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Full Length Research Paper

Productivity, nutritional quality and phenotypical stability of varieties of silage sorghum in Uberlândia, MG

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Sorghum (S*orghum bicolor* (L.) Moench) is a good alternative for silage, especially in places with water scarcity and high temperatures, due to their morphological and physiological characteristics. Proper management contributes both to productivity and to the quality of forage. The present study was conducted with the following objectives: To evaluate the agronomic and bromatological performance of varieties of sorghum silage as well as their phenotypic stability in the early and late (off) seasons of planting in the region of Uberlândia, Minas Gerais. The experiment was performed at the *Capim Branco* Experimental Farm of the Federal University of Uberlândia - UFU located in Uberlândia - MG. In this experimental area forage was planted at the normal, seasonal time and in the off season. A randomized block design was used with 25 treatments and three replications. Flowering, dry matter yield, plant height, Acid Detergent Fiber and Neutral Detergent Fiber of the cultivars were all found to be affected by the time of planting. The SF11 variety was found to be superior in terms of productivity and fiber quality regardless of the season. The evaluation of dry matter stability demonstrated superiority among the varieties: SF15, SF11, SF25, PROG 134 IPA, 1141572, 1141570 and 1141562. As for the stability of fiber quality, the 1141562 variety stood out.

Key words: Photoperiod, digestibility, genotype, *Sorghum bicolor*.

INTRODUCTION

Sorghum (*Sorghum bicolor*, L. Moench) is a crop that is increasing every day in Brazilian agriculture. As a very high energy grass, it is useful because of its high levels of productivity, digestibility, and adaptation to warm and dry environments, in comparison with other species. Sorghum adapts easily to different conditions

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of soil fertility, is tolerant of high temperatures and survives water stress (Miranda et al., 2010).

Intensification of production processes in beef and dairy cattle in Brazil have increased the need for feed, including forage that is quantitatively and qualitatively better for the animals, especially during periods of dry pasture. In this respect, the production of high quality silage is a viable alternative (Machado et al., 2011).

The production of sorghum for forage has been playing an increasingly important role in recent years in Brazil and the world, standing out as a species resistant to adverse environmental conditions (Rezende et al., 2011). Plant breeding programs have developed various cultivars adapted to various types of soil and climate. These include varieties developed by the Brazilian Agricultural Research Agency (Embrapa, 2009) and the Agricultural Research Agency of Pernambuco (IPA) (Silva et al., 2012).

The cultivation of silage sorghum outside the traditional period may allow its expansion in Brazil. However, most commercial sorghum materials were improved in Brazil for photoperiod insensitivity, only genotypes of silage sorghum are sensitive to photoperiod (Silva et al., 2005-1).

Although there has been genetic improvement in sorghum, there is still a limited availability of cultivars with desirable characteristics such as high production of fodder and high nutritional value. There is, thus, need to develop suitable cultivars that will present positive interaction with local environmental conditions. For these reasons, the aim of this study was to evaluate the agronomic and qualitative characteristics of sorghum varieties for silage as well as their adaptability and stability in the region of Uberlândia, Minas Gerais.

MATERIALS AND METHODS

The experiments were performed at the *Capim Branco* Experimental Farm of the Federal University of Uberlândia – UFU, located in Uberlândia, Minas Gerais, Brazil. The experimental area is located on the perimeter of the city of Uberlândia. The area has an altitude of 843 m, latitude 18º 54' 41" South and a tropical savanna climate (Koppen climate Rating: Aw). The soil of the area is characterized as clayey dystrophic dark Red Latosol.

The experiments used a randomized complete block design with three replications. The experimental plots were composed of two lines of 5 m, with spacing of 0.7 m between lines and a total area of each plot of 7 m². In the experiments at both planting times a total of 25 varieties of silage sorghum were evaluated, 18 from the Embrapa, Maize and Sorghum Breeding Program and 7 commercial cultivars (controls).

The soil was prepared in the conventional manner, plowed twice and disked. Fertilizer was applied at 322 kg ha^{-1} of mineral fertilizer 8-28-16. The sorghum seed was then planted, at a depth of 3 to 4 cm. Plants were thinned 10 to 15 days after emergence, for the equivalent of a population of 100,000 plants ha^{-1} and were top dressed with 250 kg ha^{-1} of urea and 250 kg ha^{-1} of Potassium chloride (KCl). Weed control was done with an herbicide (Atrazine) and manual weeding. Irrigation was used only to obtain the initial stand and then stopped, to simulate the climatic conditions of the off season. Irrigation was not necessary for harvest.

The management of caterpillars was conducted with organophosphate insecticides or pyrethroids, applied following the dosage recommended by the manufacturer. Birds were controlled by covering the panicles with nylon screens. No chemicals were necessary for disease control since the experiment used resistant cultivars.

The characteristics evaluated in the field were: flowering (number of days, measured by the time by which more than 50% of the plot had panicles with more than 50% pollen release); plant height (m, measured by average plant height from the insertion of the panicle to the ground, of flowering plants) and dry matter (t ha^{-1}). Harvest was done manually by cutting the stems at a height of 10 cm from the ground when the grains of the panicles were in the milky or “dough” stage. This time of harvest was determined because it is a time when dry matter can adequately be measured, for good quality silage. Ten plants were taken at random from each plot, crushed in a chopper and manually homogenized. A subsample of this material was used for evaluation of the dry matter. Weighing was performed immediately and the samples were placed in a forced ventilation oven at 65°C for 72 h. They were then ground in a Willey mill (1 mm sieve), for chemical analysis.

Fiber content was determined for neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to techniques described by Silva and Queiroz (2002). The chemical analysis was conducted at the Animal Nutrition Laboratory (LAMRA) of the Faculty of Veterinary Medicine - FAMEV of the Federal University of Uberlândia.

Evaluation of the data was initially carried out using ANOVA and the F test, considering only the times of planting where there were observations according to the following mathematical model for analysis of variance:

$$Yij = \mu + gi + bj + eij$$

where: Yij = observations of the plot in the block; μ = general average; gi = the effect of the genotype; bj = the effect of the block; and eij = the effect of unmeasured factors of the genotype in the block.

For the assessment of genotype environment interaction, analysis of variance was performed by the following mathematical model:

$$Yijk = \mu + gi + j + gaij + bk / aj + eijk$$

where: Yijk = observations of the plot in the block; μ = the general average; gi = the effect of the genotype; j = the environmental (time of planting) effect; gaij interaction = the effect of the genotype with the environment; bk / j = the effect of the block in the environment; eijk = the effect of unmeasured factors in the proportion received by the genotypes in the environment within block.

Analyses of variance and F tests were carried out with the help of Microsoft Excel software, according Banzato and Kronka (1992). Means for grouping the varieties used the Scott and Knott test with the Genes program (Cruz, 2013). Once the presence of genotype environment interaction G x E (significant F test) was detected, we proceeded to the analysis of phenotypic stability proposed by Annichiarico (1992). To apply this methodology, we first calculated the averages of the two environments and then obtained the percentages of cultivars in relation to the environmental averages. We then calculated the averages for each variety, in percentages, and the standard deviations of these averages. In turn, the stability parameter (ii), or confidence index was estimated by the following equation:

Table 1. Summary of the analysis of variance for flowering data (flower), plant height (height), dry matter (DM), acid detergent fiber (ADF) and neutral detergent fiber (NDF) of 25 sorghum varieties grown in season and off season environments.

Variation sources	Gl (df)	Squared averages									
		Flower		Height		Dry Matter		ADF		NDF	
		Season	Off season	Season	Off season	Season	Off season	Season	Off season	Season	Off season
Cultivar	24	253.05*	183.03*	1.64*	1.20*	49.80*	35.12*	191.85*	64.58	70.79*	170.12*
Bloc	2	1.44	0.57	0.09	0.15	0.18	3.91	14.88	22.40	28.70	0.45
Resídual	48	3.35	2.28	0.02	0.03	6.54	4.66	28.55	46.31	20.43	43.26
CV (%)		2.67	3.00	5.79	8.98	20.03	26.31	12.28	19.06	6.32	9.49

Gl: Degrees of freedom; *: Significant at 5% of error probability by F test.

Ii=Yi-Z (1-a) si; where: Yi is the average percentage of the i^{th} genotype with respect to each environmental time in question; Z (1-a) is the standardized value of the normal distribution in which a cumulative distribution function reaches the value (1-a). The level of significance adopted in this case was 0.5, and si is the standard deviation of the percentages of each genotype.

RESULTS AND DISCUSSION

Significant differences were found among all of the sorghum varieties evaluated: flowering (flower), plant height (height), dry matter (DM), acid detergent fiber (ADF) and neutral detergent fiber (NDF) in both season and off season environments, with the exception of the ADF in the off season (Table 1). Since the ratios between the highest and the average squared residuals were less than seven, it was possible to perform an analysis of variance (Banzato and Kronca, 1992). In this analysis, we observed the interaction between genotypes and environments ($p<0.01$) for all evaluated characteristics, as well as significant differences among genotypes for height and dry matter and between environments for flower, height and dry matter ($p<0.01$) (Table 2).

The coefficients of variation (CV%) ranged between 2.8 and 22.56% indicating good to moderate experimental precision. According to Pimentel Gomes (2000), in field experiments, coefficients of variation of less than 10% are considered low, that is, the experiment has high accuracy; 10% to 20% CVs are considered medium, resulting in good precision; 20 to 30% are considered high, meaning low accuracy and above 30% is regarded very high, indicating very low precision.

Coefficients of variation ranging between 14.1 and 33.4 were found by Chielle et al. (2013) in evaluating 23 cultivars of sorghum silage. Neumann et al. (2010) found CV% of 10.34% for ADF and 3.49 for NDF. Albuquerque et al. (2012) found a coefficient of variation of 6.93% for plant height, similar to the present study.

Regarding the number of days of flowering, it was found that all varieties had a longer growing period during the season, in relation to the off season, except for the 12F042224 and 12F042226 varieties which did not differ statistically between season and off season values (Table 3). In the season, the number of days to flowering ranged from 49 to 82 days. The later varieties were 1141572, 1141570 and 12F042066 and the earlier ones were 12F042224 and 12F042226.

Chielle et al. (2013) evaluated 23 silage sorghum cultivars in Rio Grande do Sul in 2011-2012 and found flowering values ranging from 65 days for the BR304 cultivar up to 84 days for FEPAGRO 18, with an average of 77 days. In the off season the number of days to flowering fluctuated between 42 and 67 and the early flowering varieties were 9929036, 9929030, FEPAGRO 18, FEPAGRO 11, 9929012, 9929026, 947216 and 947030 and the later PROG 134 IPA, SF15 and SF11.

In relation to plant height, it was noted that all varieties had higher averages when planted in November, with the exception of 12F042226, 12F042224 and PROG 134 IPA, varieties that did not differ significantly between the two periods (Table 4). The varieties of sorghum grown in the off season were earlier and shorter compared to those planted in the normal season. This can be explained by the influence of the photoperiod on the induction of flowering and hence the stoppage of plant growth. The plant height results at that time ranged from 1.26 to 3.46 m. The tallest varieties were:

Table 2. Summary of analysis of variance for flowering data (flower), plant height (height), dry matter (DM), acid detergent fiber (ADF) and neutral detergent fiber (NDF) for 25 sorghum varieties at two different times of planting.

Variation sources	Gl (Df)	Squared averages				
		Flower	Height	Dry matter	ADF	NDF
Cultivar	24	281.37	2.31*	64.66*	116.51	154.29
Time	1	12622.51*	27.34*	782.31*	2295.32	176.45
Cul x Amb	24	154.71*	0.53*	20.25*	139.94*	86.64*
Bloc	2	1.72	0.23	2.22	17.59	12.60
Resídual	96	2.81	0.03	5.60	37.43	31.84
CV (%)		2.82	7.11	22.56	15.44	8.01

Gl: Degrees of freedom; *: Significant at 5% of error probability by F test.

Table 3. Average number of days to flowering for 25 varieties of silage sorghum grown in season and off season.

Varieties	Flowering (days)	
	Season	Off season
9929036	68.66^{Ad}	42.33^{Bf}
9929030	66.00^{Ae}	42.66^{Bf}
12F042224	49.00^{Bh}	52.66^{Ac}
12F042150	73.00^{Ac}	54.66^{Bc}
FEPAGRO 18	65.66^{Ae}	42.66^{Bf}
FEPAGRO 19	71.66^{Ac}	45.33^{Be}
FEPAGRO 11	68.00^{Ad}	43.00^{Bf}
9929012	67.66^{Ad}	42.33^{Bf}
9929026	64.00^{Ae}	43.00^{Bf}
947216	70.66^{Ad}	44.66^{Bf}
947030	67.66^{Ad}	44.00^{Bf}
947254	73.33^{Ac}	47.66^{Be}
947072	54.33^{Ag}	46.00^{Be}
947252	63.33^{Ae}	49.33^{Bd}
SF15	74.33^{Ac}	66.66^{Ba}
SF 11	78.66^{Ab}	67.33^{Ba}
SF 25	74.00^{Ac}	62.00^{Bb}
PROG 134 IPA	74.66^{Ac}	65.00^{Ba}
1141572	82.00^{Aa}	52.33^{Bc}
12F042066	81.00^{Aa}	47.66^{Be}
12F042226	49.00^{Ah}	47.33^{Ae}
1141570	81.00^{Aa}	52.33^{Bc}
1141562	78.33^{Ab}	48.33^{Be}
BRS 506	60.66^{Af}	54.66^{Bc}
BRS Ponta Negra	59.33^{Af}	53.33^{Bc}

Means with the same lower case letter vertically within each time belong to the same group, according to the Scott-Knott test. Horizontally, means with the same capital letter do not differ by F test at 5% probability.

SF15, SF 11 and SF 25 and the shorter: 9929030 and 9929026. In the season there was greater plant height, with oscillations from 1.71 to 3.96 m. The varieties that were notably shorter were: 12F042226, 9929026 and 12F042224 and the notably taller ones: FEPAGRO 19, SF15, SF 11, SF 25, 1141572,

Table 4. Average plant height (m) of 25 varieties of silage sorghum grown in season and off season.

Varieties	Plant height (m)	
	Season	Off season
9929036	2.93^{Ac}	1.63^{B}
9929030	2.13^{A}	1.30^{B}
12F042224	1.81^{A}	1.86^{Ad}
12F042150	2.93^{Ac}	2.13^{Bc}
FEPAGRO 18	3.36^{Ab}	2.00^{Bd}
FEPAGRO 19	3.78^{Aa}	2.10^{Bc}
FEPAGRO 11	3.30^{Ab}	2.00^{Bd}
9929012	2.33^{A}	1.70^{B}
9929026	1.95^{A}	1.26^{B}
947216	2.50^{Ad}	1.63^{B}
947030	2.46^{Ad}	1.53^{B}
947254	2.60^{Ad}	1.86^{Bd}
947072	2.33^{A}	1.66^{B}
947252	2.26^{A}	1.50^{B}
SF15	3.73^{Aa}	3.43^{Ba}
SF 11	3.81^{Aa}	3.36^{Ba}
SF 25	3.96^{Aa}	3.46^{Ba}
PROG 134 IPA	2.80^{Ac}	3.06^{Ab}
1141572	3.68^{Aa}	2.16^{Bc}
12F042066	3.78^{Aa}	1.70^{B}
12F042226	1.71^{A}	1.83^{Ad}
1141570	3.83^{Aa}	2.20^{Bc}
1141562	3.90^{Aa}	2.23^{Bc}
BRS 506	2.83^{Ac}	2.30^{Bc}
BRS Ponta Negra	2.16^{A}	1.63^{B}

Means with the same lower case letter vertically within each time belong to the same group, according to the Scott-Knott test. Horizontally, means with the same capital letter do not differ by F test at 5% probability.

12F042066, 1141570 and 1141562. These results registered taller results than those found by Chielle et al. (2013) who obtained plant heights ranging between 1.13 and 2.54 m in the evaluation of 23 silage sorghum cultivars in Rio Grande do Sul in 2011-2012; and found by Silva et al. (2007) evaluating sorghum cultivars in Goiás with average plant heights from 1.21 to 1.55 m.

In terms of the dry matter (t ha^{-1}) it was observed that ten varieties did not differ significantly between the planting dates and the others had higher yields in the seasonal planting compared to the off season (Table 5). For seasonal plantings, yields fluctuated between 7.66 and 21.69 t ha^{-1} with the most productive varieties: SF15, SF11, 1141572, 12F042066, 1141570 and 1141562. In the off season, the results ranged from 3.91 to 15.81 t ha^{-1}. The varieties that had higher yields, SF15 and SF11, were not influenced by the environment. Silva et al. (2007) evaluated the BR 700, 1F305, Volumax, VDH 422 cultivars and Nutrigrain forage sorghum at three locations, finding an average of 5.9 t ha^{-1} of dry matter, a result well below that found in the present study.

For Albuquerque et al. (2012), the production of sorghum dry matter is directly related to plant height. Taller cultivars can achieve higher productivities. However, dry matter productivity is also associated with the management adopted and the capacity inherent to the species or variety.

Regarding the bromatological analysis, when we compared the ADF values (%) between the two planting periods there were significant differences for eleven varieties that had higher values for the seasonal than in the off season planting (Table 6). These results were explained by the lower amounts of grain at harvest, due to the attacks of birds in the area. For the seasonal planting, the ADF mean values ranged between 29.97 and 61.68%. Macedo et al. (2012) found average levels of ADF in sorghum silages ranging from 48.69 to 55.19% due to nitrogen rates.

Table 5. Average dry matter yield (t ha^{-1}) for 25 varieties of silage sorghum grown in season and off season.

Varieties	Dry matter (t ha^{-1})	
	Season	Off season
9929036	13.99^{Ab}	4.35^{Bc}
9929030	11.16^{Ab}	4.69^{Bc}
12F042224	7.66^{Ac}	8.61^{Ac}
12F042150	11.70^{Ab}	10.37^{Ab}
FEPAGRO 18	13.04^{Ab}	4.81^{Bc}
FEPAGRO 19	9.17^{Ac}	3.91^{Bc}
FEPAGRO 11	13.56^{Ab}	5.99^{Bc}
9929012	8.15^{Ac}	6.88^{Ac}
9929026	7.87^{Ac}	3.95^{Bc}
947216	13.77^{Ab}	7.19^{Bc}
947030	9.44^{Ac}	5.15^{Bc}
947254	10.80^{Ac}	6.67^{Bc}
947072	10.66^{Ac}	9.08^{Ac}
947252	9.46^{Ac}	4.98^{Bc}
SF15	17.24^{Aa}	15.03^{Aa}
SF 11	17.94^{Aa}	15.81^{Aa}
SF 25	14.46^{Ab}	10.97^{Ab}
PROG 134 IPA	11.62^{Ab}	13.07^{Ab}
1141572	21.69^{Aa}	11.61^{Bb}
12F042066	17.37^{Aa}	6.45^{Bc}
12F042226	8.00^{Ac}	7.20^{Aca}
1141570	19.72^{Aa}	12.21^{Bb}
1141562	19.26^{Aa}	8.46^{Bc}
BRS 506	12.11^{Ab}	7.83^{Bc}
BRS Ponta Negra	9.40^{Ac}	9.83^{Ab}

Means with the same lower case letter vertically within each time belong to the same group, according to the Scott-Knott test. Horizontally, means with the same capital letter do not differ by F test at 5% probability.

These values were above what is recommended.

In the off season crop, FDA values ranged from 27.27 to 44.40%. These results were close to those obtained by Cândido et al. (2002). ADF levels reported for green sorghum forage in several studies range from 28.7 to 45.6% (Gontijo Neto et al., 2004).

Higher values of NDF were found for the 12F042150, SF15, SF 25, PROG 134 IPA and 1141570 varieties. These presented higher values in the seasonal crop than in the off season. With the exception of the 9929030 variety, which showed a higher value in the off season, the others were not affected by the time of planting (Table 7). The average values of NDF at harvest ranged between 58.25 and 80.17%. In the off season, values were between 54.86 and 81.36%.

According to Gontijio Neto et al. (2004) various studies with green sorghum have reported NDA values ranging from 51.6 to 67.4%. Neumann et al. (2010) found NDF values ranging from 66.58 to 70.01% and Macedo et al. (2012) reported that with increasing doses of nitrogen, average values of NDF ranged from 62.12 to 68.17%. All of these results corroborate the present findings.

To estimate the stability of the genotypes, the Annichiarico methodology (1992) was applied. Varieties were evaluated in relation to: flowering characteristics, plant height and dry matter. Those that had superior adaptability and stability were: SF15, SF 11, SF 25, PROG 134 IPA, 1141572, 1141570, 1141562 and 12F042150 (Table 8).

The bromatological characteristics, ADF and NDF, indicated the varieties with greater adaptability and stability to be: 9929012, 947254, 947072, 947252 and 1141562, but only the last variety showed lower values, that is, optimal fiber, which directly influences the quality of silage (Table 8).

Souza et al. (2013), the cultivar BRS506 also showed general and specific adaptability and stability

Table 6. Average content of acid detergent fiber (ADF%) of 25 varieties of silage sorghum grown in season and off season.

Varieties	FDA (%)	
	Season	Off season
9929036	47.98Ab	29.85Bb
9929030	34.40Ac	37.15Aa
12F042224	33.85Ac	32.93Ab
12F042150	52.15Aa	33.60Bb
FEPAGRO 18	36.13Ac	33.68Ab
FEPAGRO 19	47.89Ab	40.71Aa
FEPAGRO 11	36.53Ac	32.98Ab
9929012	47.08Ab	43.97Aa
9929026	36.97Ac	39.03Aa
947216	37.58Ac	39.74Aa
947030	37.26Ac	33.40Ab
947254	54.49Aa	40.50Ba
947072	46.22Ab	38.52Aa
947252	41.42Ac	44.40Aa
SF15	61.68Aa	33.95Bb
SF 11	46.08Ab	35.72Bb
SF 25	53.45Aa	34.12Bb
PROG 134 IPA	45.00Ab	27.27Bb
1141572	45.43Ab	28.52Bb
12F042066	47.11Ab	34.56Bb
12F042226	29.97Ac	40.82Aa
1141570	46.59Ab	27.78Bb
1141562	51.70Aa	36.74Ba
BRS 506	31.21Ac	34.80Ab
BRS Ponta Negra	39.72Ac	37.61Aa

Means with the same lower case letter vertically within each time belong to the same group, according to the Scott-Knott test. Horizontally, means with the same capital letter do not differ by F test at 5% probability.

Table 7. Average contents of Neutral Detergent Fiber (NDF%) of 25 varieties of silage sorghum grown in season and off season.

Varieties	NDF (%)	
	Season	Off season
9929036	70.47Ab	68.07Ab
9929030	71.37Ba	81.36Aa
12F042224	68.86Ab	70.64Aa
12F042150	77.86Aa	58.99Bb
FEPAGRO 18	62.66Ab	66.50Ab
FEPAGRO 19	67.32Ab	71.31Aa
FEPAGRO 11	67.66Ab	66.61Ab
9929012	75.86Aa	75.45Aa
9929026	73.84Aa	78.77Aa
947216	72.46Aa	79.34Aa
947030	76.43Aa	74.77Aa
947254	72.70Aa	76.47Aa
947072	73.46Aa	75.69Aa
947252	80.17Aa	76.66Aa

Table 7. Cont'd.

SF15	76.34^{Aa}	63.26^{Bb}
SF 11	70.05^{Ab}	63.59^{Ab}
SF 25	76.66^{Aa}	60.06^{Bb}
PROG 134 IPA	67.17^{Ab}	54.86^{Bb}
1141572	68.42^{Ab}	70.70^{Aa}
12F042066	71.67^{Aa}	64.95^{Ab}
12F042226	70.14^{Ab}	66.63^{Ab}
1141570	69.06^{Ab}	57.09^{Bb}
1141562	74.59^{Aa}	79.10^{Aa}
BRS 506	58.25^{Ab}	62.51^{Ab}
BRS Ponta Negra	73.48^{Aa}	69.37^{Aa}

Means with the same lower case letter vertically within each time belong to the same group, according to the Scott-Knott test. Horizontally, means with the same capital letter do not differ by F test at 5% probability.

Table 8. Estimates of phenotypic stability parameters using the Annicchiarico method (1992), with a Confidence index (Wi), of 25 varieties of silage sorghum grown in season and off season.

Varieties	Confidence index				
	Flower	Height	DM	ADF	NDF
9929036	89.04	85.69	70.36	91.79	98.33
9929030	88.31	66.09	66.49	86.74	105.23
12F042224	81.61	70.90	73.79	82.24	98.05
12F042150	107.07	101.39	102.26	102.02	92.42
FEPAGRO 18	88.16	102.57	72.00	86.50	90.20
FEPAGRO 19	94.51	110.30	55.07	111.27	96.85
FEPAGRO 11	89.66	101.86	83.17	86.54	95.10
9929012	88.59	80.70	69.98	112.79	106.97
9929026	87.87	63.04	52.30	92.45	106.48
947216	93.15	81.14	93.84	94.02	105.39
947030	90.89	77.43	66.20	88.05	107.22
947254	98.48	89.51	82.33	117.07	104.35
947072	82.93	80.20	91.85	106.73	104.76
947252	94.05	74.21	64.82	104.14	111.08
SF15	115.73	139.72	149.76	109.42	96.03
SF 11	120.52	140.71	156.46	101.85	93.67
SF 25	112.55	145.76	119.50	103.95	92.97
PROG 134 IPA	115.06	112.09	111.93	84.69	83.70
1141572	108.78	111.49	150.20	87.41	97.65
12F042066	101.90	96.87	96.27	100.33	95.72
12F042226	78.36	68.03	70.36	82.82	96.74
1141570	108.33	114.19	150.56	86.79	86.74
1141562	101.63	116.01	117.77	107.80	107.35
BRS 506	94.61	101.49	95.02	79.63	84.16
BRS Ponta Negra	92.45	75.75	87.78	95.60	100.92

for favorable and unfavorable environments for the highest yield and fresh biomass. Silva et al. (2005), using another method of stability and adaptability to evaluate fresh and dry biomass yield in forage sorghum cultivars, identified BRS506, among the materials evaluated, as the most suitable to favorable and unfavorable environments, in addition to presenting the highest yield for fresh biomass (49.3 t ha^{-1}).

Conclusion

The flowering, dry matter yield, plant height, ADF and NDF are all affected by the time of planting and the variety. Regarding productivity and fiber quality, the SF11 variety was superior at both times of planting. For stability of the dry matter yield: SF15, SF11, SF25, PROG 134 IPA, 1141572, 1141570 and 1141562 stood out. As for the stability of the quality of fiber (ADF and NDF), the 1141562 variety was found to be superior.

Conflict of Interests

The authors have not declared any conflict of interests.

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Performance of an improved manual cassava harvesting tool as influenced by planting position and cassava variety

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Cassava has become an important food security crop in Ghana over the years and in most parts of sub-Saharan Africa; making it the single most important source of dietary energy. Harvesting, one of the serious bottlenecks in the cassava production value chain, has received little attention in terms of mechanisation. Earlier attempts at mechanising cassava harvesting have been challenged mainly by inappropriate method of planting, field topography and scale of cultivation. The objective of this study was to field evaluate the efficiency of an improved manual cassava harvesting tool under three different planting positions for four cassava varieties in terms of field capacity, level of drudgery and root tuber damage. Force requirement in uprooting different cassava varieties was also determined. The study was conducted at the research field of Crops Research Institute, Fumesua. Field capacity of improved manual harvesting tool ranged from 49.9 to 156 man-h/ha, root tuber breakage from 4.32 to 19.55% and harvesting energy consumption ranging from 470.34 to 773.72 W across cassava varieties and planting positions. Nkabom cassava variety was easiest in uprooting, irrespective of planting position while Sikabankye variety offered the best in terms of root tuber damage and drudgery. Again, it was faster harvesting vertically planted cassava though cassava planted slanted offered the least root tuber breakage and drudgery, regardless of cassava variety. Cassava uprooting force ranged from 86.8 to 143.3 kg, rooting depth from 22.39 to 26.86 cm and cassava yield per plant ranging from 5.84 to 13.14 kg. Further research to identify the relationship between uprooting force requirement and some cassava agronomic parameters is recommended.

Key words: Cassava, field capacity, drudgery, planting position, tuber breakage, uprooting force.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub native to Latin America (Bellotti et al., 2011; El-Sharkawy, 2012) and is primarily grown as an annual crop in the humid tropics. It is currently the world's fourth most important staple and carbohydrate rich food crop (El-Sharkawy, 2012). Sufficient cassava is consumed as food to provide one billion people with 20% of their dietary energy requirement, and more than 700 million people are highly dependent on cassava as a food (Cock, 2011). Cassava end products range from fresh roots cooked, boiled, baked or fried at the household level, to highly processed starch as a food additive (Tufan, 2013).

In Africa, cassava is the single most important source of dietary energy for a large proportion of the population living in the tropical areas (Cock, 2011). Tufan (2013) reiterated that no other continent depends on cassava to feed as many people as does Africa, where 500 million people consume it daily. Moreover, in Africa where 40% of the population consumes cassava as a staple crop, cassava is the second most important staple crop after maize; making the crop indispensable to food security in Africa. FAOSTAT (2013) indicates that out of a total world cassava production of 233,796,000 ton, Africa accounts for 51% followed by Asia with a production of 35%, and the remaining production of 14% going to the Americas. Though Africa's cassava production is largely small-scale, it accounts for more than half of the world's cassava, or about 86 million tons from over 10 million hectares (Tufan, 2013).

Currently in Ghana, serious attention is being paid to the development and promotion of some traditional starchy staples to bridge the food production gap (Amponsah et al., 2014). According to FAOSTAT (2013), Ghana is the sixth largest producer of cassava in the world in terms of value, with the crop constituting 22% of Ghana's Gross Domestic Product (GDP). Cassava has become important and popular staple for varied preparations including fufu (pounded cassava usually mixed with plantain or cocoyam), akple, ampesi (boiled cassava), yakeyake, tapioca, agbelikaklo, kokonte, gari, etc. Cassava's adaptability to most ecological zones and its hardiness to withstand extremes of weather has made it a life saver, particularly to the lower income bracket of Ghana (Amponsah, 2011). In recent times, aside the production of high quality cassava flour (HQCF) for use in wood industries, cassava has found new uses in the brewery industry for the preparation of beer and other alcoholic beverages. Despite the enormous importance of cassava for food security in Ghana and the sub-Saharan Africa as a whole, it has received relatively little research and development attention compared to other staples such as wheat, rice and maize, especially in the area of mechanisation.

Cassava harvesting, though very crucial, is one of the serious bottlenecks in the cassava production value chain. Agbetoye (2003) identified harvesting as the most difficult operation in cassava production. Studies by Addy et al. (2004) also revealed that cassava harvesting constituted the highest production cost. Harvesting too early or late also has serious consequences on the quality of harvested roots (Moore and Lawrence, 2003). Cassava is ready for harvest as soon as there are storage roots large enough to meet the requirements of the consumer, starting from six-seven months after planting, especially for most of the new cassava cultivars (Ekanayake et al., 1997). Cassava can either be harvested manually or mechanically. Manual cassava harvesting is usually done by hand; lifting the lower part of stem and pulling the roots out of the ground, then detaching them from the base of the plant by hand after the upper parts of the stem with the leaves are removed. Manual harvesting may also employ harvesting tools such as hoe, cutlass, mattock, earth chisel, etc. Studies by Amponsah et al. (2014) revealed that harvesting with an improved manual harvesting tool used to diminish about half the time required for manual cassava uprooting with bare hands. Mechanical harvesting of cassava involves the use of a harvesting implement integrally hitched to a tractor to uproot the cassava roots. Mechanical harvesting, though better, is often unavailable or unaffordable to these resource poor farmers (Amponsah, 2011).

Ghana's cassava production is predominantly small-scale (Nweke, 2005); covering just about 1 to 2 acres. Thus, even in places where such mechanised options are available, it becomes unwise to adopt because of the smaller field size. Again, most cassava fields are located in places where topography is a serious challenge, making use of tractors virtually impossible. Moreover, the farmer's practise of intercropping and the flat method of planting does not favour mechanisation (Amponsah et al., 2014). It is worth noting that in Ghana, because a larger proportion of cassava harvested on small-scale is mostly consumed domestically for varied food preparations, marketers would reject roots that are broken, damaged, cut or bruised since consumers would mostly buy and keep their cassava for a while before use. The farmer runs at a loss when damage to roots is severe. Amponsah (2011) concluded that cassava root tuber breakage or damage is therefore a major factor to consider in the selection and adoption of any type of harvesting method depending on the end use of the harvested produce. Thus, where cassava root tuber damage or breakage is of great concern, manual harvesting is preferred to mechanical harvesting method. Without doubt, developing and adopting simple but efficient energy-saving manual harvesting tools and equipment is a sure way forward in overcoming these challenges in cassava harvesting.

Different cassava planting positions or stake orientations could be followed depending upon the type and condition of soil (Ekanayake et al., 1997; CTCRI, 2012). Stakes can be planted vertically (buds facing up with two-thirds of the stake in the soil), horizontally (whole stake buried 3-5 cm in the soil) or inclined (buds facing up with two-thirds of the stake buried in the soil at an angle of about 45º). According to Ekanayake et al. (1997), when stakes are planted vertically, tuberous roots

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bulk deep into the soil. Although this gives more stability to the plant against lodging, it makes harvesting very difficult. This orientation is recommended for sandy soils. Stakes planted horizontally produce multiple stems and more tuberous roots but they are comparatively smaller in size. The roots are produced near the surface and they are easily exposed to mechanical damage and to rodents. However, in loamy and rich soils the multiple stems and roots are at an advantage resulting in high yields. Stakes that are inclined on the ridge produce tuberous roots in the same direction. The inclination of the stem and roots provide a leverage which makes harvesting easier than in the other orientations. In shallow and clayey soils, stakes should be inclined (Ekanayake et al., 1997). Studies by Abdullahi et al. (2014) and Legese et al. (2011) concluded that storage roots yield of cassava could be enhanced by planting cuttings in an inclined or slanted position. Keating et al. (1988) however, reported that planting orientation did not have significant effect on growth and yield of cassava. There's an unending controversy about the ideal planting position for cassava in Ghana and the sub- Saharan Africa as a whole. Unfortunately, there's currently no information on ideal planting position for improved yield and enhanced manual harvesting efficiency.

Furthermore, in the area of cassava harvesting in Ghana currently, there is little information on the drudgery levels, percentage tuber breakage and field capacities associated with such an improved manual cassava harvesting tool. Since its development and first field testing and evaluation in Ghana in 2010 (Amponsah, 2011), the improved manual harvesting tool has again been tested and evaluated in Trivandrum, India by Amponsah et al. (2014) after its modified design was finalised and fabricated. Moreover, there is no information on force requirement for manual harvesting under different soil conditions and cassava varieties. Due to the fact that different cassava varieties will respond differently under different planting conditions (Amponsah et al., 2014) such information will be useful to engineers in the design of appropriate harvesting tools and implements in the future.

Objective of study

The objective of the study was to assess the response of four different cassava varieties to manual harvesting under slanted, vertical and horizontal planting positions using an improved manual cassava harvesting tool. Specifically, the study sought to:

1. Evaluate the performance of the improved manual harvesting tool under different cassava planting positions.
2. Identify the ideal planting position that gives maximum manual harvesting efficiency.
3. Determine among four varieties, the cassava variety that best facilitates manual harvesting.
4. Determine the force requirement for harvesting the various cassava varieties under different planting positions.

MATERIALS AND METHODS

Study area

The study was carried out at the CSIR-Crops Research Institute research field, Fumesua (01° 28′ N 06° 41′ W), near Kumasi in the Ashanti Region of Ghana. Fumesua is classified under the moist semi-deciduous forest agro-ecological zone. It is characterized by a bimodal rainfall pattern. The major rainfall season starts in March or April and usually terminates in early August. The minor season is from September to December. The annual rainfall ranges between 1250 and 1500 mm and temperatures range between 20°C (minimum) in August and 32°C in March (maximum). Soils at the study area are predominantly Ferric Acrisol (FAO/UNESCO) or Oxic Haplustult (USDA – Soil Taxonomy) and are classified under "Bomso series" (Dedzoe et al., 2004). The soil at the study area was sandy loam in texture.

Experimental details

A split plot design with three replicates was used for this study. The main plot treatments were the four cassava varieties; *Bankyehemaa*, *Nkabom*, *Sikabankye* and *Ampong* whereas the three cassava planting positions; vertical, horizontal and slanted were the subplot treatments.

Cassava varieties and planting position

Cassava planting materials were obtained from the multiplication plots of the Root and Tuber section of CSIR - Crops Research Institute, Fumesua. Each cassava variety was planted under the flat method of planting in three different positions; vertical, horizontal and slanted (Figure 1) at a spacing of 1 m × 0.8 m (Adekunle et al., 2004).

The cassava sticks containing at least 4 to 5 nodes were cut into sizes 20 to 25 cm before planting. Cassava harvesting trials were conducted at the study site on all four cassava varieties at 12 months after planting (MAP) using the improved manual harvesting tool.

The improved manual harvesting tool

Cassava is mostly harvested by hand, lifting the lower part of stem and pulling the roots out of the ground, then detaching them from the base of the plant by hand after the upper parts of the stem with the leaves are removed. The use of manual harvesting tools helps in loosening or reducing the soil forces on the cassava root tubers in order to make it easier to uproot them (Amponsah et al., 2014). For this study, an improved manual harvesting tool (Figure 2) was used.

The harvesting tool was constructed at the CSIR-Crops Research Institute mechanical workshop with the idea of reducing the drudgery of farmers due to waist bending associated with the other harvesting tools which usually lead to waist pains and other bodily weaknesses. The original design was first adopted from the International Institute of Tropical Agriculture (IITA) in Nigeria. Several modifications have since been made to overcome some of its design constraints (Amponsah, 2011). The harvesting tool operates according to the 'grip and lift' principle. It consists of a

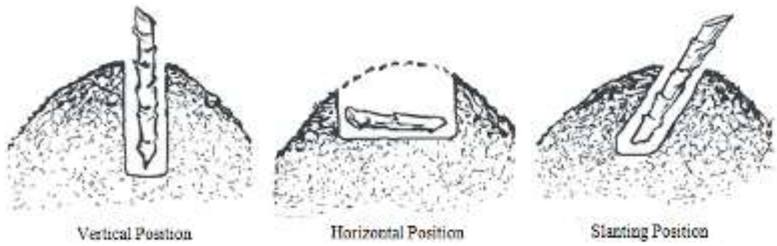

Figure 1. Different cassava planting positions.

Figure 2. The improved manual cassava harvesting tool.

Figure 3. The Eijkelkamp penetrologger in operation.

frame to which an immovable griping jaw is attached and a chisel tip which serves as the base for lifting cassava from the soil. The chisel tip can also be used to dig out cassava roots especially in hard and dry soils, where the grip and lift principle becomes difficult to employ due to the tendency of high root tuber damage or breakage. The harvesting tool, operating under the second class lever principle, has a mechanical advantage of 4.5. Its total weight of 5 kg makes it possible for even women and children to easily operate and use the tool for manual cassava harvesting.

Soil sampling

Three replicates of soil samples at harvest were randomly taken for soil moisture content and bulk density determination at depths of 0-10, 10-20, 20-30 and 30- 40 cm using a soil auger and a 5 cm diameter soil core sampler with a mallet respectively. Soil samples were oven dried at a temperature of 105˚C for 24 h for soil moisture determination (DeAngelis, 2007). Additionally, composite soil samples were also taken and analysed to determine their textural classes based on their sand (%), silt (%) and clay (%) content. Penetrometer tests using an Eijkelkamp penetrologger (Figure 3) were carried out on-site at depths of 0-10, 10-20, 20-30 and 30-40 cm at harvest to determine the soil penetration resistances (soil strength).

Harvesting force requirement

The force required for uprooting 50 plants of each cassava variety under different planting positions was determined using a force measuring apparatus (Figure 4).

The setup is composed of a metallic handle to which a modified spring balance is attached to show weight readings (in kilograms) during the cassava uprooting process. Modification of the spring balance was done by attaching a dummy dial beneath the original one. The idea is that the original dial comes back to zero at no load, thus there is the need to have a secondary (dummy) dial which will be dependent on the movement of the primary dial to assist in getting the right reading even after load is taken off the spring balance. However, the dummy dial was always reset to zero before each loading of the spring balance was done. The stem gripping

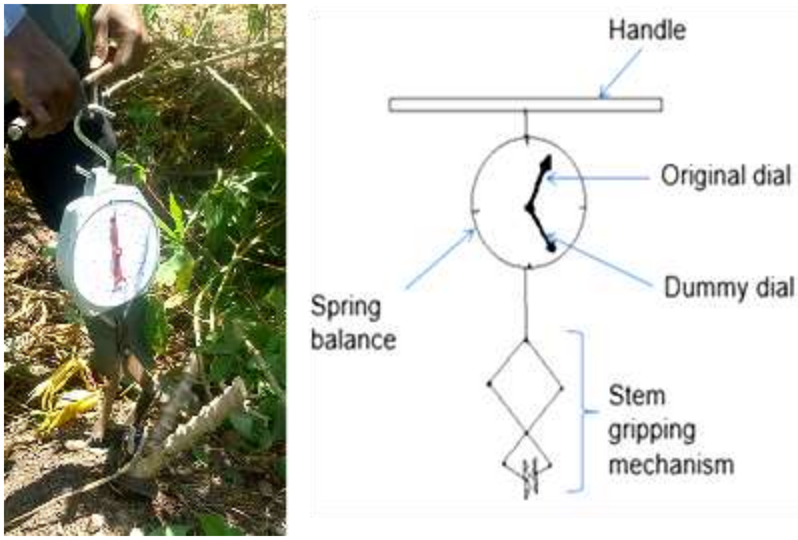

Figure 4. Cassava uprooting force measuring apparatus.

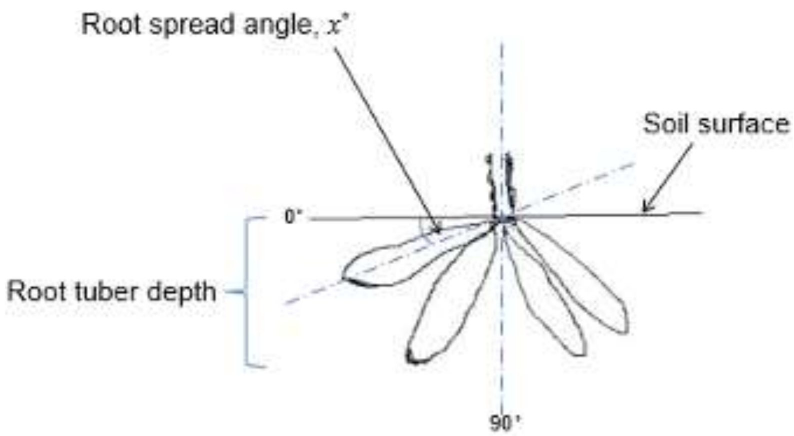

Figure 5. Cassava root orientation measurement.

mechanism is firmly attached to the cassava stem and with the help of the handle; a steady vertical force is applied to uproot the cassava. The reading as indicated by the dummy dial is then recorded after the uprooting process is ended.

Agronomic parameters

Agronomic parameters including stem girth (cm), maximum root diameter (cm) maximum root length (cm), maximum root depth (cm), number of root tubers and root spread (degrees) were determined at harvest for 30 plants of each cassava variety. Root spread was measured using a protractor with reference to the soil surface from both sides of the plant (Figure 5); stem girth and maximum root diameter were measured using a digital Vernier calliper, whereas maximum root length and depth were taken using a tape measure. Cassava root tuber yield and damaged (broken) root tubers after harvest were determined using an electronic balance. For purposes of this study however, only rooting depth and yield per plant were used.

Drudgery measurements

Polar heart rate sensing device (RS 400) was used to obtain the heart rate for each person during manual harvesting. The polar heart rate sensor is an instrument that measures the heart beat rate during every physical activity. It has a strap that is worn around the chest area and a watch (monitor) with a sensor which reads the heart rate and logs it per pre-determined interval in seconds. Data stored was downloaded onto a computer for analysis. Figure 6 shows the polar heart rate (RS 400) watch and how the chest strap (with heart beat sensor) should be worn before an activity.

Before and after any field activity, the person was allowed ten minutes' period of rest so the heart rate could be stabilized which are referred to as the rest and recovery periods respectively. Figure 7 shows a typical heart rate profile for a person before, during and after a physical activity recorded using the Polar heart rate watch and sensor (RS 400).

The period between the rest and recovery is the work period. This instrument can also calculate how much calories are burnt during any physical activity. This gives an idea of the amount of

Figure 6. The Polar (RS 400) watch and chest strap as worn by a person.

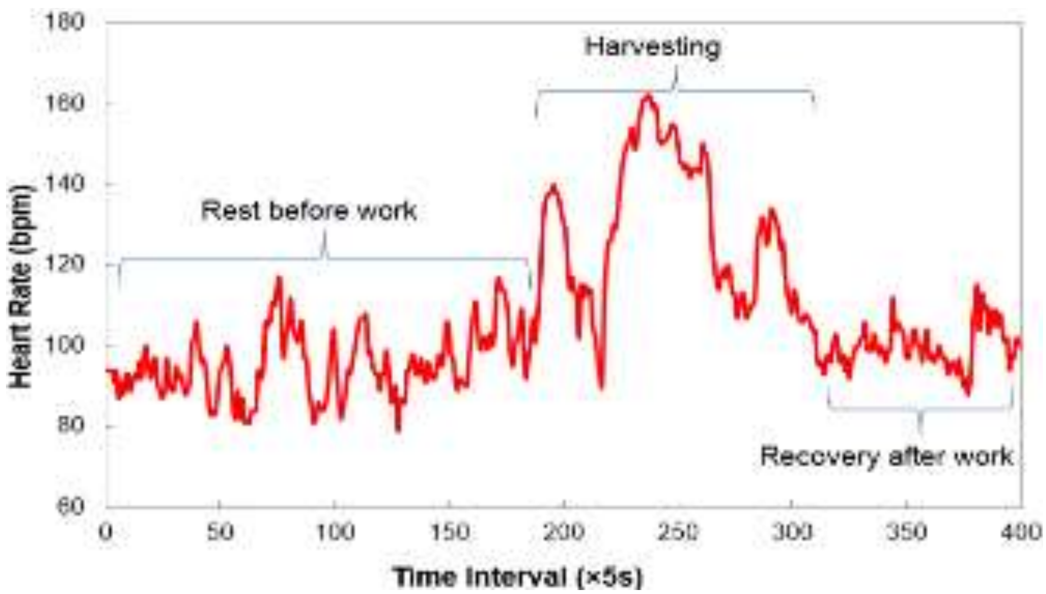

Figure 7. Typical heart rate profile before, during and after a physical activity.

energy used or the drudgery involved in carrying out any physical work. Knowledge on the amount of energy is used for carrying out a particular physical work is useful in determining the rest period (min/h) required by a person after work using Equation 1, according to Jones (1988).

$$Tr = 60 \times \left(1 - \frac{250}{P}\right) \quad (1)$$

Where, Tr = total rest period (min/h), and P = Gross energy consumption (Watts)

Using the mean heart rate obtained for a particular field activity to trace for a corresponding energy consumption value on the heart rate - energy conversion chart (Jones, 1988), the gross energy consumption (Watts) was determined.

Field capacity

Before manual harvesting, cassava plants were coppiced or cut to a stem length of about 20 to 30 cm. Three field workers were then tasked to uproot ten cassava plants of each of the four varieties under each planting position using the improved manual harvesting tool. With the help of a stop clock, time (seconds) taken to harvest the 10 plants was recorded. The capacity (timeliness of operation) for each field worker during harvesting (man-hours/ha) was determined using Equation 2 according to Amponsah et al. (2014).

$$T = \frac{10000 \times t}{n \times 3600} \quad (2)$$

Where, T = total harvesting capacity (man-h/ha); t = total time spent in harvesting (seconds), and n = number of plants harvested.

Root tuber breakage

The percentage root tuber breakage associated with each cassava variety under the different planting positions was calculated using Equation 3 according to Amponsah et al. (2014).

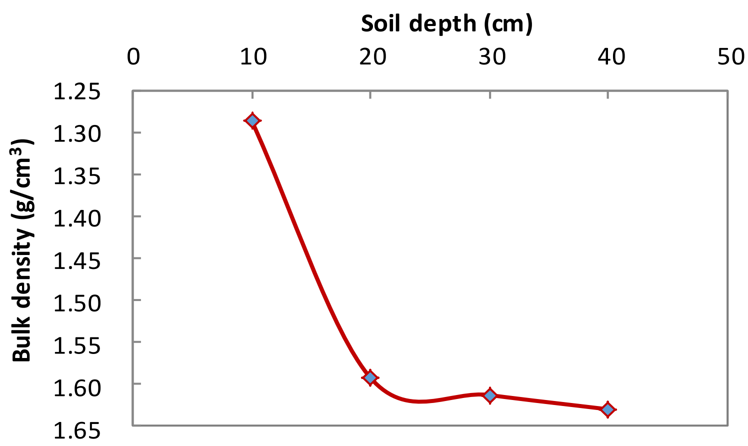

Figure 8. Mean bulk density versus soil depth.

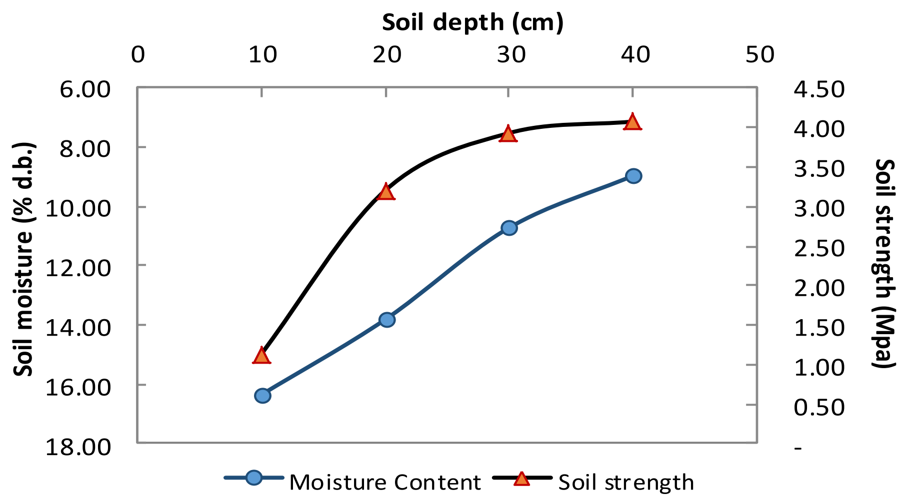

Figure 9. Mean moisture content and soil strength versus soil depth.

$$\text{Percentage Breakage} = \frac{\text{Mass of broken or damaged roots (kg)}}{\text{Total root yield (kg)}} \times 100 \quad (3)$$

Statistical analysis

The results of harvesting trials and field measurements were statistically analysed as a split plot layout in randomized complete block design (RCBD) using GenStat Discovery Edition 3 (VSN International, 2011). The least significant difference (LSD) was used at the $p < 0.05$ level of probability to test difference between treatment means. Analysis of variance (ANOVA) was performed to determine the effects of planting position and/or cassava varieties and their interaction.

RESULTS AND DISCUSSION

Soil physical properties

Figure 8 shows the mean bulk density under flat method of planting at harvest. At harvest, soil bulk density ranged from 1.29 to 1.63 g/cm^3 at increasing soil depth of 0 to 40 cm. Figure 8 generally depicts an increase in soil bulk density with increasing soil depth. This could be alluded to the decreasing moisture content down the soil profile (Figure 9), causing soil particles to be more compact due to the extra voids.

Figure 9 shows the mean soil moisture and soil penetration resistance (soil strength) at harvest under the flat method of planting. Mean soil moisture at harvest ranged from 8.97 to 16.36% d.b. whilst mean soil strength (penetration resistance) ranged from 1.13 to 4.08 MPa at increasing soil depth of 10 to 40 cm. It could also be deduced from graph in Figure 9 that soil moisture decreased with increasing soil depth whilst soil strength increased with increasing depth. This trend was expected since soil strength generally decreases with increasing soil moisture and agrees with what was reported by Utset and Cid (2001).

Table 1. Field capacity, percentage root tuber breakage, heart rate and corresponding gross energy consumption and rest period for harvesting *Bankyehemaa* (BH), *Nkabom* (NK), *Sikabankye* (SK) and *Ampong* (AM) cassava varieties under vertical (V), horizontal (H) and slanted (SL) planting positions.

Cassava variety - Planting position	Field capacity (man-h ha^{-1})**	Root tuber breakage (%)	Heart rate (bpm)	Gross energy consumption (W)	Rest period (min h^{-1})
BH-V	65.2^{cd}	10.75	96.1	479.08	28.69
BH-H	64.1^{cd}	8.73	122.0	773.72	40.61
BH-SL	64.5^{cd}	6.82	105.9	591.6	34.65
NK-V	49.9^{cd}	8.99	115.4	701.54	38.62
NK-H	50.7^{cd}	4.52	111.3	651.22	36.97
NK-SL	56.0^{cd}	5.15	112.5	665.54	37.46
SK-V	72.9^{c}	4.87	118.6	738.42	39.69
SK-H	156.1^{a*}	4.32	106.8	602.06	35.09
SK-SL	136.4^{a}	4.93	95.3	470.34	28.11
AM-V	90.9^{c}	11.76	105.1	583.45	34.29
AM-H	115.7^{ab}	19.55	115.1	696.84	38.47
AM-SL	76.4^{c}	6.38	108.8	624.82	35.99

*Values followed by the same letter(s) in the same group are not significantly different at $p < 0.05$; **Assuming 4 working hours per day, excluding rest periods.

Manual harvester performance evaluation

Table 1 shows the field performance evaluation results (field capacity, percentage root tuber breakage, heart rate and corresponding energy consumption and rest period) of the improved manual harvesting tool under flat planting method for cassava variety and planting position interaction.

From results in Table 1, it could be seen that harvesting *Sikabankye* cassava variety under horizontal planting position recorded the highest significant ($p < 0.05$) field capacity of 156 man-h ha^{-1} whereas the least significant value of 49.9 man-h ha^{-1} was recorded by *Nkabom* variety under the vertical planting position.

Similarly, harvesting *Ampong* variety under horizontal planting position recorded the highest root tuber breakage (19.55%) whilst *Sikabankye* under horizontal planting position recorded the least (4.32%). However, no significant difference ($p < 0.05$) was recorded for percentage root tuber breakage across cassava varieties and planting positions.

Again, harvesting *Bankyehemaa* cassava variety under the horizontal planting position recorded the highest heart rate (122 bpm) with a corresponding gross energy consumption of 773.72 W whilst harvesting *Sikabankye* variety under slanted planting position recorded the least value of 95.3 bpm with a corresponding gross energy consumption of 470.34 W. Generally, however, there was no significant difference ($p < 0.05$) in average heart rate and corresponding gross energy consumption across cassava varieties and planting positions. From Table 1, it could also be deduced that mean heart rate, gross energy consumption and rest period are directly proportional; the higher the heart rate, the higher the gross energy consumption, leading to longer period of rest to compensate for the used or lost energy. This relationship between energy consumption and rest period is in agreement with studies by Amponsah et al. (2014), Ericsson et al. (2006), Crouter et al. (2004) and Freedson and Miller (2000).

Table 2 shows the field evaluation summary results after harvesting with the improved tool for the different cassava varieties and planting positions under the flat planting method.

Under cassava variety in Table 2, it could be seen that *Sikabankye* cassava variety recorded the highest significant ($p < 0.05$) field capacity of 121.8 man-h ha^{-1} whereas *Nkabom* recorded the least (52.2 man-h ha^{-1}) across all planting positions. The significantly high field capacity recorded for *Sikabankye* could be attributed to its generally high uprooting force requirement and yield per plant as recorded in Tables 3 and 4. This result goes on to confirm the fact that *Sikabankye* cassava variety is high-yielding compared to the other varieties, making it difficult to uproot, particularly under the flat method of planting. Interestingly, in terms of root tuber breakage, *Ampong* cassava variety recorded the highest significant value of 12.56% whereas *Sikabankye* variety recorded the least (4.71%) across all planting positions. In terms of drudgery, though not significant ($p < 0.05$), *Ampong* cassava variety recorded the highest average heart rate (113.1 bpm) at harvest whilst *Sikabankye* variety recorded the least (106.9 bpm).

Similarly, under planting positions in Table 2, horizontally planted cassava recorded the highest significant ($p < 0.05$) field capacity of 96.6 man-h ha^{-1} during harvesting with the improved tool, whereas vertically planted cassava recorded the least (69.7 man-h

Table 2. Field capacity, percentage root tuber breakage and heart rate after manual harvesting with the improved tool for different cassava varieties (*Bankyehemaa*, *Nkabom*, *Sikabankye* and *Ampong)* and under different planting positions (vertical, horizontal and slanted).

Parameter		Field capacity (man-h ha^{-1})	Root tuber damage (%)	Heart rate (bpm)
Cassava variety	Bankyehemaa	64.6^{c}	8.77^{ab}	108.0
	Nkabom	52.2^{c}	6.22^{bc}	113.1
	Sikabankye	121.8^{a*}	4.71^{bc}	106.9
	Ampong	94.3^{b}	12.56^{a}	109.7
Planting position	Vertical	69.7^{c}	9.09	108.8
	Horizontal	96.6^{a*}	9.28	113.8
	Slanted	83.3^{b}	5.82	105.6

*Values followed by the same letter(s) in the same group are not significantly different at $p < 0.05$.

Table 3. Cassava uprooting force requirement, rooting depth and yield per plant for *Bankyehemaa* (BH), *Nkabom* (NK), *Sikabankye* (SK) and *Ampong* (AM) cassava varieties under vertical (V), horizontal (H) and slanted (SL) planting positions.

Cassava variety - planting position	Force requirement (kg)	Rooting depth (cm)	Yield (kg/plant)
BH-V	130.1^{a}	26.72	6.18^{c}
BH-H	130.7^{a}	25.09	6.68^{bc}
BH-SL	117.8^{ab}	26.86	5.84^{c}
NK-V	86.8^{bc}	23.37	6.85^{bc}
NK-H	112.3^{ab}	26.11	7.19^{bc}
NK-SL	124.8^{ab}	25.47	8.09^{bc}
SK-V	143.3^{a*}	22.83	13.14^{a}
SK-H	128.2^{a}	22.39	10.48^{b}
SK-SL	97.8^{bc}	24.30	6.69^{bc}
AM-V	118.0^{ab}	23.62	10.15^{b}
AM-H	116.8^{ab}	25.38	9.52^{b}
AM-SL	108.6^{ab}	24.26	7.31^{bc}

*Values followed by the same letter(s) in the same group are not significantly different at $p < 0.05$.

Table 4. Cassava uprooting force requirement, rooting depth and yield per plant under the flat planting method for different cassava varieties (*Bankyehemaa*, *Nkabom*, *Sikabankye* and *Ampong)* and different planting positions (vertical, horizontal and slanted).

Parameter		Force requirement (kg)	Rooting depth (cm)	Yield (kg/plant)
Cassava variety	Bankyehemaa	126.2^{a*}	26.22^{a}	6.23^{d}
	Nkabom	108.0^{b}	24.98^{a}	7.38^{c}
	Sikabankye	123.1^{a}	23.17^{ab}	10.10^{a}
	Ampong	114.5^{b}	24.42^{ab}	8.99^{b}
Planting position	Vertical	119.5	24.13	9.08^{a*}
	Horizontal	122.0	24.74	8.46^{a}
	Slanted	112.3	25.22	6.98^{b}

*Values followed by the same letter(s) in the same group are not significantly different at $p<0.05$.

ha^{-1}) regardless of cassava variety. However, in terms of root tuber breakage and drudgery, horizontally planted cassava recorded the highest value (9.28% and 113.8 bpm respectively) whilst obliquely (slanted) planted cassava recorded the least (5.82% and 105.6 bpm respectively). It is worth noting that there was no significant difference ($p < 0.05$) in percentage root tuber breakage and drudgery among the planting positions, irrespective of cassava variety. This trend of lower percentage root tuber damage and drudgery at harvest agrees with report by Ekanayake et al. (1997) that planting cassava sticks in a slanted position provide a leverage for the stem and roots which makes harvesting easier than in the other orientations.

Cassava uprooting force, rooting depth and yield

Table 3 shows the cassava uprooting force requirement, rooting depth and yield per plant under flat method of planting for cassava variety and planting position interaction.

From Table 3, it could be deduced that vertically planted *Sikabankye* cassava variety recorded the highest significant ($p < 0.05$) uprooting force requirement of 143.3 kg whereas vertically planted *Nkabom* variety recorded the least (86.8 kg). The generally low uprooting force requirement recorded for *Nkabom* cassava variety could be due to the fact that morphologically, *Nkabom* variety is bunchy (Amponsah et al., 2014) with minimal root spread in the soil, making it easier to uproot compared to the other varieties which have much wider root spread.

Cassava rooting depth, though with no significant differences ($p < 0.05$), recorded the highest value of 26.86 cm for obliquely (slanted) planted *Bankyehemaa* cassava variety with horizontally planted *Sikabankye* variety recording the least value (22.39 cm).

In terms of yield per plant however, vertically planted *Sikabankye* cassava variety recorded the highest significant value of 13.14 kg whereas obliquely (slanted) planted *Bankyehemaa* recorded the least (5.84 kg).

Table 4 shows the cassava uprooting force, rooting depth and yield per plant under the flat planting method for the different cassava varieties and planting positions.

Under cassava variety as depicted in Table 4, *Bankyehemaa* recorded the highest significant uprooting force requirement of 126.2 kg whereas *Nkabom* variety recorded the least (108 kg) irrespective of planting position under the flat planting method. It was therefore not surprising that *Bankyehemaa* again recorded the highest significant ($p < 0.05$) rooting depth (26.22 cm) with *Sikabankye* recording the least (23.17 cm). This situation reiterates the fact that cassava uprooting force requirement is significantly influenced by cassava rooting depth as reported by Amponsah et al. (2014). Conversely however, in terms of yield per plant, *Sikabankye* recorded the highest significant ($p < 0.05$) value of 10.10 kg whereas *Bankyehemaa* recorded the least (6.23 kg) regardless of planting position under the flat method of planting.

For planting position, though no significant ($p < 0.05$) difference existed among treatments, planting cassava horizontally resulted in the highest uprooting force requirement (122 kg) irrespective of cassava variety whereas planting obliquely (slanted) recorded the least (112.3 kg). In terms of rooting depth, though there was no significant difference ($p < 0.05$) among planting positions, obliquely (slanted) planted cassava recorded the highest rooting depth of 25.22 cm whereas vertically planted cassava recorded the least (24.13), regardless of cassava variety. On the other hand, vertically planted cassava resulted in the highest significant ($p < 0.05$) yield per plant (9.08 kg) whereas obliquely (slanted) planted cassava recorded the least (6.98 kg) irrespective of cassava variety under the flat method of planting. This result, however, opposes what was reported by Abdullahi et al. (2014) and Legese et al. (2011) that storage roots yield of cassava could be enhanced by planting cuttings in an inclined or slanted position.

CONCLUSIONS AND RECOMMENDATION

The improved manual cassava harvesting tool generally performed satisfactorily under the flat method of planting with field capacity ranging between 49.9 and 156 man-h ha^{-1}, root tuber breakage between 4.32 and 19.55% and heart rate with corresponding gross energy consumption ranging between 95.3 bpm (470.34 W) and 122 bpm (773.72 W) across cassava varieties and planting positions.

Generally, it required less time to harvest *Nkabom* variety using the improved manual harvesting tool compared to the other cassava varieties irrespective of planting position. However, in terms of percentage root tuber breakage and drudgery during harvesting, *Sikabankye* cassava variety is the best.

Similarly, harvesting vertically planted cassava under flat planting method using the improved manual harvesting tool provides the best timeliness of harvest irrespective of cassava variety. However, though not significant, harvesting obliquely (slanted) planted cassava is best in terms of percentage root tuber breakage and drudgery.

Cassava uprooting force ranged from 86.8 to 143.3 kg, rooting depth from 22.39 cm to 26.86 cm and cassava yield per plant ranging from 5.84 to 13.14 kg across cassava varieties and planting positions. *Nkabom* cassava variety was easier to uproot compared to the others irrespective of planting position. Though, planting cassava in a slanted position offers the best in terms of uprooting force requirement and rooting depth, it produces the poorest yield per plant, regardless of cassava variety. Best yield, however, is achieved when cassava stakes are planted vertically.

Last but not least, it could be concluded from the study that cassava uprooting force was significantly influenced by cassava rooting depth. However, as a recommendation further research should be carried out to determine the relationship between cassava uprooting force and some cassava agronomic parameters for different cassava varieties under the various planting positions and methods of planting.

Conflict of Interests

The authors have not declared any conflict of interests.

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Peat influence on Zn tolerance, bioconcentration and bioaccumulation in *Eucalyptus grandis* Hill ex Maiden

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Soil contamination with zinc occurs in mining and agricultural areas through successive applications of pig slurry. Essential to the selection and adaptation of plants to contaminated soil, is the study of species showing not only tolerance but also bioaccumulation or bioconcentration of zinc associated with amelioration. In this context, this study aimed to evaluate the influence of peat addition on the tolerance, bioconcentration and bioaccumulation of Zn in *Eucalyptus grandis* seedlings. A completely randomized experimental design was used, in a factorial arrangement (2 x 6), with and without the addition of peat (200 mL L^{-1} soil) and six doses of Zn (zero, 200, 400, 600, 800 and 1000 mg kg^{-1}) with 15 replications, conducted for 120 days. The height, stem diameter, root and shoot dry weight, concentration and Zn content in the root system and aerial parts, indices of tolerance and translocation, and bioaccumulation factors and bioconcentration were evaluated. The addition of 200 mL L^{-1} of peat provided increased tolerance and a stimulating effect on morphological parameters of *E. grandis* seedlings. This species, however, did not concentrate zinc to the extent that it could be considered a bioaccumulating plant.

Key words: Contaminated soil, phytoremediation, zinc contamination, amelioration.

INTRODUCTION

Zinc is a micronutrient, normally present in the soil in concentrations of up to 70 mg kg^{-1} plant-available Zn; however, concentrations above 100 and up to 500 mg kg^{-1} of soil are considered toxic to plants (Kabata-Pendias, 2010). Agricultural cultivation (Brunetto et al., 2014) and mining (Hooda, 2010) have been identified as factors that

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increase zinc content in the soil. Brazilian law, through Resolution CONAMA 420 2009, fixed a reference limit of 450 mg Zn kg^{-1} for intervention in agricultural areas where strategies and techniques are needed to mitigate the deleterious effect of zinc accumulation. The use of tree species is considered an important technique for the recovery of metal-contaminated areas, because trees typically have large biomass production and a long growth cycle (Domínguez et al., 2009). Among tree species, *E. grandis* is favored due to its rapid growth and high biomass production (Souza and Fiorentin, 2013). However, the accumulation of heavy metals and nutrients in eucalyptus roots has been little studied, especially due to difficulty in determination of its content (Robinson, 2003). Silva et al. (2015), working with *Corymbia citriodora*, *Eucalyptus saligna*, *Eucalyptus grandis* and *Eucalyptus dunnii* seedlings showed a reduction of, respectively, 35, 56, 60 and 81% in shoot dry mass with increasing doses of Zn. No differences in response of plant species to Zn level were found. In environments where heavy metal contents in the soil are high, establishment of plants is restricted, and the use of other techniques that mitigate the effects of contamination is required.

The possibilities for reducing contamination levels include the use of both organic and inorganic materials. One consequence of an application of organic amendments is a decrease in the availability of metals via adsorption and complexing reactions in the soil, thus reducing absorption by plants (Park et al., 2011). One example of such an amendment is peat, which, once applied to the soil, tends to make Zn less bioavailable (Pichtel and Bradway, 2008). Peat is described by George et al. (2010) as a natural organic material, stabilized and recognized for its superior ion exchange capacity. According to Santos and Rodella (2007), peat reduced available Zn by up to 12% in comparison with that in the control treatment.

Phytoremediation is the use of plant species as an alternative for the recovery of contaminated areas. Tree species, specifically young plants, are more sensitive to soil contaminated by metals (Souza et al., 2012), which facilitates studies focused on the growth of seedlings in contaminated soil. However, some questions still remain regarding the use of peat in ameliorating soil contaminated with Zn and its ability to enable greater growth, increase tolerance, and lower absorption of Zn by eucalyptus. Therefore, the hypothesis underlying the present study is that peat has an ameliorating effect in zinc-contaminated soils with the concurrent development of *E. grandis*. In this context, this study aimed to evaluate the influence of peat on the development, tolerance, bioconcentration and bioaccumulation of Zn in *E. grandis* seedlings.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse belonging to the Agricultural College of Frederico Westphalen, Rio Grande do Sul, south region of Brazil, between the months of May and September, 2015. The soil is characterized as an oxisol and was collected in the agricultural area in the Federal University of Santa Maria, campus Frederico Westphalen, Rio Grande do Sul. Green® peat was used as the organic amendment, and was characterized according to the methodology of Tedesco et al. (1995); results are presented in Table 1.

Experimental units were composed of 600 cm^3 soil in polyethylene plastic bags containing one seedling each. *E. grandis* Hill ex Maiden seeds was provided by the Forestry Research Center of the State Foundation for Agricultural Research (FEPAGRO - RS), in Santa Maria, RS. The seeds were sown in seedbeds and transplanted into experimental units upon presentation of a pair of true leaves. The experimental design was completely randomized in a factorial arrangement (2 x 6), with and without the addition of peat (200 mL L^{-1} soil (v:v)) and six doses of Zn (zero, 200, 400, 600, 800 and 1000 mg kg^{-1}) with 15 repetitions. Zn doses were applied 30 days prior to transplanting in the form of zinc acetate dihydrate solution ($C_4H_6O_4Zn.2H_2O$) diluted in 50 ml of water. In order to homogenize the soil, the plastic bag was shaken. After 15 days of soil contamination, peat was added and the soil left for another 15 days prior to transplanting seedlings. A sample from each treatment was taken for the determination of pseudototal Zn content according to the USEPA method 3050b (USEPA, 1996).

The experiment was conducted for 120 days after transplanting. During this period, daily irrigations were performed, based on the weight of the experimental units, keeping the moisture content at 80% of field capacity. Base fertilization was performed, applying the equivalent of 150 g of N, 700 g of P_2O_5 and 100 g of K_2O per cubic meter of soil. Covered fertilization was done using 100 g of N and 30 g of K_2O diluted in 10 L of water. Fertilization after transplanting was carried out in three stages: 30 days with the application of N and K; 60 days only N; and 90 days applying N and K, following the recommendations of Gonçalves and Benedetti (2005). In order to meet the requirements of design, experimental units were rotated weekly.

At the end of the experiment, the following parameters were evaluated: plant height (H), measured with graduated ruler (from seedling lap to the stem apex); stem diameter (SD), measured with a digital caliper with a precision of 0.01 mm; dry mass of the root system (DMR) and shoot (DMS), both fractions having been separated at the cervical region, dried in an oven at 60 ± 1°C to constant weight, and weighed on an analytical balance accurate to 0.0001 g. The total dry matter (TDM) was obtained as the sum of DMR and DMS. Specific surface area (SSA) of the roots was estimated using the methodology of Tennant (1975).

After weighing the root and aerial dry mass, the material was ground in a Wiley-type grinder (10 mesh sieve) to determine the concentration of Zn in the plant tissue through nitric-perchloric acid digestion (3:1) followed by determination by atomic absorption spectrophotometry as described by Miyazawa et al. (2009).

The index of tolerance (Toi) was calculated according to Equation 1 using the following parameters: TDM and zinc concentrations (mg kg^{-1}) of the root system (ZnR) and shoot (ZnS), accumulated amounts of zinc (μg $plant^{-1}$) in the root system (ZnAR) in shoot (ZnAS), and total accumulated zinc in seedlings (ZnAT) in the zero dose treatment (d0) and at doses of 200 to 1000 mg kg^{-1} (dn). The index of tolerance measures the ability of seedlings to grow in environments with high metal concentrations (Wilkins, 1978). The translocation index (Tri) was calculated according to Equation 2, and indicates is the total percentage of absorbed zinc which was transported to the shoot (Abichequer and Bohnen, 1998). Also, estimates were bioconcentration factor, by the ratio of metal concentrations in the roots (mg kg^{-1}) and the pseudototal concentration in soil (mg kg^{-1}), and the bioaccumulation factor, determined as the ratio of the metal concentration in shoots (mg kg^{-1}) and the pseudototal concentration in soil (mg kg^{-1}).

Table 1. Chemical analyses of soil and soil plus peat used for the *Eucalyptus grandis* seedling growth trial.

Substrate	pH_{water}	Ca+Mg	Al	H+Al	P	K	$Zn_{soluble}$	O.M.	Clay
	1:1	------ cmolc kg^{-1} -------			-------- mg kg^{-1} ---------			------- % ------	
Soil	5.2	4.23	0.33	5.34	2.16	61.52	1.45	1.15	65.00
Soil+peat*	5.3	15.21	0.21	6.11	43.28	368.53	1.04	4.38	57.00

*200 mL of peat L^{-1} of soil.

$$Toi = \frac{TDM_{dn}}{TDm_{d0}} \times 100 \quad (1)$$

$$Tri = \frac{ZnAS_{dn}}{ZnAT_{dn}} \times 100 \quad (2)$$

The results were subjected to variance analysis and when significant interactions were obtained, quantitative factor regression analysis was performed within each level of the qualitative factor, using the SISVAR program (Ferreira, 2011).

RESULTS AND DISCUSSION

Soil analysis after contaminant addition showed increases in the amount of zinc (Zn) in the soil with increasing doses, independent of peat application (Figure 1). The National Environmental Council, through Resolution CONAMA 420 2009, sets the reference limits for intervention in different areas, with 450 mg kg^{-1}, as the intervention threshold for Zn. In this experiment, artificial conditions of Zn contamination were efficiently created by addition of zinc acetate solution, thus enabling the realization of this work.

Using the analysis of variance, a significant interaction ($p \leq 0.05$) is evident among Zn and peat treatments for all variables measured in this study (Figures 2, 3 and 4). The height of *E. grandis* seedlings was reduced significantly by increasing doses of Zn, both with or without the use of peat. With 200 ml L^{-1} of peat in the soil, seedling height was higher as compared to the treatment without peat (Figure 2A). This effect may be attributed to the supply of additional essential nutrients provided to the plants from peat as shown in Table 1. However, Magalhães et al. (2011) studied the phytostabilization of soil contaminated with Zn in two species of *Eucalyptus* (*E. urophylla* and *E. saligna*), and observed that the application of alleviation materials to the substrate with high metal concentrations positively affected the development of the two species studied, reducing the contaminant metal effect. A high Zn content in soil greatly reduces plant growth, as compared to plants grown in uncontaminated soils (Gichner et al., 2006). In pinion pine plants, Chaves et al. (2010) also observed a height reduction in treatments with Zn, confirming the detrimental effect of Zn contamination on plant growth.

Similar to seedling height, stem diameter was significantly reduced with increasing doses of Zn, but with less intensity in the treatments with peat (Figure 2B). This effect was also observed by Chaves et al. (2010), who also showed a significant reduction of stem diameter in treatments with copper and zinc, a reduction that was less intense in the presence of peat. This increased stem diameter with the addition of peat, in spite of the presence of metal contaminants, which may be associated with additional elements essential for plant growth (Taiz and Zeiger, 2013), in this case, P and K as shown in Table 1; this effect is confirmed in the zero Zn treatment.

The dry shoot weight, root weight and specific surface area were higher in treatments with peat, and showed reductions in both treatments with increasing doses of Zn (Figure 2C, D and E). According to Pereira et al. (2010), increased root and shoot dry mass due to the addition of alleviation materials is an important feature in phytostabilization strategies of heavy metals, contributing to a greater accumulation of metals in these plants. According to Carneiro et al. (2002), the toxicity of Zn in plants reduces the production of dry matter of shoot and root biomass. On the other hand, the addition of peat can promote development of the radicular system, because in addition to the nutritional aspect, it also improves the physical properties of the soil (Franchi et al., 2003).

The root specific surface area was reduced quadratically in the treatment without peat and reached a maximum at an estimated dose of 83.33 mg Zn kg^{-1} soil with the use of peat, being significantly higher with the addition of peat and 200 mg of Zn (Figure 2F). High concentrations of Zn in the soil generally reduce root length (Hooda, 2010), directly affecting specific surface area. Carneiro et al. (2002), working with herbaceous species in soils with different contaminants, reported that the stimulation of root growth is important as part of the phytostabilization of areas contaminated with heavy metals; furthermore, greater root growth gives more protection to the soil against erosion, reduces leaching, enhances aggregation and stimulates microorganism activity. The present data show that peat stimulated root growth, therefore confirming the possibility that these associated benefits will also be observed in similar situations.

Zn content increased linearly in roots with Zn dose (Figure 3A), whereas in the shoot, there was a quadratic trend, with an increase in Zn doses of 886.93 and 908.46

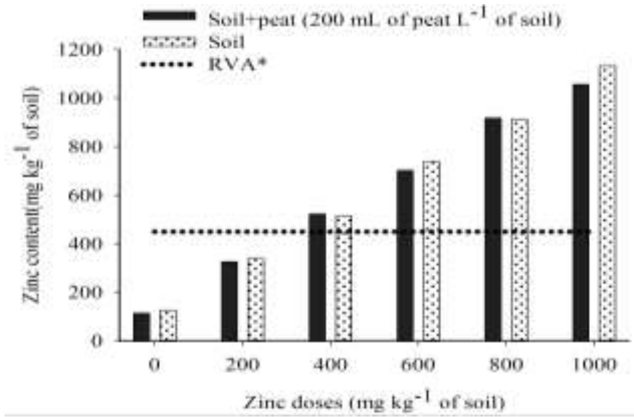

Figure 1. Zinc content in the soil due to zinc doses applied with and without addition of peat (200 mL peat L^{-1} soil).

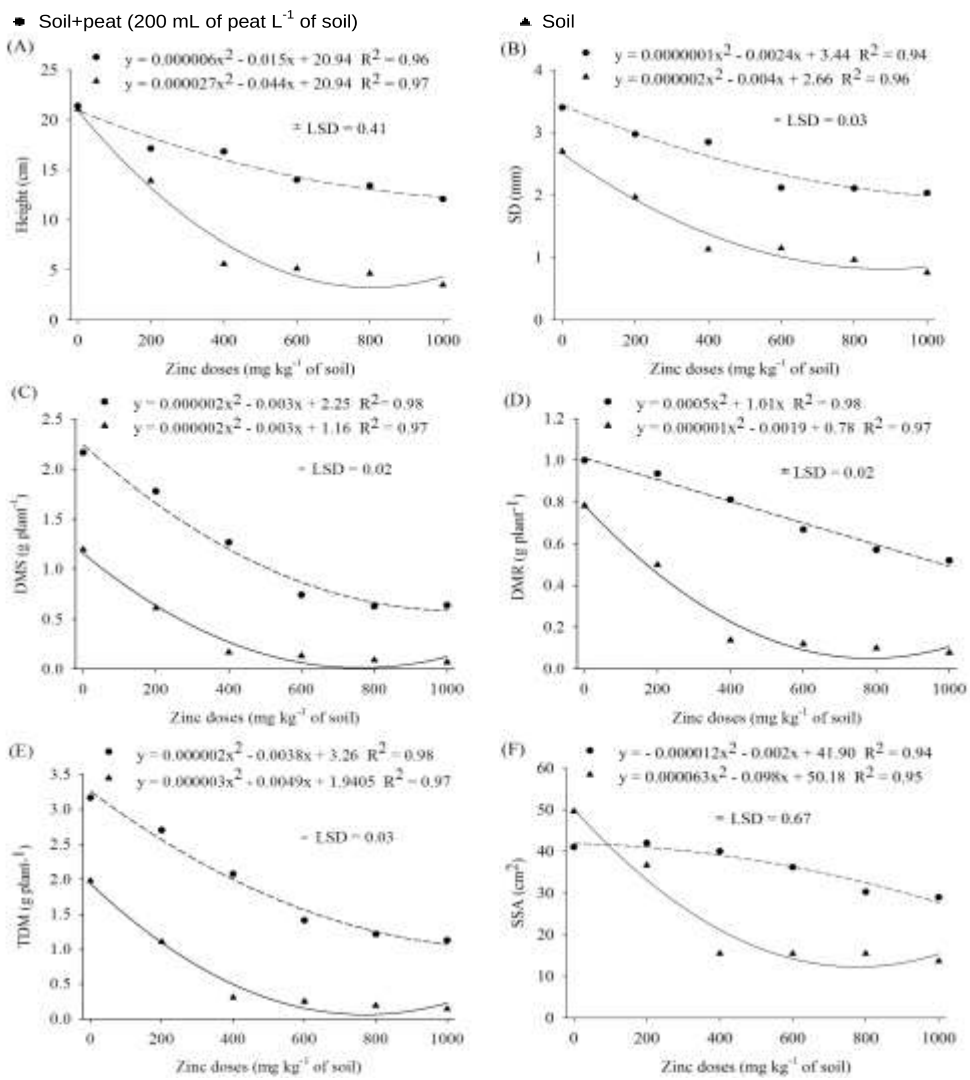

Figure 2. Regression equations for the height (A); stem diameter - SD (B); dry matter of shoots - DMS (C); dry matter of roots - DMR (D); Total dry matter - TDM (E) and surface area specific - SSA (F) in *Eucalyptus grandis* seedlings subjected to increasing Zn doses in the absence and presence of peat (200 mL L^{-1} peat soil). LSD = least significant difference.

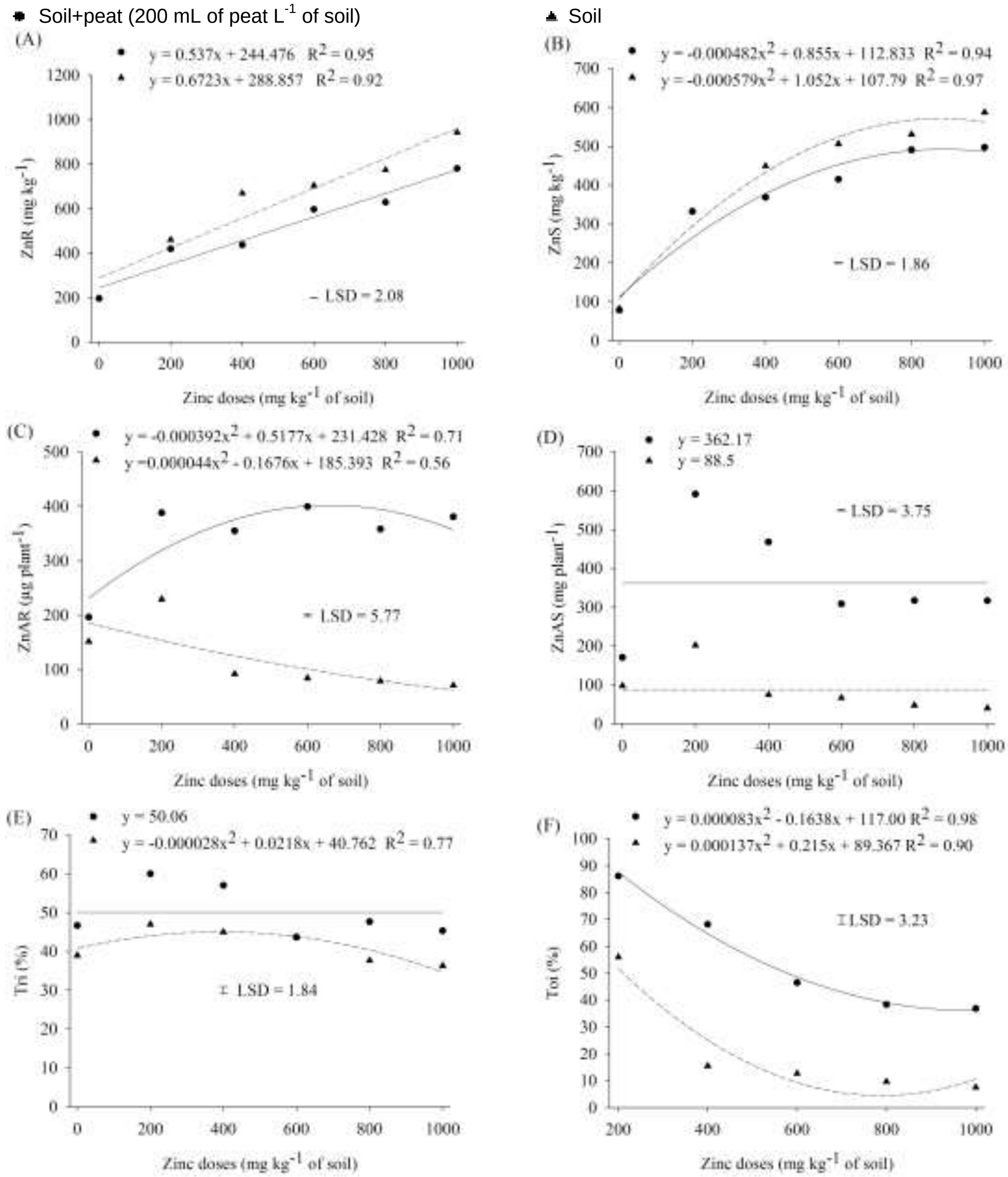

Figure 3. Regression equations for root zinc content - ZnR (A); and shoots - ZnS (B); zinc accumulated in the root - ZnAR (C); and in the shoots - ZnAS (D); translocation index - Tri (E); and tolerance index - Toi (F) in Eucalyptus grandis seedlings subjected to zinc doses in the absence and presence of peat (200 mL peat L-1 soil). LSD = least significant difference.

mg kg^{-1} soil, with and without peat respectively, being significantly lower in treatments with peat (Figure 3B). Other research has shown that increasing amounts of metals in soil increase the levels of these metals in the shoots and roots of the tree species, *Salix humboldtiana* Willd (Gomes et al., 2011), in the leaves of *Myracrodruon urundeuva* Fr. Allem (Gomes et al., 2013) and in the shoots of *E. grandis* (Silva et al., 2015). In addition, there are reports that humified organic materials reduce the availability of cations such as Zn (Jacundino et al., 2015), and Zn becomes less bioavailable with peat application (Pichtel and Bradway, 2008). This confirms the observed reduction in Zn levels in the roots and shoots of *E. grandis* seedlings upon application of 200 ml peat L^{-1} soil.

The accumulation of Zn in roots was significantly higher with the use of peat as compared to treatments without peat, reaching a maximum at a Zn application of 660 mg kg^{-1} soil, in contrast to treatments without peat, in which a

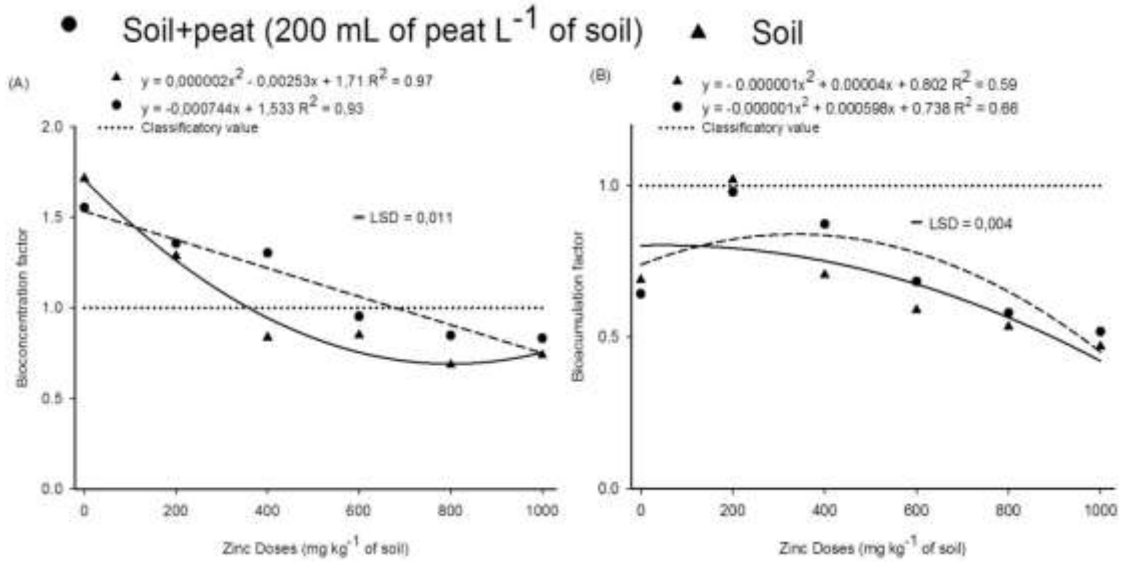

Figure 4. Regression equations for bioconcentration factor (A) and bioaccumulation factor (B) in *Eucalyptus grandis* seedlings subjected to zinc doses in the absence and presence of peat (200 mL L^{-1} soil). LSD = least significant difference.

decreasing linear tendency was observed (Figure 3C). The highest accumulation of Zn in roots in the presence of peat is related to the greater amount of root biomass produced in this treatment (Figure 2E) and is estimated as a 55.2% increase in accumulation of Zn with the use of peat and a reduction of 66.6% without peat, when the highest dose is compared to the zero dose. Plants have various mechanisms to tolerate excess metals; zinc can be immobilized onto cell walls or complexed with non-diffusible proteins in plant roots (Kabata-Pendias, 2010).

Zinc accumulation in shoots was significantly higher in the treatment with peat, however, regression equations did not produce a suitable fit (Figure 3D). This is an indication that peat did not act as ameliorating factor to zinc contamination in the soil, but rather stimulated growth and plant biomass production, leading to greater Zn content in shoots. Santos and Rodella (2007) found that peat was less efficient than a mineral-humic compound in reducing the availability of Cu, although peat did favor greater plant development.

The Zn translocation ratio observed in the present study was significantly higher in peat treatments, whereas treatment without peat reached a maximum at 389 mg kg^{-1} Zn in soil (Figure 3E). The peat had a stimulating effect on the growth of *E. grandis* and thus may have provided greater absorption of nutrients and Zn. Silva et al. (2015), in contrast, working in a Latossolo soil contaminated with Zn, found a maximum Zn translocation of 515.4 kg^{-1} mg in *E. grandis* seedlings. Branzini et al. (2012) studied the absorption and translocation of Cu, Zn and Cr in *Sesbania virgata*, and commented on the existence of mechanisms that prevent translocation of Zn to shoots in order to avoid damage to aerial parts. Thus, the translocation index values indicate low translocation of this metal in *E. grandis* seedlings, suggesting that this species may possess a physiological mechanism that prevents translocation of Zn to shoots of seedlings. The tolerance was significantly higher with peat and showed minimal points at 986 and 784 mg kg^{-1} of soil Zn with or without peat, respectively (Figure 3F).

Tolerance values between 35 and 60% are generally considered as moderate tolerance (Lux et al., 2004). Thus, in the presence of peat, *E. grandis* showed a relatively high tolerance to doses less than 451 mg kg^{-1} Zn, while without peat low tolerance is observed at dose higher than 315 mg kg^{-1} Zn. It is known that tolerant species can reduce metal toxicity by immobilization or compartmentalization in the roots (Saraswat and Rai, 2011). Thus, the addition of peat, in part due to its stimulation of root growth, provides greater tolerance to *E. grandis* seedlings in soil contaminated with Zn.

The bioconcentration factor shows a minimum in the treatment with peat at an estimated dose of 632 mg kg^{-1} Zn and a decreasing linear effect without peat, although values for bioconcentration factor without peat remained mostly above those of plants grown with peat (Figure 4A). The bioconcentration factor is greater than one at Zn doses above 387 and 679 mg kg^{-1} with and without peat, respectively. McGrath and Zhao (2003) determined that plants showing a bioconcentration factor lower than one are not recommended for phytoextraction. Thus, it is possible to use *E. grandis* as a phytoextracting plant in soils with appropriately low concentrations of Zn. However, the use of peat must be evaluated in each specific situation, as it may lower the phytoextracting potential of this species at the intermediate soil Zn levels used in the present study (approximately 300 to 800 mg kg^{-1}).

The bioaccumulation coefficient was significantly higher in the treatments without peat as compared to treatments with peat above 400 mg kg^{-1} Zn, with a maximum point at 299 mg kg^{-1} without peat and 20 mg kg^{-1} with peat (Figure 4B). The coefficient of bioaccumulation was lower than the bioconcentration factor in all treatments because all treatments were below the classificatory value, indicating that *E. grandis* has only a limited ability to absorb soil Zn and translocate it to shoots.

Conclusions

Addition of 200 mL L^{-1} of peat to soil provides increased tolerance and a stimulating effect on morphological parameters of *E. grandis* seedlings. This species, however, does not meet the necessary criteria to be considered a zinc bioconcentrating and bioaccumulating plant.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Radiological hazard of coffee to humans: a comparative study of Arabian and Turkish coffees

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Thirty-nine Arabian and Turkish coffee powder samples purchased from various markets in Saudi Arabia were analyzed by High Pure Germanium (HPGe) gamma spectrometry to determine the activity concentrations of the natural and artificial radionuclides ^{238}U, ^{226}Ra, ^{232}Th, ^{137}Cs, and ^{40}K. All samples, (except for two samples of Turkish coffee) were found to contain a high mean content of ^{40}K, ranging from 839.83 to 1197.11 Bq/kg and from 161.312 to 2411.215 Bq/kg for Arabian and Turkish coffee powders, respectively. The concentrations of ^{226}Ra and ^{232}Th were found to be 2.57 to 10.63 Bq/kg and nondetectable to 8.01 Bq/kg for Arabian coffee and nondetectable to 10.09 Bq/kg and nondetectable to 9.75 Bq/kg for Turkish coffee, respectively. Based on these values, we estimated the potential radiological hazards to consumer health from coffee powder. We determined the radium equivalent, annual effective dose rate, and external and internal hazard for each element, and all were found to be below the limit recommended by the United Nations Scientific Committee on the Effects of Atomic Radiation. However, absorbed dose rate values for some samples were higher than the permissible limit. In all samples, the ^{137}Cs concentration was below the detection limit. The average annual committed effective dose values reported in this study were far below the world average value of 0.30 mSv/yr for an individual. In addition, the limit for the threshold consumption rate was calculated. The statistical methods were applied to study the relationship between all the calculated natural radionuclides and their hazard parameters. Results indicated that the use of these types of coffee had no significant radiological health risks. This study may contribute data on coffee powder for formulating regulations related to radiological health care.

Key words: Arabian and Turkish coffees**,** natural radioactivity**,** radiological hazard parameters.

INTRODUCTION

Coffee is one of the most popular and widely consumed beverages in the world, and its consumption is increasing (Roselli et al., 2013). However, variation exists in the annual consumption between countries. In addition, coffee is grown in many countries, where the coffee trade has played a crucial role in their economic development (Roselli et al., 2013). Coffee comes from the plant genus *Coffea*, which has two primary species, *C. arabica* (Arabica) and *C. canephora* (also known as *C. robusta*, or robusta). Some naturally occurring radioisotopes and other elements present in soil are drawn into the roots of plants via ion channels or specific transporters (Sugiyama et al., 2009; Jibiri et al., 2016). Their distribution throughout the plant tissues depends on their chemical characteristics and several parameters of soil and the plants themselves (Awudu, 2012). Several studies have been performed to determine the concentrations of elements in various types of coffee and to estimate whether these concentrations contribute to toxicity (Szymczycha-Madeja et al., 2014; Welna et al., 2014; Jarošová et al., 2014; Zaidi et al., 2005).

Jarošová et al. (2014) determined the toxic elements and mineral nutrients, including Pb, Ni, Mn, Cr, Cd, Zn, Mg, Fe, Cu, and Ca, in five types of coffee by using inductively coupled plasma mass spectrometry (ICP-OES) and atomic absorption spectrometry (AAS). No significant differences were found between the results obtained through the two methods, but a multivariate analysis helped to identify variations among samples collected from different locations. Zaidi et al. (2005) used instrumental neutron activation analysis to measure 20 trace elements (toxic, essential, and nonessential) in samples of coffee beans from four different origins and two instant coffee brands consumed in Pakistan. They next estimated the daily intake of trace elements and compared those levels with tolerance limits. The intake of toxic elements was well below the recommended safety limits, but the cumulative intake of Mn was four times higher than the acceptable amount.

Knowledge of the concentrations and distributions of natural radionuclides, such as ^{40}K, ^{238}U, and ^{232}Th and their decay products, in soils, plants, sediments, and so forth is useful for monitoring environmental radioactive contamination (El-Reefy et al., 2006). Use of chemical fertilizers, especially phosphorus fertilizer, may lead to increased contamination of agricultural crops by enriching the soil with radioactive elements as well as chemical toxins (Alharbi, 2013).

Roselli et al. (2013) determined the background level of gamma emitters, including ^{212}Bi, ^{212}Pb, ^{228}Ac, ^{214}Bi, ^{214}Pb, ^{40}K, and ^{137}Cs, in 18 brands of coffee powder. The mean activity concentration of ^{40}K was found to be 907.4 ± 115.6 Bq/kg. In addition, the mean activity concentration of ^{214}Bi and ^{214}Pb, indicators of ^{226}Ra, given as the mean value of the two radionuclides, resulted in 10.61 ± 4.02 Bq/kg ^{228}Ra. ^{228}Ac indicators showed a mean activity concentration of 13.73 ± 3.20 Bq/kg. ^{212}Pb, a ^{224}Ra indicator, presented a mean activity concentration of 8.28 ± 2.88 Bq/kg. The mean activity concentration of ^{208}Tl, another ^{224}Ra indicator, was 11.03 ± 4.34 Bq/kg. Results indicated that all samples had ^{137}Cs concentrations less than the detection limit (2.0 Bq/kg).

The aim of this study was to compare the concentration of naturally occurring radioactive materials (NORMs) in Arabian and Turkish coffee powder using gamma spectrometry. The imported samples were bought from selected local markets. Additional goals were to determine the radiological hazard associated with drinking coffee made from the various coffee powders and to estimate the average annual committed effective dose via ingestion of the radionuclides in the coffee and the threshold consumption rate.

The study focused on coffee powder to enrich the radiological information in Saudi Arabia in particular and the world in general because information on the concentration of NORMs and their presence in coffee is scarce.

MATERIALS AND METHODS

Thirty-nine coffee powder samples were purchased at different markets in Saudi Arabia. Each powdered was grinded and dissolved as homogenous solution and weighing about 200 to 250 g. The samples were then transferred to polyethylene 650-mL Marinelli type beakers (of known weight) were hermetically sealed with an insulating tape to impede contact with air moisture say labeled, and packed into radon-impermeable plastic containers to prevent radon gas escape as much as possible. Containers of the same size and geometry were used for reference materials to calibrate the system for measuring radioactivity. The samples and reference materials were stored and kept for a period of 1 month to attain secular radioactive equilibrium among ^{226}Ra, ^{232}Th, and their respective short-lived decay products ^{226}Ra and its decay products in the uranium series and ^{228}Ra and its decay products in the thorium series (Kurnaz et al., 2007; Samad et al., 2012). Finally, each Marinelli container was analyzed using a HPGe detector.

The radiometric measurement

Detection of the amount of natural radioactivity for ^{226}Ra, ^{238}U, ^{232}Th, and ^{40}K in coffee powder samples was carried out with gamma spectrometry using a high-purity germanium (HPGe) detector (P-type vertical coaxial, Canberra model GC4018). The detector had 25% relative efficiency and a resolution of 1.85 at 1332 keV of ^{60}Co gamma ray. The gamma acquisition and analysis were determined by using a multichannel analyzer (16,000 channels spectral memory) coupled to a computer using Genie-2000 Basic Spectroscopy Software. Quality-assured standard samples in QCY4 solution (obtained from the International Atomic Energy Agency) were used for the calibration and the absolute efficiency of the detector. The mixture of radionuclides in the solution (with corresponding energies) included ^{60}Co (1173 and 1333 keV), ^{88}Yt (898 and 1836 keV), ^{137}Cs (662 keV), ^{85}Sr (514 keV), ^{113}Sn (391.69 keV), ^{203}Hg (279 keV), ^{139}Ce (1656 keV), ^{57}Co (122 keV), ^{109}Cd (88 keV), and ^{241}Am (60 keV). Detailed information concerning calibration of the HPGe detector and the procedures followed can be found in previous study (Tufail et al., 2006; Fawzia, 2010). To measure the environmental gamma background, an empty identical Marinelli beaker was used. All samples and the background were counted for 36,000 s. After measurement, the activity concentrations were calculated by subtraction of the background values. The ^{137}Cs and ^{40}K concentrations were measured by determining their characteristic gamma lines of energies, 661.6 and 1460.8 keV, respectively. The ^{226}Ra activity concentration was determined by averaging the calculated activity

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concentrations of three photopeaks of ^{214}Bi (1764.5, 1120.3, and 609.3 keV) and two of ^{214}Pb (295.1 and 352 keV). The activity of ^{232}Th was inferred from the weighted mean activities of the gamma peaks of ^{208}Tl (583.0 keV), ^{212}Pb (238.6 keV), and ^{228}Ac (911.2 and 338.4 keV). In addition, the activity of ^{238}U was given by the line of gamma of its product decay: ^{234}Th (63.22 and 92.78 keV) (Darko, 2015). The minimum detectable activity concentrations of ^{40}K, ^{238}U, ^{232}Th, ^{226}Ra, and ^{137}Cs using HPGe detector were 2.51, 1.0, 0.80, 0.75, and 0.85 Bq/kg, respectively.

Calculations

Absorbed dose rate and annual effective dose rate equivalent from ingestion of coffee powder

According to United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR, 2010), the external outdoor absorbed gamma dose rate (D) from natural radionuclides in the samples under study (at 1 m above the ground level) can be calculated from the following equation:

$$D(\text{nGy/h}) = 0.426A_{\text{Ra}} + 0.664A_{\text{Th}} + 0.042A_{\text{K}} \qquad (1)$$

Where, A_{Ra}, A_{Th}, and A_{K} are the activity concentrations of radium, thorium, and potassium, respectively. The absorbed dose can be converted to the annual effective dose equivalent by using the conversion factor 0.7 Sv/Gy (UNSCEAR,2000) and the indoor occupancy factor of 0.8, based on people spending an average of 80% of their time indoors and 20% outdoors (Jibiri, 2016).

The total annual effective dose (AE, the external dose rates) from the ^{226}Ra, ^{232}Th, and ^{40}K is obtained by using the following equation (Giri, 2013):

$$AE(\text{mSv/yr}) = D \times 24 \times 365.25 \times 0.8 \times 0.7 \times 10^{-6} \qquad (2)$$

The absorbed dose rate (D) and the annual effective dose (AE) for the coffee powder samples are presented in Table 4.

The radium equivalent

The radium equivalent activity for coffee powder is given by the following equation (Matiullah et al., 2004):

$$Ra_{\text{eq}}(\text{Bq/kg}) = A_{\text{Ra}} + 1.43A_{\text{Th}} + 0.077A_{\text{K}} \qquad (3)$$

Equation 3 compares the activity concentrations of coffee powder containing different amounts of ^{40}K, ^{232}Th, and ^{226}Ra. The permissible limit of this index is 370 Bq/kg as reported by UNSCEAR (2000).

The internal and external hazard indices

The internal and external exposures to gamma radiation in coffee powder were determined using the Equations 4 and 5, respectively (Fawzia, 2010):

$$H_{\text{in}} = \frac{A_{\text{Ra}}}{185} + \frac{A_{\text{Th}}}{259} + \frac{A_{\text{K}}}{4910} \qquad (4)$$

$$H_{\text{ex}} = \frac{A_{\text{Ra}}}{370} + \frac{A_{\text{Th}}}{259} + \frac{A_{\text{K}}}{4910} \qquad (5)$$

To safely consume the coffee powder, the upper limit for both the internal index (H_{in}) and the external index (H_{ex}) should be less than unity as reported by ICRP (2007).

Average annual committed effective dose or dose ingested by the consumer

Estimation of the average annual committed effective dose (AACED) due to ingestion of NORMs in coffee can be calculated using the following equation (Njinga et al., 2015; Chandrashekara and Somashekarappa, 2016):

$$E_{\text{ave}} = C_r \times DCF_i \times A_i \qquad (6)$$

where, E_{ave} (Sv/yr) is the AACED, A_i is the activity concentration of each radionuclide i , C_r is the consumption rate of radionuclide, and DCF_i is the standard dose conversion factor (2.8×10^{-4}, 2.3×10^{-4}, and 6.2×10^{-6} mSv/Bq for ^{226}Ra, ^{232}Th, and ^{40}K, respectively, for an adult) (UNSCEAR, 2000). The annual threshold consumption rate (kg/yr) for each coffee sample was obtained using the following equation:

$$C_r = \frac{3E_{\text{ave}}}{\sum_i^3 (DCF_i \times A_i)} \qquad (7)$$

Where, A_1, A_2, and A_3 are the activity concentrations of ^{226}Ra, ^{232}Th, and ^{40}K, respectively DCF_1, DCF_2, and DCF_3 are the dose coefficients for ingestion for the radionuclides (Sv/Bq) and E_{ave} = 0.3 (mSv/yr) is the threshold AACED due of NORMs in the studied samples.

RESULTS AND DISCUSSION

The activity concentrations of ^{40}K, ^{238}U, ^{226}Ra, and ^{232}Th in Arabian and Turkish coffee powders imported for Saudi markets from different countries were estimated, and the results are summarized in Tables 1 and 2, respectively. As shown in Table 1, the activity concentrations of ^{226}Ra and ^{232}Th varied from 2.57 to 10.63 Bq/kg with a mean value 6.19 Bq/kg and from nondetectable (ND) to 8.01 Bq/kg with a mean value 4.31 Bq/kg, respectively. In addition, as shown in Table 2, the activity concentrations of ^{226}Ra and ^{232}Th ranged from ND to 10.09 Bq/kg with a mean of 2.77 Bq/kg and ND to 9.75 Bq/kg with a mean of 3.12 Bq/kg, respectively. All these values are significantly lower than the permissible levels reported by UNSCEAR (2010) (33 and 45 Bq/kg for ^{226}Ra and ^{232}Th, respectively), as shown in Figures 1 and 2. The activity concentrations of ^{40}K in Arabian coffee powder ranged from 839.83 to 1197.11 Bq/kg with a mean value of 1041.24 Bq/kg. Sample CA5 had the highest activity concentration, while sample CA9 had the lowest value. For Turkish coffee powder samples, the activity concentrations of ^{40}K varied from 161.31 to 2411.22 Bq/kg with a mean of 1507.18 Bq/kg. The lowest activity was recorded for sample CT20, while the highest activity was recorded for sample CT24. With the exception of two

Table 1. Activity concentration (Bq/kg) in different types of Arabian coffee powder.

Sample code no.	Activity concentration			
	^{238}U	^{226}Ra	^{232}Th	^{40}K
CA1	45.93 ± 8.23	2.57 ± 0.42	2.66 ± 0.70	1031.93 ± 56.29
CA2	9.35 ± 1.81	7.98 ± 1.63	2.96 ± 0.79	1118.90 ± 65.99
CA3	22.11 ± 4.31	5.28 ± 1.02	2.12 ± 0.51	948.70 ± 50.82
CA4	102.39 ± 21.74	6.70 ± 1.41	4.93 ± 0.99	1086.45 ± 57.52
CA5	135.11 ± 24.61	10.63 ± 2.84	8.01 ± 1.64	1197.11 ± 61.18
CA6	37.62 ± 7.07	7.54 ± 1.34	7.06 ± 1.36	1070.23 ± 47.95
CA7	61.79 ± 8.81	8.52 ± 1.62	6.91 ± 1.12	1090.50 ± 50.00
CA8	12.64 ± 3.511	7.39 ± 1.25	5.65 ± 1.01	1004.16 ± 54.82
CA9	66.33 ± 9.62	7.863 ± 1.46	3.40 ± 1.04	839.83 ± 36.62
CA10	ND	4.97 ± 1.07	ND	956.65 ± 47.28
CA11	ND	3.10 ± 0.71	2.96 ± 0.63	1049.63 ± 36.33
CA12	52.56 ± 9.73	5.75 ± 1.07	3.52 ± 0.77	1079.09 ± 45.22
CA13	61.98 ± 8.81	5.60 ± 1.00	4.89 ± 0.91	1062.87 ± 49.82
CA14	16.73 ± 3.01	5.98 ± 1.10	5.22 ± 1.22	1041.34 ± 39.96
Mean	44.61± 7.95	6.19± 1.28	4.31± 0.91	1041.24± 49.99

Table 2. Activity concentration (Bq/kg) in different types of Turkish coffee powder.

Sample code no.	Activity concentration			
	^{238}U	^{226}Ra	^{232}Th	^{40}K
CT1	50.31 ± 10.54	10.09 ± 2.18	9.75 ± 1.86	1128.64 ± 62.92
CT2	ND	2.45 ± 0.67	3.07 ± 0.63	1138.37 ± 62.95
CT3	26.19 ± 4.67	5.50 ± 1.12	6.19 ± 1.01	1155.67 ± 50.96
CT4	28.27 ± 5.03	3.97 ± 0.64	5.76 ± 1.11	1200.03 ± 70.15
CT5	27.05 ± 5.37	1.295 ± 0.18	ND	2173.70 ± 80.32
CT6	15.36 ± 3.45	3.64 ± 0.70	4.93 ± .96	1232.48 ± 64.80
CT7	17.03 ± 3.17	4.17 ± .96	4.19 ± .97	1492.13 ± 77.96
CT8	8.68 ± 1.79	7.58 ± 1.13	7.62 ± 1.29	1070.22 ± 51.00
CT9	32.72 ± 7.08	3.65 ± 0.79	2.92 ± 0.66	1203.27 ± 63.07
CT10	57.16 ± 9.32	5.49 ± 0.96	4.65 ± 1.00	1842.68 ± 68.55
CT11	33.10 ± 7.14	ND	ND	1913.53 ± 79.06
CT12	10.08 ± 1.78	1.56 ± .26	1.32 ±0 .36	1845.82 ± 80.00
CT13	10.48 ± 2.14	ND	ND	1801.36 ± 80.13
CT14	19.75 ± 4.07	ND	ND	1805.92 ± 80.12
CT15	ND	4.66 ± 0.89	3.95 ± 0.90	1930.17 ± 82.65
CT16	31.83 ± 5.69	2.46 ± 0.71	9.53 ± 1.79	2046.99 ± 82.05
CT17	22.12 ± 5.96	1.74 ± .46	1.67 ± 0.26	1627.38 ± 75.34
CT18	18.81 ± 3.26	ND	3.18 ± 0.71	1338.38 ± 62.51
CT19	24.61 ± 6.38	ND	ND	1806.83 ± 70.60
CT20	ND	ND	ND	161.31 ± 50.71
CT21	ND	ND	ND	211.92 ± 48.80
CT22	ND	ND	ND	1899.82 ± 76.59
CT23	41.81 ± 8.65	ND	ND	1986.54 ± 83.10
CT24	46.66 ± 9.40	6.97 ±0.89	6.67 ± 1.12	2411.22 ± 83.68
CT25	40.99 ± 0.90	3.05 ± 0.66	2.52 ± 0.56	1653.16 ± 64.24
Mean	22.52± 4.00	2.77± 0.52	3.12± 0.49	1523.10± 70.09

Table 3. Radium equivalent, absorbed dose rate, annual effective dose rate, external hazard index, and internal hazard index in different types of Arabian coffee powder.

Sample code No.	Radium equivalent	Absorbed dose rate	Annual effective dose rate	External hazard	Internal hazard
CA1	85.821	45.823	0.225	0.232	0.239
CA2	98.355	52.127	0.256	0.266	0.287
CA3	81.3615	43.280	0.212	0.220	0.234
CA4	97.394	51.373	0.252	0.263	0.281
CA5	114.254	59.665	0.293	0.309	0.337
CA6	100.039	52.374	0.257	0.270	0.291
CA7	102.370	53.584	0.263	0.277	0.300
CA8	92.788	48.699	0.239	0.251	0.271
CA9	77.382	40.703	0.200	0.209	0.230
CA10	79.051	42.366	0.208	0.214	0.227
CA11	88.145	46.986	0.2301	0.238	0.246
CA12	93.874	49.781	0.244	0.254	0.269
CA13	94.434	45.066	0.245	0.255	0.270
CA14	93.628	49.340	0.242	0.254	0.269
Mean	92.778	48.655	0.241	0.251	0.268

Turkish samples CT20 and CT21, all Arabian and Turkish coffee powders had ^{40}K concentrations that were higher than the acceptable value (412 Bq/kg) (UNSCEAR, 2010). Based on Figure 3, the mean activity concentrations of ^{40}K in Arabian and Turkish coffee powders are 1.53 and 2.69 times higher than the recommended limit, respectively. In addition, the ^{40}K concentration was 46.28% higher in the Turkish coffee than in the Arabian coffee. Moreover, the current results from Arabian and Turkish coffee powders showed a mean concentration of ^{40}K that was higher than that obtained by Roselli et al. (2013), although the mean concentration of ^{226}Ra was within the range found in the same study. This finding may be linked to contaminated soils in the farming areas (Fatima et al., 2008; Faanu et al., 2016). Uranium 238 was detectible in all study samples except samples CA10, CA11, CT2, CT15, CT20, CT21, and CT22. The highest ^{238}U concentrations were found in sample CA5 (135.11 Bq/kg) and sample CT10 (57.16 Bq/kg), representing Arabian and Turkish coffee powders, respectively.

Hence, the mean activity concentration of ^{238}U in Arabian coffee was approximately 98.09% higher than the Turkish coffee. The activity concentration of ^{232}U ranged from ND to 66.33 Bq/kg with a mean value of 44.61 Bq/kg for Arabian coffee powder and from ND to 57.16 Bq/kg with a mean value of 22.52 Bq/kg. Figure 4 shows a comparison of ^{238}U in Arabian and Turkish coffee powders, in which the mean activity in Arabian samples is less than the permissible limit, but the mean activity in Turkish samples was 35.18% higher than the acceptable limit. The measurements show that all studied samples had concentrations of the artificially produced radionuclide ^{137}Cs below the detectable limit except in samples CA5 and CT19, which had the values of 3.44 and 1.26 Bq/kg, respectively.

Tables 3 and 4 list the calculated values of the radium equivalent, absorbed dose rate, annual effective dose rate, and gamma hazard indices H_{ex} and H_{in} used in Equations 1 to 5, respectively. The results showed that the radium equivalent lay in the ranges of 77.382 to 114.254 Bq/kg (CA9 and CA5, respectively) for Arabian coffee powder with a mean value 90.595 Bq/kg and from 12.421 to 202.159 Bq/kg for Turkish coffee powder with a mean value of 124.586 Bq/kg. These values are less than the permitted limit of 370 Bq/kg. The estimated values for the absorbed dose rate in Arabian and Turkish coffee powders varied from 40.703 to 59.665 nGy/h (CA9 and CA5, respectively) with a mean value of 48.655 nGy/h and from 6.727 to 107.791 nGy/h (CT20 and CT24, respectively) with a mean value of 66.709 nGy/h. According to UNSCEAR (2010), all results fell below the recommended limit for the average exposure rate (84 nGy/h). External and internal hazards were also determined based on Equations 4 and 5. Tables 5 and 6 show that the external and internal hazard indexed are less than unity for Arabian and Turkish coffee powders. With a unit consumption rate of 1 kg per annum being used, the AACED due to the ingestion of radionuclides ^{232}Th, ^{226}Ra, and ^{40}K from Arabian and Turkish coffee was estimated using Equation 6, and the results are presented in Tables 5 and 6. The AACED varied from 0.00232 to 0.00396 mSv/yr and from 0.00032 to 0.00598 mSv/yr for Arabian and Turkish coffee powders, respectively. The mean values of AACED in the study samples were far below the world AACED of 0.3 mSv/yr for ingestion of natural radionuclides detailed in UNSCEAR (2010).

Table 4. Radium equivalent, absorbed dose rate, annual effective dose rate, external hazard index, and internal hazard index in different types of Turkish coffee powder.

Sample code no.	Radium equivalent	Absorbed dose rate	Annual effective dose rate	H_{ex}	H_{in}
CT1	110.934	57.613	0.283	0.300	0.327
CT2	94.497	50.457	0.248	0.255	0.262
CT3	103.34	54.47	0.267	0.279	0.294
CT4	104.597	55.349	0.272	0.283	0.293
CT5	168.670	91.242	0.448	0.456	0.459
CT6	105.586	56.052	0.275	0.285	0.295
CT7	125.06	66.68	0.327	0.338	0.349
CT8	100.879	52.731	0.2587	0.272	0.293
CT9	100.466	53.621	0.263	0.271	0.281
CT10	153.986	82.167	0.403	0.416	0.431
CT11	147.342	79.794	0.392	0.398	0.398
CT12	145.58	78.489	0.385	0.393	0.397
CT13	138.705	75.117	0.369	0.375	0.375
CT14	139.792	75.618	0.371	0.378	0.378
CT15	158.927	85.025	0.417	0.429	0.442
CT16	173.699	92.249	0.453	0.469	0.476
CT17	129.44	69.68	0.342	0.350	0.354
CT18	107.60	57.730	0.283	0.291	0.291
CT19	141.218	76.259	0.374	0.381	0.384
CT20	12.421	6.727	0.033	0.034	0.034
CT21	16.317	8.837	0.043	0.044	0.044
CT22	146.363	79.255	0.389	0.395	0.395
CT23	153.117	82.904	0.407	0.413	0.414
CT24	202.159	107.791	0.529	0.546	0.565
CT25	133.95	71.87	0.353	0.362	0.370
Mean	124.586	66.709	0.327	0.337	0.344

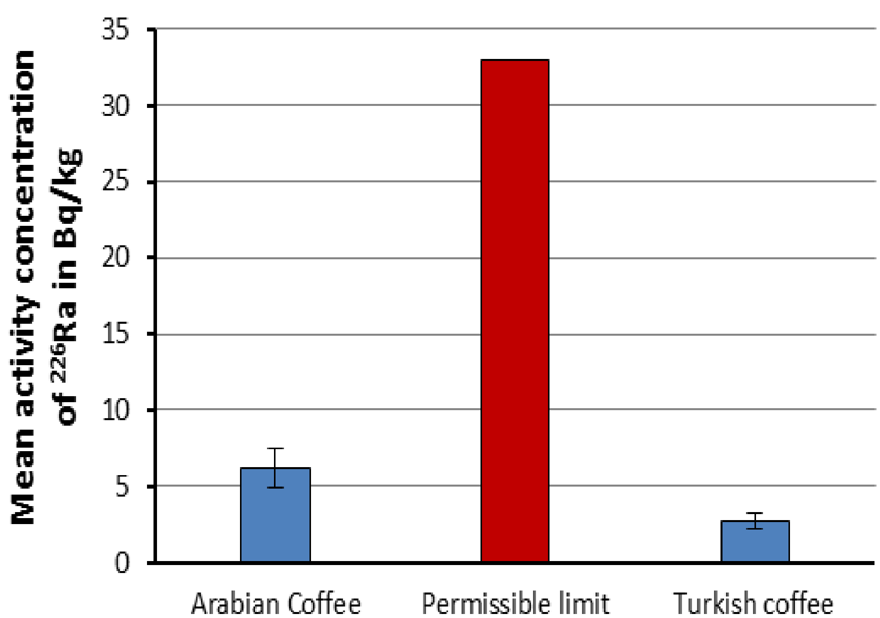

Figure 1. A comparison between the mean activity concentration of ^{226}Ra in Arabian and Turkish coffee with the permissible limit.

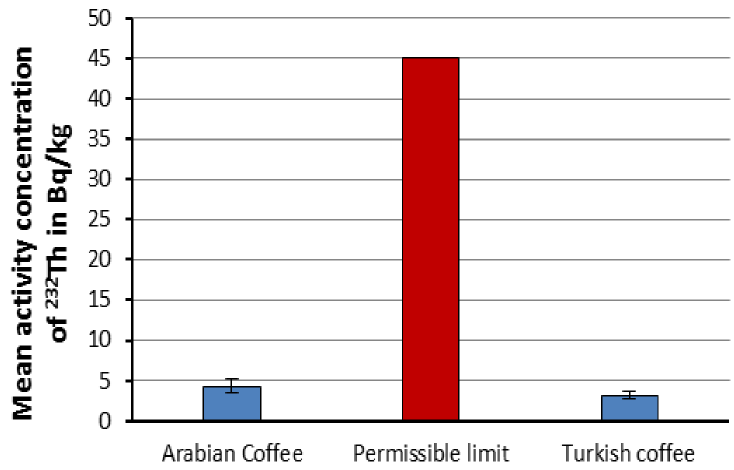

Figure 2. A comparison between the mean activity concentration of ^{232}Th in Arabian and Turkish coffee with the permissible limit.

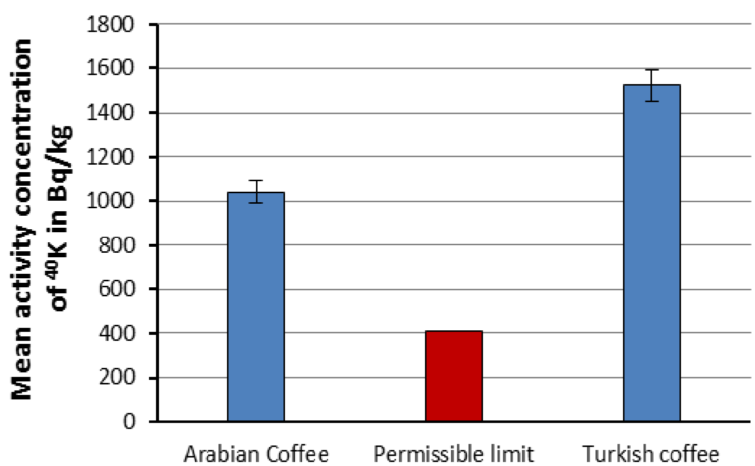

Figure 3. A comparison between the mean activity concentration of ^{40}K in Arabian and Turkish coffee with the permissible limit.

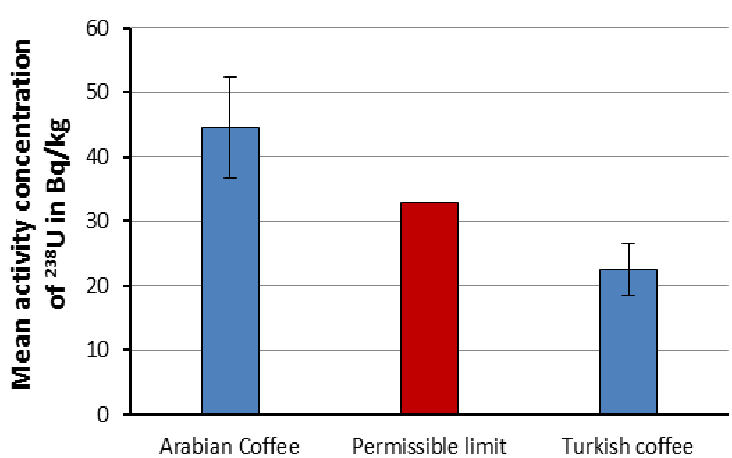

Figure 4. A comparison between the mean activity concentration of ^{238}U in Arabian and Turkish coffee with the permissible limit.

Table 5. AACED due to the ingestion of radionuclides ^{232}Th, ^{226}Ra, and ^{40}K from Arabian coffee and the threshold consumption rate.

Sample code No.	AACED for 1 kg/year (mSv/year)	Threshold consumption rate kg/year
CA1	0.00251± 0.00021	119.522
CA2	0.00313± 0.00035	95.847
CA3	0.00250± 0.00024	120.000
CA4	0.00315± 0.00033	95.238
CA5	0.00396± 0.00052	75.758
CA6	0.00338± 0.00033	88.757
CA7	0.00348± 0.00034	86.207
CA8	0.00310± 0.00031	96.774
CA9	0.00260± 0.00029	115.385
CA10	0.00232± 0.00020	129.310
CA11	0.00261± 0.00019	114.943
CA12	0.00293± 0.00025	102.389
CA13	0.00301± 0.00027	99.668
CA14	0.00291± 0.00028	103.093
Mean	0.00298± 0.00029	100.671

Table 6. AACED due to the ingestion of radionuclides ^{232}Th, ^{226}Ra, and ^{40}K from Turkish coffee and the threshold consumption rate.

Sample code No.	AACED for 1 kg/year (mSv/yr)	Threshold consumption rate kg/year
CT1	0.00394± 0.00037	76.142
CT2	0.00275± 0.00014	109.091
CT3	0.00331± 0.00016	90.635
CT4	0.00324± 0.00017	92.593
CT5	0.00445± 0.00006	67.416
CT6	0.00321± 0.00017	93.458
CT7	0.00370± 0.00033	81.081
CT8	0.00343± 0.00022	87.464
CT9	0.00296± 0.00025	101.351
CT10	0.00454± 0.00031	66.0793
CT11	0.00383± 0.00016	78.329
CT12	0.00395± 0.00022	75.949
CT13	0.00360± 0.00017	83.333
CT14	0.00366± 0.00017	81.967
CT15	0.00459± 0.00032	65.360
CT16	0.00517± 0.00025	58.027
CT17	0.00355± 0.00022	84.507
CT18	0.00308± 0.00018	97.403
CT19	0.00376± 0.00015	79.787
CT20	0.00032± 0.00011	937.500
CT21	0.00043± 0.0001	697.675
CT22	0.00381± 0.00016	78.740
CT23	0.00399± 0.00017	75.188
CT24	0.00598± 0.00034	50.167
CT25	0.00378±0.00024	79.365
Mean	0.00356±0.00021	84.270

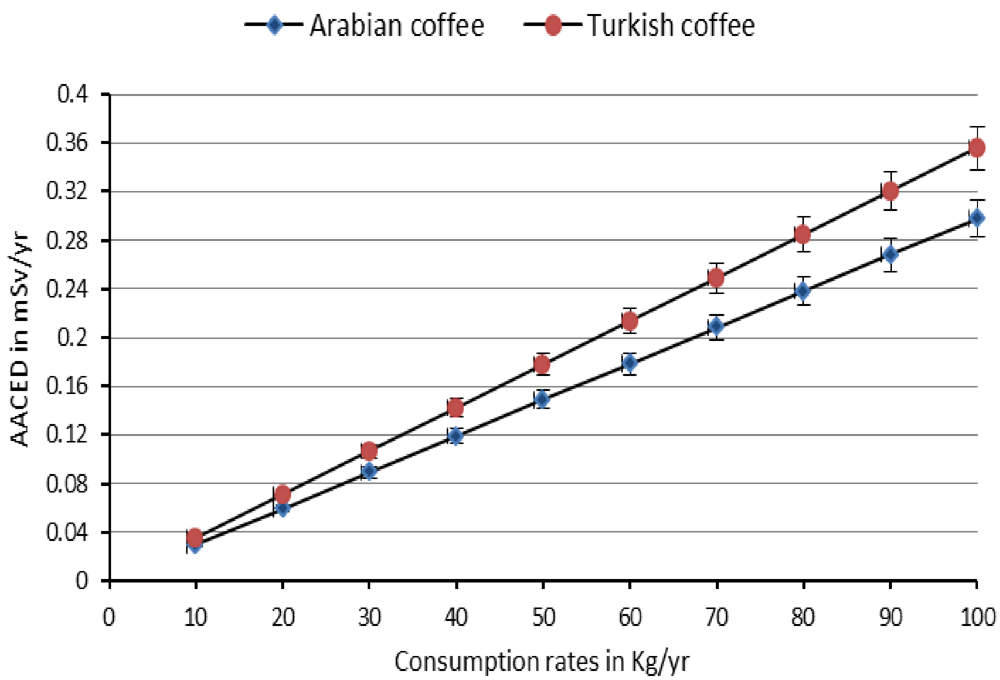

Figure 5. The AAECD as a function of various consumption rates within the range of 0 to 100 kg/year.

The highest AACED value for Arabian coffee powder was associated with sample CA5 because of the high activity concentrations of ^{232}Th, ^{226}Ra, and ^{40}K, while sample CA10 had the lowest AACED value. As shown in Tables 2 and 6, sample CT24 had the highest AACED value for Turkish coffee powder because of the high activity concentration of ^{40}K, while sample CT20 had the lowest value owing to the low activity concentrations of ^{232}Th, ^{226}Ra, and ^{40}K. Figure 5 presents the AAECD as a function of various consumption rates within the range of 0 to 100 kg/year.

The threshold consumption rate (Table 5 and 6) is the amount at which the AACED value exceeds 0.3 mSv for any of the coffee powders. Consequently, Tables 5 and 6 provide baseline data indicating that an individual with a consumption rate below the threshold values would experience an insignificant radiological health risk, but someone with a consumption rate that is slightly higher than the threshold values faces significant radiological health risk.

Statistical analysis

Descriptive statistics

Basic descriptive statistics were used to characterize the central tendency and variation of the data. The distribution of radionuclides measured in Arabian and Turkish coffees and the radiological hazards using SPSS, version16.0 for Windows (Sivakumar et al., 2014). Figures 6 and 7 shows the distribution of radionuclides (^{226}Ra, ^{232}Th, ^{40}K, and ^{238}U) with respect to sample code numbers in the Arabian and Turkish coffee. Tables 7 and 8 summarize the statistical parameters such as minimum, maximum, arithmetic mean, standard deviation, skewness, and kurtosis. The asymmetry of the probability distribution for real values of random variables can be characterized by measuring the degree of skewness. A normal distribution has a skewness of zero. Therefore, positive (or negative) skewness indicates a distribution with an asymmetric tail extending towards values that are more positive (or negative) (Adam and Eltayeb, 2012). The radionuclides in Arabian and Turkish coffees (Tables 7 and 8) have positive skewness values, indicating that the distributions are asymmetric, with the exceptions of ^{40}K, ^{232}Th, and ^{226}Ra in Arabian coffee and ^{40}K in Turkish coffee.

Kurtosis determined if the distributions for data are peaked or flat relative to a normal distribution. Positive kurtosis indicates a relatively peaked distribution, while negative kurtosis indicates a relatively flat distribution. Higher kurtosis means that more of the variance is explained by infrequent extreme deviations, as opposed to frequent slight deviations (Raghu et al., 2015). According to Tables 7 and 8, the distributions associated with ^{40}K and ^{238}U in Arabian coffee and for ^{40}K and ^{226}Ra in Turkish coffee have positive kurtosis values, indicating peaked distributions, and the others radionuclides have negative kurtosis values, indicating flat distributions.

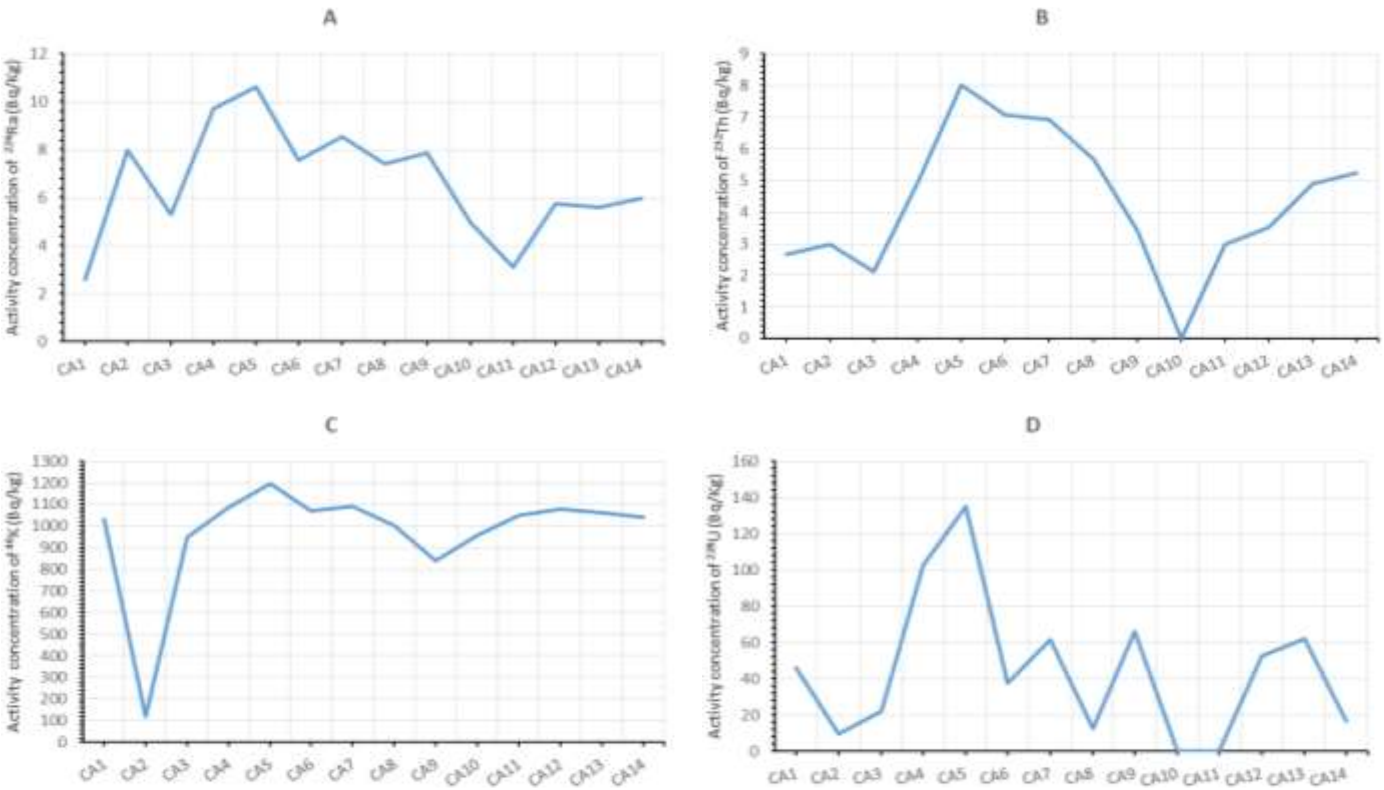

Figure 6. The distributions of ^{226}Ra, ^{232}Th, ^{40}K, and ^{238}U with respect of sample code numbers in Arabian coffee.

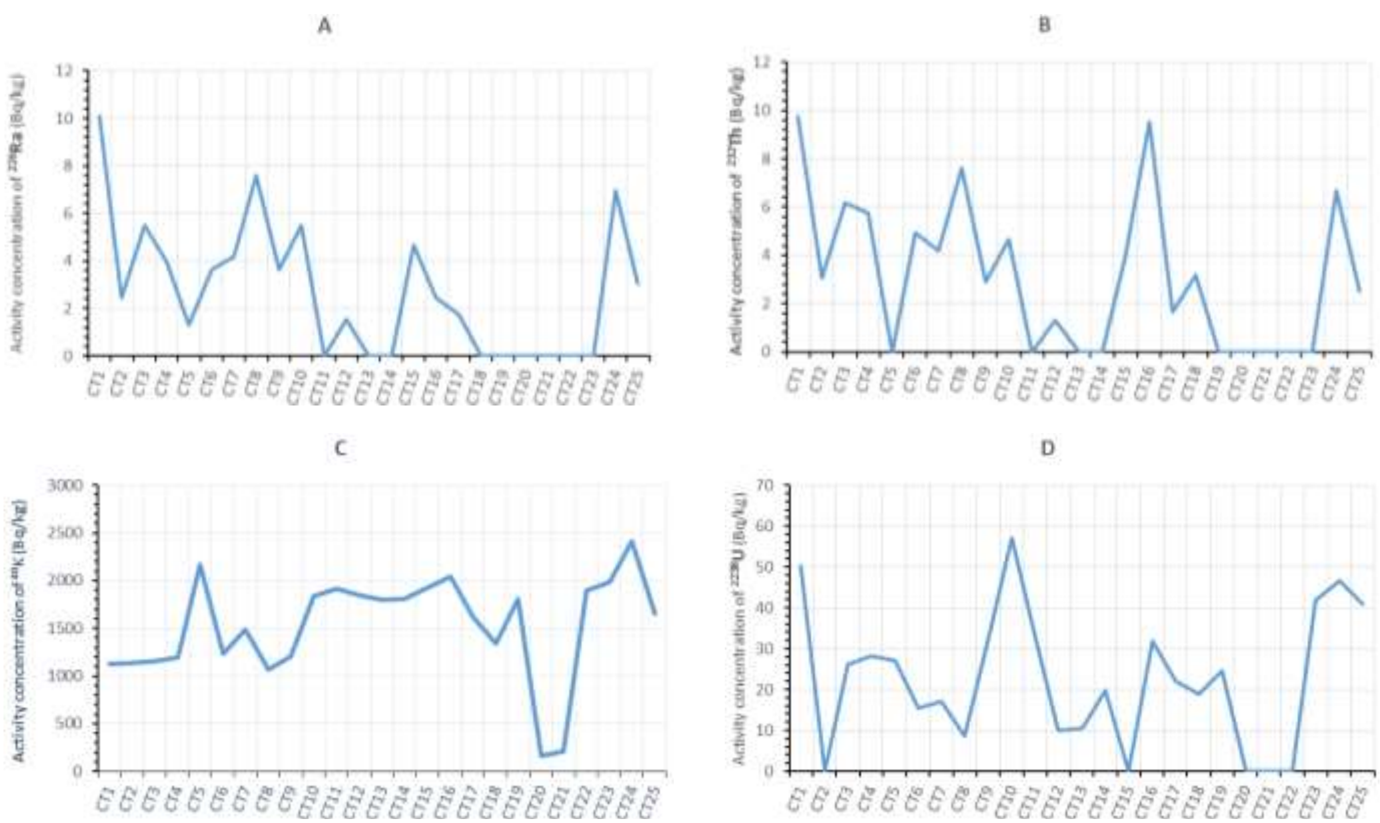

Figure 7. The distributions of ^{226}Ra, ^{232}Th, ^{40}K, and ^{238}U with respect of sample code numbers in Turkish coffee.

Table 7. Descriptive statistics of radionuclides and radiological hazard of Arabian coffee.

Parameter	Minimum	Maximum	Mean	Std. Deviation	Skewness	Kurtosis
^{40}K	118.9±36.33	1197.11±65.99	969.809±49.986	258.486	-3.112	10.633
^{232}Th	0.0±0.0	8.01±1.64	4.306±0.906	2.193	-0.067	-0.259
^{226}Ra	2.57±0.42	10.63±2.84	6.634±1.281	2.313	-0.113	-0.400
^{238}U	0.0±0.0	135.11±24.61	44.61±7.947	39.458	0.975	0.703
Radium equivalent	77.382	114.254	92.778	9.956	0.299	0.354
Absorbed dose rate	40.703	59.665	48.655	5.083	0.403	0.249
Annual effective dose rate	0.200	0.293	0.240	0.024	0.236	0.423
External hazard	0.209	0.309	0.251	0.027	0.300	0.366
Internal hazard	0.227	0.337	0.268	0.031	0.565	0.338
AACED	0.002±0.00	0.004±0.001	0.003±0.0	0.000	0.603	0.297
Threshold consumption rate	75.758	129.310	103.064	15.035	0.045	-0.584

Table 8. Descriptive statistics of radionuclides and radiological hazard of Turkish coffee.

Parameter	Minimum	Maximum	Mean	Std. deviation	Skewness	Kurtosis
^{40}K	161.31±48.8	2411.22±83.68	1523.1±48.70.09	546.8438	-0.9975	1.113
^{232}Th	0.0±0.0	9.75±1.86	3.12±0.608	3.1298	0.693	-0.5042
^{226}Ra	0.0±0.0	10.09±2.18	2.73±0.528	2.8263	0.9141	0.322
^{238}U	0.0±0.0	57.16±10.54	22.52±4.23	16.7982	0.286	-0.7029
Radium equivalent	12.421	202.159	124.5858	42.5839	-1.1515	2.237
Absorbed dose rate	6.727	107.791	66.7091	22.964	-1.1289	2.0381
Annual effective dose rate	0.033	0.529	0.3274	0.1128	-1.1296	2.0399
External hazard	0.034	0.546	0.3365	0.115	-1.1509	2.2335
Internal hazard	0.034	0.565	0.344	0.1163	-1.1858	2.5913
AACED	0.0001±0.0003	0.006±0.0004	0.0036±0.0002	0.0012	-1.2012	3.3361
Threshold consumption rate	50.167	937.5	139.5443	207.3985	3.4344	11.1314

Frequency distribution

To estimate the probability distribution of continuous variables the histograms are represented in Figure 8A to D) for Arabian coffee and in Figure 9A to D for Turkish coffee. The frequency distributions (according to the normality test for Shapiro-Wilk) for all activity concentrations in Arabian and Turkish coffees were analyzed (Tanaskovi et al., 2012). The graphs in Figure 8 show that the ^{40}K was distributed in non-normal distribution, while the ^{238}U, ^{232}Th, and ^{226}Ra were distributed in a normal (bell-shaped) distribution in Arabian coffee. All radionuclides in Turkish coffee were distributed in non-normal distribution except ^{238}U as shown in Figure 9.

Pearson's correlation analyses

Pearson's correlation analyses were performed to determine the interrelation between the natural radionuclides and the calculated radiological hazard parameters (Liu et al., 2003). The obtained correlation coefficients are presented in Tables 9 and 10. Positive correlations were observed between ^{226}Ra and ^{283}U with ^{232}Th in Arabian coffee, and also between ^{283}U and ^{226}Ra in Turkish coffee, but, it is less severe than it is in Arabic coffee. From the earlier stated observations, ^{226}Ra series and ^{232}Th series are usually found together in nature. Hence, these radionuclides contribute to the emission of gamma radiation in all regions. Positive correlations existed among of the most hazard parameters and ^{232}Th, ^{226}Ra, and ^{238}U in Arabian coffee. In addition, a strong correlation was observed between hazard parameters and ^{40}K and weak correlation between them and ^{238}U in Turkish coffee. Moreover, strong correlations among most of the radiological hazard parameters were noted and each other.

Conclusion

In this study, measurement of radioactivity in Arabian and Turkish coffee powders sold in Saudi Arabia markets was

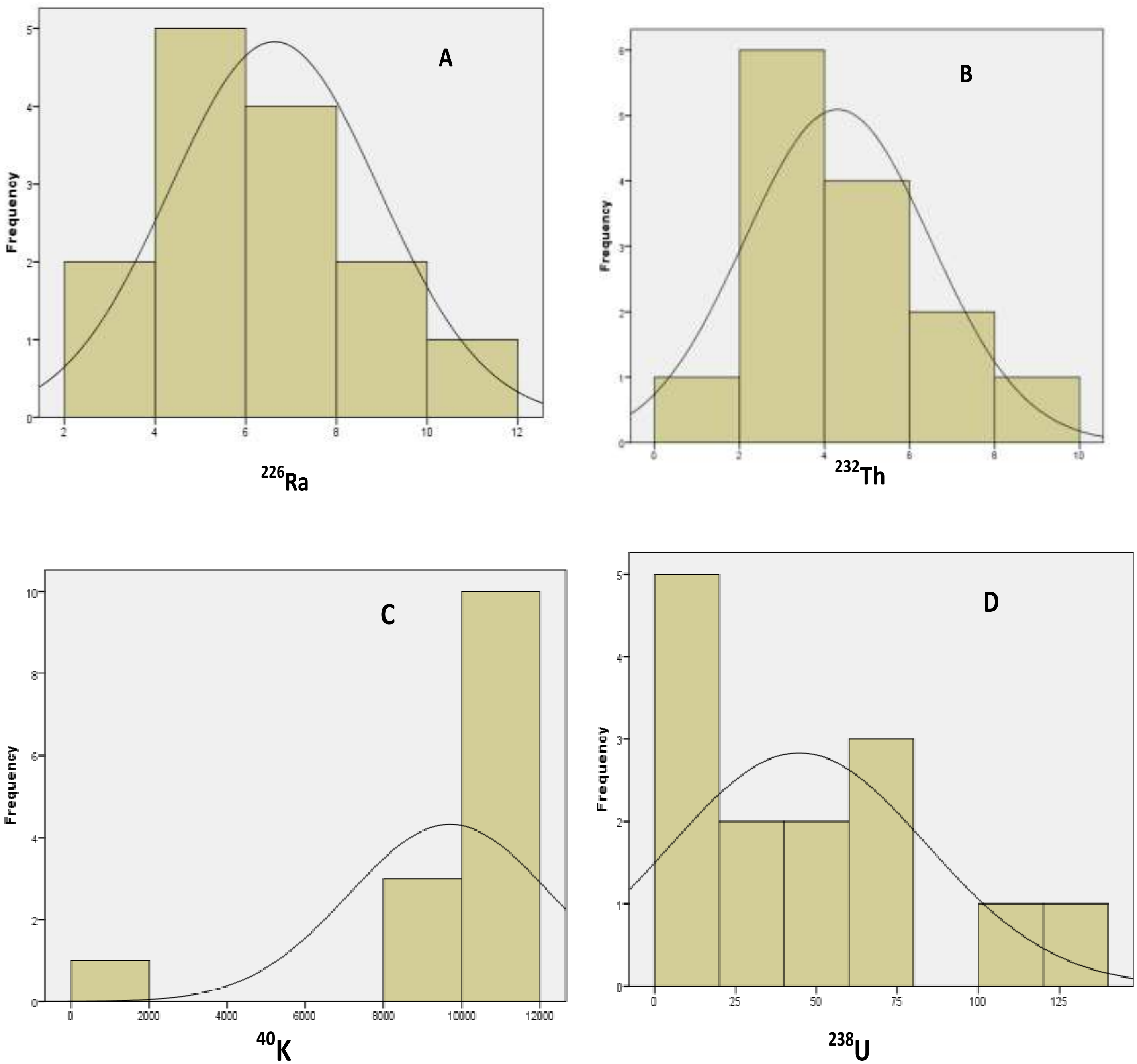

Figure 8. Frequency distributions of activity concentrations of ^{226}Ra, ^{232}Th, ^{40}K, and ^{238}U in Arabian coffee.

performed. The ^{40}K, ^{232}Th, ^{226}Ra, and ^{238}U concentrations were comparable with those reported elsewhere. The mean ^{40}K activity concentrations in Turkish coffee were greater than those in Arabian coffee, and both were higher than the allowable value. The mean ^{238}U activity concentrations in Arabian coffee were greater than in Turkish coffee, but both were less than the permissible limit. In addition, the mean activities of ^{232}Th and ^{226}Ra in Arabian and Turkish coffee powders were much less than the recommended limit. To assess the radiological risk, the mean values of the radium equivalent, absorbed dose rate, annual effective dose rate, H_{ex}, and H_{in} were estimated, and all were less than the allowable limits reported by UNSCEAR (2010). The results specified for all samples under study are far below the AACED limit (0.3 mSv/year for individual) provided by UNSCEAR (2010). Thus, these results indicate that the radiological risk related to drinking coffee is insignificant. Coffee shop

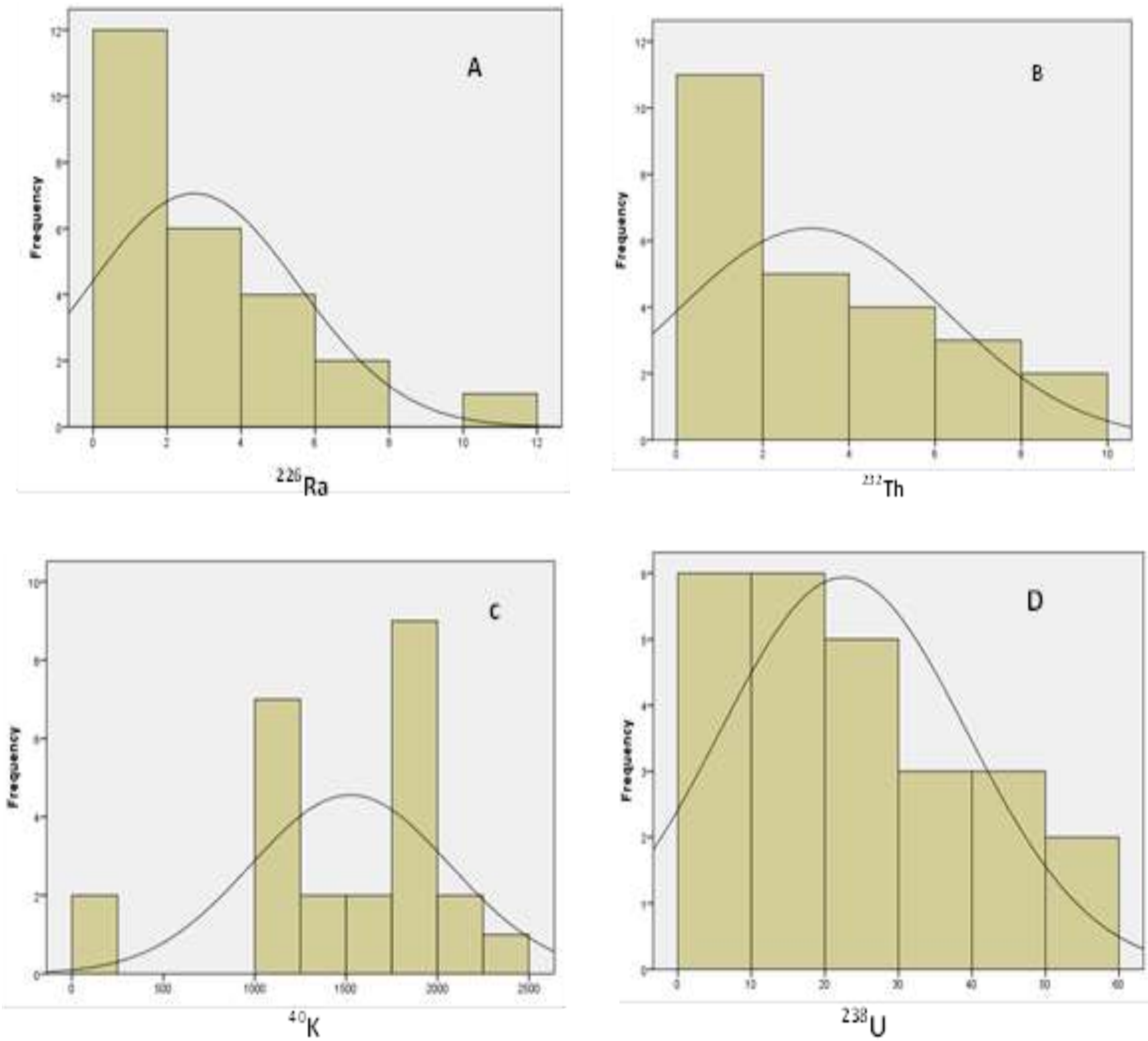

Figure 9. Frequency distributions of activity concentrations of ^{226}Ra, ^{232}Th,^{40}K, And ^{238}U in Turkish coffee.

workers may face a greater health hazard because they experience external exposure to potentially large amounts of coffee powder and may thus accumulate large doses.

The annual effective ingestion dose from coffee should be added to the total global dose from food consumption. The data obtained from this research provides information on the activity concentration of natural radionuclides and increases the knowledge about natural radioactivity. The statistical analysis also confirms that these samples under the study of Arabian and Turkish coffees do not possess significant gamma radiation effects. Coffee quality must be strictly controlled, and the study of radionuclide concentration in this matrix has great significance. In terms of the coffee market, determining the radioactivity values of the commodity is important for customers.

Conflicts of Interests

The authors have not declared any conflict of interests.

Table 9. Pearson correlation coefficients of radionuclides and radiological hazard of Arabian coffee.

Parameter	^{40}K	^{232}Th	^{226}Ra	^{238}U	Radium equivalent	Absorbed dose rate	Annual effective dose rate	External hazard	Internal hazard	AACED
^{40}K	1	0.372	-0.054	0.399	0.135	0.093	0.119	0.134	0.099	0.147
^{232}Th		1	0.656*	0.586*	0.833**	0.769**	0.805**	0.832**	0.853**	0.908**
^{226}Ra			1	0.649*	0.645*	0.637*	0.619*	0.644*	0.735**	0.786**
^{238}U				1	0.563*	0.504	0.550*	0.559*	0.588*	0.631*
Radium equivalent					1	0.967**	0.999**	1.000**	0.990**	0.960**
Absorbed dose rate						1	0.967**	0.968**	0.958**	0.919**
Annual effective dose rate							1	0.999**	0.983**	0.945**
External hazard								1	0.990**	0.959**
Internal hazard									1	0.985**
AACED										1

*Correlation is significant at the 0.05 level (2-tailed).** Correlation is significant at the 0.01 level (2-tailed).

Table 10. The Pearson correlation coefficients of radionuclides and radiological hazard of Turkish coffee.

Parameter	^{40}K	^{232}Th	^{226}Ra	^{238}U	Radium equivalent	Absorbed dose rate	Annual effective dose rate	External hazard	Internal hazard	AACED
^{40}K	1	-0.018	-0.026	0.414*	0.987**	0.991**	0.991**	0.987**	0.975**	0.915**
^{232}Th		1	0.850**	0.367	0.140	0.110	0.110	0.140	0.194	0.375
^{226}Ra			1	0.413*	0.127	0.099	0.099	0.127	0.190	0.356
^{238}U				1	0.475*	0.464*	0.465*	0.476*	0.497*	0.546**
Radium equivalent					1	1.000**	1.000**	1.000**	0.998**	0.968**
Absorbed dose rate						1	1.000**	1.000**	0.996**	0.960**
Annual effective dose rate							1	1.000**	0.996**	0.960**
External hazard								1	0.998**	0.968**
Internal hazard									1	0.982**
AACED										1

*Correlation is significant at the 0.05 level (2-tailed) .** Correlation is significant at the 0.01 level (2-tailed).

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Full Length Research Paper

Adaptability and stability of wheat cultivars sown on different dates in West Paraná

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This study aimed to assess adaptability and stability of different wheat cultivars on different sowing dates in western region of Paraná, Brazil. The assay was composed by following wheat cultivars: CD 154, CD 1252, CD 108, CD 151, CD 1550, CD 104, CD 1440, CD 116, CD 150, and CD 1104, which were sown on three different dates: April 29th 2014, May 20th 2014, and June 26th 2014. The methods used to determine stability and adaptability of production were: Traditional, Lin and Binns adapted by Carneiro, Eberhart and Russell and the Integrated. There was an agreement between Eberhart and Russell and Integrated methods for cultivars CD 104, CD 151, CD154 and CD 1440, which have general adaptability. It was also noted an agreement between methods of Lin and Binns and Eberhart and Russell on stability of cultivars CD 108, CD 1104 and CD 1440. The cultivars CD 108 and CD 1440 are considered stable and with wide adaptation and may be indicated to Western Paraná, regardless of sowing date. The cultivar CD 1104 may be indicated to be sown between April and May, while the CD 150 may be suitable for the later sowings, in late June.

Key words: Eberhart and Russell, integrated method, Lin and Binns, traditional method.

INTRODUCTION

Over time wheat breeding has led to the development of early maturity genotypes, with lower height, more tolerant to diseases, more productive and with higher grain quality (Almeida et al., 2007; Cruz et al., 2010; Sahrawat et al., 2003; Salomon et al., 2003). However, the expression of genetic potential of these genotypes varies according to environment. In this scenario, it is necessary to analyze interaction between genotype and environment to demonstrate how environmental variation may interfere in performance of each genotype. This kind of analysis may help selection of the best genotype based on the environmental average (Cargnin et al., 2006).

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Some models for analysis of adaptability and stability allow breeder to perceive the behavior of each genotype in favorable and unfavorable environments. They provide breeders necessary information to select the best genotype for each environment or a genotype that is proper in both environments (Biudes et al., 2009; Caierão et al., 2006; Silva et al., 2011).

On the Traditional method, which was suggested by Yates and Cochran (1938), a joint analysis of experiments (environments) was performed and the variance mean square of environments within each genotype is determined. The genotypes that have lower mean square of environmental variance are considered more stables. The adaptability can be defined by average of genotype (Cruz et al., 2012).

Eberhart and Russel (1966) proposed a model for analysis of adaptability and stability based on analyzed variable regression (dependent variable) in relation to environmental indexes, which are genotype average within each environment (independent variable). Therefore, by slop analysis of the line (β1), it becomes possible to identify adaptability of material to environment. Furthermore, it is possible to infer its stability by regression deviation (δ), where a more stable genotype has a lower regression deviation. The genotypes may present wide adaptability (β1=1), specific adaptability to favorable environments (β1>1) and specific adaptability to unfavorable environments (β1<1).

Methods based on regression models have been quite practical and they are widely utilized in plant breeding. However, there are some limitations in use of such methods; among this, difficulty of properly analyzed genotypes whose behavior are not linear or having complex genotype-environment interaction. In this context, centroid method, based on principal components, can overcome issues of regression-based methods, because in centroid method, the similarity between genotypes and ideotypes pre-established is calculated by Cartesian distance, in order to rank genotypes in relation to ideotypes. The ideotypes are ideotype with maximum general adaptability (ideotype I), ideotype with maximum specific adaptability to favorable environments (ideotype II), ideotype with maximum specific adaptability to unfavorable environments (ideotype III) and ideotype with a minimal adaptability (ideotype IV) (Vasconcelos et al., 2011).

The centroid method allows one to evaluate adaptability of genotypes that present a genotype-environment interaction more complex, but it does not present analysis of stability of genotypes. To fulfill this need, Vasconcelos et al. (2011) included three new ideotypes to centroid method: ideotype with maximum phenotypic stability (ideotype V), ideotype with maximal specific adaptability to favorable environments and stable in adverse environments (ideotype VI) and ideotype with maximal specific adaptability to unfavorable environments and stable in favorable environments (ideotype VII). By integrating these three new ideotypes to centroid method, Vasconcelos et al. (2011) renamed this method to Integrated method.

Lin and Binns (1988) developed a non-parametric method to evaluate adaptability and stability. In this method, reference is defined as the highest average for each environment and then distance between mean of each genotype and reference is calculated, within each environment, which is named as P_i. Lower is the P_i, higher is general adaptability of genotype.

The advantages possibility of this method of working with few environments, identify small differences between genetic materials and define a coherent analysis to data that do not fit into linear models. The disadvantage lies in possibility measuring only broad adaptability. In order to overcome this problem, Carneiro (1998) adapted model grouping environments into favorable and unfavorable and calculating P_i for each group (favorable and unfavorable), therefore allowing evaluation of specific adaptability besides general adaptability.

In general, in a breeding program of wheat, the selection of cultivars with high productivity, high stability and high adaptability, allied with superior agronomic characteristics (cycle, plant architecture, resistance to major pests and diseases and post-harvest quality) is recommended (Borém and Miranda, 2013). Therefore, mean goal of this study was to determine adaptability and stability of different wheat cultivars on different sowing dates in Western Paraná, Brazil.

MATERIALS AND METHODS

The experiment was conducted in Western Paraná, Brazil, at the coordinates 24°33’ S and 54°31’ W and at 420 m above sea level. The region climate, according to Köppen classification, was classified as Cfa, with well distributed rainfall during year and with hot summers (Caviglione et al., 2000). The soil in experimental area is classified as typical Eutophic Red Oxisol (Embrapa, 2013).

The experimental design was complete randomized blocks composed by ten wheat cultivars: CD 154, CD 1252, CD 108, CD 151, CD 1550, CD 104, CD 1440, CD 116, CD 150, and CD 1104 (Table 1), with three blocks. The plots were constituted by nine rows five meters long, with 0.17 m of row spacing. Three trials were performed simultaneously in areas, on different sowing dates (April 29th 2014, May 20th 2014, and Jun 26th 2014).

The sowing density was determined according to Coodetec (2014) recommendations for the Marechal Cândido Rondon municipality, region 3, according to Brazil (2008) classifications, which considers the region as hot, moderately dry and low altitude.

Sowing operation was performed in a no-tillage system; fertilization was based on soil analysis (Table 2) and on CBPTT (2010) recommendations. During initial crop development, weed control was carried out manually to avoid competition and weed interference.

The climate data (Figure 1) were obtained from an automatic weather station, located 50 m from experimental site. The mean temperatures during trials, sown on April 29th, May 20th and Jun 26th, were 18.15, 18.64 and 19.32°C, respectively, and daily precipitation was 5.44, 2.22 and 4.68 mm, respectively, and total precipitation were 598.80, 157.60 and 430.80 mm, respectively.

The harvest was performed manually when about 90% of ears

Table 1. Density, plant height, lodging, cycle, cycle to heading stage and germination in spike, for wheat cultivars adapted to region 3 of Parana, 2014 (COODETEC, 2014).

Cultivar	Plant m^{-1}	Height	Lodging	Cycle	Cycle to heading stage	Germination in spike
CD 104	65	Low – 80 cm	Moderately resistant	Medium	74 days	Moderately susceptible
CD 108	75	Low –67 cm	Resistant	Super Early	53 days	Moderately resistant
CD 116	70	Low – 81 cm	Moderately resistant	Early	62 days	Moderately susceptible
CD 150	65	Low – 68 cm	Moderately resistant	Early	59 days	Moderately resistant
CD 151	70	Low – 77 cm	Moderately resistant	Medium	65 days	Moderately susceptible
CD 154	70	Low – 75 cm	Moderately resistant	Medium	66 days	Moderately susceptible
CD 1104	60	No information	Moderately susceptible	Medium	No information	Moderately resistant
CD 1252	70	Low – 78 cm	Moderately resistant	Early	66 days	Moderately resistant
CD 1440	60	Medium – 82 cm	Moderately resistant	Medium	74 days	Moderately resistant
CD 1550	60	Medium – 81 cm	Moderately resistant	Medium	76 days	Moderately resistant

Table 2. Chemical properties of soil in experimental area.

Layer	P	MO	pH	Al^{3+}	K^{+}	Ca^{2+}	Mg^{2+}	Cu	Fe	Zn	Mn	SB	V
cm	mgdm^{-3}	g dm^{-3}	$CaCl_2$					$cmol_c dm^{-3}$					%
0-20	43.40	20.51	5.06	0.00	1.03	4.72	1.69	17.80	34.10	99.99	3.10	7.44	67.45

Extrators: P and K (Mehlich); Ca, Mg, Cu, Fe, Mn, Zn e Al^{+3} (KCl 1 mol).

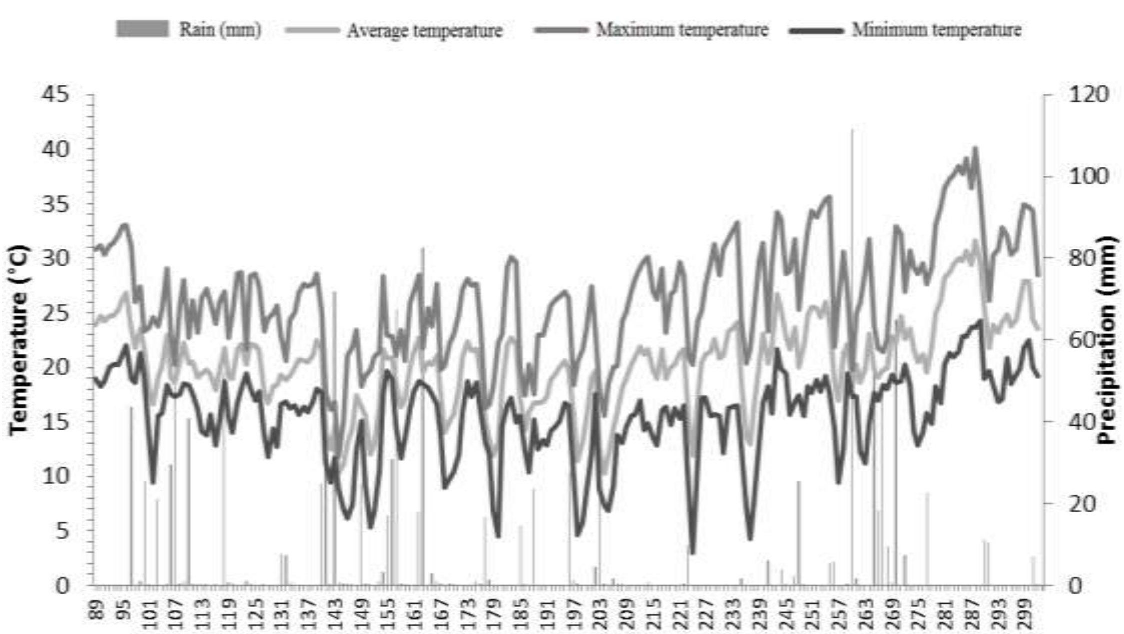

Figure 1. Rainfall distribution (mm) and average, maximum and minimum temperatures in period of Julian day number from 89 to 302 in Marechal Cândido Rondon - PR, in 2014.

were ripe and with grain moisture average of 12%. After harvest, material was subjected to mechanical threshing for grain yield determination.

Concepts and procedures of classical methods of analysis of adaptability and stability were introduced based on following methods: Traditional (Yates and Cochran, 1938), Lin and Binns (1988) adapted by Carneiro (1998), Eberhart and Russel (1966) and Integrated (Vasconcelos et al., 2011). The grain yield data were submitted to normality and homogeneity of variance test and afterwards, data were subjected to a joint analysis of variance,

Table 3. Summary of joint analysis of variance for productivity (kg ha^{-1}) of 10 wheat cultivars in 3 sowing dates, 2014, Marechal Cândido Rondon-PR.

Source of variation	df	SS	MS	F	
Blocks/Environments	6	-	-	-	
Cultivars (C)	9	16581228.43	1842358.71	1.74	ns
Environments (E)	2	932332202.84	4666101.42	36.61	**
C × E	18	19050135.67	1058340.87	3.73	**
Residual	54	15322716.45	283754.01	-	-
Total	89	145523780.16	-	-	-
Mean			1758.94	-	-
Coefficient of Variation (%)			30.28	-	-

* and **Significant on F-test at 5 and 1% of probability level, respectively.

followed by determination of adaptability and stability using software GENES (Cruz, 2013).

RESULTS AND DISCUSSION

Through individual analysis of variance of experiments, it was found that variances of residues were heterogeneous, by Hartley (1950) criteria, requiring an adjustment according to Cochran (1954) method enable joint analysis, with all test environments. In addition, it was observed that the ratio between the highest and lowest residual mean square of three sowing dates did not exceeded threshold value of seven (Pimentel Gomes, 2009). Through joint analysis of variance (Table 3), a significant interaction was found between genotypes and environments ($p \leq 0.05$), basic premise for analysis of phenological adaptability and stability of genotypes.

The coefficients of variation(CV) were 24.04, 31.07 and 15.61% in assays sown on April 29th, May 20th and Jun 26th, respectively, which are classified as high by Pimentel Gomes (2000), confirming unpredictable factors on grain yield influence. This classification has been challenged due to its range and for disregarding crop specificities and the nature of evaluated characteristics (Nunes, 2012).

When considering Traditional method (Table 4), it was observed for all cultivars that mean square of environment within genotype was significant, indicating that none of them has production stability. The ideal genotype may have been discarded, since methodology uses only one regression coefficient and deviations that should be examined in different environments, may be relatively high in relation to estimated line (Cruz et al., 2012). The highest average yield was obtained in cultivars CD 1104 (2508.06 kg ha^{-1}), CD 108 (2304.74 kg ha^{-1}), and CD 1440 (2135.09 kg ha^{-1}), which seem to be best adapted to region studied.

The cultivars CD108, CD 116, CD 150, CD 1440, and CD 1550 were considered ideal by Eberhart and Russell method (Table 4), because they exhibited general adaptability and production stability. The cultivars CD 1104 and CD 1253, despite being stable, they were classified as adapted to favorable and unfavorable environments, respectively. All cultivars obtained estimative of coefficient of determination (R^2) higher than 80%, except CD 151, showing in general a proper adjustment of data to regression line, indicating a high predictability of their behavior.

According to method of Lin and Binns (Table 4), cultivars CD 1104, CD 108, CD 1440, and CD 154 were classified as most stable and adapted because they presented smallest Pi values and the highest productivity. In decomposition of Pi described by Carneiro, cultivars CD 1104, CD 108, CD 1440, and CD 154 were classified as adapted and stable to favorable environments, whereas cultivars CD 150, CD 108, CD 151 and CD 1440 were adapted and stable in unfavorable environments. This approach has main advantage of enabling immediate knowledge of cultivars that are more stable (Pereira et al., 2009).

Using Integrated method (Table 4), the cultivars CD 104, CD 151, CD 154 and CD 1440 showed high general adaptability (Class V), whereas cultivars CD 108 and CD 1104 had specific adaptability to favorable environments (Class VI and II, respectively), and cultivar CD 150 with specific adaptability to unfavorable environments (Class VII). Other cultivars were classified as being of low adaptability (Class IV).

Methods of Eberhart and Russell and Integrated agreed with each other for cultivars CD 104, CD 151, CD 154 and CD 1440, which have general adaptability, and for cultivar CD 1104 with specific adaptability to favorable environments. These methods are complementary and they increase reliability in classification and recommendation of cultivars (Peluzio et al., 2008). An agreement was also observed between the methods of Lin and Binns and Eberhart and Russell, where cultivars CD 108, CD 1104 and CD 1440 exhibited productive stability. Similar results were found by Escobar et al. (2016) in maize and Romanato et al. (2016) in soybean. However, several studies comparing methodologies of genotypic stability and adaptability observed low

Table 4. Estimation of adaptability and stability parameters for grain yield of ten wheat cultivars, based on different methodologies: Traditional (Yates and Cochran, 1938), Eberhart and Russell (1966), Lin and Binns (1988) modified by Carneiro (1998) and Integrated (Vasconcelos et al., 2011).

Cultivars	Average	Traditional	Eberhart and Russell			Linn and Bins, adapted by Carneiro -						Integrated
		Q.M.	ß1	σ²di	R^2 (%)	Order	Pi general	Order	Pi fav.	Order	Pi unf.	Class.
CD 104	1582.48	13739353.86**	1.13^{ns}	499539.80*	87.11	5°	814910.40	5°	1186947.00	10°	70836.67	V
CD 108	2304.74	15129801.43**	1.26^{ns}	-3010.04^{ns}	98.26	2°	121187.70	2°	174002.80	2°	15557.63	VI
CD 116	1347.60	4543028.35**	0.67^{ns}	7234.05^{ns}	93.54	8°	1290692.00	8°	1902641.00	8°	66794.38	IV
CD 150	1598.31	4885834.35**	0.71^{ns}	-52522.10^{ns}	97.66	7°	945007.00	7°	1417511.00	1°	0.00	VII
CD 151	1885.40	9374642.82**	0.73^{ns}	1365160.00**	53.41	6°	867929.50	6°	1292011.00	3°	19766.94	V
CD 154	1796.57	16739450.79**	1.26^{ns}	516956.80*	89.11	4°	558656.60	4°	810401.60	5°	55166.56	V
CD 1104	2508.06	25053815.55**	1.64**	-89519.40^{ns}	99.99	1°	31526.24	1°	14938.26	7°	64702.19	II
CD 1252	1153.33	4091056.81**	0.65*	-54653.30^{ns}	97.36	10°	1590118.00	10°	2351780.00	9°	66794.38	IV
CD 1440	2135.09	13426990.40**	1.18^{ns}	53621.37^{ns}	96.78	3°	235997.30	3°	336373.60	4°	35244.49	V
CD 1550	1277.82	5298364.15**	0.74^{ns}	-38161.20^{ns}	97.09	9°	1333717.00	9°	1970802.00	6°	59546.38	IV

Class I - High general adaptability (Maximum in a favorable environment, Maximum in unfavorable environment); Class II - Specific adaptability to favorable environment (Maximum in a favorable environment, Minimum in an unfavorable environment); Class III - Specific adaptability to unfavorable environments (Minimum in a favorable environment, Maximum in unfavorable environment); Class IV - Low adaptation (Minimum in a favorable environment, Minimum in an unfavorable environment);Class V- High general adaptability (Average in favorable environment, Average in unfavorable environment); Class VI - Specific adaptability to favorable environment (Maximum in a favorable environment, Average unfavorable environment); Class VII - Specific adaptability to unfavorable environments (Average in favorable environment, Maximum in unfavorable environment).

correlation between these methodologies (Nascimento et al., 2013; Pereira et al, 2009; Silva and Duarte, 2006) indicating that combined use may provide additional information on phenotypic stability of cultivars.

Conclusion

The cultivars CD 108 and CD 1440 are considered stable and have a wide range of adaptation and may be indicated for western region of Paraná, regardless of sowing dates. The cultivar CD 1104 may be recommended for sowing in April and May, while the CD 150, for later sowing in late June.

Conflict of Interests

The authors have not declared any conflict of interests.

ABBREVIATIONS

β1, Regression coefficient; **CBPTT,** Comissão Brasileira de Pesquisa de Trigo e Triticale; **Cfa,** temperate or subtropical hot summer climates; **Coodetec,** Cooperativa Central de Pesquisa Agrícola; **CV,** coefficients of variation; Embrapa, Empresa Brasileira de Pesquisa Agropecuária; **P_i,** genotypic performance; **R^2,** coefficient of determination; **δ,** regression deviation.

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Full Length Research Paper

Effect of seed desiccation and sucrose concentration on the *in vitro* establishment of mangabeira (*Hancornia speciosa* Gomes var. *gardneri*) seedlings

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Mangabeira (*Hancornia speciosa* Gomes var. *gardneri*) is one of the most important fruit trees in the cerrado biome, its fruits being highly valued both for *in natura* consumption and for processing. Although its seeds are delicate, losing viability within a few hours after collection, seeds still represent the predominant tool of propagation multiplication of this species, since the plant encounters limitations in the formation of adventitious roots, hindering vegetative propagation. Thus, the present work aims to evaluate the *in vitro* establishment of Mangabeira seedlings as a function of the extent of natural desiccation and the sucrose concentration in the culture medium. Four sucrose concentrations (15, 30, 45 and 60 g L^{-1}) and four natural drying periods (24, 48, 96 and 192 h after the seed pulping) were used in a factorial experiment. 60 days after in vitro culturing of the naked embryos, the following variables were evaluated: germination %, germination speed index (GSI), mean germination time (MGT), oxidation index, culture medium contamination index, length of the largest root, stem diameter, seedling height and number of live and dead leaves. The interaction between desiccation times and sucrose concentrations was not significant for any of the variables analyzed. Germination was influenced only by the desiccation time, being reduced after 106 hours of natural drying. Increasing concentrations of sucrose up to 60 g L^{-1} reduced GSI and seedling height. In contrast, leaf mortality decreased, which contributed to the production of seedlings with greater ability of acclimatization to field conditions.

Key words: Germination, *in vitro* cultivation, desiccation, *Hancornia speciosa* Gomes.

INTRODUCTION

Mangabeira (*Hancornia speciosa* Gomes var. *gardneri*) is a tropical fruit tree of medium size, spontaneously vegetating in several regions of Brazil (Lorenzi, 2002; Costa et al., 2011). The fruits vary from round to oval, with a diameter of 2-6 cm, and white, soft fibrous pulp, enclosing 2-15 flattened, discoid seeds (Lorenzi, 2002; Santos et al., 2010). Fruit harvesting usually begins in November-December and extends to May-June (Vieira

Neto, 2002).

All parts of the mangabeira tree produce white or pale pinkish latex that can be exploited in the production of rubber, in folk medicine for the treatment of warts, in waterproofing of fabrics and in the production of bags (Barros et al., 2010; Silva et al., 2011). The tree can also be used as firewood and in the reforestation of deteriorated areas. However, fruits are the main product, being extremely appreciated for their aroma and nutritional value. Fruits have many uses; they can be consumed fresh or rather used in, the manufacture of soft drinks, ice creams, jams, syrups and the preparation of wine (Carnelossi et al., 2009; Barros et al., 2010).

The propagation of this species by traditional methods has been hampered by the fact that its seeds are delicate, rapidly losing their viability as soon as they are removed from the fruit (Santos et al., 2010). Another factor that hinders propagation is the inhibitory action of the fruit pulp on seed germination. Hence, there is a need for a thorough investigation to evaluate the sensitivity to desiccation of the seeds of this species (Bovi et al., 2004; Barrozo et al., 2014). Nevertheless, in view of the great diversity of the native tropical tree species in Brazil, whose seeds are intolerant to desiccation, it is necessary to develop specific technologies for conservation during storage (Maluf et al., 2003), or maintaining the viability of the seeds that are subjected immediate germination rapidly after fruit collection.

An efficient way to propagate the delicate species, such as mangabeira, is the *in vitro* establishment of seeds. This technique allows maximizing the germination rate, production of uniform seedlings with adequate genetic and phytosanitary quality (Stein et al., 2007). Moreover, *in vitro* cultured seeds exhibit greater germinability than those raised in nurseries, because the *in vitro* conditions are more suitable for germination processes and early seedling development (Noleto and Silveira, 2004). The application of tissue culture techniques to cerrado fruit trees can promote systematized multiplication of plants, exchange of genetic material, germplasm rescue and preservation of a threatened material. In these species, the *in vitro* germination has performed important achievements, allowing, in addition to other landmarks, the overcoming of dormancy within short time (Pinhal et al., 2011).

In vitro cultivation requires an exogenous source of energy to the plants, since in these environments plants have limited photosynthetic activity. Sucrose has been the most widely used carbon source, added to culture media in concentrations ranging from 20 to 40 g L^{-1} (Ferreira et al., 2002); which might affect the osmotic potential of the medium and hence the metabolism and growth of the cultured plant (Reis et al., 2008). Therefore, the objective of this study is to evaluate the impacts of both natural desiccation of seeds and the sucrose concentration in the culture medium on the *in vitro* establishment of Mangabeira (*H. speciosa* Gomes var. *gardneri* (A.DC).

MATERIALS AND METHODS

Fruits of Mangabeira (*H. speciosa* Gomes var. *gardneri*) were obtained in November 2015 from the germplasm collection of the Agronomy School of the Federal University of Goiás (EA/UFG, Brazil) established in December 2005. After collection, the fruits were sent to the EA/UFG biotechnology laboratory, where they were washed and stored in plastic containers for 6 days, at 8ºC. The fruits were then manually pulped and washed in running tap water until the pulp was completely removed and placed on paper towel for natural drying for 24, 48, 96 and 192 h at 25°C and 65% relative humidity. Seeds with no injuries or mechanical damage were selected. Twenty seeds from each drying period were randomly selected for moisture content determination in an oven at 105°C for 24 h.

Seed coat was removed by hand, and desiccated seeds were disinfected following the aseptic methodology of Vieira (2014). After seed coat removal, the naked embryos were washed in distilled water, and then in autoclaved water containing commercial detergent. Subsequently, the seeds were rinsed three times with distilled water, followed by immersion in 70% alcohol for two minutes and in 100% sodium hypochlorite for five minutes and finally by three times rinsing in distilled water to remove excess hypochlorite.

In a laminar flow chamber, the embryos were immersed for 30 min in 100% sodium hypochlorite, followed by three rinses with distilled and autoclaved water to remove excess hypochlorite. The embryos were then incubated in 200 mL glass vials containing, 30 mL of half-strength macronutrient solution of Murashige and Skoog culture medium (Murashige and Skoog, 1962), amended with 0.10 mg L^{-1} pyridoxine; 1.0 mg L^{-1} thiamine; 0.10 mg L^{-1} myo-inositol, 2 mg L^{-1} indolebutyric acid (IBA), 1 g L^{-1} activated charcoal, and different concentrations (15, 30, 45 and 60 g L^{-1}) of sucrose.

The experiment was factorial, with two factors and 10 replications in a completely randomized design. The main factors were desiccation period with four levels (24, 48, 96 and 192 h) and sucrose concentration with four levels (15, 30, 45 and 60 g L^{-1}). Each replicate was a 200 mL glass vial containing 30 mL of the modified Murashige and Skoog culture medium and inoculated with one Mangabeira embryo.

The inoculated glass vials were incubated in the dark for 48 h at a temperature of 25 ± 2°C, and then under a light period of 16 hours and photon flux density of 35 µmol m^{-2} s^{-1} for 60 days. The cultures were investigated daily, to determine the oxidation index (OI), contamination index (CI) and to identify the contaminant fungi and/or bacteria. At the end of incubation period, germination speed index (GSI), mean germination time (MGT), seedling height, stem diameter, number of live and dead leaves per seedling and length of the largest root.

Germination speed index was calculated using the following equation: $GSI = \sum_{i=1}^{n} (\frac{G}{N})$, described by Maguire (1962), where G

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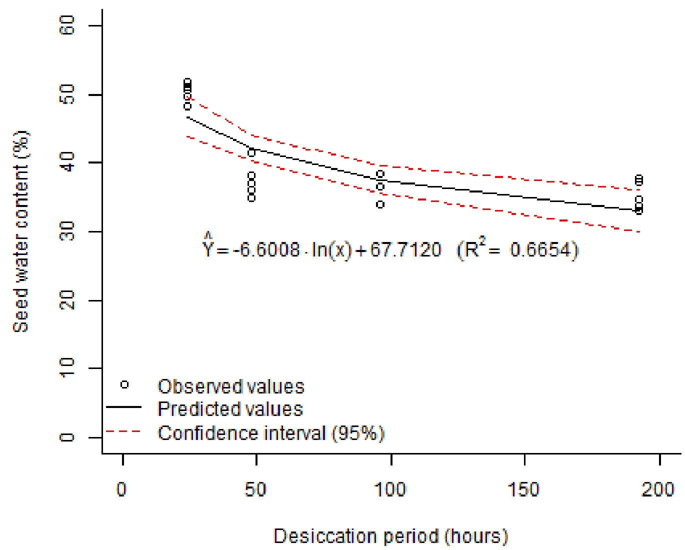

Figure 1. Water loss in mangabeira seeds (*Harconia speciosa* var. g*ardneri*) submitted to different periods of natural desiccation.

represents the number of normal seedlings germinated per day, divided by the number of days N elapsed between sowing and germination. The mean germination time was obtained from the equation: $\mathrm{MGT} = \frac{\sum_{i=1}^{n} (G \times N)}{T}$, where G represents the number of normal seedlings germinated per day, N is the number of days elapsed between sowing and germination and T is the total number of germinated seeds (Labouriau, 1983). The length of the largest root (cm) was obtained by measuring the primary roots with graduated ruler. Also, the percentage of contamination and oxidation of the medium during the experimental period was determined.

Data were submitted to analysis of variance using the statistical program R Core Team version 3.3 (2016). When there were significant differences between treatments, regression analysis was used for determination of the optimum sucrose concentration and inoculation time after pulping for the *in vitro* establishment of mangabeira seedlings.

RESULTS AND DISCUSSION

There was non-significant interaction ($p > 0.05$) between the seed desiccation period and sucrose concentration of the medium on the evaluated variables. Regression analysis revealed a pronounced loss of seed water from 50.22 to 35% with the increase in drying time from 24 to 192 h (Figure 1).

The water loss by seeds was crucial for seed germination and seedling development (in terms of the number of viable leaves) (Figure 2). A trend of quadratic behavior was observed between the extent of desiccation in one hand and seed germination and seedling development in the other hand; that is mangabeira seeds attained maximum germination potential at 106 - 110 h of natural desiccation (equivalent to 36.8% seed water content). Regarding the seedlings originating from seeds dried for periods longer than 110 h, the marked reduction in the number of viable leaves means reduced seedling vigor caused by the decrease in the water content of seeds with the progress of desiccation period.

Barros et al. (2010) found that desiccation of mangabeira seeds for periods longer than 36 h (seed water content < 25.7) reduced seedling emergence. Poor seedling vigor and difficulty in maintaining leaves viable during the *in vitro* establishment were also observed in guariroba seeds kept at a constant temperature of 37ºC for a period of 12 days after harvest (Rubio Neto et al., 2015). Salomão et al. (2004), studying the effect of desiccation of mangabeira seeds on their viability, verified that water content lower than 26% fresh weight impaired germination capacity, and values below 11% led to complete loss of viability.

The mean germination time as well as the oxidation and contamination indexes were non-significantly affected by treatments and showed average values of 19.32, 1.85 and 2.50%, respectively, regardless of desiccation period and sucrose concentration of the medium. The removal of seed coat allows high efficiency of the disinfection protocol; thus lowering the opportunity of microbial growth and guaranteeing insignificant indices of contamination of the embryo culture. Although seed coat can serve as a physical barrier for water entry and also as a shelter against attack of pathogens, yet the presence of seed coat in embryo cultures might participate in infection of the culture with recalcitrant pathogens. The maintenance of the seed coat in seeds of *Mouriri elliptica* grown *in vitro* significantly increased the rate of culture contamination, reducing the viability of the embryo (Lima et al., 2016). Although mature mangabeira seeds possess high water content, which can promote

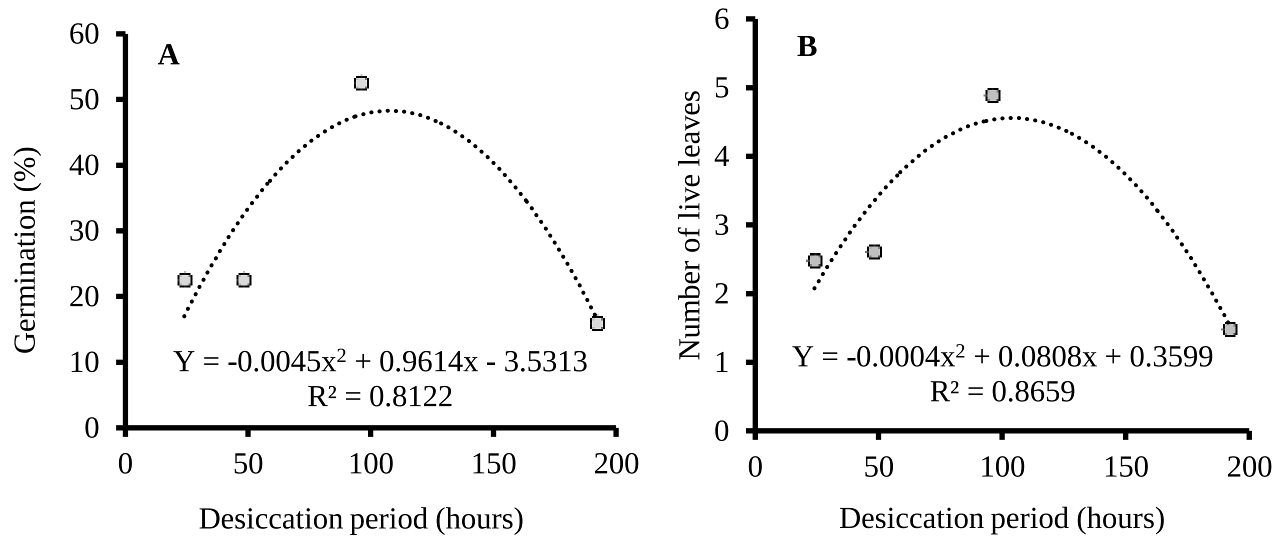

Figure 2. Germination percentage (A) and number of live leaves (B) of mangabeira seedlings grown *in vitro* up to sixty days after inoculation.

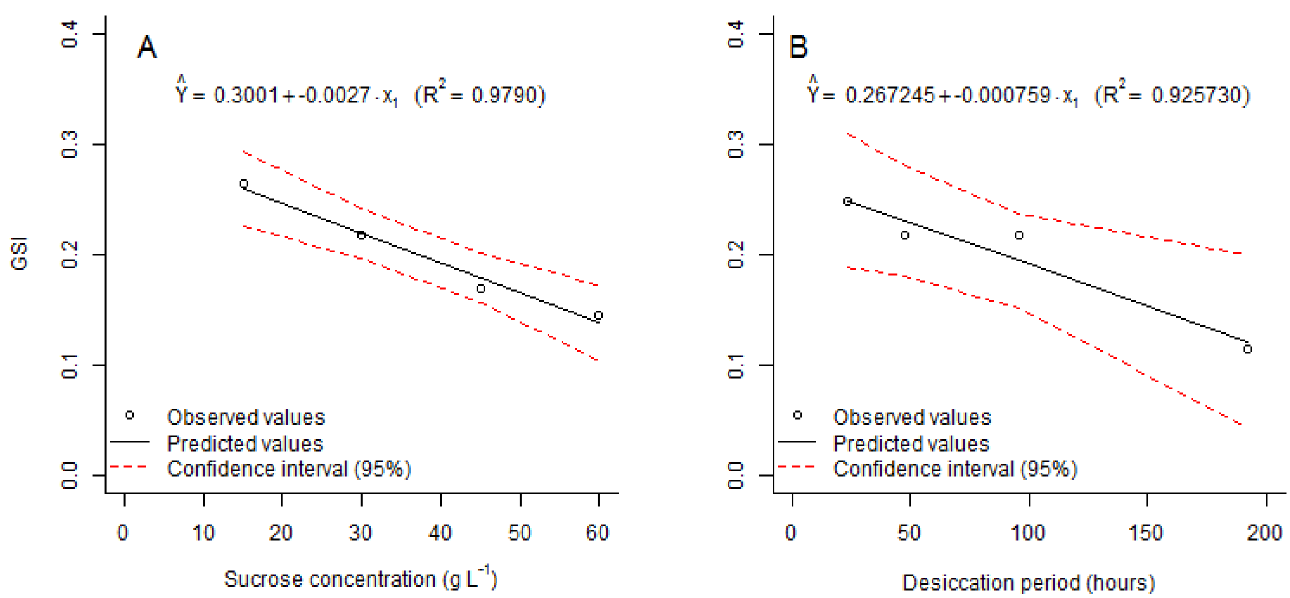

Figure 3. Influence of the increasing sucrose concentrations in the medium (A) and the seed inoculation period after pulping (B) on the germination speed index of mangabeira seedlings grown *in vitro*.

high rates of culture contamination (Rubio Neto et al., 2015), no influence of this factor was verified on the medium contamination, since, regardless of the desiccation period, the contamination index remained below 2%.

The germination speed index (GSI) decreased with increasing either the sucrose concentration or the time between fruit pulping and seed inoculation (desiccation period) (Figure 3). The addition of solutes to the culture medium, such as macronutrients, sucrose and others, leads to a considerable decrease in the osmotic potential of the medium due to the lowering of water potential (Kerbauy, 2012), however, in this study, the concentrations of these elements were not sufficient to impose water stress capable of counteract the strong forces of imbibition prevalent during germination.

Contrary to what was observed in the present study, pequi seeds (*Cariocar brasiliense* CAMB.) exhibited

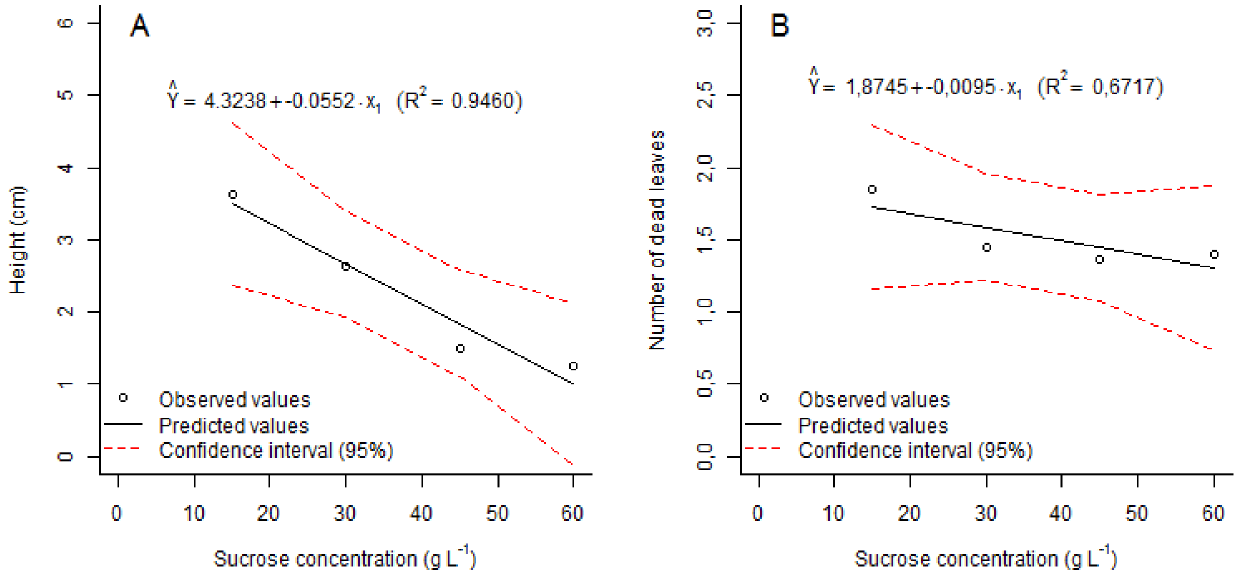

Figure 4. Height of mangabeira seedlings (A) and number of dead leaves sixty days after inoculation.

increases both in the germination percentage and the germination speed index with increase in desiccation time (reduction of seed water content) (Silva et al., 2013). These contrasting differences among native Cerrado species in response of seed germination to desiccation are mainly due to the anatomical differences among fruits and seeds and the residual water content remaining in the seeds after harvest.

Increasing sucrose concentration of the medium influenced also seedling height and the number of dead leaves. The height of the mangabeira seedlings seems a secondary response, being dependent on the presence of live leaves. This suggests that in seedlings with fewer dead leaves, sucrose consumption for maintenance of live leaves increased, which deprives the meristem from sucrose and this can partially explain the reduction in plant height (Figure 4). The reduction in the number of dead leaves with the increase in sucrose concentration of the medium is interesting, because it allows obtaining seedlings with greater photosynthetic area, which may favor their acclimatization.

Mangabeira seeds, thus, exhibit short postharvest longevity, which necessitates immediate sowing after extraction from the fruit. The seeds are also considered delicate, suffering intrinsic damage leading to loss of viability and vigor with reduction of seeds moisture. The gradual decrease of GSI and germination percentage and the increase of the number of dead leaves support this conclusion, showing that even under controlled conditions, such as the *in vitro* cultivation, the resumption of seedling development is dependent on the conditions of storage and conservation of the embryo. Seed drying, although not reducing the seed water content below 26%, was considered critical by Salomão et al. (2004), possibly causing damage to vital seed tissues, such as the embryo, which would explain the drastic reduction of seedling germination after 106 h of drying (Figure 2A).

In potato plants cultured *in vitro*, Bandinelli et al. (2013) observed that the increase of sucrose concentration in the MS medium was accompanied by a decrease in the biomass production. The dynamics of solute translocation in the phloem helps to explain the reduction in the height of seedlings cultured in medium with higher concentrations of sucrose. In the age at which the mangabeira seedlings were cultured, the photosynthetic rate could be considered null, since the leaves were not yet fully developed. Thus, the phloem loading with sucrose and other solutes from the source (culture medium) occurred in the apical direction; these solutes being intercepted by the proximal sink organs. It was observed that with the increase in sucrose concentration of the medium, there was a reduction in the number of dead leaves per seedling, concomitant with an increase in the number of young leaves which act as a sink, resulting in smaller amounts of organic solutes reaching the apical meristem. Lower seedling height can be interpreted as a consequence of maintenance of live leaves.

The length of the largest root and the stem diameter did not differ between the sucrose concentrations and the inoculation periods after pulping. This is due to the fact that the increase in sucrose concentration of the medium was accompanied by reduction in the mortality of young leaves (sink). These dynamics possibly attenuate the competition between the different sinks (young leaves and meristems), favoring growth of young leaves at the expense of limiting the amount of organic solutes reaching the apical root meristems. However, the secondary growth (stem diameter) of seedlings was less influenced by the medium conditions at the beginning of the establishment period; it may be manifested at the later developmental stages of the plant, or after the acclimatization period.

Conclusions

The natural drying of seeds of *H. speciosa* Gomes var. *gardneri* can be carried out up to 106 h after their extraction from the fruit (36.8 water content) without prejudice to the *in vitro* germination rate and the maintenance of live leaves of the seedlings. Increasing sucrose concentration up to 60 g L^{-1} reduced germination speed and seedling height. Leaf mortality was also reduced, contributing to the production of seedlings that are more capable of acclimatization to field conditions.

Conflict of Interests

The authors have not declared any conflict of interests.

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Physiological and agronomic characteristics of cassava genotypes

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This experiment was conducted with the objective of evaluating physiological and agronomic characteristics of cassava genotypes in the municipality of Cândido Sales, State of Bahia, Brazil, between October 2010 and August 2012. Complete randomized block design was used, with three replications and 28 treatments formed by genotypes Amansa Burro, Aramaris, Bom Jardim, Bromadeira, Caipira, Caitité, Caravela, Kiriris, Lagoão, Lavra Velha, Malacacheta, Mulatinha, Parazinha, Peru, Poti Branca, Salangor, Sergipe, Sergipe, Simbé, Tapioqueira, Tussuma, Verdinha, 2006-4, 2006-5, 2006-8, 2006-10 and 2006-12, coming from UESB, EMBRAPA and farmers in the region. Sergipe genotype is present in two treatments, one planted with cuttings collected from local farmers only called Sergipe, and another called Sergipe MR planted with cuttings originated from the rapid multiplication method. The crop spacing used was 1.0 x 0.6 m and each plot composed by 60 plants, being 26 plants considered useful. The evaluations were performed at the end of the first crop cycle, in July 2011, and the harvest in August 2012, twenty-two months after planting. The higher total leaf area and the high photosynthetically active radiation absorbed by the plants canopy at the end of the first crop cycle were correlated to an increase of productivity of shoot and dry weight of root. There was no change in leaf water potential among the genotypes. The genotypes Caipira, Poti Branca, Verdinha and Sergipe from cuttings obtained by the rapid multiplication method showed high productivity for tuberous roots associated to a higher dry matter values. A greater starch yield was observed in genotypes 2006-5, Verdinha, Malacacheta, Poti Branca, Caipira, 2006-10, Sergipe MR, Parazinha and Mulatinha, thus, they might be future alternatives for regional cultivation for industrial production.

Key words: *Manihot esculenta* Crantz, water potential, leaf area, varieties, tuberous root, starch.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a shrubby plant in Euphorbiaceae family that grows continuously having two alternating periods, one for growing and another for storing carbohydrates in its tuberous roots, and these periods are followed by an interval of dormancy (Alves, 2002). Brazil is considered the possible center of origin for this species (Allem, 1994), which is one of the most important crop in the tropics and the basic food for over

800 million people (Nassar et al., 2009), being cultivated on 16 million hectares worldwide (El-Sharkawy et al., 2008).

Cassava is mostly cultivated on tropical countries situated in equatorial region, between 30º North and 30º South to equator with an altitude variation from sea level to 2.000 m, and annual rainfall between 500 mm up to 2,000 mm, which shows cassava capacity to adapt to a great number of environments and ecosystems (El-Sharkawy, 2012).

In 2015, Brazil produced 23.06 million tons of roots (IBGE, 2016). According to FAO (2016), Brazil is the fourth biggest producer of cassava; behind of Nigeria, Thailand, and Indonesia. The Bahia State has the third biggest yield of cassava in Brazil, in 2015 the state had produced 2.09 million tons of roots; however, the yield has been dropped by low average of productivity, only 11.05 t ha^{-1}. In the same year, the municipality of Cândido Sales was the biggest producer of cassava in the state of Bahia (IBGE, 2016).

The city of Cândido Sales is situated in the Southwest Bahia, and presents a semi-arid climate with hydric restriction in most part of the year, and cassava is one of the few alternatives for farmers. Being tolerant to low rainfall, cassava is a crop recognized by its social importance in areas characterized by erratic rainfall and an limited water resources systematization (El-Sharkawy and Tafur, 2010).

According to Lopes et al. (2010), among the causes that contribute to the low yield of cassava in Brazil, there is a lack of varieties adapted to different conditions, inadequate cultural practices or lack of it, and the use of low quality planting material, as well as growing in regions with annual precipitation lower than 1000-1500 mm, values considered adequate to the crop (Oliveira et al., 2006).

Selection of cassava varieties is important in many parts of the world. Acquah et al. (2011) identified that there is an immediate need for new cassava varieties in Africa to replace varieties that are losing desirable traits. In this context, Tumuhimbise et al. (2014), studying the effect of genotype and environment interaction on fresh root yield and cassava related traits in Uganda, observed a high degree of genetic variation among genotypes in three different environments. Demonstrating that cassava genotypes may have different behaviors in different environments, and may have a superior variety at each site.

The cassava is cultivated in all Brazilian regions with a diversity of varieties adapted to each of these different biomes giving to the species a large genetic diversity (Galera and Valle, 2007). This diversity represents a large base for breeding programs in tropics, concentrating genes for resistance to the major pests and diseases, also adaptation for different edaphoclimatic conditions (Fukuda et al. 1999).

According to Fukuda and Silva (2003), a way to improve the yield and the production system is the use of better varieties adapted to edaphoclimatic conditions for each region, due to the high genotype x environment interaction, a genotype rarely behave in a similar manner in different ecological regions.

In addition, according to Casaroli et al. (2007), the energy emitted by the sun, which affects the surface of the leaves, together with adequate supplies of water and nutrients, can be indicators of plant productivity. These factors are part of the physiological processes responsible for capturing solar energy and its subsequent biochemical transformation into organic compounds that result in food, fiber, cellulose and energy. Even for plants of one species, the rate of photosynthesis varies with genotypes (Pereira, 1987). Therefore, physiological studies among genotypes may indicate the most efficient in the conversion of photoassimilates to the production of tuberous roots and shoots of cassava plants.

However, many of created and selected varieties had not been used by farmers, and the most common cassava varieties used are still the same that have been planted in most regions during consecutive years (Fukuda et al., 1997), as it happens in the Southwest region of Bahia.

In this region, the variety Sergipe is the most cultivated among the cassava farmers for flour production and starch extraction, and the rusticity and the high productivity attributes justified the preference of producers in the region (Carvalho et al., 2009). However, in the last ten years, this variety has been suffering gradual drop in yield, and the introduction of new varieties may result in an improvement of living conditions for local farmers.

Given the above, this work was developed to evaluate physiological and agronomic characteristics of cassava genotypes, identifying the most promising genotypes for cultivation.

MATERIALS AND METHODS

The experiment was conducted at the locality Bomba, coordinates 15º18'13'' S and 41º17'32'' W, municioality of Cândido Sales in southwest of Bahia state, Brazil. The city altitude in average is 627 m, and the semi-arid climate according to Köppen is classified as Aw type, tropical climate and dry season. The annual average temperature is 20.4ºC, and annual rainfall of 767.4 mm, being the rainy season between October and March (SEI, 2013). Figure 1

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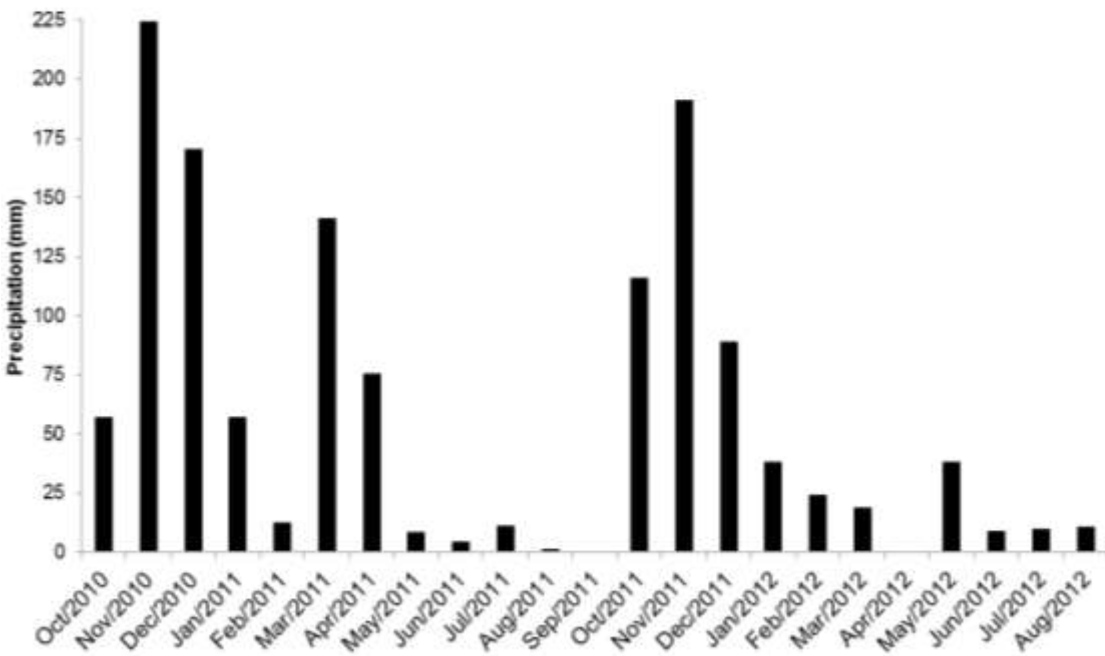

Figure 1. Monthly averages rainfall in the municipality of Cândido Sales - BA, from October 2010 to August 2012. Source: National Water Agency – ANA.

shows the rainfall data obtained during the trial period.

The soil in the experimental area was classified as Yellow Oxisol dystrophic clayey, which presented the following results for chemistry analysis in 0-20 cm depth: pH in water (1: 2.5): 4.5; P: 2.0 mg dm^{-3} ($Mehlich^{-1}$); K^{+}: 0.11 cmolc dm^{-3} ($Mehlich^{-1}$); Ca^{2+}: 0.4 cmolc dm^{-3} (KCl extractor 1 mol L^{-1}); Mg^{2+}: 0.4 $cmolcdm^{-3}$ (KCl extractor 1 mol L^{-1}); Al^{3+}: 1.0 cmolc dm^{-3} (KCl extractor 1 mol L^{-1}); H^{+}: 5.4 $cmolcdm^{-3}$ (SMP extractor solution, pH 7.5 at 7.6); Sum of Bases: 0.9 cmolc dm^{-3}; CEC effective: 1.9 cmolc dm^{-3}; CEC at pH 7.0: 7.3 cmolc dm^{-3}; Base saturation: 12%; Aluminum saturation: 52%.

The area was tilled and plowed mechanically. The planting has occurred in October 2010 and it was done with cuttings 20 cm long and 2 to 3 cm in diameter, making an average of eight gems. The spacing between rows was 1.0 m and 0.60 m between plants, totaling 26 plants per plot, in 15.6 m².

Randomized block design with 28 treatments and three replications was utilized. The harvest was performed in August 2012, 22 months after planting.

Were evaluated 28 treatments, using 27 genotypes. The genotype Sergipe, the most cultivated in the region, was planted with cuttings collected with local farmers, called by Sergipe, and also planted with originated from the rapid multiplication method, called Sergipe MR, developed by the International Center of Tropical Agriculture (CIAT) in Colombia, and later adapted to Brazilian conditions (Santos et al., 2009).

Genotypes Amansa Burro, Aramaris, Caipira, Caravela, Kiriris, Lagoão, Mulatinha, Poti Branca, Tapioqueira and Verdinha were obtained from the Cassava Active Germplasm Bank in the National Research Center of Cassava and Fruticulture of EMBRAPA (Brazilian Agricultural Research Corporation), located in Cruz das Almas, Bahia, Brazil. In this sense, for developing this bank, these genotypes were collected from different regions of the country.

Genotypes Bom Jardim, Bromadeira, Caitite, Lavra Velha, Malacacheta, Parazinha, Peru, Salangor, Sergipana, Simbé and Tussuma were obtained from the Germplasm Collection of Cassava of UESB (Southwest Bahia State University), *campus* of Vitória da Conquista, Bahia, Brazil. It was also utilized botanical seeds genotypes deriving from open pollination: 2006-4, 2006-5, 2006-8, 2006-10 and 2006-12.

At the end of the first growing season, in July 2011, nine months after planting, period characterized by mild temperatures and lower rainfall index, the foliar area was evaluated measuring all leaves of one plant in each spot, using Area Meter model LI-3100; the photosynthetically active radiation (PAR) absorbed by the canopy, between plants (RBP), and between rows (RBR), in the period from 11:00 to 12:30 h, between rows and between plants at 0.20 m by the soil level and full sun, was evaluated using ceptometer Decagon, model AccuPAR LP-80. The values were obtained from the equations: % of RBP = 100 – [(PAR between plants / PAR under full sun) x 100] and %RBR = 100 – [(PAR between rows / PAR under full sun) x 100]; The leaf water potential was determined by measuring the middle third leaves of two plants per plot, collected at 5:00 AM (predawn) and 12:00 PM (noon) using a pressure chamber (Model 1000, PMS) according to methodology proposed by Scholander et al. (1964).

At harvest, in August 2012, 22 months after planting, it was evaluated the shoot yield, the tuberous roots yield, the dry matter percentage in tuberous roots, by the hydrostatic balance method based on the equation: DM = 15.75 + 0.0564 R, being R the weight of 3 kg of roots in water (Grossmann and Freitas, 1950), and the percentage of starch in tuberous roots, calculated by subtracting from the dry matter content the constant 4.65 (Conceição, 1983).

The statistical analyzes were performed using SAEG program (System for Statistical Analysis and Genetic) version 9.1 (Ribeiro Júnior, 2001). Data were submitted to analysis of variance and average treatments grouped by the procedure proposed by Scott-Knott at 5% probability.

Data was analyzed for normality using the Lilliefors test and the variances homogeneity by Cochran's test. When necessary, data transformation was performed. The Pearson correlation was analyzed using the t-test, at 5% probability to assess the relation between dependent characteristics.

Table 1. Shoot yield (SY), tuberous root yield (TRY), dry mass percentage for tuberous roots (DM) and starch productivity (SP) of cassava genotypes. Cândido Sales, Bahia, Brazil, in 2016.

Genotypes	SY (t ha^{-1})	TRY (t ha^{-1})	DM (%)	SP (t ha^{-1})
Amansa Burro	10.76^{b}	12.74^{b}	31.32^{a}	3.40^{b}
Aramaris	6.04^{c}	12.84^{b}	30.60^{a}	3.33^{b}
Bom Jardim	7.78^{c}	8.87^{b}	30.75^{a}	2.32^{b}
Bromadeira	4.66^{c}	9.81^{b}	31.49^{a}	2.70^{b}
Caipira	8.66^{c}	19.72^{a}	31.28^{a}	5.26^{a}
Caitite	14.15^{b}	16.64^{b}	27.56^{b}	3.82^{b}
Caravela	6.71^{c}	12.90^{b}	32.10^{a}	3.55^{b}
Kiriris	6.40^{c}	16.18^{b}	29.44^{a}	4.08^{b}
Lagoão	5.92^{c}	14.23^{b}	34.13^{a}	4.21^{b}
Lavra Velha	7.49^{c}	8.12^{b}	28.50^{b}	1.94^{b}
Malacacheta	10.58^{b}	22.50^{a}	28.18^{b}	5.34^{a}
Mulatinha	20.29^{a}	15.77^{b}	32.80^{a}	4.44^{a}
Parazinha	9.85^{c}	17.01^{b}	31.39^{a}	4.54^{a}
Peru	12.25^{b}	13.82^{b}	30.26^{a}	3.55^{b}
Poti Branca	16.52^{a}	19.44^{a}	32.28^{a}	5.32^{a}
Salangor	12.02^{b}	11.13^{b}	28.91^{b}	2.70^{b}
Sergipana	9.08^{c}	10.06^{b}	28.20^{b}	2.44^{b}
Sergipe	12.44^{b}	15.02^{b}	31.00^{a}	3.91^{b}
Sergipe MR	10.06^{c}	17.50^{a}	30.26^{a}	4.61^{a}
Simbé	11.42^{b}	14.74^{b}	30.10^{a}	3.71^{b}
Tapioqueira	6.72^{c}	13.74^{b}	30.28^{a}	3.54^{b}
Tussuma	12.58^{b}	12.99^{b}	30.53^{a}	3.44^{b}
Verdinha	9.12^{c}	22.86^{a}	31.26^{a}	6.14^{a}
2006-4	6.63^{c}	18.08^{a}	23.16^{c}	3.41^{b}
2006-5	10.08^{c}	29.27^{a}	28.85^{b}	7.11^{a}
2006-8	10.91^{b}	18.05^{a}	26.69^{b}	3.98^{b}
2006-10	7.63^{c}	22.93^{a}	26.82^{b}	5.07^{a}
2006-12	7.64^{c}	15.75^{b}	25.22^{c}	3.17^{b}
Overall Average	**9.80**	**15.81**	**29.76**	**3.97**

* Averages followed by the same letter in the column do not differ, according grouping criteria of Scott-Knott at 5% probability.

RESULTS AND DISCUSSION

The agronomic characteristics evaluation of 22 months after planting (Table 1) showed that the genotypes Mulatinha and Poti Branca produced more shoot than the others, with yield of 20.29 t ha^{-1} and 16.52 t ha^{-1}, respectively, being an alternative to animal feed or to increase production for planting cuttings in the region studied.

The shoot average yield obtained in this study was 9.80 t ha^{-1}, relatively low compared to those found in the literature, as cited by Alves et al. (2011), whom reported for genotype Poti Branca and shoot yield of 43.00 t h^{-1}. Such differences can be attributed to environmental conditions, particularly rainfall and temperature. According to Sagrilo et al., (2002), the production of aerial part depends mainly on climate factors, since high temperatures with heavy rainfall not only favor the growth of stems, but as also the leaves production.

There was a positive correlation between shoot yield and the tuberous roots yield (r = 0.39 *), and between shoot yield and starch yield (r = 0.44 *), indicating that plants with more developed aerial part produce more tuberous roots and more starch, due to the higher photoassimilates production. According to Mulualem and Ayenew (2012), increments of vegetative parts have significant effect on the dry matter production of cassava.

Observing the tuberous roots yield (Table 1), a variation from 8.12 t ha^{-1} (Lavra Velha) to 29.27 t ha^{-1} (2006-5) is noticed, indicating variability for this trait. Among the 28 genotypes studied, nine were considered to be more productive with values ranging from 17.50 t ha^{-1} to 29.27 t ha^{-1}, being higher than the national average, which according to IBGE (2016), it was 15.24 t ha^{-1} in 2015.

The genotypes obtained from botanical seeds selected in UESB showed higher values for tuberous roots yield, except genotype 2006-12.

The genotype Sergipe, when propagated through the traditional method, produced less tuberous roots than when planted with cuttings obtained by the rapid multiplication method (Santos et al., 2009). In this method, the selection and the care with the cuttings lead to obtain more vigorous plants, contrasting the plants development from cuttings collected from the producers. Due the continuous cultivation for about 25 years in low natural soil fertility, without the use of fertilizer and the accumulation of pests and diseases have caused a "degeneracy" for the propagation material of Sergipe genotype, which has led to reduced productivity and the consequent reduction of area cultivated with this material. The drought experienced in the region in recent years has helped to accentuate this problem.

The harvest of cassava roots must be performed in the physiological resting period of the plant, when the roots has higher content of dry matter, differently from the growth season, when the dry matter content in the roots is reduced (Sagrilo et al., 2006; Keating et al., 1982; Guimarães et al., 2009). These conditions are found in the study area during August, a period characterized by milder temperatures and low rainfall, when the majority of local farmers harvest the roots. This leads, however, the need for storage of planting material for three to four months until the beginning of the rainy season, usually in October or November, reducing their quality.

For the characteristic of dry mass for tuberous roots, it was possible to separate the genotypes into three groups, highlighting the genotype Lagoão into the group that showed higher values (Table 1). In this group the dry matter content ranged from 29.44 to 34.13%, values that can be considered high, according to Teye et al. (2011).

The satisfactory results presented by genotypes obtained from free pollination for tuberous roots yield did not occur to dry mass of roots. Despite these genotypes forming considerable number of roots, they had higher moisture, which is not suitable for the industrialization of cassava.

It is desirable that the varieties responsible for higher productions of tuberous roots be also those which have higher dry matter content, maximizing the yield of the final product by cultivated area unit (Vidigal Filho et al., 2000). In this study, the genotypes Caipira, Poti Branca, and Sergipe from cuttings obtained by the rapid multiplication method and Verdinha stood out in both characteristics.

The cassava starch and its derivatives have been used in products for human consumption or as input from various industries such as: canned food, packaging, glues, paper, mining, textile and pharmaceutical (Cardoso and Gameiro, 2006). Genotypes that have high yield of starch may be recommended for industrial use in the region.

Nine genotypes stood out for starch yield (Table 1), showing values between 4.44 to 7.11 t ha^{-1}. The overall average of the experiment was 3.98 t ha^{-1} of starch, considering the 28 treatments. Introducing genotypes can lead to increase yield of roots by improving the income of farmers in the region. However, this work is still recent in the studied region, and further studies in different locations and years are necessary to evaluate the yield stability and subsequent recommendation for cultivation.

Separation of two genotypes groups in relation to total leaf area is observed in Table 2. The group with the lowest total leaf area was formed basically by genotypes 2006-4, 2006-5, 2006-8, 2006-10 and 2006-12, obtained from botanical seeds, deriving from open pollination selected in UESB, except the genotypes Bromadeira and Malacacheta traditionally farmed in the region.

Low temperatures and poor soil moisture change the pattern of leaf longevity, increasing the senescence process, abscission and restricting the expansion of the leaf blade (Calatayud et al., 2000). For El-Sharkawy (2006), larger leaf longevity may be important to achieve higher yields in crops such as cassava. In this crop, the development of the shoot and roots occur simultaneously, enabling the selection of cassava varieties for both traits simultaneously (Lenis et al., 2006). In this study, a correlation was found between total leaf area (TLA) and root dry weight ($r = 0.43^*$), indicating that genotypes which maintained high leaf retention had higher starch accumulation in tuberous roots.

Table 2 indicates that the RBP and RBR were similar between genotypes. The study of correlations showed that higher value of these characteristics are associated to higher starch accumulation in the tuberous root ($r = 0.34^*$ and $r = 0.50^*$, respectively).

The efficiency of genotypes for leaf area maintenance makes the plants to absorb more light energy producing more photoassimilates, accumulating it in the shoot and in the roots, as dry matter. The results corroborate with Lenis et al., (2006), which state that the increased longevity of the leaves or better leaf retention is a way to increase the cassava yield. These authors also highlight the importance for incorporating the leaf retention as an important feature to be target in breeding and selection programs to increase roots yield.

The genotypes showed similar leaf water potential (Ψw) in both the predawn period and the noon period (Table 3). This feature reflects the dynamic conditions of the water transport process in the soil-plant-atmosphere system, constituting the main component responsible for the water flow in the plant (Pereira, 2006).

Water stress usually occurs gradually in nature, and the tolerant plants have developed mechanisms to adapt to conditions of low water availability in the soil (Chaves Filho and Stacciarini-Seraphin, 2001). This study had showed no significant difference in leaf water potential between genotypes at extreme periods of day, therefore, it is concluded that the plant defense mechanisms were

Table 2. Total leaf area (TLA), photosynthetically active radiation absorbed by the canopy of plants (RBP) and between rows (RBR) at the end of the first crop cycle of cassava genotypes, in July 2011. Cândido Sales, Bahia, Brazil, in 2016.

Genotypes	TLA (cm^2)	RBP (%)	RBR (%)
Amansa Burro	2,359.67[a]	39.31[b]	38.33[b]
Aramaris	2,239.00[a]	39.38[b]	40.24[b]
Bom Jardim	2,520.67[a]	44.60[b]	44.54[b]
Bromadeira	1,231.33[b]	26.96[c]	24.56[c]
Caipira	2,998.67[a]	61.84[a]	57.77[a]
Caitite	2,124.67[a]	41.31[b]	40.25[b]
Caravela	4,001.00[a]	42.21[b]	44.87[b]
Kiriris	2,757.67[a]	32.23[c]	38.69[b]
Lagoão	3,179.67[a]	46.15[b]	48.93[a]
Lavra Velha	2,452.67[a]	44.81[b]	41.77[b]
Malacacheta	1,369.67[b]	33.44[c]	35.73[b]
Mulatinha	3,309.00[a]	57.11[a]	61.30[a]
Parazinha	4,070.33[a]	42.41[b]	36.19[b]
Peru	2,302.67[a]	60.01[a]	57.64[a]
Poti Branca	4,110.33[a]	50.96[a]	56.67[a]
Salangor	4,321.67[a]	64.01[a]	65.17[a]
Sergipana	2,768.67[a]	41.26[b]	32.19[c]
Sergipe	3,015.33[a]	52.98[a]	40.11[b]
Sergipe MR	3,698.67[a]	59.15[a]	53.48[a]
Simbé	3,170.67[a]	64.56[a]	56.11[a]
Tapioqueira	2,426.67[a]	40.00[b]	39.11[b]
Tussuma	3,294.67[a]	62.80[a]	59.48[a]
Verdinha	2,554.67[a]	58.37[a]	53.66[a]
2006-4	615.67[b]	26.31[c]	23.97[c]
2006-5	664.00[b]	20.51[c]	22.11[c]
2006-8	349.67[b]	21.78[c]	20.98[c]
2006-10	907.00[b]	30.33[c]	26.81[c]
2006-12	1,014.33[b]	33.35[c]	31.42[c]
Overall Average	**2,493.88**	**44.22**	**42.59**

* Averages followed by the same letter in the column do not differ according grouping criteria of Scott-Knott at 5% probability.

similar between the different genotypes, such as osmotic adjustment to better coexist with drought, and they did not show considerable variation among them.

Conclusions

Therefore, regarding edaphoclimatic conditions of the experiment, it is concluded that:

i) Higher total leaf area and high photosynthetically active radiation absorbed by the canopy of plants, observed at the end of the first cycle, were correlated to the increase of shoot yield and root dry weight.
ii) There was no change in leaf water potential among the genotypes.
iii) The genotypes Caipira, Poti Branca, Verdinha and Sergipe from cuttings obtained by the method of rapid multiplication showed high productivity of tuberous roots associated with higher dry matter values.
iv) Greater starch yield was observed in genotypes 2006-5, Verdinha, Malacacheta, Poti Branca, Caipira, 2006-10, Sergipe MR, Parazinha and Mulatinha and they might be future alternatives for regional cultivation for industrial production.

Conflict of Interests

The authors have not declared any conflict of interests.

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Table 3. Module of leaf water potential (Ψw) conducted in the predawn and noon periods, at the end of the first crop cycle of cassava genotypes, in July 2011. Cândido Sales, Bahia, Brazil, in 2016.

Genotypes	Ψw Predawn (MPa)	Ψw Noon [1] (MPa)
Amansa Burro	0.483[a]	1.095[a](1.199)
Aramaris	0.483[a]	0.847[a](0.717)
Bom Jardim	0.433[a]	1.152[a](1.327)
Bromadeira	0.583[a]	1.117[a](1.248)
Caipira	0.367[a]	0.888[a](0.789)
Caitite	0.783[a]	1.148[a](1.318)
Caravela	0.433[a]	1.020[a](1.040)
Kiriris	0.300[a]	1.083[a](1.173)
Lagoão	0.417[a]	1.021[a](1.042)
Lavra Velha	0.433[a]	1.030[a](1.061)
Malacacheta	0.383[a]	1.164[a](1.355)
Mulatinha	0.517[a]	1.134[a](1.286)
Parazinha	0.567[a]	1.088[a](1.184)
Peru	0.343[a]	1.088[a](1.184)
Poti Branca	0.470[a]	1.137[a](1.293)
Salangor	0.560[a]	1.051[a](1.105)
Sergipana	0.450[a]	1.251[a](1.565)
Sergipe	0.463[a]	1.125[a](1.266)
Sergipe MR	0.243[a]	1.114[a](1.241)
Simbé	0.477[a]	0.961[a](0.924)
Tapioqueira	0.510[a]	0.772[a](0.596)
Tussuma	0.533[a]	1.012[a](1.024)
Verdinha	0.583[a]	1.008[a](1.016)
2006-4	0.417[a]	1.212[a](1.469)
2006-5	0.500[a]	1.172[a](1.374)
2006-8	0.500[a]	1.323[a](1.750)
2006-10	0.400[a]	1.221[a](1.491)
2006-12	0.517[a]	1.036[a](1.073)
Overall Average	**0.470**	**1.081 (1.182)**

[1] Data transformed to $\sqrt{X}$, and not transformed averages in parentheses. * Averages followed by the same letter in the column do not differ, according grouping criteria of Scott-Knott at 5% probability.

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Spatial patterns of soil attributes in a fluvent in the semiarid region, Brazil

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Spatial variability studies on soil attributes are fundamental for adoption of best agricultural practices concerned with irrigated agricultural sustainability. This study aims to infer the spatial variability patterns of soil moisture, soils salinity and soil texture in an irrigated area of Pernambuco State (Brazil) under cultivation of carrot (*Daucus Carota* L.), during the dry period of 2012. The area is a typical system in the semiarid under small scale agricultural practices. For the study, it was adopted a 4 × 4 m regular mesh, with 49 sampling points collected at 0-0.2 m and 0.2-0.4 m layers. Data were submitted to classical statistical procedure, followed by geostatistical analysis. For soil moisture at 0.2-0.4 m layer and clay content, the degree of variability was considered low. For soil moisture at 0-0.2 m the degree of variability was moderate, while the electrical conductivity showed a high degree of variability. Occurrence of soil salinity is higher at the soil surface. Water flow occurs from the borders to the central plot, as evidenced by contour maps. Geostatistics allowed the characterization of the spatial variability patterns for the main soil attributes in an irrigated plot in the semiarid of Brazil, and it has been shown as an important tool for the prediction of soil attributes. Such results are important for issuing guidance for sustainable small scale agriculture practices in the region.

Key words: Geostatistics, soil moisture, salinity, texture, communal farming.

INTRODUCTION

Inadequate soil and water management has led many irrigated areas to become non-productive all around the world. When croplands are intensively explored, spatial and temporal variations of the various soil attributes may occur, and the agricultural economy becomes dependent on these changes (Scherpinski et al., 2010). The spatial variability studies of soil properties are fundamental to evaluate the management effectiveness, concerned with the sustainability and environmental quality (Darwish et al., 2015). Producing maps of soil properties requires a

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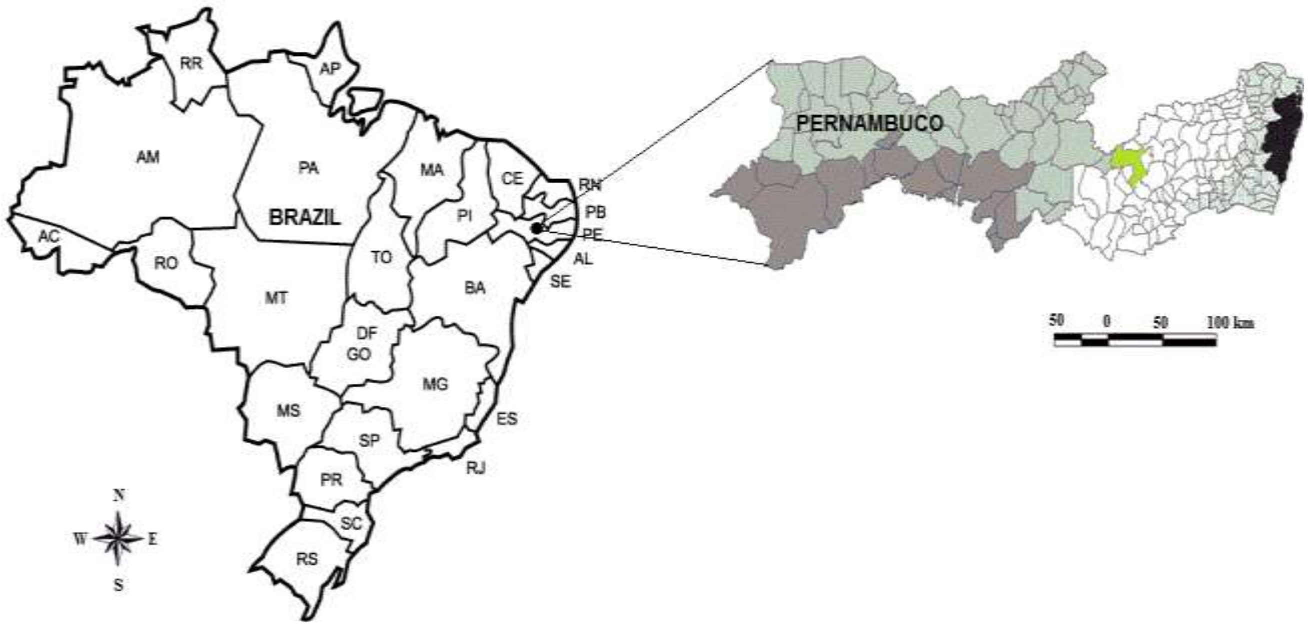

Figure. 1. Map of Brazil, Pernambuco State and Pesqueira city (green) (Source: Modified from https://portal.macamp.com.br).

large effort of sampling and evaluation of these properties, resulting in high investment of time and cost. Hence, the definition of an ideal sampling design and technique is a fundamental criterion for mapping. One tool that can assist in studies is the precision agriculture (Triphati et al., 2015). Studies that aim to understand the distribution of soil attributes minimize time and cost of sampling,and the maps can be applied in scientific areas, for example, in hydrological and climatic studies, as well as operational applications, such as decision making in agricultural practices. Among the various soil attributes, water content, electrical conductivity and soil texture can be considered as main quality indicators and their determinations have a high importance in many scientific fields and operational applications (Brocca et al., 2012; Darwish et al., 2015; Triphati et al., 2015). There are several recent studies about the spatial variability of soil attributes: spatial and temporal variability of soil moisture in the surface layer of the Loess Plateau in China (Hu et al., 2008); spatial variability of soil physical properties in a semiarid alluvial valley at Pernambuco State (Souza et al., 2008); spatial variability of hydraulic conductivity and water infiltration in the soil at Paraná State (Scherpinski et al., 2010); study in a catchment scale of soil moisture spatial–temporal variability in central Italy (Brocca et al., 2012); spatial distribution analysis of soil variables for agronomic development in North-Coastal of Egypt (Darwish et al., 2015); and spatial variability analysis of soil properties in salt affected coastal India using geostatistics and kriging (Triphati et al., 2015). Although there are many applications related to the study of the spatial variability of soil attributes, there are still few studies carried out in semiarid Brazilian regions, such as the present study.

Among management tools for sustainable development of irrigated areas, those based on the understanding of the spatial variability pattern of different soil attributes are very important. Geostatistics techniques, which can be defined as tools for studying and predicting the spatial patterns of several variables, allow understanding spatial variability in a change of natural phenomena (Jackson et al., 2007), as well as the spatial properties of soil attributes (Cambardella et al., 1994). Geostatistics offers a set of statistical tools that include the spatial coordinates of soil samples in data processing, allowing a description of spatial patterns, prediction at unsampled locations, and evaluation of the uncertainty associated to these predictions (Triphati et al., 2015). This work aims to study the spatial variability of soil moisture, salinity and texture in an irrigated area of Pernambuco State (Brazil) under cultivation of carrot (*Daucus carota* L.), during the dry period of 2012. This is a typical system in the semiarid under small scale agricultural practices.

MATERIALS AND METHODS

Study area

The study was conducted at Nossa Senhora do Rosário Farm, located in Pesqueira city, Pernambuco State (Brazil), with 08°10' S and 35°11' W coordinates and altitude of 650 m (Figure 1). The study area is within a rural settlement installed by the State Government, where small scale agriculture is practiced. The predominant soil in the region is Fluvent. The climate is BSsh (extremely hot, semiarid), according to the Köppen classification,

Table 1. Annual (1961-1990) climate normal in Pesqueira (Source: http://www.inmet.gov.br).

P_{cum} (mm)	P (mm)			E_t (mm)	T_m (°C)	RH_m (%)	W_s (ms^{-1})
	3 days	5 days	10 days				
701.5	30	20.4	9.3	1589.8	22.7	73.1	3.61

P_{cum}, Cumulative precipitation; P, Number of periods with 3 or more, 5 or more and 10 or more consecutive days without precipitation; E_t, total evaporation; T_m, Mean temperature; RH_m, mean relative humidity; W_s, wind speed.

Table 2. Physical characteristics of Fluvent at 0-0.2 and 0.2-0.4 m layers.

Layer (m)	Sand	Silt	Clay	Ds (g cm^{-3})	Dp (g cm^{-3})	P (%)
		(%)				
0 - 0.2	56.31	22.12	21.37	1.59	2.5	36.4
0.2 - 0.4	56.61	20.74	22.53			

Ds, Bulk density; Dp, particle density; P, porosity.

with an annual mean precipitation of 607 mm (Scherpinski et al., 2010). The annual climatological Normal for the period 1961 to 1990 in Pesqueira is presented in Table 1. The experimental area consists in a one hectare plot cultivated with carrot (*Daucus Carota* L.). The area is irrigated during 1.5 hours per day and the micro sprinkler system has a flow rate of 105 L h^{-1}, with a control of the applied water depth. Groundwater is used for irrigation, with electrical conductivity (EC) of 0.57 dS m^{-1}.

Samples

For the spatial analysis, it was adopted an irrigated plot of 576 m² under carrot cultivation (*Daucus carota* L.). In order to characterize the spatial dependence, a regular grid of 4 × 4 m was established, comprising 49 sampling points. The samples at 0-0.2 m and 0.2-0.4 m layers were transported to the laboratory and were air dried for 72 h and separated through sieve with a 2 mm diameter. Physical characteristics (Table 2), soil moisture evaluating, electrical conductivity and texture were performed according to Embrapa (2011). According to the textural classification of the Brazilian Society of Soil Science, the Fluvent was classified as sandy clay loam texture.

Statistical and geostatistical analysis

Data were initially analyzed using descriptive statistics, by evaluating central tendency (mean, median and mode) and dispersion measures (standard deviation, variance and coefficient of variation); and values of kurtosis, and skewness. Normal distribution was tested according to the Kolmogorov Smirnov test at 5% significance level. Spatial dependence was analyzed by fitting experimental semivariograms based on the assumption of intrinsic stationarity hypothesis (Journel and Huijbregts, 1978) to different theoretical variogram models. Coefficients of variation were observed, as suggested by Warrick and Nielsen (1998), which considers low variability when CV < 15%; moderate for 15 < CV < 50% and high variability when CV > 50%. Semivariance analysis was applied to estimate the range over which samples of the soil parameters were related. The semivariance, γ(*h*), of all observed values of a variable separated by a vector *h* were defined as:

$$\gamma(h) = \left[\frac{1}{2N(h)}\right] \sum_{\infty=1}^{N(h)} [Z_i - Z_{i+h}]^2 \quad (1)$$

where Z_i and Z_{i+h} are experimental estimates of any two points separated by the vector *h*, and N (*h*) is the number of experimental pairs separated by *h* (Goovaerts, 1999). For construction of the experimental semivariograms, the software GS+ tool (Gamma Design, 2004) was used, adopting the classic semi-variance estimator (Journel and Huijbregts, 1978). The Gaussian, exponential and spherical models were tested with the experimental data to fit the mean close to zero and standard deviation close to one, using the technique of cross-validation Jack – Knifing. The coefficients of the best fit model were then estimated. The degree of spatial dependence was observed according to the classification of Cambardella et al. (1994), where the degree of dependence lower than 25% characterizes strong spatial dependence, between 25 and 75% moderate spatial dependence, while higher than 75%, weak dependence. For the construction of contour maps, the software Surfer 9.0 was used (Golden Software, 2009).

RESULTS AND DISCUSSION

The results of statistical analysis for soil moisture, electrical conductivity and clay content at 0-0.2 m and 0.2-0.4 m layers are presented in Table 3. Kolmogorov Smirnov test indicated that the data of moisture and clay content at 0-0.2 m and 0.2-0.4 m, and soil electrical conductivity of 0-0.2 m follow Normal distribution, after removing outliers (Hoaglin et al., 1983). Electrical conductivity at 0.2-0.4 m follows a Normal behavior. Additionally, the mean, median and mode for the analyzed variables have similar values (Table 3), suggesting a symmetrical distribution. Such result indicates that the measures of central tendency are not dominated by atypical distribution values (Cambardella et al., 1994), as all the variables follow a Normal distribution. Soil moisture presented values of skewness consistent with a Normal distribution. In effect, the skewness coefficient should approach 0. It is noted that for the moisture and clay content, the values of these coefficients approached the standards required, with an electrical conductivity parameter which is distanced a

Table 3. Descriptive statistics for the soil moisture, electrical conductivity and clay content.

Descriptive statistics	Soil moisture 0-0.2 m (%)	Soil moisture 0.2-0.4 m (%)	EC 0-0.2 m (dS m^{-1})	EC 0.2-0.4 m (dS m^{-1})	Clay Content 0-0.2 m (%)	Clay content 0.2-0.4 m (%)
Mean	12.3	10.65	2.05	1.57	21.37	22.42
Median	12	11	1.61	0.95	21.48	22.64
Mode	13	11	-	-	22.64	22.64
SD	1.93	0.92	1.52	1.25	2.67	2.28
CV	15.72	8.65	74.24	79.64	12.48	10.15
Skewness	0.09	-0.47	1.23	1.10	0.03	-0.08
Kurtosis	0.21	-0.5	0.98	0.25	-0.81	0.05
1º Quartile	11	10	1.05	0.59	18.64	20.64
2º Quartile	12	11	1.61	0.95	21.48	22.64
3º Quartile	13	11	2.59	2.45	23.04	24.46
Variance	3.75	0.85	2.32	1.56	7.11	5.18

EC, Electrical conductivity; SD, standard deviation; CV, coefficient of variation.

little from zero. Variables showed a positive asymmetry, indicative that the most data tends to the minimum values. Soil moisture at 0-0.2 m, EC in both depths and clay content at 0.2-0.4 m had a positive kurtosis, which indicates that the data are dispersed, and that the distribution presents elevation (leptokurtic). Soil moisture at 0.2-0.4 m and clay content at 0-0.2 m showed negative kurtosis, where it is found that the curve is flatter than normal (platykurtic).

Other authors have studied the spatial variability of water content in different soil types and also found normality for this attribute (e.g. Hu et al., 2008; Brocca et al., 2012; García et al., 2014). Geostatistics allows the spatial dependence analysis, from the group of experimental semivariogram, according to a mathematical model, and the characterization of variability by estimating without tendency, estimating values at non-sampled locations. For setting semivariograms, normality of data is not required, but desirable. If the distribution is not normal, but is reasonably symmetrical, it may be sufficient for accepting the hypotheses for the semivariogram construction. Soil moisture at 0.2-0.4 m layer and clay content at both layers showed low degree of variability, with values respectively of 8.65, 12.48 e 10.15%. Souza et al., (2008) also found low variability degree for the clay fraction at 0-0.2 m layer in a Fluvent soil. Soil moisture at 0-0.2 m layer had a moderate degree of variability, with 15.72%. The low or moderate degree of variability found may have been influenced by irrigation performed in the study area a day before sampling. There was no antecedent precipitation in three days and the sampling and measurements were performed during the dry season in the region. Hu et al. (2008) also found moderate variability for water content in a soil in the Loess Plateau of China at 0-0.6 m layer. Brocca et al. (2012) studying the spatial and temporal variability of soil moisture at the catchment scale, found low variability at 0-0.15 m layer. According to the authors, these results can be used to estimate the average conditions of variation in a desired range and consequently, the number of required samples for spatial analysis. This was not the focus of this study, but may be further investigated. Darwish et al. (2015) studying a seacoast to the Libyan plateau, found low degree of variability for clay content.

Electrical conductivity at 0-0.2 m and 0.2-0.4 m layers showed a high degree of variability, since the coefficients of variation were respectively 74.24 and 79.64%. Other authors also found high variability for this attribute as Souza et al. (2000) working on an alluvial soil in the state of Paraíba (Brazil) and Darwish et al. (2015) studying a seacoast to the Libyan plateau. According to Souza et al. (2000), the high variability is related to the heterogeneity of these soils, caused by factors such as the processes of accumulation and distribution of its particles, they are topographically positioned in an alluvial valley, in addition to management they are subjected to, among other factors. It was observed correlation between the mean soil moisture and its standard deviation, in agreement with other recent studies (Gao et al., 2011; Brocca et al., 2012; Gao et al., 2013; Zhang et al., 2013). A positive correlation was also observed between soil moisture and the coefficient of variation, in which the decrease in the mean soil moisture along the layers reduced the spatial variability (Table 3), in agreement with Gao et al. (2011), Gao et al. (2013) and Zhang et al. (2013). The decrease on the moisture variation along the soil layer is because the subsurface layers are usually less influenced by agricultural practices, which occur at the first soil layer, mainly irrigation practices.

Except for the clay fraction at 0.2-0.4 m layer, for which the best fit was to the Gaussian model, for all other attributes the semivariogram showed pure nugget effect, indicating the randomness of the data. The pure nugget

Table 4. Parameters of semivariogram models fitted to experimental data of the study variables and parameters obtained by the validation technique.

Parameter	Soil moisture 0-0.2 m (%)	Soil moisture 0.2-0.4 m (%)	EC 0-0.2 m (dS m^{-1})	EC 0.2-0.4 m (dS m^{-1})	Clay content 0-0.2 m (%)	Clay content 0.2-0.4 m (%)
Nugget effect$_{(C0)}$	3.95	0.92	2.15	1.45	6.77	3.24
Sill$_{(C0+C1)}$	3.95	0.92	2.15	1.45	6.77	6.56
Range (A)	13.71	13.74	13.73	13.75	13.74	7.95
GD $_{(C0/\ C0+C1)\ x\ 100}$	100	100	100	100	100	49.39
Model	PNE	PNE	PNE	PNE	PNE	Gaussian
Mean	-	-	-	-	-	0.018
SD	-	-	-	-	-	0.954

EC, Electrical conductivity; GD, degree of dependence; SD, standard deviation, PNE, pure nugget effect.

effect variogram indicate that there is no spatial correlation between the variables at the sampled distance (Cambardella et al., 1994). Additionally, the nugget effect (C_0) indicates the discontinuity of a phenomenon. According to Vieira (2000), high nugget values are indicative of variation detected by the sampling process. Souza et al. (2008) set the Gaussian model for all variables, which were the soil fractions and electrical conductivity. Results of geostatistical analysis showed that the values of soil moisture, electrical conductivity at 0-0.2 and 0.2-0.4 m layers and clay content at 0-0.2 m, had weak spatial dependence, since the relationship between the nugget and the sill was equal to 100% (Table 4). The clay content at layer 0.2-0.4 m showed moderate dependence, with 49.39%. Other authors found moderate dependence on soil moisture, with values of spatial dependence of 40, 19 and 29% to layers 0-0.15, 0.15-0.30 and 0.30-0.45 m, respectively, in an irrigated Fluvisol at same region of this study, with similar hydrological characteristics (Santos et al., 2012). However, other authors found strong spatial dependence for this variable (Yang et al., 2011). Souza et al. (2008), studying a Fluvent found high spatial dependence for sand, silt and clay fractions, and moderate dependence for electrical conductivity. Authors cite Grego and Vieira (2005) and associate the strong spatial dependence of granularity fractions to excessive movement in the soil surface, which causes a change of its original structure and makes it similar nearby points. An important aspect to be highlighted is that the degree of spatial dependence considers both the variability between sampling points, as the uncertainty of the determination method in the laboratory (Silva et al., 2010). The range indicates the extent to which the studied variable is correlated, that is, represent the radius in which an area can be considered homogeneous in relation to the parameters studied (Lima et al., 2006). Semivariogram ranges found in this study were 13.71; 13.74; 13.73; 13.75; 13.74 e 7.95 m for soil moisture, electrical conductivity and clay at 0-0.2 and 0.2-0.4 m layers, respectively. For the clay content, Silva et al. (2010) studied the spatial variability of growth parameters of castor bean and physical-chemical properties in the same study area, found a range of 11.82 at 0-0.2 m. However, Souza et al. (2008) also analyzing the clay content at 0-0.2 m found a range of 50 m. These differences can be attributed degree of soil weathering and possible movement of clay in the profile. According to Montanari et al., (2008) an important concept in soil genesis is that the high degree of weathering in the surface layers are due to their greater exposure to occurrence of the weathering process. Semivariogram model fitted for clay at 0.2-0.4 m produced estimated errors with average 0.018 and standard deviations with 0.954, respectively, indicating a good fit confirmed by cross validation. In Figure 2, semivariograms with nugget effect and the Gaussian model are presented. Maps based on the respective semivariograms are shown in Figure 3.

In the contour maps the darker areas show higher values of the evaluated data. Maps for soil moisture at 0-0.2 and 0.2-0.4 m layer show that there is higher soil moisture in the surface layer, probably due to irrigation in the area prior to sample collection. The subsurface layer had low water contents. According to Santos et al. (2012), a way of maintaining the high water levels in the soil in the region would be mainly with the addition of a continuous vegetation cover on the soil, such as mulching. Soil moisture is related to many attributes and soil conditions, such as texture, density and organic matter (Busscher et al., 1997). Cohesion is a characteristic that also changes the soil moisture. Thus, when the soil is dry or has a low water content, particles are often highly aggregated, being difficult to separate them by an external force. Additionally, for soils with loam texture, water loss occurs more rapidly due to high hydraulic conductivity, related to an in efficient water retention. It can be observed that the central regions have lower soil moisture than the borders of the area, indicating that the water movement occurs from borders to the central regions. The darker areas of the maps (left) indicate values with higher soil moisture.

Regarding the maps of soil electrical conductivity, it can

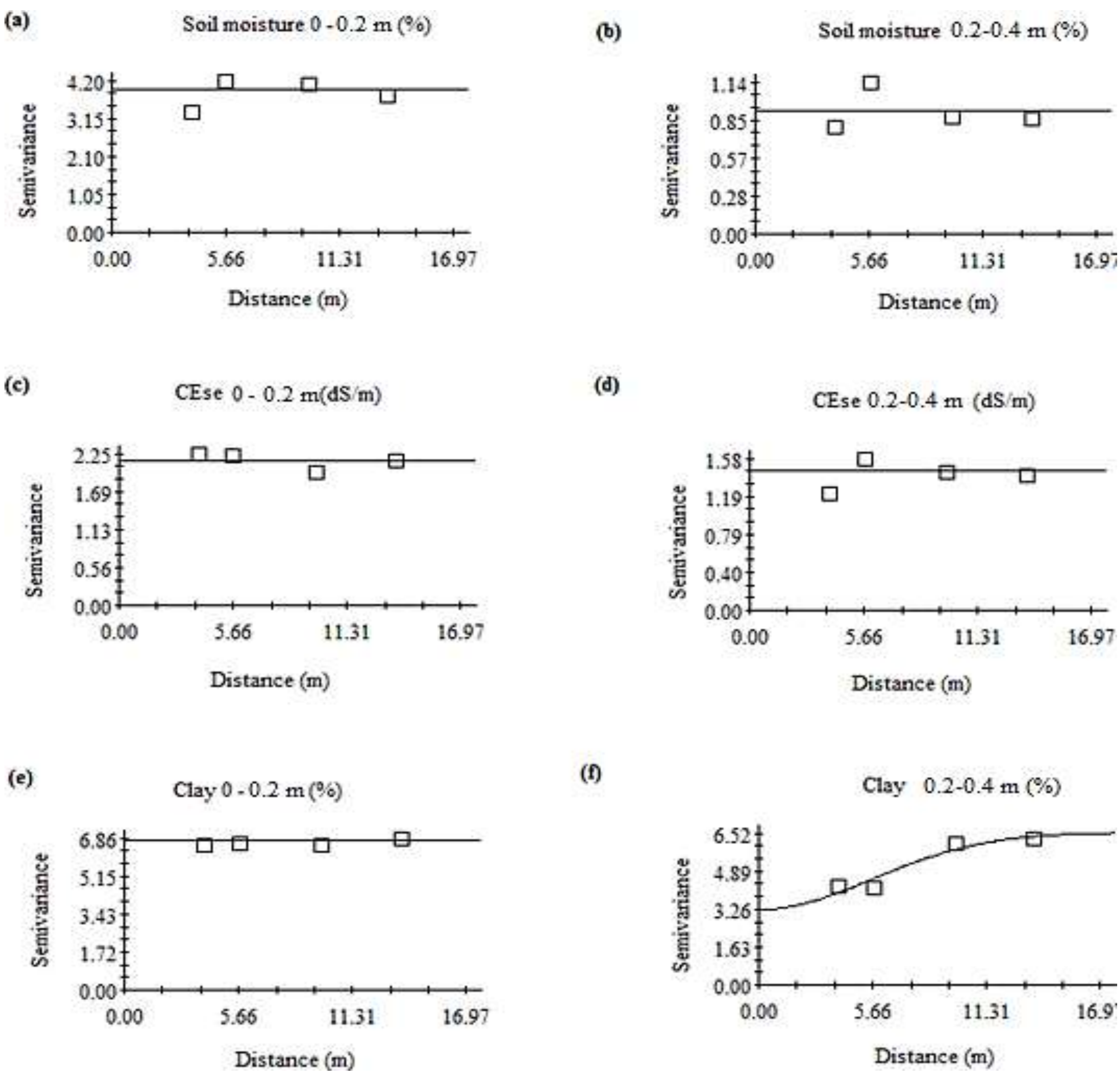

Figure 2. Semivariograms: (a) soil moisture at 0-0.2 m (%); (b) soil moisture at 0.2-0.4 m (%); (c) EC at 0-0.2 m (dS m^{-1}); (d) EC at 0.2-0.4 m (dS m^{-1}); (e) clay content at 0-0.2 m (%) and (f) clay content at 0.2-0.4 m (%).

be seen that the values are also higher in the most extreme and surface areas. According to Silva et al. (2013), the soil exposition to insolation induces capillary ascension, and consequently increases salt concentration in surface layers. Soils with high moisture have lower electrical conductivities. As commented above, the determining factor was soil moisture, because it influenced the electrical conductivity values in the soil profile. The electrical conductivity and moisture contour maps show homogeneity, have revealed a similar pattern of spatial variability. According to Fritz et al. (1998), the electrical conductivity is influenced by the water content, dissolved salts, topography, and the source material in the soil formation. In a study conducted by Silva (2016) with spatial variability of soil electrical conductivity in a cultivated area of sugarcane, the determining factor was soil moisture, because it influenced the EC values in the soil profile, as relief and the water level were the factors

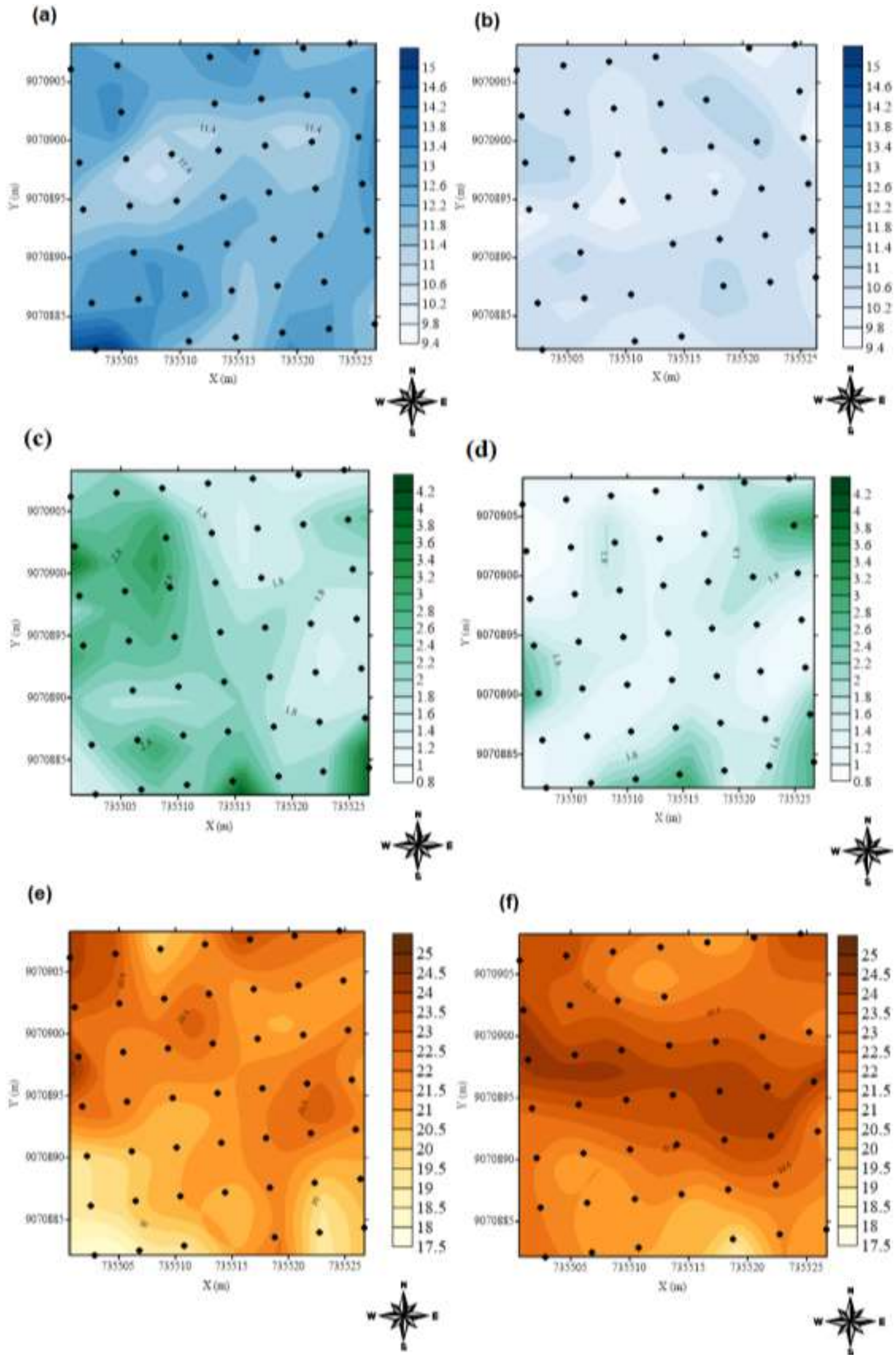

Figure 3. Maps for: (a) soil moisture at 0-0.2 m (%); (b) soil moisture at 0.2-0.4 m (%); (c) EC at 0-0.2 m (dS m^{-1}); (d) EC at 0.2-0.4 m (dS m^{-1}); (e) clay content at 0-0.2 m (%) and (f) clay content at 0.2-0.4 m (%)

that interferes with the spatial distribution of all studied attributes. Values of electrical conductivity at 0-0.2 m layer were higher than 0.2-0.4 m. It can be observed that the map 3c has less amount of clear areas in relation to the map 3d. These results are indicative that the surface layer (0-0.2 m) has higher salinity than the deeper layer (0.2-0.4 m) and this may be attributed to agriculture practiced by the local management, as well as high rates of evaporation and effect of the severe drought in the region. Considering the mean values, the average electrical conductivity of the saturation extract (EC) was equal to 2.05 dS m^{-1} at 0-0.2 m, and 1.57 dS m^{-1} for the 0.2-0.4 m layer. Silva et al. (2010) found in the same study area and before the rainy season, EC values of 1.48 dS m^{-1} and 0.65 dS m^{-1} for the 0-0.2 and 0.2-0.4 m, respectively. These results confirm that the higher salts content is in the surface layer, even using sprinkler irrigation system. Additionally, one can affirm that do not occur salinity problems in the soil, since EC values between 0-4 dS m^{-1} are classified as non-saline according Richards (1954). However, there are areas where EC values are at the limit, and so these areas can easily become saline. The carrot tolerance limits checked by Kotuby-Amacher et al. (2000) are 1.0 dS m^{-1}, with losses of 10, 25 and 50% when EC is 1.7, 2.8 and 4.6 dS m^{-1}, respectively. Also, according to the FAO (2016), the threshold is 1 dS m^{-1}, wherein carrot falls within the rating S (sensitive) salinity tolerance table. The results show that many areas of the map, due to salt levels, may can cause crop development limitations and compromise the agricultural production. Regarding the clay fraction, there is a more homogeneous distribution in the surface layer (0-0.2 m), also due to cultivation practices performed in the area, while at 0.2-0.4 m layer there is a higher amount of this fraction in the central region, also where the electrical conductivity is lower (Figures 3e, f and d). Observing Figure 3f, the clay concentration at the central region can characterize an impediment layer, with areas of lower permeability and higher water retention, soil characteristics with higher clay content. Leão et al. (2010) found that texture variability is related to the rates flow even in a small scale.

Conclusions

Geostatistics allowed the characterization of the spatial variability patterns of the main soil attributes in an irrigated plot in the semiarid of Brazil. It has been shown as an important tool for the prediction of soil attributes. The results may help to issue guidelines for agricultural management. The main results were:

1 The electrical conductivity and moisture contour maps show homogeneity, and revealed a similar pattern of spatial variability.
2 The electrical conductivity of both layers presented a high degree of variability.
3 Occurrence of soil salinity is higher at the soil surface.
4 Water flow occurs from both ends to the central plot, as evidenced by the maps.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Rooting inducers and organic substrates in the propagation of 'Paluma' guava by cutting

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To produce seedlings with quality is one of the factors that mostly contribute to increase the production chain of a fruit crop. The use of organic substrates in the production of seedlings becomes a way to reduce costs by using raw material available regionally. Then, the aim of this study was to evaluate the influence of rooting inducers and the contribution of organic substrates to improve the rooting of herbaceous cuttings of 'Paluma' guava. The experiment was completely randomized arranged in a 5×2 factorial design with 4 replications and 10 cuttings per plot. The factors comprised five substrates (S1-100% OC; S2-25% CRH + 75% OC; S3-50% CRH + 50% OC; S4-75% CRH + 25% OC; S5-100% CRH), where CRH: carbonized rice husk and OC: organic compost, and 2 rooting inducers (Radimaxi 20® and Indolbutyric Acid - IBA), with the concentration of 2000 mg L^{-1}. With regard to the analyzed variables, rooting, mortality, length of roots, and dry weight of shoots and roots did not fit with any regression model. However, the live rootless cuttings, callus, sprouting, leaf retention, and number of roots showed interaction between the inducers and the used substrates. The maximum rooting percentage obtained was 20%, independently of the type of inducer or used substrate; the use of Radimaxi 20® provides greater percentage of cuttings with callus and live rootless cuttings; the carbonized rice husk in composition S2 (25% CRH + 75% OC) is indicated to compose the rooting substrate of 'Paluma' guava cuttings; despite the satisfactory results obtained in this work, more studies are needed to clarify the rhizogenic process of guava in diversified conditions.

Key words: Indolbutyric acid, vegetative propagation. Radimaxi 20®.

INTRODUCTION

The cultivation of guava (*Psidium guajava* L.) has great economic importance in the area of fruit processing industry, with the 'Paluma' cultivar as the most widely used, with 70% of production for the industrial processing

(Pereira, 2008). However, to meet the production expectations, it is necessary plantlets with high standard of quality as result of appropriate technologies to obtain propagation material with high-quality and compatible costs (Franco et al., 2008; Altoé and Marine, 2012).

One aspect that affects the process of commercial propagation by cuttings in guava, aiming to produce fruit with quality, is the cutting type. For this crop, herbaceous cutting has been widely used, since the plantlets grows more quickly, provides a homogeneous orchard, demands less time of work, and provides high-quality plantlets at lower cost (Zietemann and Robert, 2007; Zem et al., 2015).

Another preponderant issue in the propagation of guava tree is the type of substrate. There is a wide variety of commercial substrates in the market that can be used for the rooting of fruit crops; however, small nursery owners do not have access, considering their high cost, and, furthermore, the rooting percentage for some species may be influenced by the material that composes the substrate. Thus, the mixture of raw material obtained regionally allows to lower the cost of production of seedlings. However, the materials used in this purpose must be easily accessible, continuously available, and have low cost, and that can be obtained from agricultural wastes, conditioning similar qualities or higher than those achieved with the commercial substrates (Catunda et al., 2008; Cardoso et al., 2011). An example of material of easy obtaining, availability, and very high quality is the carbonized rice husk and the organic compost (Boechat et al., 2010; Cardoso et al., 2011) that are alternative substrates that can be used in several regions.

To improve the rooting process in guava, plant growth regulators, based on synthetic auxin, is a widespread practice, especially for those species where there is difficulty in rooting, making the production of seedlings viable by the cutting method. Among the synthetic auxins used as rooting inducers, there is the indolbutyric acid – IBA (Yamamoto et al., 2010).

However, there are other compounds used by the nurseries owners, such as mixed mineral fertilizer that contains some nutrients like zinc, which acts on tryptophan synthesis, an auxin precursor (Hartmann et al., 2002). One of these products is the Radimaxi 20®, which is a mixed mineral fertilizer developed for the production of fruit crop seedlings, having in its composition 25.6% Ca, 1.8% S, 2.5% Zn and 1.5% Co, which stimulates rooting (Oliveira et al., 2010; Malvezzi, 2016[1]).

The mineral nutrition may affect adventitious rooting as it interferes in the morphogenetic responses of plants such as rhizogenesis, the density, and the modulation of their length (Assis et al., 2004). Thus, Cunha et al. (2009) showed that in the first stage of induction and formation of roots, the nutrients calcium, iron, copper, boron, zinc, and manganese are important to trigger the process, since they take part of the cell wall formation, lignification, and cell elongation, being these processes essential for root growth.

Considering these evidences and the importance of cuttings propagation in the commercial production of guava, this work aimed to verify the influence of rooting inducers and the contribution of organic substrates to improve the rooting of herbaceous cuttings of 'Paluma' guava.

MATERIALS AND METHODS

Cuttings of guava cv. 'Paluma' were collected from a five-year-old mother plants in the summer. The plants were from the Macaquinhos grange, located in the municipality of Remígio, Paraíba State. The herbaceous cuttings were taken from the apical part of the side branches, with four pairs of leaves, being involved in moistened paper and packed in plastic bags, forming a moist chamber, and afterwards, transported to the Fruit Crop Nursery of the Department of Phytotechny and Environmental Sciences, at the Centre for Agricultural Sciences of the Federal University of Paraíba.

The experimental design was completely randomized in a 5×2 factorial design, with five substrate compositions (S1-100% OC; S2-25% CRH + 75% OC; S3-50% CRH + 50% OC; S4-75% CRH + 25% OC; S5-100% CRH), where CRH: carbonized rice husk and OC: organic compost, and 2 rooting inducers (Radimaxi 20® and Indolbutyric Acid - IBA) used in the concentration of 2000 mg L^{-1}, with four replications and 10 cuttings per plot.

The preparation of the cuttings was performed in the nebulization chamber, getting these with 10 cm long making a straight cut at the apex and beveled at the base, and a couple of leaves at the apex, with the limbs reduced to half of the leaf length.

After prepared, the cuttings had 2 cm of their bases immersed for 5 s in hidroalcoholic solution of IBA and Radimaxi 20®, according to the previously defined treatments. Growth inducers were weighed and dissolved separately in 10 mL of ethanol and the volume was completed to 90 mL of distilled water to give a concentration of 2000 mg L^{-1} for both.

The cuttings were planted in plastic tubes filled with different proportions of substrates and maintained under intermittent nebulization system, and a shading coverage (50%) with temperature ranging from 39 to 20ºC. For the control of fungal diseases, the cuttings were treated with Aliette® fungicide sprays.

After 70 days of experiment installation (Yamamoto et al., 2010), the following variables were analyzed: Cuttings with callus (%), live rootless cuttings (%), number of roots, leaf retention (%), Length of roots (cm), Dead cuttings (%), rooted cuttings (%), cuttings with sprout (%), dry weight of shoots (g) and dry weight of roots (g).

The data were transformed by the square root function (y + 0.5) when obtained by counting and by logarithmic function (log + 1) for the quantitative ones. It was performed analysis of variance and regression using the F test ($p \leq 0.10$) to verify the isolated effects and the interaction between the factors. The SAS/STAT Software 9.3 (2011) was used.

RESULTS AND DISCUSSION

The variables live rootless cuttings, number of roots, cuttings with callus and leaf retention showed interaction

[1] Tereza Malvezzi: Managing partner of Fertsana agricultural specialties LTDA, responsible for the marketing of the Radimaxi 20® (Personal information, 2016).

between inducers and substrates. However, other variables showed no interaction and did not fit with any regression model.

For the percentage of cuttings with callus, there was a significant interaction between inducers and substrates (Figure 1A) observing a higher percentage of callus for IBA with 20% in the formulation S2 (25% CRH + 75% OC) and 33% for Radimaxi 20® in formulation S5 (100% CRH). When Radimaxi 20® was used as rooting inducer, the percentage of cuttings with callus increased linearly; while with the application of IBA, there was only 5.33% of cuttings with callus. As one of the origins of rhizogenesis in the cutting process is the callus, there is a possibility of formation of adventitious roots if a time greater than 70 days for completion of the assay is used. The fact that the Radimaxi 20® has zinc in its composition, what promotes auxin synthesis, may have resulted in increased callus formation.

Ferreira (2013) noted that vine cuttings treated with zinc had higher rooting than those untreated, showing that zinc may have enabled the synthesis of tryptophan, an auxin precursor. However, a higher auxin/cytokine ratio is necessary for the rooting occurrence (Hartmann et al., 2002). Probably, the levels present in the cuttings and the exogenous application of inducers have not been enough to put this ratio in a favorable rate to promote rooting of 'Paluma' cuttings.

For the live rootless cuttings (Figure 1B), there was a significant interaction between the inducers and the substrates, presenting a higher percentage of live rootless for IBA with 13.33% in the formulations S1 (100% OC) and S3 (50% CRH + 50% OC), and 40% for Radimaxi 20® in formulations S5 (100% CRH). The use of Radimaxi 20® gave a linear and increasing response with the rise of proportions of rice husks in the substrate, providing 22.67% of cuttings, which have been kept alive, while with the application of IBA was observed only 5.33%. Thus, it can be noticed that the application of Radimaxi 20® and the use of substrates with high proportions of carbonized rice husks only acted in maintaining the survival of cuttings. Probably, there would be necessary more time for the development of roots than the used herein.

For the number of roots (Figure 1C), there was an adjustment for the quadratic model for the doses effect of IBA, showing at the maximum point 3.58 roots per cutting in the substrate S4 (75% CRH + 25% OC); however, the use of Radimaxi 20® resulted in an average of 0.53. The number of roots in the cuttings was very disparate for the same cultivar, and this corroborates with Zietemann and Roberto (2007) that obtained maximum of 1.88 roots per cutting in the spring, but 4.5 in the summer to 'Paluma' guava. However, Vale et al. (2008), working with IBA and sucrose, observed an average of 12 roots per herbaceous cutting of 'Paluma' guava, using the concentration of 300 mg $L^{-1}$of IBA. The results are consistent with Altoé and Marine (2012) that obtained 3.7 roots by herbaceous cuttings, when assessing the serial minicutting technique in the rooting of 'Paluma' guava.

For the foliar retention, there was a quadratic adjustment using IBA, with the maximum retention provided by the substrate S4 (75% CRH + 25% OC); there was no effect of the substrate using Radimaxi 20®, averaging 0.72% of retention (Figure 1D).

The leaf retention in the herbaceous cutting process is important for the rhizogenesis, since it assists in the production and transport of auxin, enables the supply of carbohydrates through photosynthesis, favoring division and elongation (Vignolo et al., 2014). However, leaves do not influence directly the rhizogenesis, but increases the probability of this event happens. In herbaceous cuttings that do not have high levels of reserve compounds, the young leaves are photosynthetically active and present auxin synthesis (Hartmann et al., 2002). Vale et al. (2008) found no relationship between the leaf retention in herbaceous cuttings of 'Paluma' guava, with their rooting process. However, Pereira et al. (1993) found that the remaining leaves on the cuttings of 'Paluma' guava were effective in the rooting.

The root length was not affected by the factors studied, showing an average of 8.03 cm (Figure 2A). Zietemann and Roberto (2007) also obtained roots with length similar to the cv. Paluma rooted in the spring although it was obtained the maximum value of 12.25 cm when the rooting occurred in the summer. But, Altoé and Marine (2012) evaluating the serial minicutting technique in the rooting of 'Paluma' guava, obtained length of 5.85 cm in herbaceous cuttings, lower than that obtained herein.

There was no significant difference between the substrates formulations and rooting inducers for the mortality percentage of cuttings, showing a mean value of 65.33% of dead cuttings (Figure 2B). This high percentage of mortality may be related to the physiological condition of the mother plant, as the temperature is very high in the summer season in the Northeast region above 24ºC, which and considered ideal to stimulate cell division in rooting area (Hansen, 1989; Yamamoto et al., 2010), and the mother plants, although irrigated, may have increased the level of growth inhibitors, in order to reduce the emission of new sprouts. Although Zietemann and Roberto (2007) have obtained the higher rooting in cuttings of 'Paluma' in the summer, in the South region. This time resulted in higher mortality of cutting for this cultivar in the Northeast region.

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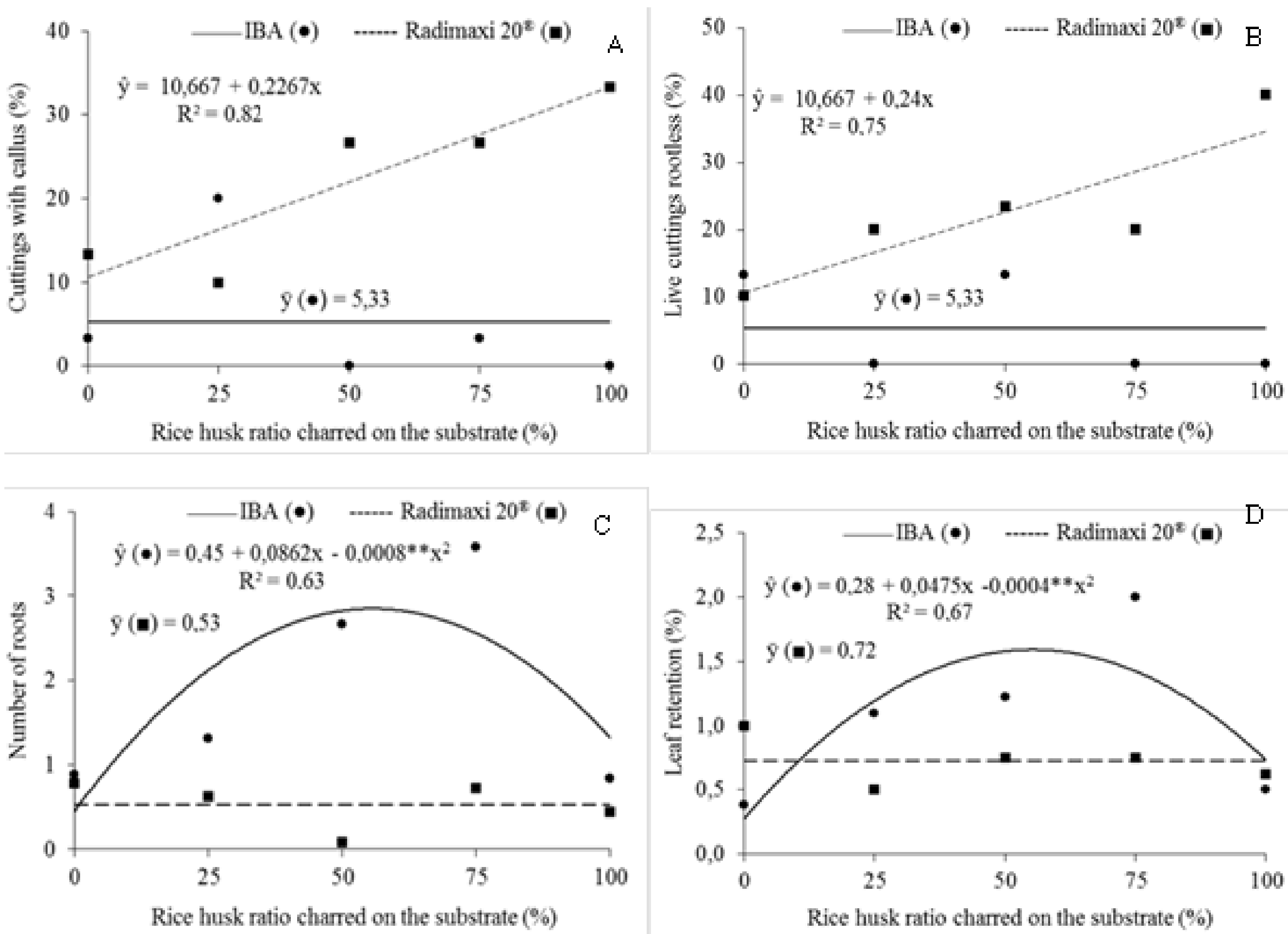

Figure 1. Cuttings with callus (A), Live rootless cuttings (B), Number of roots (C), and Leaf retention (D) in the cuttings of guava cv. Paluma grown on substrates (organic compost and carbonized rice husks) and treated with inducers at 70 days after planting.

In the Figure 2C, there is no effect of stimulators of rhizogenesis, with 20% of rooting, in average. This percentage is low and does not corroborate with those obtained by Zietemann and Roberto (2007) that evaluated the rooting of herbaceous cuttings of 'Paluma' guava collected in spring and summer periods in the north of Paraná State, using carbonized rice husk and vermiculite substrates, and doses of IBA; they obtained 53.75% of rooting, regardless the period of collection and the substrates used, but 73.75% of rooting was obtained when 2000 mg L^{-1} of IBA was applied. These authors recommend the application of carbonized rice husk substrate as the most suitable for the rooting; however, it was verified herein that the S5 (100% of carbonized rice husk) did not increase the rooting percentage. Vale et al. (2008) observed rooting of 60% at a dose of 300 mg L^{-1} of IBA 60 days after the herbaceous cutting of 'Paluma' guava.

The fact of obtaining contrasting results for the same cultivar may be related to the age of the mother plant and environmental factors such as the period of collection of cuttings because the summer in the Northeast region is characterized by high temperatures and the plants may have reduced the percentage of rooting promoters as a response for slowing the growth even being irrigated. Hartmann et al. (2002) showed that environmental and physiological conditions of the mother plant can determine the percentage of rooting, since cuttings taken from mother plants that have suffered water stress may have high levels of abscisic acid and ethylene. Therefore, the level of auxin used did not raise the levels of rooting promoters on the cuttings. Palú et al. (2013) also did not observe efficiency in root induction using the Radimaxi 20® in the rooting of passion fruit cuttings.

Regarding the percentage of cuttings with sprouts, there was an increase in the percentage of sprouts in cuttings treated with IBA, being more efficient than Radimaxi 20® (Figure 2D). The emergence of rootless cuttings with sprouts may occur if the cytokinin levels are high, resulting in low ratio of auxin/cytokinin. The high levels of cytokinin promote sprout formation and not roots (Hartmann et al., 2002), evidencing that the dose of auxin

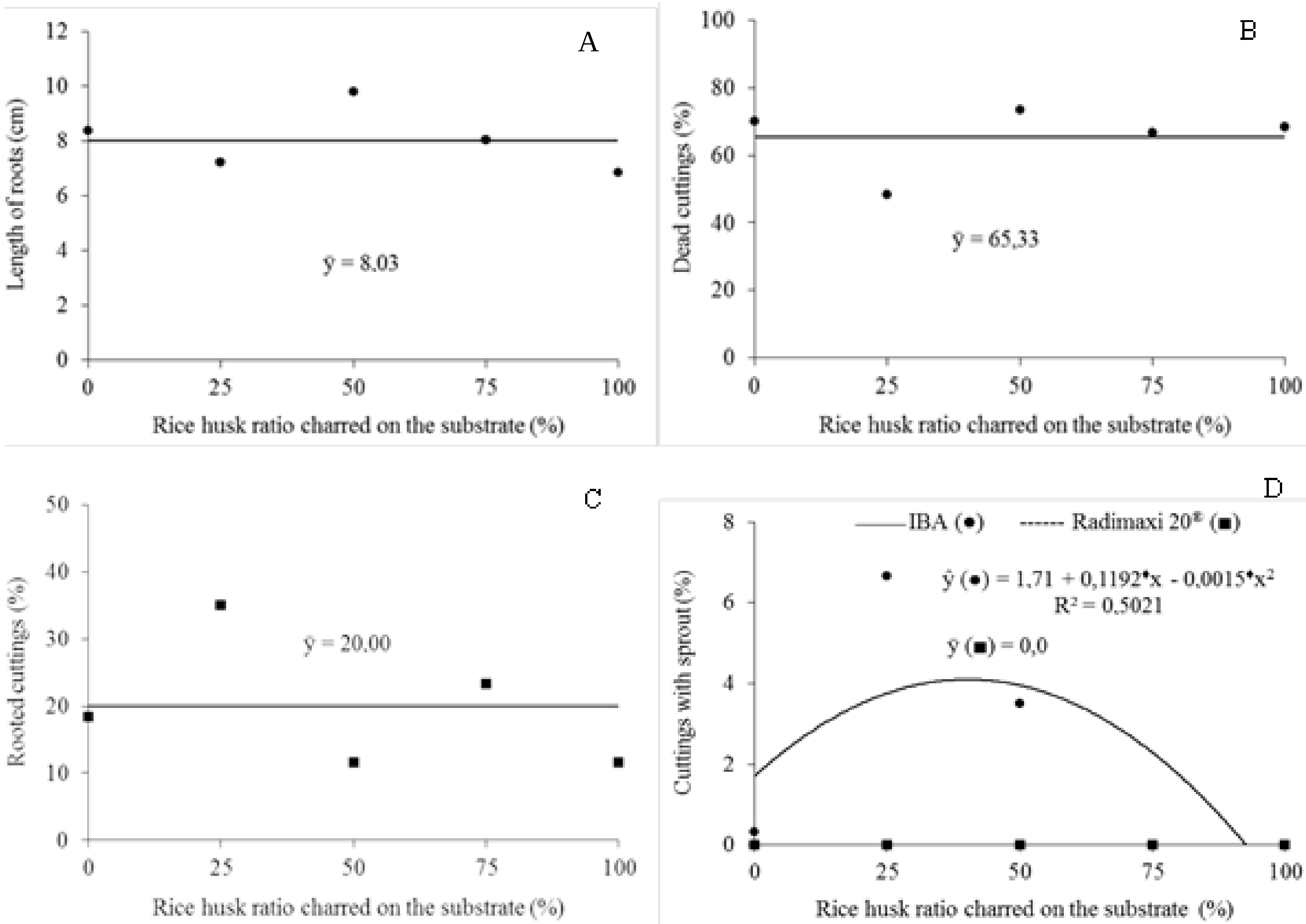

Figure 2. Length of roots (A), Dead cuttings (B), Rooted cuttings (C), and cuttings with sprout (D) in cuttings of guava cv. Paluma, grown on substrates (organic compost and carbonized rice husks) and treated with inducers at 70 days after planting.

applied did not increase this ratio to promote rhizogenesis. The absence of sprouts in the cuttings treated with Radimaxi 20® can be a result of reserves utilization in the cuttings for the formation of callus (Figure 1A).

For the dry weight of shoot, there was only isolated effect of inducers, with an average weight of 3.17 g for cuttings treated with Radimaxi 20®, and 1.18 g for the cuttings treated with IBA (Figure 3A). With regard to the dry weight of root, there was neither significant interaction nor isolated effects for the studied treatment (Figure 3B). Zietemann and Roberto (2007) also observed the lack of differences between substrates and IBA doses in the study of rooting of herbaceous cuttings of 'Paluma' guava. Milhem (2011) working with herbaceous cuttings and minicuttings of 'Paluma' guava, did not observe variations to the dry weight of root among the propagules and the used substrates.

The performance of inducers and substrates in the propagation of herbaceous cuttings of guava is highly variable due to genetic material, the edaphoclimatic condition of cultivation area, and the physiological conditions of the mother plants. These interferences allow variations in the percentage of rooting for the same cultivar, as demonstrated herein. The use of Radimaxi 20® was beneficial for the callus formation, but the time for rooting of cuttings must be longer, requiring tests to assess the changes in product concentrations in order to obtain best results.

Conclusions

Both Radimaxi 20® and Indolbutiric Acid – IBA concentration of 2000 mg L^{-1} and the organic substrates (organic compost and carbonized rice husk) do not affect the rooting of 'Paluma' guava cuttings. The use of Radimaxi 20® resulted in a higher percentage of callus

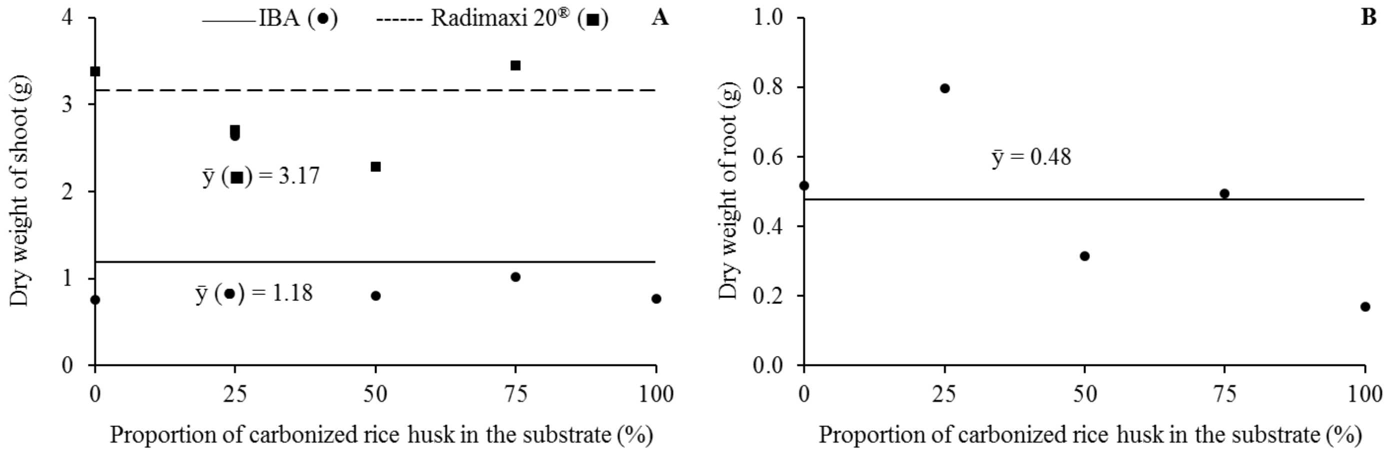

Figure 3. Dry weight of shoot (A) and dry weight of root (B) of cuttings of guava cv. Paluma, grown on substrates (organic compost and carbonized rice husks) and treated with inducers at 70 days after planting.

formation and live rootless cuttings.

Conflict of Interests

The authors have not declared any conflict of interests.

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