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Full Length Research Paper

Effect of nitric oxide on some morphological and physiological parameters in maize exposed to waterlogging stress

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Out of 28 genotypes (inbred lines) of maize (*Zea mays* L.) one waterlogging resistant (HUZM-265) and a susceptible (HUZM-55) were identified on the basis of waterlogging tolerant coefficient (WTC) by imposing waterlogging stress during early growth phase. Selected genotypes were further grown in pots and after 20 days subjected to root zone waterlogging with or without 50, 500 and 2000 µmol L^{-1} sodium nitroprusside (SNP) as a donor of NO in the flooding water. Waterlogging caused reduction in leaf number, leaf area and dry weights of plants in both genotypes. Flooding root zone with 50 µmol L^{-1} SNP, alleviated the stress effects or sensitivity (not tolerance), but to a greater magnitude in susceptible genotype. Stomatal conductance, transpiration rate, chlorophyll decreased as the waterlogging duration increased. Nitrogen content in roots and shoot of waterlogged plants also declined significantly. 500 µmol L^{-1}SNP treatment tend to alleviate the deleterious effect of waterlogging. Cell membrane injury in roots of waterlogged plant was higher in genotype HUZM-55 than in HUZM-265 and 500 µmol L^{-1}SNP were found to have mitigating role in combating it. 500 µmol L^{-1}SNP was found effective for alleviating transpiration rate, chlorophyll content and nitrogen content in both genotypes while 50 and 2000 µmol L^{-1}SNP increased stomatal conductance in HUZM-265 and HUZM-55, respectively. It is concluded that SNP mitigates the deleterious effect of waterlogging in maize. However, the effective concentration varies for different parameters and the different genotypes.

Key words: *Zea mays* L., waterlogging, nitric oxide, sodium nitroprusside, waterlogging tolerance coefficient.

INTRODUCTION

Maize (*Zea mays* L.) is widely grown in temperate to tropical regions of the world. In 2013-14 worldwide production of maize was more than 960 million tons. Global production of maize has grown at a compound annual growth rate (CAGR) of 3.40% over the last ten years; from 717 million tons in 2004-05 to 967 million

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Abbreviations: CAGR, Compound annual growth rate; **c-PTIO,** 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoneline-1-oxyl-3-oxide; **NO,** nitric oxide; **PTIO,** 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; **ROS,** reactive oxygen species; **SNP,** sodium nitroprusside;

tons in 2013-14. The area under maize cultivation has grown at CAGR of 2.20% from 146 Mha in 2004-05 to 177 Mha in 2013-14. Productivity of maize has increased at CAGR of 1.20%, from 4.90 tons h^{-1} in 2004-05 to 5.50 tons h^{-1} in 2013-14 (Anonymous, 2014). Excess soil moisture stress caused by waterlogging or high water table or heavy soil texture, is one of the most serious constraints lowering the production and productivity of maize. The extent of damage depends upon the crop growth stage and on environmental conditions at the time of waterlogging. It has been seen that the submergence of maize roots for only one day may restrict the optimum production of the crop (Singh and Ghildyal, 1980). Waterlogging stress at an early vegetative stage is detrimental for the maize crop (Shah and Srivastava, 2007) and is one of the important abiotic stresses affecting plants growth and productivity. Waterlogging causes various consequences like leaf wilting, epinasty, chlorosis, stomatal closure, reduced photosynthesis and altered carbohydrate partitioning, reduced growth rate, disruption of cell membranes, adverse effects on mineral uptake, altered growth regulator relationships and altered respiration. In recent years, nitric oxide (NO) has gained considerable importance in abiotic stresses of plants. NO is a highly reactive, membrane-permeable free radical and a highly toxic compound. Research on NO in plants has gained attention mainly due to its function in plant growth and development and also as a key signaling molecule in different intracellular processes in plants. Nitric oxide plays a vital role in diverse physiological functions in plants like regulation of plant metabolism and senescence (Guo and Crawford, 2005), induction of cell death (Pedroso and Durzan, 2000), regulation of stomatal movement (Garcia Mata and Lamattina, 2001; Bright et al., 2006), photosynthesis regulation (Takahashi and Yamasaki, 2002) and mitochondria functionality (Zottini et al., 2002). It has been seen that high levels of NO have the capacity to damage membranes and cause DNA fragmentation (Romero et al., 2004). Reduced stomatal conductance is among the earliest response to waterlogging in maize followed by leaf yellowing, inhibition of root growth, alteration in root and shoots morphology, leaf senescence and brace root development from above ground parts. Yordanova and Popova (2001) reported that flooding of barley plants for 72 h led to noticeable decrease in photosynthesis, leaf chlorophyll, and protein contents. However, the effects of NO on different types of cells have been proved to be either protective or toxic, depending on the situation and concentration. The crucial signaling role of NO in plant responses to pathogens is well established. Still knowledge about its protective function in plants exposed to abiotic stress is rudimentary. Nevertheless, there is increasing evidence indicating the involvement of NO in alleviation of harmful effects of many abiotic stresses (Del Rio et al., 2004). Not much research has been done to find out the exact mechanism of NO action when exogenously supplied during various abiotic stresses, particularly under waterlogging stress. Under waterlogging, plants suffer from nitrogen deficiency. During flooding, nutrient uptake is greatly influenced. According to Manai et al. (2012) exogenous NO is involved in prevention of Na^+ accumulation, and the increase of K^+ concentrations, also NO influence Ca^{++} absorption and increase nitrate uptake. It was hypothesized that significant genotypic differences exist in physiological and morphological processes of maize inbred lines to waterlogging stress and nitric oxide has significant role in ameliorating deleterious effect of waterlogging in maize. Therefore; in present investigation maize genotypes were screened for their relative resistance/susceptibility to waterlogging stress at early growth phase and effect of different levels of SNP (a donor of NO) on morpho-physiological process was visualized taking relatively resistant and susceptible genotypes.

MATERIALS AND METHODS

Plant materials and treatments

Twenty eight genotypes (inbred lines) of maize, *viz.* HUZM-69, HUZM-175-1, HUZM-65-1, HUZM-63, HUZM-60, HUZM-59, HUZM-58, HUZM-55, HUZM-47, HUZM-36, HUZM-46, HUZM-85-1, HUZM-184, HUZM-81, HUZM-53, HUZM-80-1, HUZM-211-1, HUZM-148, HUZM-78, HUZM-242, HUZM-71, HUZM-147, HUZM-121, HUZM-107, HUZM-265, HUZM-97-1, HUZM-355 and HUZM-88 were sown in plastic pots containing 750 g sand. Five seeds were sown in each pot. After germination thinning was done to maintain three seedlings of uniform growth. After one week of growth each pot was supplied with 100 mL normal Hoagland's nutrient solution. Waterlogging stress was imposed 20 days after sowing (DAS) by placing half set of pots of each genotype in water filled plastic containers in such a way that pots were completely submerged and water level in the containers was maintained 4 to 5 cm above the sand surface in the pots. This water level was maintained daily by adding tap water in the morning and evening. This treatment is referred to as "waterlogged" of early seedling stage. Normal plants were maintained at optimal supply of soil moisture in pots. After 7 days of waterlogging, sampling for various morphological parameters *viz.* shoot length, root length, shoot dry weight, root dry weight and leaf area were done to calculate waterlogging tolerant coefficient (WTC) of each genotype Waterlogging tolerant coefficient (WTC) was determined using the formulae given by Liu et al. (2010) as:

WTC = Mean value of a parameter in waterlogged condition/mean value of the same parameter in normal condition

The WTC values were calculated by taking parameters *viz*. shoot length, root length, shoot dry weight (SDW), root dry weight (RDW) and leaf area. Shoot and root lengths were measured using scale and dry weights of samples were determined by oven drying at 105°C for one h and then at 65°C till constant weight. Leaf area was measured by leaf area meter (CI-202, CID Bioscience, U.S.A).

Out of 28 genotypes, 2 genotypes, one relatively most resistant and the other most sensitive to waterlogging stress, were identified and sown in plastic pots containing 750 g sand. 20 days after sowing plants were subjected to waterlogging stress by putting pots in plastic containers containing water or 50, 500 and 2000 µmol L^{-1}

SNP (as a donor of NO) solution. Water/SNP solutions in containers were maintained 4 to 5 cm above the sand surface in the pots. Normal plants were maintained at optimum supply of soil moisture in pots. Various parameters were studied after 0, 3 and 7 days of imposing stress in normal and stressed plants.

Morphological parameters

These parameters were measured after 7 days of imposing stress. Plants were harvested with roots intact, washed carefully and roots and shoots were separated. Leaves, (green and dead) were counted manually. Area of green leaves was determined by leaf area meter (CI-202, CID Bioscience, USA). Dry weights of roots and shoots were taken after oven drying.

Cell membrane injury

Cell membrane injury of terminal portion of roots was done at 0, 3 and 7 days after imposing stress by the method described by Zhu et al. (2000). Freshly sampled 100 mg plant material (roots) was taken. It was washed thoroughly with glass distilled water then placed in test tubes containing 10 ml of double distilled water. These were divided in two sets. One set of test tubes were incubated at 40°C in a water bath. After 30 min of incubation test tubes were brought to room temperature and electrical conductivity of the solution (C_1) was recorded with the help of conductivity meter (Systronics model 304). Another set was boiled at 100°C for 10 min and its conductivity was also measured (C_2). Membrane injury was calculated as %:

$$\% \text{ membrane injury} = [1-(1-C_1/C_2)] \times 100$$

Stomatal parameters

Stomatal parameters including transpiration rate (E) and stomatal conductance (gs) were recorded at 0, 3 and 7 days after imposing waterlogging stress on first fully expanded leaf from top by infra-red gas analyzer; IRGA (ADC Biosynthetic Ltd.). Observations were made between 10 to 12 h. Principle and methodology involved in the operation of IRGA is elaborated by Bansal and Srivastava (2015).

Chlorophyll content

Changes in chlorophyll content in leaves of both genotypes were measured at 0, 3 and 7 days after imposing waterlogging stress with the help of SPAD meter (Minolta). The instrument directly measures chlorophyll content in intact leaves with expression unit as SPAD units. First fully expanded leaf from top was tagged initially and observations were recorded on this leaf only till the end. Amount of chlorophyll was expressed in terms of SPAD units.

Nitrogen content

Total nitrogen content in root and shoot was determined at 0, 3 and 7 days after imposing waterlogging stress by Semi-automatic Nitrogen Analyzer (Pelicon, Model, KEL 20L) adopting Kjeldahl method.

Sample digestion

Plant sample (100 mg) was taken in a Kjeldahl digestion tube containing 3 g of catalyst mixture (1:5 ratio of $CuSO_4$ and K_2SO_4) and 10 ml concentrated sulfuric acid. Tubes were put in the digestion block, fitted with manifolds and scrubber. The temperature was gradually raised to 350°C. The digestion continued till the solution became colorless. After completion, samples were brought to room temperature.

Distillation

Distillation of digested samples was done by auto distillation system (Pelicon Distil EM). Kjeldahl tubes containing digested plant samples were fitted in the assembly. Sufficient amount (20 to 30 ml) 40 % NaOH was added till the colour of the solution becomes brown. At the collection end a conical flask containing 24 ml 4% boric acid and 0.5 ml mixed indicator (0.3 g bromocresol green and 0.2 g methyl red dissolved in 400 ml of 90 % ethanol) was put. The sample was allowed to steam distilled for 9 min.

Titration procedure

The boric acid solution of the conical flask was titrated by 0.1 N HCl with the help of micro titration unit. At the end point light brown colour appeared. The amount of nitrogen in the sample was calculated as:

$$\text{N (mg g}^{-1}\text{ dry weight)} = \frac{14 \times \text{Titrant value} \times \text{normality of acid} \times 100}{\text{Sample weight (g)} \times 1000}$$

Statistical analysis

All data were taken in triplicates. For comparing within genotypes for WTC, least significant differences (LSD) were calculated at probability level ≤ 0.05 of significance by SAS software using Duncan's multiple range test (DMRT). To draw the statistically valid and significant conclusions, the data obtained by various other observations were analyzed statistically by adopting method of "Analysis of Variance" for completely randomized design factorial. Critical differences were calculated at 1% level of significance in order to compare treatment means as described by Gomez and Gomez (1984).

RESULTS

Screening of genotypes

In this experiment we attempted to identify relatively resistant and susceptible genotypes of maize to waterlogging stress at early stage of growth on the basis of WTC. The WTC was calculated on the basis of per plant shoot dry weight (SDW), root dry weight (RDW), shoot length, root length and leaf area (Table 1). High value of WTC indicates relatively resistant and low value relatively susceptible natures of genotypes to waterlogging stress. When WTC was calculated on the basis of shoot dry weight, it was the maximum for HUZM-265 (3.2503) and the minimum for HUZM-55 (0.2393) (Table 1). Different genotypes followed a similar trend when WTC was calculated on the basis of leaf area plant^{-1}. Nevertheless, WTC in studied genotypes followed

Table 1. Screening of 28 genotypes of maize under waterlogged condition on the basis of waterlogging tolerant coefficient (WTC) for different parameters.

S/N	Genotype	WTC for parameters				
		Shoot dry weight (g)	Root dry weight (g)	Shoot length (cm)	Root length (cm)	Leaf area (cm^2)
1	HUZM-55	0.2393^{d}	0.5592^{ecd}	0.8057^{c}	0.5873^{h}	0.5635^{f}
2	HUZM-175-1	0.5803^{dc}	0.3269^{e}	0.8273^{c}	0.6881^{edfhg}	1.3233^{efcd}
3	HUZM-65-1	1.4399^{bdc}	0.9950^{ecd}	1.1073^{bac}	0.7058^{edfhcg}	0.9311^{efcd}
4	HUZM-63	0.4702^{d}	0.7783^{ecd}	$0.8690^{\mathbf{c}}$	$0.7389^{ebdfhcg}$	1.0661^{efcd}
5	HUZM-60	0.5317^{dc}	0.4272^{e}	$0.9063^{\mathbf{bc}}$	0.5503^{h}	2.1059^{bc}
6	HUZM-59	0.6400^{dc}	0.6604^{ecd}	$0.9170^{\mathbf{bc}}$	0.9499^{ebdfc}	1.6306^{efcd}
7	HUZM-58	0.5897^{dc}	0.4404^{e}	$0.9927^{\mathbf{bc}}$	$0.7792^{ebdfhcg}$	0.9167^{efcd}
8	HUZM-69	0.5375^{dc}	0.7428^{ecd}	0.9117^{bc}	0.6447^{efgh}	0.7540^{f}
9	HUZM-47	0.6115^{dc}	0.6216^{ecd}	$0.8690^{\mathbf{c}}$	0.8974^{ebdfcg}	0.8128^{ef}
10	HUZM-36	0.5843^{dc}	0.6042^{ecd}	1.0553^{bac}	$0.8032^{ebdfhcg}$	1.3265^{efcd}
11	HUZM-46	0.8502^{dc}	0.5670^{ecd}	0.9983^{bc}	0.5860^{h}	0.9960^{efcd}
12	HUZM-85-1	0.5904^{dc}	0.8542^{ecd}	0.9147^{bc}	$0.8354^{ebdfhcg}$	0.8940^{efd}
13	HUZM-184	0.7236^{dc}	1.4073^{bcd}	0.8517^{c}	$0.8726^{ebdfhcg}$	0.9928^{efcd}
14	HUZM-81	0.6015^{dc}	0.4273^{e}	0.9753^{bc}	0.6954^{edfhcg}	2.0710^{bcd}
15	HUZM 53	0.8009^{dc}	0.8760^{ecd}	0.9780^{bc}	$0.8858^{ebdfhcg}$	2.0517^{bcd}
16	HUZM-80-1	2.6910^{ba}	1.4377^{bc}	1.1650^{bac}	0.6336^{fhg}	1.3583^{efcd}
17	HUZM-211-1	0.8643^{dc}	0.6722^{ecd}	1.0500^{bac}	$0.7708^{ebdfhcg}$	0.8175^{ef}
18	HUZM-148	1.3400^{bdc}	0.9138^{ecd}	0.9763^{bc}	1.0055^{bdac}	1.1753^{efcd}
19	HUZM-78	2.1670^{bac}	0.7682^{ecd}	1.0023^{bc}	1.0680^{ba}	1.4080^{efcd}
20	HUZM-242	0.8439^{dc}	0.4658^{ecd}	0.9350^{bc}	0.9820^{ebdac}	0.7892^{ef}
21	HUZM-71	1.0033^{dc}	0.6717^{ed}	1.0683^{bac}	$0.8457^{ebdfhcg}$	3.6472^{a}
22	HUZM-147	0.5009^{d}	0.4840^{ecd}	0.8823^{c}	$0.7901^{ebdfhcg}$	0.9161^{efcd}
23	HUZM-121	0.6143^{dc}	0.6635^{ecd}	0.8360^{c}	1.2825^{a}	0.7528^{ef}
24	HUZM-107	0.8463^{dc}	0.9437^{ecd}	0.9923^{bc}	0.9285^{ebdfcg}	0.8666^{efd}
25	HUZM-88	0.9207^{dc}	1.4419^{bc}	1.1500^{bac}	1.0347^{bac}	0.6392^{f}
26	HUZM-97-1	1.4623^{bdc}	2.4715^{a}	1.4783^{a}	$0.8044^{ebdfhcg}$	1.8748^{ecd}
27	HUZM-355	0.7062^{dc}	0.4020^{e}	0.7740^{c}	$0.7318^{ebdfhcg}$	0.8706^{efd}
28	HUZM-265	3.2503^{a}	1.9172^{ba}	1.3887^{ab}	$0.8439^{ebdfhcg}$	3.0290^{ba}

WTC=Waterlogging tolerant coefficient; Means followed by same letters in a column are not significantly different but different letters are significantly different (P≤ 0.05) using Duncan's multiple range test (DMRT).

variable trends when calculated on the basis of RDW, shoot length and root length, but values were always relatively higher for HUZM-265 and lower for HUZM-55.

Morphological parameters

As compared to normal plants, number of green leaves per plant in waterlogged plants decreased and number of dead leaves per plant increased, however; genotypic differences were not significant (Table 2).

Also leaf area per plant declined under waterlogged condition in both genotypes. Reduction % under waterlogged condition over normal was more in HUZM-265. SNP at 50 µmol L^{-1} tend to ameliorate the deleterious effects of waterlogging stress on leaf area; while 500 and 2000 µmol L^{-1} SNP concentrations appeared to be deleterious for both genotypes. Under waterlogged condition root and shoot dry weights per plant also declined, however; SNP at 50 µmol L^{-1} caused a marginal increase in above parameters in both genotypes as compared to other two concentrations of SNP.

It was evident that waterlogging induced membrane injury of root cells (Figure 1). Susceptible genotype HUZM-55, registered more cell injury than the resistant one and 500 µmol L^{-1} concentration of SNP was effective in ameliorating this damage. Concentration of SNP, effective in ameliorating harmful effects of waterlogging on cell membrane integrity varied in both genotypes; HUZM-265 and HUZM-55 (Figure 1).

Table 2. Different morphological parameters in maize genotypes under normal and waterlogging stress and different levels of sodium nitroprusside.

Treatment	Total leaves (plant^{-1})		Green leaves (plant $^{-1}$)		Dead leaves (plant^{-1})		Leaf area (cm^2)		Shoot dry weight (g)		Root dry weight (g)	
	HUZM-265	HUZM-55	HUZM-265	HUZM-55	HUZM-265	HUZM-55	HUZM-265	HUZM-55	HUZM-265	HUZM-55	HUZM-265	HUZM-55
N	4.00	4.00	2.66	2.66	1.00	1.00	41.52	32.17	0.25	0.11	0.14	0.11
W	3.66	4.00	2.33	2.33	2.33	2.00	37.58	31.76	0.20	0.10	0.09	0.11
W1	4.00	4.00	3.00	2.00	1.00	2.00	34.25	26.44	0.22	0.22	0.14	0.12
W2	3.66	4.00	2.00	1.66	1.66	2.33	19.22	18.24	0.20	0.10	0.06	0.07
W3	4.00	4.00	2.00	2.00	2.00	2.00	18.90	13.50	0.25	0.19	0.11	0.08
Mean	3.86	4.00	2.46	2.06	1.40	1.86	30.29	24.42	0.22	0.14	0.11	0.10
Annova	**SEm±**	**LSD≤ 0.01**	**SEm±**	**LSD≤ 0.01**	**SEm±**	**LSD≤ 0.01**	**SEm±**	**LSD≤ 0.01**	**SEm±**	**LSD≤ 0.01**	**SEm±**	**LSD ≤ 0.01**
G	0.05	NS	0.09	0.35	0.08	0.33	0.28	1.14	0.01	0.03	0.01	NS
T	0.07	NS	0.14	0.56	0.13	0.52	0.45	1.80	0.01	0.04	0.01	0.04
G×T	0.06	NS	0.11	NS	0.11	NS	0.36	1.47	0.01	0.04	0.01	NS

Plants were grown under normal condition in plastic pots. After 20 days of sowing waterlogging stress was imposed. Different parameters were analyzed after 7 days of imposing waterlogging stress. #N=normal, W=waterlogged,W_1=waterlogged with 50µm L^{-1} SNP,W_2=waterlogged with 500µm L^{-1} SNP,W_3=waterlogged with 2000µm L^{-1} SNP.

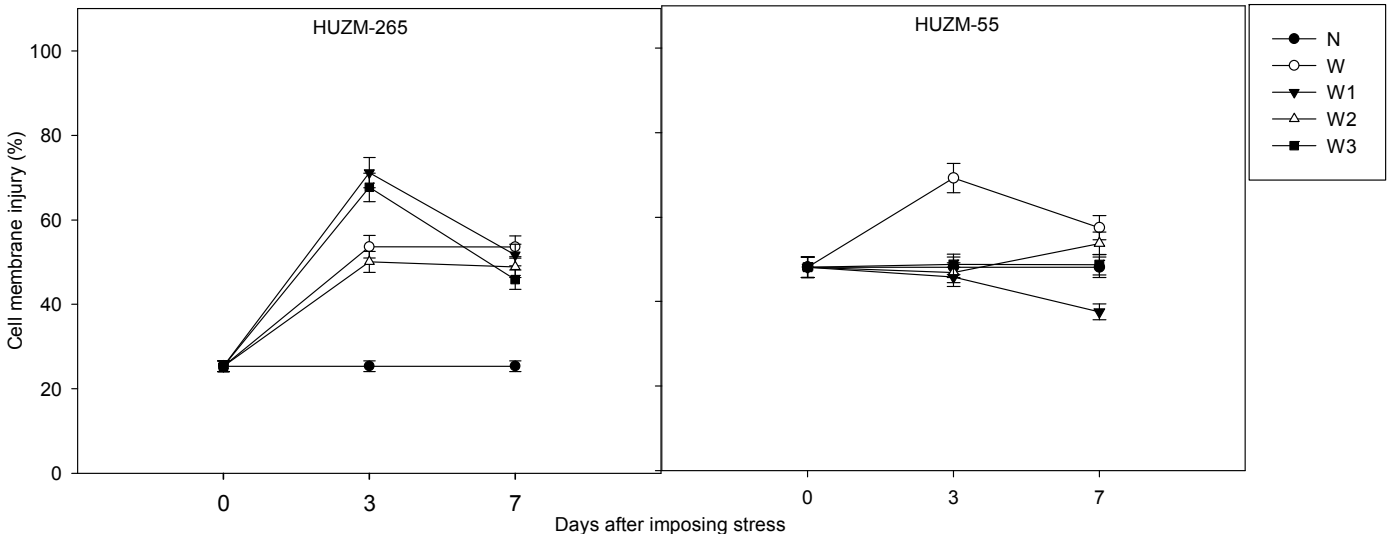

Figure 1. Changes in cell membrane injury (%) in roots of maize genotype HUZM-265 (relatively waterlogging resistant) and HUZM-55 (relatively waterlogging susceptible) under waterlogging stress with different concentrations of sodium nitroprusside (SNP). Values are means of three replicates ±SE. N=Normal, W= waterlogged, W1= waterlogged with 50 µmol L^{-1} SNP, W2= waterlogged with 500 µmol L^{-1}, SNP, W3= waterlogged with 2000 µmol L^{-1} SNP

Physiological parameters

Waterlogging caused a marked decline in stomatal conductance (gs), transpiration rate (E) and chlorophyll content in maize crop under this study. In both genotypes, exposure of waterlogged plants to SNP treatment ameliorated the deleterious effects of waterlogging at a concentration of 50 µmol L^{-1} for stomatal conductance in resistant genotype and 2000 µmol L^{-1} in susceptible one (Figure 2). Transpiration rate and chlorophyll content was found higher at SNP concentration of 500 µmol L^{-1} (Figures 3 and 4). Here, waterlogging resistant genotype HUZM-265 responded more to SNP than the susceptible genotype HUZM-55. Reduction in stomatal conductance (gs) was more in susceptible genotype than in resistant one.

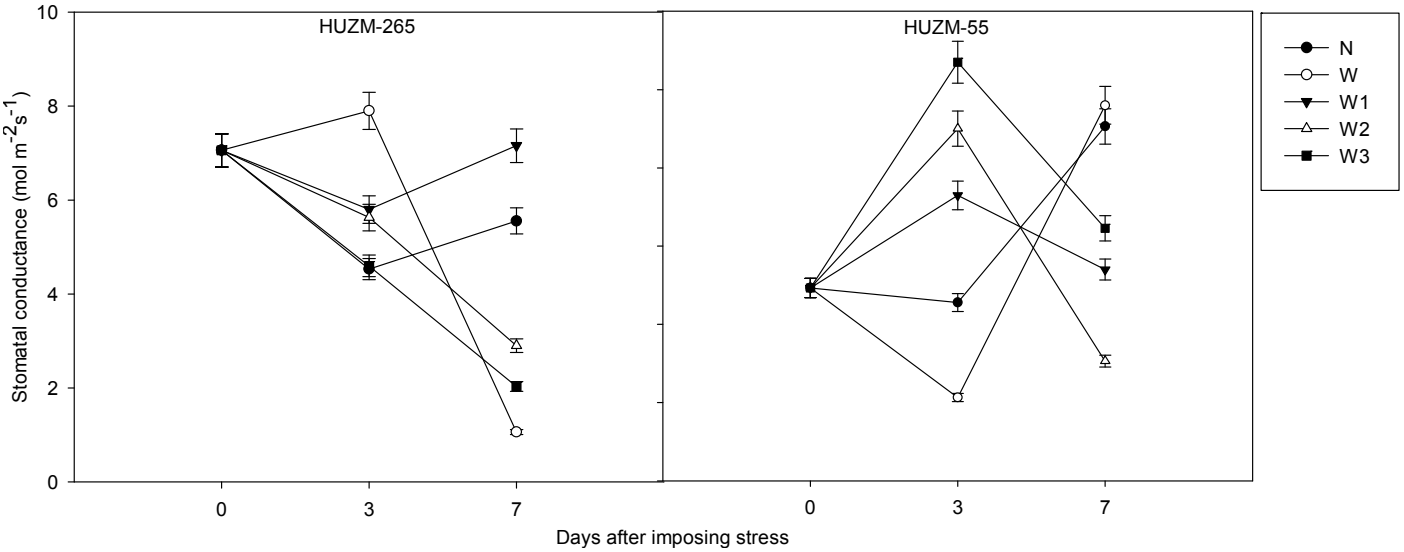

Figure 2. Changes in stomatal conductance (mol $m^{-2}s^{-1}$) in leaves of maize genotype HUZM-265 (relatively waterlogging resistant) and HUZM-55 (relatively waterlogging susceptible) under waterlogging stress with different concentrations of sodium nitroprusside (SNP). Values are means of three replicates ±SE. N=Normal, W= waterlogged, W1= waterlogged with 50 µmol L^{-1} SNP, W2= waterlogged with 500 µmol L^{-1}, SNP, W3= waterlogged with 2000 µmol L^{-1} SNP

Nitrogen content of roots and shoots decreased under waterlogging stress and the reduction was more in susceptible genotype than in the resistant. This level further declined with advancement in waterlogging duration, that is, at 7th day of stress (Figure 5). SNP treatments at 500 µmol L^{-1} ameliorated the deleterious effects of waterlogging on root nitrogen in both genotypes (Figure 5).

DISCUSSION

Screening of genotypes

Cornelious et al. (2005) reported waterlogging injury scores for the identification of quantitative trait loci (QTLs) underlying waterlogging tolerance in soybean. Qiu et al. (2007) performed QTL mapping associated with waterlogging tolerance during seedling stage in maize using traits of root length, root dry weight, plant height, shoot dry weight and total dry weight. Similar work has been carried out in wheat by Yu and Chen (2013). It is advocated that determination of WTC is a suitable parameter to identify waterlogging resistant and susceptible genotypes of maize (Liu et al., 2010). High values of WTC are associated with relatively more waterlogging resistance. Though WTC was calculated on the basis of per plant shoot dry weight, root dry weight, shoot length, root length and leaf area, but as in most crop species, plant dry matter is directly linked with their vigour and yield, therefore; WTC on the basis of shoot dry weight was chosen as the basis for identification of relatively resistant and susceptible genotypes of maize. Among studied genotypes, therefore; HUZM-265 was identified as relatively resistant (as it exhibited highest WTC value) and HUZM-55 as relatively susceptible (as it registered the lowest WTC value) to waterlogging stress (Table 1). Further experiments were carried out by taking these two genotypes.

Morphological parameters

Data indicated that waterlogging induced senescence of existing leaves more than the appearance of new leaves as in waterlogged plants leaf number per plant decreased lesser than leaf area per plant (Table 2). Such results are also reported by Yordanova and Popova (2001). SNP at 50 µmol L^{-1} tend to ameliorate the deleterious effects of waterlogging stress on leaf area; while 500 and 2000 µmol L^{-1} SNP concentrations appeared to be deleterious for both genotypes. Under waterlogged condition root and shoot dry weights per plant also declined (Table 2), however; SNP at 50 µmol L^{-1} caused a marginal increase in these parameters in both genotypes. Thus, it was evident that treatment of waterlogged maize plants with 50 µmol L^{-1} SNP ameliorated the harmful effects of waterlogging stress, but higher concentrations were detrimental to growth in maize. As the genotypic differences with respect to SNP levels were not significant, therefore; it was considered that 50 µmol L^{-1} SNP as NO donor could be used for ameliorating harmful effects of waterlogging in maize. Similar observations have been reported by Wang et al. (2011) in maize. Fan et al. (2014) also reported that spraying with 100 µM SNP markedly improved the plant height, fresh and dry

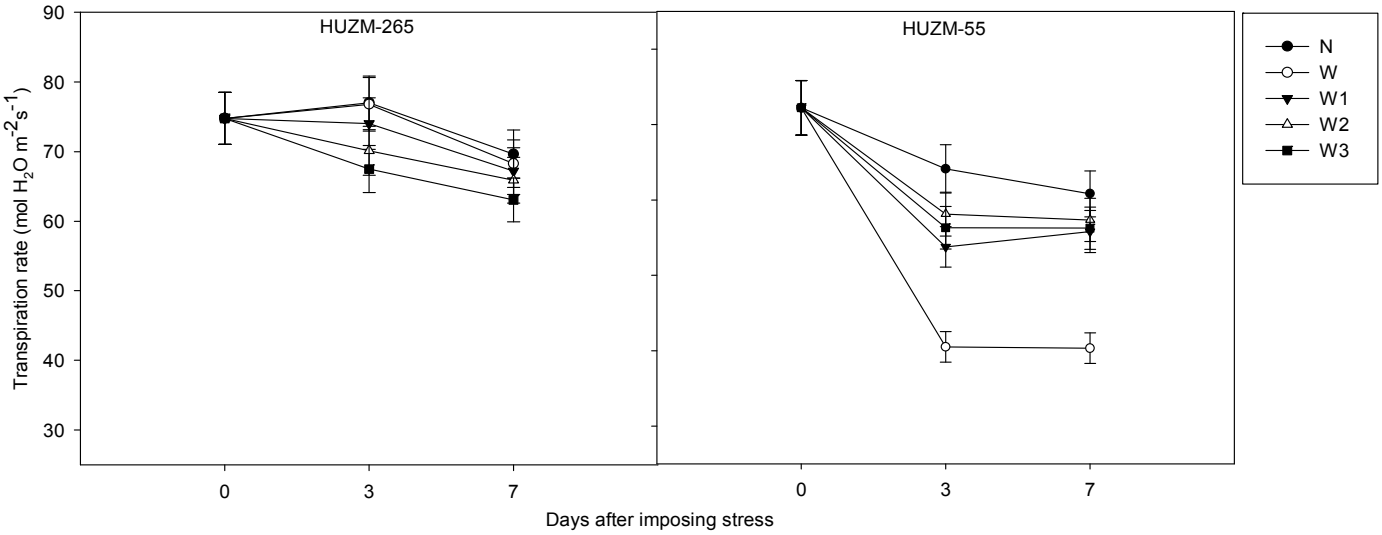

Figure 3. Changes in transpiration rate (mol $H_2Om^{-2}s^{-1}$) in leaves of maize genotype HUZM-265 (relatively waterlogging resistant) and HUZM-55 (relatively waterlogging susceptible) under waterlogging stress with different concentrations of sodium nitroprusside (SNP). Values are means of three replicates ±SE. N=Normal, W= waterlogged, W1= waterlogged with 50 µmol L^{-1} SNP, W2= waterlogged with 500 µmol L^{-1}, SNP, W3= waterlogged with 2000 µmol L^{-1} SNP

weights in cucumber seedlings exposed to waterlogging stress.

Physiological parameters

In this investigation it was evident that waterlogging induced membrane injury of root cells (Figure 1) in maize. Severity of injury increased with increased stress duration. Magnitude of NO-induced reduction of damage varied in resistant and susceptible genotypes, susceptible genotype being more protected. Therefore, genotypic differences in membrane damage under stress were found to be associated with their relative resistance and susceptibility to waterlogging. These results indicated that perhaps waterlogging susceptible maize genotypes require relatively higher levels of SNP to overcome deleterious effects of waterlogging as for as cell membrane integrity was concerned. These results are in accordance with those of Wang et al. (2011) who observed that cell membrane injury in both SNP treated and non-SNP treated maize plants under waterlogging increased rapidly to higher levels.

In this investigation we observed decline in stomatal conductance and transpiration rate in both genotypes under waterlogged condition (Figures 2 and 3). Similar observations were made by Baranwal and Singh (2002) in maize and Bansal and Srivastava (2015) in pigeonpea under waterlogging stress. Treatment of waterlogged plants to SNP ameliorated the deleterious effects of waterlogging. Effective SNP concentration in rehabilitation of stomatal conductance was 50 µmol L^{-1} in resistant genotype and 2000 µmol L^{-1} in susceptible genotype (Figure 2). Transpiration rate was higher at SNP concentration of 500 µmol L^{-1} (Figure 3). Waterlogging resistant genotype HUZM-265 responded more to SNP than the susceptible genotype HUZM-55. It has been proposed that waterlogging results in reduced water absorption by plants leading to derangement in plant-water relation parameters, closure of stomata and reduction in transpiration rate (Bansal and Srivastava, 2015). Contrary, Garcia-Mata and Lamattina (2001) showed that exogenous NO reduced transpiration and induced stomatal closure in several species such as *Vicia faba*, *Salpichroa* and *Tradescantia* species. Many different NO donors induce stomatal closure in a dose and time dependent manner and their effects was reversed by simultaneous co-incubation with the NO scavengers; 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO) or 2-(4-carboxyphenyl)- 4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) (Bright et al., 2006). Chlorophyll content also decreased in waterlogged plants of both genotypes (Figure 4). Such results are reported by other workers like Rai et al. (2004). SNP treatment of waterlogged plants increased leaf chlorophyll content. Here, waterlogging resistant genotype HUZM-265 responded more to SNP than the susceptible genotype HUZM-55. Decreased chlorophyll content per unit fresh weight of leaf as well as reduction in leaf area per plant are the proposed to be the major causes for reduction in plant dry weight under waterlogged condition. Nevertheless, reduction in bio-chemical processes associated with photosynthesis is also decreased under waterlogging stress in plants. Takahashi and Yamasaki (2002) showed that SNP did not modify the maximal quantum efficiency of PSII, but inhibited the photosynthetic linear electron transfer rate, Δ pH formation across the thylakoid membrane and

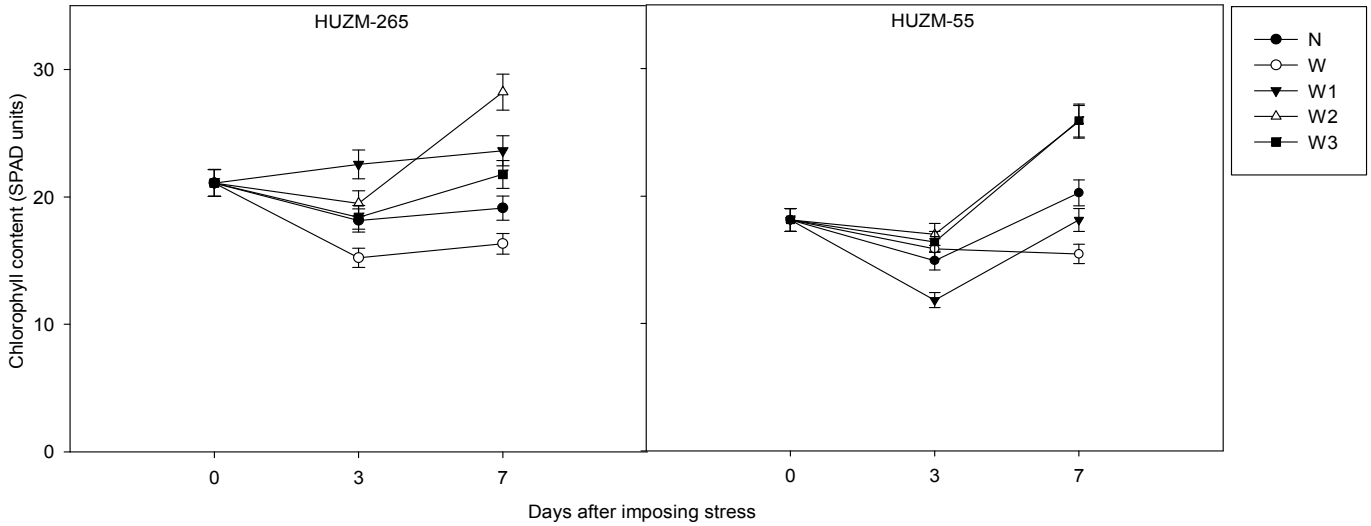

Figure 4. Changes in chlorophyll content (SPAD units) in leaves of maize genotype HUZM-265 (relatively waterlogging resistant) and HUZM-55 (relatively waterlogging susceptible) under waterlogging stress with different concentrations of sodium nitroprusside (SNP). Values are means of three replicates ±SE. N=Normal, W= waterlogged, W1= waterlogged with 50 µmol L^{-1} SNP, W2= waterlogged with 500 µmol L^{-1}, SNP, W3= waterlogged with 2000 µmol L^{-1} SNP.

decreased the rate of ATP synthesis. A moderate decrease in Fv/Fm was observed by SNP treatment in pea leaves (Wodala et al., 2005) and one possible reason for the observed changes in the rates of photosynthesis and transpiration was attributed due to the effect of NO on altered stomatal behavior. Many plant studies have used the NO donor SNP that generates cyanide. However, neither cyanide itself nor light-inactivated SNP induced stomatal closure (Takahashi and Yamasaki, 2002), the effects mediated by NO donors SNP are indeed due to the release and biological activity of NO).

Under waterlogged condition, soil redox potential decreases, which results in increase or decrease in availability of essential mineral elements (Purvis and Williamson, 1972). Waterlogging induced nitrogen deficiency leading to chlorosis and finally death of leaves, particularly of older leaves. Leaves showing yellowness under flooding stress is attributed mainly due to N deficiency (Rai et al., 2004; Srivastava et al., 2007; Steffens et al., 2005). It was interesting to note that N content in roots of SNP treated waterlogged plants was more than in roots of normal plants. Similar trend was seen for shoot N content (Figure 6). As SNP is also a source of N, therefore; it has probably supplemented additional N to waterlogged plants. This inference was further supported with the observation that SNP supplied plants generally had more chlorophyll content in leaves (Figure 4). Similar observations were made by Fan et al. (2014) who observed that chlorophyll content of waterlogged cucumber seedlings improved when plants were treated with varying concentrations of SNP. SNP treatments at 500 µmol L^{-1} ameliorated the deleterious effects of waterlogging on root nitrogen in both genotypes (Figure 5). Results are in agreement with those of Wang et al. (2011) who reported that SNP at 50 and 500 µmol L^{-1} could keep chlorophyll to a relatively higher level in maize plants. Waterlogging resistant genotype; HUZM-265, responded more to SNP application than the susceptible genotype. Shoot nitrogen declined under waterlogging and was ameliorated by using exogenous supply of SNP in rhizosphere, particularly at 500 µmol L^{-1} concentration (Figure 6). Maize roots are the organ initially damaged during initial phase of waterlogging. Longer waterlogging duration induces yellowing and decline in leaf chlorophyll content due to induction in nitrogen deficiency leading to senescence of older leaves. Waterlogging also leads to derangement in water relation parameters and other physiological and biochemical processes of plants.

Present investigation indicated genotypic differences in waterlogging resistance in maize. WTC was found to be a suitable parameter to distinguish waterlogging resistant and susceptible genotype in this crop. Differential responses in morphological, physiological and biochemical parameters in resistant and susceptible genotypes were also evident. SNP, as a donor of NO, ameliorated the harmful effect of waterlogging on plant processes; however, effective SNP concentration varied with respect to genotype and the studied plant process. Much work is still needed to visualize the potential role of SNP, a NO donor, in waterlogging stress resistance in plants.

Conflict of Interest

The authors have not declared any conflict of interest.

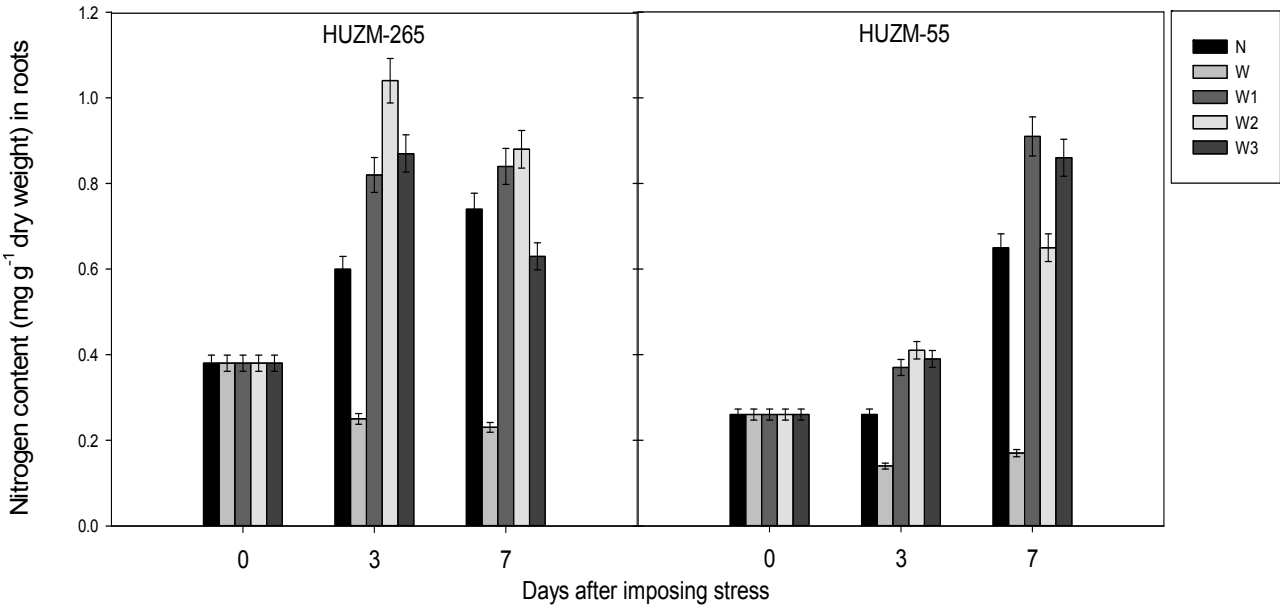

Figure 5. Changes in nitrogen content (mg g^{-1} dry weight) in roots of maize genotype HUZM-265 (relatively waterlogging resistant) and HUZM-55 (relatively waterlogging susceptible) under waterlogging stress with different concentrations of sodium nitroprusside (SNP). Values are means of three replicates ±SE. N=Normal, W= waterlogged, W1= waterlogged with 50 µmol L^{-1} SNP, W2= waterlogged with 500 µmol L^{-1}, SNP, W3= waterlogged with 2000 µmol L^{-1} SNP.

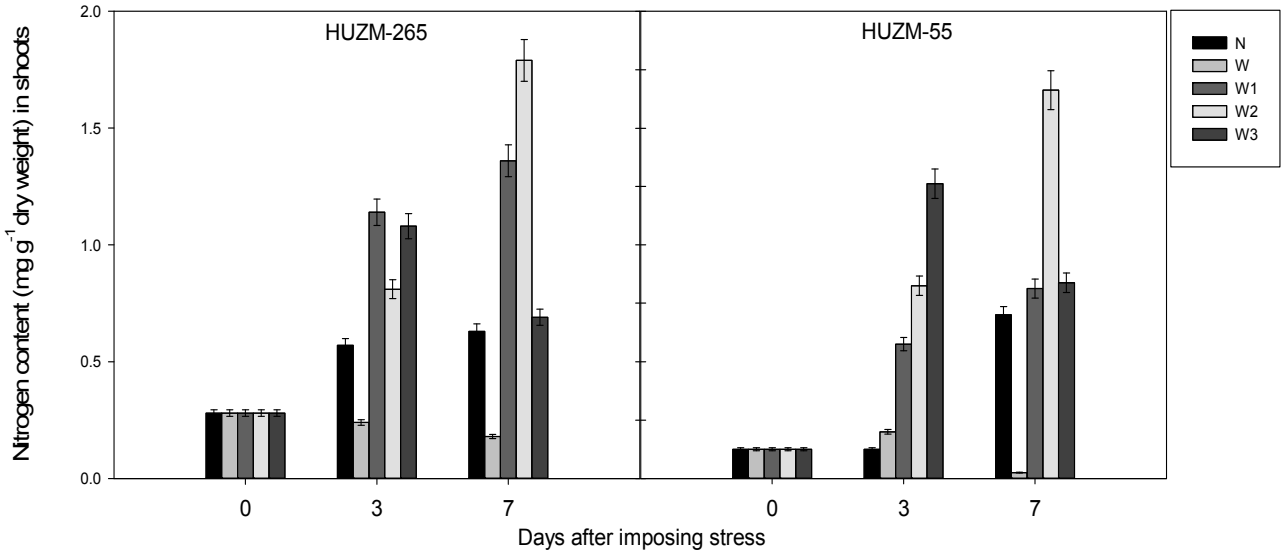

Figure 6. Changes in nitrogen content (mg g^{-1} dry weight) in shoots of maize genotype HUZM-265 (relatively waterlogging resistant) and HUZM-55 (relatively waterlogging susceptible) under waterlogging stress with different concentrations of sodium nitroprusside (SNP). Values are means of three replicates ±SE. N=Normal, W= waterlogged, W_1= waterlogged with 50 µmol L^{-1} SNP, W_2= waterlogged with 500 µmol L^{-1} SNP, W_3= waterlogged with 2000 µmol L^{-1} SNP.

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Full Length Research Paper

Germination, vigor, and fungi incidence in melon seeds treated with Thiabendazole

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Chemical treatment is known to effectively eradicate or at least reduce the presence of fungi in seeds. However, this treatment should not impair seed quality. The purpose of the present study was to evaluate the physiological and sanitary quality of Cozumel hybrid melon seeds treated with the fungicide Thiabendazole. The experiments were conducted under a completely randomized design, consisting of 15 treatments in a 3×5 factorial system (three seed lots × five Thiabendazole doses: 0, 0.12, 0.2, 0.3 and 0.4% a.i.). The commercial product used was Tecto® SC (485 g L^{-1} of Thiabendazole). Four replications of 50 seeds were used for germination and vigor tests (evaluated by germination test first count: GFC). Eight replications of 50 seeds were performed for seed pathology analysis, amounting to 400 seeds per treatment. The average results were compared by Tukey test at 5% probability. The results showed differences in germination between lots, with lower total germination in lot 84538 (90.8%). There was no difference in Thiabendazole dosage regarding both total germination (94.8% on average) and GFC (93.2% on average), showing that the fungicide did not affect the physiological quality of seeds. Pathogenic species were not detected in the sanitary analysis, only saprophytic fungal species (*Alternaria* sp., *Aspergillus* spp., *Curvularia* spp and *Penicillium* spp.) and a general reduction in fungi incidence was observed with the increase of Thiabendazole doses.

Key words: *Cucumis melo*, seed treatment, fungicide.

INTRODUCTION

The association between fungi and seeds can severely affect seed quality, reducing germination, vigor, seedling emergence and productive potential. It is not always possible to obtain seed lots 100% guaranteed free of pathogens. It is also not possible to ensure that the sown soil or substrate will be clear of fungi. Therefore, seed treatment is advisable in most cases, especially for vegetable hybrid seeds, because treatment cost is very low compared to the high price this type of seeds carries (Cardoso et al., 2015).

Seed treatment has been efficient in preventing plant disease outbreaks caused by pathogenic agents in seeds, particularly fungal agents. Chemical seed treatment aims to eradicate these pathogens and/or to protect against soil pathogens, especially during germination. Furthermore, seed treatment can help reduce the volume of fungicides needed to control the diseases. Seed treatment can eliminate the need of foliar

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use of fungicide products in field crops (Mancini and Romanazzi, 2014).

It must be emphasized that beyond the control of pathogens and/or protection of the seeds, the treatment should not impair seed physiological quality (Cardoso et al., 2015). Seed treatment effectiveness depends on, among other factors, seed species and vigor, which may vary from lot to lot (Menten and Moraes, 2010).

According to Menten and Moraes (2010), there were 19 fungicides active ingredients registered for treating seeds in Brazil, although this registration is specific for only some species. One of these active substances is Thiabendazole, a member of the Benzimidazole chemical group, registered in Brazil for seed treatment in only few cultures.

According to the Ministry of Agriculture, Livestock and Supply (MAPA, 2014), the commercial product Tecto® SC, active ingredient Thiabendazole, is a systemic fungicide registered for soybean and sunflower seed treatment; for avocado, banana, citrus, papaya, mango and melon fruits postharvest treatment and aerial part of avocado, pineapple, coconut, pea, snap pea, papaya, mango, passion fruit, melon and sweet pepper crop treatment.

Thus, considering the Cucurbitaceae family, this fungicide has only been registered for treating melon culture, to the control of fungi *Colletotrichum orbiculare* and *Didymella bryoniae*, and recommended for spraying plants or postharvest treatment in fruits. Therefore, this product has not been registered for vegetable seed treatment. It has been reported that this fungicide does not impair seed physiological quality of several species, such as alfalfa (Mendes et al., 2001), lentil (Chang et al., 2008), maize (Fessel et al., 2003; Carvalho et al., 2004), soybean (Pereira et al., 2011) and wheat crops (Garcia Júnior et al., 2008). In regard to melon, no studies on the use of this fungicide to treat seeds were found.

Thus, the purpose of the present work was to evaluate physiological (germination and vigor) and sanitary (incidence of fungi) quality in seeds of different lots of melon seeds treated with five doses of Thiabendazole.

MATERIALS AND METHODS

Seeds of three lots of Cozumel hybrid melon were analyzed concerning physiological and sanitary qualities after treatment with the fungicide Tecto® SC. This product belongs to the chemical group of Benzimidazole and the active ingredient (a.i.) is Thiabendazole (485 g/L of Thiabendazole). The experiments were conducted independently (one for sanitary and another for physiological quality), under a completely randomized design, with 15 treatments, according to a 3x5 factorial system (three seed lots: 67594, 84538 and 84539 × five fungicide doses: 0.0; 0.12; 0.2; 0.3 and 0.4% a.i.).

The application of fungicide was done in rotating pans with a central disk inside the pan located in the center, also rotating, but in the opposite direction to distribute the product. After treatment and drying, the seeds were evaluated for physiological quality and incidence of fungi.

Seed germination

Standard Germination Test (SGT) was done according to Seed Analysis Standards (ISTA, 2004; Brasil, 2009). The seeds were distributed over two pieces of paper towel dampened with 2.5 times their weight of distilled water and covered with another piece of moistened paper towel. The sheets were rolled and placed in a germination chamber in a vertical position at 25±1°C, relative humidity between 80 to 95%, without light. There were four replications with 50 seeds, amounting to 200 seeds per treatment. The counting of normal seedlings was performed on the 8th day after sowing (DAS), with values expressed in percentages.

Seed vigor

Germination First Count (GFC) tests: normal seedlings were counted on the 4th DAS during the SGT, as described previously, according to Brasil (2009), with values expressed in percentages.

Seed pathology analysis

The "blotter test" method was used to evaluate the incidence of fungi in seeds. It involved distributing ten seeds placed equidistantly over three sheets of filter paper, previously moistened, positioned on Petri dishes. Eight replications of 50 seeds were performed, totaling 400 seeds per treatments. The experimental plot consisted of five dishes with ten seeds. The dishes were kept at 20±2°C for a twelve-hour photoperiod under white fluorescent light for seven days. After incubation, the seeds were individually evaluated under a magnifier and the results were expressed in percentage of seeds with fungus. The results were converted in arc sin $\sqrt{(x/100)}$ for statistical analysis.

The data obtained for all variables evaluated were subjected to variance analysis and the averages were compared against the Tukey test at 5% probability.

RESULTS AND DISCUSSION

There was no interaction between factors (lots and fungicide doses), indicating mutual independence for germination and vigor. The results for both variables are shown in Table 1. Although, all three melon seed lots offer excellent quality with minimum germination rate above 90%, lot 84538 showed lesser germination (90.8%) compared to the other two lots (67594 and 84539) (Table 1). The rates demonstrated for germination on three lots are much higher than the minimum standard rate of 70% for melon required for marketing in Brazil by the Ministry of Agriculture, Livestock and Supply (MAPA). However, according to Cardoso et al. (2014), due to higher cost of hybrid seeds and competition between companies for the market, the official standards are outdated in relation to market practices. It is rare to find Cucurbitaceas seeds in the market with less than 85% germination rate. Even with this reference frame, all the lots can be considered to have good physiological quality.

The first seed count is considered a vigor test. The samples with faster germination and higher percentage of normal seedlings on this date are considered more vigorous (Marcos Filho, 2005; Baalbaki et al., 2009). There was no difference between lots in this feature

Table 1. Germination and vigor test of 'Cozumel' melon seed lots treated with different doses of Thiabendazole.

Treatments	Germination (%)	Vigor (%)
Seed lots		
67594	98.1[a1]	94.2[a]
84538	90.8[b]	90.6[a]
84539	95.5[a]	94.9[a]
Doses (%Thiabendazole)		
0.00	94.7[A2]	92.8[A]
0.12	96.5[A]	94.0[A]
0.20	97.3[A]	96.5[A]
0.30	93.0[A]	91.3[A]
0.40	92.5[A]	91.5[A]
F_{lots}	7.43**	0.45[ns]
F_{doses}	1.45[ns]	1.47[ns]
C.V. (%)	6.40	6.51

[1]Seed lot averages followed by the same lowercase letter do not differ by Tukey test at 5% probability. [2]Thiabendazole dose averages followed by the same uppercase letter do not differ by Tukey test at 5% probability. [ns] = non-significant by F test at 5% probability and ** = significant by F test at 1% probability.

(Table 1), with an average of 93.2% rate, indicating high seed vigor.

Considering Thiabendazole dosage (Table 1), there was no difference in germination (average of 94.8%) and vigor (first count, average of 93.2%), showing that the fungicide did not affect physiological quality of melon seeds in any lot. According to Groot et al. (2006, 2008), Lobo (2008) and Menten and Moraes (2010), sensitivity to fungicide treatment can vary according to the initial quality of seed lots. In this study, despite differences in germination among lots, none of the seeds in any of the lots were affected by the fungicide treatment, regardless of the dose.

Garcia Júnior et al. (2008) found no phytotoxic effect of this fungicide on wheat seeds, verifying germination and seedling emergence similar to untreated control seeds, as observed by Mendes et al. (2001) with alfalfa seeds and Gally et al. (2004) with soybean seeds. Yet, Fessel et al. (2003) reported that a very high concentration of fungicide could impair maize seed quality. In contrast, Carvalho et al. (2004) found higher maize seed germination in different treated lots compared to the untreated control lot, because fungicide reduced the incidence of "damping off" caused by the fungus *Stenocarpella maydis* in seeds. Also, Kaiser and Hannan (1987) verified an increase in seedling emergence for lentil seeds treated with Thiabendazole, mainly due to control of fungus *Ascochita lentis*. But, in high doses, this fungicide caused phytotoxic effects on lentil seedlings. The positive effects on germination and/or seedling emergence with seed treatment using this fungicide were also described in papaya seeds (Campos et al., 2009) and castor bean seeds (Poletine et al., 2012). These contrasting results for each crop show the importance of conducting studies for each different species because the results may not always be the same.

The treated seeds, regardless of dose and lot, showed no difference compared to untreated control seeds, both for germination test and vigor (Table 1). The discrepancy of reports on other species probably occurred for two reasons: (a) because the observed presence of fungi in seeds was small in all treatments, including in the control (Tables 2 and 3), (b) the treatment with this fungicide did not affect germination and vigor. Therefore, seed treatment with Thiabendazole, in the doses evaluated, did not affect seed germination and vigor.

Mancini and Romanazzi (2014) emphasize that vegetable seed treatment is no substitute for using pathogen free seeds. However, due to the impossibility of obtaining lots 100% free of pathogens and considering the presence of fungi in the soil or substrate where the seeds are sown, treating seeds is the most effective method to achieve maximum seedling emergence.

Regarding treated seed sanity, a pathogenic species was not detected in the seeds, notably *D. bryoniae,* one of the main seedborne pathogens in melons. However, there were saprophytic fungal species. For *Alternaria* sp., interaction between factors (seed lots and Thiabendazole doses) was non-significant. It was observed lesser incidence on lot 84539 than in lots 84538 and 67594 (Table 2). Regardless of the lot, this fungus incidence decreased as the fungicide dose increased. The lower dose (0.12%) showed no difference against untreated control seeds (Table 2).

Among other saprophytic fungal species, *Penicillium* sp.*, Aspergillus* spp. and *Curvularia* sp., the interaction

Table 2. Incidence of *Alternaria* spp. in seeds of three lots of Cozumel melon treated with Thiabendazole doses.

Treatment	*Alternaria* spp.
Seed lots	
67594	2.90[a1]
84538	3.22[a]
84539	0.61[b]
Doses (%Thiabendazole)	
0.00	5.03[A2]
0.12	3.05[AB]
0.20	1.78[B]
0.30	0.68[B]
0.40	0.68[B]

[1]Seed lot averages followed by the same lowercase letter do not differ by Tukey test at 5% probability. [2]Thiabendazole dose averages followed by the same uppercase letter do not differ by Tukey test at 5% probability.

Table 3. Incidence of saprophytic fungal species in seeds of three lots of 'Cozumel' melon treated with Thiabendazole.

Dose (%Thiabendazole)	Seed lots		
	67.594	**84.538**	**84539**
		***Penicillium* spp**	
0.00	33.69[aA1]	18.66[b A]	37.50[aA]
0.12	19.03[aB]	18.25[aA]	25.86[aB]
0.20	13.93[bBC]	19.38[abA]	25.34[aB]
0.30	5.84[aC]	12.24[aA]	10.63[aC]
0.40	5.10[bC]	9.27[abA]	15.96a[BC]
Dose (%Thiabendazole)	**Seed lots**		
	67.594	**84.538**	**84539**
		Aspergillus spp.	
0.00	6.20[cA]	29.57[aA]	19.32[bA]
0.12	6.57[bA]	24.39[aA]	3.47[bB]
0.20	3.80[aA]	8.32[aB]	4.07[aB]
0.30	3.80[aA]	9.27[aB]	2.03[aB]
0.40	1.02[aA]	7.57[aB]	1.02[aB]
Dose (%Thiabendazole)	**Seed lots**		
	67.594	**84.538**	**84539**
		Curvularia spp.	
0.00	0.00[bA]	2.03bA	6.97[aA]
0.12	0.00[bA]	0.00bA	3.74[aAB]
0.20	0.00[aA]	0.00aA	2.03[aB]
0.30	0.00[aA]	0.00aA	1.02[aB]
0.40	0.00[aA]	0.00aA	0.00[aB]

[1] For each fungus, averages followed by the same letter, uppercase letter for doses (columns) and lowercase letters for seed lots (lines) do not differ by Tukey test at 5% probability.

between factors (seed lots and Thiabendazole doses) was significant (Table 3). These fungi incidence decreased in seeds with increasing fungicide doses. Thus, the fungicide showed a positive effect on the

control of these fungi species in melon seeds.

There have been no reports on melon seed treatment with this fungicide and related control of its pathogens. But, there have been reports on controlling *Fusarium oxysporum* in alfalfa seeds (Mendes et al., 2001), *Aspergillus* sp. and *Penicillium* sp. in peanut seeds (Barbosa et al., 2013), *Ascochita lentis* in lentil seeds (Kaiser and Hannan, 1987), *Aspergillus niger, A. favus, Botrytis ricini, Curvularia* sp., *Penicillium* sp. and *Rhizopus* sp. in castor bean seeds (David et al., 2014), *Leptosphaeria maculans* in cabbage seeds (Maude et al., 1984), *Fusarium graminearum* in wheat seeds (Garcia Júnior et al., 2008), *Fusarium moniliforme* (Pinto, 2000) and *Stenocarpella maydis* (Carvalho et al., 2004) in maize seeds. These studies demonstrate that Thiabendazole is effective in controlling fungi in seeds of different species, as observed in the present study with melon seeds.

Conclusion

In general, the fungicide Thiabendazole promotes a reduction of saprophytic fungus in melon seeds without impairing their germination and vigor.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Performance of American lettuce under different plant covers for no-tillage system

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This study aimed to evaluate the agronomic performance of American lettuce "Lucy Brown" in different types of straw from dried cover crops, for a no-tillage system, under the climatic conditions of Campo Grande (Mato Grosso do Sul state, Brazil). The experiment was set up in a randomized block design with five treatments and six replications, totalizing 30 plots. The treatments were: T1 (control) - planting lettuce in bare soil; T2 - planting lettuce on millet straw; T3 - planting lettuce on white oat straw; T4 - planting lettuce on forage radish straw; and T5 - planting lettuce on sunn hemp straw. After 37 days from sprouting the plants were assessed for fresh weight. They were dried at 38 days after planting, and at 11 days after drying they were evaluated for dry weight. The transplantation of the lettuce seedlings was carried out 12 days after the cover crop was dried, leaving a space of 25 x 25 cm for cultivation of a total of 24 plants. The plots were evaluated for weed control 20 days after transplanting lettuce. At 35 days after transplantation six central plants were evaluated in each plot, assessing the following variables: Above-ground fresh mass from shoot and root, dry weight of shoot and root and head diameter. Under the conditions in Campo Grande, for no-tillage cultivation of "Lucy Brown" lettuce, hemp is the most suitable cover, followed by millet.

Key words: *Lactuca sativa, Avena sativa* L, *Pennisetum glaucum* L., *Crotalaria spectabilis* Roth, *Raphanus sativus* L, yield.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a herbaceous plant belonging to the Asteraceae family. It is the leafy vegetable that is most consumed in the Brazilian diet, which ensures that this crop has significant economic importance (Henz and Suinaga, 2009).

In Brazil, since the 1990s, there have been significant changes in the productive chain for vegetables, giving prominence to American lettuce. This is mainly due to demand from fast-food networks and because this variety presents a longer period of conservation after being harvested than do other types of lettuce (Sala and Costa, 2012).

The demand by consumers for leafy vegetables in the right quantity and quality has led to the use of high-technology cultivation systems, such as planting on straw (direct planting) (Furlani and Purqueiro, 2010). The direct planting technique is very well known in Brazil for use with grain crops, but it has been little studied for leafy

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vegetables (Purqueiro and Tivelli, 2007). Based on minimum soil turning, crop rotation and soil dressing with plant residue, it presents the advantages of improved soil structure, increased infiltration and retention of water in the soil, improved development of the plant root system, control of invasive plants, and the fact that the soil temperature remains more temperate (Henz and Suinaga, 2009; Marouelli et al., 2008). For the lettuce crop, a dead cover crop or straw is commonly used, providing a microclimate that is more favorable to crop development (Henz and Suinaga, 2009). High temperature is a factor that directly interferes in the development of lettuce plants, leading to losses of up to 60%, as well as early bolting (Sala and Costa, 2012). According to Setúbal and Silva (1992), even using hybrid cultivars, high temperatures modify the texture of lettuce leaves, making them more fibrous. As lettuce is from temperate zones, this is an important factor in the Brazilian summer, because the crop may present low yield and low quality (Filgueira, 2008).

In the state of Mato Grosso do Sul, producers face the problem of high temperatures, and so they often collect lettuce plants before these have reached their maximum vegetative development, leading to losses with low-weight plants. Crop dressing aims to reduce the oscillations in soil temperature, decrease the excessive loss of water and, as a result, improve the performance of crops (Souza and Resende, 2003).

In recent years, some researchers have carried out successful experiments with vegetables cultivated among dead plant cover (Purqueiro et al., 2009; Tivelli et al., 2010). The use of soil cover in lettuce growing has proved to be a determining factor in increasing product yields and quality (Andrade Júnior et al., 2005), and according to Carvalho et al. (2005) it has become an essential practice in hot regions. However, to optimize the use of plant cover on the soil, the most suitable species and varieties need to be identified for different regions, and they should be matched with the best management system (Ceretta et al., 1994).

It is a challenge to make planting feasible and to increase the yield of lettuce in hot regions, especially for American lettuce. This work aimed to evaluate the agronomic performance of the American lettuce “Lucy Brown”, cultivated from October to November under different dried plant covers, for the direct planting system in the soil and climate conditions of Campo Grande, Mato Grosso do Sul state (MS).

MATERIALS and METHODS

The experiment was carried out in Campo Grande, MS, in the period from August to November 2012, in soil classified as Orthic Quartizarenic Neosol (Quartzite sand) at the geographic coordinates of latitude 20°26’21” S and longitude 54°32’27” W, at an altitude of 531.2 m above sea level. According to the Köppen classification, the climate in the region is considered tropical humid, with a rainy season in summer and a dry season in winter.

Accumulated precipitation in the experimental period was 350 mm, with a mean maximum temperature of 29.2°C and a mean minimum temperature of 18.9°C (Brasil, 1992).

The experimental area was used for vegetables in the year 2010 and until September 2011. In October 2011 maize was planted and collected at the beginning of February 2012, and fertilization was applied in accordance with soil analysis and the crop being planted. After the maize had been harvested the area remained fallow for a period of four months, covered with spontaneous plant growth. The chemical and physical characteristics of the soil before the experiment was set up were determined in accordance with Embrapa (2006), and they are described as follows: pH in water 6.82, pH in $CaCl_2$ (0.01 M) 6.22; Phosphorus in Melich-1 (P) 47 mg dm^{-3}; Potassium (K^+) 136 mg dm^{-3}; Calcium (Ca^{+2}) 3.40 $cmol_+$ dm^{-3}; (Mg^{+2}) 1.30 $cmol_+$ dm^{-3}; (H^+) 2.0 $cmol_+$ dm^{-3}; organic matter (OM) 19.5 g kg^{-1}; cation exchange capacity (CEC) 6.7 $cmol_+$ dm^{-3}; base saturation (V) 71%; clay 115 g kg^{-1}; silt 30 g kg^{-1} and total sand 855 g kg^{-1}, by the pipette method.

The experiment was composed of five treatments: T1 (Blank control) – lettuce planted on soil with no plant cover, T2 – lettuce planted on millet straw (*Pennisetum glaucum* L.), T3 – lettuce planted on white oat straw (*Avena sativa* L.), T4 – lettuce planted on forage radish straw (*Raphanus sativus* L.) and T5 – lettuce planted on sunn hemp straw (*Crotalaria spectabilis* Roth.). The experimental plots were 2 m² (1 m x 2 m) with six repetitions, totaling 30 plots, and the treatments were distributed according to random block design. The plant covers for the formation of straw were sown on 08/27/2012 by scattering, without any fertilization, at the following as recommended by Embrapa (2006).

At 37 days after sowing, evaluations were carried out for fresh and dry matter of the cover crops, using a square iron frame of 20 cm placed in the center of each experimental plot, discarding the edge. The fresh and dry matter was cut within the area delimited by the square frame and weighed on a precision scale; the values were extrapolated for kg/ha. Next, chemical desiccation of the cover crops was done with glyphosate at 720 g ha^{-1} of a.i. The application was carried out with a backpack sprayer pressurized by CO_2 with a fan-type nozzle (80.03), at 8 to 9 a.m., a time of day when the temperature was 26°C, relative humidity was 55% and there was almost no breeze. The lettuce seedlings were produced in a greenhouse, using pelletized seeds of the American lettuce cultivar “Lucy Brown”, sown (one seed per cell) in a polystyrene tray with 128 cells, previously filled with commercial substrate (Plantmax®), where they remained for 29 days. For irrigation, 1000 mL of water per tray was used each day, distributed in two irrigations of 500 mL each.

The transplantation of lettuce seedlings to the straw-covered sections took place 12 days after chemical desiccation of the straw, spaced at 25 x 25 cm, totaling 24 plants per straw section. At transplantation, fertilization of the crop took place, as recommended for lettuce, at a dose of 200g of super phosphate (P_2O_5)/m^2 (Ribeiro et al., 1999). Cover fertilization with N and K_2O was applied at 10, 20 and 30 days after transplantation of the seedlings to the beds, using sources of urea and potassium chloride at doses of 15 g/m^2 of N and 10 g/m^2 of K_2O for each application. At 35 days after transplantation, the lettuce was harvested, taking the whole plant out of the soil (aboveground part and root). The six plants at the center of each plot were evaluated. After harvesting, the plants were washed, and the excess water was dried with a paper towel. Plants were sectioned, separating the aboveground part and the root and checking the following variables: aboveground fresh matter (AFM) and dry matter (ADM) and root fresh matter (RFM) and dry matter (RDM). The drying was done in an oven with forced air circulation at ± 65°C until constant weight was reached. The diameter of the head (HD) was measured individually in two positions, using a ruler graduated in cm, considering only the central compact heart of the lettuce plant.

The treatments were evaluated for infestation of weeds, 20 days

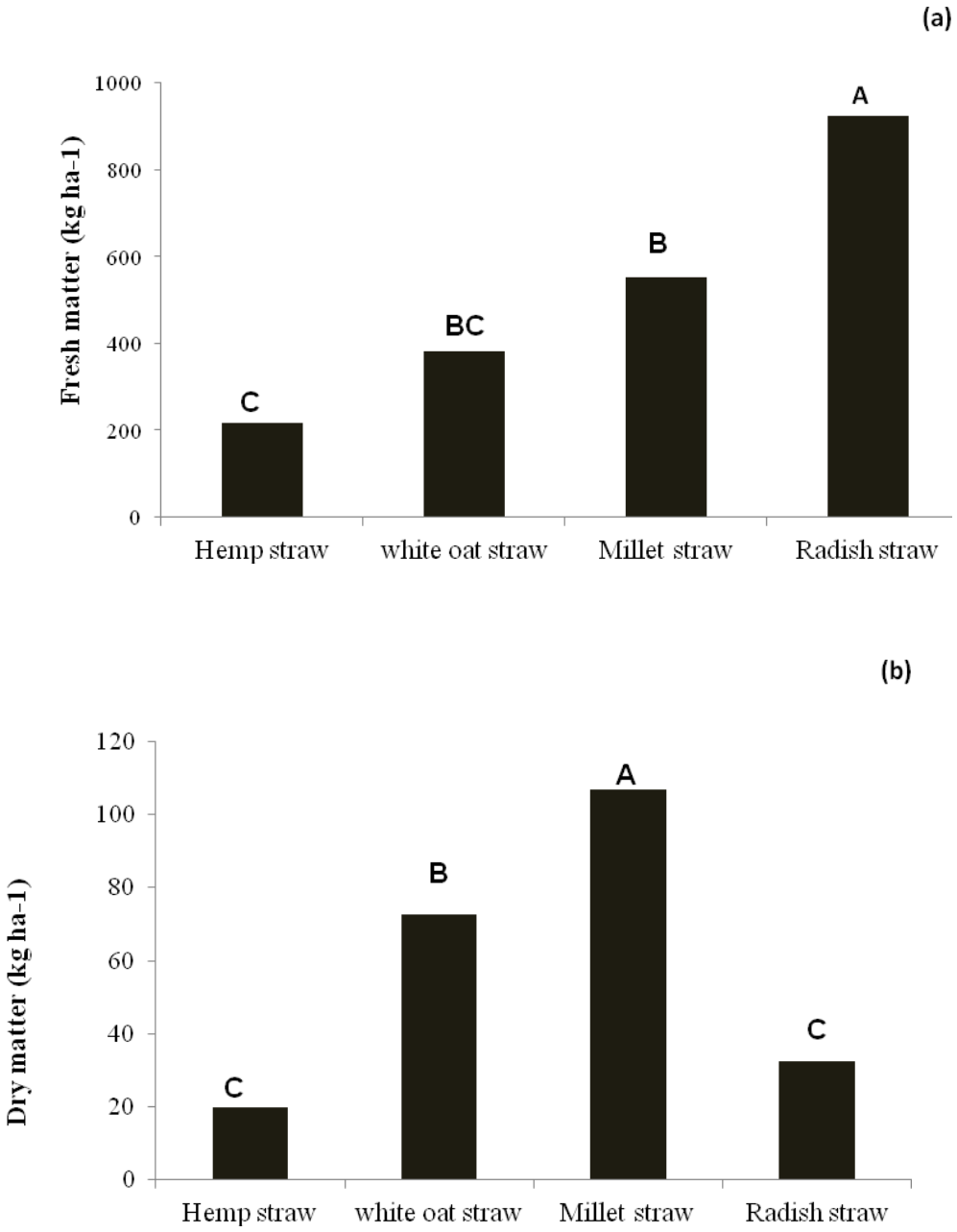

Figure 1. Means of fresh matter (a) and dry matter (b) for the plant cover crops. Bars followed by the same letter in the column do not differ among themselves by the Tukey test at 5% of probability, Campo Grande, 2013.

after the lettuce was transplanted, by a method adapted from Research Methods in Weed Science (1997). This method proposes that the efficiency of control should receive scores from 0 to 100, where: 100 (total control – death of all individuals in the population); 90 to 99 (excellent control); 80 to 89 (acceptable control - 80 is the minimum acceptable score from the point of view of agronomic efficiency); 50 to 79 (unacceptable control); 0 to 49 (insufficient control). The data were submitted to analysis of variance and to a means test by the PROC GLM procedure, using the program SAS (SAS, 2001), and the means were compared using the Tukey test at 5% of probability. Contrast was also carried out by the F test for analysis of variance among the treatments, using sunn hemp (*Fabaceae*), millet and oat (*Poaceae*) and forage radish (*Brassicaceae*).

RESULTS AND DISCUSSION

The plant covers were evaluated for fresh and dry matter, and the data can be found in Figure 1(a) and (b). Forage radish was the cover that presented the greatest yield of

Table 1. Statistical indicators for the variables analyzed in American lettuce “Lucy Brown” cultivated on different dried plant cover crops, Campo Grande, 2013.

Causes of variation	AFM	RFM	RDM	ADM	HD	CFM	CDM	WP
	Value of F							
Block	1.12ns	1.30ns	1.91ns	8.58**	2.00ns	1.47ns	1.39ns	1.62ns
Treatments	3.96**	4.17**	6.79**	3.92**	3.42*	3.77**	3.81**	7.52**
CV (%)	23.39	26.20	28.46	24.94	14.20	21.31	26.57	28.74

AFM= aboveground fresh matter RFM= root fresh matter RDM= root dry matter ADM =aboveground dry matter HD= head diameter CFM= cover fresh matter CDM = cover dry matter WP= weed presence, ns= non-significant; ** and * = significant at 1% and 5% of probability, respectively. SH= Sunn hemp; M= Millet; O= Oat; FR= Forage Radish.

Table 2. Biometric evaluations of American lettuce “Lucy Brown” cultivated on different dried plant cover crops, Campo Grande, 2013.

Treatments	AFM		RFM		RDM		ADM		HD	
	g pl^{-1}								cm pl^{-1}	
White Oat	412.01	C	7.60	B	4.92	A	22.46	BC	15.08	B
Sunn hemp	496.17	A	8.53	A	5.07	A	24.44	AB	16.40	A
Millet	422.32	BC	6.95	B	4.03	B	23.79	AB	15.18	B
Radish	430.30	BC	7.01	B	3.96	B	20.63	C	14.56	B
Control	463.82	AB	7.01	B	4.02	B	26.02	A	15.04	B

AFM= aboveground fresh matter RFM= root fresh matter RDM= root dry matter ADM = aboveground dry matter HD= head diameter. Means followed by the same letter in the column do not differ among themselves by the Tukey test at 5% of probability

fresh matter and sunn hemp the lowest (Figure 1a). For dry matter, millet yielded the most (Figure 1b). Sunn hemp, which produced least fresh matter, provided the highest yield in terms of the lettuce crop. This can be explained by the fact that sunn hemp releases a greater amount of nutrients. However, in the present study the greater yield of dry matter from millet and oat did not provide a higher yield in the next crop, in this case for the lettuce “Lucy Brown”. For the cultivation of eggplant under direct planting, Castro et al. (2005), verified that sunn hemp made more Ca, Mg and N available than did millet. In a study carried out by Andreotti et al. (2008) millet also produced less dry matter than did a species of sunn hemp, in this case *Crotalaria juncea*.

A similar result was found by Oliveira et al. (2008) with the lettuce cultivar Regina, confirming that leguminous plants, used as cover crops, release more N to the soil compared to grasses, and thus influence lettuce growth positively. The summary of the analysis of variance and its significance in function of the F test for aboveground fresh matter (AFM), root fresh matter (RFM), aboveground dry matter (ADM), root dry matter (RDM) and head diameter (HD) of the American lettuce “Lucy Brown”, in function of the different dried cover crops, is presented in Table 1. In the same table, the cover crop fresh matter (CFM), cover dry matter (CDM) and weed presence (WP), as well as the contrasts between sunn hemp, millet and white oat (SH x M x O), forage radish, millet and oat (FR x M x O) and forage radish and sunn hemp (FR x SH) are shown.

The results demonstrate that there was a significant effect of the treatments for all the analyzed variables. The means of the treatments for the variables AFM, RFM, ADM, RDM and HF are presented in Table 2. The lettuce plants presented more aboveground and root fresh matter, more root dry matter and a greater head diameter when cultivated under the sunn hemp cover. For the variable AFM there was a difference between the blank control and the cover with oat, corroborating the data of Maluf et al. (2004), in a study with American lettuce under direct planting, in which the authors confirmed greater fresh matter yield under black oat cover than with uncovered soil.

In studies of the effect of plant covers on lettuce production, various authors have observed better results for crops on covered soil. They have emphasized that cover crops promote better lettuce development, as confirmed by the data in the present study, for the cover with sunn hemp, for the variables RFM, RDM and HD (Mógor and Câmara, 2007 and Carvalho et al., 2005). The RDM of lettuces on oat cover was greater than on millet and forage radish covers. This may be related to the decomposition rate of oat, which occurs in up to 40 days (Crusciol et al., 2008). Thus, nutrients in the soil are mineralized more quickly than by millet and radish, benefiting the development of lettuce roots. With regard to the weed presence, Indian goosegrass (*Eleusine indica,* Figure 2a) and common purslane (*Portulaca oleraceae,* Figure 2b) were identified among the weeds.

The covers studied here significantly reduced the

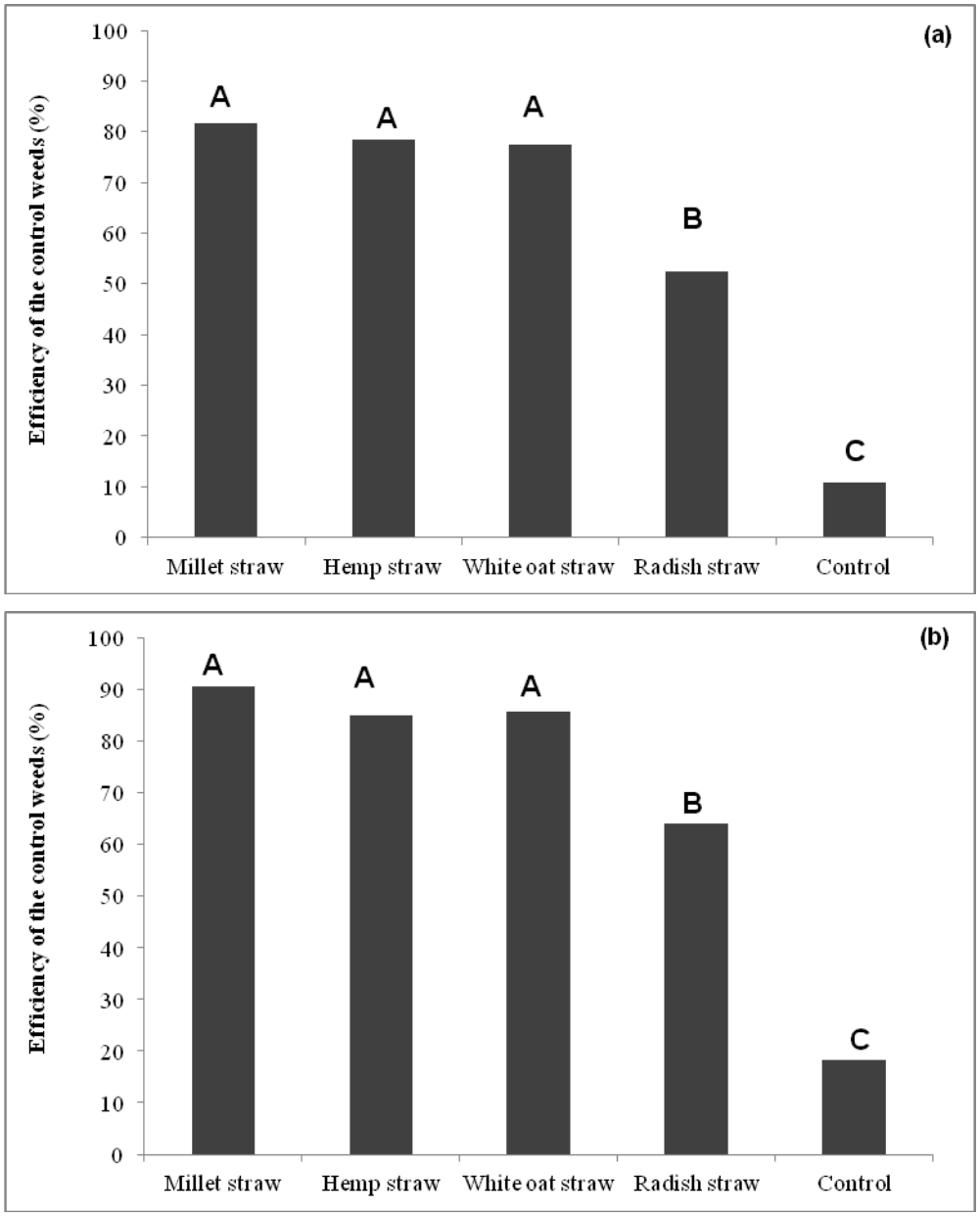

Figure 2. Efficiency of the control of weeds, (a) *E. indica* and (b) *P. oleraceae,* on different dried plant covers in the cultivation of American lettuce "Lucy Brown", Campo Grande, 2013. *Means followed by the same letter in the column do not differ among themselves by the Tukey test at 5% of probability.

density of weeds in relation to uncovered soil (blank control). Millet, despite not differing statistically from sunn hemp and oat, was the cover that presented the best control of *E. indica* (81, 67), while for *P. oleraceae* the best cover crops were millet, sunn hemp and oat. The cover with forage radish and the blank control (uncovered soil) presented higher indices of invasive plants.

Similar results were found by other authors in work with vegetable crops grown on straw cover. Among these we can cite Rezende et al. (2005), whose study confirmed that the use of dead cover in the production of the summer carrot crop was efficient in controlling *E. indica* and *P. oleraceae*; additionally, Carvalho et al. (2005) affirmed that dead cover favored the control of weeds in cultivation of lettuce cv. Regina.

Cover crops of millet, oat and sunn hemp did not present significant differences in the control of invasive plants, corroborating the data of Oliveira et al. (2008), in work with different cover crops in the cultivation of

Table 3. Comparison of biometric data of American lettuce "Lucy Brown" in function of plant cover from three families, Campo Grande, 2013.

Treatments	AFM		RFM		RDM		ADM		HD	
	g pl^{-1}								cm pl^{-1}	
Fabaceae	496.17	A	8.53	A	5.07	A	24.44	A	16.40	A
Poaceae	417.16	B	7.27	B	4.47	B	23.12	A	15.13	B
Brassicaceae	430.30	AB	7.01	B	3.96	B	20.63	A	14.56	B

AFM= aboveground fresh matter RFM= root fresh matter RDM= root dry matter ADM = aboveground dry matter HD= head diameter. Means followed by the same letter in the column do not differ among themselves by contrast using the F test of analysis of variance at 5% of probability.

lettuce, which showed that all the covers used were efficient in controlling weeds. For Kieling et al. (2009), tomato direct-planted on different straw covers underwent efficient suppression of invasive plants, and oat and forage radish covers did not differ between themselves.

The effect of controlling invasive plants may be attributed to physical and chemical alterations in the soil, provided by the cover crops. Physically, according to Constantin (2001), dead cover alters the humidity, light and superficial temperature of the soil. According to the same author, the straw also constitutes a mechanical barrier to the development of weeds. According to Pires and Oliveira (2001), many plant residues, used as plant cover, have the capacity to produce substances that have an allelopathic effect on the seeds of invasive plants, delaying and even inhibiting their germination and growth.

In this study, when we compared sunn hemp (*Fabaceae*) with millet and oat (*Poaceae*) and forage radish (*Brassicaceae*), in lettuce yield, it was observed that the first of these stood out as a soil cover, except for AFM, which did not differ from forage radish (*Brassicaceae*) and for ADM, in which the covers did not show differences among themselves (Table 3).

Oliveira et al. (2008) also worked with plant covers and confirmed better results for fresh matter and above-ground diameter for the lettuce cultivar "*Regina*", grown on a leguminous cover crop, which is in agreement with the results of the present study. Plants from species of the families *Fabaceae* and *Brassicaceae* possess faster decomposition when compared to *Poaceae*, making nutrients available to the soil in less time. Among the nutrients, nitrogen stands out, arising mainly from the association of leguminous plants with fixative bacteria. This association increases the potential performance of nitrogen in the soil, benefiting the next crop. Grasses (*Poaceae*), in turn, present a slower decomposition, thus holding back the nutrients for the subsequent crop (Barradas et al., 2001; Aita and Giacomini, 2003; Espíndola et al., 2006; Oliveira et al., 2008). Under the soil and climatic conditions of Campo Grande, MS, the American lettuce cultivar "Lucy Brown" grown on sunn hemp (*Crotalaria spectabilis* Roth) straw is the most recommended treatment for the variables AFM, RFM, RDM and HD, when compared to straw of millet (*Pennisetum glaucum* L.), white oat (*Avena sativa* L.) and forage radish (*Raphanus sativus* L.).

Conflict of Interest

The authors have not declared any conflict of interest.

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Fatty acid profiles and parameters of quality of specialty coffees produced in different Brazilian regions

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Although fatty acids are known to be important components of coffee flavor and aroma, no study relating such compounds to the quality of coffee has been conducted. Considering the importance of attaining maximum flavor and aroma in specialty coffees, we aimed to investigate the relationship between the fatty acid composition and sensory characteristics of different Bourbon genotypes cultivated under different edaphoclimatic conditions. Four genotypes of arabica coffee were evaluated on the fatty acid profiles and parameters of quality. The genotypes were evaluated in three Brazilian locations. For the first time, we showed that sensory attributes are the most suitable potential discriminators of specialty coffees. In addition, assessing the levels of fatty acids allowed us to obtain information about the key compounds that positively or negatively affect coffee beverage quality. Saturated fatty acids, including arachidic, stearic and palmitic acid are potential discriminators of the quality of specialty coffees, indicating better sensory quality. Conversely, unsaturated fatty acids, including elaidic, oleic, linoleic and linolenic acid may be related to coffees with less intense acidity, fragrance, body and flavor.

Key words: Sensory evaluation, environment, bourbon coffee, chemical compounds, gas chromatography.

INTRODUCTION

The lipid content in coffee grounds ranges from 10 to 17%. However, compared to *Coffea canephora*, higher lipid contents are found in Arabica coffees (Feldman et al.,1969). The majority of lipids are found in the oil fraction of the coffee bean endosperm. The coffee oil fraction is mainly composed of triacylglycerols, which have fatty acid proportions similar to those found in edible vegetable oils (Speer and Kölling-Speer, 2006).

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Triacylglycerols are relatively large molecules that present low volatility and, therefore, they have little contribution to formation of flavor and aroma. However, edible oils and fats from different natural sources have flavor profiles differentiated by the presence of characteristic volatile compounds, such as the byproducts of lipid oxidation and natural impurities (Dhingra et al., 1998). Fatty acids may also contribute subtle and enjoyable flavor notes. Therefore, the aroma and flavor perceived in food are usually influenced by the type and concentration of lipids. Lipids also influence the mouth feel of several foods (Damodaran et al., 2007).

Lipids play an important role in the sensory qualities of several plants, such as soy, cocoa and oats (Gutkoski and El-Dash, 1999). Triacylglycerols are important carriers of aroma in roast coffee beans (Petracco, 2005). The fatty acid (FA) fraction of triacylglycerols releases byproducts of oxidation, which are induced by temperature and mainly comprise aldehydes that react with intermediates of the Maillard reaction, providing additional flavor and aroma to the coffee (Flament, 2001). Studies relating flavor and aroma to fatty acid composition are more frequent in products of animal origin (Wood et al., 2008). Conversely, among the few scientific reports describing the influence of fatty acids on the sensory quality of products of vegetal origin, some reports are notable (Gutkoski and El-Dash, 1999; Stephan and Steinhart, 2000). Considering the relevance of sensory quality to the production of specialty coffees (Borém et al., 2013), the evaluation of the contribution of fatty acids to the flavor and aroma of coffees is justified.

The production of specialty coffees has become one of the main strategies for maintaining the economic viability of coffee production, especially in regions where high costs of production make the production of regular coffees impractical. Thus, the production of specialty coffees has been stimulated due to their higher value and demand. Brazil has an enormous diversity of coffee genotypes, which are cultivated in different regions of the country. Such genetic and environmental variability can supply demands on the production of coffee, including aspects related to quality. Coffee quality is described by its physical and sensory characteristics and is related to other aspects, such as chemical traits, crop systems, processing, etc. However, all of these aspects depend on the genotype and environmental conditions where the coffee is cultivated (Pereira et al., 2010; Pezzopane et al., 2012).

Among the available cultivars for plantations, the Bourbon cultivar is of special interest due to its high potential to produce coffee of excellent beverage quality because of its differentiated sensory characteristics. This cultivar is frequently used to produce specialty coffees in several regions of the world (Figueiredo et al., 2013).

The environmental conditions in which coffee trees are grown directly influence the chemical composition of their fruits and consequently the quality of the final product (Avelino et al., 2005; Joët et al., 2010a; Taveira et al., 2014). The influence of the climatic conditions during seed development on the final composition of fatty acids has been described in several oilseeds, particularly in terms of temperature and precipitation (Byfield and Upchurch, 2007; Fofana et al., 2006). The content of fatty acids can discriminate the origin of several plants, such as pistachio (Arena et al., 2007), hazelnut (Amaral et al., 2006) and olive (Ollivier et al., 2006). Some studies have reported the discrimination of coffees in different geographic regions based on their content of fatty acids (Bertrand et al., 2008; Joët et al., 2010b; Rui Alves et al., 2003).

However, although fatty acids are known to be important components of the flavor and aroma of coffee (Flament, 2001; Jham et al., 2008a; Petracco, 2005), to date, no study relating such compounds to the quality of coffee has been conducted. In other words, the term quality reflects the sensory characteristics of coffee, which includes fragrance, flavor, acidity, body, aftertaste, and balance. Considering the importance of attaining maximum flavor and aroma in specialty coffees and the influence of genetic and environmental factors on the final beverage quality, we investigated the relationship between the fatty acid composition and sensory characteristics of different Bourbon genotypes cultivated under different edaphoclimatic conditions.

MATERIALS AND METHODS

Chemical compounds

Palmitic acid (PubChem CID:985); Stearic acid (PubChem CID:5281); Elaidic acid (PubChem CID:637517); Oleic acid (PubChem CID:445639); Linoleic acid (PubChem CID:5280450); Arachidic acid (PubChem CID:10467); Linolenic acid (PubChem CID:5280934).

Experimental conditions

Four genotypes of *Coffea arabica* L. grown in three locations were evaluated (Table 1). The studied genotypes were grown in experimental field plots since 2005 in the southern region of the state of Minas Gerais and in the region of Mogiana in the state of São Paulo, including in the municipalities of Lavras, MG; Santo Antônio do Amparo, MG and São Sebastião da Grama, SP. The data in the present work represent the harvests of three agricultural crop seasons (2008/2009, 2009/2010, and 2010/2011).

The biome Mata Atlântica predominates in the Mogiana region, which is located in the interior of the state of São Paulo, with the presence of rupestrian fields. The southern region of the state of Minas Gerais is characterized by a transition between the biomes Cerrado and Mata Atlântica, also with the presence of rupestrian fields. Both regions are highlighted for their production of Arabica coffee on a large scale. The distinct edaphoclimatic conditions of these important Brazilian coffee producing regions were represented in this study, and their main characteristics are shown

Table 1. Studied genotypes and environments and their codes[a].

Environments	Genotypes
A1 = Lavras	G1 = Mundo Novo IAC 502/9
A2 = São Sebastião da Grama	G2 = Yellow Bourbon IAC J9
A3 = Santo Antônio do Amparo	G3 = Yellow Bourbon /Origin SSP[b]
	G4 = Yellow Bourbon /Origin CM[b]

[a]A1, A2, A3, G1, G2, G3 e G4 = codification of the genotypes and environments used in the discussion of the results.[b]SSP = São Sebastião do Paraíso, MG; CM = Carmo de Minas, MG.

Table 2. Geographic region, climatic variables and characterization of the three studied environments.

Municipality	Lavras	São Sebastião da Grama	Santo Antônio do Amparo
Region	**Southern Minas Gerais**	**MogianaPaulista**	**Southern Minas Gerais**
Altitude	919 m	1300 m	1050 m
Mean temperature	20.4°C	20°C	19.9°C
Mean annual precipitation	1460 mm	1560 mm	1700 mm
Latitude	21°14'43"S	21°44'50"S	20°56'47"S
Longitude	44°59'59"W	46°55'33"W	44°55'08"W
Soil type	Clayey Oxisol	Clayey Oxisol	Clayey Oxisol

in Table 2.

Coffee harvest and processing

The coffee fruits were manually and selectively harvested when the fruits were completely mature. Afterwards, the fruits were separated based on differences in their density in a water tank adapted with a sieve, thus guaranteeing the complete uniformity of the material from the different parcels. The higher density fruits were separated from the floating portion of lower density. Although the selective harvest of mature fruits was performed, a small portion of immature fruits was still found in the cherry portion. After the floaters were removed from the sample, another manual selection of the ripe fruits was carried out, resulting in approximately 20 L of coffee fruit, thus guaranteeing the retention of only mature fruits. Then, the coffee fruits were peeled to obtain the pulped coffee.

Drying was carried out immediately after processing. Coffee samples were dried in 1 m² sieves (wooden frame and grille mesh size 2.00 x 1.00 mm, manufactured in polyethylene yarn) in a paved yard. Seven liters of pulped coffee was uniformly distributed per sieve and stirred 20 times a day. The coffee samples were kept spread out and uncovered on the first night, and on the following nights they were covered with black canvas. The thickness of the layer was maintained at 7 L.m² until the coffee attained a constant dryness with a water content of approximately 25% wet basis (w.b.). Then, the thickness of the coffee layer was doubled. This procedure was repeated until the coffee attained a water content of 11% (w.b.). All procedures for harvesting and processing were performed according to Borém (2008).

Sample preparation

After drying, the samples were packed in paper bags and covered with plastics bags, identified, and stored in chambers at a controlled temperature of 18°C for 60 days. Then, the samples were benefited and the defects were removed in order to standardize the samples and minimize interferences unrelated to the genetic material or the environment. Chemical analysis and roasting were performed in beans retained on sieves 16 and higher (16, 17 and 18/64 inches). For the chemical analyses, raw beans were milled for one minute in an 11A basic mill (IKA, Brazil) by adding liquid nitrogen to facilitate the milling and avoid sample oxidation. After milling, the samples were kept in a freezer at -80°C until analysis.

Roasting and sensory evaluation

All procedures were performed according to the protocol described by the Specialty Coffee Association of America - SCAA (Lingle, 2011). In total, 100 g of each coffee sample was roasted in the Probat TP2 (Curitiba, Brazil) for no longer than 24 h before tasting. The roasting was terminated when the coffee samples attained the desired roasting, which was visually determined using a system of color classification employing standardized discs (SCAA/Agtron Roast Color Classification System; reference color number 65 for milled beans and 55 for whole beans). The temperature and time of roasting were monitored by a thermometer and cronometer, respectively, with the time range of roasting between 8 and 12 min.

Samples were weighed to obtain a pre-determined ratio of 8.25 ±0.25 g per 150 ml of water and then milled in a Mahlkönig Guatemala (Hamburg, Germany). Ten sensory attributes were evaluated by a panel of trained judges and scored on a scale of 10 points according to SCAA (Lingle, 2011).The sensory attributes included the aroma, uniformity, absence of injuries, sweetness, flavor, acidity, body, balance, completion and overall impression. The final sensory grade was generated from the sum of all of the evaluated attributes. For each evaluation, five cups of coffee representing each genotype were evaluated, with one session of sensory analysis for each repetition and a total of three repetitions. Each environment was evaluated separately, and the results of the sensory analyses were scored on a scale representing the quality level in intervals of 0.25 points.

In addition to the final grade obtained from the sensory evaluation, the attributes of aroma, acidity, body and flavor were also analyzed statistically in order to complement the analysis, considering that these are the main attributes responsible for distinguishing the different sensory profiles of the coffee.

Extraction of lipids

The samples containing green coffee beans (~ 0.25 g) were weighed in 1.5 ml microcentrifuge tubes, and 1.0 ml of hexane was added to each one. The tubes were then placed in an ultrasonic bath for 10 min to extract the lipids. Afterwards, the lipids were centrifuged at 6,000 rpm for 2 min. Aliquots of 0.5 µl of each supernatant in a 2.0 ml cryogenic tube were evaporated, hydrolyzed, methylated and analyzed by gas chromatography.

Hydrolysis of lipids In total, 10 mg of the oil was dissolved in a 2.0ml cryogenic tube in 100 µl of an ethanol (95%)/potassium hydroxide 1 mol/L (5%) solution. After vortexing for 10 s, the oil was hydrolyzed (Silva and Ferraz, 2006). After cooling, 400 µl of 20% hydrochloric acid, a spatula tip of NaCl, and 600 µl of ethyl acetate were added. After vortexing for 10 seconds and resting for 5 minutes, an aliquot of 300 µl of the organic layer was removed, placed in a microcentrifuge tube and dried by evaporation to obtain the free fatty acids (Christie, 1989).

Methylation of fatty acids

The free fatty acids were methylated with 100 µl BF3/methanol (14%) and heated for 10 min in a water bath at 80°C, then diluted with 300 µl of methanol and analyzed by gas chromatography.

Gas chromatography

Analyses were performed in a gas chromatograph HP5890 equipped with a flame ionization detector. An SP-2380 column (Supelco; 30 m x 0.25 mm) was used with a temperature gradient of 150°C for 1 min, 7 °C/min to 220°C; with an injector (split 1/50) at 250°C and a detector at 250°C; with hydrogen as the carrier gas (2 ml/min); and with an injection volume of 2 µl. Peak identification was made by comparing standards of methylated fatty acids analyzed on a Supelco37 column. The results regarding the content of fatty acids refer to the harvests from 2010 and 2011. The final content of fatty acids is given as the percentage of dry matter (% d.m.). The following fatty acids were quantified by the normalization area method (Christie, 1989): palmitic (C16: 0), stearic (C18: 0), elaidic (C18: 1T), oleic (C18: 1c), linoleic (C18: 2), arachidic (C20: 0) and linolenic (C18: 3).

Statistical analyses

Four genotypes of arabica coffee were evaluated in three production environments. The three experiments were installed following a randomized block design with three repetitions in the field and plots comprising ten plants. The data for the sensory attributes and the content of fatty acids were initially submitted to analysis of variance (ANOVA), and when significant differences by the F test were detected, the Scott-Knott test was applied at a 5% significance level using the software SISVAR®(Ferreira, 2011).

To better understand the effects of the studied variables, the data were also submitted to multivariate analysis. Discrimination among samples was performed using principal component analysis (PCA) based on interactions between the genotype and the environment; groupings were made according to the sensory attributes and chemical compositions using the software Chemoface (Nunes et al., 2012).

RESULTS AND DISCUSSION

Chemical and sensory composition

Figure 1 presents a typical chromatogram of the analyzed samples of coffee. The peaks from the fatty acids and their respective retention times can be observed. The retention time varied from approximately 4.5 to 8 min. A similar chromatogram was obtained by Martín et al. (2001). Table 3 shows the contents of fatty acids (% m. s.), the grades of the analyzed sensory attributes and the final grade of the sensory analysis for each studied genotype and environment as well as the interaction between these factors.

Linoleic (C18:2) and palmitic (C16:0) acids predominated. Moderate amounts of stearic (C18:0), oleic (C18:1c) and arachidic (C20:0) acids and values lower than 1.6% of linoleic (C18:3) and elaidic (C18:1t) acid were also found (Table 3). The compositions of fatty acids obtained from raw grounds of coffee in the present study are in accordance with the values reported in Bertrand et al.(2008); Jham et al.(2008a); Joët et al.(2010a) and Martín et al. (2001). Most of the analyzed fatty acids did not differ among the studied genotypes ($p>0.05$), except for linoleic acid (C18:2) and arachidic (C20:0) acid (Table 3). Jham et al. (2008) also did not find any significant differences among the content of fatty acids in coffee.

Martín et al. (2001) determined the content of fatty acids in coffee by capillary gas chromatography. The fatty acid contents allowed the differentiation of arabica coffee from canephorus. The fatty acids that primarily contributed to the differentiation of the species were oleic, linolenic, linoleic and myristic acids (Martín et al., 2001). The acid profiles differed statistically among the different environments, except for elaidic (C18:1t) and arachidic (C20:0) acid. Environment 3 (A3) had the most different fatty acid profile (Table 3).

Studies of the influence of climatic conditions on the fatty acid compositions of seeds sensitive to cold, such as coffee, are rare (Joët et al., 2010a). In the present work, there were significant differences in the contents of linoleic and oleic acids among the studied environments. Among all of the studied fatty acids, linoleic acid was the only potential marker for differentiating the coffee samples from the three environments. For some fatty acids, the interaction between genotype and environment was significant, thus allowing the distinction of some genotypes in the studied environments (Table 3). There was also a significant interaction between genotype and environment for all of the sensory attributes (fragrance, flavor, acidity and body) and for the final sensory grade, thus emphasizing the effect of the interaction between genotype and environment on the final quality of the coffee.

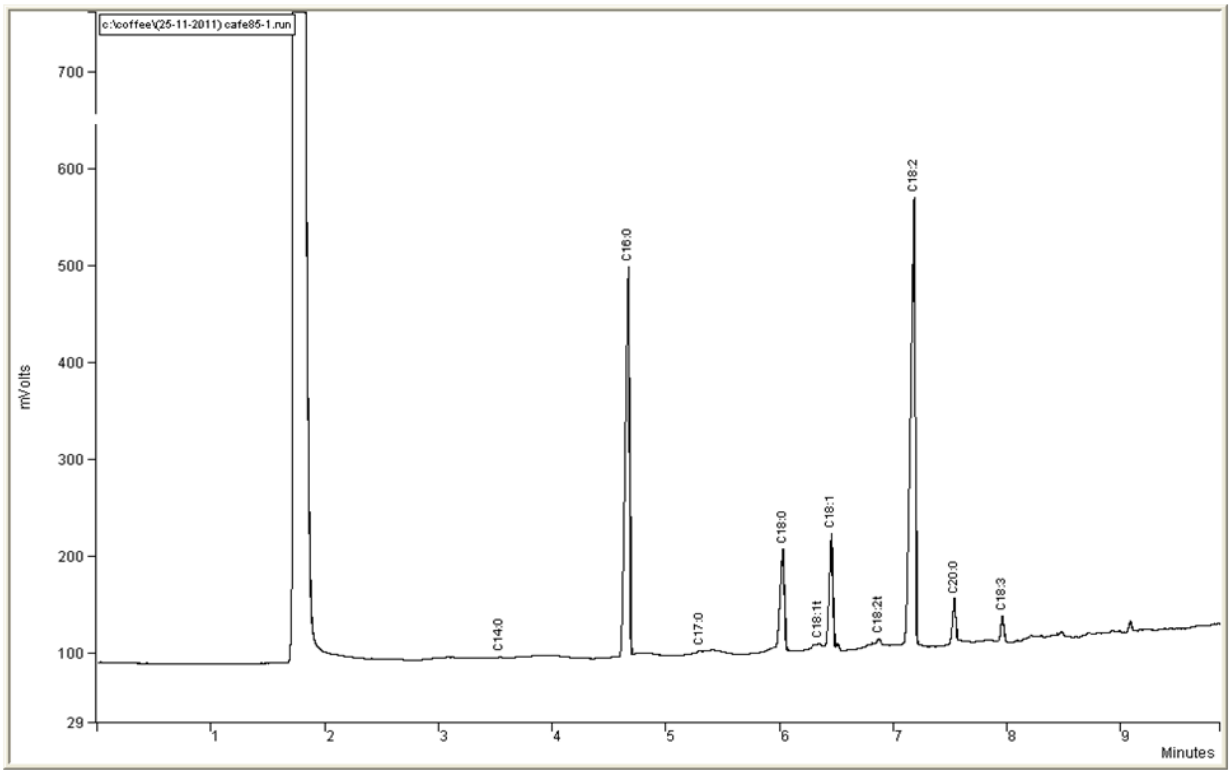

Figure 1. Typical chromatogram of the fatty acids quantified in the analyzed coffees, including palmitic (C16: 0), stearic (C18: 0), elaidic (C18: 1T), oleic (C18: 1c), linoleic (C18: 2), arachidic (C20: 0) and linolenic (C18: 3) acids.

Several factors may influence quality, such as sensory attributes, chemical composition, crop systems, etc. However, all of these factors are encompassed by the general considerations of genetics and plant breeding, which depend on the genetic constitution or genotype, the environmental conditions to which the genotype is subjected, and the interaction between them (Pereira et al., 2010).Because of the complexity of the sensory aspects involved in the characterization of specialty coffees and their relationship to the analyzed fatty acids, the ability of multivariate analysis to interpret the results is limited. Therefore, the data were analyzed by means of PCA, as shown in Figure 2.

Principal component analysis (PCA)

Principal component analysis was applied to interpret the results of the chemical and sensory analyses of the samples of four genotypes (G) of coffee cultivated in three environments (A). A biplot was generated (Figure 2) as a function of the content of fatty acids, the final sensory grade and the sensory attributes.

The first two components explained 73.21% of the variability among the genotypes, among which 46.52% corresponded to the first component and 26.69% corresponded to the second (Figure 2).The data at each point (interaction between genotype χ environment, A_xG_y) are presented as the means of scores, which were calculated based on the three repetitions. Points with similarities in one or more aspects (content of fatty acids and/or sensory attributes) are next on the graph. The representative vectors of each studied variable directed to the points (A_xG_y) detected by the principal components indicate which aspects were determinants for the groupings. The equations of principal components were estimated according to each correlation coefficient presented in Table 4. Such results show, for the first time, a correlation between the sensory characteristics and the composition of fatty acids in the genotypes of Bourbon coffee, which has important implications for the production of specialty coffees.

Three distinct groups were formed when observing the first axis, PC1 (Figure 2): the first, with points on the left part of the biplot (A1G1, A1G4, A3G1, A2G4 and A3G4); the second (II), with points on the central part of the biplot (A3G3 and A3G2); and the third (III), with points on the right part of the biplot (A2G3, A1G3, A2G1, A1G2 and A2G2). The sensory attributes contributed the most in discriminating the groups as a function of the first principal component (Table 4). The coffees in group 1 had a less intense body, fragrance and flavor and lower final sensory grades, contrary to the coffees in group III (Figure 2). Bourbon genotypes were grown in the same

Table 3. Effect of genotype, environment and the interaction between them on the sensory attributes, the final sensory grade, and the content of fatty acids[a] of coffee beans. The means and probability of significance (*F*) were determined by analysis of variance (ANOVA) for the three environments and four genotypes.

Genotype/ environment[b]		C16:0	C18:0	C18:1t	C18:1	C18:2	C20:0	C18:3	Fragrance	Flavor	Acidity	Body	Final
G1		34.47	8.93	1.29	8.94	39.70[a]	3.03[a]	1.51	7.25[a]	7.11[a]	7.25[a]	7.37[b]	80.38[a]
G2		34.53	9.29	1.03	8.66	39.25[b]	3.10[a]	1.60	7.60[b]	7.39[b]	7.38[b]	7.37[b]	81.61[b]
G3		35.02	9.40	1.11	8.67	38.61[b]	3.13[a]	1.55	7.58[b]	7.44[b]	7.43[b]	7.33[b]	81.76[b]
G4		34.03	9.13	1.30	8.54	40.28[a]	2.82[b]	1.54	7.26[a]	7.07[a]	7.15[a]	7.17[a]	79.87[a]
F		0.09	0.05	0.18	0.06	0.00	0.00	0.06	0.00	0.00	0.00	0.01	0.00
A1		35.56[a]	9.29[a]	1.15	8.63[a]	38.37[a]	3.09	1.51[a]	7.36	7.20	7.22	7.24	80.59
A2		35.02[a]	9.37[a]	1.16	8.47[a]	39.33[b]	2.98	1.49[a]	7.52	7.35	7.37	7.36	81.42
A3		32.95[b]	8.91[b]	1.25	9.02[b]	40.68[c]	2.99	1.65[b]	7.38	7.22	7.32	7.33	80.70
F		0.00	0.00	0.70	0.00	0.00	0.20	0.00	0.11	0.20	0.07	0.12	0.12
A1	xG1	35.08[a]	9.00	1.35	8.85	38.58[a]	3.22[a]	1.48	7.09[a]	6.95[a]	7.12[a]	7.25[b]	79.64[a]
	xG2	35.68[a]	9.29	0.87	8.67	38.40[a]	3.12[a]	1.60	7.53[b]	7.25[b]	7.27[b]	7.31[b]	80.93[b]
	xG3	36.73[b]	9.64	1.19	8.42	36.68[b]	3.23[a]	1.47	7.58[b]	7.51[b]	7.46[b]	7.36[b]	81.96[b]
	xG4	34.75[a]	9.27	1.17	8.56	39.83[a]	2.79[b]	1.50	7.22[a]	7.07[a]	7.02[a]	7.05[a]	79.86[a]
F		0.02	0.23	0.29	0.39	0.00	0.00	0.11	0.01	0.01	0.00	0.05	0.03
A2	xG1	34.95	9.10	1.12	8.66	39.81	2.90[a]	1.46	7.62[b]	7.40[b]	7.54[b]	7.40	81.89[b]
	xG2	34.94	9.75	1.10	8.50	38.68	3.13[b]	1.48	7.62[b]	7.45[b]	7.35[b]	7.37	81.76[b]
	xG3	34.98	9.42	0.95	8.60	39.31	3.08[b]	1.55	7.68[b]	7.51[b]	7.45[b]	7.37	82.28[b]
	xG4	35.24	9.22	1.48	8.12	39.50	2.79[a]	1.47	7.19[a]	7.03[a]	7.15[a]	7.30	79.77[a]
F		0.96	0.16	0.21	0.16	0.56	0.04	0.33	0.01	0.04	0.03	0.87	0.02
A3	xG1	33.40	8.71	1.40	9.33	40.72	2.96	1.61	7.04[a]	6.99[a]	7.10	7.47[a]	79.63[a]
	xG2	32.97	8.86	1.12	8.80	40.67	3.06	1.73	7.64[b]	7.48[b]	7.52	7.42[a]	82.15[b]
	xG3	33.35	8.93	1.21	9.00	39.85	3.07	1.62	7.48[b]	7.29[b]	7.36	7.26[b]	81.06[b]
	xG4	32.12	9.16	1.26	8.94	41.50	2.86	1.64	7.36[b]	7.12[a]	7.28	7.15[b]	79.98[a]
F		0.19	0.52	0.73	0.22	0.26	0.42	0.14	0.00	0.04	0.06	0.02	0.02

[a]fatty acids in percentage of dry matter (% d.m.): palmitic (C16: 0), stearic (C18: 0), elaidic (C18: 1T), oleic (C18: 1c), linoleic (C18: 2), arachidic (C20: 0) and linolenic (C18: 3). [b]G1 = Mundo Novo IAC 502/9, G2 – Yellow Bourbon IAC J9, G3 = Yellow Bourbon/Origin CM, G4 = Yellow Bourbon/Origin CM, A1= Lavras, A2= São Sebastião da Grama, A3= Santo Antônio do Amparo. Test at the 5% significance level.

municipalities in which our study was performed, and São Sebastião da Grama showed a high potential to produce high quality coffees (Figueiredo et al., 2013). The possibility of differentiating coffee beans of different genotypes grown in different environments according to their chemical profiles was also highlighted by Taveira et al. (2014).

The fatty acids that presented the strongest correlation with the first component were arachidic (C20:0), elaidic (C18:1t), stearic (C18:0) and palmitic (C16:0) acid (Table 4). The coffee beans with better sensory qualities (group III) were positively correlated with arachidic, stearic and palmitic acids and negatively correlated with elaidic acid. The inverse behavior was observed for the coffees in group I (Figure 2, Table 4).The fatty acid composition depends on several factors,

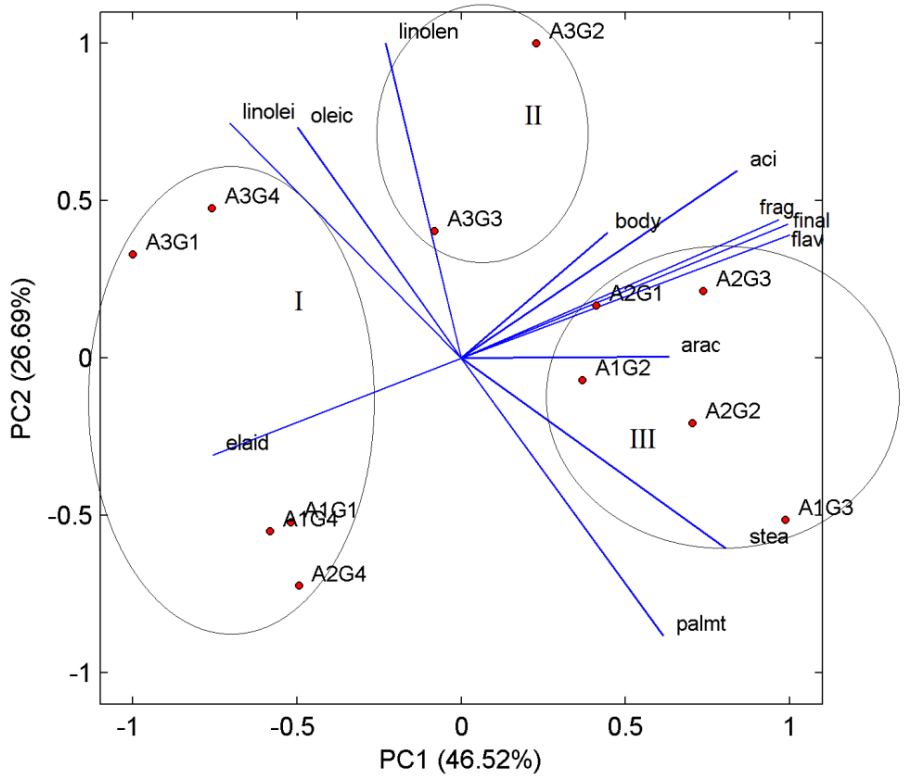

Figure 2. Biplotof the first axes of the principal component analysis for the data from the four genotypes (G) and three environments (A) as a function of the content of fatty acids, the final sensory grade and the sensory attributes. Fatty acids: palmt (C16: 0), stea (C18: 0), elaid (C18: 1T), oleic (C18: 1c), linolei (C18: 2), arac (C20: 0) and linolen (C18: 3). Frag = fragrance, flav = flavor, aci = acidity. G1 = Mundo Novo IAC 502/9, G2 = Yellow Bourbon IAC J9, G3 = Yellow Bourbon/Origin SSP, G4 = Yellow Bourbon/Origin CM, A1= Lavras, A2= São Sebastião da Grama, A3= Santo Antônio do Amparo.

mainly the species and variety (Amaral et al., 2006). Therefore, comparing standards of fatty acids is a useful tool for differentiating coffees (Dagne and Jonsson, 1997). In the present study, arachidic (C20:0), elaidic (C18:1t), stearic (C18:0) and palmitic (C16:0) acids were correlated with the sensory characteristics of coffees, suggesting that they may be possible discriminators of coffee quality.

Stearic acid is a common component in several foods, such as red meats and dairy products. It has many desirable flavor and texture characteristics that are common among long chain saturated fatty acids (Monsma and Ney, 1993). It was previously reported that lack of flavor and flavor imbalance in foods are associated with low levels of saturated fatty acids, such as butanoic and hexanoic acid (Banks et al., 2007).

All of the fatty acids that presented a negative correlation with the first principal component and consequently with the sensory quality were unsaturated fatty acids (elaidic, oleic, linoleic and linolenic acids) (Table 4). The propensity of unsaturated fatty acids to oxidize has been reported; this oxidation leads to the development of rancidity and in many cases to the formation of undesirable aromas, both in vegetal (Jham et al., 2008b) and animal oils (Wood et al., 2008). Our results suggest the association of unsaturated fatty acids with less intense acidity, fragrance, flavor and body, which are highly valued in specialty coffees.

Elaidic acid was the most distinct among the unsaturated fatty acids. Its levels were inversely related to the final sensory grade (Figure 2). All coffees in group I presented higher contents of elaidic acid, less intense sensory attributes, and lower final sensory grades. Elaidic acid is a trans isomer of oleic acid. The trans isomers of unsaturated fatty acids are formed in the frying process as well as during the refining of oils and hydrogenation processes by thermally induced mechanisms (Sebedio et al., 1996). Such compounds are widely studied in relation to technological and nutritional aspects (Stender et al., 2008), but there have been no studies relating elaidic acid to the sensory characteristics of foods. Although it is present in low concentrations in coffees (Table 3), in the present study, the content of elaidic acid was strongly

Table 4. Correlations between the evaluated parameters (fatty acids, final sensory grade and sensory attributes) and the first two principal components.

Parameter	PC1 (46.52%)	PC2 (26.69%)
Fragrance	0.97	0.44
Flavor	1.00	0.39
Acidity	0.84	0.59
Body	0.44	0.40
Final	0.99	0.42
Palmitic	0.62	-0.88
Stearic	0.80	-0.60
Elaidic	-0.76	-0.31
Oleic	-0.50	0.73
Linoleic	-0.61	0.75
Arachidic	0.63	0.00
Linolenic	-0.23	1.00

correlated with the sensory aspects of the evaluated coffees.

The attribute of body was positively correlated with the first principal component (PC1) as well as with the levels of stearic, arachidic and palmitic acids (Figure 2, Table 4). The oil of coffee is mainly composed of triacylglycerols with fatty acids in proportions similar to those found in edible vegetal oils (Speer and Kölling-Speer, 2006). The oils present on the coffee have the capacity to cover the tongue during ingestion, thus providing the oily and creamy mouth feel that is characteristic of the beverage (Illy and Viani, 2005). This study verified the contributions of stearic and arachidic acid to the sensory attribute of body in coffee and to the increase in flavor. The oil of coffee also contains aromatic compounds present in the beverage that may increase or decrease the beverage quality depending on composition (Avelino et al., 2005). Because they are positively correlated with coffee fragrance, stearic and arachidic acid may also be associated with aromatic compounds beneficial to quality. In contrast, elaidic acid may be related to aromatic compounds that are detrimental to the quality of coffee.

Linoleic, oleic and linolenic acid presented more significant contributions to the second principal component (PC2) (Table 4). This second component allowed the differentiation of the points (genotype χ environment) as a function of that fatty acids. Linoleic, oleic, linolenic and palmitic acid allowed the differentiation of environment 3 (A3) from the others. Independent of the evaluated genotype, the coffees cultivated in this environment were positively correlated with the contents of linoleic (C18:2), oleic (C18:1c) and linolenic acid (C18:3) and negatively correlated with the content of palmitic acid (C16:0).

Evaluating the effect of different genotypes and environments and their interaction on the composition of fatty acids in extracts of green coffee beans, Bertrand et al. (2008) observed a high potential of most of the studied fatty acids (palmitic, margaric, stearic, linoleic, linolenic, arachidic and eicosenoic) to differentiate the environments in which the coffees were grown. This ability of fatty acids to discriminate crop origin has also been demonstrated in other fruits and beans, for example, in pistachio (Arena et al., 2007), hazelnut (Amaral et al., 2006) and olive (Ollivier et al., 2006). The influence of climatic conditions during the development of seeds and on the final composition of fatty acids has been reported in many oilseeds (Byfield and Upchurch, 2007; Fofana et al., 2006) and model species of plant. Therefore, it was possible to verify the potential of linoleic, oleic and linolenic acids to both discriminate environment 3 and characterize this environment in relation to the evaluated sensory attributes by determining the negative correlation of these fatty acids with the attributes of acidity, fragrance, body and flavor.For the first time, we showed that sensory attributes are the most suitable potential discriminators of specialty coffees. In addition, assessing the levels of fatty acids allowed us to obtain information about the key compounds that positively or negatively affect coffee beverage quality. Saturated fatty acids, including arachidic, stearic and palmitic acid are potential discriminators of the quality of specialty coffees, indicating better sensory quality. Conversely, unsaturated fatty acids, including elaidic, oleic, linoleic and linolenic acid may be related to coffees with less intense acidity, fragrance, body and flavor.

Inferior sensory quality can also be related to fatty acids, such as elaidic acid, which was present in higher levels in the coffee samples of worse quality. Fatty acids can also potentially differentiate coffee growing environments. In this study, we demonstrate that oleic, linoleic and linolenic acids strongly contribute to the discrimination of the environment in Santo Antônio do Amparo.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Top-dressing nitrogen management decision in potato using the "UFV-80" color chart and SPAD readings

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The chlorophyll meter (SPAD-502) and "UFV-80" color chart can be used to diagnose the nitrogen (N) nutritional status of potato plants to determine the need for fertilizer-N topdressing. The present investigation was focused on how chlorophyll meter and "UFV-80" color chart help in determining optimal N management through real time N application for improving yield and profitable rate in potato crop. The experiments, in two planting seasons (dry and wet), were carried out a split-plot design in a randomized complete blocks with four repetitions. In the whole plot, five N fertilization rates (0, 50, 100, 200 and 300 kg ha^{-1} of N) were applied before planting. In the half of plot area, N fertilization rates (42, 152, 152, 152 and 152 kg ha^{-1} of N at dry season and 133, 93, 69, 58 and 205 kg ha^{-1} of N in wet season) were or did not applied in top-dressing, depending on the UFV-80 color chart reading and SPAD chlorophyll meter reading in the terminal leaflet of the fourth leaf, at 21 day after the plant emergency (DAE). At 77 and 70 DAE for the dry and wet seasons, respectively, the plants were harvested to determine the commercial tuber yield. The SPAD chlorophyll meter reading and "UFV-80" color chart can be used to determine N nutritional status in potato crops in both planting seasons. Both planting seasons, the commercial production was not influenced by the N fertilization rates in pre-planting and top-dressing.

Key words: *Solanum tuberosum* L. fertilization, diagnosis, profitable rate.

INTRODUCTION

Nitrogen (N) is one of the most important nutrient since it has a positive effect on chlorophyll concentration, photosynthetic rate, plant height and dry matter accumulation and higher potato tuber yields (Sinfield et al., 2010; Tremblay et al., 2011), and increase the chance of nitrate contamination on surface and ground water (Muñoz-Huerta et al., 2013). Due to the low availability of N in the superficial layer of the soil and high demand by N of potato plants makes the N one of the most limiting nutrients for plant growth and increase tuber yield.

Potato plants have a high demand on soil nutrients to obtain maximum tuber yield. Proper N management is one of the most important factors required to obtain high

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tuber yields. However, soil N supply is often limited. Then, optimum N fertilization rate applied in top-dressing to obtain high tuber yields is necessary. Until now, many researchers have worked with the SPAD chlorophyll meter, and proposed that SPAD readings could estimate leaf N concentration and potato yield prognosis. In this study, we hypothesized that SPAD chlorophyll meter reading and "UFV-80 color chart" in potato leaves can be used to determine N fertilization rates applied in top-dressing.

Various farmers increase the amount of N fertilizers in order to achieve better crop yield without any criteria, fact that can cause unnecessary expenditure on the part of farmers or deficiency this nutrient in potato plants. Then, according of this hypothesis, it is necessary to have methods for the evaluation of N nutritional status, preferably in real time (Fontes and Araujo, 2007; Busato et al., 2010).

Several diagnostic methods can be used to determine N nutritional status in crops, such as destructive and non-destructive techniques (Fontes, 2001; Muñoz-Huerta et al., 2013). N concentration and critical N dilution curve have been used as destructive techniques. Non-destructive techniques based on leaf reflectance have been proposed as robust alternative and simple methods for pigment quantification in leaves (Silva et al., 2009; Busato et al., 2010; Coelho et al., 2012). The most commonly used equipment for the evaluation of N nutritional status in real time are: the analysis of the N-NO_3 content in the sap of the petiole using microelectrode (Brink et al., 2002) and the indicator strip selected for nitrate (Parks et al., 2012). Additionally, SPAD chlorophyll meter readings have been shown to correlate well to laboratory-extracted chlorophyll (León et al., 2007), and to N status in culture of lettuce (Fontes et al., 1997), wheat (Tremblay et al., 2010), potato (Gil et al., 2002; Silva et al., 2009, Busato et al, 2010), tomato (Fontes and Araujo, 2006) and rice (Cabangon et al., 2011). Other equipment have been used as a tool to evaluate N nutritional status are Dualex® (Coelho et al., 2012) and the digital camera (Li et al., 2010; Wang et al., 2014). Furthermore, the chlorophyll meter conducts fast readings without destroying leaves; however, it has relatively elevated costs for the small farmer (Fontes and Araujo, 2007).

The SPAD chlorophyll meter readings can be an indication of the need for N application in top-dressing, since the critical level below critical is known in which the plant would deprive of this element, and other factor should also be considered to affect the SPAD chlorophyll meter readings, such as: edafoclimatic conditions, attack of plagues, diseases and weeds, growth status and plantation system (Arregui et al., 2000). Due to the high price of equipments for plant N status estimation limits its use by individual income-poor farmers (Yang et al., 2003). Another tool faster and non-destructive for plant N status estimation is a leaf color chart, in analogy to the one originally proposed for Asian rice farmers (Yang et al., 2003; Nachimuthu et al., 2007). The high price of SPAD limits its use by individual income-poor farmers. The color chart is easy, simple to use and has low cost; it is an alternative tool to the chlorophyll meter to establish N nutritional status at any given moment.

In a research conducted by Fontes and Silva (2006) culminated in the development of a color chart for potato crops, not seen before anywhere. This color chart was proposed to growth Monalisa and was called "UFV-80", a tribute to the 80th anniversary of the Federal University of Viçosa.

Initial studies with Monalisa crop have shown that the color green number 4, determined in the terminal leaflet of the fourth leaf completely expanded from the apex at the moment of clumping, it is the "critical color" or when it is not necessary to apply the N in top-dressing (Fontes and Silva, 2006). This recommendation was not tested in the field. However, information on chlorophyll meter and leaf color chart to diagnose N nutritional status of potato plants and determine the need of N in topdressing are scarce. Therefore, the present investigation was focused on how chlorophyll meter and "UFV-80" color chart help in determining optimal N management through real time N application for improving yield and profitable rate in potato crop.

MATERIALS AND METHODS

Two experiments were conducted in the field. One experiment was performed in dry season (105 mm of rainfall), from June 26th to September 26th of 2008, cold weather, with supplementary irrigation; and another experiment was performed during hot and rainy season (1,075 mm of rainfall), from November 4th of 2008 to February 4th of 2009. The experiments were performed in the field of adjacent areas of the Federal University of Viçosa (UFV), in Viçosa, Minas Gerais State, Brazil, located at 693 m altitude, latitude 20°45′ S and longitude 42°51′ E. Fields were located on a Cambic Red-Yellow Argisol clay (Typic Hapludult) (Embrapa, 2006) which characteristics are 1.50 and 1.40 mg kg^{-1} of N-NO_3, 4.52 and 4.21 dag kg^{-1} of organic matter, 170 and 175.4 mg dm^{-3} of available P, 165 and 163 of available K mg dm^{-3}, 4.57 and 5.70 $cmol_c$ dm^{-3} of exchangeable Ca, 0.77 and 0.81 $cmol_c$ dm^{-3} of exchangeable Mg, 0.00 and 0.00 $cmol_c$ dm^{-3} of exchangeable Al, 280 g kg^{-1} and 280 g kg^{-1} of sand, 610 g kg^{-1} and 610 g kg^{-1} of clay and 110 g kg^{-1} and 110 g kg^{-1} of silt, at a layer of 0-20 cm depth, in dry and wet seasons, respectively.

The experiments were carried out in a split-plot design, in a randomized block design with four replications. In both planting seasons, five N fertilization rates (0, 50, 100, 200, and 300 kg ha^{-1} of N) were allocated in the plots, and the five N fertilization rates were applied in top-dressing in dry season (42, 152, 152, 152, and 152 kg ha^{-1} of N) and in wet season (133, 93, 69, 58, and 205 kg ha^{-1} of N). The potato plants were fertilized with ammonium sulfate (20% N). Experiments performed in both growing season, half of the plot was fertilized with N and the other half did not receive the N fertilizer. This was based on the study of true/false diagnosis describe by Beverly and Hallmark (1992).

The SPAD chlorophyll meter readings and "UFV-80 color chart" were carried out in the morning, between 8:00 and 11:00 a.m at 21 days after the completely emergency (DAE). On the same day, the N fertilization rates applied in top-dressing were performed. Twenty-

two days after plant emergence (DAE), the plants were hilled by hoeing soil up around the plant stems. The N fertilization rates applied in top-dressing were calculated based on SPAD chlorophyll meter reading according to results obtained by Silva et al. (2009). The SPAD chlorophyll meter readings obtained in this experiments, color of terminal leaflet of the fourth leaf completely expanded from the apex, measured with the "UFV-80" color chart, and critical level of SPAD chlorophyll meter readings are shown in Table 1.

The decision to fertilize (yes or no) with N was based on the "UFV-80 color chart" (Fontes and Silva, 2006). According to authors, images of terminal leaflet of the fourth leaf completely expanded from the apex were captured using a digital still color camera (Nikon 135 TL) with high resolution. Soon after, the images were digitalized in scanner, model HP 1315, with resolution of 300 dpi. All images from the experiments were saved in JPEG and stored in CorelDRAW 12 software. With this program, images were attributed degree of colors from 1 to 5 to terminal leaflet of the fourth leaf completely expanded from the apex from each subplot, using the color scale, in which 1= yellowed-green, 2= light green, 3= opaque green, 4= dark green and 5= very dark green.

Immediately before N application in top-dressing, SPAD index was determined using chlorophyll meter SPAD-502 to calculate N fertilization rates in top-dressing at 21 DAE (Table 1). The SPAD chlorophyll meter readings were measured in the terminal leaflet of the fourth leaf completely expanded from the apex, in four plants of each subplot. Each leaf five readings were performed, from which the average was calculated.

The ammonium sulphate (20% of N) was the source of N applied in the experiments. The application of N fertilization rates in pre-planting was made in the planting grooves. The N fertilization rates applied in top-dressing was distributed next to the plants, throughout the rows, at 21 DAE, making the clumping afterwards.

Plots (5 m × 3 m) were consisted of four rows with 20 plants each, spacing of 0.75 m between rows and 0.25 m between plants in the row. The two outer rows and the outermost two plants at each end of the inner rows were used as borders, totalizing 32 useful plants per plot. Half of the subplot was fertilized with N and the other half did not receive N fertilizer.

The soil was prepared with a mouldboard plow and two passages of leveling blade. In both planting seasons, the quantity of fertilizers applied in the planting grooves were: 2000 kg ha^{-1} of simple super phosphate (18% of P_2O_5), 500 kg ha^{-1} of potassium chloride (60% of K_2O), 200 kg ha^{-1} of magnesium sulphate (9% of Mg), 10 kg ha^{-1} of borax (11% of B), 10 kg ha^{-1} of zinc sulphate (22% of Zn), 10 kg ha^{-1} copper sulphate (24% of Cu) and 250 g ha^{-1} sodium molybdate (39% of Mo). During the experimental period, pulverization was made for plague control and diseases, as needed.

The irrigation, when needed, was made using an aspersion system. The irrigation blade was established using the evaporation-transpiration estimate and the culture coefficient (Kc). The evaporation-transpiration estimate was calculated by the Penman-Monteith method. The soil was kept with moisture close to field capacity.

The precision in diagnosis was verified following the procedure cited by Fontes (2001) and adapted by Beverly and Hallmark (1992). Basically, the method was based on the comparison between the decision of fertilizing or not, and the plant's response to the application of N. In the proposition, the criterion of false (F) or true (V) incidence of diagnosis was used.

One week after complete drying of the areal part, which occurred at the 77^{th} and 70^{th} day after the emergency, for dry and wet seasons respectively, the plants in the useful area of each subplot were harvested to determine the production of commercial tubers.

Economic analysis of the production was also made; prices of tubers and ammonium sulphate were also checked in several establishments in the city of Viçosa, Minas Gerais State, in both planting seasons. The prices of potato paid to the farmer varied from R$ 0.80 and 1.00 kg^{-1}, and the N fertilizer had the purchase values from R$ 6.60 and 3.0 kg^{-1}, in dry and wet seasons, respectively. The production costs were based on Fontes (2005) that considered the value of R$ 10.000,00 ha^{-1} in both planting seasons.

The data was submitted to variance analysis (ANOVA) and regression. The regression models were chosen based on the biological occurrence of the response, the significance of the regression coefficients, using the "t"-test up to 10% of probability, and on the equation's coefficient of the determination value. Tukey's multiple range test ($P<0.05$) was used to separate the means when the ANOVA *F-test* indicated a significant effect on the treatment. The statistical software used to perform the statistical analysis was the System for Statistical and Genetic Analysis (SAEG version 9.1).

RESULTS AND DISCUSSION

There was a significant correlation between the SPAD chlorophyll meter readings performed in terminal leaflet of the fourth leaf completely expanded from the apex with "UFV-80 color chart" for both growing seasons. These results are presented in Figure 1. In this figure, it is shown a good and high correlation coefficient for both planting seasons ($r=0.96$ and $r=0.97$, for the dry and wet seasons, respectively) regardless of the N fertilization rates. Then, "UFV-80 color chart" could substitute for SPAD readings in estimating leaf N status in leaf potato plants. These results are in accordance with Cabangon et al. (2011), that reported $r=0.84$, in two years of crops, in rice crops, as well as the results presented by Yang et al. (2003). These authors reported that for diagnosing leaf N status in real-time N management, leaf color chart and SPAD readings can be used directly for determining the timing of N topdressing without adjusting for specific leaf weight (dry matter/leaf area), and if leaf color chart or SPAD readings is used to estimate N (per unit dry weight), specific leaf weight has to be considered.

Although in both planting season the critical color of the leaves obtained value 4, the values of SPAD chlorophyll meter readings were 45.3 and 38.9 in both dry and wet seasons, respectively (Table 1). In wet season, SPAD chlorophyll meter readings were 14.5% lower when compared to dry seasons. This probably occurred due to the higher volume of precipitated rain in the wet season, which may have led to losses of N by leaching. Cabangon et al. (2011) reported that for rice crop the critical values of 3 and 3.5 for leaf color chart corresponded SPAD chlorophyll meter readings of 35 and 38, respectively. Furthermore, the foliar color chart as well as the SPAD chlorophyll meter readings can diagnose N nutritional status of the potato in a practical and non-destructive manner.

The SPAD chlorophyll meter readings of 47.3 is above the SPAD chlorophyll meter readings of 44.9 (N fertilization rates of 144.3 kg ha^{-1} of N in pre-planting) found by Gil et al. (2002) during the winter in Viçosa, Minas Gerais State. Difference in N fertilization rates and the number of SPAD chlorophyll meter readings in the terminal leaflet of the fourth leaf completely expanded

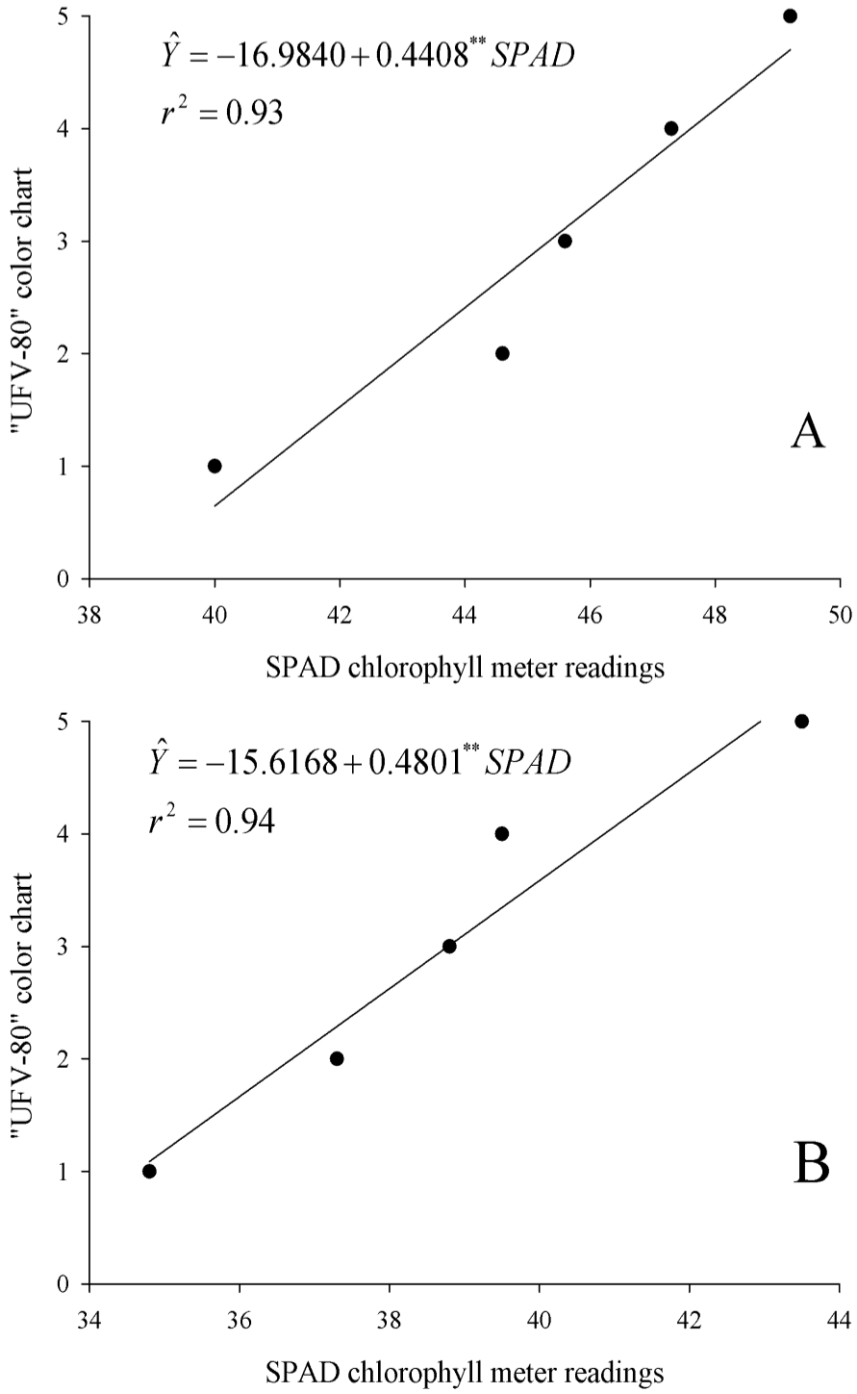

Figure 1. Relation between the "UFV-80" color chart and SPAD chlorophyll meter readings in the terminal leaflet of the fourth leaf completely expanded from the apex of the 'Monalisa' potato, at 21 DAE, in the dry (A) and wet (B) seasons. **: Statistically significant by the 't' test (P<0.01).

from the apex could have caused a variation in the data of the present work in comparison to the results from Gil (2001). However, Arregui et al. (2000) suggests the reading to be made in each leaflet of the potato without reaching the main nerve of the leaf, since it can cause variation in SPAD chlorophyll meter readings. In this study, SPAD chlorophyll meter readings was performed in leaflet of terminal leaflet of the fourth leaf completely expanded from the apex, in which each leaflet was measured four times, totaling 16 readings with SPAD

Table 1. Nitrogen (N) fertilization rates applied in pre-planting, SPAD chlorophyll meter readings obtained, color of terminal leaflet of the fourth leaf completely expanded from the apex measured with the "UFV-80" color chart (LCC), critical level (CL) of the SPAD chlorophyll meter readings, and N fertilization rates applied in top-dressing in the "Monalisa" cultivar at 21 days after the emergency, during dry and wet seasons.

N rates in pre-planting (kg ha^{-1})	SPAD Index at 21 DAE	LCC	CL SPAD at 21 DAE	Difference between the index and CL SPAD	N rates in top-dressing (kg ha^{-1})
			Dry Season		
0	40.0	1	42.1 *	2.1 #	42 &
50	44.6	2	42.1	- 2.5	152
100	45.6	3	42.1	- 3.5	152
200	47.3	4•	42.1	- 5.2	152
300	49.2	5	42.1	- 7.1	152
			Wet Season		
0	34.8	1	43.1	8.3	133
50	37.3	2	43.1	5.8	93
100	38.8	3	43.1	4.3	69
200	39.5	4	43.1	3.6	58
300	43.5	5	43.1	- 0.4	205

• Critical color of the terminal leaflet of the fourth leaf completely expanded from the apex, according to (Fontes and Silva, 2006). * Critical level SPAD obtained in Silva et al. (2009). # Negative value indicates no need for N application, however, applied for the true/false study (Beverly and Hallmark, 1992). & The quantity of N applied in top-dressing followed the recommendations of Fontes (1999) (152 kg ha^{-1} of N), except when the dose 0 kg ha^{-1} of N was applied, according to Silva et al. (2009) 1 unit of SPAD is equal to 20 and 16 kg ha^{-1} of N in the dry and wet seasons, respectively.

Table 2. Potato marketable yields (g/plant) and economic analysis of "Monalisa" cultivar as influenced by N fertilization rates applied in pre-planting and in top-dressing in dry and wet seasons.

N rates in pre-planting (kg ha^{-1})	Potato marketable yields (g/plant		Expenditure with N (R$ ha^{-1})	Total cost	Gross profit	Net profit
	With	Without		(R$ ha^{-1})		
			Dry season			
0	681.75^{a}*	677.50^{a}	0.00#	8,746.00	28,906.40	20,160.40
50	772.75^{a}	792.25^{a}	330.00	9,076.00	33,802.66	24,726.66
100	685.00^{a}	823.75^{a}	660.00	9,406.00	35,146.66	25,740.66
200	732.75^{a}	798.75^{a}	1.320.00	10,066.00	34,080.00	24,014.00
300	619.75^{a}	585.50^{a}	1.980.00	10,726.00	24,981.33	14,255.33
			Wet season			
0	554.25^{a}	462.00^{a}	0.00	8,746.00	24,640.00	15,894.00
50	620.25^{a}	551.75^{a}	150.00	8,896.00	29,426.66	20,530.66
100	627.75^{a}	551.75^{a}	300.00	9,046.00	29,426.66	20,380.66
200	660.00^{a}	552.75^{a}	600.00	10,030.00	29,480.00	19,450.00
300	645.75^{a}	563.75^{a}	900.00	10,570.00	30,066.66	19,496.66

*Averages in the lines followed by the same letter do not differ statistically between them by the Tukey test ($P<0.05$). # The economic analysis was only made when N fertilizer was used in pre-planting, seen that there was no significant statistical difference when the N was applied in top-dressing.

chlorophyll meter readings in each subplot, however Gil et al. (2002) conducted only 6 readings per subplot.

For both planting seasons (dry and wet), there was no interaction between N fertilization rates applied in pre-planting and in top-dressing over the productivity (Table 2). It was noticed that each N fertilization rates in pre-planting, the commercial production did not differ statistically, in relation to the N fertilization in top-dressing. Therefore, the N applications in pre-planting as well as the N applications in top-dressing did not influence the production of commercial tubers of potatoes.

In Table 2, the expenditure with N and the total cost has increased linearly with the applied N fertilization rates

Table 3. Nitrogen (N) fertilization rates applied in pre-planting to verify the diagnosis of N nutritional status (DENN) of the potato plant at 21 DAE, need for application of N in top-dressing (NANC), response of the commercial production, false or true and their implication, in the dry and wet seasons.

N rates in pre-planting (kg ha^{-1})	DENN	NANC	Production response	False or true	Implications
		Dry season			
0	Deficient	Yes	No *	F + [#]	FAD [&]
50	Non deficient	No	No	V -	NND
100	Non deficient	No	No	V -	NND
200	Non deficient	No	No	V -	NND
300	Non deficient	No	No	V -	NND
		Wet season			
0	Deficient	Yes	No	F +	FAD
50	Deficient	Yes	No	F +	FAD
100	Deficient	Yes	No	F +	FAD
200	Deficient	Yes	No	F +	FAD
300	Non Deficient	No	No	V -	NND

*Values of commercial production of the potato in Table 2. [#] Adapted from Beverly and Hallmark (1992). [&] FAD=Fertilizer applied unnecessarily and NNF=No need for fertilizer.

in pre-planting, in both planting seasons. Regarding the variables of gross and net profits, in dry season the dose that provided the highest gross and net profits was the dose of 100 kg ha^{-1} of N in pre-planting. However, in wet seasons the best N fertilization rates were 300 and 50 kg ha^{-1} of N for gross and net profits, respectively.

Compared to the average yield in Minas Gerais State in 2007, which was 29.90 ton ha^{-1} (Agrianual, 2009), the values found in the present work were superior, with an average commercial yield of 38.23 and 30.88 ton ha^{-1} in dry and wet seasons, respectively. This is due to the accounting of Agrianual (2009) of yields in every region in Minas Gerais State, using several N fertilization rates in different planting seasons, many crops of potatoes, distinct soils and seeds.

The average commercial yield was practically 20% lower in the wet season, when compared to the dry season (Table 2), probably due to more elevated temperature conditions, which has favored a larger growth of the vegetative part, in detriment to the productive part of the potato, the tuber. According to Ewing (1997), temperature higher to 20°C is a limiting factor to the tuberization, the average temperature in the experiments during dry and wet seasons were 17 and 22°C, respectively. Furthermore, Parr et al. (2014) reported that the effect of different planting dates on infestation and damage of various sweet potato cultivars can be a sustainable way to obtain good yields coupled with low Cylas sp. damage.

According to Table 2, in both planting seasons, it is only recommended to apply N in pre-planting, despite the savings by the farmer when N is only applied in the foundation, inconveniences might occur if only this application is made. The reason is that the application of all N in pre-planting makes corrections impossible according to the current requirements of the crops during the entire plantation period (Olivier et al., 2006), consequently, it is not possible to manage the application of the N in top-dressing.

The answer of the culture yield to a determined nutrient can be affected at the planting season, inefficient application of the fertilizer, absorption and interaction of the nutrients, plant's characteristic, or stress caused by draught (Beverly and Hallmark, 1992).

The highest net profit of the potato (R$ 25.740,66 ha^{-1}) became evident in dry season due to the pre-planting application and in top-dressing of 100 and 0 kg ha^{-1} of N, respectively, which provided the production of 823.75 g plant^{-1}. However, it did not differ when applied N rates of 152 kg ha^{-1} of N in top-dressing, obtaining the yield of 685 g plant^{-1} (Table 2). In wet season, the application in pre-planting and in top-dressing was 50 and 0 kg ha^{-1} of N, respectively, providing the yield of 551.75 g plant^{-1}, giving the highest net profit (R$ 20.530,66 ha^{-1}), in this planting season. These differences are due to productions, cost of fertilizer and value of the potato paid by the rural producer, in which prices fluctuate every planting season.

In Table 3 in dry season, only N rates of 0 kg ha^{-1} of N in pre-planting caused a deficient N nutritional status diagnosis (DENN), considering that the difference between the index and the SPAD critical level was positive (Table 1), thus the need for the application N fertilizer, however, due to the false/true study, N in top-dressing was applied only in half of the plot (with or without N in top-dressing) (Table 2), nevertheless, no difference was seen between the commercial production in dry season, with or without application of N in top-

dressing, implying unnecessary application of fertilizer.

In wet season, only N rates of 300 kg ha^{-1} of N in pre-planting caused a non-deficient N nutritional status diagnosis (DENN) (Table 3), considering that the difference between the index and the SPAD critical level was negative (Table 1), therefore, there would be no need to apply N fertilizer, but, due to the false/true study, remained only one half of the plot (subplot) without fertilizer and the other half the N was applied (Table 2), there was no statistical difference between the commercial production of these subplots.

It was verified that there was no deficient DENN, application of fertilizer (yes), production response (yes) and true (V +), however, according to Beverly and Hallmark (1992) it would imply in a decrease in yield (Table 3). This occurred, probably due to the use of a very high critical level (Table 1), which caused a low probability of response to the nutrient and no frequency of diagnosis V + (Beverly and Hallmark, 1992).

The use of a deficient DENN, that is, very low rate of N in the terminal leaflet of the terminal leaflet of the fourth leaf completely expanded from the apex, cause a high probability of response to the N, therefore, bringing lower frequency of F + and V - diagnosis. However, there will be a higher frequency of F – and V + diagnosis. This can reduce the opportunity of fertilize and consequently elevate the production (Fontes, 2001).

Conclusions

The 'UFV-80' color chart for Monalisa potato can be used to diagnose N nutritional status of the potato plant in both planting seasons. The higher net profit was obtained when applied 100 kg ha^{-1} of N in pre-planting plus 152 kg ha^{-1} of N in top-dressing for the dry season and 50 plus 93 kg ha^{-1} in pre-planting and top-dressing, respectively, in wet season. The commercial production was not influenced by the N fertilization rates in pre-planting and in top-dressing in dry and wet seasons.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Accelerated ageing test to study the relative storage potential of hybrid sunflower-RSFH-130 (*Helianthus annuus*)

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Deterioration of oil seeds is more rapid when compared to the other crop seeds, the deterioration will depend on the chemical composition of the seed and also storage environment. Accelerated ageing (a.a) is one test to study the storage potential of the seeds where the seeds have been exposed to the artificial ageing conditions such as the different relative humidity and temperature. Sunflower hybrid seeds are exposed to different temperatures and relative humidity against the time. These accelerated aged seeds are stored for about 2 to 4 months and even after the 4 months of storage the seeds of the treatment T-1(a.a at 90 to 95% RH and 41°C for 48 h) showed the minimum seeds certification standards compared to all the treatments. There is a significant reduction in the germination as the accelerated ageing increases which corresponds to the increase in the temperature, relative humidity and timing. Seeds can be stored at this extreme temperature and relative humidity for about 4 months without losing the minimum seed certification standard germination.

Key words: Sunflower, RSFH-130, accelerated ageing, storage.

INTRODUCTION

Cultivation of the sunflower has been increased over the years due to its wide adoptability to climatic conditions and also its importance in consumption of the oil. The supply of the quality seeds to the farmers is also one of the main important factors the problem in the sunflower seeds is low storability and also the storage environment is also contributing for the storage of the sunflower seeds. The two most important environmental factors influencing the rate of deteriorative processes in seed ageing are the relative humidity of the air, which controls seed moisture content, and the temperature (McDonald, 1999). Accelerated ageing (a.a) technique is a widely used tool to test the seed quality. This ageing test of seed vigor can give better indications of probable field emergence for vegetable crop seeds than germination and growth tests (Pandey et al., 1999) a.a. Initially

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proposed as a method to evaluate seed storability and later this test is considered as a rapid, inexpensive and simple technique study the relative storability of the seeds (Burris, 1980) a.a techniques have great potential for understanding the mechanism of ageing and associated deterioration processes of seeds (McDonald, 1999). Meanwhile, the process of deterioration under accelerated ageing conditions are essentially similar to those under normal conditions where we can predict the rate of deterioration of the seeds through the a.a test so that the relative storability of the seeds can be estimated (Aiazzi et al., 1996; Goel et al., 2002).

The sensitivity of seeds to accelerated ageing is dependent on temperature and on their moisture content. At a constant temperature, loss of seed viability is faster with increasing moisture content, seed moisture and storing temperature plays a key role in seed longevity (McDonald, 1999). At the cellular level, seed ageing is associated with various alterations including loss of membrane integrity, reduced energy metabolism, impairment of RNA and protein synthesis, and DNA degradation (Kibinza et al., 2006). During storage, a number of physiological and physicochemical changes occur, termed ageing (Sisman, 2005). The rate at which the seed ageing process takes place depends on the ability of seed to resist degradation changes and protection mechanisms, which are specific for each plant species (Sisman and Delibas, 2004; Mohammadi, 2011). In seed ageing damage at cellular membranes, decrease in mitochondrial dehydrogenises activities, chromosomal aberrations and DNA degradation increases. The sunflower hybrid RSFH-130 seeds are subjected to accelerated ageing test to know the relative storability of the seeds.

MATERIALS AND METHODS

The experiment was carried out by using the sunflower hybrid RSFH-130 which is one of the potential hybrids released from the University of Agricultural Sciences, Raichur, Karnataka, India. The seed production was taken in one of the Agricultural research station on the university the seeds are dried to the safe moisture storage. Seeds were sterilized using 5% sodium hypochlorite solution for 3 min and rinsed thoroughly in distilled water, then seeds were dried at room temperature for overnight in the laboratory and kept in room temperature until the study (Khan et al., 2003).

Accelerated ageing treatment

The sunflower hybrid RSFH-130 seeds were given treatment to study the accelerate ageing with the following treatments T0(Control No AA), T1(a.a at 90 to 95% RH and 41°C for 48 h),T2(a.a at 90 to 95% RH and 41°C for 96 h), T3(a.a at 90 to 95% RH and 41°C for 144 h), T4(a.a at 90 to 95% RH, 41°C 192 h) and T5(a.a at 90 to 95% RH and 41°C for 240 h). The accelerated ageing was done with the accelerated ageing chamber for each treatment about one kilogram of hybrid seeds were treated and after the seed treatment initial observations such as the germination, vigour and field emergence was recorded and stored in room temperature for the storage studies after the treatment.

Germination

Germination test was conducted using between paper (BP) method with 100 seed per 3 replication and then the germination paper was kept in between plastic sheets to maintain the relative humidity. The sheets were rolled and placed vertically in a plastic beaker in a germinator (ISTA, 1993). Seeds were considered as germinated when radicals reached at 5 mm length.

Germination percentage = (No. of germinated seeds / Total No. of seeds sown) X 100)

Seedling vigor index

The seedling vigor index (SVI) = (Seedling length (cm) X Germinated percentage) / 100

Field emergence

The treated seeds at sown in the field to know the field emergence 100 seeds for each of the treatments are sown and counted after 10 to 15 days and expressed in percentage.

Storage

The treated seeds are stored for 2 to 4 months in the normal room temperature and studied the storability of the seed material.

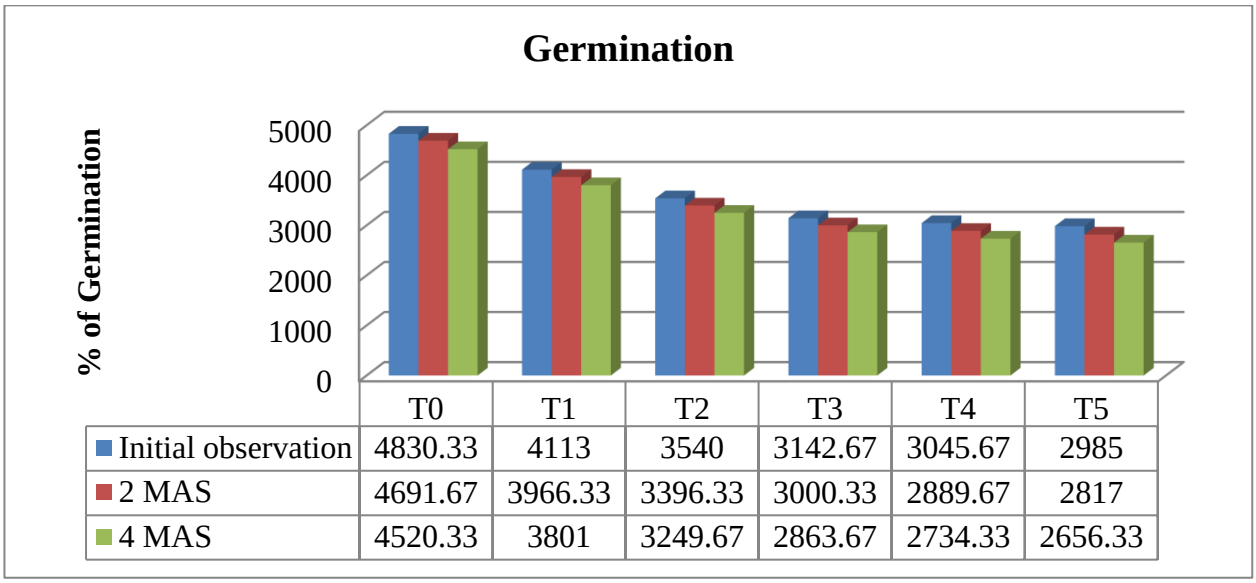
Statistical analysis

The experiment was statistically analyzed for its significance with complete randomized design.

RESULTS AND DISCUSSION

Germination

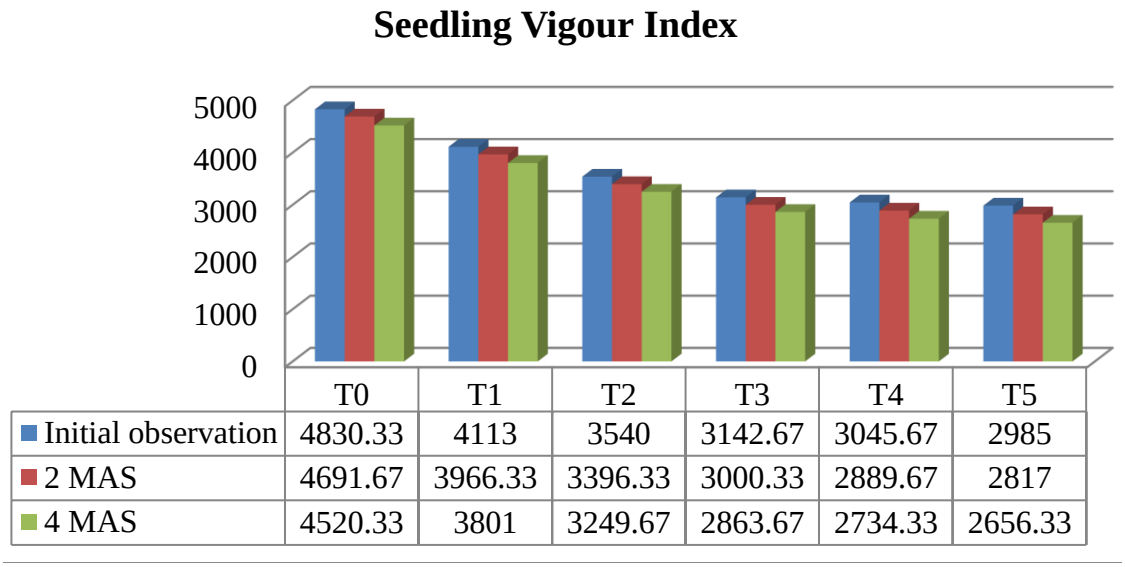
The initial germination percentage showed that the as accelerated ageing in hour's increases the germination percentage has been decreases. The possible reason of this reduction might be the lowering of biochemical activities in seeds. Ageing have damaging effect on enzymes that are necessary to convert reserve food in the embryo to usable form and ultimately production of normal seedling (Iqbal et al., 2002). Alternatively, the reduction in germination might be due to degradation of mitochondrial membrane leading to reduction in energy supply necessary for germination (Gidrol et al., 1998). The decline in shoot length, root length and seedling vigor index might be attributed to DNA degradation with ageing which leads to impaired transcription causing incomplete or faulty enzyme synthesis essential for earlier stages of germination (Kapoor et al., 2002) (Table 1).It also shows that during the storage period the germination was decreased significantly. The treatment T2 is showing the highest germination (77.36%) which is



	T0	T1	T2	T3	T4	T5
Initial observation	4830.33	4113	3540	3142.67	3045.67	2985
2 MAS	4691.67	3966.33	3396.33	3000.33	2889.67	2817
4 MAS	4520.33	3801	3249.67	2863.67	2734.33	2656.33

2 MAS: 2 Months after storage, 4MAS: 4 Months after storage.

Figure 1. Effect of accelerated ageing on the germination.



	T0	T1	T2	T3	T4	T5
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2 MAS: 2 Months after storage, 4MAS: 4 Months after storage.

Figure 2. Effect of accelerated ageing on the vigour index.

statistically superior and significant (Figure 1).

Seedling vigour index

Testing of vigour index is the measure in which seeds can produce the normal seedlings in the adverse situation in the field. The vigour index is another aspect related to viability found to decrease gradually in all the treatments combinations with increase in storage period. The a.a and vigour are negatively correlated to each other as the a.a increases the viability decreases (Figure 2).

This result could be explained by when sunflower RSFH-130 seeds are submitted to accelerated ageing for 2 days, the plasma membrane remains undamaged.

Table 1. Showing the results of accelerated aged seeds after the 4 months after the storage.

Treatment	Germination			Vigour Index			Field emergence		
	Initial observation	2 Months after storage	4 Months after storage	Initial observation	2 Months after storage	4 Months after storage	Initial observation	2 Months after storage	4 Months after storage
T0	96.74(79.60)	93.43(75.15)	90.0(71.58)	4830.33	4691.67	4520.33	91.67	89.67	86.33
T1	84.01(66.43)	80.7(63.94)	**77.36(61.59)**	4113.00	3966.33	3801.00	75.00	73.00	69.33
T2	73.97(59.33)	69.67(56.59)	66.36(54.55)	3540.00	3396.33	3249.67	66.33	62.67	60.33
T3	66.32(54.53)	62.68(52.35)	59.68(50.58)	3142.67	3000.33	2863.67	63.67	56.33	53.33
T4	61.34(51.55)	59.01(50.19)	57.0(49.02)	3045.67	2889.67	2734.33	57.67	55.00	51.67
T5	61.01(51.36)	57.0(49.02)	53.33(46.91)	2985.00	2817.00	2656.33	54.00	50.67	47.67
Average	75.71(60.47)	71.71(57.87)	68.26(55.7)	3609.44	3406.22	3304.22	68.66	64.56	61.44
SEm±	1.01	1.70	1.87	56.44	51.84	65.41	2.28	2.92	2.83
CD at 1 %	3.19*	5.38*	5.93*	178.89*	164.31*	207.31	7.22*	9.27*	8.97*
CV	1.67	2.95	3.41	1.62	1.84	2.42	4.10	5.55	5.64

These results suggest that, at least within the first 2 days of treatment, the lipid reserve in sunflower seeds might act as a detoxifying trap, protecting membranes from excessive damage (Gidrol et al., 1998). Finally, sunflower seed deterioration during accelerated ageing is closely related to a decrease in the activities of detoxifying enzymes and to lipid peroxidation (Gidrol et al., 1998). The activities of superoxide dismutase and peroxidase decreased during sunflower seed ageing and it was especially pronounced when accelerated ageing was applied to the seeds (Balesevic et al., 2005).

All enzyme activity is positively correlated with germination of seed as ageing progressed germination also decreased and enzyme activity also decreased which showed significant deterioration in both accelerated as well as in natural aged seed lot. All seeds undergo ageing process during long-term storage which leads to deterioration in seed quality, especially in the humid tropical regions. However, the rate of seed deterioration can vary among various plant species (Merritt et al., 2003). Aged seeds show decreased vigour and produce weak seedlings that are unable to survive once reintroduced into a habitat (Aiazzi et al., 1996).

Conclusion

The sunflower hybrid (RSFH-130) seeds were under gone for accelerated ageing to know the relative storability of the sunflower seeds and that to an adverse environment conditions, germination of the seeds has been reduced over the number of hours has been increased for the treatments. After 4 months of storage the T1 (a.a at 90 to 95% RH and 41°C for 48 h) has been maintained the minimum seed certification standards. As the storage increases the accelerated aged seeds are decreased in the minimum seed certification standards and the treatments found statistically superior and if the same environmental factor we can store the seeds for about 4 months. These findings corresponded well to those reported elsewhere that unfavourable storage conditions (high air temperature and high humidity of air) accelerate seed deterioration, causing seed quality losses and therein lower germ inability percentage of stored seed (Burris, 1980; Tewari and Gupta, 1981; Al-Yahya,1995; Depaula et al., 1996; Beratlief and Iliescu, 2005).

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

A preliminary study of the orange (*Citrus sinensis*) fruit value-chain in Chimanimani Rural District, Zimbabwe

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The study identified the players in the sweet orange value-chain and interviewed them to quantify postharvest losses incurred along the sweet orange value-chain in Rusitu Valley. A sample of 100 farmers in Rusitu Valley was selected using a snow balling sampling technique. A Value-Chain Priority Test was conducted to determine farmers' priorities between oranges and bananas using a five point hedonic-scale. Interviewer administered questionnaires were used to gather socio-demographic data, sweet orange trading information, and farmers' perceptions on the causes and estimation of postharvest losses in the Valley. The study estimated that postharvest losses of 36%, 3% and 42% occurred; in the field, during transportation and at the market, respectively. These amounted to a total of 81% postharvest losses with an estimated monetary value of US$ 11 003 126.40. There was a significant positive association (Pearson r = 0.29, p < 0.05) between the farmers' score of pest and disease incidence in their sweet orange field and the reported postharvest losses. The present findings of the study clarified the process by which the physical flow of oranges move within the value-chain, the marketing alternatives to farmers, and constraints faced by primary actors in the chain.

Key words: diseases, orange, pests, postharvest loss, production value-chain.

INTRODUCTION

The sweet orange (*Citrus sinensis*) is a member of the citrus family (Rutaceae), along with other fruits such as mandarins, lemons, grapefruits and limes. Oranges account for the greatest value followed by grapefruits, lemons, mandarins and limes. In the pre-historic era, sweet orange was cultivated in several locations including areas now occupied by the modern China, India, Bhutan, Burma, and Malaysia (Leibbrandt, 1897; Webber, 1943). Globally, the leading producer of sweet oranges is Brazil followed by the European Union and China. In 2014, Brazil produced 17 340 MT followed by China [7 600 MT], United States [6 291 MT], and European Union [6 075 MT]. In Africa, Egypt was leading with a production of 2 570 MT followed by South Africa [1 600 MT] and Morocco [1 000 MT] (Anonymous, 2014). Orange production in Southern Africa is ranked the third

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regarding importance after vegetables and deciduous fruits with commercial production mainly concentrated in South Africa, Swaziland, Mozambique, and Zimbabwe (RSA, 2011).

Zimbabwe's geographic position and climate makes it ideal to produce early maturing varieties of oranges which reach the target markets earlier than neighbouring competing countries (Heri, 2000). Oranges are mainly produced in areas within or surrounding Limpopo Valley, Save Valley, Mazowe Valley, and Rusitu Valley in Zimbabwe (Dzingai, 2010). In Rusitu Valley, communal farmers benefited from the 1982 European Union's Lome Convention funding program that supported Manicaland Smallholder Coffee and Fruit projects (Brown, 2002). The program transformed a number of "backyard" orange orchards into viable commercial orchards. However, these orchards deteriorated due to depressed markets in Zimbabwe's economy from 2007 to 2009 (Chinembiri, 2009). By 2007, Zimbabwe's orange exports had declined from 78 to 42% (FAOSTAT, 2009). Zimbabwe was ranked number 35 in the world's orange-area harvested resulting in a world share of 0.3% (FAOSTAT, 2009). Musemwa and Mushunje (2011) noted a decrease in sweet orange production between 2000 and 2004, clearly indicating a huge difference in citrus management at the production levels between former commercial farmers (old value-chain primary actors) and the resettled small-scale farmers (new value-chain primary actors) who lack capital investment for pre-and post-harvest management of oranges (Chinembiri, 2009).

Small-scale fruit production plays an important role in income generation, poverty alleviation and in improving the nutrition and livelihood security of the rural population in the developing world. In developing countries, the horticulture sector suffers greatly from postharvest losses which are estimated to be more than 30%, especially in sub-Saharan Africa (Ladaniya, 2008; Tschirley, 2011; Kereth et al., 2013). In Rusitu Valley, more than 30% orange fruit post-harvest losses were reported for the 2011/12 farming season (Musasa et al., 2013). These postharvest losses are a result of the degradation of aesthetic and market value of fruits due pests, diseases and physical and chemical deterioration (Sudheer and Indira, 2007; Watson, 2013). Fruit flies were perceived as the major cause of fruit postharvest losses in Rusitu Valley (Musasa et al., 2013). Other causes of postharvest losses in the fruit production value-chain include inadequate fruit storage facilities, poor post-harvest fruit handling and lack of access to markets (Kader, 2002; Ladaniya, 2008; Tschirley, 2011). The aim of the present study was to characterise the sweet orange value-chain and determine the nature and extent of postharvest losses at various points of the value-chain in Rusitu Valley, in Chimanimani district, Manicaland Province in Zimbabwe. Information from this study may provide insights on critical factors that need to be addressed along the value-chain to reduce postharvest losses in sweet oranges and increase profitability and investment in post harvest infrastructure and management to the benefit of smallholder producers and other players in the sweet orange value-chain in Rusitu Valley in Zimbabwe.

MATERIALS AND METHODS

Study site

The study area was Rusitu Valley (latitudes of 20ºS 032ºE; an altitude of about 460 m above sea level), located in Chimanimani rural district shown in Figure 1. Chimanimani rural district has a population of 133 810 and 96.2% of the district population resides in the rural areas (Anonymous, 2013). The Valley receives moderately high rainfall (>1000 mm) almost throughout the year making it suitable for horticultural production (Vincent and Thomas, 1960; Rukuni and Eicher, 2006). The soils have high agricultural productivity, which is also a characteristic of agro-ecological region I. Their particles are well graded and consolidated making them less vulnerable to erosion, enabling farmers to plough and grow crops on slopes and hilly places. The livelihood of most rural district population depends on horticulture especially fruit production. The major produced fruits are bananas, sweet oranges, naartjies and avocadoes.

Data collection

A survey was conducted to identify the major players in the value chain and estimate the post harvest losses incurred at each point of the value chain and their causes in Rusitu Valley. Yamane's formula (Yamane, 1967) at 90% confidence interval (e = 0.1) was used to calculate the sample size of 100 farmers from the total population of 133,810 smallholder farmer households in Rusitu Valley, Chimanimani district, in Zimbabwe. A snow balling sampling technique was used to select the study sample of 100 farmers from the four sweet orange producing wards (Burns, 1994). The snowballing sampling technique was used because of the steep and hilly terrain in the Rusitu Valley that made access to a random sample extremely difficult. A Value Chain Priority Test was conducted during focus group discussion with two groups of 10 farmers per ward; to determine farmers' priorities between orange and banana production which are the major perennial fruits produced in the valley. A five point hedonic-scale was used in scoring for the priority tests; a score of 1 meaning that the sweet orange best met that criterion and a score of 5 meaning that sweet orange did not meet that criterion ranked against the other fruits. Interviewer administered questionnaire was used to gather information on: socio-demographic data, postharvest loss estimates, trading information, transportation, and attitudes towards postharvest management (Harry and Boone, 2012).

The collected data were categorised and analysed using SPSS version 20 (Field, 2011). A student t-test was used to test the significance of priority setting differences at $\alpha = 0.01$, Pearson correlation test was used to test the significance of relationship between scoring priorities for bananas and oranges at $\alpha = 0.01$ and regression analysis to test the relationship between postharvest losses and occurrence of pests and diseases was performed at $p < 0.05$. Key informant interviews and focus group discussions engaging traditional leaders and community leaders were used for fruit value chain priority settings within Rusitu Valley. Data from the key informant interviews and focus groups were synthesised and analysed for significance using student t-test computed on GraphPad Prism 6.

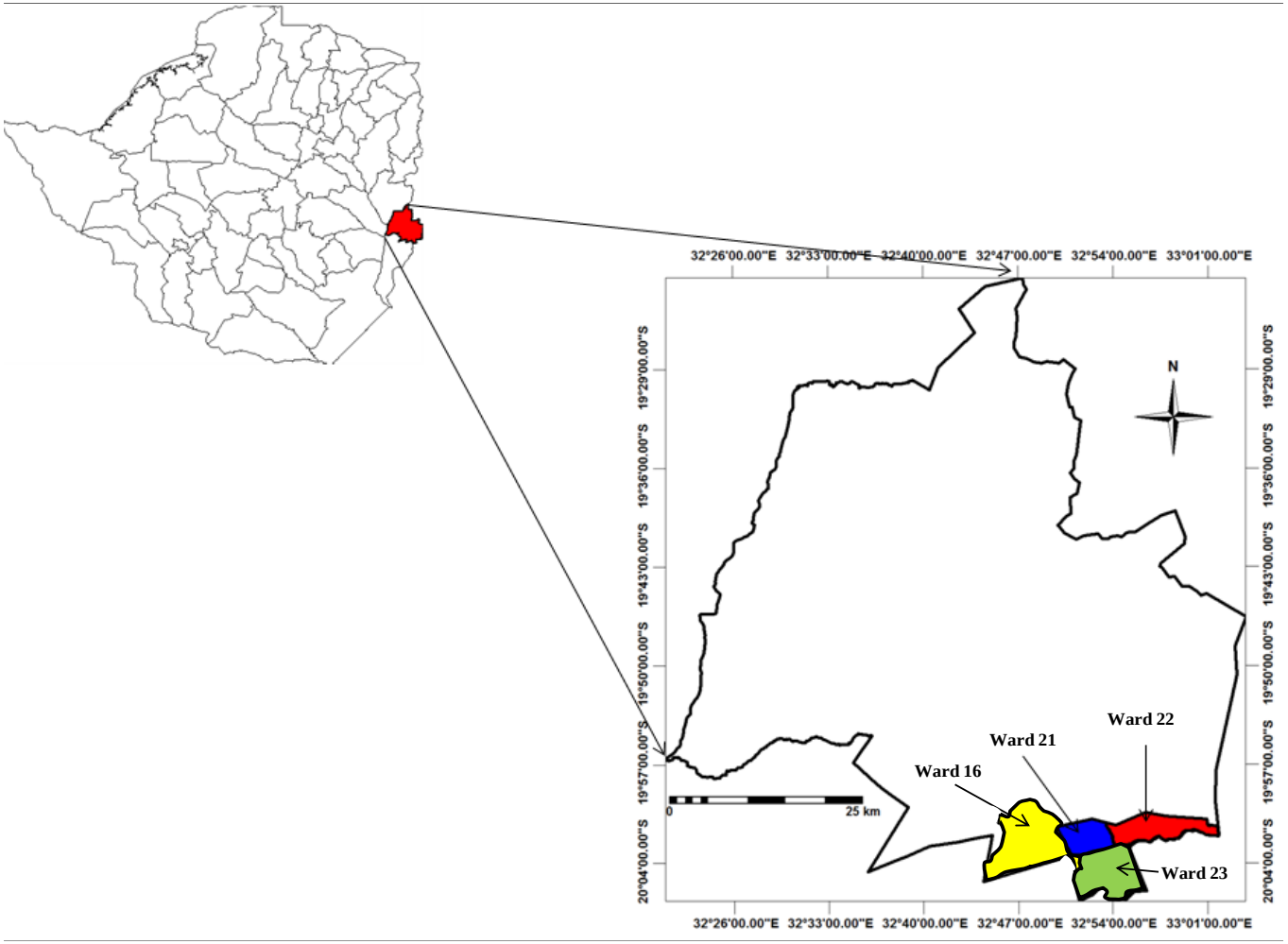

Figure 1. Map of Rusitu Valley wards studied, Chimanimani Rural District of Zimbabwe.

RESULTS AND DISCUSSION

A total of 100 questionnaires were administered in the four orange producing wards (Ward 16, 21, 22, and 23) in Rusitu Valley. The respondents were derived from a total of 12 villages representing the mentioned wards and the study reflected that the villages, Muchadziya, Dzingire, Mukondomi, Muterembwe, Musareketa, and Dherudhe had a greater representation of sweet orange farmers in the Valley as depicted in Table 1. The gender representation of the study

Table 1. Response with respect to location (N = 100).

Ward	Village	% Response
16	Dherudhe	14
	Zayawe	3
	Ndadzingwa	6
21	Gadzingo	2
	Mukondomi	11
	Dzingire	14
22	Matendera	5
	Muitire	1
	Muchadziya	16
23	Chishiri	1
	Musareketa	12
	Muterembwe	13

respondents was 48% female and 51% being males. Of these respondents 61% were married, 23% widows or widowers, 7% single, and 1% divorced as shown in Table 2.

Most of the study respondents were aged above 50 years (31%) with 30% of the respondents being in the range of 30 to 50 years whilst 28% refused to reveal their age. Majority of the respondents (49%) attended secondary education, 38% primary school, 3% tertiary education and 5% never attended school at all. Thus, the majority of the farmers can read and write resulting in efficient knowledge sharing with other orange production value-chain actors such as Zimbabwe Farmers' Union (ZFU) who were perceived as important fruit production knowledge providers in the valley by 83% of the study respondents. The study revealed that 95% of farmers depend on farming as a major livelihood source and that 72% of the farmers grow both bananas and oranges as a major source of income whilst 22% grow bananas only and 3% oranges only (Table 2). The study also revealed a positive Pearson correlation ($r = 0.31$) at 0.01 significant level existed between the level of education farming as a major source of income in Rusitu Valley.

Value chain priorities test

The Value Chain Priorities Test (VCPT) revealed that bananas were the most preferred with an average priority ranking of 2.0625 than oranges which had an average priority ranking of 2.3125 (Table 3). Though a student t-test confirmed that these priority setting differences were significant at $\alpha = 0.01$; a weak correlation ($r = 0.2828$) existed between scoring priorities for bananas and oranges and that this relationship was not significant since P (two tailed) = 0.3731 at $\alpha = 0.01$. Thus, respondents perceived that oranges were of less importance compared to bananas in Rusitu Valley and they attributed this to oranges' high fruit fly infestation rate compared to bananas. Therefore it was important to examine the production value-chain and proffer for sustainable strategies of improving orange postharvest quality and shelf-life in order to enhance orange production preference by local farmers in Rusitu Valley.

Sweet orange fruit production value-chain in Rusitu Valley

In Rusitu Valley the core processes characterising the sweet orange fruit production-chain include; the primary production stage characterised by smallholder farmers and secondary stage characterised by informal middleman traders (Figure 2). The secondary stage of the value – chain was highly dominated by the middleman traders as shown in Table 4. The middleman traders transport the oranges to urban markets especially Masvingo, Bulawayo, Mutare, Chipinge, Gweru and Harare as shown in the geographical flow of sweet oranges from Rusitu Valley in Figure 3. The farmers perceived that these middleman traders solely rely on buying fruits from Rusitu Valley and selling them to urban markets. The study revealed that 69% of the farmers sold their orange fruits to middleman traders and that 23% sold to local vendors in the Valley (Table 4).

From the study, 71% of the respondents strongly perceived that orange losses in Rusitu Valley resulted from of pests and diseases prevalence followed by deterioration of the orange quality parameters. Pests and diseases prevalence, harvesting methods, and deterioration in quality parameters were perceived as the major causes of postharvest losses as shown in Table 5.

Table 2. Response with respect to demographic data (N = 100).

	Gender		Age		Marital status		Level of education		Farming as a major source of income	
Response	Male	51%	<30	11%	Married	67%	Primary	38%	Yes	95%
	Female	48	30 - 50	30%	Single	7%	Secondary	49%	No	4%
			>50	31%	Divorced	1%	Tertiary	3%		
					Widow	23%	Never Attended	5%		
Std Deviation	0.521		1.194		1.280		0.833		0.278	
Variance	0.272		1.425		1.639		0.694		0.077	

Table 3. Rusitu fruit production value chain priority test.

Type of impacts		Banana (Average Score)	Sweet orange (Average Score)
Poverty and sustainability	Availability of resources	2	5
	Potential for labour intensity technology	3	1
	Number of households involved in the sector	1	1
	Future potential	1	1
Structure of chain	Extent of value-adding potential	2	1
	Number of different value-chain actors	5	4
	Length of marketing chain	1	4
	Maturity of fruit production industry in the region	3	3
	Marketing potential	1	1
	Lack of previous research	3	4
	Potential for lessons learned/replication of the mechanism	3	1
	Production information availability	1	3
Average ranking		2.0625	2.3125

The literacy rate of farmers and middleman traders allows for better flow of product information and knowledge within the value chain. In Rusitu Valley, the middleman traders determine the prices of sweet oranges as was revealed by most of the farmers. The farmers perceived that 90% of the middleman traders were not setting prices basing on the cost of production but instead they offer very low prices thus taking advantage of the failure by farmers to handle large quantities of sweet oranges when in season. Thus, the middleman traders use the poorly developed farm infrastructure to their advantage by buying oranges at low prices.

Farmers in the Valley only receive producer - trade information from traders unlike in other developing countries such as Tanzania where fruit and vegetable value-chains are well organised

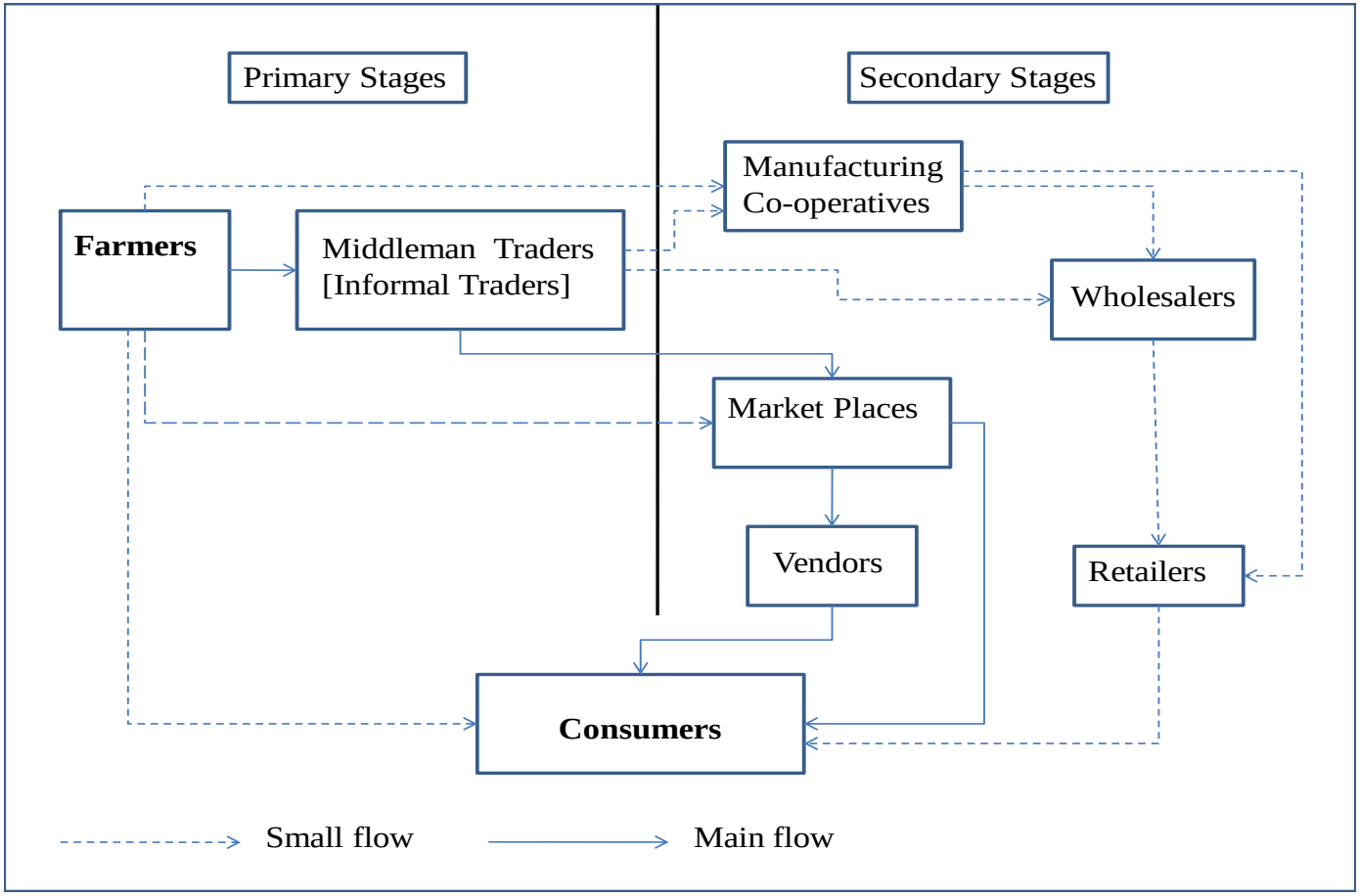

Figure 2. Rusitu Valley sweet orange production value chain.

and supported by different actors especially the government (Izamuhaye, 2008). As a result of this anomaly, during the 2013/2014 season 81% of the farmers sold their oranges to the middlemen traders at prices ranging from $1, 00 to $2, 00 per 15 kg pocket and 12% of the farmers sold at $3, 00 per 15 kg pocket directly to vendors within the Valley (Table 4). Therefore, the average sweet orange price in Rusitu Valley was $0, 13/kg during the 2013/2014 season which was lower than the banana price at $0, 20/kg during the same season. Though the orange value chain in Rusitu Valley was dominated by an average of 3 164 communal farmers, major flow of sweet oranges was being handled by middlemen traders. These middleman traders are not registered companies but individuals or informal traders. The dominating communal farmers lack the capacity to handle the abundant orange produce since they tend to ripen almost at once causing seasonal gluts.

The survey revealed that orange fruit tree

Table 4. Sweet orange tree owned/farmer and orange prices for 2013/14 season (N=100).

	Orange trees owned/farmer		Orange buyers		Orange prices (US$)	
Response	<50	77%	Middleman	68%	<1	1%
	50 - 100	14%	Local Vendors	28%	1-2	80%
	101 - 150	7%	Cooperatives	2%	>2	6%
	>50	2%				
Std deviation	0.699		1.44		0.568	
Variance	0.489		2.075		0.323	

Table 5. Perceptions of farmers on the major causes of postharvest losses in Rusitu Valley (N=100).

Perceived causes of losses	Strongly agree (%)	Agree (%)	Indifferent (%)	Disagree (%)	Strongly disagree (%)	Std Deviation	Variance
Pests and diseases	71	27	2	0	0	0.506	0.256
Transportation to the market	13	35	16	35	1	1.102	1.215
Harvesting methods	11	68	1	20	0	0.916	0.838
Farming and marketing practices	31	62	3	4	0	0.682	0.465
Deterioration in the sweet orange quality parameters	49	40	3	8	0	0.87	0.758

population in the Valley reduced from 2011/2012 season's 174 020 to 145 544 trees during the 2013/2014 season. The study also highlighted that orange fruit production capacity per tree reduced to 700 kg from the 2011/2012 season's 1200 kg/tree as most of the farmers (71%) now owned trees less than 50 in their orchards (Table 4). Pests, diseases, and tree aging were noted as the major causes of reduction in the production capacity and quantity of sweet oranges (Table 5). Basing on the total number of sweet orange farmers the study revealed that a total of 101 880,8t of sweet oranges were produced in Rusitu Valley during the 2013/2014 season. Of this produce 36% deteriorated in the field, 3% during transportation, and 42% at markets. Thus, the total postharvest losses in this value chain amounts to 81% of the total produce (82 523,448t of sweet oranges with a monetary value of US$11 003 126.40). The study also revealed that a positive correlation ($r = 0.22$, significant at the 0.05 level (2-tailed)) existed between the varieties farmers grow and the total postharvest losses incurred during the 2013/2014 season.

Farmers who grow both Navel and Late Valencia varieties incurred more postharvest losses than farmers growing Navel variety only. The median for farmers who grow both Navel and Late Valencia varieties is higher (Figure 4, Graph a) than those of the rest of farmers reflecting that farmers growing the two varieties incurred greater losses during the 2013/2014 season. The lower quartile for farmers growing Navel, Late Valencia, and other varieties is actually larger than the rest of farmers, which means that there is more variability in the lower 25% of their postharvest scores than the other farmers. The box plots in Figure 4 show that the range of postharvest losses amongst farmers was different during the 2013/2014 season. In Figure 4, Graph a is showing an asymmetrical distribution of postharvest loss scores, Graph b resembling the distribution postharvest losses with respect to orange varieties, and in graph c the p-p plots are showing that the postharvest losses do not follow a normal distribution. It can be concluded that the variability of postharvest losses with respect to orange varieties grown by communal farmers in Rusitu Valley do not follow a normal distribution.

Majority of the farmers strongly agreed with the perception that postharvest losses incurred on

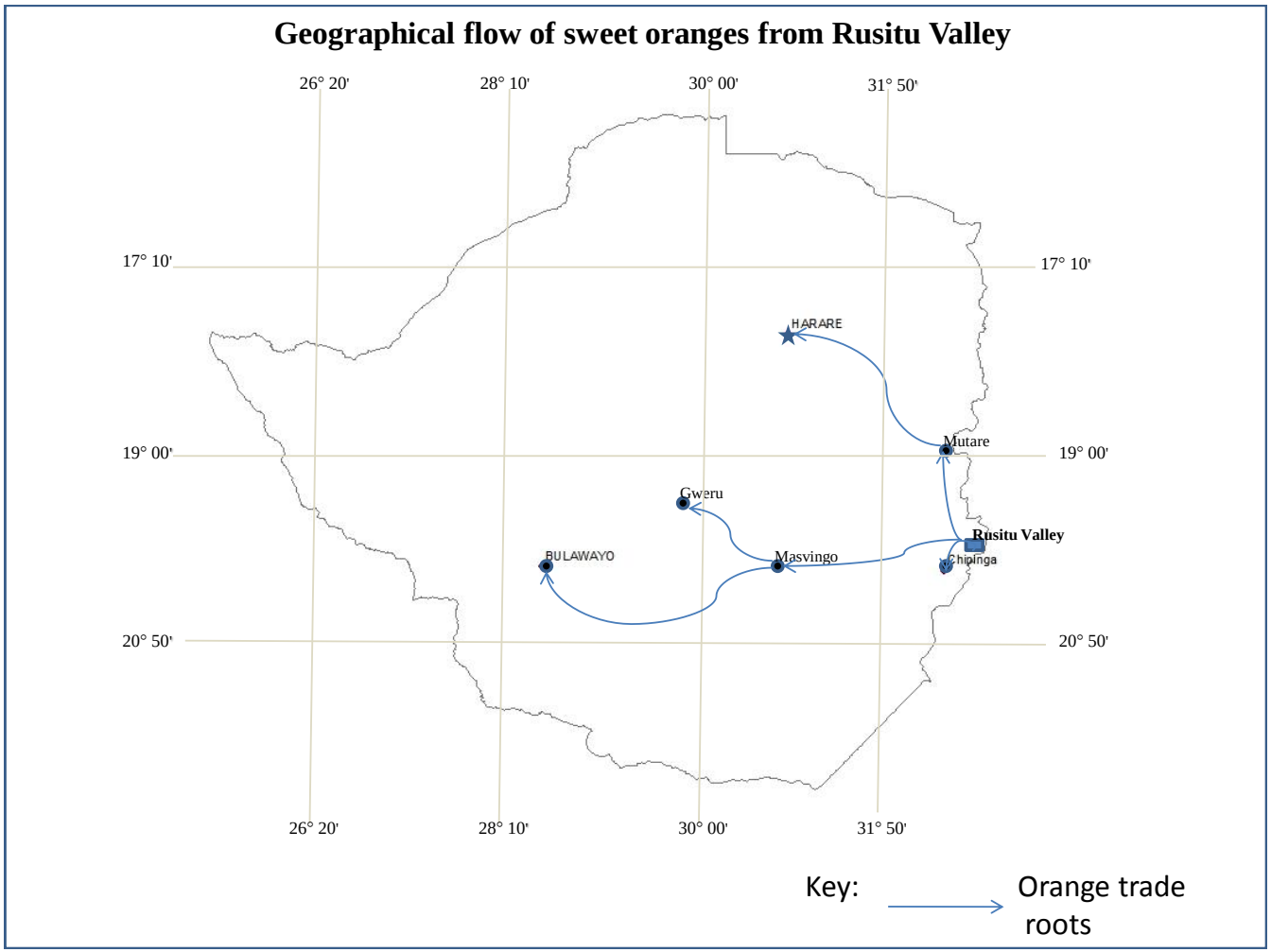

Figure 3. Sweet orange trade routes.

sweet orange production during the 2013/2014 season were caused by pests and diseases, followed by deterioration in orange quality parameters (Table 5). A regression analysis on the relationship between postharvest losses and prevalence of pests and diseases confirmed that correlation existed ($r = 0.29$) and was significant at $p < 0.05$. The regression analysis also established that prevalence of pests and n diseases accounts for 8.5% ($R^2 = 0.085$) of the total post harvest losses thus there are other variables that are contributing to postharvest losses in Rusitu Valley.

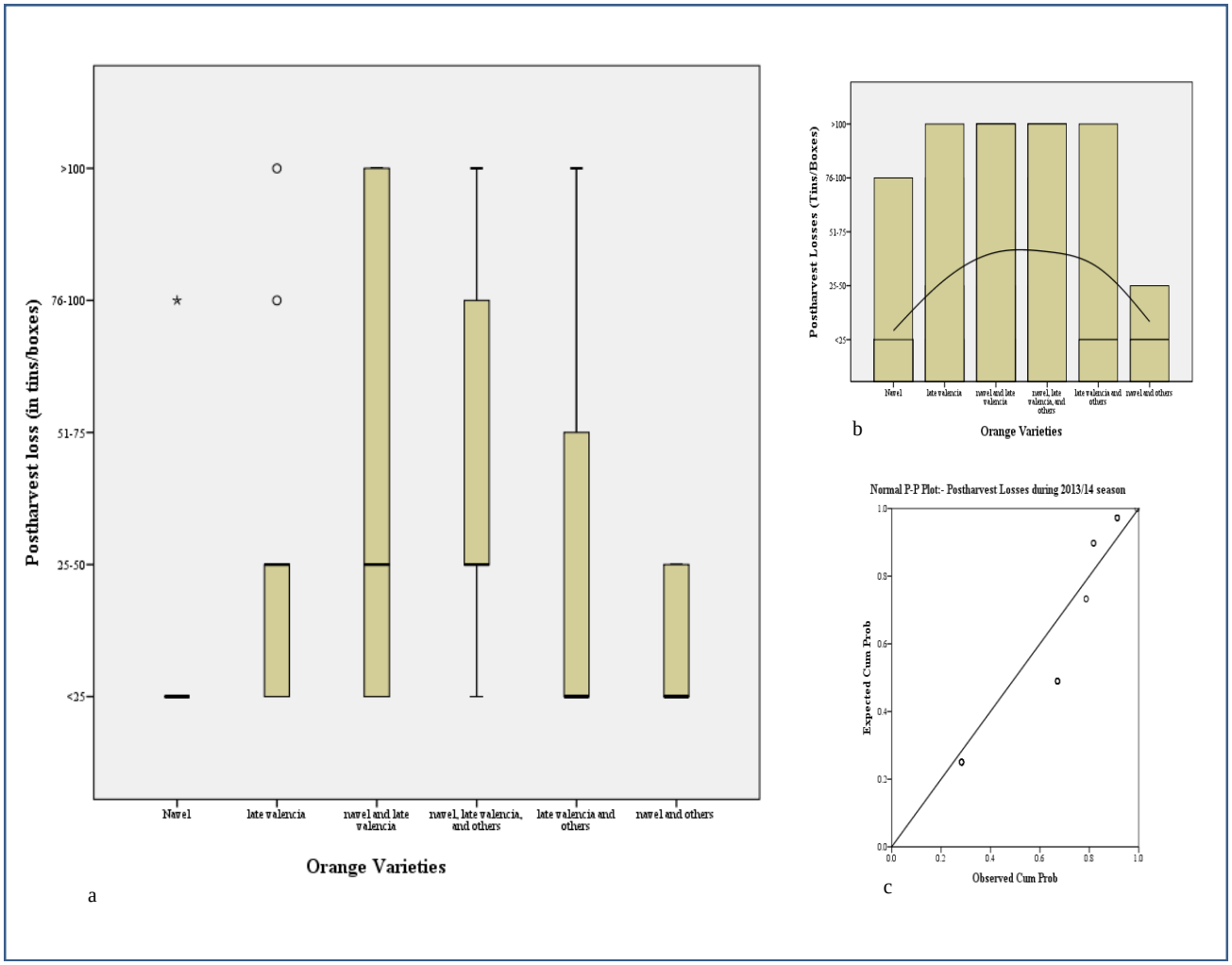

Figure 4. Sweet orange varieties and postharvest loss distribution tests for postharvest losses.

These variables include harvesting methods, farming and marketing practices, deterioration in quality parameters, and transportation of sweet oranges.

CONCLUSIONS AND RECOMMENDATIONS

From the study, the main flow of oranges is distributed through informal middleman traders who transport produce to urban market places where there are poor storage facilities hence huge losses amounting to 42% of the total produce are incurred. Farmers in Rusitu Valley are forced to

sell their orange produce to informal middleman traders at prices below the production costs, since the farmers lack capacity and market information to handle the seasonal gluts of sweet oranges.

The study also revealed a decline in the quantity of Rusitu Valley orange produce and an increase in postharvest losses; resulting from poor postharvest management and prevalence of pests and diseases. The distribution of postharvest losses was greater for farmers who own both Navel and Late Valencia varieties compared to farmers owning Navel variety only. It can be concluded that reduction in these postharvest losses improves the livelihoods and development of Rusitu Valley communities. In light of the study findings, it was suggested that the main flow of oranges should be distributed through registered collection agents linked to the manufacturing industry. There is also need for investment on proper postharvest management within the orange value chain especially in assisted value addition technologies since all the actors are lacking investment capital.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Bioactivity of trypsin inhibitors from sesame seeds to control *Plodia interpunctella* larvae (Hübner) (Lepidoptera: Pyralidae)

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Biochemical and feeding assays were performed in order to assess the bioactivity of trypsin inhibitors from sesame seeds to control *Plodia interpunctella* larvae. The biochemical assays were based on the ability of protease inhibitors (PI) present in sesame seeds to inhibit bovine pancreatic trypsin and digestive enzymes of insect larvae. The stability of the inhibitors was also assessed at different pH and temperature. The feeding bioassays were carried out in plates containing second instar larvae fed on diet with sesame protein extract. It was observed that all genotypes presented inhibition when tested with BPT, ranging from 51 to 90%, among them BRS Seda, CNPA G3 and CNPA G4 showed average of 69% of inhibitory activity for intestinal homogenate. The FII-fractions in these three genotypes showed thermal and pH stabilities at 40°C and 8.5, respectively. In feeding bioassay, *P. interpunctella* larvae were susceptible to SPCE of all three genotypes, revealing average mortality of 74% to SPCE and 45% to FII-fractions. Among all genotypes, BRS Seda was more effective to inhibition of digestive enzymes of insects and considered the more suitable genotype for further using in sesame breeding program to resistance to *P. interpunctella*.

Key words: *Sesamum indicum* L., serine protease, stored grain pest, Lepidoptera.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is an oilseed grown in many parts of the world and is widely used in food and cosmetics industries (Sharma et al., 2014). The annual worldwide grain production is around 4 million tonnes (FAO, 2014). Sesame is a short cycle crop with broad adaptation to semiarid conditions. The main problem in management is the occurrence of insect-pests, especially at post-harvest season, including *Sitophilus* spp.,

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Abbreviations: CNPA, Centro Nacional de Pesquisa do Algodão; **PIs**, protease inhibitors; **SPCE**, seed protein crude extract.

Table 1. Inhibitory rate of seed protein crude extracts on bovine pancreatic trypsin in different sesame genotypes.

Genotypes	Inhibition (%)
CNPA G4	90.76[a]
CNPA G3	87.57[ab]
BRS Seda	87.02[ab]
ECGSG01	79.45[c]
ECGSG02	77.43[c]
ECGSG03	80.08[c]
ECGSG04	76.54[c]
ECGSG05	64.40[d]
ECGSG07	66.26[d]
ECGSG06	51.85[e]

Means followed by the same letter do not differ by Tukey test ($p < 0.05$).

Tribolium spp. and *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) (Arriel et al., 2009). The extent of damage depends on the degree of infestation in warehouses.

P. interpunctella is a harmful grain storage pest it affects the quality of the grain (Michereff Filho et al., 2013). The control is based on chemical pesticides, but it found limitation due to level of infestation and also resistance of inset to some chemical insecticides, including classes of organophosphates and pyrethroids (Arthur and Phillips, 2003). Resistance via transgenesis could be an alternative; however, Herrero et al. (2001) have found high level of resistance to Cry1 Ab and Cry1 Ac toxins, from *Bacillus thuringiensis*, in insect populations.

Considering these limitations, several researches have focused on identification of natural metabolites with potential to control insect-pests. Massive information is available in literature reporting about effective plant bioactives with toxicity to lepidoptera and coleopteran insects, which are the more harmful to commercial crops.

Several leguminous and oleaginous seeds contain proteins with insecticidal property, such as protease inhibitors (PIs), that can inhibit the activity of proteolytic enzymes present in insect guts, causing severe physiological disorders, such as malnutrition, delayed in development and even death (Martins et al., 2014; Lima et al., 2004). PIs are widely distributed in the plant kingdom; they are proteins able to inhibit the activities of trypsin, chymotrypsin, amylase, carboxipetidase, among others.

PIs are classified according to the specificity of interaction with amino acids groups that compound the proteolytic enzymes: Serine, cysteine, aspartic, metallo, threonine and glutamic proteases (Ryan, 1990; Powers et al., 2002; Rawlings et al., 2012). The serine and cysteine proteases have been the most studied, with proven interference in the life cycle of the insect, weight reduction, decreased rate of posture and mortality (Aghaali et al., 2013).

Several works are available in literature involving inhibitory activity of seed-PIs from oleaginous crops, such soybean and peanut (Martins et al., 2014; Oliva et al., 2011). In sesame, El-Bramawy (2011) studied antinutritional factors of seeds, including tannins, phytates and trypsin inhibitors (TI), in order to further use them as selection criteria to disease resistance. Despite the little information available, it is suggested that PI expression in sesame is genotype-dependent, similarly to the findings in other species as *Arachis hypogaea*, and also that the bioactivity varies according to the target insects (Martins et al., 2014).

The Brazilian Company of Agricultural Research (Embrapa) holds a Sesame Germplasm Collection, with over 1500 national and international accessions, some of which are used in breeding program for development of cultivars and top lines to semiarid environment (Arriel et al., 2009). Some traits such as grain yield, earliness, oil quality and tolerant to pest and disease are quite useful in selection procedures (Arriel et al., 2009). The identification of high-PIs accessions may be an additional activity to contribute with the sesame breeding to tolerance to stored grain-pests. Then, the present study aimed to investigate the bioactivity of seed sesame-trypsin inhibitors (TI) on gut enzymes of *P. interpunctella*, based on biochemical and feeding experiments.

MATERIALS AND METHODS

Protein extraction and fractioning

The sesame seeds used in this study were harvested in 2013 in Barbalha, CE, (07° 18'18 "S, 39° 18'07" W, 414 m), in a semiarid region of Brazilian northeastern. Seeds (3 g) of ten sesame genotypes (Table 1) were used for total protein extraction, starting with delipidation (Bland and Lax, 2000). Delipidated samples (100

mg) were homogeneized in 1 ml Tris-HCl 0.05 M buffer, pH 8.5 and centrifuged at 12.000 x g for 20 min at 4°C. The supernatant was recovered and incubated at 4°C for 16 h. On the next day, it was performed a new centrifugation and the supernatant was collected and stored at -20°C. The seed protein crude extracts (SPCE) were fractionated by sequential precipitation with ammonium sulfate at 0-30%, 30-60% and 60-90% saturation levels, according to the Green and Hughes method (1955). These fractions were dialyzed in Tris-HCl 0.05 M buffer, pH 8.5 and termed FI (0-30%), FII (30-60%) and FIII (60-90%), according to their respective degrees of saturation, and were kept at -20°C. The partially purified extracts and SPCE were quantified using Bovine Serum Albumin (BSA) as standard (Bradford, 1976), in a spectrophotometer (Femto, Model 700S) at 595 nm.

Determination of antitrypsin activity in *P. interpunctella*

Midguts collected from fifty 5th instar (10 days) larvae of *P. interpunctella* were sectioned and transferred to microtubes (1.5 mL) containing 200 µL of Tris-HCl 0,05 $M.L^{-1}$ buffer, pH 8.5 and homogenized. The homogenate was centrifuged at 12.000 x g, for 30 min, at 4°C, and the supernatant was quantified (Bradford, 1976) and used in inhibition enzyme assays. Briefly, the antitryptic activity was based on the following steps (Martins et al., 2014): |(a) Bovine pancreatic trypsin assay with SPCE; (b) Bovine trypsin assay with trypsin inhibitor partially purified from sesame seeds; (c) in vitro assays with digestive enzyme of insects and SPCE, and (d) in vitro assays with digestive enzyme of insects with trypsin inhibitor partially purified from sesame seeds.

The reactions were performed in a microtube (1.5 mL) containing the following components (Kakade et al., 1969): 5 µL of bovine pancreatic trypsin (BPT, 1 µg/µL) or 5 µL of insect digestive enzyme preparations (4 µg/µL), 20 µL of seed protein crude extract or partial purified seed trypsin inhibitor (3.5 µg protein/µL), 125 µL of 50 mM Tris-HCl buffer pH 8.5. The reaction was pre-incubated at 37ºC for 20 min and 200 µL azocasein (1.5%, m/v) was added, after which it was incubated again at the same temperature and period. The reaction was discontinued with 300 µL of 20% trichloroacetic acid. After 5 min at room temperature, the samples were centrifuged at 12.000 x g for 10 min. An aliquot of 250 µL of supernatant was collected and added to 250 µL of 2 mM NaOH; the reading was performed in a spectrophotometer at 440 nm. All assays were performed in triplicate with three biological repetitions and with blanks. Reagents from Sigma Aldrich (USA) were used in these assays.

Thermal and pH stability of the inhibitory extract

These tests were conducted with partially purified protein extract (fraction FII) from seeds of the genotypes BRS Seda, CNPA G3 and CNPA G4, following the methodology described by Gomes et al. (2005). To estimate the thermal stability, the samples (1 mL) were previously incubated for 30 minutes at temperatures of 40, 60, 80 and 100°C; and to determine the pH stability, the samples (1 mL) were previously dialyzed at different pHs using the following buffers: 50 mM sodium phosphate, pHs 6.5 to 7.5, and 50 mM Tris-HCl, pHs 8.5 to 10.5. Then, the samples were incubated at 37°C and dialyzed again for 4 h in 50 mM Tris-HCl, pH 8.5. The antitrypsin activities were determined according to Kakade et al. (1969), using the preparation of digestive enzymes of *P. interpunctella* and BPT. All assays were performed in triplicate with three biological replicates.

In vivo bioassays using *P. interpunctella*

Fraction FII and SPCE from genotypes BRS Seda, CNPA G3 and CNPA G4 were used in feeding assay with 2nd larvae (4 days) of *P. interpunctella*. Both extracts were lyophilized (Liotop, L10L model) for 24 hours and added to the artificial diet (Amorim et al., 2008) at 0.1, 0.3, 1, 1.5, 3 and 6%. Bioassays were performed in 24-cell plates (1 larva/cell, with 12 larvae/treatment) containing 200 mg of diet. The plates were incubated in Biochemical Oxygen Demand (BOD) chamber at 25°C, 65 to 70% relative humidity and photoperiod of 12:12 h. The assay was completely randomized with 4 replications. The mortality rate of the larvae was estimated until 30 days.

Statistical analyses

The data were statistically analyzed using the Sisvar software system (Version 5.1). The means were compared by Tukey test (p<0.05). The regression curves were generated and the lethal concentrations were defined (LC 50%) for bioassays.

RESULTS

Inhibitory activity of SPCE on BPT and digestive enzymes of *P. interpunctella*

The inhibitory activity of SPCE on BPT from all genotypes assessed ranged among 51 to 90%, revealing average rate of 88% to cvs. BRS Seda, CNPA G3 and CNPA G4 (Table 1). In order to estimate the inhibitory activity of SPCE on digestive enzymes of *P. interpunctella* larvae, the extracts of these three genotypes were used, showing inhibition rate of 77, 60 and 67%, respectively (Figure 1).

Inhibitory activity of fractions FI, FII and FIII on BPT and digestive enzymes of *P. interpunctella*

Figure 2 shows the inhibition rates obtained from FI, FII and FIII protein fractions from BRS Seda, CNPA G3 and CNPA G4 seeds on BPT and digestive enzymes of *P. interpunctella.* The FII fraction provided high rate of inhibitory activity as tested on BPT and digestive enzymes of larvae, in all genotypes with averages of 78, 60 and 70% (Figure 2a) and 68, 55 and 59% (Figure 2b), respectively.

Thermal stability of FII fraction on bovine pancreatic trypsin and digestive enzymes of *P. interpunctella*

The thermal stability of FII fractions on bovine pancreatic trypsin was quite similar in both BRS Seda and CNPA G3, with inhibition rate between 58 and 38% at 40 to 100ºC. CNPA G4 was more stable showing rates of 63 and 40% at 40 to 100ºC (Figure 3a). On digestive enzymes of *P. interpunctella*, the thermal stability was similar in all three cultivars, with inhibition rate of 55% at 40ºC, with further gradual reduction and keeping in about 45 at 100ºC (Figure 3b).

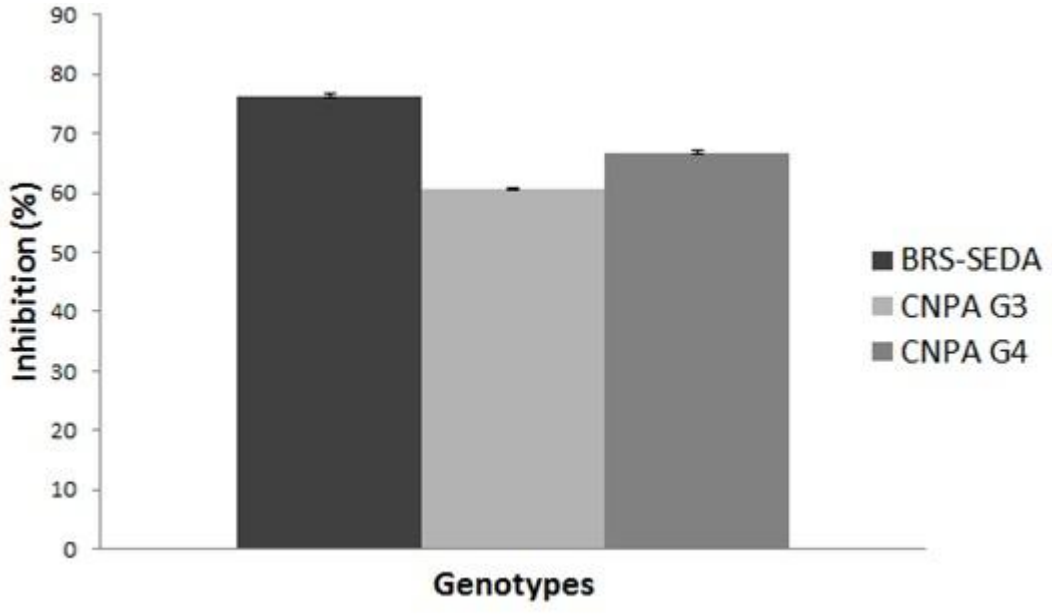

Figure 1. Inhibition rate of digestive enzymes of larvae of *P. interpunctella,* by using seed protein crude extracts of BRS Seda, BRS G3 and BRS G4 sesame genotypes. Data are mean values ± SD of three biological replicates.

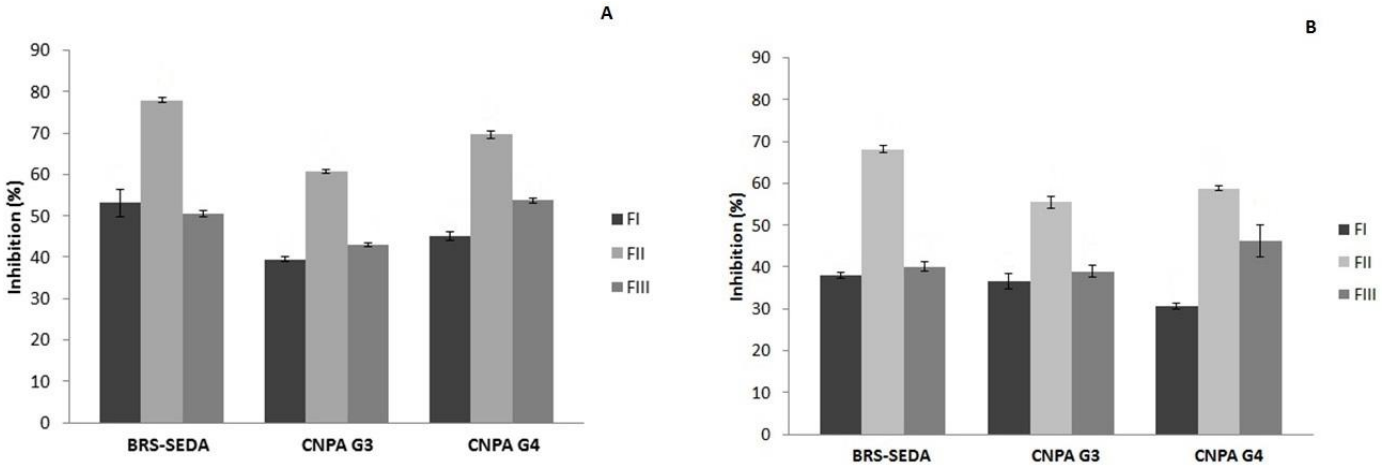

Figure 2. Inhibition of bovine pancreatic trypsin (A) and digestive enzymes of *P. interpunctella* (B) by using FI, FII and FIII fractions from seed protein crude extract in different sesame cultivars. Data are mean values ± SD of three biological replicates.

The inhibition rates on bovine pancreatic trypsin and digestive enzymes of *P. interpunctella* for FII fraction at different pHs are shown in Figure 4. The highest inhibition rates for both bovine pancreatic trypsin (Figure 4a) and digestive enzymes of *P. interpunctella* (Figure 4b) were obtained at pH 8.5, with means of 73 and 63%, respectively, in all three cultivars. The inhibition rate for bovine pancreatic trypsin decreased from pH 9.5, reaching less than 20% at pH 10.5. On the other hand, for digestive enzymes, that rate remained close to 45% at pHs above of 8.5.

Feeding bioassays with *P. interpunctella*

In bioassays with *P. interpunctella*, 2nd instar larvae were fed on SPCE and FII fraction of BRS Seda, CNPA G3 and CNPA G4, at different concentrations. As seen in Table 2, statistically significant difference was found only for concentrations tested. No effect of interactions arising from genotypes was observed.

The average of mortality rates of *P. interpunctella* in the treatments with SPCE and FII fraction of the three cultivars were 74 and 45%, respectively (Tables 3 and 4).

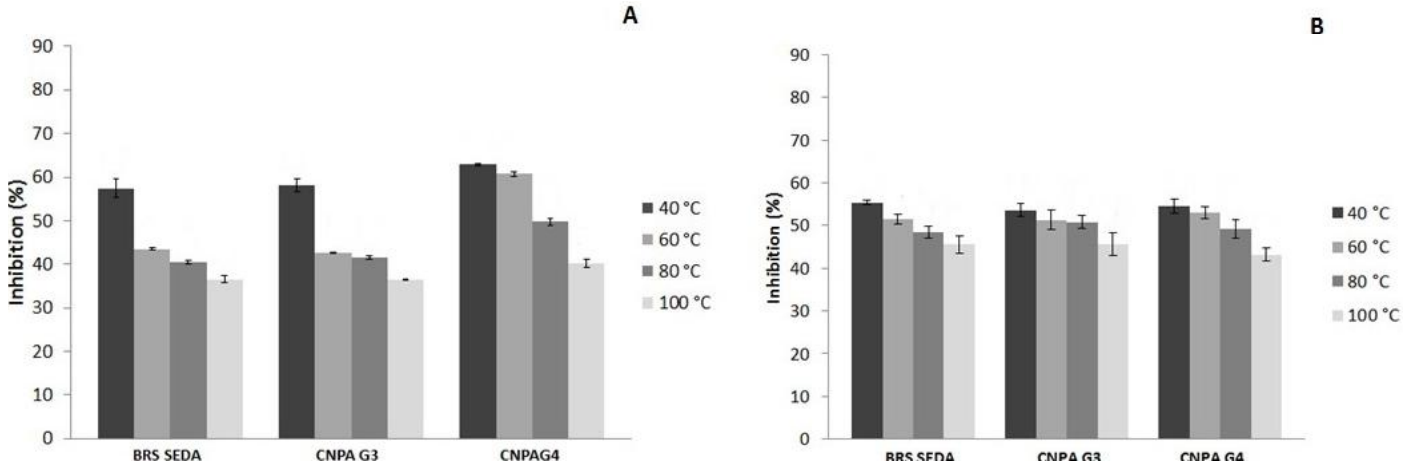

Figure 3. Thermal stability of FII fraction of seed protein crude extract on bovine pancreatic trypsin (A) and digestive enzymes of *P. interpunctela* (B). Data are mean values ± SD of three biological replicates.

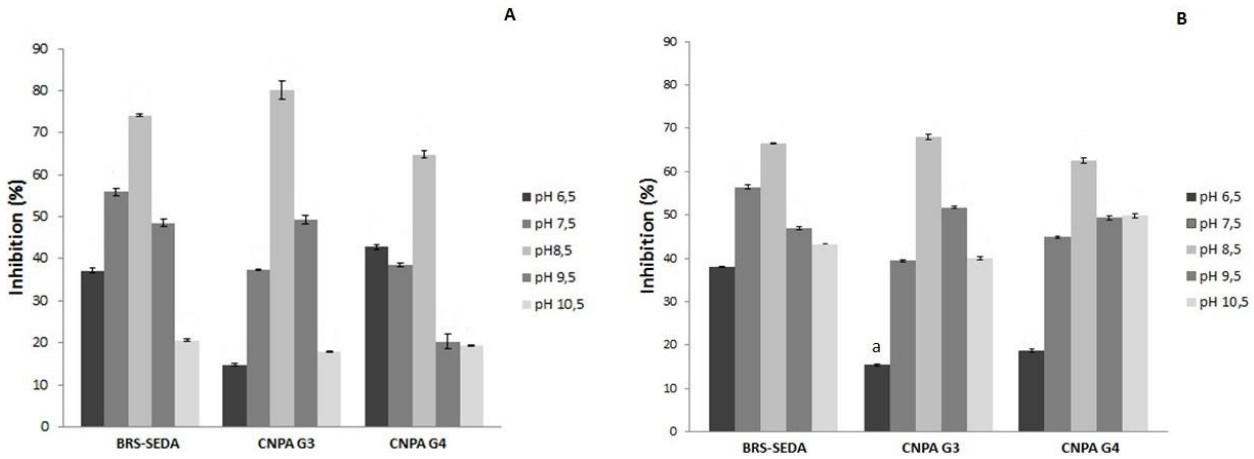

Figure 4. PH stability of FII fraction of seed protein crude extracts on bovine pancreatic trypsin (A) and digestive enzymes of *P. interpunctella* (B). Data are mean values ± SD of three biological replicates.

Figure 5 shows the regression curves related to mortality rate in both treatments. The estimated CL_{50} of FII fraction was 0.55% to BRS Seda and 0.70% to CNPA G3 and CNPA G4. However, in the treatments with SPCE, CL_{50} could not be determined due to high mortality rate.

DISCUSSION

Plant PIs, known as anti-enzymes, are widely studied in various biological processes, including those related to plant defense against pathogens and insect pests. These metabolites could be a strategic alternative to pest control, either by natural or transgenesis means (Bariani et al., 2012; Macedo et al., 2013; Ranjbar et al., 2014).

In this work, PIs of sesame seeds were investigated for their ability to inhibit bovine pancreatic trypsin and digestive enzymes of *P. interpunctella* based on *in vitro* and *in vivo* assays. In *in vitro* assays, it was found that the SPCE of three sesame cultivars, developed by Embrapa, allowed high rate of inhibition both for bovine pancreatic trypsin and digestive enzymes of the insect.

The activity of these extracts on digestive proteases of *P. interpunctella* larvae was verified by feeding bioassays, which demonstrated mortality rate of 74% in larvae fed on diet containing SPCE, and only 45%, when FII fraction were provided. This reduced mortality rate suggests that, although the highest rate of inhibition on digestive enzymes of *P. interpunctella* has been detected in FII fraction, both FI and FIII must have some intrinsic functions that could contribute to raise the mortality rate of insect. As they were absent in the extract, the mortality rate was 39% lower, considering the average observed

Table 2. Analysis of variance for mortality rates of *P. interpunctella* fed on SPCE and FII fraction from different sesame genotypes, at different concentrations.

SV	DF	MS	SPCE		FII		
			F	P>F	MS	F	P>F
Genotype (G)	2	0.013	1.024	0.366 ^{ns}	0.001	0.070	0.932^{ns}
Dose (D)	5	0.334	26.23	0.000**	0.166	11.34	0.000**
G*D	10	0.003	0.200	0.995^{ns}	0.001	0.059	1.000^{ns}
Error	54	0.013			0.015		
CV (%)		10.84			13.01		

SV: Source of variation; DF: Degree of freedom; MS: Mean square; F: F test; P: Probability test; CV: Coefficient of Variation. ns- No significant, **significant at 1% probability.

Table 3. Mortality rate (%) of *P. interpunctella* fed on SPCE from different sesame genotypes, at different concentrations.

Genotypes	Concentration (%)					
	0	0.1	0.3	1	1.5	3
BRS Seda	0.0^{Ab}	67^{Aa}	83^{Aa}	83^{Aa}	83^{Aa}	83^{Aa}
CNPA G3	0.0^{Ab}	58^{Aa}	58^{Aa}	67^{Aa}	75^{Aa}	83^{Aa}
CNPA G4	0.0^{Ab}	67^{Aa}	67^{Aa}	75^{Aa}	75^{Aa}	83^{Aa}
Average	0.0	64	69	75	78	83

* Means followed by the same letter in the line and column do not differ statistically by the Tukey test (p <0.05). Capitalized letters compare genotypes and lowercase letters, concentrations.

Table 4. Mortality rate (%) of *P. interpunctella* fed on FII fraction from different sesame genotypes, at different concentrations.

Genotypes	Concentration (%)					
	0	0.1	0.3	1	1.5	3
BRS Seda	0.0^{Ab}	33^{Aa}	50^{Aa}	50^{Aa}	50^{Aa}	58^{Aa}
CNPA G3	0.0^{Ab}	33^{Aa}	42^{Aa}	42^{Aa}	50^{Aa}	58^{Aa}
CNPA G4	0.0^{Ab}	33^{Aa}	42^{Aa}	50^{Aa}	50^{Aa}	58^{Aa}
Average	0.0	33	45	47	50	50

Means followed by the same letter in the row and column do not differ statistically by the Tukey test (p <0.05). Capitalized letters compare genotypes and lowercase letters, concentrations.

with SPCE. These results allow inferring that interactions between these fractions may lead to potential benefits for plant protection against insects, especially because, as a complex of proteins, specific components in the extract may affect the binding and inhibition of proteases associated to digestion (Linser et al., 2009; Vinokurov et al., 2006; Dow, 1992).

The three cultivars evaluated in this study showed potential to control *P. interpunctella,* however, considering the results of inhibitory activity of SPCE and inhibition of digestive enzymes of insect with the fractions of the extract, BRS Seda seems to be the most suitable for further studies involving tolerance to storage grain pests. Moreover, the CL_{50} obtained from FII fraction of this cultivar was only 0.55%, demonstrating that it is an interesting candidate to sesame breeding program.

Another relevant result seen in BRS Seda is that the protein extracts retained the inhibitory activity above 50% at pH ranging between 7.5 and 8.5. It means that the extract from the seeds could be able to fight various species of insects if the intestinal pH is at this range of alkalinity. It must be highlighted that BRS Seda, along with CNPA G4, presented inhibitory activity at pH 6.5, although the rate did not exceed 40%. This confirms one of the characteristics of trypsin inhibitors, which can act in media ranging from neutral to alkaline, and thus become more relevant for plant defense against insects, since the intestinal lumen may also vary in the same proportions (Terra and Ferreira, 1994; Lopes et al., 2006).

Some species of coleoptera have acidic pH in the

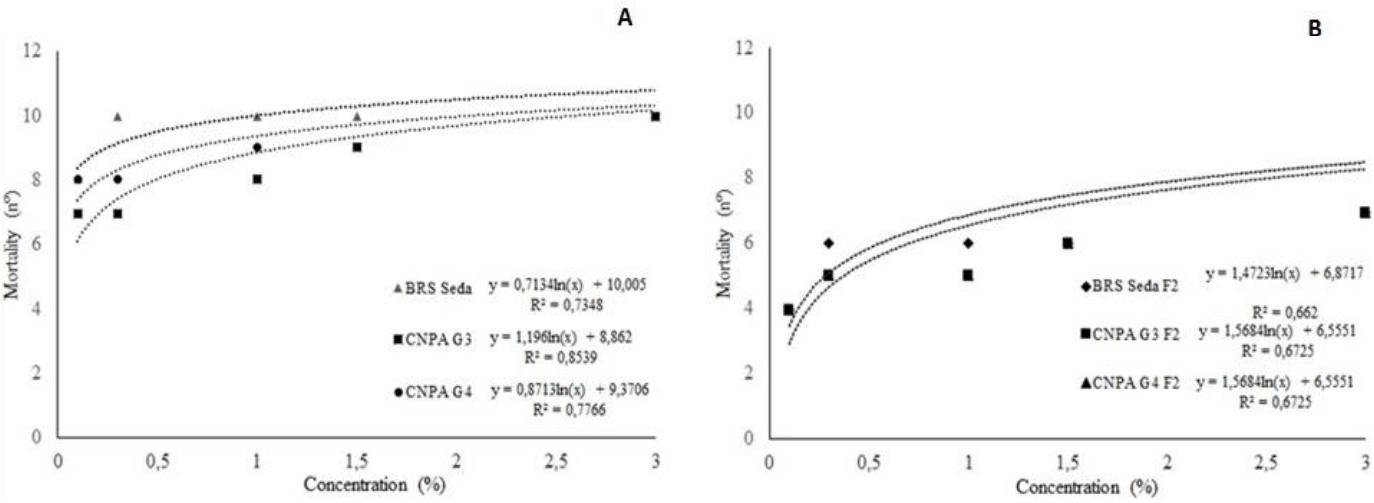

Figure 5. Mortality rate of *P. interpunctella* larvae fed on diet containing different concentrations of SPCE (A) and FII fraction (B) of different sesame genotypes.

anterior midgut (pH 5.2-5.6), where cysteine proteases predominate, and slightly alkaline (pH 7.8-8.2) in the posterior region, where serine proteases are in activity (Vinokurov et al., 2006; Terra and Cristofoletti, 1996). In lepidopteran, most of the contents of the intestinal lumen (endoperitrophic region) has alkaline pH, while the ectoperitrophic portion (outside the membrane) has pH next to neutrality (Rossi et al., 2009; Rossi et al., 2012; Ranjbar et al., 2014). Thus, since the protein extracts of the cultivars investigated in this study showed better inhibitory activity at pH 8.5 for serine proteases, it is suggested that all of them could be used in a sesame program aiming to tolerance to *P. interpunctella* or even other lepidopteran insects. Further studies are necessary to attest these assumptions.

As to temperature, results found here showed that the inhibitory activity of FII fraction of three cultivars maintained a rate above 40% up to 80°C, in both experiments with bovine pancreatic trypsin and digestive enzymes of *P. interpunctella*, revealing a subsequent decreasing of 30% at most, when submitted to 100ºC. This is an interesting result since the inhibitory capacity of proteins depends on temperature. Above 40°C, the activity of some proteins is reduced or even inactivated as result of denaturation. It could be a limiting factor to its use to insect control in semiarid environments where the temperatures of the soil and the air are often high. In thermostability assays using protein extracts from peanut seeds tested on bovine trypsin, Martins et al. (2014) reported maintenance of inhibitory activity above 60%, at 40 to 100ºC. In *Peltophorum dubium* Spreng, however, Macedo et al. (2013) tested the thermostability of the protein extract and found that trypsin inhibitors attenuated the inhibitory capacity from 80ºC. According to Bariani et al. (2012), these different responses can be explained by the three-dimensional structure of each protein, considering the formation of amino acids and peptide bonds that constitute the structure and provide functional stability.

Conflict of Interest

The authors declare no conflict of interest between the partners with the dissemination of results.

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Full Length Research Paper

Relationship between sunflower productivity and soil´s chemical properties by geo-statistical techniques

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Classical geostatistic techniques and geostatistics are important tools to co-relate linearly and spatially vegetal productivity to the soil´s properties. Spatial and Pearson´s co-relationships between the attributes of the sunflower plant and the soil´s properties were used in Campo Novo do Parecis MT Brazil in 2013 to determine the most significant co-relationship between cause and effect (soil and plant). A geostatistic network was established to collect data from the soil and plant from 100 sample points in plot with sunflower plants. Soil was classified as Red-Yellow Dystrophic Latosol (Typic Tropudox) and the capacity for cation exchange by Pearson´s linear co-relationships was the sole attribute of the soil to estimate the productivity of achenes. Since it had a direct spatial relationship with APL, and, in its turn, APL with PR, the soil´s pH provided a better performance in the limitation of regions with distinct growth and productive potentials of sunflowers. Conservational managements of the soil that aim at raising organic matter rates which, in their turn, affect directly and positively cation exchange capacity, are required to obtain the highest productivity of sunflower achenes due to their close relationship with plant´s height and achene mass.

Key words: Soil fertility, *Helianthus annuus* L., regression, kriging map, semivariogram.

INTRODUCTION

Sunflower crops (*Helianthus annuus* L.) have highly interesting agronomic characteristics for production systems in the Brazilian savannah. They comprise great tolerance to droughts, cold and heat when compared with most species cultivated in Brazil (Leite et al., 2005). Owing to its high demand by industrial and commercial sectors, the sunflower is a highly relevant economical alternative in crop rotation, intercropping and succession in grain-producing regions (Porto et al., 2007). According to data by Conab (2014), estimates on sunflower

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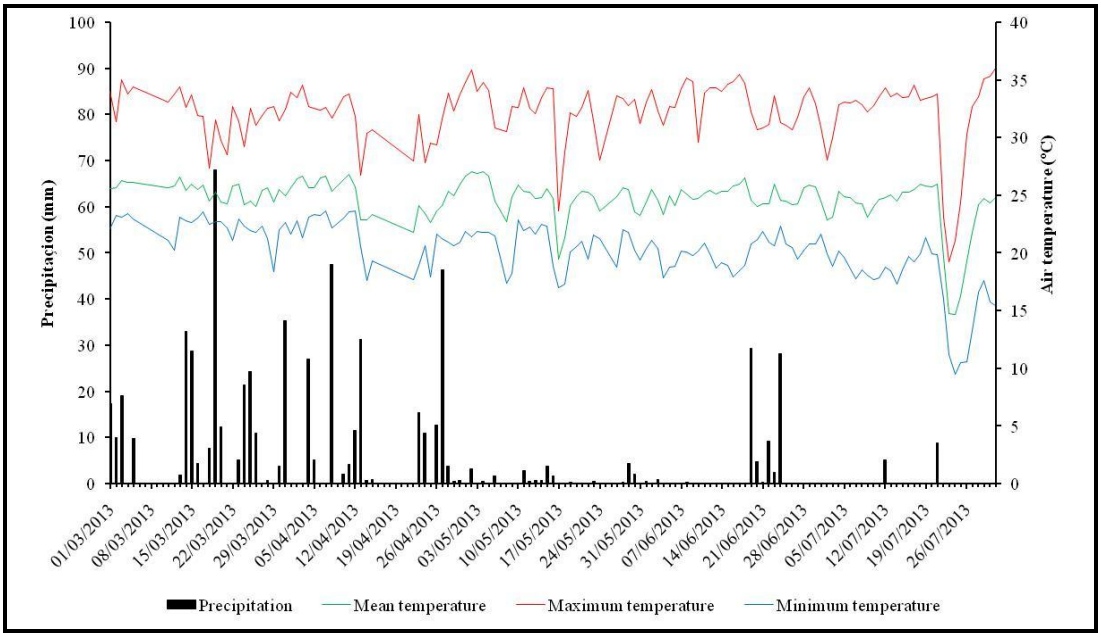

Figure 1. Rainfall and temperature averages at the experimental area between March and July 2013.

production in Brazil for the 2014/14 harvest amount to 108.8 thousand tons of grain, with the share of the state of Mato Grosso featuring 78.2%, or 85.1 thousand tons. Cultivated area occupies 68.7 thousands of hectares, with mean productivity of 1585 kg ha^{-1}, with 1679 kg ha^{-1} for the state of Mato Grosso.

Research-provided information has been decisive for technological support in crop development with better productivity and competitive economic gains. Among the several technologies developed for the production of sunflower, the choice of the genotype is the main item for the crop´s production system (Porto et al., 2007). Further, increase in crop productivity also involves mineral nutrition and the correct supply of essential elements that comply with the requirements at each phenologic stage. The sunflower´s nutritional requirements (Zobiole et al., 2010) are higher than those of other second harvest crops, such as corn (Pinho et al., 2009) which is the main crop cultivated in autumn in the Parecis region in the state of Mato Grosso, Brazil.

Geostatistics analyzes the spatial dependence of geo-referenced data from which a semivariogram is derived, represented by a semi-variance graph due to distances between observations. A kriging map may be thus prepared for each soil or plant attribute which represents the data´s spatial variability. However, a co-kriging maps may also be provided for the main attribute, more interesting, albeit more difficult to obtain, due to the secondary attribute which is normally easier to obtain (Molin et al., 2007; Montanari et al., 2010; Dalchiavon et al., 2011). The secondary attribute may spatially provide estimates for the first attribute and eventual interventions of soil management targeting the primary attribute.

Research has recently been developed to discover the relationship between the soil´s chemical attributes (secondary) and the crop´s productivity (primary), with special reference to Lima et al. (2010), Dalchiavon et al. (2011,2013a,b), Carvalho et al. (2012) and Montanari et al. (2013a) respectively for eucalyptus, jack beans, eucalyptus, sugarcane, rice and beans. Since the studies above reveal the scantiness of research on the sunflower, it is highly relevant that research co-relates its development with the soil´s chemical characteristics, with special focus on the regions in which the crop may be employed during autumn to replace corn. In fact, producers in the Parecis region may minimize production costs and the social and environmental impacts of monoculture. Current research utilizes spatial and Pearson´s co-relationships to investigate the sunflower plant´s productivity as a function of the soil´s chemical attributes.

MATERIALS AND METHODS

Current study was developed at the Instituto Federal de Mato Grosso (IFMT) in 2013, in Campo Novo do Parecis MT Brazil, at 13°40'31" S; 57°53'31" W; 574 m high. Climate is A_w (tropical wet climate, with rainy summers and dry winters). Figure 1 shows mean rainfall and temperature during the crop period.

Soil is Red-Yellow Dystrophic Latosol (Typic Tropudox), characterized by 506 g kg^{-1}clay, 134 g kg^{-1} silt and 360 g kg^{-1}sand at 0 to 0.20 m layer. Initial chemical features of the soil (2012) at 0 to 0.20 m layer revealed the following rates: pH ($CaCl_2$) = 5.7; MO = 26 g dm^{-3}; P (resin) = 5.9 mg dm^{-3}; K, Ca, Mg and H+Al = 1.5; 32; 11 and 40 $mmol_c$ dm^{-3}, respectively, with V = 54.8%. After soybean

Table 1. Descriptive analysis of *Helianthus annuus* L. attributes in a Red-Yellow dystrophic Latosol in direct tillage system. Campo Novo do Parecis MT Brazil, 2013.

Attribute[(a)]	Descriptive statistical measurements									
	Mean	Median	Rate		Standard deviation	Coefficient			Probability of test[(b)]	
			Minimum	Maximum		Variation (%)	Kurtosis	Asymmetry	Pr<w	DF
PA (kg ha^{-1})	1330.6	1350.0	411.8	2398.8	406.85	30.6	-0.165	-0.060	0.662	NO
MHP (m)	1.55	1.60	1.11	1.83	0.164	10.6	0.494	-0.964	10^{-4}	ND
BDS (mm)	17.3	17.7	10.2	22.8	2.57	14.9	0.662	-0.597	0.006	ND
CD (mm)	96.7	96.9	56.9	142.5	15.80	16.3	0.307	-0.021	0.880	NO
CM (g)	70.26	68.40	15.80	153.50	24.74	35.2	0.638	0.512	0.154	NO
MA (g)	46.21	45.65	8.80	94.60	16.49	35.7	0.089	0.334	0.418	NO
AI	0.658	0.664	0.557	0.731	0.036	5.5	0.272	-0.729	0.003	ND
M100 (g)	6.06	5.78	4.21	8.56	0.95	15.7	-0.615	0.222	0.114	NO

[(a)]PA, MHP, BDS, CD, CM, MA, AI and M100 are respectively achene productivity, height of plant, diameter of stem, diameter of chapter, mass od chapter, mass of achenes, index of achenes and mass of one hundred achenes; [(b)] DF = distribution of frequency, NO and ND are respectively normal and non-determined type.

harvest, Hybrid Syn 045 sunflower crop was sown in a 5,525 m^2 (65 x 85 m) plot on 13/03/2013, with 0.45 x 0.33 m spacing and basing fertilization at 300 kg ha^{-1} featuring 10-30-20. Data collection network was established on 25/07/2013, with 10 N-S transections, spacing 7 m, with ten sampling point each, spaced 9 m, with a total of 100 points. Soil and plant attributes for each sampling point were collected between 25/07/2013 and 30/07/2013.

Soil attributes at a depth between 0 to 0.20 m were collected between the lines of the central point, for rates of phosphorus (P), organic matter (OM), pH ($CaCl_2$), potassium (K), calcium (Ca), magnesium (Mg), potential acid (H+Al), sum of bases (SB), capacity for cation exchange (T), saturation per base (V%) and boron (B). The soil´s chemical attributes were obtained following Raij et al. (2001). All analyses were performed at the Laboratory of Physics and Soil Fertility of the Engineering Faculty of Ilha Solteira (UNESP) SP Brazil.

Plants´ attributes, productivity of achenes (PA), mean height of plant (MHP), basal diameter of the stem (BDS), chapter diameter (CD), chapter mass (CM), mass of achenes (MA), achene index (AI) and mass of one hundred achenes (M100) were determined from data collected in 4 rows of 1.80 m crop height.

Calculation of PA (kg ha^{-1}) for each point was based on 3.24 m^2 (1.8 x 1.8 m) by manual harvest of the chapters at stage R9 after natural drying, mechanical pathway and weighing. MHP (m) was assessed by a sample of five continuous plants measured between the plant´s base and the insertion of the chapter in full florescence (R5.5); BDS (mm) was measured by digital caliper at 5 cm of soil level at the end of full florescence; CD (mm) was also measured by digital caliper. CM (g) was evaluated by mean weight of 5 chapters, whereas MA (g) was measured by weighing the achenes only. AI was calculated by MA/MC; M100 (g) was determined by random collection and weight of a samples with 100 achenes. Grain humidity was 11% for PA and M100 (humid base – h.b.).

Statistical analysis was performed with SAS (Schlotzhaver and Littell, 1997) and Excel sheets, following Dalchiavon and Carvalho (2012). The attributes´ descriptive analysis was performed by calculating mean, median, minimum and maximum rates, standard deviation, coefficient of variance, kurtosis, asymmetry and the analysis of frequency distribution by Shapiro and Wilk´s test. A correlation matrix was established among all the researched attributes comprising all possible paired combination to detect Pearson´s significant correlations among the attributes (dependent variables x independent variables). PA´s simple and multiple linear regressions were undertaken as a function of the soil´s or plant´s attributes to trace which would function as quality indicator when PA had to increase. SAS was employed for multiple linear equations at 10% probability for the inclusion and exclusion of variables in the model, following Dalchiavon and Carvalho (2012). Gamma Design Software 7.0 (GS$^+$, 2004), was employed for geostatistic analysis following procedures described by Dalchiavon and Carvalho (2012). Spatial dependence was analyzed for each attribute by calculating the semivariogram where adjustments were previously made by the initial selection of (a) the lowest sum of the square of the deviations (RSS); (b) highest coefficient of determination (r^2); and (c) highest evaluator of spatial dependence (ESD). Interpretation proposed for ESD followed Dalchiavon and Carvalho (2012): a) ADE < 20% = very low dependence spatial variable (VLD); b) 20% ≤ ESD < 40% = low dependence (LD); c) 40% ≤ ESD < 60% = fair dependence (FD); d) 60% ≤ ESD < 80% = high dependence (HD) and e) 80% ≤ ESD < 100% = very high dependence (VHD). Co-krigings were performed especially between PA and the soil´s and plant´s attributes to discover an attribute (soil and/or plant) that would spatially function as quality indicator, or rather, when the productivity of achenes had to be increased.

RESULTS AND DISCUSSION

Normal frequency distribution (FD) is the best for statistical studies (regression and/or geostatistic analyses) since it is the typical representation of the plant´s data; otherwise, normality is reached by logarithmic transformation (Molin et al., 2007). Table 1 shows that the attributes PA, CD, CM, MA and M100 had normal frequency distribution whose coefficients of kurtosis and asymmetry varied respectively between -0.615 and 0.662 and e -0.964 and 0.512. The above was expected since they were biological attributes (Lima et al., 2010; Montanari et al., 2010, 2013a; Dalchiavon and

Table 2. Descriptive analysis of chemical attributes in a Red-Yellow dystrophic Latosol in direct tillage system. Campo Novo do Parecis MT Brazil, 2013.

Attribute[(a)]	Descriptive statistical measurements									
	Mean	Median	Rate		Standard deviation	Coefficient			Probability of test[(b)]	
			Minimum	Maximum		Variation (%)	Kurtosis	Asymmetry	Pr<w	DF
P (mg dm^{-3})	15.7	11.5	5.0	50.5	11.46	72.8	2.308	1.753	10^{-4}	ND
OM (g dm^{-3})	26.6	27.0	21.0	32.0	2.18	8.2	0.135	-0.484	0.002	ND
pH	5.6	5.6	4.9	6.1	0.26	4.6	-0.116	-0.427	0.009	ND
K ($mmol_c$ dm^{-3})	1.1	1.1	0.2	2.4	0.44	39.6	0.382	0.522	0.053	ND
Ca ($mmol_c$ dm^{-3})	20.5	20.0	10.0	33.0	4.68	22.9	-0.411	0.128	0.628	NO
Mg ($mmol_c$ dm^{-3})	16.2	16.5	7.0	25.0	4.13	25.5	-0.507	-0.135	0.144	NO
H+Al ($mmol_c$ dm^{-3})	30.8	31.0	16.0	45.0	6.43	20.9	-0.244	-0.290	0.046	TL
Al ($mmol_c$ dm^{-3})	0	0	0	0	0	0	0	0	0	ND
SB ($mmol_c$ dm^{-3})	37.8	38.0	18.3	60.8	8.59	22.7	-0.280	0.012	0.931	NO
T ($mmol_c$ dm^{-3})	68.6	68.5	52.5	84.8	6.07	8.9	0.247	0.022	0.895	NO
V% (%)	54.8	54.0	29.0	73.0	10.06	18.4	-0.586	-0.230	0.143	NO
B (mg dm^{-3})	0.18	0.18	0.05	0.35	0.050	27.0	0.667	0.360	0.080	NO

[(a)]P, OM, pH, K, Ca, Mg, H+Al, Al, SB, T, V%1 and B are respectively phosphorus, organic matter, pH, potassium, calcium, magnesium, potential acidity, exchangeable aluminum, sum of bases, capacity of cation exchange, saturation per bases and boron; [(b)] DF = distribution of frequency, ND, NO and TL respectively non-determined, normal and prone to lognormal.

Carvalho, 2012, 2013a,c), where as the other attributes (MHP, BDS and AI) had a non-determined type of distribution.

Low (AI), medium (MHP, BDS, CD and M100) and very high (PA, CM and MA) data variability occurred when analyzed by the coefficient of variance (Table 1) and contrasted data by Martin et al. (2012) for attributes PA, MHP, BDS and CM. This may have been related to the fact that the authors worked with different spatial distributions among the plant rows. PA rate of 1331 kg ha^{-1} was low when compared to rate 1671 kg ha^{-1} of medium PA for the state of Mato Grosso in the 2012/2013 harvest (Conab, 2014); similarly to PA rate of 1555 kg ha^{-1} reported by Höhn et al. (2012) for cultivar Syn 045. The results above may be due to difficulties in controlling weeds in the post-emergence condition, which caused competition for the available environmental resources, and to the lack of B fertilization, an essential nutrient to the crop´s numberless metabolic processes. On the other hand, PA exceeded the rate (1287 kg ha^{-1} for the same cultivar Syn 045) registered by Embrapa (2011) in an experiment network in the state of Rondônia, Brazil. Other medium rates were respectively 96.7 mm; 70.26, 46.21 and 6.06 g for CD, CM, MA and M100. In the case of attributes with non-determined frequency distribution, median rates were 1.60 m (MHP), 17.7 mm (BDS) and 0.664 (AI), similar to data by Höhn et al. (2012), featuring MHP with 1.68 m; CD with 120 mm and M100 with 5.10 g, and by Embrapa (2011), featuring MHP with 1.26 m.

In the case of soil attributes (Table 2), normal (Ca, Mg, SB, T, V% and B) and non-determined DF (P, MO, pH, K and Al) occurred, with lognormal – TL (H+Al). As a rule, the coefficients of variation remained between low (pH and Al) and very high (P and K), similar to those of kurtosis, between -0.586 and 2.308, and asymmetry, between -0.484 and 1.753, as described by Dalchiavon et al. (2012, 2013a,b) and Montanari et al. (2013a) for DF and coefficients of variation. In the case of soil fertility and taking into account mean/median rates of their chemical attributes, they represented low (P, pH, K), medium (MO and V%) and high (Ca and Mg) rates, following limits reported by Dalchiavon et al. (2012c), justifying partially PA. Rates of the soil´s chemical attributes agreed with those mentioned by the same authors when they investigated the spatial variability of fertility of a Red Dystroferric Latosol under direct tillage in Selvíria MS Brazil.

Interesting and significant correlations for the pairs of attributes were 1) PA x MHP (r = 0.557**); 2) PA x BDS (r = 0.558**); 3) PA x CD (r = 0.630**); 4) PA x CM (r = 0.664**); 5) PA x MA (r = 0.671**); 6) PA x M100 (r = 0.217*)and 7) PA x T (r = 0.196*), or rather, similar to the positive correlations by Amorim et al. (2008) and Martin et al. (2012) for PA x CD and PA x M100, respectively. It should be underscored that all correlations had a direct cause-effect behavior. In other words, any alteration in the independent variables´ rates (cause) caused a similar change in the variable response (PA).

Coefficients of correlation between the plant´s attributes were high ($p<0.01$) and positive, featuring a good direct relation among the attributes analyzed. The T

Table 3. Parameters of simple and cross semivariograms adjusted for *Helianthus annuus* L. attributes and chemical parameters of a Red-Yellow Dystrophic Latosol in direct tillage system. Campo Novo do Parecis MT Brazil, 2013.

Attribute[(a)]	Adjustment parameters										
	Model[(b)]	C_o	C_o+C	A_o (m)	r^2	SSR[(c)]	ESD[(d)]		Cross validation		
							%	Class	A	b	r
					γ(h) simple – plant						
#MHP	exp (81)	$9.22.10^{-3}$	$1.85.10^{-2}$	67.8	**0.688**	$2.45.10^{-5}$	50.3	ME	0	1.000	0.416
					γ(h) simple – soil						
P	gau (110)	2.45.10	7.85.10	12.6	**0.443**	$7.32.10^{2}$	68.8	H	$4.00.10^{-2}$	1.002	0.520
pH	exp (104)	$1.14.10^{-2}$	$6.08.10^{2}$	41.7	**0.916**	$8.47.10^{-5}$	81.3	VH	$7.20.10^{-1}$	0.871	0.485
K	gau (97)	$1.32.10^{-1}$	$1.96.10^{-1}$	16.2	**0.838**	$3.83.10^{-4}$	32.9	LA	$6.10.10^{-1}$	0.453	0.138
Ca	gau (103)	2.80	1.93.10	14.4	**0.753**	2.52.10	85.5	VH	5.90	0.716	0.527
Mg	gau (106)	5.97	1.42.10	14.1	**0.558**	1.39.10	58.0	ME	2.36	0.859	0.398
H+Al	sf (87)	$9.00.10^{-1}$	3.54.10	15.9	**0.800**	5.64.10	97.5	VH	1.53.10	0.501	0.329
SB	sf (102)	7.40	6.69.10	18.4	**0.851**	$1.37.10^{2}$	88.9	VH	1.06.10	0.727	0.448
V%	sf (108)	$4.00.10^{-1}$	8.31.10	14.2	**0.423**	$1.15.10^{3}$	99.5	VH	1.71.10	0.689	0.428
					γ(h) cross – plant x soil						
#MHP=f(pH)	sf (42)	$8.70.10^{-4}$	$7.54.10^{-3}$	30.5	**0.396**	$4.41.10^{-5}$	88.5	VH	0	0.058	0.179

[(a)] # MHP = mean height of plant, P = phosphorus, pH = hydrogenionic potential, K = potassium, Ca = calcium, Mg = magnesium, H+Al = potential acidity, SB = sum of bases; V% = saturation per base; # = attribute with data residues; parentheses following the model = number of pairs in the first *lag*; [(b)] exp = exponential, gau = gaussian and sf = spheric; [(c)] SSR = sum of squares of residues; [(d)] ESD = evaluator of spatial dependence, ME = medium, H = high, VH = very high; L = low.

was the only significant soil attribute with PA, with a low but positive coefficient of correlation ($p<0.05$), mainly due to being a classic example of dependent (PA) against and independent (T) variable, and to the high number of observations (n=100), common in geostatistic studies, as has been reported by Carvalho et al. (2012) and registered by other researchers (Molin et al., 2007; Dalchiavon et al., 2013a,b,c; Montanari et al., 2013a,b,c). Adjusted equations were the following:

$PA = 562.26xMHP^{1.87**}$.....(r = 0.600**) (1)

$PA = 30.33xBDS^{1.313**}$.....(r = 0.603**) (2)

PA =-238.52+16.22**xCD.....(r = 0.630**) (3)

PA = 563.29+10.92**xCM.....(r = 0.664**) (4)

PA = 565.77+16.55**xMA.....(r = 0.671**) (5)

$PA = 498.06xM100^{0.518*}$.....(r = 0.227*) (6)

PA = 430.28+13.133*xT.....(r = 0.196*) (7)

$PA =-686.41+903.80**xMHP+13.32**xMA(r^2 = 0.565**)$(8)

Equations (1), (2) and (6) showed the potential direct influence of MHP, BDS and M100 on PA, whilst Equations (3), (4), (5) and (7), albeit direct, revealed linear models among the independent (CD, CM, MA and T) and dependent (PA) attributes. Equation (5) provided the best adjustment (with a higher "r") among the equations for plant´s attributes. Thus, it has been highly recommended to estimate PA at 1330 kg $ha^{-1}$when MA has 46.21 g (Table 1). However, T was the only soil attribute to estimate PA (Equation 7) which will be 1331 kg ha^{-1} when T is 68.6 $mmol_c$ dm^{-3}. Alternately, Equation (8), multiple linear regression obtained stepwise, may be employed to estimate productivity, which is currently 1375 kg ha^{-1}. Consequently, conservation managements of soil, such as direct tillage to raise the rates of organic matter which, in turn, affect T directly and positively, are required to obtain the highest productivity of sunflower achenes since the attribute is closely related to MHP and MA (Equation 8).

Geostatistic analysis (Table 3; Figure 2) showed that, in the case of attributes lacking gold standard, MHP derived in a coefficient of high (0.668) spatial correlation (r^2), medium (50.3%) spatial dependence (ESD) and angular coefficient(b) of cross validation equal to 1, with a high quality of experimental semivariogram of the exponential type and differing from the Guassian semivariographic model reported by Carvalho et al. (2012), who analyzed MHP of *Eucalyptus camaldulensis*, and reported very high r^2 (0.965), medium ESD (59.5%) and angular coefficient of cross validation equal to 0.986.

In the case of soil attributes, spatial correlation coefficients remained between medium (V%; 0.423) and very high (pH; 0.916), spatial dependencies between low (K; 32.9%) and very high (V%; 99.5%) and angular coefficients between 0.453 (K) and 1.002 (P). Geostatistic data in Table 3 had a similar behavior to

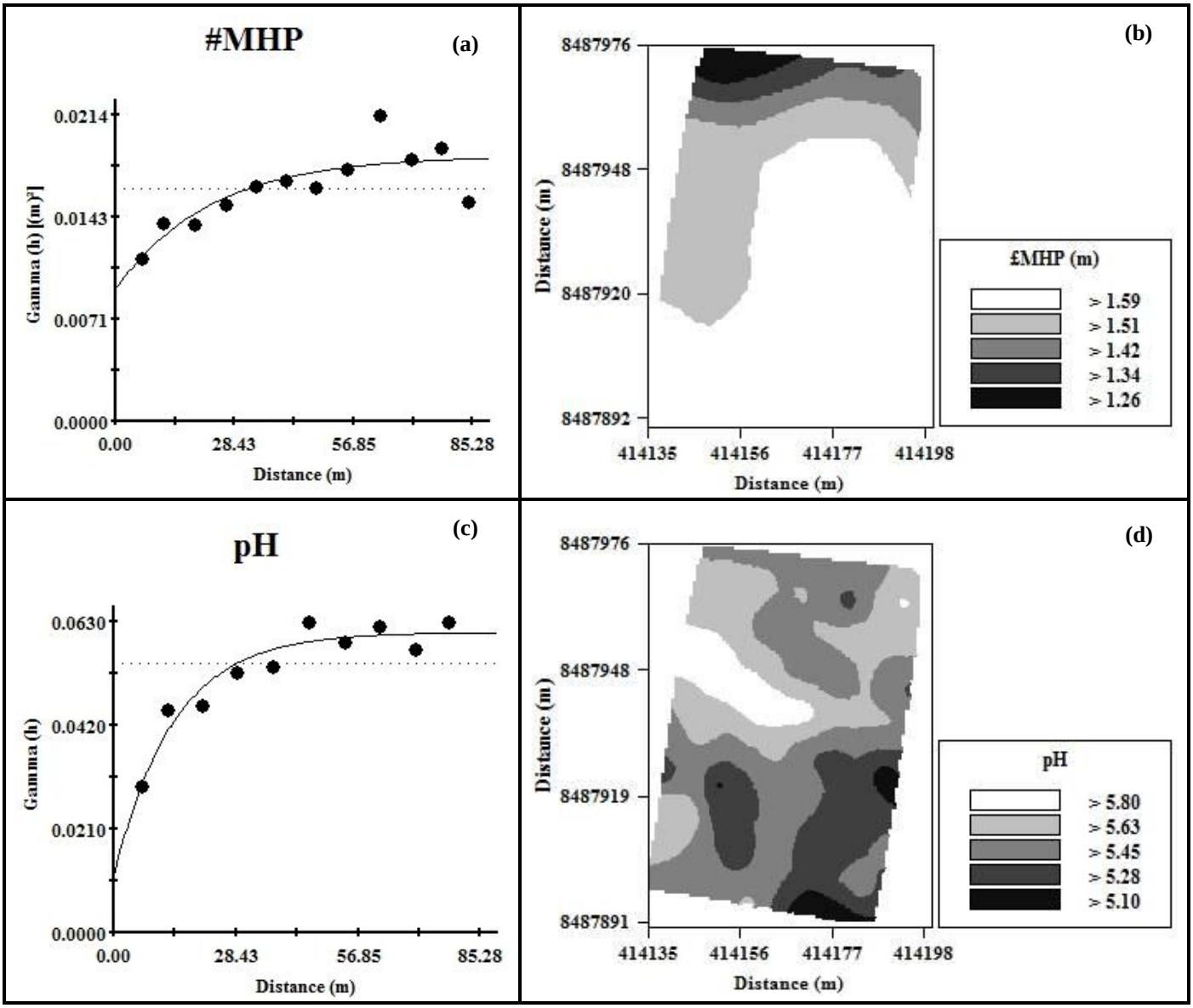

Figure 2. Semivariograms and kriging maps of plant height of *Helianthus annuus* L. and pH of Red-Yellow Dystrophic Latosol in direct tillage system. Campo Novo do Parecis MT Brazil, 2013.

results reported by Molin et al. (2007), Lima et al. (2010), Carvalho et al. (2012) and Dalchiavon et al. (2011, 2012c, 2013a,b,c), when these authors investigated the spatial variability of the soil´s chemical attributes with different annual and perennial crops.

Cross semivariogram (Table 3; Figure 3) provided a low coefficient of spatial determination (r^2=0.396) for the sphere-type only semivariographic model [#APL=f(pH)]. From the spatial point of view, a direct MHP correlation occurred with pH. In their research on the relationship of the soil´s physical and chemical attributes with the eucalyptus´s dendrometric characteristics, Lima et al. (2010) reported that the volume of timber in the species was spatially explained by the strict bonds with the soil´s pH. On the other hand, Dalchiavon et al. (2011) studied the spatial variability of the bean´s productivity correlated with the soil´s chemical attributes under direct tillage system and registered spatial correlation between the grains´ productivity and the soil´s pH. Gauss-type cross semivariogram with r^2=0.925 could be modeled. In an analogous way, Montanari et al. (2013a) verified that, when they analyzed the relation between the productivity of bean grains and the chemical attributes of a type of soil with direct tillage system, the productivity of beans could be explained by the soil´s pH special rates. The studies above clearly reveal the influence of the soil´s pH

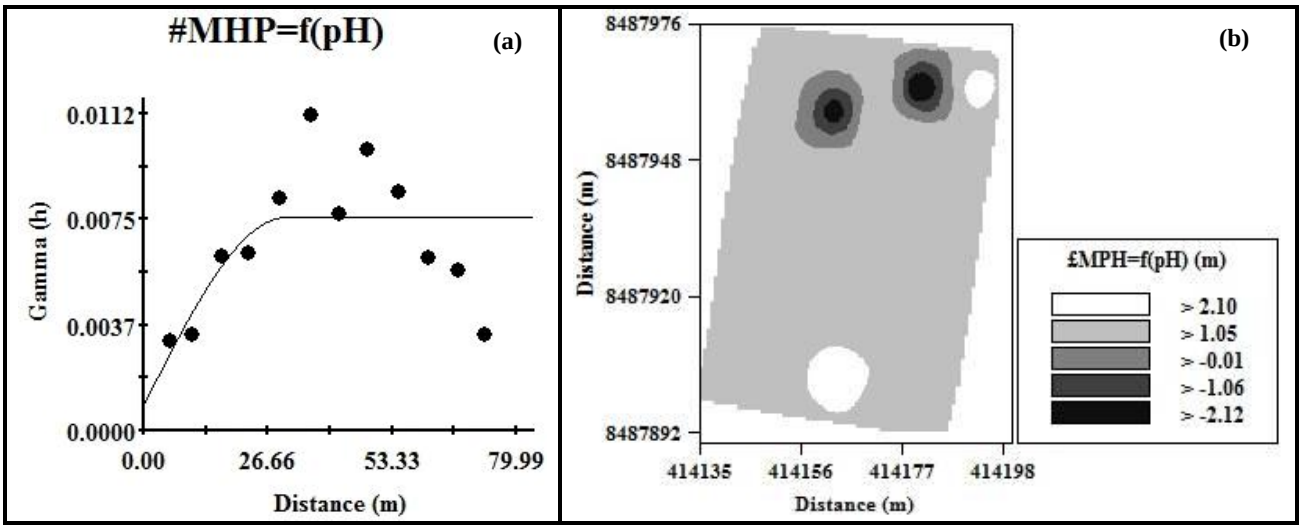

Figure 3. Cross semivariogram and co-kriging map of height of plant of *Helianthus annuus* L. as a function of pH of a Red-Yellow Dystrophic Latosol in direct tillage system. Campo Novo do Parecis MT Brazil, 2013.

on the attributes of vegetal production.

In Figures 2 and 3, co-kriging #APL=f(pH) showed the lowest £APL (1.26 to 1.42 m) at the sites of the lowest pH rates (5.10 to 5.45). On the other hand, the highest £APL (1.42 to 1.59 m) occurred within the regions with the highest pH rates (5.45 to 5.80). Since the soil´s pH had a spatial relation with MHP and the latter with PA by Pearson´s correlation (Equation 1), there was a good performance in the limitation of two regions with different plant growth when evaluated by their heights. Whereas MHP of one region lay between 1.26 and 1.42 m, the MHP of the other region was found to be between 1.42 and 1.59 m. Therefore, the latter had the highest PA. When £APL and PA should be increased, pH may be used as a reference.

Conclusion

The capacity for cation exchange within Pearson´s linear correlations was the sole attribute to assess achene productivity. However, since the soil´s pH has a direct spatial relation with £APL and the later with PA, it provided a better performance in the limitation of regions with distinct growth and production potentials of sunflower plants. Soil conservation managements raise organic matter rates which, in their turn, comprise directly and positively cation exchange capacity, are required to obtain the highest production of sunflower achenes since they are closely related to plant height and achene mass.

Conflict of Interest

The authors have not declared any conflict of interest.

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Nutrient demand of the carrot crop

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The existing literature for the recommendation of fertilizers and diagnosis of the nutritional status of the carrot crop is outdated because it contemplates productivities lower than those currently obtained. The objective of this research was to characterize the nutritional demand of the carrot crop by estimating the dry matter content of the roots, the coefficient of biological utilization of the nutrients and the harvest index of dry matter and of the mineral nutrients, in order to indicate fertilizers according to the desired productivity for winter and summer cultivations. We sampled 210 carrot plots located in the Alto Paranaíba region, Minas Gerais, Brazil, during 2012 and 2013. We determined the content of dry matter of the roots, the coefficient of biological utilization of the nutrients in the roots and leaves and the harvest index of dry matter and nutrients in the crop. Data were grouped in two groups of cultivations: Winter and summer. The harvest index of dry matter and of nutrients was bigger for the winter cultivars. Regardless of the growing season, the N, P, K, Mg and B were retained in greater amounts in the roots. Phosphorus had the highest harvest index, and the Cu, the lowest. The differences were insignificant in the nutritional demand of N, P, Ca, S, B, Cu and Zn between winter and summer cultivars for the average productivity obtained in each season. In the summer cultivars, the carrot accumulates greater amounts of Fe and lower of K, Mg and Mn when compared to winter. The modeling of the nutritional demand of the carrot crop can be carried out depending on the desired productivity and growing season.

Key words: Nutrients balance, *Daucus carota*, nutritional demand, nutrients recommendation.

INTRODUCTION

In the last years, there was an evolution in the cultivation of carrot due to the introduction of new techniques in the production system, and consequently, rapid evolution of productivity was achieved. Besides the introduction of new cultivars, phytosanitary and nutritional managements evolved to provide high productivity.

Fertilizer recommendations are made based on information available in tables published in state manuals; however, some drawbacks can be cited about this method of recommendation. The regional applicability, the non-constant updates in relation to new cultivars/hybrids that appear on the market and the scope of productivity generally lower than those obtained in technified crops represent the main negative points of this method of recommendation. In the state of Minas Gerais, Brazil, for example, the official recommendation

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was published in 1999 and includes productivity of up to 40 Mg ha^{-1} of roots (Ribeiro et al., 1999).

In this context, the use of nutritional balance models can be a strategic way of recommending fertilizers and correctives by taking into consideration numerous factors, in particular, the productivity (Haefele et al., 2003). The obtainment of information (attributes) necessary to calculate the nutrient demand is the critical point for the use of the nutritional balancing system in the carrot crop due to the lack of data in the literature.

The nutritional balance system comprises mathematical models which allow the estimation of the requirement of nutrients by the crop and the supply of nutrients by the soil, and thus, the recommendation comprises the difference between the demand of the crop and the soil supply (available nutrients in the soil plus the ones coming from the mineralization of crop residues).

Although efficient, the method of nutritional balance still cannot be used in carrot crop due to the lack of information of the nutritional demand of this species for high yields. To estimate the nutritional demand of the new cultivars you must know some attributes as dry matter content in the roots (DM), the coefficient of biological utilization (CBU) of nutrients in different organs of the plant and the harvest index (HI) of dry matter and nutrients. The CBU is the ratio of the accumulation of biomass and the accumulation of a particular nutrient (Fageria, 1998; Kurihara et al., 2013). The HI is the percentage of dry matter or nutrient, which is found in the harvested organ (tuberous root in the carrot crop) in relation to the whole plant biomass.

Thus, this study aimed to characterize the nutritional demand of the carrot crop by estimating the dry matter content of the roots, the coefficient of biological utilization of the nutrients and the harvest index of dry matter and of the mineral nutrients, in order to indicate fertilizers according to the desired productivity for winter and summer cultivations.

MATERIALS AND METHODS

To determine the attributes needed to estimate the nutritional demand of the carrot crop we generated a database with information of 210 commercial plots located in the region of Alto Paranaíba, Minas Gerais, Brazil. To achieve this, samples were taken during the growing seasons 2012 and 2013 and covered crops in the municipalities of Rio Paranaíba, São Gotardo and Campos Altos. In these places, the carrot fields were in the altitude of approximately 1100 m and in an environment with the prevailing Cwa climate, according to the Köppen-Geiger classification. This climate is characterized by a dry season and a well-defined rainy season that occurs between October and March. Regarding the type of soil, very clayey yellow, red and red-yellow latosols predominated. Fertilizers recommendations were made based on soil analysis. Irrigation and phytosanitary crop management followed the technical recommendations characteristic of the crop.

In the plots that were evaluated, we determined the root productivity, the dry matter content in the roots, the accumulation of dry matter of roots and leaves and the nutrient content in the plant. Samples of leaves and roots collected at harvest were dried in a greenhouse with forced air at 70ºC for 72 h. Then they were ground in a Willey mill equipped with sieve of 1.27 mm. The nutrients content was determined according to methods described by Malavolta et al. (1997).

The extraction of nutrients was obtained from the sum of the content of nutrients in the roots and leaves. This, in turn, was obtained by the product of the accumulation of the dry matter and nutrient concentration in each part of the plant (root or leaf). The CBU was calculated by dividing the accumulation of DM and accumulation of specific nutrients in each organ of the plant and expressed in kg kg^{-1} and kg g^{-1} for macro and micronutrients, respectively. We calculated the HI with the ratio between the accumulation of DM or nutrient in the commercial body (root) and the total accumulation of the crop, which was expressed in percentage. Data were grouped into two cultivation systems: Summer or winter.

The demand for nutrients was calculated by dividing the content of nutrients in the tubers and the nutrient harvest index. The content of nutrients in the tubers was obtained from the ratio between the dry matter produced from tubers and the CBU of the nutrient for each cultivar, according to the equations:

$$DEM\ X = 100.\frac{EXP\ X}{HI\ X} \quad (1)$$

$$EXP\ X = \frac{10\ .\ Prod\ .\ DM}{CUB\ X_{root}} \quad (2)$$

Wherein: DEM X: demand of the nutrient X (kg ha^{-1}); HI X: harvest index of the nutrient X (%); EXP X: export of the nutrient X (kg ha^{-1}); Prod: desired productivity of roots (t ha^{-1}); DM: dry matter content in the roots (%); CBU X $_{root}$: coefficient of biological utilization of the nutrient X in the root (kg kg^{-1}).

The data were submitted to outliers` analysis, eliminating the values dissonant from the average. Descriptive statistics tools were employed to characterize the database and present the necessary attributes for modeling the nutrient demand of the carrot crop.

RESULTS AND DISCUSSION

The analysis of the chemical properties of the cultivation soil showed that they had corrected acidity (high pH) and adequate levels of macronutrients (adequate levels of P and K and less levels of Ca, Mg and S) (Table 1). In contrast, the soils showed imbalances as for the micronutrients, once, on average, the contents of Mn were considered low, the ones of B average, the ones of Fe good and the ones of Cu and Zn high, according to the classification proposed by the Ribeiro et al. *(*1999).

The soils presented average levels of organic carbon (2 dag kg^{-1}) and low remaining P (10.6 mg L^{-1}). The organic carbon content can be the result of the handling adopted in the properties in the region, where the carrot is within the crops rotation comprising other vegetable crops, such as garlic, onion and potatoes. Thus, these soils annually undergo intense turnings to condition the cultivation of these species and consequently, the mineralization of the organic matter of the soil is increased. In relation to the remaining P, the low value indicates that soils are much buffered for this nutrient, that is, addition of large amounts of this nutrient is required on soil to increase a small fraction of P available to the plant (Bedin et al.,

Table 1. Average and standard deviation of the main soil attributes in the layer 0 to 20 cm depth.

Attribute	Unit	Extractor/ method	Average	Standard deviation
pH	-	H_2O	6.3	0.3
Organic carbonic	dag kg^{-1}	$K_2Cr_2O_7$/ Walkley-Black	2.0	0.3
P - rem	mg L^{-3}	-	10.6	3.2
Phosphorus (P)	mg dm^{-3}	Mehlich-1	28.0	15.1
Potassium (K^+)	$mmol_c$ dm^{-3}	Mehlich-1	3.1	0.8
Calcium (Ca^{2+})	$mmol_c$ dm^{-3}	KCl	33.9	5.8
Magnesium (Mg^{2+})	$mmol_c$ dm^{-3}	KCl	10.7	3.0
Sulfur (SO_4^{2-})	mg dm^{-3}	$Ca(H_2PO_4)_2.H_2O$ in AcOH	7.5	4.5
CEC (T)	$mmol_c$ dm^{-3}	-	82.3	8.2
Base saturation (V)	%	-	58.0	7.0
Boron (B)	mg dm^{-3}	Hot water	0.52	0.21
Copper (Cu)	mg dm^{-3}	Mehlich-1	2.5	1.4
Iron (Fe)	mg dm^{-3}	Mehlich-1	38.0	12.2
Manganese (Mn)	mg dm^{-3}	Mehlich-1	3.2	2.3
Zinc (Zn)	mg dm^{-3}	Mehlich-1	6.8	3.0
Ca saturation	%	-	41.2	4.9
Mg saturation	%	-	13.0	3.4
K saturation	%	-	3.8	1.1

Table 2. Number of plots, cultivated area, total productivity of roots and cycle of carrot hybrids.

Cultivar	Number of plots		Area		Total productivity		Cycle	
	Nº	%	ha	%	Mg ha^{-1}	CV (%)	Day	CV (%)
Baltimore	18	8.6	67.5	10.1	83.4	25.2	123	8.7
Belgrado	10	4.8	20.3	3.0	83.4	13.0	118	7.1
Concerto	8	3.8	19.1	2.9	90.9	24.2	131	4.0
Maestro	27	12.9	118.6	17.8	82.1	18.1	127	6.3
Músico	13	6.2	40.3	6.1	86.4	19.5	130	4.4
Nancy	10	4.8	35.4	5.3	87.3	21.0	121	8.8
Nandrin	20	9.5	87.4	13.1	81.5	24.7	115	9.4
Soprano	16	7.6	85.4	12.8	87.0	18.8	129	6.7
Winter cultivars	155	73.8	495.3	74.4	81.6	24.3	125	7.9
Juliana	16	7.6	140.6	21.1	63.2	12.5	100	6.4
Poliana	7	3.3	20.4	3.1	56.5	16.4	101	9.3
Summer cultivars	55	26.2	170.6	25.6	60.9	14.3	105	8.1
General (winter and summer)	210	100.0	665.9	100.0	75.4	24.6	120	10.8

2003; Broggi et al., 2011).

There were more winter cultivars (8 major hybrids) than summer (2 major hybrids) (Table 2). This fact is related to climate requirements of the carrot, which are better contemplated in the winter season (mild temperatures, short days and less rainfall). The mild temperatures for the summer conditions in the region of the Alto Paranaíba and cultivars resistant to foliar diseases allow the cultivation of carrot during this time; however, with minor importance and productive potential than in winter cultivars.

The winter cultivars showed productivities 34% higher than those obtained during the summer (81.6 Mg ha^{-1} against 60.9 Mg ha^{-1}), while concerning the cycle, winter cultivars presented cultivation periods 14% higher than summer (125 and 105 days of cycle, respectively). The highest temperatures recorded during the summer induce greater accumulation of DM in the shoot due to unfavorable climatic conditions for root growth (Hussain et al., 2008) and thus reduces the productivity of the roots due to the change in biomass partition. Furthermore, higher temperatures tend to reduce the cycle of the carrot

Table 3. Maximum, average and minimum content of dry matter of tuberous roots of carrot hybrids.

Cultivar	Content of dry matter in roots			
	Maximum (%)	Average (%)	Minimum (%)	CV(%)
Baltimore	11.1	9.7	8.0	9.5
Belgrado	10.9	8.6	6.3	17.9
Concerto	10.7	8.9	7.0	13.0
Maestro	11.5	9.2	4.5	14.1
Músico	12.1	8.8	6.7	18.5
Nancy	10.7	8.4	5.7	18.5
Nandrin	12.3	9.2	6.6	13.5
Soprano	11.1	9.0	7.0	13.7
Winter cultivar	12.3	9.1	4.5	14.4
Juliana	10.9	8.0	4.7	22.3
Poliana	10.0	8.1	6.0	18.6
Summer cultivar	11.5	9.0	4.7	18.5
General (Winter and Summer)	12.3	9.0	4.5	15.4

due to unfavorable environmental conditions for crop growth. According to Thiagaranjan et al. (2012), temperatures higher than 24°C significantly reduce the net photosynthesis of the carrot crop due to thermal stress caused to the species. Thus, the shortest time the plants remain in the field also contributes to the reduction of productivity in summer cultivars compared to winter.

The average yields obtained (75.4 Mg ha^{-1}) can be considered high in relation to the estimated national average in 2012 (28.9 Mg ha^{-1}) (FAO, 2014). In a study conducted by Cecílio Filho and Peixoto (2013) in 2004 in the municipality of São Gotardo in Alto Paranaíba – MG the average productivity obtained was 72 Mg ha^{-1}, similar to the average obtained in this work. In the international context, the yields achieved in Alto Paranaíba are above the world average (30.9 Mg ha^{-1} in 2013) (FAO, 2014) and similar to the ones obtained by Seljasen et al. (2012) in Norway (65.4 Mg ha^{-1}) and Tesfaendrias et al. (2010) in Holland (82.5 Mg ha^{-1}).

The contents of DM in carrot roots ranged from 4.5 to 12.3%, averaging 9.0% (Table 3). Similar average contents (9.9%) were obtained by Seljasen et al. (2012) in studies in Norway. The coefficients of variation (CV) obtained for the content of DM in the roots of the main hybrids of the carrot can be considered low. This parameter shows that the content of DM of the carrot roots does not tend to have large variations within the genetic material, even with the diversity of cultural handlings to which the sampled plots were submitted.

There was virtually no difference in the average levels of DM in winter (9.1%) and summer (9.0%) cultivars (Table 3). However, the main cultivars of carrots grown in summer (Juliana and Poliana) showed average levels of DM below the average for this time of cultivation. Among the main features that make genetic breeders and producers choose certain genetic material one can cite productivity. In the case of the Juliana and Poliana cultivars, the lower content of DM in the roots can provide higher yields, since for the same accumulation of DM in the roots there will be higher fresh mass of roots accumulation. Thus, it is possible that the fact that these cultivars present lower content of DM in the roots is related to the selection performed during the breeding processes of the species and, or, by selection of production traits by farmers. Regarding the winter cultivars, the average content of DM of the main cultivars revolved around the overall average for this time (Table 3).

The CBU showed high variability (CV) for cationic micronutrients (Cu, Fe, Mn and Zn) in both organs of the crop (roots and shoots) and, in some cultivars, the CBU of the root also presented great variability for Ca, Mg and S (Tables 4 and 5). Regarding the cationic micronutrients, part of this variation may be a consequence of the high variability of the contents of these elements in the plant tissue (data not shown). For other nutrients (macronutrients and B), the average CV of the leaf and root CBUs was close to 30%. The lower variability is interesting for the proposition of the model to estimate the demand for nutrients in line with productivity. This is because it allows a single model for the crop and not for the cultivar.

The averages of the CBUs in the root system of winter cultivars for N, P, K, S, Cu and Zn are greater than those of the summer (Table 4). In the shoot, winter cultivars showed the highest CBUs only for Ca, Fe and Zn (Table 5). The higher CBU indicates that these cultivars is more efficient in the use of the respective element, that is, there is greater accumulation of DM per unit absorbed from the nutrient.

Comparing the CBUs between the hybrids of each season it is observed that there is low variation in this

Table 4. Maximum, average, minimum values and coefficients of variation of the coefficient of biological utilizations of macro and micronutrients of carrot hybrids.

Cultivar	Parameter	Coefficient of biological utilization										
		N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
		kg kg^{-1}						g kg^{-1}				
Baltimore	Maximum	131.1	605.2	39.8	1492.5	1342.2	3656.6	42.9	1945.6	23.1	169.5	241.5
	Average	86.3	357.3	28.2	336.0	801.1	1661.6	27.4	351.7	8.4	98.4	73.0
	Minimum	59.2	209.1	18.5	140.1	548.5	771.3	20.6	57.6	3.7	50.0	20.8
	CV (%)	23.5	35.1	21.1	91.6	28.6	48.9	22.4	127.7	65.1	38.6	95.2
Belgrado	Maximum	111.1	513.2	39.7	258.0	1041.7	1585.5	34.5	385.3	8.9	344.8	185.2
	Average	84.5	272.4	27.9	195.6	519.0	1213.9	22.3	142.1	4.5	131.3	59.7
	Minimum	65.8	160.0	20.9	151.0	345.1	623.0	14.6	56.8	2.6	67.5	21.7
	CV (%)	18.4	46.4	20.7	16.6	39.2	29.0	26.6	90.8	56.1	64.7	95.0
Concerto	Maximum	106.0	719.4	50.1	819.7	650.7	4200.0	39.3	635.7	19.3	250.0	186.4
	Average	87.6	422.3	29.7	312.2	547.4	2014.9	29.3	281.5	10.6	114.2	77.3
	Minimum	64.0	232.6	19.4	174.2	377.8	1098.7	22.6	87.6	3.8	72.9	31.7
	CV (%)	15.4	38.3	36.0	67.1	18.9	57.9	19.9	65.3	52.5	52.8	69.0
Maestro	Maximum	103.8	629.0	33.7	295.9	815.0	4023.1	50.0	1907.2	27.1	203.1	697.1
	Average	70.4	361.8	27.2	239.7	564.6	1427.9	29.3	371.5	11.8	136.5	169.4
	Minimum	48.6	191.3	20.6	167.3	401.1	550.0	21.4	45.7	4.2	83.4	23.4
	CV (%)	18.9	31.2	17.1	15.7	21.8	62.1	23.0	118.9	49.1	22.5	95.3
Músico	Maximum	88.7	505.8	37.6	295.9	820.1	5928.4	40.2	1192.0	13.5	158.8	286.1
	Average	71.7	294.4	29.3	228.7	552.3	1765.0	28.0	312.0	9.6	114.9	114.8
	Minimum	53.3	195.5	20.4	140.7	372.9	518.9	18.3	66.6	5.5	67.8	18.6
	CV (%)	17.4	29.3	19.5	18.2	22.5	82.8	22.5	97.2	31.1	21.7	64.5
Nancy	Maximum	244.4	489.1	37.7	337.4	797.3	3782.3	37.5	328.5	13.2	190.1	313.6
	Average	96.0	308.2	26.8	261.3	565.8	1776.3	25.7	136.9	6.3	111.5	98.1
	Minimum	71.1	171.4	19.0	189.1	419.5	747.9	19.8	57.4	1.9	64.2	27.1
	CV (%)	54.7	37.2	23.9	21.8	21.6	51.8	25.0	65.2	60.8	30.9	102.1
Nandrin	Maximum	97.4	782.4	39.4	378.9	1015.4	2643.5	44.9	1366.9	20.6	232.4	1353.1
	Average	78.5	408.4	27.8	265.6	692.6	1686.2	28.3	423.6	10.7	141.1	275.1
	Minimum	56.4	184.0	15.6	162.7	398.5	731.1	16.8	118.8	4.1	70.4	26.4
	CV (%)	17.2	43.7	23.1	22.1	24.7	38.4	31.6	88.6	42.1	33.1	116.7
Soprano	Maximum	104.7	488.8	56.5	308.7	3276.7	2878.6	46.2	422.3	23.7	136.2	1353.1
	Average	75.9	259.7	29.8	253.0	836.5	1602.3	25.3	168.8	8.0	96.5	199.2
	Minimum	55.9	153.7	17.4	185.0	417.9	781.4	16.1	51.0	2.8	63.8	13.7
	CV (%)	18.0	31.8	28.7	16.5	79.8	44.3	34.5	60.5	67.6	25.7	141.9
Winter cultivars	Maximum	244.4	782.4	56.5	1538.5	3276.7	8510.1	50.0	1945.6	27.1	536.3	1353.1
	Average	78.7	325.3	27.4	293.8	649.2	1676.8	26.9	278.4	8.2	125.8	114.8
	Minimum	46.7	93.5	15.6	140.1	345.1	517.3	14.6	37.6	1.8	47.4	11.9
	CV (%)	26.2	40.4	23.6	79.2	43.5	62.1	26.9	112.2	62.1	49.3	138.9
Juliana	Maximum	97.5	399.3	58.7	402.7	1020.4	2445.1	41.5	1376.0	22.1	416.7	1353.1
	Average	74.7	273.8	31.2	256.5	645.3	1580.9	29.5	296.9	6.7	138.0	252.9
	Minimum	49.5	186.1	18.0	191.4	461.4	833.3	18.3	73.1	2.7	40.5	21.1
	CV (%)	17.0	27.4	43.2	22.4	24.9	32.3	30.3	114.6	98.8	69.9	121.1
Poliana	Maximum	96.6	281.1	31.3	1075.3	1492.5	2873.7	45.1	2384.0	7.9	454.5	1353.1
	Average	66.2	217.6	22.5	361.6	844.8	1818.0	21.0	950.5	4.4	218.3	262.8

Table 4. Contd.

	Minimum	48.1	161.7	14.8	178.8	517.4	900.9	12.2	169.7	2.9	106.8	30.2
	CV (%)	24.1	23.1	24.8	88.2	40.6	40.4	44.5	90.9	42.8	61.6	116.1
Summer cultivars	Maximum	97.5	661.9	58.7	1369.9	2244.8	6041.5	41.5	2384.0	22.1	555.6	960.0
	Average	65.2	275.9	25.5	414.2	815.6	1447.6	28.0	466.1	5.5	230.7	103.8
	Minimum	41.2	161.7	14.8	161.8	441.5	775.2	15.8	73.1	1.8	40.5	21.1
	CV (%)	24.7	39.2	36.2	85.0	39.0	57.6	21.7	85.4	83.3	61.5	138.7
General (Summer and winter)	Maximum	244.4	782.4	58.7	1538.5	3276.7	8510.1	50.0	2384.0	27.1	555.6	1353.1
	Average	75.0	312.4	26.9	326.2	693.9	1616.2	27.2	327.8	7.5	154.6	111.7
	Minimum	41.2	93.5	14.8	140.1	345.1	517.3	14.6	37.6	1.8	40.5	11.9
	CV (%)	27.2	40.9	27.4	84.6	43.5	61.8	25.7	106.2	68.7	66.5	139.4

Table 5. Maximum, average and minimum values and coefficients of variation of macro and micronutrients coefficients of biological utilization from shoot of carrot hybrids.

Cultivar	Parameter	Coefficient of biological utilization										
		N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
		kg kg^{-1}								kg g^{-1}		
Baltimore	Maximum	56.3	1077.8	41.1	67.6	666.7	654.5	22.3	97.3	3.8	37.7	31.4
	Average	47.0	658.5	19.1	44.8	302.4	414.3	18.9	34.6	1.9	18.6	19.4
	Minimum	35.3	318.3	13.4	30.3	198.3	260.9	16.1	7.2	0.7	7.0	11.6
	CV (%)	13.2	33.3	33.6	20.4	36.0	28.9	9.4	100.5	50.8	49.6	31.4
Belgrado	Maximum	67.6	1052.6	57.7	56.3	625.0	1021.6	25.6	33.6	3.8	54.3	42.2
	Average	50.5	518.4	22.1	40.4	363.2	538.0	19.2	10.8	1.2	21.2	21.5
	Minimum	37.8	356.1	14.7	28.8	267.9	314.8	13.2	4.9	0.4	5.5	13.9
	CV (%)	15.5	38.6	57.8	21.2	30.4	40.7	23.6	82.5	83.2	71.8	45.8
Concerto	Maximum	57.0	952.4	43.2	43.6	416.7	679.3	23.4	38.3	2.5	60.2	39.6
	Average	52.9	779.4	20.7	34.1	342.2	532.4	17.2	17.2	1.7	21.7	21.3
	Minimum	45.0	506.4	13.5	22.8	264.6	416.7	14.3	7.4	0.7	5.0	13.0
	CV (%)	8.6	17.7	47.6	18.8	16.4	23.4	15.9	64.0	38.8	96.9	39.4
Maestro	Maximum	61.6	1457.8	48.5	59.0	546.0	1566.0	26.4	106.9	5.7	75.2	87.4
	Average	47.1	666.1	24.9	38.7	329.6	544.0	19.0	21.9	2.6	18.4	35.8
	Minimum	36.8	399.0	12.3	30.8	218.2	278.5	13.9	4.2	1.0	5.2	14.8
	CV (%)	12.8	33.8	39.9	18.7	25.6	57.7	16.7	113.6	50.7	83.0	52.4
Músico	Maximum	63.7	1438.8	42.3	41.6	459.6	1274.2	23.3	38.2	3.3	51.8	82.8
	Average	51.2	796.3	25.6	32.7	355.7	721.5	18.6	17.7	2.0	18.6	47.5
	Minimum	42.5	549.9	16.9	26.6	258.9	478.9	14.5	6.7	1.0	5.1	13.5
	CV (%)	11.4	32.1	31.6	13.9	15.6	45.7	14.1	62.5	41.3	77.9	51.6
Nancy	Maximum	60.7	1188.2	50.5	54.3	361.2	521.0	22.5	23.3	3.8	29.9	58.4
	Average	48.1	664.8	24.7	42.9	301.9	357.5	17.2	14.3	2.5	17.9	29.3
	Minimum	37.7	354.9	14.3	34.8	223.0	248.1	11.8	5.0	1.4	10.4	10.6
	CV (%)	15.8	33.5	46.4	15.3	14.8	26.0	17.4	53.6	38.2	36.1	53.4
Nandrin	Maximum	52.0	909.0	44.1	73.3	434.4	645.4	24.8	81.1	3.9	25.1	51.6
	Average	42.4	618.9	21.4	49.7	332.7	402.9	19.1	23.2	2.6	15.2	31.1
	Minimum	29.8	368.3	12.2	34.5	258.9	260.8	14.5	3.6	1.4	8.8	11.6
	CV (%)	13.3	24.3	34.3	20.9	14.4	26.2	15.6	103.2	26.9	31.7	34.3
Soprano	Maximum	59.6	1041.7	32.8	49.2	400.0	1240.9	23.9	42.0	4.0	79.4	56.4

Table 5. Contd.

	Average	47.7	626.1	20.3	36.6	260.6	656.5	17.7	14.8	2.1	28.0	21.4
	Minimum	39.8	290.1	15.3	26.2	202.1	369.1	11.2	4.7	0.7	5.7	8.2
	CV (%)	11.8	33.9	29.6	17.4	21.3	34.5	22.0	82.3	45.7	80.7	67.1
	Maximum	71.9	1457.8	66.0	73.3	625.0	1566.0	26.4	106.9	5.7	68.0	89.1
Winter cultivars	Average	47.0	623.0	24.3	40.4	321.3	481.8	18.6	19.9	2.0	20.5	28.2
	Minimum	29.8	290.1	12.2	22.8	198.3	200.3	11.2	3.6	0.3	5.0	8.2
	CV (%)	15.9	33.3	42.1	22.4	23.4	46.5	17.2	101.2	53.6	63.3	59.3
	Maximum	73.5	1315.8	51.8	51.3	735.3	1101.6	35.4	29.1	3.5	149.3	43.2
Juliana	Average	43.1	414.5	29.1	39.5	483.5	555.7	19.5	11.4	1.2	34.0	21.7
	Minimum	34.0	257.1	18.5	26.4	300.0	262.8	14.6	5.6	0.5	7.2	12.6
	CV (%)	21.1	62.5	36.3	17.3	24.0	44.3	27.9	66.6	72.2	113.3	45.0
	Maximum	64.9	1098.9	33.3	48.1	588.2	835.3	29.0	13.4	3.2	119.0	38.2
Poliana	Average	52.1	566.9	26.5	34.8	450.2	668.8	20.5	9.4	1.7	43.5	24.7
	Minimum	42.7	272.0	22.1	21.2	367.8	434.8	15.9	6.9	1.1	10.4	15.7
	CV (%)	15.4	56.9	14.3	26.2	17.9	27.6	22.1	23.4	60.7	91.8	34.4
	Maximum	73.5	1315.8	55.9	69.2	735.3	1101.6	35.4	526.3	3.5	149.3	44.9
Summer cultivars	Average	50.6	686.0	26.4	36.9	511.3	537.1	21.5	75.0	1.0	53.3	25.0
	Minimum	34.0	257.1	16.6	21.2	255.2	262.8	12.9	5.2	0.2	7.1	12.6
	CV (%)	20.8	53.4	32.5	30.8	22.9	34.7	24.8	203.3	72.9	67.1	39.9
	Maximum	73.5	1457.8	66.0	73.3	735.3	1566.0	35.4	526.3	5.7	149.3	89.1
General (Summer and Winter)	Average	47.8	649.7	24.7	39.3	376.9	497.6	19.5	35.1	1.7	30.3	27.1
	Minimum	29.8	257.1	12.2	21.2	198.3	200.3	11.2	3.6	0.2	5.0	8.2
	CV (%)	17.9	41.0	39.8	25.1	33.8	42.5	21.1	241.2	64.0	87.5	56.2

attribute, except for the CBU of Cu in the root system of the Poliana cultivar (summer cultivation). For this cultivar, the CBU for the Cu was high (950.5 kg g^{-1}) when compared with the overall average of the summer cultivars and the Juliana cultivar, indicating that this is the most efficient in the use of this nutrient.

The winter cultivars showed export tax of dry matter 16% higher than summer cultivars (74% against 64%), that is, higher HI (Table 6). As a result of this greater HI of DM, winter cultivars had higher HI of nutrients to all the quantified elements when compared to the summer cultivars.

As for the nutrients accumulated preferably in the root (HI > 50%) N, P, K, Mg and B stood out in both growing seasons. Similar results were obtained by Cecílio Filho and Peixoto (2013), who, by analyzing only the macronutrients, concluded that N, P, K, Mg and S accumulate preferentially in the roots. However, according to the results shown in Table 6, S is accumulated preferentially in the leaves (HI = 45%). Phosphorus is the nutrient that has the highest HI (83%), while Cu is the nutrient with the smallest exported fraction (21%). Cecílio Filho and Peixoto (2013) also concluded that P is the macronutrient with the highest HI (86.1%).

The demand for nutrients can be calculated by the ratio between export and harvest index for each element. The export in turn can be calculated as the product of productivity, content of dry matter of root and inverse of CBU of the nutrient for the root system. Based on this model, to obtain 80 Mg ha^{-1} of roots of winter cultivars, extractions vary from 114 to 163 kg ha^{-1} of N, 23 to 32 kg ha^{-1} of P, 338 to 411 kg ha^{-1} of K, 77 to 106 kg ha^{-1} of Ca, 17 to 20 kg ha^{-1} of Mg, 7 to 11 kg ha^{-1} of S, 383 to 483 g ha^{-1} of B, 44 to 280 g ha^{-1} of Cu, 1446 to 4259 g ha^{-1} of Fe, 180 to 244 g ha^{-1} of Mn and 62 to 246 g ha^{-1} of Zn. For summer cultivars, the extractions of nutrients to obtain 60 Mg ha^{-1} of roots vary from 129 to 147 kg ha^{-1} of N, 25 to 31 kg ha^{-1} of P, 253 to 361 kg ha^{-} of K, 68 to 98 kg ha^{-1} of Ca, 12 to 14 kg ha^{-1} of Mg, 8 to 9 kg ha^{-1} of S, 299 to 459 g ha^{-1} of B, 65 to 244 g ha^{-1} of Cu, 2728 to 3896 g ha^{-1} of Fe, 81 to 123 g ha^{-1} of Mn and 52 to 131 g ha^{-1} of Zn. For variations in nutrient extraction we considered the differences in CBUs, content of DM and HI of the nutrients of each cultivar.

By comparing the nutritional demands of winter and summer cultivars with yields of 80 and 60 Mg ha^{-1} of roots (averages of both growing seasons), respectively, it was observed that there are virtually no differences in the extractions of N, P, Ca, S, B, Cu and Zn. In contrast, the summer cultivars tend to present higher demand for Fe

Table 6. Harvest indexes of dry matter, macronutrients and micronutrients of carrot hybrids.

Cultivar	Parameter	Harvest index (%)											
		DM	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
Baltimore	Maximum	81.6	71.4	93.9	82.9	48.9	66.2	62.7	78.3	44.9	52.6	49.7	74.1
	Average	73.2	59.6	82.8	64.6	31.8	50.6	41.4	66.5	22.1	41.1	32.3	52.7
	Minimum	63.8	40.8	67.6	45.9	9.2	39.7	19.3	57.4	6.8	20.9	15.4	18.0
	CV (%)	7.0	14.3	8.1	18.2	28.9	13.7	35.7	8.9	51.9	21.4	32.2	29.5
Belgrado	Maximum	77.6	62.2	92.5	90.5	38.4	71.1	68.2	78.8	41.2	51.8	58.6	66.7
	Average	67.7	56.0	79.1	59.4	30.6	60.0	48.5	64.0	17.3	35.6	25.5	46.8
	Minimum	62.9	49.6	60.8	44.9	25.6	52.0	30.7	54.3	4.3	7.8	11.6	18.7
	CV (%)	6.5	7.7	12.0	20.9	12.9	8.5	24.6	10.2	58.4	35.2	52.6	30.3
Concerto	Maximum	88.5	82.6	92.6	91.1	58.7	86.4	51.9	85.4	61.6	58.3	75.4	82.0
	Average	78.6	69.4	87.7	70.6	35.4	69.8	39.0	68.6	21.1	43.2	42.9	53.2
	Minimum	61.8	51.4	80.1	55.5	4.3	54.3	28.1	58.3	6.9	30.4	13.8	25.9
	CV (%)	9.3	12.1	4.1	16.3	40.9	12.9	25.2	11.9	81.9	23.9	66.4	36.2
Maestro	Maximum	84.5	80.0	94.4	89.1	48.5	80.8	77.6	81.7	47.2	62.1	52.7	88.1
	Average	75.3	67.7	84.7	72.2	34.1	63.9	54.8	66.4	19.2	43.0	25.6	47.5
	Minimum	64.4	59.1	74.9	51.8	25.2	51.2	35.0	52.6	2.5	25.4	9.1	17.5
	CV (%)	7.8	7.7	7.0	12.6	19.8	13.0	21.7	11.5	61.1	21.6	45.0	40.1
Músico	Maximum	81.4	75.2	94.0	85.0	37.2	73.9	73.1	75.0	46.0	48.4	57.8	64.7
	Average	74.4	67.2	88.5	71.1	29.1	64.7	59.6	65.6	20.0	35.1	34.1	49.7
	Minimum	68.4	61.6	84.9	62.3	22.6	58.5	41.8	58.0	7.3	21.3	9.9	35.9
	CV (%)	4.4	6.3	3.1	10.6	14.1	7.3	17.2	8.2	59.0	21.4	74.9	22.6
Nancy	Maximum	81.2	70.8	92.5	86.2	39.7	69.4	62.5	78.2	45.9	71.6	49.5	67.6
	Average	75.0	61.6	85.9	69.5	33.6	61.5	36.3	66.2	25.6	55.5	33.5	52.3
	Minimum	66.2	38.2	79.9	52.1	26.7	57.2	20.7	50.0	18.8	46.3	18.4	24.1
	CV (%)	6.4	14.8	4.7	18.2	13.5	6.2	35.7	12.3	36.9	13.9	31.4	30.4
Nandrin	Maximum	78.3	67.5	88.6	89.6	46.0	71.3	58.2	80.6	60.4	56.4	37.4	68.8
	Average	71.1	57.5	79.0	64.3	31.9	54.6	38.2	63.1	39.9	38.6	22.0	35.0
	Minimum	64.2	49.3	55.7	44.3	25.8	44.3	19.5	48.2	1.9	19.7	11.2	7.5
	CV (%)	5.8	9.2	10.2	14.9	16.6	13.7	27.9	13.3	102.8	26.5	35.0	56.4
Soprano	Maximum	81.6	72.9	91.1	83.3	35.9	62.5	75.0	79.4	61.9	61.6	57.8	75.7
	Average	74.8	65.4	87.4	66.5	31.5	51.2	55.0	67.9	20.9	45.9	36.7	58.5
	Minimum	66.7	47.2	80.8	37.1	24.5	22.7	30.3	45.8	3.3	30.0	15.5	33.9
	CV (%)	6.2	10.3	3.7	16.0	9.5	20.1	22.1	12.7	62.4	21.8	41.7	22.5
Winter cultivars	Maximum	88.5	82.6	94.5	94.9	58.7	86.4	77.6	89.5	61.9	71.6	75.4	88.1
	Average	73.9	63.3	84.4	69.0	31.6	59.5	45.9	66.7	21.8	42.1	30.6	50.5
	Minimum	61.8	38.2	55.7	37.1	4.3	22.7	10.1	45.8	1.9	7.8	9.1	7.5
	CV (%)	7.5	12.4	8.2	16.6	28.1	16.2	32.8	12.1	62.8	28.5	46.5	37.6
Juliana	Maximum	73.3	77.2	93.9	78.7	31.6	64.3	60.1	76.9	39.8	56.5	61.9	69.8
	Average	63.2	50.0	70.6	60.7	21.3	56.5	37.1	54.5	11.6	26.3	28.3	36.4
	Minimum	49.0	38.2	55.1	33.2	14.2	43.4	20.0	40.8	2.1	6.6	6.4	2.0
	CV (%)	9.1	20.6	15.8	22.7	22.9	11.8	34.3	20.3	94.8	48.4	57.0	58.8
Poliana	Maximum	66.3	72.2	91.0	67.9	17.6	48.3	48.7	65.5	3.7	45.6	33.5	52.5
	Average	55.6	49.8	72.7	60.0	13.7	41.5	35.6	50.3	2.1	28.2	18.6	34.4
	Minimum	43.4	37.2	50.7	46.4	3.6	33.9	24.2	29.7	0.7	16.0	7.3	15.3
	CV (%)	15.4	25.9	19.3	12.4	32.1	12.6	26.4	24.6	60.4	41.7	48.1	39.6

Table 6. Contd.

Summer cultivars	Maximum	78.5	80.2	93.9	83.2	43.8	69.1	72.2	76.9	71.4	61.0	61.9	69.8
	Average	64.5	58.2	78.7	64.3	19.2	54.2	42.8	58.3	17.7	25.2	28.8	39.6
	Minimum	43.4	37.2	50.7	33.2	3.6	27.1	18.5	29.7	0.7	6.6	6.4	2.0
	CV (%)	10.8	22.8	15.9	17.1	48.1	15.8	29.4	18.6	124.1	53.2	43.2	39.9
General (Summer and winter)	Maximum	88.5	82.6	94.5	94.9	58.7	86.4	77.6	89.5	71.4	71.6	75.4	88.1
	Average	71.3	61.9	82.9	67.8	28.2	58.0	44.9	64.4	20.7	37.4	30.1	47.3
	Minimum	43.4	37.2	50.7	33.2	3.6	22.7	10.1	29.7	0.7	6.6	6.4	2.0
	CV (%)	10.2	16.0	11.0	17.0	37.4	16.6	32.0	15.0	79.8	39.0	45.8	39.7

and lower demand for K, Mg and Mn.

Summer cultivars produced fewer roots to the same accumulated quantity of the nutrients N, P, Ca, S, B, Cu and Zn, as compared to winter cultivars. This indicates that winter cultivars have higher agronomic efficiency of use of these nutrients. The higher agronomic efficiency of winter cultivars may be related to the biomass partitioning (HI of DM), because for the same amount of produced roots, summer cultivar generates greater accumulation of DM in the shoot, and consequently, greater accumulation of nutrients in this organ.

According to the estimated accumulations we verified the following nutrients extractions order for the winter and summer cultivars, respectively: K > N > Ca > P > Mg > S > Fe > B > Mn > Zn > Cu and K > N > Ca > P > Mg > S > Fe > B > Zn > Mn > Cu. The decreasing order of nutrient accumulation of winter and summer cultivars is identical for macronutrients; however, there is change in the order of accumulation of Mn and Zn. The accumulation of macronutrients in “Forto” carrots – verified by Cecílio Filho and Peixoto (2013) - was similar to that seen in this work, except that the S accumulation is greater than that of Mg.

Different cultivars of winter and summer promote low effect on the nutritional demand of the carrot crop in each growing season, except for the nutrients K, Mg and Mn, as discussed above. Thus, the nutritional demand of the carrot crop can be estimated as a function of the desired productivity and the growing season.

Conclusions

The harvest index of dry matter and nutrients is greater for winter cultivars. The differences are insignificant in the nutritional demand of N, P, Ca, S, B, Cu and Zn between winter and summer cultivars for the average yields obtained in each season. The summer cultivars accumulate larger amounts of Fe and lower amounts of K, Mg and Mn when compared to the winter cultivars. The modeling of the nutritional demand of the carrot crop can be performed depending on the desired productivity and growing season.

Conflict of Interest

The authors have not declared any conflict of interest.

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Development and longevity of Citrus mealybug *Planococcus citri* (Risso, 1813) (Insecta: Homoptera: Pseudococcidae) associated with grapevine

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The Citrus mealybug *Planococcus citri* has a wide geographical distribution and has been described as a pest of economic importance in several crops. The present work determined the developmental and biological aspects of the Citrus mealybug in order to obtain information that may support the integrated pest management (IPM) of grapevine (*Vitis vinifera* L.) cv. Syrah in the Lower Basin of the São Francisco Valley region. The research was conducted at the Laboratory of Entomology of Embrapa Semiarid, Petrolina-PE, on leaves of grapevine kept in a controlled environment (25 ± 1°C, 60 ± 10% R. H. and a photoperiod of 12L:12D). The first two instars had higher mortality, indicating high susceptibility in these nymphal periods. The overall nymphal period of females and males is similar at 22.52 ± 0.46 and 23.5 ± 0.29 days, respectively, with viability of 39%. The adult longevity of females is nearly 30 times greater than that of males, indicating that females of *P. citri* are mainly responsible for damage and injury to grapevine. The sex ratio was 0.64, indicating that females make up the majority of the adult population of *P. citri*. We conclude that the species in question completes its lifecycle on leaves of grapevine and reaches the adult phase in a short time interval.

Key words: Mealybugs, life cycle, grape.

INTRODUCTION

Viticulture of the semiarid region in the Lower Basin of the São Francisco Valley (LBSFV) is characterized by high productivity and quality of grapes and wines, and especially by the environmental conditions and its

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integrated grape production system. It is one of the major centers of fruit production in Brazil, being a pioneer in the production of grapes, juice and wine in tropical conditions (Silva et al., 2009; Camargo et al., 2011).

However, due to the expansion in cultivated area and constant changes in the agroecosystem of the grapevine some problems of a phytosanitary nature, such as the occurrence of pests, especially mealybugs, are being reported in the region (Oliveira et al., 2008). According to the literature, among the pests associated with grapevine, mealybugs stand out for causing frequent crop damage, since they limit the quality and yield of fruits (Daane et al., 2008; Bertin et al., 2010; Formolo et al., 2011; Ghini et al., 2011; Bordeu et al., 2012).

Mealybugs of the Pseudococcidae family are characterized by presenting a body covered by a thin layer of white waxy secretion. They are small sucking insects that feed on sap from the phloem of plants, live in colonies and can be found on different parts of the host plant, thus constituting an important group of insect pests associated with different cropping systems and agronomic crops (Santa-Cecília et al., 2007; Daane et al., 2008; Cid et al., 2010; Daane et al., 2012).

On ingesting their food mealybugs excrete a sugar-rich substance known as honeydew, often associated with the development of fungi and the presence of ants. The accumulation of honeydew on leaves and fruits results in direct damage to the grapevine, and may even, in some cases, cause death of the plant. Fungi commercially depreciate the clusters, resulting in the disposal of the product (Culik and Gullan, 2005; Daane et al., 2008; Cid et al., 2010; Ahmed and Abd-Rabou, 2010).

Many species of mealybugs coexist and infest vineyards of important wine centers in various countries, hindering sustainable pest control. In this sense, each species has its own biological features, it being necessary initially to know them in order to deploy control and integrated pest management programs (Daane et al., 2008; Mahfoudhi and Dhouibi, 2009; Cid et al., 2010; Bordeu et al., 2012). Among the species that stand out in Brazil is *Planococcus citri* (Risso, 1813), which is most abundant in the vineyards of the region Serra Gaúcha, Rio Grande do Sul (Morandi Filho et al., 2008).

Given the above, the objective of this research was to determine the development and longevity of *P. citri* associated with grapevine, aiming to provide information to support the Integrated Pest Management of the grapevine (IPM-grape).

MATERIALS AND METHODS

Identification of *Planococcus citri*

The identification of *P. citri* was done by researchers Dr. Cherre Sade Bezerra da Silva and Dr. Caroline Viana Morgante using molecular markers developed for identifying pseudococcids (Rung et al., 2008).

Rearing and maintenance of *Planococcus citri*

Rearing of *P. citri* was done from insects collected from grapevine clusters on a commercial farm in the municipality of Petrolina-PE. As a cleaning process, pumpkins were washed in running water, and then a neutral detergent was applied with sponges moistened with water, followed by rinsing. The pumpkins were again washed in running water and dried at room temperature. The insects were then transferred with the aid of fine tip brushes to the pumpkins (*Cucurbita moschata Duschesne*) of the cultivar Jacarézinho.

The pumpkins were kept in small plastic pots (10 × 15 cm) and placed in wooden cages (53.5 × 43 × 47.5 cm), with a glass upper surface, sides of nylon mesh screen and a front surface covered by "voile"-like material, at 25 ± 1°C, 60 ± 10% RH and a photoperiod of 12 h.

Host plant

Grapevine leaves (*V. vinifera*) of the cultivar Syrah were collected in a vineyard at the Bebedouro Experimental Station (09° 09'S, 40° 22" W) of Embrapa Semiarid in Petrolina, PE. In the collection area and proximity there was no application of insecticides.

The leaves were washed in running water, then dried with a paper towel and observed under binocular loupes (with 22-fold increase) to check for the presence of opportunistic arthropods.

Determination of development and longevity

The work was conducted at the Laboratory of entomology of Embrapa Semiarid, Petrolina-PE, in climatic chambers of the B.O.D. type (25 ± 1°C, 60 ± 10% R. H. and photoperiod of 12L:12D). The determination of the biology of *P. citri* was made from newly hatched nymphs, maintained on leaves of grapevine. To obtain nymphs, adult females in the reproductive phase were collected and placed in Petri dishes containing water-agar (2%) and grapevine leaf discs (7 cm diameter). After hatching, the first instars were placed on leaf discs (3 cm diameter), which were placed in Petri dishes (9 cm diameter) with the abaxial side up. To maintain turgor, the leaves were placed under a layer of water-agar (2%). Soon after, the plates were sealed with PVC film and taken to the B.O.D.

To reduce the risk of contamination by fungi and other pathogens, caused by exudates from the leaf discs, and to maintain and provide food to the insects, the Petri dishes were replaced and the water-agar solution and leaf discs were renewed at five day intervals. In addition, to prevent damage to the mouthparts and allow the natural movement of the mealybug a cut was made, with a scalpel, of a small leaf area around the insect and with the aid of forceps it was moved to the new leaf disc (Santa-Cecilia et al., 2008; Correa et al., 2011).

The leaf discs containing second and third instar male nymphs were transferred to plates without water agar because at this stage the insect's mouthparts atrophy and it does not drink (Correa et al., 2005; Santa-Cecilia et al., 2009; Ross et al., 2012).

Parameters evaluated

The parameters evaluated were duration and viability of the nymphal period, longevity and sex ratio.

The assessment of the change of instar of males and females was based on the release of exuviae, being done daily with the aid of magnifying glasses. After registration, the exuviae were removed from the plates with the aid of brushes.

During the first and second instars the replicates were constituted of individuals of unknown sex because there is no overt sexual

Table 1. Duration in days (mean ± SE) of the development of *Planococcus citri* on leaves of grapevine (*Vitis vinifera* L.) cultivar Syrah. Laboratory conditions of 25 ± 1° C, 60 ± 10% R. H. and photoperiod of 12L:12D.

Sex	1st instar	2nd instar	3rd ínstar	4th instar	Nymphal period	Adult longevity
Female	8.20±0.24[a]	6.60±0.44[a]	7.72±0.25[a]	*	22.52±0.46[a]	63.68±5.45[a]
Male	7.93±0.22[a]	7.50±0.23[a]	2.86±0.29[b]	5.21±0.42	23.50±0.29[a]	2.07±0.25[b]

* No 4th instar occurs; Means followed (± SE) by the same letter in the column do not differ by Tukey test (p <0.01).

differentiation. Sexual dimorphism becomes more evident at the end of the second instar, when the males form cocoons to complete their development (Silva et al., 2010; Correa et al., 2011).

Analysis of experimental data

The experimental design was fully randomized with 100 replications. Initially, each replicate contained two nymphs of the same age. After the setting of these nymphs one was removed. The data for the parameters evaluated were submitted to analysis of variance and the means were compared by Tukey test (p<0.01) using the program BioEstat 5.0 (Ayres et al., 2007).

RESULTS AND DISCUSSION

When kept on grapevine, males have four instars, whereas females have three instars (Table 1), corroborating other studies of the biology of mealybugs in which males and females have four and three instars, respectively (Chong et al., 2008; Morandi Filho et al., 2008; Santa-Cecilia et al., 2009; Vennila et al., 2010; Bertin et al., 2013; Fand et al., 2014).

There was not a significant difference, in days, in the duration of the first two instars for males and females. This period corresponded to approximately 14 and 15 days for females and males, respectively (Table 1).

The nymphs, principally of the first instar, were characterized by yellow coloration, little white wax, substantially reduced size and great mobility compared to later instars, corroborating the characteristics presented by Correa et al. (2005) and Santa-Cecilia et al. (2007). The small size and great mobility of the nymphs is a notable characteristic of the first two instars, favoring their spreading to other structures of the host plants, thereby hindering their location (Daane et al., 2008; Cid et al., 2010; Ross et al., 2010, 2012).

The duration of the first instar in males varied from 7 to 9 days and in females from 7 to 11 days. In the second instar, the duration in males varied from 6 to 9 days, whereas in females this variation was from 4 to 14 days. In studies of the biology and development of *P. citri*, Correa et al. (2005), Morandi Filho et al.(2008) and Santa-Cecília et al. (2009) observed that the first instar is the longest and can last up to four days longer than the second instar. However, the duration of the first instar found by these authors was longer than that observed in this study. In the first instar, Correa et al. (2005) found 10.4 ± 3.1 days for males and 11.6 ± 2.6 days for females on sweet orange (*Citrus sinensis* L.) cv. Bahia. In grapevine Morandi Filho et al. (2008) found 11.17 ± 0.18, 11.20 ± 0.16 and 11.06 ± 0.20 days in the cultivars Cabernet Sauvignon, Itália (*Vitis vinifera* L.) and Isabel (*Vitis labrusca* L.), respectively.

The inverse situation, in which the duration of the second instar is longer than the first or similar, can also be observed in *P. citri* or other pseudococcids, as reported by Ahmed and Abd-Rabou (2010) and Francis et al. (2012) in *Planococcus minor*, Bertin et al. (2013) in *Dysmicoccus brevipes*, and Fand et al. (2014) in *Phenacoccus solenopsis*. This difference is probably due to, among other factors, the use of different substrates and the nutritional quality that this furnishes to the insect, since the experimental conditions were similar.

The duration of third and fourth instars in males were of 2 to 5 days and 2 to 7 days, respectively. It was observed that the period in which the males remained in cocoons was similar to the duration of the third instar in females, which showed duration of 4 to 10 days (Table 1). Both the results are different to those found by Correa et al. (2005), who observed a mean duration of 6.3±1.9 days for third instar females and 10.7±2.7 days for males during the period that they remained in cocoons. Morandi Filho et al. (2008) observed that the development of males, especially during the third and fourth instars, can be inferior to that of females when fed on grapevine; moreover, when the development occurred on grapevine roots, the duration was similar or superior in males.

The viability presented by females and males during the first two instars (Table 2) showed that in this period the insects are more fragile, corroborating other studies, where high mortality of pseudococcids is observed during the first and second instars (Chong et al., 2008; Morandi Filho et al., 2008; Vennila et al., 2010; Fand et al., 2014).

In the third instar there was not a significant difference between the viability of females and males. Survival results similar to those found in our study were reported by Francis et al. (2012) and Fand et al. (2014). It was observed that even in the cocoon the males are susceptible, since mortality occurred in the passage from the third to the fourth instar. This result differs from those found by Correa et al. (2005), in which mortality did not occur in the change of male instars. Comparing our results for longevity of males to those reported in the scientific literature, there is evidence that the presence of

Table 2. Viability (mean ± SE, %) of *Planococcus citri* on leaves of grapevine (*Vitis vinifera* L.) cultivar Syrah. Laboratory conditions of 25 ± 1° C, 60 ± 10% R. H. and photoperiod of 12L:12D.

Sex	1st ínstar	2nd instar	3rd instar	4th instar	Nymphal period
Female	70.00±5.77**	71.48±3.12**	79.17±7.42[a]	-*	39.00±4.92**
Male			77.77±9.03[a]	100	

* No 4th instar occurs; **Both sexes, males and females; Means followed (± SE) by the same letter in the column do not differ by Tukey test ($p < 0.01$).

cocoons does not seem to be a factor that ensures survival, whether in the third or fourth instar, since the presence of a cocoon also did not impede the occurrence of mortality in the studies conducted by Morandi Filho et al. (2008), Francis et al. (2012) and Fand et al. (2014)

In the adult phase, the longevity of females was much greater than that of males, nearly 30 times greater (Table 1). Maximum durations of 99 and 4 days of longevity were registered for females and males, respectively, in this phase. For females, the result found in this study is much longer than that reported by Morandi Filho et al. (2008), Vennila et al. (2010), Francis et al. (2012), Bertin et al. (2013) and Fand et al. (2014). Possibly this result occurred due to the absence of the reproductive period of *P*. *citri*.

The total cycle, here understood as the nymphal phase and the adult phase, also differed between females and males, which presented approximately 86 and 25 days respectively, such that in females the cycle was 3.4 times greater than that of males. In this case, the difference is due to the adult longevity of the females, which was much greater than that of the males (Table 1).

It was observed that in both of the sexes the period of nymphal development is similar, although the number of instars is different. In studying the biology of *P*. *citri* and *P. minor*, respectively, Correa et al. (2008), Francis et al. (2012) and Fand et al. (2014) report that the duration of nymphal states as well as the nymphal-adult cycle of females and males can be close or similar, even at different temperatures. However, Morandi Filho et al. (2008) and Correa et al. (2005) observed a prolongation of 7 to 8 days in the nymphal period of females.

Assessment of viability in the nymphal period shows a low rate of survival associated with high mortality during the first two nymphal instars; after this period the mortality was reduced, demonstrating that the later instars are less susceptible. Similar results with a low rate of survival were described by Chong et al. (2008), Morandi Filho et al. (2008) and Vennila et al. (2010).

The sex ratio obtained was 0.64; this shows the greater number of females in the adult phase relative to the number of males. This corroborates the reports of Chong et al. (2008) and Vennila et al. (2010), where there was a greater proportion of females relative to males. In agreement with Francis et al. (2012), females can correspond to between 60 and 73% of the population of mealybugs.

The similarities or differences between the results found in this study compared with the literature can be attributed to different factors, such as temperature and host plant. According to Chong et al. (2008), Lazzari and Zonta-de-Carvalho (2009), Vennila et al. (2010), Francis et al. (2012) and Fand et al. (2014), these are among the main factors that exert influence on the biology of insects, especially sap sucking insects, as is the case for mealybugs. Notwithstanding, understanding the biology of *P. citri* from the results obtained is the basis for the beginning of integrated pest management programs in vineyards of the LBSFV, since despite being pioneering research in the region, concrete and precise data are presented about the biology of the pest in question, generating information for future work.

Conclusions

The mealybug *P. citri* completes its life cycle in leaves of grapevine (*Vitis vinifera* L.) cultivar Syrah showing high longevity for adult females. In grapevine, the nymphal period of females and males of *P. citri* is similar.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Initial assessment and nutritional status of hybrid *eucalyptus* sp. in the municipality of Colorado Do Oeste, Rondônia State - Brazil

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The objective of this research was to determine the initial growth, nutrition and soil fertility of *Eucalyptus* plantation in Rondônia State, Brazil. The treatments consisted of four hybrids (VM1, H13, GG100 and I144) and four replications, each plot contained 30 plants, totaling 480 plants. The VM1 clone was obtained from the hybrid of *Eucalyptus urophylla* x *Eucalyptus camaldulensis*, and the others, from the hybrid of *E. urophylla* x *Eucalyptus grandis,* all of which are minicutting source courtesy of a nursery in the region. The experiment was a completely randomized block design, as the area is homogeneous. Biometric evaluations consisted of measuring the BD (base diameter), total height and survival at six and 10 months of age. In September 2014, 20 newly mature leaves of every tree were collected from branches located halfway up the canopy, addressed to the quarter cardinal points. In this analysis, 10 trees of each working area of the plots, of each treatment, formed 16 composite samples for the analysis of macro- and micronutrients. Once collected, the leaves were placed in paper bags and sent for laboratory analysis. Hybrids showed no differences in the concentration of Fe, Mn and B (P> 0.05). The hybrid VM1 exhibited higher concentrations of K, Ca, Mg, S and N and intermediate concentration of P. Clones of *E. camaldulensis* x *E.urophylla* presented higher survival, height and diameter than clones of *E. urophylla* x *E. grandis.* There were differences between treatments for the height at six and 10 months after planting. For the base diameter, there was no significant difference at six months, but at 10 months, a significant difference was detected between treatments. The differences identified in this study for height growth, nutritional status, base diameter and survival in the seedling stage suggest the possibility of selecting genotypes of this crop for the region.

Key words: Development, genotypes, survival, clone, *Eucalyptus.*

INTRODUCTION

Silviculture, part of forest science, aims to the production and maintenance of forest stands in order to achieve the proposed goals within the prescribed period, to provide the benefits of forestry. The demand for forest products

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has existed since the dawn of history, whether to obtain energy, resins and pigments, and for the construction of houses. Consumption of wood and its derivatives is increasing and requires the introduction of reforestation using forest species with high productivity, enabling a cutting cycle within a short period of time. In this context, *Eucalyptus* sp. represents an alternative to supply this need. It is important to seek information to achieve better productivity in eucalyptus, noting the great diversity clones and hybrids used in different regions, which may generate different results. According to Pinto et al. (2011), the high number of species and clones of eucalyptus provides a high possibility of geographic and economic expansion, since these genetic materials are adapted to many different environmental and soil conditions and meet numerous types of economic exploitation. A large part of eucalyptus plantations is in degraded areas with low fertility, which requires a proper nutrient balance to achieve high productivity. The increase in productivity is related to breeding, system management and nutritional balance. Forest species differ in the ability to uptake, translocate, accumulate and use nutrients that influence their growth. These differences occur between species, origins, progenies, and even between clones of a given species (Godoy and Rosado, 2011). Among the species of eucalyptus, *Eucalyptus urophylla* and *Eucalyptus grandis* are the most used in controlled breeding program, which generate a commonly called genotype "urograndis", which allows the combination of the good traits of both in the segregating generation (Muro_Abad, 2000). According to Silva and Matos (2003), 11% of eucalyptus forest plantations are formed by hybrid *Eucalyptus urophylla* and *Eucalyptus grandis*.

Brazil has about 7.2 million hectares of planted forests, especially with species of *Eucalyptus* and *Pinus*, representing 92.8% of the total. This area corresponds to only 0.84% of the country area and 1.55% of the total area of forests (Abraf, 2013). Businesses in the Amazon region have few studies on eucalyptus, especially those intended for energy purposes, and the existing information is rarely released. Thus, the research of genetic materials is essential for the success of the activity in the region (Matos et al., 2012). In 2012, the Rondônia State, consumption of sawn logs was 2,234,206 m^3 and production was 1,328,945 m^3, generating a deficit of 905,261 m^3 (IBAMA, 2012). The distribution of forests planted with *Eucalyptus* and *Pinus* in the Rondônia State not even appeared in the surveys of ABRAF (2013), base year 2012, on the other hand the need for timber is evident. This observation demonstrates the need to strategically prepare the region for the foreseeable future, so that this can at least supply its domestic market. As a strategy to solve this problem, effective mechanisms in research and technology should be taken into, such as the Socio-Economic-Ecological Zoning -ZEE of the state. According to the ZEE Law of the Rondônia State (2000), Article 7 – The zone 1 composed of areas intended for agriculture, agroforestry and forest covers 120,310,48 km^2, equivalent to 50.45% of the total area of the State. These areas are preferably intended for forest plantations, mainly because they are classified as fragile soils of low fertility. Another point underlined in this law is the incentive to develop primary activities on deforested or inhabited lands, so the eucalyptus farming, as well as any other primary activity should not be encouraged in areas of natural forests but in areas already disturbed. In this way, it is important to evaluate the initial development of seedlings of *Eucalyptus* sp. and the concentration of nutrients in these for future soil amendment and guidance on the best hybrids for commercial planting in line with local conditions. Given the above, this study evaluated the initial development and nutritional status of seedlings of *Eucalyptus* sp. at six and 10 months old, in the Rondônia State.

MATERIALS AND METHODS

This work was conducted in the experimental limited to geographic coordinates 13°06'56"S and 60°29'22", municipality of Colorado do Oeste, Rondônia State, Brazil. The climate, according to Köppen classification, is Aw (Alvarez et al., 2013), tropical hot and humid, the months with higher rainfall are from January to March, with an average annual rainfall of 2,234 mm. The average annual temperature is 24°C, maximum of 36°C and minimum of 12ºC. The soil in the area is Red Argisol (EMBRAPA, 2006). The characterization of chemical properties and the particle size analysis was performed for 20 sampling points, wherein each five collections formed a composite sample for each depth collected (0-10, 10-20, 20-40, 40-60 cm), taken at random 30 days before planting (Table 1) and then sent for laboratory analysis.

Four hybrids of *Eucalyptus* were used, namely GG100 (Gerdau Group), I144 (Acesita) and VM1 (Vallourec and Mannesmann Brazil) and H13 (IP). The VM1 clone was obtained from the hybrid of *Eucalyptus urophylla* x *Eucalyptus camaldulensis*, and the others, from the hybrid of *Eucalyptus urophylla* x *Eucalyptus grandis,* all of which are minicutting source courtesy of a nursery in the region. The experiment was a completely randomized block design, as the area is homogeneous. The treatments consisted of four hybrids (VM1, H13, GG100 and I144) and four replications, each plot had 30 plants, totaling 480 plants. The distance between rows in the tree rows was 3 m, and the distance between the tree rows (number of trees arranged in the same row) of 10 m (distance chosen so that the machinery available in the institution could freely move in cases of cultural managements), with 2.5 m distance between trees in the row and three rows per tree rows. Biometric evaluations consisted of measuring the BD (base diameter), total height and survival at six and 10 months of age. In September 2014, 20 newly mature leaves of every tree were collected from branches located halfway up the canopy, addressed to the quarter cardinal points. In this leaves analysis, 10 trees of each working area of the plots, of each treatment, formed 16 composite samples for the analysis of macro and micronutrients. Once collected, the leaves were placed in paper bags and sent for laboratory analysis.

Regarding the elements N, P, K, Ca, Mg, S, Fe, Cu, Mn, Zn and B, chemical analyses were performed at private laboratories after sulfuric digestion of nitrogen, dry digestion of boron and perchloric digestion of the other elements. Nitrogen was determined by micro-Kjeldahl method, P and B elements were determined by

Table 1. Chemical characteristics of the soil used in the experiment, pH (hidrogenionic potential), OM (Organic Matter), P, K, Ca, Mg, Al, H + Al, S.B (sum of bases), CTC (Cation Exchange Capacity), V (base saturation), Cu, Fe, Mn, Zn.

Chemical characteristic	Unit	Depth (cm)			
		0-10	10-20	20-40	40-60
pH (in H_2O)	pH	6.40	6.40	6.60	6.60
OM*	g kg^{-1}	34.00	33.00	19.00	13.00
P	mg dm^{-3}	14.40	12.70	9.70	3.30
K	cmolc dm^{-3}	89.00	87.00	89.00	87.00
Ca	cmolc dm^{-3}	15.21	13.24	9.73	8.28
Mg	cmolc dm^{-3}	2.29	2.21	2.20	1.75
Al	cmolc dm^{-3}	0.00	0.00	0.00	0.00
H+Al	cmolc dm^{-3}	3.00	3.00	1.50	1.88
S.B.	cmolc dm^{-3}	17.70	15.70	12.20	10.30
CTC (pH 7.0)	cmolc dm^{-3}	20.70	18.70	13.70	12.10
V	%	85.50	83.90	89.00	84.50
Cu	mg dm^{-3}	6.50	7.20	6.80	5.40
Fe	mg dm^{-3}	155.00	176.00	119.0	109.00
Mn	mg dm^{-3}	88.70	88.30	86.50	80.90
Zn	mg dm^{-3}	15.20	18.60	11.20	9.00
Sand	g kg^{-1}	507.00	507.00	461.0	446.00
Silt	g g^{-1}	161.00	176.00	177.0	161.00
Clay	g kg^{-1}	332.00	317.00	362.0	393.00

* Recommendations for nitrogen fertilization is given by the percentage of organic matter in the area.

Table 2. Growth in diameter (GD) and height (H), survival (Surv) of origins of *Eucalyptus urophylla* × *Eucalyptus grandis* and *Eucalyptus urophylla* × *Eucalyptus camaldulensis* at six and 10 months of age, in Colorado do Oeste, Rondônia State.

Clone	GD (cm)		H (cm)		Surv (%)	
	6 months	10 months	6 months	10 months	6 months	10 months
VM1	13.41[a]	23.57[a]	122.12[a]	189.05[a]	100.00[a]	99.16[a]
GG100	10.67[a]	14.51[b]	92.44[b]	123.63[b]	100.00[a]	99.19[a]
H13	9.09[a]	13.20[b]	76.01[b]	98.89[b]	90.83[b]	88.35[a]
I144	10.78[a]	14.32[b]	81.68 [b]	104.71[b]	91.66[ab]	89.16[a]
Mean	10.98	16.4	93.06	129.07	95.63	94.79
CV(%)	12.85	18.66	24.60	15.81	4.47	6.89

colorimetry, K by flame photometry and S by turbidimetry. Ca, Mg, Fe, Cu, Mn and Zn were determined by atomic absorption spectrophotometry indicated by Malavolta et al. (1997). Data were subjected to analysis of variance by F test (5%). Differences between the means were tested by Tukey's test at 5% probability.

RESULTS AND DISCUSSION

In the field, the clones showed a mean survival over 94% in the evaluation period (Table 2), indicating that they showed excellent ability to adapt to the environmental conditions of the site. These values are similar to those observed by Matos et al. (2012), who evaluated the initial development and nutritional status of eucalyptus clones in the northeastern Pará State at five and 18 m of age and found 3% mortality at five months and 11% at 18 m.

The lowest survival of the clones H13 and I144, which are genetic materials of *E. grandis* × *E. urophylla*, may be related to difficulties in adapting to the environmental conditions in the region or indicating that the water deficiency, common in the study region from April to October, was more limiting to the development of these genetic materials. Table 2 shows that the growth in height and base diameter revealed significant variations. In general, clones of *E. camaldulensis* × *E. urophylla* presented average height, base diameter and survival rate superior to those obtained in clones *E. grandis* × *E. urophylla.* Considering all the clones, values of mean height at six and 10 months were 0.93 and 1.29 m, respectively. Matos et al. (2012) found average height at

Table 3. Concentration of micronutrients in hybrids of eucalyptus VM1 (*E. urophylla* x *E. camaldulensis*) and H13, GG100, I144 (*E. urophylla* x *E. grandis*).

Hybrid	Micronutrients (mg kg^{-1})					
	B	Cu	Fe	Mn	Na	Zn
VM1	30.77^{a}	2.12^{b}	56.50^{a}	228.7^{a}	100^{b}	23.65^{a}
H13	32.37^{a}	3.75^{a}	87.25^{a}	186.42^{a}	377.7^{a}	19.20^{b}c
GG100	51.17^{a}	3.33^{ab}	75.25^{a}	181.27^{a}	371.5^{a}	15.32c
I144	71.10^{a}	2.42^{ab}	72.25^{a}	310.35^{a}	296.7^{a}	20.17^{ab}
CV^{a} (%)	46.99	23.99	25.84	40.29	29.40	9.67

Means followed by different letters in the same column are significantly different by Tukey's test at 5%. ^{a}Coefficient of variation, B, Cu, Fe, Mn, Na and Zn.

five months of 0.96 m, a result similar to the present study.

Accordingly, Macedo et al. (2000) stated that the potential capacity of establishment of fast-growing species can be usually found in the field in the first post-planting periods, evaluated by their survival percentage. Under field conditions, in general, seedlings of different tree species differ in their phenotypic expressions of adaptation and vigor. For Macedo et al. (2006), the differences observed between the average heights of clones evidence that they have different genetic capacities for exploiting the productive potential of the *habitat* of introduction, being probably related to their phenotypic plasticity. Martinez et al. (1993) point out that the different behavior as for the growth of plants of different varieties, grown under the same conditions, may indicate differences in the internal factors responsible for the nutritional efficiency of these genetic materials.

The hybrids presented different concentration of the micronutrients Cu, Na and Zn ($P < 0.05$). The lowest concentration of Zn was found in GG100, followed by I144 and H13, which maintained an intermediate concentration of this element (Table 3). The lowest concentration of Zn may be because such micronutrient has low mobility in the plant, and may be at appropriate levels in soil and plant. Sgarbi et al. (1999) verified similar results when examined biomass production in eucalyptus species under deficiency of B and Zn, in which although Zn presents low mobility in the plant, the amount supplied until the first years was enough to maintain the concentration of this micronutrient at adequate levels in leaves.

The VM1 had lower concentration of Na and intermediate of Cu. The low content of Na can be associated with the traits related to use and uptake of nutrients, and thus the plant can absorb greater amounts of other nutrients. Caldeira et al. (2004) reported that Na has relatively low efficiency of use compared to other micronutrients, due to the high concentration in green leaves and internal translocation, returning to the soil through leaf litter, and again integrated into the biogeochemical cycle. Hybrids did not show differences in the concentration of Fe, Mn and B ($P > 0.05$). This result is similar to that observed by Silva et al. (2008), which analyzed nutrients in eucalyptus treated with sewage sludge and reported no significant difference for Fe content in leaves between treatments. These results can be attributed to greater dilution of the element in the biomass of eucalyptus and the ability of trees to maintain a balance between the levels of this nutrient in the leaves, even with higher availability of the element in soil.

Boron (B) is very important for eucalyptus crops, because part of the plantations is intended to low fertility areas, with low boron concentration in the soil. Hybrids studied did not differ in relation to the concentration of that element, on the contrary to that observed by Barretto et al. (2007), who studied clones of *E. grandis x E. urophylla* and detected differences in boron use efficiency, with variation among clones and differently in each concentration of boron applied. This may be related to the concentration of this nutrient in soil; in this study area it can be properly distributed in the planting site, being absorbed uniformly by hybrids.

Moreover, there were differences in the concentrations of P, K and Ca ($P < 0.05$) between hybrids (Table 4). The highest concentration of P and Ca and the lowest of K were found in the hybrid GG100. The Hybrid I144 showed lower absorption of P and K, followed by the hybrid H13 with intermediate values for P and K, both coming from *E. urophylla x E. grandis*. These variations may be related to the difference in absorption and translocation of nutrients among eucalyptus species, since they may behave differently according to soil and climatic conditions of the site chosen for planting.

As to macronutrients, the hybrid VM1 exhibited higher concentrations of K, Ca, Mg, S and N and intermediate concentration of P, compared to the other hybrids. This hybrid has its origin from the crossing between *E. urophylla* and *E. camaldulensis*, indicating that this species reached a better adaptation to the planting site, efficiently absorbing the nutrients available in the soil. For Pinto et al. (2011), regarding the translocation of nutrients to the shoot, VM1 stood out among the other eucalyptus clones, with the highest efficiency in the translocation of

Table 4. Concentration of macronutrients in hybrids of eucalyptus VM1 (*E. urophylla* x *E. camaldulensis*) and H13, GG100, I144 (*E. urophylla* x *E. grandis*).

Hybrid	Macronutrients (g kg^{-1})					
	N	P	K	Ca	Mg	S
VM1	12.92^{a}	1.7^{ab}	12.75^{a}	12.17^{a}	3.67^{a}	0.80^{a}
H13	15.00^{a}	1.6^{ab}	10.85^{ab}	9.17^{b}	3.17^{a}	0.75^{a}
GG100	15.35^{a}	2.12^{a}	9.97^{b}	11.95^{a}	2.92^{a}	0.47^{a}
I144	16.55^{a}	1.47^{b}	9.37^{b}	13.90^{a}	3.05^{a}	0.57^{a}
CV^{a} (%)	14.5	16.35	9.41	11.14	42.22	51.89

Means followed by different letters in the same column are significantly different by Tukey's test at 5%. ^{a}Coefficient of variation, N, P, P, Ca, Mg and S.

all macronutrients for the shoots of seedlings. In this sense, Zákia et al. (1983) studied the concentration of nutrients in eucalypt canopy and observed factors that could explain the differences in nutrient concentrations between the conventional and improved species, leading to the belief that improved trees compete strongly for water and light. This as in agroforestry systems, the trees are under constant competition, and so despite the high availability of nutrients from fertilization, only certain trees would have been able to metabolize them, and these trees are better adapted to the climate and soil conditions.

For N, Mg and S, there were no differences between hybrids (P> 0.05). In this way, the results may be related to the age of the plants, in which in the early years of development, the plants absorb more nutrients for the initial development. N is a key element for the initial development of the crop, accompanied by Mg that is part of the chlorophyll molecule and is essential for the photosynthesis and the development of the species. Cunha et al. (2009) point out that the assessment of nutritional status of eucalyptus under the influence of different genetic materials and the age of the tree showed that N deficiency is greater at the beginning of the crop cycle and it is required in higher concentrations, especially in division and cell elongation processes. Similar results were registered by Pinto et al. (2011), where clones with high efficiency in the uptake and translocation of nitrogen and sulfur are the hybrids GG100, I144 and VM1.

Conclusions

The differences identified in this study for growth in height, base diameter and survival at the seedling stage suggest the possibility of selecting genotypes of this crop for the region. The hybrid *E. urophylla* x *E. camaldulensis* showed better results for uptake of macronutrients, height, base diameter and survival compared to the other hybrids. The differences identified in this study as to the concentration of nutrients of *Eucalyptus* clones at the seedling stage allow the selection of genotypes for different conditions of soil fertility.In conclusion, there are differences in the uptake of micronutrients by the different hybrids studied; some of them have better ability to use these nutrients available in the soil. Thereby, adequate fertilization and proper management prevent the uptake of the nutrient pool of the soil by plants, with an efficient control of hybrids for the different regions.

Conflict of Interest

The authors have not declared any conflict of interest.

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The influence of financial crisis on inefficiency and nonlinearity on Brazilian soybean prices

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We investigated the effect of the 2007 to 2008 Brazilian financial crisis on nonlinearity and the prediction accuracy of artificial neural networks on monthly soybean prices in Brazil. To determine the exogenous variable, the commodity's logarithm return was calculated. The best period for the series simulation was then identified, simulations carried out and the model validated. Model forecasting results were satisfactory for all samples. A group method of data handling (GMDH) methodology was capable of demonstrating the returns' non-randomness, denoting marketing inefficiency, arbitrage opportunities and abnormal return to investors, especially after the financial crisis of 2007 to 2008.

Key words: Financial crisis, predictability, nonlinearity, soybean.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.], with its countless and varied uses, is an important crop at the global level. Its seeds are rich in oil - approximately 20% - and protein - approximately 40% - (Singh, 2010). Soybean is one of the oldest food sources known to humans. In 2011, the total cultivated area of soybean in the world was 102.99 million ha and the total production was 260 Tg year^{-1} (FAO, 2013), of which 75 Tg year^{-1} were produced in Brazil. Global soybean production and trade has changed dramatically in the past 30 years (Chianu et al., 2010). These changes have been driven by an increasing demand for soybean meal, a component which accounts for 65% of animal feed bulk (Ash et al., 2006).

Growing economies such as those of China, India and other developing countries have dramatically increased the demand for livestock products, which, in turn, has increased the demand for soybean meal (Delgado, 1999). In 2007, the global area, production and productivity of soybean were 90.1 million ha, 220 Tg and 2.44 Mg ha^{-1}, respectively (Singh, 2010). According to this author, the USA, Brazil, Argentina, China and India are the major soybean-producing countries. IGC (2015) noted of that for a world soybean production of 316 Tg year^{-1} projected for 2014/2015, 265 Tg year^{-1} would come from the exporting countries of Brazil, Argentina and the USA, and the remainder from other countries.

Both worldwide and in Brazil, soybean is the crop having shown the greatest perceptual growth in last few years. According to USDA data, global soybean production grew from 44 Tg year^{-1} in 1970, to over 220 Tg year^{-1} in 2008. Considering the favourable

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weather conditions for the American harvest, August 2009 saw a projected (2010/2011) 15% increase (32 Tg y $year^{-1}$) in global production, with a projected American production for 2010/2011 of 261 Tg $year^{-1}$, representing a four-fold growth. This represents substantial growth, compared to other crops: *e.g*., 300 to 792 Tg y $year^{-1}$ (1.6-fold) of wheat (*Triticum æstivum* L.), and 310 to 432 Tg $year^{-1}$ (40%) for rice (*Oryza sativa* L.), over the same period (Trennepohl and Paiva, 2011) {Trennepohl, 2011 #216}.

Accounting for 25.14% of global soybean production, in 2011 Brazil's produced 66 Tg $year^{-1}$ of soybean on 2.5×10^8 km^2, and area equivalent to all the UK territory (FAOSTAT, 2014). Still in 2010, soybean accounted for only 9% of all Brazilian exports, 5.6% of the nation's agricultural GDP and 1.25% of its overall GDP.

Many factors influence soybean prices (Jun and Chao, 2010), *e.g*., meteorological conditions, the family consumption level, the consumption structure, offer and demand, as well as national and international stock in the futures market and the soybean circulation system. This circulation system has non-linear features typical of a dynamic system and of the evolution law. This is sustained by the application of the chaotic sequence in order to study fluctuation and price forecast law. All of these features influence the price efficiency.

Based on different observed references with respect to information type, Fama (1970) stated the efficient market hypothesis to be comprised of three forms: weak, semi-strong and strong. Weak form efficiency is based on a set of information that only includes price or stocks return history. The semi-strong form considers a set of information that only includes the public knowledge available to all participants in the market, while the strong form includes any information obtained by any participant in the market.

Other definitions of market efficiency have been suggested (Rubinstein, 1975; Jensen, 1978; Beaver, 1981; Black, 1986; Dacorogna et al., 2001; Malkiel, 2003; Timmermann and Granger, 2004; Milionis, 2007). Since there is no consensual definition for the pattern of market efficiency, we adopted the version enounced by Fama (1970) that emphasises both speed and precision of price adjustments to new information.

Interest in predicting the behavior of prices is probably as old as the markets themselves, so the literature on this matter is wide and significant. Recent studies, such as that of Righi and Ceretta (2011), implementing time series analysis, showed daily quotations for some Brazilian commodities [soybean, cotton (*Gossypium hirsutum* L.), coffee (*Coffea arabica* L.) and sweet corn (*Zea mays* L.)] do not follow anticipated market efficiency, thus generating opportunities arbitrage.

In Brazil, the study of efficiency and predictability in the agricultural commodity markets is important to government as well as producers and purchasers. For the government, an efficient market is a better alternative than market intervention through policies. For processors and marketers, predictability provides a reliable forecast of prices allowing them to effectively manage their market risks. It is also in the interests of international market participants from countries like Canada, the USA, Australia and the European Union, who are major grain exporters.

Considering these issues, we defined the following research problem: "Did the financial crisis of 2007 to 2008 influence the nonlinearity and prediction accuracy of soybean price paid in Brazil?"

MATERIALS AND METHODS

In order to answer the research problem, we performed a time series (January 1990 to May 2014) analysis using the logarithmic return of the prices paid to producers in Brazil as the exogenous variable. In order to calculate return, we used a secondary data base (IPEADATA, 2011).

Tsay (2005) states that two main reasons exist why most studies on financial time series use returns rather than assets themselves: (i) For the average investor, assets return is an adequate indicator when comparing investments opportunities and, (ii) Return series are easier to deal with than a price series, since returns show more attractive statistical features, including the fact that non-bias is common in non-stationary data series. Given that, according to the assumed hypothesis, asset returns are independently and identically distributed (i.i.d.) with an average μ and variance σ^2, using logarithmic returns is well suited to financial studies (Tsay, 2005).

After calculating the logarithmic returns, we performed a random walk test to assess whether or not the data series presented non-random features. Results of this analysis indicated these features to be non-random, therefore offering the opportunity to perform modelling in an effort towards time series forecasting. In order to analyse the level of predictability of soybean price, we used the sample determination coefficient (R^2), which measures the proportion or percentage of variation in *y* anticipated by models:

$$R^2 = 1 - \frac{\sum_1^N \left(\hat{y}_i\right)^2}{\sum_1^N \left(y_i - \bar{y}\right)^2}. \quad (1)$$

where, *N* is number of observations; y_i is return during *i period;* $\hat{y}_i$ is computed values according to the model, and $\bar{y}$ is the mean value

Two other indicators were used, namely the mean standard error (MSE) and mean absolute error (MAE):

$$MSE = \sqrt{\frac{1}{N}\sum_1^N \left(y_i - \hat{y}_i\right)^2}. \quad (2)$$

$$MAE = \frac{1}{N}\sum_1^N \left|\sqrt{y_i^2} - \sqrt{\hat{y}_i^2}\right|. \quad (3)$$

We also analysed the Theil inequality coefficients, also known as *U*. The denominator for *U* is *MSE,* but the scale for the denominator is such that *U* exists in the interval from 0 to 1; where *U*=0 constitutes

a perfect forecast of observed values, and *U*=1 the model's worst possible predictive performance:

$$U = \frac{\sqrt{\frac{1}{N}\sum_1^N \left(y_i - \hat{y}_i\right)^2}}{\sqrt{\frac{1}{N}\sum_1^N \left(y_i\right)^2} + \sqrt{\frac{1}{N}\sum_1^N \left(\hat{y}_i\right)^2}}. \quad (4)$$

Besides the Theil inequality coefficient, we analysed the bias proportion and variance proportion (U^M and U^S, respectively), allowing us to break down the error into its characteristics sources. The U^M addresses possible systematic error, since it measures how much the average values for the simulated and effective series deviate from each other (Pindyck and Rubinfield, 1991). Whatever the value of *U*, U^M is expected to be close to zero. An elevated value for U^M (above 0.1 or 0.2) would be worrying, since it would indicate the presence of systematic bias, requiring the model to be modified accordingly. The U^M and U^S are calculated as:

$$U^M = \frac{\left(\bar{y}^S - \bar{y}^A\right)^2}{(1/T)\sum\left(\bar{y}_t^S - \bar{y}_t^A\right)^2}. \quad (5)$$

$$U^S = \frac{\left(\sigma_S - \sigma_A\right)^2}{(1/T)\sum\left(\bar{y}_t^S - \bar{y}_t^A\right)^2}. \quad (6)$$

where, $\bar{y}^S$, $\bar{y}^A$ are the means of observed and estimated values, respectively, and σ_S and σ_A are the standard deviations of observed and estimated values, respectively

The variance proportion U^S, indicates the capacity to replicate the rate of variability rate of the variable of interest (Pindyck and Rubinfield, 1991). A high value of U^S would indicate that the effective series floated a great deal. That would also be worrying and could lead to revising the models. To further evaluate the forecasts' success the δ^2 was calculated (Ivakhnenko et al., 1993):

$$\delta_i^2 = \frac{\sum_1^N \left(y_i - \hat{y}_i\right)^2}{\sum_1^N \left(y_i - \bar{y}\right)^2} \rightarrow \min. \quad (7)$$

Adequate performance would be reflected in cases where $\delta^2 \leq$ 0.05, while a satisfactory performance would be reflected in cases where $0.5 < \delta^2 < 0.8$, while $\delta^2 >$ 1.0 indicate inadequate performance and the need to revisit the modelling process.

To compare the accuracy of forecasts against random walk predictions, we used the Diebold-Mariano test (Diebold and Mariano, 1994) . When comparing two forecasts, the question of whether the predictions of a given model, *A*, are significantly more accurate, in terms of a loss function $g(\cdot)$, than those of the competing model, *B* arises. The Diebold-Mariano test aims to test the null hypothesis of equality of expected forecast accuracy against the alternative of differing forecasting ability across models. The null hypothesis of the test can be, thus, written as:

$$d_t = E\left|g\left(e_t^A\right) - g\left(e_t^B\right)\right| = 0 \quad (8)$$

where, e_t^i is the forecasting error of model *i* when performing *h*–step-ahead forecasts.

The Diebold-Mariano test uses the autocorrelation-corrected sample mean of d_t in order to test the null hypothesis (Equation 8). If *n* observations and forecasts are available, the test statistic is, therefore,

$$S = \left[\hat{V}\left(\bar{d}\right)\right]^{-1/2}\bar{d}, \quad (9)$$

where

$$\hat{V}\left(\bar{d}\right) = \frac{1}{n}\left[\hat{\gamma}_0 + 2\sum_{k=1}^{h-1}\hat{\gamma}_k\right], \quad (10)$$

and

$$\hat{\gamma}_k = \frac{1}{n}\sum_{t=k+1}^{n}\left(d_t - \bar{d}_t\right)\left(d_{t-k} - \bar{d}_t\right) \quad (11)$$

Under the null hypothesis of equal forecasting accuracy, *S* is asymptotically normally distributed.

RESULTS AND DISCUSSION

Before analyzing predictability of Brazilian soybean return prices, it was necessary to choose the period to analyze. We therefore tried to identify the structural breaks in the analyzed series. The problem of detecting structural changes in linear relationships has been an important topic in econometric and statistical research (Zeileis et al., 2001), considering that a careless analysis can result in incorrect inferences in causality tests, co-integration and acceptance of incorrect models (Covas, 1997). The latter stated that these tests can determine the way in which exogenous shocks or political regime changes are felt in the behavior of some economic indicators.

In order to adequately treat the time series, some authors have presented several tests that make it possible to identify and estimate the moments for structural breaks. Among the first works to be published, we can find tests by Chow (1960) and cumulative sum control charts (CUSUM; Brown et al., 1975). The former tests had the inconvenience of implying an *a priori* knowledge of where the structural break was, while the latter test, part of a different class of tests, allows one to detect breaks of several types for parameters of interest, and for which one is not required to specify the number of breaks in the series (Covas, 1997).

In the present study we used a more sophisticated model to estimate the structural breaks in the data series. The Bai and Perron (1998) method allows one to simultaneously estimate multiple breaks as well as determine their previously unknown dates. Initially, we tested the hypothesis of the existence of structural breaks in the Brazilian soybean returns prices on a monthly

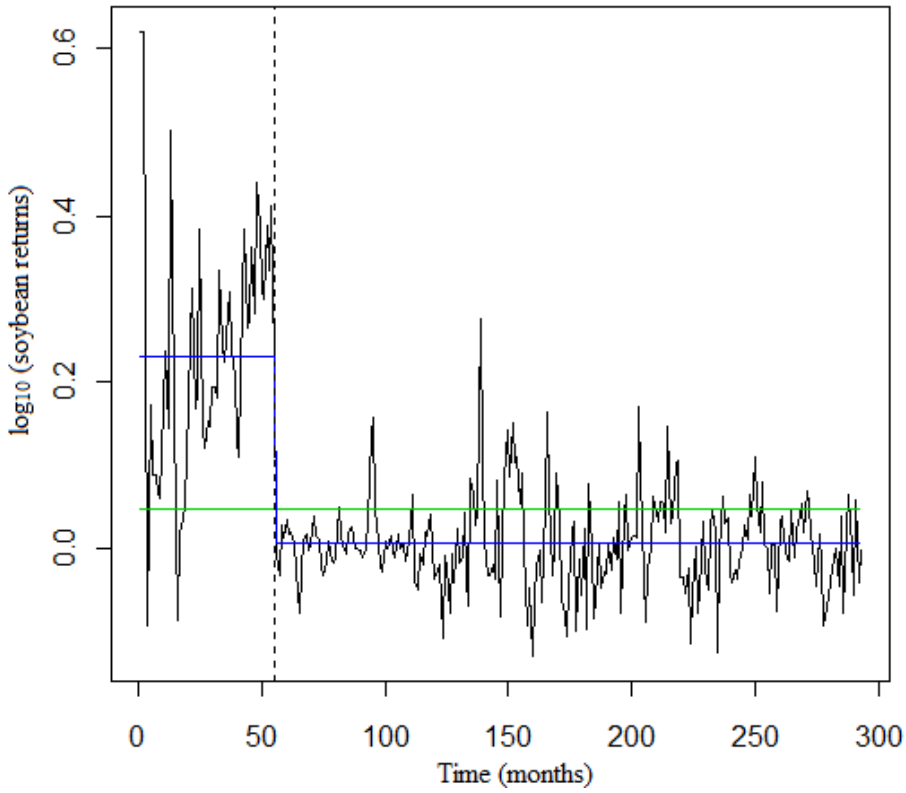

Figure 1. Structural break for the monthly logarithmic returns of soybean (January 1990 to May 2014).

basis. Based on the monthly negotiation prices for soybean, we calculated the logarithmic returns and rejected the null hypothesis stating that the vector *b* variance was constant throughout the whole series (*F* stats = 20.8434, sig. 0.000). This indicated the existence of structural breaks in the time series (Figure 1).

A single rupture in the monthly logarithmic returns of Brazilian soybean prices occurred between January 1990 and May 2014, as the change in behaviour of this series at the 55th observation attests (Figure 1). We therefore performed the simulation excluding the first 55 observations (January 1990 to June 1994).

Excluding the first 55 observations (January 1990 to June 1994) can be explained from an economics perspective: Brazil initiated the Plano Real, a program strictly limiting government spending, creating a new currency, and implementing many other fiscal reforms in June 1994. This significantly modified inflation memory within the Brazilian society, enhancing the Brazilian productive processes, especially the agribusiness sector and soybean trading.

A well-known problem in modelling is the search for an optimal model, which essentially depends on the adopted methodology. Prior to testing the predictability of the series, it was necessary to choose an appropriate method. The linearity test is a determinant criterion when choosing the methodology to be adopted when modelling a time series (Steyerberg, 2009). A similar problem occurs when one examines different transformations, jeopardizing the variable's linearity.

Several tests have been proposed for assessing the need for nonlinear modeling in time series analysis (Cryer and Chan, 2008). Some of these tests, such as those of Keenan (1985) and Tsay (1986), can be interpreted as Lagrange multiplier tests for specific nonlinear alternatives.

Keenan (1985) derived a test for nonlinearity analogous to Tukey's one degree of freedom for nonadditivity test. Keenan's test seeks to approximate a nonlinear stationary time series by a second-order Volterra expansion:

$$Y_t = \mu + \sum_{\mu=-\infty}^{\infty} \theta_\mu \varepsilon_{t-\mu} + \sum_{\nu=-\infty}^{\infty} \sum_{\mu=-\infty}^{\infty} \theta_{\mu\nu} \varepsilon_{t-\mu} \varepsilon_{t-\nu} \quad (12)$$

where, Y_t is the process in time *t*, ε is the error of the process at the past (μ or ν).

In this case, $\{\varepsilon_t, -\infty<t<\infty\}$ is a sequence of independent and identically distributed zero-mean random variables. The process is linear if the double sum on the right hand

side of Equation (12) vanishes (Nazlioglu and Soytas, 2010). Keenan's test is equivalent to a test of n=0, according the regression model (Cryer and Chan, 2008):

$$Y_t = \theta_0 + 1 + \varphi_1 y_{t-1} + \ldots + \varphi_m y_{t-m} + \exp\left\{ n\left(\sum_{j=1}^{m} \varphi_j y_{t-j} \right)^2 \right\} + \varepsilon_t \quad (13)$$

where, Y_t is the process in time t, θ is a constant, φ_1,..., φ_m and η are the parameters of y_t at time t, and ε_t is the error at time t.

In this case, $\{\varepsilon_t\}$ are independent and normally distributed with zero mean and finite variance.

If $\eta \neq 0$, the model is non-linear. Keenan's test is both conceptually and computationally simple and only has one degree of freedom, which makes the test very useful for small samples (Cryer and Chan, 2008). However, Keenan's test is only powerful in detecting nonlinearity in the form of the square of the approximating linear conditional mean function. Tsay (1986) extended Keenan's approach by considering more general nonlinear alternatives. A more general alternative to nonlinearity may be formulated by replacing the term:

$$\exp\left\{ n\left(\sum_{j=1}^{m} \varphi_j y_{t-j} \right)^2 \right\} + \varepsilon_t \quad (14)$$

By

$$\begin{aligned} &\exp(\ \delta_{1,1} y_{t-1}^2 + \delta_{1,2} y_{t-1} y_{t-2} + \ldots + \delta_{1,m} y_{t-1} y_{t-m} \\ &+ (\delta_{2,2} y_{t-2}^2 + \delta_{2,3} y_{t-2} y_{t-3} + \ldots + \delta_{2,m} y_{t-2} y_{t-m} \ldots \\ &+ (\delta_{m-1,m-1} y_{t-m+1}^2 + \delta_{m-1,m} y_{t-m+1} y_{t-m} + \ldots + \delta_{m,m} y_{t-m} y_{t-m}) + \varepsilon_t \end{aligned} \quad (15)$$

where, ε_t is a white noise, φ_j and δ_m are the parameters at time j or m, for example.

Using the approximation $\exp(x) \approx 1 + x$, the nonlinear model can be approximated as a quadratic AR model, and whether or not all the $m(m+1)/2$ coefficients $\delta_{i,j}$ are zero can be tested by an F-test (Cryer and Chan, 2008).

In order to test each regime's non-linearity, we used the Tsay Test (Tsay, 1986), which assesses the existence of non-linearity on average, and considers the residuals ($\hat{\varepsilon}_i$) of the auto-regressive process:

$$\hat{y}_i = \hat{\beta}_i y_{i-1} \cdots + \hat{\beta}_p y_{i-p+1} + \hat{\varepsilon}_i \quad (16)$$

where,

$\hat{\varepsilon}_i$ represents the estimated residuals of the model, p is the number of lags, $\hat{y}_i$ is the estimated dependent variable, and y_{i-1} is the lagged dependent variable in t-1.

For each y_t observation, we built a vector z_t of the lagged variables' cross products, that is, y_{t-i}, y_{t-j} for i, j = 1, ..., p where $i > j$. For instance, if p = 2 then $z_t = \left[y_{t-1}^2, y_{t-1} y_{t-2}, y_{t-2}^2 \right]^T$. Subsequently, the parameters are estimated according to:

$$\hat{y}_i = \hat{\phi}_1 y_{i-1}^2 + \hat{\phi} y_{i-1} y_{i-2} + \hat{\phi}_3 y_{i-2}^2 + \hat{\eta}_i \quad (17)$$

where, $\hat{\phi}_i$ represents the model's estimated parameters, and $\hat{\eta}_i$ represents the model's estimated residuals.

We then determined the regression for the estimated residuals $\hat{\varepsilon}_i$ in $\hat{\eta}_i$ as:

$$\hat{\varepsilon}_i = \gamma_0 + \gamma_1 \hat{\eta}_{t-1} + \gamma_2 \hat{\eta}_{t-2} \cdots + \gamma_p \hat{\eta}_{t-p} + \hat{\xi}_t \quad (18)$$

where, γ_0 represents the estimated parameters, and $\hat{\eta}_{i-p}$ are the estimated residuals lagged in p. Based on the steps of Eqs. 16-18, we calculated the Tsay Test statistics, as:

$$\hat{F} = \frac{\left(\hat{\varepsilon}^{\mathrm{T}} \hat{\eta}\right)^{\mathrm{T}} \left(\hat{\eta}^{\mathrm{T}} \hat{\eta}\right)^{-1} \left(\hat{\eta}^{\mathrm{T}} \hat{\varepsilon}\right) / m}{\left(\hat{\varepsilon}^{\mathrm{T}} \hat{\varepsilon}\right) / \left(n - p - m - 1\right)} \quad (19)$$

where, $m = p(p+1)/2$ and the null hypothesis of a linear series, that is, $H_0 : \gamma_1 = \gamma_2 = \cdots \gamma_p = 0$, is tested.

While Keenan's test and Tsay's test for nonlinearity are designed for detecting quadratic nonlinearity, they may not be sensitive to threshold nonlinearity. Here, we discuss a likelihood ratio test with the threshold model as the specific alternative. The null hypothesis is an AR(p) model versus the alternative hypothesis of a two-regime TAR model of order p with constant noise variance, that is; $\sigma_1 = \sigma_2 = \sigma$. With these assumptions, the general model can be rewritten as:

$$\begin{aligned} y_t &= \varphi_{1,0} + \varphi_{1,1} y_{t-1} \ldots + \varphi_{1,p} y_{t-p} \\ &+ \left\{ \varphi_{2,0} + \varphi_{2,1} y_{t-1} \ldots + \varphi_{2,p} y_{t-p} \right\} I(y_{t-d} > r) + \sigma\, e_t \end{aligned} \quad (20)$$

where the notation $I(y_{t-d} > r)$ is an indicator variable that equals 1 if and only if the enclosed expression is true, and zero otherwise. In practice, the test is carried out with fixed p and d values. The likelihood ratio test statistic can be shown to be equivalent to:

Table 1. Results of Tsay, Keenan and Threshold nonlinearity test, for return of Brazilian soybean prices (on a monthly basis) for before and after 2008 crises.

Period	Tsay´s test		Keenan´s test		Threshold	
	Statistics	***p*-value**	**Statistics**	***p*-value**	**Statistics**	***p*-value**
Before 2008 crises	23.8900	0.0000	76.475	0.0062	33.596	<0.0001
After 2008 crises	0.7795	0.3806	0.1472	0.7028	46.897	<0.0001

$$T_n = (n-p)\log\left\{\frac{\hat{\sigma}^2(H0)}{\hat{\sigma}^2(H1)}\right\} \quad (21)$$

where $n - p$ is the effective sample size. The test statistic is the maximum likelihood estimator of the noise variance from the linear AR(p) fit and from the TAR fit with the threshold searched over some finite interval. Under the null hypothesis ($\varphi_{2,0} = \varphi_{2,1} = \ldots = \varphi_{2,p} = 0$) the (nuisance) parameter r is absent. Hence, the sampling distribution of the likelihood ratio test under H_0 is no longer approximately $\chi 2$ with p degrees of freedom.

The results of Tests for Nonlinearity for before and after the 2008 crises (on a monthly basis) are shown on Table 1.

As Tsay's test for quadratic nonlinearity in a time series considers a null hypothesis that the process is linear, when we reject the null hypothesis we reject linearity for the given time series. Accordingly our results indicate the soybean market showed linearity after but not before the 2008 crisis (Table 1). Keenan's test analyses a series' non-linearity against a the null hypothesis that the time series follows some AR process. Keenan's Test shows series nonlinearity after, but not before, the 2008 crises (Table 1). In order to confirm this fact, we carried out the Threshold test for non-linearity (Table 1). The null hypothesis of the Threshold test for non-linearity is an AR(p) model *vs.* an alternative hypothesis of a two-regime TAR model of order p and with constant noise variance, that is; $\sigma_1 = \sigma_2 = \sigma$. This test suggests that the series returns are highly non-linear after the 2008 crisis (p <0.0001). Thus it is necessary to use a nonlinear method to forecast such a time series. Consequently a GMDH model was used.

Based on an algorithm that dates back to the 1960s, the Group Method of Data Handling (GMDH) is a mathematical method that allows one to estimate states in a system, along with controllers' exits and performers' functions (Ivakhnenko, 1969). The algorithm can be considered self-organized and of inductive propagation in the solution of practical and complex problems. Moreover, it is possible to obtain a mathematical model for a given process from data sample observations, that will be used when identifying and recognizing patterns, or even to describe the process itself.

The use of GMDH-like self-organizing networks has been successfully applied to a wide range of fields of study (Ahmadi et al., 2007). Mottaghilab et al. (2010) reported good results when this type of network was applied in specific areas, particularly such as Engineering and Economics. Most GMDH algorithms use polynomial reference functions. A general connection between entry and exit variables can be expressed by the Volterra functional series, an analogue of the Kolmogorov-Gabor polynomial:

$$y = \beta_0 + \sum_{i=1}^{n}\beta_i x_i + \sum_{i=1}^{n}\sum_{j=1}^{n}\beta_{ij} x_i x_j + \sum_{i=1}^{n}\sum_{j=1}^{n}\sum_{k=1}^{n}\beta_{ijk} x_i x_j x_k + \xi. \quad (22)$$

where, x_i, x_j, x_k are endogenous variables, β_0, β_{ij} and β_{ijk} are the polynomial coefficients, and ξ is the error term.

The content of Ivaknenko's algorithm was developed as a vehicle to identify linear and non-linear relationships between inputs and outputs, thereby generating a structure tending towards an optimum, through a successive process of several data manipulations, via the incorporation of new layers.

The GMDH model can be analyzed as a combination of neural networks and stochastic concepts (Valença, 2005). GMDH networks are implemented with activating functions in the neurons of the hidden layers, and present a selection criterion in order to decide how many layers will be built. In the original formula, each neuron of the hidden layer to be built receives two entries and must activate a 2nd degree polynomial. As a consequence, a polynomial exit function will be generated via the combination of each pair of these entry neurons; the complexity of such a polynomial depends on the number of layers, that is, if there are two layers, we have a 4th degree polynomial function; for three layers, there will be an 8th degree function, and so on. Thus, such networks are called polynomial, for the resulting model is a polynomial function.

For the period between October 2003 and May 2014, we carried out 127 forecasts, all for t+1 months, that is, only one step (month) ahead. It is important to note that in this period the American crisis occurred (that is, a credit crisis in the banking sector). Symptoms were however perceived in other sectors, especially the agricultural production sector.

Table 2. Accuracy of forecast of the logarithmic return of the soybean monthly price paid to producers in Brazil's Paraná state, during and after the 2008 American crisis,

Category	R^2	Correlation	Signals	MSE	MAE	U	U^M	U^S	Ivakhnenko
Full period	0.0566	0.2379	0.5906	0.0027	0.0416	0.0410	0.0028	0.0006	0.9444
Before the crisis	0.0078	0.0884	0.4915	0.0038	0.0504	0.0521	0.0034	0.0012	1.0009
During the crisis	0.0380	0.1949	0.4444	0.0017	0.0374	0.0303	0.0739	0.0003	0.9684
Post-crisis	0.1741	0.4172	0.7119	0.0018	0.0335	0.0298	0.0093	0.0003	0.8462

In this regard, Krugman et al. (1999) states that there is no universally-accepted formal definition for the concept of a financial crisis, but we know them when we see them. According to them, the basic element is a type of circular logic, where investors run away from an investment because they fear that it can go down, and where many, but not necessarily all pressures for the investment going down arise precisely from the flight of capital. They further note that such crises have been a recurring feature in international economy, since gold and silver coins were replaced by coin and paper.

A systemic global crisis arising in the USA strongly affected the Brazilian economy, both in terms of external trade and financial flux, particularly in terms of commercial credit lines and market application of Brazilian equity (De Freitas, 2009). In Brazil, the most immediate effect was the downfall in stock markets, caused by significant selling off of stocks to foreign speculators that literally stepped over each other to repatriate their equity in order to cover their losses in their own countries. Consequently, there was an strong rise in the American dollar rise which directly influenced the Brazilian agribusiness sector.

In order to limit periods, we used a theoretical limit based on the work of De Freitas (2009), who stated that the period of greatest crisis-induced turbulence occurred from September 2008 to May 2009 – a period of nine months. This period was termed "during the crisis". The period consisting of the 59 months preceding the crisis (October 2003 to August 2008) was termed "before the crisis," while the "post-crisis" period was defined as occurring between June 2009 and May 2014 (59 months). Forecasts results for these periods, and overall are shown in Table 2.

Based on the Ivakhnenko criterion (Equation 8), the logarithmic return forecast for soybean price was effective for the full period, as well as during and after the crisis. Positively, we also note that the Theil U, the variance proportion (U^M) and the error bias proportion (U^S) were also adequate, indicating the absence of a systematic error in the forecast, which would denote that significant information – contained in the original series – had not been well modelled.

However, for the period prior to the 2008 American crisis, prediction results were poor, the Ivakhnenko criterion (IC = 1.0009) showing the forecasts to be unsatisfactory and the results erroneous (Table 2). Yet in the period after the crisis (June 2009 to May 2014), forecasts were satisfactory (IC = 0.8462). Based on the signals (Table 2), the forecasts were right in 71.19% of cases, and the R^2 was highest at 0.1741. Other post-crisis indicators, such as the MSE and MAE were similar or better that those for before or during the crisis.

These results denote a new behaviour of soybean prices paid to producers in Brazil after the 2007/2008 crisis. The Diebold-Mariano test shows that predictability after the 2007/2008 American crisis was greater than before the crisis (DM-statistic = 2.7501 p = 0.0030). It is important to emphasise that the Diebold-Mariano test aims to test the null hypothesis of equality of expected forecast accuracy against the alternative hypothesis of one series (before *vs.* after crisis) being predicted more accurately than the other. The after crisis series was shown to be predictable with the GMDH model. This research corroborates the work of Righi and Ceretta (2011), who have demonstrated that there is a mild inefficiency for the Brazilian soybean prices series, thus opening the possibility for arbitrage procedures and abnormal returns for this type of investments, as well as opportunities for the farmer to plan how to sell this commodity in moments that are more favourable.

Conclusions

In the present study, we tried to assess the predictability of the monthly return of soybean price paid to producers in Brazil. The logarithmic return of this series was calculated, and the hypothesis that returns would follow a random walk, preventing predictability, was tested. Soybean price returns show different features depending on the period – before implementing the Real plan (June 1994) and after. We used 127 months in order to simulate the modelling parameters and another 112 months to carry out forecasts (October 2003 to May 2014). The forecast results were satisfactory for all samples. The GMDH model was able to demonstrate the returns' non-randomness, denoting inefficiency for this market, and therefore arbitrage opportunities and abnormal returns for investors, as well as the opportunity for producers in that region to plan their sales in more favourable periods.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Effects of water management on growth, irrigation efficiency and initial development of *Aspidosperma polyneuron* seedlings

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The amount of available water for nursery irrigation is forecasted to decline on this decade and over or deficit watering of seedlings may have adverse consequences. To produce quality seedlings for accelerate the restoration of ecosystems and prevent further environmental damage, nursery water management should be reformed, especially for endangered species, such as *Aspidosperma polyneuron* Müll. Arg. The objective of this work was to evaluate the effect of three gross irrigation depths (8, 11 and 14 mm) applied daily and two irrigation frequencies (two and four times a day) on growth, irrigation efficiency and initial development of *Aspidosperma polyneuron* seedlings. Irrigation efficiency, in *A. polyneuron* seedlings, is related to the shoot part development degree (height and shoot dry mass). Increases in irrigation efficiency do not necessarily produce a greater development of morphological parameters and root system quality in the nursery. The 11 mm irrigation depth produces the same amount of seedlings with able root systems that the 14 mm irrigation depth, and uses 21% less water. The 11 mm irrigation depth applied in two irrigation frequencies produce *A. polyneuron* seedlings with optimum roots system and proper morphological development in the nursery, which continues after planting.

Key words: Peroba-rosa, land degradation, tropical forest, gross irrigation depth, irrigation frequency, runoff, forest nurseries, development after planting.

INTRODUCTION

The original Brazilian Atlantic Forest has been lost, and only 11.73% of the original vegetation (16,377,472 ha) remains (Ribeiro et al., 2009). In these cases, tree seedling plantings are a potential option to accelerate the restoration of this ecosystem and prevent further environmental damage (Modna et al., 2010).

Due to the reduction of native populations caused by extensive logging, the tropical species *Aspidosperma polyneuron* Müll. Arg. has been included on the Red List of Endangered Species (IUCN, 2015). This species is native of Brazilian Atlantic forest and is recommended to recover ecosystems and restore degraded riparian areas in soils not subject to flooding (Carvalho, 2003).

To optimize plantings, especially under harsh conditions, it has become increasingly obvious that a change of focus towards seedling quality is needed

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(Lindqvist and Ong, 2005), improving the morphological, physiological and genetic quality of seedlings (Wilson and Jacobs, 2006).

In nurseries, the main factors that affect the development and quality of seedlings are the quality of its genetic materials, water management, nutrition, the type of container and the substrates used (Silva et al., 2012). Water management is defined as the process of determining how much to apply (irrigation volume) and timing (when to apply) (Warren and Bilderback, 2005).

Frequently the water management in most Brazilian nurseries is applied by micro-sprinklers (Augusto et al., 2007) and over or deficit watering of seedlings may have adverse consequences. Overwatering may lead to nutrient leaching which may affect environmental quality and increase production costs, while water deficit can deleteriously affect potential growth and cause seedling death (Bauerle et al., 2002; Montague and Kjelgren, 2006). Thus, is necessary to define water management in the nursery to suit environmental laws and improve the quality of the seedlings (Silva et al., 2004).

The objective of this work was to evaluate the effects of gross irrigation depths and irrigation frequencies on growth, irrigation efficiency and initial development of *A. polyneuron* seedlings.

MATERIALS AND METHODS

Plant material and experimental conditions

This experiment was conducted between October 2011 and October 2012 in a suspended and sectorized nursery located in Botucatu, São Paulo State, Brazil (22°1'S, 48°25'W). The climate of the region is Cwa according to the Köppen climate classification.

Seeds of *A. polyneuron* were collected in September 2011 with climbing techniques in a forest fragment located in Botucatu, Brazil. The seeds were packed in polyethylene bags and transported to the nursery. In the nursery, the seeds were stored for 30 days at a temperature of 10 ± 2°C and a relative humidity between 8 and 12%, where they remained until sowing. Plastic tubes (92 cm^3) were used in seedling production. The plastic tubes were placed in 108 cells of each polypropylene tray and filled with a substrate consisting of *Sphagnum* peat, vermiculite and carbonized rice chaff (2:1:1; volume basis).

Substrate physical analyses were conducted according to methods described by Guerrini and Trigueiro (2004), and chemical analyses were conducted according to methods described by Brasil (2007) (Table 1).

The soluble fertilizers Yoorin® Master 1S and Fosmag® 500B and the controlled release fertilizer Osmocote® with NPK (19:6:10) were added to the substrate. These fertilizers provide macronutrients in dosages of 42.3, 69, 31.3, 25.2, 48.2 and 18 mg per plastic tube of N, P, K, S, Ca and Mg, respectively, and dosages of micronutrients 0.3, 0.1, 0.6, 18.4 and 1 mg per plastic tube of B, Cu, Mn, Si and Zn, respectively.

Sowing was performed manually by placing a seed in each plastic tube. The trays were transferred to an automated greenhouse with temperature control (less than or equal to 30°C) and relative humidity (greater than 80%, maintained through nebulization) with a 7 L h^{-1} flow nozzle, triggered automatically by an electric panel for 10 s, every 15 min, from 9:00 am to 4:00 pm. After sowing, the seedlings remained in this environment for 37 days after which they were transferred to a shade house (with 50% light reduction) where they were irrigated with micro-sprinklers with a 200 L h^{-1} flow nozzle, triggered automatically by an electric panel for 20 s, every 30 min, from 9:00 am to 4:00 pm, and where they remained for 42 days.

Irrigation treatments

The experiment was laid out in completely randomized design with a factorial scheme that consisted of three daily gross irrigation depths (8, 11 and 14 mm), split into two and four irrigation frequencies by micro-sprinklers (Table 2).

Each treatment consisted of 4 replicates (trays). In each tray, the percentage occupancy of the seedlings was 25%. In each repetition, the 12 central seedlings were the useful seedlings, and the 18 other surrounding seedlings constituted the boundary, totaling 48 useful seedlings per treatment.

Before starting the treatments, seedlings were selected in which to homogenize the repeats, ensuring that height and stem diameter averages did not statistically differ ($p < 0.05$). The mean values and standard deviations of height and stem diameter were 5.3 ± 0.8 cm and 1.33 ± 0.16 mm, respectively.

To begin the treatments, the repetitions were distributed in a completely randomized design in three outdoor beds, covered with a plastic light diffuser, in the sunlit area of the nursery. Each outdoor bed received the two treatment repetitions, which were automatically applied by the electric panel-powered irrigation system.

The side dressing fertilization was performed twice a week for 85 days after the start of the treatment application. In each fertilization, the 4 mm irrigation depth of nutrient solution was applied *via* fertigation in all treatments. The solution comprised the fertilizers according to described by Silva and Silva (2015). The hardening fertilization was performed twice a week from 85 until 120 days after the beginning of treatments. In each fertilization, the 4 mm irrigation depth of nutrient solution was applied *via* fertigation in all treatments. The fertilizer solution was composed according to described by Silva and Silva (2015).

Nursery plant and irrigation analysis

To evaluate the quality of the seedlings in the nursery, the following morphological parameters were measured 120 days after the start of treatments: Height (cm) and stem diameter (mm) (these two parameters were evaluated in the 12 useful seedlings per replication), as well as, shoot, root and total dry mass (g). The roots and shoots measurement was conducted on 6 useful seedlings per repetition. From the combination of morphological parameters, the total dry mass (g) and the Dickson quality index (DQI) was determined using the following equation:

$$DQI = \frac{\text{Total dry mass (g)}}{\left(\frac{\text{Height (cm)}}{\text{Stem diameter (mm)}}\right) + \left(\frac{\text{Shoot dry mass (g)}}{\text{Root dry mass (g)}}\right)}$$

The quality of the root system was evaluated according to Silva et al. (2013), in the same seedlings.

The irrigation efficiency (IE) of each treatment was assessed at 120 days after the start of the treatment application, in two useful seedlings per replication, totaling eight seedlings per treatment, from the equation of Fain et al. (1998):

Table 1. Physical and chemical properties of substrate used in this experiment.

Physical properties	Porosity (%)			Water retention (ml per plastic tube)
	Macro	Micro	Total	
	24.2	59.3	83.4	54.6
Chemical properties	**Electrical conductivity (mS cm^{-1})**			**pH**
	0.5			6.5

Table 2. Irrigation treatments.

Treatment	Compositions
ID8F2	4 mm 10:00 am and 4 mm 2:00 pm
ID8F4	2 mm 9:00 am, 2 mm 11:00 am, 2 mm 1:00 pm and 2 mm 3:00 pm
ID11F2	5.5 mm 10:00 am and 5.5 mm 2:00 pm
ID11F4	2.75 mm 9:00 am 2.75 mm 11:00 am, 2.75 mm 1:00 pm and 2.75 mm 3:00 pm
ID14F2	7 mm 10:00 am and 7 mm 2:00 pm
ID14F4	3.5 mm 9:00 am, 3.5 mm 11:00 am, 3.5 mm 1:00 pm and 3.5 mm 3:00 pm

ID, irrigation depth; *F,* irrigation frequency.

$$IE = \frac{\text{Water applied (mL)} - \text{Water drained (mL)}}{\text{Water applied (mL)}} \times 100$$

Initial development conditions and analysis

After the end of nursery phase, the seedlings were kept in their treatments for more 20 days, when six seedlings from each treatment were planted in pots of 7 L, containing 8 kg of soil each. The soil was collected from the surface layer (0-20 cm), corresponding a dystrophic Red Latosol, medium texture. The fertilizer NPK (4:14:8) in dosages 2 kg of fertilizer per cubic meter and limestone, in the same dose, were added to the soil and mixed for 5 min in mixer. Before and immediately after planting, each pot was irrigated, respectively, with 2 and 1 L of water. The plants were kept in a completely randomized design in the greenhouse covered with transparent plastic for 120 days and also irrigated with 0.5 L every nine days. The height (cm) and stem diameter (mm) of plants were evaluated immediately after planting and thereafter at intervals of 30 days. At 120 days after planting, shoot and root dry mass of plants (g) were evaluated.

Statistical analysis

An analysis of variance was performed to compare the effect of irrigation depths and irrigation frequency on the parameters analyzed in the nursery. When the value of the F test indicated a significant effect, we used the Tukey's test ($p < 0.05$) to compare differences between means of treatments. In the analysis of initial development, when the value of the F test indicated a significant effect, we used regression analysis over time (height and stem diameter) and Tukey's test ($p < 0.05$) (shoot and root dry mass).

RESULTS AND DISCUSSION

Nursery and irrigation results

The 11 and 14 mm irrigation depths applied in two irrigation frequencies formed seedlings with greater height, stem diameter and shoot and total dry mass compared to the same irrigation depths applied in four irrigation frequencies. In the root dry mass and DQI, only the 14 mm irrigation depth applied in two irrigation frequencies overcome the four irrigation frequencies (Table 3).

These results are in agreement with those obtained by Warren and Bilderback (2005), who stated that the cycles may vary from two to twelve per day, but about two at an appropriate time application, as is appropriate.

In two irrigation frequencies, the 11 mm irrigation depth overcome 8 mm the irrigation depth in all morphological parameters and compared to the 14 mm irrigation depth provided equal development. This indicated that the seedlings irrigated with 8 mm irrigation depth showed this behavior because they were likely saturated just a few inches below the surface layer of the substrate, causing water deficits during seedling production in the nursery and the 14 mm irrigation depth was excessive during the treatment application, which may have lead to the nutrient leaching. The purpose of irrigation is to artificially supply water to meet plant water needs for economical crop production. As population and industrial development increase, water supply will become a major constraint to future irrigation development. Therefore, future irrigation systems must utilize water resources more efficiently (Irmak et al., 2001).

The 8 mm irrigation depth produced a greater and smaller amount of seedlings with "poor" and "able" root systems, respectively, showing that the 8 mm irrigation depth is insufficient to produce seedlings with root system quality (Figure 1).

According to Taiz and Zeiger (2004), the proliferation of

Table 3. Effects of the interaction between irrigation depths and the irrigation frequencies on some morphological parameters in *A. polyneuron* seedlings 120 days after the beginning of treatments.

Irrigation depths (mm)	Height			Stem diameter		
	Frequencies		CV (%)	Frequencies		CV (%)
	2x	4x		2x	4x	
8	14.1^{Bb}	19.1^{Aa}	15.4	4.01^{Bb}	4.78^{Aa}	9.21
11	24.3^{Aa}	18.9^{Ab}	15.3	5.18^{Aa}	4.77^{Ab}	9.8
14	23.5^{Aa}	17.0^{Bb}	19.3	5.36^{Aa}	4.52^{Bb}	12.4
CV (%)	15.1	19		9	12.2	
Irrigation depths (mm)	**Shoot dry mass**			**Root dry mass**		
	Frequencies			**Frequencies**		
	2x	**4x**	**CV (%)**	**2x**	**4x**	**CV (%)**
8	1.67^{Bb}	2.59^{Aa}	13.9	0.86^{Bb}	1.07^{Aa}	17.2
11	3.58^{Aa}	2.57^{Ab}	17.8	1.17^{Aa}	1.11^{Aa}	19.6
14	3.54^{Aa}	2.23^{Bb}	20.8	1.33^{Aa}	0.99^{Ab}	21.6
CV (%)	20.3	15.4		21.3	18.1	
Irrigation depths (mm)	**Total dry mass**			**DQI**		
	Frequencies			**Frequencies**		
	2x	**4x**	**CV (%)**	**2x**	**4x**	**CV (%)**
8	2.53^{Bb}	3.66^{Aa}	14.2	0.46^{Bb}	0.58^{Aa}	18.7
11	4.75^{Aa}	3.68^{Ab}	17.7	0.63^{Aa}	0.59^{Aa}	22.1
14	4.87^{Aa}	3.22^{Bb}	20.5	0.70^{Aa}	0.53^{Ab}	23.2
CV (%)	20.1	15.5		23.5	19.7	

Means followed by the same capital letter in the column and the same lowercase letter across the row are not significantly different according to the Tukey's test ($p < 0.05$).

roots depends on the availability of water and nutrients in the microenvironment surrounding the root, called the rhizosphere. If the rhizosphere is nutrient-poor or too dry, root growth is slow.

The 11 mm irrigation depth, that did not differ the 14 mm irrigation depth, produced a smaller and greater amount of seedlings with "poor" and "able" root systems, respectively. This indicated that the 14 mm irrigation depth was excessive and unnecessary during the treatment application. The highest plant growth is not always associated with the highest rates of irrigation (Fox and Montague, 2009). The application of water and fertilizers during plant production places an onus on container nurseries to minimize the potentially harmful effects of nutrient contamination of runoff water. Experiments that quantify runoff volume and nutrient content during production provide insight as to the relative roles various production practices play in utilizing water and nutrients most efficiently (Million et al., 2007).

The water managements showed no significant effects on the category "good" of root system quality. The 11 and 14 mm irrigation depths applied in two irrigation frequencies formed the same amount of seedlings with optimum root systems, showing the possibility of saving water in irrigation of this species in the nursery. According to Montague and Kjelgren (2006), excess watering can lead to the nutrient leaching, causing damage to the environment, low plant growth and increased maintenance costs (Table 4).

Only the irrigation depths influenced the irrigation efficiency (Table 5).

The 8 mm irrigation depth had increased the irrigation efficiency. This may be because they were likely saturated just a few inches below the surface layer of the substrate. According to Warren and Bilderback (2005), the key to increased irrigation efficiency is in control the application rate, application duration, and interval between applications and if low water volumes are used without taking into account the maintenance of adequate water in the container, stomatal closure may occur, reducing photosynthesis and consequently reducing plant growth.

The 11 and 14 mm irrigation depths did not differ in irrigation efficiency. This result was possible because the seedlings produced in the 14 mm irrigation depth had less amount of shoot part (height and shoot dry mass), reducing water capitation and consequently reducing runoff.

According to Lea-Cox et al. (2001) and Million et al. (2007), the size of the seedlings and the capitation of water is an important factor that may influence irrigation efficiency.

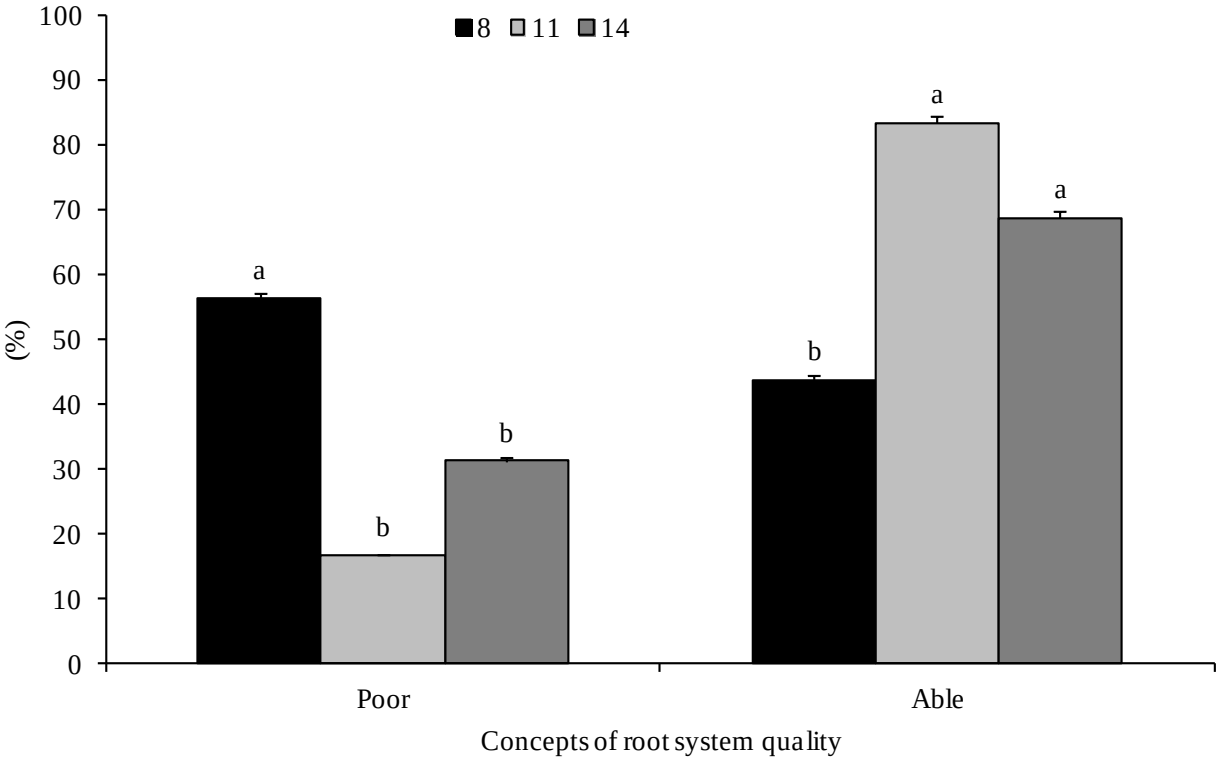

Figure 1. Effect of irrigation depths (mm) on the categories "poor" and "able" of root system quality of *A. polyneuron* seedlings at 120 days after the beginning of treatment. *Bars* represent mean ± standard error. The same letter in the same category is not significantly different according to the Tukey's test ($p < 0.05$).

Table 4. Effects of the interaction between the irrigation depths and the irrigation frequencies on the category "optimum" of root system quality of *A. polyneuron* seedlings 120 days after the beginning of treatments.

Irrigation depths (mm)	Optimum (%)		
	Frequencies		
	2x	4x	CV (%)
8	8.3^{Ba}	25^{Aa}	2.2
11	58.3^{Aa}	29.2^{Ab}	1.1
14	62.5^{Aa}	12.5^{Ab}	1.1
CV (%)	1	1.9	

Means followed by the same capital letter in the column and the same lowercase letter across the row are not significantly different according to the Tukey's test ($p < 0.05$).

Initial development results

The effect of all treatments on height and stem diameter of the seedlings after planting showed linear behavior (Figure 2). The 11 mm irrigation depth applied in two irrigation frequencies, which produced greater seedlings at nursery phase, continued providing greater heights and stem diameters after planting, showing the influence of seedlings quality in the initial development. With the greater development of the seedlings in the subsequent months after planting, there has been a decrease in the need for cleaning of plantations, which implies a considerable reduction of implementation costs (Carneiro, 1995).

The 8 mm irrigation depth applied in two irrigation frequencies, which produced smaller seedlings at nursery phase, continued providing smaller heights and stem diameters after planting. Before any deficit irrigation regime is applied on nurseries, it is essential that irrigation uniformity is optimized, that the water use of

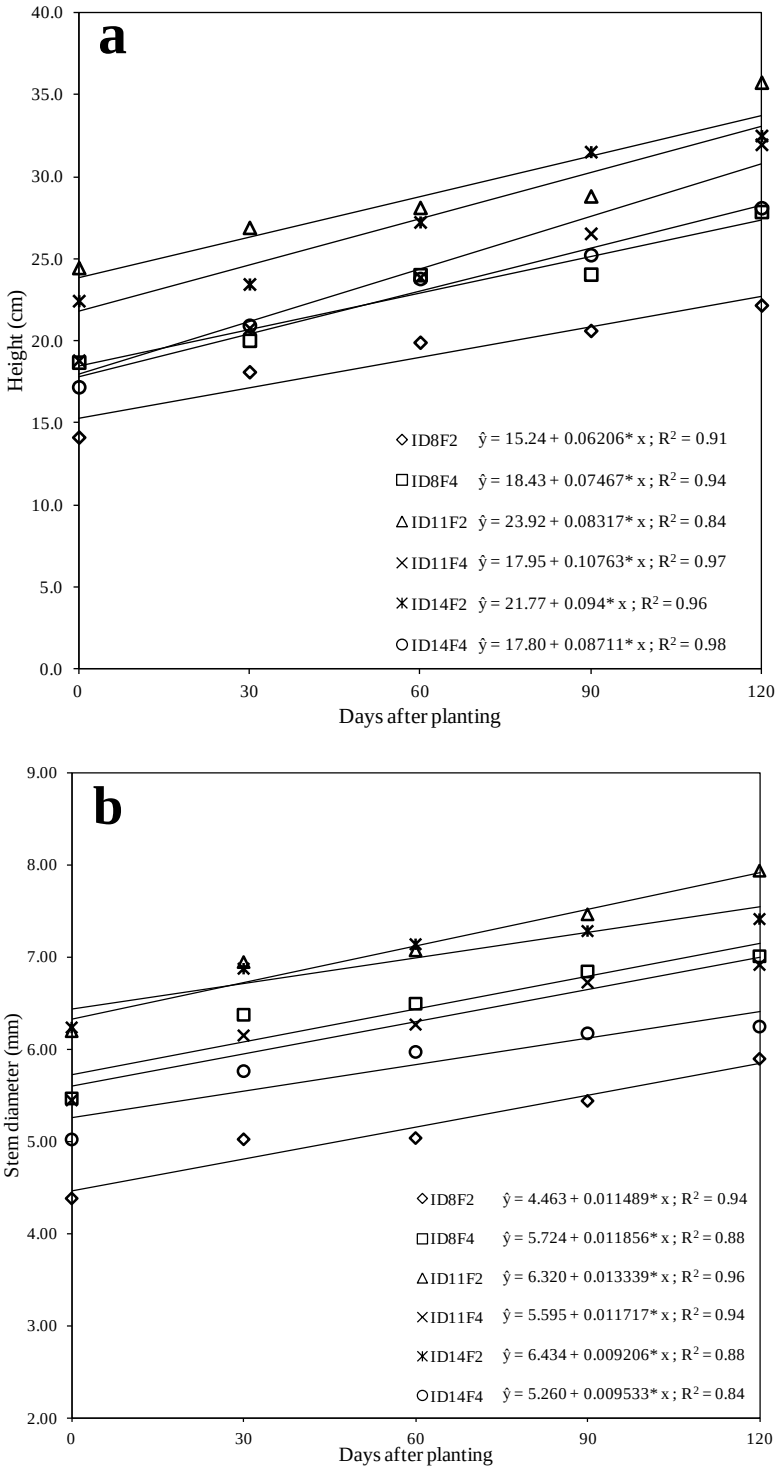

Figure 2. Effect of irrigation depths and irrigation frequencies applied in the *A. polyneuron* seedlings in the nursery phase on the height (**a**) and stem diameter (**b**) 120 days after planting. *Significant according to the F test (p < 0.05). *ID,* irrigation depth; *F,* frequency.

container crops is well understood, and that the effective methods exist for determining irrigation requirements (Grant et al., 2009).

The seedlings produced with 14 mm irrigation depth applied in two irrigation frequencies showed greater stem diameters until day 30th day, however, from that period,

Table 5. Effect of irrigation depths on irrigation efficiency when applied to *A. polyneuron* seedlings 120 days after the beginning of treatments.

Irrigation depths (mm)	Irrigation efficiency (%)	CV (%)
8	62.2[a]	8.2
11	59.2[b]	8.7
14	57.2[b]	9.0

Means followed by the same letter are not significantly different according to the Tukey's test ($p < 0.05$).

Table 6. Effects of the interaction between irrigation depths and the irrigation frequencies applied in the *A. polyneuron* seedlings in the nursery phase on the shoot and root dry mass (g) at 120 days after the planting.

Irrigation depths (mm)	Shoot dry mass			Root dry mass		
	Frequencies		CV (%)	Frequencies		CV (%)
	2x	4x		2x	4x	
8	3.98[Ba]	4.64[Aa]	10.8	1.13[Bb]	1.73[Aa]	16.4
11	7.09[Aa]	3.56[Ab]	29.6	2.72[Aa]	1.79[Ab]	18.2
14	6.84[Aa]	3.97[Aa]	13.4	2.57[Aa]	1.90[Ab]	8.9
CV (%)	21	17.4		14.9	15	

Means followed by the same capital letter in the column and the same lowercase letter across the row in the same parameter are not significantly different according to the Tukey's test ($p < 0.05$).

were overcome by the seedlings produced with 11 mm irrigation depth applied in this same irrigation frequency, showing the possibility of saving water in irrigation of this species in the nursery. Because there is a lack of scientific information regarding irrigation requirements of landscape and nursery tree species, nursery and landscape trees are frequently irrigated in excess (which may result in water logged soil, poor plant growth, increased runoff, leached nutrients, increased water bills, and misuse of irrigation water) amounts (Montague and Kjelgren, 2006).

In root and shoot dry mass parameters, the 11 mm irrigation depth applied in two irrigation frequencies in the nursery resulted in greater growth after planting compared to the same irrigation depth applied in four irrigation frequencies (Table 6).

The success of planting is largely dependent to the ability of plants quickly generate new roots to maximize the absorption of water and compete with the local vegetation (Burdett, 1990; Haase and Rose, 1993; Grossnickle, 2005; Riley and Steinfeld, 2005; Mañas et al., 2009).

After planting, the shoot dry mass of seedlings produced in the 14 mm irrigation depth did not differ with respect irrigation frequency. Furthermore, there was no difference between the irrigation depths applied in four irrigation frequencies in shoot and root dry mass.

The 11 and 14 mm irrigation depths applied in two irrigation frequencies overcome 8 mm irrigation depth and formed the same root and shoot dry mass, showing the possibility of saving water in irrigation of this species in the nursery. Thus, the 11 mm irrigation depth applied in two irrigation frequencies produce *A. polyneuron* seedlings with proper morphological development in the nursery and after planting. This result is in accordance with those obtained by Silva and Silva (2015) that worked with water management in *Piptadenia gonoacantha*, other tropical species of Brazilian Atlantic forest.

Conclusions

The following conclusions were supported by the present study:

1. Irrigation efficiency, in *A. polyneuron* seedlings, is related to the shoot part development degree (height and shoot dry mass).
2. Increases in irrigation efficiency do not necessarily produce a greater development of morphological parameters and root system quality in the nursery.
3. The 11 mm irrigation depth produces the same amount of seedlings with able root systems that the 14 mm irrigation depth, and uses 21% less water.
4. The 11 mm irrigation depth applied in two irrigation frequencies produces *A. polyneuron* seedlings with optimum roots system and proper morphological development, which continues after planting.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Olive (*Olea europaea* L.) seed germination as affected by different scarification treatments

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In Olive (*Olea europaea* L.) a critical balance of concentration and time was necessary to achieve high germination percentages without loss of viability of the seeds. Therefore olive cvs. Coratina and Pendolino seeds were subjected to chemical treatment with HCl, GA_3, NaOH and water for two dips time (12 and 24 h) to determine the most appropriate treatment to improve seed germination and to reduce seed germination time. Among studied treatments (GA_3 500 ppm for 12 h dip, GA_3 500 ppm for 24 h dip, HCl 1N for 12 h dip, HCl 1 N for 24 h dip, NaOH 1 N for 12 h dip, NaOH 1 N for 24 h dip, Water 12 h, Water 24 h, Control) tested, the best results were recorded in both cultivars with GA_3 500 ppm for 12 h dip as compared to control in both the cultivars suggesting that this treatment could be most the effective to enhance of seed germination and minimize the seed germination time.

Key words: Seed germination, scarification, olive.

INTRODUCTION

Olive (*Olea europaea*) is undoubtedly one of the world's oldest cultivated crop originated from Asia Minor, it has tremendous potential in India especially in mid hills and warm temperate regions of North Western Himalaya. It is a crop of Mediterranean region but grows well even under mild temperate conditions if chilling requirements are met. Olive can be propagated by seed, cutting, grafting and suckers, but most common method is the rooting of stem cuttings under mist system. Stem cuttings of selected cultivars are hard to root (Fabbri et al., 2004). The efficiency of propagation is low and the root systems of rooted cuttings are also shallow spreading to 0.9 to 1.2 meter even in deep soils (Bandino et al., 1999). Growing seedlings and grafting the selected cultivars on them may be suitable alternative for olive propagation. Olive seed germination is erratic, very slow and proceeds for 2 to 3 years (Sotomayor-Leon and Caballero, 1990; Zuccherelli and Zuccherelli, 2002) and germination percentage might not exceed 10% in many cultivars (Acebcdo et al., 1997). The major barrier for olive seed germination is the stony endocarp in addition to other causes of dormancy including seed coat, endosperm and embryo itself (Lagarda et al., 1983a; Lagarda and Martin, 1983; Prista et al., 1999 and Lagarda et al., 1983b). Seed germination of 'Manzanillo olive was improved by using stoneless seeds (Crisosto and Sutter, 1985). However, low germination percentage was obtained with other cultivars using stonless seeds (Acebedo et al., 1997). It was reported that 28% of olive seed dormancy is imposed by the endocarp and 56% by the endosperm (Sotomayor-

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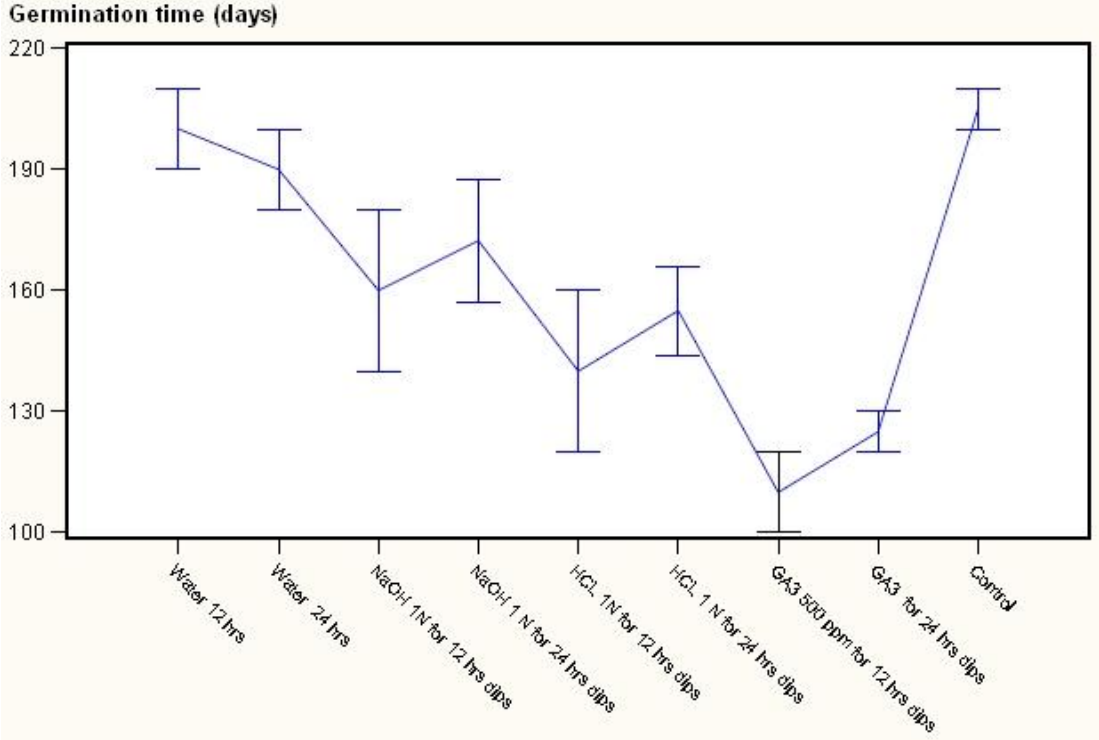

Figure 1. Effect of chemical scarification on germination time in olive cv. Coratina**.**

Leon and Caballero, 1994). For commercial olive seed germination, breaking olive endocarps described by Sotomayor-Leon and Caballero (1990) does not always work well. Chemical scarification has been widely used to overcome physical seed dormancy (Hartmann and Kester, 2002). Germination percentage of three olive cultivars was improved after the stony seeds were scarified with 0.1 N NaOH and H_2SO_4 at 0.1 N or 1 N concentrations (Bandino et al., 1999). Chemical agents such as norflurazon and continuous washing in running water have also been used to overcome olive seed dormancy (Sotomayor- Leon and Altisent, 1994). The aim of this work was to determine the effect of different treatments on the germination of Coratina and Pendolino olive cultivars seeds.

MATERIALS AND METHODS

Fruits from ' olive cv. Coratina and Pendolino from olive research block at the Central Institute of temperate Horticulture, Srinagar were harvested in October, 2010 and 2011 when the color was changed from yellow green to violet. Fruit flesh was separated by soaking them for 15 min in 4% NaOH. The stony seeds were cleaned with sand and water, and were then soaked in running water for 12 h to remove residues. Seed were immediately sown with the following treatments like GA_3 500 ppm for 12 h dip, GA_3 500 ppm for 24 h dip, HCl 1 N for 12 h dip, HCl 1 N for 24 h dip, NaOH 1N for 12 h dip, NaOH 1 N for 24 h dip, Water 12 h, Water 24 h, Control (without any soaking treatment) . Fifty seeds were used in each treatment with five replicates for each cultivar as per methods described by Gomez and Gomez (1984). The stony seeds in the 9 treatments were left to germinate in plates filled with sand containing 90% moisture and placed in a mist chamber at 20 to 25°C. Germination time is calculated from sowing to emergence of the hypocotyle and germination percentage was determined by the emergence of the number of hypocotyls. Statistical analysis was done using SAS software package (SAS Inst. 2012), for mean differences the analysis of variance was followed by DMRT test at 1% probability level.

RESULTS AND DISCUSSION

Both average times taken for germination and average germination percentage were influenced by cultivars and treatments; however, treatments did not always produce the same effects in the two cultivars. In Coratina cultivar stony seeds soaked in GA_3 500 ppm for 12 h dips was found most effective (Figure 1) and statistically significant in terms of early germination (Figure 2) of stony seeds with a maximum germination percentage. This increase in seeds germination percentage might be related to the initial enzyme induction and to the activation of reserve food – mobilizing systems by Gibberellins which have also been used to enhance germination and stimulate early seedling emergence and growth (Hopkins and Hüner, 2004). Hopkins and Hüner (2004) stated that gibberellins prominently involved in seed germination and mobilization of endosperm reserves during early embryo growth as well as flower and fruit development. However

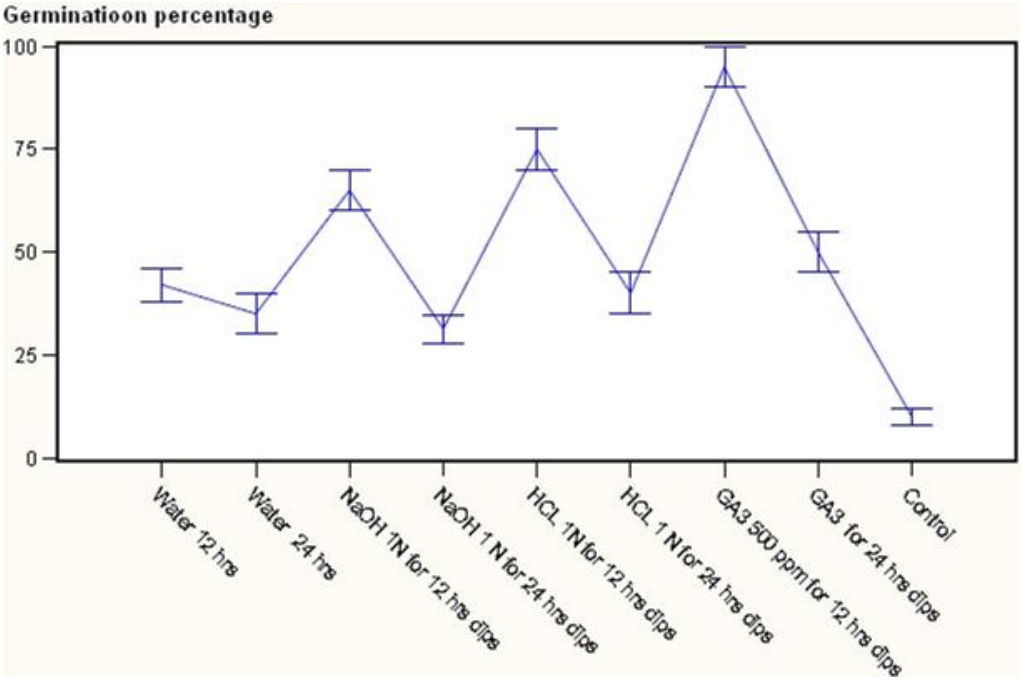

Figure 2. Effect of chemical scarification on germination % in olive cv. Coratina.

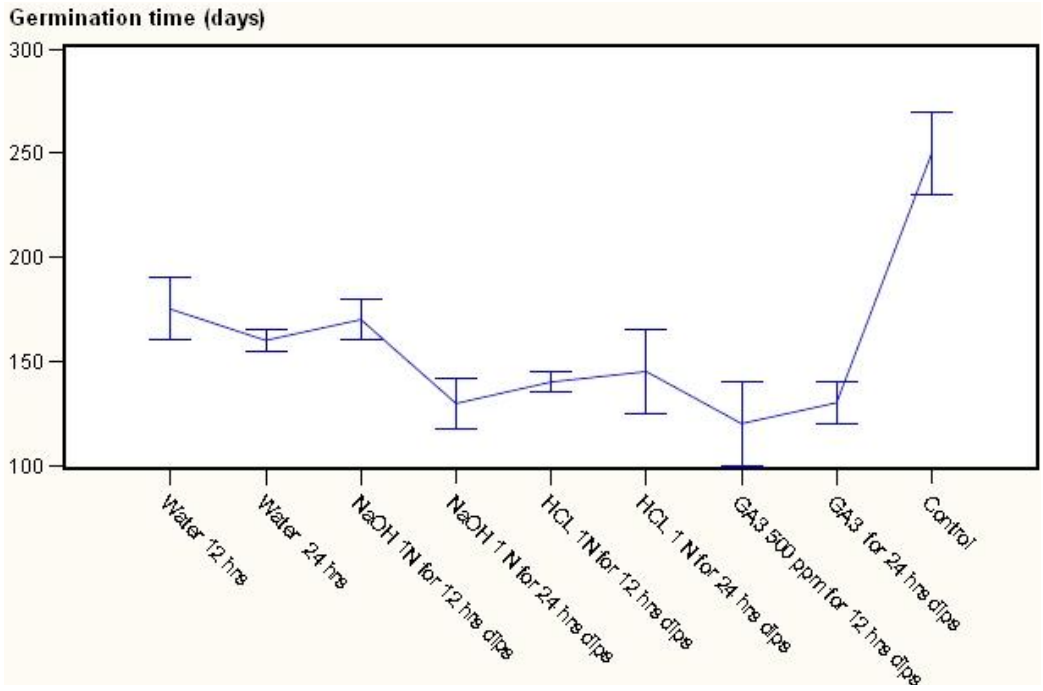

Figure 3. Effect of chemical scarification on germination time in olive cv. Pendolino.

as the seeds soaking time increased the germination percentage was decreased. Treatments NaOH 1 N for 24 h dips, GA_3 for 24 h dips also found at par with the best treatments for seed germination and found similar at significant level. In Pendolino cultivar also minimum (110.00) time taken for germination (Figure 3) of stony seeds was recorded with the treatment of GA_3 12 hrs as compared to control (205 days) showing in the same time the highest germination (Figure 4) percentage (95%). Second best treatment was found HCl 1N for 12 h dips

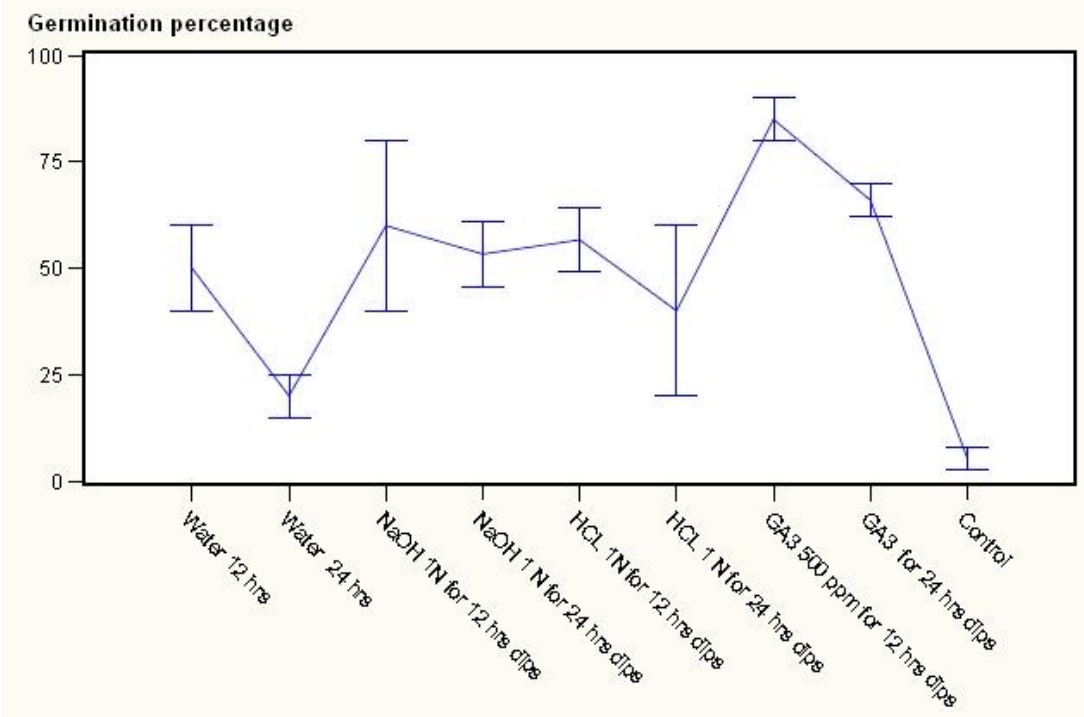

Figure 4. Effect of chemical scarification on germination % in olive cv. Pendolino.

Table 1. Interaction effect of treatments and cultivars on germination time and percentage germination.

Treatments	Germination days			Germination percentage		
	Coratina	Pendolino	Means	Coratina	Pendolino	Means
GA_3 500 ppm for 12 h dips	120.00	110.00	115.00	85.00	95.00	90.00
GA_3 500 ppm for 24 h dips	130.00	125.00	127.50	66.00	50.00	58.00
HCL 1 N for 12 h dips	140.00	140.00	140.00	56.66	75.00	65.83
HCL 1 N for 24 h dips	145.00	155.00	150.00	40.00	40.00	40.00
NaOH 1 N for 12 h dips	170.00	160.00	165.00	60.00	65.00	62.50
NaOH 1 N for 24 h dips	129.66	172.33	150.99	53.33	31.33	42.33
Water 12 hrs dips	175.00	200.00	187.50	50.00	42.00	46.00
Water 24 hrs dips	160.00	165.33	162.66	20.00	35.00	27.50
Control	250.00	205.00	227.50	5.33	10.00	7.66
Means	157.74	159.18		48.48	49.25	
CD at 5% Cultivars		NS			NS	
Treatments		20.86			9.84	
Treatments x Cultivars		29.50			13.92	

however time taken for germination was little higher than GA_3 for 24 h dipped. Pendolino seeds germinated faster than Coratina cultivars, with an average germination time of 110 days as compared to 250 days for Coratina. The acid and base treatment was found not as effective as GA_3 in increasing germination percentage and reducing the germination time that might be due to the their corrosive effect which may caused damages to the embryo or the treatment was not as effective as GA_3. In treatment cultivar interaction (Table 1) the cultivars did not differ significantly however among the treatments, minimum germination time (115 days) and maximum seed germination percentage (90%) were recorded with GA_3 500 ppm for 12 h dips and significantly differ as compared to control that showed a germination time of 227.5 days and a germination rate of 7.65%.

Thus, the increased percentages of germination obtained when olive seeds are treated with GA_3 soaking overnight would improve the feasibility of developing breeding programs for olives and would enable nurserymen to grow olives from seed without serious loss.

Conflict of Interest

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

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