African Journal of Agricultural Research

Volume 11 Number 15 14 April 2016
ISSN 1991-637X

Academic Journals

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Vol. 11(15), pp. 1282-1288, 14 April, 2016
DOI: 10.5897/AJAR2015.10473
Article Number: 306250C58039
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Viability and enzyme activity of coffee seeds subjected to LERCAFE test

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Received 1 October, 2015; Accepted 17 February, 2016

The LERCAFE test consists in immersing coffee seeds in an active chlorine solution, which reacts with the seeds' endosperm, thus staining the viable parts dark green. The goal was to adapt the LERCAFE methodology to coffee seeds and assess the isoenzymatic profile of seeds subjected to the test. Two experiments were conducted, the first with adequacy of the LERCAFE test methodology, and the second experiment adequacy of the LERCAFE test methodology with the content of active chlorine quantified. A completely randomized design was used, with four replications of 50 seeds in a factorial scheme 4x4 (4 cultivars and 4 treatments of LERCAFE test). In the first experiment, it was possible to sort the cultivars into two quality levels by means of the treatments at 2.5% for 3 h, 3.5% for 2 h and 3 h. In the second experiment, it was found that the test enables determining the coffee seeds' physiological potential by using 2 and 3% active chlorine for 5 and 3 h, respectively. The coffee seeds subjected to LERCAFE test show changes in the activity of esterase, malate dehydrogenase, superoxide dismutase, catalase and alcohol dehydrogenase enzymes, and the activation or deactivation of these enzyme systems vary with the concentration and immersion time in the solution of active chlorine.

Key words: Viability, sodium hypochlorite, *Coffea arabica*.

INTRODUCTION

There are several factors that contribute to a successful implementation of a coffee farming, among them, the use of healthy and well developed seedlings, which are the base of support for the establishment of culture, mainly because it is a perennial crop (Carvalho et al., 2012). The time for seedling formation becomes larger, due to the coffee seeds possessing slow and uneven germination (Lima et al., 2012).

Studies have been intensified regarding LERCAFE test, because it allows obtaining results concerning the viability of coffee seeds in a short period of time as well as being easy to perform, but little is known about the mechanisms of action involved between the coffee seeds´ endosperm and the active chlorine presents in the

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sodium hypochlorite solution.

The methodology of LERCAFE test consists in immersing coffee seeds in a sodium hypochlorite solution. The active chlorine and the active principle of the sodium hypochlorite solution reacts with the seeds´ endosperm. From the evaluation of the demarcated region location, it is possible to sort the seeds as viable or non-viable (Reis et al., 2010). Reis et al. (2010) concluded that treatment in which seeds were immersed in a sodium hypochlorite solution with 2.5% of active chlorine for a period of 3 h at 25°C is efficient in the estimation of the viability by the test, as Zonta et al. (2010) concluded that the treatments of 2.5% of active chlorine with immersion time of 3 h at 35°C and 3.5% of active chlorine with immersion period of 2 h at 30°C are also efficient in estimating the viability by the test.

Zonta et al. (2008) used the LERCAFE test to estimate germination and to characterize damage in coffee seeds. According to the authors, the test is efficient in detecting damage caused by drying at high temperature and by shoot borer attack. The test was also used to evaluate and characterize mechanical damage in coffee seeds, proving to be efficient (Zonta et al., 2011).

As it is a test that combines the reaction of active chlorine with possible killed/injured regions of the seeds´ endosperm, the discovery of possible enzymatic processes involved in the test can support the understanding of the reactions that occur between the seed´s endosperm and the active chlorine. A wide variety of proteins and structural enzymes is responsible for the integrity and cellular metabolism and, so, the activity of certain enzymes, such as superoxide dismutase, esterase, the malate dehydrogenase and alcohol dehydrogenase, associated with reserves breaking or new tissue biosynthesis, can determine the deterioration stadium of seeds (Carvalho et al., 2000).

Thus, this research objective is to standardize the LERCAFE test methodology for assessment of coffee seeds quality (*Coffea arabica* L.), and to verify the behavior of seeds enzymatic systems after LERCAFE test.

MATERIALS AND METHODS

The research was carried out in the Federal University of Jequitinhonha and Mucuri Valleys' Seed Laboratory and in the Federal University of Lavras' Seed Central Analysis Laboratory, with coffee seeds originating from the Três Pontas Experimental Farm, provided by Agricultural Research Corporation of the State of Minas Gerais (EPAMIG), in two experiments, as described:

Experiment 1 - Adequacy of the LERCAFE test methodology

Coffee seeds batch (*Coffea arabica* L.) of the cultivars was used: Catuaí Amarelo IAC 44, Mundo Novo IAC 376-4, Travessia MGS and Rubi MG 1192, from the 2009/2010 crop. The moisture content was determined by oven method at 105°C for 24 h (Brasil, 2009), with two replicates of 30 g of seeds, whose parchment was manually removed. The germination test was performed using seeds without parchment (manual removal). The results were expressed as percentage of normal seedlings counted after 15 days (first count) and 30 days (final count) (Brasil, 2009). The germination speed index (GSI) was calculated according to the formula proposed by Maguire (1962).

The LERCAFE test was performed in coffee seeds without the parchment (manually removed) for all cultivars. Seeds were subjected to immersion treatments in aqueous solution of sodium hypochlorite at concentrations of 2.5, 3.5, 5.0 and 6.0% of active chlorine, during periods of 2, 3 and 6 h at 30°C, with the aim to determine the best concentration and immersion time for the experimental evaluation. The active chlorine concentrations were obtained from the dilution of commercial sodium hypochlorite, with 10% content of active chlorine, in distilled water. The test was performed following the methodology proposed by Reis et al. (2010).

After this step, the seeds were placed on a properly sterilized workbench for visual assessment and photographic record. They were classified, according to Reis et al. (2010) (Figure 1). After visual evaluation, the seeds were subjected to germination test. A completely randomized design was used, with four replications of 50 seeds in a factorial scheme 4x4 (4 cultivars and 4 treatments of LERCAFE test). The four treatments were 2.5% / 3 h, 2.5% / 6 h, 3.5% / 2 h and 3.5 / 3 h, obtained by the pre- test. Data were subjected to analysis of variance and the means were compared by Tukey test at 5% probability. Statistical analyzes were performed with the aid of SISVAR ® statistical program (Ferreira, 2010).

Experiment 2 - Adequacy of the LERCAFE test methodology with the content of active chlorine quantified

Coffee seeds batch (*Coffea arabica* L.) of the cultivar Catuaí Vermelho IAC 99, from the 2010/2011 crop was used. The moisture content, the germination test, the first count and the germination speed index (GSI) were performed as described in experiment 1. For the adequacy of the LERCAFE test methodology, sodium hypochlorite solutions were tested with concentrations of 1, 2, 3, 4 and 5% respectively of active chlorine for the periods of 1, 2, 3, 4 and 5 h of immersion in a germination chamber at 30°C, and the contents of active chlorine were obtained from the dilution of commercial sodium hypochlorite (ready for analysis) in distilled water quantified according to Brazil (2005). The coffee seeds were classified as germinable and non-germinable following the classification presented in experiment 1 (Figure 1). For the identification of effective treatments in the estimation of viability by LERCAFE test, the unilateral left Dunnett test was applied, with 5% of probability, finding, thus, significant results compared to the control treatment (germination test). For this, data were installed in a completely randomized design with four replications of 50 seeds and subjected to analysis of variance.

To assess the enzymatic activity, the behavior of seeds subjected to effective treatments in determining the coffee seeds viability was evaluated; for this, four replications of 50 seeds were used, representing each treatment. Into the enzyme assessment, it was also inserted the control treatment (T), in which the seeds were not subjected to the LERCAFE test, but wetted for 3 h on paper substrate (moistened equivalent to 2.5 times the weight of the substrate). The seeds of these treatments were crushed using a TE613/1 model Rebnal mill, cooled at 4°C in the presence of antioxidant PVP (Polyvinylpyrrolidone) and liquid nitrogen in a mortar. After milling, the material was stored at - 86°C, for the isoenzyme analysis. The extraction of the protein was performed by adding 100 mg of the seed powder to 280 µl of extraction buffer (Tris 0.2), homogenized by vortexing and, then, kept in the refrigerator for 1 h. The samples were centrifuged at 14,000 rpm at 4°C for 1 h.

The electrophoresis in polyacrylamide gels was performed in a

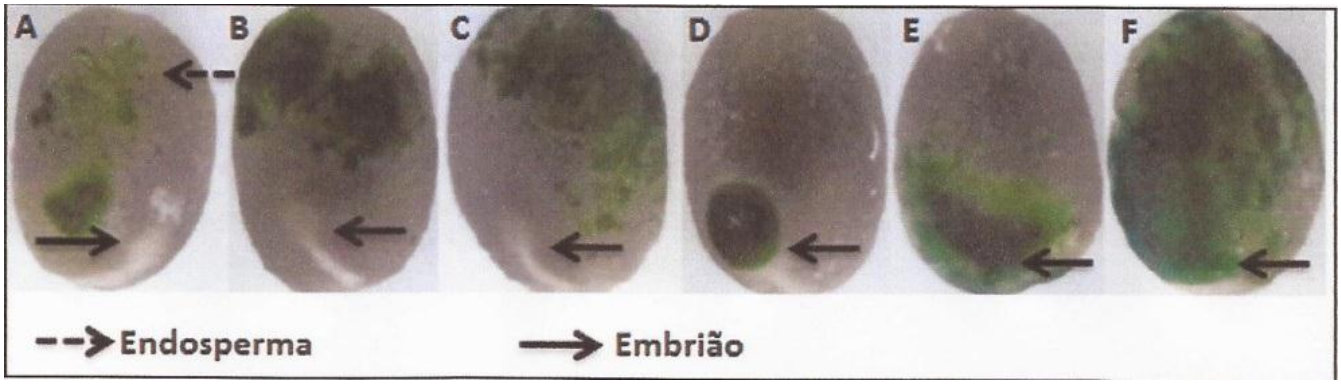

Figure 1. Categories of coffee seeds subjected to LERCAFE test. Categories of viable seeds - seeds with dark green coloration in the endosperm in a distant region of the embryo (A, B, C). Categories of non-viable seeds – seeds with dark green coloration on the embryo (A, B, C). Categories of non-viable seeds – seeds with dark green coloration on the embryo or full color (D, E, F). The dotted arrow indicates the region of endosperm and the solid arrow indicates the position of the embryo.

Table 1. Results in percentage of normal seedlings obtained in the first count (FC), germination test (G) and germination speed index (GSI) for coffee cultivars.

Cultivars	Tests		
	FC (%)	G (%)	GSI
Catuaí Amarelo IAC 44	40[a]	72[c]	4.45[a]
Mundo Novo IAC 376-4	42[a]	80[ab]	4.61[a]
Travessia MGS	36[a]	77[bc]	4.73[a]
Rubi MG 1192	47[a]	86[a]	4.91[a]
CV(%)	14.27	4.07	7.37

Means followed by the same lower case letter in the column do not differ by Tukey test at 5% probability.

discontinuous system (7.5% separating gel and 4.5% concentration gel). The gel / electrode buffer system used was Tris-glycine pH 8.9. To perform the eletrophoretic run, 60 µl of the supernatant of the extracted material were applied to the gel channels and the run was performed at 4°C, 150 V, for 6 h. At the end of the run, the gels were revealed for the following enzyme systems: esterase (EST), malate dehydrogenase (MDH), superoxide dismutase (SOD), catalase (CAT) and alcohol dehydrogenase (ADH), according to the methodology described by Alfenas, (2006).

In order to compare the germination potential of the coffee seeds submitted to the LERCAFE test, and to the control treatment (T), Tukey test at 5% probability was used. Data were subjected to analysis of variance and the means were compared by Tukey test. The experiment was installed in a completely randomized design with four replications of 50 seeds. Statistical analyzes were performed with the aid of SISVAR® statistical program (Ferreira, 2010).

RESULTS AND DISCUSSION

Experiment 1: Adequacy of the LERCAFE test methodology

The moisture content of coffee seeds at the time of testing was 16% for Catuaí Amarelo and Mundo Novo, 15% for Rubi and 17% for Travessia. By listing the cultivars batches (Table 1), it is observed that the germination rate did not vary among cultivars, however a superiority was observed in the germination of the cultivar Rubi compared to Catuaí Amarelo and Travessia.

In Table 2, assessments of viability obtained by LERCAFE test are observed (visual evaluation). Treatments of 2.5% of active chlorine for 3 h and 3.5% of active chlorine for 2 and 3 h allowed distinguish cultivars at two levels of quality, being Rubi, Travessia and Mundo Novo superior compared to the cultivar Catuaí Amarelo, with inferior quality.

The coffee seeds immersed for 2 h in a sodium hypochlorite solution containing 2.5% of active chlorine, did not have greenish coloration in the endosperm, precluding their assessment (Table 2). Zonta et al. (2010) when testing the efficiency of LERCAFE test, failed to achieve satisfactory results for the treatments in which the coffee seeds were immersed in a sodium hypochlorite solution containing 2.5% of active chlorine for the periods of 1 and 2 h, because there was absence of staining for these treatments. For treatments of 3.5% of active chlorine for 6 h, 5 and 6% of active chlorine for 2, 3 and 6

Table 2. Estimates of viability (%) of coffee cultivars by LERCAFE test.

Treatment concentration/period (%)	Estimates of viability (%) of cultivars			
	Catuaí amarelo IAC 44	Mundo novo IAC 376-4	Travessia MGS	Rubi MG 1192
2.5 /3 h	76^{Ba}	88^{Aa}	83^{Aa}	86^{Aa}
2.5 /6 h	61^{ABb}	53^{CDb}	69^{Ab}	46^{Bb}
3.5 /2 h	75^{Ba}	86^{Aa}	81^{Aa}	85^{Aa}
3.5 /3 h	79^{Ba}	89^{Aa}	80^{Aa}	88^{Aa}
CV(%)		9.38		

Means followed by the same letter, capital in the line and lower case in the column, do not differ, by Tukey test at 5% probability. * No staining. ** Excess staining.

Table 3. Percentage of germination obtained by germination test of coffee seeds cultivars after being subjected to the LERCAFE test.

Treatment Concentration/period (%)	Cultivars			
	Catuaí amarelo IAC 44	Mundo novo IAC 376-4	Travessia MGS	Rubi MG 1192
2,5 /3 h	72^{Ba}	83^{Aa}	80^{Aa}	83^{Aa}
2,5 /6 h	23^{Bb}	38^{Ab}	45^{Ab}	18^{Ab}
3,5 /2 h	70^{Ba}	80^{Aa}	79^{Aa}	80^{Aa}
3,5 /3 h	68^{Ba}	81^{Aa}	80^{Aa}	83^{Aa}
CV(%)		11.09		

Means followed by the same letter, capital in the line and lower case in the column, do not differ, by Tukey test at 5% probability. * No staining. ** Excess staining.

h, an intense dark green color was observed, occupying a large part of the seeds´ endosperm, this complicated their assessment by LERCAFE test, and data were not computed.

When observing the data in the germination test after the LERCAFE test, only the cultivar Catuaí Amarelo was distinguished as inferior to the other (Table 3). In the treatment of 2.5% for 6 h, it was difficult to distinguish the quality of seeds with excessive staining of the endosperm, preventing the assessment of seeds viability by LERCAFE test. For this treatment, low percentage of germination by the germination test (Table 3) was observed, indicating that the immersion period of 6 h affected negatively seeds´ physiological quality. It is observed, in general, that the LERCAFE test overestimates the results of seed viability. The results of the LERCAFE and germination tests match, however, these results may show considerable discrepancies, due to possible infestation with pathogens in the batch. So, not all abnormalities found in seedlings can be observed in the embryo and, as a result, the LERCAFE test can provide superior results.

The choice of the appropriate methodology for the employment of LERCAFE test should be based on the ease of differentiation of viable and non-viable tissue, and on the ability to differentiate batches with different physiological qualities. Another factor that must be taken into account in the assessment of seed viability is the execution time of the test, since a rapid evaluation provides advantages such as the possibility of dropping batches with inadequate quality, also requiring adjusting the concentrations of active chlorine solution.

For this experiment, commercial sodium hypochlorite solutions were used, containing 10% of active chlorine. According to Brazil (2005), the sodium hypochlorite in concentrated solutions degrades under the influence of light and heat; so, the quantification of this solution is essential for standardization of the test. To standardize the LERCAFE test, it is essential to standardize the sodium hypochlorite solution. In order to find a treatment that allows the distinction of batches in different levels of physiological quality, making the test reproducible is important. Thus, it was the second experiment that was performed in which the content of active chlorine present in the sodium hypochlorite solution was quantified. For this, the efficiency of the LERCAFE test with seeds immersed in sodium hypochlorite solution with contents of 1, 2, 3, 4 and 5% respectively of active chlorine for the periods 1, 2, 3, 4 and 5 h at 30°C were assessed.

Experiment 2: Adequacy of the LERCAFE test methodology with the content of active chlorine quantified

The moisture content of the coffee seeds of the cultivar

Table 4. Results of the estimation of coffee seeds viability by LERCAFE test, performed with different concentrations of active chlorine and immersion periods.

	Percentage (%)	Immersion periods (h)				
		1 h	2 h	3 h	4 h	5 h
Level of active chlorine (%)	1	-	-	-	-	-
	2	96^{ns}	93^{ns}	91^{ns}	91^{ns}	87*
	3	96^{ns}	93^{ns}	87*	91^{ns}	45^{ns}
	4	94^{ns}	92^{ns}	73^{ns}	+	+
	5	94^{ns}	94^{ns}	61^{ns}	+	+

*Means equal to the control treatment (82% of germination) by Dunnett test at 5% probability. ns - not significant. Absence (-) and excess (+) of staining of the endosperm.

Table 5. Percentage of germination of coffee seeds, Catuaí Vermelho IAC 99, control treatment (T) and treatments of seeds submitted to LERCAFE test.

Treatment	Germination (%)
Control	82^{a}
2% of active chlorine / 1 h of immersion	78^{a}
2% of active chlorine / 3 h of immersion	79^{a}
2% of active chlorine / 5 h of immersion	81^{a}
3% of active chlorine / 1 h of immersion	82^{a}
3% of active chlorine / 3 h of immersion	79^{a}
3% of active chlorine / 5 h of immersion	53^{b}

Means followed by the same letter do not differ, by Tukey test at 5%.

Catuaí Vermelho IAC 99 was 16%, the germination percentage was 82%, and the germination speed index equal to 4.31. Treatments in which seeds were immersed in solution containing 2 and 3% of active chlorine for the periods of 5 and 3 h, respectively, were effective in the estimation of viability by LERCAFE test, because they had means statistically equal to the control (Table 4).

For the other immersion periods, effective treatments were not observed in the estimation of the viability by LERCAFE test. The treatments in which seeds were immersed in a sodium hypochlorite solution with levels of 4 and 5% for periods of 4 and 5 h did not allow the evaluation by LERCAFE test, due to excessive coloration of the endosperm (Table 4). As for all immersion periods in sodium hypochlorite solution with level of 1% of active chlorine, it was not possible to assess seeds by LERCAFE test, because these treatments were not enough to color the coffee seeds' endosperm, precluding their evaluation.

The results showed that increasing the immersion period, associated to the increase of the active chlorine concentration, cause excessive staining of the endosperm, precluding visual assessment by LERCAFE test. Zonta et al. (2010) also failed to achieve satisfactory results for LERCAFE test when sodium hypochlorite solution with high levels of active chlorine combined with prolonged immersion periods was used. They reported that the test underestimated the results of coffee seeds germination under these conditions.

In order to associate the enzyme activity with what was observed by LERCAFE test, it the enzymatic behavior of the treatments which were effective in estimating the viability by LERCAFE test in experiment 2 (Table 4) was assessed. For better interpretation of the results, treatments were added with immersion periods above and below those found for effective treatments. So, the enzymatic behavior of the following treatments was assessed: control (seeds not subjected to LERCAFE test) treatments in which seeds were immersed in with levels of 2 and 3% of active chlorine for the periods 1, 3 and 5 h.

Comparing the germination percentage of the control treatment (T) with germination results found for the treatments in which seeds were subjected to LERCAFE test, it was observed that only the treatment in which seeds were immersed in a sodium hypochlorite solution, containing 3% of active chlorine for a period of 5 h, showed germination statistically lower than the other treatments (Table 5).

Figure 2 shows the zymogram for the esterase enzyme (EST). According to Santos et al. (2004), this enzyme participates in the hydrolysis of esters reactions, and is directly linked to the lipid metabolism and to the degenerative process of membranes. For treatments with

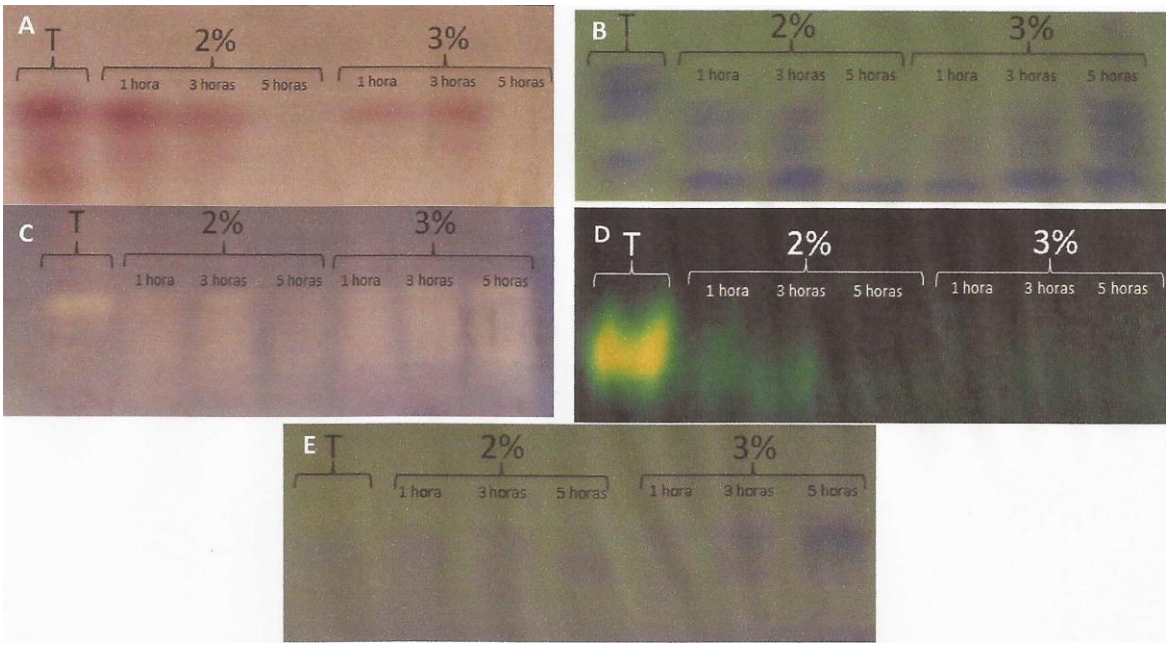

Figure 2. Enzymatic patterns of coffee seeds, Catuaí Vermelho. A - EST, B - MDH, C - SOD, D - CAT, E - ADH. Control treatment (T) and treatments in which seeds were subjected to LERCAFE test (immersed in sodium hypochlorite solution with levels of 2 and 3% of active chlorine for the periods 1, 3 and 5 h of packaging).

5 h of immersion in concentrations of 2 and 3%, there was no activity of the EST. This absence may be due to the effect of prolonged immersion period of seeds in sodium hypochlorite solution. The seeds under treatment of 3% for 5 h showed low germination, indicating that the degradation processes were possibly activated (Baker, 1962). However, the seeds under treatment of 2% for 5 h did not show low germination percentage, indicating the effect of exposure of seeds to treatments with high levels of active chlorine, as well as prolonged immersion periods in the activity of EST (Table 1).

The reduction of the esterase isoenzymes activity can be related to the self-oxidation of fatty acids (Flood and Sinclair, 1981) and loss of integrity of the membrane system and release of lipid or denaturing of the enzyme (Machado, 2000). The malate dehydrogenase enzyme (MDH) catalyzes the last reaction of the Krebs cycle (Tunes et al., 2011). This is an important enzyme for cellular respiratory process, the increase of its activity may be due to the increase of its expression in different cell compartments and / or to the induction of enzyme activity expressed by a higher intensity of the bands. This may have occurred due to the increase of respiration in seeds that were in deteriorating process, since the enzymes involved in respiration may be activated in seeds with reduced quality as in the study of Shatters et al. (1994). It was noted that treatments with higher germination had a stable or decreased enzyme activity. As the treatment of 3% for 5 h had a greater influence of active chlorine (Figure 2). The superoxide dismutase (SOD) and catalase (CAT) enzymes are efficient mechanisms in cellular detoxification process, participating in removing process of free radicals (Taveira et al., 2012).

Among the enzymes responsible for the defense system, the SOD is one of the most important (Corte et al., 2010). This group of metalloenzymes catalyzes the formation of hydrogen peroxide from superoxide radicals, protecting the cell from oxidative processes (Taveira et al., 2012). However, the accumulation of peroxide can also be toxic to the cell, and can kill it, especially in the presence of iron (Eanton, 1991). Looking at Figure 2, it is noted that there was intense activity of SOD for all treatments, with low activity of this enzyme for the control treatment (T), showing the effect of treatment of LERCAFE test on SOD activity, that possibly activated the antioxidant system, as consequence to the stress generated by immersing the seeds in sodium hypochlorite solution. The treatment of 3% for 5 h showed the most intense band for this enzyme, and the seeds subjected to this treatment were the ones that showed the greatest drop in germination potential (Table 1).

Through the oxidation-reduction cycle, the CAT acts as a key enzyme in the removal process of hydrogen peroxide, participating in the control of these endogenous peroxides (Ataíde et al., 2012). Thus, the reduction of CAT activity reduces the ability to prevent against oxidative damage. As the CAT is an enzyme being able to perform the detoxification of $O_2^{\cdot}$ and H_2O_2 (Taveira et al., 2012). But, looking at Figure 2, it is noted that there were no changes in the enzyme profile for this enzyme,

being that the CAT activity wasn't visible for the control treatment (T). Probably the stress caused by immersing seeds in sodium hypochlorite solution caused the inhibition of this antioxidant system (Figure 2).

In Figure 2, the progressive increase in the alcohol dehydrogenase enzyme (ADH) activity was observed, as it increased the content of active chlorine of the sodium hypochlorite solution, as well as the immersion period. This enzyme acts on anaerobic metabolism, reducing acetaldehyde to ethanol and oxidizing NADH to NAD^+ ADH (Buchanan et al., 2005). Tunes et al. (2011) describe that acetaldehyde is responsible for the acceleration of deteriorating process in seeds, which coincides with what was observed in the treatment of 3% for 5 h.

Conclusion

The LERCAFE test allows the determination of coffee seeds' physiological potential. The coffee seeds subjected to LERCAFE test show changes in the activity of EST, MDH, SOD, CAT and ADH enzymes, and the activation or deactivation of these enzyme systems vary with the concentration and immersion time in the solution of active chlorine.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The author sincere thanks goes to Agricultural Research Corporation of the State of Minas Gerais (EPAMIG) for providing the seeds, and also to the Research Support Foundation of the State of Minas Gerais (FAPEMIG) for their financial support.

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academicJournals

Vol. 11(15), pp. 1289-1298, 14 April, 2016
DOI: 10.5897/AJAR2015.10100
Article Number: 4AB9E4D58041
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Morpho-biochemical responses to salinity tolerance in common bean (*Phaseolus vulgaris* L.).

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Received 2 July, 2015; Accepted 6 October, 2015

The effect of salinity stress during germination, early seedling and vegetative growth on morphological and biochemical traits was evaluated for 18 genotypes of common bean *(Phaseolus vulgaris* L.) at 0, 60, 120, and 180 m*M* NaCl. Analysis of variance showed that the salinity stress had significant effect on all traits except shoot to root length and dry weight ratios. Though salinity stress delayed germination in all accessions, three local landraces, 'Naein', 'Lordegan' and 'Talash' germinated fastest under high salinity (120 *mM* NaCl). The Na uptake among the cultivars studied suggested that 'COS-16' (1.12 mg/g) and 'Naein' (1.07 mg/g) were most tolerant to salinity. Conversely, 'Cardinal' (1.89 mg/g) and 'Talash' (1.89 mg/g) that had the highest Na uptake were considered as the most susceptible cultivars. Seeds that germinated rapidly at 60 m*M* NaCl also germinated rapidly at 120 m*M* NaCl. At 180 m*M* NaCl, several accessions reached 50% germination by 6 days, demonstrating high genetic potential within *P. vulgaris* for salinity tolerance during germination. The biomass of radicles plus hypocotyls decreased with increasing salinity. Cluster analysis separated the accessions into three groups. Group I included salt sensitive accessions with late germination, high sensitivity index, and reduced seedling growth. Group II included salt tolerant accessions with rapid germination, high sensitivity index, and enhanced seedling growth. Group III only included cultivated accessions corresponding to the CIAT gene pool with rapid germination, low sensitivity index, and intermediate seedling growth.

Key words: *Phaseolus vulgaris* L., salinity stress, Na^+ ions, morphological and biochemical traits.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important source of dietary protein in many developing countries. Common bean like many other leguminous crops is sensitive to salinity, and suffers reduced yield even if it is grown at soil salinity less than 2 dS m^{-1} (Maas and Hoffman, 1977). Plants growing under saline conditions are stressed basically in three ways; (1) Reduced water potential in the root zone causing water deficit; (2) Phytotoxicity of ions such as Na^+ and Cl^-, and (3) Nutrient imbalance by depression in uptake and/or shoot transport (Lauchli, 1984; Munns and Termatt, 1986; Gama et al., 2007). This is attributed to the fact that Na^+ competes with K^+ for binding sites essential for cellular function. This role makes K^+ an important element as more than 50

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enzymes are activated by K^+, and Na^+ can not substitute in this role. On one hand, the latter implication of these two macronutrients in salinity is thought to be one of the factors responsible for reduction in the biomass and yield components. Several studies such as genetic variability of cultivated *Phaseolus* bean cultivars exposed to salinity at germination stage, seedling stage (Bayuelo-Jimenes et al., 2002a) and early vegetative growth (Bayuelo-Jimenes et al., 2002b) have been conducted.

One approach to reducing the deleterious effects of soil salinity on crop production is the development of salt-tolerant cultivars (Epstein et al., 1980). In certain species, this may be achieved by exploiting intra specific variability. However, when such variability is limited, as occurs in many crop species, genes may be transferred from closely related wild species adapted to high salinity. A large number of accessions of cultivated species of Leguminosae have been evaluated for salt tolerance. These include faba bean *(Vicia faba* L.) (Abdel-Ghaffar et al., 1982), chickpea *(Cicer arietinum* L.), mung bean *[Vigna radiata* (L.) Wilczek] (Lauchi, 1984), pigeon pea *[Cajanus cajan*(L.) Millsp.] (Subbarao et al., 1991), and common bean *(P. vulgaris)* (Moreno-Limon et al., 2000). However, few salt tolerant genotypes were identified in these studies.

Evidence collected from various species suggests that salt tolerance is dependent on the stage of development; such that tolerance at one stage of development may not be correlated with tolerance at other developmental stages. The objective of this study was to evaluate morphological and biochemical characteristics responses of eighteen common bean varieties to salinity stress and was undertaken to characterize variability for NaCl salinity tolerance in, during seed germination, early seedling and vegetative growth.

MATERIALS AND METHODS

Two independent split plot experiments were conducted in the form of complete randomized block design in growth chamber and greenhouse. State the arrangement and number of replications.

Germination assay and early seedling growth

In this study, 18 *Phaseolus* accessions were evaluated for salt tolerance during germination and early seedling growth at 0, 60, 120, and 180 *mM* NaCl concentration (with electrical conductivity values of < 0.1, 5.2 , 11.1, and 17.0 dS m^{-1} and a water potential of the salt solution of -0.05, -0.28, -0.57, and -0.85 MPa, respectively). Seeds were manually scarified by removing approximately 1 mm of the testa with a scalpel. Before scarification, seeds were surface sterilized with 10% sodium hypochlorite solution for 5 min, rinsed with sterile distilled water several times, and briefly blotted onto sterile paper towels. Ten seeds were used for germination in covered, sterilized disposable Petri dishes (110×110×10) containing germination paper (Anchor Paper Co., St. Paul, MN) moistened once with 10 mL of distilled water or NaCl solution. The Petri dishes were tightly sealed with Parafilm (American Can Co., Greenwich, CT) (O_2 permeable) to prevent evaporation of water, thus minimizing changes in concentration of solutions. A randomized complete block design with a split plot arrangement of treatments and three replications was used with NaCl levels as the main plots and accessions (as a group of ten seeds per dish) randomized within each main plot. The Petri dishes were placed in a dark growth chamber. The mean temperature was 30°C and relative humidity was 80%. Temperature and relative humidity were measured and controlled automatically in a computerized growth chamber.

Seeds were considered germinated when the emergent radicle reached 2 mm in length. Percentage germination was recorded each 12 h for 6 day. On the 7th day, fresh weights of radicles and hypocotyls were measured. Subsequently the radicles and hypocotyls were dried at 65°C for 72 h, and weighed. Mean radicle dry weight was calculated based on total radicle dry weight related to each Petri dish in the number of germinated seeds in each Petri dish to evaluate speed of germination (Cotyledons were not included in fresh and dry weight comparisons, since they reflect imbibition rather than growth). In calculating the time of germination (that is, time from imbibition to radicle emergence), seeds that germinated within an interval were presumed to have germinated at the midpoint of that interval. The control treatment was used to estimate potential germination of seeds within each accession.

Establishment and vegetative growth

Seeds were sown in bed for germination and seven days old seedlings of uniform size from each genotype were transferred to rectangular containers of 90×60×25 cm size which were filled with half strength Hoagland's solution. Spacing was 10 cm between and within rows. Roots were slipped through a hole in the grid and the plants were held in place with a wrapping of Dacron batting around bases. The internal surface of the grid was covered with foil to prevent algal growth in the solution. The pH of the solution was periodically adjusted (usually once a day) to pH 5.8 ± 0.2. The plants were grown on this control solution until the emergence of the first trifoliate leaf (6-7 days after transplanting), and then salt stress treatments were initiated. Nutrient solution for plants with salt stress was identical to that for controls except for the addition of NaCl to the appropriate concentration. In the salt stress treatment, the first increment of salt, containing 60 *mM* NaCl was added 7 days after transplanting and additional increments of the same composition were added daily until the salt concentration reached the final treatment level of 180 *mM* NaCl. Treatments were replicated 3 times and arranged into spilt plot in the form of randomized complete block design. Finally, 3 weeks after conduction of salinity treatment, data were collected on plant height, root length, shoot dray weight, root dray weight, shoot to root biomass ratio, plant height to root length ratio, uptake and K^+/Na^+ and Na^+/Ca^{2+} ratio.

Ion analysis

In order for ion analysis, 0.5 g of finely grounded shoot samples were burned at 550 for 5 h, then cooled and 5 ml HCL 6 N added, heated for few minutes. The resulting filtrates were stored at a temperature of 4°C until measurement. Sodium ions were determined using flame emission spectrophotometer AA6700 (Shimadzu Corporation, Kyoto, Japan). Ca^{2+} concentration was estimated based on titration approach.

Statistical analysis

Data from two experiments were independently analyzed. Before analysis of variance, data of mean values of tolerance for each accession for each variable were subjected to tests for heterogeneous error variances by the Bartlett's test. Error variances were homogeneous thus data were not transformed. Statistical differences were ascertained from the SAS Generalized Linear Models Procedure. A protected least significant difference (PLSD) was constructed when the *F-tests* indicated statistically significant differences among genotypes *(P*< 0.05). Ward's minimum variance clustering method was used to classify the accessions into discrete clusters. The optimum number of clusters was determined by MANOVA procedure (Sorkhe et al., 2007).

RESULTS

Morphological characteristics related to salinity tolerance

Comparison of mean values of genotypes showed high variability, ranging from minimum (0.619 g) to maximum (1.729 g) for 'Aligodarz' and 'Khomain-2' landraces, respectively. Maximum RaFW was obtained for 'G-14088' (2.965 g), 'Khomain-2' (2.202 g) and 'MCD-4024' (0.621 g) genotypes at salinity levels of 60, 120 and 180 mM NaCl, respectively (Table 1). In addition, 'Khomain-2' had maximum RaDW at 0 (0.381 g) and 60 (0.289 g) m*M* NaCl. However, differences of genotypes for RaFw and RaDW at 180 m*M* NaCl were not significant (Table 1).

Comparison of mean values showed that genotypes for germination speed (Table 1) were significantly different for GP in different salinity levels, but the difference between genotypes was only significant at 120 and 180 m*M* NaCl for GS. 'Kohdasht' local landrace showed minimum GS (0.01) at 120 and 180 m*M* NaCl salinity level. However, cultivars 'CRAN75' with 0.74 and 'Talash' with 0.34 were maximum for this trait at 120 and 180 m*M* NaCl salinity level, respectively (Table 1). Root growth was reduced in all genotypes as the salinity level increased. The local landrace 'Naein' produced maximum root dry weight (RDW) of 1.05, 0.88, 0.87 and 0.83 g in 0, 60, 120 and 180 mM NaCl salinity level, respectively (Table 1). Root length (RL) was also reduced as salinity level increased. Mean per all genotypes was ranged from 7.26 to 12.87 cm at 180 mM NaCl and control, respectively. 'Naein' landrace produced longer roots relative to other genotypes at all salinity levels with maximum 20.6 cm in control, while minimum RL (4.3 cm) was produced by 'Aligodarz' at 180 mM NaCl (Table 1). Genotypes were significantly different for plant height. The landrace 'Naein' (41.76 cm) and 'Mich Map' (16.93 cm) showed maximum and minimum PH in control. In addition, 'Naein' landrace showed maximum PH in all salinity levels (Table 1). The SDW reduced as result of increasing salt concentration, ranging from 4.99 g for 'Talash' in control to 0.65 g for 'Daneshju' in 180 mM NaCl , respectively (Table 1). The values obtained for SDW/RDW ranged from 2.61 to 8.43 (Table 1). Higher and lower values for this ratio were observed in 'G-14088' and 'COS-16', respectively (Table 1).

Biochemical characteristics related to salinity tolerance

Sodium analysis showed that when used overall salinity levels are, 'COS-16' with 1.12 mg/g and 'Naein' with 1.07 mg Na^+ per gram dry leaf accumulated less amount of Na^+ than other genotypes. Conversely, 'Cardinal' and 'Talash' with 1.89 mg/g had the highest amount of Na^+ uptake. Non significant differences were observed among genotypes at control, however, genotypes showed significant differences in the salinity level (Table 2). The 'Naein' accumulated the least amount of Na^+ at 60 mM (0.43 mg/g) and 120 mM (1.23 mg/g) salinity. The amount of Na^+ accumulation of 'Naein' landrace at 180 mM salinity was also low (Table 2). Among genotypes, 'CRAN75' and 'Cardinal' were the most and the least K^+ accumulating at all salinity levels, respectively (Table 2). Assessment of genotypes in different salinity levels showed that in control condition 'Aligodarz' had the least amount of Ca (0.33 mg/g) and 'Talash' had the highest amount (3.04 mg/g) of Ca uptake. The amount of Ca accumulation of 'Naein' in 60 and 120 m*M* NaCl was also high (Table 2). Evaluation of genotypes for Na^+/Ca^{2+} ratio showed that 'Khomain-5'with mean 0.57 had the least and 'G-14088' with mean 1.97 had the highest amount. Differences of genotypes for this ratio were non-significant at control level, but were highly significant in other salinity levels (Table 2). The results obtained for K^+/Na^+ ratio indicated highly significant differences among genotypes, salinity levels and interaction of genotypes into salinity (Table 2). Genotypes 'CRAN75' 220.97 and 'Kohdasht' 63.13 had the highest and the least values of this ratio in control. However, difference of genotypes in other salinity level was not significant (Table 2).

Correlation

Correlation coefficients were positive and highly significant *(P* < 0.01) for root dry weight (RDW) and shoot dry weight (SDW) with plant height (PH), root length (RL), germination percent (GP) and speed of germination (GS). Correlation coefficients between radicle fresh weight (RaFW) and radicle dry weight (RaDW) with germination percentage and speed of germination were positive and highly significant *(P* < 0.01). A positive association was found between plant height, root length and shoot dry weight with Ca content. A negative association was found between amount of K and RDW, SDW, PH and RL (Table 3).

Cluster analysis

The MANOVA method was used in this study to cluster

Table 1. Mean comparison for radicle fresh weight (RaFW), radicle dry weight (RaDW), root length (RL) and root dry weight (RDW) of 18 common bean genotypes evaluated at 0, 60, 120 and 180 mM NaCl salinity levels.

Genotype	Salinity levels							
	0	60	120	180	0	60	120	180
Naien	§1.520^{abc} 20.60^{a}	0.985^{defg} 13.30^{a}	0.843^{bcde} 12.03^{a}	0.415^{a} 12.43^{a}	ᵑ0.248^{cdef} 1.05^{b}	0.155^{ef} 0.88^{b}	0.105^{defgh} 0.87^{a}	0.043^{a} 0.83^{b}
Mich map	1.575^{bcd} 18.50^{ab}	1.575^{cde} 12.60^{abc}	0.963^{bcd} 9.90^{ab}	0.244^{a} 6.90^{cde}	0.355^{ab} 0.62^{a}	0.253^{abc} 0.35^{e}	0.155^{cd} 0.26^{ef}	0.053^{a} 0.23^{def}
COS-16	2.44^{ab} 17.90^{abc}	1.323^{cdef} 11.6^{abc}	0.399^{de} 9.10^{abcd}	0.356^{a} 6.23^{cde}	0.257^{cde} 0.36^{fgh}	0.138^{f} 0.26^{fghi}	0.049^{ghi} 0.21^{efghi}	0.032^{a} 0.23^{def}
CRAN75	1.504^{bcd} 12.80e^{fg}	1.693^{fg} 9.13^{bcde}	1.183^{bc} 7.70^{bcdef}	0.552^{a} 7.56^{cde}	0.256^{cde} 0.28^{i}jk	0.261^{ab} 0.21^{i}j	0.142^{cde} 0.21^{efg}	0.064^{a} 0.19^{efg}
Sharekord	1.328^{cd} 8.1jk	0.885^{fg} 7.03^{de}	0.405^{de} 5.30^{ef}	0.248^{a} 4.73^{e}	0.197^{efg} 0.22kl	0.061^{g} 0.21^{i}j	0.039^{hi} 0.17^{gh}	0.027^{a} 0.16^{fg}
MCD-4024	1.660^{bcd} 9.06^{hi}jk	1.676^{cde} 7.33^{de}	1.463^{b} 6.30^{bcdef}	0.621^{a} 5.22^{de}	0.288^{bcd} 0.35^{ghi}	0.200^{bcdef} 0.26^{fghi}	0.166^{cd} 0.22^{efg}	0.074^{a} 0.2^{efg}
Cardinal	1.940^{abc} 12.03e^{fgh}	0.559^{g} 11.13^{abc}	0.437^{de} 9.35^{abc}	0.258^{a} 5.43^{de}	0.293^{bc} 0.32^{ghi}j	0.134^{f} 0.30^{efg}	0.191^{bc} 0.22^{efg}	0.028^{a} 0.19^{efg}
Khomain-5	1.801^{bc} 16.60^{bcd}	0.201^{defg} 11.40^{abc}	0.276^{cde} 9.16^{abcd}	0.081^{a} 8.50^{bcd}	0.217^{defg} 0.77^{c}	0.144^{f} 0.50^{d}	0.304^{hi} 0.47^{c}	0.007^{a} 0.40^{c}
Tylor	2.076^{ab} 16.43^{bcd}	1.048^{defg} 10.50^{abcd}	0.716^{cde} 9.85^{ab}	0.294^{a} 8.16^{bcde}	0.218^{defg} 0.54^{e}	0.145^{f} 0.50^{d}	0.118^{defg} 0.46^{cd}	0.038^{a} 0.41^{c}
Aligodarz	1.085^{d} 6.40k	1.059^{defg} 6.25^{e}	0.185^{e} 4.36^{f}	0.149^{a} 5.13^{cde}	0.174^{fg} 0.19l	0.166^{def} 0.16^{i}j	0.318^{hi} 0.14^{h}	0.007^{a} 0.12^{g}
MCD-4017	2.060^{ab} 8.35^{i}jk	2.383^{ab} 7.50^{de}	0.470^{de} 6.00^{cdef}	0.389^{a} 5.68^{de}	0.309^{bc} 0.28^{hi}jk	0.239^{abcd} 0.28^{fghi}	0.041^{ghi} 0.22^{efg}	0.033^{a} 0.22^{def}
Daneshju	1.253^{cd} 11.6e^{fgh}	1.090^{defg} 10.40^{abcd}	0.848^{bcde} 5.60^{def}	0.351^{a} 6.72^{cde}	0.145^{g} 0.43^{f}	0.140^{f} 0.23^{hi}j	0.073^{efghi} 0.18^{fgh}	0.042^{a} 0.19^{efg}
Khomain-2	2.214^{ab} 10.90^{fghi}	1.894^{bc} 9.00^{cde}	2.202^{a} 7.06^{bcdef}	0.572^{a} 6.76^{cde}	0.381^{a} 0.29^{hi}j	0.289^{a} 0.24^{ghi}	0.273^{b} 0.23^{efg}	0.068^{a} 0.24^{de}
G-O1437	1.534^{bcd} 12.03e^{fgh}	0.912^{fg} 10.60^{abcd}	0.722^{cde} 6.00^{cdef}	0.292^{a} 7.20^{cde}	0.260^{cde} 0.39^{fg}	0.181^{cdef} 0.29^{efgh}	0.308^{a} 0.22^{efg}	0.062^{a} 0.16^{efg}
Talash	2.092^{ab} 14.63^{cde}	0.977^{efg} 12.70^{ab}	0.873^{bcde} 12.30^{a}	0.057^{a} 11.03^{ab}	0.319^{bc} 0.79^{c}	0.162^{def} 0.59^{c}	0.128^{cdef} 0.4^{d}	0.005^{a} 0.35^{c}
G-14088	2.516^{a} 9.88^{ghi}j	2.965^{a} 9.06^{bcde}	0.210^{e} 8.36^{bcde}	0.083^{a} 8.00^{bcde}	0.298^{bc} 0.36^{gh}	0.233^{abcde} 0.33^{ef}	0.021^{i} 0.28^{e}	0.001^{a} 0.273^{d}
Kohdasht	1.273^{cd} 11.91e^{fgh}	1.253^{cdefg} 9.30^{bcde}	0.328^{de} 7.23^{bcdef}	0.063^{a} 5.86^{cde}	0.067^{h} 0.24jkl	0.126^{fg} 0.24^{ghi}	0.057^{fghi} 0.15^{gh}	0.005^{a} 0.14^{g}
Lordegan	1.088^{d} 14.00^{d}e^{f}	1.347^{cdef} 12.10^{abc}	0.857^{bcde} 9.47^{ab}	0.350^{a} 9.10^{bc}	0.167^{g} 1.12^{a}	0.156^{ef} 0.96^{a}	0.119^{defg} 0.66^{b}	0.045^{a} 0.50^{b}
Naien	†0.96^{a} 41.76^{a} 4.94^{ab}	0.60^{bc} 37.00^{a} 4.36^{a}	0.36^{ef} 32.36^{a} 3.41^{a}	0.30^{de} 30.60^{a} 3.28^{ab}	‡0.72^{a} 4.58^{b} 2.10^{def}	0.74^{a} 3.68^{a} 2.86^{b}	0.41^{def} 2.97^{a} 2.73^{abc}	0.34^{a} 2.68^{a} 2.52^{ab}
Mich map	0.83^{c}	0.60^{bc}	0.46^{cd}	0.60^{a}	0.67^{a}	0.44^{a}	0.41^{def}	0.13^{d}

Table 1. Contd.

	16.93^{g}	14.90^{gh}	17.78^{de}	15.6^{de}	2.81^{cd}	2.33^{c}	1.46^{cde}	1.31^{cd}
	4.55^{ab}	6.79^{a}	5.91^{a}	6.082^{ab}	1.89^{defg}	1.74^{cde}	1.79^{defg}	2.35^{abc}
	063^{f}	0.53^{d}	0.36^{ef}	0.20^{fg}	0.69^{a}	0.90^{a}	0.26^{ghi}	0.22^{abcd}
COS-16	19.80^{g}	16.30^{fgh}	12.08^{f}	11.56^{efg}	3.02^{c}	1.19^{fg}	1.00^{efghi}	0.88^{def}
	3.64^{ab}	4.49^{a}	4.87^{a}	3.94^{ab}	1.46^{fg}	1.46^{e}	1.38^{g}	2.004^{abc}
	0.56^{g}	0.50^{d}	0.53^{b}	0.56^{a}	0.24^{a}	0.33^{a}	0.74^{a}	0.33^{ad}
CRAN75	20.06^{g}	16.46^{fgh}	14.85^{def}	12.33^{efg}	1.62^{f}	1.42^{ef}	1.17^{efgh}	0.98^{cdef}
	5.73^{ab}	6.90^{a}	5.65^{a}	5.45^{ab}	1.10^{g}	1.82^{cde}	1.97^{cdefgi}	1.672^{bc}
	0.63^{f}	0.56^{cd}	0.40^{de}	0.20^{fg}	0.30^{d}	0.34^{a}	0.52^{cd}	0.16^{cd}
Sharekord	21.43e^{fg}	14.76^{gh}	12.63^{ef}	9.63^{fg}	1.60^{f}	1.28^{fg}	1.06^{efghi}	1.01^{cdef}
	7.56^{ab}	6.28^{a}	6.28^{a}	8.43^{ab}	1.612^{fg}	2.13^{bcde}	2.45^{abcde}	2.06^{abc}
	0.93^{a}	0.63^{b}	0.53^{b}	0.30^{de}	0.51^{a}	0.42^{a}	0.52^{cd}	0.22^{bcd}
MCD-4024	17.00^{g}	13.50^{h}	10.70^{f}	10.18^{efg}	1.56^{f}	1.32^{fg}	1.22^{defg}	1.01^{cdef}
	4.54^{ab}	5.29^{a}	5.63^{a}	4.74^{ab}	2.63^{bcd}	1.85^{cde}	1.703^{efg}	1.95^{abc}
	0.73^{d}	0.33^{f}	0.56^{b}	0.20^{fg}	0.50^{a}	0.51^{a}	0.36^{efg}	0.23^{abcd}
Cardinal	26.30^{d}e	24.20^{dc}	18.51^{cd}	14.58^{efg}	1.81^{f}	1.58^{ef}	1.10^{efghi}	0.74^{ef}
	5.82^{ab}	6.24^{a}	4.95^{a}	3.8^{ab}	2.24^{cdef}	2.25^{bcde}	2.014^{bcdefg}	2.73^{a}
	0.5^{g}	0.40^{e}	0.266^{h}	0.23^{f}	0.27^{a}	0.55^{a}	0.25^{ghi}	0.22^{abcde}
Khomain-5	34.70^{bc}	18.93^{fgh}	23.13^{bc}	21.06^{bc}	3.15^{c}	2.31^{c}	1.73^{c}	1.20^{cde}
	4.15^{ab}	4.63^{a}	3.69^{a}	2.9^{ab}	2.10^{def}	2.53^{bcd}	2.48^{abcde}	2.5^{abc}
	0.86^{bc}	0.66^{b}	0.50^{bc}	0.23^{f}	0.62^{a}	0.52^{a}	0.47^{cde}	0.15^{cd}
Tylor	27.60^{d}	19.10^{efg}	13.42^{def}	12.10^{efg}	3.16^{c}	2.75^{b}	1.31^{cdef}	1.00^{cdef}
	5.87^{ab}	5.63^{a}	2.89^{a}	2.61^{ab}	1.68^{efg}	1.99^{bcde}	1.54^{fg}	1.603^{c}
	0.66e^{f}	0.50^{d}	0.46^{cd}	0.30^{de}	0.40^{a}	0.45^{a}	0.33^{fgh}	0.12^{d}
Aligodarz	18.73^{g}	15.25^{gh}	12.613^{ef}	12.14^{efg}	1.42^{f}	1.4^{ef}	0.78^{ghi}	0.68^{f}
	7.69^{ab}	8.19^{a}	5.62^{a}	5.88^{ab}	3.09^{abc}	2.48^{bcd}	2.903^{ab}	2.49^{abc}
	0.66e^{f}	0.53^{d}	0.53^{b}	0.26^{ef}	0.46^{a}	0.64^{a}	0.33^{fgh}	0.26^{abc}
MCD-4017	25.50^{d}e^{f}	19.53^{efg}	14.68^{def}	12.9^{efg}	1.83^{f}	1.26^{fg}	0.97^{fghi}	1.04^{cdef}
	8.02^{ab}	4.84^{a}	4.88^{a}	5.12^{ab}	3.11^{ab}	2.62^{bc}	2.468^{abcde}	2.58^{ab}
	0.66e^{f}	0.50^{d}	0.30^{fg}	0.20^{fg}	0.03^{a}	0.75^{a}	0.54^{bc}	0.13^{d}
Daneshju	26.80^{d}	17.60^{fgh}	11.06^{f}	11.13^{efg}	2.53^{de}	1.46^{ef}	0.66^{i}	0.65^{f}
	5.74^{ab}	6.63^{a}	3.67^{a}	5.20^{ab}	2.54^{bcde}	1.67^{de}	1.96^{cdefg}	1.75^{bc}
	0.90^{ab}	0.83^{a}	0.76^{a}	0.46^{b}	0.32^{a}	0.66^{a}	0.51^{cd}	0.26^{abc}
Khomain-2	26.60^{d}e	25.70^{cd}	16.1^{def}	15.00^{defg}	2.43^{de}	1.76^{de}	0.76^{ghi}	0.77^{ef}
	8.25^{a}	7.44^{a}	3.42^{a}	4.35^{ab}	2.76^{bcd}	2.85^{b}	2.309^{abcdef}	2.312^{abc}
	0.83^{c}	0.66^{b}	0.46^{cd}	0.36^{cd}	0.55^{a}	0.53^{a}	0.31^{fgh}	0.23^{abcd}
G-O1437	25.43^{d}e^{f}	21.60^{def}	14.08^{def}	15.16^{ef}	2.54^{de}	2.08^{cd}	0.72^{hi}	0.81^{ef}
	7.4^{ab}	7.99^{a}	3.33^{a}	5.44^{ab}	2.17^{def}	2.09^{bcde}	2.437^{abcde}	2.177^{abc}
	0.70^{d}e	0.33^{f}	0.40^{cdef}	0.13^{h}	0.52^{a}	0.50^{a}	0.64^{ab}	0.34^{ab}
Talash	37.86^{ab}	34.10^{ab}	26.30^{b}	25.83^{b}	4.99^{a}	3.91^{a}	2.75^{ab}	2.56^{ab}
	6.37^{ab}	7.07^{a}	7.52^{a}	7.94^{ab}	2.76^{bcd}	2.79^{b}	2.165^{abcdefg}	2.386^{abc}
	0.46^{h}	0.43^{e}	0.26^{i}	0.16^{gh}	0.24^{a}	0.25^{a}	0.23^{hi}	0.26^{abcd}
G-14088	37.78^{ab}	33.2^{ab}	25.26^{b}	21.13^{c}	2.32^{e}	2.24^{c}	1.62^{cd}	1.42^{c}
	6.62^{ab}	6.93^{a}	6.43^{a}	5.28^{ab}	3.83^{a}	3.65^{a}	3.47^{a}	2.672^{a}
Kohdasht	0.50^{gh}	0.40^{e}	0.20^{fghi}	0.10^{h}	0.16^{a}	0.16^{a}	0.01j	0.01^{e}

Table 1. Contd.

	20.63^{fg}	15.83^{g}	10.66^{f}	9.46^{g}	0.89^{g}	0.91^{g}	0.69^{i}	0.67^{f}
	4.08^{ab}	3.53^{a}	4.51^{a}	4.94^{ab}	1.74^{efg}	1.71^{de}	1.494^{fg}	1.61^{c}
	$0.66e^{f}$	0.60^{bc}	0.36^{defg}	0.40^{bc}	0.63^{a}	0.44^{a}	0.16^{i}	0.32^{ab}
Lordegan	32.30^{c}	29.60^{bc}	24.24^{b}	19.95^{cd}	2.99^{c}	2.17^{c}	2.44^{b}	2.21^{b}
	2.62^{b}	2.96^{a}	3.78^{a}	4.93^{ab}	2.13^{def}	2.52^{bcd}	2.67^{abcd}	2.19^{abc}

[§]The number including RaFW and RL, respectively; [n] The number including RaDW and RDW, respectively; Values with different letters showed statistically significant differences (α=5% Duncan Test); [†] The number, indicating GP, PH and SDW/RDW ratio, respectively; [‡] The number indicating GS, SDW and PH/RL ratio, respectively.

Table 2. Evaluation of biochemical traits including Na^+, K^+, Ca^{2+}, K^+/Na^+ and Na^+/Ca^{2+} in the 18 common bean genotypes separated for 0, 60, 120 and 180 mM NaCl salinity levels.

Genotype	Salinity levels							
	0	**60**	**120**	**180**	**0**	**60**	**120**	**180**
Naien	$^{†}0.07^{a}$ 1.22^{e}	0.42^{g} 1.90^{abc}	1.23^{de} 2.193^{a}	2.56^{efg} 1.95^{a}	$^{‡}7.50^{efg}$ 102.74^{cdef} 0.06^{a}	6.20^{efg} 14.67^{a} 0.22^{e}	5.46^{de} 4.42^{a} 1.03^{def}	5.52^{cd} 2.12^{a} 1.31^{h}
Mich map	0.07^{a} 1.46^{de}	0.56^{efg} 1.84^{abc}	1.74^{abcd} 1.23^{abcd}	2.35^{fgh} 1.54^{abcd}	10.46^{c} 150.72^{b} 0.047^{a}	10.62^{b} 18.93^{a} 0.31^{e}	8.43^{b} 4.84^{a} 1.40^{cde}	8.48^{b} 3.61^{a} 1.52^{gh}
COS-16	0.11^{a} 2.24^{bc}	0.54^{fg} 2.20^{ab}	1.54^{cde} 1.16^{def}	2.31^{gh} 0.94^{def}	11.72^{b} 108.34^{cde} 0.04^{a}	8.36^{c} 15.45^{a} 0.24^{e}	8.37^{b} 5.41^{a} 1.32^{cde}	8.37^{b} 3.61^{a} 2.46^{de}
CRAN75	0.06^{a} 1.14^{e}	0.48^{fg} 1.63^{bcd}	1.51^{cde} 1.25^{bcde}	3.01^{de} 1.30^{bcde}	13.7^{a} 220.97^{a} 0.05^{a}	13.05^{a} 27.14^{a} 0.29^{e}	11.79^{a} 7.63^{a} 1.19^{cde}	10.67^{a} 3.54^{a} 2.31^{ef}
Sharekord	0.07^{a} 1.33^{de}	0.48^{fg} 1.06^{defgh}	1.620^{bcde} 0.93^{def}	3.56^{abc} 0.99^{def}	7.66^{defg} 111.01^{cd} 0.05^{a}	6.74^{e} 13.87^{a} 0.45^{de}	5.31^{de} 3.28^{a} 1.74^{bcd}	5.24^{def} 3.65^{a} 3.60^{c}
MCD-4024	0.11^{a} 1.52^{de}	1.06^{cde} 0.62^{h}	1.63^{bcde} 0.93^{ab}	3.09^{cde} 1.89^{ab}	8.56^{de} 74.05^{gh} 0.08^{a}	6.51^{ef} 6.14^{a} 1.71^{b}	5.23^{de} 3.21^{a} 1.73^{bcd}	5.00^{def} 1.61^{a} 1.63^{fgh}
Cardinal	0.06^{a} 1.25^{e}	0.73^{defg} 1.02^{defgh}	1.49^{cde} 0.80^{cdef}	2.44^{fg} 1.21^{cdef}	$4.8i$ 80.00^{fgh} 0.05^{a}	$3.11i$ 4.26^{a} 0.71^{cde}	2.83^{f} 1.89^{a} 1.86^{bc}	2.04^{g} 1.13^{a} 2.02^{cd}
Khomain-5	0.01^{a} 2.96^{a}	0.960^{def} 2.42^{a}	1.20^{e} 2.41^{a}	2.84^{ef} 1.31^{abcde}	6.84^{fg} 71.25^{gh} 0.03^{a}	5.17^{gh} 5.38^{a} 0.39^{de}	4.73^{de} 1.67^{a} 0.49^{f}	4.05^{ef} 1.43^{a} 2.11^{efg}
Tylor	0.06^{a} 2.17^{bc}	1.08^{cd} 1.27^{cdefg}	1.370^{de} 1.45^{bcde}	3.48^{abc} 1.35^{bcde}	6.51^{gh} 109.78^{cd} 0.03^{a}	5.37^{fgh} 4.96^{a} 0.85^{cde}	5.64^{de} 4.10^{a} 0.94^{ef}	5.30^{cd} 1.52^{a} 2.58^{de}
Aligodarz	0.07^{a} 0.33^{f}	0.43^{g} 1.37^{cdef}	2.23^{a} 1.55^{abcde}	2.94^{e} 1.35^{bcde}	8.81^{d} 120.68^{c} 0.22^{a}	6.47^{ef} 15.02^{a} 0.31^{e}	5.78^{de} 2.59^{a} 1.43^{cde}	4.77^{def} 1.62^{a} 2.18^{efg}
					5.50^{he}	5.30^{gh}	5.78^{de}	4.09^{f}

Table 2. Contd.

MCD-4017	0.06^{a} 2.23^{bc}	0.60^{defg} 1.06^{defgh}	1.95^{abc} 1.16^{def}	2.70^{efg} 1.16^{def}	84.66^{cdgh} 0.03^{a}	8.79^{a} 0.56^{de}	2.92^{a} 1.68^{bcd}	1.51^{a} 2.31^{ef}
Daneshju	0.06^{a} 2.27^{bc}	0.65^{defg} 2.18^{ab}	2.05^{ab} 0.99^{def}	2.68^{efg} 1.09^{def}	4.70i 78.38^{gh} 0.03^{a}	4.93^{h} 7.51^{a} 0.30^{e}	3.27^{f} 1.59^{a} 0.48^{f}	2.21^{g} 0.83^{a} 2.45^{de}
Khomain-2	0.08^{a} 1.40^{de}	0.88^{defg} 1.03^{defgh}	1.68^{bcde} 1.05^{ef}	3.44^{bcd} 0.76^{ef}	6.77^{fg} 89.08^{defg} 0.05^{a}	4.36^{h} 4.95^{a} 0.85^{cde}	2.52^{f} 1.50^{a} 1.6^{bcde}	2.14^{g} 0.62^{a} 4.51^{b}
G-O1437	0.09^{a} 1.94^{cd}	1.45^{bc} 1.35^{cdef}	1.35^{de} 0.93^{cdef}	2.88^{efg} 1.19^{cdef}	6.90^{fg} 72.66^{gh} 0.04^{a}	6.34^{efg} 4.35^{a} 1.07^{bcd}	5.06^{de} 3.73^{a} 1.45^{bcde}	5.88^{cd} 2.04^{a} 4.42^{de}
Talash	0.01^{a} 3.04^{a}	1.49^{bc} 0.93^{efgh}	2.02^{abc} 0.94^{f}	3.97^{a} 0.60^{f}	7.38^{efg} 76.87^{gh} 0.03^{a}	6.43^{ef} 4.31^{a} 1.60^{b}	4.49^{e} 2.22^{a} 2.15^{ab}	4.05^{f} 1.02^{a} 6.59^{a}
G-14088	0.08^{a} 1.03^{e}	0.64^{defg} 0.72^{gh}	1.90^{abc} 0.72^{ef}	3.58^{abc} 0.83^{ef}	8.20^{de} 107.89^{cde} 0.07^{a}	7.16^{de} 11.20^{a} 0.89^{cde}	7.13^{c} 3.75^{a} 2.64^{a}	6.44^{c} 1.79^{a} 4.31^{b}
Kohdasht	0.10^{a} 1.39^{ab}	1.82^{ab} 1.40^{cde}	1.52^{cde} 1.11^{abc}	3.61^{ab} 1.81^{abc}	6.50^{gh} 63.13^{h} 0.07^{a}	5.35^{gh} 2.93^{a} 1.30^{bc}	5.39^{de} 3.55^{a} 1.37^{cde}	5.99^{cd} 1.63^{a} 2.00^{efg}
Lordegan	0.10^{a} 2.76^{bc}	2.04^{a} 0.76^{fgh}	1.54^{bcde} 0.90^{ef}	1.96^{h} 0.86^{ef}	7.91^{def} 75.12^{gh} 0.04^{a}	7.93^{cd} 3.88^{a} 2.69^{a}	5.21^{de} 3.36^{a} 1.71^{bcd}	5.55^{cd} 2.82^{a} 2.28^{ef}

†The numbers indicating Na^{+} and Ca^{2+}, respectively. ‡ The numbers indicating K^{+}, K^{+}/Na^{+} and Na^{+}/Ca^{2+}, respectively.

analysis and the cutting point was 0.45. The stability of nodes on the dendrogram was estimated with a bootstrap procedure. The distance coefficients for genotypes of common bean varied from a maximum of 27.201 (between 'Naein' landrace and 'Aligodarz') to a minimum of 3.133 (between 'COS-16' and 'Taylor'), with average of 13.859. The dendrogram distance coefficient of 0.90, consisted of three clusters, that is, three groups of genotypes; 9, 3, 11, 12, 13, 7, 17, 14, 4, 6, 5 and 10 (cluster I); and 18, 15, 8, 2, and 1 (cluster II); and cluster III only consisted of genotype 16. Cluster I divided into two subgroups in distance of 0.72, for which subgroup Ia contained 3, 14, 4, 6, 5, 1, 9, 17 and subgroup IIb contains genotypes 7, 10, 11, 12, and 13. In addition, cluster II also divided into two subgroups in distance of 0.66, for which subgroup Ic contained genotypes 2, 8, 15, 18 and subgroup IId only contained genotype 1 (Figure 1).

DISCUSSION

In the context of this discussion, the term salt tolerance during seed germination is used only to refer to situations where the seed germinates rapidly under salt stress conditions. No distinction is made between osmotic and ionic effects of the salinity stress. Likewise, salt tolerance during early seedling growth is assessed on the absolute growth at a given salt concentration as well as the percentage of growth under salt stress relative to growth under non-stress conditions. On the basis of these two criteria, our results demonstrated genetic variation in seed germination and early seedling growth responses to salinity among *P. vulgaris* genotypes. This study indicated that 'CRAN75', 'Naein' and 'Talash' had superior germination performance at 120 and 180 m*M* NaCl levels of salt stress. A high correlation between mid germination time at 120 and 0 m*M* indicated that germination processes that facilitate rapid germination under salt and non-stress conditions possibly were controlled by similar genetic and physiological mechanisms (Foolad, 1996). Conversely, several accessions germinated rapidly under control conditions but germinated poorly at the highest salt stress levels, thus exhibiting high sensitivity indices. Consequently, in

Table 3. Correlation analysis of different traits.

Traits	RaFW	RaDW	GP	GS	RDW	SDW	PH	RL	PH/RL	SDW/RDW	Ca^{2+}	K^{+}	Na^{+}	K^{+}/Na+	Na^{+}/Ca^{2+}
RaFW	1.00														
RaDW	0.87**	1.00													
GP	0.53**	0.64**	1.00												
GS	0.41**	0.47**	0.36**	1.00											
RDW	0.13**	0.20**	0.52**	0.29**	1.00										
SDW	0.28**	0.41**	0.74**	0.34**	0.81**	1.00									
PH	0.39**	0.44**	0.68**	0.29**	0.72**	0.85**	1.00								
RL	0.29**	0.43**	0.63**	0.37**	0.69**	0.73**	0.69**	1.00							
PH/RL	-0.60**	-0.66**	0.79**	0.03**	-0.22**	-0.38**	-0.36**	-0.50**	1.00						
SDW/RDW	-0.28**	-0.33**	-0.38**	-0.009	-0.29**	-0.19**	-0.19**	-0.33**	0.46**	1.00					
Ca^{2+}	-0.36**	0.31**	-0.28**	0.09**	-0.09**	0.108	0.24**	0.36**	-0.37**	-0.21**	1.00				
K^{+}	-0.55	0.61**	0.71**	-0.087	-0.27**	-0.42**	-0.44**	-0.44**	0.81**	0.38**	0.35**	1.00			
Na^{+}	-0.40**	-0.46**	0.48**	0.41**	-0.02	-0.21**	-0.25**	-0.06	0.06	0.27**	0.30**	-0.59**	1.00		
K^{+}/Na^{+}	0.42**	0.49	0.63**	0.059	0.18**	0.35**	0.34**	0.38**	0.73**	0.32**	0.38**	-0.85**	-0.55**	1.00	
Na^{+}/Ca^{2+}	-0.53**	-0.59**	0.45**	0.36**	-0.22**	-0.13**	-0.39**	-0.43**	0.02	0.07	0.48**	0.36**	0.82**	0.50**	1.00

*, ** indicates significance at $P < 0.05$ and $P < 0.01$ respectively.

these accessions, the physiological processes required for germination were sensitive to salt. Thus, these accessions might be deficient in genetic elements required for coping with salinity (Foolad and Jones, 1993).

'Naein' landrace was the most tolerant to salinity stress, as indicated by rapid germination, relative stability, and greater seedling growth. In contrast, 'Kohdasht' landrace was less salt tolerant in terms of early seedling growth. These results demonstrate that tolerance to salinity in *P. vulgaris* genotypes might also vary with developmental stages. Salt tolerance at germination and at the seedling stage appears to be controlled by more than one gene and is highly influenced by salt concentration (Foolad and Jones, 1993). Salt stress inhibited the growth of hypocotyls more than radicles in all *Phaseolus* taxa. Similar observations have been reported in pigeon pea, *C. cajan* (Subbarao et al., 1991), and tepary bean, *Phaseolus acutifolius* A. Gray (Goertz and Coons, 1991). The consequent increase in root to shoot ratio may be helpful for salinized seedlings by improving water relations.

Reductions in the biomass of *P. vulgaris* under saline condition were indicative of severe growth limitations. Salinity had adverse effects not only on the biomass, but also on other morphological parameters such as plant height, number of leaves, root length and shoot/root ratio. In several legumes, such as faba bean (Zahran and Sprent, 1986) and P*. vulgaris* (Wignarajah, 1992), salinity was reportedly found to reduce shoot and root weights.

Our results showed that landrace of 'Naein' exhibited lower Na uptake than the others, while 'Cardinal' and 'Talash' had comparatively, the highest Na uptake. This suggests that 'Naein' is more resistant genotype because common bean is known to exclude Na^{+} from the shoot by re-absorption of Na^{+} from the xylem, but takes up Cl^{-} in proportion to external NaCl concentrations (Jacobi and Ratner, 1984). The genotypes 'Cardinal' and 'Talash' with the highest Na uptake had a low survival rate with distinct visual symptoms of salinity damage. This observation tends to confirm the report which identified correlations of high shoot Na^{+} concentrations with shoot damage as a physiological marker during screening for salinity tolerance (Gama et al., 2007). The low survival rates noticed for other cultivars could be explained by the fact that high concentrations of sodium ions in the protoplasmic constituents not only effectively inhibit metabolic functions (Gama et al., 2007), but also result to high viscosity in the cell, therefore increasing the chances of molecular interactions that cause

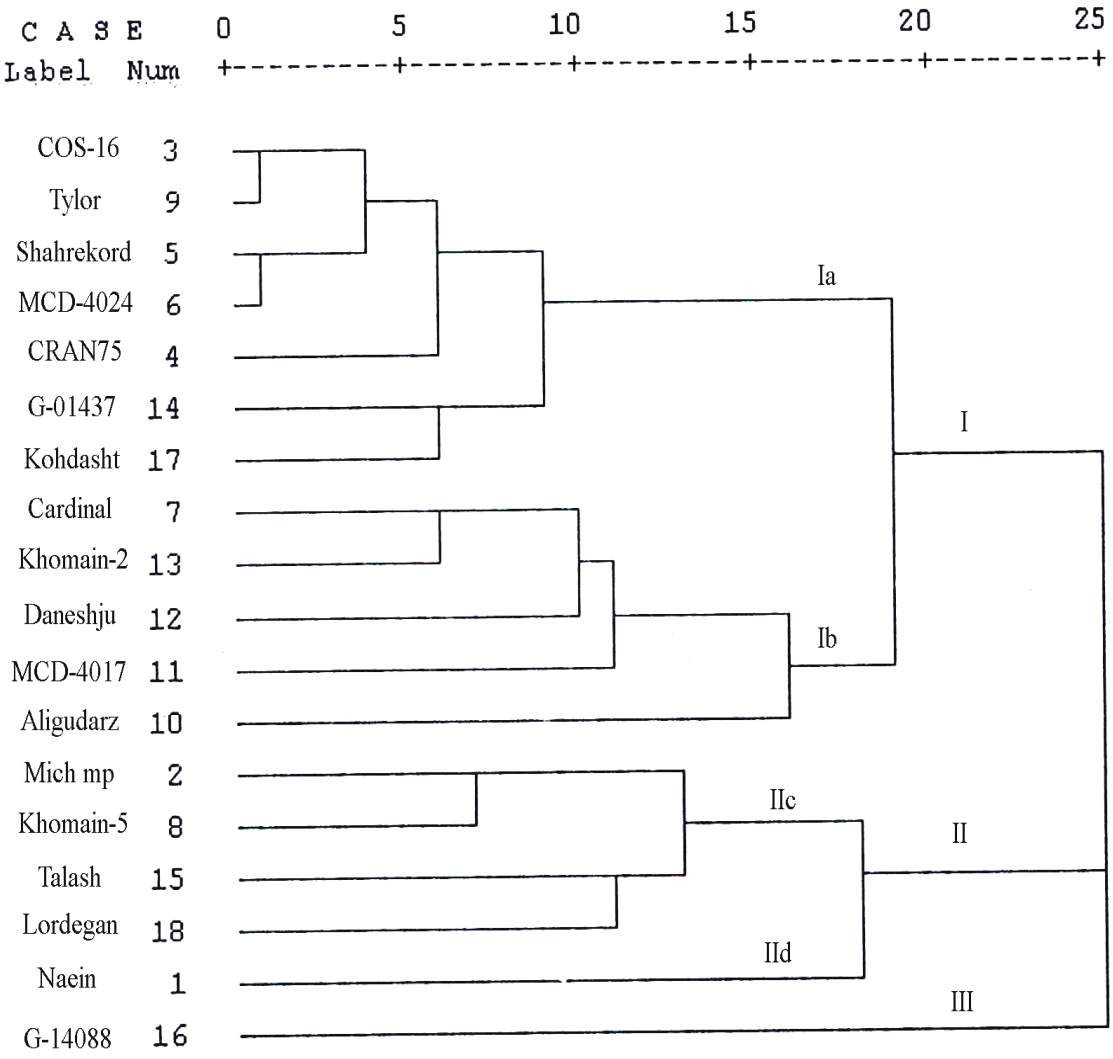

Figure 1. Ward's Minimum Variance Dendrogram of 18 *Phaseolus Vulgaris* L. genotypes. Optimum number of cluster was determined by MANOVA procedure (Sorkheh et al., 2007).

protein denaturation and membrane fusion. One interesting phenomenon about *Phaseolus* is that it tends to show signs of salinity shock at the 5th day of salt exposure and recovery in case of the salt tolerant landrace of 'Naein' that is in agreement with results that had obtained by Gama et al. (2007). The results here are unlikely in favor of findings of Bayuelo-Jiménez (2002a) and others that most of the cultivars of *P. vulgaris* compared to their wild relatives were sensitive to salinity stress because the response of 'Naein' to salinity by maintaining high dry weight and a low Na^+ concentration in shoot tissues is a unique characteristic in cultivated beans. Thus, this provides more evidence that some of the cultivated cultivars of common bean in Iran have substantially higher degree of tolerance to salinity. This is probably due to wide crosses with wild relatives for disease resistance. These retrogressed disease resistant traits, therefore, might also be of multiple or diverse importance to other environmental stresses such as salinity.

However, to evaluate biochemical, physiological and morphological responses of locally adapted common bean varieties to salinity stress, we suggest more robust methodologies, in terms of time and resources, for screening common bean for salinity tolerance. These include physiological markers such as survival rates, ion concentrations, SDW and RDW, SDW/RDW ratio and relative growth rate as essential parameters for screening for salinity. However, other morphological characters like plant height, number of leaves, leaf area, and root length and density are difficult to correlate to salinity tolerance where cultivars have different growth pattern (Gama et al., 2007).

The accessions which make up group Ia and Ib in the cluster analysis correspond to the salt sensitive genotypes. These accessions grow in tropical and

temperate subhumid climates, on rocky or sandy soils associated with tropical deciduous, although the climatic and environmental range of accessions seem not to be associated with the pattern of incidence of hot semiarid climates and saline soils (Bayuelo-Jimenez et al., 2002a).

The accessions which makes up groups IIc and IId correspond to the Iran and CIAT gene pool, respectively. These cultivated genotypes mostly distinguished by the highest RDW, RL, PH and SDW. The available range of variability for salinity tolerance in these accessions could come largely from seed size. Large seeded genotypes have more seed reserves to support seedling growth during stress periods. A high correlation coefficient between seedling growth and seed size conform that cultivated accessions having the largest seeds, exhibit the greatest seedling growth under salt stress. These results according with Bayuelo-Jimenez et al. (2002a) but in our study the correlation coefficient was relatively moderate to high. Although increased seedling growth was positively related to seed size under salt stress, such tolerance may vary with plant ontogeny. Cultivated accessions identified in this study as the most tolerant, despite results had obtained by Bayuelo-Jimenez et al. (2002a), during germination and early seedling and vegetative growth, specifically the local landrace of 'Naein' is most tolerant during different stages. Thus particular species may be differentially affected at various physiological stages of development and may not produce tolerant adult plants, for example in this study, landrace of 'Aligudarz' and 'Daneshju' from Iran gene pool clustered in subgroup Ib. The resulting information will be useful in improving the understanding of the diversity of cultivated and wild common bean. The morphological characters and ionic analysis such as Na^+ underlying theses groups provide a useful aid to target the search for new germplasm needed for future crop improvement.

In conclusion, the results of this study demonstrate that salt tolerance during germination and early seedling growth exists within *P. vulgaris* genotypes. The local landrace 'Naein' represents a genetic resource for improvement of salt tolerance in common bean.

Conflict of Interests

The authors have not declared any conflict of interest.

ACKNOWLEDGMENTS

The authors are grateful to Shahr-e-Kord University for financial assistance. Thanks to Agricultural research, Education and extension organization, seed and plant improvement institute of Tehran, Iran, for access to genotypes of common bean.

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Vol. 11(15), pp. 1299-1308, 14 April, 2016
DOI: 10.5897/AJAR2016-10870
Article Number: 3FA16D858043
ISSN 1991-637X

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African Journal of Agricultural Research

Full Length Research Paper

Physiological quality of quinoa seeds submitted to different storage conditions

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Received 3 February, 2016; Accepted 17 March, 2016

Quinoa has grown importance in the world due to the nutritional quality of its grains and crop adaptability to diverse climatic conditions. One problem that limits its cultivation is the reduced viability of seeds during storage and the information is rather scarce. This work aimed at evaluating the physiological quality of quinoa seeds along time when submitted at storage conditions and packaging. An entirely randomized experiment was conducted on factorial scheme 2 x 3 x 6 with four repetitions. The treatments consisted of 2 storage conditions: lab environment and Biochemical Oxygen Demand (B.O.D.) chamber set at 4±2°C and 90% relative humidity (RH); 3 package types: permeable, semi-permeable and impermeable; and 6 evaluations: before storage (0), 60, 120, 180, 240 and 300 days after storage. Seed viability was determined by the standard germination test while vigor by accelerated aging test, emergence in sand and emergence speed index. The use of impermeable packaging kept at low temperature maintained the physiological quality of seeds during 300 days of storage. The seeds kept in permeable or semi-permeable packaging under uncontrolled temperature and humidity conditions were viable only for 180 days. The permeable package using kraft paper was the least efficient to conserve physiological quality of quinoa seeds. It was demonstrated that quinoa seeds are rather sensitive to high temperature, loosing viability in short time.

Key words: *Chenopodium quinoa*, seed vigor, seed viability, seed conservation, packaging.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willdenow) is a pseudocereal of the Amaranthaceae family originated from the Andes of South America where it has been cultivated since more than 5,000 BC (Abugoch, 2009). The protein of its grains has a balanced amino acid composition, with higher quantities of lysine (5 to 8%) and methionine (2.4 to 5.1%) than most cereals (Stikic et al., 2012). The grains are also rich in minerals and vitamins being gluten free and most utilized by celiac patients (Nascimento et al., 2014). Moreover, the content of fiber is 25% higher than the one found in wheat and maize (Lamothe et al., 2015).

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The largest world producers are Peru and Bolivia reaching respective 52,129 and 50,489 metric tons in 2013 (FAOSTAT, 2014). This represents only a small fraction of the world's demand. For this reason there has been growing interest to adapt and cultivate quinoa in North America, Europe, Asia, Africa and Australia (FAO, 2011). It was first introduced in Brazil in the 1990's aiming at diversifying cropping systems in the savannahs. Studies have been undertaken to select genotypes adapted to the growing conditions of the Brazilian grain cropping areas, culminating with the release of BRS Syetetuba. It has shown favorable characteristics as grain yield of 2.3 Mt ha^{-1} phenotypic homogeneity and relatively large grains, with the 1,000 seed weight varied between 2.5 and 3.3 g (Spehar et al., 2011).

The ample adaptation and commercial production of quinoa in Brazil depends, however, of seed quality studies. One of the major problems restricting the quinoa crop in sub-tropical and tropical regions of the world is the seed quality. The end products of quinoa are aquene type fruits, with the shape of flat cylinders. They have a layer of dead cells surrounding the seeds. They are highly hygroscopic, presenting root protrusion in short time, 6 to 10 h after imbibing (Parsons, 2012). Therefore seeds can deteriorate rapidly in wet and high temperature environments (Ceccato et al., 2011).

The essential practice common to grain crops is the storage of seeds until next crop season. Their deterioration can be prevented by suitable storage to keep seed viability (Krohn and Malavasi, 2004; Lins et al., 2014). In the storage environment air relative humidity followed by temperature are the factors affecting physiological quality of seeds, interfering directly with their metabolic processes (Sravanthi et al., 2013).

Relative air humidity affects directly the water content in seeds and, when combined to high temperatures, intensifies seed respiration (Marcos, 2005). The consequences of higher respiration are the humidification and the warming up of seed mass, aggravated by the action of micro-organisms and insects (Baudet and Vilella, 2006). Seeds consume internal reserves, causing weight loss and drastic decline of germination (Carvalho and Nakagawa, 2012).

The packaging of seeds during storage could be valuable in maintaining their physiological maturity, depending on their intrinsic characteristics as permeability. The types of packages used in storage could have direct effect on the quality by preventing or not humidity exchange between seeds and the environment (Medeiros and Zanon, 2000). The main function of packaging seeds is to retard their deterioration by reducing respiration (Hong and Ellis, 2003; Tonin and Perez, 2006). The storage conditions and onion (*Allium cepa* L.) seed viability was studied utilizing cloth and paper, rigid polyethylene and paper, rigid polyethylene, flexible polyethylene, aluminum foiled flexible polyethylene and tin. Seed vigor at 20°C and 50% RH was not affected by package type, while at uncontrolled room temperature cloth, polyethylene and rigid polyethylene seeds reduced vigor (Caneppele et al., 1995). Crambe (*Crambe abyssinica* H.) seeds stored in tin at room temperature had better performance than in polyethylene terephthalate (PET) bottles, polystyrene box, and polyethylene bags (Cardoso et al., 2012). In *Cajanus cajan* L., PET bottles and polyethylene bags were more efficient than Kraft paper turning evident that this was associated with low temperature (Lisboa et al., 2014).

Every plant species has its particularities of seed viability and conservation, mostly related to the environment it was domesticated and adapted. Such is the case of quinoa, originated in the Andean Mountains at 3,800 m above the sea level. Therefore, in adapting its cultivation to the low altitude high temperature in tropics seed quality is a setback. This study aimed at evaluating the effect of packaging and environments in maintaining the physiological quality of *C. quinoa* seeds.

MATERIALS AND METHODS

The work was conducted in the Laboratory of drying and storage plant products of the Agricultural Engineering Couse, State University of Goiás, Anapolis, GO, Brazil, between February and November 2012.

Quinoa fruits

The quinoa fruits, and treated here as seeds, were of cultivar BRS Syetetuba, grown in the 2011 summer cropping at Emater Extension Service farming and experimentation area in Anápolis Goiás, Brazil. It is located at an altitude of 980 m above sea level, 48°18'23"WM and 16°19'44"SL. At physiological maturity, seeds had 20% wet basis (w.b.) moisture. After harvest, the seeds were dried down in forced ventilation greenhouse at approximately 60 m^3 min^{-1} m^{-2} and temperature of 35°C until moisture level reached 13.5% w.b.

Experimental design and treatments

The experimental design was entirely randomized on 2 x 3 x 6 factorial scheme with 4 repetitions. The treatments were: natural laboratory conditions and Biochemical oxygen demand (B.O.D.) chamber set at 4±2°C e 90% R.H. Three packaging types were used: impermeable – 250 ml, 0.126 mm PET bottles sealed with paraffin; semipermeable – 0.125 mm aluminum foil sealed with permeable sticking tape; and permeable – double-foiled Kraft paper bags sealed with sticking tape. The six evaluations were made at 0, 60, 120, 180, 240 and 300 days after beginning of experiment.

The respective maximum, (Tmax), mean (Tmean) and minimum (Tmin) temperatures and relative humidity in the storage environment during the experiment are presented (Figures 1 and 2).

Seed physiological quality

From the beginning of experiment up to 300 days seeds were evaluated by the following tests: i) water content – seed samples

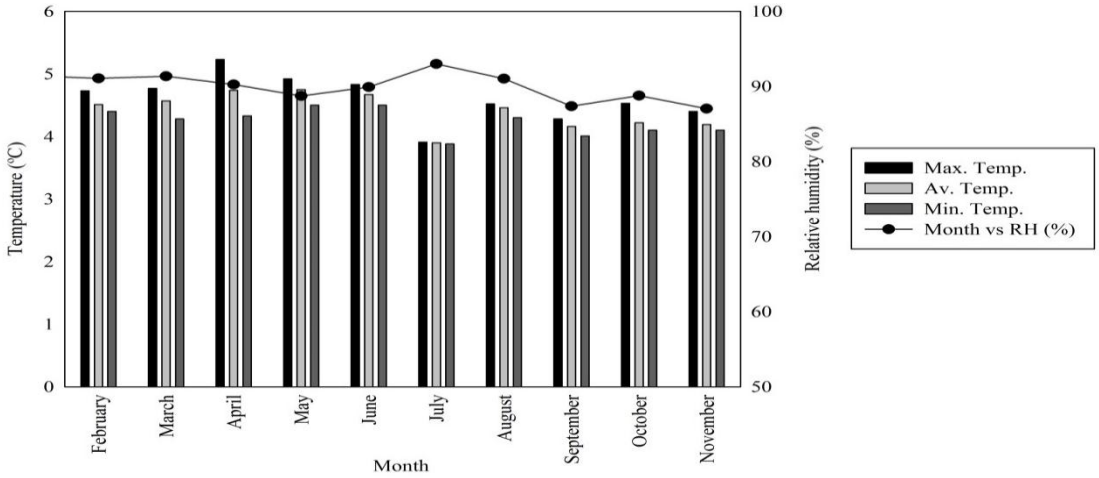

Figure 1. Maximum, average and minimum temperature and relative humidity in the B.O.D. chamber during the storage of quinoa seeds.

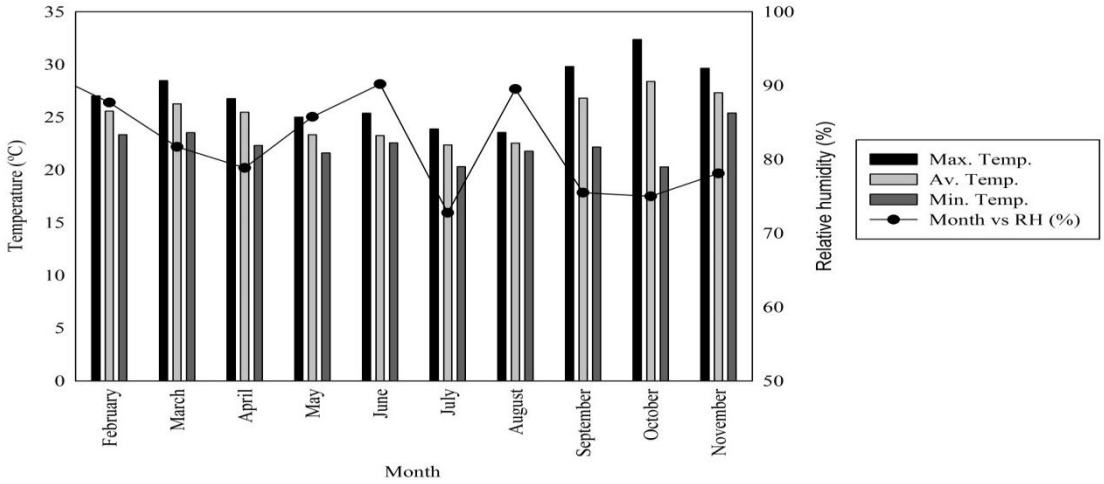

Figure 2. Maximum, average and minimum temperature and average relative humidity in the laboratory environment, in Anápolis, Goiás, Brazil, during the storage of quinoa seeds.

were placed on greenhouse at 105 ± 3°C for 24 h, on three replicates following the standard procedure test (BRASIL, 2009); ii) germination – a sample of 200 seeds from each storage package was divided into 4 replicates and sown on transparent plastic boxes (11 x 11 x 3.5 cm) containing distilled water soaked filter paper 2.5 times the weight. The boxes were placed in the dark with alternated temperatures of 25 to 30°C for 8 to 16 h. (Dias et al., 2003). Normal seedlings were scored on the 10th day following the standard procedure test (BRASIL, 2009); iii) first germination count – it was conducted simultaneously with the germination test with evaluation of normal seedlings rate on the 7th day (BRASIL, 2009); iv) accelerated aging: 12 g seeds were uniformly distributed on wire mesh placed in transparent plastic boxes (11 x 11 x 3.5 cm) containing 40 ml NaCl saturated solution (40 g .100 ml^{-1} in distilled water). The boxes were covered and kept in B.O.D. a 45°C for 48 h, being subsequently submitted to germination test, followed by evaluation of normal plants on the 7th day; v) emergence – from each storage treatment four replicates of 50 seeds were sown in autoclaved washed sand at 120°C, in 10 cm long furrows 1.5 cm deep. The trays were kept in the laboratory and daily irrigated by micro-sprayers to keep the substrate highly moist. Evaluation was conducted on the 10th day, expressing the results in percent of normal plants (Krzyzanowski et al., 1999); vi) emergence speed index (ESI): the test was conducted simultaneously with emergence, by scoring daily and at the same time the number of emerged plants. At the end of test, ESI was calculated by the Maguire (1962) formula - IVE = $E_1+E_2+E_3+...+E_n/N_1+N_2+...+N_n$, where E1, E2,. En = number of emerged plants at each day and N1, N2,... Nn = number of days from sowing until last count.

Statistical analysis

Analysis of variance was conducted for the observations and the values expressed in percentage were transformed in arcsen $\sqrt{x/100}$. Means were compared by Tukey test at the 0.05

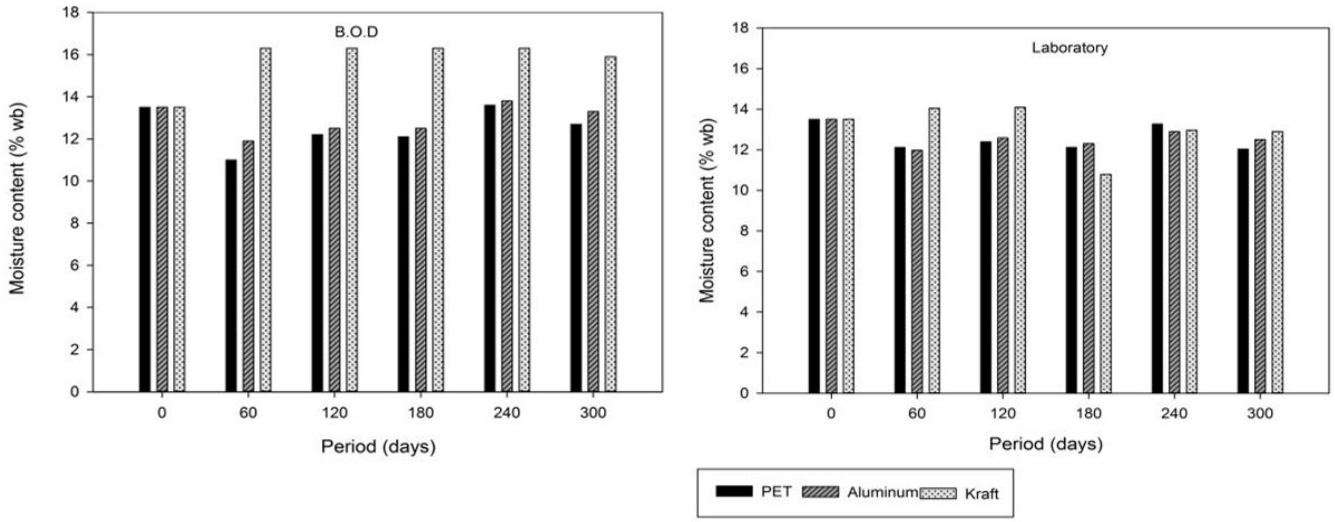

Figure 3. Moisture content of the quinoa seeds (% w.b.) stored in different environments and packages during 300 days.

probability level. The interaction of storage period x packaging type was submitted to regression analysis, at 5% de probability by F test. All statistical analysis utilized Sisvar 5.3 programme (Ferreira, 2011).

RESULTS AND DISCUSSION

The seed water content for packaging and environments are presented in Figure 3. In the laboratory environment, as would be expected, showed higher oscillation in temperature and relative humidity as related to seasonal variations along the year. In B.O.D. the relative humidity was constantly high and related to low temperature (4 ±2°C). Association of low temperature in storage and increase in relative humidity has been demonstrated by Regalo and Brena (2006).

The higher water content in B.O.D. stored seeds was related to increased relative humidity. Therefore, seeds packed in permeable Kraft paper had the equilibrium reached at 60 days of beginning, presenting average value of 16% w.b. The hygroscopic equilibrium has occurred when the water vapour pressure in seeds equals to the air water vapor pressure, after they were exposed to a long storage period (Amaral and Baudet, 1983; Silva et al., 2008). In environments with constant variations in humidity seeds are exposed to fluctuations in water content. This was verified in seeds maintained in Kraft paper packages at laboratory condition. At 180 days from beginning of experiment, in July when relative humidity was 73%, 10.8% w.b. was obtained. It should be worth emphasizing quinoa seeds are aquene fruits that possess a permeable outer layer of dead cells turning them prone to exchange moisture (Spehar et al., 2015).

In both laboratory and B.O.D. environments the seed water content of impermeable package (PET bottle) altered during storage periods. This could be related to increased respiration rate and intrinsic biological factors proper to each seed type (Almeida et al., 1999; Carvalho and Nakagawa, 2012). Experiments with seeds of *Copaifera multijuga* and *Caesalpinia pyramidalis*, kept in impermeable packages showed similar pattern in storage (Silva et al., 2011; Oliveira et al., 2011). The analysis of variance for physiological seed quality of quinoa during storage showed that environment (E), packaging (P), period (PE) and the interactions ExP and ExPE had an effect on all observations ($p<0.05$). Except for germination, the interaction ExP influenced significantly the results with other seeds tests ($p<0.05$). The interaction ExPxPE influenced significantly only the emergence speed index and accelerated aging ($p<0.05$).

The high relative humidity in B.O.D., independently of package type, did not affect germination rate of seeds during storage. In the laboratory, the germination decreased steadily turning the seeds unviable at 300 days (Figure 4). Physiological quality of seeds could be maintained for some time under controlled condition storage, but what was lost cannot be recovered unless there is dormancy (Carvalho and Nakagawa, 2012), which is not the case.

The rate of normal plants decreased along time in all packages tested. However, this was more evident for seeds kept in Kraft paper which had reduced seed viability as soon as 60 days comparatively lower than PET bottles and aluminum foil with decrease viability at 120 days (Figure 5). Seeds of sunflower and pigeon pea also decreased germination when stored in Kraft paper, compared the seeds stored in semi-permeable and impermeable (Lins et al., 2014; Lisboa et al., 2014).

The first count and emergence tests for seeds kept in B.O.D. showed seed viability during all the storage

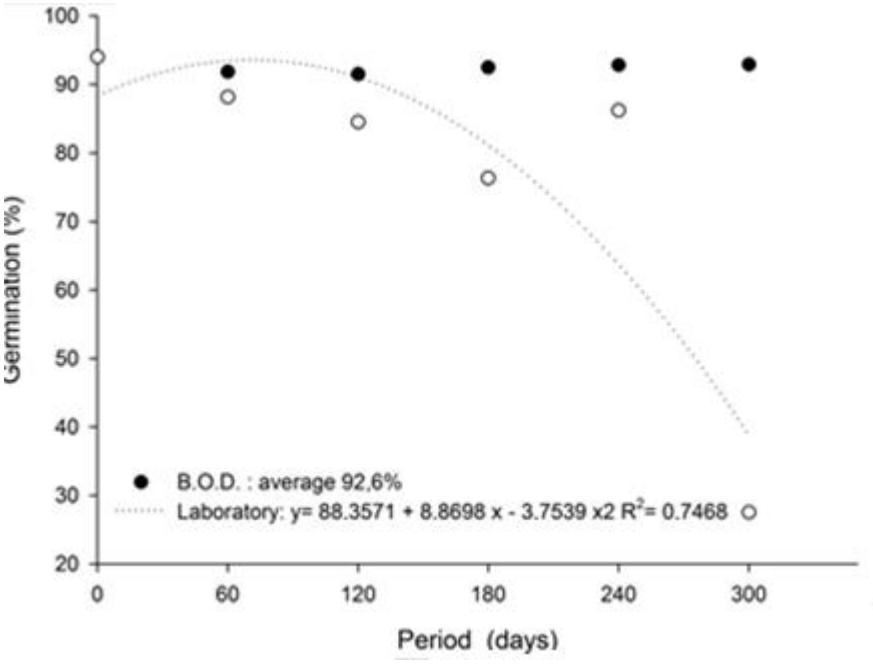

Figure 4. Quinoa seed germination from storage in different environments during 300 days. ** Significant at 0.01 probability.

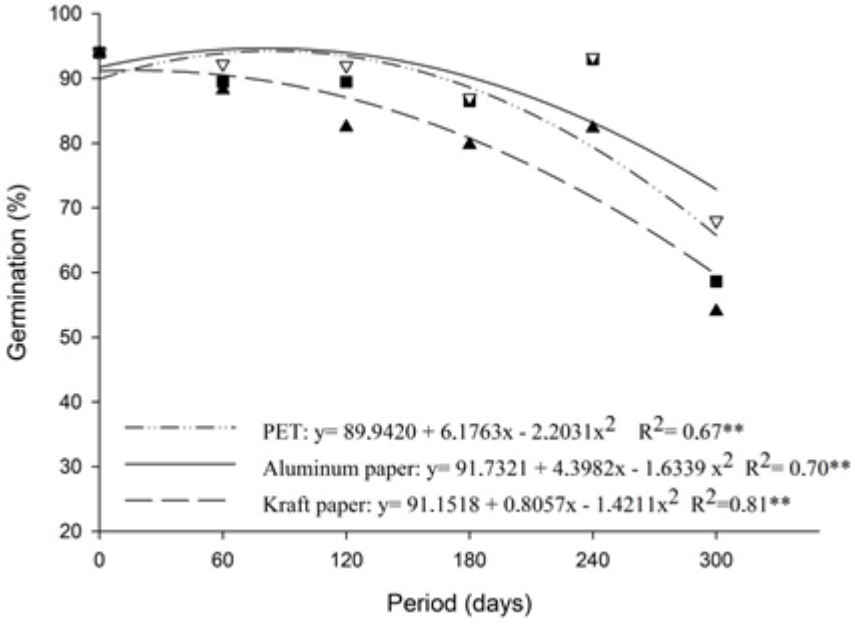

Figure 5. Seed germination of quinoa kept in different packages during 300 days. **Significant at 0.01 probability.

period, contrary to what was verified for the seeds kept in laboratory, which presented sharp decrease in vigor (Figures 6 and 7). The reduction in seed vigor in this case may have been related to variations in temperature and relative moisture during storage (Marcos, 2005). Seeds kept in impermeable PET bottle and semi-permeable aluminum foil had similar performance in the first count and emergence test (Figure 8), being superior to permeable Kraft paper package. The result could be associated to the thickness of semi-permeable aluminum foil, 0.25 mm maximum value for this type of packaging material (Baudet, 2003). Seed moisture had the same

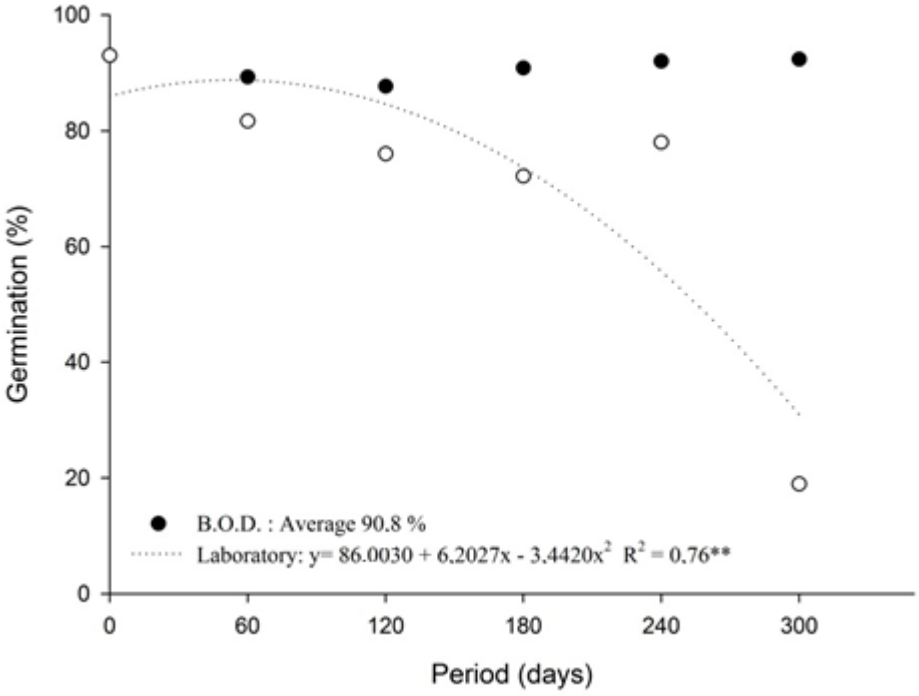

Figure 6. First count germination of quinoa seeds stored at laboratory and B.O.D. condition during 300 days. ** Significant at 0.01 probability.

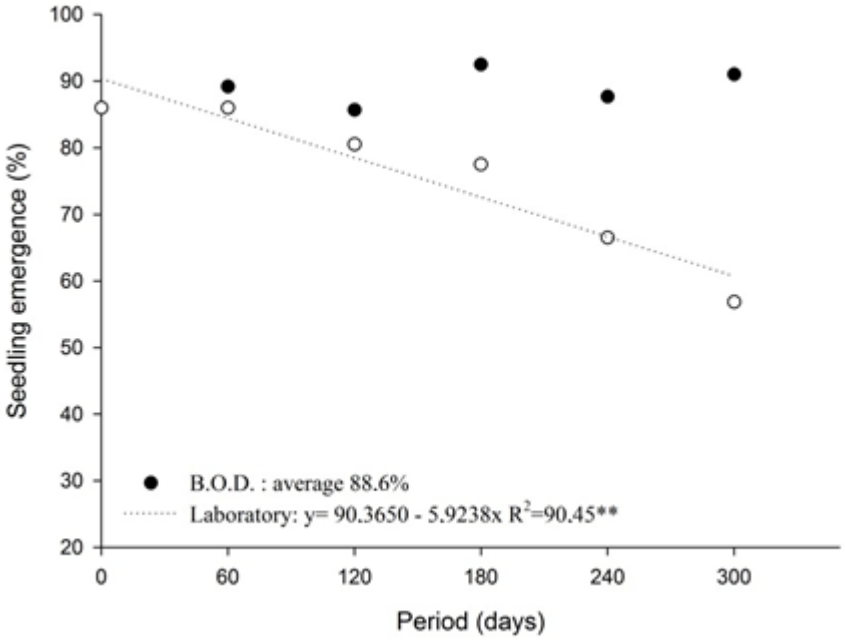

Figure 7. Seedling emergence of quinoa seeds stored at laboratory and B.O.D. condition during 300 days. ** Significant at 0.01 probability.

trend as in PET bottle, turning evident vigor is directly influenced by water content (Rao and Singh, 2006).

First count and emergence tests were also sensitive to detect significant differences for E x P interaction (Tables 1 and 2, respectively). Vigor was superior for all packaging types when stored in B.O.D. chamber. When

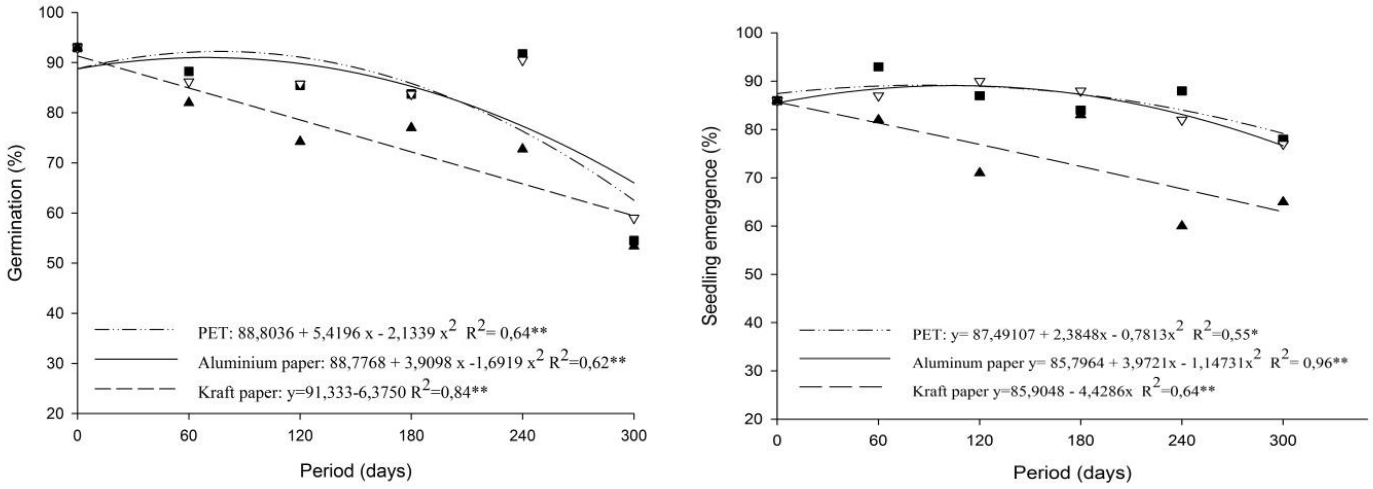

Figure 8. Germination at first count and emergence of seedlings from quinoa seeds kept in different packages during 300 days storage. Significant at *0.05 and **0.01 probability.

Table 1. Germination at first count of quinoa seeds kept in different environments and packages.

Storage environment	Package		
	PET	Aluminum foil	Kraft paper
B.O.D.	90^{Aa}	93^{Aa}	90^{Aa}
Natural (laboratory)	75^{Ba}	74^{Ba}	61^{Bb}

Means followed by the same capital letter in column and low case letter in the line are not statistically different (Tukey's test, p <0.0 5).

Table 2. Emergence of quinoa seedlings from seeds stored in different environments and packages.

Storage environment	Package		
	PET	Aluminum foil	Kraft paper
B.O.D.	90^{Aa}	90^{Aa}	86^{Ab}
Natural (laboratory)	82^{Ba}	80^{Ba}	64^{Bb}

Means followed by the same capital letter in column and low case letter in the line are not statistically different (Tukey's test, p<0.0 5).

emergence is observed, there was no significant difference for packaging, while the Kraft paper had significant effect in reducing it. Under uncontrolled laboratory environment, PET bottle and aluminum foil, showed no significant differences, although superior to Kraft paper package. The latter are permeable to water vapor, justifying the fluctuations in seed water content and reduction in vigor (Marcos, 2005).

The emergence speed index was more sensitive to detect significant difference in the environment x packaging x period. From 180 days after beginning of storage, there appeared a significant difference between laboratory and B.O.D. chamber conditions. In the latter, vigor reductions were lower than room conditions for the three package types during the time evaluations were made (Figure 9). This can be attributed to gradual deterioration in B.O.D. related to lower seed respiration and metabolic processes under reduced and constant temperature (Das et al., 1998).

In the first 120 days of storage, seeds for all packaging types kept in B.O.D. conditions and kept in PET bottle and aluminum foil in laboratory, had similar results (Figure 9), except the ones kept in Kraft paper package, which declined rapidly from 60 days, turning unviable at 180 days after beginning of experiment, similar to seeds of *Adenanthera pavonina* L. and *Sebastiania*

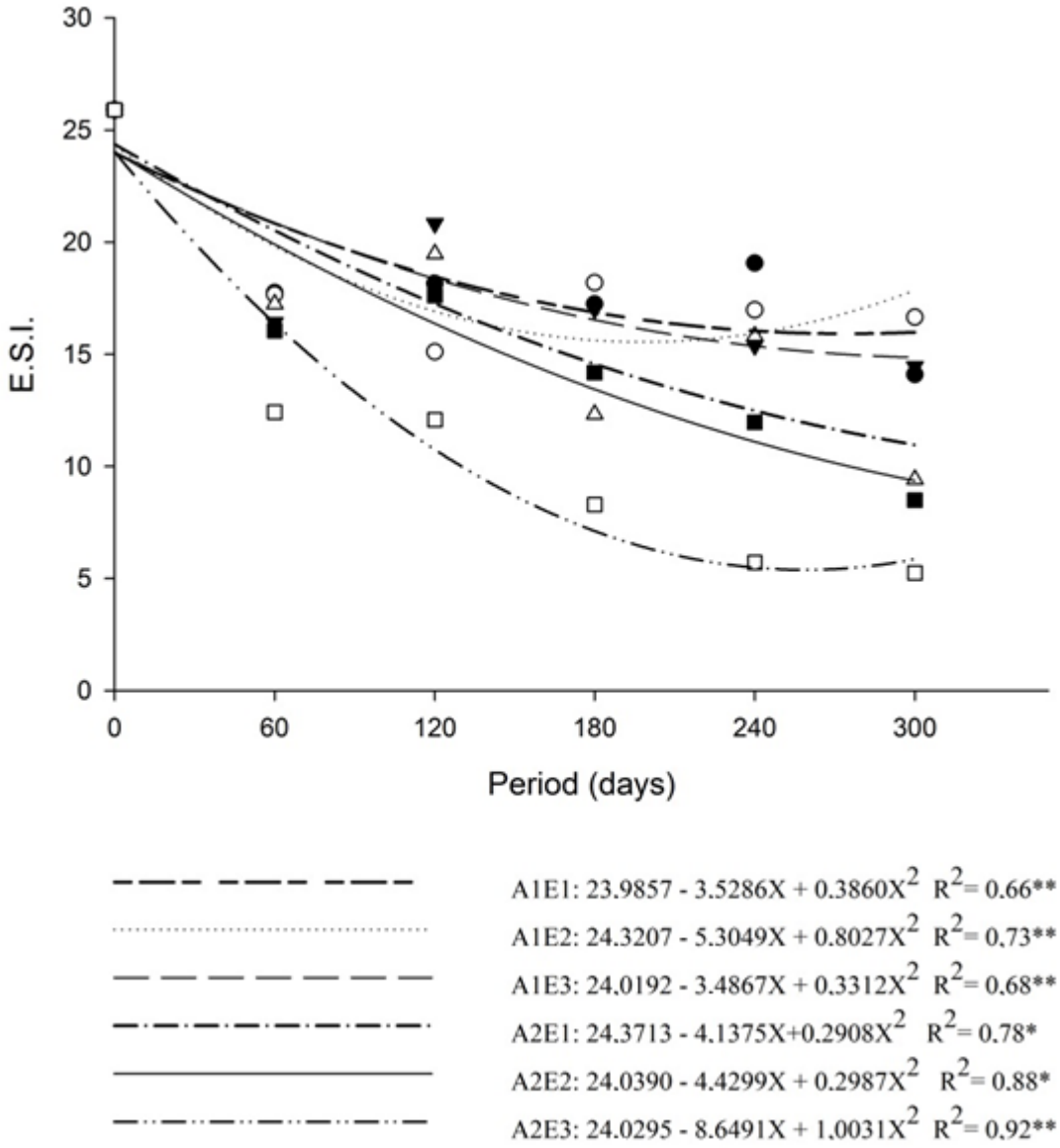

Figure 9. Emergence speed index (E.S.I.) of quinoa seedlings coming from seeds stored in different environments and packages during 300 days. (A1: B.O.D.; A2: laboratory; E1: PET botle, E2: aluminum foil and E3: Kraft paper). Significant at * 0.05 and ** 0.01 of probability according to test F.

commersoniana (Oliveira et al., 2012; Santos and Paula, 2007).

In the accelerated aging test there was also significant interaction for environment x packaging x period (Figure 10). The interactions that maintained the vigor, by the accelerated aging test, for all storage periods, were in lots of the kept seeds in PET bottle and aluminum foil in B.O.D. In the other treatments seed vigor decreased as early as 60 days. As in the other tests, seeds were more vigorous for all types of packages when kept in B.O.D. controlled low temperature, with the exception of the ones kept in Kraft paper reducing to zero at 240-300 days. These were kept viable until 180 days but in the uncontrolled laboratory conditions they became unviable at 120 days.

Conclusion

Quinoa seeds maintain physiological quality for long period (300 days) when kept in impermeable package and low temperature (4±2°C). Under uncontrolled temperature and moisture semi-permeable and impermeable package seeds are viable until 180 days of storage. Permeable package as Kraft paper is the least efficient in conserving physiological quality of quinoa seeds.

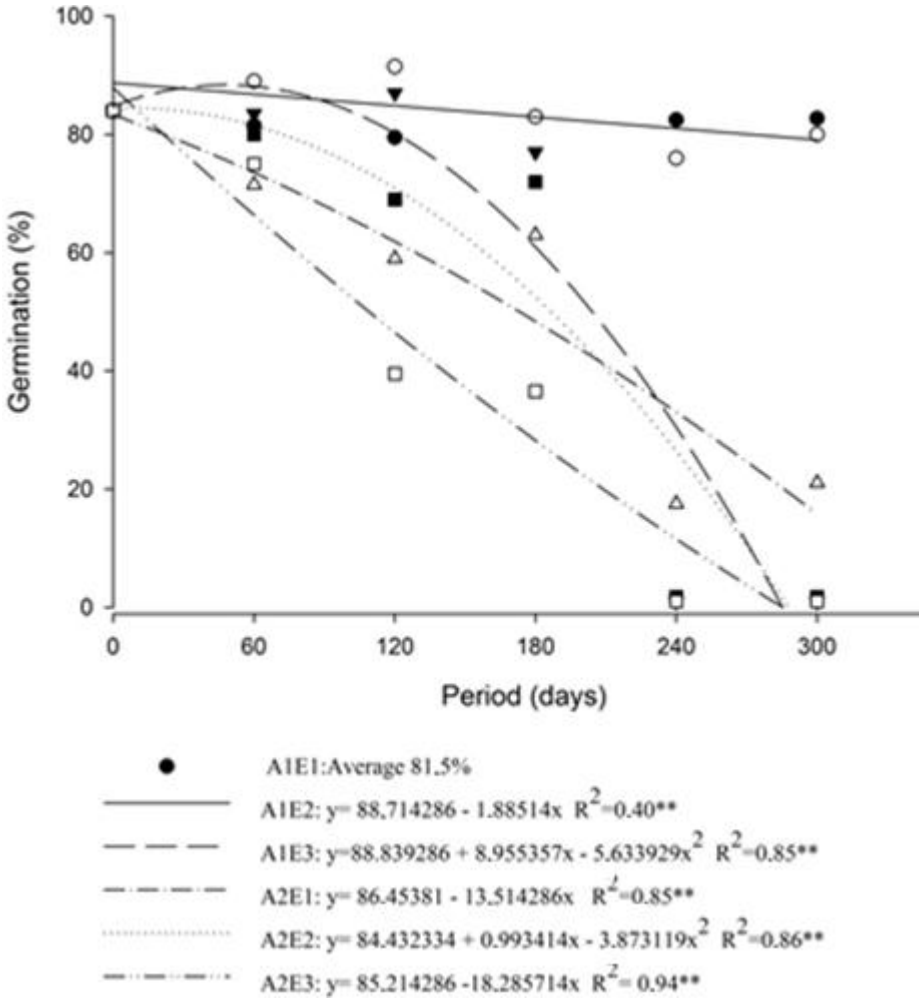

Figure 10. Germination assessed from the accelerated aging test for quinoa seeds stored in different environments and packages during 300 days. (A1: B.O.D.; A2: laboratory; E1: PET bottle, E2: aluminum foil and E3: kraft paper). ** Significant at 0.01 probability.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are thankful to CAPES and the State University of Goiás for scholarship and support to the research.

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academicJournals

Vol. 11(15), pp. 1309-1325, 14 April, 2016
DOI: 10.5897/AJAR2015-10713
Article Number: D43D97658045
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Competitive strategies applied to agribusiness in South-Eastern Paraná, Brazil

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Received 8 December, 2015; Accepted 27 January, 2016

Agriculture plays a key role in the economy and quality of life. How to best employ available resources in order to improve productivity, profitability and sustainability of these processes has been the subject of many studies. This study aims to analyse by multi-index methodology the expected return and risks associated with investment in agribusiness, particularly on what concerns the implantation viability of a vegetable-washing machine. It thus listed the production and maintenance costs of carrot and cucumber cultures, as well as cleaning costs and the profitability and inherent risks of the implementation. Having in mind that this research requires empirical and analytical evidences as well as a case study, a company located in South-eastern Brazil was chosen by means of an applied research. Data from a Minimum Acceptable Rate of Return (MARR) of 6% per year was collected by documental research and semi-structured interviews systematized in a cash flow projected within the respective deadlines. Results indicate an Additional Return Over the Investment (AROI, 16.06%) higher than the MARR for carrots and 14.94% higher for cucumbers. These results show that when a competitive strategy of vegetable cleaning through a machine is employed, with expectation for return in 24 months, the impacts are positive, signalling high profitability and compatible risks with the expected return, reinforcing the soundness of such an investment in agribusiness.

Key words: Agribusiness, agriculture, investment, multi-index methodology.

INTRODUCTION

Agriculture is one of the most important segments in the global economy. Agriculture is by far the biggest user of water, accounting for more than 70% of all water utilization worldwide and 90% of water utilization in

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developing countries (Dwivedi et al., 2015). The agriculture development strategies of most of these countries depend on the possibility of maintaining, improving, and expanding irrigated agriculture (Siebert et al., 2006). However, as the pressure on water resources increases, irrigation is facing growing competition from other water-use sectors and becoming a threat to the environment in an increasing number of regions. Despite the current problems and negative perceptions in many sectors of society (Hoffman and Evans, 2007), it is certain that irrigation and the proper use of water will continue to be essential to the welfare and development of the world.

A part of this segment, the vegetable sector, is a branch of agribusiness in rapid growth, mobilizing millions of Brazilian reais (BRL) annualy throughout all of its supply chain, from the production to the final customer (Kureski et al., 2015; Zhong et al., 2015). In Brazil, according the Instituto Brasileiro de Geografia e Estatística – IBGE, annual vegetable consumption per capita is around 27.08 kg, split in three groups: (i) leafy and floral vegetables: average 3.22 kg, (ii) fruity vegetables: average 12.60 kg, and (iii) root bulb vegetables and others: average 11.26 kg (IBGE, 2008). The southern region, a focal point of this study, is according to the IBGE Data (2008), a region that is more highlighted, having an average annual consumption of 38.60 kg per capita, followed by the south-eastern region with 27.99 kg, central-western with 26.65 kg, north-eastern with 22.07 kg, and northern region with 19.41 kg (IBGE, 2008).

The vegetables commercialized in Paraná's state in 2012 amounted to 552,418.70 tons, which were worth R$ 705,797990.00, at an average price of R$1.27/kg (CEASA-PR, 2012). The fruit group amounted to 512,196.30 tons, with average price of R$1.57/kg and the total value of R$ 804,534.560.00 (CEASA-PR, 2012). Even with a tendency for growth in the region, a city located in South-eastern Paraná, Campo do Tenente, had a significant reduction in its production (58.71%), caused by the waver and/or reduction in the amount of local products, particularly, roots and tubercles, which are no longer commercialized in CEASA-PR Units (2012). It was in this context that the problem which guides this study arose: how to increase vegetable sales in this region? Considering the growth of the vegetable sector in other regions, measures to increase and/or modernize available machinery and equipment are necessary, aimed at increasing revenue and consequently the growth of companies operating in the sector. The adoption of irrigation technologies and water-management practices and their resulting costs, with wise resource usage, can affect the production of goods, farm profitability, and environmental quality, as well as customer satisfaction with regard to product price and quality.

This study has the objective of analysing the return expectations and risks related to the investment in a vegetable-washing machine, looking at the main indicators, verifying which benefits this investment will bring to the company under analysis and if the acquisition of such a machine can add value to the production sale.

The main contribution of this study for the literature and practical implications is the importance in indicating what is the most convenient among investments in agribusiness, in the vegetables sector, with evidences of practical information that help the managers take decisions. This way, it presents options that will bring a higher return in a reduced timespan, with the implementation of competitive strategies based on the internal resources of the firm.

MATERIALS AND METHODS

The market of fruits, greenery and vegetables (FGV)

The concern with the consumption of fresh and healthy food has been rapidly increasing, in the same rate at which concerns with beauty and mainly health, increase. The costumers are ever more aware of medicinal and nutritional discoveries of food, seeking quality in what is consumed, which in turn collaborates with the sale increase in the FGV sector (Bublitz and Peracchio, 2015).

A research from Agriculture and Livestock Confederation of Brazil about the consumption of fruits and vegetables showed that a reduced rate of only 18.2% of Brazilians ingest the recommended amount of fruits proposed by the World Health Organization (WHO), that is 400 g per day or 146 kg per year. Another data from the research is that Brazilians spend, on average, only 6.2% of their income with the acquisition of fruits, greenery, and vegetables. The habit of consuming fruits is not very strong even though Brazil is the world's third largest fruit producer (CNA, 2011).

The retailers have some barriers to overcome to get better results in the FGV's trading, starting from the difficulties found in their own products supply, where the quality, the variety, and the prices are often below expectations. In the products' delivery, flaws worthy of note include products harvested out of season and their resulting inferior quality in the form of poor size and appearance; poor harvests that raise the products' price and faulty supplying that makes the product unavailable to the customer, among others (SEBRAE, 2012). A research from Associação Paulista de Supermercados (APAS, 2006) showed that the sale of FGV grew up much in the retail and currently, represents between 8 and 12% of the supermarket's revenue, which can become an opportunity for the retailer who knows how to take advantage from assortment and strategies of sector trading.

The process of food washing

The process of food washing consists in the activity post-crop, aiming to add value to the product sale. The washing is the operation where the vegetables pass by a detailed process of cleaning made by specific machines and equipment.

The process occurs in the following way: the vegetables harvested are forwarded to a deposit as they are. In this deposit lie the cleaning machines where the cleaning is done and vegetables are packed for transportation up to consumer centres, as shown in the flowchart Figure 1.

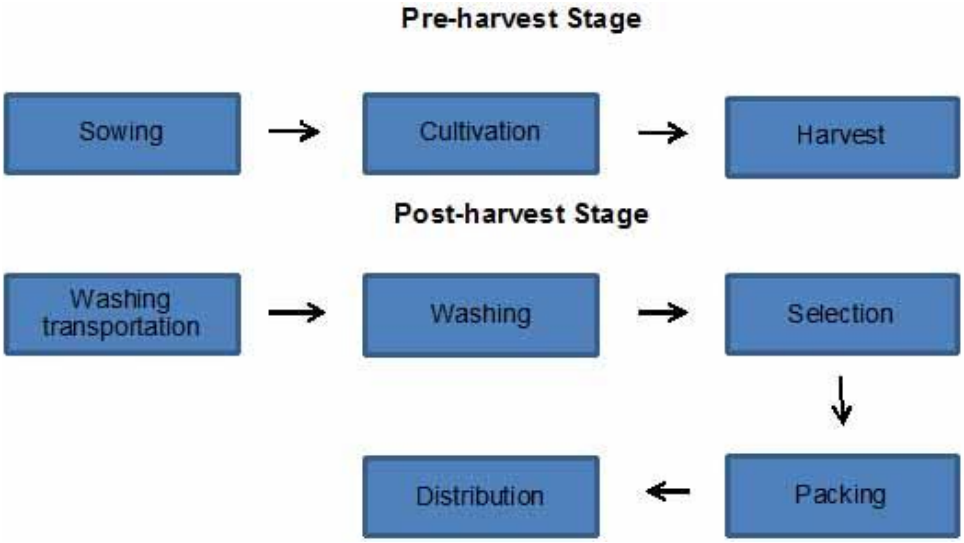

Figure 1. Activities/Tasks flowchart.

Figure 2. Washing process.

Picture 1: Activities/Tasks flowchart

In the first stage, the washing process happens when the products to be washed are inserted in a conveyor belt, as shown in Figure 2. The washing system consists of brushes with soft nylon bristles; such as, brushes overlap and are accompanied by a water shower, both being responsible for the cleaning, while the items are in movement. This process allows the products to undergo complete cleaning before proceeding to the classification belts.

Picture 2: Washing process

In the second stage, the products go by the belt towards manual selection, where they are handpicked for good quality and presentation before being forwarded for sale. Products with poor quality are disposed, as shown in Figure 3.

Picture 3: Selection process

In the third stage, the products are packed, usually in boxes, and forwarded to consumption centres, as shown on Figure 4.

Picture 4: Packing process

With the investment and implantation of vegetable-washing machines, the producer can: (i) wash; (ii) classify; (iii) pack and trade the produce. As an added benefit, the producer can create his own brand and packages, a fact that can add value and allow better control and significant reduction of waste in the production.

Competitive strategies

Because of the competitiveness, strongly boosted by globalization, companies must be flexible in order to react quickly to changes in the competition and market in general. The basis for a competitive strategy is to create differentials from the main competitors in the market. It means to choose in a deliberate way a different group of activities to provide a higher value mix (Porter, 2005). In this respect, it is necessary to know that the adoption of incorrect competitive strategies can compromise the results of an industry.

Figure 3. Selection process.

Figure 4. Packing process.

Nevertheless, to prevent this, Porter (2005, p. 46) affirms it is fundamental that the companies foster essential competences in the race to remain ahead of other market competitors.

In the search for market participation, the competition does not only happen in relation to the contestant, but in all of the supply chain. The competition's state in a sector depends on five basic powers, which are: (i) bargaining power with clients and (ii) suppliers; (iii) threats of new entrants and (iv) surrogate products. Lastly, the (v) rivalry among the contestants. The set of these competitive powers determines the profitability and/or maximum potential profit of a sector (Porter, 2005).

In the context of this research, the competitive strategy applied will be the internal resources implantation (Barney, 1991) controlled by the companies; a vegetable-washing machine through which the producer can add value to the final product sale, distinguishing oneself among the main competitors of the sector.

Rural accounting

Rural accounting is still little used by rural entrepreneurs as well as accountants. This often happens due to the lack of knowledge regarding the importance of information obtained by means of accountancy, and how this information can help in decision-taking (Abib et al., 2015; Freitas Filho et al., 2002).

The importance of rural accounting is given by supplying information about expansion conditions, about urges for cost and expense reduction, needs in resource gathering having planning strategies as the objective. Furthermore, the accounting information is interesting for the investors, suppliers, banks, financial institutions, clients, and rural companies' managers too. This information can tell if the investment is safe and if there is possibility of a rapid return (Crepaldi, 2012).

Accountancy can be studied in a general way, for all the companies, or in a particular one, being applied in a determined field of activity or economy sector. When applied in a specific field, it is usually denominated according to the activity of that field. Thus, rural accounting is the general accounting applied to rural companies. The rural companies are those that explore the soil's productive capacity by means of land cultivation, livestock farming and the transformation of determined agricultural products (Marion, 2014).

Costs and expenditures in agricultural activity

Agricultural accounting needs, in any system, to distinguish between costs and expenditures. The distinction is easy: costs are expenses (or economic sacrifices) relating with assets transformation, for example, the consumption of inputs or salary

payments. The expenditure consists of expenses that provoke reduction of patrimony, for example taxes, sales commissions, among others (Crepaldi, 2012). In Marion's (2014) opinion, the difference between crop costs and periodic expenditures to the agricultural activity is based on: (i) the crop costs, (ii) period's expenditure, (iii) harvest, (iv)storage, and (v) losses.

Investment projects

An investment project can be interpreted as an effort to raise the information level regarding all the implications, whether desirable or undesirable, in order to reduce the risk level (Souza and Clemente, 2008). The development and improvement of the project aims primarily at the reduction of the uncertainty level, but may also result in the alteration of estimated gains of each opportunity and even in the spotting of new opportunities. Regarding investment, the term can be defined in a wide way like money application in insurances, shares, properties, machinery, among others, with the purpose of obtaining gain (Hoji, 2011).

An investment for a company is a disbursement made aiming at a flow of future benefits, usually more than a year away from the present time. In a broad sense, the term is employed in the purchase of machines, equipment and properties for the installation of productive units, as well as for the purchase of financial titles, for example (Souza and Clemente, 2008). The objective of an investment analysis is to compare investment options and observe which present a better return, with the lower possible risk for the organization. The investment is always made with the intention of generating profit and bringing some improvement for the company, mainly long term ones.

Risk and return

The risk can be understood as an uncertainty measure associated with expected returns of an investment decision. Therefore, the risk is a cost always involved in the business, so it must be quantified (Assaf Neto, 2010). The word risk is used when the available information is enough to determine the possible events and assign them probabilities. That is why the decisions of investments never happen under conditions of full certainty. The analysis will be in confrontation with situations of uncertainty and risk, in bigger or smaller levels (Abib et al., 2015; Souza and Clemente, 2008).

Every rational financial decision is taken based on analysis between risk and return. The investments do not offer certainty related to their future results, and can generate high and low returns. When the results do not float so much, it is understood that the decision presents a lower risk. For example, applications in fixed income insurances produce more stable and predictable returns than the returns in shares; because of this, they are admitted as having a lower risk.

The return is the amount of resources that one receives or expects to receive when making any capital expenditure, in other words, investments (Ross et al., 2011). There is no possibility for expecting high returns in assets of low risk. A major level of risk must offer major return to the investor, in such a way as to compensate for the high risk business. The relation between risk and return is proportional, and an additional compensation should always be provided for running greater risks (Assaf Neto, 2010).

Multi-index methodology

The multi-index methodology applied in this study was proposed by Souza and Clemente (2008). It consists of supporting the decisive process related to acceptance or rejection of certain investment projects by means of using many indicators. The first group consists of the indicators: PV (present value), NPV (net present value), ANPV (annual equivalent net present value), BCI (benefit/cost index and Aditional Return Over the Investment (AROI)). The additional return resulting from the investment is used to improve the perception of projected financial returns. The second group is formed by: Decison Risk (MARR/IRR as a proxy of P(NPV <=0). Period of Return on Investment/Period (Payback/N), Degree of Revenue Commitment (DRC), Risk Management (RM), and Business Risk (BR), used to improve the project risk perception. The essence according to Souza et all (2015), of multi-index methodology consists of:

- Not incorporating the risk premium as a spread about the MARR;
- Expressing the project profitability by means of AROI as an additional return beyond what would be earned by applying the capital in low risk insurances;
- Using the environmental analysis to deepen the evaluation about involved risks;
- Confronting the expected gains with the risks perception in each project.

In the multi-index methodology, five risk indicators are used to evaluate the perceived project's risk: (i) Index MARR/IRR as probability's proxy to get bigger return in financial applications of low risk than in the project; (ii) Pay-back index as probability's proxy of not recovering from the loss of invested capital; (iii) Degree of Revenue Commitment to evaluate the operational risk, in other words, to evaluate the perception of maximum revenue that is compromised with the payment of costs and expenditures; (iv) Management Risk to evaluate the manager group competence level to accomplish the enterprise successfully; (v) Business Risk for quantifying, even subjectively, the classical analyses: PEST, 5 Porter's Powers, and SWOT. These indicators help in the perception of expected behaviour between risk and return; in other words, bigger risks may increase the expected return. These indicators have their concepts presented in Board 1.

Monte Carlo simulation: Crystal ball

The Crystal Ball software runs predictions and risk analysis by probability, removing the uncertainty in decision-taking. By means of a technique called Monte Carlo simulation, Crystal Ball predicts all the possible results for the analysed situation and also shows its levels of confidence so that one can know the probability of occurrence of any specific event. The Monte Carlo simulation is a sort of simulation in spreadsheet that generates random values to uncertain variables repeatedly, simulating thus a model. The analysis of a risk spreadsheet uses the model and also the simulation to analyse the variation effects of entrances in results of the modelled system (Charnes, 2007). This analysis tool helps in taking decisions through simulations in models of spreadsheet. The predictions which result from these simulations help quantify the risk areas; decision takers can thus have all possible information to take the best decisions. With the Monte Carlo simulation, Crystal Ball demonstrates the results' forecast chart that shows us all the possible results and the probability of achieving each one of these results, and presents all the possible predicted scenarios.

RESEARCH CHARACTERIZATION

In this study, the research related with its nature is revealed as applied research, because it is focused on the solution of specific problems (Gil, 2010). Regarding the objectives, the research

Board 1. Indicators-Multi-index methodology (Casarotto Filho et al., 2010; Souza and Clemente, 2008).

Net Present Value - NPV	The most known and used analysis technique of investment. The NPV is the concentration of all the expected values of a cash flow in date zero. If the NPV is positive, it means the initial investment was recovered as well as the revenue that one would have had this capital been applied to MARR. The NPV value must be sufficient to cover the project's risks and attract the investor.
Annual Net Present Value - ANPV	The ANPV is a method variation of Net Present Value. While the NPV concentrates all the values of cash flow in date zero, in an ANPV the cash flow representative of investment project is transformed in a uniform series.
Benefit/Cost Index - BCI	The Benefit/Cost Index is a mean of how much the company expects to gain by each unit of invested capital. The implicit hypothesis in the calculation of BCI is that the released resources throughout the lifetime of the project be reverted to the minimum acceptable rate of return.
Aditional Return Over the Investment - AROI	The AROI is the best estimate of profitability for an investment project. It represents, in percentage terms, the wealth generated by the project. The AROI derives from the equivalent rate to BCI for each project period.
Minimum Acceptable Rate of Return - MARR	It is understood as the best rate, with a low level of risk, available for capital application in analysis. The minimum acceptable rate of return is the rate from which the investor considers that he is obtaining financial gains. It is an associative rate in a low risk and highly liquid, in other words, any box remnant can be applied, in the worst of hypotheses, in MARR.
Internal Rate of Return - IRR	It is a rate that turns the NPV from a cash flow equal to zero. The method of Internal Rate of Return requires the rate calculation, which can zero the Present Value of cash flows, of alternatives. The investments with IRR bigger than MARR are considered profitable and are admissible of analysis.
Investment Recovery Period - Payback	The payback of accordance represents the necessary time for the project benefits to recover the invested value. It can be interpreted as a risk measure of project, because the projects whose payback is near the end of its economic life, present a high degree of risk.
Management Risk - MR	The managing risk is associated to the knowledge and competence level of the management group. The knowledge and experience accumulated about the productive process, commercialization process, channels of distribution and mainly in the conduction of negotiations help the company in turbulent and adverse times.
Business Risk - BR	The Business Risk is associated to conjectural factors and not controllable, which affects the project's atmosphere. The Business Risk is applied to quantify, even if subjectively, the classical analyses: PEST, 5 Porter's Powers and SWOT.

presents a descriptive content, because it has as its objective the description, interpretation, and analysis of the data about the expectations of return and risk on investing in a vegetable-washing machine. The approach strategy used in this study has as typology a case study, having as its objective informing the researcher about the situation, facts, values, and behaviours in the analysed cases. On what concerns the technical procedures for data gathering, the research can be defined as documental (Beuren, 2008). For the time section, the research tells about a longitudinal study and temporal aspect, framing in the analysis of quantitative data, because it was made with an application for study of risk variables and return, survey calculations and analysis of the investment return (Richardson, 1999).

Collection, treatment and data analysis

For the data collection, a checklist was elaborated prior to all, else guiding the data survey process (Beuren, 2008). For effective data collection, interviews with the rural producer were conducted, within the studied property, during the months of August, September, October, and November of 2014. After the interviews, electronic spreadsheets were created with the EXCEL software to ascertain and calculate the indicators PV, NPV, ANPV, AROI, BCI, IRR, and Payback, used in the analysis survey of investment in an asset; in this case, the vegetable-washing machine.

This study highlights the application of Multi-Index Methodology proposed by Souza and Clemente (2008), which consist of supporting the decision process related to the acceptance or rejection of investment project using many indicators. For the study composition, the Crystal Ball software, which runs predictions and risk analysis by probability, was used, thus eliminating the uncertainty in decision-taking, therefore achieving the presented results.

RESULTS PRESENTATION

The current study identifies the production costs of carrot

Table 1. Cost of mechanized operations for production of carrot/há.

Description	Mechanized operations			
	Specification	Unit value (R$)	Quantity	Unproductive phase formation batch 120 days (R$)
Soil preparation	**Machine hour**	**Per hour**	**Hours**	
Decompacting soil	Tractor 132 hp + subsoiler	105.26	1	105.26
Liming	Tractor 85 hp + limestone machine	102.06	1	105.26
Evener harrowing	Tractor 85 hp + grating leveling	102.06	0.5	51.03
Fertilization of plantation	Tractor 85 hp + lancer spreader	102.06	1	102.06
Bed survey	Tractor 85 hp + rotating roe	102.06	3	306.18
Plantation	Tractor 85 hp + seeder	102.06	1	102.06
Other services	Limestone transportation, carrier and access	102.06	1	102.06
Subtotal	-	-	-	870.71
Total	-	-	-	870.71

Source: Authors (2014).

Table 2. Cost of mechanized operations for production of cucumber/ha.

Description	Mechanized operations			
	Specification	Unit value (R$)	Quantity	Unproductive phase formation batch 120 days (R$)
Soil Preparation	Machine hour	Per hour	Hours	
Decompacting soil	Tractor 132 hp + subsoiler	105.26	1	105.26
Liming	Tractor 85 hp + limestone machine	102.06	1	105.26
Evener harrowing	Tractor 85 hp + grating leveling	102.06	0.5	51.03
Fertilization of plantation	Tractor 85 hp + lancer spreader	102.06	0.5	51.03
Plantation	Tractor 85 hp + seeder	102.06	1	102.06
Other services	Limestone transportation, carrier and access	102.06	1	102.06
Subtotal	-	-	-	513.50
Total	-	-	-	516.50

Source: Authors (2014).

and cucumber crops and subsequently the value-enhancement by implantation and purchase of a vegetable-washing machine. This research comprehends, initially, the operational cost of labour and equipment to prepare the land, sowing and post-planting care and consumed inputs. These were measured for the production of 1 hectare of carrots and 1 hectare of cucumber.

For the production and maintenance costs survey, mechanized and manual operations were separated from the production itself. The mechanized operations were calculated from tractor use, based in the cost per hour, according to Marion (2014), added of depreciation and other inputs as shown in Tables 1 and 2.

The manual operations were calculated from working hours, based in the hour/man cost, according to Marion (2014), added of charges. Also aggregated were the inputs applied in this stage, displayed on Tables 3 and 4. In Tables 5 and 6, the mechanized operations for carrots and cucumber maintenance, for example, spraying, top dressing, among others, are displayed. In Tables 7 and 8, there is a demonstration of the cost for manual operations for carrot and cucumber maintenance, for example, thinning/slashing harvest and also the inputs that are applied in this stage. Discriminated on Tables 9 and 10 is the sale price practiced for commercialization of unwashed carrots and cucumbers. Table 11 shows the cash flow with the results of the commercialization of unwashed carrots and cucumbers. In Table 12, the indicators found for the commercialization of unwashed carrots and cucumbers are listed.

With the objective of implementing a competitive

Table 3. Cost of manual operations and inputs for production of carrot/ha.

Description	Manual operations			
	Specification	Unit value (R$)	Quantity	Unproductive phase formation batch 120 days (R$)
Fertilizers	-	-	-	-
Limestone	kg	0.09	2.000	180.00
Fertilizer planting	bag of 50 kg	45.00	50	2,250.00
Subtotal	-	-	-	2,430.00
Others				
Carrot seeds	kg	1,000.00	2.3	2,300.00
Subtotal	-	-	-	2,300.00
Total	-	-	-	4,730.00

Source: Authors (2014).

Table 4. Cost of manual operations and inputs for production of cucumber/ha.

Description	Manual operations			
	Specification	Unit value (R$)	Quantity	Unproductive phase formation batch 60 days (R$)
Fertilizers				
Limestone	kg	0.09	2.00	180.00
Fertilizer planting 00-20-00	Bag of 50 kg	45.00	10.00	450.00
Subtotal	-	-	-	630.00
Others				
Cucumber seeds	Can with 87 g	90.00	15.00	1,350.00
Subtotal	-	-	-	1,350.00

Description	Machinery and equipment used			
	Unity	Unit value (R$)	Quantity	Unproductive phase formation batch 60 days (R$)
Hoe for cleaning	-	20.15	10	201.50
Subtotal	-	-	-	201.50
Total	-	-	-	2,181.50

Source: Authors (2014).

strategy, based on investments in firm's internal resources, the acquisition of a vegetable-washing machine was conducted. The implantation costs were calculated from the expenditures related to the equipment acquisition itself, to the shack and the floor construction, demonstrated in Table 13.

Tables 14 and 15 bring the washing costs of 2.000 carrot boxes and 2.000 cucumber boxes and they are: (i) washing, (ii) crate for packing and the (iii) transportation to consumer's centres according to Tables 16 to 19. Tables 16 and 17 show sale prices practiced for the commercialization of clean carrots and cucumbers. Table 18 presents the cash flow of clean products, adding the value of production cost, maintenance, and washing. Finally, Table 19 demonstrates the indicators found for commercialization of carrots and cucumbers clean/washed.

Result analysis

The investment analysis in a vegetable-washing machine was elaborated having as base an initial investment of R$

Table 5. Cost of mechanized operations for maintenance of carrot/ ha

Description	Mechanized operations			
	Specification	Unit value (R$)	Quantity	Unproductive phase formation in 120 days - Total (R$)
Cultural tracts	-	-	-	-
Sprays	Tractor 85 hp + sprayer	102.06	2.5	255.15
Top dressing	Tractor 85 hp + spreader	102.06	0.5	51.03
Irrigation	Central pivot	52.14	10.0	521.40
Harvest	Tractor 85 hp + cutting blade	102.06	3.0	306.18
Harvest loading	Tractor 85 hp + lorry	102.06	16.0	1,632.96
Subtotal	-	-	-	2,766.72
Total	-	-	-	2,766.72

Source: Authors (2014).

Table 6. Cost of mechanized operations for maintenance of cucumber/ha.

Description	Mechanized operations			
	Specification	Unit value (R$)	Quantity	Unproductive phase formation in 60 days - Total (R$)
Cultural tracts	Machine hour	Per hour	Hours	-
Sprays	Tractor 85 hp + sprayer	102.06	2.5	255.15
Top dressing	Tractor 85 hp + spreader	102.06	0.5	51.03
Irrigation	Central pivot	52.14	7.0	364.98
Harvest loading	Tractor 85 hp + lorry	102.06	16.0	1,632.96
Subtotal	-	-	-	2,304.12
Total	-	-	-	2,304.12

Source: Authors (2014).

125,500.00 from a MARR of 6% per year. It is important to highlight that although the machine has a long service life; these indicators were calculated having as base a return expectation in 24 months. Regarding return indicators, when opting for the investment in a vegetable-washing machine, from a MARR of 6% per year, the expectation of recovery of the investments made is confirmed, from a Present Value of R$ 40,965.57 for carrots and R$ 34,373.88 for cucumbers, generating a Net Present Value of R$ 18,389.79 and R$14,679.86 respectively.

The Benefit/Cost Index (BCI), an indicator which measures the return expectation for each capital unity invested; the results show that R$ 1.81 is made for every R$1.00 invested in carrot washing and R$ 1.75 for every R$ 1.00 invested in cucumber washing. The Additional Return of Investment (AROI) associated with implantation of the vegetable-washing machine estimated at 16.06% for carrots washing and 14.94% for cucumbers washing.

Related to the risk indicators, the Internal Rate of Return (IRR) found was 29.99% for carrots and 39.09% for cucumbers, overcoming the MARR used. The index MARR/IRR found were 20.01% for carrots and 15.35% for cucumbers.

The management risk that is associated to experiences and knowledge of the production and commercialization process that the producer has about the issue, can be considered 0.50, in relation to the availability of public or private technical orientation in this segment. Regarding business risk, the levels was also that of 0.50 due mainly to the weather, because the lack of rain can hinder the vegetable production.

Viability analysis by means of Monte Carlo simulation

In the simulation, the uncertain variables utilized were the number of produce boxes (2.000 in the observed harvest) and their respective sales price in each situation (unwashed and washed), denominated presupposition. For the definition of variables: box quantity/ha, the density and triangular probability functions were chosen, being the originally surveyed values considered the most likely ones, with the minimum and the maximum

Table 7. Cost of manual operations for maintenance of carrot/ha.

Description	Manual operations			
	Specification	Unit value (R$)	Quantity	Unproductive phase formation batch 60 days (R$)
Implementation				
Thinning	Days/man	70.00	10	700.00
Subtotal	-	-	-	700.00
Harvest				
Manual harvest	Bag of 50 kg	72.00	12	864.00
Subtotal	-	-	-	864.00
Fertilizer				
Fertilizer coverage NPK	Days/man	70.00	30	2,100.00
Subtotal	-	-	-	2,100.00
Fitosanitaries				
Insecticide Furadan 50 GR	kg	8.50	80	680.00
Herbicide Afalon SC	l	110.00	1.5	165.00
Fungicide Cantus	kg	520.00	0.3	156.00
Fungicide Rovral SC	l	80.00	3	240.00
Fungicide Cabrio Top	kg	41.00	5	205.00
Fungicide Sumilex 500 WP	kg	103.00	3	309.00
Fungicide Nativo	l	76.00	1.4	106.40
Subtotal	-	-	-	1,861.40
Total	-	-	-	5,525.40

Source: Authors (2014).

estimated at 10%, there being no need to use the historical data to support the distribution.

On the variable sale price, the probability density function was chosen, attributing the minimum value of R$ 18.90 and the maximum of R$ 23.10 for commercialization of cleaned vegetables, since all the values between the minimum and the maximum are equally probable of occurring, characterizing such as a distribution of uninterrupted probability. For prediction variables, the NPV, IRR and AROI were chosen. The quantity of repetitions considered for the result executed was of 5,000.00. After the simulation execution, it was possible to obtain the frequency graphics, with the minimum values, medium and maximum of variables, median, variance and standard deviation, among other information.

In the next pages, one can visualize the graphics related to the sales price practiced for commercialization of unwashed carrots and cucumbers. Figure 5 demonstrates that the average for the NPV is R$ 16,775.00 for carrots and R$ 13,925.00 for cucumbers, and these values are very near from those found in the Multi-index which are R$16,772.39 and R$13,931.07 respectively. The minimum value was R$11,294.00 for carrots and R$ 9,092.00 for cucumbers, and maximum R$ 22,760.00 for carrots and R$ 19,130.00 for cucumbers.

Figure 6 demonstrates that the average for IRR is 44.50%, while for carrots it is 45.57% as well as for cucumbers, values which are very near from those found in the Multi-index, which are 33.51% and 45.71%, respectively. The minimum value was 33.40% for carrots and 34.71% for cucumbers, and the maximum 55.31% for carrots and 55.72% for cucumbers.

Figure 7 demonstrates that the average AROI is 21.20% for carrots and 19.94% for cucumbers, values very close to those found with the Multi-index, which are 21.86 and 20.01%, respectively. The minimum value was 15.66% for carrots and 14.31% for cucumbers, and maximum of 26.50% for carrots and 25.25% for cucumbers.

In the next graphics, the results related to the sale price practised for commercialization of carrots and cucumbers clean/washed can be analysed. Figure 8 demonstrates that the average for the NPV is R$ 18,361.00 for carrots and R$ 16,568.00 for cucumbers; values similar to

Table 8. Cost of Manual operations for maintenance of cucumber/ha.

Description	Manual operations			
	Specification	Unit value (R$)	Quantity	Unproductive phase formation batch 60 days (R$)
Implementation				
Thinning	Days/man	70.00	3	175.00
Subtotal	-	-	-	175.00
Harvest				
Manual harvest	bag of 50 kg	72.00	10	700.00
Subtotal	-	-	-	700.00
Fertilizer				
Fertilizer coverage NPK	Days/man	70.00	100	7,000.00
Subtotal	-	-	-	7,000.00
Fitosanitaries				
Insecticide Furadan 50 GR	kg	8.50	80	680.00
Herbicide Afalon SC	l	110.00	1.5	165.00
Fungicide Cantus	kg	520.00	0.3	156.00
Fungicide Rovral SC	l	80.00	3	240.00
Fungicide Cabrio Top	kg	41.00	5	205.00
Fungicide Sumilex 500 WP	kg	103.00	3	309.00
Fungicide Nativo	l	76.00	1.4	106.40
Subtotal	-	-	-	1,861.40
Total	-	-	-	9,736.40

Source: Authors (2014).

Table 9. Commercialized carrot sale price unwashed/ha.

Description	Sale batch 120 days - 1 hectare - 2.000 boxes approximately			
	Specification	Unit value (R$)	Quantity boxes	Total value (R$)
Carrot box	Box with 30 kg	16.00	2.000	32,000.00
Subtotal	-	-	-	32,000.00
Total	-	-	-	32,000.00

Source: Authors (2014).

those found in the Multi-index, which are R$ 18,389.79 and R$ 14,679.86, respectively. The minimum value was R$11,510.00 for carrots and R$ 10,842.00 for cucumbers, and the maximum R$ 26,127.00 for carrots and R$ 23,236.00 for cucumbers.

Figure 9 demonstrates that the average for IRR is 29.85% for carrots and 44.50% for cucumbers, values close to those found in Multi-index, which are 29.99 and 39.09%, respectively. The minimum value was 20.83% for carrots and 33.78% for cucumbers, and the maximum of 38.74% for carrots and 55.39% for cucumbers.

Figure 10 demonstrates that the average for the AROI is 15.99% for carrots and 17.83% for cucumbers, values not unlike those found in the Multi-index, which are 16.06 and 14.94%, respectively. The minimum value was 10.92% for carrots and 12.72% for cucumbers, and the maximum of 21.06% for carrots and 23.08% for cucumbers.

FINAL CONSIDERATIONS

The object t of this research was to analyse return

Table 10. Commercialized cucumber sale price unwashed/ha.

Description	Sale batch 120 days - 1 hectare - 2.000 boxes approximately			
	Specification	Unit value (R$)	Quantity Boxes	Total value (R$)
Cucumber box	Box with 30 kg	18.00	2.000	36,000.00
Subtotal	-	-	-	36,000.00
Total	-	-	-	36,000.00

Source: Authors (2014).

Table 11. Net cash flow statement of commercialization of carrot and cucumber unwashed/ha.

Month	Carrot / Ha			Cucumber / Ha		
	Disbursement	Revenue	Cash flow	Disbursement	Revenue	Cash flow
0	(5,600.71)		(5,600.71)	(2,695.00)		(2,695.00)
1	(2,073.03)		(2,073.03)	(3,000.66)		(3,000.66)
2	(2,073.03)		(2,073.03)	(3,000.66)		(3,000.66)
3	(2,073.03)		(2,073.03)	(3,000.66)		(3,000.66)
4	(2,809.03)	32,000.00	29,190.97	(3,828.66)	36,000.00	32,171.35

Source: Authors (2014).

Table 12. Commercialization indicators of carrot and cucumber unwashed/ha.

Indicators		Carrot	Cucumber
		1 Ha	1 Ha
Return	Present value of cash flow of investments	-14,439.47	-12,970.23
	Present value of cash flow of benefits	31,840.80	26,901.29
	Net present value	16,772.39	13,931.07
	Benefit/Cost index	4,245.64	4,020.39
	NPV Equivalent ha/year	2.21	2.07
	Annual ARI	21.86%	20.01%
Risk	Annual internal rate of return	33.51%	45.71%
	MARR/IRR Annual	17.91%	13.13%
	Management risk	0.50	0.50
	Business risk	0.50	0.50

Source: Authors (2014).

expectations and the risks associated to the implantation of a vegetable-washing machine, bringing to comparison the commercialization of unwashed vegetables and washed/clean ones, through the machine implantation for washing. The Multi-index methodology, proposed by Souza and Clemente (2008), was employed for data analysis. As metrics for validity and comparison, Monte Carlo's simulations were conducted to verify and confirm the decision of investment in this agribusiness.

The risk indicators for the commercialized vegetables, unwashed/dirty, are NPV of R$ 16,772.39, IRR of 33.51% and AROI of 21.86%, both for carrots. For cucumbers, the indicators found were NPV of R$ 13,931.07, IRR of 45.71% and AROI of 20.01%, all confirmed by the Crystal Ball software.

When a competitive strategy based on the firm's internal resources was applied by means of the vegetable-washing machine, with return expectations for 24 months, the impact for carrots are NPV of R$ 18,389.79, IRR of 29.99% and AROI of 16.06%. For cucumbers, the indicators found were NPV of R$ 14,679.86, IRR of 39.09% and AROI of 14.94%.

It is worth highlighting that the management and business risks were considered of medium size, given

Table 13. Total cost of implantation of vegetable-washing machine.

Description	Washer Construction			
	Unity	**Unit value (R$)**	**Quantity**	**Total (R$)**
Shack	Unity	40,500.00	1	40,500.00
Vegetable washing machine	Unity	85,000.00	1	85,000.00
Floor construction	Unity	25,000.00	1	25,000.00
-	Sub total	-	-	150,500.00
-	Total	-	-	150,500.00

Source: Authors (2014).

Table 14. Washing cost for 2.000 carrot boxes.

Description	Mechanized operations			
	Specification	**Unit value** (R$)	**Quantity**	**Total value** (R$)
Transport to consuming centres	Truck	241.00	4	964.00
	Sub total	-	-	964.00
	Total	-	-	964.00
Description	**Manual operations**			
	Specification	**Unit value (R$)**	**Quantity**	**Total value (R$)**
Washing	Days/man	70.00	25	1,750.00
	Sub total	-	-	1,750.00
Packing wooden boxes	Unity	1.50	2.000	3,000.00
	Sub total	-	-	3,000.00
	Total	-	-	4,750.00

Source: Authors (2014).

Table 15. Washing cost for 2.000 cucumber boxes.

Description	Mechanized operations			
	Specification	**Unit value (R$)**	**Quantity**	**Total value (R$)**
Transport to consuming centres	Truck	241.00	4	964.00
	Sub total	**-**	**-**	**964.00**
	Total	**-**	**-**	**964.00**
Description	**Manual operations**			
	Specification	**Unit value (R$)**	**Quantity**	**Total value (R$)**
Washing	Days/man	70.00	25	1,750.00
	Sub total	**-**	**-**	**1,750.00**
Packing wooden boxes	Unity	1.50	2.000	3,000.00
	Sub total	**-**	**-**	**3,000.00**
	Total	**-**	**-**	**4,750.00**

Source: Authors (2014).

Table 16. Sale price of commercialized carrot clean/ha.

Description	Sale batch 120 days - 1 hectare - 2.000 boxes aproximately			
	Specification	Unit value (R$)	Quantity	Total value (R$)
Carrot box	Box with 30 kg	21.00	2.000	42,000.00
	Sub total	-	-	42,000.00
	Total	-	-	42,000.00

Source: Authors (2014).

Table 17. Sale price of commercialized cucumber clean/ha

Description	Sale batch 120 days - 1 hectare - 2.000 boxes aproximately			
	Specification	Unit value (R$)	Quantity	Total value (R$)
Cucumber box	Box with 30 kg	23.00	2.000	46,000.00
	Sub total	-	-	46,000.00
	Total	-	-	46,000.00

Source: Authors (2014).

Table 18. Net cash flow statement of commercialization of carrot and cucumber clean/ha.

Month	Carrot 1 Há			Cucumber 1 Ha		
	Disbursement	Revenue	Cash flow	Disbursement	Revenue	Cash flow
0	(5,600.71)	-	(5,600.71)	(2,695.00)	-	(2,695.00)
1	(3,977.70)	-	(3,977.70)	(4,905.32)	-	(4,905.32)
2	(3,977.70)	-	(3,977.70)	(4,905.32)	-	(4,905.32)
3	(3,977.70)	-	(3,977.70)	(4,905.32)	-	(4,905.32)
4	(5,379.46)	42,000.00	36,620.54	(6,399.09)	46,000.00	39,600.91

Source: Authors (2014).

Table 19. Net cash flow statement of commercialization of carrot and cucumber clean/ha.

Indicators		Carrot / Ha	Cucumber / Ha
Return	Present value of cash flow of investments	-22,575.78	-19,694.02
	Present value of cash flow of benefits	40,965.57	34,373.88
	Net Present Value	18,389.79	14,679.86
	Benefit/Cost Index	5,307.14	4,236.48
	NPV Equivalent ha/year	1.81	1.75
	Annual ARI	16.06%	14.94%
Risk	Annual Internal Rate of Return	29.99%	39.09%
	MARR/IRR Annual	20.01%	15.35%
	Management Risk	0.50	0.50
	Business Risk	0.50	0.50

Source: Authors (2014).

there is availability of public or private technical orientation for the agricultural segment and that this agribusiness is exposed, mainly, to weather interference. Moreover, it was not considered for the marketing of

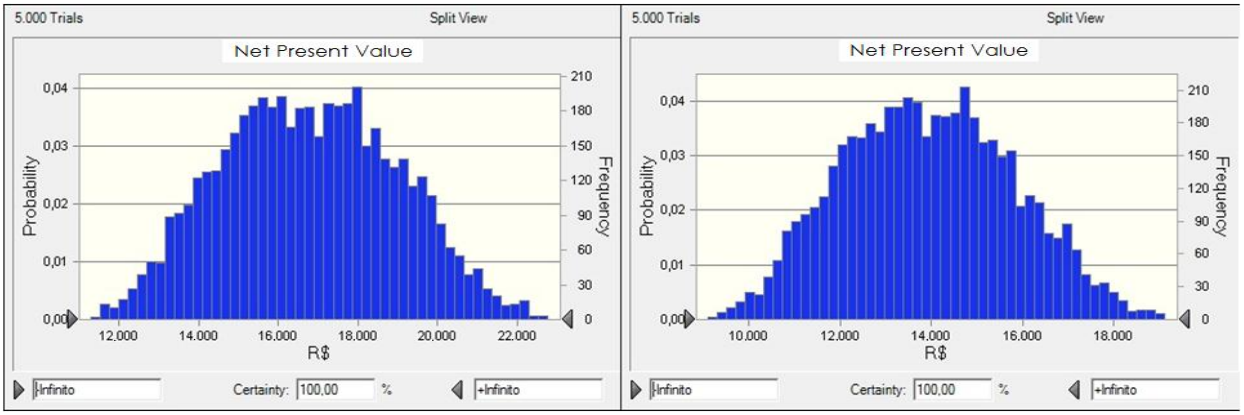

Figure 5. Graphic of frequency and statistic of output variable Net Present Value (NPV)-unwashed carrot and cucumber.

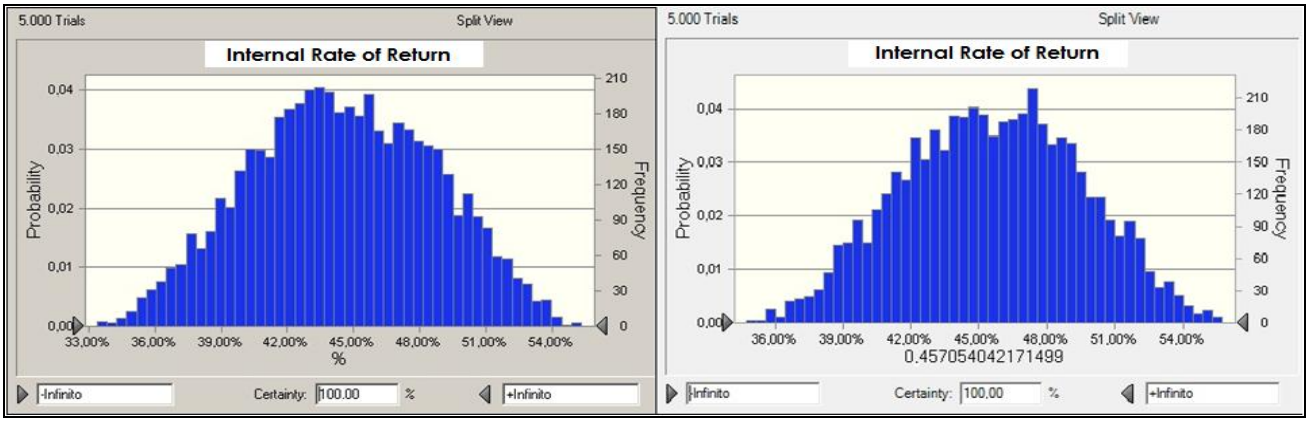

Figure 6. Graphic of frequency and statistic of output variable IRR (Internal Rate of Return) – Unwashed carrot and cucumber

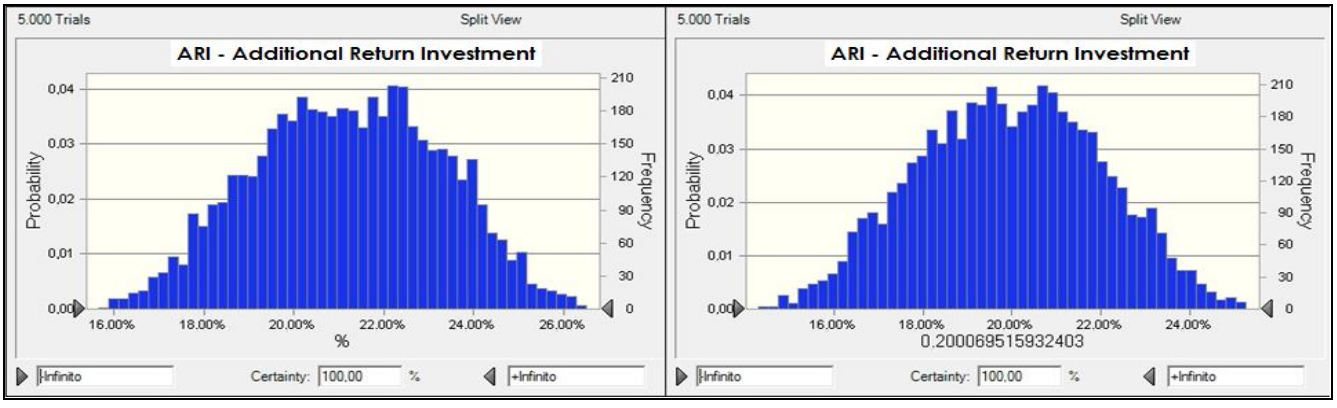

Figure 7. Graphic of frequency and statistic of output variable AROI (Additional Return Over the Investment) - Unwashed carrot and cucumber

products under review the impact that the appearance of the washed product can bring in negotiating with customers.

The research proposes that the use of Multi-index methodology, its group of indicators for analysis, the return evaluation of investment and the associated risks

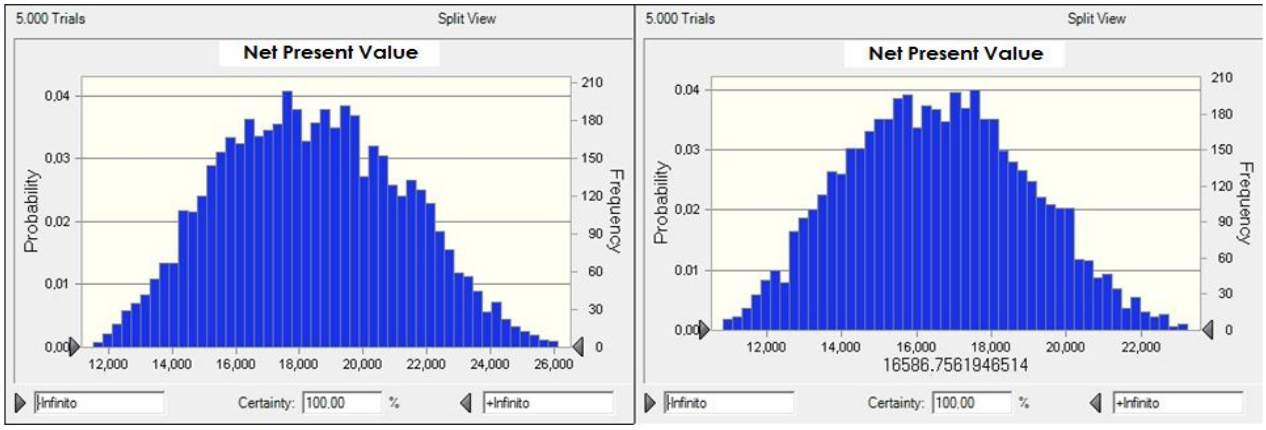

Figure 8. Graphic of frequency and statistic of output variable NPV (Net Present Value) - Clean carrot and cucumber

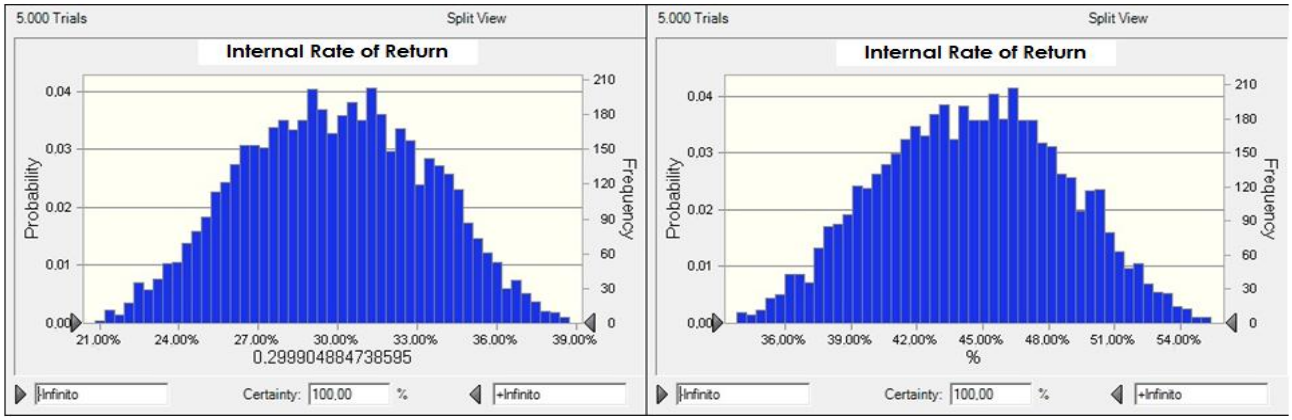

Figure 9. Graphic of frequency and statistic of output variable IRR (Internal Rate of Return) - Clean carrot and cucumber

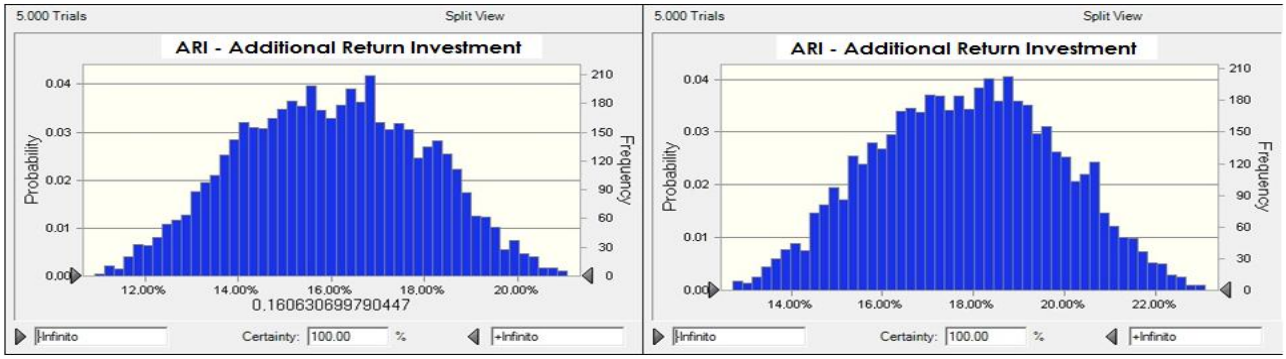

Figure 10. Graphic of frequency and statistic of output variable AROI (Additional Return Over Investment) - Clean carrot and cucumber.

enhance the rural manager perception, contributing with satisfactory results in his investment portfolio demonstrating the theoretical implication. The obtained results in this study indicate that the investment in a vegetable-washing machine brings a rapid return and constitutes a profitable activity, demonstrating the practical implication of the study.

As for research limitations, some caution is needed

when analysing the investment return in vegetable-washing machines, because there are possible variations verified in the agricultural area, such as price of inputs and seeds, which are restricted to the prices dictated by the manufacturers; these in turn can impact in the final product price and consequently in the analysis of return and risk in the business. As suggestions for future research, it is recommended that this research structure be applied on other kinds of vegetables, as well as in the same sector, for the next years and in different regions as well.

Conflict of Interests

The authors have not declared any conflict of interest.

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academicJournals

Vol. 11(15), pp. 1326-1335, 14 April, 2016
DOI: 10.5897/AJAR2015-10643
Article Number: 2E5AD1F58049
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Soil physical and hydraulic changes in different yielding zones under no-tillage in Brazil

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Received 13 November, 2015; Accepted 17 March, 2016

Soil physical and structural degradation may influence crop productivity over time in long-term no-tillage system areas. A field study was conducted at two sites, Palmeira das Missões and Não-Me-Toque, in southern Brazil to quantify soil physical/hydraulic and structural changes in zones with different yield potentials. The sites have been managed under no-tillage system without soil disturbance for more than 10 years. Soils were classified as Oxisols (Hapludox). Each site was divided into three zones with low, medium and high yield potentials based on overlapping of yield maps obtained from harvesters with precision agriculture tools. Within each yielding zone soil samples were collected to determine bulk density, porosity and aggregate stability. In addition, water infiltration rate and initial time for starting surface runoff were measured using a sprinkler infiltrometer (Cornell Sprinkler infiltrometer). Our findings showed that soils under low-yielding zones presented higher bulk density, lower macro-aggregate stability and water infiltration rate as well as shorter time for starting surface runoff compared to high-yielding zones. Therefore, these results suggest that soil physical and structural degradations have induced crop yield losses under long-term no-tillage areas. Macro-aggregate stability (>4.76 mm) and water infiltration rate were efficient parameters for distinguishing yielding zones in Oxisols managed under long-term no-tillage system in southern Brazil.

Key words: Soil compaction, Cornell Sprinkler Infiltrometer, soil aggregation, water infiltration.

INTRODUCTION

The perpetuation of conservationist tillage systems is directly related to the soil and to the adopted crop management practices, which can enable the maintenance and improvement of soil structure quality (Dumanski, 2015) and crop yield (Pittelkow et al., 2015). Thus, the success of no-tillage systems depends on the maintenance of the following physical-hydraulic conditions: i) suitable soil water and air balance for plant growth (Lanzanova et al., 2010; Jemai et al., 2013; Palm et al., 2014, Nunes et al., 2015); ii) high rate of water infiltration into the soil (Golabi et al., 2014); iii) reduction of surface runoff (Sun et al., 2015).

In contrast, intensive machinery traffic during crop management practices is one of the major drivers of deleterious impacts on soil structural quality (Raper, 2005; Drescher et al., 2011; Barik et al., 2014). Soil aggregation is globally used as an indicator of soil structural quality changes induced by land use and management practices (Vezzani and Mielniczuk, 2011; Karlen et al., 2013). Macro-aggregates play crucial role on the stabilization and protection of soil organic matter (Six et al., 2000a), which increase the soil resilience to structural degradation and water dynamics in the soil (Vezzani and Mielniczuk, 2011). Studies have shown that increases of soil aggregation is positively correlated to higher yields of corn (Song et al., 2015), soybean (Corbin et al., 2010) and other cereals (Hou et al., 2012).

The determination of soil physical-hydraulic properties such as water infiltration rate can be important for understanding the causes of variability in crop productivity. According to Jégo et al. (2015), the knowledge of the spatial variability of crop yields requires a large amount of data about soil physical properties and their relationships that influence plant development. The relationship between soil water dynamics and crop yield can provide complementary information for understanding critical levels of soil porosity, resistance to penetration and bulk density (Kılıç et al., 2004; Whalley et al., 2008; Reichert et al., 2009).

Soil water infiltration can be determined using several methods, such as: tension infiltrometers or disc permeameters, pressure infiltrometers and sparkler infiltrometers. The method of concentric cylinders (pressure infiltrometer) uses a hydraulic load on the soil surface and has been used as a standard determination of infiltration, although some authors consider that this method overestimates the real water infiltration (Cheng et al., 2011). On the other hand, van Es and Schindelbeck (2003) stated that a sprinkler infiltrometer (Cornell Sprinkler infiltrometer) allows the determination of several important hydrological features, such as, the initial time for surface runoff, infiltration rate, accumulated infiltration and water consumption which are relevant characteristics in the evaluation of soil hydro-physical quality. These authors also mention the advantages of this method compared to double concentric rings, being of low cost in acquisition; convenience in transportation (small size and low weight), rapid assessment and operated by one person, easy calibration for different rainfall intensities, and low water consumption. Despite these advantages, the high coefficients of variation observed in the soil water infiltration data (Warrick and Nielsen, 1980) have limited use in commercial areas, since an accurate assessment requires a high number of field replications. In this sense, the adoption of precision agriculture tools makes possible to identify homogeneous areas with different yielding potential (Amado et al., 2009; Santi et al., 2013), and to investigate soil physical and hydraulic property changes within each one these yielding zones (Keller et al., 2012).

Although many technical papers have been published, changes on physical and hydraulic properties within different yielding zones in Brazil are still poorly documented. An on-farm study was conducted in southern Brazil, a pioneer region for the adoption of no-tillage (NT) system and precision agriculture tools. The objective of this study was to quantify soil physical/hydraulic and structural changes within zones with high-, medium- and low-yield potential. This research hypothesized that zones with higher crop yield have better soil physical/hydraulic and structural quality compared to zones of lower crop yield. Water infiltration rate assessed by sprinkler infiltrometer (Cornell Sprinkler infiltrometer) method is able to distinguish zones with different yield potentials.

MATERIALS AND METHODS

Experimental field sites

The study was carried out in the Eastern Plateau of Rio Grande do

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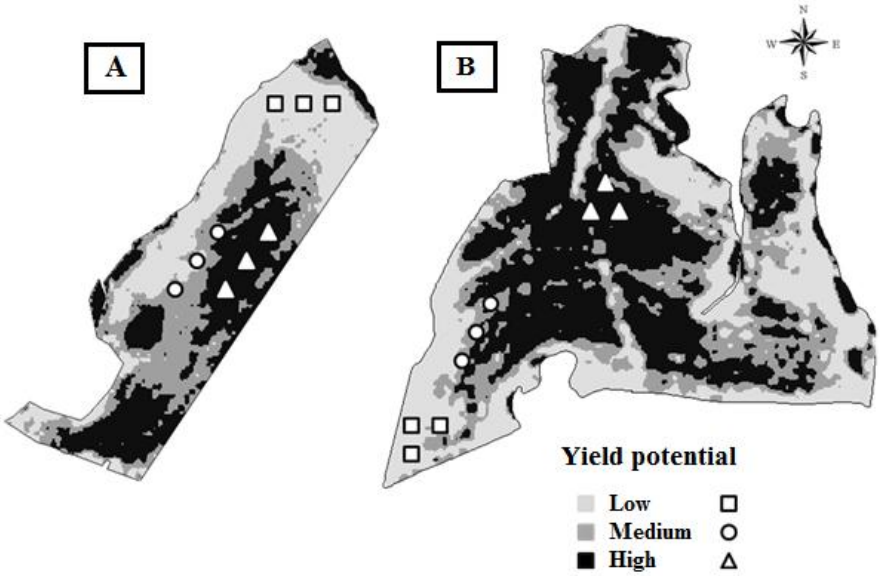

Figure 1. Experimental areas, Palmeira das Missões (A) and Não-Me-Toque (B), divided into three zones according to the yield potential (low, medium and high). Symbols represent the locations where were undertaken evaluations (soil physical characterization and water infiltration test), Rio Grande do Sul – Brazil.

Sul, Brazil at two experimental sites: i) Palmeira das Missões (PM), with area of 57.4 ha, at medium geographic coordinates, 27°53'S and 51º18'W and altitude of 600 m; and ii) Não-Me-Toque (NMT), with area of 132 ha, at coordinates 28°48'S and 52º77'W, and altitude of 550 m (Figure 1). The soils were classified as Oxisols (Hapludox) at both study sites (Santos et al., 2013). The average clay contents within each site were: i) PM - 730; 741 and 785 g kg^{-1}; ii) NMT - 572; 616 and 518 g kg^{-1} within high-, medium- and low-yielding zones, respectively. The regional climate according to the Köppen´s global climatic classification is humid subtropical (Cfa), with an average annual temperature of 18.1 ºC and annual rainfall of the order of 1,900 mm.

Both study sites have been managed with precision agriculture tools such as yield monitoring, systematic soil sampling and variable rate applications of fertilizers for five years and under NT system for more than ten years without interruption. In this work, we used the methodology described in Santi et al. (2013) overlapping the following sequences of crop yield maps for PM: soybean-corn-soybean-wheat-soybean-corn and NMT: corn-soybean-wheat-corn.

From the overlap of yield maps, each area were divided into three homogeneous zones according to their yield potential (high, medium and low), which was considered a low-yielding zone when showing up to 95% of the average crop yield in the total area, medium-yielding zone between 95 and 105% and high-yielding zone above 105% (Santi et al., 2013). Average grain yield of crops observed in both experimental areas were: i) PM: 2000/01 soybean (3,180 kg ha^{-1}); 2001/02 corn (7,800 kg ha^{-1}); 2002/03 soybean (3,240 kg ha^{-1}); wheat 2003 (3,540 kg ha^{-1}); 2003/04 soybean (2,220 kg ha^{-1}) and corn 2004/05 (6,000 kg ha^{-1}); ii) NMT: 2001/02 corn (5,640 kg ha^{-1}); 2002/03 soybean (3,720 kg ha^{-1}); wheat 2003 (2,700 kg ha^{-1}) and corn 2004/05 (7,680 kg ha^{-1}).

Sampling and determination of physical soil properties

In each study site, three representative sampling points within each yielding zone (high, medium and low yield) were selected (Figure 1). Undisturbed soil samples were taken using a metal cylinder (height 0.05 m x internal diameter 0.05 m), from the 0-0.05 and 0.05-0.10 m layers. At each zone five samples (replications) were collected, providing a total of 45 samples at each site. In addition, soil blocks of 0.05 x 0.05 m to 0.05 m depth were collected from the 0-0.05 and 0.05-0.10 m layers at each sampling point for soil aggregate stability analyses.

In the laboratory, the undisturbed soil samples were weighted (initial moisture), saturated with water and weighted again. The determination of the water content at -6 kPa (60 cm of water column) matrix potential was made on tension tables (Ball and Hunter, 1988). Afterwards, the soil samples were dried at 105ºC for 48 h and weighed. Bulk density (BD) was calculated dividing the soil dry weight by volume of cylinder. The soil total porosity (TP, m^3 m^{-3}) was calculated by: TP = 1 - (BD/PD), where PD is particle density (2.65 Mg m^{-3}). Soil macroporosity (Ma, m^3 m^{-3}) was estimated from the difference between water content in saturated soil and at -6 kPa. Soil microporosity (MiP) was estimated as the retained water content at -6 kPa soil matrix potential.

Field-moist soil was gently passed through an 8 mm sieve by breaking up the soil along natural planes of weakness, and air dried. The aggregate samples (25 g) were moistened slowly by capillarity and after 10 min, subjected to aggregate stability analysis using the standard wet-sieving method (Kemper and Chepil, 1965), in a vertical oscillator (Yoder, model MA-148 unit), at a speed of 30 oscillations per min for 10 min. The vertical oscillator consists of set of four sieves (4.76, 2.0, 1.00, and 0.21 mm mesh sizes). The

Table 1. Soil bulk density (BD), macroporosity (Ma), microporosity (Mi) and total porosity (TP) within different yielding zones (High, Medium, Low) under no-tillage system in Palmeira das Missões and Não-Me-Toque. Rio Grande do Sul, Brazil.

Yielding zones	Palmeira das Missões - PM				Não-Me-Toque - NMT			
	BD	Ma	Mi	TP	BD	Ma	Mi	TP
	Mg m^{-3}	m^3 m^{-3}			Mg m^{-3}	m^3 m^{-3}		
	0.00 to 0.05 m layer							
High	1.336 [a]	0.162 [ns]	0.438 [ns]	0.600 [ns]	1.188 [a*]	0.194 [ns]	0.352 [ns]	0.532 [ns]
Medium	1.386 [b]	0.159	0.433	0.592	1.281 [ab]	0.168	0.341	0.509
Low	1.373 [b]	0.159	0.457	0.616	1.337 [b]	0.185	0.321	0.506
	0.05 to 0.10 m layer							
High	1.497 [ns]	0.105 [ns]	0.455 [ns]	0.561 [a]	1.431 [a]	0.113 [ns]	0.371 [a]	0.484 [ns]
Medium	1.505	0.117	0.468	0.585 [ab]	1.501 [ab]	0.129	0.326 [b]	0.495
Low	1.490	0.125	0.477	0.602 [b]	1.554 [b]	0.127	0.325 [b]	0.452

*Means following for same letter in each soil layer do not differ themselves according to Tukey`s test ($p<0.05$). ns: non-significant.

aggregates were then separated into the following classes: 8.00-4.76; 4.76-2.00; 2.00-1.00; 1.00-0.21 and < 0.21 mm. The aggregates retained on each sieve were dried at ± 105 °C for 48 h for dry mass determination. After, the aggregates were chemically dispersed (NaOH 2% for 30s) for quantification of the mass of inert materials. Inert materials were subtracted from the respective aggregate size fractions.

Soil hydraulic properties

Around sampling points where undisturbed soil samples were taken, tests of water infiltration into soil were performed, with five replicates, providing a total of 45 measurements at each site. Water infiltration assessment was performed through a sprinkler infiltrometer ("Cornell Sprinkler infiltrometer"), described by van Es and Schindelbeck (2003). This infiltrometer is a portable rainfall simulator with a capacity of 20.6 liters composed of 69 drippers at the bottom, with a diameter of 0.00063 m and 0.19 m long each. This assembly is mounted on a cylinder with a diameter of 0.24 m, simulating different rain intensities by an air inlet regulation system.

To determine the water infiltration rate and the initial time for surface runoff a simulated rain of 300 mm h^{-1} was established for 60 min, as proposed by van Es and Schindelbeck (2003). Although the intensity is high for regional conditions, we chose to follow the original standard established by these authors. The rainfall intensity was determined from the expression:

$$Ri = (H1 - H2) / Ti \quad (1)$$

Where: Ri = rainfall intensity (mm h^{-1}); H1 and H2= initial and final reading (mm) of the water volume in the infiltrometer ruler, respectively; Ti= time interval (hours) between readings (we used 0.05 h in this study).

The surface runoff was determined by:

$$SR = [(V_t \times 1000) / (45730 \times t)] \quad (2)$$

Where: SR= surface runoff (mm h^{-1}), Vt= water volume collected (ml); 45730= ring area (mm^2); t= time interval (hours) between the runoff collections (we used 0.05 h in this study).

The water infiltration rate was determined by:

$$WI = Ri - SR \quad (3)$$

where: WI= water infiltration rate (mm h^{-1}), Ri = rainfall intensity (mm h^{-1}), SR= surface runoff (mm h^{-1}).

At the moment of water infiltration measurements, the soil surface was fully covered by plant residues from previous winter crops and the volumetric soil water content was very similar among yield zones (that is, PM – 0.243, 0.234 and 0.241 m m^{-3}; NMT – 0.253, 0.266 and 0.259 m m^{-3} at low- medium- and high-yielding zone, respectively).The equations of water infiltration rate and cumulative water infiltration were adjusted by non-linear and linear regressions, respectively, based on average values observed in each treatment, using Statistical Analysis System v.8.0 software (SAS Inc, Cary, USA). The surface runoff time was determined by intersection point between infiltration rate and surface runoff curves.

RESULTS AND DISCUSSION

Soil physical properties

Bulk density was significantly lower under high-yielding zones compared to low-yielding zones for both sites at surface soil layer (0-0.05 m) and only for NMT at the deeper soil layer (0.05-0.10 m) (Table 1). Bulk density is widely used to investigate soil compaction and its deleterious impacts on plant growth (da Silva et al., 1994; Reynolds et al., 2009; Stolf et al., 2011; Salem et al., 2015). Within low- and medium-yielding zones bulk density reached values >1.2 Mg m^{-3} for both 0-0.05 and

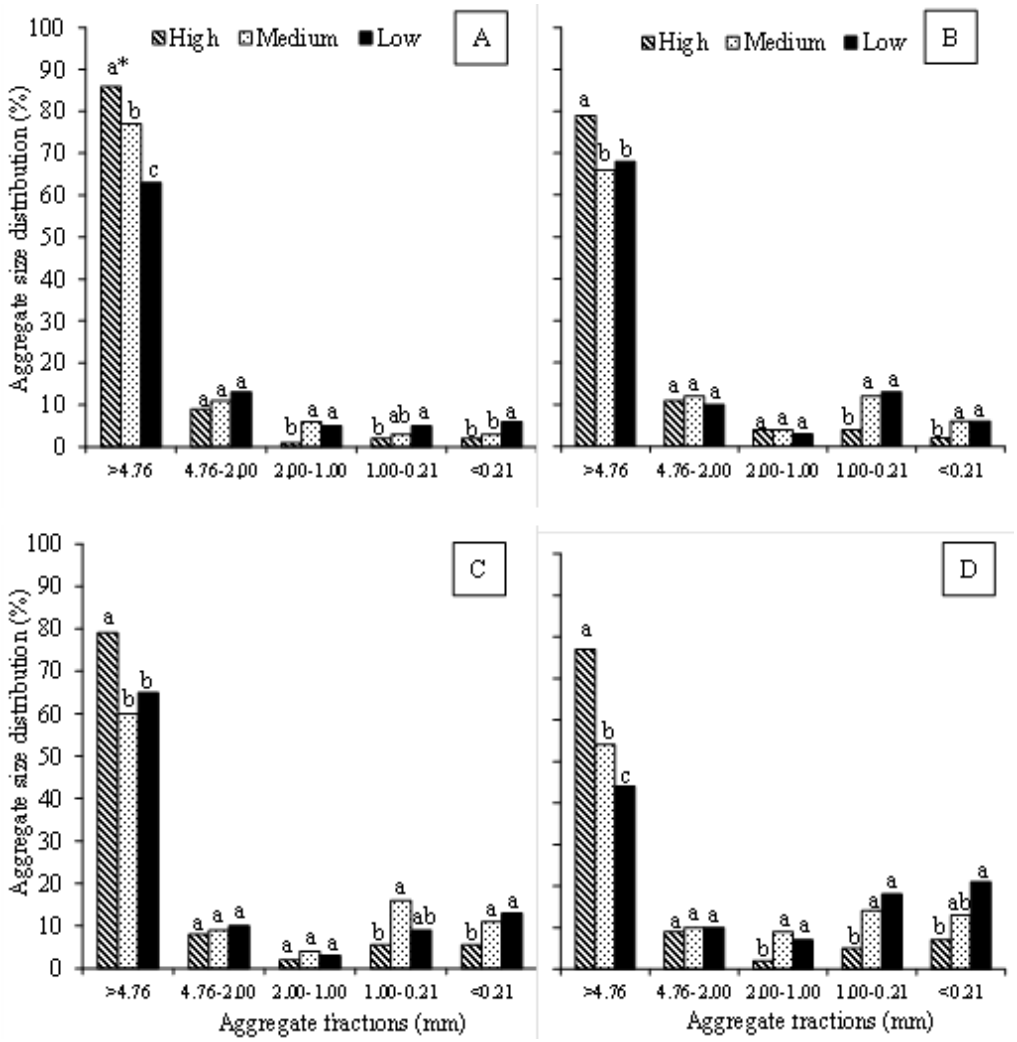

Figure 2. Aggregate size distribution (%) in high-, medium- and low-yielding zones for the 0-0.05 m (A;B) and 0.05-0.10 m (C;D) soil layers in Palmeira das Missões (left) and Não-Me-Toque (right). *Mean values within of aggregate fractions followed by the same letter do not differ among themselves according to Tukey's test (p<0.05).

0.05-0.10 m layers, which is considered a critical limit to suitable plant growth in clay soils (Reynolds et al., 2009). Therefore, soil compaction is likely a key factor that drives the productive potential losses in these zones. Under long-term no-tillage the absent of soil disturbance associated with intensive machinery traffic have induced critical soil compaction (Nunes et al., 2015), that leads to decreasing crop growth and yield, as reported by Pittelkow et al. (2015). Decreased productivity of the crops also decreases atmospheric CO_2 uptake by the biomass (above and belowground), resulting in lower organic C inputs to soils over time, and to progressive process of soil physical degradation. Soil porosity attributes had small changes among yielding zones, though an overall non-significant tendency of higher macroporosity and total porosity in the high-yielding zones was verified (Table 1).

Overall, macro-aggregation stability was high regardless of the yielding zone for both sites (Figure 2). These results are typically reported in studies conducted in weathered Brazilian soils (Madari et al., 2005; Moraes et al., 2014; Cherubin et al., 2015), being associated primarily with mineralogical composition dominated by Fe and Al oxides and 1:1 minerals present in these soils

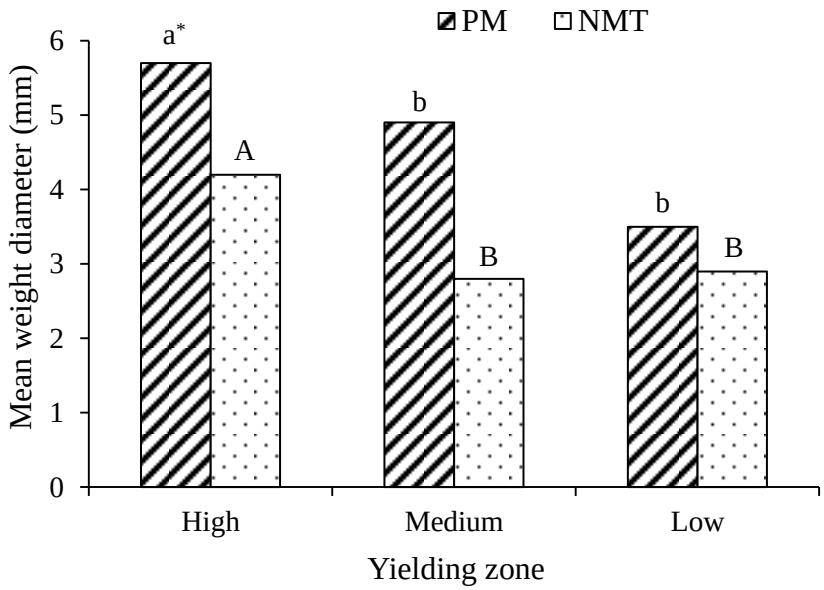

Figure 3. Mean weight diameter (mm) of soil aggregates for the 0-0.05 m layer in high-, medium- and low-yielding zones in Palmeira das Missões (PM) and Não-Me-Toque (NMT). *Mean values followed by the same lower letter and upper letter do not differ among themselves in PM and NMT, respectively, according to Tukey's test (p<0.05).

(Silva and Mielniczuk, 1997; Six et al., 2000b). Despite that, large macro-aggregate fractions (>4.76 mm) were highly sensitive indicators to detect zones with different yield potentials, agreeing to reported values by O'Brien and Jastrow (2013). Soil macro-aggregates protect physically some organic matter fractions, resulting in pools with longer turnover times (Six et al., 2000a) that mediate soil physical processes related to water and air dynamics and providing resistance against soil erosion. In contrast, smaller aggregates fractions (<2 mm), which are units with lower structural complexity, increased under medium- and low-yielding zones. Therefore, significantly greater macro-aggregate values (Figure 2) and mean weight diameter values (Figure 3) under high-yielding zones for both sites indicate that soil physical and structural degradation have been negatively impacted on the productive potential of crops. Our findings suggest that improved soil structural quality provides better conditions for plant growth (roots and shoots) and consequently higher grain yield. Therefore, these areas have greater annual inputs of organic C into the soil, which acts as cementing agents throughout soil aggregation processes (Tisdall and Oades, 1982), and plants with larger and deeper root systems, which release exudates that have a cementing effect on soil particles, beyond the physical action in microaggregate formation via compressing action of growing roots and in the entanglement of soil particles to form and stabilize macro-aggregates (Tisdall and Oades, 1982; Bronick and Lal, 2005). In addition, greater biomass production led to higher amount of residues on soil surface, improving soil resistance to degradation (Wegner et al., 2015).

Water infiltration into the soil

The water infiltration rate and cumulative water infiltration within yielding zones at PM and NMT are shown in Figure 4. A significant decrease in water infiltration rates from high- to medium- and low-yielding zones was verified. Previously, Amado et al. (2009) also reported that zones of low yield potential had lower macroporosity and lower water availability for plants. Girardello et al. (2011) mentioned that zones of low yield potential had lower physical quality, expressed by higher soil density and lower total porosity, respectively, at 0.15-0.20 m depth, conditioning low water infiltration into the soil. These results indicate the distinction of current environmental offer between different yielding zones, confirming that in areas with a historical of high productivity have the highest water infiltration rates. Similarly, Keller et al. (2012) measured significantly lower

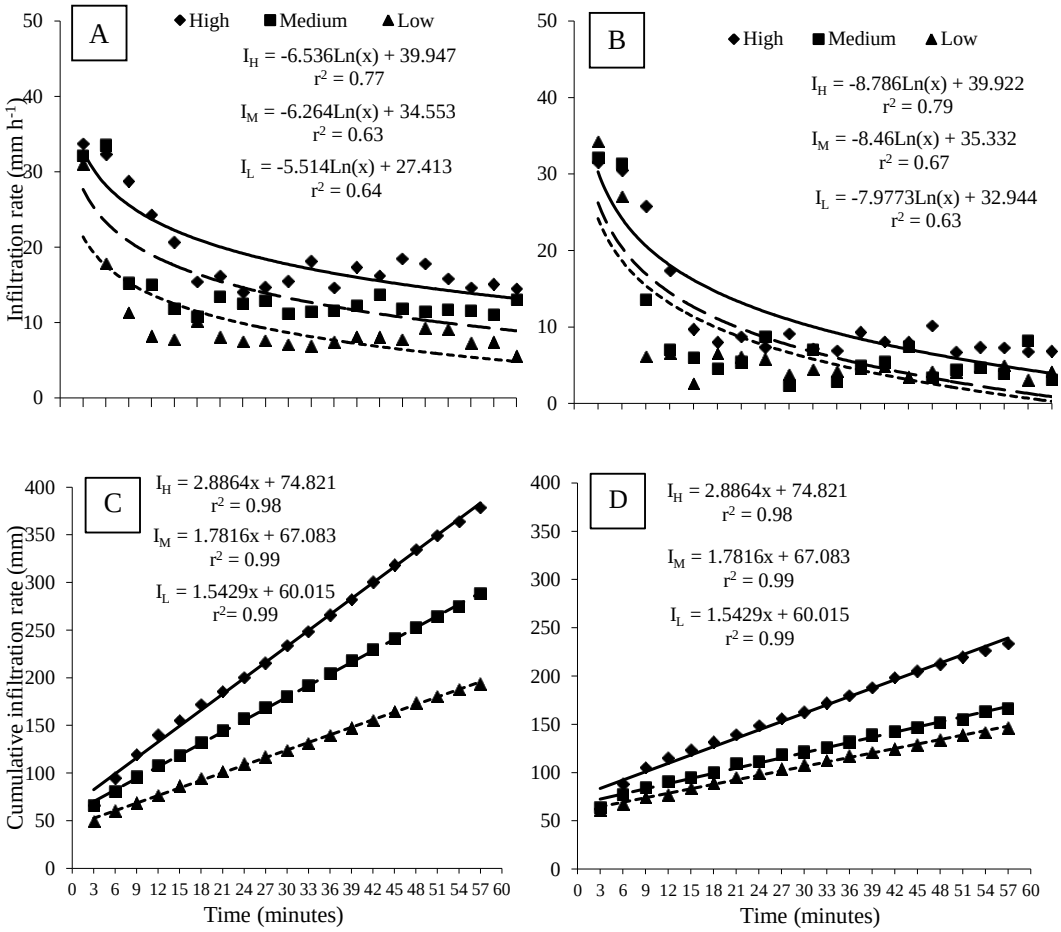

Figure 4. Water infiltration rate (mm h^{-1}) and cumulative water infiltration (mm) in soil obtained by a sprinkler infiltrometer (Cornell Sprinkler infiltrometer) in areas with different yield potentials in Palmeira das Missões (A and C) and Não-Me-Toque (B and D), Rio Grande do Sul, Brazil.

field-saturated hydraulic conductivity in low-yielding zones than in high- and medium-yielding zones. Thus, water infiltration into soil is established as an important indicator of soil physical quality (Alves et al., 2007; Amado et al., 2009; Reichert et al., 2009).

The coefficients of determination of the fitted regressions obtained in the two sites were > 0.60. These values are classified as satisfactory for water infiltration data, due to high spatial and temporal variability, assumed by the interrelationships involving intrinsic and extrinsic soil factors, which are undergo differentiated changes in time and space (Warrick and Nielsen, 1980; Tavares-Filho et al., 2006). According to Vieira et al. (2010) water infiltration in soil is usually quite variable due to variability of integrating texture, soil structure, biological activity, plant growth and uniformity of soil management practices.

In general, similar water infiltration rates were verified in PM and NMT, however, greater differences among yielding zones were observed in PM. The cumulative infiltration was higher in PM than in NMT, and again, greater differences among yielding zones was observed in PM. Both water infiltration rate and cumulative water infiltration values were higher within high-yielding zones than those quantified within medium- and low-yielding zones. In regard to sites, cumulative water infiltrations were lower in NMT compared to PM. This result may be associated with higher soil compaction in NMT area, at the time of assessment. According to Drescher et al. (2011), compacted soils have several hydrophysical changes with consequent damage to crop production capacity. The water infiltration is one of the phenomena

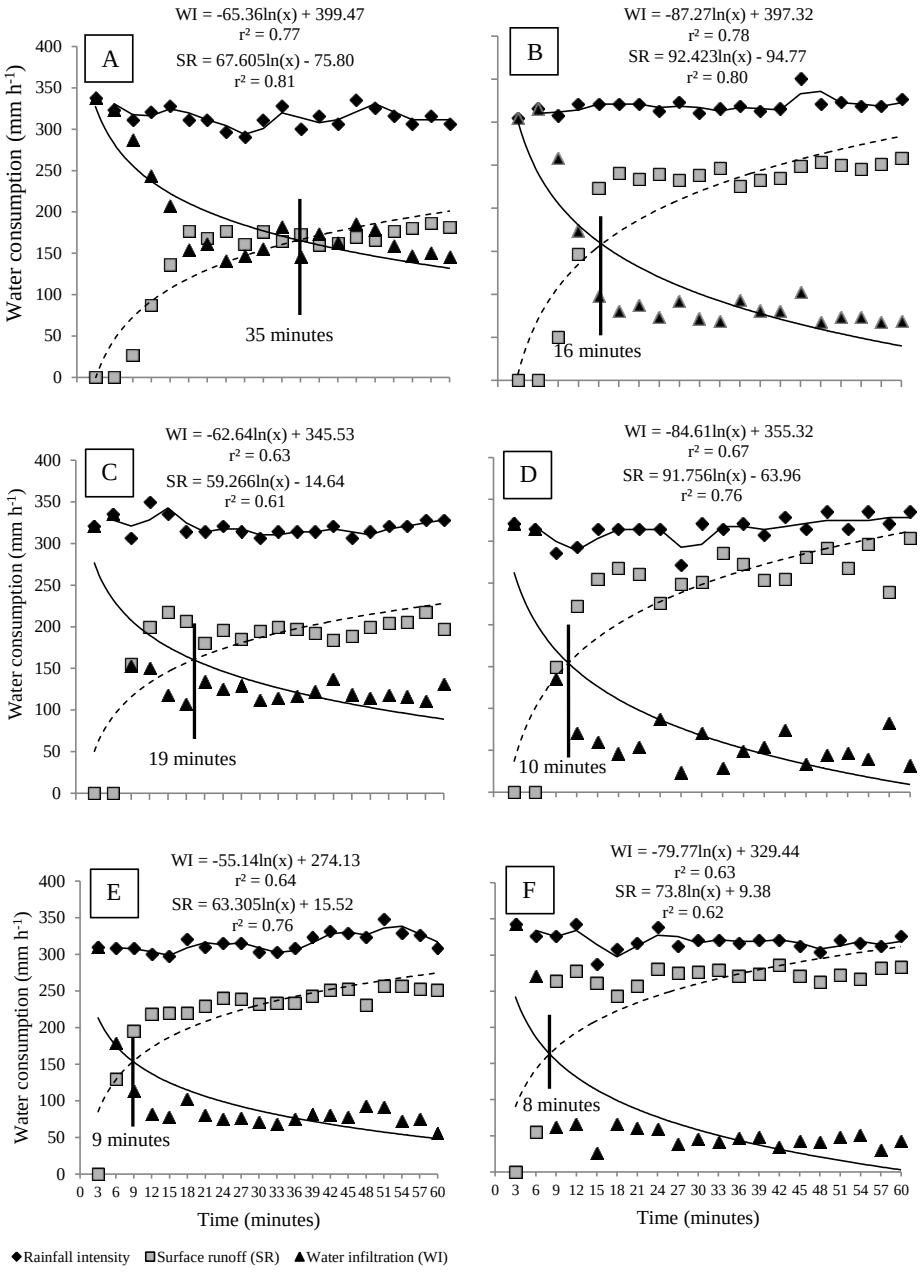

Figure 5. Simulated rainfall intensity, surface runoff (SR) and water infiltration rate (WI) into the soil (mm h^{-1}), determined by a sprinkler infiltrometer in high- (A and B), medium- (C and D) and low-yielding zones (E and F) in Palmeira das Missões (left) and Não-Me-Toque (right), Rio Grande do Sul, Brazil.

that reflect hydrophysical soil conditions, because the structural quality leads to a pore size distribution favorable to root growth and water infiltration (Alves et al., 2007).

Surface runoff water

The initial time for surface runoff for each yielding zone at PM and NMT is shown in Figure 5. At PM, a simulated

rainfall of 300 mm h^{-1} induced surface runoff after 35, 19 and 9 min within high-, medium- and low- yielding zones, respectively. At NMT, the surface runoff stated after 16, 10 and 8 min. These results suggest that under high-yielding zones water partition initially favors infiltration and reduces losses by runoff. These results gave more evidences that high-yielding zones have a greater soil physical structural quality and consequently improved soil quality, influencing positively crop yield (Amado et al., 2009).

Based on our findings, we suggest that management strategies including cover crop with tap root system, crop rotation, or even mechanical chiseling should be adopted by farmers, especially under medium- and low-yielding zones, to improve soil physical and structural qualities, and consequently, to increase water infiltration rate, water storage and reduce surface runoff, as consistently reported in the literature (Lanzanova et al., 2010; Jemai et al., 2013; Sun et al., 2015).

Conclusion

This field study showed that soils under low-yielding zones presented higher bulk density, lower macro-aggregate stability and water infiltration rate as well as shorter time for starting surface runoff, compared to higher-yielding zones. Therefore, these findings suggest that soil physical and structural degradation have induced crop yield losses over time under long-term no-tillage areas.

Macro-aggregate stability (>4.76 mm) and water infiltration rate estimated by a sprinkler infiltrometer were efficient parameters for distinguishing yielding zones in Oxisols managed under long-term no-tillage system.

Conflict of Interests

The authors have not declared any conflict of interests.

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Vol. 11(15), pp. 1336-1343, 14 April, 2016
DOI: 10.5897/AJAR2016.10806
Article Number: 6A6888E58051
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Volumetric models for Eucalyptus grandis x urophylla in a crop-livestock-forest integration (CLFI) system in the Brazilian cerrado

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Received 14 January, 2016; Accepted 1 March, 2016

The influence of the forest component in the crop-livestock-forest integration system depends on several factors, among which are the plant species used and the row spacing established in system deployment. Therefore, the objective of this study was to characterize the tree component dendrometrically using Eucalyptus grandis x urograndis individuals from the CLFI system and to determine the model fit with volumetric models of homogeneous stands. The study area consists of a six year old CLFI system of Eucalyptus grandis x urophylla, located at the municipality of Cachoeira Dourada – GO. Forest inventory and volume measurement were carried out through the Smalian method. The hypsometric relations of *Eucalyptus urograndis* were adjusted to seven volumetric models. The arrangement proposed in the crop-livestock-forest integration system (CLFI) was efficient. The models tested (Näslund, Ogaya, Schumacher & Hall, Spurr logarithmic, Honner, Takata and Husch) showed adjustments above 87%, where the models Näslund (99.53%) and Ogaya (99.17%) had the best fit.

Key words: Näslund, Ogaya, wood production.

INTRODUCTION

The economic growth in Brazil is highly connected to the agricultural and livestock sector (Macedo, 2009). Activities involving agricultural and livestock production are the main sources of financial income and, consequently, the main land use conversion and human occupation factors since the 70s in the Cerrado biome (Klink and Machado, 2005; Sano et al., 2008).

The agricultural and livestock sector has undergone major transformations due to the increase in production costs and more competitive market, demanding

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increased productivity, quality and profitability, without harming the environment. An alternative that has stood out in the last years to reach these objectives is to use an integration system that incorporates agricultural, livestock and forestry activities, in a single spatial and/or temporal dimension, seeking a synergistic effect between the agro-ecosystem components for the sustainability of the production unit, while contemplating its environmental suitability and the enhancement of the natural capital (Balbino et al., 2011).

The area planted with trees in Brazil reached 7.60 million hectares in 2013. In the same year, Brazilian consumption of wood from planted trees for industrial use was 185.3 million cubic meters (m³) (IBÁ, 2014). Different planting systems have been adopted in the last decades to combine production and economic growth, mitigating the negative impacts which the agricultural production can exert on natural ecosystems (Macedo, 2009). The crop-livestock-forest integration (CLFI), also known as agrosylvopastoral system, is one of the systems recognized as an alternative to encourage the recovery of degraded areas and environmental conservation, in addition to the financial gains from agricultural and livestock production (Macedo, 2009; Paciullo et al., 2011) Mixed crop-livestock farming systems comprise a key element of the world's land use and agricultural production. About 25 M km^2 of land is used worldwide for mixed farming (de Haan et al., 1997; Euclides et al., 2010), including most of the world's croplands and 30-40% of its grazing area. Rainfed mixed farming systems alone produce just under half the beef, a third of sheep-meat and half of milk of the world (Steinfeld et al., 2006). In Australia, mixed crop-livestock farming has also been a major and longstanding feature of agricultural land use; in 2010, about 0.35-0.40 M km^2 of land, a third of the agricultural zone, was occupied by farms, operating both cropping and livestock enterprises (Bell and Moore, 2012).

The crop-livestock (CLI), crop-forest (CFI), livestock-forest (LFI), crop-livestock-florest (CLFI) integrated production systems are different production systems, intentionally combined, which enable the diversification of economic activities on the property (same physical and temporal space), however large, medium or of family farms. Thereby, such integrated systems provide viable and sustainable production. The integration consists entirely on the diversification and integration of different production systems (EMBRAPA, 2015).

The CLFI consists primarily of intercropping products derived from the three components (crop, livestock and forest) in the same area, where each activity tends to benefit each other and facilitate the maintenance of ecosystem balance, in addition to the recovery of degraded areas (especially degraded pastures). The model also provides income diversification, as it provides marketing of more than one product (Macedo, 2009; Paciullo et al., 2011; Balbino et al., 2012; Castro, 2013).

The species (tree, pasture or culture) to be used must have a cooperation behavior, where one species complements, completes or assists the development of the other. The species interact in influencing nutrient cycling for each, the availability of lighting each plant strata receive and improving microclimatic conditions becoming more favorable environment for the development for all groups. The influence of the forest component (trees) on a system depends on several factors, among which are the plant species used and the spacing established in system deployment (Paciullo et al., 2011; Pezzopane et al., 2015).

Therefore, the main objective of this study was to characterize the dendrometry and adjustment of volumetric models for Eucalyptus grandis x urograndis in a crop-livestock-forest integration system (CLFI) located in southern State of Goiás.

MATERIALS AND METHODS

Study area

This study was conducted in a Technology Reference Unit (TRU; Unidade de referência Tecnológica) of Embrapa, located in the Boa Vereda Farm, municipality of Cachoeira Dourada – GO (Southern state of Goiás, Brazil; latitude 18°29'30" and longitude 49°28'30"). The farm is located at an average altitude of 459 m with regards to sea level, within the Cerrado biome.

Climatic characteristics

According to Köppen, the climate is Aw (tropical weather with a dry season in winter). This climate type is typical of the tropical humid climates, with two well defined seasons – dry winter and wet summer, average annual temperature of 24°C and average annual precipitation of 1.340 mm occurring from October to March (Alvares et al., 2013).

Land use and characteristics

The prevailing soil of the area is a clayey Oxisol. The study area is 15 hectares, and consists of a crop-livestock-forest integration system (CLFI). In this CLFI, six years old eucalyptus occur, planted in triple lines (3 x 2 m), with brachiaria pasture {*Urochloa brizantha* (Stapf) Webster} in the space between the three triple lines (14 m) and grazing animals, rotated with other areas, at three heads per hectare (Figure 1).

Forest inventory

The forest component studied was an Eucalyptus grandis x urograndis clone planted in February 2009, following an arrangement of rows in three lines of trees spaced three meters between rows, two meters between plants and 14 meters between lines. The forest inventory of the crop-livestock-forest integration system, comprising the tree component (eucalyptus clones) was carried out in November 2014. The diameter at breast height (DBH: diameter at 1.30 m in height relative to ground level) was measured. Height measurement and estimation values were obtained as follows. Three specimens were randomly selected,

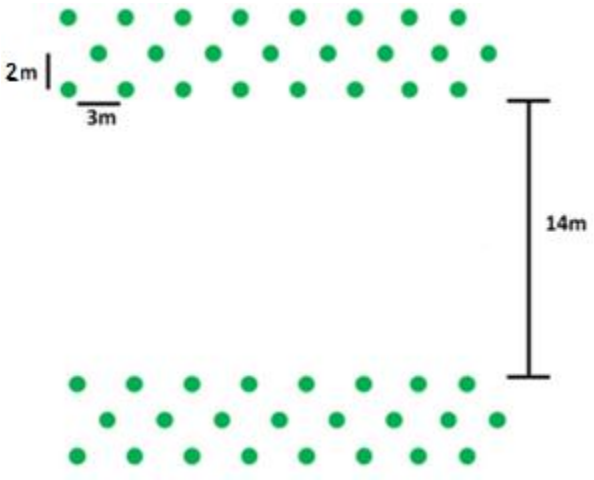

Figure 1. Outline of the area showing the arrangement of the eucalyptus trees in the crop-livestock-forest integration system at Cachoeira Dourada, Goiás.

disregarding plants located at the edges. Then, the diameter of the three specimens and height of a specimen located between the three were obtained.

The total height of the trees was estimated using an electronic inclinometer, (Haglof), and the DBH using a bevel gauge (adapted from Venturoli, 2015). Regular intervals of 12 m between each site were used to determine sampling sequence. Tree density was calculated, based on the spacing among trees (between rows and lines).

Rigorous tree scaling and volume

The trees were separated into five classes according to the rule of Sturges (Machado et al., 2010) after the forest inventory. The first class comprises trees of DBH values ranging from 5 to 10 cm, the second DBH from 10 to 15 cm, the third 15 to 20 cm the fourth 20 to 25 cm and the fifth class had DBH from 25 to 30 cm. The first class was excluded for having only two individuals. Three individuals were considered to represent each of the other classes, namely the trees with DBH with values of the extremities and the center of the class. The trees were harvested and subjected to a rigorous tree scaling, following Smalian (Soares et al., 2006), with sections of 1.0 m in length until the total height of each tree is obtained.

Statistical data analysis

The DBH and height of each plant were related to determine the hypsometric relations of the *Eucalyptus urograndis*. A linear regression (95% significance) was conducted to assess the relationship between the variables, observing the regression coefficient and residue distribution. A total of seven models were adjusted. The DBH was measured at 1.30 m height (X_1; cm), and the total tree height (X_2, m) were the independent variables and the total and stem volumes, with the bark were the dependent variables. The volumetric models used are described below:

Näslund: $Y= \beta_0 X_1^2 + \beta_1 X_1^2 X_2 + \beta_2 X_1 X_2^2 + \beta_3 X_2^2$;
Ogaya: $Y=X_1^2 (\beta_0+\beta_1 X_2)$;
Schumacher & Hall: $Y= \beta_0 X_1^{\beta 1} X_2^{\beta 2}$;
Logarítimica de Spurr: $Y= \beta_0 (X_1^2 X_2)^{\beta 1}$;
Honner: $Y=X_1^2/(\beta_0+\beta_1 X_2)$;
Takata: $Y= (X_1^2 X_2) / (\beta_0+\beta_1 X_1)$;
Husch: $Y= \beta_0 X_1^{\beta 1}$.

Where:
X_1 = Diameter at breast height (DBH; cm); X_2 = height (m); β_0 = estimated height value when the diameter is zero; β_1 = slope of the line, corresponding to the value of the first derivative; β_2 = rate of change in volume (m^3) as height (m) variation occurs, with constant DBH (cm); β_3 = coefficient of the multivariate model.

The adjusted coefficient of determination, the corrected and percentage residual standard error, and graphical analysis of residues were used as model selection criterion. O coefficient of determination (R^2) shows how much of the variation of the dependent variable is explained by the independent variables. Therefore, coefficient of determination values close to 1 indicates better fit and other criteria.

The residual standard error (Syx) measures the average dispersion between the observed and estimated values along the regression line. Smaller residual standard error values indicate better fit. The residual standard error had to be transformed in the models where the characteristic of interest or dependent variable undergoes transformation.

The coefficient of determination and the residual standard error were not used alone to assess the model accuracy, given that these measures may provide distorted information regarding model adjustment. A graphic residue analysis was used in complement. This analysis is decisive in assessing model quality, once it enables the detection of possible bias in estimating the dependent variable along the regression curve.

RESULTS

Dendrometric characterization

The crop-livestock-forest integration system has 845 trees per hectare (177 dead and 668 live trees per hectare). Most *E. urograndis* trees (327 individuals per hectare) in the system occur within the 20 - 25 cm DBH class, followed by the 15 - 20 class (289 individuals) (Table 1). The individuals of these two classes comprise 91% of the trees of the integration system.

The average DBH of the trees was 18.63 cm and the average estimated height was 23.88 m. The average volume per tree is 0.378m^3, total increase of 0.063 m^3/year/tree (Figure 2).

Volumetry

The average volume per tree at six years of age in the CLFI integrated system is 0.378 m^3. The total volume is 259.93 m^3/ha, with an annual increase of 43.32 m^3/ha/year. The annual increase in volume per tree is 0.063 m^3/year. The DBH class 20 - 25 cm had the highest contribution (Table 2).

About 52.24% of the trees in the eucalyptus plantation exhibited DBH values in the 20 - 30 cm class, and

Table 1. Characteristics of the inventory of the eucalyptus forest of the crop-livestock-forest integration system of Cachoeira Dourada, Goiás.

Characteristics	Values
Number of trees (N/ha)	845
Number of dead trees (N/ha)	177
Average DBH (cm/tree)	18.63
Average height (m/tree)	23.88
Average volume (m³/tree)	0.378
Total volume (m³)	259.93

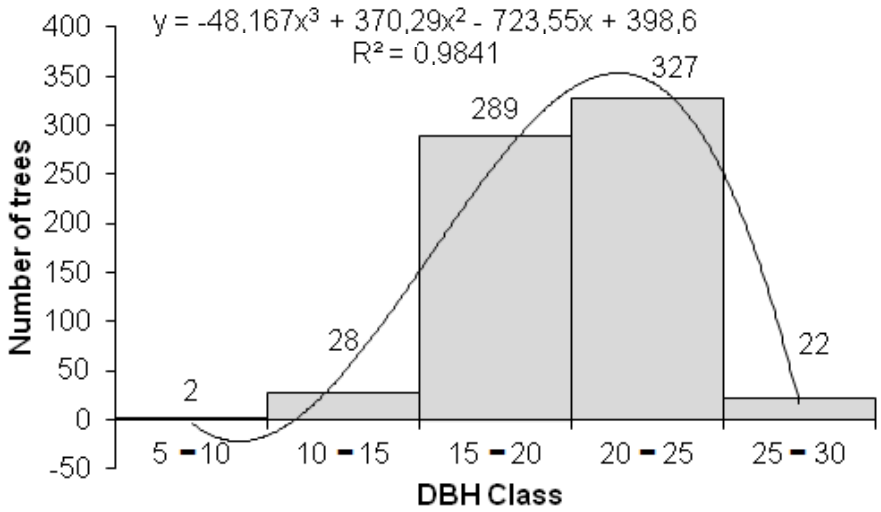

Figure 2. Distribution of the diameter (DBH) of the eucalyptus trees of the crop-livestock-forest integration system of Cachoeira Dourada, Goiás.

Table 2. Wood volume for the Eucalyptus of the integrated crop-livestock-forest system located at Cachoeira Dourada, Goiás.

Class	Number of trees	Volume (m³)	Volume (m³/ha)	Volume (m³ - population)
5 - 10	2	-		-
10 - 15	28	0.127	3.58	5.97
15 - 20	289	0.284	82.12	136.86
20 - 25	327	0.491	160.78	267.97
25 - 30	22	0.611	13.44	22.40
Total	668		259.93	433.22

43.26% in the 15 - 20 cm class. Therefore, over 95% of the trees had diameter between 15 and 30 cm. Volumetry by DBH class in the crop-livestock-forest integration system (CLFI) is shown in Figure 3.

Hypsometric relations

Figure 4 shows the hypsometric equations for Eucalyptus grandis x urograndis and the DBH residue distribution. The adjustments for volumetric models for *E. urograndis* in the crop-livestock-forest integration system (CLFI) are shown in Table 3. The models of Näslund (99.5%) and Ogaya (99.1%) were considered the most predictive and efficient to predict wood volume in the CLFI. Still, all tested models have model adjustments above 87%, with statistical significance and low standard error. Residue distribution is shown in Figure 5.

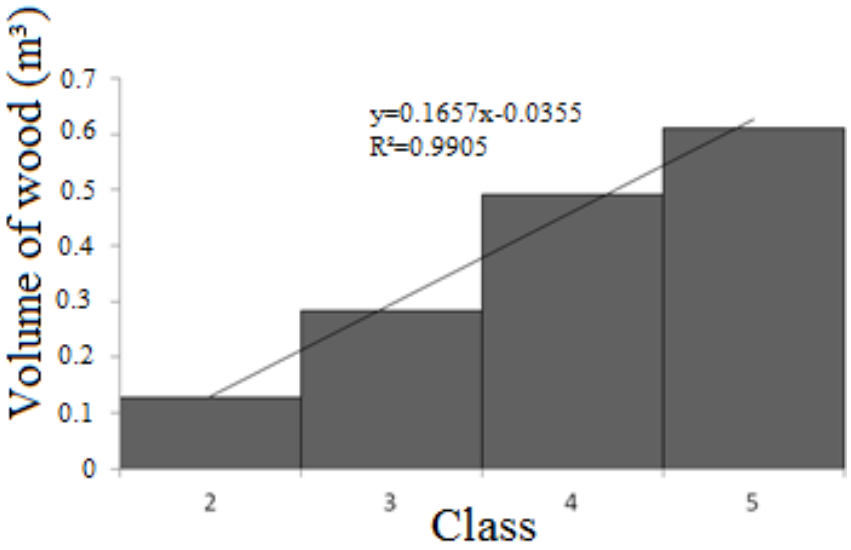

Figure 3. Volume per class of Eucalyptus of the crop-livestock-forest integrated system in Cachoeira Dourada, Goiás.

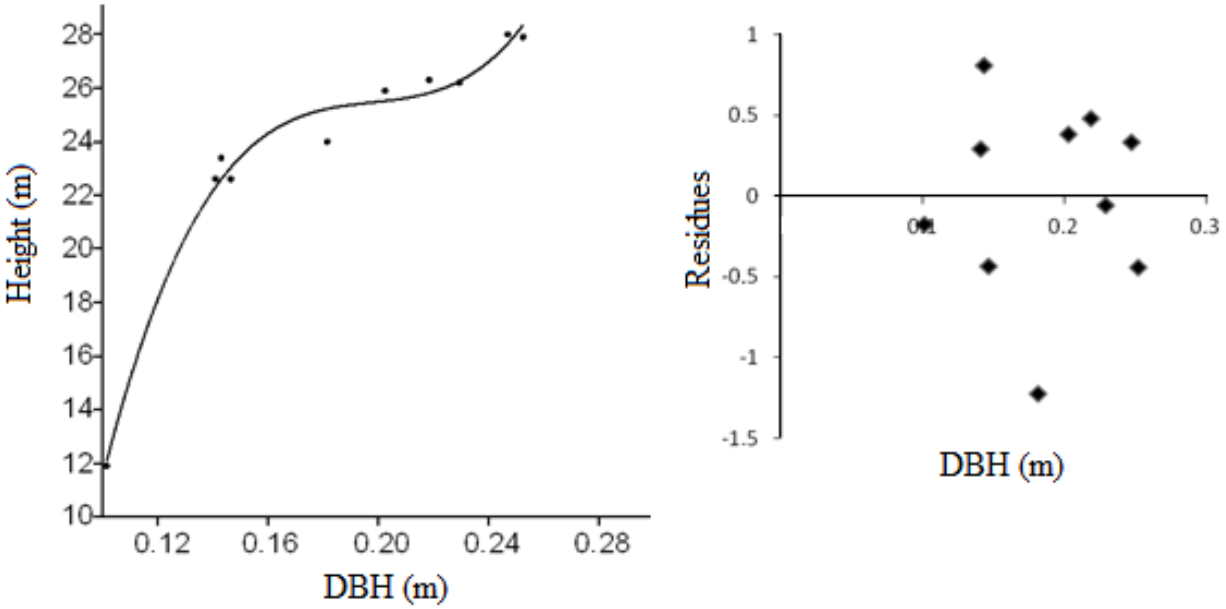

Figure 4. A. Hypsometric equations for Eucalyptus grandis x urograndis (X: DBHS; Y: Height $Y=13832.2X^3-8204.41X^2+1633.21X-83.64$; $R^2= 0.9838$ p= 0.000). B. Distribution of the residues of the DBH of Eucalyptus grandis x urograndis in a crop-livestock-forest integration system at Cachoeira Dourada, Goiás.

DISCUSSION

The eucalyptus volume in the crop-livestock-forest integration system is 259.93 m^3/ha for the entire area of the system. However, the volume increases to 433.22 m^3/ha when only the Eucalyptus area is accounted for separately. Vieira et al. (2012), studying an 18-month old population of the hybrid *Eucalyptus urograndis*, found a volume of only 21.2 m^3/ha, explained by the low age of the plants. Thus, there is an estimated gain in volume of approximately 79 m^3/ha/year considering the current volumetry in this study, using a six and a half old population. In a later study, Vieira et al. (2013) found a volume of 444.3 m^3/ha in a ten year old population of *E.*

Table 3. Adjustments of volumetric models for *E. urograndis* in a crop-livestock-forest integration system located at Cachoeira Dourada, Goiás.

Models	Coefficients				R^2	S_{yx} (m^3)
	β_0	β_1	β_2	β_3		
Näslund: $Y= \beta_0 DBH^2 + \beta_1 DBH^2H + \beta_2 DBH*H^2 + \beta_3 H^2$	3.98651	-0.5114	0.00936	0.0008	0.9953	0.0130
Ogaya: $Y=DBH^2 (\beta_0+\beta_1 H)$	0.01637	0.33212	-	-	0.9917	0.0173
Logarithmic Schumacher & Hall: $Y= \beta_0 DBH^{\beta 1} H^{\beta 2}$	0.47260	2.07027	0.56456	-	0.8793	0.2594
Spurr Logarithmic: $Y= \beta_0 (DBH^2H)^{\beta 1}$	-1.0474	0.90263	-	-	0.8990	0.2372
Honner: $Y=DBH^2/(\beta_0+\beta_1 H)$	-0.1534	327.848	-	-	0.9583	0.0389
Takata: $Y= (DBH^2H) / (\beta_0+\beta_1 DBH)$	-0.1310	0.0999	-	-	0.9892	0.0197
Husch: $Y= \beta_0 DBH^{\beta 1}$	-0.3510	3.66667	-	-	0.9607	0.0378

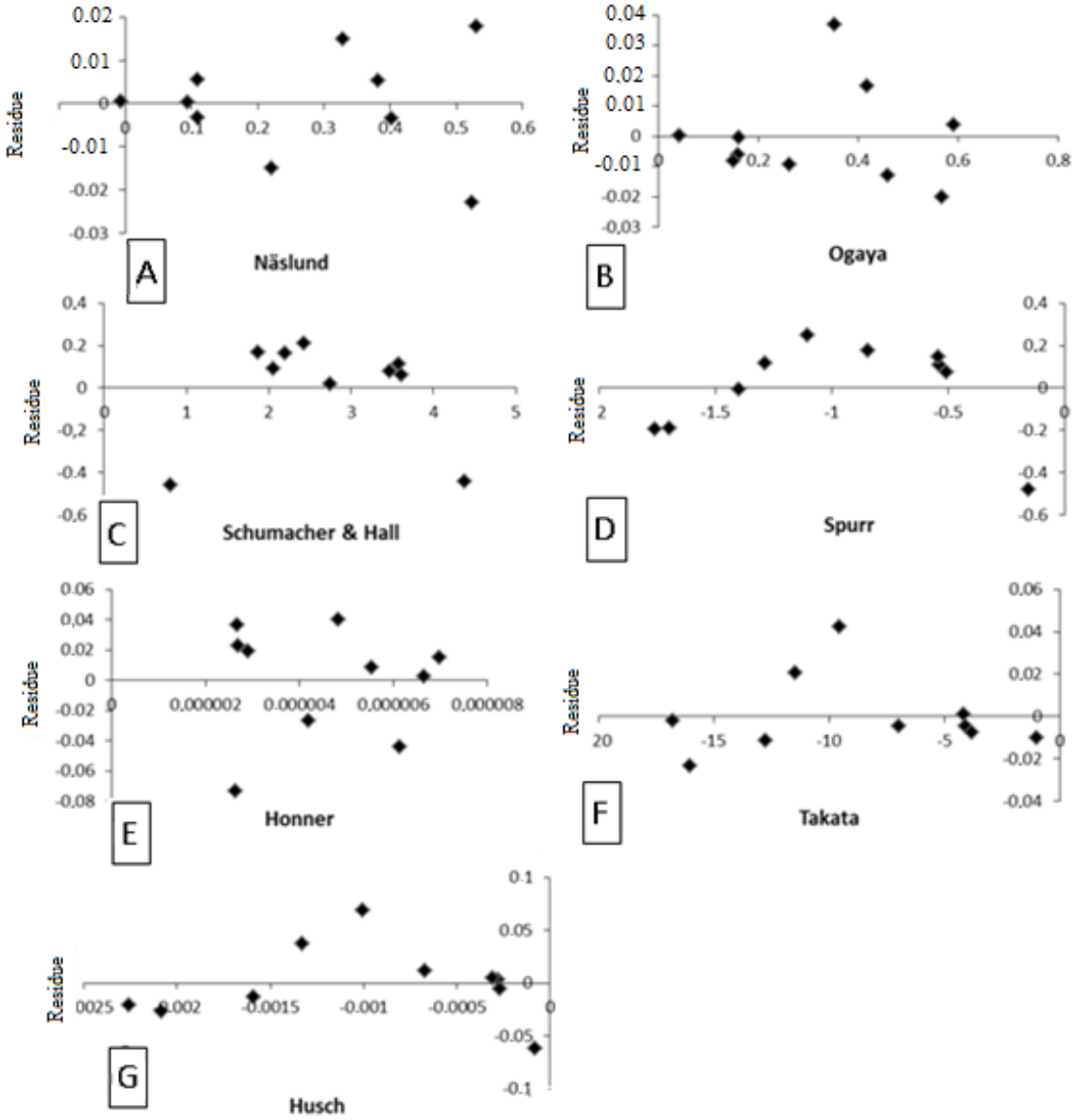

Figure 5. Residue distribution for the volumetric models for *E. urograndis* from a crop-livestock-forest integration system located at Cachoeira Dourada, Goiás.

urophylla x *E. globulus*. Different *Eucalyptus* species yielded volumes of 344.4 m³/ha for six-year-old populations and 414.0 m³/ha for eight-year-old populations in small farms of Santa Catarina (Schumacher et al., 2011).

Different genetic material of six-year-old *E. grandis* and *E. saligna*, in different regions of the state of São Paulo had a volume of wood ranging from 228 to 473 m³/ha (Santana et al., 1999). This pioneering study shows how variable the dendrometric characteristics of the plantation is, taking into account the chosen genetic material and cultural management strategies suitable for the region. Thus, knowing the characteristics of the integrated system becomes relevant to enhance silvicultural practices and obtain improved production yields.

The dynamics existing among the components influences the growth and dynamics of each component within an agrosylvopastoral system (similar to the CLFI). The tree system is a key component, strongly influenced by light entrance and nutrient cycling (Carvalho et al., 2002). Müller et al. (2005) recorded ten year old *E. grandis* producing near 40 m³ of wood for 60 trees (0.67 m³ per trees) in an agrosylvopastoral system located at the zona da mata (state of Minas Gerais) area. These results resemble the ones recorded in this study, and show the relevance of this production system providing results that exceed productivity with regards to volume and biomass when compared with dense stands of eucalyptus.

Modelling procedures are used in decision making processes held to implement and manage native and exotic forest species. Thus, overestimating or underestimating the volume of wood of an enterprise may compromise decision-making. Modelling procedures are the most indicated to evaluate the economic viability of an agroforestry system, obtaining the wood volume data required for evaluation (Salles et al., 2012).

The non-logarithmic models yielded the best results among the models adjusted to estimate the volume of *E. urograndis* in the crop-livestock-forest integration system with regards to fit and predictability, probably due to the homogeneity of eucalyptus trees (clones) and low number of available trees for slaughtering and rigorous scaling (Venturoli and Morales, 2013).

Salles et al. (2012) prefer the Clutter model to adjust tree volumes in CLFI systems, considering it is efficient to estimate volumes. Therefore, the Clutter model shows the relevance of the adjustment of other models and the need to improve prediction and estimations of volumes of tree of agroforestry systems, in order to improve decisions regarding management of the tree component of this system. The adjusted models in this work may be adjusted for other data sets providing information to reduce costs and optimize the management of agricultural systems integrated with planted forests. Thus, these results are the basis for timber volume estimates and biomass of tree in integrated systems and can be used as a reference for other systems even with other forest species at different spacing.

Conclusion

The arrangement proposed in crop-livestock-forest integration system (CLFI), proves to be efficient. The models tested (Näslund, Ogaya, Schumacher & Hall, Spurr Logarithmic, Honner, Takata and Husch) exhibited adjustment values higher than 87%, whereas the models of Näslund (99.53%) and Ogaya (99.17%) were the best.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The research group is grateful to the Postgraduate Program in Agronomy of the Federal University of Goiás (UFG), the Foundation of the State of Goiás Research (FAPEG) for project financing and Dr. Abilio Rodrigues Pacheco, a researcher at the Brazilian Agricultural Research Corporation (EMBRAPA) for all the support.

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Vol. 11(15), pp. 1344-1352, 14 April, 2016
DOI: 10.5897/AJAR2015.9723
Article Number: BA8FA8458053
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Physicochemical quality of Murici covered with starch-based coverings and stored at different temperatures

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Received 17 March, 2015; Accepted 17 February, 2016

The aim of this work was to evaluate the physicochemical characteristics of murici (*Byrsonima crassifolia* (L.) Rich.) covered with starch-based coverings and stored at different temperatures. The fruits were harvested on EMBRAPA experimental farm, in the city of Pacajus-Ceará, where they were taken to the lab, washed, sanitized and naturally dried. Afterwards, they were treated with coverings made out of different concentrations of manioc starch solution and conditioned at 12 and 25°C. We carried out assessments on: pH, titratable acidity, soluble solids, ratio, ascorbic acid, average weight, color, humidity and water activity. The murici fruits were able to maintain their physical and chemical properties for ten days of storage at a temperature of 12°C. Out of the different concentrations of manioc starch-based coverings, the fruits treated with 4% solutions kept their good quality for commercialization.

Key words: Murici fruits, temperatures, starch.

INTRODUCTION

Brazil is one of the largest citrus producers in the world, producing more than 128,000 tons a year (FAO, 2012). Citrus fruits are consumed all year long, *in natura*, or in the form of processed juice, compote and jelly (Rodrigo et al., 2013); however, they are perishable products and tend to postharvest degradation and reduced quality due to their physical aspects, such as loss of mass, and their chemical characteristics, like vitamin C (Mannheim and Soffer, 1996). The quality of citrus fruits is extremely important for marketing reasons, either for *in natura* consumption or industrial processing. The quality characteristics refer to citrus aspect, taste, smell, texture, and nutritive value. Recently, due to people's increasing concern about human health and environmental Brazil is the third largest fruit producer in the world, following India and China. In 2008, Brazil produced more than 43 million

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tons of fruits (4.5% more than the previous year), and this growth is a milestone in Brazilian agribusiness. By the end of 2013, the state of Ceará alone produced 1,650 thousand tons of fruit, according to data from IBGE (2013).

Tropical fruits are mainly produced in semi-arid regions. By stimulating this industry in these historically fragile areas, local economies can be developed, jobs created and manpower enhanced, in addition to improving the income in local communities (Quintino et al., 2010).

Murici (*Byrsonima crassifolia* (L.) Rich.) is one of the most important fruit in these areas, being native to Cerrado. These berries are found from December through March in the mountainous areas of the southeastern region of Brazil, in cerrados in Mato Grosso and Goiás and on the coast of North and Northeast Brazil, where they are consumed in many ways, such as: canned goods, candies, ice creams, snacks, juices and liqueurs (Avidos, 2000). It is estimated that 250,000 plant species have been described worldwide and Brazil contains approximately 55,000 – 60,000 species, being considered the richest biodiversity in the world (22%) (Aragão et al., 2002). However, not all species are consumable or domesticated.

Murici fruits are spherical or pyriform (1 to 2 cm in diameter) and are extremely appreciated by local populations because of their typical characteristics, such as their slight cheese-like scent. Furthermore, other parts of the plant (leaves, seeds and fruits) can be used as medicinal products for gastrointestinal affections, gynecological inflammations, skin infections and snakebites (Mariutti et al., 2013; Ferreira, 2005). They are rich in phenolic compounds, carotenoids and other bioactive agents, and studying them may open fields for the discovery of new products that may interest the population and contribute with the producing region (Mariutti et al., 2014). Nevertheless, there are few studies on this fruit; therefore, knowledge on its physicochemical, chemical and biological properties is scarce.

During storage, fruits are susceptible to a series of chemical, physical and biochemical alterations, which decrease their quality, lead to their senescence and change their characteristics, causing them to be unsuitable for consumption. Regarding conservation, refrigeration is one of the most used methods for long-term storage of fresh fruits, in addition to the application of coverings and other postharvest techniques (Vasconcelos and Melo filho, 2010). Thus, the aim of this study was to assess the postharvest characteristics of murici fruits (*B. crassifolia* (L.) Rich.) stored at different temperatures and covered with manioc starch-based covering solutions at different concentrations.

MATERIALS AND METHODS

Sample preparation

The ripe fruits (yellow) used in this study were collected from seven Murici trees on the Empresa Brasileira de Pesquisas Agropecuárias (EMBRAPA) experimental farm in the city of Pacajus, Ceará, Brazil, in early 2013, and taken to the Laboratory of Food Quality Control and Drying from the Department of Food Technology in the Federal University of Ceará, where they were selected, washed and sanitized with a solution of sodium hypochlorite at 1%.

Covering application

The fruits were divided into the following four groups: (1) control, only washed and sanitized fruits; (2) Manioc starch at 2%; (3) Manioc starch at 4% and (4) Manioc starch at 6%. All concetrations of the soluctions were for suspension manioc starch in destilled water and heating to 70°C under constant stirring and after left to reach until 25°C. We immersed the fruits into the respective treatments for two minutes; then they were removed and placed on nylon sieves (ø 21 cm) to dry for 4 h at room temperature. Afterwards, 40 fruits from each treatment were placed on polyethylene trays, wrapped in PVC film (density 20 mm) and stored at different temperatures (12 and 25°C).

Sample storage

The fruits were divided into two groups: the first one was stored at room temperature (25 ± 2°C) and controlled relative humidity (46 ± 5%), whereas the second group was stored under refrigeration in B.O.D. TE-390 (Tecnal, Brazil), at 12 ± 2°C, and controlled relative humidity (85 ± 5%). We stored the fruits for a period of 10 days, during which we carried out physicochemical analyses every two days.

pH

A potentiometry was used to determine pH and the results were read directly in a pHmeter “Hanna Instruments”, model HI221. This methodology was in accordance with Method No. 981.12 by (AOAC, 1997).

Titratable acidity (TA)

This determination was quantified by titration with NaOH 0.1 M standardized, the pH of the solution was monitored by potentiometer to the pH ranger (8.2 - 8.4). 5 g of pulp of Murici fruit was diluted with destilled water and homogenized for titration. The results are expressed as % of citric acid, according to methodology from (Brasil, 2005).

Soluble solids (SS)

This determination was carried out with concentrated juice, the aid of a digital refractometer (Pocket) PAL – 1, brand ATAGO. The results were expressed as ˚Brix, according to method No. 932.12 – (AOAC, 1997).

Ratio (SS/TA)

Ratio was calculated by the method described by Brasil (2005), which demonstrates the relation between SS and TA.

Ascorbic acid

The Tilmans methods was used to determine ascorbic acid content.

Table 1. Average pH values for Muricis (*B. crassifolia*) (L.) Rich.) treated with different coverings and temperatures during storage.

Time (Days)	pH (12°C)			
	Control	Starch 2%	Starch 4%	Starch 6%
0	3.58^{aA}	3.58^{aA}	3.58^{aA}	3.58^{aA}
2	3.45^{bA}	3.56^{aA}	3.43^{aA}	3.47^{aA}
4	3.39^{bA}	3.45^{aA}	3.54^{aA}	3.56^{aA}
6	3.48^{aA}	3.77^{aA}	3.78^{aA}	3.52^{aA}
8	3.43^{bA}	3.56^{aA}	3.47^{aA}	3.51^{aA}
10	3.35^{bA}	3.52^{aA}	3.45^{aA}	3.50^{aA}
CV(%)		3.7		

Time (Days)	pH (25°C)			
	Control	Starch 2%	Starch 4%	Starch 6%
0	3.59^{aA}	3.58^{aA}	3.58^{aA}	3.58^{aA}
2	3.47^{bA}	3.30^{cC}	3.27^{cC}	3.60^{aB}
4	3.41^{cB}	3.42^{bB}	3.19^{dC}	3.47^{bA}
6	3.29^{dB}	3.30^{cB}	3.33^{bB}	3.47^{bA}
8	..	..	..	..
10	..	..	..	..
CV(%)		2.10		

Different lowercase letters in the same column have significant differences between them by Tukey test ($p<0.05$). Different uppercase letters in the same line and in the same temperature have significant differences between them by Tukey test ($p<0.05$). cv (%) = Coefficient of variation .(..) Treatments discarded due to rotten fruits.

5 g of pulp of Murici fruit was diluted with oxalic acid, homogenized and titration with 2,6-diclorophenolindophenol until turning point that indicates the degradation os ascorbic acid. The results were expressed as mg/100 g of pulp (Brasil, 2005).

Average weight

Every two days, until the end of the storage period, the fruits were weighed in a semi-analytic scale brand Labstore model PK 200. The results were calculated for weight difference and expressed as grams (g) according to the methodology described by Citadin et al. (2005).

Color determination

The color of murici fruits was determined by colorimeter model CR – 10, brand Konica Minolta and recorded as *L, *C and *H color system, where *L consists of luminance or lightness component; *C defines the chromaticity or chroma, where values range from 0 (neutral colors) to 60 (intense colors) and *H represents hue angle from 0 to 360° (0°: red; 90°: yellow; 180°: green and 270°: blue). The analysis was made according to Vaillant et al. (2005).

Moisture

We assessed humidity by using a hot house (brand Marconi, model MA 035) with forced hot air circulation, under conditions of controlled temperature (60 to 70°C). The samples were weighed several times until they reached constant weight (AOAC, 1997).

Water activity (aw)

The sample was homogenized and analyzed in Aqualab (model CX-2, Decagon. Inc.) was used to analyze water activity, which was expressed by the ratio between the sample's water steam pressure and pressure of water steam, duly calibrated, according to Harris (1995).

Statistical analysis

All analyses were carried out in triplicates and reported as average ± standard deviation. Then, they were submitted to analysis of variance (ANOVA) and, in order to compare the averages, we used Tukey test at the level of 5% of probability, using the statistical software SISVAR (Ferreira, 2008). The plots were generated with Software Origin 5.0.

RESULTS AND DISCUSSION

pH

The average results for murici (*B. crassifolia* (L.) Rich.) fruits were significantly different, measured from 3.19 and 3.77 (Table 1). It is observed decreased of pH values, indicating increased organic acid and the treatments were not influenced during storage. Canuto et al. (2010), while studying several Amazonian fruits, obtained a pH average of 3.70, for Murici pulps, while Guimarães and

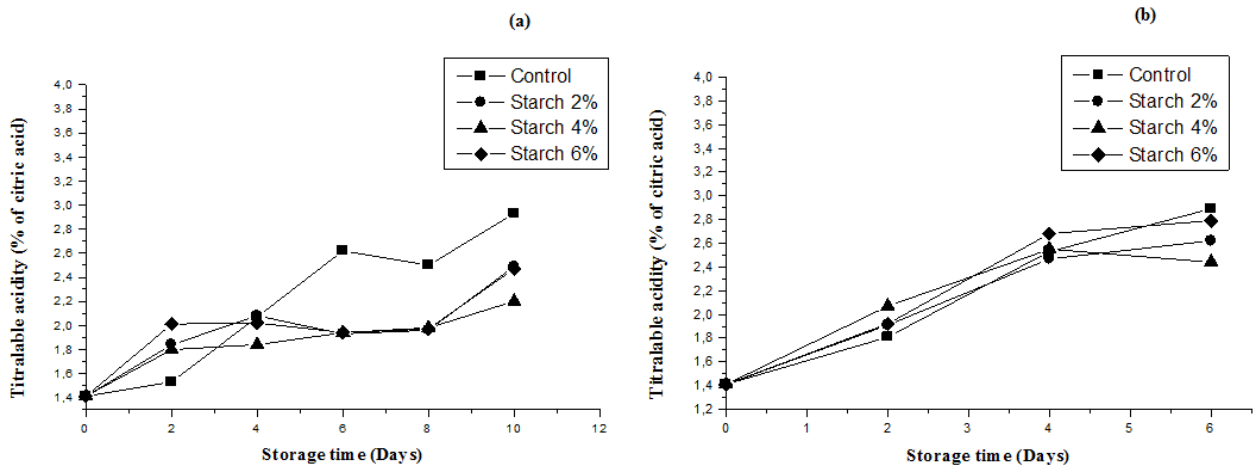

Figure 1. Average values of titratable acidity (% of citric acid) in Murici fruits submitted to different coverings and temperatures of (a) 12°C and (b) 25°C during storage.

Silva (2008), with fresh and dehydrated Murici fruits, var. *Byrsonima verbascifolia,* found numbers that remained below 4.50.

Titratable acidity (TA)

The stored fruits showed values between 1.41 to 2.93% of citric acid, and during storage, there was an increase, regardless of the treatment, as it can be observed in Figure 1. The increase in concentration of organic acids in fruit content can explain such situation, since it points out that the respiratory process consumed other compounds. Belisário and Coneglian (2013) found values of 1.68% of citric acid for Murici fruits stored at 12°C and 1.47% for fruits stored at 25°C. The author also observed a decrease in acidity on the eighth day of storage in both temperatures. These results were similar to the ones found in the study.

Soluble solids (SS)

The average values for soluble solids of Muricis fruits in both treatments remained between 9.67 and 22.00 °Brix (Table 2). The coverings and temperatures applied did not influence the averages during storage, when compared to the control treatment, which showed non-uniform values. At the end of storage, at both temperatures, some treatments tended to a slight decrease in averages of SS, which can be explained by the consumption of sugars in fruit respiration (Souza et al., 2008). The content of soluble solids is an important quality factor for flavor, and the average content superior to 9% is quite desirable from the commercial point of view. Thus, the results obtained in this experiment prove that Murici fruits are great for commercialization and industrialization (Menezes et al., 2001).

Ratio (SS/TA)

This relation is one of the most used forms to evaluate flavor, since it is more representative than the isolated measure of sugars or acidity. Moreover, it shows the balance between those two components and indicates sweetness in foods (Chitarra and Chitarra, 2005). In our results (Table 3), the values varied between 4.64 and 11.13, at 12°C, and 5.62 and 9.80, at 25°C. The Murici fruits decreased quality at the end of the storage. Their averages were 5.72 and 8.10, respectively.

Ascorbic acid

The values of ascorbic acid decreased from 128.00 mg/100 g and 20.24 mg/100 g during the storage days (Figure 2). There was no significant difference between the various concentrations of covering. We still need to consider that the temperature was the determining factor for the results, since the temperature of 12°C retarded the degradation of ascorbic acid. Ascorbic acid content in Murici fruits is equivalent to *Anacardium occidentale* L., which has around 119.70 mg/100 g, and *Psidium guajava* L. fruits, with 99.20 mg/100 g (TACO, 2011).

Average weight (g)

During the days of storage, the average values decreased from 2.60 to 1.83 g (Figure 3). The control treatment showed the highest loss, at both temperatures, whereas the treatments with starch, regardless of concentration, did not lose as much weight, which influenced

Table 2. Average values of soluble solids in Muricis (*Byrsonima crassifolia*) (L.) Rich.) treated with different coverings and temperatures during storage.

Time (Days)	Soluble solids (12 ºC)			
	Control	**Starch 2%**	**Starch 4%**	**Starch 6%**
0	13.67^{cA}	13.67^{cA}	13.67^{cA}	13.67^{dA}
2	9.67^{dC}	19.00^{aB}	17.00^{abB}	22.33^{aA}
4	16.33^{cB}	19.00^{aA}	19.00^{aA}	17.33^{bcAB}
6	19.33^{aA}	16.33^{bB}	15.67^{bcB}	16.33^{cB}
8	17.67^{abAB}	16.33^{bB}	17.00^{abAB}	18.67^{cA}
10	13.67^{cB}	17.67^{abA}	17.67^{abA}	19.00^{bA}
CV(%)		6.38		

Time (Days)	Soluble solids (25 ºC)			
	Control	**Starch 2%**	**Starch 4%**	**Starch 6%**
0	13.67^{bA}	13.67^{cA}	13.67^{cA}	13.67^{cA}
2	11.00^{cC}	18.67^{bA}	16.00^{bB}	16.00^{bB}
4	21.33^{aA}	20.33^{abA}	21.00^{aA}	21.00^{cA}
6	20.00^{aB}	22.00^{aA}	15.67^{bC}	15.67^{bC}
8	..	..	..	..
10	..	..	..	..
CV(%)		4.98		

Different lowercase letters in the same column have significant differences between them by Tukey test ($p<0.05$). Different uppercase letters in the same line and in the same temperature have significant differences between them by Tukey test ($p<0.05$). cv (%) = Coefficient of variation (..) Treatments discarded due to rotten fruits.

the final results. The results published by Damiani et al. (2008), while studying fruits of *Caryocar brasiliense*, Camb., demonstrated that the higher the temperature, the larger the weight loss, Nevertheless, the same result was not observed in this study, given the fact that there were no significant differences. Fruit mass loss during storage occurs mainly because of two factors: transpiration and respiration. Transpiration is the major cause of mass loss because it is the mechanism by which water is lost due to differences of water steam pressure between the atmosphere and the fruit surface. Respiration also causes mass reduction because the fruit loses carbon atoms whenever a molecule of CO_2 is released to the atmosphere, thus altering fruit quality (Browmk and Pan, 1992; Castricini et al., 2010).

Color

Color indicates whether or not the fruit suffered many alterations during the days of storage. Canuto et al. (2010) evaluated pulp color in different Amazonian fruits, and nances had an average for L* (Lightness) of 45.8 (Figure 4), which are similar values to the ones presented in this study. As for *Spondias mombin* L., a fruit that presents a yellow coloration similar to Murici's, they found average values of 47.9. Chroma (C*) (Figure 4S) stands for color intensity, and Muricis had a vivid and intense color on the first days of storage; the averages varied between 40.65 and 58.78 for fruits stored at 12°C, and from 40.72 to 56.28 for fruits stored at 25°C. On the last days of storage, these values were lower at both temperatures. °Hue is a parameter that represents degree of coloration, going on a scale from 0° to 360°. The results we found for the fruits stored at both temperatures dropped during storage, ranging from 94.00 to 80.00 (yellow), and there were no significant differences between the applied coverings (Figure 4). According to Silva (2000) fruit color must remain attractive with the application of different conservation methods; otherwise consumers will neither taste nor consume the food. We finished our experiment when more than 50% of the fruits had wilted or damaged.

Moisture

In all treatments, the averages range between 82.48 and 84.81%, which are values that were significantly different (Table 4), but apparently were not influenced by the treatments. We can observe that fresh fruits have high moisture content and some values similar to these were found for *Persea americana* Mill. (84%) and *Malus domestica* (82%) (TACO, 2011). Moisture content in a given food is related to its stability, quality and composition, which are factors that must be taken into

Table 3. Average values of Ratio in Muricis treated with different coverings and temperatures during storage in days.

Time (Days)	*Ratio* (12 ºC)			
	Control	**Starch 2%**	**Starch 4%**	**Starch 6%**
0	9.72^{aA}	9.72^{aA}	9.71^{abA}	9.71^{abA}
2	6.33^{bcB}	10.45^{aA}	7.74^{bB}	11.13^{aA}
4	8.13^{abB}	9.26^{abAB}	10.38^{aA}	8.59^{bAB}
6	7.44^{abB}	8.49^{abAB}	9.59^{abA}	9.54^{abA}
8	7.14^{bB}	8.36^{abAB}	9.72^{abA}	9.57^{abA}
10	4.64^{cB}	7.12^{bA}	8.04^{aA}	7.70^{bA}
CV(%)	11.1			

Time (Days)	*Ratio* (25 ºC)			
	Control	**Starch 2%**	**Starch 4%**	**Starch 6%**
0	9.72^{aA}	9.72^{aA}	9.71^{aA}	9.71^{aA}
2	6.08^{cC}	9.80^{aA}	7.75^{bB}	8.33^{bB}
4	8.44^{bA}	8.26^{bA}	6.91^{bcB}	7.82^{bAB}
6	6.94^{cB}	8.41^{bA}	6.49^{cBC}	5.62^{cC}
8	..	..	..	..
10	..	..	..	..
CV(%)	6.48			

Different lowercase letters in the same column have significant differences between them by Tukey test ($p<0.05$). Different uppercase letters in the same line and in the same temperature have significant differences between them by Tukey test ($p<0.05$). cv (%) = Coefficient of variation (..) Treatments discarded due to rotten fruits.

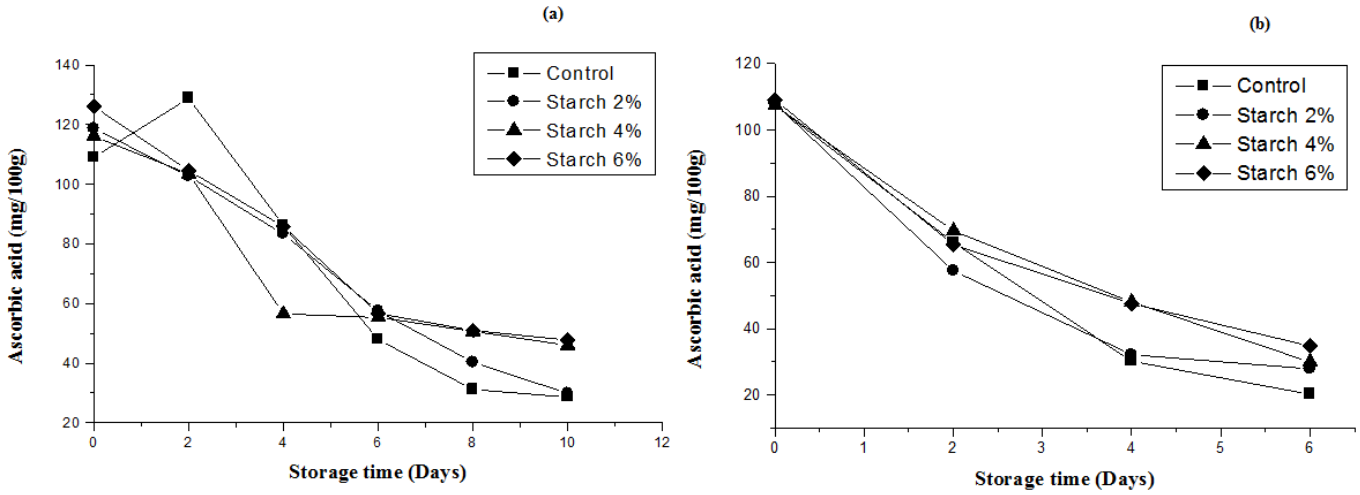

Figure 2. Average values of ascorbic acid (mg/100 g) in Murici fruits submitted to different coverings and temperatures of (a) 12°C and (b) 25°C during storage.

consideration while choosing methods to conserve them.

Water activity (aw)

The water activity predicting the stability and safety of Murici. This parameter has interaction to the chemical, physical and biological characteristics of fruits than moisture content, thus have an effect on reactions and microorganisms proliferations. There were no significant differences among the fruits, which can be characterized as foods with high free water content (aw > 0.9). The average values in both treatments and storages remained unaltered, ranging between 0.96 and 0.97

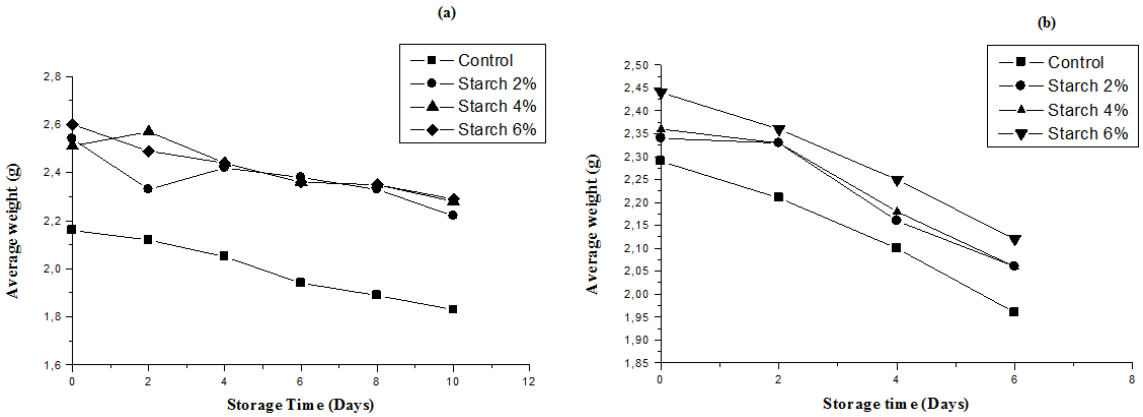

Figure 3. Average values of weight (g) for murici fruits submitted to different coverings and temperatures of (a) 12°C and (b) 25°C during storage.

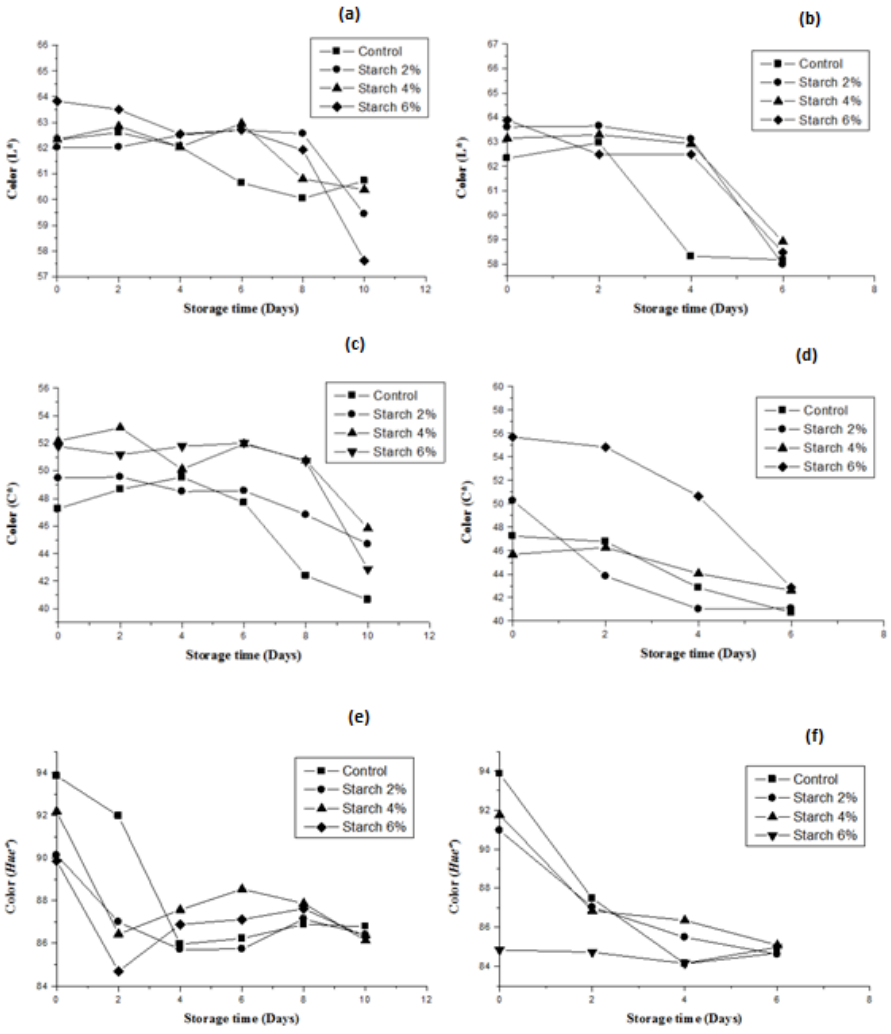

Figure 4. Average values of color (L*) for murici fruits submitted to different coverings and temperatures of (a) 12°C and (b) 25°C during storage. Average values of color (C*) for murici fruits submitted to different coverings and temperatures of (c) 12°C and (d) 25°C during storage. Average values of color (*Hue*º) for murici fruits submitted to different coverings and temperatures of (e) 12°C and (f) 25°C during storage.

Table 4. Average moisture values (%) of Muricis fruits (*Byrsonima crassifolia*) (L.) Rich.) treated with different coverings and temperatures during storage.

Time (Days)	Moisture (12 ºC)			
	Control	**Starch 2%**	**Starch 4%**	**Starch 6%**
0	83.48^{abA}	83.46^{abA}	83.48^{aA}	83.48^{abA}
2	83.50^{abA}	84.81^{bA}	83.85^{aA}	83.99^{abA}
4	82.74^{aA}	83.81^{abAB}	84.21^{aB}	84.42^{bB}
6	84.14^{abB}	82.71^{aA}	83.93^{aAB}	84.41^{bB}
8	84.51^{bA}	83.45^{abA}	83.92^{aA}	84.44^{bA}
10	83.00^{abAB}	84.06^{abB}	84.31^{aB}	82.69^{aA}
CV(%)[1]		3.89		
Time (Days)	**Moisture (25 ºC)**			
	Control	**Starch 2%**	**Starch 4%**	**Starch 6%**
0	83.48^{aA}	82.71^{aA}	83.48^{aA}	83.48^{aA}
2	83.42^{aA}	82.74^{aA}	83.94^{aA}	83.74^{aA}
4	84.04^{aA}	83.97^{aA}	83.14^{aA}	83.50^{aA}
6	84.51^{aB}	84.49^{aB}	84.37^{aB}	82.48^{aA}
8	..	..	..	..
10	..	..	..	..
CV(%)[1]		4.6		

Different lowercase letters in the same column have significant differences between them by Tukey test ($p<0.05$). Different uppercase letters in the same line and in the same temperature have significant differences between them by Tukey test ($p<0.05$). cv (%) = Coefficient of variation .(..) Treatments discarded due to rotten fruits.

Table 5. Average values of water activity (aw) in fruits of Muricizeiro (*Byrsonima crassifolia*) (L.) Rich.) treated with different coverings and temperatures during storage (n = 3).

Time (Days)	Aw (12°C)			
	Control	**Starch 2%**	**Starch 4%**	**Starch 6%**
0	0.97^{abA}	0.97^{abA}	0.97^{abA}	0.97^{abA}
2	0.97^{abA}	0.97^{aA}	0.97^{aA}	0.97^{aA}
4	0.96^{abA}	0.97^{abA}	0.97^{aA}	0.97^{aA}
6	0.97^{abA}	0.96^{abA}	0.96^{bA}	0.96^{bA}
8	0.96^{bB}	0.97^{abAB}	0.97^{aA}	0.96^{bB}
10	0.97^{aA}	0.96^{bB}	0.97^{abAB}	0.96^{bB}
CV(%)		0.35		
Time (Days)	**Aw (25°C)**			
	Control	**Starch 2%**	**Starch 4%**	**Starch 6%**
0	0.97^{aA}	0.97^{aA}	0.97^{abA}	0.97^{aA}
2	0.96^{aA}	0.96^{aA}	0.97^{aA}	0.96^{aA}
4	0.96^{aB}	0.97^{aAB}	0.96^{bcB}	0.97^{aA}
6	0.96^{aB}	0.97^{aA}	0.96^{cB}	0.97^{aA}
8	..	..	..	..
10	..	..	..	..
CV(%)		0.41		

Different lowercase letters in the same column have significant differences between them by Tukey test ($p<0.05$). Different uppercase letters in the same line and in the same temperature have significant differences between them by Tukey test ($p<0.05$). cv (%) = Coefficient of variation .(..) Treatments discarded due to rotten fruits.

(Table 5). Similar results were observed by Guimarães et al. (2008), while studying Murici (0.98).

Conclusion

The physical and chemical properties of Muricis (*B. crassifolia*) (L.) Rich.) not showed interaction between treatments during storage of the evaluation time (10 days) at a temperature of 12°C. For the temperature 25°C, the same showed lower shelf life. Out of the concentrations of covering solutions applied to the fruits, those with manioc starch at 4% maintained the best quality during the period under analysis.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The authors would like to thank Universidade Federal do Ceará for their help with the Muricis analysis.

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academicJournals

Vol. 11(15), pp. 1353-1360, 14 April, 2016
DOI: 10.5897/AJAR2013.7953
Article Number: 1221D1E58055
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Evaluation of *Spirulina platensis* as microbial inoculants to enhanced protein levels in *Amaranthus gangeticus*

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Received 20 September, 2013; Accepted 9 December, 2015

The demand for increase in food production for increasing population with adequate bioavailable nutrients has become a challenge for the agriculturists, nutritionists, biotechnologists to meet the requirements of mankind. The microbial biofertilizers are applied in the form of seaweed liquid extracts, microbial inoculants, biostimulators and biofortification agents. All these categories of microbial biofertilizers are involved in the enhancement of plant nutrient uptake and result in increase of vitamin and nutrient contents in plants producing high yields. *Spirulina platensis* is a blue green alga of cyanobacterial member and it is rich in protein. In the present study, *Spirulina platensis* is used as a biofortification agent to enhance leaf protein levels in crops such as *Amaranthus gangeticus*. Different experimental methods were followed including soaking seeds in different concentrations of *Spirulina* (5 to 30 g); soaking seeds in *Spirulina* at different time intervals (1 h – overnight); *Spirulina* in combination with biofertilizers, chemical fertilizer, organic fertilizer and Vermicompost in various proportions (25:75; 50:50; 75:25). The protein content of the yield was estimated and the study results indicated that there was significant increase in protein with biofortification of *S. platensis.*

Key words: *Spirulina platensis, Amaranthus gangeticus,* protein, dietary supplements and biofortification.

INTRODUCTION

Spirulina platensis also called as *Arthrospira* is a microscopic and filamentous cyanobacterium (Blue green algae) that has a long history of use as food. Its name derives from the spiral or helical nature of its filaments (Becker, 1993). *S. platensis* has been used as food for centuries by different populations and only rediscovered in recent years. It grows naturally in the alkaline waters of lakes in warm regions. Measuring about 0.1 mm across, it generally takes the form of tiny green filaments coiled in spirals of varying tightness and number, depending on

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the strain (Abdulquader et al., 2000). *S.platensis* is cultivated worldwide, used as a dietary supplement as well as whole food and is available in the forms of cakes, tablets, powder. It is also used as a food supplement in the aqua culture, aquarium and poultry industries (Vonshak, 1997).

S. plantensis is rich in protein (65 to 71%) and it is a complete protein with all essential amino acids. The protein of *Spirulina* can be compared to that of legumes. It has about 7% of lipids by weight. It is rich in gamma-linolenic acid (GLA), alpha-linolenic acid (ALA), linolenic acid (LA), stearidonic acid (SDA), ecicosapentaenoic acid (EPA), decosahexaenoic acid (DHA) and arachidonic acid (AA). It also possesses vitamins like B1, B2, B3, B6, B9, vitamin C, D, A and E. It is a good source of minerals like Potassium, Calcium, Chromium, Copper, Iron, Magnesium, Manganese, Phosphorous, Selenium, Sodium and Zinc. *Spirulina* contains pigments like β-carotene, Zeaxanthin, Chlorophyll, Xanthophylls, Echinenone, Myxoxanthophyll, Canthaxanthin, Diatoxanthin, 3`-hydroxyechinenone, β-cryptoxanthin and Oscillaxanthin which are phytochemicals and antioxidants (FAO, 1981).

It possesses phycobiliproteins like C-phycocyanin and Allophycocyanin (Vonshak, 1997) which are phytonutrients and antioxidants that combat cancers effectively. *Spirulina* is a natural super food, because it does not contain any preservatives, cultivated with no use of pesticides and the important thing is that it is naturally green in color and no artificial colors are added to it (Henrikson, 1994).

In the current scenario, the diet and nutrition around the world has lot of effect on wellbeing of humans. The crash diets, insufficient nutrient uptake and even the junk food culture have become order of the day and leading to health hazards. When the low nutritious foods lacking in essential trace elements loaded to human digestive system, enough quantity and quality nutrients cannot be assimilated. These conditions would lead to over eating resulting in Obesity. To this problem *Spirulina* would be a good solution with complete wholesome nutrients allowing the body to absorb (Switzer, 1982).

The increased utility of the chemical fertilizers, the cost of organic fertilizers has necessitated the production of *Spirulina* in bulk quantities to replace minerals in soil in required quantity. Organic farming methods using vermicompost and either of the two methods mentioned above have not given satisfactory results over the years (Madan et al., 1988). Hence, use of appropriate combination of fertilizers is the suggestible practice in Agriculture in order to reduce cost and get good yields (Kollerstrom and Staudenmaier 2001).

Amaranthus gangeticus is grown as leafy vegetable, and ornamentals. *A. gangeticus* shows a wide variety of morphological diversity among and even within certain species. Although the family is distinctive, the genus has few distinguishing characters among the 70 species included (Juan et al., 2007). *A. gangeticus* leaves are good sources of dietary minerals including calcium, iron, magnesium, phosphorous, zinc, copper and manganese (Tucker, 1986). Vegetables, especially leafy vegetables are important in the diet as they are micro-nutrient dense foods, rich in carotene, and minerals such as calcium, iron and Phosphorous (Ali and Tsou, 2001). In this context the present study aimed to evaluate the effect of *S. platensis* as a cost effective microbial inoculant to increase the protein content in *A. gangeticus* which is edible green leafy vegetable.

MATERIALS AND METHODS

Culturing of *Spirulina*

Spirulina culture was obtained from department of Microbiology, Andhra University Visakhapatnam, Andhra Pradesh, India. To obtain a good amount of growth, *Spirulina* was cultured in Zarrouk's medium. The medium was prepared as per composition and inoculated with *Spirulina*. Incubation was carried out up to 21 days. After 21 days the media along with culture is filtered using a Whatman filter paper No:1. The culture thus obtained as a residue on the filter paper, was sun dried for two days and weighed.

The experimental design followed for the present study was randomized block design and all measures were taken care of to reduce the measurement error. Experimental design with various methods and combinations were given in Table 1.

Estimation of protein level in *A. gangeticus*

The protein content in *Spirulina* and also in the yield on dry basis was analyzed by Kjeldahl method Pelican Kelplus – KES 12 INL (Ravi et al., 2010).

Molecular studies

The protein content of the leaf yield of *Amaranthus* plants were analyzed for both experimental and control set-ups. The set-up which has shown the highest protein in the yield was further subjected to molecular analysis by SDS–PAGE.

MALDI–MS procedure

The bands obtained from SDS-PAGE were extracted and digested for further analysis by MALDI-MS (Rauser et al., 2010). MALDI is expanded as Matrix Assisted Laser Desorption/ Ionization and by using this process the peaks were blasted.

Statistical analysis

The data obtained from the present study is processed and analysis was carried out with SPSS package and MINI tab (Version 16).

RESULTS AND DISCUSSION

Protein in diet is an important nutrient constitute and

Table 1. Field experimental set-ups.

S/N	Name of Set up	Variations						
1.	Time period Soaking (5 g of *Spirulina* in 100 ml of sterile water)	1 h	2 h	3 h	4 h	5 h	Over night	C
2.	Seed soaking in different concentrations (In 100 ml sterile water)	5 g	10 g	15 g	20 g	25 g	30 g	C
3.	*Spirulina*+Biofertilizer (S:B)	25:75	50:50	75:25	-	-	-	C
4.	*Spirulina*+Vermicompost (S:B)	25:75	50:50	75:25	-	-	-	C
5.	*Spirulina*+Organicmatter (S:B)	25:75	50:50	75:25	-	-	-	C
6.	*Spirulina*+Chemical fertilizer (S:B)	25:75	50:50	75:25	-	-	-	C
7.	Spray method (g/L)	25/5	50/5	75/5	100/5	-	-	C

*C: Control.

proteins are known as the building blocks. Sufficient protein levels are required to carry out the metabolic functions of body cells. Protein malnutrition is a major problem faced by the developing countries ((Kaniszewki and Elkner, 1990; Sainju et al., 2000).

Protein can be obtained in diet through plant and animal origins. The animal protein is highly expensive and hence dependence on plant protein has become inevitable (Butt and Rizwana, 2010). The quality and the quantity of protein either conventional or fabricated have to meet the protein requirements and improve the status both in the plants as well as human population (Maheswarulu, 2011).

Table 2 show the protein content (g/100 mg) of *Amaranthus* yield grown with *Spirulina* as microbial inoculant in various concentrations and combinations along with biofertilizer, vermicompost, organic manure, chemical fertilizer and spray method. The results were represented as mean ± standard deviation. The results of percent increase when compared with reference value were also shown in graph (Figure 1). It is evident from the tables that the protein content has been increased in *Amaranthus* in various treatments when compared with reference value of 4.98 g/100 g according to National Institute of Nutrition (NIN), Hyderabad, India.

In set up – I where the seeds were soaked for increasing time intervals that is, from 1 to 5 h and overnight, the highest protein content of leaf yield was noted in 4 h soaked sample. The highest protein in set up – II, III, IV, V, VI, and VII were observed for 20 g, 25:75, 75:25, 50:50, and 25 g/5 L concentrations and combinations respectively. In set up-I due to less surface area of the seeds the penetration of *S. platensis* hydrolysate into the seed was maximum up to 4 h. The fluctuations in this set up – I has to be subjected to further studies. In set up – II as the concentration of *S. platensis* increased there was an increase in protein till 15 g and after there was a decrease, because of saturation point.

In set up – III 25:75 ratio showed the highest protein which indicates that biofertilizers is playing a major role. As the ratio of *Spirulina* is increased the percent protein decreased, because of the synergistic action of biofertilizers. In the combination of *S. platensis* vs vermicompost highest protein percent was observed in 75:25 ratio which indicates that as the ratio of *Spirulina* increased the percent protein increased. In set ups – IV to VI equal effect of *Spirulina* and combination of fertilizers can observed (Table 2).

In the last set up that is the spray method more protein level can be observed with less concentration of *Spirulina* that is, 25 g/5 L. This effect may be attributed to the effective absorption of nutrients from *Spirulina* into the leaf directly.

Seeds of *Amaranthus*, when soaked in *Spirulina*, there was a positive increase that is, as the concentration of *S. platensis* increased, protein content of leaf yield was found to be increased. The same trend was observed for *Spirulina* + Biofertilizer, *Spirulina* + Chemical fertilizer and with spray method. However, same trend was observed in *Spirulina* + vermicompost treatment. The positive results shown from the present study has been supported by the earlier studies done by Shozeb and Aruna (2013) that is, high protein content was observed in urad (black gram) plants grown in soil treated with biofertilizer + chemical fertilizer than the plants grown in soil treated with biofertilizer alone. However, the quality of the protein has to be checked by analyzing the amino acid composition. However, the increments in leaf protein of *Amaranthus* need the bioavailability studies.

The protein in the *Amaranthus* yield was estimated by using SDS-PAGE. The results of the

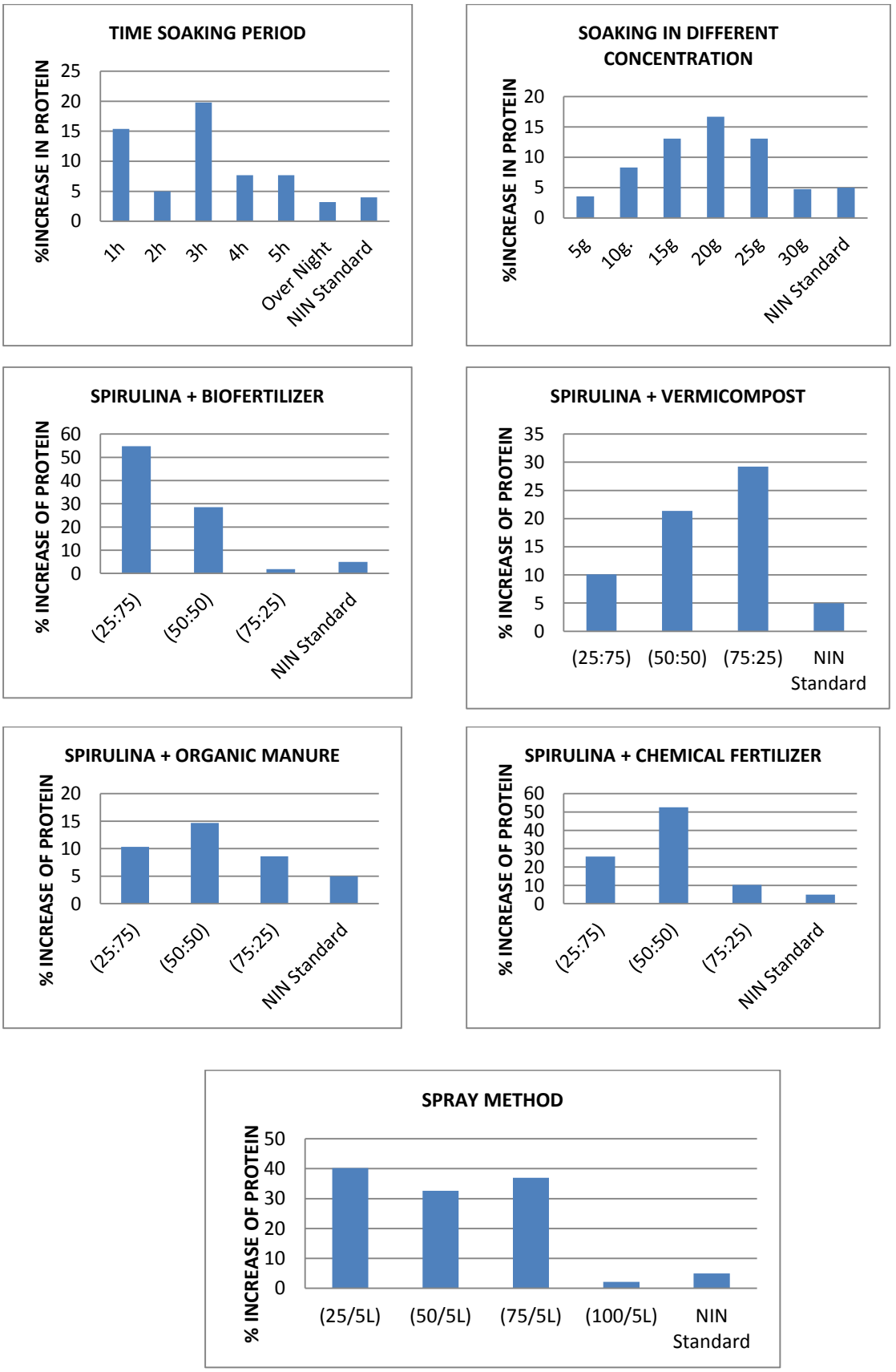

Figure 1. Percent increase of protein in different set ups as compared to reference standard.

Table 2. Protein content of the leaf yield of *Amaranthus* treated with *Spirulina*.

S/N	Treatments	Protein%
	SET-I Time period soaking	
1	1 h	7.7±0.00
2	2 h	3.2±0.00
3	3 h	7.3±7.07
4	4 h	**9.8±0.00**
5	5 h	8.4±7.07
6	Overnight	9.4±0.00
7	Control	9.1±0.00
8	NIN standard	4.98
	SET-II Soaking in different concentration	
1	5 g	8.7±0.00
2	10 g.	9.1±0.00
3	15 g	**9.5±0.00**
4	20 g	7.0±0.00
5	25 g	7.3±0.00
6	30 g	8.0±0.00
7	Control	8.4±7.07
8	NIN Standard	4.98
	SET-III Biofertilizer (S:B*)	
1	(25:75)	**13.0±0.00**
2	(50:50)	10.8±0.00
3	(75:25)	9.4±0.00
4	Control	8.4±0.00
5	NIN Standard	4.98
	SET-IV Vermicompost (S:V*)	
1	(25:75)	8.0±0.00
2	(50:50)	10.8±0.00
3	(75:25)	**11.5±0.00**
4	Control	8.9±0.00
5	NIN Standard	4.98
	SET-V Organic manure (S:O*)	
1	(25:75)	12.8±0.00
2	(50:50)	**13.3±0.00**
3	(75:25)	12.6±0.00
4	Control	11.6±0.02
5	NIN Standard	4.98
	SET – VI Chemical fertilizer (S:C*)	
1	(25:75)	9.8±0.00
2	(50:50)	**11.9±0.00**
3	(75:25)	7.0±0.00
4	Control	7.8±0.00
5	NIN Standard	4.98
	SET-VII Spray method (S/W*)	
1	(25/5 L)	**12.9±0.00**
2	(50/5 L)	12.2±0.00
3	(75/5 L)	12.6±0.00
4	(100/5 L)	9.4±0.00
5	Control	9.2±0.00
6	NIN Standard	4.98

Table 3. Band appearance on gel (in kda): Interpreted from semi log graph of mol wt vs rm.

M	C	AO	AV	AB	AC
	110	110	110	110	110
97.4					
	92	92	92	92	92
	72	72	**72**	72	72
	70	70	70	70	70
66					
	55	55	55	55	55
	45	45	45	45	45
43					
	34	34	34	34	34
29					
	14.5	14.5	14.5	14.5	14.5
	12.5	12.5	12.5	12.5	12.5

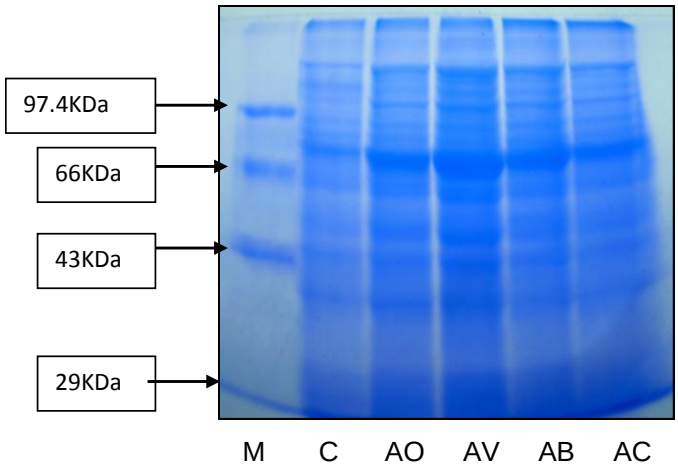

Figure 2. SDS PAGE gel showing protein bands of yield in different experimental set ups. M -Molecular weight marker (Bovine serum albumin (BSA); AO –*Amaranthus* treated with experimental set up containing *Spirulina* + organic manure in 50:50 proportions; AV-*Amaranthus* treated with experimental set up containing *Spirulina* + Vermicompost in 50:50 proportions; AB -*Amaranthus* treated with experimental set up containing *Spirulina* + Biofertilizer in 50:50 proportions; AC -*Amaranthus* treated with experimental set up with *Spirulina* + Chemical fertilizer in 50:50 proportions; C-*Amaranthus* control without adding any type of *Spirulina.*

SDS-PAGE were obtained in the form of protein bands (Table 3, Figure 2).

The protein was run on SDS PAGE has shown clearly marked bands after electrophoresis. The bands shown in the Figure 2 exhibit the highest molecular weight protein and were indicated in Table 3.

All the samples run have shown distinct bands. The bands of all samples were found prominently between 97.4 KDa regions to 66 KDa region when compared with marker protein. Among all the samples the thick band was observed for the sample AV. The molecular weight of this band was 92 KDa. After AV the order of band thickness was observed in AB with molecular weight -72 KDa, AO has molecular weight of 70 KDa and AC has molecular weight of 69 KDa respectively. All the samples run exhibited increased protein expression when compared with control. The expression levels were concluded on the basis of thickness of bands as well as intensity of stain taken (Figure 2).

The protein bands isolated by SDS-PAGE were further subjected to analysis to determine the molecular weight of proteins by MALDI. The MALDI technique results were obtained in the form of spectra with intensity shown on y-axis and the mass/charge ratio of proteins (m/z values) taken on x-axis (Figure 3a, b and c) and Table 4.

The peptide peaks obtained in MALDI spectra has shown the similarity with the *Spirulina* Phycocyanin α and β subunits with regards to molecular weight. This may be due to the supplementation of *Spirulina* to plants in combinations with other methods of fertilization (Wang et al., 2013). In the MALDI-MS spectra for sample containing *Spirulina* + Vermicompost (AV) the peptides were found to have molecular weight >10,000 Da (15103.5, 13239.0 Da) and had shown similarity with an unidentified protein CAB 69331 with molecular weight 18066 Da and another protein-peptidyl prolyl isomerase of *Escherichia coli K*-12 strain having the molecular weight 20, 418 Da (Thammasorn et al., 2009). The MALDI-MS mass spectra for the sample containing *Spirulina* + Biofertilizer (AB), the theoretical mass of the peptide was estimated as 10437.8 Da which has similarity with an *Enolase* protein from *E. coli* AAA 24486 with molecular weight 12523 Da. The control sample has the MALDI-MS spectra with molecular weight 10398.4 Da has been found to be similar with Plant protein- Albumin (nsLTP1) having molecular weight of 9748.29 Da (Del et al., 2003) was clearly shown in Table 4. As *Spirulina* and *E. coli* both are prokaryotic organisms, the protein expressed in the experimental samples might be similar with the protein found in *E. coli*.

Conclusion

S. platensis treated plants have shown increase in protein content when compared to the control and reference value. From the present study analysis, among all the different variations and combinations of *S. platensis* treated plants, the effect was best observed in the plants that were treated with soaking the seeds in different concentrations, and in the combinations of *S. platensis* with Biofertilizer and Vermicompost. At the end it is concluded that *S. platensis* which is a blue green algae can be helpful in agriculture as a biofortification agent when compared with chemical fertilizer as an enhancer of plant growth in terms of protein content.

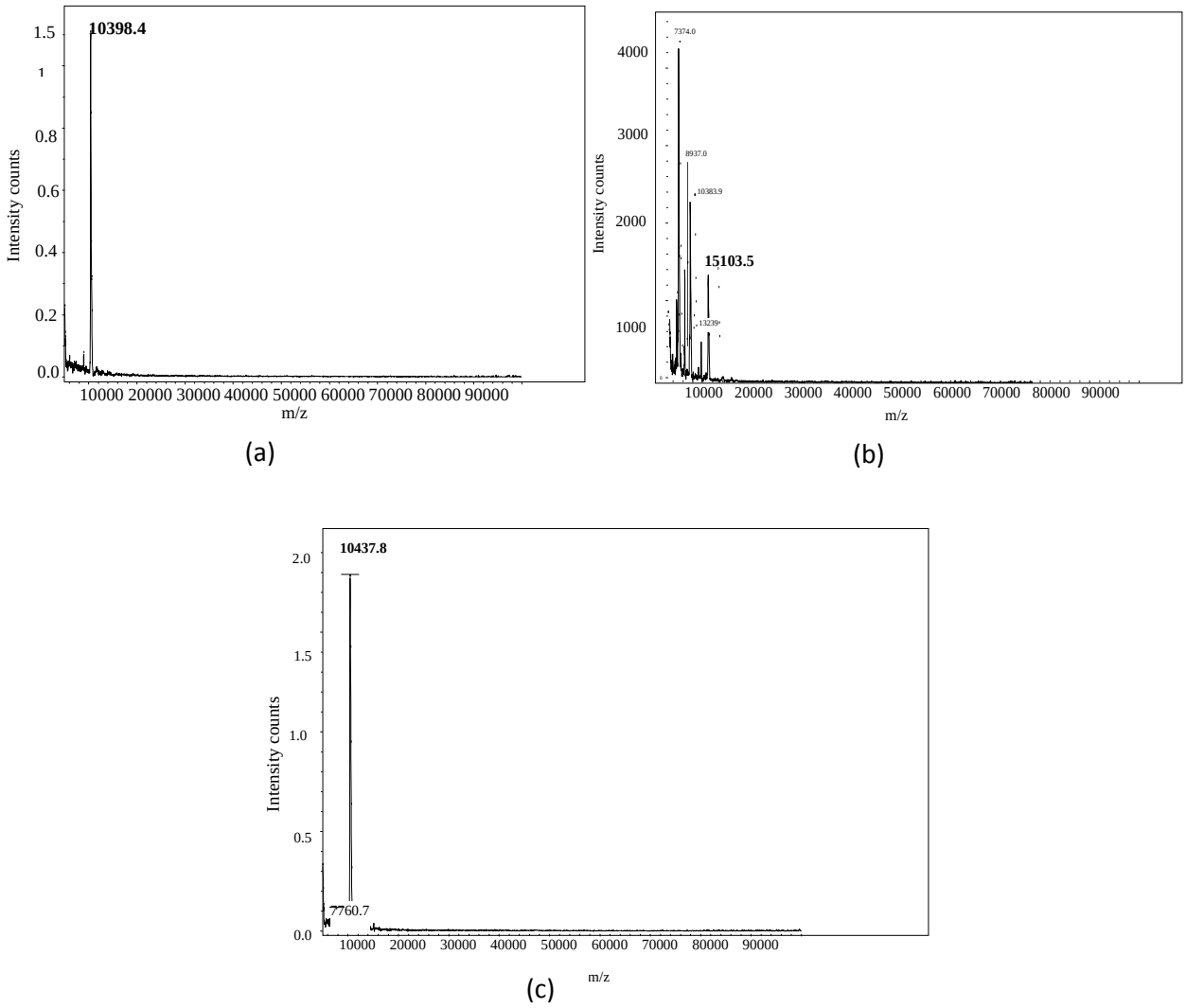

Figure 3. (a) MALDI-MS spectrum of *Amaranthus* sample control, **(b)** MALDI-MS spectrum of *Amaranthus* sample with experimental treatment containing *Spirulina* + vermicompost, **(c)** MALDI-MS spectrum of *Amaranthus* sample with experimental treatment containing *Spirulina* + biofertilizer.

Table 4. Matched sequence of protein fragment from experimental samples.

CAB69331	SEQUENCE 1 FROM PATENT WO9845454 (fragment) – unidentified	18066
AAA24486	ECOPYRG NID: - *Escherichia coli*	12523
nsLTP1	Plant protein- Albumin	9748.29

Conflict of Interests

The authors have not declared any conflict of interests.

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academicJournals

Vol. 11(15), pp. 1361-1372, 14 April, 2016
DOI: 10.5897/AJAR2015.10756
Article Number: 20586B058057
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Farmers' adaptive measures to climate change induced natural shocks through past climate experiences in the Mekong River Delta, Vietnam

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Received 22 December, 2015; Accepted 1 March, 2016

This study examined farmers' adaptive measures to climate change induced natural shocks through past climate experiences in the Mekong River Delta (Vietnam) from a data set of 330 farmers. Seemingly unrelated regression model was used to identify the determinants of farmers' adaptive measures. Results showed that male household head, education of the household head, marital status of the household head, production assets, firm size, availability of credit, access to market, temperature and rainfall had significant impacts on choices of adaptation. Results also indicated that past climate experiences was the most important determinant of adaptive measures. Policy messages enhanced access to credit, to markets, and created awareness on climate change. Other policy options could also be suggested, including: strengthening education level of farmers, facilitating cheap technologies, spurring irrigation investment through public - private partner, and supporting the land reform such as farmers' cooperation in large-scale production.

Key words: Climate change induced natural shocks, adaptative measures, past climate experiences, Mekong River Delta.

INTRODUCTION

The Mekong River Delta, the major agricultural region of Vietnam, is identified as significantly vulnerable to climate change (Yusuf and Francisco, 2010; Asian Disaster Preparedness Center, 2003). Agricultural production remains the main source of livelihoods for most farmers in this area (Nguyen and Le, 2012; Le Anh et al., 2014; Asian Disaster Preparedness Center, 2003). Climate change has greater negative impacts on farm households as they have the lowest capacity to adapt to changes in climatic conditions (Yu et al., 2010; Asian Disaster Preparedness Center, 2003).

Adaptation measures are therefore important to help farmers to better face extreme weather conditions and associated climatic variations (Valipour et al., 2015; Adger et al., 2003; Kandlinkar and Risbey, 2000).

A better understanding of current adaptation measures and their determinants will be important to inform policy for future successful adaptation. Some related studies

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conducted in the last few years focused on farmers' past climate experiences. Niles et al. (2015a), using farmer survey data from New Zealand, showed that limiting factors mediated the effect of past climate experiences on the adoption of adaptation strategies differently in two regions with water acting as a limiting factor in Hawke's Bay and water and temperature as a limiting factor in Marlborough. Le Dang et al. (2014) addressed the limited understanding of how rice farmers appraise their private adaptive measures and influential factors in the Mekong River Delta of Vietnam. Authors found that belief in climate change, information and objective resources were found to influence farmers' adaptation assessments. Geoff (2014) also stressed that farmers' climate change beliefs affect adaptation to climate change.

Nicholas and Gina (2012) explored commercial farmers' perceptions of and responses to shifting climates in the Little Brak River area along South Africa's south coast and found that farmers' experience with shifting climates has played a large part in driving their adaptive decision-making. This study adds to these analyses by evaluating farmers' past climate experiences and distinguishing determinants of adaptation to climate change induced natural shocks among farmers in the Mekong River Delta. Evaluating past climate experiences of and response to climate change includes exploring what these perceptions are, how they are formed and how perception affects response (Bryant et al., 2000; Vedwan and Rhoades, 2001).

DATA AND METHODOLOGY

Research site and data

Long An, Ben Tre, Can Tho, Soc Trang, Kien Giang, and Ca Mau are the six provinces randomly selected from 13 provinces in the Mekong Delta which are defined at different agro-ecological systems which enabled representation of the Mekong Delta region. One district from each province and two communes from each district were randomly chosen. In total, there are 12 communes and commune centres in the survey. From the official household lists of the twelve communes, farm households were selected by simple random sampling. The face-to-face structured interviews were conducted in July, 2014. Four teams of 10 interviewers each had been involved in two intensive training sections, one before and one after the pre-test. The interviewers visited 335, but interviewed 330 farm households, 50 in each commune. Each interview was around two hours in duration. In this study, the farm household was the unit of analysis and the household heads or their spouses were the interviewees.

The structured questionnaire mainly covers perception of past climate change, climate change adaptation assessment, and a number of influential factors. The questionnaire was refined and finalized based on the information from three focus group discussions in provinces such as Long An, Ben Tre, Can Tho and of the questions were also tested through the pre-tests with 30 randomly chosen farm households in Ben Tre Province. The data used in this paper are specified from questions about climate change, adaptation assessment, farm characteristics, income, assets, infrastructure and institutional factors.

The principal systems in the survey areas are mono-rice, shrimp-mixed rice, fish, shrimp, cereal-root crop mixed, and fruit. Out of 330 farm households, 32% of them cultivate rice, 1% of them shrimp-mixed rice, 2.5% of them fish, 11.3% of them shrimp, 5.5% of them annual crops, and 10.5% of them fruit. Farmers have average land size of about 1.1 ha. About 70% of farming housholds have less than 1.2 ha. Average size of rice land, land for annual crops and land for fruit are 2.5, 2.4, and 1.9 ha, respectively. Average shrimp-mixed rice land size is 4.3 ha. Paddy yield in the study area is around 6.09 tones/ha. Average income of farmers in the study area is about 76.348 thousand Vietnam Dong (about 3.455 USD). Several Mekong River Delta related studies and reports by the (United Nations Development Programme (UNDP), 2008; EU/MWH, 2006; ADPC/GTZ, 2003) clarify the trends of climate change in terms of higher temperature, salt water intrusion, eroded shorelines, exacerbated coastal flooding, rainfall increasingly concentrated over fewer months in the rainy season, while the dry season will be more prolonged. This will lead to more frequent and intense floods and droughts simultaneously. In addition, tropical cyclone and typhoon occurrence are expected to alter and become more intense under a warmer climate as a result of higher sea-surface temperatures.

Model specification

Common approach uses a univariate technique such as probit/logit analysis for discrete choice dependent variables to model each of the adaptation measures individually as functions of the common set of explanatory variables. The shortfall of this approach is that it is prone to biases caused by ignoring common factors that might be unobserved and unmeasured and affect the different adaptation measures. In addition, independent estimation of individual discrete choice models fails to take into account the relationships between adoptions of different adaptation measures. Farmers might consider some combinations of adaptation measures as complementary and others as competing. By neglecting these common factors the univariate technique ignores potential correlations among the unobserved disturbances in adaptation measures, and this may lead to statistical bias and inefficiency in the estimates (Lin et al., 2005; Belderbos et al., 2004; Golob and Regan, 2002).

A multinomial (MNL) discrete choice model is another alternative to the multivariate model with more than two endogenous discrete choice variables. In the multinomial discrete choice model the choice set is made up of all combinations of adaptation measures. The shortfall of this technique is that interpretation of the influence of the explanatory variables on choices of each of the original separate adaptation measures is very difficult. The shortfall of this technique is that all multinomial replications of a multivariate choice system have problems in interpreting the influence of explanatory variables on the original separate adaptation measures (Golob and Regan, 2002).

This study follows Zellner's Iterative Seemingly Unrelated Regressions (ISUR) to overcome the shortfalls of using the univariate and multinomial discrete choice techniques. The ISUR technique provides parameter estimates that converge to unique maximum likelihood parameter estimates. The resulting model has stimulated countless theoretical and empirical results in econometrics and other areas (Zellner, 1962; Srivastava and Giles, 1987). The benefit of this model is that the ISUR estimators utilize the information present in the cross regression (or equations) error correlation and hence it is more efficient than other estimation methods such as the univariate and multinomial discrete choice techniques.

The methodology used in this paper for misspecification testing follows Godfrey (1988) and Shukur (2002). White (1980) test was used (including cross products of the explanatory variables) to test for heteroscedasticity and Ramsey's (1969) RESET test to test for functional misspecification (Ramsey, 1969) Table 1. Definition of variables. Table 2 provides the variables hypothesized to determine

Table 1. Definition of variables.

Variable	Description	Value	Expected sign
Household characteristics			
Age	Age of the household head	Years	Cannot be signed a priori (+ or -)
Education	Number of years of formal schooling attained by the household head	Years	Positive
Gender	Gender of the household head	1= male, 0= female	Cannot be signed a priori (+ or -)
Household size	Number of family members	years	Positive
Wealth	An index of production assets was constructed using production assets including tractor, pesticide sprayers, grain harvesting machine, rice milling machine, feed grinding machine, plough	Numbers	Positive
Farm characteristics			
Farm size	Land area	Numbers	Positive
Institutional factors			
Credit	If household has access to credit from any sources	1=yes, 0= no	Positive
Off-farm employment	Income from off-farm activities during the survey year		Cannot be signed a priori (+ or -)
Tenure	Proportion of land use with Land Right Certificate or	Numbers	Positive
Climate conditions			
Sunshine	Total hours of sunshine	hours	Cannot be signed a priori (+ or -)
Rainfall	Total level of rainfall	mm	Cannot be signed a priori (+ or -)
Climate change induced natural shocks' perception			
Wind storm	Perceived by wind storm	1=yes, 0= no	Cannot be signed a priori (+ or -)
Drought	Perceived by drought	1=yes, 0= no	Cannot be signed a priori (+ or -)
Flood	Perceived by flood	1=yes, 0= no	Cannot be signed a priori (+ or -)
Untimely rain	Perceived by untimely rain	1=yes, 0= no	Cannot be signed a priori (+ or -)
Pestilent insect	Perceived by pestilent insect	1=yes, 0= no	Cannot be signed a priori (+ or -)
Water shortages	Perceived by water shortages	1=yes, 0= no	Cannot be signed a priori (+ or -)

Source: Author's compilation

adaptation behaviour, a brief description of each variable, its value, and expected sign. Following Filmer and Pritchett (2001), principal component analysis (PCA) was used to assign weights to each asset. The overall wealth index is calculated by applying the

Table 2. Adaptation measure to climate change induced natural shocks in the Mekong River Delta, 2014.

Code	Category	Adaptation measure (%)
A	**Water use management (n)**	**44**
01	Build/repair cistern	0.05
02	Build/repair well	0.08
03	Water saving technology	0.03
B	**Adjustments of crops and varieties (n)**	**162**
04	Change varieties	0.39
05	Change crops/livestock	0.08
06	Change crop structure	0.07
C	**Adjustments of planting techniques (n)**	**175**
07	Change crop cultivation	0.14
08	Change fertilizer/stimulus	0.16
09	Change pesticides/herbicides	0.30
10	Change crops quantity	0.13
11	Change farmyard manure	0.04
D	**Adjustments of planting calendar (n)**	**48**
12	Change irrigation schedule	0.07
13	Change crop rotation	0.07
E	**No adaptation (n)**	**43**

Source: Climate change survey in the Mekong River Delta (2014).

following formula:

$$w_j = \sum_{i=1}^{k} [b_i(a_{ji} - x_i)]/s_i$$

Where *w* is the wealth index, *b* is the weights from PCA, *a* is the asset value, *x* is the mean asset value, and *s* is the standard deviation of the assets.

Dependent variables

Intergovernmental Panel on Climate Change (IPCC's) report, which is based on the social sciences, states that "adaptation refers both to the *process* of adapting and to the *condition* of being adapted" (Smit and Pilifosova, 2001). Specifically, adaptation is the process of improving society's ability to cope with changes in climatic conditions across time scales, from short term (for example, seasonal to annual) to the long term (for example, decades to centuries). TheIPCC (2007) defines adaptive capacity as the ability of a system to adjust to climate change (including climate variability and extremes), to moderate potential damages, to take advantage of opportunities, or to cope with the consequences. The goal of an adaptation measure should be to increase the capacity of a system to survive external shocks or change.

Important adaptation options in the agricultural sector include: crop diversification, mixed crop-livestock farming systems, using different crop varieties, changing planting and harvesting dates, mixing less productive, drought-resistant varieties and high-yield water sensitive crops (Adger et al., 2003; Bradshaw et al., 2004). Agricultural adaptation involves two types of modifications in production systems. The first is increased diversification that involves engaging in production activities that are drought tolerant and or resistant to temperature stresses as well as activities that make efficient use and take full advantage of the prevailing water and temperature conditions, among other factors. Crop diversification can serve as insurance against rainfall variability as different crops are affected differently by climate events (Orindi and Eriksen, 2005; Adger et al., 2003). The second strategy focuses on crop management practices geared towards ensuring that critical crop growth stages do not coincide with very harsh climatic conditions such as mid-season droughts. Crop management practices that can be used include modifying the length of the growing period and changing planting and harvesting dates (Orindi and Eriksen, 2005). Use of irrigation has the potential to improve agricultural productivity through supplementing rainwater during dry spells and lengthening the growing season (Baethgen et al., 2003; Orindi and Eriksen, 2005). It is important to note that irrigation water is also subject to impacts from climate change. Use of irrigation technologies need to be accompanied by other crop management practices such as use of crops that can use water more efficiently. Important management practices that can be used include: efficient management of irrigation systems, growing crops that require less water, and optimizing of irrigation scheduling and other management techniques that help reduce wastage (Loë et al., 2001).

The adaptation measures in this study are based on asking farmers about their perceptions of past climate change induced natural shocks and the actions they take to counteract the negative impacts of these shocks. We follow Maddison (2006) and Hassan and Nhemachena (2008) by assuming that farmers' adaptation actions are driven by climatic factors.

A list of private adaptive measures to climate change was initially developed from the literature (Bradshaw et al., 2004; Bryan et al.,

2009; Deressa et al., 2009; Hassan and Nhemachena, 2008; Thomas et al., 2007). To ensure the appropriateness, these measures were raised for discussion in focused group discussions. Typical farmers, participants of the focus grouped discussions, were asked to choose the measures that have been used or available in their areas. The same request was given to agricultural province-level officers interviewed. The adaptive measures had finally been refined by the pre-tests before they were actually included in the questionnaire. Those 14 adaptive measures in table were considered as farmers' adaptive responses to climate change induced natural shocks. These adaptive measures were grouped into five groups: (A) Water use management, (B) Adjustments of crops and varieties, (C) Adjustments of planting techniques, (D) Adjustments of planting calendar, and (E) No adaptation. Most of previous studies considered "no adaptation" as lacking of information on climate change and dropped from analysis. In this paper, we treated "no adaptation" as one of choices by farmers since climate change can also bring positive effects on agriculture production.

In general, measures such as "Build/repair cistern" (5%), "Build/ repair well" (8%), and "Water saving technology" (3%) in water use management were not very commonly used. The limited use of these adaptations could be attributed to need for more capital.

While a high proportion of farmers used measure of "Change varieties" (39%) as an adjustment of crops and varieties, a lower proportion of farmers use "Change crops/livestocks" (8%), "Change crop structure" (7%) in response to climate change. Local farmers may be lacking skills, motivation and opportunities for other crops and/or livestocks. While a high proportion of farmers used measure of "Change pesticide/herbicides" (30%) as an adjustment of planting techniques, a lower proportion of farmers use "Change crop cultivation" (14%), "Change fertilizer input/stimulus" (16%), "Change crop quantity" (13%), and "Change farmyard manure" in response to climate change. These adaptations could be associated with the less expense and ease of access by farmers than that of adjustments of crops and varieties.

Less proportion of farmers use "Change irrigation schedule" (7%), and "Change crop rotation" (7%) in response to climate change. This is probably because farmers access to climate change information is rather limit. No farmer bought insurance as an adaptive measure to climate change and only 1 percent of farmers combined agriculture and forestry. Those numbers implied a lacking of suitable mechanism that can secure farmers from extreme climate events associated with climate change in the Mekong River Delta. Moreover, about 13% of the surveyed farmers reported not to have taken any adaptation method above.

Explanatory variables

With respect to household characteristics such as household head's gender, on the one hand, various studies have shown that gender is an important variable affecting adoption decisions at the farm level. According to Bayard et al. (2007) female farmers are more likely to adopt natural resource management and conservation practices. It was also emphasized by Burton et al. (1999) that female farmers are indeed important in the choice of agricultural practices to adopt, particularly in regard to conservation or sustainable technology. According to Nhemachena and Hassan (2007) the possible reason for female to adapt is that in most rural smallholder farming communities, men are more often based in towns, and much of the agricultural work is done by women. Therefore, women have more farming experience and information on various management practices and how to change them, based on available information (Anim, 1999). On the other hand, according to Asfaw and Admassie (2004), male-headed households are more likely to get information about new technologies and undertake risky businesses than female-headed households. Moreover, Tenge and Hella (2004) argue that having a female head of household may have negative effects on the adoption of soil and water conservation measures, because women may have limited access to information, land, and other resources due to traditional social barriers. There is a line of argument in between by Bekele and Drake (2003) who believe that gender has no significant factor in influencing farmers' decision to adopt climate change adaptation measures. They stressed that there is a significant difference in farmer's ability to adapt to climate change due to major differences between them in terms of access to assets, education, credit, technology and input supply.

Many research works have shown that education increases one's ability to receive, decode, and understand information relevant to making innovative decisions (Maddison, 2006; Lin, 1991; Igoden et al., 1990). On the contrary, Clay et al. (1998) found that education was an insignificant determinant of adoption decisions. The influence of age on adaptation has been mixed in the literature. For example, Bekele and Drake (2003) in their study of Eastern highlands of Ethiopia found that age had no influence on farmer's decision to participate in soil and water conservation activities. Others such as Nyangena (2008) and Dolisca et al. (2006), however, found that age is significantly and negatively related to farmers' decisions to adopt in soil and water conservation and forestry management programs, respectively. Studies by Maddison (2006) and Nhemachena and Hassan (2007) indicate that experience in farming increases the probability of uptake of adaptation measures to climate change.

Regarding farm characteristics, according Nhemachena and Hassan (2007) the empirical literature shows that household size has mixed impacts on farmers' adoption of agricultural technologies. Larger family size is expected to enable farmers to take up labour intensive adaptation measures. Alternatively, a large family might be forced to divert part of its labour force into non-farm activities to generate more income and reduce consumption demands.

Farm and nonfarm income and assets represent wealth. It is regularly hypothesized that the adoption of agricultural technologies requires sufficient financial wellbeing (Knowler and Bradshaw, 2007). Other studies that investigate the impact of income on adoption found a positive correlation (Franzel, 1999). The occupation of the farmer is an indication of the total amount of time available for farming activities. Off-farm employment may present a constraint to adoption of technology because it competes for on-farm managerial time (McNamara et al., 1991).

Among institutional factors, access to credit is an important factor affecting adoption of measures. Access to affordable credit increases financial resources of farmers and their ability to meet transaction costs associated with various adaptation options they might want to take (Nhemachena and Hassan, 2007). Similarly, land tenure can contribute to adaptation, because landowners tend to adopt new technologies more frequently than tenants, an argument that has justified numerous efforts to reduce tenure insecurity (Lutz et al., 1994; Shultz et al., 1997). Land ownership is widely believed to encourage the adoption of technologies linked to land such as irrigation equipment or drainage structures. Land ownership is likely to influence adoption if the innovation requires investments tied to land.

Among infrastructure factors, it is hypothesized that as distance to output and input markets increases, adaptation to climate change decreases. Proximity to market is an important determinant of adaptation, presumably because the market serves as a means of exchanging information with other farmers (Maddison, 2006). Perceived change in climate variables and access to climatic change information are also important pre-conditions to take up adaptation measures (Niles et al., 2015a; Geoff, 2014; Nicholas and Gina, 2012; Nhemachena and Hassan, 2007; Maddison, 2006). Accordingly, it is hypothesized that farmers that perceive change in

Table 3. Summary statistics of independent variables in models.

Variable	Mean	Std. Dev.	Min	Max
Male-headed household (male: 1; female: 0)	0.89	0.31	0	1
Years of education by household head (years)	6.23	3.34	0	16
Marital status of household head (married: 1; other: 0)	0.90	0.30	0	1
Household size (persons)	4.19	1.40	1	8
Production asset index	0.01	1.32	-0.52	10.30
Proportion of cultivation income in total income (%)	0.27	2.31	-39.93	3.64
Proportion of aquaculture income in total income (%)	0.20	0.44	-1.75	2.5
Proportion of non-agriculture income in total income (%)	0.24	0.44	-1.29	3.35
Land area (log)	0.45	1.22	-4.42	4.03
Access to loan (1: Yes; 0: No)	0.21	0.41	0	1
Proportion of land with long-term use right	0.95	0.18	0	1
Distance from plot(s) to house (km)	0.69	1.69	0	20
Distance from plots(s) to nearest commune road (km)	2.97	3.92	0	35
Total hours of sunshine	2313.83	237.32	1952.6	2592
Total level of rainfall	1503.56	450.83	1018.4	2262
Climate change induced natural shocks perceived				
Through wind storm (1: Yes; 0: No)	0.15	0.35	0	1
Through drought (1: Yes; 0: No)	0.23	0.42	0	1
Through higher temperature (1: Yes; 0: No)	0.25	0.43	0	1
Through flood (1: Yes; 0: No)	0.19	0.39	0	1
Through untimely rain (1: Yes; 0: No)	0.23	0.42	0	1
Through salt water intrusion (1: Yes; 0: No)	0.04	0.19	0	1
Through eroded shorelines (1: Yes; 0: No)	0.02	0.12	0	1
Through pestilent insect (1: Yes; 0: No)	0.70	0.46	0	1
Through water shortages (1: Yes; 0: No)	0.08	0.27	0	1

Source: Author's estimation; N=329.

climatic conditions and farmers who have access to climate change information have higher chances of taking adaptive measures in response to observable changes (Nhemachena and Hassan, 2007). The explanatory variables in this study include household characteristics such as education, gender, age of the household head, household size. Farm characteristics include farm size, farm and nonfarm income; institutional factor such as access to credit; infrastructure includes distance to input and output markets, climate conditions, and past climate experiences (Le Dang et al., 2014; Nicholas and Gina, 2012; Patrick and Richard, 2012; Nhemachena and Hassan, 2007; Maddison, 2006). In the empirical model, each explanatory variable is included in all four equations to help test if the impacts of variables differ from one adaptation option to another. Table 3 gives the descriptive statistics of variables in the models.

RESULTS OF ANALYSIS AND DISCUSSION

Farmers' past experiences of climate change induced natural shocks

Farmers' past climate experiences and variability is a pre-requisite for devising subsequent adaptation strate-gies. Therefore, it is important to understand how farmers perceive climate change and variability in the context of agriculture production in the Mekong River Delta. Studies in Mekong River Delta have found that farmers' perception of climate change corresponds with local climate data (Le Dang et al., 2014; Nguyen and Le, 2012; Nguyen, 2007; UNDP, 2008, EU/MWH, 2006; ADPC/GTZ, 2003). In this study, farmers' perceptions of the changes were considered in terms of nine past climate experiences - wind storm, drought, flood, higher temperature, untimely rains, salt water intrusion, eroded shorelines, pestilent insect, and water shortages. The key informants were asked to assess the frequencies of the main climate changes they have seen/observed over the last 5 years. A list of options was provided with 5 levels of occurrences. From Figure 1, recurrent droughts, flood, changes in temperature, and pestilent insect have been confirmed by their often frequencies annually in the Mekong River Delta. Higher temperature and pestilent insect are the two most often past climate change phenomena.

Past climate change induced natural shocks' impacts

The respondents were asked to rank from "very severe"

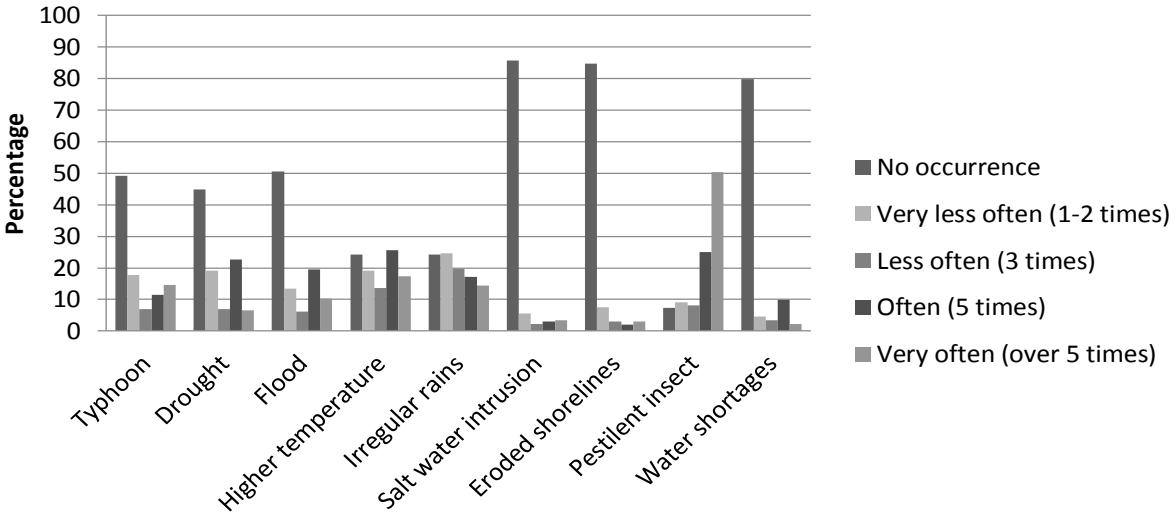

Figure 1. Climate change induced natural shocks (frequencies in 5 recent years). Source: Author's estimation.

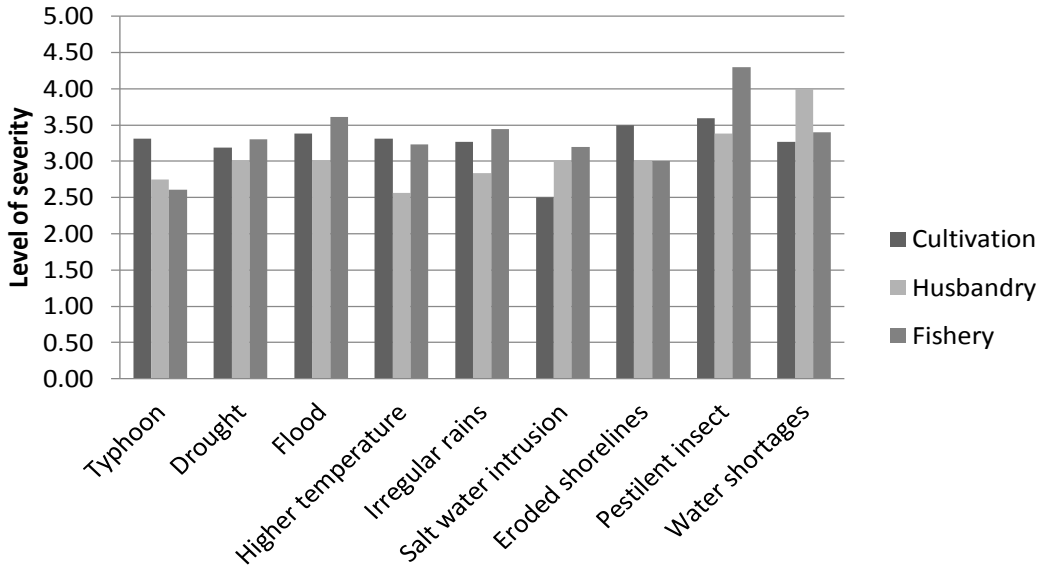

Figure 2. Climate change induced natural shocks' impacts on agricultural activities. Source: Author's estimation.

(5) to "not severe at all" (1) from options provided by the impacts they have noticed on different agricultural activities. From

Figure 2, typhoon, drought, flood, higher temperature, untimely rain, eroded shoreline, pestilent insect, and water shortages are the most severe climate change phenomena in cultivation (average points of above 3.0). Pestilent insect and water shortages are the most severe climate change phenomena in husbandry (average points of above 3.0). Drought, flood, higher temperature, untimely rain, pestilent insect, and water shortages are the most severe climate change phenomena in fishery (average points of above 3.0).

Regression results

Despite the fact that the majority of the farmers interviewed claimed that they have perceived at least one change in climatic attributes, some of the farmers who perceived climate change did not respond by taking adaptation measures. Here it is argued that farmers who perceive and responded (or did not respond) share some common characteristics, which assist in better understanding the reasons underlying their response

(failure to respond) as captured by the ISUR probit model. The R^2 and F test of four ISUR models are shown in Table 4 (Note that one choice of adaptation - Water use management - was not significant and dropped from the results). Four models with alternative choices are in columes, namely: (1) Adjustments of crops and varieties, (2) Adjustment of planning techniques, (3) Adjustments of planting calendar, and (4) No adaptation.

Table 4. Model summary.

Parameter	Adjustments of crops and varieties	Adjustments of planting techniques	Adjustments of planting calendar	No adaptation
R squared	0.14	0.16	0.20	0.19
Breusch-Pagan test for independent equations (Chi squared and p value)	54.61 (0.0001)	63.47 (0.0000)	84.47 (0.0000)	76.03 (0.0000)
Goodness-of-fit test (Pearson chi-square and p value)	323.55 (0.25)	323.01 (0.25)	325.15 (0.23)	299.04 (0.62)
Test for multicollinearity (mean VIF)	1.32	1.32	1.32	1.32
Test for model specification -Ramsey test (p value of squared coefficient)	0.27	0.89	0.09	0.74

Source: Author's estimation.

The R^2 for all models indicated that the statistically significant explanatory variables can explain around 14 to 20% of the variation of farmers' adaptation assessments. Breusch-Pagan test for independent equations were highly significant with values less than 0.001, implying that equations are correlated. Goodness-of-fit test indicates that four models fit the data well. No multi-co-llinearity problems were detected as the variance inflation factor (VIF) for all explanatory variables were less than 1.4. Tests for model specification (Ramsey test) pass for all four models. The regression coefficients in the four ISUR regression models are presented in Table 5. Bootstrap estimates were conducted. We used bias corrected bootstrapped (n =1000) results because they have been shown to perform the best with regards to power and Type I error results (Briggs, 2006), particularly for smaller sample sizes (Preacher and Hayes, 2008).

DISCUSSION

With respect to household characteristics, the estimated coefficients for the household head's gender, years of education by household head, and marital status of household head were statistically significant. Male headed households have more probability of specifically adapting to climate change which is revealed by the fact that a unit change from being headed by a female household to male increases the probability of adapting adjustments of planting techniques to climate change by 23.0%, *ceteris paribus*. This result is in line with the argument that male-headed households are often considered to be more likely to get information about new technologies and take risky businesses than female headed households (Asfaw and Admassie, 2004). However, male headed households have less probability of choosing no adaptation to climate change by 22.3%, *Ceteris paribus*.

A unit increase in the education of the head of the household will have the impact of raising the probability of making adjustments of planting techniques to climate change by 2.2%, *ceteris paribus*. This is in line with studies of Maddison (2006), Lin (1991) and Igoden et al. (1990). Although a series of adaptive measures has been used by many households, the above findings may imply causes of inefficient adaptation in local areas. Poor education can be one possible cause. Our estimation also showed that education level of household head has less probability of choosing no adaptation to climate change by 1.7%, *Ceteris paribus*.

With respect to farming characteristics, farm size was statistically significant and positively related to adaptation. Farm households who owned more land conducted adjustments of crops and varieties more by 5.8%, *Ceteris paribus*. The probable reason for the positive relationship between adaptation and farm size could be due to the fact that adaptation is subject to economies of scale. The result is in accordance with study by Nhemachena and Hassan (2007). If large-scale cultivation is an advantaged in conducting adaptation measures, in general, government should support some implementations of the land reform such as farmers' cooperation in cultivating in large-scale fields. In the Mekong River Delta in recent years, this trend has turned to be common. It was also found that more land households have less probability of choosing no adaptation to climate change by 3.9%, *Ceteris paribus*.

Households with higher production assets (but may not necessarily have most land) increases the probability of adjustments of planting calendar to climate change by

4.3%, *Ceteris paribus*. This is in line with Nhemachena and Hassan (2007), Knowler and Bradshaw (2007) and

Table 5. Results of ISUR probit analysis of determinants of adaptation measures.

Variable	Adjustments of crops and varieties	Adjustments of planting techniques	Adjustments of planting calendar	No adaptation
Male-headed household (male: 1; female: 0)	0.070 (0.097)	0.220** (0.097)	0.064 (0.071)	-0.223** (0.087)
Years of education by household head (years)	0.005 (0.009)	0.022*** (0.008)	0.001 (0.005)	-0.017** (0.007)
Marital status of household head (married: 1; other: 0)	0.008 (0.099)	-0.169 (0.105)	-0.0323 (0.074)	0.193*** (0.066)
Household size (persons)	-0.022 (0.021)	-0.029 (0.020)	-0.011 (0.015)	0.005 (0.014)
Production asset index	0.002 (0.023)	0.043** (0.018)	-0.003 (0.013)	-0.016** (0.008)
Proportion of cultivation income in total income (%)	-0.018 (0.032)	0.007 (0.036)	0.001 (0.024)	0.009 (0.023)
Proportion of aquaculture income in total income (%)	-0.285*** (0.075)	-0.067 (0.077)	-0.056 (0.036)	0.087* (0.048)
Land area (log)	0.058* (0.029)	0.006 (0.028)	0.004 (0.019)	-0.039* (0.021)
Access to loan (1: Yes; 0: No)	0.031 (0.072)	0.109 (0.069)	0.109** (0.055)	-0.0557 (0.040)
Proportion of non-agriculture income in total income (%)	0.015 (0.066)	-0.081 (0.067)	0.039 (0.045)	0.083 (0.052)
Proportion of land with long-term use right	0.143 (0.159)	0.112 (0.174)	-0.0619 (0.129)	-0.0453 (0.124)
Distance from plot(s) to house (km)	-0.033** (0.016)	-0.006 (0.020)	-0.001 (0.012)	0.036** (0.015)
Distance from plots(s) to nearest commune road (km)	0.014* (0.008)	-0.001 (0.008)	-0.001 (0.004)	-0.006 (0.004)
Total hours of sunshine	0.00003 (0.000)	0.001*** (0.000)	0.00003 (0.000)	-0.001** (0.000)
Total level of rainfall	0.0001 (0.00007)	-0.0001* (0.00007)	-0.0001*** (0.00005)	0.0001 (0.00004)
Climate change induced natural shocks				
Perceived through wind storm (1: Yes; 0: No)	0.001 (0.079)	0.054 (0.076)	-0.091 (0.058)	0.038 (0.051)
Perceived through drought (1: Yes; 0: No)	-0.038 (0.069)	-0.012 (0.073)	0.177*** (0.059)	-0.040 (0.038)
Perceived through flood (1: Yes; 0: No)	-0.065 (0.072)	-0.034 (0.069)	0.072 (0.052)	0.059 (0.049)
Perceived through untimely rain (1: Yes; 0: No)	0.266*** (0.064)	0.112* (0.066)	0.066 (0.051)	-0.082** (0.033)
Perceived through pestilent insect(1: Yes; 0: No)	-0.005 (0.063)	0.046 (0.073)	-0.006 (0.039)	-0.022 (0.046)
Perceived through water shortages (1: Yes; 0: No)	-0.083 (0.102)	0.105 (0.106)	0.218** (0.091)	-0.020 (0.072)

Bootstrap standard errors in parentheses;*** p<0.01, ** p<0.05, * p<0.1; N = 329. Source: Author's estimation.

Franzel (1999). As mentioned in Nhemachena and Hassan (2007), with access to technology farmers are able to vary their planting dates, switch to new crops, diversify their crop options and use more irrigation, apply water conservation techniques. However, production assets requires large capital stock that can be a constraint and thus ensuring availability of cheap technologies for farmers, especially smallholders, can significantly increase their use of other adaptation options. Our estimation finds that households with higher production assets decrease the probability of choosing no adaptation to climate change by 1.6%, *Ceteris paribus*.

Our results indicate that farm system types alone may not determine climate change's responses; these systems are also imbedded with institutional factors, infrastructure, climate conditions and varying climate experiences as well. With respect to institutional factors, farmers with access to credit have higher chances of adapting to changing climatic conditions as found in Nicholas and Gina (2012) and Nhemachena and Hassan (2007). Household with access to credit will have the impact of raising the probability of making adjustments of planting calendar to climate change by 10.9%, *Ceteris paribus*.

According to Nhemachena and Hassan (2007), access to affordable credit increases financial resources of farmers and their ability to meet transaction costs associated with the various adaptation options they might want to take. With more financial and other resources at their disposal farmers are able to change their management practices in response to changing climatic and other factors. Farmers with limiting access to market have higher probability of adjustments of crops and varieties to changing climatic conditions by 1.4%, *Ceteris paribus*. With access to markets farmers are able to buy new crop varieties, new irrigation technologies, and other important inputs they may need to change their practices to suit the forecasted and prevailing climatic conditions (Nhemachena and Hassan, 2007). However, household with plots in longer distance from house will have the less probability of making adjustments of crops and varieties to climate change by 3.3%, *Ceteris paribus*. In addition, household with plots in longer distance from house will have the more probability of choosing no adaptation to climate change by 3.6%, *Ceteris paribus*. Overall, the improvement of both the accessibility and usefulness of local services is deemed a necessity for adaptation strategies.

With respect to climate conditions, increasing temperature increases the probability of adapting adjustments of planting techniques to climate change by 0.1%, *Ceteris paribus*. The fact that adaptation to climate change increases with increasing temperature is in line with the expectation that increasing temperature is damaging to agriculture and farmers respond to this through the adoption of different adaptation methods (Nguyen and Le, 2012; Nhemachena and Hassan, 2007). It was also found that increasing temperature also decreases the probability of choosing no adaptation to climate change by 0.1%, *Ceteris paribus*.

Annual average precipitation is negatively related to adaptation. Increasing rainfall decreases the probability of adapting adjustments of planting techniques and adjustments of planting calendar to climate change by 0.01%, *Ceteris paribus*. The probable reason for the negative relationship between average annual precipitation and adaptation could be due to the fact that agriculture in the Mekong River Delta is water scarce and faces high temperature and increasing precipitation will not constrain agricultural production or does not promote the need to adapt (at least using the main adaptation options considered in this study).

ISUR estimates show that past climate experiences increases the probability of uptake of adaptation measures (Niles et al., 2015b; Nicholas and Gina, 2012; Maddison, 2006). Farmers who are aware of changes in climatic conditions have higher chances of taking adaptive measures in response to observed changes. Specifically, increasing drought increases the probability of farmers to respond to changes in terms of adjustments of planting calendar (Niles et al., 2015b and Patrick and Richard, 2012) by 17.7%, *Ceteris paribus*.

Increasing untimely rain increases the probability of farmers changing their management practices, in particular, adjustments of crops and varieties (including changes in varieties, crops/livestocks, and crop structure) by 26.6%, *Ceteris paribus*, and adjustments of planting techniques (including changes in crop cultivation, fertilizer/stimulus, pesticides/herbicides, crop quantity, and farmyard manure) by 11.2%, *Ceteris paribus*. Resulting water shortages leads to adjustments of planting calendar, including changes in irrigation schedule, and crop rotation (Niles et al., 2015b) by 21.8%, *Ceteris paribus*. However, increasing untimely rain decreases the probability of farmers making no adaptation by 8.2%, *Ceteris paribus*. This can be explained that farmers' experiences of climate change induced natural shocks lead their choice of no adaptation since this kind of shock benefits their agriculture production (Geoff, 2014). Generally, if perception of climate change induced natural shocks are the most salient for farmers, it likely has significant implications for assessing how short-term responses can influence long-term adaptations and the subsequent policies that may be needed to accompany such actions (Carlo et al., 2015; Le Dang et al., 2014; Park et al., 2012). In addition, because climate variability in higher temperature and accompanied by drought and water shortages, irrigation investment needs from the viewpoint of Public - Private Partner (PPP) should be reconsidered to allow farmers increased water control to counteract adverse impacts from climate variability and change.

CONCLUSION AND IMPLICATIONS

A better understanding of current adaptation measures and their determinants will be important to inform policy for future successful adaptation. This study was based on farm-level analysis of farmers' past climate experiences and distinguished determinants of adaptation to climate change induced natural shocks in the Mekong River Delta of Vietnam. This research has shown that the majority of farmers used adaptive measures that mostly related to their farming practices such as adjustments of crops and varieties (including changes in varieties, crops/livestocks, and crop structure), adjusting planting techniques (such as changes in crop cultivation, fertilizer/stimulus, pesticides/herbicides, crop quantity, and farmyard manure) and adjusting planting calendar (such as changes in irrigation schedule, and crop rotation). On top of that, no adaptation is also considered as a choice of adaptation. The adaptive measures farmers followed were those that they perceived climate change induced natural shocks such as wind storm (typhoon), drought, flood, higher temperature, untimely rain, salt water intrusion, eroded shorelines, pestilent insect, and water shortages.

This paper further explored the determinants of different adaptive measures using an ISUR probit model. The model allows for the simultaneous identification of the determinants of all adaptation options, thus limiting potential problems of correlation between the error terms. Correlation results between error terms of different equations were significant (positive) indicating that various adaptation options tend to be used by farmers in a complementary fashion, although this could also be due to unobserved farm-level socioeconomic and other factors. ISUR probit results confirm gender of the farm head being male, education of the farm head, marital status of the farm head, production assets, firm size, availability of credit, access to market, and temperature and rainfall have significant impact on choices of adaptation to climate change. Results also indicate that awareness of past climate experiences is the most important determinants of farm-level adaptation.

The findings suggest some directions for adaptation policies. Sources and quality of information can be of important consideration due to the potential influences on farmers' past climate experiences and their adaptation assessments. Additionally, awareness creation on climate change and adaptation methods should be focused. On top of that, improvement of both the accessibility and usefulness of local services, such as credit and infrastructure, are deemed a necessity for successful adaptation strategies in the Mekong River Delta. Other policy options could also be suggested, including: strengthening education level of farmers, facilitating cheap technologies for farmers, spurring irrigation investment through public – private partner. Last but not least, government should support some implementations of the land reform such as farmers' cooperation in large-scale production.

Conflict of Interests

The author certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

ACKNOWLEDGMENTS

Sincere gratitude to the Departments of Agriculture and Rural Development of 6 districts: Duc Hoa (Long An Province), Thanh Phu (Ben Tre Province), Chau Phu (An Giang Province), Hon Dat (Kieng Giang Province), Phong Dien (Can Tho Province), and Dam Doi (Ca Mau Province) for their great help and support in organising farmer interviews. Thanks also to the Southern Center of Agricultural Rural Policy and Strategy (SCAP) staff and 20 undergraduate students of Nong Lam University, local guides and 335 farm households in the Mekong Delta in helping and supporting the interviews conducted on July, 2014.

This paper is made possible under the sponsor of The National Foundation for Science and Technology Development of Vietnam (NAFOSTED) under the Project No II.6.1-2012.14.

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Vol. 11(15), pp. 1373-1380, 14 April, 2016
DOI: 10.5897/AJAR2015.10759
Article Number: F29914858059
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Effect of different irrigation levels with different qualities of water and organic substrates on cultivation of pepper

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Received 22 December, 2015; Accepted 10 February, 2016

The amount of water needed to meet the water requirement of crops and use of alternative organic substrates affect the development of the plants' nutrients. The objective of this research is to evaluate the cultivation of pepper (*Capsicum chinense*) under different organic substrates and irrigation levels with supply water and wastewater. The experiment was conducted in a greenhouse at Campina Grande, Paraíba, Brazil. Biquinho BRS Moema pepper species was used. The experimental design was randomized block in a factorial design of 2 x 5 x 2. Two types of water (water supply and wastewater) and five water levels (L) were used based on the water requirement (WR) of the crop as follows: 100% WR (L5), 80% WR (L4), 60% WR (L3), 40% WR (L2) and 20% WR (L1); and two kinds of substrates (caprine and bovine) were also used, all in three replicates with two plants each. The germination percentage for bovine substrate averaged 44%, while for the caprine substrate, it was 18%. 24% increase in the number of leaves at level 4 was compared with that of level 5, where there was 20% reduction of WR of the plant. The pepper "Biquinho" irrigated with wastewater had better average, making it an alternative for irrigation. For the growth of pepper, it is not suitable to use irrigation values less than 40% of the water requirement for culture, because it influences negatively the development of the culture. For pepper, the ideal irrigation level is 80% of water requirement.

Key words: Water reuse, water demand, BRS Moema.

INTRODUCTION

The most commercialized vegetable crop consumed in the world is *Capsicum* pepper, and about a quarter of the world population consumes pepper as natural, liquid sauces, canned or dehydrated (Silva et al., 2015). Also smaller sizes are used as ornamental plant; it is used as family farms, and for agribusiness (Xavier et al., 2006).

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Pepper is a crop with good productivity, and generally profitable for producers; it has greater rusticity on the field and longer cycle and may be harvested for more than one year (Hortifruti Brazil, 2015).

In Brazil pepper is the second most exported vegetable, contributing 13.5% of total production. Due to the increased consumption and interest of exporters, pepper cultivation is a trade of big importance, involving various sectors, from small to multinational farmers (Informa Economics F. N. P., 2012). In this way, pepper cultivation should be studied to provide quality and alternatives in its management. This information will be beneficial to its producers.

Organic substrates are an alternative to reduce costs in pepper cultivation. Almeida et al. (2012) state that the ideal substrate should be easily acquired, transported and have nutrient (Kusdra et al., 2008). The proper pH of the substrate must have good texture and structure (Silva et al., 2012).

The substrate must have bovine manure, because according to Silva et al. (2007), it is the main organic fertilizer used to improve the fertility of the Brazilian semiarid region soils. However, the use of caprine manure is a promising alternative for substrate composition (Pereira et al., 2012).

For pepper cultivation, the types of substrate and water used are important because they provide the proper environment and nutrients for the plant development. In regions with water shortage like the Brazilian semiarid regions, irrigation with wastewater is an alternative that helps the production of pepper, and also the environment, because it makes necessary the reuse of water. For Medeiros et al. (2008), the benefits of water reuse are conservation of available water, its wide availability, possibility of intake and nutrient recycling, preservation of the environment. There are few studies on pepper cultivation. It is important to consider the type of substrate and the amount of water needed at each stage of the cycle of the plant in order to obtain maximum development during the entire cycle in a sustainable manner with use of organic substrates and water reuse.

The efficiency of the use of water in irrigation reduces water loss and also supplies the water requirement of the plant for its best development. Irrigation management aims to supply the water requirement of the crop to some extent without deficit or excess. It is very important that irrigation management is done properly, to obtain success in yield and also preserves the environment (Gomes and Testezlaf, 2007).

The research was carried out to evaluate the cultivation of pepper (*Capsicum chinense*) under different organic substrates and irrigation levels with water supply and wastewater.

MATERIALS AND METHODS

The investigation was carried out in a greenhouse, at Campina Grande, PB, Brazil, in 2014. The greenhouse is located at an altitude of 550m above mean sea level and it is 7º15'18" latitude and 35°52'28" longitude. The climate is classified as Bswh according to the Köppen classification system, with an average annual temperature of 26.8°C and an average annual rainfall of 360 mm. Cultivar BRS Moema pepper "Biquinho" (*C. chinense*) seeds planted in a substrate by the company ISLA Seeds, were used in this experiment. For the propagation, seeds were planted directly on the cultivation site, 0.5 cm deep, as recommended by the company.

For the experiment 60 black plastic pots (no. 17) were used with a capacity of approximately 1.6 L, with the following dimensions: top diameter, 15 cm; bottom diameter, 9 cm and height, 14 cm. For the drainage six holes were made and a protection screen was placed at the bottom of the pots. They were then filled with crushed stones (no. 0), covering all the bottom and the substrate (soil: manure, 7:3); there was 70% soil and 30% manure, on a volume basis.

The experiment was laid out in a randomized block, in a 2 x 5 x 2 factorial design; it is composed of two types of water: supply water (A1) and wastewater (A2), five water levels based on crop water requirement and two types of substrate: bovine manure (S1) and caprine manure (S2). The five irrigation levels (L) using supply water and wastewater were treated by an up flow anaerobic sludge blanket reactor (UASB + WETLAND), based on crop water requirement (WR): 100% of WR (L5), 80% of WR (L4), 60% of WR (L3), 40% of WR (L2) and 20% of WR (L1).

They were evaluated daily until 14 days after sowing (DAS); the germination percentage (GP) was based on Labouriau and Valadares (1976) and the Emergence Speed Index (ESI) was determined by Maguire (1962). From 23 days after sowing (DAS) the following evaluations were carried out weekly: growth and development of the plant, plant height (PH) measured from the soil until the apex of the plant; stem diameter (SD) close to the soil; number of the leaves (NL).

Data were analyzed using the statistical software SISVAR (Ferreira, 2014) and the difference between treatment means was assessed by the Tukey test, at 5% probability.

RESULTS AND DISCUSSION

Figure 1 shows the germination percentage for bovine substrate averaged 44.4%, while for the caprine substrate, it was 18.64%. These results show the efficacy of bovine substrate in the early stages of the pepper. The use of bovine substrate increases the percentage of germination in about 26%. After 30 days of sowing percentage of germination of the varieties "Girl Finger" (TECNOSEED), "Tabasco" (TOP SEED), "Volcano 2011" and "Bishop Hat 2012" had, respectively, 34, 25, 5 and 0% germination (Silva et al., 2013).

While 14 days after sowing, the "Biquinho" pepper in organic substrates at different water levels was 36.3%, as shown in Figure 2. For the emergence speed index (ESI) the highest average, according to Figure 2, was L1 (20% WR) with 1.44 germination/day. Similar results of ESI were obtained by Magalhães et al. (2011) studying the emergency of Chili pepper at several concentrations of CO_2, with 2.23 to seedlings/day; whereas, for the "Girl Finger" pepper was 0.92 to 1.42 seedling/day, varying according to the carbon dioxide concentrations (550 and 360 ppm).

In the germination of Biquinho pepper, it was observed that only 20% of the water requirement of culture was

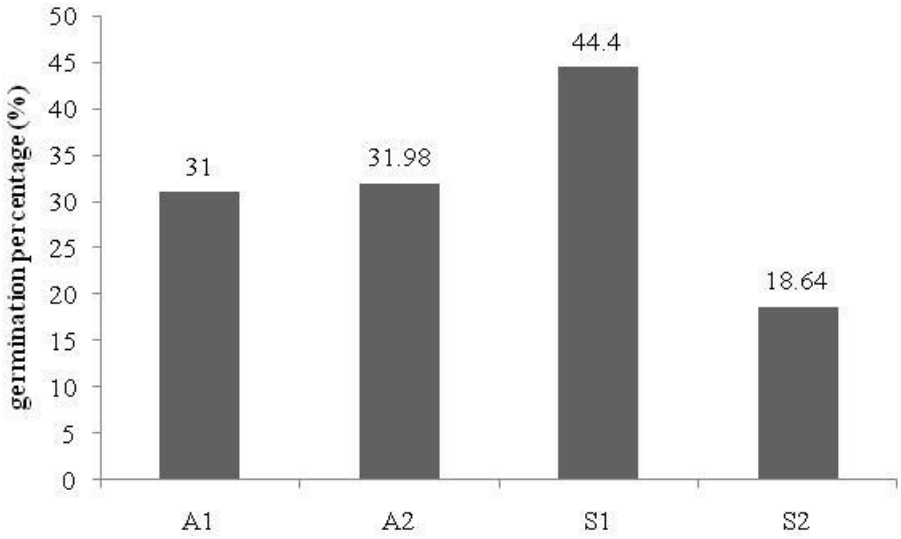

Figure 1. Variation of germination percentage (GP) of pepper Biquinho considering the mean of treatments.

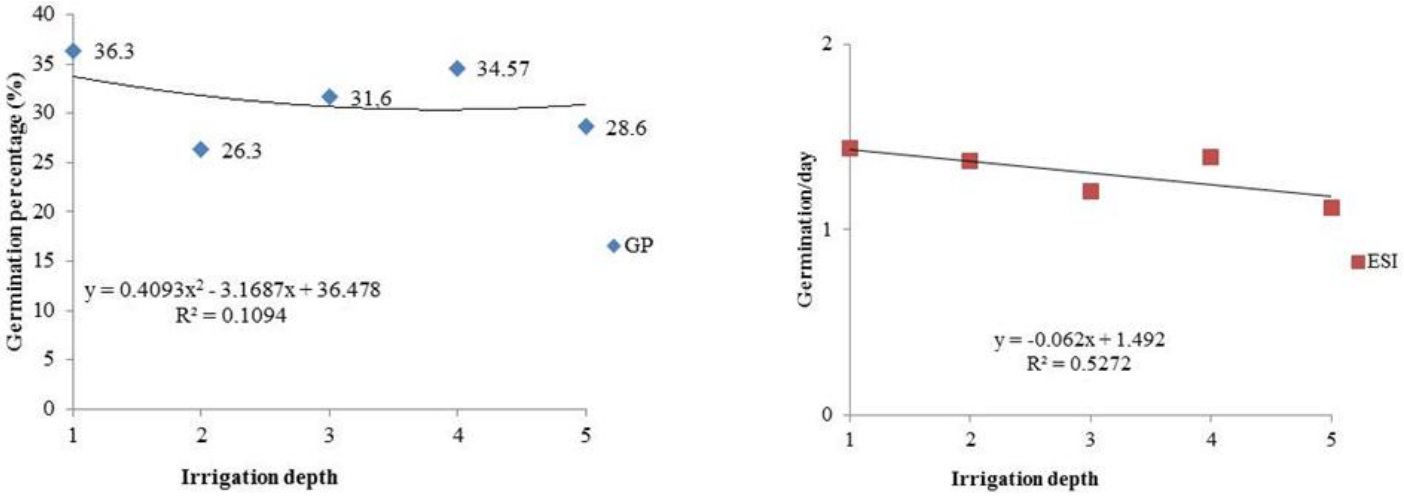

Figure 2. Germination percentage (GP) and emergence speed index (ESI) of “Biquinho” pepper seeds with organic substrates irrigated with different levels of treated wastewater and supply water.

sufficient in tolerating decreased water content in the soil; thus making the seeds to germinate under deficits water. Similar results were obtained by Silva et al. (2014) on the germination of irrigated Biquinho pepper with water blades and wastewater; they found best results at 20 to 80% of the water requirement. According to Paiva et al. (2012), irrigation using wastewater for chili resulted in better performance.

The use of wastewater and bovine substrate resulted in better average results for plant height in all assessments, with continued growth (Table 1). Using wastewater to produce pepper seedlings, okra and tomato, Oliveira et al. (2012a) and Alves et al. (2012) had higher crops. In producing pepper seedlings Malagueta and Tequila Sunrise, Oliveira et al. (2012b) had better results, using 75% of wastewater. This study found positive effects with higher averages in plants irrigated with wastewater treated.

With bovine substrate, the plant height average was higher than that of caprine substrate, as shown in Table 1. Evaluating different substrate compositions (topsoil, washed sand, commercial substrate, caprine and bovine manure) in the yield of ornamental pepper (*Capsicum annuum* L.), Da Silva et al. (2013) achieved lower results for the height of pepper. Silva et al. (2010) achieved lower mean for chili height (10.7 cm) in substrates whose

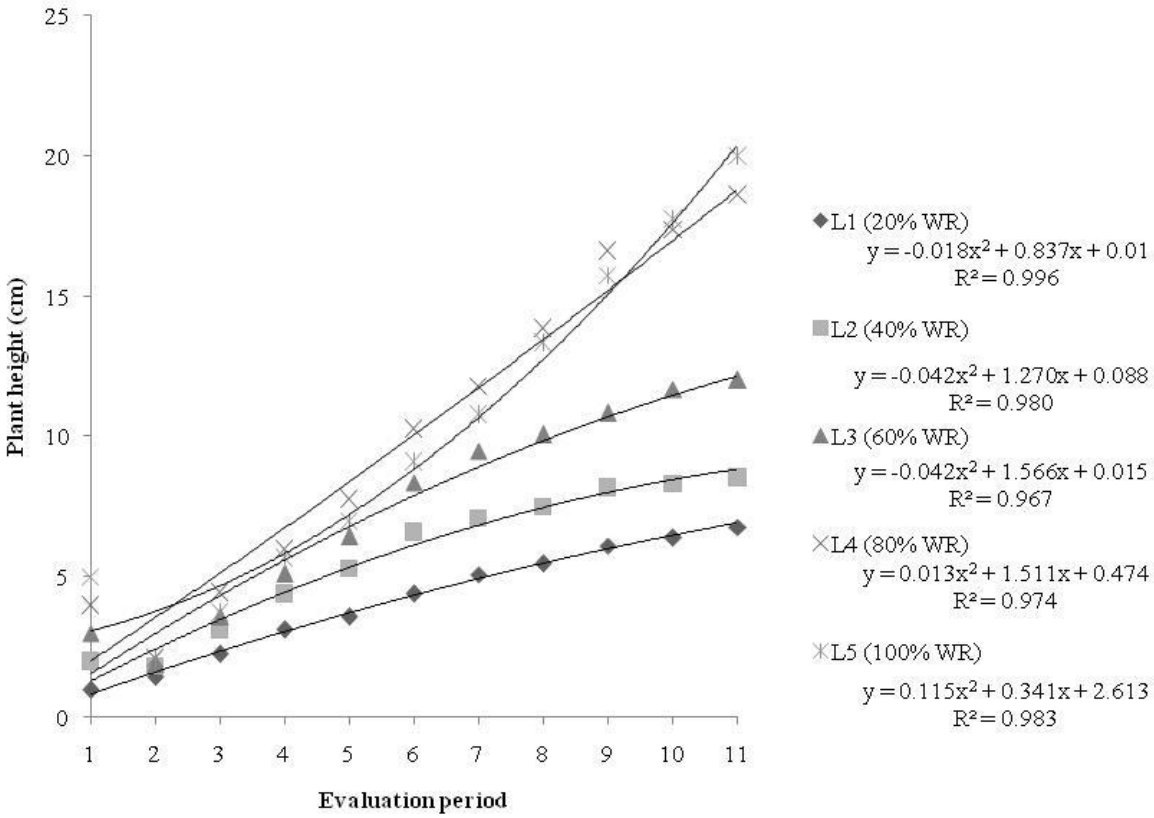

Figure 3. Regression of pepper "Biquinho" with high organic substrates irrigated with different water levels, and evaluation times of 30 (1), 44 (2), 58 (3), 72 (4), 86 (5), 100 (6), 114 (7), 128 (8), 142 (9), 156 (10) and 170 DAS (11).

Table 1. Plant height (PH) of pepper BRS Moema ("Biquinho"), with organic substrates irrigated levels with water supply and wastewater treated.

	Mean square plant height[1]										
Source of variation	**PH_1**	**PH_2**	**PH_3**	**PH_4**	**PH_5**	**PH_6**	**PH_7**	**PH_8**	**PH_9**	**PH_{10}**	**PH_{11}**
Type of water											
Supply water (A1)	1.84[a]	3.32 [a]	4.51[a]	5.45[a]	6.91[a]	8.00[a]	9.11[a]	10.63[a]	11.60[a]	12.45[a]	13.39[a]
Wastewater (A2)	1.93[a]	3.54 [a]	5.27[a]	6.68[b]	8.63[b]	9.71[b]	11.06[b]	12.37[a]	13.03[a]	13.98[a]	15.33[a]
Type of substrate											
Cattle substrate (S1)	2.25[b]	4.72[b]	6.95[b]	8.47[b]	10.26[b]	11.25[b]	12.01[b]	13.23[b]	13.93[b]	14.47[a]	15.24[a]
Goat substrate (S2)	1.52[a]	2.14[a]	2.84[a]	3.67[a]	5.28[a]	6.46[a]	8.06[a]	9.78[a]	10.70[a]	11.97[a]	13.48[a]

Means followed by the same letter in the column do not differ by Tukey test. 1Option of transformation: square root of Y + 1.0 - SQRT (Y + 1.0). Evaluation: 30 DAS (PH1), 44 DAS (PH2), 58 DAS (PH3), 72 DAS (PH4), 86DAS (PH5), 100 DAS (PH6), 114 DAS (PH7), 128 DAS (PH8), 142 DAS (PH9), 156 DAS (PH10) e 170 DAS (PH11). Plant height (cm).

mixture had humus. Nascimento et al. (2015) analyzed the influence of water stress on the nozzle of pepper cultivated with goat substrate; they observed similar results with an average height of 14.53 to 13.70 cm using wastewater and supply water at 177 DAS.

As shown in Figure 3, the irrigation levels applied are directly proportional to plant height, and its linear growth. The highest means were obtained in L5 (100% WR) and L4 (80% WR), providing better development in plants. Reduction of irrigation levels resulted in plants of smaller sizes. This result is interesting, because smaller plants are easily managed, and also for ornamental purposes. At 170 DAS (11), the pepper plant's height ranged from 7.43 to 23 cm (67.69%); it was checked to compare the

Table 2. Variable number of leaves (NL) of pepper BRS Moema ("Biquinho"), with organic substrates irrigated with water supply levels and wastewater treated.

	Mean square number of leaves[1]										
Source of variation	**NF_1**	**NF_2**	**NF_3**	**NF_4**	**NF_5**	**NF_6**	**NF_7**	**NF_8**	**NF_9**	**NF_{10}**	**NF_{11}**
Type of water											
Supply water (A1)	2.0[a]	4.4[a]	6.8[a]	8.63[a]	9.75[a]	10.78[a]	13.23[a]	13.35[a]	15.60[a]	13.86[a]	14.0[a]
Wastewater (A2)	2.3[a]	5.3[b]	7.95[a]	10.4[a]	13.03[b]	12.38[a]	15.08[a]	15.86[a]	14.76[a]	13.80[a]	12.90[a]
Type of substrate											
Cattle substrate (S1)	3.0[b]	6.71[b]	10.2[b]	13.4[b]	15.0[b]	13.8[b]	15.4[a]	14.81[a]	13.33[a]	10.16[a]	7.46[a]
Goat substrate (S2)	1.3[a]	2.98[a]	4.53[a]	5.67[a]	7.73[a]	9.36[a]	13.63[a]	14.40[a]	17.33[b]	17.50[b]	19.43[b]

Means followed by the same letter in the column do not differ by Tukey test. [1]Option of transformation: square root of Y + 1.0 - SQRT (Y + 1.0). Evaluation: 30 DAS (LN_1), 44 DAS (LN_2), 58 DAS (LN_3), 72 DAS (LN_4), 86DAS (LN_5), 100 DAS (LN_6), 114 DAS (LN_7), 128 DAS (LN_8), 142 DAS (LN_9), 156 DAS (LN_{10}) e 170 DAS (LN_{11}). Number or leaves in units.

smaller and larger irrigation depth. It shows that by reducing the amount of water available to plant for drought stress, the metabolism of the culture is affected. The irrigation level for a higher average height "Biquinho" pepper is 100% WR in this study; it is similar to that achieved by Aragão et al. (2011), who studied different irrigation levels of Magali R. They observed a higher average height, using up to 100% of Eto. Barcelos et al. (2015) submitted that the red sweet pepper nozzle potassium doses reach 3.7 cm height.

In Table 2, the analysis of variance for the number of leaves (NL), and the source of water was significant at NL_2 (30 DAS) and NL_5 (86 DAS) reviews. Biquinho grown peppers with goat substrate and irrigated treated wastewater and supply averages were superior at 51 and 93 DAS; supply water had the best average until the end of the experiment; at 177 DAS, it ranges from 16.33 to 19.13 leaves per plant (Nascimento et al., 2015).

In research conducted with commercial substrate in the yield of seedlings of three black pepper genotypes, Serrano et al. (2012) observed the number of leaves for an average of 11.1 (Gajarina), 11.0 (Iaçara) and 12.9 (Singapore). Corroborating with the results of these authors, the "Biquinho" pepper with bovine substrate produced an average of 15.4 leaves at 144 DAS, and with caprine substrate an average of 19.34 leaves at 170 DAS; indicating that organic substrates used propitiated conditions favorable to the crop.

As shown in Figure 4, there was a quadratic regression trend in the number of leaves. The L4 level (80% WR) and L5 level (100% WR) had better results when compared to other irrigation levels. By regression analysis, there was a 24.44% increase in the number of leaves in the L4 when compared with L5, where there was reduction of 20% of water available to the plant. When comparing L3 with L4, improving water availability by 20%, there is an increase in the number of leaves of 55.2%. The L4 irrigation level was considered adequate influencing positively the amount of pepper leaves. In the number of sheets of the nozzle pepper there was an increase in the number of sheets when there is increased availability of water based on the water requirement (80% (L4) and 100% (L5)).

By studying the tomato growth rates at different irrigation levels, Soares et al. (2011) had lower results; they reported an increase in the number of leaves, increase in water availability (20%) of the reference evaporation. There was an increase of 29.45% in the amount of leaves between irrigated plants. Silva et al. (2012), studying lettuce growing culture, saw a greater amount in number of leaves of plants irrigated with the blade, 125% ET0.

The plants irrigated with wastewater had the best averages and bovine substrate had superior results when compared to the substrate goats. At 170 (SD11), the nozzle pepper irrigated with wastewater had an average of 5.39 mm, and when irrigated with the average supply of water it is 5.17 mm and approximately 0.22 mm difference (Table 3). The wastewater had in all evaluations higher values when compared to the water supply. Costa et al. (2009) confirmed it on studying the stem diameter of the corn stalk. SD was higher for plants receiving wastewater compared to the results of plants that received water supply.

Comparing the values obtained with the stem diameter of the "Biquinho" pepper under different types of fertilizers obtained by Pagliarini et al. (2014), there was an average of 3.07 to 4.44 mm; while the values obtained in this experiment with bovine and caprine substrate under different water levels were higher. Silva et al. (2013), in producing ornamental pepper (*C. annuum* L.) with different substrate compositions, observed that the stem diameter of the pepper changed from 2.2 of 4.4 mm. According to Figure 5, L4 (80% WR) and L5 (100% WR) had better average as compared to other levels. To reduce 20% of the water requirement, there was a decrease of 15.8% in stem diameter in L4 levels and L3 levels. Comparing L2 with L1, there was an increase of 13.16% in stem diameter. It is noted that the highest means were obtained with irrigation levels of 80% of the

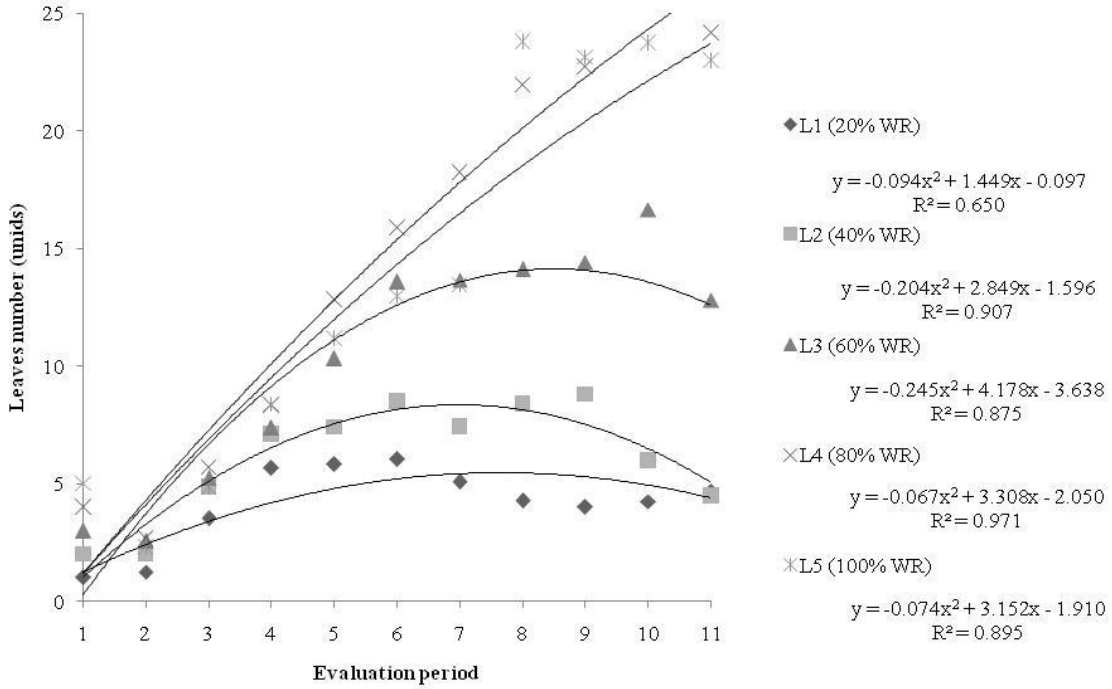

Figure 4. Regression of number of leaves of "Biquinho" pepper with organic substrates irrigated with different water levels, and evaluation times of 30 (1), 44 (2), 58 (3), 72 (4), 86 (5), 100 (6), 114 (7), 128 (8), 142 (9), 156 (10) and 170 DAS (11).

Table 3. Means the stem diameter (SD) of pepper BRS Moema ("Biquinho"), with organic substrates irrigated with water supply levels and wastewater treated.

	Mean square stem diameter[1]										
Source of variation	**SD_1**	**SD_2**	**SD_3**	**SD_4**	**SD_5**	**SD_6**	**SD_7**	**SD_8**	**SD_9**	**SD_{10}**	**SD_{11}**
Type of water											
Supply water (A1)	1.03^a	1.38^a	1.78^a	2.02^a	2.59^a	2.91^a	3.42^a	3.87^a	4.28^a	4.54^a	5.17^a
Wastewater (A2)	1.06^a	1.45^a	1.87^a	2.12^a	2.72^a	3.10^a	3.65^a	4.07^a	4.64^a	5.10^b	5.39^a
Type of substrate											
Cattle substrate (S1)	1.13^b	1.70^b	2.34^b	2.64^b	3.40^b	3.79^b	4.33^b	4.85^b	5.18^b	5.51^b	5.96^b
Goat substrate (S2)	0.96^a	1.13^a	1.32^a	1.50^a	1.91^a	2.21^a	2.73^a	3.09^a	3.75^a	4.12^a	4.60^a

Means followed by the same letter in the column do not differ by Tukey test. [1]Option of transformation: square root of Y + 1.0 - SQRT (Y + 1.0). Evaluation: 30 DAS (SD_1), 44 DAS (SD_2), 58 DAS (SD_3), 72 DAS (SD_4), 86DAS (SD_5), 100 DAS (SD_6), 114 DAS (SD_7), 128 DAS (SD_8), 142 DAS (SD_9), 156 DAS (SD_{10}) e 170 DAS (SD_{11}). Stem diameter in mm.

water requirement of the crop.

Silva et al. (2012), studying the effect of percentages (125, 100, 75, 50 and 25%) on the actual daily evapotranspiration of lettuce, Saia Veia (*Lactuca sativa* L.), it was found that plants irrigated with different water levels had significantly different stem diameter in treatments 25, 50 and 125% of Eto; there was increased stem diameter in the blade at 125% of Eto. The nozzle pepper increased in all slides, but the greatest diameter was for the replacement of 80 and 100% of the water requirement.

Reducing the diameter of the stem is directly related to increased water replacement levels. Soares et al. (2011) found similar results for tomato cultivation; so drought in the vegetative and reproductive phase provides lower height of plants, number of leaves and stem diameter. Analyzing the development of the coffee conilon it was found that drought reduced the stem diameter of this species (Dardengo et al., 2009). In rosemary-pepper cultivated at different irrigation levels, Alvarenga et al. (2012) and Figueiredo et al. (2009) observed better growth occurring with greater water availability.

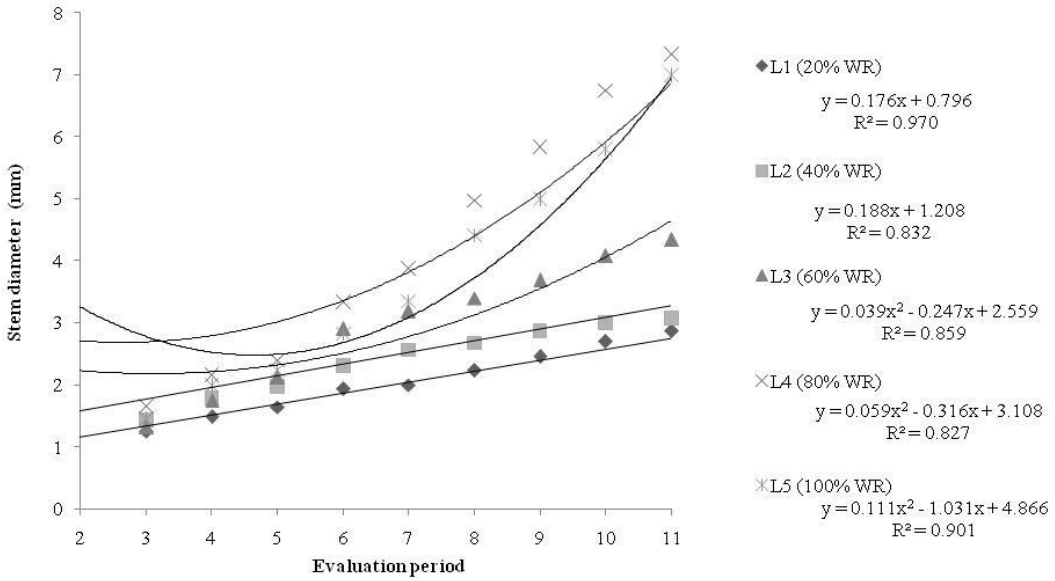

Figure 5. Regression of stem diameter of "Biquinho" pepper with organic substrates irrigated with different water levels and evaluation times of 30 (1), 44 (2), 58 (3), 72 (4), 86 (5), 100 (6), 114 (7), 128 (8), 142 (9), 156 (10) and 170 DAS (11).

Conclusions

The bovine substrate used in BRS Moema pepper cultivation had the best results for the development of the crop. The caprine substrate can be applied, but the pepper trees would be small, making it ideal for ornamentation.

The wastewater used for irrigation of pepper is an alternative of water reuse in agriculture, reducing costs, providing nutrients for the plants and resulting in better averages, so, more shrubby pepper. The L4 irrigation (80% WR) and L5 (100% WR) were the water supply needs of pepper BRS Moema; they are favorable conditions for the crop. So, the plants irrigated with 80% WR allows for proper development of the pepper, saving water.

Conflict of Interests

The authors have not declared any conflict of interests.

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Vol. 11(15), pp. 1381-1393, 14 April, 2016
DOI: 10.5897/AJAR2015.10770
Article Number: EA76B6E58063
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Clustering analysis of several peanut varieties by pre and post-harvest and biochemistry parameters

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Received 26 December, 2015; Accepted 2 March, 2016

Peanut (*Arachis* species) plants originated in South America where they have existed for thousands of years. Successively, peanut culture has been introduced in many African countries and was incorporated into local traditional food cultures. Numerous studies showed peanut nutritive importance and capacity to prevent several human diseases. The target of the present survey aimed to create a germplasm benchmark of peanut varieties in the north region of Côte d'Ivoire (West Africa country) since this plant is weakly studied in this geographic area. For this purpose, six peanut varieties were processed and pre and/or post-harvest measurements have been brought on seedlings. In addition, biochemical composition of peanut seed for each considered varieties were measured. Statistical analysis based on several R software functions showed a good quality of collected peanut data and proposed post-harvest parameters as an adequate factor clustering the present analyzed peanut varieties. Then, statistical analysis performed in this study, allowed to cluster analyzed peanut varieties in two different groups. Moreover, the same survey evidenced a strong agreement between both post-harvest and biochemistry parameters assessing the difference between the two detected peanut variety groups (p-value < 0.05). Finally, the findings exhibited protein, glucose as well as ash biochemistry parameters as decent indicators selecting and clustering the present managed peanut varieties (p-value <0.05). In conclusion, this study proved a methodology demarche suggesting the possibility to hypothesize peanut germplasm benchmark in the savanna region of Côte d'Ivoire.

Key words: Peanut (*Arachis* species) varieties, groundnut, pre and post harvesting parameters, biochemical composition.

INTRODUCTION

Peanut (*Arachis* species), also known as groundnut or earth nut, is an important legume of underground fruiting. It is native to eastern South America region. Groundnut is now grown worldwide in the tropical and temperate zones primarily as an oil seed crop (Weiss, 1983; Bansal et al., 1993; Clavel, 1997) and was introduced by Portuguese

explorers in the 16th century in West Africa. Peanut was widely cultivated in more than 100 countries and over 26.4 million hectares with an average productivity of 1.4 ton/ha (FAO, 2003; Barraud and Maury, 2004; Ntare et al., 2008). It is the fifth most important oilseed crop in the world after soybean, cotton seed, rape seed, and sunflower seed (Nwokolo and Smartt, 1996; FAO, 2003). In 2012, world production of groundnuts was estimated at 40.5 million/ton (FAOSTAT, 2012). Africa supplies about 27.4% of this production mainly with Nigeria, Sudan and Senegal (FAOSTAT, 2012). For consumption, legumes like peanuts (*Arachis hypogea*), bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), and soybean (*Glycine max*) have an important contribution for daily diet of humans and animals. Some beans are eaten, cooked or served as mainly additive, and others provide vegetative oil. Groundnut is consumed as seeds, oil, and butter found in many foods, because of its common use for its nutritional qualities as an additive or for its technological skills. The fat content in groundnut has been largely studied. In general, groundnuts contain 50 to 55% fat of which approximately 30% is linoleic acid and 45% is oleic acid. Groundnut seed contain 20 to 25% protein on a dry seed basis and is a rich source of minerals (phosphorus, calcium, magnesium and potassium) and vitamins (Annerose, 1990; Savage and Keenan, 1994; FAO, 2003). Considering their organoleptic qualities and high fat content, Rakotovao (1994) and Schilling (1996) classified peanuts in three group: mouth peanuts are characterized by a clear and uniform color pods, large seeds (weight 100 seeds> 80 g) and at least two big seeds per pod with a fat content of around 50%; in oil peanuts, raw material for the production of oil and high performance husking (about 70%) have a high fat content (>50%) and 100 seed weight <50 g; and finally, peanuts which present two purpose have the characters at a time for the production of oil and for consumption state. They are characterized by low-fat (<50%) and a higher seed weight (100 seed weight <40 g) (Schilling, 1996). Despite its high potentialities producing several cereal crops, Côte d'Ivoire contributes at 0.26% (low contribution) of world output of groundnuts (FAOSTAT, 2012). Therefore, groundnut is seen in Côte d'Ivoire as a traditional peasant culture, mainly for self-consumption and domestic marketing. In other words, groundnut culture is weakly practiced in this country. Indeed, the bulk of production is concentrated in the savanna regions of the north, but culture is also present in the southern forest regions.

Thereafter, all these geographical parameters as well as climates, and the variability of ecological contexts considerably influence peanut plants morphologic aspects and their growth development. These ecological changes are superimposed on large differences in production systems, from slash and burn areas to those where production is partially mechanized, and since the boxes crops carefully maintained to field crops, or low crops case for local markets. However, it is believed that the improvement of the comprehension of groundnut or peanut plant cultures through morphological, biochemical as well as genetic selection can represent a useful approach improving quantitatively and qualitatively this culture worldwide and in particular in north region of Côte d'Ivoire. Hence, it was proposed through the present study to establish a peanut germplasm benchmark in this part of the world by collecting the latter's morphological data from different experimental sites of the Peleforo Gon Coulibaly University of Korhogo. Here, six peanut varieties from both Dikodougou and Korhogo localities refereed as Arachide Dikodougou (ARD) and Arachide Korhogo (ARK), respectively were processed for a clustering analysis basing on the statistical integration of their pre-harvest, post-harvest and biochemistry parameters.

MATERIALS AND METHODS

Source of raw materials and experimental design

Experiments were carried out on six peanut varieties from Dikodougou (AR_1D, AR2D AR_3D, and AR4D) and Korhogo (AR_1K, AR_2K). The study was conducted in the experimental site of the University of Korhogo (north of Côte d'Ivoire). This site are located between an altitude of 392 m between -5° 34 '31" and -5° 29'34" West longitude and between 9° 31' 23" and 9° 31'32" latitude North and 5° 38'83.2" at an average altitude. The weather in this area is characterized by two types of Sudanese seasons: a dry season (November to April) and rainy season (from May to November). Annual precipitation varies between 1100 and 1600 mm (Diarrassouba et al., 2015). The present assays have been performed during the rainy season (from July to October). The experimental design was randomized at complete block with three (3) repetitions. In each repetition, each variety was shown on 3 lines of 10 m length corresponding to an elemental parcel. The distances are 80 cm between lines and 40 cm on the line.

Crop management

Showing was carried out in July 2013 during the rainy season. The

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seeds were treated in a mixture fungicide/insecticide and sown by two in each hill. After germination, thinning of plantlet was done leaving only a single plantlet per hill. No phytosanitary treatment was done during the plant growth; no input of fertilizers was made. Harvest was done manually, 120 days after sowing. Then the plants harvested from each plot were left to dry on the ground for 10 days. After 10 days, pods were removed from the boots for analyzes.

Parameters measurement

Pre-harvest, post-harvest and physicochemical parameters were measured for each experimental repetitions (three repetitions).

- It was started with pre-harvest parameters measurement, two weeks after showing. However, the number of branch per plant as well as plant height parameters were measured 30 and 60 days after sowing, respectively. Post-harvest parameter average values have been archived on 15 plants in each experimental site for each considered peanut variety. Furthermore, peanut maximum germination state was estimated assessing plant number two weeks after showing.
- Post-harvest measurements were performed on pods ten days after harvest. They were brought on yields of pods, the rate of single-seed pods, two seeds and three seeds in a kilogram of pods per variety.

Biochemistry parameters measurement

Biochemistry parameters were made on whole intact seeds (seed coat, embryo and cotyledons).

(1) Moisture content: Moisture was determined by drying the sample at 105°C for 24 h according to AOAC (1995). Samples were then cooled in desiccators and weighed. The loss in weight expressed as a percentage of the initial weight of the samples give their moisture content.

(2) Ash content: Ash was obtained after incineration at 550°C for 6 h according to AOAC (1995). 5 g sample was weighed into a previously dried and weighed porcelain crucible. The crucible with its content was placed in a furnace at 550°C for 6 h. After cooling in desiccators, the crucible with its content was weighed. The weight of the ash was expressed as a percentage of the initial weight of the sample.

(3) Fat content: Fat was determined based on the Sohxlet extraction method of AOAC (1995). Five gram (5.0 g) of the sample was introduced into a cartridge of Whatman. An empty flask reweighed and containing 60 ml of hexane was placed on the heating block of the Soxhlet apparatus and heated at 110°C. After 6 h of extraction, the flask was removed from apparatus and then the solvent was evaporated on a Rotary Evaporator. The flask containing the fat and residual solvent was placed on a water bath to evaporate the solvent followed by a further drying in an oven at 60°C for 30 min to completely evaporate the solvent. It was then cooled in desiccators and weighed. The fat obtained was expressed as a percentage of the initial weight of the sample.

(4) Protein content: Protein was determined by determination of total nitrogen according to the Kjeldahl method (BIPEA, 1976). The principle under the action of NAOH and after sulfuric mineralization in the presence of catalyst ($CuSO_4$), ammoniac formed was neutralized. The ammonia in the sample solution was then distilled into the boric acid until it changed completely to bluish green. The distillate was then titrated with 0.1 N HCl solutions until it became colorless. The percent total nitrogen and crude protein were calculated using a conversion factor of 6.25.

(5) Total carbohydrate content: Total carbohydrate content is determined by different method [100% - (% moisture + % ash + % fat + protein %)].

(6) Energy content: Energy is calculated with 4 kcal/g carbohydrates, 4 kcal/g protein and 9 kcal/g lipids according to Livesey and Elia (1995).

VE = [(9 × %Fat) + (4 × %Protein) + (4 × %Carbohydrates)]

Statistical analysis

Data normalization process

Data analysis was performed by our previous developed computational statistical pipeline (Noel et al., 2016). In statistics, normalization refers to the creation of shifted and scaled versions of statistics, where the intention is that these normalized values allow the comparison of corresponding normalized values for different datasets in a way that eliminates the effects of certain gross influences, as in an anomaly time series (Dodge, 2003). Here, logarithm transformation of analyzed pre or post-harvest and biochemistry data with the purpose to simplify their comparison and integration were performed.

Bioinformatics and biostatistical pipeline content

The biostatistical pipeline used for the present survey includes several functions and scripts implemented in R statistical package environment (R Core Team, 2013). For this study, the following mentioned scripts and functions used are: Heat-map (gplots package, version 2.12.1. License: GPL-2) and phylogeny dendrogram based on Euclidian distance of Person correlation; N factors function in R environment determining the number of factors to extract for the present analysis (Horn, 1965; Franklin et al., 1995); Corrplot package from R software (R Core Team, 2013), testing the aforementioned and detected factors or/and components by parallel principal component analysis (parallel PCA) assessing variance difference and/or similitude between considered parameters and samples.

Descriptive and inferential statistic tests (Fisher and student tests) were performed in the present study assessing (i) data quality control (repetition data reliability) and (ii) data variability among analyzed peanut sample varieties by their biochemistry composition (biochemistry parameters).

RESULTS

Boxplot analysis assessing quality control of peanut collected data

Experimental design of the present survey includes three repetition fields (three experimental sites). Each repetition

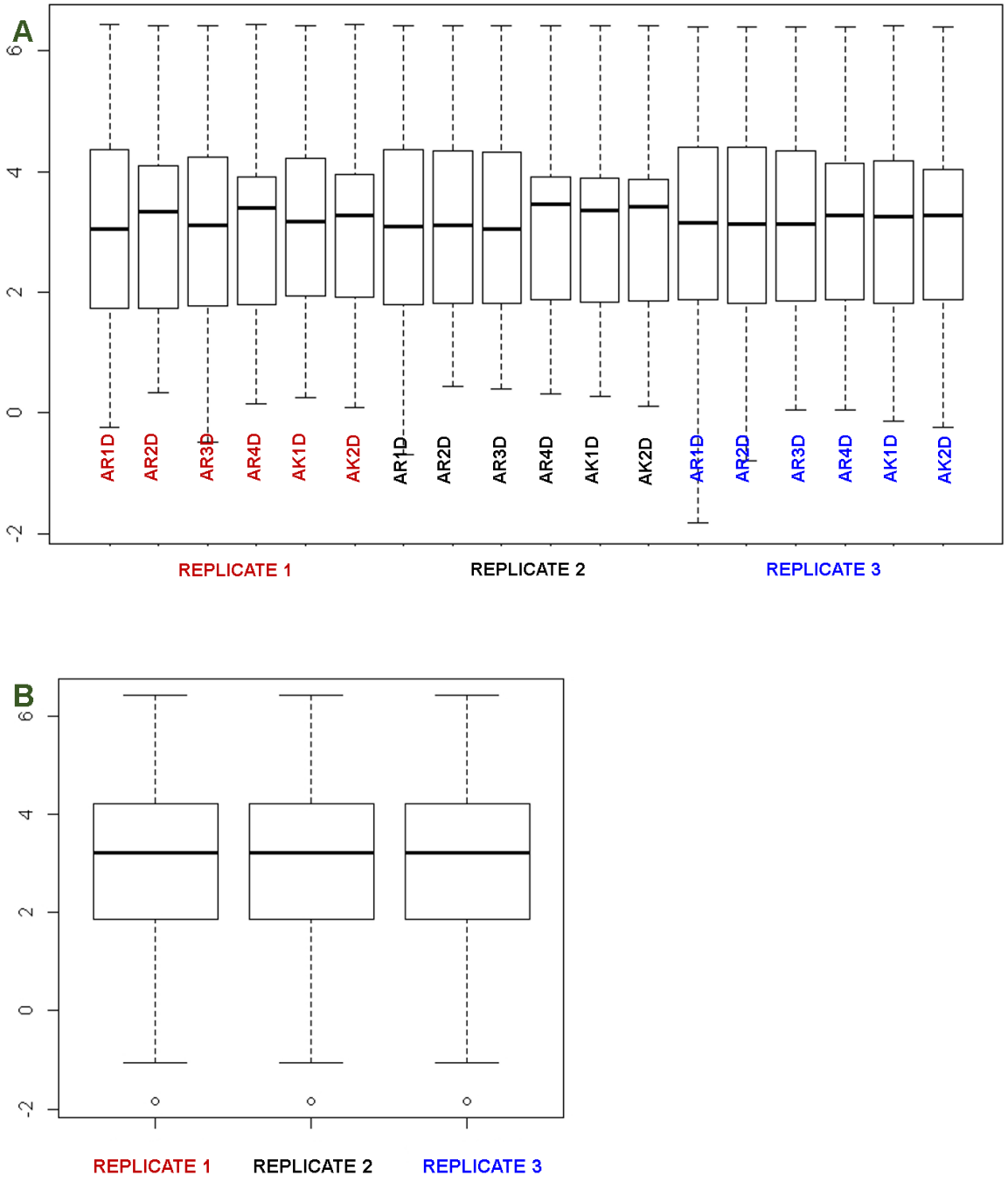

Figure 1. Boxplot showing the variability between the six analyzed peanut varieties in the three experimental sites (A); and between the three experimental sites assessing peanut varieties clustering analysis (B).

included 6 peanut samples. Hence, data were collected and grouped in three replicate before being processed for analysis. Here, a quality control data analysis was performed, aiming to assess the reliability of peanut replicate samples considering both (i) pre and post-harvest and (ii) biochemistry parameters. Indeed, the variability coefficient between the three considered experimental sites was assessed. Then, even if panel A of Figure 1, evidenced the difference between the six analyzed peanut varieties in each considered experimental

Table 1. Descriptive statistic merging all parameters of analyzed peanut samples in each experimental site (three experimental repetition data).

Parameter	Data replicate 1	Data replicate 2	Data replicate 3
Mean	74.69	73.74	73.06
Median	23.53	22.27	25.17
Standard deviation	154.38	152.70	150.59
Minimum value	0.62	0.5	0.16
Maximum value	619.2	615.14	610.9

site, panel B of the same figure suggested a low variability between the latter's (experimental sites). Next, the variance difference between the aforementioned experimental sites performing a Fishertest was evaluated. The results of this analysis reinforced the low variability observed between collected datafrom each analyzed experimental sites (p-value>0.80) as previously suggested by boxplot graph (Figure 1). Moreover, descriptive statistical analysis evidenced that processed samples replicates were comparable each to other (Table 1). Taking together, this analysis presumed that the present collected data were suitable for a statistical analysis reducing the potential errors due to replicate data variability.

Peanut plant clustering analysis by pre and post-harvest parameters

As previously supported, 6 varieties of peanut (AR_1D_j, AR_2D_j, AR_3D_j, AR_4D_j, AR_1K_j and AR_2K_j) have been considered for the present clustering analysis. Here a phylogeny analysis evaluating Euclidian distance of Pearson correlation with the goal to assess the capacity of both pre and post-harvest parameters explaining analyzed peanut variability was stimulated. Pre and post-harvesting feature data were normalized by logarithm transformation before processing them for statistical analysis. Pre-harvest parameters (morphological parameters) suggested high heterogeneity behaviors between analyzed peanut sample varieties as opposed to post-harvest parameters (Figure 2). Indeed, pre-harvest stage allowed to group peanut features in four diverse groups, while the post-harvest stage tended to reduce them in two groups (Figure 2). Taking together, these results suggested that, during their growing process, the present peanut varieties incline to cluster in two distinct groups. However, it is noteworthy to underline that the Pearson correlation values among all analyzed peanut features inside each discriminated group were significantly high (p-value<0.05) confirming their tendency to bunch in two great groups (Figure 2).

Heat-map evaluation of the similitude and/or dissimilitude between peanut samples by biochemistry parameters

A heat-map analysis was achieved based on the Pearson correlation test between peanut sample features considering their biochemistry parameters with the aim to confute difference and/or similitude between the previous detected peanut groups (Figure 2; panel B). As suggested in materials and methods session, 6 different peanut varieties (AR_1D, AR_2D, AR_3D, AR_4D, AR_1K and AR_2K) on three different experimental sites were processed for statistical analysis. Based on their biochemistry contents, the present analyzed peanut samples exhibited a high agreement between themselves (R>0.99; p-value<0.05). However, despite this high concordance, heat-map correlation analysis, clearly evidenced two tendencies in peanut clustering survey, suggesting its agreement with the previous results session. Then, statistical analysis processing peanut biochemistry parameters tend to oppose peanut samples collected in Dikodougou (AR_iD_j group) with those pooled in Korhogo (AR_iK_j group) locality. Moreover, the present analysis suggested a relative high variability in AR_iD_j peanut varieties as opposed to AK_iD_j peanut category (Figures 2 and 3). Considered as whole, these analyses suggested that despite their high similitude in term of Pearson correlation weighing biochemistry parameters, the present processed peanut samples tend to cluster in two distinct groups (Figure 3). In other words, it is possible to cluster the present analyzed peanut varieties in 2 great groups based on their biochemistry composition. This section is in agreement with the previous one confirming the high variability in AR_iD_j peanut samples as opposed to AK_iD_j (Figure 3).

Relationship between analyzed peanut samples and biochemistry parameters by using biplot and principal component analysis

The biplot analysis highlights the heterogeneity behaviors

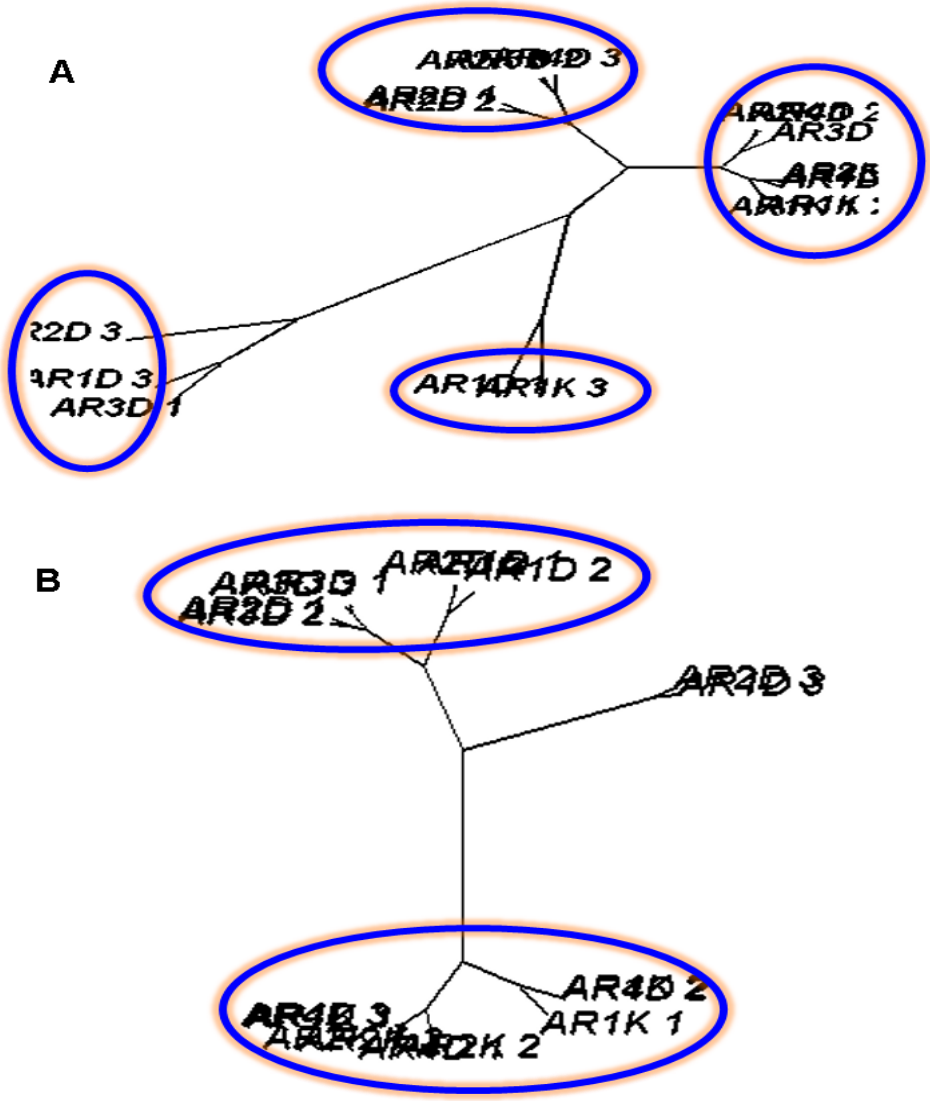

Figure 2. Phylogeny analysis based on Pearson correlation values showing (A) Pre and (B) Post-harvest parameters aptitude clustering processed peanut varieties.

between the 6 analyzed peanut varieties. This analysis also established the high variability among samples collected in Dikodougo land with respect to those collected in Korhogo land as previously shown (Figures 2 and 3). However, the present relationship analysis showed that replicate samples inside each analyzed peanut features (3 replicate × 6 peanut samples) exhibited a good clustering and a great agreement among themselves (Figure 4). In other word, the present analysis evidenced a low intra features data variability suggesting a good quality of the present collected data (Figure 1). In addition, principal component survey showed that energy source provided by peanut varieties, was strongly associated with their lipid biochemistry contents (Figure 4). The same analysis suggested a good agreement between energy source and peanut protein component. By contrast peanut glucose component exhibits low concordance with energetic nutritive component (Figure 4). Taking together, we were able to show that glucose component was not a good predictor of energy parameter in the present analyzed peanut sample features as opposed to both lipid and protein biochemistry components (Figure 4). Also, and as expected, it was shown that ash element was not a good

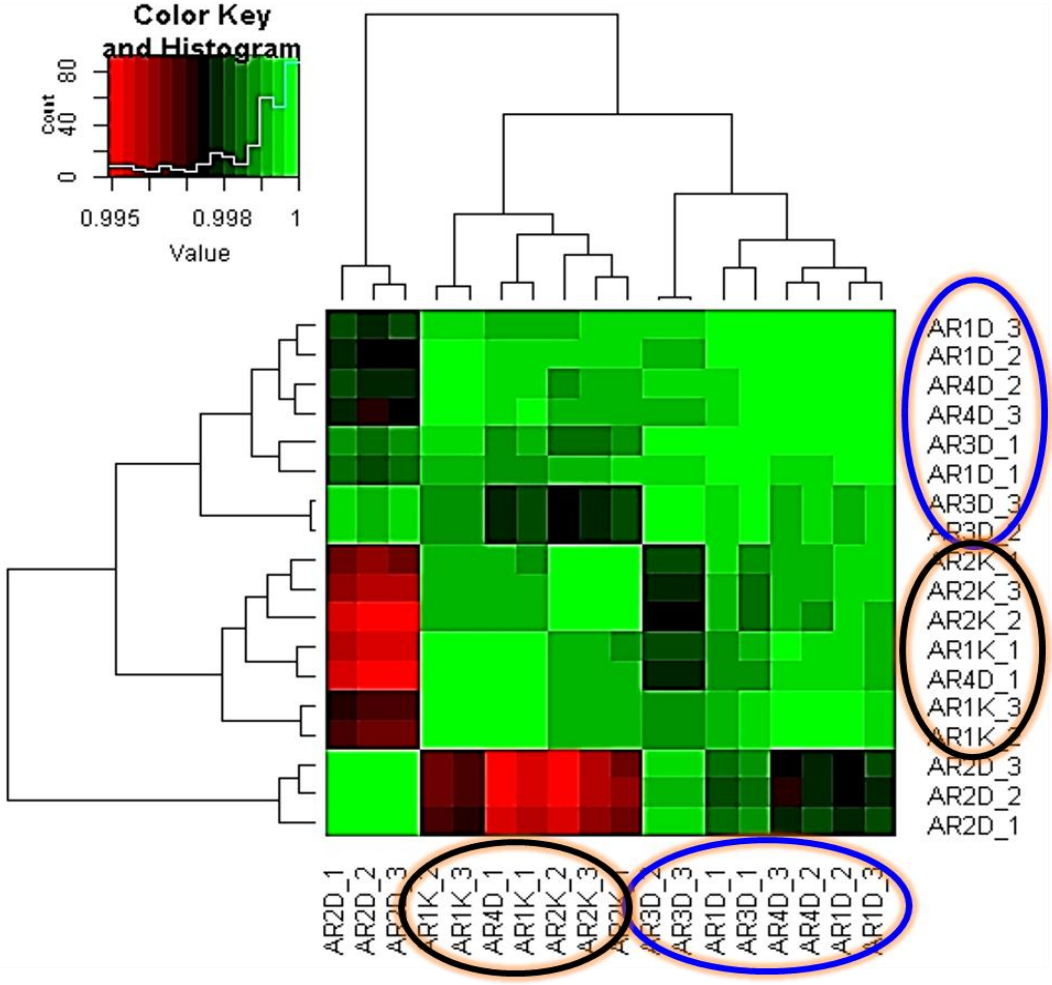

Figure 3. Heat-map of Pearson correlation analysis evaluating agreement and/or disagreement between analyzed peanut sample features by their biochemistry parameters.

predictor of humidity (Figure 4). Moreover, these two components (ash and humidity) contrast with considered peanut feature samples energy parameter (Figure 4). At this point, it could be interesting to investigate how biochemical composition of the present analyzed peanut varieties could influence their relative observed heterogeneity and/or similitude.

Parallel analysis evaluating right number of needed principal component examining peanut samples varieties

Parallel analysis in PCA survey is a useful method to establish the number of principal component needed in a multi-variant statistical analysis. In this analysis, a theoretical estimate variance is computed and compared to the observed and/or real variance. The output file of this analysis is a scree graph (Figure 5) in which both theoretic (gray) and observed (black) values have been reported and compared. For the present survey, only two components and/or biochemistry parameters (Components 1 and 2) exhibit their observed variance values higher than their respective theoretic variance (experimental data in black were compared with theoretic median value in gray). Generally, right component in a parallel PCA analysis must favor observed data with respect to theoretic data. Based on this observation and on Table 2 results, it was established that humidity, lipid, ash and energy variant parameters (4 components) appear to favor theoretic values displaying their estimated values under the threshold measure (Figure 5).

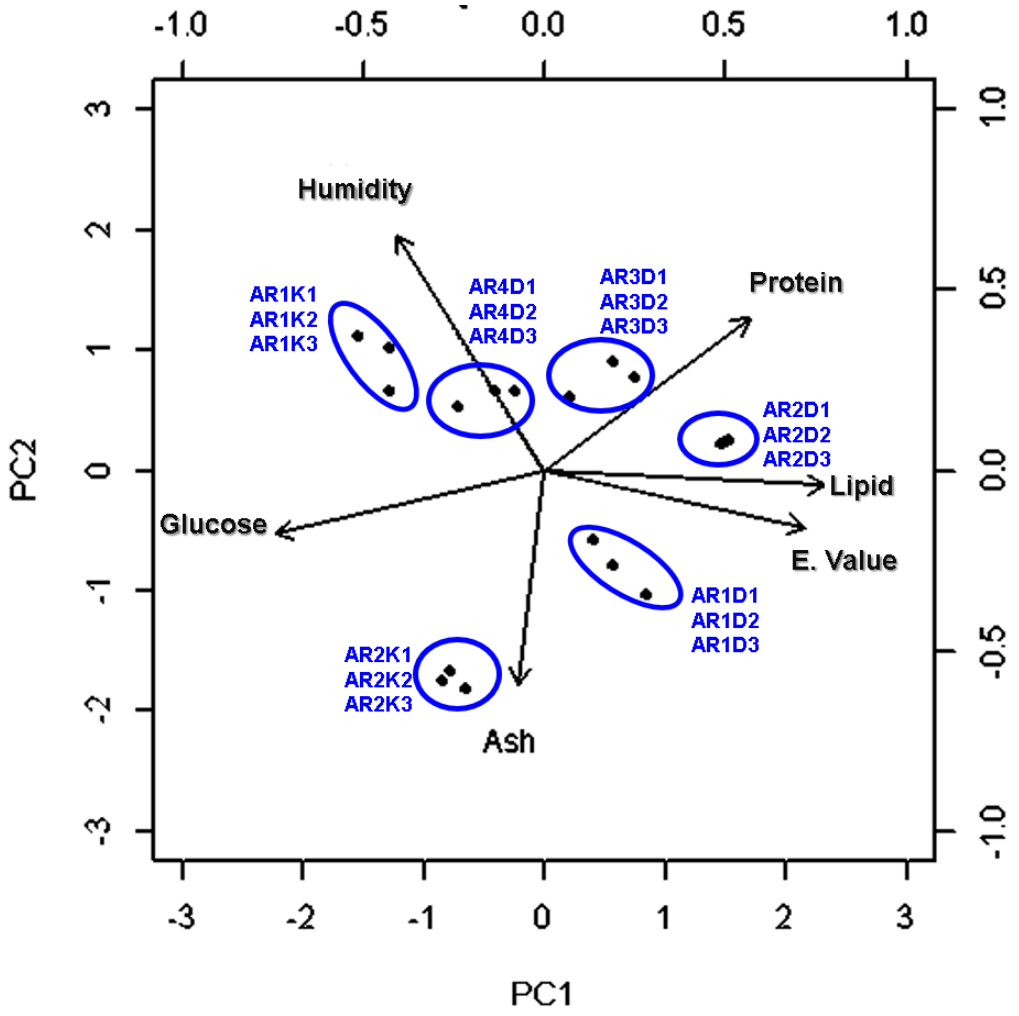

Figure 4. Peanut sample features clustering analysis by biochemistry parameters using a combination of both Biplot and principal component analysis (PCA).

In other words, these biochemistry parameters were not able to considerably assess the difference between the two detected peanut group varieties. Next based on the standard deviation parameter values, variance proportion was shown to be associated to previous mentioned components 1 and 2 are 0.95 and 0.034, respectively (p-value<0.0001). Then, the cumulative proportion of variance among these two components is estimated to 0.99 (p-value<0.0001). Then, both detected components 1 and 2 are statistically able to explain the differences observed between the 6 analyzed peanut varieties through their biochemistry parameters. Merging these results with those reported in Table 2, it can be suggested that both glucose and protein biochemistry parameters as components 1 (Comp 1) and 2 (Comp 2), respectively, were easily able to explain the difference observed between the two previous detected peanut varieties.

Principal component analysis network assessing the relationship between peanut samples biochemistry parameters by 2 components

The present network analysis evidenced a high concordance between lipid, protein and energy source parameters by the first component (bold green arrow associated with Comp 1 in Figure 6). In other words, lipid, protein and energy composts display a good concordance assessing the difference among analyzed peanut varieties (Figure 6). The same investigation showed that humidity, ash and glucose biochemistry

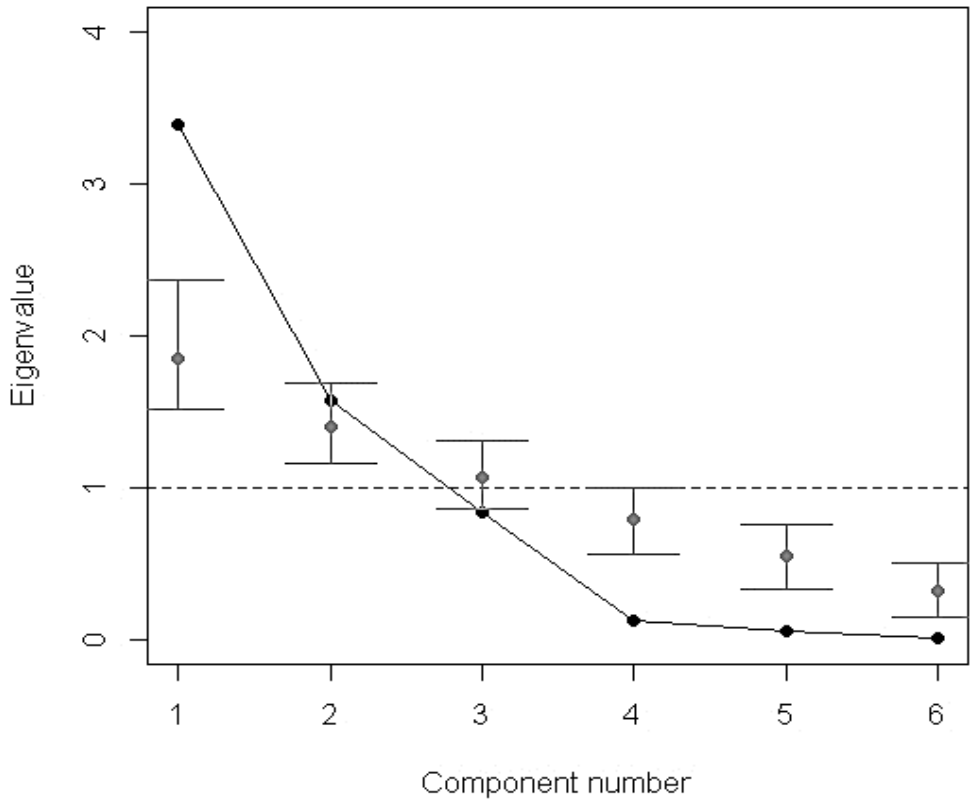

Figure 5. Parallel Principal Component Analysis (PCA) discriminating the number of statistical needed component for peanut sample features clustering analysis.

Table 2. Summary of peanut biochemistry parameters descriptive statistic estimating the difference and/or similitude between the 18 analyzed peanut sample features on the three experimental site.

Parameter	Humidity	Lipid	Protein	Ash	Glucose	Energy value
Mean row data	5.93	48.18	21.26	2.10	22.75	609.72
Standard deviation (Row Data)**	0.26	1.44	1.52	0.12	2.79	7.54
Mean normalized data (Logarithm Data Transformation)	0.77	1.68	1.33	0.32	1.35	2.79
Standard deviation (Normalized Data)**	0.02	0.01	0.03	0.02	0.06	0.00

**Statistical data normalization process is useful to prevent outlier in statistical analysis. Here data normalization procedure reduced data standard deviation coefficient. This process makes straightforward the relationship analysis between heterogeneous parameters.

parameters exhibited an opposite behavior with respect to lipid, protein and energy source parameters (bold red arrow against bold green arrow in association with Comp1 in Figure 6). These results suggested that differences observed between the two detected peanut varieties (AR_iD_j and AR_iK_j) could be explained in both (i) energy and/or lipid and/or protein and (ii) glucose and/or humidity and/or ash biochemical parameters categories. Moreover, based on the principal component 2 (comp 2), the present principal component network analysis tend to associate protein and humidity parameters (red arrow) suggesting a potential agreement between these two biochemistry elements assessing the comparison between the 6 peanut sample varieties. However, considering that the difference between the two discriminated peanut groups were explained by both

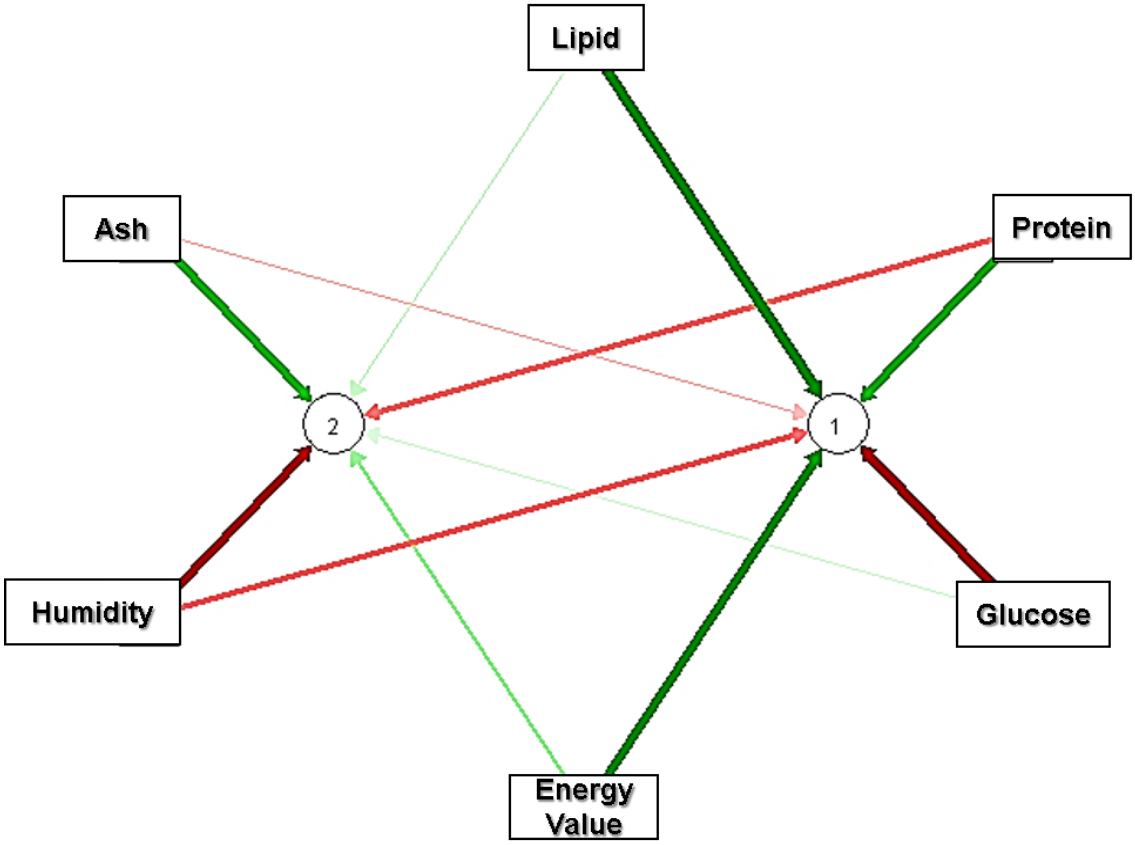

Figure 6. Biochemistry parameters principal component network analysis based on two component (Comp1 and Comp2) evaluating the relationship between detected peanut variety groups.

components 1 and 2, the present analysis supposed that ash component could reasonably act as a good biochemistry parameter explaining the difference between previously mentioned peanut groups as opposed to lipid, energy and humidity (Figure 6). In the same tendency, the present investigation suggested both peanut glucose and protein composition as valuable parameters highlighting the dissimilarities observed between the two discriminated peanut varieties (Figures 3 and 6). Considering as whole, glucose and protein parameters as well as peanut ash constituent should exhibit a significant statistical difference between the two detected peanut group varieties (AR_iD_j and AR_iK_j).

Statistical analysis assessing the difference between both AR_iD_j and AR_iK_j peanut varieties by biochemistry components

A statistical analysis was implemented based on both student and Fisher test assessing the difference between $ARiD_j$ (peanut form Dikodougou) and $ARiK_j$ (peanut from Korhogo) peanut varieties by all processed biochemistry parameters ((i) Glucose, (ii) Protein, (iii) Ash, (iv) Lipid, (v) energy variant, and (vi) humidity). As expected, both Fisher and student tests confirmed the significant difference in glucose and protein components (p-value associated to glucose tests reached from 0.01 to 0.0003, while those associated to protein fluctuated between 0.15 and 0.01) evaluating the variance between the two discriminated peanut varieties (Table 3). In the same tendency, ash component has been detected as significantly differentially modulated between the two analyzed peanut groups (p-value<0.05). The same investigation considering both lipid and energy parameters, because of their high link with protein, showed that both lipid and energy parameters were reasonably differentially modulated between the two analyzed categories of peanut groups (t test in Table 3; p-value <0.057). However, estimated variance between both AR_iD_j and AR_iK_j peanut ecotypes by the latter's (lipid and energy value) was not significant (p-value>0.05,

Table 3. Summary of statistical tests applied to the 6 analyzed peanut samples biochemistry parameters.

Parameter	Protein	Glucose	Lipid	Energy	Ash	Humidity
Mean value AR_iD_j	21.76	21.46	49	614	2	5.89
Mean value AR_iK_i	20.24	25.30	46.6	601.5	2.2	6.01
p-value	0.01***	0.0002***	7.517e-06***	0.002***	0.05***	0.45^{NS}
Fisher test (F) ratio of variance	3.85	13.61	2.72	0.52	0.23	0.33
p-value	0.10**	0.01***	0.27^{NS}	0.35^{NS}	0.04***	0.12**

^{NS} no statistical significance; **p-value<0.2 (statistical significance at 0.2) and ***p-value<0.1 (statistical significance at 0.1).

Table 3). These results could exclude both lipid and energy component as good indicator in the present peanut germplasm clustering analysis. However, the present analysis suggested ash component as a good parameter (as opposed to lipid and energy parameters) evaluating the difference between the two aforementioned peanut varieties groups. Better, ash biochemistry parameter displays a significant variability among both detected AR_iD_j and AR_iK_j peanut groups as opposed to peanut lipid and energy component (p-value<0.05; Table 3). The present result also established and confirmed both glucose and protein biochemistry parameters as top principal component evaluating AR_iD_j and AR_iK_j peanut samples differences. In addition, AR_iK_j peanut varieties were shown to record the highest performance producing glucose substance as opposed to AR_iD_j group, while the latter exhibits a significant high level of protein (Table 3). Considering as a whole, this analysis demonstrated that peanut variety collected in Dikodougou (AR_iD_j) favors glucose biochemistry compost biosynthesis, while those pooled in Korhogo (AR_iK_j) appears to good turn protein biosynthesis.

DISCUSSION

The genus *Arachis* is native to a region that includes Central Brazil and neighboring countries. Cultivated peanut is grown worldwide as rich-source of oil and protein. However, cultivated peanut exhibits a considerable amount of variability for various morphological, physiological, and agronomic traits. Little is known about both morphological and biochemical composition of peanut variety cultivated and recorded in Côte d'Ivoire since peanut cultures in this area of the world is not fully practiced. The understanding of both morphologic and genetic diversity of cultivated and wild species of peanut (*Arachis* spp.) is essential to develop strategies of collection, conservation and use of the germplasm in variety development. The identity of the ancestor progenitor species of cultivated peanut has also been of great interests (Moretzsohn et al., 2004). However, considering that in Côte d'Ivoire the bulk of peanut production is concentrated in the northern savannah regions, a peanut clustering analysis was realized in this geographical area aiming to develop the latter's local germplasm variety in prelude to future genetic gathering investigation. Indeed, for this investigation, six different peanut sample groups and/or variety with 3 replicate each were processed by both pre or post-harvest and biochemistry parameters since peanut present several important nutritional proprieties (Ensminger et al., 1986; Blomhoff et al., 2006; Talcott et al., 2005). Moreover, peanuts are an excellent source of biotin, copper, manganese, niacin, molybdenum, folate, vitamin E, phosphorus, vitamin B1, and protein as previously indicated (Nutritional Labeling of Food, 2010; USDA National Nutrient Database, 2010). Numerous studies showed health benefit of peanut preventing several cancers and diseases as well as their potential antioxidant effects (Blomhoff et al., 2006; Mathers, 2002; Awad et al., 2002; Alper et al., 2003). Considering as whole, it is evident that diligent initiatives directing to help quantitative and qualitative yield of peanut (*Arachis* spp.) as promoted by this study could represent a high nutritional and health preventing opportunity for worldwide populations in general and in particularity for the populations in the north of Côte d'Ivoire. Then, the present investigation focusing on phylogenic survey by Pearson correlation analysis suggested that morphologic approaches based on pre-harvest parameters were not adequate factors evaluating peanut varieties differences as opposed to post-harvesting parameters (Figure 2). In fact, phylogenic analysis based on post-harvesting parameters weighing the variability between the six considered peanut varieties, evidenced two tendencies (Figure 2; panel B). This analysis allowed to clustered whole analyzed peanut features (six peanut varieties replicated three time; 18 peanut sample features in total) in two distinct groups (AR_iD_j and AR_iK_j). Further, Person correlation analysis by heat-map graphic (Figure 3) considering peanut varieties, biochemistry composition

confirmed these two groups (p-value>0.05). These observations suggested that the two detected peanut varieties could assume selective and distinct physical and biochemical characteristics and compositions during their respective growing process. Moreover, evaluating the relationship between detected peanut groups and their biochemistry parameters, it was possible to show a good quality of the present collected data (Figures 1 and 4). In other words, the present investigation suggested low variance variability among considered peanut varieties replicate (low intra features data variability). The same analysis also showed a strong agreement between both peanut lipid and energy variant parameters, suggesting that energy or nutritive calories resource provided by the present analyzed peanut samples meanly arise from their lipid component (Figure 4). Indeed, this observation is in agreement with the aforementioned energy variant (EV) equation (materials and methods session). Further, both lipid and energy variant parameters in the present survey exhibited a low variance difference between the two detected peanut variety suggesting that investigated peanut categories yield the same amount of lipid and provide the same nutritive calorie value (p-value>0.05). Considering as whole, lipid and energy variant biochemistry factors resulted inadequate parameters discriminating the difference between the two detected peanut groups AR_iD_j and AR_iK_j. However, peanuts have been recognized as a protein source (Singh and Singh, 1991) since peanut butter became sought after at the time of Second World War when meat was not readily available. A one ounce serving, about a handful, is considered an excellent source of protein by the Food and Drug Administration (FDA). Peanuts are actually a legume and have more protein than any other nut with levels comparable to or better than a serving of beans (U.S. Department of Agriculture Research Service, 2009). The principal component analysis developed in the present study opposed both proteins and glucose biochemistry parameters measuring peanut varieties variability (Figure 4). This contrast has been used as pretext to estimate variance difference between both selected AR_iD_j and AR_iK_j peanut varieties (Figures 5 and 6). Indeed, it is noteworthy to observe that, in addition to protein and glucose component, ash biochemistry parameter revealed significant differential behaviors between the two detected peanut varieties groups (Figure 6 and Table 3). The present results are essential and useful to understand and develop strategies of peanut collection, conservation as well as to improve peanut germplasm variety expansion in the North region of Côte d'Ivoire. Moreover, our findings revealed that peanut ARiDj peanut variety appears to favor protein biosynthesis, while AR_iK_j peanut varieties record a consistent amount of glucose (p-value<0.05). Then, peanut collected in Dikodougou land (AR_iD_j) seems to promote protein biochemistry compost as opposed to those collected in Korhogo locality (p-value<0.05). These observations provided strong biochemical indicators (protein and glucose) in peanut varieties clustering analysis performed in the savanna region in North of Côte d'Ivoire. Taking together, these findings presumed that peanut capacity to synthetize both protein and glucose biochemical composts could depend and/or influenced by environmental and ecosystem components. This study also highlighted high variability inside peanut variety collected in Dikodougou (AR_iD_j) (Figures 2, 3 and 4) without mentioning the profound reasons of this changeability. However, it is believed that further analysis in increasing collected data size (that is, more experimental repetitions) as well as data integration by new parameters such as physical-chemical features considering that peanuts are an excellent source of numerous inorganic elements, could substantially improve the present peanut germplasm analysis quality. Also, based on the fact that the current analyzed data have been collected several years ago (three year ago), the development of a statistical stimulation platform combined with the integration of other measured parameters is trusted (physical-chemical parameters), to help in updating the present investigation results.

Conclusion

Nutritive importance of peanut has been fully demonstrated by previous studies. Peanut plant varieties clustering process appears to be a good start point understanding the importance of these plants since it could provide useful information regarding (i) pre, post-harvest behaviors and (ii) biochemical composition. The present survey, integrating both pre and post-harvest and biochemistry parameters, provided an excellent analysis model in peanut clustering analysis and allowed to discriminate two different peanut varieties in the present analyzed area. Finally, this study proposed both protein and glucose biochemistry components as well as ash compost as relevant and acceptable indicators selecting and clustering peanut sample features in the North region of Côte d'Ivoire.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors would like to thank Mr Ouattara Daoda,

Coulibaly Micheal Pefoundgodjomon and MChristGn for their practice assistance in preparing experimental sites.

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Vol. 11(15), pp. 1394-1397, 14 April, 2016
DOI: 10.5897/AJAR2013-7131
Article Number: 6A60CC058065
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Effect of scab disease on the yield of intercrop systems of cowpea maize and sorghum

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Received 28 March, 2013; Accepted 20 November, 2015

Three field sites were selected during the raining season (July to October) of 2004, 2005 and 2006 at the Institute for Agricultural Research, Ahmadu Bello University, in Samaru, Zaria, Nigeria to determine the effect of scab on the yield of cowpea variety (Ife brown) intercrop with cereals. This variety (Ife Brown) selected was highly susceptible to scab. Any reduction in yield is attributed to the disease since it affects all the above ground parts of the plant. Pod abortion was observed and pods were deformed and transformed into mummies. The yields obtained from the different cropping patterns were calculated using land equivalent ratio (LER) to compare the yield from the different cropping patterns with the yield of sole cowpea. Sole cropped cowpea had a higher yield than the other cropping patterns. Improved practices yielded more for both cowpea and cereals than farmers' practices. Strip cropping proved better for improved practices and inter-row for farmers' practices. Cowpea grain yield from farmers' practices were lower in quantity and of poor quality (being wrinkled) than yield from the other cropping patterns.

Key words: Scab, cowpea, intercrop, maize, sorghum.

INTRODUCTION

Cowpea, which is valued for its high nutritive quality, has a protein content of about 25% (Davis et al., 1991; Obuinya, 1997; Singh et al., 1995). The seeds can be boiled as fresh vegetable, canned or frozen, mashed and fried as cowpea cakes or tied on leaves and boiled.

Current estimate indicates that West Africa accounts for about 9.3 million hectares with annual production of 5 million tones. The major cowpea producing countries in the region are Nigeria with about 4.4 million hectares and 3 million tones production, followed by Niger with about 4.1 million hectares and produces 0.69 million tones (FAO, 2006). However, cowpea is also an important crop

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in Sudan, Somalia, Burkina Faso, Cameroon, Egypt, Benin Republic, Ghana, Kenya, Malawi, Mali, Senegal, South Africa and Togo (Singh et al., 1997).

Reduction in the yield of cowpea can be attributed to many factors amongst which are diseases, parasitic weeds, insect pests, drought and low soil fertility (Olufajo and Singh, 2002; Niringiye et al., 2005). Scab is one of the most destructive diseases of cowpea in the Guinea savanna belt of West and Central Africa including Burkina Faso, Nigeria and Cameroon (Emechebe and Shoyinka, 1985; Mungo et al., 1995). In eastern and southern Africa, the disease is recognized from Ethopia, Kenya, Uganda, Tanzania, Zambia (Iceduna, 1993; Edema et al., 1997; Edema and Adipala, 1996; Tumwegamire et al., 1998) and Rwanda (Price and Cishahayo, 1985). Despite its wide geographical distribution, it appears that the disease is ecologically restricted to semi-arid environments. In Nigeria, scab is seldom encountered outside a narrow latitudinal belt of about $10°30^{1}$-$12°30^{1}$N which corresponds approximately to the extent of the Guinea savanna. Cowpea scab is absent in the sub-humid forest, the northern Sudan savanna and the Sahel Zones of Nigeria. The disease affects all the different plant parts of the cowpea. Yield losses of 60-100% due to severe infections has been reported from Nigeria (Emechebe, 1980; Emechebe and Shoyinka, 1985; Mungo et al., 1995).

MATERIALS AND METHODS

Site selection

Three field sites were selected during the raining season (July to October) of 2004, 2005 and 2006 at the Institute for Agricultural Research, Ahmadu Bello University, in Samaru, Zaria, Nigeria. One variety of cowpea (Ife brown- highly susceptible to scab) and two varieties of cereals (maize, TZEEW-extra early and sorghum, ICSV111-extra early) were used for this study. The field layout was factorial concept in randomized complete block design (RBCD) with four replicates. Five cropping patterns (mixed cropping at different ratios for both farmers' and improved practices versus monocropping) and the cultivars grown were noted.

Each treatment plot consisted of eight, 75 cm ridges, 6 m long. The plots were separated by border rows consisting of one ridge along the length and 2 m along the width. Cowpea and maize were sown at two seeds per stand and sorghum at eight seeds per stand and were later thinned to two plants per stand two weeks after germination to give approximately the same population of plants/ha in the various plots. Plant spacing for inter-row mixed cropping of maize and sorghum was 0.5 m and cowpea was 25 cm. Plant spacing for intra-row mixed cropping of maize and sorghum was 1 m and cowpea was 25 cm in-between the maize and sorghum, respectively. Plant stand establishment was taken at 14 DAS and fertilizer was applied 14 DAS for both cowpea and cereals. A second dose of fertilizer was applied on the cereals two weeks after the first application. All plots were weeded thrice and cowpea plants were protected from insect damage by spraying biweekly with insecticide (Uppercott- Cypermethrin + Dimethoate at 1 L/ha) until 75% podding.

Data collection and analyses

Disease incidence and severity for scab and Septoria leaf spot were recorded at weekly intervals starting from the first appearance of the symptoms. Data for the different plant parts infected by scab and Septoria leaf spot were recorded from four middle ridges for each plot.

Produce from the different cropping systems were harvested, sun-dried, threshed and winnowed, and the weights were recorded. The yields of each of the crops in the different cropping patterns were calculated using land equivalent ratio (LER) to compare yield from sole cowpea with that from the different cropping pattern. The data obtained was subjected to analysis of variance (ANOVA) and mean separation was by Student Newman Keuls Test (SNK).

RESULTS

Cowpea variety Ife brown is highly susceptible to scab and a reduction in yield of this variety with respect to the different cropping pattern is attributed to the disease since it affects all the above ground parts of the variety. The yields obtained from the different cropping patterns were calculated using land equivalent ratio (LER) to compare the yield from the different cropping patterns with the yield of sole cowpea. In 2004 and 2005, the effect of cropping patterns on the yield of cowpea variety Ife brown for both cowpea: maize and cowpea: sorghum were statistically similar. As compared to sole cropped cowpea that had a higher pod and grain yield, improved practices (double-row and strip cropping) followed with a higher pod and grain yield than farmers' practices (inter-row and intra-row). Strip cropping proves better for improved practices and inter-row for farmers' practices. Intra-row for farmers' practices had the lowest pod and grain yield as compared to the other cropping patterns. Yield from farmers' practices were lower in quantity and of poor quality. In 2006, a significant ($P \leq 0.05$) different was observed on pod yield on the different cropping patterns for cowpea: maize with intra-row having a lower pod yield as compared to the other cropping patterns. Pod and grain yields from the other cropping patterns for cowpea: maize and cowpea: sorghum were statistically similar and the trend was the same as in 2004 and 2005 (Table 1).

The effect of cropping patterns on cowpea variety Ife brown in all the years combined showed significant ($P \leq 0.05$) differences for cowpea: maize and cowpea: sorghum with intra-row having the lowest pod and grain yields as compared to the other cropping patterns. The results gotten from the combined years followed the same trend with what was obtained in 2004, 2005 and 2006. The general range of increase in pod and grain yields for all the cropping patterns in all the years combined was in the order of sole>strip>double-row>inter-row>intra-row (Table 1). Reduction in yield in the different cropping patterns was due to scab infection, which resulted in pod abortion with some of the pods being deformed and transformed into mummies.

The effects of cropping pattern on grain yield of maize were not significantly different from each other in 2004, 2005 and 2006 but improved practices had a higher maize yield than farmers' practices. A significant ($P \leq 0.05$)

Table 1. Effect of cropping pattern on the yield of cowpea variety Ife brown in 2004, 2005, 2006 and combined.

Cropping patterns	Cowpea Pod yield (kg/ha) in:				Grains yield (kg/ha) in:			
	2004	2005	2006	Combined	2004	2005	2006	Combined
Cowpea: Maize								
Inter-row	1400.0^{cb}	722.2^{a}	1324.2^{a}	1148.8^{b}	979.2^{b}	527.8^{a}	1027.9^{a}	844.9^{ab}
Intra-row	1219.5^{cb}	666.7^{a}	541.7^{b}	809.3^{c}	862.5^{b}	507.0^{a}	421.3^{b}	596.9^{c}
Double-row	1418.1^{cb}	750.0^{a}	1231.6^{a}	1133.2^{b}	1080.6^{b}	562.5^{a}	935.6^{ab}	844.4^{ab}
Strip	1611.1^{cb}	854.2 ^{a}	1389.0^{a}	1284.8^{ab}	1187.5^{b}	590.3^{a}	1129.7^{a}	969.2^{ab}
Cowpea: Sorghum								
Inter-row	1500.0^{cb}	701.4^{a}	1222.3^{a}	1141.3^{b}	1137.5^{b}	541.7^{a}	963.0^{ab}	880.7^{ab}
Intra-row	1166.7^{c}	687.5^{a}	805.6^{ab}	886.6^{c}	805.6^{b}	513.9^{a}	629.7^{ab}	649.7^{c}
Double-row	1486.1^{cb}	729.2^{a}	1361.2^{a}	1192.1^{b}	1020.9^{b}	520.9^{a}	1074.2^{a}	812.5^{b}
Strip	1655.6^{b}	638.9^{a}	1370.5^{a}	1305.0^{ab}	1166.7^{b}	687.5^{a}	1045.7^{a}	966.6^{ab}
Sole cowpea	2095.9^{a}	1007.0^{a}	1157.5^{a}	1420.1^{a}	1479.2^{a}	743.1^{a}	898.2^{ab}	1040.2^{a}

Figures with the same letters within a column for the same cropping patterns are not significantly different at P≤0.05 (SNK Test).

Table 2. Effect of cropping pattern on the yield of cereals (maize and sorghum) in 2004, 2005, 2006 and combined.

Cropping patterns	Maize yield (kg/ha) in			
	2004	2005	2006	Combined
Inter-row	1426.4^{a}	2015.3^{a}	1370.5^{a}	1716.6^{a}
Intra-row	1211.9^{a}	1708.5^{a}	638.9^{a}	1186.4^{b}
Double-row	1916.7^{a}	2388.9^{a}	1037.1^{a}	1790.2^{a}
Strip	1944.5^{a}	2505.6^{a}	1222.3^{a}	1881.5^{a}
Sorghum yield (kg/ha)				
Inter-row	2048.7^{a}	2451.4^{a}	194.5^{a}	1564.8^{a}
Intra-row	774.4^{b}	1441.1^{a}	97.23^{b}	770.9^{b}
Double-row	1965.3^{a}	2257.0^{a}	129.6^{ab}	1450.6^{a}
Strip	2104.2^{a}	2555.6^{a}	213.0^{a}	1624.2^{a}

Figures with the same letters within a column for the same cropping patterns are not significantly different at P≤0.05 (SNK Test).

different was observed for sorghum yield in 2004 and 2006 with intra-row having a lower yield as compared to the other cropping patterns. Sorghum yield from the other cropping patterns were statistically similar but higher for double-row and strip cropping than for inter-row. In 2005, sorghum grain yield was not significantly different from each other. Generally, the grain yields obtained for maize and sorghum followed the same trend with strip cropping having a higher yield for improved practices and intra-row having the lowest yield for farmers' practices (Table 2).

Generally, cropping pattern showed a significant (P≤0.05) different for both maize and sorghum yields in all the years (combined) with intra-row having the lowest yield as compared to yield obtained from the other cropping patterns which were statistically similar. Maize and sorghum yields obtained from intra-row were lower in quantity but of good quality. The results obtained in the combined years followed the same trend with the results obtained in the individual years with strip cropping been better for improved practices and inter-row for farmers' practices. The general range of increase for maize was in the order of strip>double-row>inter-row>intra-row and for sorghum, the order was strip>inter-row>double-row>intra-row (Table 2).

DISCUSSION

Cropping patterns significantly affected the yield of Ife brown for both cowpea: maize and cowpea: sorghum with sole cowpea having a higher yield than the other cropping patterns. This report confirms those of Carsky and Vanlauwe (2002) that a greater cowpea yield was obtained from sole cropping than when cowpea was intercropped with maize. Study by Niringiye et al. (2005) and Atuahene-Amankwa et al. (2004) also confirms that sole-cropped bean yielded more than bean: maize intercrop. Similar results by Myaka et al. (2002) agreed that sole cowpea had a higher yield than cowpea intercropped with cotton. Considering the different cropping patterns, strip cropping had a higher yield for improved practices and inter-row for farmers' practices for both cowpea: maize and cowpea: sorghum. Intra-row had the lowest yield as compared to the other cropping patterns. A reduction in yield may be due to a favourable moisture regime that favoured disease development in cowpea variety Ife brown. Similar reports by Niringiye et al. (2005) suggested that a reduction in yield of bean intercrop with maize might be due to favourable moisture

present among maize plant population density that could lead to adverse effects. Ife brown showed marked differences in its reaction to scab. In this study, Ife brown was highly susceptible to scab. A reduction in yield of this variety may be attributed more to scab infection because the disease affects all the above plant parts of the crop. Similar reports by Alabi (1994) showed that Ife brown was highly susceptible to brown blotch. Grain yields obtained from intra-row intercrop were lower in quantity and of poor quality as compared to grain yield from inter-row, improved practices and sole cowpea. Some pods were deformed and transformed into mummies and also pod abortion was observed.

Cropping patterns also showed significant differences on the yield of maize and sorghum with improved practices having a higher grain yield than farmers' practices. Strip cropping had higher grain yield and intra-row had the lowest grain yield for all the cropping patterns. Reduction in yield may be due to drought stress and competition among the plant population density. This confirms similar reports by Niringiye et al. (2005) that plant population density and drought stress significantly affects the yield of maize intercrop. Grain yield from intra-row intercrop were of good quality than those from the other cropping patterns. This may be attributed to intra-row spacing of maize and sorghum seeds that were sown at 1 m apart as compared to the other cropping patterns where seeds were sown at 0.5 m apart

Conflict of Interests

The authors have not declared any conflict of interests.

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