan, phenylalanine and tyrosine synthesis, which is inhibited by glyphosate, or in glycine, serine, cysteine and methionine. When the first branch is inhibited, the second one is more induced. It is possible to find the presence of methionine in it, which is the precursor of ethylene synthesis (Yamada and Castro, 2007).

The abscission of flowering structures; abscission after ethephon application may occur due to auxin transport inhibition caused by ethylene liberation that happens when the product contact the plant tissue. Auxin is produced in the leaves and an auxin gradient from the leaf blade to the stem keeps the petiole abscission zone insensitive to ethylene. The ethylene diminishes the auxin activity for the reduction of its synthesis and transport as well as raise of its destruction (Taiz and Zeiger, 2013). The physiological effect of IAA, in this process, is to reduce the sensibility of the abscission zone to ethylene. On low IAA concentrations, the "active" ethylene, after the application, result in abscission (Bangerth, 2000).

Germination percentage

At the beginning of flowering stages, it can be noted that with or without application, there are no significant differences, however, the germination being lower than in the later stages (Table 3).

Analyzing the stage closed flowers; the ripeners have results similar to the control. On open flowers, the treatments as a whole, caused reduction related to the control plot, of 26%. At fruit stage, the ripeners do not differ among themselves or the control plot.

Germination on the beginning of the flowering structures may have occurred due the dormancy mechanism characteristic of some weed species, e.g. primary dormancy, which, following Viana et al. (2008), is

Table 3. Interaction effect between treatments and reproductive stages on germination percentage of morning glory seeds.

Treatments	Beginning of flowering organs	Closed flowers Open flowers		Fruits	F
		Germination (%)			
Ethephon	17.0 ^{Ac}	65.0 ^{ABb}	79.0 ^{Bab}	89.0 ^{Aa}	45.81**
Fluazifop-P-butyl	14.0 ^{Ac}	62.0 ^{Bb}	64.0 ^{Bb}	86.0 ^{Aa}	41.56**
Glyphosate	7.0 ^{Ac}	82.0 ^{Aa}	49.0 ^{Bb}	76.0 ^{Aa}	52.61**
Sulfometuron-methyl	16.0 ^{Ac}	73.0 ^{ABab}	63.0 ^{Bb}	82.0 ^{Aa}	38.31**
Trinexapac-ethyl	10.0 ^{Ac}	74.0 ^{ABab}	62.0 ^{Bb}	81.0 ^{Aa}	46.61**
Control	13.0 ^{Ab}	78.0 ^{ABa}	86.0 ^{Aa}	94.0 ^{Aa}	62.68**
F	0.65 ^{ns}	2.38*	6.40**	1.98 ^{ns}	

Means followed by different letters differ through Tukey test at 5% probability. Upper-case letters for treatments and lower-case letters for stages. ** Significant at 1% probability through F-test. * Significant at 5% probability through F-test. F (treatments x stages) = 2.20**; VC(%) plots=15.92; VC(%) split-plots=15.84.

Table 4. Interaction effect between treatments and reproductive stages upon germination velocity index of morning glory.

Treatments	Beginning of flowering organs	Closed flowers	Open flowers	Fruits	F
Ethephon	15.2 ^{Abc}	18.5 ^{Aab}	11.8 ^{Bc}	20.8 ^{Aa}	9.02**
Fluazifop-P-butyl	11.2 ^{ABb}	12.6 ^{Bb}	13.9 ^{Bab}	18.6 ^{Aa}	5.96**
Glyphosate	5.2 ^{Cc}	18.0 ^{Aa}	10.4 ^{Bb}	16.6 ^{Aa}	20.28**
Sulfometuron-methyl	14.2 ^{Aab}	17.0 ^{ABab}	12.4 ^{Bb}	17.6 ^{Aa}	3.56*
Trinexapac-ethyl	6.1 ^{BCb}	17.1 ^{ABa}	13.0 ^{Ba}	15.8 ^{Aa}	14.32**
Control	7.1 ^{BCb}	17.0 ^{ABa}	19.8 ^{Aa}	21.3 ^{Aa}	23.98**
F	9.47**	2.27 ^{ns}	5.46**	2.53*	

Means followed by different letters through Tukey test at 5% probability. Upper-case letters for treatments and lower-case letters for stages. ** Significant at 1% probability through F-test. * Significant at 5% probability through F-test. F (treatments x stages) = 5.35**; VC (%) plots=22.43; VC (%) split-plots=17.87.

intrinsic of weed seeds, developed while still attached to the mother-plant, remaining after the dispersion. Likewise, dormancy is a perpetuation and defense mechanism, producing seed lots with variation on the germination times (Marcos Filho, 2005).

It is notable that in the fourth stage the mean germination percentage was higher than in the initial stages. Knowing that after the fecundation, seeds grow fast in size and reaches a maximum growth in a short period (Carvalho and Nakagawa, 2012), the usage of cell division inhibitors, which molecules are ready for translocation to the meristem tissues (Shaner et al., 1984) and highly movable to the meristematic areas with high growth rates (Rodrigues, 1995) explain, the efficiency of ripeners on the initial stages of reproductive development.

Velocity index

For the beginning of flowering structures, sulfometuron-methyl and ethephon are the ones that presented the highest values among all treatments, taking a longer time for germination processes (Table 4).

On the closed flowers stage, the ripeners showed results similar to those obtained from the control plot, while the open flowers stage showed mean values higher than the control, as it happened to the germination percentage. The effect of treatments on the fruits stage shows no significant difference.

The germination velocity index was similar to the pattern attained in the germination percentage for every treatment, except for ethephon on the closed flowers stage, being the lowest values for velocity for the also lowest values of germination percentage. On the open flowers stage, the values obtained from ripener application treatments also don't go over the control plot's. The vigor level of the seed may affect the plant development and proportionate lower competition capacity of these for light, water and nutrients with neighboring plants. According to Oliveira et al. (2009) the germination velocity test is based on the principle of seeds that have lesser velocity are less vigorous.

Mean germination time

The effect of treatments on the mean germination time

Table 5. Interaction effect between treatments and reproductive stages upon mean germination time (days) of morning glory.

Treatments	Beginning of flowering organs	Closed flowers	Open flowers	Fruits	F
	Mean germination time				
Ethephon	1.5 ^{Ca}	1.3 ^{Aa}	1.7 ^{Aa}	1.3 ^{Aa}	0.86 ^{ns}
Fluazifop-P-butyl	1.5 ^{Ca}	1.4 ^{Aa}	1.6 ^{Aa}	1.6 ^{Aa}	0.20 ^{ns}
Glyphosate	2.2 ^{BCa}	1.4 ^{Ab}	1.7 ^{Aa}	1.7 ^{Aa}	1.93 ^{ns}
Sulfometuron-methyl	1.4 ^{Ca}	1.4 ^{Aa}	2.2 ^{Aa}	1.5 ^{Aa}	2.56 ^{ns}
Trinexapac-ethyl	3.1 ^{Aab}	1.3 ^{Ab}	1.6 ^{Ab}	2.0 ^{Ab}	10.87 ^{**}
Control	3.6 ^{Aa}	1.7 ^{Ab}	1.3 ^{Ab}	1.2 ^{Aa}	23.03 ^{**}
F	15.20 ^{**}	0.54 ^{ns}	1.51 ^{ns}	1.35 ^{ns}	

Means followed by different letters through Tukey test at 5% probability. Upper-case letters for treatments and lower-case letters for stages. ** Significant at 1% probability through F-test. * Significant at 5% probability through F-test. F (treatments x stages)= 5.15^{**}; VC (%) plots= 29.0; VC (%) split-plots= 27.0.

Table 6. Interaction effect between treatments and stages upon the aerial parts length (mm) of morning glory seedlings.

Treatments	Beginning of flowering organs	Closed flowers	Open flowers	Fruits	F
	Aerial parts length (mm)				
Ethephon	43.5 ^{Cc}	45.9 ^{Abc}	69.1 ^{Ba}	52.2 ^{ABb}	36.74 ^{**}
Fluazifop-P-butyl	49.7 ^{BCb}	48.5 ^{Ab}	82.1 ^{Aa}	43.1 ^{Cb}	86.77 ^{**}
Glyphosate	48.6 ^{BCa}	34.8 ^{Bb}	49.5 ^{Ca}	44.9 ^{BCa}	12.46 ^{**}
Sulfometuron-methyl	54.5 ^{ABbc}	49.3 ^{Ac}	89.9 ^{Aa}	57.8 ^{Ab}	93.05 ^{**}
Trinexapac-ethyl	59.8 ^{Ab}	47.7 ^{Ac}	72.9 ^{Ba}	47.7 ^{BCc}	39.98 ^{**}
Control	48.5 ^{BCb}	45.7 ^{Ab}	65.6 ^{Ba}	45.6 ^{BCb}	25.54 ^{**}
F	8.72 ^{**}	7.71 ^{**}	52.97 ^{**}	8.25 ^{**}	

Means followed by different letters through Tukey test at 5% probability. Upper-case letters for treatments and lower-case letters for stages. ** Significant at 1% probability through F-test. * Significant at 5% probability through F-test. F (treatments x stages) = 12.24^{**}; VC (%) plots = 10.3; VC (%) split-plots = 9.95.

shows no significant difference, except in the beginning of the flowering structures stage there is difference for glyphosate, Fluazifop-P-butyl, sulfometuron-methyl and ethephon, which exhibit the lowest time to germination (Table 5). The comparison among the stages regarding the treatments show the beginning of flowering structures stages has a slower germination than the other treatments.

Aerial parts and root length

The interaction results between stages and treatments upon the aerial parts length are on Table 6. Glyphosate treatment showed the lowest length when applied on closed and open flowers, with a 24% variation, when it was compared to control.

It is valid to point that for trinexapac-ethyl, fluazifop-P-butyl and sulfometuron-methyl, these treatments added to the increase in length, in some stages. Trinexapac-ethyl increases 9.0% when applied in the beginning of flowering structures while fluazifop-P-butyl and sulfometuron-methyl increased 25 and 37% in the open

flowers. These treatments did not show significant differences on the other stages compared to the control plot. For ethephon treatment, in all stages, the results were similar to the control plot results.

Table 7 revealed the interaction results between treatment and stages for root length. Glyphosate shows lower results for every stage applied than any other. When set against the control plot, the reductions are of 68, 10, 80 and 87%, for each of the treatments, respectively.

Trinexapac-ethyl treatment, when compared to the control plot, shows reduction of 18% (beginning of flowering structures) and 22% (fruit). However, when in the closed flowers, the root length was 67% higher.

As for the effect of fluazifop-p-butyl on the length, it is noted that, when applied in the beginning of flowering structures and open flowers, there were 39 and 16% lower than the control plot. Yet, when applied on the closed flowers, the length was 77% higher. The results from sulfometuron-methyl and ethephon were similar to the control.

During the root length evaluation (third and fourth stages), the seedlings from glyphosate application did not

Table 7. Interaction effect between treatments and stages upon the root length (mm) of morning glory seedlings.

Treatments	Beginning of flowering organs	Closed flowers	Open flowers	Fruits	F
	Root length (mm)				
Ethephon	37.3 ^{CDb}	28.9 ^{BCc}	46.4 ^{ABa}	36.3 ^{ABb}	14.31**
Fluazifop-P-butyl	31.2 ^{Db}	36.7 ^{ABab}	43.0 ^{Ba}	35.1 ^{ABb}	6.70**
Glyphosate	16.4 ^{Eab}	18.7 ^{Da}	10.3 ^{Cbc}	5.4 ^{Cc}	10.06**
Sulfometuron-methyl	47.3 ^{Ab}	37.5 ^{Ab}	42.5 ^{Bab}	30.3 ^{Bc}	14.65**
Trinexapac-ethyl	41.8 ^{BCa}	34.6 ^{ABb}	45.3 ^{ABa}	33.4 ^{Bb}	9.04**
Control	50.9 ^{Aa}	20.7 ^{CDc}	51.3 ^{Aa}	42.9 ^{Ab}	57.55**
F	37.39**	16.18**	52.90**	40.67**	

Means followed by different letters through Tukey test at 5% probability. Upper-case letters for treatments and lower-case letters for stages. ** Significant at 1% probability through F-test. * Significant at 5% probability through F-test. F (treatments x stages) = 13.86**; VC (%) plots= 19.84; VC (%) split-plots= 15.87.

Table 8. Interaction effect between treatments and stages of the aerial parts dry matter content (g) of morning glory seedlings.

Ripener	Beginning of flowering organs	Closed flowers	Open flowers	Fruits	F
	Aerial parts dry matter (g)				
Ethephon	0.07 ^{BCb}	0.12 ^{Ca}	0.14 ^{Aa}	0.13 ^{Ab}	35.73**
Fluazifop-P-butyl	0.09 ^{ABbc}	0.13 ^{BCa}	0.07 ^{Bc}	0.11 ^{Ab}	9.61**
Glyphosate	0.10 ^{Ab}	0.16 ^{ABa}	0.11 ^{ABb}	0.12 ^{Ab}	8.29**
Sulfometuron-methyl	0.04 ^{Cc}	0.12 ^{Ca}	0.08 ^{Bb}	0.09 ^{Ab}	15.43**
Trinexapac-ethyl	0.07 ^{BCc}	0.17 ^{Aa}	0.07 ^{Bc}	0.12 ^{Ab}	35.73**
Control	0.06 ^{Cc}	0.10 ^{Cbc}	0.15 ^{Aa}	0.12 ^{Ab}	19.46**
F	8.04**	10.57**	20.69**	3.42**	

Means followed by different letters through Tukey test at 5% probability. Upper-case letters for treatments and lower-case letters for stages. ** Significant at 1% probability through F-test. * Significant at 5% probability through F-test. F (treatments x stages)= 8.93**; VC (%) plots= 19.21; VC (%) split-plots= 21.28.

show primary root development and didn't emit secondary roots either. Pline et al. (2001) reported that 30% of absorbed glyphosate may be retained in the reproductive tissues. Viator et al. (2003) reported that squares and bolls of resistant cotton retained 0.2 to 3.7% of the applied glyphosate. Funguetto et al. (2004) presented results alike, studying the glyphosate effects on non GMO soy plants, which had reduction in the root structures due the inhibition of primary roots and the secondary roots emission, being the hypocotyls proportionally bigger than the primary root, characterizing an anomaly.

The limitation on the root length may be explained by the diminishing of IAA production during the seedling development. As the translocation of glyphosate through the plant occurs through symplastic way, the application of agrochemicals on source organs (mature leaves) enable the translocation of said product to sink organs (growing organs) on the rest of the plant, simultaneously to the photoassimilates (Peterson et al., 1978; Caseley and Coupland, 1985; Franz et al., 1997), and so, glyphosate would be translocated to the forming seeds in the mother-plant, and by accumulating on the seeds, may

cause the inhibition of IAA hormone (indoleacetic acid auxin) during the plant development. The IAA, hormone produced on the tip regions of a plant (roots and stem), is a substance related to the growth regulation, derived from triptofan through many phases, and, also, independent of triptofan, as does its precursor the indol-3-glycerol phosphate, which depends on chorismate for its formation. Glyphosate inhibits the synthesis of chorismate as well as triptofan synthesis (Yamada and Castro, 2007).

Aerial parts and roots dry matter content

On Table 8 are the interaction results between treatments and stages upon aerial parts dry matter. For all treatments, in fruits stage, the results did not differ from control plot. When flowers were open, the dry matter content for all treatments, except for ethephon, decreased around 25% (glyphosate) and 50% (fluazifop-P-butyl, sulfometuron-methyl and trinexapac-ethyl).

In the beginning of the flower structures and in the closed flowers ethephon and sulfometuron-methyl

Table 9. Interaction effect between treatments and stages upon the roots dry matter content (g) of morningglory seedlings.

Ripener	Beginning of flowering organs	Closed flowers	Open flowers	Fruits	F
	Roots dry matter (g)				
Ethephon	0.03 ^{Ba}	0.05 ^{ABa}	0.02 ^{BCb}	0.02 ^{Ab}	12.80**
Fluazifop-P-butyl	0.05 ^{Aa}	0.07 ^{Aa}	0.05 ^{Aa}	0.02 ^{Ab}	20.80**
Glyphosate	0.01 ^{Cc}	0.07 ^{Aa}	0.02 ^{BCb}	0.01 ^{Ac}	40.40**
Sulfometuron-methyl	0.02 ^{Bb}	0.04 ^{Ba}	0.03 ^{BCb}	0.02 ^{Ab}	7.53**
Trinexapac-ethyl	0.03 ^{Bb}	0.06 ^{ABa}	0.01 ^{Cb}	0.03 ^{Ab}	19.00**
Control	0.01 ^{Cc}	0.05 ^{ABa}	0.04 ^{ABab}	0.02 ^{Abc}	14.51**
F	13.40**	4.34**	10.73**	1.59**	

Means followed by different letters differ through Tukey test at 5% probability. Upper-case letters for treatments and lower-case letters for stages. ** Significant at 1% probability through F-test. * Significant at 5% probability through F-test. F (treatments x stages)= 5.85**; VC (%) plots= 33.95; VC (%) split-plots= 35.76.

treatments showed the same results to control plot. As for glyphosate and fluazifop-P-butyl, it increases the mass when applied to the beginning of the flower structures (about 58%) and in closed flowers, where both accumulated 60% (glyphosate) and 30% (fluazifop-P-butyl) more than the control.

Trinexapac-ethyl, in the beginning of the flower structures, did not differ from control plot, while in the closed flowers, it accumulated 70% more dry matter content than control plot.

Table 9 shows the interaction results between treatments and stages of roots dry matter. For the fruit stage, as it happened in the aerial parts dry matter content, no results were different. In the beginning of the flowering stages, trinexapac-ethyl, fluazifop-P-butyl, sulfometuron-methyl and etephon have more dry matter than the control. Fluazifop-P-butyl is the treatment that most increased this parameter.

The application of these ripeners on the closed and open flowers have similar results to the control plot, except to trinexapac-ethyl treatment, on the open flowers application, that provided the least dry matter accumulation between the treatments, 75% lower than control. The glyphosate results were similar to the control in all stages.

The difference between the results of dry matter may be related to the product accumulation in the seeds when it receives the photoassimilates upon the seed formation stage, and, though, the malformation of these during the development of the mother-plant, causing negative effects on the seed quality. It is also important to highlight that the application of the products was before the fruits reached the physiological maturity; therefore, the photoassimilate transport was not finished (Carvalho and Nakagawa, 2012).

The data found for the ripeners' applications on the reproductive stage indicate that those ripeners may affect the initial development of the seedlings which originated from the mother-plant, and, hence, provide lesser competition characteristics for light, water and nutrients

with the desirable crop. Besides, in having reduced fruit and seed production, as well as increasing the abscission of reproductive structures diminishes the weeds seed bank resupplying.

Conclusion

Glyphosate (216 g a.e ha⁻¹) and etephon (480 g a.i ha⁻¹) may reduce the egress of *I. hederifolia* to the ground. Trinexapac-ethyl (300 g a.i.ha⁻¹), glyphosate (216 g a.e ha⁻¹) and fluazifop-P-butyl (75 g a.i.ha⁻¹) applied when flowers are open, reduced the seeds viability.

The application of glyphosate (216 g a.e ha⁻¹) during the reproductive stages of *Ipomoea hederifolia* may affect the seedlings development.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Phenotypic variability in Nigerian castor (*Ricinus communis* L.) accessions

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Ten accessions of *Ricinus communis* L. were grown in derived savannah agro-ecological zone for two cropping seasons under rain fed conditions. The work aimed at phenotypic characterization of *R. communis* for different qualitative and quantitative traits. The field characterization of the genotypes was done using randomized complete block design with three replications at the new site of Botanical garden, University of Nigeria, Nsukka. The different genotypes were randomly assigned to each of the ten plots within a block. Data collected on morphological and flowering attributes was subjected to statistical analysis. The results on phenotypic traits showed variations in fruit texture and stem color. Seedling emergence across the accessions was uniform in both years. The number of branches appeared stable within genotypes in years showing non-significant genotype by year mean square values. The number of leaves showed a significant variation ($p \leq 0.05$) among genotypes in 2013. There was a significant difference in the effects of years on the number of capsule and seed weight.

Key words: Castor, *Ricinus communis*, phenotypic traits.

INTRODUCTION

Castor (*Ricinus communis* L.), the single member of the African genus *Ricinus*, presents a wide variation regarding vegetative traits such as leaf and stem colors, number and size of leaf lobes and presence of wax. Castor bean plant occurs in dense stands and is frequently found along roadsides. It is both self- and cross-pollinated by wind, but controlled crossing studies suggested that out crossing is a frequent mode of reproduction (Meinders and Jones, 1995).

It originates from India and is cultivated in the tropical and sub-tropical climates of the world (Weiss, 2000). India is the world's largest producer of castor seed and meets most of the global demand for castor oil; other

major producers being China, Brazil, Ethiopia and Paraguay (FAO, 2013).

According to Weiss (2000), the castor bean plant varies greatly in its growth habit, colour of foliage, stems, seed size, colour and oil content, and varieties often bear little resemblance with each other. Colour differences in leaves, stems and inflorescences aid in the selection of horticultural and ornamental plants (Koutroubas et al., 1999). In its widespread naturalized state, castor is usually a fairly tall, mainly branched perennial but when cultivated commercially, it is short-lived, erect, little branched and treated as an annual crop (Ogunniyi, 2006).

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Castor crop can be grown on a wide range of soils, provided they are fairly deep and well drained. Heavy clays, with poor drainage and marshy soils are unsuitable. The highly suitable soils for castor are deep, moderately fertile, with slightly acidic conditions (pH 5.0 – 6.5), well drained sandy loams. Excessively fertile soils are not desirable, as they favor excessive vegetative growth at the expense of seed yield (Weiss, 2000).

Castor flowers occur most periods of the year in dense terminal clusters with female flowers just above the male flowers. This species is clearly monoecious, with separate male and female flowers on the same individual. There are no petals and female flower consists of a little spiny ovary (which develops into the fruit or seed capsule) and a bright red structure with feathery branches that receives pollen from male flowers. Each male flower consists of a cluster of many stamens which literally smoke as they shed pollen in a gust of wind (Weiss, 2000).

The capsule is composed of three sections or carpels which split at maturity. Each carpel contains a single seed and as the carpel dries and spilt open, the seed is often ejected with considerable force (Mensah and Ocran, 2005). Castor seeds are nearly flattened and oval but differ in size and colour. The seeds may be white, black, brown or have several colours occurring as very attractive mottling on testa (Mensah and Ocran, 2005; Salunkhe and Desai, 1986). Li et al. (2008) described the seeds as shiny brown seeds with darker streaks or spots and resemble a blotted tick. A number of colours are usually found in castor seeds. The most striking ones are red, white and grey. Others are faint chocolate, deep chocolate and purple (Ikisan, 2000). The sizes of castor seed have been reported to vary (Weiss, 2000). The seed vary greatly in size, from 10 to about 250 mm long in giant types and from 5 to 16 mm in breadth. The 100 seed weight may vary from 10-100 g, averaging about 30 g in dwarfs (Li et al., 2008; Salunkhe and Desai, 1986). There is a great variation in seed sizes; the smallest seed noticed is 5.30 × 9.95 mm, while the largest is 13.50 × 20.95 mm. The common cultivated types have seed size ranging from 7.79 × 11.19 to 9.80 × 13.54 mm. The mean weight of castor seeds has been reported to vary from 10 - 100 g; for instance, seeds found in Nigeria and Kenya have been reported to weigh 69.3 and 59.2 g, respectively.

There are a very few documented evidences describing morphological diversity in castor. Earlier taxonomists and botanists studied morphological diversity with the purpose of classifying the genus *Ricinus*. Moshkin (1986) reported existence of diverse morphological variants in many parts of the world for plant height, branching, stem colour, leaf size, waxy coating, length, shape and compactness of raceme, pedicle length, size and shape of capsule and seed. Woodend (1993) described white, black and dark brown seed with varying mottling intensity, dark green and dark red colour stem, prostrate to

columnar growth habit, weak-framed, robust and tree-like plant types among Zimbabwe collections.

The seeds of castor bean contain more than 45% oil and its oil is rich (80 to 90%) in an unusual hydroxyl fatty acid, ricinoleic acid. The oil is characterized by a high viscosity, owing to the hydrogen bonding of its hydroxyl group. It is rich in unique hydroxyl fatty acid. Ricinoleic acid is a major component in castor oil. Ricinoleic acid is widely used for its lubricating properties and medicinal purposes. It is widely used for its lubricating properties and medicinal purposes in industry. It is also used for manufacturing soaps, lubricants, hydraulic and brake fluids, paints, dyes, coatings, inks, cold resistant plastics, waxes and polishes, nylon, pharmaceuticals and perfumes (Duke, 1998).

Uncultivated wild and semi-wild castor plants are widespread not only in its centres of origin, but also outside. They represent the tremendous variability existing in the species. Castor had been adapted to diverse ecological niches. Therefore, ecological heterogeneity, stresses, natural selection and its interaction with other evolutionary forces including mutation, migration and genetic drift might have contributed greatly to genetic diversity according to circumstances in the natural niches. Studies on genetic diversity are necessary to elucidate and categorize the naturally existing variability. Genetic diversity in castor was assessed mostly by using agro-morphological traits and to some extent by molecular techniques. The vast worldwide castor collections reported were poorly studied and barely tapped for castor genetic improvement. The development of new cultivars with traits of interest and adaptation to specific microclimates is only possible when there is available knowledge on the extent of genetic diversity among the species (Gepts, 2004). Due to increased demand for castor bean in many countries, improvement of varieties is drawing attention from breeders (Sujatha et al., 2008). Success in breeding for high yield is limited by a low genetic variability and lack of geographically structured genetic populations, for productivity traits and sources of resistance to diseases and pests (Weiss, 2000; Hegde et al., 2003; Allan et al., 2008; Foster et al., 2010). It is therefore necessary to characterize the genetic diversity present in *R. communis* germplasm from different geographic regions to develop a genotyping scheme that links castor bean to a particular source, geographic region or batch (Hinckley, 2006). Information on diversity and genetic structure is essential in organizing accessions in germplasm collections because it allows effective maintenance of distant genotypes, thus reducing costs (Allan et al., 2008).

Although, the phenotypic traits have been extensively studied in castor in other parts of the world, such information is virtually unavailable for castor under Nigerian environmental conditions. Hence, the aim and objective of this work was to characterize *R. communis*

genotypes by field evaluation for different phenotypic traits.

MATERIALS AND METHODS

The experimental design was randomized complete block design (RCBD) measuring 390 m² with three replications. Each block measuring 3 by 4 m was subdivided into ten plots with 0.5 m spacing between plots. A space of 1 m was allowed between blocks with row spacing of 30 cm. The different genotypes were randomly assigned to each of the ten plots. Randomization was repeated for each block. Standard cultural practices were applied to all plots. The data collected were analyzed using RCBD format. The traits observed in this work were randomly selected. Statistically significant differences were detected using LSD at P = 0.05 levels of significance. The following quantitative traits were observed in the course of the work:

Days to emergence

The number of days it took a seedling to emerge was recorded. The data were randomly selected.

Days to 50% emergence

Emergence count started when the seeds sowed have emerged. 50% emergence is when half of the accessions sown have emerged.

Plant height (cm)

The height of castor plants started after four weeks and was done using meter rule and measuring tape. This was taken at four weeks (4 weeks) interval throughout the duration of the work (16 weeks).

Number of branches

The number of branches was taken into record after four weeks and these continued throughout the duration of the work at the interval of four weeks. These data was collected by counting.

Number of leaves

The number of leaves was counted at interval of four weeks.

Days to first flower

The number of days to flowering was counted from number of days after emergence to first flower.

Days to 50% flowering

The number of days in which 50% of the plants have flowered per plot was also recorded.

Days to maturity of castor bean plant

Days to maturity was calculated by counting the number of days from sowing date to the day when capsules colour changed from

green to brown.

Number of capsules per plant

The number of capsules per plant was determined by dividing the total number of the capsule by the number of plants that emerged.

Seed weight

The seeds of each accession were weighed at the end of the experiment at the Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka. The weighing machine used was mettle P1200.

Stem and leaf color

In order to ascertain the exact color of the stem and leaf of castor plant, the traits were subjected to eye estimation.

Data analysis

The data collected were analyzed using RCBD format. Statistically significant differences were detected using least significant difference (LSD) at P = 0.05 levels of significance.

RESULTS AND DISCUSSION

The data collected on the evaluation of castor accessions under rain fed conditions were analyzed and the means are reported. The phenotypic traits showed marked variations. The stem colour varied from light green to reddish. Most of the accessions had mean values of 7 days in both years on days to emergence. All the accessions had the lowest 50% emergence in 2012 evaluation season (Table 1). The value of number of days to 50% emergence ranged from 8 to 13.67. Accession 3 had the highest mean value of 13.67 on days to 50% emergence (Table 1). Ac 1 collected from Ibagwa-Aka started emerging 7 days after planting and within another two days (8.67), it attained 50% emergence. The year effects on days to emergence and 50% emergence was shown (Table 2). From the data presented, days to emergence and 50% emergence were statistically insignificant (Table 2). The lowest number of days to 50% emergence was recorded in 2012 evaluation season. In the second year, however, the gap between days to emergence and a number of days to 50% emergence widened, giving 7 to 12 days gap between days to emergence and number of days to 50% emergence. In the environmental condition of year two, accession 10 had the shortest emergence and could be selected in a breeding strategy to escape drought. The uniform emergence reported among the accessions aids the crop in avoiding drought and environmental stress. Uniformity in emergence recorded in this study could be attributed to earliness in sowing castor seed. Different sowing dates have profound influence on the germination

Table 1. Interactive mean effect of castor accessions on agro-morphological parameters.

Year	Accession	DOE	50% E
2012	AC ₁	7±0.01	8.67±0.88
	AC ₂	7±0.01	12.67±0.33
	AC ₃	7±0.01	13.67±1.76
	AC ₄	7±0.01	10±1.00
	AC ₅	7±0.01	10.67±3.18
	AC ₆	7±0.01	12.33±2.60
	AC ₇	7±0.01	13±2.08
	AC ₈	7±0.01	8.33±0.88
	AC ₉	7±0.01	11±0.58
	AC ₁₀	8±0.58	12.67±0.33
2013	AC ₁	7±0.01	10±1.53
	AC ₂	7±0.01	8±0.58
	AC ₃	7±0.01	10±0.58
	AC ₄	7±0.01	10±0.01
	AC ₅	7±0.01	11±0.58
	AC ₆	7±0.01	12.33±0.67
	AC ₇	7±0.01	9.67±1.20
	AC ₈	7±0.01	9.67±0.88
	AC ₉	7±0.01	11.67±0.67
	AC ₁₀	8±0.01	11.33±0.67
LSD(axy)		n.s	3.75

DOE= days to emergence, 50%E= 50% emergence, LSD= least significant differences of accession by year interaction, n.s= not significant.

Table 2. Year effects on days to emergence and 50% emergence.

Year	DOE	50% E
2012	7.1±0.07	11.3±0.54
2013	7±0.01	10.37±0.31
LSD (P≤0.05)	Ns	Ns

DOE= days to emergence, 50% E= 50% emergence, n.s= not significant.

of the seeds.

Variations exist within genotypes in a particular year and across the different growing seasons for plant height (Table 3). The lowest plant height was recorded in 2012 growing season. Accession 1 collected from Adani attained the plant height of 180.6 cm at 16 weeks after planting (WAP) in 2012 growing season. Similar effect was recorded in accession one in 2013 growing season at 16 weeks after planting (16WAP). This height of plant recorded is disadvantageous as it blocked other crop from sunlight thereby reducing their growth and photosynthetic activity in the leaves. The perennial growth habit of castor plants limits mechanical harvest because the plant grows very tall when the environmental

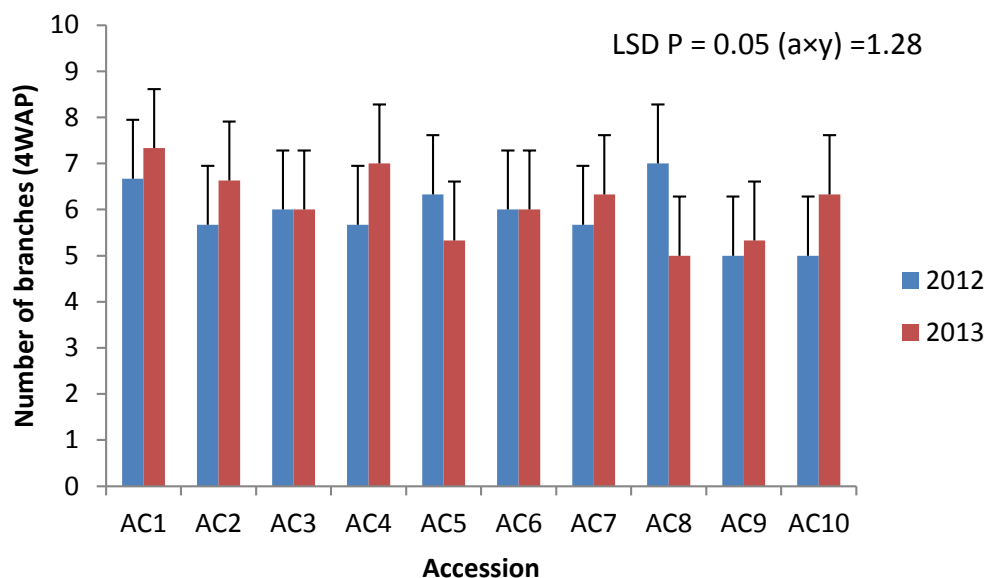
conditions are desirable. Different temperature and moisture levels may have affected the plant height to some extent within the genetic potential of the plant. Overall plant height remained the same over years which imply the existence of dwarf and tall genotypes.

The number of branches had the highest value in 2012 growing season except at 4WAP (Figure 1). The number of branches at 4 weeks after planting (DAP) varied from 5 to 6 in 2012 and 5 to 7 in 2013. Ac 1 had the highest significant number of branches (7) in 2013. Accessions 7 and 10 had the same number of leaves (6) in 2013 (Figure 1). Ac 8 had significantly the highest value (15) in number of branches at 8WAP in 2012 (Figure 2). The number of branches at 8WAP ranged from 8 to 15 in 2012 and 6 to 8 in 2013. A similar number of branches were recorded in ac 2 in 2012 and ac 1 in 2013 (Figure 2). AC 6 had the highest number of branches in 2012 at 12WAP. The number of branches ranged from 9 – 16 in 2012 and 6 – 8 in 2013. Similar number of branches was attained in accessions 7 and 8 in 2013 while in 2012; accessions 8 and 9 had the same value (Figure 3). There was a significant variation in the number of branches among the accessions in sixteen weeks after planting (Figure 4). The number of branches at 16WAP ranged from 10 to 15 in 2012 and 8 to 11 in 2013. In 2013, accessions 3 and 9 had the similar value (8). Accessions

Table 3. Effect of accessions on plant height (cm).

Year	Accession	4 WAP	8 WAP	12 WAP	16 WAP
2012	AC ₁	12.17±1.0	58.9±2.8	113.0±15.0	180.6±16.3
	AC ₂	9.74±0.5	55.7±6.8	103.5±10.2	158.5±17.4
	AC ₃	12.19±2.8	60.3±8.2	128.7±24.5	192.3±39.2
	AC ₄	12.26±1.0	47.7±7.0	115.6±17.5	167.6±11.7
	AC ₅	13.81±3.5	57.2±3.73	112.0±19.6	173.5±19.8
	AC ₆	7.94±1.4	53.4±7.6	61.2±10.7	110.9±19.1
	AC ₇	9.04±0.7	49.4±9.5	89.4±17.3	141.5±26.5
	AC ₈	18.79±1.7	92.3±5.7	127.1±14.8	221.1±37.1
	AC ₉	19.19±5.6	44.3±10.2	123.7±14.7	185.2±15.1
	AC ₁₀	9.47±1.0	42.6±3.8	85.2±16.7	147.1±13.4
2013	AC ₁	20.32±3.5	52.1±14.3	92.8±17.2	166.2±31.7
	AC ₂	31.73±5.6	60.0±9.4	91.6±15.6	129.4±19.3
	AC ₃	22.80±3.4	50.0±14.1	82.2±27.1	117.0±21.8
	AC ₄	33.79±8.1	90.5±10.3	148.9±15.3	171.6±20.4
	AC ₅	33.32±2.8	62.4±15.6	97.2±31.6	137.7±33.2
	AC ₆	25.10±4.5	51.7±9.5	82.5±21.2	118.1±21.4
	AC ₇	31.99±2.4	58.8±10.3	68.0±16.2	128.5±20.7
	AC ₈	34.00±1.0	90.0±4.5	150.3±10.2	229.5±18.4
	AC ₉	25.88±2.7	44.0±9.0	72.6±15.5	103.1±19.8
	AC ₁₀	26.01±2.4	42.1±4.4	64.7±7.5	99.5±10.5
LSD(axy)		1.81	15.78	25.14	30.81

WAP= weeks after planting, LSD= least significant differences of accession by year interaction.

**Figure 1.** Effect of accession on number of branches four weeks after planting.

6 and 9 had the highest significant number of branches (15) in 2012 while accessions 5 and 10 had same value (11) (Figure 4).

Generally, the number of branches performed better in

2012 growing season. The number of branches seemed stable within genotype in years but varied among genotypes. The number of branches had the highest value in 2012 while Accession 1 had the highest

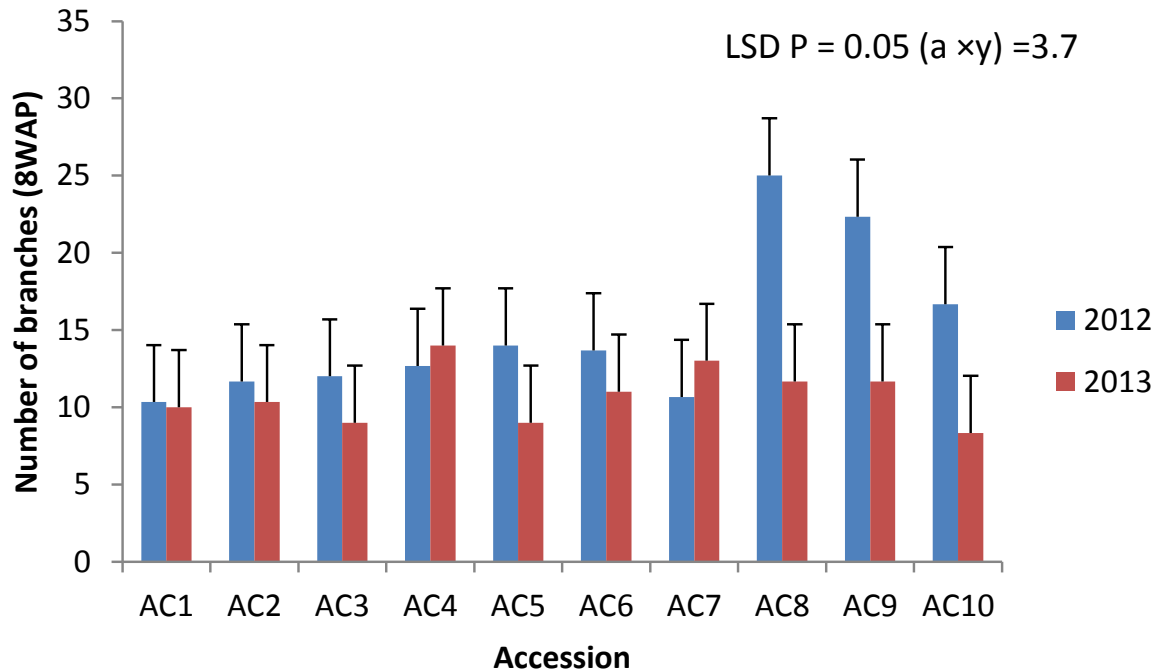


Figure 2. Effect of accession on number of branches eight weeks after planting.

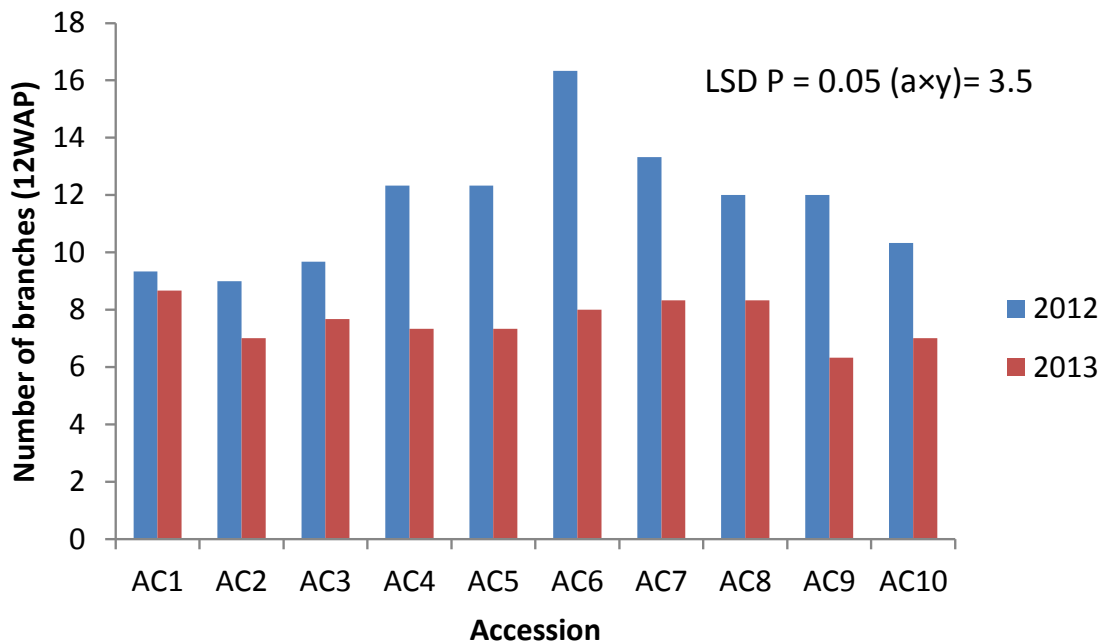


Figure 3. Effect of accession on number of branches twelve weeks after planting.

significant number of branches. The accessions seemed to be stable in year one but varied across the different growing seasons.

Accession 10 had the highest significant number of leaves (8) in 2013 at 4WAP (Figure 5). At 8, 12 and 16WAP, accession 8 had the highest significant number

of leaves of 19, 25 and 36, respectively (Figures 6 to 8) in 2012. The number of leaves in both cropping season was attacked by Semi looper *Achaea janata* Linn. The smooth greyish-brown caterpillars feed on the leaves and sometimes cause wholesale devastation of the crop-damage to defoliation. Older larvae are voracious feeders

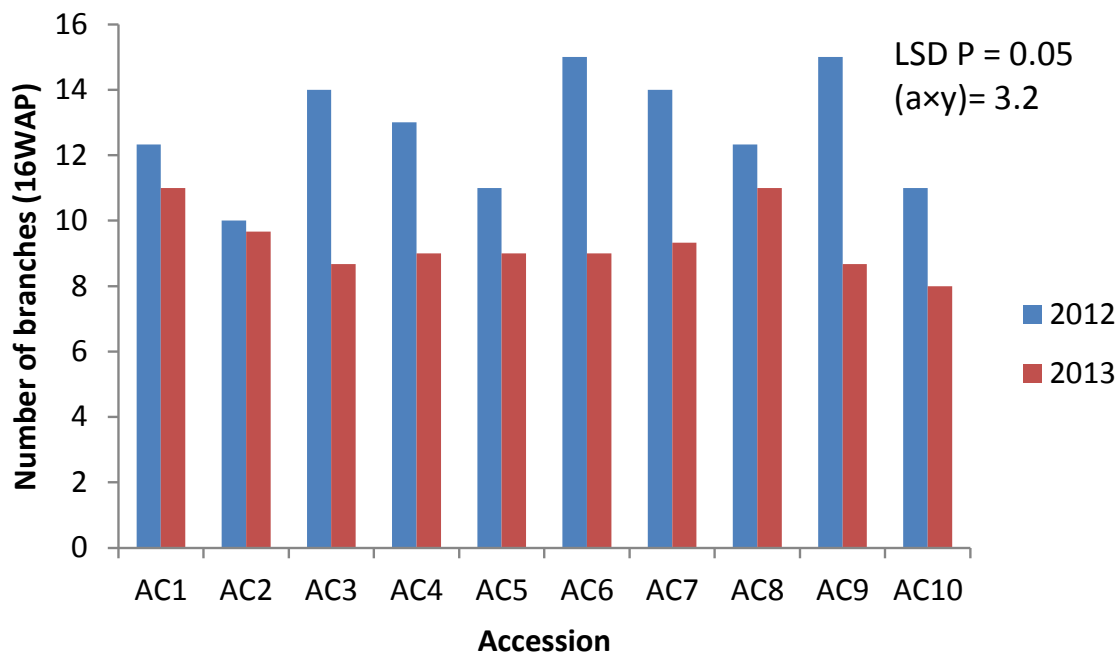


Figure 4. Effect of accession on number of branches sixteen weeks after planting.

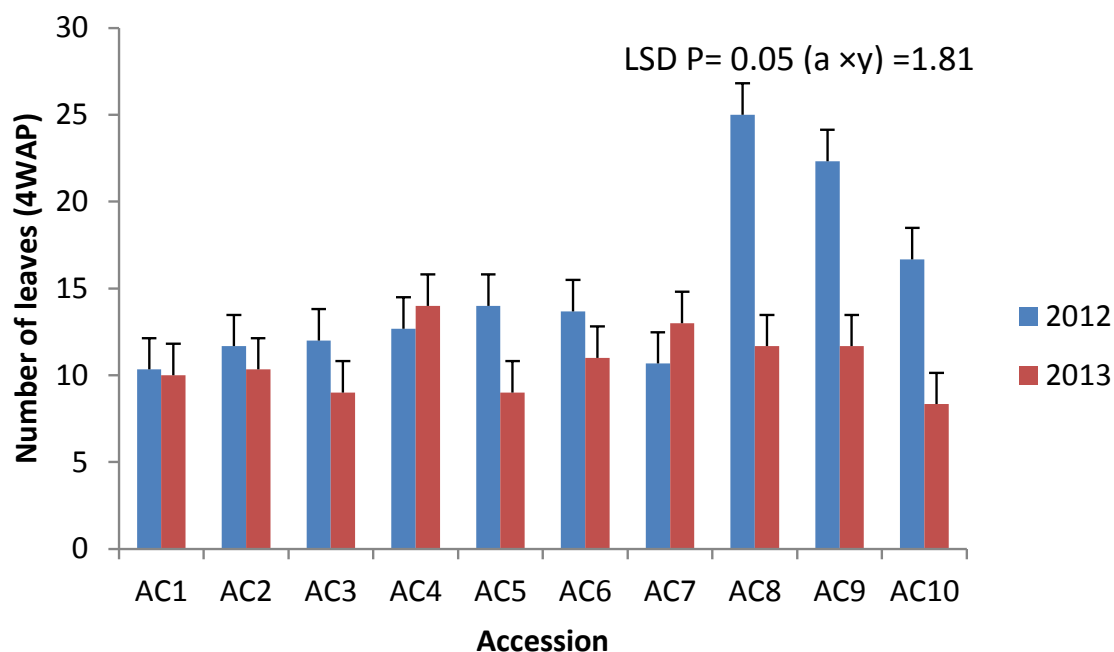


Figure 5. Effect of accession on number of leaves four weeks after planting.

and leave bare stems veins. This was controlled by hand picking of older larvae during early stages. The number of leaves was affected in year two by the tall plant height as it limits the amount of sunlight; thereby reducing its photosynthetic process. This also leads to significant difference in leaf area, plant height and higher seed yield.

The use of green manure with inorganic fertilizer enhances the growth, morphological indices and yield of castor as compared to the application of either of them. Days to first flower had the highest value in 2013. The number of days to first flower was 72 days in accessions 4, 5 and 9 which had the same days to first flower. The

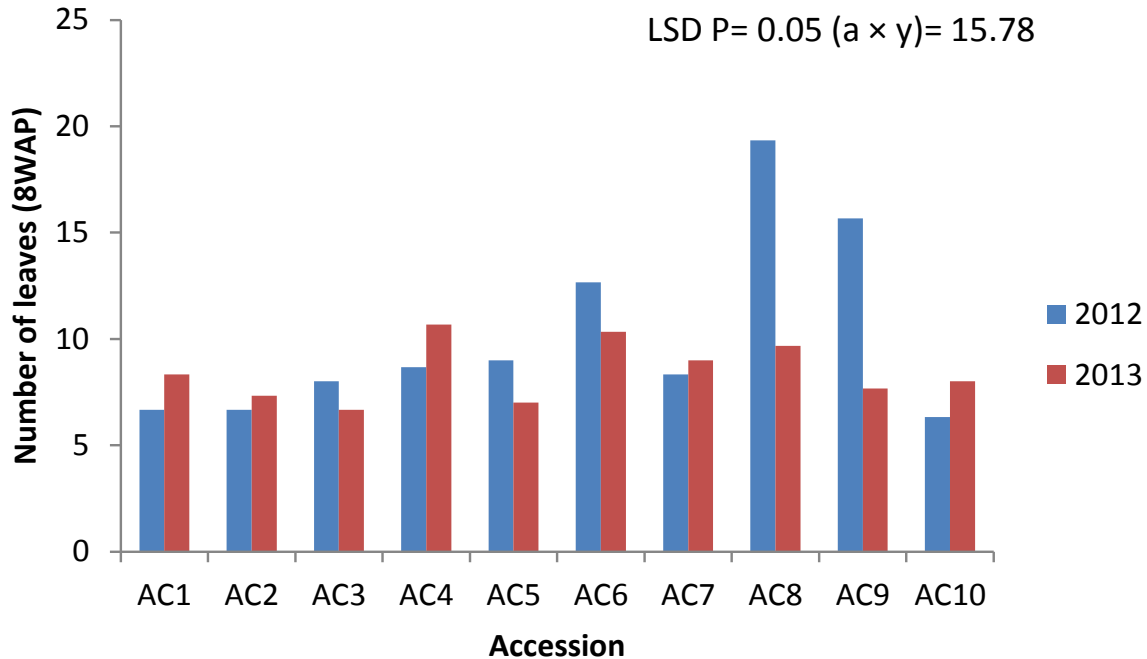


Figure 6. Effect of accession on number of leaves eight weeks after planting.

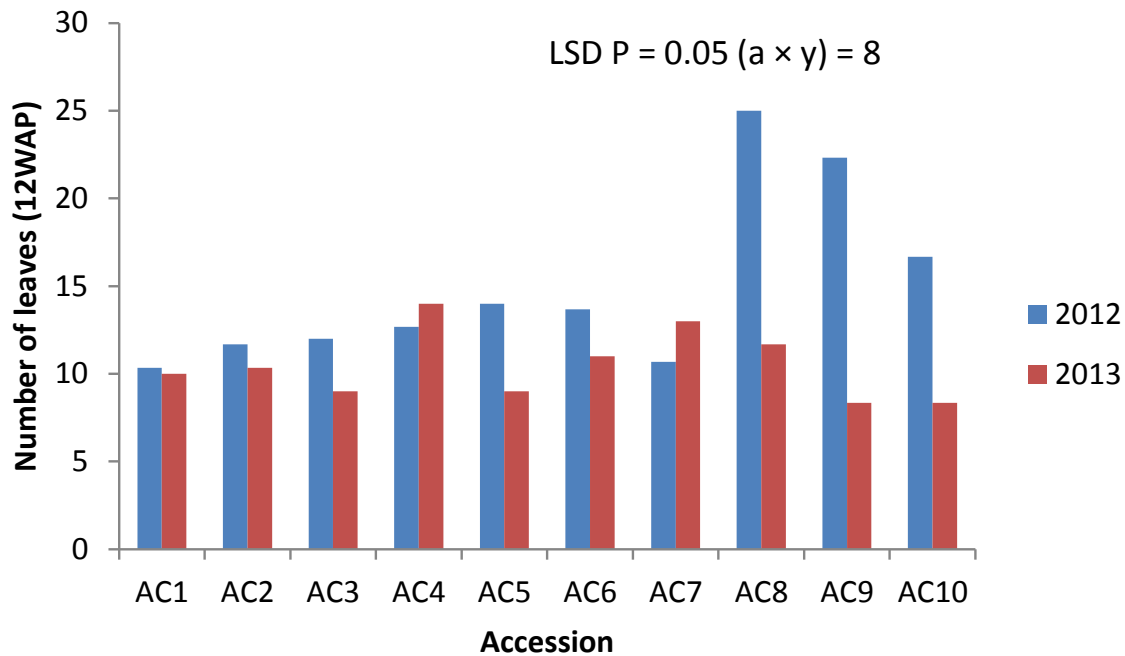


Figure 7. Effect of accession on number of leaves twelve weeks after planting.

value of this trait did not vary significantly (Table 4). Variation in days to 50% flowering was not significant in most of the accessions for the two years the accessions were evaluated but ac 7 had the highest significant days to first flowering in 2012 evaluation season. Stability in

the manifestation of these traits was observed across years for the ten genotypes. Days to maturity of castor fruit did not vary (Table 4). All the genotypes had the highest number of days to maturity in 2013 evaluation season (Table 4). The flowers are borne in terminal

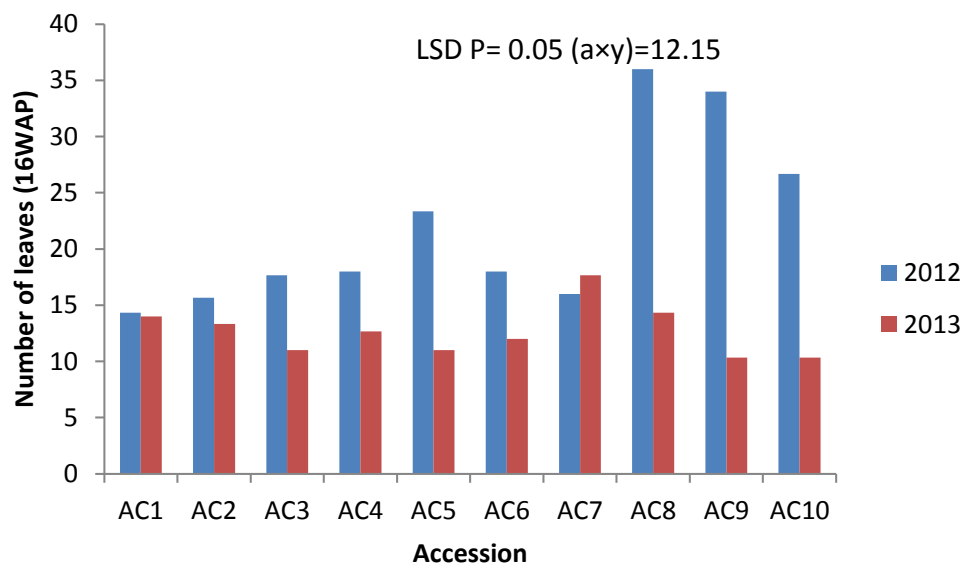


Figure 8. Effect of accession on number of leaves sixteen weeks after planting.

Table 4. Effect of accession on days to first flower, 50% flowering and days to maturity.

Year	Accession	DOFF	DO50%F	DM
2012	AC ₁	50.3±4.4	62.3±3.3	120±8.7
	AC ₂	64.7±3.8	75.3±4.8	141±1.7
	AC ₃	62.3±0.7	77±1.0	140±1.7
	AC ₄	60.3±2.6	77±6.1	121.3±7.2
	AC ₅	63.7±2.7	74.7±2.2	132.7±7.2
	AC ₆	57±2.5	73±2.3	130.3±3.4
	AC ₇	67±4.4	82±3.5	133±7.6
	AC ₈	65.3±5.2	76.7±7.0	143.3±3.5
	AC ₉	60.3±6.2	71.7±6.0	132.3±2.4
	AC ₁₀	59±1.5	72.3±2.0	148.3±22.0
2013	AC ₁	72±10.1	77.7±9.1	135.7±6.4
	AC ₂	63±2.5	67.7±1.9	135.3±2.8
	AC ₃	57.7±4.7	65±6.6	132.3±2.3
	AC ₄	70±5.5	75.3±4.2	135.7±2.3
	AC ₅	70.3±5.2	80±1.5	136.7±5.4
	AC ₆	63.7±14.0	69±13.1	143.7±8.0
	AC ₇	62.7±7.5	66.7±6.1	128.3±2.5
	AC ₈	71.7±3.5	73.7±3.5	134±1.0
	AC ₉	70±1.2	76±1.2	141.7±1.0
	AC ₁₀	66.7±6.2	74.7±3.9	139.3±4.1
LSD(axy)		15.37	14.97	20.47

DOFF = days to first flower, DO50%F = days to 50% flowering, DM = days to maturity, LSD = least significant differences of accession by year interaction.

panicle-like inflorescences of green or, in some varieties, shades of red monoecious flowers without petals. The male flowers are yellowish-green with prominent creamy stamens and are carried in ovoid spikes up to 15

centimeters long; the female flowers, borne at the tips of the spikes, have prominent red stigmas. Days to first flower had the highest value in 2013. Variation in 50% flowering was not significant in most of the accessions for

Table 5. Effect of accession on number of capsules per plant and seed weight per plant.

Year	Accession	Number of capsules per plant	Seed weight per plant
2012	AC ₁	58±2.4	88.9±2.4
	AC ₂	41.7±3.2	100.2±3.1
	AC ₃	55.0±2.4	81.0±1.7
	AC ₄	31.7±2.5	72.2±2.3
	AC ₅	60.7±7.4	81.9±1.2
	AC ₆	51.7±1.7	121.4±4.2
	AC ₇	44.7±2.8	77.5±3.2
	AC ₈	61.3±4.3	74.7±3.4
	AC ₉	53.6±2.3	33.6±2.5
	AC ₁₀	48.0±3.0	103.3±7.1
2013	AC ₁	45.0±2.1	102.5±3.4
	AC ₂	26.0±1.4	98.9±2.7
	AC ₃	40.3±3.8	84.5±4.3
	AC ₄	27.3±4.5	88.1±7.4
	AC ₅	37.7±2.4	96.5±3.7
	AC ₆	31.0±4.1	83.8±2.5
	AC ₇	29.7±5.6	87.2±4.1
	AC ₈	37.7±2.6	88.9±3.2
	AC ₉	23.2±3.4	97.4±3.3
	AC ₁₀	28.0±4.7	86.9±2.4
	LSD	24.90	36.48

LSD = Least significant differences of accession by year interaction.

Table 6. The year effects on the number of capsules per plant and seed weight per plant.

Year	Number of capsules per plant	Seed weight per plant
2012	45.3±0.6	83.5±0.5
2013	32.6±0.4	91.5±0.7
LSD	7.88	5.70

LSD = Least significant differences of year.

the two years the genotypes were evaluated. Stability in the manifestation of these traits was observed across years for the ten genotypes.

The effect of the genotype on the number of capsules per plant and seed weight was not statistically significant (Table 5). There was significant difference in the year effects on seed weight and number of capsules (Table 6). The highest number of days to maturity was recorded in accession 10 in 2012 and several accessions seemed to attain maturity within 130 to 136 days. Accession 2 produced the highest seed weight and all accessions produced heavier seeds in 2012 evaluation seed. Accessions 4 and 8 in 2013 had the same seed weight. Capsule weight is one of the important components contributing to the yield of castor bean crop. Accession 8 had the highest significant number of capsules per plant in 2012. For traits associated with yield, the spacing seems to have a significant effect on several of the study

variables during both cropping seasons. The capsule weight during the cropping season decreased linearly. This could be attributed to competition for nutrients, reduced amount of light and less space available for plant development, thus less development of capsule.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Treatment of bean seeds with plant extracts for controlling *Zabrotes subfasciatus* and its effects on physical and physiological quality during storage

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Bean crop occupies a prominent place in medium and large farming units in Brazil. Most of the time, the success of this crop depends, among other factors, on the use of good quality seeds at sowing; which requires strict quality control, harvesting, and storage. The objective of this study is to evaluate the effect of leaves and shells extracts of *Aspidosperma pyriforme* Mart., *Anadenanthera colubrina* and myrtle (*Licania rigida* Benth) in controlling *Zabrotes subfasciatus* associated with bean seeds and to evaluate the seeds treated and stored in pet bottles for a period of 180 days. The experiments were conducted in the Agricultural Products Storage and Processing laboratory of the Federal University of Campina Grande. It was observed that for leaf extracts, germination decreased with storage time for all the extracts and the best germination percentage (95.00%) occurred in 5 ml of *A. pyriforme* Mart. leaves extract. These results found for the bark extract *A. pyriforme* Mart. showed that germination was 94.00% in the seeds treated with 5 ml dose of this extract. On the other hand, the extracts that had lower efficiency on germination were the extract of myrtle leaf and bark which showed 64.00 to 65.40% germination in 180 days of storage. It is concluded that low percentages of infestation show the efficiency of the extracts tested in the control of insects. The efficiency of these extracts may be due to the action of their secondary metabolites; especially the extracts of *A. colubrina*'s leaves and bark.

Key words: Bioactive plants, bean weevil, stored seeds.

INTRODUCTION

In recent years, Brazil is the world's third leading producer of beans (*Phaseolus vulgaris* L.), the

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largest producer is Myanmar, followed by India (Fao, 2013). In the scenario of the northeastern agricultural production, given the infrequent rainfall, beans, because of their short cycle and tolerance to water stress, holds special relevance in the food supply and the composition of family income. Due to the observation, that there is lower occurrence of losses and hand labor during seasonal periods, as in the case of cultivation in a second season crop, because of the use of wet or irrigated lowlands (Frota and Pereira, 2000).

Beans can lose their physical and physiological quality after harvest, especially if incorrectly stored. This can happen due to fungal contamination, insect infestation and/or metabolic processes that reduce germination and vigor, causing, among other defects; seed coat darkening (especially the Carioca group); due to oxidation of phenols in the presence of oxygen (Lazzari, 2005). Therefore, the adoption of conservation practices is critical to the maintenance of seed quality during storage, to prevent loss in germination and vigor, as well as pest attack.

The presence of insects causes qualitative and quantitative losses. The insects consume the substrates and the respiration of the insects creates hot spots. These hot spots cause increase in temperature and inter granular humidity which create an environment for fungal growth (Lazzari, 1997; Rupilho et al., 2006). The losses of grains and seeds, resulting from contaminated storage in Brazil, are 10% (Viebrantz et al., 2016).

The grains and seeds can rot and become useless for consumption due to the appearance, smell and taste. According to Lorini et al. (2002), reducing water content of the beans up to 13% by drying, helps reduce losses due to fungal attack. Although without chemical control, this does not prevent the presence of insect pests during storage. In this context, extracts of plants can be used as an alternative to control insects and may also be used together with other pest control practices. The advantages are their rapid biodegradation which reduces the environmental contamination risk; and they are easy to obtain.

Given the unavailability of technical information on the control of *Zabrotes subfasciatus* L. using natural insecticide produced by plants, this work aims to evaluate the effect of leaves and barks extracts (*Aspidosperma pyrifolium* Mart. and *Anadenanthera colubrina*) and myrtle (*Licania rigida* benth) in the control of *Z. subfasciatus* associated to seed bean. It also aims to evaluate the seed treated during storage in pet bottles for a period of 180 days.

MATERIALS AND METHODS

The experiment was conducted in Agricultural Products Storage and Processing Laboratory) of the Academic Unit of Agricultural Engineering (UAEA) at the Federal University of Campina Grande (UFCG) Paraiba. Bean seeds were obtained from producers of Belo Jardim, PE, Brazil and seeds were obtained in the 2010/2011 crop

season.

The initial quality of the seeds was evaluated by the purity analysis and the determination of moisture content, germination, vigor and infestation percentage of beans by *Z. subfasciatus*.

The moisture content was conducted in accordance with the Rules for Testing Seeds (Brazil, 2009). Moisture content was determined with two replications of 10 g of seeds and by using the oven method at 105 for 24 h. After treatment, the seeds were packed in pet packaging and stored in laboratory conditions without temperature and relative humidity control for a period of 180. They were evaluated every 45 days for moisture content, germination and first count of germination infestation, and weight loss.

The procedures of germination were carried out according to the Rules of Seed Analysis (BRASIL, 2009), but 200 seeds were used per treatment instead of 400. In this case, four replicates of 50 seeds were used per treatment (leaf and bark extracts and doses of 0, 1, 2, and 3 ml). The seeds were placed on germitest paper and the paper rolls were kept in a growth chamber at 25°C. The first count (vigor test) was performed together with the germination test by counting the normal seedlings found on the fifth day after sowing. Final germination was evaluated at 9 days (Brasil, 2009).

The infestation of *Z. subfasciatus* present in the stored beans was evaluated by observing the seeds per 100 g. The damaged seeds were then separated from the intact ones and were calculated over the total number of samples. The calculation was performed using the equation suggested by Almeida and Villamil (2000).

The percentage of beans' weight loss under storage conditions was conducted using 100 g of intact seeds and 100 of damaged seeds, and then calculated by the equation suggested by Almeida and Villamil (2000).

$$PP = (I - D/I) \times 100$$

where PP is the weight loss (%), I is the seeds loss integrity (g), and D is the weight damaged seeds (g).

The experiment of the extracts of leaves were separated, weighed and dried in an oven with forced air circulation for 72 h. Then, the plant materials were ground separately and placed in amber glass container containing 8 L of ethanol at 70% for maceration under occasional shaking. The extracted liquid passed through a first filtration filter paper under reduced pressure and a second filtration by gravity to hopper closed with cotton. Therefore, the filtrate was concentrated on evaporator under reduced pressure at a temperature of $\pm 80^\circ\text{C}$, for separating ethanol. The obtained solution was placed in water bath at a constant temperature of 65°C to evaporate the water and obtain solid material (Costa et al., 2011).

The hydro alcoholic extracts were used in doses that are sufficient to kill the insects, within seven days of life. The extracts were applied by the pipetting method directly on the mass of beans. Homogenizing was done by manual agitation and one plot was used for control. Then, the seeds were spread on polyethylene trays for a period of 24 h at room temperature in order to increase extract absorption for the seed mass.

After this time, seeds were distributed in plastic pet containers, with capacity of 500 g which were infested with 30 adult insects of *Z. subfasciatus* each. Insect were collected from bean seeds which are stored in plastic bottles. They were multiplied after selection, separation and identification using a microscopic. Insects were inoculated into a mass of beans, previously purged, inside a glass container of 300 ml capacity, sealed at the top with organdy cloth to allow natural ventilation.

The same procedure was used for untreated seeds. They were then stored in laboratory without temperature conditions and relative humidity control, for a period of 180 days. After this period, the percentage of infested seeds, weight loss, germination and the moisture content were performed. The control did not receive

Table 1. Control of infestation (adult emergency) of *Zabrotes subfasciatus* using hydroalcoholic extracts of leaf and bark of *A. pyrifolium* mart, *A. colubrina* and *L. rigida* in inoculated and non-inoculated bean seeds after 180 days of storage in pet packaging.

Extracts	Doses (ml)			
	0	1	3	5
<i>A. pyrifolium</i> (leaves)	9.00 ^{bB}	13.68 ^{aA}	6.82 ^{aC}	9.22 ^{abB}
<i>A. colubrina</i> (leaves)	9.09 ^{bA}	7.11 ^{bB}	6.48 ^{aB}	7.45 ^{bcB}
<i>L. rigida</i> (leaves)	9.30 ^{bB}	13.46 ^{aA}	2.88 ^{bD}	7.07 ^{cC}
<i>A. pyrifolium</i> (barks)	11.99 ^{aA}	7.00 ^{bB}	3.96 ^{bC}	7.22 ^{bcB}
<i>A. colubrina</i> (barks)	9.39 ^{bA}	6.69 ^{bB}	6.34 ^{aB}	8.79 ^{abcA}
<i>L. rigida</i> (barks)	9.60 ^{bAB}	6.56 ^{bC}	7.95 ^{aBC}	10.64 ^{aA}

DMS in the columns = 2.13; DMS in the lines =1.92; Classific.c/lower case; Classific.c/capital letters.

treatment and was not infested (they did not received any hydro alcoholic solution)

The experiment was conducted in a completely randomized design, in which the experiments were arranged in a factorial design with four replications and the means were compared by Tukey test at 1 and 5% probability. Effects of quantitative factors were analyzed using regression analysis: linear and quadratic equations for all variables and their interactions were examined. The data were evaluated with the software ASSISTAT version 7.6 (Silva and Azevedo, 2009).

RESULTS AND DISCUSSION

The seeds were initially stored with 13.29% moisture content and 96% germination was observed. The results of the evaluation of the initial quality of the seeds prior to storage with respect to moisture content was in the range suggested for storage by Delouche et al. (1976), Popinigs (1985) and Carvalho and Nakagawa (2000).

According to the results shown in Table 1, it appears that the infestation reduced from 9.49 (control) to 6.82, 6.48 and 2.88% with a dose of 3 ml of the leaves extracts of *A. pyrifolium* Mart., *A. colubrina* and *L. rigida*, respectively and similar behavior was observed in extracts from barks of the same species. It can be noted that the 3 ml dose was the best for controlling insect infestation and extracts from *L. rigida* leaf and bark of *A. pyrifolium* Mart. showed the best results in controlling the infestation of *Z. subfasciatus* present in seed mass of stored beans. It was also observed that extracts of *L. rigida* (2.88) and *A. pyrifolium* Mart. barks (3.96) in 3 ml dose, showed no statistic difference in controlling insect infestation.

The low percentages of insect infestation in controlled bean seeds, reveal the efficiency of the extracts used in the control of insect infestations. This efficiency is probably due to its secondary metabolite action, especially in *Anadenanthera colubrina* extracts (bark or leaf); which it does by leaving a thin protective waterproof film. This thin lubricant layer serves as a barrier to insect. According to Silva et al. (2013), repellent effects are observed in insect sensory system when they are exposed to undesirable substances.

Costa (2011) studying *Z. subfasciatus* infestation in a mass of beans, treated with extracts of jackfruit (*Artocarpus heterophyllus* L.) and *Chenopodium ambrosioides* L. verified reduction of infestation by 90% using doses of 6 to 8 ml, during 120 days of storage. Ali et al. (2011) observed that the amount of damage to grains caused by weevils increased with storage time and this devalued the product sale.

Garcia et al. (2000) noted that the treatment of beans with ground black pepper with doses 4 and 6 g per kg⁻¹ had absolute control on *Z. subfasciatus* during the storage period. It is noted that, it is feasible to use alternative plant extracts in order to control insect infestations in seeds and grains during storage.

For interaction with extracts procedure as shown in Table 2, it is noted that when inoculated with *A. colubrina* and myrtle leaves extracts or *A. pyrifolium* Mart. and myrtle shells, no statistic differences were observed. The best results were observed in extracts from *A. pyrifolium* Mart. leaves and *A. colubrina* barks. However, uninoculated beans showed better control of *Z. subfasciatus* with *A. pyrifolium* Mart. leaves and myrtle bark extracts which did not differ statistically; this result was followed by *L. rigida* leaves (Silva et al., 2013).

When the bean seeds were inoculated with *A. colubrina* and myrtle leaves extracts, no statistical differences were observed.

The low percentage of infestation demonstrates the efficient action of the extracts in the study. However, we noticed an emollient action on stored seeds treated with myrtle extracts, this action promoted a strong bond between the seeds. These residues are probably due to the chemical compounds (saponins) present in the myrtle extract, indicating that these seeds should not be stored for more than 180 days under these conditions, when treated with myrtle extracts.

Garcia et al. (2003) studying the bioactivity of *A. colubrina* and myrtle extracts, observed results which show that the extract had active substances against insects (mortality). Barbosa et al. (2000) observed resistance associated with species of bean arcelin *Z. subfasciatus* protein, implying that some substances

Table 2. Efficiency (mortality) of hydro alcoholic extracts of leaf and bark *A. pyrifolium* Mart., *A. colubrina* and *L. rigida* applied in bean seeds inoculated and non- inoculated with *Z. subfasciatus* in 180 days of storage in pet type packaging.

Extracts	Treatments	
	Inoculated	Non-inoculated
<i>A. pyrifolium</i> (leaves)	10.84 ^{aA}	8.77 ^{aB}
<i>A. colubrina</i> (leaves)	8.73 ^{bA}	6.54 ^{bcB}
<i>L. rigida</i> (leaves)	8.92 ^{bA}	7.53 ^{abcB}
<i>A. pyrifolium</i> (barks)	9.02 ^{bA}	6.07 ^{cB}
<i>A. colubrina</i> (barks)	9.51 ^{abA}	6.15 ^{cB}
<i>L. rigida</i> (barks)	9.30 ^{bA}	8.02 ^{abB}

DMS para columns= 1.51; DMS in the lines = 1.03; Classific.c/lower case; Classific.c/capital letters.

Table 3. Mean values (%) of the infestation procedure of bean seeds, inoculated and non-inoculated with *Zabrotes subfasciatus* and treated with plants extracts in pet packaging, for a period of 180 days.

Procedure	Periods (days)			
	45	90	135	180
Inoculated	4.61 ^{aD}	6.66 ^{aC}	11.22 ^{aB}	15.04 ^{aA}
No Inoculated	3.57 ^{bD}	5.50 ^{bc}	8.32 ^{bb}	11.33 ^{ba}

DMS in the columns = 0.84; DMS in the lines = 1.11; Classific.c/lower case; Classific.c/capital letters.

Table 4. Mean value (%) of *Zabrotes subfasciatus* infestations in inoculated and non-inoculated bean seeds, treated with different doses of plant extracts, stored in pet containers, in a period of 180 days.

Doses (ml)	Periods (days)			
	45	90	135	180
0	3.83 ^{aD}	6.55 ^{bc}	10.27 ^{bb}	15.68 ^{aA}
1	2.10 ^{bc}	2.28 ^{cc}	6.66 ^{cb}	10.91 ^{ca}
3	5.06 ^{ac}	6.24 ^{bc}	9.88 ^{bb}	12.41 ^{bcA}
5	5.38 ^{ac}	8.27 ^{ab}	12.26 ^{aA}	13.73 ^{ba}

DMS in the columns = 1.57; DMS in the lines = 1.26; Classific.c/lower letters; Classific.c/capital letters.

derived from the secondary metabolism of plants can affect the biology of insects.

Analysis of the data contained in Table 3, exhibits equal statistics of doses in controlling *Z. subfasciatus* present in beans paste stored at room condition for 180 days in pet packaging. However, for procedures within each dose (column), the dose of 3 ml showed the best control of *Z. subfasciatus*, where the infestation after 180 days of storage was 5.73% (average). These results are due, probably, to the quantity of the dose as secondary plant constituents present in the extracts; this dose operates with higher efficiency due to better distribution upon its application to the seeds.

According to Costa (2011), when the extracts *A. heterophyllus* and *C. ambrosioides* were used to control the infestation of *Z. subfasciatus*, in *P. vulgaris*, for a storage period of 120 days, equal statistical result was

observed in both the inoculated and non-inoculated procedure with doses of 6 and 8 ml.

As shown in Table 4, for interaction with the procedure time, there is increased infestation of *Z. subfasciatus* in seed stored in the mass, with the passage of storage time, gradually, in both procedures with superiority in the inoculated seeded process. It appears also that after three months (90 days) of storage, the percentage of infestation was 6.66% for the mass of seeds infested with *Z. subfasciatus*. It can be seen that during storage the extracts were losing their bioactivity due to the effects of their volatile constituents, which might explain the increased infestation with time. The insecticide effect of plant extracts was also analyzed by Pessoa (2004) for corn seed storage for popcorn with positive results in controlling infestation of *Sitophilus zeamais*. Santos et al. (1998) and Costa (2011) also controlled infestation of *Z.*

Table 5. Mean value (%) of *Zabrotes subfasciatus* infestations in inoculated and non-inoculated bean seeds, treated with different doses of plant extracts, stored in pet containers, in a period of 180 days.

Extracts	Time (days)			
	45	90	135	180
<i>A. pyrifolium</i> (leaves)	5.05 ^{aD}	8.07 ^{aC}	11.16 ^{aB}	14.94 ^{abA}
<i>A. colubrina</i> (leaves)	2.91 ^{cD}	4.88 ^{bC}	9.16 ^{bB}	13.58 ^{bcA}
<i>L. rigida</i> (leaves)	4.14 ^{bD}	6.36 ^{abC}	9.53 ^{bB}	12.87 ^{bcA}
<i>A. pyrifolium</i> (bark)	4.21 ^{bB}	5.53 ^{bB}	10.12 ^{aA}	10.31 ^{dA}
<i>A. colubrina</i> (bark)	4.47 ^{bC}	6.25 ^{abC}	9.03 ^{bB}	11.56 ^{cdA}
<i>L. rigida</i> (bark)	3.78 ^{bC}	5.40 ^{bC}	9.62 ^{bB}	15.85 ^{aA}

DMS in the columns = 2.13; DMS in the lines = 1.92; Classific.c/lower case; Classific.c/capital letters.

Table 6. Germination percentage (%) of bean seeds treated with hydroalcoholic extracts of leaves and barks of *Aspidosperma pyrifolium* Mart., *A. colubrina* and *L. rigida*, inoculated and not inoculated with *Zabrotes subfasciatus* for 180 days of storage packaging pet.

Extracts	Procedures	
	Inoculated	Not inoculated
<i>A. pyrifolium</i> (leaves)	91.00 ^{abA}	92.00 ^{aA}
<i>A. colubrina</i> (leaves)	88.00 ^{bcB}	90.00 ^{aA}
<i>L. rigida</i> (leaves)	78.00 ^{dB}	93.00 ^{aA}
<i>A. pyrifolium</i> (bark)	92.00 ^{aA}	92.00 ^{aA}
<i>A. colubrina</i> (bark)	88.00 ^{cb}	92.00 ^{aA}
<i>L. rigida</i> (bark)	80.00 ^{dB}	91.00 ^{aA}

DMS in the columns = 2.91; DMS in the lines= 2.00; Classific.c /lower case; Classific.c/capital letters.

subfasciatus in bean seed with plant extracts.

Silva Junior (2011) working with hydroalcoholic extracts of sugar apple and black pepper on the infestation of corn with *S. zeamais*, inoculated the seed mass; after 180 days in storage, he observed lower infestation for the highest levels of these extracts, which partly agree with this research.

From the results obtained in Table 5, it appears that the behavior of infestation by *Z. subfasciatus* is progressive and increases with time regardless of the dose. It was observed that the best controls were those with higher doses of 1 and 3 ml, respectively (line), with a dose of 1 ml, the infestation of *Z. subfasciatus* was controlled more efficiently within the time (column) when compared to the dose of 3 ml. It appears that the lower volume of extract was the most effective in controlling infestation, probably by the interaction of its chemical constituents in the form of application and speed of exposure of extracts on the seeds.

Resende et al. (2008) studied bean storage, observed after 84 days, a significant increase of 91.67% in grain population of insect pests stored in a mass of seeds treated with plant extracts.

In this study, the maximum infestation of *Z. subfasciatus* after 180 days of storage was 13.73% with a 5 ml dose compared to 10.91% with a dose of 1 ml. Therefore, these two doses are confirmed as being the

most efficient for the control of weevil in beans seeds.

Harborne (1980) stated that the leaves of angiosperms accumulate flavonoids which serve as insect repellent. A similar result was found in *A. colubrina* and *A. pyrifolium* Mart. which are present in the extracts of the leaves and flavonoids of the barks.

The *A. colubrina* leave extract was also more effective in the control of *Z. subfasciatus* at the rate of 2.91, 4.88 and 9.16% in 45, 90, and 135 days, respectively. The bark extracts from myrtle was also found to be more efficient, it showed a result of 3.78 and 5.40% infestation in 45 and 90 days of storage, respectively. This level of effectiveness is followed by *A. pyrifolium* Mart. extract.

As shown in Table 6, there is infestation of *Z. subfasciatus* in seed mass, during storage and the extracts of *A. colubrina* leaves showed higher results in infestation control at all times. This result is followed the by *A. colubrina* bark.

The percentage of infestation of leaf extracts was statistically equal for all times, however, there was no statistical difference for all leaves and bark extracts analyzed during the 180 days of storage. There was a lower infestation occurrence in *A. pyrifolium* Mart. bark with 10.31%, followed by *A. colubrina* bark with 11.56%. Neves et al. (2000) used botanical products to control *Z. subfasciatus* in bean seeds. Assessments were performed at 30, 60 and 90 days after infestation and

Table 7. Average germination values (%) of interaction in bean seeds treated with doses of leaves hydro alcoholic extracts ,bark of *Aspidosperma pyrifolium* Mart., *A. colubrina* and *L. rigida*, inoculated and not inoculated with *Zabrotes subfasciatus* for 180 days in storage with packaging pet.

Extracts	Doses (ml)			
	0	1	3	5
<i>A. pyrifolium</i> (leaves)	88.00 ^{aC}	90.00 ^{bcBC}	92.00 ^{aAB}	95.00 ^{aA}
<i>A. colubrina</i> (leaves)	88.00 ^{aA}	87.00 ^{cA}	90.00 ^{aA}	91.00 ^{bA}
<i>L. rigida</i> (leaves)	85.00 ^{abB}	91.00 ^{abcA}	84.00 ^{bB}	82.00 ^{cB}
<i>A. pyrifolium</i> (bark)	88.00 ^{abB}	94.00 ^{aA}	93.00 ^{aA}	94.00 ^{abA}
<i>A. colubrina</i> (bark)	87.00 ^{abB}	93.00 ^{abA}	93.00 ^{aA}	84.00 ^{cB}
<i>L. rigida</i> (bark)	88.00 ^{aA}	89.00 ^{bcA}	84.00 ^{bB}	81.00 ^{cB}

DMS in the columns = 4.12; DMS in the lines = 3.71; Classific.c/lower case; Classific.c/capital letters.

they had the best results with a mixture of neem oil with oil *Piper hispidinervum*. The result was a 100% reduction in the numbers of eggs and damaged grains, at 90 days.

Navickiene et al. (2007) after testing organic extracts from the seeds, leaves and stalks of *Piper tuberculatum*, ascertained that these extracts showed potential insecticidal activity, showing a process of rapid poisoning against *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) causing 80% mortality when doses greater than 800 insect⁻¹ cg were administered.

According to Torres and Marcos Filho (2001), the susceptibility of plants to insects extracted from plant allelochemicals, depends on the organ and plant species, form of extraction and insect species. According to Chagas et al. (2003), a range of different compounds can be isolated depending on the solvent used to obtain the extract.

In the analysis of germination percentage of stored bean seeds (Table 7) and their interaction with extract products, it appears in the inoculated procedure that the highest percentage of germination was given to the shell *A. pyrifolium* Mart. extract (92.00%) followed by *A. pyrifolium* Mart. leaf extract (91.00%), then the *L. rigida* bark (80.00%) and the lowest for the *L. rigida* leaf extract (78.00%).

In the non-inoculated procedure, all extracts showed the same efficiency in maintaining the viability of seeds, this is revealed by the average germination rate which was 91%. The efficiency showed by all extracts was with the exception of the *A. pyrifolium* Mart. extracts sheet and *A. pyrifolium* Mart. shell, which though showed the same germination in the non-inoculated procedure, the seeds remained higher than that of the inoculated procedure. This shows that the presence of *Z. subfasciatus* caused damage to the beans seeds.

According to Olanda et al. (2011), when the leaf extract of *Casearia sylvestris* Sw. was used to treat bean seeds in a concentration of 0.78%, the germination percentage of 12.5 was observed but from 25 to 50%, complete death of the seeds was observed. Thus, a high concentration of *Echinodorus macrophyllus* (Kunth) Micheli bark extract showed phytotoxicity on the bean

seeds. However, when applied in lower concentrations, the extract showed a beneficial effect on germination; thus, demonstrating the potential of *E. macrophyllus* (Kunth) Micheli in bean seed treatment.

The addition of millet extracts reduced the percentage of germination rate in bean seeds (Farias et al., 2009). Karunakaran et al. (2001) viewed fungal growth in wheat seeds stored with 19% moisture content at temperatures between 20 and 35°C, only after the germination of the seeds have reduced the percentage below 90%.

Matioli et al. (1978) found that in corn seeds infested by *Sitophilus oryzae*, there was increased insect population and reduced weight and seed germination. Garcia et al. (2000) found effects of ground black pepper extract at a dose of 4 g/1 kg of seeds, as the most efficient treatment in the control of *Z. subfasciatus* in bean seeds.

It can be seen that seed germination was not influenced by *A. pyrifolium* Mart leaf and *A. colubrina* bark extracts. The percentages of germination of bean seeds were statistically similar at doses of 1, 3 and 5 ml. There was statistical similarity in the germination of bean seeds with doses 1, 3 and 5 ml. In general, germination was more affected by *L. rigida* leaf and bark extracts, which was statistically similar at doses of 3 and 5 ml, but within each dose (column) the 3 ml dose proved to be the overall best in the treatment of the seed in order to obtain seed quality. Whereas, the following *A. pyrifolium* Mart. with *A. colubrina* leaf and *A. pyrifolium* Mart. with *A. colubrina* bark resulted in a higher percentage of seed germination (92.00%) (Table 8).

It is noted that in the procedures, equal statistic results were observed, except for the dose of 1 ml in the inoculated procedure which has the highest percentage of germination. It is also observed that there is difference in 3 and 5 ml doses with higher germination percentage for the non-inoculated procedure (Table 9).

In summary, it can be stated that germination was not affected in seeds treated with doses of these extracts. It is also observed through graphic representation that there is high coefficient and quality. This validates the use of equations in the conditions under which the study did; when you want to estimate intermediate points of the

Table 8. Average values (%) of germination of the interaction procedure with dose in bean seeds treated with hydroalcoholic extracts of leaves and bark of *Aspidosperma pyrifolium* Mart., *A. colubrina* and *L. rigida*, inoculated and not inoculated with *Zabrotes subfasciatus* for 180 days of storage packaging pet.

Procedure	Doses (ml)			
	0	1	3	5
Inoculated	85.00 ^{bB}	90.00 ^{aA}	86.00 ^{bB}	83.00 ^{cB}
Non inoculated	89.00 ^{bA}	92.00 ^{aA}	93.00 ^{aA}	92.00 ^{aA}

DMS in the columns = 2.14; DMS in the lines= 1.63; Classific.c/lower case; Classific.c/capital letters.

Table 9. Average values of germination percentage (%) for interaction of extract with bean seeds treated with hydroalcoholic extracts of leaves and bark *Aspidosperma pyrifolium* Mart., *Anadenanthera colubrina*, and *L. rigida* leaves inoculated and not inoculated with *Zabrotes subfasciatus* in pet type storage packaging for 180 days.

Extracts	Periods (days)			
	45	90	135	180
<i>Aspidosperma pyrifolium</i> Mart. leaves	98.00 ^{aA}	92.00 ^{aB}	92.00 ^{aB}	83.00 ^{bC}
<i>Anadenanthera colubrina</i> leaves	97.00 ^{aA}	94.00 ^{aA}	86.00 ^{bcB}	79.00 ^{cC}
<i>L. rigida</i> leaves	95.00 ^{aA}	93.00 ^{aAB}	90.00 ^{aB}	64.00 ^{dC}
<i>Aspidosperma pyrifolium</i> Mart. barks	98.00 ^{aA}	93.00 ^{aB}	89.00 ^{abC}	88.00 ^{aC}
<i>Anadenanthera colubrina</i> barks	97.00 ^{aA}	92.00 ^{aB}	90.00 ^{abB}	80.00 ^{bcC}
<i>L. rigida</i> barks	98.00 ^{aA}	94.00 ^{aB}	85.00 ^{cC}	65.00 ^{dD}
Inoculated	96.00 ^{aA}	93.00 ^{aB}	87.00 ^{bC}	67.00 ^{bD}
No Inoculated	98.00 ^{aA}	92.00 ^{aB}	90.00 ^{aC}	86.00 ^{aD}

DMS in the columns = 4.12; DMS in the lines= 3.71; Classific.c/lower case; Classific.c/capital letters.

Table 10. Percentage of germination (%) for the interaction procedure in bean seeds treated with hydroalcoholic extracts of leaves and bark of *Aspidosperma pyrifolium* Mart., *Anadenanthera colubrina* and *L. rigida* inoculated and not inoculated with *Zabrotes subfasciatus* in storage packaging type pet for 180 days.

Procedures	Periods (days)			
	45	90	135	180
Inoculated	96.00 ^{aA}	93.00 ^{aB}	87.00 ^{bC}	67.00 ^{bD}
No Inoculated	98.00 ^{aA}	92.00 ^{aB}	90.00 ^{aC}	86.00 ^{aD}

DMS in the columns = 1.63; DMS in the lines = 6.51; Classific.c/lower case; Classific.c/capital letters.

experiment.

Brito (2010) found that *Mimosa tenuiflora* (Wild) extract negatively influenced all the variables studied mainly in corn germination, supporting the thesis that corn can be used as a model in bioassays in tests on allelopathy (Macias et al., 2000).

Silva Junior (2011) reported the interaction of inoculated and uninoculated procedures in the treatment of corn seeds within a period of 180 days, showing a different result on germination. There was a record of 72% germination at the end of the storage period for inoculated seeds. This shows the efficiency of the extracts.

It appears that time was decisive in the germination results of stored bean seeds; those that were observed between 45 and 90 days showed similar results for the

extracts. That is, significant effects of extracts on seed germination were only observed after 90 days of storage (column). However, after 180 days of storage, the *A. colubrina* leaf extract which showed 64.00% result and myrtle bark which showed 65.00% showed the worst germination result. This is followed by *A. colubrina* leaf extract (79.00%) (Table 10).

L. rigida bark extracts (85.00%) and *A. colubrina* leaf (86.00%) were the least efficient, with the former being statistically, in the maintenance of seed germination after 135 days of storage. However, it was observed that the tough *A. pyrifolium* Mart. extract (88.00%) kept seed germination to the end of the storage period. It was also observed that the *A. pyrifolium* Mart. leaf extract (92.00%) and *L. rigida* leaf (90.00%) showed similar results at 135 days of storage.

Table 11. Percentage (%) of germination for dose interaction in bean seeds treated with hydroalcoholic extracts of leaves and bark of *Aspidosperma pyriforme* Mart., *Anadenanthera colubrina* and *L. rigida*; inoculated and not inoculated with *Zabrotes subfasciatus* in pet packaging storage for 180 days.

Doses (ml)	Periods (days)			
	45	90	135	180
0	94.00 ^{ba}	91.00 ^{bb}	87.00 ^{bc}	77.00 ^{bd}
1	98.00 ^{aA}	94.00 ^{aB}	86.00 ^{cC}	85.00 ^{aC}
3	98.00 ^{aA}	95.00 ^{aB}	90.00 ^{abC}	76.00 ^{bd}
5	99.00 ^{aA}	92.00 ^{abB}	92.00 ^{aB}	69.00 ^{cC}

DMS in the columns = 3.03; DMS in the lines = 3.03; CV% = 6.51; Classific.c/lower case; Classific.c/capital letters.

This result is due to the presence of natural substances with potential insecticide, present in the hydro alcoholic extracts used. This potential is revealed in the phytochemical study, where steroids and tannins showed bioactive effects on *L. rigida* and *A. colubrina* extracts.

Medeiros et al. (2007) evaluated dry and green leaves of neem on the quality of cowpea seeds and they concluded that the extracts affected seed germination; the treatments which received neem seed powder differed significantly from the control. Silva (2007) observed reduced germination in corn seeds treated with extracts of *M. tenuiflora* (Wild Poiret). Haven given effect to the woody part, it was observed that germination of seeds of other grasses was not affected when treated with leaf extract of *M. tenuiflora* (Wild) Poiret).

Silva and Aquila (2006) verified the effect of *Erythroxylum argentinum* extracts, *Luehea divaricata*, *Mysine guianensis* and *Ocotea peberula* on the initial germination of lettuce. They observed that the treatment showed significant difference from the control group despite that those species presented allelopathic potential. Lima et al. (2007) observed that aqueous extracts of the aerial part of *Crotalaria juncea*, *Canavalia ensiformis* (L.) DC. and *Sesamum indicum* reduced the final germination of *Bidens pilosa* in a concentration of 20%.

It was also observed that there was reduction in seed germination with advance deterioration during storage time for both the inoculated and non-inoculated, statistically, equal to the procedures within 45 times and 90 days.

But in the final two times (135 and 180 days), the inoculated procedure (87.00 and 67.00%) was lower than the uninoculated procedure (90.00 and 86.00%), respectively (Table 11).

Almeida et al. (2008) found that *Croton sonderianus* of aqueous extracts also promoted a reduction in the percentage of germination of *Cassia tora*.

Germination decreased within storage period of 45 and 90 days (column), there were no significant statistical differences, but seed germination showed different behavior at 135 and 180 days. In addition, at 180 days,

there was lower seed germination for the dose of 5 ml 69.00% (Table 12).

This result shows that the doses tested for this test are effective in controlling *Z. subfasciatus* and did not inhibit the germination of seed bean.

Promising results in the control of *Z. subfasciatus* were verified by Queiroga (2010) with vegetable oils in the treatment of stored beans. The insects were controlled and after 150 days of storage, there was an observation of 57.00% seed germination.

Almeida (2003) studied the effects of plant extracts in the control of *Callosobruchus maculatus* and its effects on the bean *Vigna unguiculata*. From the research, it was concluded that the vigor and germination of seeds treated with *Conospermum caeruleum* extracts and *Piper nigrum* were positive, while this decreased over storage time. While the best extract in preserving the seed was *P. nigrum*.

Conclusions

Plant extracts showed insecticidal activity against *Z. subfasciatus*, killing them and/or inhibiting their development. In the treatment of bean seeds, the dose of 3 ml was the best to control the incidence of *Z. subfasciatus*, with better results from the use of *L. rigida* leaf extracts and *A. pyriforme* Mart. bark. The procedures adopted in seed treatment with the extracts of plant species were efficient in maintaining the viability and did not affect germination during 180 days of storage. Germination was affected by insufficient storage time (less than 180 days), and the doses 1 and 3 ml were the most efficient for the maintenance of seed germination.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Bioherbicide based on *Diaporthe* sp. secondary metabolites in the control of three tough weeds

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Brazilian growers are facing difficulties to manage the weeds *Lolium multiflorum*, *Conyza* sp. and *Echinochloa* sp. using commercially available agrochemicals in most crops. This study aims to evaluate the use of a bioherbicide based on fermented broth containing secondary metabolites of *Diaporthe* sp. to control *L. multiflorum*, *Conyza* sp. and *Echinochloa* sp. The bioherbicide activity in pre-emergence and post-emergence of these weed species and phytotoxicity on soybean, wheat and rice plants were evaluated. In the pre-emergence test using all weed species, the bioherbicide showed 100% control efficiency in comparison with the control group. Phytotoxic symptoms were observed on soybean leaves and horseweed was efficiently controlled using the bioherbicide.

Key words: Bioherbicide, weeds, bioproducts.

INTRODUCTION

Several factors can restrict the production yield in agricultural species and among them, weeds are a tough competitor, which usually can be controlled using different methods, such as mechanical, physical, biological and chemical (Silva and Silva, 2012). Combining different methods that reduce weed development and its effects on agricultural species is seen as a promising strategy (Nunes et al., 2010). However, weed management in recent decades has been accomplished mostly through chemical control (Oliveira et al., 2011), leading to the

selection of resistant biotypes (Christoffoleti et al., 1997).

The high number of weed species resistant to herbicides calls for new modes of action and herbicide classes. However, an herbicide with a new mode of action has not been launched on the market in the last 20 years (Dayan and Duke, 2014). In this context, biological molecules are considered important sources to be explored for the development of new products for weed management (Duke et al., 2002). According to Duke et al. (2000) there are many toxins of natural origin still

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unexplored and which have herbicide action with different modes of action from those currently known. Fungi are one option and often are found in nature colonizing and causing severe damage in some species of weeds (Barreto and Evans, 1998). This has led to an increased number of studies using these microorganisms and their metabolites as weed control agents, as well as the discovery of new sites of action and bioactive molecules (Dayan and Duke, 2014). Several studies have shown herbicidal activity of metabolites produced by microorganisms on weeds. Varejão and Demuner (2013) identified compounds produced by *Alternaria euphorbiicola* showing herbicidal activity on *Euphorbia heterophylla*. Khattak et al. (2014) found metabolites of *Aspergillus* spp. and *Penicillium* spp. negatively interfering in the growth of *Lemna minor* and in the seed germination of *Silybum marianum*.

In a previous study, our research group collected thirty-nine phytopathogenic fungi from infected tissues of weeds and isolated them in the laboratory looking for the production of metabolites with herbicidal action. Among them, a strain belonging to the *Diaporthe* sp. genus was screened due to herbicidal effects of its secondary metabolites on target plants (Souza et al., 2016a). In a second study, the production of the bioherbicide in a bench-scale bioreactor with working volume of 3 L was assessed (Souza et al., 2015b). The bioherbicide efficiency rate reached 100% in pre-emergence, since there was no seed germination after its application. *Diaporthe* has been reported often as producing secondary metabolites for biological control of weeds (Ash et al., 2010; Andolfi et al., 2015). Phomentrioloxin B caused small necrotic spots on a number of plant species, whereas gulpyrone A caused leaf necrosis in *Helianthus annuus* plantlets (Andolfi et al., 2015). Cimmino et al. (2013) tested several compounds produced in liquid culture by *Phomopsis* sp. (teleomorph: *Diaporthe gulyae*) for the control of the annual weed *Carthamus lanatus*.

The main objective this study was to evaluate the application of fermented broth containing secondary metabolites of *Diaporthe* sp. to control *Lolium multiflorum*, *Conyza* sp. and *Echinochloa* sp. The herbicidal activity was evaluated in post-emergence and pre-emergence of these weed species and also on *Glycine max*, *Triticum aestivum* and *Oryza sativa*.

EXPERIMENTAL SECTION

Weeds and cultures used in the bioassays

Two bioassays were carried out to evaluate the herbicidal effect of secondary metabolites of *Diaporthe* sp. obtained by submerged fermentation in the pre-emergence of soybean (*Glycine max*), wheat (*Triticum aestivum*), rice (*O. sativa*), rye grass (*L. multiflorum*) and sorghum (*Sorghum halepense*) and in the post-emergence of soybean (*Glycine max*), wheat (*T. aestivum*), rice (*Oryza sativa*), rye grass (*L. multiflorum*), horseweed (*Conyza* sp.)

and rice grass (*Echinochloa* sp.).

Location of bioassays

Pre-emergence assays were carried out in an incubator with controlled humidity (85%) and temperature (28 °C), while the post-emergence evaluation was carried out in a greenhouse, located at the coordinates 29° 43'39.79"S and 53°33'40.06"W, Santa Maria – Brazil.

Production of metabolites by submerged fermentation

The strain of *Diaporthe* sp. was isolated from plants of the weed *Solanum americanum* Mill in the Pampa biome and was used in this study for bioherbicide production by submerged fermentation. The strain of *Diaporthe* sp. was kept in a potato dextrose agar (PDA) at 4°C and subcultured every 15 days. Cell production for pre-inoculum was incubated in a Petri dish containing PDA for 8 days at 28°C (Souza et al., 2016a). Afterwards, a 6 mm disc of fungal mycelium was transferred to a 250 mL Erlenmeyer flask, which contained 100 mL of fermentation medium at 28°C at 120 rpm for 7 days (Inova 44R, NewBrunswick) for inoculum. The fermentations were carried out in a batch bioreactor (BIOTEC-C, Tecnal, Brazil), containing 3.0 L of the optimized culture medium, using a 10% (v/v) inoculum at an initial pH of 6.0, incubated at 28°C for 7 days at 40 rpm. The culture medium was composed of corn steep liquor 33% (v/v), sucrose 18 g.L⁻¹, (NH₄)₂SO₄ 2 g.L⁻¹, MgSO₄.7H₂O 0.5 g.L⁻¹, FeSO₄.7H₂O 1 g.L⁻¹ and MnSO₄.H₂O 1 g.L⁻¹. After fermentation, the cell mass was separated from the fermentation broth by centrifugation at 4000 rpm for 10 minutes and the supernatant used in the bioassays.

Herbicidal activity in pre-emergence

The activity of the bioherbicide obtained from *Diaporthe* sp. was evaluated in the pre-emergence of dicotyledon seeds (*G. max*) and monocotyledon seeds (*T. aestivum*, *O. sativa*, *L. multiflorum* and *S. halepense*). For this purpose, 10 mL of fermented broth without the cells were sprayed on a germitest paper containing 100 seeds of each culture and maintained at 28°C (POL-EKO, model KK) 350. A control test was carried out by replacing the fermented broth with distilled water. Seed germination was evaluated during 7 days, according to the Brazilian rules for seed analysis (Brasil, 2009). Afterwards, the germinated and non-germinated seeds were counted. All seeds that presented primary root protrusion higher than 2 mm were considered to be germinated.

Herbicidal activity in post-emergence

For the evaluation of herbicidal activity in post-emergence, seven different concentrations of fermented broth were sprayed on the upper part of *G. max*, *T. aestivum*, *O. sativa*, *L. multiflorum*, *Conyza* sp. and *Echinochloa* sp. plants. The concentrations were 0D (water), 1/4D, 1/2D, 1D, 2D, 4D and 8D, where D corresponds to undiluted fermented broth. A mineral oil 0.5% (v/v) was added to all treatments.

The experimental design was completely randomized with five replicates. The units were 500 ml polyethylene pots, filled with commercial substrate Macplant®. Four seeds of each species evaluated were manually sown in each pot. After plant emergence only one plant was maintained per pot. For the fermented broth sprays, a backpack sprayer was used, which was pressurized by CO₂, provided with a bar pattern with four tips, model Teejet XR

110.02, with a pressure of 40 lbf and spacing between tips of 0.5 m. The walking speed during spray was 1 m s⁻¹ and a volume of 200 L ha⁻¹ was used. At spray time, the plants had between 2-3 leaves and the temperature and relative air humidity were 31.2°C and 62%, respectively.

In each treatment, shoot dry matter, weed control and injury according to Frans et al. (1986) were evaluated. The injury for the species *G. max*, *T. aestivum* and *O. sativa* was determined at 7 and 15 days after the fermented broth spray (DAA). The control of *L. multiflorum*, *Conyza* sp. and *Echinochloa* sp. was evaluated at 15 DAA. After the determination of control and injury at 15 DAA, the aerial part of plants was sectioned, packed in paper bags and dried at 70°C during 72 h to obtain shoot dry matter.

Statistical analysis

All data were submitted to analysis of variance and Tukey's test was used to verify significant differences between sprays ($p < 0.05$) using the software Assistat[®] 7.7 beta.

RESULTS

Herbicidal activity in pre-emergence

Table 1 presents the herbicidal activity in pre-emergence of fermented broth. Seeds of *G. max*, *T. aestivum*, *O. sativa*, *L. multiflorum* and *S. halepense* did not present root protrusion. The germination inhibition reached 100% for all species, whereas in the control test, germination varied by species, but reached values higher than 60% (Figures 1 and 2).

Herbicidal activity in post-emergence

The effect of fermented broth on shoot dry matter mainly for species of *Conyza* sp. and *Echinochloa* sp was exhibited in Figure 3. The best results for *Conyza* sp. were obtained when 4D and 8D were applied, however, plant death did not occur.

The effect of the bioherbicide on injury of crop species shown in Table 2. Only *G. max* presented injury symptoms, which was verified at 7 and 14 days after application (DAA) for concentrations higher than 2D and most severely for 8D. Plants showed symptoms, such as yellowing and blight on youngest leaves, whereas the oldest leaves showed partial necrosis at the tips, as presented in Figure 4.

DISCUSSION

Herbicidal activity in pre-emergence

These results confirm that metabolites of *Diaporthe* sp. have herbicidal activity in pre-emergence of some weed species, providing an important alternative for the management of weeds resistant to chemical herbicides. The pre-emergent control has the advantage of reducing competition from weeds with the crop especially in the

Table 1. Pre-emergence activity of secondary metabolites of *Diaporthe* sp.

Species	Germinated seeds (%)	
	Fermented broth	Control
<i>Lolium multiflorum</i>	0	68
<i>Triticum aestivum</i>	0	78
<i>Oryza sativa</i>	0	95
<i>Glycine max</i>	0	87
<i>Sorghum halepense</i>	0	91

initial phase of its establishment as a result of lower germination of weeds, besides enabling a reduction in the seed bank.

The metabolites of *Diaporthe* sp. showed a broad spectrum of action in pre-emergence, since activity was verified in both dicotyledonous (*G. max*) and monocotyledonous seeds (*T. aestivum*, *O. sativa*, *L. multiflorum* and *S. halepense*). Souza et al. (2015b) obtained an efficiency of 100% in the pre-emergence of *Cumumis sativus* (dicotyledonous) and *Sorghum* sp. (monocotyledonous) seeds when fermented broth of *Diaporthe* sp. was used. The control of different plant families, as well as different species, is considered beneficial from an agricultural viewpoint, since simultaneous growth of different species of weeds is common in crops and an herbicide should have the broadest action possible.

The inhibition of germination by the action of secondary metabolites of *Diaporthe* sp. may be related to molecules from the terpenoid groups which are produced by a wide range of fungi. Castro et al. (2001) reported that terpenoids have hormonal functions, inhibiting the growth and acting in active-transport through the membrane. However, the confirmation of this hypothesis should be based on more in-depth studies.

Herbicidal activity in post-emergence

In practice, the increased concentrations increased the amount of molecules with herbicide activity, which are very diluted in the fermentation medium (Varejão and Demuner, 2013). As a consequence, a suppressive effect was verified instead of control, as shown in Figure 5 for *Conyza* sp. For this weed, 4D and 8D resulted in about 50% reduction in weight of shoot dry matter, when compared with the control treatment. Even though significantly less, it can be seen that the fermented broth caused a reduction in the weight of shoot dry matter for *Echinochloa* sp. at all doses used. The 8D concentration of fermented broth also resulted in a significant reduction of weight of shoot dry matter for the *G. max* species. This confirms that phytotoxins are highly diluted in the fermentation broth, so that an effect only occurs when it is used at higher concentrations.

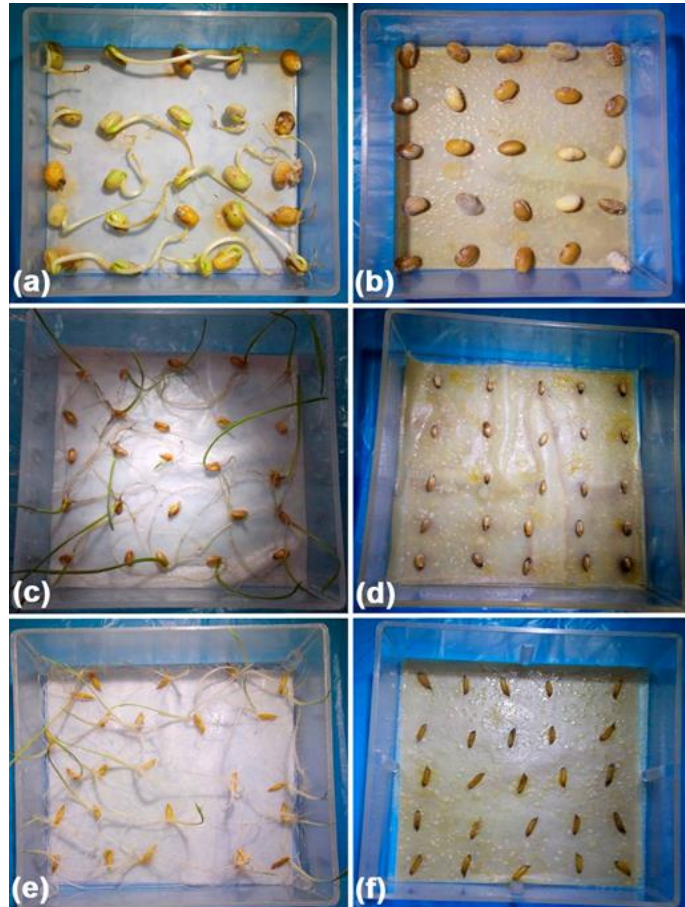


Figure 1. Germination of *Glycine max*, *Triticum aestivum*, *Oryza sativa* in the control test (a, c and e) and using bioherbicide fermented broth (b, d and f), respectively.

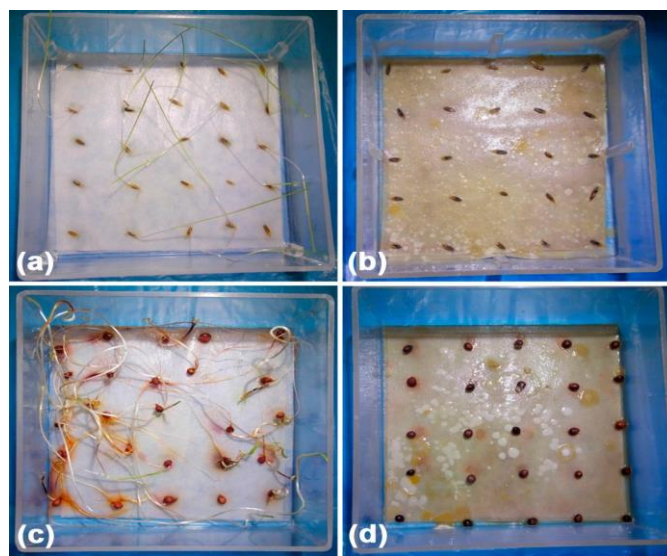


Figure 2. Germination of *Lolium multiflorum* and *Sorghum halepense* in the control test (a and c) and using bioherbicide fermented broth (b and d), respectively.

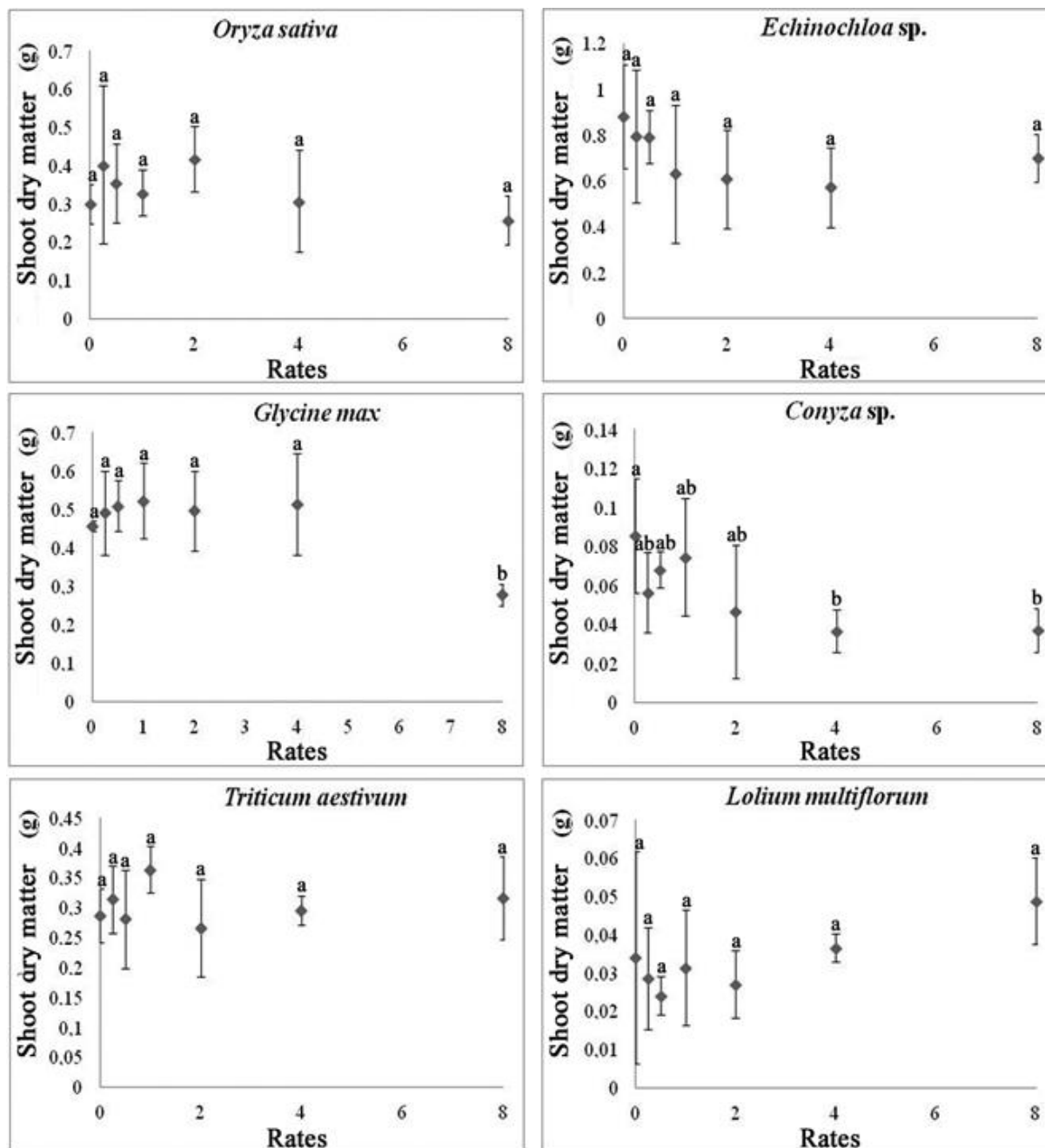


Figure 3. Influence of bioherbicide on shoot dry matter.

In addition, the plants presented significant shoot dry matter reduction as a result of injury caused by the application of fermentation broth. The observed symptoms suggest that phytotoxins present systemic action, as both *G. max* and *Conyza sp.* plants presented a retardation of plant growth. One hypothesis related to the retarded development of plants and consequent reduction of shoot dry matter may be related to interference of the phytotoxin in the biosynthesis of amino acids that are essential in plant growth, such as valine, leucine and isoleucine.

Conclusions

In this study, the application of fermented broth of *Diaporthe sp.* as a bio-herbicide in pre- and post-emergence of different crop species (*O. sativa*, *G. max* and *T. aestivum*) and weeds (*Echinochloa sp.*, *Conyza sp.* and *L. multiflorum*) was investigated. In pre-emergence, the fermented broth showed an efficiency of 100%, since no seeds of any species germinated. In post-emergence, the most promising results were obtained for *Conyza sp.* and *Echinochloa sp.* species,

Table 2. Phytotoxic effect of bioherbicide on crop species.

Treatment	Phytotoxicity (%)	
	7 DAA	15 DAA
<i>Glycine max</i>		
0	0	0
¼ D	0	0
½ D	0	0
1 D	0	0
2 D	10	10
4 D	40	20
8 D	80	70
<i>Triticum aestivum</i>		
0	0	0
¼ D	0	0
½ D	0	0
1 D	0	0
2 D	0	0
4 D	0	0
8 D	0	0
<i>Oryza sativa</i>		
0	0	0
¼ D	0	0
½ D	0	0
1 D	0	0
2 D	0	0
4 D	0	0
8 D	0	0

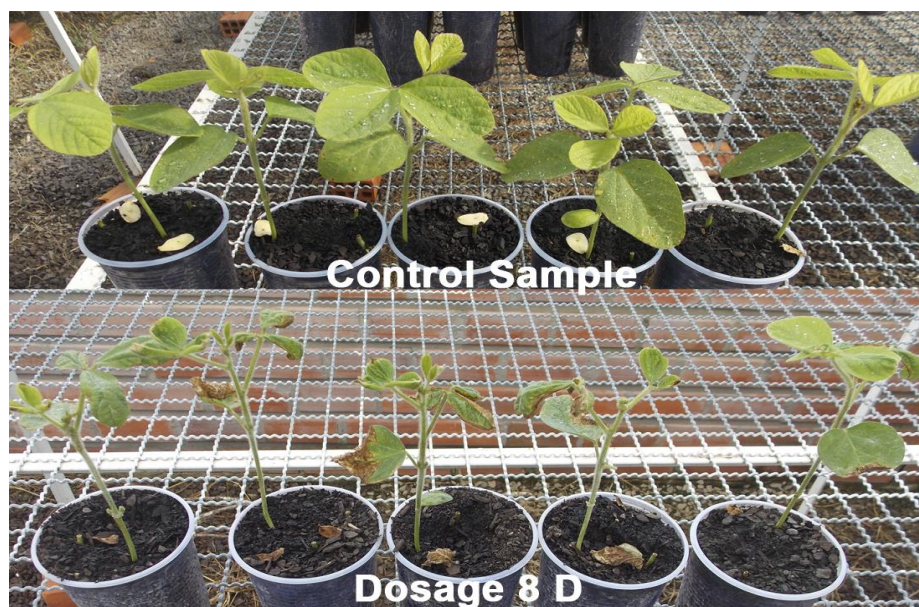
**Figure 4.** At the top of the figure, plants of *Glycine max* exempted from metabolites *Diaporthe* sp., at the bottom plants under the highest concentration of metabolites.



Figure 5. Influence of bioherbicide in the control of *Conyza* sp.

because plant growth was suppressed. *G. max* showed injury to metabolites when concentrations were higher than 2D at 7 and 15 DAA. The biomolecules produced by *Diaporthe* sp. are promising for as a natural herbicide.

Conflicts of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Growth and production of a Japanese cucumber crop under pulse irrigation

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Water is the most important production factor in agriculture, since even small restrictions to the water supply can result in decreased productivity. Due to climatic reasons, water supply is limited in many parts of the world, resulting in a need to develop techniques that increase the water use efficiency. Pulse irrigation consists of the application of an irrigation depth, relative to the actual irrigation needed, split throughout the day. The objective of this study was to verify the effects of pulse irrigation on cucumber plants that were either subjected to a water deficit or were sufficiently supplied with water, considering the hypothesis that the application of water during times of greater evapotranspiration demand will promote benefits to the crop in comparison with the continuous irrigation in the early hours of the day. A completely randomised design was used and the treatments were distributed in 3×4 and 4×4 factorial scheme in the first and second cycles respectively. The first factor was the replenishment of the irrigation depth relative to the crop evapotranspiration, while the second factor was the number of pulses; there were a total of 48 and 64 plots in the first and second cycles, respectively. The application of the treatments was started in the vegetative phase and in the reproductive phase for the first and second cycles respectively. It was concluded that smaller irrigation depths than the crop requires can be applied by pulses without resulting in a reduction in the vegetative growth in Japanese cucumber.

Key words: Drip irrigation, water savings, reduced irrigation depth, *Cucumis sativus*, protected environment.

INTRODUCTION

One of the challenges when irrigating is to reconcile increased productivity with the use of diminishing amounts of water. The interest in producing more with less water is warranted as water is a limiting factor for production in various parts of the world (Ali and Talukder, 2008). For

some regions of Brazil, water is considered an economic asset and its use is associated with the collection of taxes through river basin committees (CBH) (Kelman and Ramos, 2005). It is important to perform irrigation management to achieve the greatest productivity per unit

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Table 1. Physical and chemical variables of the soil in the experimental area.

Variable	Value
Soil bulk density (Mg m^{-3})	1.01
Coarse sand content (g kg^{-1})	50.0
Fine sand content (g kg^{-1})	72.60
Silt content (g kg^{-1})	120.60
Clay content (g kg^{-1})	756.80
CaCl_2 Ph	6.00
Organic matter content (g dm^{-3})	8.03
Phosphorous content (mg dm^{-3})	32.41
Potassium content ($\text{cmol}_c \text{ dm}^{-3}$)	0.31
Calcium content ($\text{cmol}_c \text{ dm}^{-3}$)	6.16
Magnesium content ($\text{cmol}_c \text{ dm}^{-3}$)	1.68
Aluminium content ($\text{cmol}_c \text{ dm}^{-3}$)	0
Cation exchange capacity ($\text{cmol}_c \text{ dm}^{-3}$)	11.00
Base saturation (%)	74

of water applied, resulting in reduced costs and environmental impact. The application of water should be judicious, based on measurements of climatic and soil phenomenon, in accordance with the method of irrigation utilised.

The water use efficiency (WUE) can be understood as the relationship between the productivity and the applied irrigation depth (Oliveira et al., 2011). The increase in WUE is interesting if it is associated with acceptable levels of productivity. In other words, the WUE needs to be optimised without incurring decreased productivity. Various irrigation techniques have been investigated in order to increase the WUE, the partial irrigation of the root system and deficit irrigation have both shown promise in this objective, even though these techniques have resulted in lower productivity (Ali and Talukder, 2008).

The relationship between cucumber crop yield and the total irrigation depth follows a quadratic model and it presents a maximum productivity (Oliveira et al., 2011). It is suggested that the excessive vegetative growth arising from the supply of excessive water can result in lower root activity, an increase in disease incidence due to the growth of the canopy and a lower harvest index, calculated by the ratio between the production and total biomass. Thus, the increase in biomass production comes at the cost of increased irrigation depths that can result in a reduction in the water use efficiency (Ali and Talukder, 2008).

Irrigation through pulses enables the reduction of the irrigation depth without reducing the crop yield. In lettuce the replenishment of 75% of the crop evapotranspiration, divided into six pulses with a 50 min interval, resulted in greater water use efficiency and a non-significant difference in yield in relation to a continual replenishment of 100% of the ET_c (Almeida et al., 2015). The pulsed

irrigation also results in a reduction in the irrigation depth required in potato and in the occurrence of emitter blocking (Abdelraouf et al., 2012).

The cucumber (*Cucumis sativus*) has great economic and social importance among the vegetables grown by Brazilian agribusiness. The Brazilian annual cucumber production exceeds 200.000 t. Furthermore, cucumber has nutraceutical properties and can be used in cosmetics and medicines (Carvalho et al., 2013).

Greenhouse farming has advantages comparing to field farming. Growing in a protected environment allows controlling adverse conditions of climate and incidence of diseases and pests (Streck et al., 2003). However, greenhouse crop growth hinders access of pollinators to the crop. Japanese cucumber is a partenocarpic crop and it is well adapted to greenhouse conditions because it does not require pollination (Carvalho et al., 2013).

The hypothesis of this work was that the splitting of the application of irrigation depth throughout the day would result in an increase in cucumber growth, yield and WUE, since the water application would be conducted for a longer period during the day and, consequently, during the moments of highest evapotranspiration demand.

The objective of this work was to verify the effects of pulsed irrigation, irrigation depth and its interactions on Japanese cucumber growth and production.

MATERIALS AND METHODS

The experiment was conducted in a protected environment at the Irrigation Technical Center (CTI) of the Department of Agronomy of the State University of Maringá (UEM), located in Maringá, PR, between the months of 07/2014 and 01/2015. The soil analysis (Table 1) was performed at the Laboratório Rural de Maringá.

The partial soil water retention for the layer 0 to 0.3 m (Equation 1) was determined according to Almeida et al. (2010) and Tavares et al.

(2008). The water potential measurements were made by nine tensiometers installed at a depth of 0.15 m, while soil moisture estimates were made using nine Time Domain Reflectometer (TDR) probes installed at the same depth with an inclination of 45°. Moistening of the soil was accomplished through continuous irrigation for three days and readings were taken six hours after cessation of the irrigation. Taking into account Equation 1, it was calculated that to raise the water potential from -30 to -10 kPa it was necessary to apply a 24 mm irrigation depth to the 0 to 0.3 m layer. It justified disregarding losses through deep percolation in the water balance equation, since the greatest irrigation depth applied on the same day was equal to 20.1 mm.

$$\theta = 0.2191 + \frac{0.4334 + 0.2191}{\left[1 + (0.1738|\Psi|)^{0.6911}\right]^{1.9320}} \quad (1)$$

Which are θ = soil moisture ($\text{m}^3 \text{m}^{-3}$), and Ψ = water potential (kPa). The saturation pressure (e_s) was calculated every 30 min from 06:00 until 21:00. The calculation of e_s (Equation 2) was based on the recommendation by Allen et al. (1998).

$$e_s = 0.618 \cdot \exp^{\frac{17.27T_{\text{med}}}{237.3 + T_{\text{med}}}} \quad (2)$$

Which are T_{med} - mean of the daily mean temperatures of each time from all the days of irrigation ($^{\circ}\text{C}$). The actual vapour pressure (e_a) for each time was calculated through Equation 3.

$$e_a = \frac{\text{RH}_{\text{min}} \cdot (0.618 \cdot \exp^{\frac{17.27T_{\text{max}}}{237.3 + T_{\text{max}}}}) + \text{RH}_{\text{max}} \cdot (0.618 \cdot \exp^{\frac{17.27T_{\text{min}}}{237.3 + T_{\text{min}}}})}{2} \quad (3)$$

Which are RH_{min} - mean of the minimum relative humidity values expressed in decimals; RH_{max} - mean of the maximum relative humidity values expressed in decimals; T_{max} - mean of the highest temperatures ($^{\circ}\text{C}$); T_{min} - mean of the lowest temperatures ($^{\circ}\text{C}$).

The preparation of Japanese cucumber seedlings, cv. Hokushin, was performed in trays with 162 cells. A row of six seedlings spaced 0.5 m apart were planted in each plot, which resulted in the spacing 1.0 x 0.5 m. The planting was conducted when the seedlings had their first true leaf completely expanded. The nitrogen and potassium fertilisations were conducted every two weeks according to Ribeiro et al. (1999). The nutrient sources utilised were urea and potassium chloride.

In each plot a polyethylene irrigation pipe 3 m in length and 16 mm in diameter with self-compensating drippers *on line*, with a flow rate of 8 L h⁻¹ spaced 0.2 m apart. In addition, a regulator was installed at the start of the irrigation pipe, which allowed the irrigation of each plot individually. The flow of each emitter in the experimental area was measured to obtain the Emission Uniformity (Keller and Karmeli, 1975), whose value was equal to 94%. A schematic map of the experimental area is represented in Figure 1.

The calculation of the reference evapotranspiration (ET_o) was performed through the Penman-Monteith-FAO equation on a daily basis (Allen et al., 1998): The meteorological parameters from within the protected environment were monitored through a Campbell automatic station, adjusted to collect data every 2 s and provide the average of each variable at 30 min intervals. The data were recorded using a datalogger CR1000. The crop coefficient (K_c) was estimated using the equation adjusted by Blanco and Folegatti (2003).

Irrigation management of the area was conducted using the

water balance method. The wall, 0.5 m in height, that demarcated the protected environment, the presence of a mesh with a pore size of 40 mesh and the growing conditions with raised beds permitted the surface runoff, the water flow in the soil and the precipitation to be disregarded. For this reason, Equation 4 was used for the water balance and can be expressed as:

$$\Delta A = IETc \quad (4)$$

Which are ΔA = variation in the water storage of the 0 to 0.3 m layer (mm per day); I = irrigation depth (mm per day); ET_c = crop evapotranspiration (mm per day).

Before the application of the treatments, the soil in the experimental area was irrigated until the humidity was near field capacity in the layer 0 to 0.30 m deep. From this point on, irrigation was conducted three times per week; these were designed to replenish the deficit derived from evapotranspiration according to the treatments. The irrigation water utilised was sourced from a semi artisanal well and exhibited an electrical conductivity between 0.2 and 0.4 dS m⁻¹.

A completely randomised design was used and the treatments were distributed in a factorial design (Table 2). Each treatment had four replications, totalling 48 and 64 in the first and second cycle respectively. Each plot consisted of six plants, and the response variables were measured for the four central plants.

In the first cycle, the application of the treatments started 25 days after planting (DAP), when at least 50% of the plants had two completely expanded leaves. In the second cycle, the treatments were applied 35 DAP, when the plants had 1.80 m, at least 50% of the plants had five completely opened leaves and one flower on the main branch.

The irrigation time of the day was the same to all treatments in each cycle and it was calculated considering the replenishment of water storage deficit in the plot was provided by the drippers, according to the equation:

$$T_i = \frac{DA}{qn} \quad (5)$$

Which are T_i = irrigation time of the day (min); D = water storage deficit in the 0 to 0.3 m layer (mm); A = surface area of the plot (m^2); q = flow rate of the dripper (L min⁻¹); n = number of drippers per plot.

The pulse irrigation time was calculated considering the IDRE and number of pulses according the treatment. Therefore, treatments with higher number of pulses had smaller pulse irrigation time. Similarly, treatments with higher IDRE had higher pulse irrigation time, according to the equation:

$$T_p = T_i \frac{\text{IDRE}}{P} \quad (6)$$

Which are T_p = pulse irrigation time (min); IDRE = irrigation depth replenishment relative to the crop evapotranspiration expressed in decimals; P = number of pulses;

In the first cycle the plant height and number of nodes 61 days after planting (DAP), the diameter of the stem (mm) 100 DAP and the fruit mass (g) produced up to 100 DAP were measured. In the second cycle, the total fruit mass (g) produced up until 83 DAP and root mass (g) after the end of the cycle was measured. The water use efficiency (g mm^{-1}) was calculated as the ratio between the mass of harvested fruits in the plot (g) and the total irrigation depth (mm) in the two cycles.

The root samples were obtained using a sampler 0.20 x 0.25 x 0.30 m. The soil volume collected was washed in a 2 mm sieve to separate the roots. The root samples of each plant were placed in

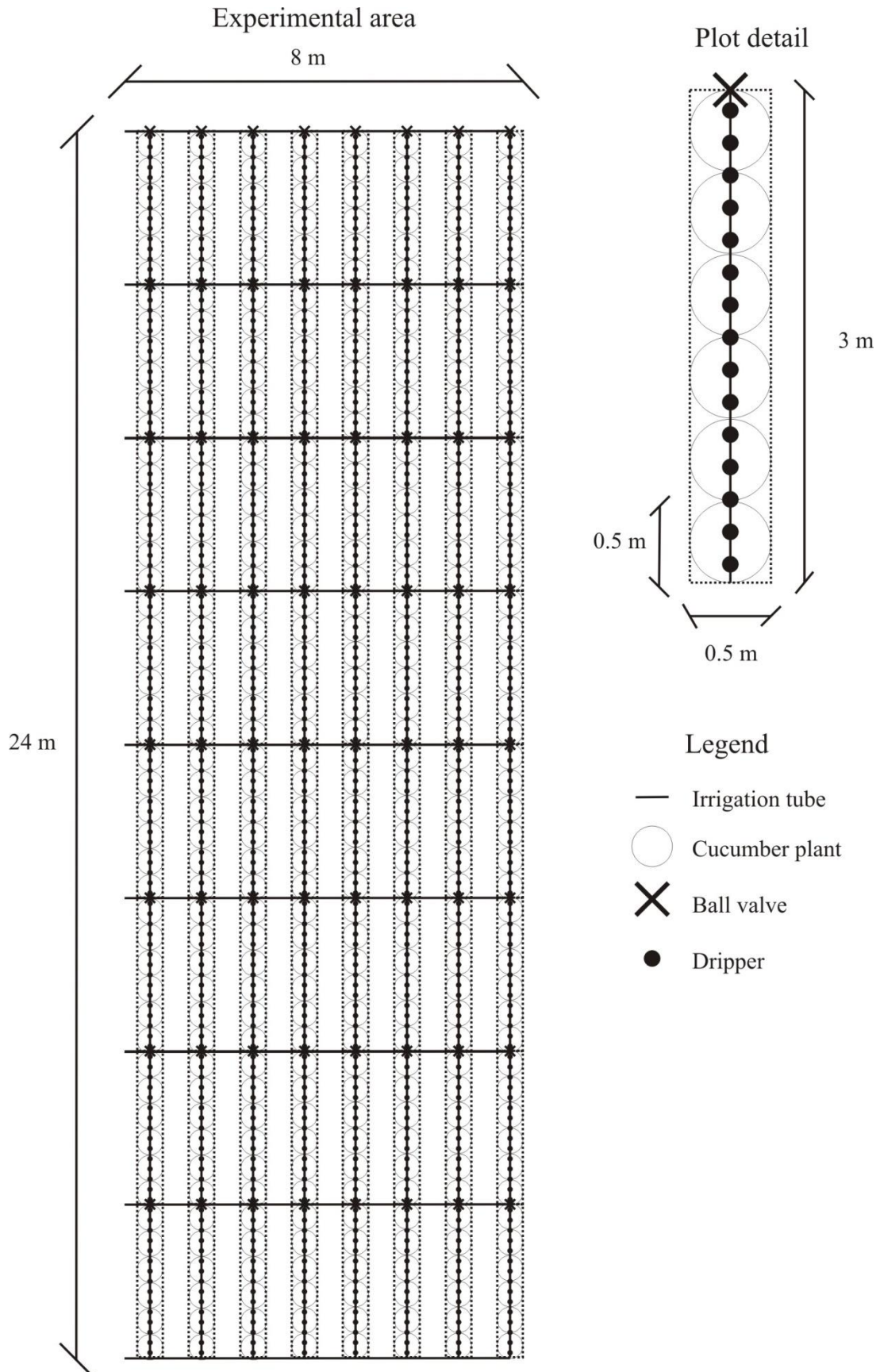


Figure 1. Esquematic map of the experimental area and a plot detail.

Table 2. Description of first and second cycle treatments.

Cycle	IDRE ¹ (%)	Number of pulses	Treatment	Interval between pulses (min)	Relative pulse irrigation time ² (%)	Time interval of first pulse application of the day ³
1	50	1	1T50-1	-	100	09 h 15 min - 10 h 00 min
		2	1T50-2	240	50	08 h 35 min - 09 h 00 min
		4	1T50-4	120	25	08 h 15 min - 08 h 35 min
		8	1T50-8	60	12,5	08 h 00 min - 08 h 15 min
	75	1	1T75-1	-	100	09 h 15 min - 10 h 00 min
		2	1T75-2	240	50	08 h 35 min - 09 h 00 min
		4	1T75-4	120	25	08 h 15 min - 08 h 35 min
		8	1T75-8	60	12,5	08 h 00 min - 08 h 15 min
	100	1	1T100-1	-	100	09 h 15 min - 10 h 00 min
		2	1T100-2	240	50	08 h 35 min - 09 h 00 min
		4	1T100-4	120	25	08 h 15 min - 08 h 35 min
		8	1T100-8	60	12,5	08 h 00 min - 08 h 15 min
2	75	1	2T75-1	-	100	09 h 40 min - 10 h 40 min
		2	2T75-2	320	50	08 h 45 min - 09 h 20 min
		4	2T75-4	160	25	08 h 20 min - 08 h 45 min
		8	2T75-8	80	12,5	08 h 00 min - 08 h 20 min
	100	1	2T100-1	-	100	09 h 40 min - 10 h 40 min
		2	2T100-2	320	50	08 h 45 min - 09 h 20 min
		4	2T100-4	160	25	08 h 20 min - 08 h 45 min
		8	2T100-8	80	12,5	08 h 00 min - 08 h 20 min
	125	1	2T125-1	-	100	09 h 40 min - 10 h 40 min
		2	2T125-2	320	50	08 h 45 min - 09 h 20 min
		4	2T125-4	160	25	08 h 20 min - 08 h 45 min
		8	2T125-8	80	12,5	08 h 00 min - 08 h 20 min
150	1	2T150-1	-	100	09 h 40 min - 10 h 40 min	
	2	2T150-2	320	50	08 h 45 min - 09 h 20 min	
	4	2T150-4	160	25	08 h 20 min - 08 h 45 min	
	8	2T150-8	80	12,5	08 h 00 min - 08 h 20 min	

¹Irrigation depth replenishment relative to the evapotranspiration; ²Ratio between the pulse irrigation time and total irrigation time of the day; ³that is first irrigation pulse of 1T50-8, 1T75-8 and 1T100-8 treatments were applied between 08 h 00 min and 08 h 15 min.

paper bags and dried at 60°C until achieving a constant weight. Multiple regressions were performed when the interactions between number of pulses and IDRE were significant ($p < 0.05$). The selection of the variables was performed using the Stepwise procedure, with $p < 0.10$ to enter a variable to the model and $p < 0.05$ to keep a variable in the model. The statistical analysis and graphs were made using SISVAR (Ferreira, 2008) and Sigma Plot 11.0 software.

RESULTS

The majority of the maximum daily temperatures showed values higher than the optimum maximum temperature, while the minimum daily temperatures measured in the first cycle were below the optimum minimum temperature (Figure 2A and B). The mean daily temperatures observed in the second cycle were, on average, higher than the first cycle, and showed values in the optimum temperature range (Carvalho et al., 2013). There were no

frosts during the experimental period. It was observed that the pressure deficit tended to increase up to 15:00 during the first cycle (Figure 2C) and up to 13:30 during the second cycle (Figure 2D). The irradiance was greater close to 12 h 00 min during the two cycles.

The difference between the accumulated water depths in the treatments regarding the irrigation depth replenishment relative to the crop evapotranspiration (IDRE) tended to increase with the temperature (Figure 3). However, there was no variation of accumulated applied irrigation depth between the levels of the factor number of pulses at the same level of the factor IDRE. Twenty-seven irrigations were performed during the first cycle, totalling 120.5; 180.7 and 240.9 mm of water applied for the levels 50, 75 and 100% of the factor IDRE, respectively. In the second cycle, 20 irrigations were performed, totalling 175.6; 234.1; 292.7 and 351.2 mm of water applied for the levels 75, 100, 125 and 150% of the

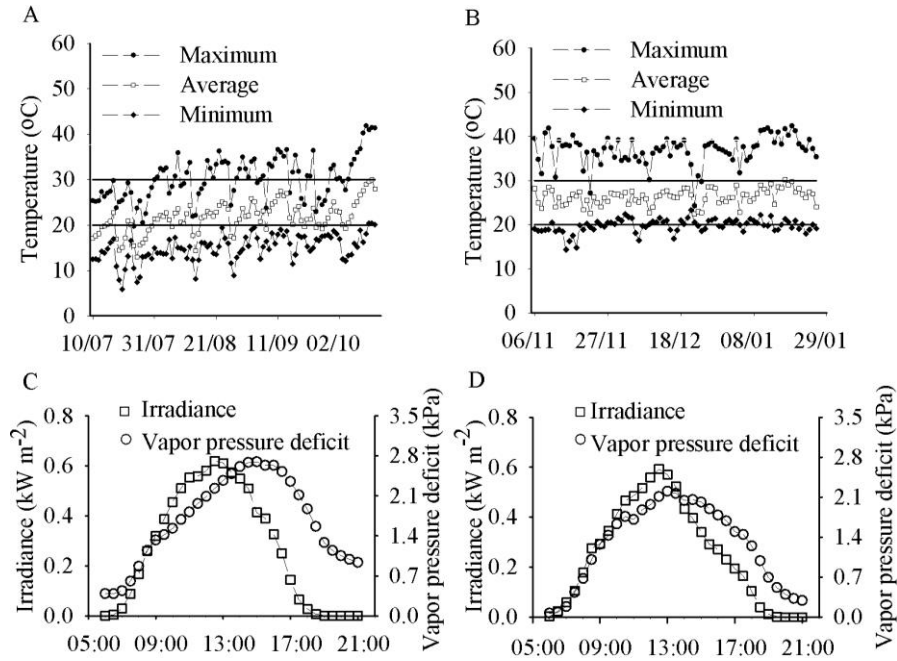


Figure 2. Temperatures within the protected environment during the first (A) and the second (B) cycles in comparison with the optimum range for the development of the crop, and average values for pressure deficit and irradiance on irrigation days during the first (C) and second (D) cycles.

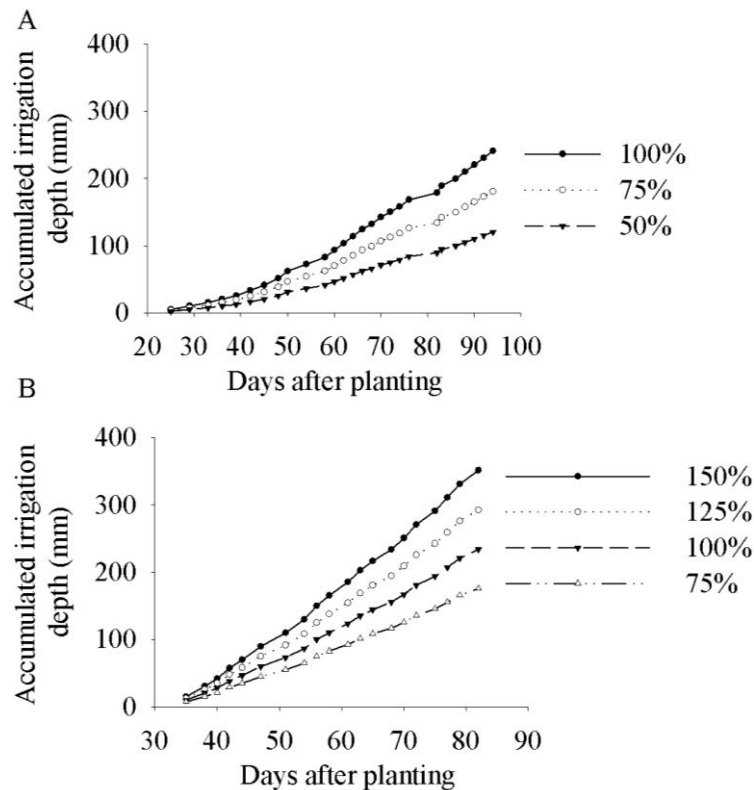


Figure 3. Accumulated irrigation depth in the treatments relating to the replenishment of the irrigation depth relative to crop evapotranspiration in the first (A) and second (B) cycles.

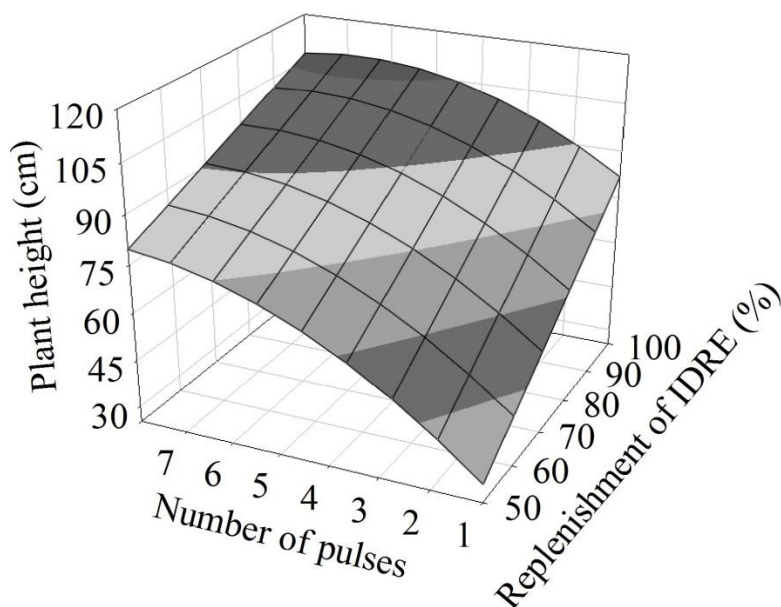


Figure 4. Influence of the number of pulses and of the replenishment of the irrigation depth relative to the ET_c on plant height in the first crop cycle (07/2014 to 10/2014).

factor IDRE, respectively.

For the same level of IDRE, the variation of the plant height with number of pulses (Figure 4) occurred in a quadratic form. Under water deficit conditions, that is, with the replenishment of 50% IDRE, there was an increase from 30.7 to 79.7 cm, that is, 160% with the application split over the day. Under conditions of adequate water supply, that is, with the replenishment of 100% of the IDRE, there was an increase from 81.8 to 107.4 cm, that is, 31%. The model suggests that, despite the fact that splitting the application of the irrigation depth into various pulses during the day resulted in increased plant height independently of the IDRE response, the effect was more notable under water restriction conditions.

Considering the replenishment through a single pulse, the increase in the application of the irrigation depth from 50 to 100% of the ET_c resulted in an increase from 30.7 to 81.7 cm, that is, a 166% increase in plant height. When considering the application through eight pulses, the increase in irrigation depth resulted in an increase in plant height of 79.7 to 107.4 cm, that is, 35%. This analysis allows us to conclude that the increase in irrigation depth shows a greater effect on plant height when applied in instalments throughout the day.

Height of the plants irrigated with 50% of the IDRE through eight pulses was equal to 79.7 cm, while plants irrigated with 100% of the IDRE through a single pulse were 81.7 cm. This means that the use of half of the necessary irrigation depth applied through instalments resulted in plant heights similar to the plants with 100% of

the necessary water needs supplied through a single pulse.

Effect of the splitting of the water application on number of nodes and stem diameter was not significant. On the other hand, the data relating to the IDRE levels could be significantly fitted to a linear model. It was expected that the growth variables would increase with the replenishment of the soil moisture up to 100% of the crop needs (Figure 5).

Fruit mass harvested in the first cycle was significantly affected by the studied factors (Figure 6). Splitting application of 100% of the IDRE into 7 pulses provides the largest predicted value for fruit mass. The same irrigation depth applied through eight pulses results in a lower fruit mass of 0.8%. For this reason, it is possible to consider that the increase in the number of pulses for values greater than the optimum value, within the interval relating to the levels of the factor number of pulses, showed a low significant effect on the fruit mass harvested.

It was observed that the level of the factor number of pulses showed an effect on the fruit mass, which was described by a polynomial model. When replenishing 50% of the IDRE, there was a 163% increase in the fruit mass with the increase in the number of pulses, while at the same time the 100% replenishment of the IDRE caused an increase equal to 30% (Figure 6). The analysis allowed us to conclude that the increase in production with irrigation by pulses is greater under conditions of water restrictions than under adequate water supply conditions.

The increased replenishment of the IDRE resulted in a linear increase in the fruit mass (Figure 6). For the

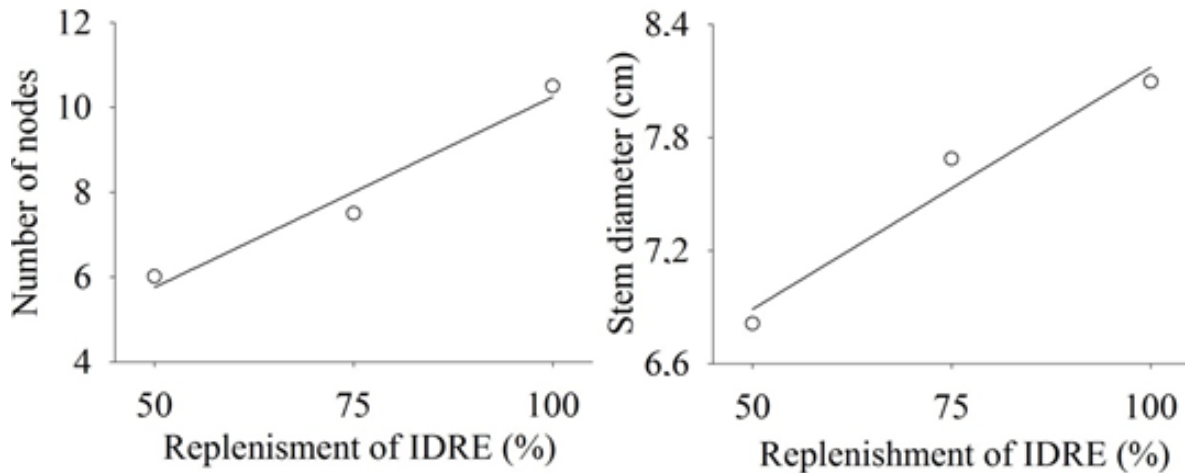


Figure 5. Influence of the number of pulses and of the replenishment of the irrigation depth relative to the ETc on number of nodes and stem diameter in the first crop cycle (07/2014 to 10/2014).

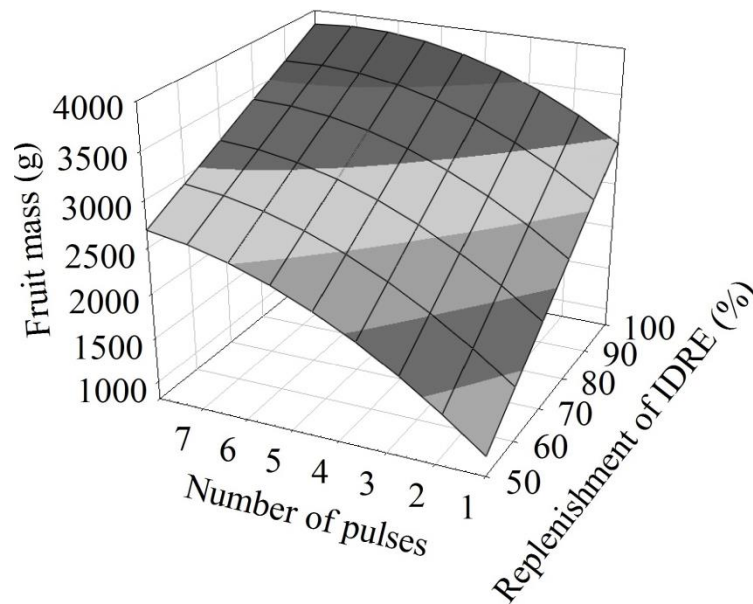


Figure 6. Influence of the number of pulses and of the replenishment of the irrigation depth relative to the ETc on fruit mass in the first crop cycle (07/2014 to 10/2014).

irrigations through one and eight pulses, the increases were 190 and 43%, respectively, which allowed us to conclude that the increase in irrigation depth was more effective if conducted through a single irrigation pulse. Plants subjected continuously to water deficit tend to reduce their cycle and productivity (Ambachew et al., 2014).

Plants with 50% of the IDRE split into eight pulses and those with 100% of the IDRE applied through one pulse were estimated to produce 2689 and 2964 g of fruit respectively, that is, the pulse irrigation with deficit resulted in less production in relation to irrigation that

supplied all the water needs of the crop through a single pulse.

In Figure 7 it was observed that number of pulses had a quadratic effect on the WUE of the plants in the first cycle. With the replenishment of 50 and 100% of the IDRE, the increases were 158 and 23% respectively, showing that the splitting of the water application has a greater effect under water restriction conditions.

For the irrigation through one pulse, increasing the replenishment of the IDRE increased the WUE by 42%. This also suggests that the water deficit results in a water

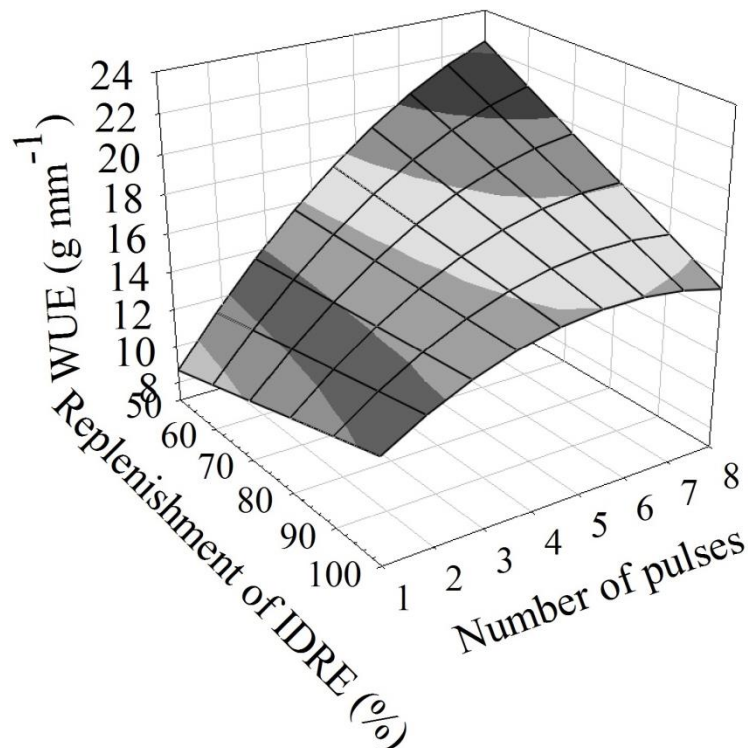


Figure 7. Influence of the number of pulses and of the replenishment of the irrigation depth relative to the ETC on water use efficiency in the first crop cycle (07/2014 to 10/2014).

Table 3. Correlation matrix between the response variable of the first cycle.

Parameter	Plant height	Fruit mass	Number of nodes	Stem diameter
Plant height	1.00	0.82	0.49	0.43
Fruit mass	-	1.00	0.52	0.41
Number of nodes	-	-	1.00	0.66
Stem diameter	-	-	-	1.00

balance favourable to the vegetative growth and maintenance of turgor in detriment to the production of fruit. One of the probable causes of the detriment to production is the slower growth and emission of branches under such conditions.

When considering the water application split into eight pulses, the increased replenishment of the IDRE resulted in linear decrease in WUE by 32%. The greatest WUE was observed with the replenishment of 50% of the IDRE through eight pulses. This suggests that the plant, despite showing reduced growth characteristic with such a level of replenishment, presented a balance of water use favourable to the production of fruit in detriment to vegetative growth. Plants that had their water needs met through the application of eight pulses showed more vegetative growth, which could result in balance of water

use more favourable to the maintenance of the vegetative part.

The variables plant height and fruit mass in the first cycle could be significantly fitted to similar multiple regression models, which suggests the existence of a correlation between them. In the correlations matrix (Table 3), it was found that the correlation between the above variables is greater than between the others, which suggests that the main branch height is related to the vegetative vigour of the plant, greater emission of branches and, consequently, female flowers from which the fruit originate. Treatments were applied later in the second cycle than in first cycle in order to prevent effect of reduced growth on production.

The water deficit presented a greater effect on the reduction of the productivity when imposed on the

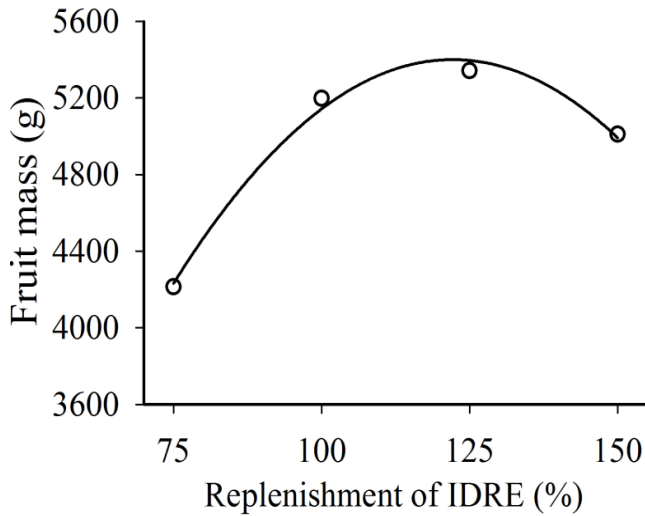


Figure 8. Influence of the replenishment of the IDRE on the fruit mass in the second cycle (12/2014 to 01/2015).

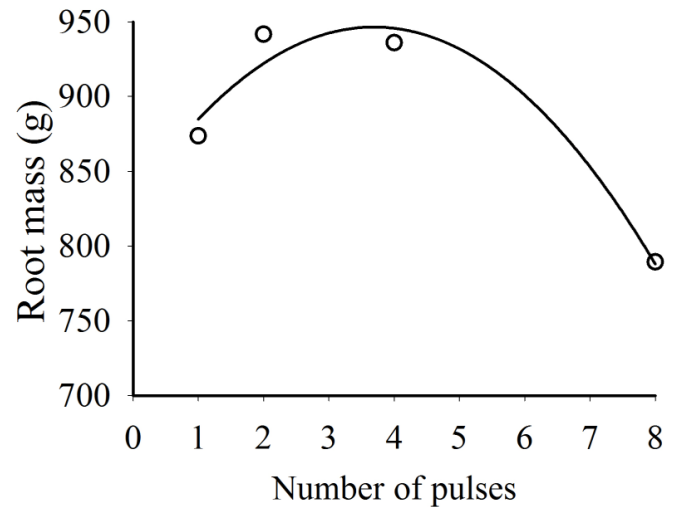


Figure 9. Influence of the number of pulses on root mass in the second cycle (12/2014 to 01/2015).

reproductive stages of the crop. However, such effects are milder if the deficit occurs in the vegetative stage. Therefore, it is feasible as a strategy to conserve water, at least in some crops, by irrigating with less irrigation depth than is required during the vegetative stages and irrigating with proper irrigation depth during the reproductive stages (Ambachew et al., 2014). In the second cycle, the treatment applications commenced when all the plants had the same height, with completely open flowers on the main branch.

The fruit mass measured in the second cycle was significantly affected only by the factor replenishment of IDRE (Figure 8). The greatest value for fruit mass is obtained through the replenishment of 129% of the IDRE. The crop of Japanese cucumber grown in the protected environment presented a variable production in relation to the replenishment of the irrigation depth in the soil, with both the deficit and the excess being detrimental (Oliveira et al., 2011).

Root mass was significantly affected by the splitting of the irrigation (Figure 9). Root mass obtained by 4 pulses is higher than that obtained by any other number of pulses.

DISCUSSION

The growth of the plant tissue is related to cellular expansion and division. The cellular expansion is the physiological process most affected by water deficit. As the severity of the deficit increases, other processes related to cellular division are affected, such as the synthesis of cell walls and proteins (Taiz and Zeiger, 2013). It is likely due to these reasons that the plant height was lower with the 50% replenishment of IDRE in

relation to the complete irrigation.

Pulse irrigation with deficit resulted in a reduction in water losses not associated with production, such as deep drainage and evaporation. For this reason, the ratio between the irrigation depth absorbed by the roots and the irrigation depth is greater in the pulse irrigation with deficit than in the pulse irrigation with 100% of ET_c . This result is associated with lower soil moisture levels, which shows that under such humid conditions, the roots are conditioned to absorb water at lower water potentials. The pulse irrigation with deficit is a promising technique for increasing the efficiency of irrigation (Phogat et al., 2013).

The water deficit is associated with the reduction of turgidity plants, the reduction of nutrient absorption and stomatal closure (Allen et al., 1998), while excess water can cause nutrient leaching and reduce oxygen availability to the roots, which can damage processes related to respiration and trigger cell death (Colmer, 2003). To achieve the optimal development of the crop, the porosity of the soil should be filled with air and water in a proportion that permits the water and aeration needs of the crop to be met.

Drip irrigation is designed to maintain soil moisture, in the layer explored by the root system, at optimal levels for the crop (Frizzone et al., 2012). The reduction of the water depletion period in the soil is beneficial for the development of the crop (Araújo et al., 2012). Further to this, daily irrigation maintains relatively stable soil moisture, which results in an increase in the emission of fine roots and cucumber crop growth (Liang et al., 2014). However, it would be more suitable to analyse the benefits of maintaining the moisture levels while taking into account the times of greatest transpiration demand.

The application through a single pulse occurred for a period of 45 min, while the irrigation through eight pulses occurred intermittently for a period of 7 h 00 min and 09 h 30 min in the first and second cycle respectively. It is likely that the intermittent water application maintains the water potential values in the surface layer of the soil at a relatively high level, resulting in water absorption during the periods of high demand with greater ease. On the other hand, irrigation through a single pulse allowed the decrease in water potential values throughout the day due to the water interaction time with the soil matrix. The distribution of the water in the soil results in greater energy expenditure for absorption during the periods of greater vapour pressure deficit.

The cucumber crop evapotranspiration in the protected environment is related to the air temperature, vapour pressure and solar radiation (Zhang et al., 2010). The vapour pressure deficit includes the effects of the three variables in evapotranspiration, because of this the actual vapour pressure and air temperature are used to calculate it, both of which are influenced by solar radiation.

The intensity of the application in the drip irrigation is, frequently, greater than the infiltration capacity of the soil at the point of the drip. As a result of the irrigation in these conditions, there is an accumulation of water on the surface of the soil in the region close to the dripping point and an elevation of the moisture up to saturation. Once the water application has ended, the soil water tends to distribute due to the water potential gradient. The wet bulb volume tends to increase at the same time as the water potential tends to diminish, which results in a reduction in the water potential gradient. The wet bulb expansion ends when the water potential gradient is not sufficient.

During the bulb formation process, albeit temporarily, water is held to high water potentials. For this reason, it can be absorbed more easily by the plants. Irrigation by pulses allows the water in the layer relating to the depth of the effective root system to stay longer at high water potentials, favouring the absorption during periods of higher vapour pressure deficit. In other words, the water is in a higher energy state in the soil during times of increased demand for absorption.

Conclusions

Water is the most important production factor in agriculture and its scarcity is a reality in many parts of the world. Irrigation new strategies must be researched in order to reduce the water consumption without reducing productivity. Pulse irrigation has the potential to save about half irrigation water in greenhouse. The results showed that the splitting of the irrigation is advantageous if carried out before cucumber plants reach the top wire

of the staking. Plants irrigated with half of the irrigation depth through eight pulses throughout the day tended to grow and produce to a similar extent as plants with 100% of the irrigation depth required through a single pulse. However, pulse irrigation presents no advantages if initiated when Japanese cucumber plants reach the top wire of the staking.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Characterization of ‘Cabernet Sauvignon’ wine made with grapes from Campanha– RS Region

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Campanha region in the state of Rio Grande do Sul (RS) has received attention on grape production specifically ‘Cabernet Sauvignon’, ‘Merlot’ and ‘Tannat’. Due to the edafoclimatic peculiarities such as sandy soil, good thermal amplitude and sunshine, and low rainfall during the ripening period, it is supposed that the wine of this micro-region, in especially Bagé, might have particular typicity. However, as being a recent activity in the region, the results on grape ripening and wine quality are few. The objective of this work was to characterize Cabernet Sauvignon wines made with grapes from the Region of Campanha, state of Rio Grande do Sul – Brazil, specifically from the municipality of Bagé. As control parameters, it was also vinificated, grapes of the same cultivar from vineyards of Serra do Sudeste and Serra do Nordeste of the state of Rio Grande do Sul. The grape of each vineyard was harvested and submitted to microvinification. In one of the vineyards in 2007 (in the region of Bagé) was measured the late harvesting of grapes on wine quality. In general, the results of Bagé wines from the 2004 vintage pointed to good alcohol content and dry extract, but no efficiency on intensity and shade of color. This is probably due to the decrease of acidity during vinification reducing the stability of anthocyanic compounds. The over-ripeness of ‘Carbenet Sauvignon’ grapes during 2007 vintage is a positive alternative of both color and alcohol increment in the wines of Bagé/RS. There was no effect on total acidity whether compared to conventional harvesting or industrially recommended. The high levels of K detected might be one of the factors that contributed to the low total acidity, high pH and color.

Key words: *Vitis vinifera*, grape quality, color, ripening.

INTRODUCTION

Seeking to expand and differentiate the Brazilian vitiviniculture, the wine sector is investing in new potentially apt regions to produce grapes and wines of particular quality. From this context, the South Meso-Region of Rio Grande do Sul, specifically Região da Campanha where Bagé is located, has presented edafoclimatic potential to become an interesting Brazilian winery resort. This micro-region is situated on 31°S, having dry climate, good sunshine and thermal amplitude, and low rainfall right before harvesting. The average values of the main climate parameters are: minimum air temperature (annual average of 12,3°C) and maximum air temperature (annual average of 24,3°C) (Tonietto and Mandelli, 2003), annual rain precipitation of 1.388 mm, air relative humidity of 76% and sunshine of 2.372 h. In general, these conditions allow the development of enological ripening and provide quality to the grapes and wines (Miele and Miolo, 2003). 'Cabernet Sauvignon' is a late-ripening variety and, although, the variants as *terroir* and vinification conditions, the wines have good structure and a particular character. The wine is dark red, bodied and with a great persistent aroma. The young wine is a little hard, astringent, tannic and astringent. The ageing process is slow and demands a long time of storage (Souza, 2000).

Nevertheless, the wine is not just a variety result, but especially it is a complex of natural and human factors that determines the qualitative and quantitative potential of the cepagem, vineyard and region. The natural features (climate, soil, topographic exposition, etc.) are independent variables that affect the product quality and differentiation. The management does not have direct action on these processes, except rare exceptions; once chosen the region, they continue during the whole productive life of the vineyard, without possible rectification practices. It is evident that management interferences may occur as soil correction, irrigation, vineyard mulching, etc (Hidalgo, 1980; Mandelli, 2002). According to Martínez-Peláez (1994), the climate establishes or does not favor the eco-physiological conditions of the vine culture, whereas the mesoclimate determines the particularities in each region. He also points out that it is not possible to idealize that a country might develop a wine industry based on the rusticity of the specie or appeal to diverse genotypes. There must be an adjustment between ecosystem conditions of each region and their wine typicity as cited by Mandelli (2002). Diaz (1992), in commenting on the main relationships between environment and wine biochemicals characteristics, affirmed that the specificities of wine characteristics are determined by grape quality.

Nevertheless, the grape quality at first depends upon the cultivar and then the environment where it is produced. The others aspects are accomplished by the management of grape production and the enologic process (Mandelli, 2002).

Several studies have been carried out aiming to characterize the wines of the main Brazilian wine-growing regions with focus on the Serra Gaúcha (Rizzon et al., 1998; Rizzon and Miele, 2001). In this region it has been verified that, regarding its edafoclimatic and management conditions, the main red wine varieties (Merlot, Cabernet Sauvignon, Cabernet Franc and Tannat) have provided wines with high acidity, good color and structure but incomplete phenolic ripening. Among the cited varieties, Merlot has been pointed out as the more equilibrated wine (Rizzon et al., 1998; Rizzon and Miele, 2004; Rizzon and Miele, 2003; Rizzon and Miele, 2002). Because of the lower rainfall and higher sunshine in the Region of the Campanha, it is believed that the grapes might acquire good ripening providing wines with intense color and adequate phenolic ripening. However, there may be difficulties as for acidity preservation. In spite of good thermal amplitude, there are some periods with excessively high night temperatures that results in wines with high pH levels. The objective of this work was to characterize Cabernet Sauvignon wines made with grapes from the Region of Campanha, State of Rio Grande do Sul – Brazil, specifically from the municipality of Bagé. As reference parameters it was also vinificated grapes from Santana do Livramento, Encruzilhada do Sul and Bento Gonçalves. Furthermore, the effect of late harvesting of grapes on wine quality was studied.

MATERIALS AND METHODS

The experiment was carried out with 'Cabernet Sauvignon' grapes cultivated at different vineyards situated at the Region of the Campanha (one at Santana do Livramento and two at Bagé – São Xico and Cerrito), Serra do Sudeste (one at Pinheiro Machado and one at Encruzilhada do Sul) and Serra Gaúcha (Vale dos Vinhedos, Bento Gonçalves). The vineyards were between four and eight years old. The vines were planted at 1.20 x 3.30 m and trained on simple trellis system. From each vineyard, three boxes of grapes weighing about 16 kg each were harvested. The grapes were harvested on 3rd March, 2004 with three microvinifications of three replications for each vineyard.

Microvinification

For each experimental unit, the grapes were crushed and the musts were acondicionated into 20 L glass flasks topped with a Müller valve. The must was enriched with 40 mg/L of sulphur dioxide and 15 mg/L of pre-active dry yeast. The must remained on

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Table 1. Basic physico-chemical characteristics of must of 'Cabernet Sauvignon' grape, 2004 vintage of the regions Bagé, Pinheiro Machado, Encruzilhada do Sul, Santana do Livramento and Bento Gonçalves.

Vineyard	°Brix	Total Acidity (meq/L)	pH
Bagé – São Xico	19.07	100	3.43
Bagé – TrêsCerros	20.1	77	3.45
Santana do Livramento	18.84	90	3.5
Pinheiro Machado	19.75	109	3.2
Encruzilhada do Sul	20.4	90	3.29
Bento Gonçalves	20.37	98	3.43

fermentation at 23 and 25°C. The fermentation time, in presence of skins and seeds, was of six days with daily pumping over. The first separation was done after the end of alcohol fermentation. The wines continued in flasks adapted with the same Müller valves until the end of malolactic fermentation. In the way, next the wines were separated and again it was added the sulphur dioxide (50 mg/L). The tartaric stabilization was done at temperature of 4°C for a period of ten days. After the tartaric stabilization was completed, the wines were bottled.

Wine physico-chemical analysis

The physico-chemical evaluations (density, alcohol concentration, total acidity, volatile acidity, pH, dry extract, reducing sugars, reduced dry extract, ashes, alkalinity of ashes) were done with conform methods described by Ribéreau-Gayon et al. (1976). The anthocyanins contents were determined by the method of Ribéreau-Gayon and Stonestreet (1965). The optical density (OD) at 420 nm, 520 nm and 620 nm and the total polyphenols were determined by spectrophotometry, previously calibrated by Rizzon and Miele (2002) with cuvests of 1 mm and 10 mm of optical path for color indexes and total polyphenols, respectively.

Statistical analysis

The data were submitted to analysis of variance and the comparison of means by Tukey test at 1% level of significance. The statistical analyses were performed by using Sanest program (Zonta and Machado, 1991).

RESULTS AND DISCUSSION

The mash showed very different results (Table 1) to the different regions, probably related to soil type and climatic conditions imposed by the altitude difference. But all followed the same line that increasing the total sugars, decreased acidity. They followed which is widespread in the literature which provides increased maturation of sugars, but the cannibalization of malic acid, there is a reduction in major or minor proportion of the total acidity. The wine density (Table 2) settled between 0.9973 and 0.9945, which is considered normal for dry red wines (Rizzon and Miele, 2002). The alcohol concentration of the wines varied from 10.7% to 12.39% (v/v) (Table 2) and there was not chaptalization. This indicates that with the improvement on vine culture, the grapes accumulated

sufficient sugar levels to obtain the minimum alcohol contents required. This is backed up by Castro et al. (1991) who cited that a good phytotechnic management increases sugars levels. This aspect is important since, in general, there is still the need for corrections as for the addition of sugar as for the concentration or combination of methods (Manfroi et al., 2006). Comparing the alcohol contents and residual sugar of wines (Table 2) with the °Brix of the respective musts (Table 1) the relation is coherent with the yield estimated in the alcohol fermentation of red wines.

The total acidity varied significantly (Table 2). The higher concentrations were obtained in wines of Encruzilhada do Sul and Pinheiro Machado. This result is expected in these regions for having good thermal amplitude and nights of low temperatures during the grape ripening period. Nevertheless, the region of Serra Gaúcha is known for producing grapes with high total acidity (Rizzon and Miele, 2002; 2004; Manfroi et al., 2006). Therefore, the acidity of the wine was 55 mEq / L in the region of Bento Gonçalves, which is similar to the wine regions of Bagé and Santana do Livramento (48 and 56 mEq / L, respectively). Although, the musts had presented initial total acidity between 77 and 109 mEq/L (Table 1), there was a reduction in values to 51 and 66 mEq/L in the stabilized wine. The decrease of total acidity during the tartaric stabilization process is normal because of the precipitation of potassium acid tartrates and bitartrates (Rizzon et al., 1998; Manfroi et al., 2006). But, the exact causes of low total acidity were not demonstrated. A possible explanation for low acidity rates is the high potassium content found (Table 3) between 1263 and 1947.1 mg / L, which would favor a higher precipitation of potassium bitartrate decreasing, tartaric acid content influencing acidity. Potassium is the main nutrient required by the vine, being responsible for the proper development of the plant and fruit, also being required by the yeast during fermentation and interfering with the pH of the must and wine. It was observed that the higher potassium content (Table 3), the higher pH found in wine (Table 2). The pH is one of the most important variables in the equilibrium of wines and wine responsible for providing microbiological stability, color and sensory balance, directly interfering extractability and

Table 2. Classic analysis in Cabernet Sauvignon wines of the regions of Santana do Livramento, Bagé (São Xico and TrêsCeros), Encruzilhada do Sul, Pinheiro Machado and Bento Gonçalves, 2004.

Variable	Wine Grower Regions						General Average	VC%
	Santana do Livramento	São Xico (Bagé)	TrêsCeros (Bagé)	Encruzilhada do Sul	Pinheiro Machado	Bento Gonçalves		
Density (20/20°C)	0.9973 a*	0.9964 ^{ab}	0.9952 ^{bc}	0.9945 ^c	0.9951 ^{bc}	0.9954 ^{bc}	0.9956	0.03
Alcohol content (% v/v)	10.7 ^a	10.65 ^a	12.0 ^a	12.08 ^a	11.36 ^a	12.39 ^a	11.5	2.8
Total acidity (meq/L)	48.00 ^b	51.00 ^b	56.00 ^b	66.00 ^a	65.00 ^a	55.00 ^b	56.8	2.7
Volatile Acidity (meq/L)	10.5 ^{ab}	7.5 ^b	8.0 ^b	12.0 ^a	9.5 ^{ab}	11.5 ^a	9.3	5.9
pH	4.2 ^a	4.19 ^a	4.11 ^a	3.89 ^b	3.83 ^b	4.16 ^a	4.1	0.5
Dry Extract (g/L)	24.2 ^{ab}	22.94 ^{abc}	22.68 ^{bc}	20.82 ^c	21.60 ^c	24.98 ^a	22.9	1.8
Reducers sugars (g/L)	1.73 ^{cd}	1.33 ^d	1.95 ^{bc}	2.26 ^{ab}	2.46 ^a	1.76 ^{cd}	1.9	4.3
Reduced Dry Extract (g/L)	23.47 ^a	22.62 ^{ab}	21.74 ^{abc}	19.57 ^c	19.83 ^{bc}	24.22 ^a	21.9	2.5
Alcohol in weight / Reduced dry Extract	3.35 ^c	3.77 ^{bc}	4.42 ^{ab}	4.93 ^a	4.52 ^{ab}	4.09 ^{bc}	4.2	3.3
Ashes (g/L)	4.15 ^a	3.88 ^{ab}	3.75 ^{ab}	3.50 ^{ab}	2.93 ^b	4.13 ^a	3.7	4.8
Ashes Alkalinity (meq/L)	45.25 ^a	40.50 ^b	37.75 ^b	32.25 ^c	30.75 ^c	40.50 ^b	37.8	2

*Means with the same letter in line are not significantly different by Tukey Test ($\alpha = 0.01$).

Table 3. Analysis of phenolic compounds, tartaric acid and potassium in Cabernet Sauvignon wines of the regions of Santana do Livramento, Bagé (São Xico and TrêsCeros), Encruzilhada do Sul, Pinheiro Machado and Bento Gonçalves, 2004.

Variable	Wine Grower Regions						General Average	VC%
	Santana do Livramento	São Xico (Bagé)	TrêsCeros (Bagé)	Encruzilhada do Sul	Pinheiro Machado	Bento Gonçalves		
O.D. I 420	0.172 ^c	0.368 ^{ab}	0.323 ^{ab}	0.353 ^{ab}	0.396 ^a	0.233 ^{bc}	0.308	8.5
O.D. I 520	0.176 ^c	0.385 ^{abc}	0.390 ^{abc}	0.471 ^{ab}	0.612 ^a	0.268 ^{bc}	0.384	12.3
O.D. I 620	0.053 ^c	0.139 ^{ab}	0.115 ^{abc}	0.127 ^{ab}	0.143 ^a	0.074 ^{bc}	0.109	11.2
Color Intensity (O.D.420 + O.D.520 + O.D.620)	0.401 ^c	0.892 ^{ab}	0.828 ^{abc}	0.951 ^{ab}	1.153 ^a	0.575 ^{bc}	0.8	10.6
Color (O.D.420 / O.D.520)	0.979 ^a	0.958 ^a	0.828 ^{ab}	0.757 ^{bc}	0.647 ^c	0.868 ^{ab}	0.84	3.5
Tannin (g/L)	1.42 ^c	1.53 ^c	1.72 ^{bc}	2.68 ^{ab}	2.86 ^a	1.36 ^c	1.9	10.3
Totals Anthocyanins (g/L)	244.83 ^c	393.63 ^b	493.73 ^{ab}	567.45 ^a	568.42 ^a	438.44 ^b	451.1	4.1
Totals Polyphenols (g/L)	29.0 ^d	36.55 ^{bc}	38.65 ^b	45.8 ^a	48.05 ^a	31.55 ^{cd}	38.3	2.6
Tartaric Acid (g/L)	3.9 ^a	3.05 ^a	3.5 ^a	2.46 ^a	2.83 ^a	3.27 ^a	3.2	11
Proline (mg/L)	2168.25 ^a	1927.40 ^a	1893.10 ^a	442.75 ^b	905.55 ^b	1888.50 ^a	1537.6	5.5
Potassium (mg/L)	1925.7	1947.1	1894.7	1351.0	1263.0	1839.7	1703.5	11.9

*Means with the same letter in line are not significantly different by Tukey Test ($\alpha = 0.01$).

stability of anthocyanins. These pigments are very important and are involved in color development and stabilization of wine (Pechamat et al., 2014). The high potassium concentration found may be related to soil conditions, fertilization and variety of the rootstock. Therefore, it is necessary to study and monitoring of the K levels in the soil of the vineyards, as well as monitoring the progress of this cation on the vine.

Measuring the tartaric acid in the wines, the final concentration between 2.46 g/L (Encruzilhada do Sul) and 3.9 g/L (Santana do Livramento) is considered satisfactory (Rizzon et al., 1998; RizzonandMiele, 2001)

to maintain the total acidity of red wines. But, this is not found in this work, because even with good contents of tartaric acid there was detection of high pHs up to 4.2 (Santana do Livramento). Contrary to expectation, the wines with the highest final concentration of tartaric acid showed high pH (Santana do Livramento) and the lowest ones showed low pH (Pinheiro Machado and Encruzilhada do Sul). The apparent cause of high levels of pH in wines of Santana do Livramento may be associated with the higher concentrations of K, which would result in higher proportion of soluble tartaric salts in the wine. It also has generated a higher precipitation of

acids tartrate during fermentation and tartaric stabilization. Regarding volatile acidity it may be affirmed that the fermentation and wine treatments of Bagé were conducted adequately (values between 7.5 and 8.0 mEq/L). To the other wines the volatile acidity also settled under 20 mEq/L, which level is recognized as the maximum value by Brazilian legislation. Nevertheless, the values between 10.5 and 12 mEq/L found in the wines of Encruzilhada do Sul, Bento Gonçalves and Santana do Livramento are an indication of alert because they mean a tendency to wines alterations.

The wines pH variations had opposite behavior to the total acidity, which is normal (Manfroi et al., 2006). In other words, the wines from regions of lower total acidity showed high pH levels. In general, red wines from the classics regions of Brazilian wine-growing are situated at pHs between 3.3 to 3.8. In this aspect, the wines of the studied vineyards require assessments to avoid increase in pH. It is proved that the inductor causes of high pH also cause low total acidity, excess potassium, high night temperature and/or interaction of these factors. Nevertheless, these hypotheses need to be proved. All the musts (Table 1) showed pH values in the recommended range, but the stabilized wine had higher values of pH. This pH increasing is coherent with the reduction of total acidity observed as transforming musts (Table 1) into stabilized wine (Table 2). The reduction of the total acidity was in average 40%. The average dry extract settled above 20 g/L, indicating that the particular year allowed the soluble solids and sugar accumulation, which resulted in wines of good structure on these components. From the total of dry weight more than 90% is reduced dry extract (dry extract-residual sugars), which was expected for dry red wine. The relation of alcohol (mass)/reduced dry extract always set below 5.20 that it is the maximum limit permitted by Brazilian legislation for a great wine equilibrium.

Concerning ashes concentration, normally the encountered value corresponds approximately to 10% of the dry extract (Manfroi et al., 2006). This contribution was in average 17% of the total dry extract in these wines, indicating that the mineral matter significantly participated of the total dry extract. In analyzing the ashes alkalinity the wines of lower total acidity and higher pH also showed higher values of this variable, suggesting that there is an effective formation of salts in these wines probably with K for being the main cation of wines (Rizzon et al., 1998). Assessing the indicator variables of yellow (OD 420), red (OD 520) and violet (OD 620) color, it was verified that the wines from São Xico (Bagé-RS) and Santana do Livramento had higher relation OD 420/OD 520 close to 1.0, value commonly observed in older wines (Ribéreau-Gayon and Stonestreet, 1965; Manfroi et al., 2006). As all wines were vinified in the same date and process the color differences must be due to grapes origin. It is provable that the higher OD₄₂₀ alterations (yellow color indicator) are due to the low total acidity and high pH of these wines. It is widely known

that the acidity and pH strongly affect the anthocyanins stability in wines (Boulton, 1980a; Boulton, 1980b; Champagnol, 1988,1986; Rizzon et al., 1998). This affirmation is confirmed by the higher contents of anthocyanins and total phenol compounds found in the wines of higher acidity and lower pH (Encruzilhada do Sul and Pinheiro Machado). Sims and Morris (1985) had already proved that high pH permits the formation of "tostada" color (brown) in wine. The shade "tostada" or "brick" color is the result of increasing yellow color together with violet and red colors. In fact, wines from regions with high pH levels also presented lower indexes of color intensity (OD 420 + OD 520 + OD 620) and higher proportional participations of OD 420.

Besides nutritional and managements aspects, the climatologic conditions also influence the syntheses and amount of grapes pigments (Leeuwen et al., 2004; Jones and Davis, 2000). As an example, Buttrose et al. (1971) verified that high temperatures affect positively increasing pigmentation in bagas of 'Cabernet Sauvignon', but not necessarily in ripen vineyards. Tabares et al. (2002) found high concentrations of phenols compounds in vineyards situated in higher altitudes. The grapes of 'Cabernet Sauvignon' grown in high-altitude climates showed remarkable and particular characteristics of the complete phenol maturation (Rosier, 2007). These factors could also have influenced the wine color intensity in this experiment. For example, the wines from Pinheiro Machado and Encruzilhada do Sul, situated at average altitude of 200 to 600 m, presented higher color intensity, whereas, Bagé and Bento Gonçalves are situated at 100 to 200 m (Miele and Miolo, 2003). However, the wine of Bento Gonçalves, produced at an altitude of approximately 600 m, does not respond in the same way. Higher levels of proline might be an indicator of vineyard ripening whether combined to good alcohols contents and adequate acidity levels. The amount of proline in wines is a genetic characteristic of the vine, thus, it is strongly influenced by climate conditions, nitrogen fertilizing and sanity degree of the grape (Bisson, 1991; Sponholz, 1991; Rizzon et al., 1993). Cultivar is one of the most important factors that interfere on proline content (Kliwer, 1969, 1970; Sponholz, 1991). Cabernet Sauvignon, Cabernet Franc and Merlot wines show elevated contents of proline in relation to *Vitis Labrusca* and hybrids (Bisson, 1991; Sponholz, 1991; Rizzon et al., 1993). According to Rizzon et al. (1993), the wines of 'Cabernet Sauvignon' present more elevated contents of proline than 'Cabernet Franc' and 'Merlot'. The regions of Santana do Livramento and Bento Gonçalves showed proline contents articulated with vineyard age. But, Santana do Livramento did not reach adequate levels of alcohol, total acidity and pH. Regions of Bagé, the wines had high proline contents but with pH above 4, low total acidity and medium alcohol content. The problem is the the measurement year; the vineyards are four years old and are in their second year of production. Contrary to previous situations, the vineyards of Pinheiro Machado

and Encruzilhada, also with four years implantation, had inferior values of proline, good alcohol content, suitable acidity and low pH in the wines compared to other vineyard, supposing it to be vineyards of high enologic potential and in evolution phase, due to the lower proline contents.

According to Leeuwen et al. (2004), the climate influence was superior at most of wine parameters, followed by soil and cultivar. Therefore, it could be affirmed that for the typification of 'Cabernet Sauvignon' in the different assessed regions, it is only possible after years of repetition of the same experiment carried out in the 2004 vintage. Regarding ashes alkalinity (Table 2), the four regions that surpassed the technical limits (Santana do Livramento, Bagé- São Xico, Bagé-Três Cerros and Bento Gonçalves) varied from 25 to 35 mEq/L. They are the same regions that showed pHs above 4, demonstrating a strong correlation between the two factors. This is coherent, since potassium is responsible for more than 40% of the ashes composition, reinforcing the hypotheses of the big influence of this cation on both wine pH and ashes alkalinity. It is also observed (Table 1) that the ashes amounts are high whether compared to the amounts encountered by Rizzon and Miele (2002), even that there is no technical limits for this variable. Comparing the wine ashes contents of the different regions, Pinheiro Machado that showed inferior ashes amounts to other regions had also lower pH. Normally, in wines of ripen grapes, the dry extract and alcohol content show a directly proportional relation, but it was not observed (Table 2). Only Bento Gonçalves satisfied this relation. The others regions showed a high amount of dry extract and low alcohol content (Santana do Livramento and Bagé – São Xico) or an opposite situation, low dry extract and high alcohols contents (Bagé – Três Cerros, Pinheiro Machado and Encruzilhada). The exact causes were not clarified but it is likely that excessive salt precipitation during cold stabilization has resulted in the reduction of dry extract. The reduced sugars levels (Table 2) demonstrate that all wines underwent adequate and complete alcohol fermentation. In Santana do Livramento and Bagé - São Xico, the values of alcohol in weight/reduced dry extract ratio were inversely proportional to alcohol content.

Conclusion

The wines Campanha Region of the State of Rio Grande do Sul, have a good structure. However, depending on the vintage, there might be decrease of total acidity and increase of pH, and consequently, problems on color stability during ageing.

Conflict of Interests

The authors have not declared any conflict of interests.

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