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Full Length Research Paper

Development and parasitism of *Encarsia hispida* (Hymenoptera: Aphelinidae) on *Bemisia tabaci* biotype B in cotton

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The biological control of pests is essential for the use of Integrated Pest Management in agricultural environments. In this context, the objective of this study was to identify biological parameters and quantify the parasitism index of *Encarsia hispida* on *Bemisia tabaci* biotype B nymphs in cotton plants. The research was conducted at the Entomology Laboratory of the Federal University of Paraíba, in Areia, Paraíba State, Brazil. For the first bioassay, the treatments consisted of cotton cultivars BRS H8 and BRS Topázio to evaluate the biological development of the parasitoid in its host. In the second bioassay, these cultivars were used to assess the impact of the biological agent in a greenhouse. In the first experiment, there were only female parasitoids with longevity of 24.61 and 22.61 days in BRS H8 and BRS Topázio, respectively. However, they were not statistically different. The life cycle of the parasitoid (egg to adult) was 35.68 and 33.71 days in BRS H8 and BRS Topázio, respectively, and they did not differ from each other. In the second bioassay, there were *E. hispida* parasitism indexes around 34.33 and 29.63% in BRS H8 and BRS Topázio, respectively. The parasitoid *E. hispida* develops properly when the nymphs of the host were from the two cotton cultivars. The parasitoid *E. hispida* has an application potential in the biological control of *B. tabaci* biotype B whiteflies.

Key words: Biological control, whitefly, biological parameters, parasitoid.

INTRODUCTION

The cotton plant (*Gossypium hirsutum* L. var. latifolium Hutch) is significant in the Brazilian Agricultural Theater. It is produced in all five regions of Brazil, distributed in more than half of its federal units (Oliveira et al., 2012). The states of Mato Grosso, Bahia and Goiás are the most relevant (IBGE, 2014). However, the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) biotype B is a very important pest, for it causes significant losses in the agricultural production of various cultures in the world (Begum et al., 2011).

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The whitefly weakens the host plant during feeding and excretes sugary substances on the surface of leaves and fruits (Horowitz et al., 2011), causing sooty mold, which interferes with photosynthesis (Xu et al., 2013). In addition, it may inoculate the host plant with viruses, causing diseases in various cultures (Navas-Castillo et al., 2011). The control of *B. tabaci* biotype B is made exclusively by chemical methods, inducing the selection of resistant populations (Shadmany et al., 2013) and interfering with the survival of beneficial agents.

Among the most promising contributors to the control of the whitefly population, the species *Encarsia hispida* De Santis (Hymenoptera: Aphelinidae) may be mentioned (Hernández-Suárez et al., 2003; Lourencão et al., 2007; Torres et al., 2014). However, studies related to the influence of the whitefly's (*B. tabaci* biotype B) host plant on the biological aspects of *E. hispida* are needed. Takahashi et al. (2008) evaluated the biology of *Encarsia formosa* (Hymenoptera: Aphelinidae) in *B. tabaci* biotype B in different host plants and found that, when whitefly nymphs fed on tomato, they provided a more prolonged development of the parasitoid compared to those fed on cabbage. The information in this context is a relevant prior knowledge for the adoption of biological control programs.

The species *E. hispida* has an application potential in biological control of whiteflies. However, in the literature, there is no information about its biology as parasitoids of whitefly nymphs from agricultural and ornamental plants, although the potential of this parasitoid has been noted in records of success in controlling different species of whiteflies in field and at greenhouses. The objective of this study was to identify biological parameters and quantify the parasitism index of *E. hispida* in *Bemisia tabaci* biotype B nymphs in cotton plants.

MATERIALS AND METHODS

The research was conducted at the Entomology Laboratory of the Federal University of Paraíba (LEN/UFPB), Areia Campus, Paraíba State. BRS H8 (white) and BRS Topázio (colored) cotton cultivars used in this study were from the Brazilian Agricultural Research Company at the National Center for Cotton Research (EMBRAPA/CNPA). The whitefly *B. tabaci* biotype B and the parasitoid *E. hispida* were obtained from cabbage plants (*Brassica oleracea* L. var. acephala) at Campus II of UFPB.

Rearing of *B. tabaci* biotype B

The rearing of *B. tabaci* biotype B was in a greenhouse with poinsettia plants (*Euphorbia pulcherrima* Willd) using pots with a 10-liter capacity and a substrate with a blend of vegetable soil, manure and sand (1:2:1 ratio, respectively). The host plants were obtained by vegetative propagation. Three months after the emergence of shoots, they were infested with whiteflies. The plants were involved in circular galvanized metal frame cages with an anti-aphid "*voil*" tissue mesh (50 × 26 cm). As for the suspension of cages, a wooden structure with galvanized wire on the sides was mounted, thus allowing cage height adjustment following the development of the plant.

After approximately 15 days, numerous whitefly nymphs were collected to find the colony. The environmental conditions were 26 ± 2°C, relative humidity 70 ± 10%, and photoperiod of 12 h. However, the maintenance of the whitefly population was made by free poinsettia plants.

Rearing of *E. hispida*

Female parasitoids were collected from poinsettia using 00 gelatin capsules (Medeiros, 2009) released among poinsettia plants that would be colonized. They contained whitefly nymphs in their 3rd and 4th instars in the above-mentioned environment. After the release of the parasitoid, 3rd instar larvae of this biological agent were expected to excrete the meconium so that the darkening process to pupation would occur. After this process, the pupae were transferred along with leaves to the laboratory. Then, the pupae were removed with an entomological pin and placed in Petri dishes (9.0 × 1.5 cm) coated with plastic film until emergence.

After emergence, the adult insects were captured in 00 gelatin capsules and accommodated in test tubes (2.5 × 8.5 cm) with a honey solution (20%) distributed on the sides of the tubes to feed the parasitoid. Food was supplied every three days and the exchange of containers was made every 15 days. The containers were sealed with plastic wrap.

Development of *E. hispida* on *Bemisia tabaci* biotype B in cotton

The bioassay was made by adapting the methodology proposed by Antony et al. (2003). Cotton cultivars BRS H8 and BRS Topázio, 30 days after planting, were placed in plastic bags with 1 kg of the above-mentioned substrate. The plants were placed at the laboratory at 25 ± 2°C, relative humidity 70 ± 10%, and photoperiod of 14 h. These plants were infested with 20 couples of whiteflies for oviposition using a cage (18 × 13 cm) with a "*voil*" tissue involving the leaves of the two cultivars for 24 h. After infestation, it was expected that whitefly nymphs reached the 3rd instar. Four nymphs on each leaf were selected for parasitoid infestation.

For the oviposition of the parasitoid, one individual with up to 24 h of age fed with honey was used. It was collected and released using 00 gelatin capsules (Medeiros, 2009) in clip cages (2.0 cm), allowing contact with the hosts for 24 h. After oviposition, parasitized nymphs were daily recorded using stereomicroscopy through the cuticle of whitefly nymph. Upon reaching the pupal stage, they were removed with an entomological pin and placed in containers (9.0 × 1.5 cm) waiting for the emergence of the parasitoid. After emergence, they were captured and transferred to test tubes (8.5 × 1.5 cm) containing food.

The parameters identified were the corresponding development periods of oviposition to larva, pupa period, pre-imago period, female longevity, oviposition to adult and sex ratio.

Parasitism of *E. hispida* on *Bemisia tabaci* biotype B in cotton

For the record of the incidence of parasitoids in *B. tabaci* biotype B nymphs in cotton cultivars BRS H8 and BRS Topázio in a greenhouse, the methodology proposed by Simmons and Abb-Rabou (2005) suffered adaptations. Three leaves of each cultivar per pot were collected randomly. Each container contained three plants, totaling 30 leaves. The cotton plants were used 60 days of age in an environment at a temperature of 26 ± 2°C and relative humidity of 70 ± 10% with a 12 h photoperiod. The leaves were taken to the LEN/CCA for stereomicroscopy.

The observation of parasitism on nymphs by the parasitoids was recorded by their holes with their ovipositor on the host integument

Table 1. Biological parameters of *Encarsia hispida* parasitizing *Bemisia tabaci* biotype B in two cotton cultivars.

Cultivars	Egg to larva (days)	Pupa (days)	Pre-imago (days)	Egg to adult (days)	Longevity of ♀ (days)	Sex ratio
BRS H8	6.01±0.09[a]	5.06±0.08[a]	11.07±0.13[a]	35.68±1.67[a]	24.61±1.67[a]	1.0
BRS Topázio	6.05±0.12[a]	5.05±0.09[a]	11.10±0.17[a]	33.71±1.16[a]	23.61±1.17[a]	1.0
CV (%)	7.79	7.56	6.13	18.63	27.37	

Means followed by the same letter in columns do not significantly differ from each other by F test (P = 0.05). Untransformed data ± mean standard error.

and the visualization of the larval development of this parasitoid inside the whitefly.

Statistical analysis

The experiments were arranged in a completely randomized design (CRD). For the experiment I (development), the repetition consisted of four nymphs of 3rd instar whiteflies with 20 repetitions per cultivar. In experiment II (parasitism), using the data analyzed, it was calculated by the Simmons and Abb-Rabou (2005) equation:

$$P = \frac{NPP + NP + NA}{NN2 + NN3 + NN4 + NPP + NP + NA} \times 100$$

NPP = number of pre-pupae of the parasitoid; NP = number of pupae of the parasitoid; NA = number of adults of the parasitoid; NN2 = number of 2nd instar nymphs of whitefly; NN3 = number of 3rd instar nymphs of whitefly; NN4 = number of 4th instar nymphs of whitefly.

Data were subjected to analysis of variance and means of the treatments were compared by F test at 5% probability. Data were analyzed by the software Assistat 7.7 (Silva and Azevedo, 2002).

RESULTS AND DISCUSSION

Development of *E. hispida* on *Bemisia tabaci* biotype B in cotton

The evaluated biological parameters of *E. hispida* were not affected when *B. tabaci* biotype B nymphs were developed in cotton cultivars BRS H8 and BRS Topázio. For the larva to egg period, the pupa duration, the pre-imago period, the egg to adult period and longevity had no significant differences from each other. Regarding the variable sex ratio, only female individuals of *E. hispida* were recorded when host nymphs were from both cotton cultivars (Table 1).

It was found that the parasitoid broke the integument of the host using the ovipositor to feed from the hemolymph. The sucking of the hemolymph of the host by parasitoids enables them to acquire nutrients. However, this process destroys the parasitoid's oviposition opportunity for the development of their offspring (Shah et al., 2015).

The 3rd instar larva of the parasitoid, upon releasing meconium, begins the sclerotization process of its cuticle in a matter of days. When this is completed, the pupal stage begins. After its formation, it shows small movements within its host in a matter of hours, decreasing when the emergence of adults is close. Before emergence of the adult, it is observed that the individual changes its body position to perform an opening in the host. According to Antony et al. (2004), this type of adult insects creates an opening in the cuticle of the host to enable its emergence.

The values corresponding to the periods egg to larva and pre-imago of this study differ from those found by Azimi et al. (2014) for *Encarsia formosa*, and Pessoa et al. (2016) for *Encarsia desantisi* (Hymenoptera: Aphelinidae) in non-BT cotton. Thus, researchers have shown that species from the genus *Encarsia* may reduce or extend its life cycle in function of the host plant where the whitefly developed.

The results for the egg to adult period of *E. hispida* which developed in both cotton cultivars indicate that host nymphs of *B. tabaci* biotype B, allow a proper biological development of the parasitoid. According to Talaei (2009), the plant host is an important factor for the adequacy of hosts to parasitoids.

The longevity of the female parasitoid was 24.61 and 22.61 days for BRS H8 and BRS Topázio, respectively. Pessoa et al. (2016), evaluating the natural agent *E. desantisi* in *B. tabaci* nymphs from cotton cultivars DeltaOpal and FM 993, found longevity values of 19.3 and 22.3 days, respectively. According to Hódar et al. (2002), the quality of food is one of the main factors influencing longevity, body size and abundance of fertility.

According to Giorgini et al. (2009), a characteristic of the genus *Encarsia* is its asexual reproduction, thelytokous parthenogenesis, where the presence of males is rare or unknown. This characteristic of parasitoids, that is, breeding female individuals, is of great importance in biological control programs. There is a possibility of the presence of the symbiont *Cardinium hertigii*, which induces parthenogenesis and breeds exclusively female individuals of *E. hispida*. They are located with a larger quantity in follicular and nutritive cells, and to a lesser extent in parasitoid oocytes (Zchori-Fein et al., 2004). Thus, the symbiont influenced sex ratio, which was 1.0 in both cultivars assessed.

Parasitism of *E. hispida* on *Bemisia tabaci* biotype B in cotton

The parasitism index of *E. hispida* in *B. tabaci* biotype B

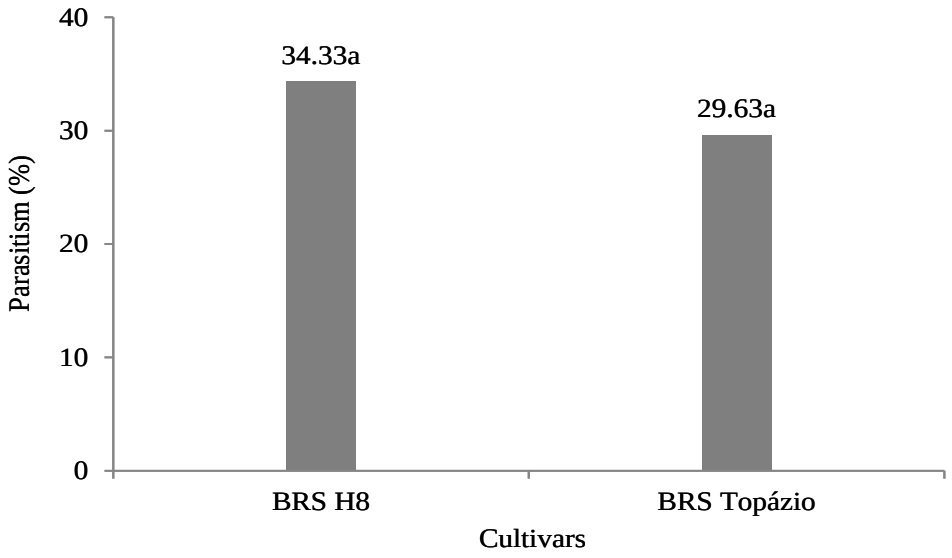

Figure 1. Parasitism of *E. hispida* when whitefly nymphs were from the two cotton cultivars.

nymphs from both cotton cultivars were not statistically different from each other (F = 3.9623, P = 0.0618) (Figure 1). In total, 1.230 and 933 parasitoids from whitefly nymphs in BRS H8 and BRS Topázio cultivars, respectively, were recorded. Among these parasitoids, there was the presence of individuals from both sexes in both cultivars. They are visually different in color. The female has a light yellow color all over the body, while the male has a brown color (Myartseva and Evans, 2008).

Graff et al. (2006) reported the performance of this natural agent regarding its parasitism in *B. tabaci* pests in vegetable plants. They concluded that its effect on the density of this pest's nymphs varied only in tomatoes, while in pepper and cucumber plants it had the same parasitism rate on the host. These authors analyzed the use of *E. hispida* for *B. tabaci* on hibiscus plants from 2004 to 2005 to control the plague, and found high rates. Yet the parasitoid behavior was influenced by abiotic and biotic factors. Furthermore, the use of synthetic products affected this agent regarding the control of *B. tabaci*. In Brazil and in the world, the species *E. hispida* has been reported affecting different host species of several ornamental plants, vegetables and crops, resulting in socioeconomic damage (Hernandez-Suarez et al., 2003; Oliveira et al., 2003; Lourencão et al., 2007; Torres, 2014).

Conclusion

The parasitoid *E. hispida* develops properly when *B. tabaci* biotype B nymphs are from the two cotton cultivars BRS H8 and BRS Topázio. The parasitoid *E*. *hispida* has an application potential in the biological control of the whitefly *B. tabaci* biotype B.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Screening melon genotypes for resistance to *Meloidogyne enterolobii*

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Melon (*Cucumis melo* L.) is a cucurbitaceous of great appreciation worldwide. The intensive cultivation of melons has favored the increase of plant health problems in several producing regions. Among these problems, the root-knot nematode (*Meloidogyne* spp.) stands out. The development of genetically resistant cultivars is consolidated as an effective strategy for the management of these pathogens, it is then essential to screen cultivars and accessions for later identification of genetic sources of resistance. This study aimed to evaluates the reaction of melon genotypes to *Meloidogyne enterolobii*. The essay was conducted at the Sector of Vegetable Crops and Aromatic-Medicinal Plants of UNESP-FCAV Jaboticabal Campus, in greenhouse, from March to June, 2015. It was evaluated 18 melon genotypes, two commercial cultivars 'Fantasy' and 'Louis', and as susceptibility control, the tomato 'Santa Cruz Kada'. A completely randomized design was adopted, with 21 treatments and 7 repetitions. The total number of eggs and juveniles in the roots (TNEJ) and the reproduction factor (RF) were obtained in order to determine the reaction of each genotype evaluated. The accessions PI 414723, AC 29, and PI 124112 are resistant to *M. enterolobii* and are therefore promising for breeding programs.

Key words: *Cucumis melo*, reproduction factor, plant breeding, root-knot nematode.

INTRODUCTION

The melon (*Cucumis melo* L.) is a cucurbitaceous of great appreciation worldwide, which uses it in many ways, for the preparation of juices, fruit salads and for fresh consumption. A diverse offering of fruits of this species is a differential, and these vary in shape, flavor, flesh color, aroma, among other aspects. The cropping systems are also diverse, depending basically on the type of melon intended to be commercialized. The noble melons, on account of its high commercial value, are commonly grown in greenhouses (Peil, 2003; Ito et al.,

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2014). In turn, the yellow melons are open-field grown, in large extension areas.

The intensive cultivation of cucurbits has promoted the development of nematodes that results in significant losses in highly infest crops (Gallati et al., 2015). For cucurbits, the most common species are *Meloidogyne incognita*, *Meloidogyne javanica,* and *Meloidogyne arenaria* (Pinheiro and Amaro, 2010).

Recently, another species, *Meloidogyne enterolobii*, although it occurs less frequently in relation to *M. incognita* and *M. javanica*, is becoming increasingly important, since many reported melon genotypes as resistant to major root-knot nematodes show no resistance to this species (Brito et al., 2007; Cetintas et al., 2007; Cantu et al., 2009; Kiewnick et al., 2009; Eppo, 2011; Melo et al., 2011; Westerich et al., 2011; Castagnone-Sereno, 2012; Singh et al., 2013).

Some authors believe that the cultivation of plants resistant to other species of nematodes can cause, eventually, a selection pressure in favor of *M. enterolobii,* and can take it to the status of primary economic importance. In some countries, *M. enterolobii* is classified as a quarantine pest (Castagnone-Sereno, 2012; Elling, 2013).

The nematode belonging to *M. enterolobii* species was first described by Yang and Eisenback (1983) from the roots of *Enterolobium contortisiliquum* (Vell.) Morong. Rammah and Hirschmann (1988) classified the same nematode as *Meloidogyne mayaguensis,* from eggplant roots (*Solanum melongena* L.). Later, with the use of more sophisticated methodologies, it was established that *M. mayaguensis* is actually a young form of *M. enterolobii*, being these two species synonyms and the second nomenclature should be adopted (Xu et al., 2004; Karssen et al., 2012).

Symptoms of *M. enterolobii* are characterized by leaf yellowing, reduced plant growth and root galls (Eppo, 2011), and interactions may also occur with other pathogens (Pinheiro and Amaro, 2010). Although there are few reports (Pinheiro et al., 2014; Bitencourt and Silva, 2010) in the literature regarding *M. enterolobii* in melon, the occurrence of this pathogen in other cucurbits is indicative that this nematode can potentially cause economic damage to melon crops. The identification of sources of resistance to *M. enterolobii* is therefore of fundamental importance for breeding programs. The use of genetically resistant plants is the most sustainable method to control *Meloidogyne* spp., being a challenge the search for sources of resistance (Molinari, 2011). Alternatively, however, in a short-term, the use of resistant rootstocks would be feasible, as practiced for other crops (Louws et al., 2010; Thies et al., 2012; Galatti et al., 2013; Guan et al., 2014). Nevertheless, this practice would have greater applicability in noble melons cultivated in greenhouses, because of the high commercial value-added. This work aimed at select melon genotypes resistant to *M. enterolobii*, in order to use the sources of resistance to start breeding programs.

MATERIALS AND METHODS

The experiment was conducted at the Sector of Vegetable Crops and Aromatic-Medicinal Plants of UNESP- FCAV Jaboticabal Campus, in greenhouse, from March to June 2015. A randomized complete block design was adopted, with 21 treatments and seven replicates. It was considered as repetition a plant inoculated with *M. enterolobii* per pot. As control of susceptibility, the tomato 'Santa Cruz Kada' was used.

The melon genotypes used were Vendrantais, PI-140471, PI-432398, PI 420150, PI 5322830, PMR-5, PI-157082, WMR-29, Charentais Fom 1, PI-420145, C160, CNPH 01- 930, Nantais Oblong, AC 29, PMR-45, PMR-6, PI 414723, PI 124112, used as differentiators of powdery mildew races and gummy stem blight and the commercial cultivars Louis and Fantasy. The inoculum were obtained from a subpopulation of *M. enterolobii*, extracted from guava 'Paluma' roots, coming from Taquaritinga, Sao Paulo State, Brazil . The species were identified at the Nematology Laboratory of UNESP-FCAV Jaboticabal Campus, using a photonic microscope TNB-40T-PL. The identification was based on the morphological characters of the perineal pattern, prepared as Taylor and Netscher (1974), on the morphology of the males lip region (Eisenback et al., 1981) and on the esterase isozyme phenotype, obtained by the technique Esbenshade and Triantaphyllou (1990), using a traditional vertical electrophoresis system Mini Protean II from BIO-RAD.

The subpopulation was previously multiplied in potted eggplant (*Solanum melongena* L.) 'Anápolis', in greenhouse. In order to obtain the initial inoculum, after 90 days of inoculation, eggplant plants were removed from pots and the roots were washed and pounded in a blender with 0.5% sodium hypochlorite (Hussey and Barker, 1973). The estimation of eggs and juveniles population presents in suspension was carried out with the aid of Peters counting chamber, using a photonic microscope, with subsequent adjustment of the concentration at 1000 eggs and second stage juveniles/mL and inoculation of 5 ml of this suspension per seedling. The melon and tomato seedlings were produced in 128 cells polystyrene trays using the commercial substrate Bioplant®, in greenhouse equipped with sprinkler irrigation system. It was seeded two seeds per cell, with subsequent thinning.

When the seedlings were 25 days-old, the transplant was held. It was used two-liters plastic pots. The substrate was composed of a mixture of soil, sand and cattle manure, at the ratio 1:1:1. This mixture was previously autoclaved (120°C, 1 atm, 1 h). At transplanting, it was also held up the inoculation of the suspension containing eggs and second stage juveniles of *M. enterolobii.* The nematode species identity was confirmed at the Nematology Laboratory of UNESP-FCAV Jaboticabal Campus. For this, it was used the perineal pattern as Taylor and Netscher (1974), and morphology of male lip region, according to Eisenback et al. (1981). All inoculated plants were analyzed at 60 days after transplanting and inoculating the nematodes.

All roots were gently washed in a bowl with water, in order to remove the excess of soil. It was then processed for the extraction of nematodes eggs and juveniles, according to Hussey and Barker (1973). The final population of each suspension was derived from individually processed root systems and estimated by counting eggs and juveniles with the aid of Peters counting chamber, using a photonic microscopy. This population was used for determining the reproduction factor (RF), whereas plants with FR<1 were considered as resistant, and those with FR≥1, susceptible to the nematode, according to Oostenbrink (1966).

The data were transformed to $\sqrt{x}$. Analyses were performed

Table 1. Summary of the analysis of variance of melon genotypes reaction to *Meloidogyne enterolobii*. Unesp – FCAV – Jaboticabal (SP), 2015.

Source	DF	TNEJ[y]	RF[z]
Treatment	20	13120,70**	2,53**
Error	126		
Total	146		
General average		97,98--	1,63--
CV (%)		30,08--	30,21--
Phenotypic variance		1874,38--	0,36--
Environmental variance		124,15--	0,03--
Genotypic variance		1750,24--	0,33--
Ratio CVg/Cve		1,42--	1,15--

** Significant effect by F test at 1% probability, [ns] Not significant at 1% probability, [y] Total number of eggs and juveniles of second stage, [z] RF reproduction factor.

using the statistical software Genes (Cruz, 2013), and averages were grouped by the Scott and Knott test (p <0.01). Phenotypic, genotypic and environmental variances, as well as the ratio CVg/CVe were estimated.

RESULTS

There were differences (p<0.01) among genotypes for the total number of eggs and juveniles (TNEJ) and reproduction factor (RF) when inoculated with *M. enterolobii.* Therefore, this analysis assumes that chance produces only small deviations, and the major differences are generated by real causes. For this reason, we used this type of analysis) (Table 1). The environmental variation coefficients for total number of eggs and juveniles and reproduction factor were 30.08 and 30.21, respectively.

The variables TNEJ and RF showed values of CVg/CVe of 1.42 and 1.15, respectively, indicating that a selection of resistant genotypes through phenotypic traits, would be effective. *M. enterolobii* inoculation was efficient, since there was multiplication of the nematode in tomato 'Santa Cruz Kada', which presented averages FR>1 and TNEJ larger than what was inoculated (Table 2). For TNEJ, it was established three groups according to average grouped by the Scott and Knott test. The susceptible control (tomato 'Santa Cruz Kada') had the highest average (43598) and access ‘PI 124112', the lowest average (1594).

For RF, it was formed four groups of averages by the Scort and Knot test, with the tomato 'Santa Cruz Kada' presenting the highest value and 'PI 124112’ the lowest, averaging 8.7 and 0.3, respectively. Based on the reproduction factor (RF), as Oostenbrink (1966), the accessions that presented RF<1 were considered resistant to *M. enterolobii*, namely: ‘PI 414723’, ‘AC 29’, and ‘PI 124112'. Similarly, other materials were considered susceptible for presenting RF≥1. All materials classified as resistant were not immune to *M. enterolobii*, having, as the other tested materials, hosted the nematode. Comparing TNEJ, seven materials did not differ statistically from the three genotypes considered as resistant, namely: ‘Charentais Fom 1’, 'PI-420145', 'C160', 'CNPH-01930', ‘Nantais Oblong ', ‘PMR-45’, and ‘PMR-6’.

DISCUSSION

The mean square analysis of variance is significant, which may be indicative of the existence of variability between genotypes. The higher coefficient of variation can be explained by the greater environment influence on the characteristic in question, since the variable response results from interaction between two biological factors (nematodes x plants). There are reports of several works in which were also obtained high coefficients of variation, being characteristic of this type of essay (Wilcken et al., 2005; Freitas et al., 2008).

It is observed that, for both evaluated characteristics, most of the phenotypic variance was attributed to genetic effects (Table 1). This result expresses the reliability of the obtained results, by indicating that the responses had low environment influence and are highly determined by genetic effects. Based on the favorable results in some genotypes, for resistance to *M. enterolobii*, it is noted that there is the possibility of selection for resistance in subsequent generations. One way to increase the efficiency of breeding programs would make selection based on the average of progenies selection (Carvalho Filho et al., 2011).

When the reason CVg/CVe presents greater or equal to one values indicates that the gains from selection are favorable for a certain characteristic, due to the positive difference of genetic variation compared to the environmental variation (Vencovsky and Barriga, 1992).

Silva et al. (2002) points out that since the melon breeding involves various interest features, it is interesting to study the genetic, phenotypic and

Table 2. Reaction of melon genotypes to *Meloidogyne enterolobii*. Unesp – FCAV – Jaboticabal (SP), 2015.

Genotype	TNEJ[x*]	RF[y*]	Reaction[z]
Tomato S[ta] Cruz Kada	43598[a]	8.7	S
Vendrantais	40011[a]	8.0	S
Fantasy	14235[b]	2.8	S
Louis	15339[b]	3.0	S
PI 140471	13098[b]	2.6	S
PI 432398	11013[b]	2.2	S
PI 420150	10913[b]	2.1	S
PI 5322830	11484[b]	2.2	S
PMR-5	8222[b]	1.6	S
PI 157082	14241[b]	2.8	S
WMR-29	13794[b]	2.7	S
Charentais Fom 1	6763[c]	1.3	S
PI 420145	6506[c]	1.3	S
C160	5128[c]	1.7	S
CNPH 01- 930	5753[c]	1.1	S
Nantais Oblong	5242[c]	1.0	S
PMR-45	5805[c]	1.2	S
PMR-6	5830[c]	1.1	S
PI 414723	4597[c]	0.6	R
AC 29	3814[c]	0.6	R
PI 124112	1594[c]	0.3	R

[x] TNEJ: Total number of eggs and juveniles of second stage, [y] RF: reproduction factor, [z] Reaction: (R: resistant; S: susceptible), * To perform statistical analyses, data were transformed to $\sqrt{x}$. Means followed by the same letter in the column do not differ by the Scott-Knott test ($p < 0.05$).

environmental correlations. Thus, as the work related to resistance to this pathogen progress, it has the ability to verify these correlations featuring more effectively the resistance of melon genotypes to *M. enterolobii*.

In view of the reported resistance to *M. enterolobii* in three genotypes, it is possible to perform selection for that characteristic, since the genotypes described in this work are also being used in other lines of research, which enables the incorporation of more interest features to the genotype within the breeding programs.

Although some studies have reported resistance in yellow melon to *M. incognita* and *M. javanica* (Bitencourt and Silva, 2010; Marques et al., 2012; Galatti et al., 2013; Ito et al., 2014; Lopez-Gomez and Verdejo-Lucas, 2014), there are few studies that show resistance, or even susceptibility for melons in relation to *M. enterolobii*. The results are promising in view of the scarcity of studies related to this nematode in melon. There are several reports of cultivars of various species resistant to most root-knot nematodes, but susceptible to *M. enterolobii*. Cantu et al. (2009) evaluated eight tomato rootstocks (*Solanum lycopersicum* L.) informed as resistant to *M. incognita*, *M. javanica* and *M. arenaria*, and observed that the rootstocks behaved as susceptible to *M. enterolobii*. A work on the parasitism of this nematode held in cowpea (*Vigna unguiculata* L.) 'IPA-9', 'IPA 206' and the tomato cultivars 'Santa Cruz' and 'Viradoro' demonstrated susceptibility reactions (Guimarães *et al.*, 2003).

In species of *Capsicum* spp., only *C. frutescens* was considered resistant *M. enterolobii* (Oliveira, 2007). In lettuce, variations of reaction to some root-knot nematode species are also reported (Gomes et al., 2000; Maluf et al., 2002; Carvalho Filho et al., 2008; Silva et al., 2008; Bitencourt and Silva, 2010). According to Yang and Eisenback (1983), melon is a good host for *M. enterolobii*, making it difficult to manage this crop in fields infested by this pathogen. The evaluation of resistant materials, such as those obtained in this study, allows the identification of promising materials for the rootstocks or to the transfer of resistance genes in subsequent works. According to Peil (2003) grafting has been used in Cucurbitaceae vegetable crops (watermelon, melon, cucumber and pumpkin) in Brazil, which have features that enables grafting.

In this study, the genotypes (Vendrantais, PI 140471, PI 432398, PI 420150, PI 5322830, PMR-5, PI 157082, WMR-29, Charentais Fom 1, PI 420145, C160, CNPH 01- 930, Nantais Oblong, PMR-45, PMR- 6, along with the cultivars 'Louis' and 'Fantasy') increased the initial

population, being classified as susceptible, and three genotypes (PI 414723, AC 29, and PI 124112) did not increased the initial population, and are therefore resistant. Thus, it was found resistant genotypes to this nematode, however none is immune. Immunity is characterized by interactions between the host and pathogen, being the host the plants exposed to the pathogen, which produces defense substances (Williamson, 1999; Williamson and Kumar, 2006). The resistance of melon genotypes can be further assessed in heritage studies, as the resistance genes are usually specific, contemplating few species of *Meloidogyne*, at where the resistance can be conferred by one, a few or many genes (Williamson and Roberts, 2009). From the results obtained in this work, further studies on melon breeding programs with PI 414723, AC 29, and PI 124112, resistant to *M. enterolobii*, is possible.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Potential use of herbicides in different sorghum hybrids

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The sorghum crop in Brazil has expanded substantially. Among the factors that interfere in sorghum yield is the interference imposed by the presence of weeds. The objective of this study was to assess the potential of different herbicide treatments applied in pre-emergence or post-emergence of sorghum in terms of selectivity and weed control. Two experiments were conducted, one for each application modality: experiment 1: pre-emergence; experiment 2: post-emergence. The experimental design was a randomized block design with four replications, in split plots. For experiment 1, the pre-emergence herbicides applied constituted the plots, and the different sorghum hybrids (50A10, 50A40, 50A50, 1G100, 1G233, SS318) constituted the subplots. For experiment 2, the post-emergence herbicides applied constituted the plots, and the different sorghum hybrids (50A10, 50A40, 50A50, 1G100, 1G233, SS318) constituted the subplots. Based on the results obtained, and on the discussion done, it is possible to conclude that herbicides and their respectively doses that had potential for use in sorghum crop in pre-emergence were: atrazine (1000 and 2000), mesotrione (100), tembotrione (75), atrazine + mesotrione (1000+100 and 2000+100) and atrazine + trifluralin (1000+1000 and 2000+1000). Meanwhile in post-emergence the best options were: atrazine (1000 and 2000), mesotrione (50 and 100), bentazon (720), fluroxypyr (100), mesotrione + atrazine (50+1000) and mesotrione + fluroxypyr (50+100). All of those treatments provided less than 25% of plant injury which means less potential to reduce the sorghum grain yield.

Key words: phytotoxicity, weed control, *Sorghum bicolor*, selectivity.

INTRODUCTION

In Brazil, grain sorghum has expanded to some areas where farmers have the ability to grow two crops on the same field per year. Sorghum is usually grown after soybean crop as a successional crop. In season 2013/2014, the area sown with sorghum exceeded 800,000 hectares, especially in the “Cerrado” area which accounted for more than 482,000 ha (Conab, 2015). Sorghum crop has a very well adaption in this region because its temperature and rainfall requirements.

Among the factors that interfere in sorghum yield is the interference imposed by the presence of weeds in the crop. In studies described in the literature, yield losses due to this interference may reach 85% for grain sorghum and 81% for forage sorghum (Andres et al., 2009;

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Rodrigues et al., 2010).

Despite the importance that sorghum has taken in recent years, and the significant yield loss when this crop remains in coexistence with weeds, there are few herbicides registered for use in the crop in pre-emergence and/or post-emergence. Currently, only atrazine, simazine and 2,4-D are registered for use in Brazil (MAPA, 2015), which restricts the farmers to control weeds in sorghum crop.

The sorghum crop is very sensitive to herbicides and therefore herbicide residual activity studies use sorghum like a bioindicator plant for testing the behavior of herbicides in the soil (Guerra et al., 2014). On the other hand, some studies in the literature have reported herbicides with a potential for use in sorghum in post-emergence, such as tembotrione (Dan et al., 2010), mesotrione (Abit et al., 2011), bentazon (Stahlman and Wicks, 2000), fluroxypyr (Horky and Martin, 2005), pendimethalin, trifluralin (Grichar et al., 2005) and metsulfuron (Brown et al., 2004; Hennigh et al., 2010).

For pre-emergence applications, the lack of herbicide options with a potential for use is even greater. Geier et al. (2009) assessing the application effects of acetochlor and s-metolachlor, alone or in mixture with atrazine, found that these herbicides can be safely applied in sorghum only when the seeds are treated with the fluxofenim safener. In the United States, sorghum seeds are usually protected with safener to allow acetamide herbicide application.

Thus, it is important to conduct research to find new herbicide solutions that can be selective and effectively used in this crop. Another factor to consider is the degree of tolerance of each sorghum hybrid to herbicides and also the dose of the herbicides, which should be selective to the sorghum but sufficient to control weeds. Abit et al. (2011) have assessed the response of 85 sorghum hybrids to the mesotrione application in 0, 52, 105, 210 and 315 g a.i. ha^{-1} when plants had three to four leaves. They found a differential response of sorghum hybrids to mesotrione.

Within this context, the aim of this study was to assess the potential of using of different herbicide treatments applied in pre-emergence or in post-emergence of the grain sorghum crop based on selectivity and weed control.

MATERIALS AND METHODS

The experiments were performed in an agricultural area located in the Brazilian municipality of Mogi Mirim, SP, at 22°26'44''S and 47°04'13''O.

Two experiments were conducted in two different fields, one for each modality of application: Experiment 1: pre-emergence; Experiment 2: post-emergence. The soil experimental field had: pH ($CaCl_2$) of 5.2; 1.6 $cmol_c$ of $H^+ + Al^{+3}$ dm^{-3} of soil; 4.3 $cmol_c$ dm^{-3} of Ca^{+2}; 1.3 $cmol_c$ dm^{-3} of Mg^{+2}; 129 mg dm^{-3} of K^+; 46.7 mg dm^{-3} of P; 1.2 dag kg^{-1} of organic matter; 42% of sand; 5% of silt; and 53% of clay (clay texture). Before installation of the experiments, the emerged weeds present in the experimental area were controlled by an application of paraquat (N,N′-dimethyl-4,4′-bipyridinium dichloride) (400 g a.i. ha^{-1}).

Sowing was carried out distributing fifteen seeds per linear meter via grain drill, sown to a depth of 1 to 2 cm. The fertilizer used in the planting furrow was 200 kg ha^{-1} of the commercial formula 5-20-20 (N-P-K). The experimental area was equipped with sprinkler type irrigation. Whenever necessary, an irrigation of approximately 10 mm was applied.

The experimental design was a randomized block design with four replications, in split plots. For experiment 1, the different sorghum hybrids (50A10, 50A40, 50A50, 1G100, 1G233 and SS318) constituted the subplots, and the plots constituted by pre-emergence herbicides treatments (g a.i. or a.e. ha^{-1} only for acetochlor): atrazine (1000), atrazine (2000), mesotrione (100), tembotrione (75), nicosulfuron (50), chlorimuron (20), s-metolachlor (800), acetochlor (2300), atrazine + trifluralin (1000+1000), atrazine + mesotrione (1000+100), atrazine + tembotrione (1000+75), atrazine + s-metolachlor (1000+800), atrazine + nicosulfuron (1000+50), atrazine + trifluralin (2000+1000), atrazine + mesotrione (2000+100), atrazine + tembotrione (2000+75), atrazine + s-metolachlor (2000+800), atrazine + nicosulfuron (2000+50) and untreated.

For experiment 2, the different sorghum hybrids (50A10, 50A40, 50A50, 1G100, 1G233 and SS318) constituted the subplots, and the plots constituted by post-emergence herbicides treatments (g a.i. or a.e. ha^{-1} only for fluroxypir-meptyl): atrazine (1000), atrazine (2000), mesotrione (50), mesotrione (100), tembotrione (37.5), tembotrione (75), nicosulfuron (50), fluroxypir-meptyl (100), bentazon (720), metsulfuron (2), mesotrione + atrazine (50+1000), mesotrione + fluroxypyr-meptyl (50+100), mesotrione + nicosulfuron (50+50), tembotrione + atrazine (37.5+1000), tembotrione + fluroxypyr-meptyl (37.5+100), tembotrione + nicosulfuron (37.5+50), atrazine + nicosulfuron (1000+50), cloransulam (33.6) and untreated.

For both experiments, the experimental units comprised two rows of each hybrid (subplot), totaling twelve sowing rows (plot) spaced 0.45 m, 4 m long, with a total area of 21.60 m^2 per plot. Each plot corresponded to twelve rows except 0.5 meters at the ends of the sowing rows.

The applications were done with a CO_2 backpack sprayer at a constant pressure (45 psi), fitted with six AIXR 110.015 type spray nozzles, spaced 0.5 m, providing an application volume equivalent to 100 L ha^{-1} of spray solution.

For experiment 1, application was done one day after sowing, in pre-emergence of the crop and of the weeds. The application conditions were: moist soil; average temperature of 26°C; average relative humidity of air of 78%; average wind speed of 0.5 km h^{-1} and clear sky with few clouds.

For experiment 2, application occurred sixteen days after sowing, in post-emergence of the crop and of the weeds. At the time of applying, the crop had 3-4 fully expanded leaves, while weeds *Panicum maximum* and *Bidens pilosa* were at the 2 to 4 leaf stage. These two weed species were seeded on the field because the low infestation of weeds. The application conditions were: moist soil; average temperature of 25.8°C; average relative humidity of air of 71.0%; average wind speed of 0.8 km h^{-1} and clear sky with few clouds.

Phytotoxicity assessments for each hybrid were conducted in both experiments, in which 0% means no plant injury, and 100% means all plants death. For experiment 1, the pre-emergence control of *Eleusine indica*, *Brachiaria plantaginea*, *Euphorbia heterophylla* and *Ipomoea grandifolia* was assessed at 7, 14 and 21 days after emergence of the sorghum (DAE). A stand assessment at 3 DAE has also taken place, counting the number of emerged sorghum plants in 2 linear meters of each hybrid within each plot. For purposes of analysis, the average value per meter sampled was considered. As for experiment 2, the control in post-emergence of weeds present at the time of application at 7, 14 and

Table 1. Average number of emerged plants of six sorghum hybrids at three days after emergence (DAE) due to the application of different herbicide treatments during pre-emergence. Mogi Mirim (SP), 2016.

Treatment	Dose (g a.i. ha^{-1})	Number of plants per linear meter											
		50A10		50A40		50A50		1G100		1G233		SS318	
Atrazine	1000	12.1	a	12.9	a	13.2	ab	14.0	a	14.1	a	14.4	ab
Atrazine	2000	14.0	a	10.7	a	14.0	ab	13.5	a	12.9	a	14.9	ab
Mesotrione	100	13.2	a	13.1	a	14.9	a	13.9	a	12.1	a	13.0	ab
Tembotrione	75	13.2	a	11.9	a	15.2	a	12.7	a	14.0	a	12.4	ab
Nicosulfuron	50	13.7	a	10.5	a	11.0	ab	11.6	a	11.6	a	10.6	b
Chlorimuron	20	14.1	a	12.5	a	13.6	ab	11.1	a	12.1	a	13.5	ab
S-metolachlor	800	11.0	a	10.7	a	11.2	ab	11.0	a	11.5	a	11.1	ab
Acetochlor	2300	13.5	a	9.4	a	9.9	b	11.5	a	10.6	a	12.2	ab
Atrazine + trifluralin	(1000 + 1000)	11.4	a	11.1	a	12.0	ab	12.2	a	13.0	a	13.9	ab
Atrazine + mesotrione	(1000 + 100)	14.2	a	13.4	a	13.6	ab	11.7	a	12.9	a	15.9	a
Atrazine + tembotrione	(1000 + 75)	13.6	a	12.0	a	13.6	ab	11.7	a	13.1	a	13.9	ab
Atrazine + s-metolachlor	(1000 + 800)	11.0	a	9.6	a	11.0	ab	11.5	a	10.0	a	11.7	ab
Atrazine + nicosulfuron	(1000 + 50)	14.4	a	12.4	a	11.4	ab	11.2	a	12.0	a	11.6	ab
Atrazine + trifluralin	(2000 + 1000)	11.2	a	10.7	a	13.5	ab	12.4	a	13.0	a	14.9	ab
Atrazine + mesotrione	(2000 + 100)	13.6	a	11.7	a	14.5	a	13.0	a	12.0	a	14.2	ab
Atrazine + tembotrione	(2000 + 75)	12.6	a	11.9	a	12.0	ab	11.4	a	12.5	a	15.0	ab
Atrazine + s-metolachlor	(2000 + 800)	12.1	a	11.1	a	10.9	ab	11.2	a	10.1	a	12.4	ab
Atrazine + nicosulfuron	(2000 + 50)	12.6	a	12.6	a	11.4	ab	12.7	a	10.9	a	13.2	ab
Untreated	-	13.4	a	12.9	a	12.7	ab	11.2	a	12.5	a	14.5	ab
F	-	1.7		2.2		3.3		1.3		1.0		2.4	
CV (%)	-	13.5		13.3		23.6		13.8		18.6		14.1	
LSD (least significant difference)	-	4.5		4.0		4.5		4.3		5.9		4.9	

*Treatments followed by the same letter in the columns do not differ by Tukey test at 5%.

21 days after application (DAA) was assessed.

All data were submitted to analysis of variance and, when detecting a significant effect among the tested factors or the levels of each factor, the means comparison Tukey test at 5% significance was applied.

RESULTS AND DISCUSSION

Experiment 1: Pre-emergence

According to the variance analysis, the herbicide versus hybrid interaction was not considered statistically significant for any of the response variables. Thus, only the effects of herbicides were compared within each hybrid.

The application of the different herbicide treatments did not affect the emergence of the sorghum plants at 3 DAE (Table 1). What was observed was a delayed emergence of some hybrids when subjected to the application of some of the herbicide treatments, such as, for example, hybrids 50A50 and SS318, subjected to application of acetochlor and nicosulfuron, respectively.

At 7 DAE, the phytotoxicity in the treatments containing acetochlor, nicosulfuron, chlorimuron and s-metolachlor was extremely severe, especially when these same herbicides were applied in association with atrazine (Table 2). In a second level of phytotoxicity it is possible to include trifluralin + atrazine, mesotrione, tembotrione and their combinations with atrazine. Applying atrazine alone caused a little injury in the sorghum plants. Hybrids 1G233 and SS318A were considered sensitive to the application of high doses of atrazine + s-metolachlor.

The phytotoxicity observed at 21 DAE, in most treatments, was less than that observed in the first assessment, which indicates a recovery of these plants to the symptoms of injuries (Table 3). On the other hand, in the treatments containing nicosulfuron alone or mixed with atrazine there were very high levels of crop injury (>90%).

Some treatments such as atrazine, mesotrione, tembotrione, atrazine + trifluralin and atrazine + mesotrione had low levels of phytotoxicity at 21 DAE, which can be a potential indication for use in this crop. Importantly, no treatment showed a high degree of selectivity, other than the application of atrazine alone.

In general, hybrids did not show marked differences in crop response to the application of these herbicides. However, it is noteworthy that hybrids 1G233 and SS318

Table 2. Phytotoxicity percentage in six sorghum hybrids at 7 days after emergence (DAE), due to the application of different herbicide treatments during pre-emergence. Mogi Mirim (SP), 2016.

Treatment	Dose (g a.i. ha^{-1})	% of phytotoxicity (7 DAE)											
		50A10		50A40		50A50		1G100		1G233		SS318	
Atrazine	1000	5.0	fg	4.5	gh	4.8	de	3.0	f	5.0	f	4.8	e
Atrazine	2000	3.3	fg	6.0	fgh	2.3	de	2.3	f	2.8	f	2.2	e
Mesotrione	100	16.3	efg	15.0	efgh	12.5	cde	21.3	def	16.3	ef	11.7	de
Tembotrione	75	26.3	defg	25.0	defgh	23.8	cde	26.3	cdef	32.5	cdef	23.7	cde
Nicosulfuron	50	71.3	abc	74.5	ab	72.0	a	74.5	ab	75.0	ab	74.0	a
Chlorimuron	20	51.3	abcde	52.5	abcd	41.3	bc	48.8	bcd	47.5	bcde	56.3	ab
S-metolachlor	800	48.8	abcde	35.0	defg	32.5	bcd	26.3	cdef	32.5	cdef	30.0	bcde
Acetochlor	2300	62.0	abcd	68.8	abc	58.8	ab	55.8	abc	65.0	abc	58.3	ab
Atrazine + trifluralin	(1000 + 1000)	22.5	efg	17.5	efgh	11.3	cde	11.3	ef	13.8	ef	15.0	de
Atrazine + mesotrione	(1000 + 100)	16.8	efg	14.3	efgh	15.0	cde	14.3	ef	14.8	ef	9.5	de
Atrazine + tembotrione	(1000 + 75)	37.5	cdefg	28.8	defgh	23.8	cde	27.5	cdef	27.5	def	23.8	cde
Atrazine + s-metolachlor	(1000 + 800)	39.0	bcdef	37.5	cdef	24.5	cde	24.3	def	40.8	cde	39.3	bcd
Atrazine + nicosulfuron	(1000 + 50)	76.3	ab	79.5	a	80.0	a	79.5	a	79.5	ab	81.3	a
Atrazine + trifluralin	(2000 + 1000)	26.3	defg	25.5	defgh	15.0	cde	22.0	def	22.5	ef	21.8	cde
Atrazine + mesotrione	(2000 + 100)	23.0	efg	23.8	defgh	20.0	cde	18.8	def	26.3	def	20.0	de
Atrazine + tembotrione	(2000 + 75)	25.0	defg	30.0	defgh	27.5	cde	33.8	cde	31.3	cdef	30.0	bcde
Atrazine + s-metolachlor	(2000 + 800)	48.8	abcde	42.5	bcde	36.3	bc	35.0	cde	59.5	bcd	51.8	abc
Atrazine + nicosulfuron	(2000 + 50)	79.5	a	79.0	a	78.8	a	78.3	ab	83.3	a	81.5	a
Untreated	-	0.0	g	0.0	h	0.0	e	0.0	f	0.0	f	0.0	e
F	-	11.0		16.5		18.3		18.1		15.9		19.1	
CV (%)	-	41.3		35.9		38.3		36.8		36.8		36.2	
LSD (least significant difference)	-	38.4		32.5		30.5		30.4		34.2		31.6	

*Treatments followed by the same letter in the columns do not differ by Tukey test at 5%.

showed greater sensitivity to S-metolachlor application applied both alone and in combination with atrazine. Abit et al. (2011) have assessed the response of 85 sorghum hybrids to the application of different herbicides and also found differential responses across sorghum hybrids, which corroborates the results obtained in this experiment.

At 14 DAE, the treatments with atrazine effectively controlled the assessed weeds, especially when this herbicide was applied at higher doses (Table 4). It is noteworthy that the control of grasses was better when atrazine was applied in combination with other herbicides, especially for *B. plantaginea*. Acetochlor and S-metolachlor gave a satisfactory control of grass only, whereas chlorimuron and nicosulfuron controlled (>80%), especially for broadleaves. Mesotrione and tembotrione alone did not provide acceptable levels of control.

At 21 DAE, it was observed that the lowest dose of atrazine maintained control of the assessed weeds, except for *B. plantaginea*. However, control of grasses was better when this herbicide was applied at higher doses (2000 g a.i. ha^{-1}) or in combination with mesotrione and tembotrione (Table 4). Nicosulfuron provided excellent levels of control of *E. heterophylla* and *I. grandifolia*, while chlorimuron also provided a satisfactory control of *E. heterophylla* only. The other herbicides applied alone were not effective in controlling these weeds in pre-emergence.

Experiment 2: Post-emergence

The hybrid factor of the hybrid versus herbicides interaction showed no significant interaction in the assessments performed. This indicates that there was no differential response of the assessed hybrids to the application of the herbicides during post-emergence. On the other hand, the hybrid factor showed significance, which means different levels of selectivity of these herbicides for the sorghum hybrids used.

At 7 DAA, treatments atrazine (1000 and 2000), bentazon and fluroxypyr showed very low levels of phytotoxicity (<5%) (Table 5). In a second level of selectivity are mesotrione (50), mesotrione (100), mesotrione + atrazine and mesotrione + fluroxypyr. These treatments caused mild symptoms of chlorosis in plants, which accounted for these percentages of phytotoxicity. The other treatments gave high percentages of phytotoxicity, especially with nicosulfuron (>60%). Treatments containing tembotrione alone or in

Table 3. Phytotoxicity percentage in six sorghum hybrids at 21 days after emergence (DAE), due to the application of different herbicide treatments during pre-emergence. Mogi Mirim (SP), 2016.

Treatment	Dose (g a.i. ha^{-1})	% of phytotoxicity (21 DAE)											
		50A10		50A40		50A50		1G100		1G233		SS318	
Atrazine	1000	0.7	de	2.0	d	0.0	d	0.0	d	2.5	e	0.0	d
Atrazine	2000	6.2	cde	3.7	d	0.0	d	1.2	d	3.7	e	5.5	d
Mesotrione	100	7.5	cde	6.2	cd	3.7	cd	2.5	d	6.2	de	5.0	d
Tembotrione	75	17.5	bcde	12.5	cd	2.5	cd	8.7	bcd	15.0	cde	10.7	cd
Nicosulfuron	50	95.7	a	95.5	a	92.2	a	95.0	a	96.2	a	95.2	a
Chlorimuron	20	35.0	bc	32.5	bc	25.0	bc	28.7	bc	37.5	bc	36.2	b
S-metolachlor	800	18.7	bcde	17.5	bcd	10.0	bcd	10.0	bcd	25.0	bcde	21.2	bcd
Acetochlor	2300	37.5	b	42.5	b	33.0	b	31.2	b	46.2	b	33.7	bc
Atrazine + trifluralin	(1000 + 1000)	18.0	bcde	20.0	bcd	10.0	bcd	10.5	bcd	15.5	cde	13.7	bcd
Atrazine + mesotrione	(1000 + 100)	8.7	bcde	13.7	cd	5.7	cd	2.5	d	12.2	cde	6.0	d
Atrazine + tembotrione	(1000 + 75)	20.0	bcde	18.7	bcd	10.7	bcd	21.2	bcd	18.7	bcde	11.7	bcd
Atrazine + s-metolachlor	(1000 + 800)	23.0	bcde	25.0	bcd	7.5	cd	10.0	bcd	35.0	bcd	22.5	bcd
Atrazine + nicosulfuron	(1000 + 50)	93.2	a	95.7	a	87.0	a	94.2	a	92.0	a	95.5	a
Atrazine + trifluralin	(2000 + 1000)	13.2	bcde	19.2	bcd	5.0	cd	6.7	bcd	10.5	cde	5.0	d
Atrazine + mesotrione	(2000 + 100)	7.2	cde	11.2	cd	3.7	cd	4.2	cd	15.5	cde	10.0	cd
Atrazine + tembotrione	(2000 + 75)	14.2	bcde	18.7	bcd	11.2	bcd	23.7	bcd	18.2	bcde	17.5	bcd
Atrazine + s-metolachlor	(2000 + 800)	30.0	bcd	23.7	bcd	15.0	bcd	15.0	bcd	46.2	b	32.5	bc
Atrazine + nicosulfuron	(2000 + 50)	95.7	a	95.7	a	93.5	a	96.7	a	97.2	a	97.7	a
Untreated	-	0.0	e	0.0	d	0.0	d	0.0	d	0.0	e	0.0	d
F	-	30.3		35.2		49.0		43.4		29.4		45.1	
CV (%)	-	38.8		36.1		41.5		41.0		37.1		35.3	
LSD (least significant difference)	-	29.5		27.5		23.7		26.1		30.3		25.2	

*Treatments followed by the same letter in the columns do not differ by Tukey test at 5%.

combination showed severe chlorosis.

Chlorosis observed in treatments containing herbicides from the group of carotenoid synthesis inhibitors has been minimized in the course of time, being observed only in older leaves (Table 6). It should be remembered that this chlorosis was more severe for tembotrione than for mesotrione. The symptoms of chlorosis in these treatments are due to the oxidative degradation of the chlorophyll and of the photosynthetic membranes, since carotenoid synthesis that protect them does not occur (Grossmann and Ehrhardt, 2007; Pataky et al., 2008).

For maize, what is observed is the opposite, because when assessing mesotrione and tembotrione herbicides in the selectivity for maize crop, Bollman et al. (2008) have found that mesotrione was what caused most phytotoxicity compared to tembotrione.Another point noted in this assessment is that the hybrids have shown high sensitivity to treatments containing ALS-inhibiting herbicides such as nicosulfuron, metsulfuron and cloransulam, which prevents their use in crops. At the last assessment of phytotoxicity (21 DAA), it was observed that most treatments recovered from the injuries seen inother assessments, indicating a potential use of these treatments in crops postemergence (Table 6). Herbicides that showed potential for use in crops were: atrazine, mesotrione, bentazon, fluroxypyr, mesotrione + atrazine and mesotrione + fluroxypyr. Bentazon and fluroxypyr are shown as viable options for controlling broadleaves, which decreases the dependence of atrazine and 2,4-D. Moreover, under the conditions of this study, fluroxypyr can be applied in more advanced stages of crops (V3-V4), which does not occur in the case of 2,4-D, which has the limitation of being applied before the crop reaches the V2 stage. In the other treatments, despite this reduction in symptoms of phytotoxicity, phytotoxicity levels can be an indication that these herbicides cause reductions in crop yield.

Dan et al. (2010) report that herbicide tembotrione showed high levels of phytotoxicity to the sorghum crop. They also state that there was greater potential for phytotoxicity when this herbicide was applied in the earlier stages of sorghum cultivar AG-1040. The same authors also state that there are different levels of selectivity, which can vary depending on dose and time used for application. In the case of this experiment, the studied sorghum hybrids showed great tolerance to tembotrione, demonstrating potential for use in that crop. A plausible explanation for this discrepancy may be the differentiated response that hybrids have to the application of tembotrione. Nevertheless, studies to

Table 4. Control percentage of weeds at 14 and 21 days after emergence (DAE) due to the application of different herbicides during pre-emergence. Mogi Mirim (SP), 2016.

Treatment	Dose (g a.i. ha^{-1})	% of control (14 DAE)								% of control (21 DAE)							
		EPHHL		IPMGR		ELEIN		BRCPL		EPHHL		IPMGR		ELEIN		BRCPL	
Atrazine	1000	94.7	ab	100.0	a	88.7	ab	75.0	a	96.5	a	95.7	a	80.5	ab	60.0	abcd
Atrazine	2000	98.7	a	99.2	a	94.5	ab	82.0	a	90.5	a	100.0	a	87.0	ab	85.7	abc
Mesotrione	100	25.0	c	57.5	b	15.0	de	21.2	cd	12.5	d	35.0	cd	17.5	fg	25.0	de
Tembotrione	75	62.5	b	67.5	ab	58.7	c	73.7	a	35.0	bcd	55.0	bc	42.5	de	53.7	bcd
Nicosulfuron	50	95.5	ab	90.0	ab	72.7	bc	80.0	a	99.0	a	96.0	a	50.0	cde	62.5	abcd
Chlorimuron	20	81.0	ab	78.7	ab	31.2	d	37.5	bc	82.0	ab	65.0	abc	26.2	ef	48.7	cd
S-metolachlor	800	20.0	c	23.7	c	82.2	ab	80.0	a	30.0	cd	40.0	c	63.7	bcd	66.2	abc
Acetochlor	2300	71.2	ab	77.5	ab	85.0	ab	83.2	a	66.2	abc	70.0	abc	68.0	abc	71.2	abc
Atrazine + trifluralin	(1000 + 1000)	77.5	ab	91.2	a	90.0	ab	73.0	ab	68.2	abc	72.5	abc	70.0	abc	75.0	abc
Atrazine + mesotrione	(1000 + 100)	86.0	ab	100.0	a	88.7	ab	90.5	a	81.5	ab	88.7	ab	84.2	ab	77.5	abc
Atrazine + tembotrione	(1000 + 75)	66.2	ab	86.2	ab	88.7	ab	82.5	a	66.0	abc	80.0	ab	75.0	ab	77.5	abc
Atrazine + s-metolachlor	(1000 + 800)	72.0	ab	86.2	ab	95.0	a	90.5	a	50.0	bc	78.7	ab	82.0	ab	67.5	abc
Atrazine + nicosulfuron	(1000 + 50)	91.0	ab	100.0	a	81.2	ab	82.5	a	92.5	a	95.0	a	74.7	ab	72.5	abc
Atrazine + trifluralin	(2000 + 1000)	96.2	ab	100.0	a	96.7	a	92.7	a	72.5	abc	100.0	a	87.0	ab	87.2	abc
Atrazine + mesotrione	(2000 + 100)	96.5	ab	100.0	a	96.7	a	95.7	a	94.2	a	100.0	a	91.2	a	83.7	abc
Atrazine + tembotrione	(2000 + 75)	89.0	ab	97.5	a	95.0	a	93.2	a	89.0	a	92.5	ab	83.2	ab	88.2	ab
Atrazine + s-metolachlor	(2000 + 800)	90.5	ab	99.7	a	94.5	a	95.5	a	87.2	a	91.7	ab	90.5	a	94.5	a
Atrazine + nicosulfuron	(2000 + 50)	96.2	ab	100.0	a	96.0	a	93.0	a	98.7	a	99.7	a	86.7	ab	86.2	abc
Untreated	-	0.0	c	0.0	c	0.0	e	0.0	d	0.0	d	0.00	d	0.0	g	0.0	e
F	-	19.5		18.8		49.3		14.6		9.3		13.7		32.4		9.9	
CV (%)	-	17.5		15.6		10.8		18.2		28.4		19.3		14.1		21.8	
LSD (least significant difference)	-	34.0		33.5		21.6		35.6		51.3		76.6		24.5		38.5	

*Treatments followed by the same letter in the columns do not differ by Tukey test at 5%. EPHHL: *Euphorbia heterophylla*; IPMGR: *Ipomoea grandifolia*; ELEIN: *Eleusine indica*; BRCPL: *Brachiaria plantaginea*.

Table 5. Percentage of phytotoxicity in six sorghum hybrids at 7 days after application (DAA), depending on the application of different herbicide treatments applied during post-emergence. Mogi Mirim (SP), 2016.

Treatment	Dose (g a.i. ha^{-1})	% of phytotoxicity (7 DAE)											
		50A10		50A40		50A50		1G100		1G233		SS318	
Atrazine	1000	3.0	g	1.2	f	1.2	f	1.7	g	3.2	fg	1.2	g
Atrazine	2000	4.0	g	1.5	ef	0.7	f	0.0	g	2.5	fg	2.0	g
Mesotrione	50	15.0	fg	10.5	def	9.2	ef	10.0	fg	13.0	efg	7.5	fg
Mesotrione	100	26.2	ef	22.5	cd	18.7	def	20.0	efg	23.7	de	20.5	efg

Table 5. Contd.

Tembotrione	37.5	46.2	cd	45.0	ab	40.0	abc	37.5	abcd	43.7	bc	40.0	cd
Tembotrione	75	48.7	bcd	55.0	a	46.2	ab	46.2	ab	47.5	ab	43.7	bc
Nicosulfuron	50	65.0	ab	61.2	a	57.5	a	55.0	a	60.0	a	55.7	abc
Fluroxypyr	100	2.0	g	1.0	f	2.5	f	1.2	g	1.7	fg	0.5	g
Bentazon	720	1.0	g	1.2	f	0.0	f	0.0	g	2.0	fg	0.0	g
Metsulfuron	2	57.5	abc	53.7	a	51.2	a	47.5	ab	51.2	ab	51.2	abc
Mesotrione + atrazine	(50 + 1000)	35.0	de	33.7	bc	28.7	bcd	30.0	bcde	30.0	cd	27.5	de
Mesotrione + fluroxypyr	(50 + 100)	21.2	ef	18.7	dce	16.2	def	17.5	efg	17.5	def	15.0	efg
Mesotrione + nicosulfuron	(50 + 50)	61.2	abc	60.0	a	53.7	a	52.5	a	60.0	a	58.7	ab
Tembotrione + atrazine	(37.5 + 1000)	50.0	abcd	53.7	a	42.5	abc	43.7	abc	50.0	ab	48.7	abc
Tembotrione + fluroxypyr	(37.5 + 100)	47.5	cd	50.0	ab	41.2	abc	38.7	abc	43.7	bc	42.5	cd
Tembotrione + nicosulfuron	(37.5 + 50)	58.7	abc	57.5	a	51.2	a	48.7	a	58.7	ab	55.0	abc
Atrazine + nicosulfuron	(1000 + 50)	66.2	a	60.5	a	56.2	a	52.5	a	58.7	ab	60.0	a
Cloransulam	33.60	56.2	abc	57.5	a	50.0	a	47.5	ab	50.0	ab	51.2	abc
Untreated	-	0.0	g	0.0	f	0.0	f	0.0	g	0.0	g	0.0	g
F	-	58.0		56.9		37.4		40.1		57.5		58.2	
CV (%)	-	18.5		19.4		24.9		23.4		18.9		20.1	
LSD (least significant difference)	-	16.9		17.2		19.2		17.6		15.9		15.9	

*Treatments followed by the same letter in the column do not differ by Tukey test at 5%.

assess the crop yield in response to these variables are needed to infer conclusions about the selectivity of these herbicides. In the control of *B. pilosa* and *P. maximum*, it was observed that at 7 DAA, some treatments such as atrazine, metsulfuron, bentazon, mesotrione + atrazine, tembotrione + atrazine, atrazine + nicosulfuron and cloransulam already had a satisfactory control of *B. pilosa* (Table 7). On the other hand, only tembotrione + atrazine provided a satisfactory control of *P. maximum* at 7 DAA, indicating a high difficulty of control of grasses by these treatments.

The mesotrione + fluroxypyr treatment provided a percentage of control of *B. pilosa* above 80% in this assessment. It should be noticed that for the treatments containing mesotrione or tembotrione, when the control of *P. maximum* is unsatisfactory (<80%) there is a significant suppression of this grass imposed by these herbicides. This suppression is even higher when these treatments are in combination with atrazine and nicosulfuron.

At 21 DAA, some treatments provided control that was higher than that observed in the previous assessment (Table 7). For the control of *B. pilosa*, treatments such as atrazine, bentazon, metsulfuron, mesotrione + atrazine, mesotrione + fluroxypyr, tembotrione + atrazine, atrazine + nicosulfuron and cloransulam were considered satisfactory. As for *P. maximum*, the number of options is smaller and only tembotrione + atrazine and atrazine + nicosulfuron are the effective treatments for this species.

Importantly, the treatments with mesotrione in general were selective, except when this herbicide was applied in combination with nicosulfuron. In addition, the combination of this herbicide with a post-emergence for broadleaves is an excellent option for the tillage of monocotyledon and dicotyledon weeds. Although these treatments have not obtained a satisfactory control of *P. maximum*, the effectiveness of mesotrione in early post-emergence has been observed in several grasses such as *Digitaria horizontalis* and Cenchrurs echinatus (Dan et al., 2011). Furthermore, the synergism between the pre-

Table 6. Percentage of phytotoxicity in six sorghum hybrids at 21 days after application (DAA), depending on the application of different herbicide treatments applied during post-emergence. Mogi Mirim (SP), 2016.

Treatment	Dose (g a.i. ha^{-1})	% of phytotoxicity (21 DAE)											
		50A10		50A40		50A50		1G100		1G233		SS318	
Atrazine	1000	0.0	g	0.0	f	0.0	g	0.0	g	0.0	h	0.0	c
Atrazine	2000	0.0	g	0.0	f	0.0	g	0.0	g	0.0	h	0.0	c
Mesotrione	50	0.0	g	0.5	f	0.5	g	0.0	g	0.0	h	0.0	c
Mesotrione	100	3.5	fg	3.5	ef	3.5	fg	3.5	fg	3.0	gh	2.5	c
Tembotrione	37.5	16.2	cde	16.2	cd	15.0	cde	13.0	de	14.2	def	14.2	bc
Tembotrione	75	18.7	cd	18.7	c	17.5	cd	17.5	cd	17.5	cde	15.0	bc
Nicosulfuron	50	98.2	a	98.2	a	97.7	a	98.2	a	98.0	a	83.5	a
Fluroxypyr	100	1.2	g	1.2	f	1.2	g	1.2	g	1.2	h	1.2	c
Bentazon	720	0.0	g	0.0	f	0.0	g	0.0	g	0.0	h	0.0	c
Metsulfuron	2	25.0	c	20.0	c	22.5	c	22.5	c	25.0	c	23.7	bc
Mesotrione + atrazine	(50 + 1000)	7.5	efg	7.5	def	7.5	efg	6.2	efg	7.5	fgh	7.5	c
Mesotrione + fluroxypyr	(50 + 100)	3.7	fg	3.7	ef	3.0	fg	3.5	fg	3.0	gh	3.0	c
Mesotrione + nicosulfuron	(50 + 50)	97.7	a	97.7	a	97.7	a	98.2	a	97.7	a	97.7	a
Tembotrione + atrazine	(37.5 + 1000)	22.5	cd	22.5	c	21.2	cd	18.7	cd	21.2	cde	21.2	bc
Tembotrione + fluroxypyr	(37.5 + 100)	13.0	def	13.5	cde	12.2	def	12.2	def	11.2	efg	11.2	c
Tembotrione + nicosulfuron	(37.5 + 50)	97.5	a	96.7	a	97.0	a	97.0	a	97.5	a	97.7	a
Atrazine + nicosulfuron	(1000 + 50)	97.7	a	97.7	a	98.0	a	97.5	a	97.7	a	97.5	a
Cloransulam	33.60	46.2	b	46.2	b	45.0	b	45.0	b	45.0	b	42.5	b
Untreated	-	0.0	g	0.0	f	0.0	g	0.0	g	0.0	h	0.0	c
F	-	336.1		341.4		408.1		497.6		497.1		38.4	
CV (%)	-	15.0		15.0		13.9		12.7		12.6		45.5	
LSD (least significant difference)	-	10.8		10.7		9.8		8.9		8.9		30.4	

*Treatments followed by the same letter in the column do not differ by Tukey test at 5%.

Table 7. Control of *Bidens pilosa* and *Panicum maximum* at 7, 14 and 21 days after application (DAA), depending on the application of different herbicide treatments applied during post-emergence. Mogi Mirim (SP), 2016.

Treatment	Dose (g a.i. ha^{-1})	% of control (7 DAA)				% of control (21 DAA)			
		BIDPI		PANMA		BIDPI		PANMA	
Atrazine	1000	100.0	a	15.0	gh	100.0	a	0.0	k
Atrazine	2000	100.0	a	23.2	hij	100.0	a	0.0	k
Mesotrione	50	5.0	fg	30.0	fg	0.0	e	23.7	j
Mesotrione	100	5.0	fg	42.5	de	0.0	e	42.5	i
Tembotrione	37.5	4.5	fg	45.7	de	0.0	e	55.0	gh
Tembotrione	75	4.5	fg	50.0	cd	0.0	e	58.7	fgh
Nicosulfuron	50	55.0	c	13.0	hij	68.7	c	70.0	cde
Fluroxypyr	100	52.5	cd	5.0	jkl	77.2	bc	0.0	k
Bentazon	720	86.7	b	1.5	kl	85.0	b	0.0	k
Metsulfuron	2	93.2	ab	16.2	gh	99.5	a	0.0	k
Mesotrione + atrazine	(50 + 1000)	100.0	a	58.7	bc	100.0	a	61.2	efg
Mesotrione + fluroxypyr	(50 + 100)	52.5	cd	46.2	cd	85.5	b	51.2	hi
Mesotrione + nicosulfuron	(50 + 50)	36.2	e	21.2	fg	77.2	bc	78.5	bc
Tembotrione + atrazine	(37.5 + 1000)	100.0	a	81.5	a	100.0	a	84.7	b
Tembotrione + fluroxypyr	(37.5 + 100)	42.5	de	46.2	cd	70.0	c	67.5	def
Tembotrione + nicosulfuron	(37.5 + 50)	36.2	e	36.2	de	67.5	c	75.7	bcd

Table 7. Contd.

Atrazine + nicosulfuron	(1000 + 50)	100.0	a	61.2	b	100.0	a	95.0	a
Cloransulam	33.60	82.0	b	12.5	hij	99.7	a	16.2	j
Untreated	-	0.0	g	0.0	l	0.0	e	0.0	k
F	-	280.1		112.7		252.5		403.3	
CV (%)	-	8.7		13.8		8.2		8.8	
LSD (least significant difference)	-	12.2		11.1		13.6		9.0	

*Treatments followed by the same letter in the column do not differ by Tukey test at 5%.

emergence and post-emergence applications of atrazine plus HPPD inhibitors on others weed species have already been related for other authors (Armel et al., 2005; Williams et al., 2011). The results obtained in this study indicate a number of treatments with potential use in crops, both as regards to the selectivity, as to the control of weeds during post-emergence. Nevertheless, it is noteworthy the recommendation that, for these treatments to be safe, further studies are needed to quantify the yield of grains of the crops when subjected to these applications.

The control of grasses remains a problem, since atrazine alone is not sufficient to ensure that the crop is in the clean during its development. To this end, as discussed in this paper, there are viable alternatives to complement the control in post-emergence. Thus, studies that assess "managements systems" are essential for progress in the weed management in sorghum crop.

Conclusions

Based on the results obtained, the herbicides and their respectively doses that had potential for use in sorghum crop in pre-emergence were: atrazine (1000 and 2000), mesotrione (100), tembotrione (75), atrazine + mesotrione (1000+100 and 2000+100) and atrazine + trifluralin (1000+1000 and 2000+1000). Meanwhile in post-emergence the best options were: atrazine (1000 and 2000), mesotrione (50 and 100), bentazon (720), fluroxypyr (100), mesotrione + atrazine (50+1000) and mesotrione + fluroxypyr (50+100). All of those treatments provided a satisfactory control of weeds, and presented less than 25% of plant injury which means less potential to reduce the sorghum grain yield.

Conflict of Interests

The authors have not declared any conflict of interests.

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Gas exchange, growth and yield of cowpea genotypes under different irrigation strategies

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Water availability is a major limiting factor for cowpea bean crops, especially in semiarid regions, where it is necessary to adopt more productive and tolerant genotypes and efficient strategies for water use. Thus, an experiment was carried under field conditions in the semiarid region of Pombal city, PB, Brazil. Using a completely randomised blocks design experiment and four replications, in a factorial scheme. The first factor was formed by four cowpea beans genotypes (Costela de Vaca, Pingo-de-Ouro, Paulistinha and BRS Marataoã), and the second factor consisted of five different irrigation strategies (40, 60, 80, 100 and 120% of actual evapotranspiration (ETr)). Gas exchange was evaluated at the V4 stage, dry biomatter formation at the R2 stage and crop yield until 90 days after sowing. The gas exchange from cowpea genotypes was reduced by lower irrigation amounts. For dry biomass formation, greater values in the Pingo-de-Ouro genotype were observed when irrigated with 120% of ETr. Thus, the treatment of 120% ETr improved the growth in dry matter independently of the genotype. The Costela de Vaca genotype had better CO_2 assimilation rates. Paulistinha had the highest productivity among genotypes, and Costela de Vaca had the greatest water use efficiency.

Key words: Assimilation rate, *Vigna unguiculata*, water productivity.

INTRODUCTION

Beans have contributed significantly to the food and economic establishment of humankind due to their market potential, directly and indirectly generating income for small farmers, especially family farms (Agrianual, 2006). The Brazilian north-eastern region has an average cowpea bean yield of about 330 kg ha^{-1} (Freire et al., 2005), which is considered low, since yield potential can reach 3000 kg ha^{-1}, depending on the cultivar (Oliveira et al., 2001; Salgado et al., 2011).

In reality, some studies have pointed out yield improvements when using appropriate irrigation levels (Andrade Junior et al., 2002; Tagliaferre et al., 2013; Dutra et al., 2015). Thus, for good production, irrigation should be used to give crops water they need or techniques should be used to maintain soil moisture to sustain plants growth and production cycles.

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Thus, cultivation in dry season is made possible by irrigation systems, which have great advantages compared to rainfed agricultural systems that causes environmental problems and encumber the cost of irrigated crops (Moura and Oliveira, 2013; Almeida and Costa, 2014).

It is also known that some crops produce economically viable yields even under soil water deficit, while others are sensitive to relatively low water scarcity. This difference could be due to factors related to the root system, especially elements that determine its growth, such as soil physical characteristics, plant genetic characteristics and irrigation systems management (Bernardo et al., 2008). It should be noted that identification of germplasm with water stress tolerance is of interest to breeding programmes, and knowledge of mechanisms related to such differential responses is important.

Therefore, it is important to study potential genotypes through physiological, growth and crop production characteristics to select drought-tolerant genotypes using these variable types (Shaker et al., 2013; Dutra et al., 2015).

Taking into account the importance of cowpea production in the semiarid region of the Brazilian Paraíba state and the need for improved water use efficiency in irrigated production systems, it is necessary to intervene in order to identify improved genotypes, allying productive potential and drought tolerance cultivars, which can optimised water use.

In order to study the ecophysiological behaviour of cowpea genotypes, it is necessary to identify and classify the genotypes regarding their water stress tolerance through growth, gas exchange and yield production while identifying the genotype that provides the greatest water use efficiency.

MATERIALS AND METHODS

The experiment was carried out at the Center of Agrifood Science and Technology - CCTA, Federal University of Campina Grande - UFCG, Pombal city, PB state (6°47'20" S latitude and 37°48'01" W longitude, altitude of 194 m). According to the Köppen classification system, the region has a BSh (hot and dry semiarid) climate, common in semiarid regions.

The experimental design was laid out in a randomized complete block with treatments distributed in a factorial scheme, 4 × 5, corresponding to four cowpea genotypes (Costela de Vaca, Pingo-de-Ouro, Paulistinha and BRS Marataoã) and five irrigation strategies (40, 60, 80, 100 and 120% of actual evapotranspiration (ETr)), with four replications. Irrigation depth differentiation was initiated 15 days after sowing (DAS), and lasted until 90 DAS, consisting of vegetative (V) and reproductive (R) crop stages. The fruit maturation cycle can last up to 90 days depending on the cultivar (Freire et al., 2005).

Costela de Vaca, Pingo-de-Ouro and BRS Marataoã genotypes have indeterminate growth habits and grow in a prostrate manner. The Paulistinha genotype has determinate growth and grows in an erect manner. It should be noted that the BRS Marataoã cowpea genotype came from the breeding programme of Embrapa Meio Norte. The other genotypes were acquired from local producers, as they were commonly grown in the region.

Reference evapotranspiration determination was conducted through soil moisture balance from daily readings using a dielectric diffusivity moisture meter. A sensor was installed in each plot that was designed to receive the 100% ETr irrigation strategy. Thus, irrigation amount (Li) corresponded to the difference between maximum moisture ($cm^3\ cm^{-3}$) (Θcc) and current humidity ($cm^3\ cm^{-3}$) (Θa). The result was multiplied by the root system depth (Z) and expressed in millimetres, using expression 1 (Equation 1).

In order to determine irrigation amounts in treatments relative to strategies of 40, 60, 80 and 120% of ETr, the value obtained in Eq. 1 was multiplied by coefficients of 0.4, 0.6, 0.8 and 1.2, respectively. The 20 treatments totalled 80 experimental plots with dimensions of 10.8 m^2 (3.6 m × 3.0 m). Sowing involved using double-row spacing, 0.6 × 0.3 × 0.2 m, which allowed for deployment of 144 plants per plot (10.8 m^2), totalling a planting density of 111111 plants ha^{-1}. However, assessments were conducted in four plants per plot, constituting, in sum, an experimental area of 864 m^2. A soil sample was then taken from the 0 to 20-cm-deep layer for chemical characterisation; soil data are shown in Table 1 and were used for plant nutritional management.

During nutritional management, basal dressing was conducted through soil analysis results using 64 g of single superphosphate per linear metre, as recommended by Freire et al. (2005). It is noteworthy that there was no nitrogen or potassium top-dressing application in order to stimulate *Nitrobacter* growth to supply nitrogen. A drip irrigation system was installed inside the double rows using drip tapes with a flow rate of 1.62 L h^{-1} per dripper, with 0.2-m spacing on the tape. After installation, a distribution uniformity test (DUT) was carried out following the methodology by Bernardo et al. (2008), obtaining a DUT of 92%.

Before each irrigation event, soil moisture sensors were taken in the plots from the control treatment (100% ETr) in order to calculate irrigation amounts. In addition, sensors were installed to monitor humidity, obtaining data of available water during the experimental period (Figure 1A). A decrease in available water was observed over time due to an increase in plant absorption, which was replaced by the irrigation water (Figure 1B), as can be seen in the different amounts applied with each irrigation. Regarding moisture behaviour during the evaluation period, overlap in the values was observed, especially in the 100 and 120% irrigation levels, suggesting that the soil only retains its maximum capacity (field capacity). Values above maximum capacity were caused by water loss due to percolation. In addition, during the experimental period, a rain event occurred 7 mm at 52 DAS, which has increase in available water (Figure 1A).

The irrigations amounts are presented in Table 2, with the mean between genotypes. Water demands of 135.7, 203.6, 271.5, 339.4 and 407.3 mm in the irrigation strategies of 40, 60, 80, 100 and 120% of ETr, respectively, which were obtained by the sum of the intake throughout the crop production cycle were presented. Among agronomic practices, weeding was conducted using specific herbicides for cowpea crops. This occurred in addition to pest and disease control with preventive applications of pesticides.

Gas exchange measurements were determined at the V4 stage when the plants had four definitive trifoliates. Specifically, CO_2 assimilation rate (*A*) ($\mu mol_{CO2}\ m^{-2}\ s^{-1}$), transpiration (*E*) ($mmol_{H2O}\ m^{-2}\ s^{-1}$), stomatal conductance (*gs*) ($mol_{H2O}\ m^{-2}\ s^{-1}$) and internal CO_2 concentration (*Ci*) ($\mu mol\ mol^{-1}$) in the first mature leaf from the apex, using the infrared gas analyser of an ADC Bio Scientific Ltd. LCpro+, were assessed. With these data, instantaneous water use efficiency (*iWUE*) (*A/E*) [($\mu mol_{CO2}\ m^{-2}\ s^{-1}$) ($mmol_{H2O}\ m^{-2}\ s^{-1}$)$^{-1}$] was quantified (Brito et al., 2012).

When the R2 flowering stage was reached (45 DAS), with flowers in the bean pod stage, plants were assessed with respect to biomatter and nodulation. Two plants were removed from each plot, regardless of those used in growth assessments, partitioned,

Table 1. Chemical characteristics of soil used for evaluation of cowpea genotypes under irrigation strategies. Pombal, PB, 2015.

pH	EC	P	N	K	Na	Mg	Al	Ca
$CaCl_2$ 1:2.5	dS m^{-1} 1:5	mg dm^{-3}	%	----------cmolc dm^{-3}----------				
6.13	0.09	102	1.70	0.50	0.09	3.35	0.10	5.15
H + Al	**SB**	**(t)**	**(T)**	**V**	**m**	**NaRS**	**MO**	**-**
----------cmolc dm^{-3}----------				-----%---------				-
2.97	9.00	9.10	12.06	74.63	0.83	0.75	29.00	-

EC: Electrical conductivity; P: phosphorus; N: nitrogen; K: potassium; Na: Sodium; Mg: Magnesium; Al: Aluminium; Ca: Calcium; SB: Sum of bases; t: efetive Cation Exchange Capacity; T: Cation Exchange Capacity; V: percent base saturation; m: percent aluminium saturation; NaRS: sodium rate saturation; OM: organic matter.

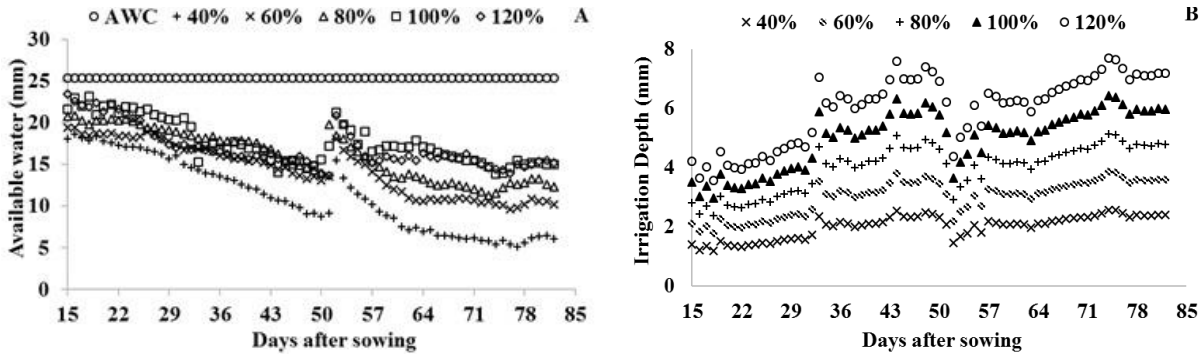

Figure 1. Daily available water in the soil (mm) (A) and irrigation depth (mm) (B) applied daily to cowpea bean genotypes subjected to different irrigation strategies (Pombal, PB, 2015. AWC = available water content; strategies: 40, 60, 80, 100 and 120% of actual evapotranspiration (ETr)).

Table 2. Irrigation amounts (mm) from irrigation strategies applied to cowpea bean genotypes. Pombal, PB, 2015.

Variable	Irrigation strategies (% ETr)				
	40 (%)	60 (%)	80 (%)	100 (%)	120 (%)
Irrigation amount (mm)	135.7	203.6	271.5	339.4	407.3

placed inside a forced air circulation oven at 65°C for 72 h and weighed afterward on an analytical balance in order to determine leaf (LDB), petiole (PDB), stem (SDB), root (RDB) and nodule (NDB) dry biomatter. The sums of these biomatter values and total biomatter (TDB) were determined and data expressed in grams per plant.

It should be noted that root collection to determine RDB was performed through the removal of a soil volume corresponding to the plant area (0.6 × 0.3 × 0.2 m) at a depth of 30 cm. The material was washed and sieved in order to keep only roots, same procedure were adopted for all plots.

Yield was assessed in dried beans. Therefore, pods of four plants per plot were harvested and stored during the production cycle until 90 DAS. Dried pods were collected at intervals of 7 days. In each collection, grain weight per plant was obtained. At the end of the experiment, the whole grain yield per plant was summed. Yield value was estimated with the multiplication of grain weight per plant by the number of plants per hectare, in which data were shown as kilograms per hectare.

Data variability were analyse using ANOVA. With F-test for significance, regression analysis was used for irrigation strategies. For the genotype factor, Tukey's test was used at the 5% probability level, using SISVAR software (Ferreira, 2011).

RESULTS AND DISCUSSION

Relative to cowpea plant gas exchange under water stress, was observed a significant interaction effect to *gs*, *E*, *A* and *iWUE* variables, according to ANOVA (Table 3). However, an isolated effect of factors was not observed

Table 3. Summary of analysis of variance for internal CO_2 concentration (*Ci*), stomatal conductance (*gs*) (mol_{H2O} m^{-2} s^{-1}), transpiration (*E*) ($mmol_{H2O}$ m^{-2} s^{-1}), assimilation rate (*A*) (µmol m^{-2} s^{-1}) and instantaneous water use efficiency (*iWUE*) (*A/E*) [(μmol_{CO2} m^{-2} s^{-1}) ($mmol_{H2O}$ m^{-2} $s^{-1})^{-1}$] from cowpea genotypes under different irrigation amounts at the V4 vegetative stage. CCTA/UFCG, Pombal, PB, 2015.

Control factor	DF	Mean square				
		Ci	***gs***	***E***	***A***	**iWUE**
Genotype (G)	3	2.687^{ns}	0.001^{ns}	0.027*	0.231^{ns}	0.008^{ns}
Depth (ID)	4	4.228^{ns}	0.024**	0.158**	3.028**	0.088^{ns}
G × ID	12	5.890^{ns}	0.002**	0.018**	0.869**	0.154**
Block	3	6.597^{ns}	0.002*	0.146**	0.715**	0.231*
Error	57	3.085	0.001	0.007	0.109	0.058
CV (%)		12.49	2.83	4.10	6.77	8.25
Mean		140.66	11.68	20.09	48.63	29.19

DF = degrees of freedom; CV = coefficient of variation; **, * and ns = significance to 1%, 5% and non-significant by F-test, respectively.

in the latter two variables. In addition, differences in *E* ($mmol_{H2O}$ m^{-2} s^{-1}) among genotypes ($p \leq 0.05$) were observed. As for the irrigation amount factor, isolated effects stood out regarding *gs* (mol_{H2O} m^{-2} s^{-1}), *E* ($mmol_{H2O}$ m^{-2} s^{-1}) and *A* values (μmol_{CO2} m^{-2} s^{-1}) ($p \leq 0.05$).

The aforementioned variables were measured at the V4 growth stage, which occurred near 30 DAS, about 15 days after differentiation of irrigation strategies began. This shows the importance of gas exchange evaluation in describing water stress effects on the CO_2 influx process. From studying the effects of irrigation amounts on *gs* of each genotype (Figure 2), an increasing linear behaviour was observed in all genotypes, with the exception of Paulistinha. Specifically, 0.058, 0.056 and 0.052 mol_{H2O} m^{-2} s^{-1} from 20% ETr irrigation increased *gs* values of Costela de Vaca, Pingo-de-Ouro and BRS Marataoã genotypes, respectively. In Paulistinha, there was quadratic behaviour, with maximum conductance obtained by applying an irrigation level equivalent to 93.5% ETr.

However, in general, all studied genotypes, even with a 40% Etr irrigation strategy application, showed higher results than those reported by Nascimento et al. (2011), who found values from 0.03 to 0.11 mol_{H2O} m^{-2} s^{-1} when plants were under stress in the reproductive stage. However, the differences may be related to time of stress, as the authors conducted plant evaluations at 43 DAS. Moreover, as noted by the results, water deficit tends to reduce the water flow and, consequently, cell turgidity, providing stomatal closure, which implies *E* and CO_2 influx reductions, as explained by Taiz and Zeiger (2013).

By studying *E* (Figure 2), it was observed that all genotypes were significantly influenced by irrigation levels. In Costela de Vaca and Pingo-de-Ouro, increasingly linear behaviour was observed, with 0.144 and 0.196 $mmol_{H2O}$ m^{-2} s^{-1} increases in *E* values, respectively, for every 20% increase in ETr. Such an *E* increase may indicate higher *A*. However, if it does not occur, water use efficiency tends to decrease. Thereby, net photosynthetic data should be considered when assessing whether this increase is interesting for plants. There is a tendency for water to transform from liquid to gas depending on the water vapour concentration difference between the leaf intercellular spaces and outer air mass (Taiz and Zeiger, 2013), which is optimised with increased water availability.

Regarding *E*, quadratic behaviour was observed for Paulistinha and BRS Marataoã genotypes, with maximum *E* obtained when irrigated with 101.75 and 99.83% ETr, respectively, which can be explained by the fact that plants showed an *E* rate decrease above ideal humidity conditions. This may be attributed to water saturation in the soil, which may have limited the absorption by roots, since water inflow depends on gas exchange conditions in the soil (Taiz and Zeiger, 2013). Even limitations regarding nitrogen accumulation and fixation in the plant may occur (Guimarães et al., 2015).

When analysing *A* (Figure 2), quadratic behaviour can be observed in Costela de Vaca, Paulistinha and BRS Marataoã genotypes, with maximum photosynthetic rates obtained when irrigation was conducted with levels estimated at 83, 100, and 105% ETr, respectively, obtaining 28.97, 26.994 and 25.6 µmol m^{-2} s^{-1}, respectively. These results are higher than those found by Dutra et al. (2015), who studied gas exchange in cowpea under different water levels and found means between 15 and 21 µmol m^{-2} s^{-1} while studying BRS Marataoã. This fact may be attributed to the experimental conditions, as these authors varied levels in relation to reference evapotranspiration, while actual evapotranspiration was used in this paper, indicating that crop water demand should be lower than the atmospheric demand (that is, the crop coefficient (Kc) must be lower than 1.0). It is worth noting that the aforementioned genotypes,

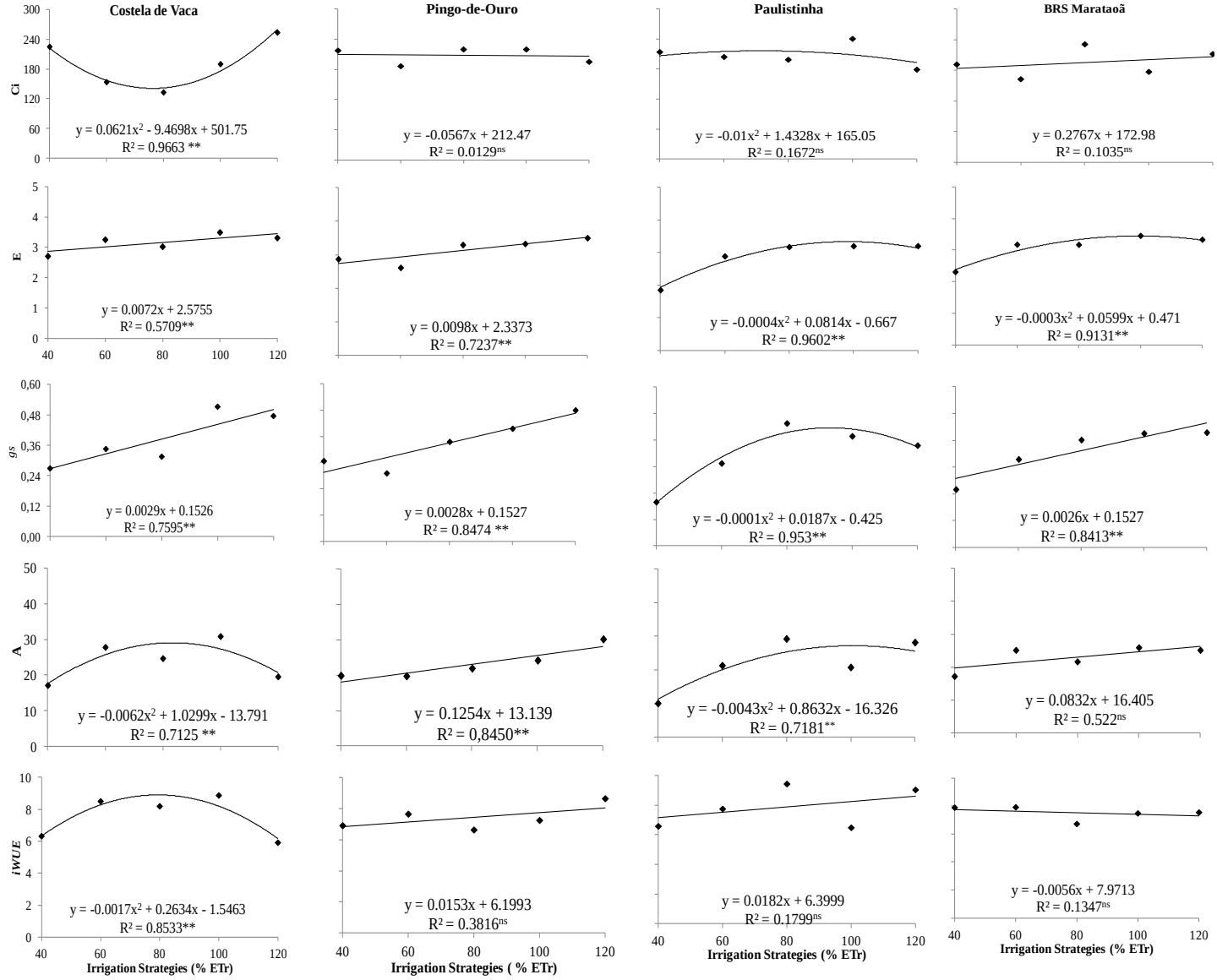

Figure 2. Regression analyses relative to internal CO_2 concentration (*Ci*), stomatal conductance (*gs*) (mol_{H2O} m^{-2} s^{-1}), transpiration (*E*) ($mmol_{H2O}$ m^{-2} s^{-1}), assimilation rate (*A*) (μmol_{CO2} m^{-2} s^{-1}), instantaneous water use efficiency (iWUE) (*A*/*E*) [(μmol_{CO2} m^{-2} s^{-1}) ($mmol_{H2O}$ m^{-2} s^{-1})$^{-1}$] and instantaneous carboxylation efficiency (EiCi) (*A*/*Ci*) from cowpea genotypes under different irrigation amounts at the V4 vegetative stage 45 DAS. CCTA/UFCG, Pombal, PB, 2015.

under stress conditions by deficit and excess water, had a tendency to reduce *A*, corroborating information by Freire (2005), who highlighted that lack of or excess water directly harms plant development.

Regarding the Pingo-de-Ouro genotype, it can be inferred that the 20% linear increase in irrigation amount allowed for an increase of 2.508 µmol m^{-2} s^{-1} in *A*. This result can be explained by the fact that the genotypes were conditioned to higher available water in the soil, increased *gs* and *E*, as noted by Ferraz et al. (2012). This result is interesting, as it may mean greater production potential in growing conditions in which there are no water restrictions.

With respect to water use efficiency, quadratic behaviour was observed for the Costela de Vaca genotype, with maximum efficiency estimated at the 77% ETr level, with a value of 8.65 ((μmol_{CO2} m^{-2} s^{-1}) ($mmol_{H2O}$ m^{-2} s^{-1})$^{-1}$). For Jaimez et al. (2005), the relationship between photosynthetic rate and *E* indicates the *iWUE*, in which values relative to carbon fixed in the plant by each water unit lost are observed. It can be seen that an increase in stomatal chamber conductance allowed for *A* up to the mentioned level, which is due to low CO_2 concentrations, reducing *iWUE*, which is, in turn, related

to increases in *gs* and *E*. In a similar situation, Nascimento et al. (2011) observed a reduction in *gs* values when cowpea plants were maintained under low hydric potential, resulting in lower production.

In Pingo-de-Ouro and Paulistinha genotypes, increasing linear behaviour was observed. Specifically, increases of about 0.0153 and 0.0182 ((μmol_{CO2} m^{-2} s^{-1}) (mol_{H2O} m^{-2} $s^{-1})^{-1}$) with each unit increase in ETr strategy were observed. This fact is interesting especially for Pingo-de-Ouro, in which photosynthesis and *E* increases also were observed, indicating that this genotype can produce better under higher irrigation amounts. Thus, Pingo-de-Ouro is more suitable for conditions without water availability restrictions. Moreover, in all genotypes, mean values were higher than those found by Ferraz et al. (2012), who observed an average of 4.3 ((μmol_{CO2} m^{-2} s^{-1}) ($mmol_{H2O}$ m^{-2} $s^{-1})^{-1}$) in the time from 9 a.m. to 10 a.m., showing the potential of these genotypes and of the region for cowpea cultivation.

By studying biomatter formation of cowpea genotype plants under water stress through ANOVA (Table 4), there was no significant interaction between factors of any variable studied. However, significant differences were observed between cowpea genotypes for PDB, SDB, RDB, NDB and TDB. A significant effect of irrigation amount also was observed in all variables, with the exception of RDB. Thus, cowpea biomatter sensitivity is observed when exposed to low water availability in the soil, providing variables that are recommended to determine water stress in cowpea.

Sensitivity may be related to plant adaptation mechanisms to tolerate stress by reducing the leaf area and reducing photosynthetic area and biomatter formation, avoiding increased *E* and controlling its temperature in the environment (Taiz and Zeiger, 2013). Biomatter formation effects also were observed by Dutra et al. (2015), who studied cowpea under different irrigation levels, and by Vale et al. (2012), who evaluated water stress tolerance in common bean, confirming the importance of these variables in the definition of stress conditions. It also should be noted that biomatter was evaluated at the R2 stage, corresponding to 45 DAS, which confirms that water stress affects cowpea plants with an increased exposure period.

By studying PDB, SDB, RDB and TDB formation (Figure 3) in relation to genotypes, significant differences are noted. The highest means were observed for Pingo-de-Ouro for all of these variables, with the exception of NDB, which had the lowest mean. Moreover, it should be noted that BRS Marataoã and Costela de Vaca genotypes did not differ from Pingo-de-Ouro in SDB and TDB variables. These results, therefore, confirm the biomatter formation potential of genotypes with indeterminate growth characteristics, which was more marked in Pingo-de-Ouro. This is of great importance if producing matter for incorporation into the soil is desired.

Although the lowest TDB mean was observed in the Paulistinha genotype, this may be due to its the 'determinate' growth type, which has the advantages of mechanised and regular harvest possibilities. It is therefore necessary to evaluate production aspects and production system objectives before choosing the most suitable variety. Although greater dry matter formation was observed for Pingo-de-Ouro, it was noted that this genotype had the lowest NDB, which indicates these plants had more efficient nodulation. Thus, the plants form more dry matter with fewer nodules, which is interesting for breeding programmes or identification studies for the corresponding microorganisms. Regarding irrigation level effects, there was increasing linear behaviour with increasing water availability to plants in all matter accumulation variables studied (Figure 4).

The 120% ETr level provided the most dry matter accumulation, with increases in the order of 95.5, 45.9, 62.3, 120, 13.1 and 70.7% between the lowest and the highest water levels for LDB, PDB, SDB, RDB, NDB and TDB, respectively. Thus, it was observed that higher water availability ensures higher water influx and cellular turgor maintenance, providing conditions for plant growth by cell division and expansion (Taiz and Zeiger, 2013). Significant effects of genotype × irrigation strategy interaction were observed for cowpea yield as well as isolated effects of studied factors (Table 5). For Cordeiro et al. (1998), the cowpea filling stage is the most sensitive to water stress, which justifies such interaction effects.

By studying yield, differential behaviours of genotypes when subjected to irrigation strategies (Figure 4) were observed. In this sense, the highest yields were observed when irrigation was conducted with levels equivalent to 120% ETr in Pingo-de-Ouro and Paulistinha genotypes, relative to 407.2 mm during the production cycle, which provided an estimated yield of 2025 and 3000 kg ha-1, respectively. In relation to Pingo-de-Ouro, it is emphasised that linear behaviour was observed in most physiological variables, indicating that this genotype needs more water to express its productive potential. However, by applying the same water amount, higher yield was obtained with the Paulistinha genotype, which may be indicated as a cowpea genotype for semiarid climates, where there are water restrictions.

In the BRS Marataoã and Costela de Vaca genotypes, quadratic behaviour was observed, with maximum yields expressed with levels estimated of 97 and 92% ETr, respectively, resulting in estimated values of 1835.23 and 2634.09 kg ha^{-1}, respectively, demonstrating the potential of these genotypes, although they were lower than those obtained with Paulistinha.

On the other hand, the Costela de Vaca genotype produced 2634.09 kg ha^{-1} using a 92% ETr level, which equals 312.2 mm, while Paulistinha produced 3000 kg ha^{-1} with 407.2 mm. Thus, Costela de Vaca produced 0.843 kg of grain for each 1.0 m^3 of water consumed, while Paulistinha produced 0.736 kg for each 1.0 m^3 of

Table 4. Summary of analysis of variance for dry biomatter of leaves (LDB), petioles (PDB), stems (SDB), roots (RDB) and nodules (NDB) and total dry biomatter (TDB) expressed as grammes per plant from cowpea genotypes under different irrigation amounts until 45 days after sowing. CCTA/UFCG, Pombal, PB, 2015.

Control factor	DF	Mean square					
		LDB	PDB	SDB	RDB	NDB	TDB
Genotype (G)	3	1.629^{ns}	5.717**	5.021**	0.175**	0.008*	6.460**
Depth (ID)	4	4.019**	3.502**	1.472**	0.024^{ns}	0.015**	5.785**
G × ID	12	0.550^{ns}	0.058^{ns}	0.324^{ns}	0.015^{ns}	0.002^{ns}	0.801^{ns}
Block	3	0.125^{ns}	0.086^{ns}	0.913^{ns}	0.041^{ns}	0.004^{ns}	0.799^{ns}
Error	57	0.601	0.040	0.253	0.024	0.002	0.812
CV (%)		18.62	9.05	14.15	8.85	4.11	15.12
Mean		4.16	2.22	3.56	1.75	1.10	5.96

DF = degrees of freedom; CV = coefficient of variation; **, * and ns = significance to 1%, 5% and non-significant by F-test, respectively.

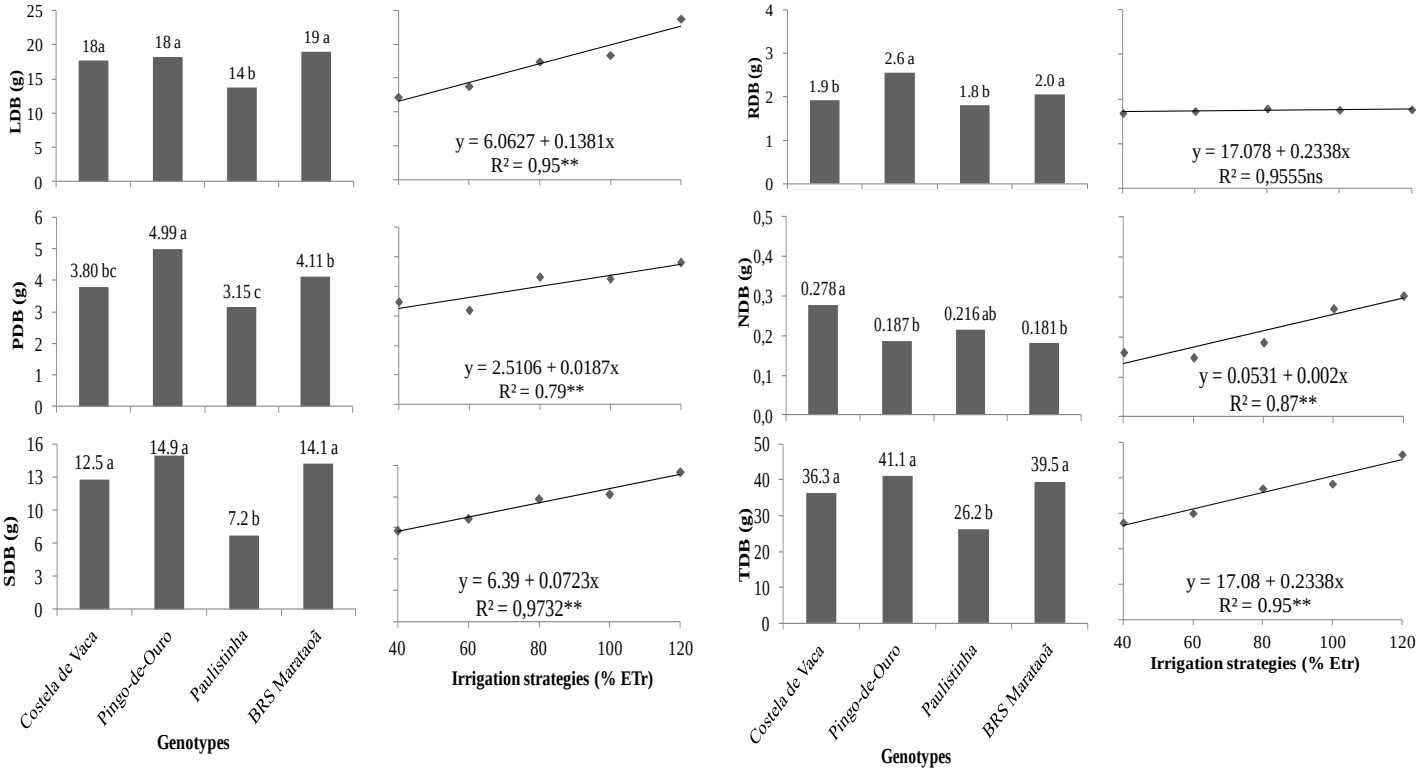

Figure 3. Means based on Tukey's test (p > 0.05) between cowpea genotypes and regression analyses regarding irrigation strategies for leaf (LDB) (g), petiole (PDB) (g), stem (SDB) (g), root (RDB) (g) and nodule (NDB) (g) dry biomatter and total dry biomatter (TDB) (g) until 45 days after sowing. CCTA/UFCG, Pombal, PB, 2015.

water, with better water use efficiency with Costela de Vaca.

Furthermore, BRS Marataoã yield values were slightly higher than those observed by Dutra et al. (2015), who obtained a maximum yield of 1715 kg ha^{-1} when levels equivalent to 100% ETr were applied to the same genotype. In general, the results observed in this study were higher than those observed by Silva and Neves (2011), who found values ranging from 668.70 to 1070.3 kg ha^{-1}, and higher than those reported by Nascimento et al. (2011), who observed a mean yield of 1167 kg ha^{-1}, with variation from 663 to 1529 kg ha^{-1} between genotypes without water stress. Those comparisons show the cultivation potential of these varieties in semiarid regions and the potential of appropriate water use in irrigation.

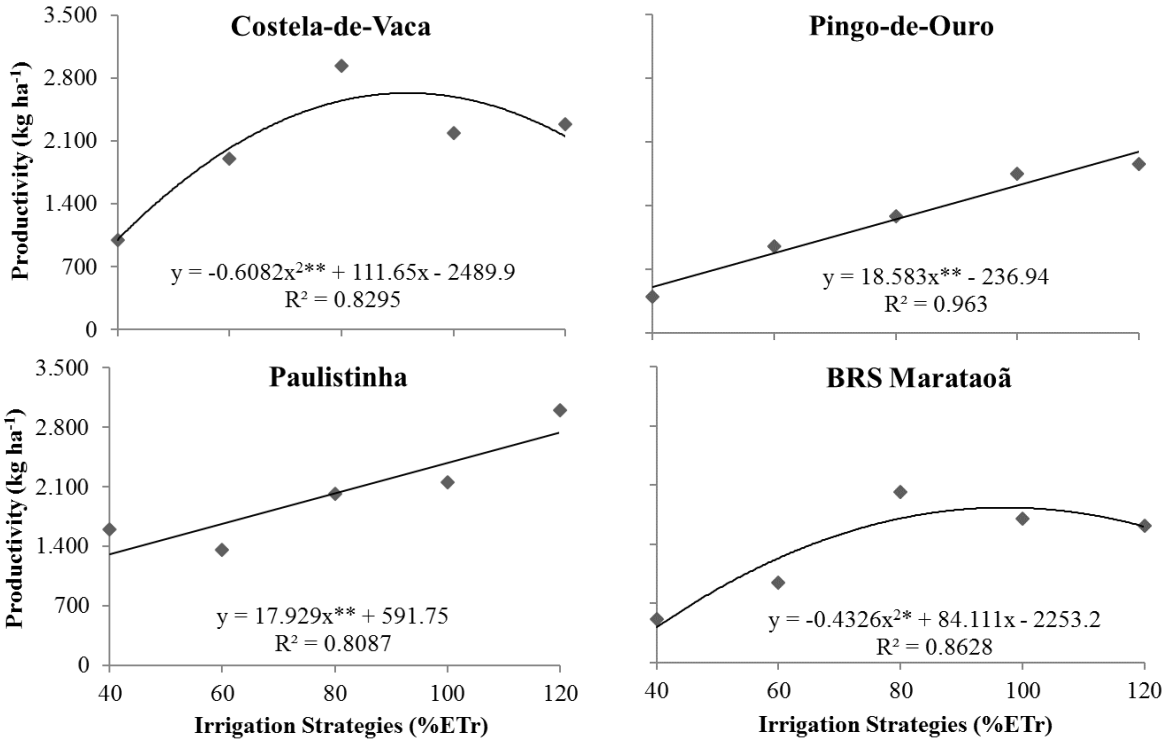

Figure 4. Regression analyses relative to yield (kg ha^{-1}) from cowpea genotypes under different irrigation strategies until 90 days after sowing. CCTA/UFCG, Pombal, PB, 2015.

Conclusions

The highest growths in cowpea were observed in Costela de Vaca and Pingo-de-Ouro genotypes for leaf and dry biomatter formation, respectively. Among the evaluated characteristics, the leaf formation on cowpea genotypes was found to be the most sensitive to water stress. The use of 120% ETr water levels provided the highest growth of total dry biomatter on genotypes. The genotype Costela de Vaca had the highest physiological potential based on photosynthetic rates. The Pingo-de-Ouro genotype needed more water to express its productive potential than other genotypes. Higher productivity was achieved with the Paulistinha genotype (that is, 3000 kg ha^{-1}) when irrigated with 120% ETr, which equals to 407.2 mm in the production cycle. The Costela de Vaca genotype presented the highest water use efficiency (that is, 0.843 kg m^{-3}).

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

New substrates for the cultivation of *Pleurotus ostreatus* using exhausted compost

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Several materials have been used in the cultivation of the edible mushroom *Pleurotus ostreatus*. However, little is known about the reuse of the exhausted compost. This study evaluated the utilization of used substrates. Four formulations of composts were evaluated: C1 with no exhausted compost, and C2, C3 and C4 with 26, 45 and 64% of exhausted compost, respectively. Loss of organic matter, biological efficiency and mass of basidiomata were evaluated by means of the results of the chemical analysis of the initial and final composts and the nutritional assessment of the basidiomata. The data obtained were submitted to statistical analysis. The results of the chemical analysis of the composts show an increase of nitrogen between the initial and the exhausted compost and a decrease of the carbon/nitrogen ratio. The loss of organic matter and biological efficiency of composts C2, C3 and C4 were lower than the traditional compost. The mass of fresh basidiomata of composts 1 and 2 were not significantly different, being superior to other treatments. Treatment C3 showed a higher amount of protein. The conclusion was that the exhausted compost can be reused up to a certain amount without affecting the production and the nutritional value.

Key words: Residues, utilization, productivity, mushrooms.

INTRODUCTION

With the advent of the second-generation ethanol, crushed sugarcane, one of the main substrates used in mushroom cultivation in the State of São Paulo, has become scarcer in the market, once it has been used by industries as an energy source in boilers. Thus, fungi producers have found difficulties to obtain this product and, when it is available, its price has become continuously inviable.

Another common problem in mushroom cultivation regions is the correct discharge of the exhausted compost in order to avoid environmental damages. After harvesting, it is very common to find producers who pile all the exhausted substrate somewhere else in the property, attracting flies and other agronomic pests which can eventually cause damages for the next mushroom cultivations, according the distance from the production site and the environmental factors (rain, wind, humidity, etc.).

Several materials have been used in the preparation of the compost for the cultivation of *Pleurotus ostreatus*,

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such as sawdust, banana tree straw, coffee husks and several cellulosic residues (Bonatti et al., 2004; Fan et al., 2006; Tisdale et al., 2006; Das and Mukherjee, 2007; Sales-Campos et al., 2010a; Carvalho et al., 2010). However, little is known about the reuse of these materials for new cultivation cycles. Royse (1993) evaluated the performance of exhausted compost used to produce *Shiitake* by adding wheat bran and corn in the production of *Pleurotus sajor-caju*. Kilpatrick et al. (2000) used formulations in the cultivation of *Lentinula edodes* added with exhausted compost of *Agaricus*, with several grains, wheat flour and calcium carbonate ratios. Mamiro and Royse (2008) evaluated portions of exhausted substrate added to the traditional compost of *A. bisporus*.

Thus, the objective of the present work was to find a noble destination for the exhausted compost (mushrooms production) and reduce the accumulation of this material in the environment by using it in new cultivation cycles of *P. ostreatus*.

MATERIALS AND METHODS

The experiment was carried out in two stages: 1. Composting and pasteurization carried out at the Faculdade de Ciências Agronômicas (FCA/UNESP), Botucatu, São Paulo, Brazil. 2. Incubation and harvesting carried out at the Universidade do Sagrado Coração (USC), Bauru, São Paulo, Brazil. Three formulations of composts were tested, named based on the exhausted substrate and compared to traditional compost (without the addition of exhausted compost - control) for the cultivation of *P. ostreatus*.

The *P. ostreatus* strain used in the experiment was POS-09/101, obtained from the mycology collection of the Módulo de Cogumelos, FCA/UNESP, Botucatu. The inoculum was prepared by using the methodology proposed by Minhoni et al. (2005).

Phase I of the composting was performed in an open shed, with galvanized sheet roof and concrete floor. Before forming the plots, the sugarcane straw was moistened at 75% of average humidity and revolved every two days for a total period of six days (pre-wetting).

After Phase I of composting, the plots were formed by a moistened straw layer (20 cm high), followed by an exhausted compost layer (20 cm high) (with exception of the control) until reaching 1.8 m of height × 1.8 m of width, respectively.

Limestone, plaster and wheat bran were added in all plots according to each treatment (Tables 1 to 4). All the materials used were previously analyzed in order to obtain a calculated C/N ratio of 67/1, which is the most recommended for the cultivation of *P. ostreatus*. Limestone was used to correct the pH of the compost. Gypsum was added to improve the physical characteristics of the compost.

The composts were turned over and water was added manually with a hose to keep humidity between 70 and 75%. In Phase I, three overturns were performed in a total of six days. In Phase II, the composts were transferred to lattice boxes and then arranged randomly inside a climatic chamber (Dalsem Mushrooms) for pasteurization (8 hours at 62 ± 2°C) and conditioning (4 days at 48 ± 2°C).

The inoculation of the compost with *Spawn* from strain POS-09/101 of *P. ostreatus* was performed manually, inside a Dalsem climatic chamber. The tools used (tray, scissors and dosing glass of *Spawn*) were cleaned with alcohol 70%. The inoculum ratio used was 40 g Kg^{-1} of fresh mass of the compost.

The incubation was performed in an experimental greenhouse at USC for 30 days at an average temperature of 25°C and relative humidity of 55 to 70%. The four treatments named C1, C2, C3 and C4 were control, 26% of exhausted compost, 45% of exhausted compost and 64% of exhausted compost (Tables 1 to 4), respectively; they were randomly arranged on shelves and represented by four treatments with twenty repetitions each. After the incubation period, harvesting and weighing of mushrooms were carried out daily for 60 days.

The nutritional analyses of the basidiomata were performed at the Food Laboratory of the USC, in Bauru, São Paulo. Three whole samples of basidiomata from each treatment were dehydrated and ground for analysis. A total of 12 samples of mushrooms were analyzed for raw protein, ash and lipids, according to the methodology of Silva and Queiroz (2002), with some adjustments mentioned ahead.

To evaluate humidity, dry weighting bottles were used in a greenhouse at 105°C for half an hour and then cooled with their respective lids in a desiccator. After cooling, the bottles were weighed empty in analytical scales and then approximately 3.5 g of sample of each treatment were added and the bottles were weighed again. Next, the samples were dried in a greenhouse at 105°C for 6 h, removed, weighed again and placed back inside the greenhouse until reaching constant weight. Ash was determined by incinerating a 5 g sample of each treatment in a muffle at 550°C. The samples were manipulated by using a clamp and a crucible and then cooled in a desiccator and weighed again.

The raw protein content was evaluated by the Kjeldahl method by extracting the total amount of nitrogen of the samples and multiplying them by the correction factor (PB% = N × 4.38).

Usually, the correction factor used in this type of analysis is 6.25, considering that proteins have 16% of nitrogen. However, this value is 4.38 for fungi because they have non-digestible nitrogen composts, such as the chitin, in the cell wall (Furlani and Godoy, 2005).

Approximately 0.2 g of each sample was weighted to extract the nitrogen. Ten glass beads in the Kjeldahl tube, 5 ml of H_2SO_4 and 2.5 g of a catalyzed mixture of $CuSO_4$ and K_2SO_4 were placed inside the digester; the temperature was gradually increased until reaching 400°C.

7 ml of distilled water and 3 drops of methyl red indicator were dropped in the Kjeldahl tube. 10 ml of 4% boric acid and 3 drops of mixed indicator were poured in an Erlenmeyer flask.

20 mL of 40% NaOH were added to the receptacle of the equipment and the tap was opened in order to allow a slow dripping over the Kjeldahl tube of the equipment already adapted.

The button on the left of the equipment was turned on in the beginning of the reflow; the Erlenmeyer flask was placed and left to distill until reaching the volume of 50 ml. The button on the left was turned off when the desired volume was reached; the Erlenmeyer was removed and titrated with standardized HCl 0.1 M until the color changed.

The N percentage was calculated by using the formula “nitrogen % = 0.014 × N × f × V (ml) spent × 100/weight of the sample”.

The determination of lipids was performed by using a Soxhlet extractor; approximately 3.5 g of each sample were weighed and placed in Soxhlet cartridges and weighed in a flat bottom round glass bottle. The equipment was assembled with enough petroleum ether for the occurrence of siphonage and left for 8 h with 4 to 5 drops per second; the round glass botlle was dried in a stove at 105°C for half an hour and weighed again.

At the end of Phases I and II of composting, three samples of compost from each plot were removed and dehydrated at 65°C during 48 h for carbon, nitrogen, organic material and pH analysis. The same procedure was repeated at the end of the cultivation cycle. These analyses were performed at the Fertilizers and Correctives Chemical Analyses Laboratory of the Department of Natural Resources of the School of Agronomic Sciences - UNESP,

Table 1. Formulation of C1 compost.

Material	Humidity (%)	Wet weight (Kg)	Dry weight (Kg)	C (%)	C (Kg)	N (%)	N (Kg)
Sugarcane straw	6	200.00	188.00	48.00	90.24	0.50	0.94
Wheat bran	6	24.00	22.56	46.00	10.38	2.50	0.65
Total		224.00	210.65		100.92		1.50
Limestone (3%)		6.3					
Plaster (1%)		2.1				C/N_{final}	67

Table 2. Formulation of C2 compost.

Material	Humidity (%)	Wet weight (Kg)	Dry weight (Kg)	C (%)	C (Kg)	N (%)	N (Kg)
Sugarcane straw	6	175.00	164.50	48.00	78.96	0.50	0.82
Wheat bran	6	21.00	19.74	46.00	9.08	2.50	0.46
Exhausted compost	36.45	100.00	63.55	36.45	23.16	0.53	0.34
Total		296.00	247.79		111.20		1.65
Limestone (3%)		7.5					
Plaster (1%)		2.5				C/N_{final}	67

Table 3. Formulation of C3 compost.

Material	Humidity (%)	Wet weight (Kg)	Dry weight (Kg)	C (%)	C (Kg)	N (%)	N (Kg)
Sugarcane straw	6	150.00	141.00	48.00	67.78	0.50	0.71
Wheat bran	6	19.00	46.00	46.00	8.22	2.50	0.45
Exhausted compost	36.45	200.00	36.45	36.45	46.33	0.53	0.67
Total		369.00			122.22		1.83
Limestone (3%)		8.7					
Plaster (1%)		2.9				C/N_{final}	67

Table 4. Formulation of C4 compost.

Material	Humidity (%)	Wet weight (Kg)	Dry weight (Kg)	C (%)	C (Kg)	N (%)	N (Kg)
Sugarcane straw	6	100.00	94.00	48.00	4.12	8.50	0.47
Wheat bran	6	13.00	12.22	46.00	5.62	2.50	0.31
Exhausted compost	36.45	300.00	190.65	36.45	69.49	0.53	1.01
Total		413.00	296.87		120.23		1.79
Limestone (3%)		9					
Plaster (1%)		3				C/N_{final}	67

Botucatu, SP, according to Lanarv's (1988) methodology.

According to Rajarathnam and Bano (1989), the loss of organic matter (LOM) is the index that evaluates the decomposition of the substrate by the fungus, which occurs during the cultivation. This index is based on the loss of organic matter decomposed by the fungus and it is determined by the difference between the dry mass of the initial substrate and the dry mass of the residual substrate (post-harvest).

Productivity was expressed by means of the biological efficiency (BE), which represents the conversion percentage of the substrate into fungal biomass (basidiomata).

Data were submitted to the analysis of variance and the averages were compared by the Tukey test (5%) (Snedecor and Cochran, 1972) using the SISVAR 4.2 software developed by the Department of Exact Sciences from the Federal University of Lavras, Minas Gerais, Brazil (UFLA).

RESULTS AND DISCUSSION

The chemical analyses of the composts used for the

Table 5. Chemical analysis of the substrate at the end of phase I.

Treatments	N (%)	O.M (%)	C (%)	C/N (%)	Humidity	pH
C1	0.30[a]	26.6[a]	14.6[a]	49.0[a]	68.3[a]	7.2[a]
C2	0.33[a]	28.0[a]	15.6[a]	47.6[a]	65.0[a]	7.3[a]
C3	0.30[a]	20.6[a]	11.6[a]	39.0[b]	64.6[a]	7.5[a]
C4	0.46[a]	31.6[a]	17.6[a]	37.0[b]	50.3[a]	7.5[a]
CV	23.3	24.5	23.7	7.08	12.2	2.4
MSD	0.21	17.2	9.2	8.02	19.2	0.47

C1, Control treatment, with sugarcane straw; C2, treatment with 26% of exhausted compost; C3, treatment with 45% of exhausted compost; C4, treatment with 64% of exhausted compost; CV, coefficient of variation; MSD, Minimum significant difference; N%, Nitrogen percentage; OM%, Organic matter percentage; C%, Carbon percentage; pH, hydrogen potential. Averages followed by equal letters in each column are not different among each other (Turkey, 5%).

Table 6. Chemical analysis of the substrate at the end of phase II.

Treatments	N (%)	O.M (%)	C (%)	C/N	Humidity	pH
C1	0.26[a]	19.33[a]	10.66[a]	40.66[a]	76.00[a]	7.3c
C2	0.30[a]	25.33[a]	14.00[a]	46.66[a]	68.00[b]	7.8[b]
C3	0.30[a]	20.00[a]	11.00[a]	37.00[b]	67.33[ab]	8.0[ab]
C4	0.36[a]	16.66[a]	10.66[a]	29.33[b]	61.33[b]	8.4[a]
CV	13.24	17.43	18.14	15.12	6.83	2.36
MSD	0.10	9.51	4.49	15.20	12.17	0.48

C1, Control treatment, with sugarcane straw; C2, treatment with 26% of exhausted compost; C3, treatment with 45% of exhausted compost; C4, treatment with 64% of exhausted compost; CV, coefficient of variation; MSD, Minimum significant difference; N%, Nitrogen percentage; OM%, Organic matter percentage; C%, Carbon percentage; pH, hydrogen potential. Averages followed by equal letters in each column are not different among each other (Turkey, 5%).

Table 7. Chemical analysis of the substrate at the end of the cultivation cycle. (exhausted).

Treatments	N (%)	O.M (%)	C (%)	C/N	Humidity (%)	pH
C1	0.30[b]	14.33[c]	8.00[c].	27.00[ab]	74.66[a]	6.36[b]
C2	0.43[a]	22.00[a]	12.33[a]	28.66[a]	61.00[a]	5.43[b]
C3	0.36[ab]	13.66[c]	7.66[c]	21.00[c]	55.00[a]	8.73[a]
C4	0.40[ab]	17.33[b]	9.66[b]	24.33[bc]	67.33[a]	6.9[b]
CV	10.89	6.64	6.16	6.16	16.93	8.18
MSD	0.10	2.92	4.06	4.06	27.4	1.46

C1, Control treatment, with sugarcane straw; C2, treatment with 26% of exhausted compost; C3, treatment with 45% of exhausted compost; C4, treatment with 64% of exhausted compost; CV, coefficient of variation; MSD, Minimum significant difference; N%, Nitrogen percentage; OM%, Organic matter percentage; C%, Carbon percentage; pH, hydrogen potential. Averages followed by equal letters in each column are not different among each other (Turkey, 5%).

cultivation of *P. ostreatus* at the end of the composting Phases I and II and at the end of the cultivation cycle (exhausted compost) are shown in Tables 5 to 7.

The results of the four treatments at the end of Phases I and II of composting were not significantly different among each other (Tables 5 and 6). There was a little decrease in the nitrogen values in this period. On the other hand, the values presented in the substrate after the cultivation showed an increase in the nitrogen percentage. Sales-Campos et al. (2010b) noticed an increase in the amount of nitrogen in the exhausted substrate of *P. ostreatus.* It is possible to observe the reduction of organic material (OM%) and carbon (C%) among the three stages (Table 5 to 7).

Organic matter and carbon values were not significantly different between treatments at the end of phases I and II, but these values varied for the exhausted substrate, showing a non-uniform consumption of carbon and

Table 8. Biological efficiency (BE%), loss of organic matter (LOM%), mass of fresh basidioma (MFB Kg).

Treatments	BE (%)	LOM (%)	MFB (Kg)
C1	54.20[a]	95.50[a]	18.33[a]
C2	42.87[b]	66.03[b]	16.90[a]
C3	20.11[c]	41.62[c]	10.98[b]
C4	3.85[d]	7.23[d]	2.25
CV	12.45	28.89	0.29
MSD	5.14	20.75	4.87

C1, Control treatment, with sugarcane straw; C2, treatment with 26% of exhausted compost; C3, treatment with 45% of exhausted compost; C4, treatment with 64% of exhausted compost; CV, coefficient of variation; MSD, Minimum significant difference; N%, Nitrogen percentage; OM%, Organic matter percentage; C%, Carbon percentage; pH, hydrogen potential. Averages followed by equal letters in each column are not different among each other (Turkey, 5%).

organic matter among the four treatments (Table 7).

The C/N ratio of the treatments varied between 49.0 and 37.0 at the end of Phase I. These values declined in the end of Phase II and in the end of the cultivation cycle due to the carbon consumption from the fungal capability of degrading lignin and cellulose, with a posterior release of CO_2 and H_2O.

The highest values of the C/N ratio were provided by the treatments C1 (40.66) and C2 (46.66), indicating that these values are influenced by the amount of exhausted compost mixed with the traditional one.

According to Sales-Campos et al. (2010a), the ideal value of the C/N ratio for *P. ostreatus* is 80/1 in axenic cultivation (without composting) and around 25 to 50/1 for agroindustrial waste taken to composting and pasteurization process, according to Duprat (2012).

A decrease in the substrate humidity was verified in the results of the three composting phases (Tables 6 to 8); however, it was not significantly different in the analyses in the end of Phase I and in the end of the cultivation cycle. It was observed that this decrease occurs as the amount of exhausted substrate increases.

PH decreased during the colonization of the substrate by *P. ostreatus*, except for Treatment C3, which showed an increase. According to Chang and Miles (1989), the decrease of pH during the colonization process of the substrate by the fungus occurs due to the production of substances, such as fatty acids. The increase of pH in treatment C3 may be explained by the production of metabolites by *P. ostreatus*, such as the fatty acids affecting the concentration of the compost.

The biological efficiency (BE%) and the loss of organic matter (LOM%) were significantly different among each other (Table 8); the highest averages were obtained by treatment C1 (54.20%). There was a gradual decrease in terms of BE% and LOM% as the levels of exhausted substrate increased. However, the mass of fresh basidiomata (MFB) were not significantly different between treatments C1 and C2.

The decreasing rates of BE% and LOM% might be related to the decreasing rates of the C/N ratio in the end of Phase II, once this was the moment in which the inoculation of *P. ostreatus* occurred.

In an experiment carried out with sugarcane straw and bocaiuva straw, Cardoso et al. (2013) noticed that the decrease of BE% was proportional to the decrease of the C/N ratio. Bernardi (2010), testing the efficiency of various substrates for *P. ostreatus* and *P. sajor-caju*, noticed that *P. ostreatus* showed a better mycelial growth in elephant grass with a high C/N ratio: 162:1.

However, the results for biological efficiency and productivity showed better results in waste of castor bean cultivation (with a C/N ratio of 37:1) and elephant grass mixed with waste of castor bean (with a C/N ratio of 73:1); the biological efficiency was not significantly different.

These results might be compared the treatment C2, which did not obtain a significant BE% when compared to treatment C1, but presented good results in terms of mass of fresh basiodiomata (MFB). It was observed that both treatments showed higher values of C/N ratio in the end of Phase I.

Pardo-Giménez and Pardo-Gonzáles (2009) evaluated the efficiency of the cultivation of *P. ostreatus* in exhausted substrate of *P. ostreatus* mixed with the exhausted substrate of *Agaricus* sp, in different proportions when compared to the commercial substrate. It was observed that, in this study, the C/N ratio decreased as the amount of exhausted substrate of *P. ostreatus* decreased and proportionally to the decrease of BE%. However, the productivity of basidiomata was not significantly different to a certain extent, in terms of the amount relation of the *P. ostreatus* exhausted substrate and the C/N ratio, as in this study.

The result of the nutritional analysis of the basidiomata can be seen in Table 9. Treatment C3 had the highest values of proteins and ash. On the other hand, treatments C1 and C2 were not significantly different from each other in the parameters analyzed. However, this result showed itself different for ash in the dry base, being the best

Table 9. Nutritional analysis of the basidiomata.

Treatment	Proteins (%)	Lipids (%)	Ash (%)	Humidity (%)
C1	18.09[a]	1.29[a]	8.41[b]	8.38[a]
C2	19.45[ab]	1.21[a]	8.31[b]	7.72[a]
C3	21.06[a]	1.23[a]	8.58[a]	9.40[a]
C4	16.23[b]	1.33[a]	8.08[c]	9.05[a]
CV	8.78	8.78	0.50	11.60
MSD	0.29	0.29	0.10	2.62

C1, Control treatment, with sugarcane straw; C2, treatment with 26% of exhausted compost; C3, treatment with 45% of exhausted compost; C4, treatment with 64% of exhausted compost; CV, coefficient of variation; MSD, Minimum significant difference; N%, Nitrogen percentage; OM%, Organic matter percentage; C%, Carbon percentage; pH, hydrogen potential. Averages followed by equal letters in each column are not different among each other (Turkey, 5%).

result obtained for treatment C3. Treatments C1 and C2 are right below this value and were not significantly different from each other. The treatment with the lowest value was treatment C2. The results regarding lipids and humidity were not statistically different from each other.

An increase in the protein content was observed in treatment C3. This increase might have occurred due to the increase in the amount of exhausted substrate; however, this value decreased in treatment C4, showing that this assumption is not completely trustful. According to Furlan and Godoy (2005), the type of substrate used is one of the main factors that influence the proteins content of the mushrooms. The nitrogen content in the substrate in the end of phase II did not vary in the present study; however, the C/N ratio was different among the treatments (Table 6). It is theoretically known that the amount of total nitrogen and organic matter are closely related, probably influencing the amount of proteins and ash in this study.

Conclusions

1. The use of exhausted substrate for the cultivation of *P. ostreatus* is viable until the amount of 26% mixed to the traditional substrate;
2. The biggest average of raw protein in the basidiomata was provided in the compost with 45% of exhausted substrate mixed to the traditional; 21.06%;
3. The C/N ratio influenced the biological efficiency and the loss of organic material proportionally; the initial composts with higher C/N ratios provided a higher biological efficiency and loss of organic matter.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Growth, chlorophyll index and production of common and cowpea beans using different fertilizations

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Beans are a the major component in the Brazilian diet population, mainly in the northeast of Brazil, though yield is considered low due to the low technological content that is conducted in most producing regions, making it necessary for fertilizations to increase this feature. Considering the above, this study aimed to evaluate growth, chlorophyll index and the production of cowpea bean and common bean under different fertilizations. The experiment had been conducted in the Centre of Human, Social and Agricultural Sciences at The Federal University of Paraíba. The experimental design was a randomized block in factorial arrangement 2 × 4 with seven repetitions. The treatments consisted of two-bean varieties (*Phaseolus vulgaris* L. and *Vigna unguiculata* L.) and four fertilization (Leaf biofertilizer, organic compost made with goat manure, mineral fertilization and a without fertilizer treatment). The variables analyzed was growth, chlorophyll index and bean production. The bean cultivar Sempre Verde obtained higher growth and chlorophyll index *a, b* and total in relation Carioca. Fertilization with organic compost provided higher productivity of bean cultivars. The organic compost may be indicated as fertilizer alternative to the bean in the Paraíba swamp region.

Key words: *Phaseolus vulgaris L., Vigna unguiculata* L., Fertilization, chlorophyll, productivity.

INTRODUCTION

Bean is a high quality nutritional food because of its high protein content (20-25%), high lysine content, and low fat and high fiber contents, making it a major component of the Brazilian diet (Costa, 2008). Despite being a culture little competitive and despite a strong competition with products targeted to the foreign market, beans are still in a prominent position in the Brazilian agribusiness, playing an important role in generating employment and income in Brazil (Carvalho, 2009).

The main species of beans cultivated are *Phaseolus vulgaris* L. (common bean, grown throughout the country), and *Vigna unguiculata* L. (cowpea, Macassa or Macassar bean, grown mainly in the Northeast region and in the Amazon region).

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The use of organic compost in the agricultural production is a practice adopted worldwide, and its efficiency depends on the system and the way its preparation process and raw material used is managed. There may be several quality variations. The nutritional and biological richness that organic compost provide to the soil and plants helps in its cultivation, improving the chemical, physical and biological properties of the soil (Melo et al., 2007). It also provides increased growth, dry matter accumulation and chlorophyll index of crops (Cavalcante et al., 2016).

The use of Leaf biofertilizer is a practice increasingly being adopted by producers using alternative materials in their crops (Pereira et al., 2010). Organic solutes from bovine biofertilizers may provide more suitable conditions for plant cell elongation because of a physical improvement of the soil environment and stimulation of the action of organic protein and solutes, resulting in increased microbial activity (Freire et al., 2010).

The use of biofertilizers may be a viable alternative for providing nutrients, especially for short-cycle crops such as beans. Studies conducted by Mendes et al. (2007) indicate that it is possible to produce beans with an organic production system, achieving yields similar to those obtained with a conventional system.

Biofertilizers provide improvements in physical, chemical and biological properties of the soil and, when applied on leaves, contribute to a balanced supply of macronutrients and micronutrients to plants (Alves et al., 2009; Patil, 2010), allowing the plant to develop all its genetic and productive potential. Its use as a liquid provides an increased absorption of nutrients by the plants (Souza and Resende, 2003).

Considering the importance of bean crops to farmers, the low crop productivity in most producing states, the low adoption of efficient technologies adapted to local conditions, and the capitalization of small farmers to purchase inputs derived from petrochemicals, the study of the use of alternative fertilizing by means of biofertilizers and organic compost is needed as a fertilization and soil conditioning technique involving maximizing the use of existing natural resources in the agro-ecosystem and a less dependence on large industrial conglomerates, holders of chemical-mechanical technologies. Considering the above, this study aimed to evaluate growth, chlorophyll index and the production of cowpea bean and common bean under different fertilizations.

MATERIALS AND METHODS

The experiment was conducted from April to July 2014 at the Humanities, Social and Agricultural Center located in Bananeiras, Paraíba state, a municipality belonging to the Agreste mesoregion and Brejo Paraibano microregion, Brazil (IBGE, 2013).

The soil of the area was classified according to the criteria of the Brazilian System of Soil Classification (SiBCS) (EMBRAPA, 2013) as a Dystrophic Yellow Latosol. The rainfall and the maximum and minimum temperature of the city of Bananeiras-PB recorded during the experiment are shown in Figure 1.

A randomized block design was adopted in a 2 × 4 factorial design with seven replications. The factors under study consisted of two bean cultivars (*Phaseolus vulgaris* L. and *Vigna unguiculata* L.) and four types of fertilization (Leaf biofertilizer, organic compost made with goat manure, mineral fertilization and a without fertilizer treatment) with seven replications. The biofertilizer was prepared according to Penteado (2007) and weekly applied 15 days after emergence. In the first two sprays, the concentration was 5 and 10%. The organic fertilization consisted of organic compounds prepared with goat manure, being applying two liters per hole. For the mineral fertilization, 5.62 g/hole of P_2O_5 were used according to soil analysis (Table 1).

The experimental unit consisted of 16 plants in a 0.50 × 0.25 m spacing, with a total area of 2 m^2, considering four plants as a use area. At forty days, bean plants were in full bloom, and stem diameter, plant height, chlorophyll index *a*, *b* and total were evaluated. The production was harvested at ninety days, and the number pods per plant, number of seeds per pod, weight of 100 seeds, pod length and productivity were evaluated.

To measure plant height, a centimeter-graduated ruler was used from the base of the plant until the end of the main stem. The stem diameter was measured with a precision digital caliper at the base of the plant two centimeters from the soil. Chlorophyll *a*, *b* and total index were measured with a portable chlorophyll meter, ClorofiLOG CFL1030, with readings performed in the flowering period on fourth leaf of the main stem, evaluating the three leaflets exposed to solar radiation.

The number of pods per plant was determined in four plants per sample plot. The number of seeds per pod was determined by counting the grains of 20 random pods per plot. For weight of 100 seeds, after harvesting and threshing the beans, the grains were weighed with 11% humidity. The pod length was measured using a ruler graduated in centimeters.

Data were submitted to analysis of variance and the comparison of means was performed by Tukey test at 5% probability using the statistical software ASSISTAT version 7.7 beta (Silva and Azevedo, 2002).

RESULTS AND DISCUSSION

The greatest plant height was observed for the Sempre Verde bean cultivar, and the fertilization with organic compost provided an increase of this variable (Table 2). The organic inputs contribute to the improvement of the growth of agricultural crops by providing improvements in chemical and physical characteristics of the soil (Barros et al., 2013; Adejobi et al., 2014), making it economically viable and ensuring the productivity of cultures without causing a long-term potential threat to the environment (Nur et al., 2013).

The cultivar Sempre Verde stood out in relation to the Carioca cultivar with a greater stem diameter. This response may be a result of genetic differences existing between species (Table 3). The use of organic fertilizers with organic compost and biofertilizer provided an increase in stem diameter of Sempre Verde bean plants. The organic inputs from plants and animals may have beneficial effects on physical characteristics. They were expressed by the increase in the stability of aggregates and soil total porosity (Mellek et al., 2010). They also act in the chemical improvement, providing nutrients and

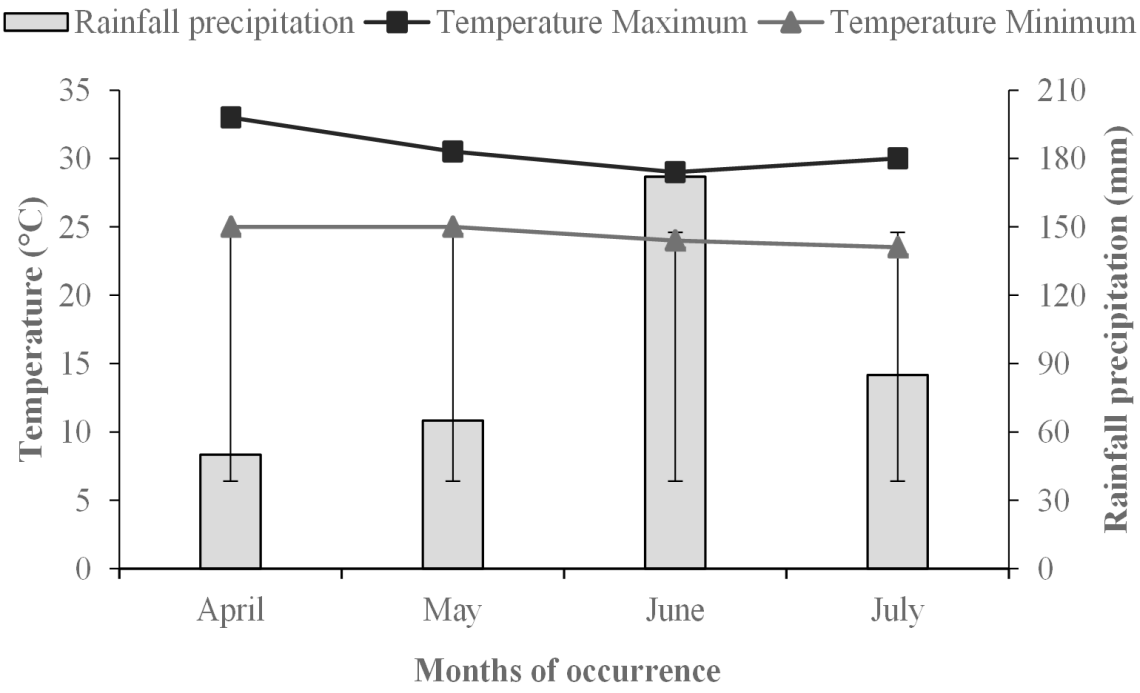

Figure 1. Rainfall precipitation and temperature of the city of Bananeiras-PB between April and July 2014.

Table 1. Characterization of chemical and soil fertility, organic compost and biofertilizer.

Sources	**pH H_2O	P	K^+	Na^+	H^+Al^{3+}	Al^{3+}	Ca^+	Mg^{2+}	BS	CEC	V	m	OM
		Mg dm^{-1}					$cmol_c$ dm^{-3}				---%---		g kg^{-1}
*S	6.51	6.94	0.29	0.03	1.32	0.00	4.45	1.65	6.42	7.75	82.96	0.00	19.38
OC	6.83	136.8	9.53	1.22	4.62	0.00	8.50	5.45	24.68	29.30	84.23	0.0	141.1
	***pH	N	P		K^+	B			S		ORG. C.		OM
B.		g/kg									%		
	3.27	15.93	0.40		0.52	153.58			10.47		47.25		81.46

*S = soil; OC = organic compost; B. = biofertilizer. **pH = active acidity, P = phosphorus available, K^+ = available potassium, Na^+= exchangeable sodium, H^+Al^{3+} = potential acidity, Al^{3+}= exchangeable acidity, Ca^+ = exchangeable calcium, Mg^{2+} = exchangeable magnesium, BS = base sum, CEC = effective cation exchange capacity, V = base saturation, m = Al^{3+} saturation, OM = organic matter. B. = biofertilizer, ***pH = active acidity, N = available nitrogen, P = available phosphorus, K^+ = available potassium, B = available boron, S = available sulfur, ORG. C. = organic carbon, OM = organic matter.

Table 2. Plant height of cowpea *[V. unguiculata* (L.) Walp], cultivar Sempre Verde, and common bean (*P. vulgaris* L.), cultivar Carioca, in function of different fertilizations.

Treatments	Cultivars height (cm)		
	Sempre Verde	Carioca	Mean
Leaf biofertilizer	37.21	33.14	35.17^b
Organic compost	40.54	35.37	37.95^a
Mineral fertilizer	35.98	34.47	35.22^b
Without fertilizer	37.05	37.05	34.59^b
Mean	37.70^A	33.77^B	-
CV (%)			6.86

Means followed by the same letter, lowercase on the columns and uppercase on the row do not differ by Tukey test at 5% probability.

Table 3. Diameter stem plants of cowpea *[V. unguiculata* (L.) Walp], cultivar Sempre Verde, and common bean (*P. vulgaris* L.), cultivar Carioca, in function of different fertilizations.

Treatments	Cultivars stem diameter (mm)		
	Sempre verde	**Carioca**	**Mean**
Leaf biofertilizer	10.38^{bA}	9.14^{aA}	9.76
Organic compost	14.08^{aA}	9.10^{aB}	11.59
Mineral fertilizer	9.61^{bA}	8.37^{aA}	8.99
Without fertilizer	10.97^{bA}	7.07^{aB}	9.02
Mean	11.26	8.42	--
CV (%)			18.62

Means followed by the same letter, lowercase on the columns and uppercase on the row do not differ by Tukey test at 5% probability.

Table 4. Chlorophyll *a* index of cowpea [*V. unguiculata* (L.) Walp], cultivar Sempre Verde, and common bean (*P. vulgaris* L.), cultivar Carioca, in function of different fertilizations.

Treatments	Cultivars		
	Sempre Verde	**Carioca**	**Mean**
Leaf biofertilizer	37.21	33.14	35.17^{b}
Organic compost	40.54	35.37	37.95^{a}
Mineral fertilizer	35.98	34.47	35.22^{b}
Without fertilizer	37.05	37.05	34.59^{b}
Mean	37.70^{A}	33.77^{B}	-
CV (%)			6.86

Means followed by the same letter, lowercase on the columns and uppercase on the row do not differ by Tukey test at 5% probability.

improving the ability of the soil's cation exchange (Benbouali et al., 2013; Cavalcante et al., 2016), and provide an increase in the diversity of soil fauna (Sall et al., 2015).

Chlorophyll *a* index were higher in fertilization with organic compost. This input may have improved the soil fertility and the availability of nitrogen and magnesium, nutrients that are part of the chlorophyll molecule (Table 4). For Ndubuisi-Nnaji et al. (2011), organic fertilizers provide a greater diversity of nutrients to the soil and can provide a better nutritional balance of the culture. The Sempre Verde bean cultivar had higher chlorophyll *a* index compared to the Carioca cultivar, possibly a genotypic response. For sources of fertilization, the response may have happened because the amount of nitrogen supplied by the organic compost was higher and met the needs of this nutrient by plants, favoring the higher chlorophyll content. For Taiz and Zeiger (2013), plants with a high concentration of chlorophyll are potentially capable of achieving higher photosynthetic rates due to its light energy capture value per time unit.

Fertilization did not significantly affect the chlorophyll *b* index of the cultivar Carioca, a behavior different from what was observed for the cultivar Sempre Verde, where the goat compost performed better when compared to the other fertilizers (Table 5). Among cultivars, Sempre Verde captured the most quantity of quantum lights. According to Scalon et al. (2003), the activity of chlorophyll *b* is an important feature because this chlorophyll pigment captures energy from other wavelengths and transfers them to chlorophyll *a*, which effectively operates the photochemical reactions of plant photosynthesis.

As Table 6 shows, the total chlorophyll index accumulated in bean leaves followed the same tendency of chlorophylls *a* and *b*, in which fertilization with organic compost and the Sempre Verde bean cultivar had the best results (Table 6). The results can be explained by the greater availability of compost nutrients, by the benefits provided by the physical properties of soil, and by the possible increment of humic substances to the substrate, according to the results found by Cavalcante et al. (2013). On the other hand, Silva et al. (2015) found similar results for a lima bean crop when different organic substrates were incorporated into the substrate with the foliar application of cow urine.

There was a significant effect of type of fertilization regarding the variable number of pods per plant. It is observed that the organic compost provided the greatest number of pods per plant, probably due to the beneficial characteristics that the use of the compost provides to

Table 5. Chlorophyll *b* index of cowpea [*V. unguiculata* (L.) Walp], cultivar Sempre Verde, and common bean (*P. vulgaris* L.), cultivar Carioca, in function of different fertilizations.

Treatments	Cultivars		
	Sempre Verde	Carioca	Mean
Leaf biofertilizer	10.38^{bA}	9.14^{aA}	9.76
Organic compost	14.08^{aA}	9.10^{aB}	11.59
Mineral fertilizer	9.61^{bA}	8.37^{aA}	8.99
Without fertilizer	10.97^{bA}	7.07^{aB}	9.02
Mean	11.26	8.42	-
CV (%)			18.62

Means followed by the same letter, lowercase on the columns and uppercase on the row do not differ by Tukey test at 5% probability.

Table 6. Chlorophyll total index of cowpea [*V. unguiculata* (L.) Walp], cultivar Sempre Verde, and common bean (*P. vulgaris* L.), cultivar Carioca, in function of different fertilizations.

Treatments	Cultivars		
	Sempre verde	Carioca	Mean
Leaf biofertilizer	47.60	42.28	44.94^{b}
Organic compost	54.62	44.47	49.55^{a}
Mineral fertilizer	45.60	42.84	44.22^{b}
Without fertilizer	48.02	39.20	43.61^{b}
Mean	48.96^{A}	42.20^{B}	-
CV (%)			8.35

Means followed by the same letter, lowercase on the columns and uppercase on the row do not differ by Tukey test at 5% probability.

Table 7. Number of pods per cowpea plant [*V. unguiculata* (L.) Walp], cultivar Sempre Verde, and common bean (*P. vulgaris* L.), cultivar Carioca, in function of different fertilizations.

Treatments	Cultivars		
	Sempre verde	Carioca	Mean
Leaf biofertilizer	13.63^{aB}	28.73^{aA}	21.18
Organic compost	14.11^{aB}	28.72^{aA}	21.42
Mineral fertilizer	14.79^{aB}	21.76^{bA}	8.27
Without fertilizer	13.68^{aB}	22.46^{bA}	18.07
Mean	14.05	25.42	-
CV (%)			11.34

Means followed by the same letter, lowercase on the columns and uppercase on the row do not differ by Tukey test at 5% probability.

the soil (Table 7). Pereira et al. (2013), in a research adding to the soil 2.45 kg of goat manure per hole, observed 29.64 pods per plant in *Vigna* beans. Beltrão Jr. et al. (2012) observed that the addition of organic inputs to the soil provided a decrease in the number of cowpea pods, different from this research.

The Carioca cultivar had the highest increase in the number of pods per plant. Hawerroth et al. (2011) also observed differences in the number of pods per plant upon assessing six common bean cultivars with seed inoculation with Rhizobium, obtaining an amount of 29 pods per plant for the Carioca cultivar.

The Sempre Verde bean cultivar obtained the highest number of seeds per pods compared to the Carioca cultivar. This is a genetic trait (Table 8). There were no significant effects of fertilizations on this feature in the

Table 8. Number of seeds per pod of cowpea [*V. unguiculata* (L.) Walp], cultivar Sempre Verde, and common bean (*P. vulgaris* L.), cultivar Carioca, in function of different fertilizations.

Treatments	Cultivars		
	Sempreverde	**Carioca**	**Mean**
Leaf biofertilizer	18.15^{aA}	10.73^{aB}	14.44
Organic compost	18.40^{aA}	10.91^{aB}	14.65
Mineral fertilizer	18.38^{aA}	10.75^{aB}	14.57
Without fertilizer	15.72^{bA}	10.34^{aB}	13.03
Mean	17.66	10.68	-
CV (%)			5.84

Means followed by the same letter, lowercase on the columns and uppercase on the row do not differ by Tukey test at 5% probability.

Table 9. Length pods of cowpea [*V. unguiculata* (L.) Walp], cultivar Sempre Verde, and common bean (*P. vulgaris* L.), cultivar Carioca, in function of different fertilizations.

Treatments	Cultivars pods length (cm)		
	Sempre Verde	**Carioca**	**Mean**
Leaf biofertilizer	18.15^{aA}	10.73^{aB}	14.44
Organic compost	18.40^{aA}	10.91^{aB}	14.65
Mineral fertilizer	18.38^{aA}	10.75^{aB}	14.57
Without fertilizer	15.72^{bA}	10.34^{aB}	13.03
Mean	17.66	10.68	-
CV (%)			5.84

Means followed by the same letter, lowercase on the columns and uppercase on the row do not differ by Tukey test at 5% probability.

Carioca cultivar. For the Sempre Verde cultivar, the highest number of seeds per pod was observed in the treatments with goat compost and mineral fertilization.

Studying *Phaseolus vulgaris* L. beans, some authors also found no significant differences in the number of seeds per pod with the increase in doses of fertilizer, as is the case studied by Andrade et al. (2004) using mineral fertilization, Carvalho et al. (2011) using different doses of organic waste and mineral fertilizer, and Viana et al. (2011) using fertilization with nitrogen and phosphorus.

Table 9 shows that the Sempre Verde cultivar stood out in relation to the Carioca cultivar regarding pod length, probably a genetic trait, little influenced by other production factors. Smaller pods were observed in cultivar without fertilizer treatment. Results similar to those of this study were found by Araújo et al. (2001) with snap beans, in which there was no significant response for pod length with the use of increasing doses of swine manure and NPK. Using phosphorus fertilization, Zucareli et al. (2006) found no significant differences between the doses tested for pod length in common beans.

The lowest weight of 100 seeds was observed for the Sempre Verde bean cultivar. As for fertilization, treatments with foliar biofertilizers and goat compost were those that provided the highest weight of 100 seeds accumulations (Table 10). Silva et al. (2011) also did not observe an influence on weight of 100 grains using different mineral sources and the inoculation of cowpea bean seeds. Alves et al. (2009), in a study with cowpea, observed that there was no significant effect of the increase in biofertilizer doses on the treatments when compared to the without fertilizer treatment.

There was no significant difference between the productivity of bean cultivars (Table 11). However, the fertilized treatments had higher yields, especially the treatment with organic compost, which provided a productivity higher than that obtained by Moreira et al. (2013), who used nitrogen doses up to 120 kg ha^{-1}, and Galvão et al. (2013) upon evaluating cowpea productivity in different managements and residual potassium fertilization systems.

According to Galbiatti et al. (2011), biofertilizer fertilization provides a seeds yield similar to the mineral fertilizer, corroborating the present study. However, the addition of two liters of compost per hole provided an increase in grain yield possibly because it favored nutritional balance, improved the physical characteristics of the soil and increased the diversity of soil fauna, thus

Table 10. Weight of 100 seeds of cowpea [*V. unguiculata* (L.) Walp], cultivar Sempre Verde, and common bean (*P. vulgaris* L.), cultivar Carioca, in function of different fertilizations.

Treatments	Cultivars weight (g)		
	Sempre Verde	Carioca	Mean
Leaf biofertilizer	13.63^{aB}	28.73^{aA}	21.18
Organic compost	14.11^{aB}	28.72^{aA}	21.42
Mineral fertilizer	14.79^{aB}	21.76^{bA}	8.27
Without fertilizer	13.68^{aB}	22.46^{bA}	18.07
Mean	14.05	25.42	-
CV (%)			11.34

Means followed by the same letter, lowercase on the columns and uppercase on the row do not differ by Tukey test at 5% probability.

Table 11. Productivity of cowpea [*V. unguiculata* (L.) Walp], cultivar Sempre Verde, and common bean (*P. vulgaris* L.), cultivar Carioca, in function of different fertilizations.

Treatments	Cultivars productivity (kg ha^{-1})		
	Sempre Verde	Carioca	Mean
Leaf biofertilizer	2471.57	2240.00	2355.78 ^{ab}
Organic compost	2993.28	3126.71	3060.00 ^{a}
Mineral fertilizer	3304.71	2254.42	2779.57 ^{ab}
Without fertilizer	2136.28	2154.28	2145.28 ^{b}
Mean	2726.46 ^{A}	2443.85^{A}	-
CV (%)			18.82

Means followed by the same letter, lowercase on the columns and uppercase on the row do not differ by Tukey test at 5% probability.

improving the development of the culture (Sall et al., 2015).

Conclusion

The Sempre Verde bean cultivar have a higher growth and a higher accumulation of chlorophyll index *a, b* and total contents than the Carioca cultivar. Fertilization with organic compost provides a better development of these variables in relation to the other fertilizations. Fertilization with organic compost provides a greater productivity of bean cultivars. The organic compost may be indicated as a fertilization alternative for family farmers of the Paraíba state swamp region.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Seedling of development and tolerance of eggplant cultivars under saline stress

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This study aimed to evaluate the initial growth and tolerance of eggplant cultivars under saline water irrigation. The experiment was carried out in protected environment (greenhouse) at the Federal University of Campina Grande - UFCG, located in the municipality of Pombal-PB, Brazil. The experiment was set in a completely randomized design, in a 2 × 5 factorial scheme, corresponding to two eggplant cultivars (C_1 - 'Comprida Roxa' and C_2 - 'Preta Comprida/Enbu') and five levels of irrigation water salinity (0.6, 1.2, 1.8, 2.4 and 3.0 dS m^{-1}), with four replication and five plants per replication. Plants were grown for 30 days on trays with 30 cells, with capacity for 0.1 dm^3 of substrate, monitored in relation to emergence, growth and phytomass accumulation, and evaluated with respect to the salinity tolerance index. Emergence, growth and dry matter accumulation of eggplant cultivars were negatively affected by the increase in irrigation water salinity. The cultivar 'Comprida Roxa' showed higher tolerance to irrigation water salinity in comparison to 'Preta Comprida/Enbu'.

Key words: *Solanum melongena* L., irrigation, saline water, plant emergence.

INTRODUCTION

Eggplant (*Solanum melongena* L.) is an herbaceous plant from the Solanaceae family, with annual cycle, and its centers of origin are the tropical regions of the East. In Brazil, areas cultivated with eggplant have expanded and surpassed 1500 ha, due to its medicinal properties, such as the potential to reduce cholesterol levels, and for being an important source of minerals and vitamins (Gonçalves et al., 2006).

This crop is cultivated in all regions of the country, especially in the Northeast, where it plays a fundamental role in the generation of jobs and income in family farming. However, this region faces problems with the quantitative and qualitative scarcity of water resources and thus has demanded the use of alternatives for the irrigation of crops, such as the use of water with concentrations of dissolved salts. In spite of that, studies on eggplant are scarce under salinity conditions (Bosco et al., 2009; Lima et al., 2015) and, with respect to the

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Table 1. Chemical characteristics of the components of the substrates used in eggplant cultivation.

Substrate	EC	pH	P	K^+	Ca^{+2}	Mg^{+2}	Na^+	Al^{3+}	H^++Al^{3+}	CEC	OM
	dS m^{-1} (1 : 2.5)	H_20	mg dm^{-3}	$cmol_c dm^{-3}$							G kg^{-3}
A	0.09	8.07	3.00	0.32	6.40	3.20	0.18	0.00	0.00	10.49	16.0
B	1.65	5.75	86.00	1.67	11.60	28.50	17.84	0.00	11.88	71.49	570.0

EC = electrical conductivity; CEC = cation exchange capacity; OM = organic matter; A = Soil; B = commercial substrate.

Table 2. Chemical analysis of the freshwater used in the preparation of the solutions.

EC	pH	K^+	Ca^{+2}	Mg^{+2}	Na^+	SO_4^{-2}	CO_3^{-2}	HCO_3^-	Cl^-	RAS
(dS m^{-1})	$(mmol_c L^{-1})$									$(mmol\ L^{-1})^{0.5}$
0.3	7.0	0.3	0.2	0.6	1.4	0.2	0.0	0.8	1.3	2.21

EC = electrical conductivity; SAR = Sodium adsorption ratio.

tolerance of eggplant cultivars to saline stress, such studies are absent in literature.

In general, the limit of tolerance to saline stress depends on the concentration of the salt in solution, time of exposure and the developmental stage of the plants (Munns and Tester, 2008). The eggplant crop is classified as moderately sensitive to salinity and shows threshold salinity of 1.5 dS m^{-1} (Ünlünkara et al., 2010).

Nonetheless, the results given by many authors in the literature show divergence with respect to the limit of tolerance to salinity in the case of this crop: Bosco et al. (2009) reported significant reduction in growth and production of shoots and roots for threshold salinity of 4.08 dS m^{-1} with the cultivar 'Florida Market'; Lima et al. (2015) observed that the salinity above 0.5 dS.m^{-1} reduced plant growth and fruit production in eggplant. According to these authors, the crop is sensitive to salinity. On the other hand, Queiroz et al. (2013) has reported that in eggplant cultivation with the application of nutrient solutions with salinity levels ranging from 0.5 to 6.0 dS m^{-1}, did not show any significant effect of salinity on plant growth. Such divergence in the case of the reports corroborates that salinity tolerance varies depending on genetic factors of the cultivars, adopted cultural management and local edaphoclimatic conditions where the crop is grown (Moura and Carvalho, 2014; Oliveira et al., 2014; Lima et al., 2015) and evidences the importance of studying potential cultivars more tolerant to salinity in each region. Given the above, this study aimed to evaluate the initial growth and tolerance of eggplant cultivars under saline water irrigation.

MATERIALS AND METHODS

The experiment was carried out from August to September 2014 in a protected environment (green house), at the Center of Science and Agrifood Technology (CCTA) of the Federal University of Campina Grande (UFCG) located in the municipality of Pombal-PB, Brazil (6º47'20'' S; 37º48'01'' W; 194 m).

The experiment was set in a completely randomized design, in a 2 × 5 factorial scheme, which corresponded to two eggplant cultivars (C_1 - 'Comprida Roxa' and C_2 - 'Preta Comprida/Enbu') and five levels of irrigation water salinity (0.6, 1.2, 1.8, 2.4 and 3.0 dS m^{-1}), with four replicates and five plants per replicate.

Eggplant plants were cultivated on trays with 30 cells, with the capacity for 0.1 dm^3 of substrate, until 30 days after sowing (DAS). The substrate used for the production of seedlings was composed of soil (Entisol fluvisol) and commercial substrate, mixed at the proportion of 1:1, and its chemical characterization is presented in Table 1.

For sowing, five tray cells were used in each treatment, so that each cell received two seeds, in a total of 10 seeds per treatment. At the end of plant emergence, thinning was performed, leaving only the most vigorous plant per cell. The seeds of both cultivars were obtained at a commercial house, with 99% of purity and 95% of germination.

Irrigation was daily performed in order to maintain the soil close to its maximum holding capacity, based on the drainage lysimetry method, and the applied water depth was summed to a leaching fraction of 20%. The applied volume (V_a) per container was obtained by the difference between the previously applied volume (V_{prev}) and the drained volume (d), divided by the number of containers (n), as indicated in Equation 1.

$$V_a = \frac{V_{prev} - D}{n(1 - FL)} \quad (1)$$

The preparation of irrigation waters corresponding to the respective salinity levels was based on the relationship between EC_w and the concentration of salts (10 * meq L^{-1} = 1 dS m^{-1} of EC_w), according to Rhoades et al. (1992), valid for EC_w of 0.1 to 5.0 dS m^{-1}, which encompasses the tested levels. Freshwater from the local supply system (EC_w = 0.3 dS m^{-1}), whose chemical characteristics are shown in Table 2, was used in the preparation of the other irrigation waters, after mixing with NaCl, according to necessity. The desired level of electrical conductivity was measured using a portable microprocessor-based conductivity meter, with automatic temperature adjustment.

After preparation, the waters corresponding to each salinity level were stored in 30-L plastic containers, which were covered to avoid evaporation, entry of rainwater and contamination with materials that could compromise quality.

During the experiment, plants were monitored with respect to emergence through the daily count of emerged plantlets, that is, with the cotyledons above the soil level, generating a cumulative

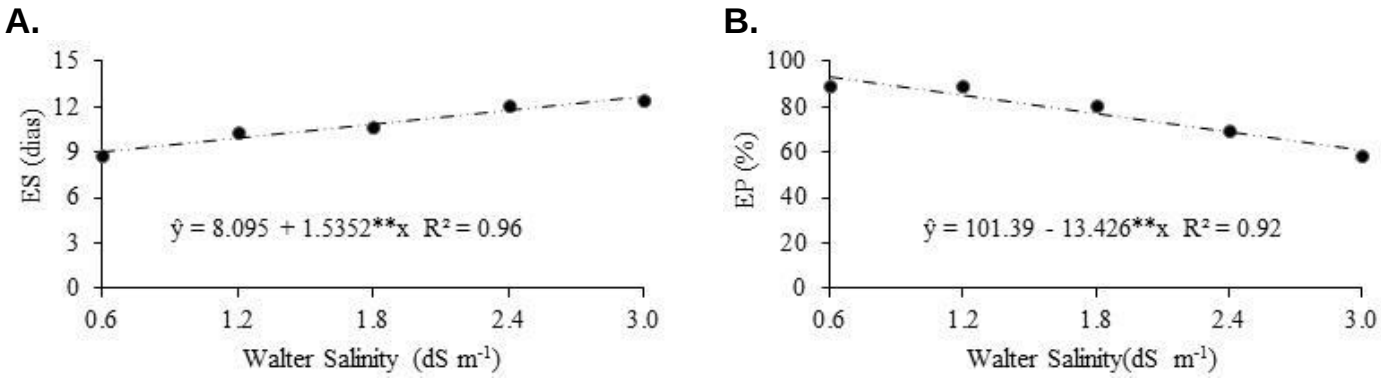

Figure 1. Emergence speed (ES) (A) and emergence percentage (EP) (B) of eggplant cultivars under different levels of

value. Thus, the number of emerged plantlets for each count was obtained by the subtraction of the value read. With the value read on the previous day and the number of emerged plants referring to each reading, emergence speed (ES) (days) was calculated according to Equation 2, described in Schuab et al. (2006).

$$ES = \frac{(N_1G_1)+(N_2G_2)+...+(N_nG_n)}{G_1+G_2+...+G_n} \quad (2)$$

Where, ES = emergence speed (days); G = number of emerged plantlets observed in each count; N = number of days from sowing to each count.

After stabilization of emergence, emergence percentage (EP) (%) was determined through the relationship between the number of emerged plants and the number of planted seeds.

Morphological evaluation of plantlet growth, at 30 DAS, was performed with the determination of plant height (PH) (cm), measured with a graduated ruler as the distance from the soil to the apex of the plant, stem diameter (SD), measured with a digital caliper, 1 cm high from the soil surface, and number of leaves (NL), through the count of mature leaves. After morphological analyses, plants were collected and separated into shoots and roots, which were dried in a forced-air oven at 65°C until constant mass, for the determination of shoot dry matter (SDM) (g) and root dry matter (RDM) (g) on an analytical scale. Total dry matter (TDM) (g) corresponded to the sum of SDM and RDM.

The data of total dry matter production were used to calculate the percentages partitioned between the vegetative organs and the salinity tolerance index (STI), comparing the saline treatments with the control (EC_w = 0.6 dS.m^{-1}) through Equation 3.

$$STI(\%) = \frac{\text{TDM production in the saline treatment}}{\text{TDM production in the control treatment}} x100 \quad (3)$$

The data were subjected to analysis of variance by F test and, when significant, regression analyses were applied for the factor levels of irrigation water salinity and Tukey test for the factor cultivars, both at 0.05 probability level, using the statistical program SISVAR® (Ferreira, 2011).

RESULTS AND DISCUSSION

Emergence speed (ES) data were best fitted to a linear model and increased as the levels of irrigation water salinity increased; at the highest level (3.0 dS m^{-1}), there was an increment of 42% in the ES of eggplant plants (Figure 1A). As to emergence percentage (EP), a linear reduction was observed as salinity increased, which was equal to 52.4% (58.3%) when plants were irrigated with EC_w of 3.0 dS m^{-1}, in comparison to the control (0.5 dS m^{-1}) (Figure 1B).

Considering that the germination process depends on the absorption of water and energy, through heat, the reduction in the osmotic potential due to the increase in NaCl contents in the soil decreases soil water potential, reducing the energy of the water in the soil and causing the plant to perform osmotic adjustment (Sá et al., 2013). In addition, the increase in the concentration of NaCl ions causes toxicity to plants and may cause damages to the radicle, thus limiting the seed imbibition process and the absorption of water by the plantlet (Munns and Tester, 2008; Voigt et al., 2009; Taiz and Zaiger, 2013). Similar results were observed in other vegetables such as melon (Secco et al., 2010), broccoli (Lopes et al., 2014), beet (Oliveira et al., 2015a) and cabbage (Oliveira et al., 2015b).

For the variables plant height (PH), stem diameter (SD) and number of leaves (NL), there were progressive reductions in the data, which were best fitted to a linear model, with decreases of 76% (1.87 cm) in PH (Figure 1A), 14.1% (1.06 mm) in SD (Figure 2C) and 69.1% (2.17) in NL (Figure 1D) for plants under EC_w of 3.0 dS.m^{-1}, in comparison to the control (0.6 dS m^{-1}). The inhibition of growth caused by salinity is due to the osmotic effect, because it promotes physiological drought. Likewise, there may be a toxic effect, resulting from the concentration of ions in the protoplasm. Hence, the reduction in the water potential of the tissues caused by the excess of salts in the soil solution leads to restrictions in elongation and cell division rates, thus reducing plant growth (Munns and Tester, 2008; Queiroz et al., 2013; Taiz and Zaiger, 2013; Sá et al., 2013; Oliveira et al., 2015a).

The factor cultivars influenced the variables shoot dry matter (SDM), root dry matter (RDM) and total dry matter

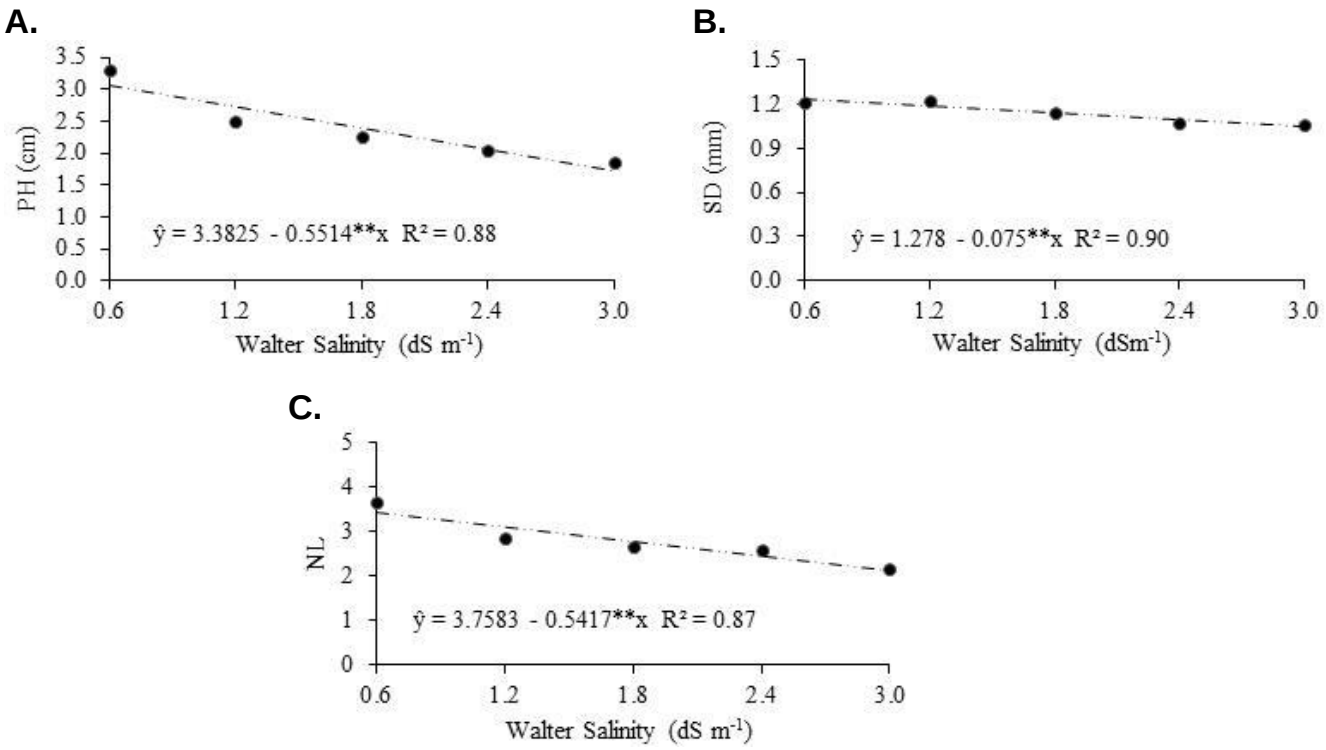

Figure 2. Plant height (PH) (A), stem diameter (SD) (B) and number of leaves (NL) (C) of eggplant cultivars under different levels of irrigation water salinity. ** = Significant at 0.01 probability level.

(TDM). For these variables, in the comparison between cultivars, it was observed that the cultivar 'Preta Comprida/Enbu' stood out with accumulations of 0.0086, 0.0028 and 0.0114 g for SDM, RDM and RDM, respectively (Figure 3B, D and F). Additionally, for the factor Salinity, according to the regression equations, the linear model indicated decreases in dry matter production with the increment in irrigation water EC, which were equal to 29.7% (0.0074 g) in SDM (Figure 3A), 129.4% (0.0017 g) in RDM (Figure 3C) and 48.3% (0.0091 g) in TDM (Figure 3E), in plants under EC_w of 3.0 dS m^{-1}, in relation to the control.

Considering that saline water irrigation increases the index of salinization of the soils and the abundant presence of toxic ions in these soils, due to the accumulation of salts, especially Na^+ salts, there might occur nutritional imbalance, modification in the osmotic potential of the plant and physiological alterations that interfere with the accumulation of photoassimilates and, consequently, with the accumulation of dry matter (Munns and Tester, 2008; Esteves and Suzuki, 2008; Garcia et al., 2012; Sá et al., 2013; Silva et al., 2013; Lima et al., 2015). Similar results have been reported in the literature. Lima et al. (2015), studying the tolerance of the eggplant hybrid 'Ciça' to irrigation water salinity, observed that the crop was sensitive to salinity. These authors reached such a conclusion after observing that crop development was already negatively affected at salinity levels above 0.6 dS m^{-1}. On the other hand, in a study with the same hybrid, Silva et al. (2013) observed significant reduction in dry matter production of eggplant only at salinity levels above 3.3 dS m^{-1}.

As to the root/shoot ratio (R/S), according to the regression equation, the data were best fitted to a decreasing linear model, and the highest level of irrigation water salinity (3.0 dS m^{-1}) led to a reduction of 78.3% (0.23) in R/S, compared with the control (Figure 4). For Sá et al. (2013), this response is related to the greater reduction in root growth, compared with the shoots, aiming to reduce the absorption of salts from the environment, especially in environments with higher salinity levels. This fact was confirmed in the present study, considering the drastic reductions observed in RDM accumulation (Figure 3B). Similar results were observed by Oliveira et al. (2015b), evaluating phytomass accumulation of cabbage plants under saline stress.

Regarding the salinity tolerance index (STI), there were reductions in the tolerance of the cultivars as irrigation water salinity increased, reaching approximately 48.1% in plants irrigated with EC_w of 3.0 dS m^{-1}, in relation to the control (Figure 5A). For the factor Cultivars, it can be noticed that the cultivar 'Comprida Roxa' obtained the highest indices of tolerance, equal to 83.99 and 6.87% higher than that of 'Preta Comprida/Enbu' (Figure 5B). Although higher phytomass accumulations were observed in the cultivar 'Preta Comprida/Enbu', these plants

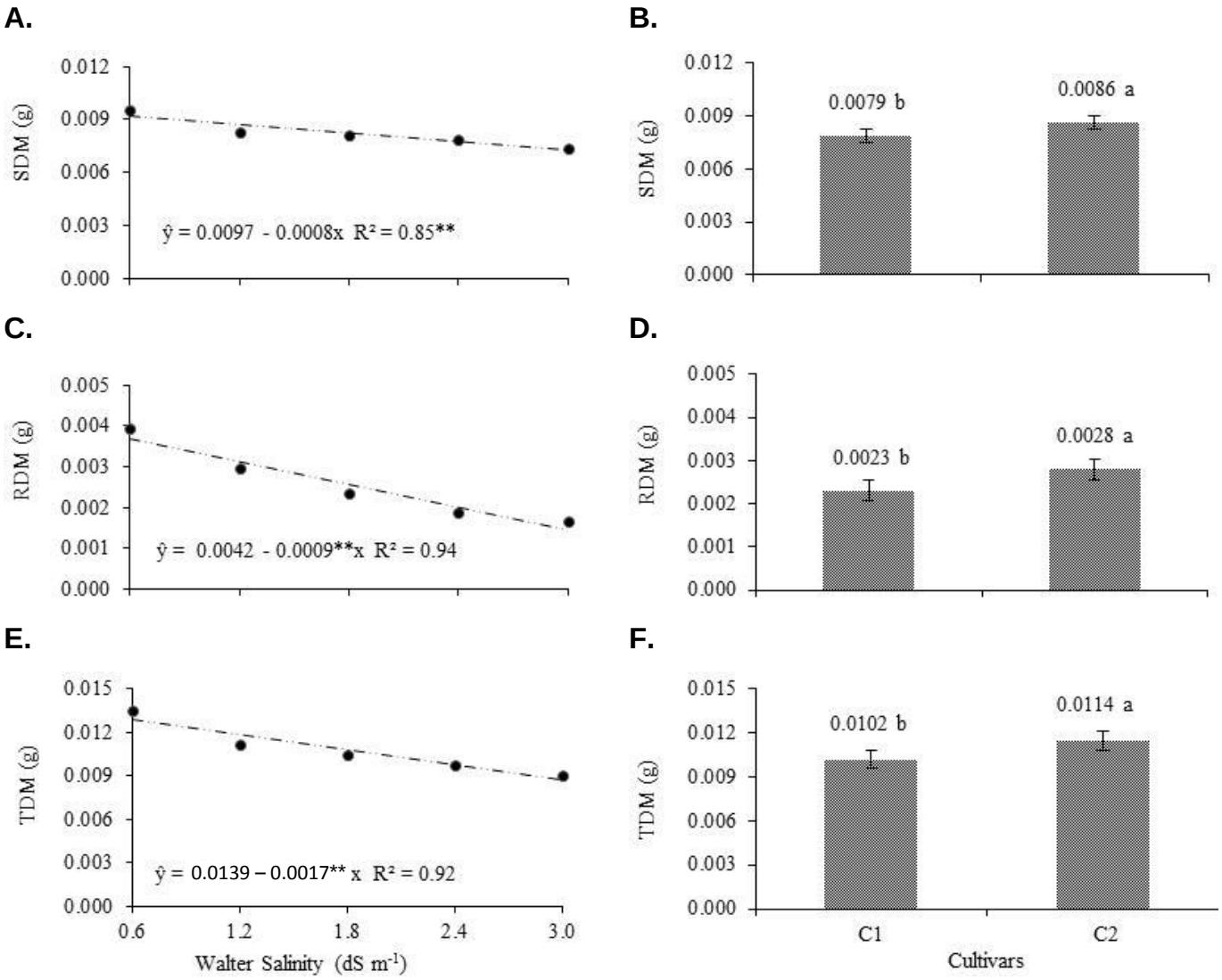

Figure 3. Shoot dry matter (SDM) (A and B), root dry matter (RDM) (C and D) and total dry matter (TDM) (E and F) of eggplant cultivars (C_1 - 'Comprida Roxa' and C_2 - 'Preta Comprida/Enbu') under different levels of irrigation water salinity. ** = Significant at 0.01 probability level; Equal letters do not differ by Tukey test at 0.05 probability level.

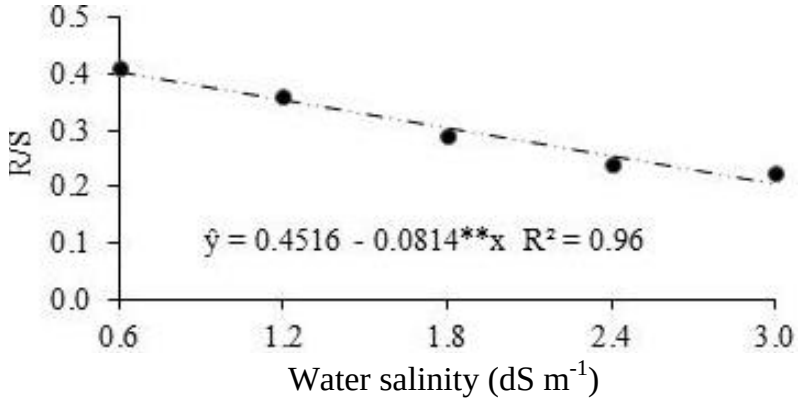

Figure 4. Root/shoot ratio (R/S) of eggplant cultivars under different levels of irrigation water salinity. ** = Significant at 0.01 probability level.

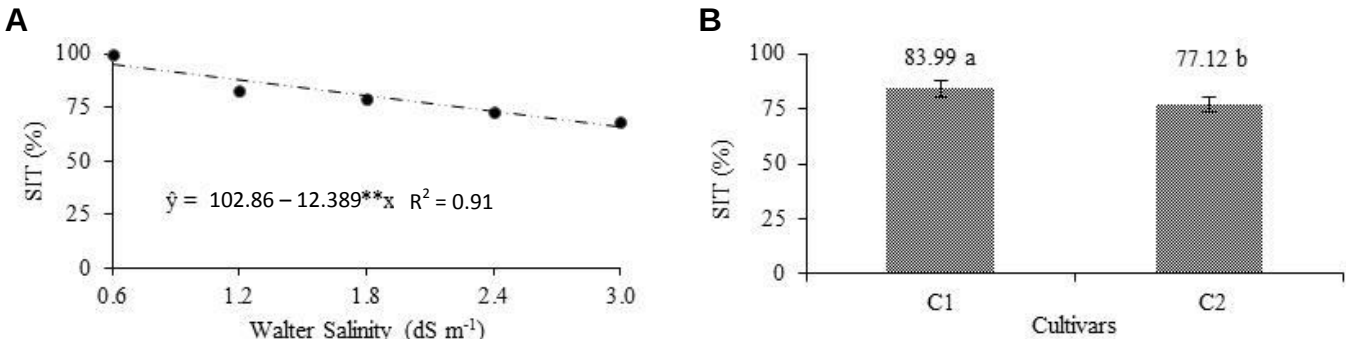

Figure 5. Salinity tolerance index (STI) of eggplant cultivars (C_1 - 'Comprida Roxa' and C_2 - 'Preta Comprida/Enbu') under different levels of irrigation water salinity. ** = Significant at 0.01 probability level; Equal letters do not differ by Tukey test at 0.05 probability level.

greater losses in phytomass accumulation as water salinity progressively increased. These reductions were higher than those observed in the cultivar 'Comprida Roxa', which presents itself as more tolerant to salinity.

Conclusions

Emergence, growth and dry matter accumulation of the eggplant cultivars were negatively affected by the increase in irrigation water salinity. The cultivar 'Comprida Roxa' shows higher tolerance to irrigation water salinity in comparison to 'Preta Comprida/Enbu'.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Phenotypic profiles of different accessions of sweet potato (*Ipomoea batatas* L. Lam) in the coastal savanna agro-ecological zone of Ghana

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Twenty accessions of sweet potato (*Ipomoea batatas* L. Lam) cultivated under rain-fed conditions were evaluated based on their agromorphological traits to assess diversity in yield, morphology and other key agronomic characteristics of the accessions under study. The accessions consisted of 13 local and 7 exotic breeding lines grown in the research farm of the Biotechnology and Nuclear Agriculture Research Institute during the rainy and dry seasons of 2011. The Randomised Complete Block Design (RCBD) was used with four replicates. Results indicate high genetic variability among the 20 accessions based on the agromorphological and yield characteristics. The exotic accession (US 020) recorded the highest total root yield and harvest index of 56.32 t/ha and 57.11%, respectively, indicating its superiority over the local accessions. Two accessions (ER 001 and HMA 2) were found to be possible duplicates. This study provides valuable information that can be utilised in a breeding programme to ameliorate local clones of sweet potato in Ghana.

Key words: Sweet potato, accessions, agromorphological characteristics, harvest index, total root yield, percent dry matter, Ghana.

INTRODUCTION

Sweet potato (*Ipomoea batatas* L. Lam), is a hexaploid (2n = 6x = 90), and usually considered the only species of economic significance within the genus *Ipomoea* (Sossah et al., 2014; Zhang et al., 2000). Sweet potato is

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generally cultivated for its tuberous roots and leaves, useful for human consumption, animal feed and for industrial purposes (Lebot, 2009). Sweet potato is the world's seventh most important food crop after wheat, rice, maize, potato, barley and cassava, and second most important tuber crop after cassava with a yearly production of 106 million tonnes (FAOSTATs, 2010; Loebenstein, 2009; Hijmans et al., 2001). It is widely adapted in the tropics, sub-tropical and warm temperate regions and is easily propagated in both high and low input agricultural systems (Kapinga et al., 1995). In Ghana, sweet potato cultivation and consumption are very prominent (Otoo et al., 1995) and rapidly becoming more important attributable to its high yielding ability, high energy and nutrient content, especially vitamin A in orange-fleshed and its capacity to grow on marginal soils (Sossah et al., 2014).

With annual production of 0.13 million tonnes in 2010, Ghana ranks the fourth largest producer of sweet potato in West Africa (Food and Agricultural Organisation (FAO), 2010) with an extensive production at almost all agro-ecological zones in the country, yielding 1.75 t/ha on average per annum. However, low yields are realised by Ghanaian farmers with poor quality of produce occasioned by the paucity of high-yielding varieties, pests and diseases infestation (especially viruses), fluctuating agro-climatic conditions and poor agronomic practices (Sossah et al., 2014; Ndunguru et al., 2009; Otoo et al., 2001). Previous improvement programmes in the country has been limited to evaluation of local and exotic varieties at different agro-ecological regions, which led to the release of 8 varieties (6 white and 2 orange-fleshed) to farmers with enhanced attributes for food quality and marketability (Akoroda, 2009; Otoo et al., 1995, 1998). These locally ameliorated genotypes offers higher yields, but these qualities have declined over the years particularly in the face of change in climate in the local agroecology. Moreover, considerable variation of local names has characterised naming of both local and released genotypes (Sossah et al., 2014).

Agromorphological characterisation is an important first step in the assessment of genetic diversity in crops including sweet potato (Amoatey et al., 2015; Ahiakpa et al., 2013). Major variation has been reported in the vine, leaf, flower and storage root characteristics (Tairo et al., 2008; Tsegaye et al., 2007). Several other researchers have used morphological and agronomic characters to distinguish between and among sweet potato accessions, assess comparative reaction and susceptibility to pests, diseases and other stress factors resulting from change in climate and appraise genetic variability (Elameen et al., 2011; Yada et al., 2010; Tairo et al., 2008; Tsegaye et al., 2007; Veasey et al., 2007). Morphological characters are easy to study, relatively cheap to evaluate and can be visually detected. Agromorphological characterisation is not only useful in describing each accession but potentially useful for clonal identification and estimation of genetic distance (Ahiakpa et al., 2013; Elameen et al., 2011); therefore, the need to characterise existing local and introduced accessions of sweet potato, identify duplicate accessions and evaluate their phenotypic diversity for effective utilisation in breeding programmes.

MATERIALS AND METHODS

Study site

The study was conducted at the research farm of Nuclear Agriculture Research Centre, Biotechnology and Nuclear Agriculture Research Institute (NARC-BNARI) of the Ghana Atomic Energy Commission (GAEC), during the minor and major seasons of 2011. The study site is located at 05°40′ N, 0° 13′ W, 76 m above sea level within the Coastal Savannah agro-ecological zone of Ghana. The soil at the site is the Nyigbenya-Haatso series, which is a typically well-drained savannah Ochrosol (Ferric Acrisol) derived from quartzite Schist (FAO/UNESCO, 1994). The maximum and minimum average temperatures for the period of the study were 30.7 and 23.2°C, respectively, with mean annual rainfall and relative humidity of 220 mm and 40.54%, respectively (Local Weather Station, 2012).

Germplasm assembly

A total of twenty (20) accessions of sweet potato were collected for the study comprising 13 local and 7 new introductions from Cuba, South Africa, United Kingdom, and United States of America (Table 1).

Experimental design and layout

A total land area of 70 m x 39 m was ploughed, harrowed to turn the soil, break the soil clods, and provide a fine tilth. Ridges were made with a ridge size of 0.7 m and 0.8 m distance between ridges. A plot consisted of one row (ridge) of 8 m. The Randomised Complete Block Design (RCBD) was used with four replicates consisting of 20 plants. The planting distances were 0.4 m within rows and 1 m between centres of the ridges. Each replicate was separated by a 2 m path from the other. Cultivation of the plants were done manually. Weed control was done manually by hoe. No fertilisers or pesticides were applied. The study was done under rain fed conditions.

Data collection

Morphological characters of all the 20 accessions were scored using CIP-standard descriptors of sweet potato (Hijmans, 1991). A total of 37 characters (25 aerial and 12 storage root characters) were evaluated for each accession (Table 2) and scored using a scale of 0-9 at 90-120 days after planting.

Data recorded for aerial parts were average expressions of characters of at least 3 leaves, 3 internodes located in the middle portion of the main stem for 3 plants. Storage root descriptors were recorded considering the most representative expression of the character shown in medium-to large sized storage roots of five plants. Agronomic traits recorded include Root Form (RF), degree of Damage of Storage Roots (DaMR), Weevil Damage at First Evaluation (WED1), Percent Dry Matter (%DM), Number of Non-Marketable (small) Roots (NSR), Number of Marketable (large) Roots (NLR), Weight of Non-Marketable (small) Roots (WSR), and

Table 1. Name and collection sites of Sweet potato genotypes used in the study.

Accessions	Source	Type
CR001	Ghana (Central Region)	Local
CR002		
ER 001	Ghana (Eastern Region)	
FREEMA	Ghana (Greater Accra Region)	
HMA1		
HMA2	Ghana (Volta Region)	
HMA3	Ghana (Greater Accra Region)	
LOCAL 1		
LOCAL 2		
UE 007	Ghana (Upper East Region)	
CRI001	Ghana (Ashanti Region)	
CRI027		
CRI 054		
DOAK 08-007	Cuba	Introductions/exotic
CEMSA 74-228		
SA/BNARI	South Africa	
UK/BNARI	UK	
US 004	USA	
US 020		
US 029		

Table 2. Agromorphological characters used for evaluating the 20 accessions of sweet potato.

Plant organ	Characters scored
Vine	Plant type (PTY), vine internode length (VIL), vine internode diameter (VID), predominant colour of vine (PVC), and secondary colour of vine (SVC).
Leaf	General outline of leaf (GOL), leaf lobe type (LLT), leaf lobe number (LLN), shape of central leaf lobe (SCLL), mature leaf size (MLS), abaxial leaf vein pigmentation (ALVP), mature leaf colour (MLC), immature leaf colour (ILC), petiole pigmentation (PP), and petiole length (PL).
Flower	Flower (FLR), flower colour (FCL), flower length (FL), flower width (FW), shape of limb (SLB), sepal shape (SS), sepal apex (SA), sepal colour (SLL), colour of stigma (CST), colour of style (CSL), stigma exertion (SE).
Storage root	Storage root arrangement (SRA), storage root shape (SRS), storage root defects (SRD), predominant skin colour (PSC), intensity of predominant skin colour (IPC), secondary skin colour (SSC), predominant root flesh colour (PFC), secondary flesh colour (SFC), distribution of secondary flesh colour (DSF).
Agronomic traits	Root form (RF), Damage of storage roots (DaMR), Weevil damage at first evaluation(WED1), percent dry matter (%DM), number of small roots per plant (NSR/P), number of large roots per plant (NLR/P), weight of small roots (WSR), weight of large roots (WLR).

Source: Huamán (1991).

Weight of Marketable (large) Roots (WLR). A number of variables, which are useful in evaluating the performance of clones, were calculated from the raw data of the agronomic traits. These include Percent of Plants without Storage Roots (%PWSR), Large Root Yield (LRY) (t/ha), Small Root Yield (SRY) (t/ha), Total Root Yield (TRY) (t/ha), Foliage Yield (FY) (t/ha), Root Dry Matter Yield (RDMY) (t/ha), Fresh Biomass Yield (t/ha), Number of Large Roots Per Plant (NLR/P), Number of Small Roots Per Plant (NSR/P) and Harvest Index (HI).

Determination of storage root dry matter (DM) content was done according to the method described by Carey and Reynoso (1999) using an oven and a balance with an accuracy of 0.1 g. To avoid post-harvest changes in DM content prior to DM determination, initial steps were done within 24 h after harvest. Medial sections of 3 undamaged market-size roots were chopped into small flakes and mixed thoroughly out of which a 150 g sample was taken for the next step. The samples of 150 g fresh weight were placed in paper bags and dried at 60°C for 72 h to a stable weight. The dried samples were weighed and the resulting value used for estimating dry matter content as

$$\%DM = \frac{Dry\ weight}{Fresh\ weight} \times 100\ \%$$

Table 3. Variability in agromorphological characters and percentage of accessions in each class.

Character	Score	% Accessions	Character	Score	% Accessions	Character	Score	% Accessions	Character	Score	% Accessions
	3	5		1	10		0	10		1	55
	5	25		3	40		1	5		3	45
	7	45		5	35		2	10			
	9	25		7	15		3	5			
PTY			LLN			SRS	4	15	VIL		
							5	30			
							7	5			
							8	10			
							9	10			
	1	20		0	25		1	40		1	5
	2	20		1	5		3	25		2	15
	3	15		3	25		4	10		3	20
SCLL	4	30	SRD	4	10	PVC	6	20	ILC	6	35
	5	15		5	25		8	5		7	5
				6	5					8	5
				7	5					9	15
	0	25		1	15		2	25		0	10
	1	10		3	35		3	5		2	35
SVC	2	25	PP	4	30	ALVP	5	5	PSC	5	5
	5	20		8	10		6	5		6	20
	6	20		9	10		7	15		8	30
							8	45			
	3	45		0	10		1	40		0	10
	4	15		1	10		3	35		1	5
	5	5		2	20		5	10		3	45
GOL	6	35	PFC	4	35	LLT	7	15	SRA	5	40
				6	5						
				7	10						
				8	10						

*Values in the score column represent the scores for each character evaluated. PTY = plant type; LLN = leaf lobe number; SRS = storage root shape; VIL = vine internode length; SCLL = shape of central leaf lobe; SRD = storage root defects; PVC = predominant colour of vine; ILC = Immature leaf colour; SVC = secondary colour of vine; PP = petiole pigmentation; ALVP = abaxial leaf vein pigmentation; PSC = predominant skin colour; GOL = general outline of leaf; PFC = predominant root flesh colour; LLT = leaf lobe type; SRA = storage root arrangement.

Statistical data analyses

Correlation analysis was performed to delineate the degree of association among the accessions. Furthermore, the principal components analysis (PCA) was done to assess the percentage contribution of each trait to total genetic variation among the accessions. Cluster analysis based on similarity matrices (CLA) was also employed to assess the relatedness among the accessions. All the data collected were analysed for variation in each character scored. The General statistical package (Genstat, ver. 9.2), Statgraphics Plus (XV.I) were used for the Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) for mean separation. Microsoft Excel was used for collation of all data gathered.

RESULTS AND DISCUSSION

Variation in quantitative traits among the 20 sweet potato accessions

Table 3 shows 16 qualitative traits exhibiting most variation in the collection. The accessions exhibited significant variation with respect to 15 characters. Vine internode length (VIL) exhibited the least variability with 55% of the accessions being very short and 45% short. Five characters, plant type (PTY), general leaf outline (GOL), type of leaf lobe (LLT), number of leaf lobes (LLN)

and storage root arrangement (SRA) exhibited four classes of variability in all the 20 accessions studied. The rest of the characters had classes ranging from five to nine. Storage root shape (SRS) displayed the highest number of variation consisting of nine classes in all the accessions. The numbers in the score column represent the scores for each character (Table 3) and the percentages refer to percentages of accessions per score. Thus, high level of genetic diversity was exhibited in the sweet potato accessions. Some morphological characters were highly variable among the accessions studied. The high variability in morphological traits by the 20 sweet potato accessions are consistent with reports by Elameen et al. (2011), Yada et al. (2010), Vimilar and Hariprakash (2010), Tsegaye et al. (2007), Veasey et al. (2007) and Islam et al. (2002) who recorded significant variations in VIL, PTY, GOL, LLT, LLN and SRA in sweet potato.

General leaf outline were cordate (45%), triangular (15%), hastate (5%) and lobed (35%) confirming reports by Yada et al. (2010), Tsegaye et al. (2007) and Veasey et al. (2007). According to Yada et al. (2010), the lobed leaves could perhaps be an adaptation for decreasing insect pest damage. Only 45% of the accessions flowered, with variation in stigma exertion ranging from inserted (stigma shorter than the longest anther), same height as highest anther, slightly exerted, to exerted (stigma longer that the longest anther). Similarly, Veasey et al. (2007) reported that, sweet potato cultivars vary in their ability to flower, and some cultivars may not flower or produce very few flowers, whereas others flower profusely under normal field conditions. The variation in stigma exertion can be ascribed to the occurrence of heterostyly in sweet potato, which probably reinforces the self-incompatibility system within the crop, useful as morphological marker in inheritance studies (Vimilar and Hariprakash, 2010).

The predominant skin colour were cream (35%), brownish orange (5%), pink (20%), and purple red (30%). 10% of the accessions failed to produce storage roots. Similarly, flesh colour also ranged from predominantly white to dark orange with 10% of the accessions unable to produce storage roots. The colour of root skin and flesh colour is determined by pigments such as carotenoids and anthocyanin, the combination of which produces different skin and flesh colour depending on the cultivar (Vimilar and Hariprakash, 2010; Gasura et al., 2008). These traits could be controlled by several genes with epistatic interactions and complementary gene actions as reported by Gasura et al. (2008). On the other hand, 8 classes of storage root shape was detected. 30% of the accessions were obovate, 5% were round, 10% were round elliptic, 5% were elliptic, 15% were ovate, 5% were long oblong, 10% were long elliptic and 10% long irregular or curved; as 10% of the accessions had no storage roots. The presence of numerous intermediate in storage root shape clearly reveals incomplete dominance as well as occurrence of multiple alleles for this trait. This may have accounted for this observation which is consistent with report by Vimilar and Hariprakash (2010).

Variability in quantitative traits among the 20 accessions of sweet potato

Table 4 shows the performance of 20 sweet potato accessions evaluated based on 11 quantitative traits. The accessions revealed significant variation with respect to the 11 traits evaluated. The percent of plants without storage root (%PWSR) was highest in CR 002 (a local accession) followed by the introduced accession CRI 027 with significant difference (Table 4). However, differences in percent of plants without storage roots (%PWSR) for all the other accessions were not statistically significant. The highest large root yield was observed in US020 (an introduced accession) (46.88 t/ha), followed by FREEMA (local accession) (27.90 t/ha) and UK/BNARI (introduced accession) (23.48 t/ha) while the lowest were CR002 (0), CRI027 (1.97 t/ha) and DOAK.08-007 (3.48 t/ha). However, the highest and lowest small root yields were recorded in Local 2 (25 t/ha) (local accessions) and CR002 (0.0t/ha), respectively.

US 020 produced the highest total tuber yield which was significantly different from those of FREEMA, Local 2 and UK/BNARI. Conversely, FREEMA yielded significantly higher total root yield than UK/BNARI while Local 2 was not statistically significant compared to the rest of the accessions. Two accessions (CR 002 and CRI 027) which gave the lowest total root yield also recorded the highest foliage yield of 157.13 t/ha and 84.19 t/ha, respectively. The foliage yield of FREEMA, Local 1, UE 007, DOAK.08-007, UK/BNARI, US 020 and US 029 were high but not significantly different from one another. The fresh biomass for all the accessions ranged from 29.66 t/ha-57.13 t/ha, with no significant differences among HMA 2, HMA 3, UE 007, US 029, SA/BNAR and US 004. FREEMA produced the highest number of large roots per plant (1.44) followed by Local 2 and UE 007 at 1.38 and 1.19, respectively. There were no significant differences in the number of large roots among most of the accessions. Also, CR 001 had the highest mean score for number of small roots per plant (4.31) followed by HMA 2 (3.44) and UK/BNARI (3.0). There was no significant difference in the number of small roots among most of the accessions under study.

ER 001 recorded the highest dry matter (36.65%) with corresponding increase in root dry matter yield. In contrast, UK/BNARI had the least dry matter content (14.79%) with parallel decrease in root dry matter yield. US 020, UK/BNARI, US 004, FREEMA, SA/BNARI and Local 2 recorded high harvest indices (57.11, 49.47, 46.51, 45.07, 44.37 and 40.29%, respectively). However, there was no significant difference between harvest indices for FREEMA and any of SA/BNARI, Local 2, UE

Table 4. Variability in quantitative traits among 20 accessions of *Ipomoea batatas* L.

Accessions	%PWSR	LRY (t/ha)	SRY(t/ha)	TRY (t/ha)	FY (t/ha)	FB (t/ha)	NLR/P	NSR/P	%DM	RDMY (t/ha)	HI (%)
CR001	6.25^{c}	12.06^{bcde}	12.01^{abc}	24.06^{bcdef}	67.93^{bcd}	91.99^{bc}	0.56^{bcde}	**4.31^{a}**	32.34^{bcde}	7.65^{bcde}	24.08^{cdefg}
CR002	**100.00^{a}**	0.00^{e}	0.00^{c}	0.00^{f}	**157.13^{a}**	**157.13^{a}**	0.00^{e}	0.00^{e}	0.00^{i}	0.00^{e}	0.00^{h}
ER001	12.50^{c}	2.90^{de}	11.92^{abc}	14.82^{ef}	60.43^{bcde}	75.25^{cdef}	0.19^{de}	2.75^{acbd}	**36.65^{a}**	5.46^{de}	18.68^{efgh}
FREEMA	6.25^{c}	27.90^{b}	16.99^{ab}	44.89^{ab}	41.29^{defgh}	86.18^{bcd}	1.44^{a}	1.81^{bcde}	31.99^{bcde}	14.17^{abc}	45.07^{abcd}
HMA 1	6.25^{c}	14.73^{bcde}	13.10^{abc}	27.83^{bcde}	54.24^{bcdef}	82.07^{bcde}	0.63^{abcde}	3.44^{ab}	32.18^{bcde}	9.318^{bcd}	32.92^{bcde}
HMA 2	0.00^{c}	8.93^{cde}	5.16^{bc}	14.09^{ef}	48.66^{cdefg}	62.75^{cdefg}	0.88^{abcd}	1.81^{bcd}	30.70^{cdef}	4.28^{de}	26.56^{bcdef}
HMA 3	6.25^{c}	8.93^{cde}	10.62^{abc}	19.55^{cdef}	54.24^{bcdef}	73.79^{cdefg}	0.94^{abcd}	1.74^{bcde}	34.89^{ab}	6.81^{cde}	23.42^{cdefgh}
LOCAL 1	12.50^{c}	7.37^{cde}	3.14^{bc}	10.51^{ef}	34.49^{defgh}	44.99^{defg}	0.38^{cde}	1.44^{cde}	30.34^{def}	4.62^{de}	19.51^{efgh}
LOCAL 2	18.75^{c}	17.99^{bcde}	**25.00^{a}**	42.98^{abc}	80.73^{bc}	123.72^{ab}	**1.38^{ab}**	1.46^{cde}	34.61^{abc}	15.15^{ab}	40.29^{abcde}
UE007	0.00^{c}	19.74^{bcd}	4.36^{bc}	24.00^{bcdef}	35.76^{defgh}	59.76^{cdefg}	1.19^{abc}	2.38^{abcd}	27.37^{fg}	6.73^{cde}	36.67^{abcde}
CRI001	6.25^{c}	8.57^{cde}	6.80^{bc}	15.38^{def}	20.57^{fgh}	35.94^{fg}	0.50^{cde}	2.13^{bcd}	31.09^{bcdef}	4.74^{de}	39.31^{abcde}
CRI027	75.00^{b}	0.00^{e}	1.97^{bc}	1.97^{f}	84.19^{b}	86.16^{bcd}	0.00^{e}	0.81^{de}	0.00^{i}	0.00^{e}	2.42^{gh}
CRI054	0.00^{c}	2.23^{de}	5.25^{bc}	7.49^{ef}	24.11^{fgh}	31.60^{fg}	0.44^{cde}	2.13^{bcd}	26.03^{g}	1.98^{de}	25.40^{cdefg}
DOAK 08-007	18.75^{c}	0.00^{e}	3.48^{bc}	3.48^{ef}	40.18^{defgh}	43.66^{defg}	0.00^{e}	1.68^{bcde}	32.35^{bcde}	1.02^{e}	7.23^{fgh}
CEMSA 74-228	18.75^{c}	7.37^{cde}	2.65^{bc}	10.00^{ef}	27.99^{efgh}	38.00^{efg}	0.50^{cde}	1.56^{bcde}	33.32^{abcd}	3.42^{de}	26.85^{bcdef}
SA/BNARI	0.00^{c}	9.38^{cde}	6.02^{bc}	15.40^{def}	14.27^{h}	29.66^{g}	0.81^{abcde}	1.81^{bcde}	24.44^{g}	3.81^{de}	44.37^{abcd}
UK/BNARI	0.00^{c}	23.48^{bc}	16.65^{ab}	40.13^{abcd}	41.03^{defgh}	81.16^{bcde}	0.75^{abcde}	3.00^{abc}	14.79^{h}	5.95^{de}	49.47^{ab}
US004	18.75^{c}	10.18^{bcde}	4.56^{bc}	14.73^{ef}	15.40^{gh}	30.13^{g}	0.94^{abcd}	0.88^{de}	34.32^{abcd}	5.26^{de}	46.51^{abc}
US020	0.00^{c}	**46.88^{a}**	9.44^{bc}	**56.32^{a}**	36.16^{defgh}	92.48^{bc}	0.94^{abcd}	1.19^{cde}	33.07^{abcd}	**18.29^{a}**	**57.11^{a}**
US029	0.00^{c}	8.26^{cde}	4.65^{bc}	12.91^{ef}	35.49^{defgh}	48.40^{cdefg}	0.44^{cde}	2.31^{bcd}	28.43^{efg}	3.56^{de}	22.08^{defgh}
Mean	15.30	11.80	8.20	20.00	48.70	68.70	0.644	1.93	27.45	6.11	29.40
P value	***<.001***	***<.001***	***0.048***	***<.001***	***<.001***	***<.001***	***0.004***	***0.015***	***<.001***	***<.001***	***<.001***
STD	26.01	11.27	6.49	15.38	32.13	33.32	0.43	0.96	10.53	4.88	16.85
CV (%)	117.50	40.20	75.20	57.30	15.50	21.60	35.60	30.30	3.20	59.70	38.60

Means in the same column followed by the same letter are not significantly different at P ≤ 0.01. %PWSR = Percent of plant without storage roots; LRY = Large root yield; SRY = Small root yield; TRY = Total root yield; FY = Foliage yield; FB = Fresh Biomass; NLR/P = Number of large roots per plant; NSR/P = Number of small roots per plant; DM = percent dry matter; RDMY = root dry matter yield; HI = Harvest Index.

007 and CRI 001, HMA 2 and CEMSA 74-228, and CR001 and CRI 054. Most of the accessions had harvest indices ranging from 18 to 27%. Similarly, other workers, Tumwegamire et al. (2011) and Laurie (2010) recorded high coefficient of variations (CV). The CV values obtained in this study were however higher than those by Otoo et al. (2001) except percent dry matter (%DM) and fresh biomass (FB). Caliskan et al. (2007), Abidin et al. (2005), Grüneberg et al. (2005) all reported varying CVs and attributed this to high sensitivity of sweet potato to environmental variations as affirmed in this current study. Also, many authors have reported the presence of significant genotype x environment (G X E) interactions in the crop in both yield and quality traits (Caliskan et al., 2007; Abidin et al., 2005; Grüneberg et al., 2005). The implication of high CV or the presence of significant G X E interaction is useful to the plant breeder to develop widely or specifically adapted genotypes and/ or diversify resources for yield and

auxiliary qualities (Grüneberg et al., 2005).

Accession CR 002 had the highest percentage of plants without storage roots. US 020 recorded the highest total root yield (TRY) (56.23 t/ha) followed by FREEMA (44.89 t/ha). A high percentage of plants without storage roots may be attributed to lack of adaptation or lateness of a clone (Carey and Reynoso, 1999). FREEMA also recorded the highest number of large roots per plant (1.44) while CR 001 recorded the highest number of small root per plant (4.31). These results are consistent with those by Ssebuliba et al. (2006) who reported higher number of plants per root for local accessions compared to introduced orange-fleshed varieties. Total root yield for most of the accessions are much higher than those evaluated by Otoo et al. (1995, 2001). Gasura et al. (2008) reported that root yield depends on the number of storage roots per plant. Therefore, tuber number could be useful for estimating yielding potential of given cultivars. In sweet potato, large numbers of small roots may indicate potential for higher yields at later harvests (Carey and Reynoso, 1999). The total root yield for the local accessions were generally higher than the introduced accessions. This may be attributable to the adaptability of the landraces to the local environment.

At large, the local accessions produced higher foliage yield (FY) and fresh biomass (FB) than the introduced accessions. CR 002 recorded the highest foliage yield (FY) and fresh biomass (FB) (157.13 t/ha) but with no storage roots even after 170 days after planting. Similarly, Tairo et al. (2008) and Lebot (1986), recorded high foliage yield (FY) with no storage roots after 180 days post-planting. Generally, accessions with the highest foliage yield (FY) produce lower total root yields (TRY). This may be ascribed to variances in rate of photosynthate translocation to storage roots ensuing in yield differences among the accessions. CR 002, CRI 027 and LOCAL 2 which recorded both high foliage yield (FY) and fresh biomass (FB) may be recommended for fodder production for livestock feed formulation (Otoo et al., 2001).

Again, percent dry matter (%DM) content among the 20 accessions varied from 14.49 to 36.65%. It was generally higher for the white-, cream- and yellow-fleshed accessions compared to the orange-fleshed accessions (SA/BNARI, UK/BNARI and US 029) which are all exotic lines. UK/BNARI nonetheless recorded the lowest %DM content of 14.49%. Brabet et al. (1998) reported that orange-fleshed sweet potato genotypes have lower %DM than the white/cream and yellow-fleshed genotypes which is consistent with findings of this study. In the same vein, high %DM content contributed significantly to root dry matter yield (RDMY) among the accessions. US 020 registered the highest (18.29 t/ha) RDMY while the rest of the accessions ranged from 15.15 to 1.02 t/ha. The local accessions generally produced more RDMY than the introduced accessions, which were comparatively higher than reports by Otoo et al. (2001) in the coastal savannah zone of Ghana however less than what was reported in the forest zone of Ghana (Otoo et al., 1995).

Harvest indices (HI) for the exotic lines were relatively higher than those of the local accessions. The highest HI was recorded by US 020 (57.11%), an introduction from USA. Among the local accessions, FREEMA recorded the highest HI of 45.0.7%. High HI of genotypes could be indicative of the level of tuber photosynthetic efficiency to draw photo-assimilates (Otoo et al., 2001).

Genetic relationship among 20 accessions of *I. batatas* L. using both qualitative and quantitative traits

Figure 1 shows the relatedness among the accessions generated using qualitative and quantitative agromorphological traits. The accessions were separated into two clusters at a genetic similarity index (GSI) of 61.6%, and further regrouped into 6 sub-clusters at levels up to 100% similarity. These accessions are related by presence of flowers, flower colour, sepal shape, sepal colour and colour of style. The clustering pattern of the 20 sweet potato accessions are consistent with reports by Yuan et al. (2011), Li et al. (2009) and Yan et al. (2009) who recorded 6 sub-clusters of genetic similarity among their collections. Characters of sweet potato flowers can serve as tool to detect duplicates among collections (Reynoso et al., 1999). Traits such as general outline of leaf and shape of the central leaf lobe have been recognised as crucial in the study of sweet potato diversity (Karuri et al., 2010, 2009; Tairo et al., 2008; Gichuru et al., 2006), which contrasts findings of this study.

Seven local accessions (CR001, FREEMtableA, HMA 1, ER001, HMA 2, LOCAL 1 and UE 007) were grouped into IIF sub-cluster at 87.8% similarity. The pattern of clustering of these local accessions showed possible relationship to a common geographic origin. With exception of UE 007 from the forest agro-ecological zone, all the other accessions were from the coastal savannah agro-ecological zone of Ghana. This is in consonance with Zhang et al. (2000) and He et al. (1995), who detected clustering of several accessions together based on their geographic origin. In contrast, Karuri et al. (2010), Yada et al. (2010), Tairo et al. (2008) and Veasey et al. (2007) reported no distinct relationships between clusters generated based on their geographic origins. However, for accessions to be considered as possible duplicates, their genetic similarity index should be equal or greater than 95% (Andersson et al., 2007). Accessions ER 001 and HMA 2 exhibited the closest resemblance at a similarity index of 97.1% (possible duplicates). Some workers also identified possible duplicates in their sweet potato collections (Karuri et al., 2010; Yada et al., 2010; Veasey et al., 2007; Huamán et al., 1999a and b). Only one of the duplicates could be used in plant breeding and

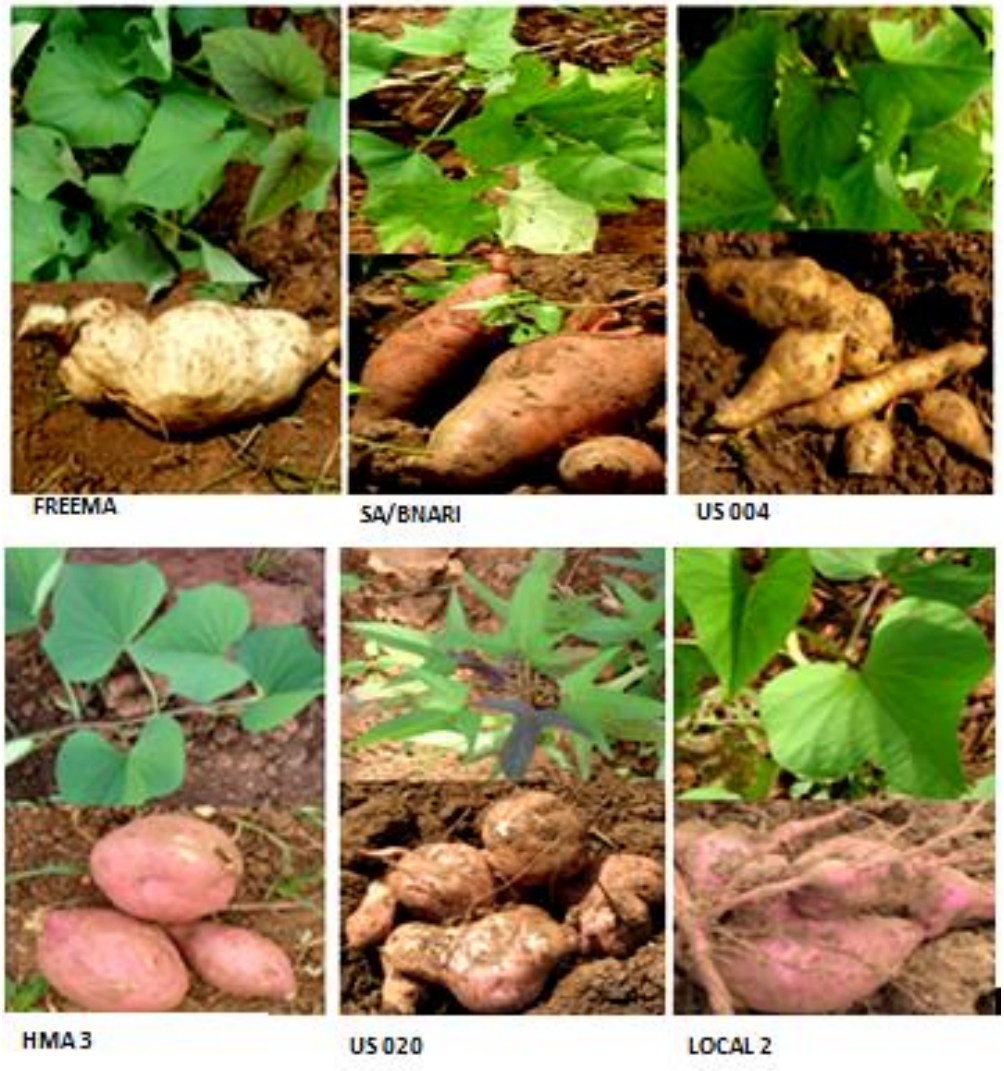

Figure 1. Photographs of leaves and tubers of some accessions on the field.

in germplasm conservation to save cost. CR001 and US 020 were the most diverse accessions.

The second major sub-cluster II (74% genetic distance) contained the highest number of accessions (13) which subsequently regrouped into five sub-sub clusters (1 - 5) at a genetic distance of 71.4%. Sub-cluster 5 contained CRI 027 and CR 002 at a genetic distance of 89% and were clustered based on similar foliar characters (plant type, vine internode diameter, mature leaf size, mature leaf colour and petiole length) and storage root characters (thus, absence of storage roots). Sub-cluster 4 housed four accessions namely, SA/BNARI, CEMSA 74 - 228, CRI 054 and DOAK 08-007 all grouped at a genetic distance of 83.3% (Figure 2). SA/BNARI and CEMSA 74-228 were regrouped at a genetic distance of 89.1% based on vine internode length and damage by weevils on first evaluation. CRI 054 and DOAK 08-007 were individually grouped at a genetic distance of 83.3 and 83%, respectively. CRI 054 was separated based plant type and secondary flesh colour with erect plant type and orange secondary flesh colour as unique traits.

Principal components analysis for 18 quantitative traits of *Ipomoea batatas* L.

Table 5 shows the eigenvalues, percentage variation and cumulative percent variations of 5 principal components of 18 quantitative traits scored among 20 sweet potato accessions. The first five principal component axes (PC_1, PC_2, PC_3, PC_4 and PC_5) in the PCA analysis had eigenvalues greater than 1.0, with cumulative variance of 84.29%. Principal component one (PC_1), with eigenvalue of 6.72, contributed 37.35% to total genetic variability,

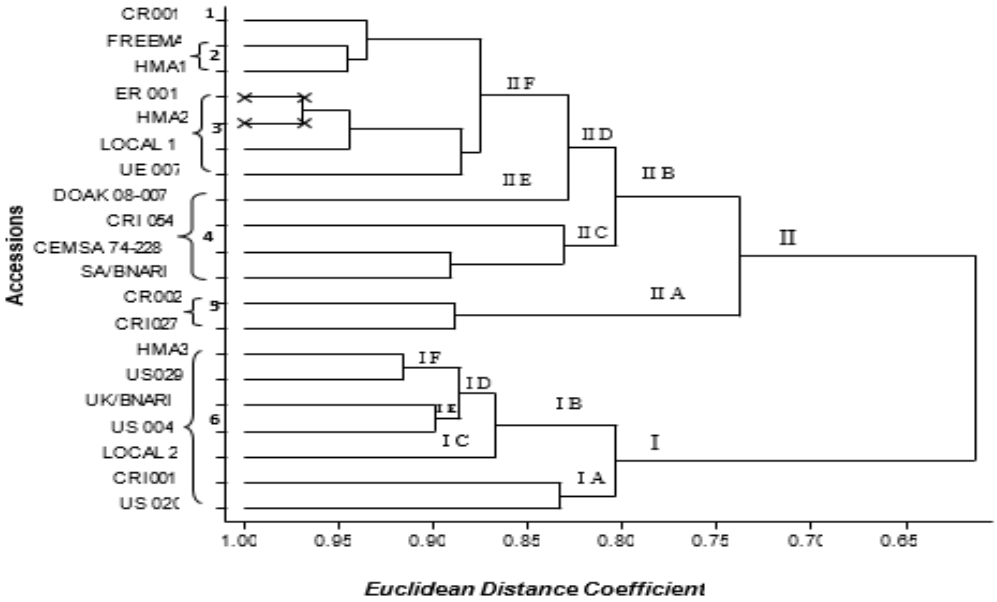

Figure 2. Average-linked dendrogram based on Euclidean distance coefficient of 20 accessions of *I. batatas* L. generated by qualitative and quantitative traits.

Table 5. Principal components analysis of 18 quantitative traits for 20 Accessions of *I. batatas* L.

Traits	PC_1	PC_2	PC_3	PC_4	PC_5
PTY	-0.151	0.260	0.347	0.282	0.247
VID	0.043	0.355	0.163	-0.421	0.051
VIL	-0.041	-0.004	0.444	0.378	0.406
MLS	0.142	0.169	0.252	0.040	-0.478
PL	-0.038	0.248	0.394	-0.292	0.210
FW	0.270	0.104	-0.045	-0.396	0.231
FL	0.276	0.099	-0.036	-0.375	0.218
%PWSR	-0.283	0.267	-0.263	0.031	0.020
LRY(t/ha)	0.314	0.136	-0.049	0.218	0.259
SRY(t/ha)	0.268	0.238	-0.007	0.009	-0.298
TRY(t/ha)	0.342	0.181	-0.055	0.177	0.056
FY(t/ha)	-0.187	0.423	-0.133	0.098	-0.142
FB (t/ha)	-0.022	**0.491**	-0.154	0.176	-0.111
NLR/P	0.316	-0.005	-0.185	0.147	-0.010
NSR/P	0.151	-0.043	**0.493**	***0.000***	-0.400
%DM	0.244	-0.234	0.160	0.014	-0.146
RDMY(t/ha)	0.331	0.162	-0.074	0.245	0.025
HI	0.328	-0.117	-0.098	0.118	0.158
Eigenvalue	**6.723**	3.504	1.965	1.584	1.398
% Variance	**37.352**	19.467	10.915	8.798	7.765
% CV	37.352	**56.819**	**67.734**	**76.532**	**84.297**

% CV = Percent cumulative variance; Values bolded made substantial contribution to total genetic variance.

while PC2, with eigenvalue of 3.50, accounted for 19.47% of total variability among the 20 sweet potato accessions. PC_3, PC_4 and PC_5 had eigenvalues of 1.97, 1.58 and 1.40 contributing 10.92, 8.80 and 7.77% to the

total genetic variance, respectively.

The relative discriminating power of the principal axes as indicated by the eigenvalues was high (6.72) for axis 1 and low (1.40) for axis 5. In PC_1, traits that accounted for most of the observed variability among the 20 accessions include flower width (FW) with vector loading of 0.270, flower length (FL) (0.276), large root yield (LRY) (0.314), small root yield (SRY) (0.268), total root yield (TRY) (0.342), number of large root per plant (NLR/P) (0.316), number of small root per plant (NSR/P) (0.151), percent dry matter (%DM) (0.244), root dry matter yield (RDMY) (0.331), and harvest index (HI) (0.328).

PC_2, PC_3, PC_4 and PC_5 were positively correlated with plant type (PTY). Characters that were mostly correlated with the PC_2 were fresh biomass yield (FB), foliage yield (FY) and vine internode diameter (VID). Number of small roots per plant (NSR/P), vine internode length (VIL) and petiole length (PL), vine internode length (VIL) and root dry matter yield (RDMY), vine internode length (VIL) and large root yield (LRY) correlated with PC_3, PC_4 and PC_5, respectively. In PC_4 and PC_5, PTY, VIL and LRY contributed substantially to total genetic variation. These results confirm the results of studies of the association between root yield and other agromorphological traits (Easwari et al., 1999). The current study reveals that root yield is significantly correlated with plant type, petiole length and number of roots per plant. Plant type in turn is highly correlated with petiole length and number of roots per plant. Vine internode length and vine internode diameter showed significant association as shown in PC_2, PC_3, PC_4 and PC_5. In addition, root yield and petiole length are highly correlated with number of roots per plant as shown in PC_3 and PC_5.

The total contribution of the five principal component axes of this study was higher (84.3%) than those detected by other workers (Amoatey et al., 2015; Ahiakpa et al., 2013; Afuape et al., 2011; Tairo et al., 2008) where the principal component axes contributed 76, 52.5 and 70.09% to total variation, respectively. In the present study, all the eigenvalues except that for PC_1 were higher than observed by Afuape et al. (2011). Hence, based on the factor scores of the 18 characters, accessions which recorded high scores for the component traits in PC_1 could be selected as parents in any future hybridisation programme.

Pearson correlation analysis of 18 quantitative traits in 20 accessions of *Ipomoea batatas* L.

Table 6 displays association among eighteen (18) quantitative traits of the various accessions of sweet potato. Vine internode length (VIL) and petiole length (PL) showed a poor to very low positive/negative correlations among all the traits. Similarly, five traits; plant type (PT), vine internode diameter (VID), mature leaf size (MLS), fresh biomass (FB) and number of small root per plant (NSR/P) showed poor to low positive and negative correlations among all traits except plant type (PT) and vine internode length (VIL), vine internode diameter (VID) and petiole length (PL), mature leaf size (MLS) and number of small root per plant (NSR/P) which showed moderate positive correlations (r = 0.59; 0.63 and 0.56), respectively. Interestingly, flower width (FW) and flower length (FL) recorded poor to very low positive/negative correlations to all other traits except storage yield determinants and flower length where low to moderate and perfect positive correlations were recorded, respectively.

Also, very low to high negative correlation was observed between foliage yield (FY) and percent of plants without storage root (%PWSR) with all other traits except fresh biomass (FB) and foliage yield (FY) which recorded high positive correlation. Finally, all the storage root traits showed very low to very high positive correlations among all other traits except foliage yield for which there was very low negative correlation. The correlation matrix generally showed a markedly low and negligibly positive/negative (±0.00 - ±0.10) correlation to low positive/negative (±0.30 - ±0.50) correlation between storage root traits and shoot traits (plant type (PTY), vine internode diameter (VID), vine internode length (VIL), mature leaf size (MLS), petiole length (PL), flower width (FW), and flower length (FL)). This result is consistent with those reported by Afuape et al. (2011) and Yada et al. (2010) but contrasts report of Tsegaye et al. (2007) who recorded moderate positive correlation in shoot traits to root traits. Many economically important traits of plants are usually related to one another in one or several ways. Correlations are measures of the degree of associations between these traits (Steel and Torrie, 1984). Selection for one trait results in progress for all characters that are positively correlated but reduces for traits that are negatively correlated. Therefore correlation analysis enables the breeder to understand the mutual component characters on which selection can be based for genetic improvement.

All the root traits were low to highly negative correlations with percentage of plants without storage roots (%PWSR); thus, increase in %PWSR automatically reduces storage root yield. Foliage yield (FY) and fresh biomass (FB) showed moderate to highly positive correlations (r = 0.57 and r = 0.81) with %PWSR. Also, the root traits were poorly correlated to FY and FB. According to Lewthwaite and Triggs (2000), storage root yield depends on leaf photosynthesis. Hence, canopy type might have influenced the net assimilation rate (Sasaki et al., 2005). The transport of photo-assimilates from the leaves to the root stalk is prejudiced by storage root growth, as storage root cell must be formed and expanded prior to storage of assimilates. Therefore, increased foliage yield without considerable storage root cells development would spontaneously induce reduction in tuber yield, hence the negative correlation between

Table 6. Pearson correlation coefficients between 18 quantitative traits of 20 *I. batatas* L. accessions evaluated at NARC in Ghana.

Traits	PTY	VID	VIL	MLS	PL	FW	FL	%PWSR	LRY(t/ha)	SRY(t/ha)	TRY(t/ha)	FY(t/ha)	FB (t/ha)	NLR/P	NSR/P	%DM	RDMY(t/ha)
PTY																	
VID	0.21																
VIL	**0.59***	-0.14															
MLS	0.05	0.13	-0.10														
PL	0.39	**0.63****	0.20	0.21													
FW	-0.27	0.34	-0.14	0.17	0.12												
FL	-0.26	0.32	-0.13	0.21	0.10	**1.00*****											
%PWSR	0.37	0.09	-0.10	-0.15	0.09	-0.37	-0.38										
LRY(t/ha)	-0.08	0.13	0.07	0.24	0.05	**0.52***	**0.53***	-0.43									
SRY(t/ha)	-0.13	0.44	-0.18	0.41	-0.06	0.46	0.47	-0.32	0.49								
TRY(t/ha)	-0.14	0.25	-0.04	0.34	-0.03	**0.55***	**0.57***	-0.46	**0.94*****	**0.76****							
FY(t/ha)	0.47	0.30	-0.05	0.08	0.20	-0.22	-0.24	**0.81****	-0.23	0.07	-0.16						
FB (t/ha)	0.39	0.40	-0.06	0.23	0.18	0.04	0.03	**0.57***	0.21	0.42	0.31	**0.89*****					
NLR/P	-0.38	-0.08	-0.18	0.23	-0.18	0.46	0.47	**-0.53***	**0.68****	**0.57***	**0.75****	-0.32	0.04				
NSR/P	-0.01	0.10	0.19	**0.56***	0.10	0.13	0.15	**-0.61****	0.12	0.40	0.26	-0.28	-0.15	0.11			
%DM	-0.37	-0.20	0.03	0.19	-0.17	0.31	0.32	**-0.77****	0.27	0.36	0.35	**-0.60****	-0.42	0.46	0.39		
RDMY(t/ha)	-0.15	0.12	0.01	0.38	-0.07	0.51	**0.52***	-0.42	**0.88*****	**0.73****	**0.94*****	-0.12	0.32	**0.74****	0.17	0.48	
HI	-0.38	-0.12	-0.09	0.16	-0.24	0.49	**0.52***	**-0.65****	**0.79****	0.37	**0.76****	**-0.61****	-0.24	**0.75****	0.21	0.44	**0.68****

* = significant (P<0.05); ** = very significant (P<0.001); *** = highly significant (P<0.0001) computed using standard linear Pearson *correlation*. PTY = Plant type; VID = vine internode diameter; VIL = vine internode length; MLS = mature leaf size; PL = petiole length; FW = flower width; FL = flower length; %PWSR = percent plant without storage root; LRY = large root yield; SRY = storage root yield; TRY = total root yield; FY = foliage yield; FB = fresh biomass; NLR/P = number large root per plant; NSR/P = number of small root per plant; %DM = percent dry matter; RDMY = root dry matter yield; HI = harvest index.; NARC = Nuclear Agriculture Research Centre.

foliage yield (FY) and fresh biomass (FB) to storage root traits. The low positive to moderate positive correlations between flower width (FW) and flower length (FL) and storage yield determinants could be attributed to *MADS-box genes* found in sweet potato flowers and storage roots (Ravi et al., 2009; Ku et al., 2008; Kim et al., 2002, 2005). These *MADS-box genes* are expressed in relation with anthocyanin accumulation in both flowers and pigmented root periderm and cortex tissue (Lalusin et al., 2006) or may impact the different stages of storage root development (Ku et al., 2008; Kim et al., 2005).

There was moderate to high positive correlations between total root yield and large root yield (LRY) and small root yield (SRY) and also positive correlations between total root yield and number or large roots per plant (NLR/P) and number of small roots per plant (NSR/P). These results corroborate that of Afuape et al. (2011), but contrast the results of Islam et al. (2002) and Tsegaye et al. (2007) who reported negative correlations between total root yields (TRY) and, number of large roots per plant (NLR/P) and number of small roots per plant (NSR/P). Root dry matter yield (RDMY) and harvest index (HI) had moderate to very high positive correlations with total root yield (TRY), large root yield (LRY), small root yield (SRY), and number of large root per plant (NLR/P). This is consistent with results by Felenji et al. (2011). There was low positive correlation between percent dry matter (%DM) and total root yield (TRY), large root yield (LRY), small root yield (SRY), number of large roots per plant (NLR/P) and number of small root per plant (NSR/P). These results are consistent with findings made by Felenji et al. (2011) but inconsistent with those by Tairo et al. (2008). These results therefore suggest that total root yield in sweet

potato is a composite character with contributions from a number of traits. Thus, total root yield trait can be improved by simultaneous selection for other traits positively correlated to it.

Conclusion

There were significant genetic variability among the 20 accessions of sweet potato studied based on the agromorphological characters evaluated. Hierarchical cluster analysis grouped accessions into two clusters at a genetic similarity index of 61.6%. Accessions, ER 001 and HMA 2 were found to be possible duplicates. Accession US 020 recorded the highest total root yield and harvest index of 56.32 t/ha and 57.11%, respectively. The PCA showed characters contributing differently to the 84.29% of total genetic variability with only PC_1 contributing 37.35% to the total variability. Key component traits contributing to total root yield (TRY) include large root yield (LRY), number of large root per plant (NLR/P), percent dry matter (%DM), root dry matter yield (RDMY) and Harvest index (HI). This study provides valuable information that can be utilised in a breeding programme to ameliorate local clones of *I. batatas* L in Ghana. Further studies using molecular markers are needed to delineate useful genetic information at the molecular level.

Conflict of Interests

The authors have not declared any conflict of interest.

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Path analysis for yield traits in F_2 generation and molecular approaches for breeding rice tolerant to drought and submergence

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Drought and submergence are the two major limiting factors that reduce rice production. In this study, the relevance of yield traits through path analysis under drought and submergence conditions to improve grain yield of rice, from dry season 2014-2015 and genotypic analysis using SSR markers was evaluated, during 2015-2016. Path analysis indicated that the number of panicles/clusters had the highest and a direct positive effect on the grain yield, followed by the number of filled-grain/panicle, and the harvest index compared to other component traits. These traits could be used as selection criteria for high yield and drought tolerance in populations of rice. There were two markers including RM201 (210-225 bp) and RM219 (210-215 bp) chosen to select parents in backcrossing because production of polymorphic bands relevant to submergence and drought tolerance genes. By the BC_1F_1 and BC_2F_1 generations of the cross OM6162/Swarnasub1//OM6162, primers RM201 and RM219 were identified drought and submergence tolerant individuals. These lines will be used in breeding programme for release of both drought and submergence tolerant with considerable yield in next step. Findings of this study are promising to develop rice cultivars tolerant to both drought and submergence, and may therefore help to reduce detrimental impacts from climate changes to rice production.

Key words: Correlation, direct selection, grain yield, marker assisted selection.

INTRODUCTION

Rice is currently grown in varied environmental conditions where it shows different levels of response to abiotic stress, depending on the environmental condition of origin and cultivation (Rananwake and Hewage, 2014). The climate change, such as drought, flooding, salinity and high temperature have detrimental impacts on rice production, especially in developing countries. Abiotic stress as drought and submergence have been identified as the two constraints to cause most rice loss (Bernier et al., 2008; Devereux, 2007; Dey and Upadhyaya, 1996;

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Gauchan and Pandey, 2012; Pandey and Bhadari, 2009; Venupeasad et al., 2007). Flood are major cause of low yields in rainfed lowed land areas of Mekong Delta Vietnam, which occupy more than 1 million ha. Excess water is a problem for about haft of rainfed areas. Rice production is damaged by both short-term submergence (up to 2 weeks) and by longer term stagnant flooding at water depth above 40 cm. Adaptability of rice to the drought and submergence stresses is the most important objective of the rice breeding program. Additionally, rice yield can be improved with a comprehensive combination of both conventional and molecular breeding techniques (Khush, 2005).

Marker-Assisted Selection (MAS) is a method proposed by Tanksley (1983) to investigate the introgression of tolerant genes (Melchinger, 1990). It includes the Marker-Assisted Evaluation of breeding materials, Marker-Assisted introgression, and Marker-Assisted pyramiding. To improve the selection for early generations, MAS can decrease the number of plants retained due to their early generation performance and can ensure a high probability of retaining superior lines (Eathington et al., 1997). The important prerequisites for successful selection of the early generation with MAS are the population size and heritability level of the selected traits (Lande and Thompson, 1990). Kuchel (2007) and Bonnett et al. (2005) noted that maximum grain in crops can be achieved at a much lower cost with the aid of MAS, compared with the conventional breeding. MAS have successfully introduced the bacterial blight resistance gene *Xa21* (Chen et al., 2000) and *waxy* gene (Zou et al., 2003) to target commercial rice cultivars.

Studies in genetics of rice showed that the submerged-tolerant trait of the FR13A variety is controlled by a polygene and effected by environment, designated *sub1* (Xu and Mackill, 1996). This gene was identified recently as an ethylene responsive like factor (ERF) and designated *Sub1A* (Xu et al., 2006). The most widely submerged-tolerant donors are IR64sub1 and Swanasub1. Swanasub1 was pyramided with the *sub1* gene for tolerance both drought and submergence. *Sub1* versions of popular rice varieties were developed through the Marker-Assisted Backcrossing (MABC) approach (Neeraja et al., 2007; Septinnighsih et al., 2009; Iftekharuddaula et al., 2015; Lang et al., 2015). Nguyen et al. (2004) developed 85 markers for mapping of QTL regions for drought tolerance in rice and identifying putative candidate genes. One QTL region controlling osmotic adjustment on chromosome 3 and 14 affects root traits which are located on chromosomes 1, 2, 4, 5, 6, 7, 8, 9, 10 and 12. In a previous study, Kumar et al. (2014) reported that two markers, RM201 and RM328, were linked with drought-tolerant genes ($qDTY_{1.1}$, $qDTY_{2.1}$, and $qDTY_{3.1}$).

Path analysis appears as the best method to evaluate the relationship between yield and relevant traits (Board et al., 1997). Path analysis permits estimation of direct effects of various traits on yield as well as their indirect effects via other components traits. In crop breeding, the diallel theory which was first developed by Hayman (1954), is widely used for path analysis (Krisha Veni and Shobha Rani, 2005; Eradasappa et al., 2007). Path coefficient analysis partitions into direct and indirect matrix presenting correlation in a more meaningful way in breeding (Mohsin et al., 2009). The diallel analysis is useful to get information about the genetic structure of populations and helps to explore the genetic mechanism of various traits in crops plants such as rice (Griffing, 1956; Rahimi et al., 2010; Muthuramu et al., 210). In rice, information on correlation coefficient has been helpful as a basis for selection in a breeding programme.

Development of high yield cultivars that combine drought and submergence tolerance could be the ideal to reduce detrimental effects of climate change on rice production. IRRI has started drought and submergence breeding programs to develop germplasm for this target population (Kumar et al., 2008; Septiningsih et al., 2009). Therefore, the introduction of both drought and submerged-tolerant characteristics to target rice cultivars is an important task for rice breeders. Thus, the objectives of this study were (1) to clarify direct and indirect effects of yield traits under drought and submergence stresses and (2) to evaluate genotypic using SSR markers for background selection of drought and submergence tolerant.

MATERIALS AND METHODS

Plant materials

The materials consisted of 36 F_2 combinations by crossing of six parents IR64sub1, OMCS2000, OM6162, OM1490, Swarnasub1, and IR78933-B-24-BB-4 in a diallel mating design. The variety OM6162 was crossed with Swannasub1 which was used as the donor for both *qDTY* and *Sub1* genes to obtain a backcross population for MAS.

Path analysis

Evaluation of agronomic characters and grain yield of rice under drought stress

Seeds of the F_2 diallel lines were soaked, germinated in an incubator, and sown into plastic trays. After 15 days, they were transplanted into cement basins. The row-to-row and plant-to-plant space of 20 cm x 15 cm was maintained. Ten days after transplanting, water was not provided until flowering. Fertilizer was applied at the rate 100-40-30 kg of N-P_2O_5-K_2O ha^{-1}. The record plant recovery for each entry followed the 0-9 score of the standard evaluation system (IRRI, 1996) with scores 0-3: tolerance and score 5-9: susceptible, and agro-morphological characters and grain yield were recorded.

Screening for submergence tolerance

Seeds of the F_2 diallel lines were soaked, germinated in an incubator, and sown into plastic trays. Ten-day-old seedlings were transplanted

using 1 plant/hill and with space of 20 x 15 cm in submergence tanks. At the seventh-day after transplanting, plants were completely submerged for 14 days at 10 cm water depth which was then increased by 10 cm at every 10-day interval. Finally, 50 cm water level was maintained up to the soft dough stage. Four plants were tagged for tiller counting. Surviving plants were counted just after the recession of water and their tillers were counted before and after submergence at 7-day intervals.

The standard evaluation system (SES) scores for submergence tolerance followed by IRRI (1988), 1 to 9 (1: all plants survive; 9: all plants completely dead).

Agro-morphological character evaluation

All agro-morphological traits including panicle/cluster, filled grain/panicle, the weight of 1000 grains (g), root length (cm), yield/cluster (g) were recorded. Biomass-weight of 10 plants harvested from each accession per replication was also recorded. Harvested plants were dried before weighing for calculating the Harvest Index as follows;

Harvest Index = Economic yield/Biological yield x 100

where economic yield is the total weight of grain harvest from 10 plants per accession per replication, and biological yield is the total grain weight and biomass from 10 plants per accession per replication.

The correlation coefficient (*r*) among traits was calculated by using SAS 9.1 program. The correlation coefficient is a measure of the association between two or more variables. It is a measure of symmetrical association between variables and does not measure the dependence of one variable on another.

Marker-Assisted selection

Microsatellite primers were used to survey polymorphism on the samples based on information of the gene mapping of Lang and Buu (2008). For submerged-tolerant genes, the molecular markers were evaluated based on the genetic mapping information of the International Rice Research Institute (IRRI) and the study of Lang et al. (2015). Sixteen microsatellite primers were selected from microsatellite primers mentioned above (Table 1).

In BC_1F_1 and BC_2F_2 generations, selection was initially carried out by markers through screening parental polymorphism at both *qDTY* and *Sub-1* loci.

DNA extraction

Leaves were collected 2-3 weeks after planting for extraction of DNA. Standard molecular grade chemicals and general techniques for preparing stock solutions, buffers, reagents, and equipment were followed according to Sambrook et al. (1989).

DNA extraction was prepared according to a method described by McCouch et al. (1997) and conducted at the Genetics and Plant Breeding Department of Cuu Long Rice Research Institute, Can Tho, Vietnam.

DNA quality was checked using 1% agarose (melting 3 g agarose in 300 ml TAE buffer). The mixture was heated in the microwave for 5-6 min and then cooled to around 55-60°C. This was then poured into a prepared electrophoresis box with combs. Gels were ready and the combs were removed after about 45 min. Seven microliters of DNA sample and 3 µl loading buffer (Tris 1M pH = 8.0, glycerol, EDTA 0.5 M pH = 8.0, xylene cyanol 0.2%, bromophenol blue 0.2% and distilled water) were mixed and placed in the wells. The electrophoresis program was run at 70-80 V, 60 mA for 45 min or until loading buffer dye moved far from the wells. Gel was then taken out and stained with ethidium bromide. The gel image was visualized under UV light.

Amplification of microsatellites and detection of their polymorphisms

PCR amplification was performed in a mixture of 10 mM Tris-HCl (pH=8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1 unit of TAKARA *Taq*, 4 nmole of dNTPs, 10 ρmole of primers, with 30 ng of genomic DNA per 25 µl using a thermal cycler 9600 (Perkin-Elmer, USA). The PCR reactions were denatured at 94°C for 4 min, followed by 35 cycles at 94°C for 1 min, 55°C for 1 min and 72°C for 2 min. The final extension was at 72°C for 5 min. After PCR, 13 µl of loading buffer (98% formamide, 10 mM EDTA, and 0.025% bromophenol blue, 0.025% xylene cyanol) were added. Polymorphisms in the PCR products were detected by ethidium bromide staining after electrophoresis on 3% agarose gel.

RESULTS

Path analysis

The correlation coefficient was conducted out 6 to 36 crosses of diallel from the varieties IR64sub1, OMCS2000, OM6162, OM1490, Swarnasub1, and IR78933-B-24-BB-4 (Table 2). The trait of panicle/cluster showed a strong and positive association with root length, drought tolerant at seedling, and yield. Positive and high phenotypic correlations of yield with the number of panicles/cluster were obtained in this study. Wherever, negative significant association with 1000 weight was observed. Association among yield traits revealed that filled grains/panicle showed significant positive association with HI and root length, whereas, significant negative correlation was observed with the weight of 1000 grains and drought tolerance at the flowering stage. The number of filled grains/panicle also showed a strong positive and high phenotypic correlation on grain yield. The weight of 1000 grains was recorded to have a positive significant association with drought tolerance at the flowering stage and significant negative association was recorded with drought tolerance at the seedling stage. The weight of 1000 grains and submergence at seedling was not correlated with grain yield. Correlation between the HI and drought responses at the flowering stage was significantly negative, and phenotypic correlation of HI with yield trait was 0.69. Therefore, the HI could be used as a reliable criterion for improving yield. The trait root length exhibited a significant and positive association with drought tolerance and was associated with yield trait. The trait test for drought tolerance at the flowering stage showed a positive and significant association with drought tolerance at the seedling stage, significant negative association with yield, and strong positive and high phenotypic ccorrelation (0.65). Whereas, drought tolerance at the seedling stage did not show significant phenotypic association with yield.

Path analysis permits estimation of direct effects of

Table 1. The list of information molecular markers used in diagnosis of submergence and drought.

No.	Markers	Sequence code (5' - 3')	Chromosome	Repeating sequence
1	RM201	F: ctcgtttattacctacagtacc R: ctacctcctttctagaccgata	9	(CT)17
2	RM511	F: cttcgatccggtgacgacac R: aacgaaagcgaagctgtctc	12	(GAC)7
3	RM11125	F: ccaagaaccctagctccctctcc R: tcgacgagatcctcctcgtaaacc	1	(CT)22
4	RM10713	F: atgaacccggcgaactgaaagg R: ctggctccctcaaggtgattgc	1	(AGA)12
5	RM3252	F: ggtaactttgttcccatgcc R: ggtcaatcatgcatgcaagc	1	(CT)13
6	RM10115	F: acaagacgaggtaacacgcaagc R: gcgaaggatcaacgatgatatgg	1	(CTT)24
7	RM105	F: gtcgtcgacccatcggagccac R: tggtcgaggtggggatcgggtc	9	(CCT)6
8	RM219	F: cgtcggatgatgtaaagcct R: catatcggcattcgcctg	9	(CT)17
9	RM23662	F: gagaggacgatggcactattgg R: cgaggaacttgattcgcatgg	9	(GGC)10
10	RM23877	F: tgccacatgttgagagtgatgc R: tacgcaagccatgacaattcg	9	(CA)30
11	RM547	F: taggttggcagacctttcg R: gtcaagatcatcctcgtagcg	8	(ATT)19
12	RM249	F: ggcgtaaaggttttgcatgt R: atgatgccatgaaggtcagc	5	(AG)5A2(AG)14
13	RM24103	F: actgacgagagagacatggatgg R: ccggcacacaatgaataggg	9	(AC)17
14	RM25181	F: aaagagcttccctaatggcttcg R: gagagaatgacctctcccaagacc	10	(TTC)22
15	RM1125	F: ggggccagagttttcttcag R: gtacgcgcagaaaatgagag	10	(AG)12
16	RM328	F: catagtggagtatgcagctgc R: ccttctcccagtcgtatctg	9	(CAT)5

various traits on yield as well as their indirect effects via other component traits. The number of panicles/cluster was found to have a maximum direct positive effect on grain yield (Table 3), followed by the number of filled grains/panicle, HI and weight of 1000 grains, which indicated that these traits were contributors towards yield in these combinations, but there has not been stability in the results in various experiments or in different populations.

Marker assisted selection approach

A total of 16 markers were used to screen for drought and submergence in parent varieties (Table 1). The parental polymorphic survey was performed among the parental genotypes OM6162 and Swarnasub1. Two SSR markers RM201 and RM219 produced polymorphic bands (Figures 1 and 2). These markers clearly distinguished drought, submergence susceptible and tolerant parents. Homozygous plants were selected for backcrossing generation.

In the BC_1F_1 generation of OM6162/Swarnasub1//OM6162, plants were screened for drought tolerance by the robust tightly-linked marker RM201, a marker linked to drought tolerance QTL. There were two amplified bands, type P1 of the 225 bp band and type P2 of the 210 bp band. Out of 38 plants, eight lines showed “B” score similar homozygous donor allele by the 210 bp band, 15 lines showed heterozygous “H” score

Table 2. Correlations coefficients among the traits with yield of F_2 diallel generation.

Traits	Panicle/Cluster	FG	W-1000	HI	RL	SubS	DF	DS	Yield	
									r	Pr
Panicle /Cluster	1	0.20	-0.28*	0.19	0.59**	-0.13	0.15	0.43**	0.78**	0.88
FG		1	-0.38*	0.68**	0.79**	-0.04	-0.68**	0.25	0.76**	0.67
W-1000			1	-0.19	-0.07	-0.04	0.82**	-0.39*	-0.24	0.37
HI				1	0.08	0.25	-0.17	-0.69**	-0.89**	0.69
RL					1	-0.04	0.28	0.37**	0.84**	-0.39
SubS						1	0.06	0.11	-0.14	-0.08
DF							1	0.99**	-0.68**	0.65
DS								1	-0.20	-0.19

: significant at P<0.01. *: significant at P<0.05; r: correlations coefficients; Pr:** phenotype correlation coefficients. DF**:** Drought at flowering stage; DS: Drought at seedling stage; RL: Root length; SubS: Submergence at seedling; HI: harvest index; W-1000: Weight of 1000 grains: FG: Filled grain/panicle.

Table 3. Analysis of correlation system by path (path analysis) between the grain yield traits of rice in F2 diallel generation.

Traits	Panicle/Cluster	FG	W-1000	HI	RL	SubS	DF	DS	r total
Panicle /Cluster	0.85	0.15	-0.10	0.06	0.00	0.00	0.00	0.00	0.68
FG	0.19	0.67	-0.11	0.34	-0.14	0.07	0.11	-0.01	0.73
W-1000	-0.27	-0.20	0.46	-0.10	0.02	0.04	0.00	0.01	-0.21
HI	0.15	0.37	-0.06	0.59	-0.01	-0.01	0.00	0.02	0.41
RL	0.44	0.27	-0.02	0.01	-0.44	0.00	0.00	-0.01	0.52
SubS	-0.12	-0.03	-0.01	0.13	0.02	-0.06	-0.00	0.00	-0.16
DF	0.09	-0.31	0.29	-0.05	-0.07	0.00	0.00	-0.03	0.15
DS	0.16	0.11	-0.10	-0.41	-0.40	-0.10	0.01	-0.06	0.17

DF: Drought at flowering stage; DS: Drought at seedling stage; RL: Root length; SbS: Submergence at seedling; HI: harvest index; W-1000: Weight of 1000 grains: FG: Filled grain/panicle.

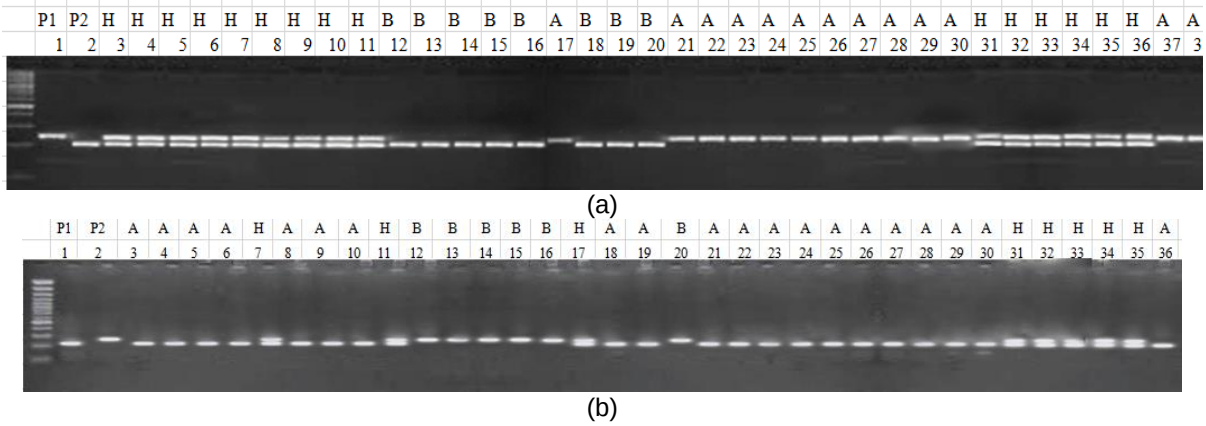

Figure 1. PCR profiles of some lines genotype in BC_1F_1 of OM6162/Swarnasub1//OM6162; (a) RM211 linked drought-tolerant gene: single band linked lane 2 as P1: the recipient parent (225 bp), P2: the donor parent (210 bp), A: similar homozygous recipient allele B: homozygous donor allele and H: double band indicated heterozygous allele; (b) RM219 linked submergence-tolerant gene: same as "a"; P1: the recipient parent (210 bp), P2: the donor parent (215 bp).

and 15 lines showed "A" score (Figure 1a). Thus, eight plants from cross OM6162/Swarnasub1//OM6162 were self-ed to develop BC_2. These plants with the "H" score for tightly linked marker were subjected for phenotypic selection.

In the BC_2F_1 generation, segregation of plants into

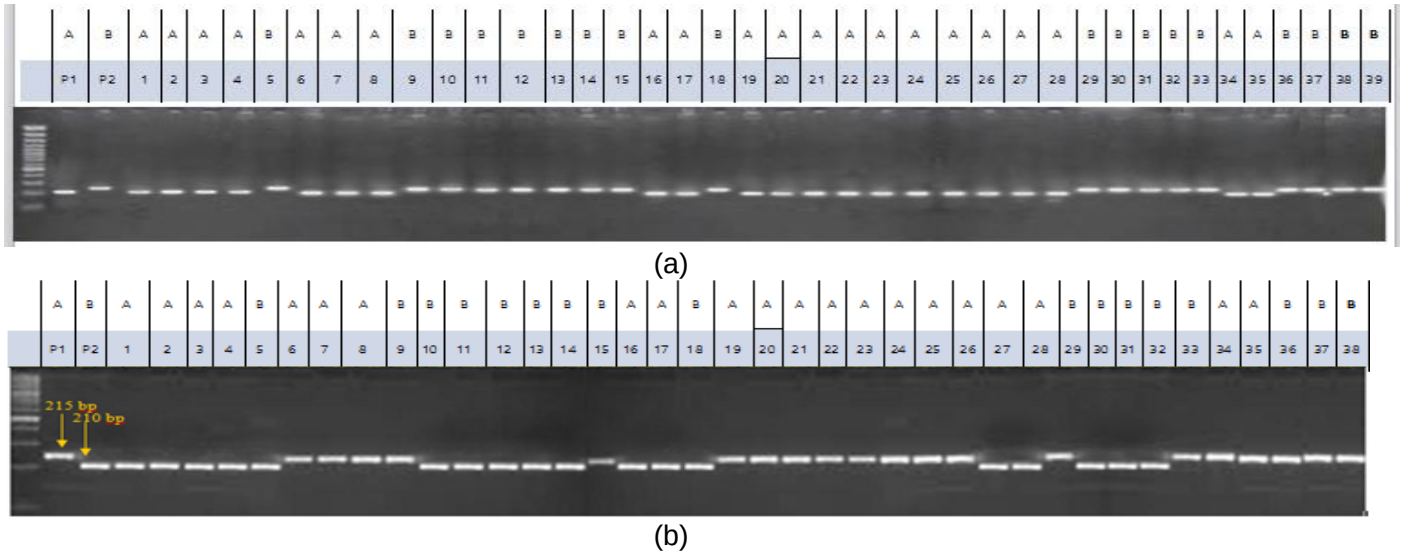

Figure 2. PCR profiles of some lines genotype in BC_2F_1 of OM6162/Swarnasub1//OM6162; (a) RM211 linked drought-tolerant gene: single band linked lane 2 as P1: the recipient parent (210 bp), P2: the donor parent (2105 bp), A: similar homozygous recipient allele B: homozygous donor allele and H: double band indicated heterozygous allele; (b) RM219 linked submergence-tolerant gene: same as "a"; P1: the recipient parent (215 bp), P2: the donor parent (210 bp).

drought tolerant and susceptible can be seen clearly in the gel picture with linked RM201 by type P1 of 215 bp band and type P2 of 210 bp band. The 21 plants with " A" score similar similar homozygous recipient allele. Eighteen plants with "B" score as homozygous donor allele of Swarnasub1 were produced due to accidental failure of backcrossing (Figure 2a).

In the case of RM219, a marker linked to the submergence tolerance QTL *sub1*. there were two amplified bands, 210 bp, and 215 bp band. The twenty plants showed "A" score, 8 plants showed heterozygous "H" score and only six plants of OM6162/Swarnasub1//OM6162 had the band similar to Swarnasub1 variety. (Figure 1b). In the BC_2F_1 generation of OM6162/Swarnasub1//OM6162, the marker RM219 linked to type P1 of 210 bp band and type P2 of 215 bp band. A total of eighteen plants had homozygous donor allele as Swarnasub1 (Figure 2b).

DISCUSSION

The number of panicles/cluster was found to have a maximum direct positive effect on grain yield. The results of the importance of the direct effect of panicles per plant were reported by (Bagheri et al., 2011; Madhavilatha et al., 2005; Yogameenaskshi and Vivekanandan, 2010). Here, the analysis aimed to determine important traits directly correlated to the yield or indirectly through other traits because they are able to help improve rice yield. Vaishali (2003) showed that grain yield exhibited strong significant positive correlation with the number of productive tillers per plant. Significant genetic variability in some root traits has been demonstrated and implicated for improving drought tolerance in crop plants (O'Toole and De Datta, 1986; Thangaraji et al., 1990; Sharma et al., 1994; Sinclair and Muchow, 2001). Jeena and Mani (1990) studied root traits and grain yield on some upland rice varieties and indicated that high root length density and root weight were important for breeding drought tolerance genotypes.

In summary, according to the principle of correlation, the system was evaluated by path analysis, and the results are displayed in Table 3. If the correlation coefficient among the cause and the result are equivalent to its direct value, the correlation can be explained as a really close relationship and direct selection through this traits. If the correlation is positive, but directly affected values are negative or negligible, the indirect values can be seen as the causes of the correlation. In this case, the indirect causes must be simultaneously considered in the selection. For example, for the length of roots, we must consider its indirect factors simultaneously if the traits for selection are a number of panicles/cluster, filled grains/panicle and drought tolerance at the flowering and seedling stages. Commonly, phenotype correlation of grain yield, yield, and drought related traits provides the information (to determine the direction of association (Sunderraj et al., 1972).

Molecular markers can be used in many steps of rice breeding program. Markers are also used to examine parental polymorphism with desirable genes and gene combinations. This approach has the potential to make parental selection more efficient, to expand the gene pool of modern cultivar and to speed up the development of new varieties. Lang and Buu (2008) studied that the

markers RM201 and RM328 were linked to drought-tolerant traits. Under drought stress treatment, it was confirmed that this root length QTL with target segment on chromosome 9 was segregated in the BC population of OM1490/WAB 880-1-38-18-20P1-HB; OM1490/WAB881 SG9, and OM4495/IR65195-3B-2-2-2-2 (Lang et al., 2013). If BC_1F_1 generation more than on individual satisfying the strong condition is found, selection between them can be performed on the basis of analysis of other marker loci to determine the most desirable individual for producing BC_2 (Tanksley et al., 1989). SSR marker, RM219 has been mapped for 3.4 cmRM219 to *sub 1* locus (Xu et al., 2004). Rathnayake et al. (2012) studied that 220 bp of allele of RM219 was used as diagnostic alleles or gel bands to monitor Sub-1 in IRRI119/Bw363 cross. For Swarna variety, a combination of three QTLs ($qDTY_{1.1}$, $qDTY_{2.1}$, and $qDTY_{3.1}$) was pyramided *Sub1*, the large effected QTL for tolerance of submergence (Kumar et al., 2014).

Exploitation of the initial materials are very important in breeding. Based on the drought and submergence tolerant gene are multi-gene, therefore evaluation of initial materials to select the parents serving studies of hybridization is urgently to select good hybrid material for achieving targets in breeding. Currently, at least one popular determined the usefulness of the two markers (RM201 and RM219) for selection both of submergence and drought tolerance genes. These evaluated lines with genotypes will be reference to pick for the next generation. At the same time, phenotypic testing of final products of the MAS exercise needs to be performed in order to confirm the transfer of QTL.

Conclusions

Most of the above traits showed that traits as root length, the number of panicles/cluster, and a number of filled grains/panicles at harvest had a strong and positive correlation with grain yield. Based on path analysis, trait number of filled grains/panicles, the number of filled-grain/panicle, and harvest index had strong and direct positive effect correlation with grain yield.

The present study established the utilization of marker assisted selection for developing new varieties by combinations between drought and submergence tolerance. Fortunately, both *qDTY* and *Sub1* can be combined in the same variety. These best lines will be used for development of further breeding. This type of variety was approached as a first step to develop new varieties for gathering genes of drought and submergence tolerance.

Abbreviations

MAS, Marker-assisted selection; **MABC,** marker-assisted backcrossing; **IRRI,** International Rice Research Institute; **SES,** standard evaluation system; **BC,** backcross.

Conflict of Interests

The authors have not declared any conflict of interest.

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Current status of fodder production, conservation and marketing in the arid and semi-arid lands of Tharaka Nithi County, Kenya

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The purpose of the survey was to document the current status of fodder production, conservation and marketing in the arid and semi-arid land (ASAL) Divisions of Tharaka Nithi County, Kenya. The survey covered specifically Nkondi, Igambang'ombe and Tharaka Central divisions. A sample of 74 livestock farmers selected through stratified random sampling was engaged in the study. The study adopted a descriptive research design and data was collected using a structured questionnaire to obtain farm level information from livestock farmers. The data was analyzed using descriptive statistics and inferential statistics. Chi-square statistics was used to test the relative significance between land owned and fodder production. The majority of the respondents (68%) owned between 1 and 6 acres. The results indicated that most farmers did not grow fodder crops. The main type of fodder produced by farmers in the study area was Napier grass (cultivated by 10% of the respondents). Although a number of livestock farmers grew napier grass, it was not adequate for marketing and conservation. The results further indicated that only 1% of the respondents grew fodder on a piece of land between 1 and 3 acres thus implying that the amount of fodder grown was too little and could not cater for the livestock feeds required. There was a significant association between land sizes and fodder production ($p < 0.05$). Thus preference was given to crop cultivation due to limited land and approximately 80% of the respondents conserved maize stalks and other crop residues for their livestock. Fodder production, conservation and marketing were very low despite the high potential for its production and the possibility of becoming an income generating enterprise. The study therefore recommended for outreach programmes to train farmers on fodder production, conservation and marketing through Chuka University in collaboration with the area extension agents.

Key words: Arid and semi-arid lands (ASAL), fodder production, fodder conservation, marketing.

INTRODUCTION

In Kenya, about, 56% of the rural dwellers live below the poverty line while 48% are food insecure (Government of Kenya, 2004). They derive their livelihood largely from agriculture which contributes 25% of the gross domestic

product (GDP) and provides livelihood to over 80% of the population (Alila and Atieno, 2006). Livestock, on the other hand, contributes approximately 30 and 10% of the agricultural and overall GDP's, respectively. Thus, livestock production plays an important role in the national economy, especially in the subsistence and semi-commercial smallholder farming systems, dominated by resource poor farm households to improve their households' food security and livelihoods (Lanyasunya et al., 2005).

Livestock is raised in the mixed livestock-crop system and the arid and semi-arid lands (ASAL) where pastures, fodder crops, crop residues and agro-industrial by-products represent the bulk of animal feed resources in the country. The areas have biannual rainfall pattern with long rains between March and May and short rains from October to December. The rains are unreliable and characterized with periodic droughts. Seasonal variation in the nutrient content and nutritive value of feeds has been reported in most parts which lead to inadequate dry matter (DM) intake and limited organic matter digestibility (McDowell, 1987). High population growth rate, together with the traditional land inheritance norms and the Government's policy of resettlement, have culminated in subdivision of land which exert high pressure on animal feed resources (Zemmelink et al., 1999). Apart from pastures and fodder crops many tropical regions are endowed with leguminous fodder trees and shrubs that are deep rooted for survival during the dry season while providing livestock feed resource base (Abdulrazak et al., 2001). Although, they require little or no cash investment or land taken away from producing food or other crops, the adoption of this technology in feeding systems for ruminants is low mainly due to limited knowledge (Franzel and Wambugu, 2007). Consequently, the scarcity and low quality feed and fodder resources, in addition to the shortage of water, contribute significantly to low production of milk and meat in these regions (Chinogaramombe et al., 2008; Mapiye et al., 2006). On the contrary, many farmers do not know how to produce, conserve and manage fodder, despite the high demand of feeds. However, fodder production and conservation is an appropriate intervention in cushioning pastoralists and agro-pastoralist against the impact of droughts by providing more food and income to improve their livelihoods (African Development Solutions, 2012).

Tharaka Nithi county has a large population of livestock but the productivity of milk and other livestock product per animal is very low compared to other many counties in the country. This is attributed to severe shortage of feeds which affects, the future growth of livestock that can be sustainable primarily through enhanced animal productivity and not on increased number of animals (Tharaka Nithi County, 2013). According to Ministry of Agriculture, Livestock and Fisheries (2013) pasture and browse situation is fair to poor in ASAL divisions and fair in rain fed and mixed farming zones. In view of this shortage, livestock owners in the lower parts of the county access pasture in Meru National Park during the dry spell where they are charged Kenya shillings (KES) 100 (1.1 US$) for cattle and KES. 40 (0.44 US$) for small stock every month. Scarcity of animal feeds has been associated with massive losses of livestock and livelihood assets, despondency and rising poverty (Tharaka Nithi County, 2013). Considering this scarcity of feed / fodder resources, it is important to emphasise on fodder development programmes for augmenting fodder/feed supply, when formulating livestock development strategies. The study aimed at gathering information on the current status of fodder production and marketing within three targeted ASAL divisions in Tharaka Nithi County.

MATERIALS AND METHODS

Description of the study area

Tharaka Nithi County borders the Counties of Embu to the South and South West, Meru to the North and North East, Kirinyiga and Nyeri to the West and Kitui to the East and South East (Figure 1). The County lies between latitude 000 07' and 000 26' South and between longitudes 370 19' and 370 46' East. The total area of the County is 2,662.1 km^2, including Mt Kenya forest which is estimated at 360 km^2. The County is divided into four administrative Sub Counties namely Tharaka North, Tharaka South, Meru South and Maara. The lower altitude is classified as semi-arid. The study mainly focused on 3 areas in the lower altitude region namely Marimanti (Tharaka Central Division), Nkondi (Tharaka Central Division) and Igambang'ombe (Meru South). The region has a bimodal rainfall distribution pattern with the long rains falling between March and May and the short rains between October and December. The average rainfall ranges between 200 and 800 mm per year. The ambient temperatures range between 22 and 27°C, with the lowest temperatures being in July and the highest in January. Temperatures of up to 40°C are experienced at certain periods (Ministry of Agriculture, Livestock and Fisheries, 2013).

Data collection

The study adopted a descriptive research design as it was aimed at describing the status of fodder production and conservation in the County. According to Polit and Hungler (2004) descriptive design describes data and characteristics about the population or phenomenon being studied. A stratified random sampling was used to select 74 livestock farmers in target area. Data was collected using a structured questionnaire to obtain farm level information from livestock farmers on the production, conservation and marketing of forages.

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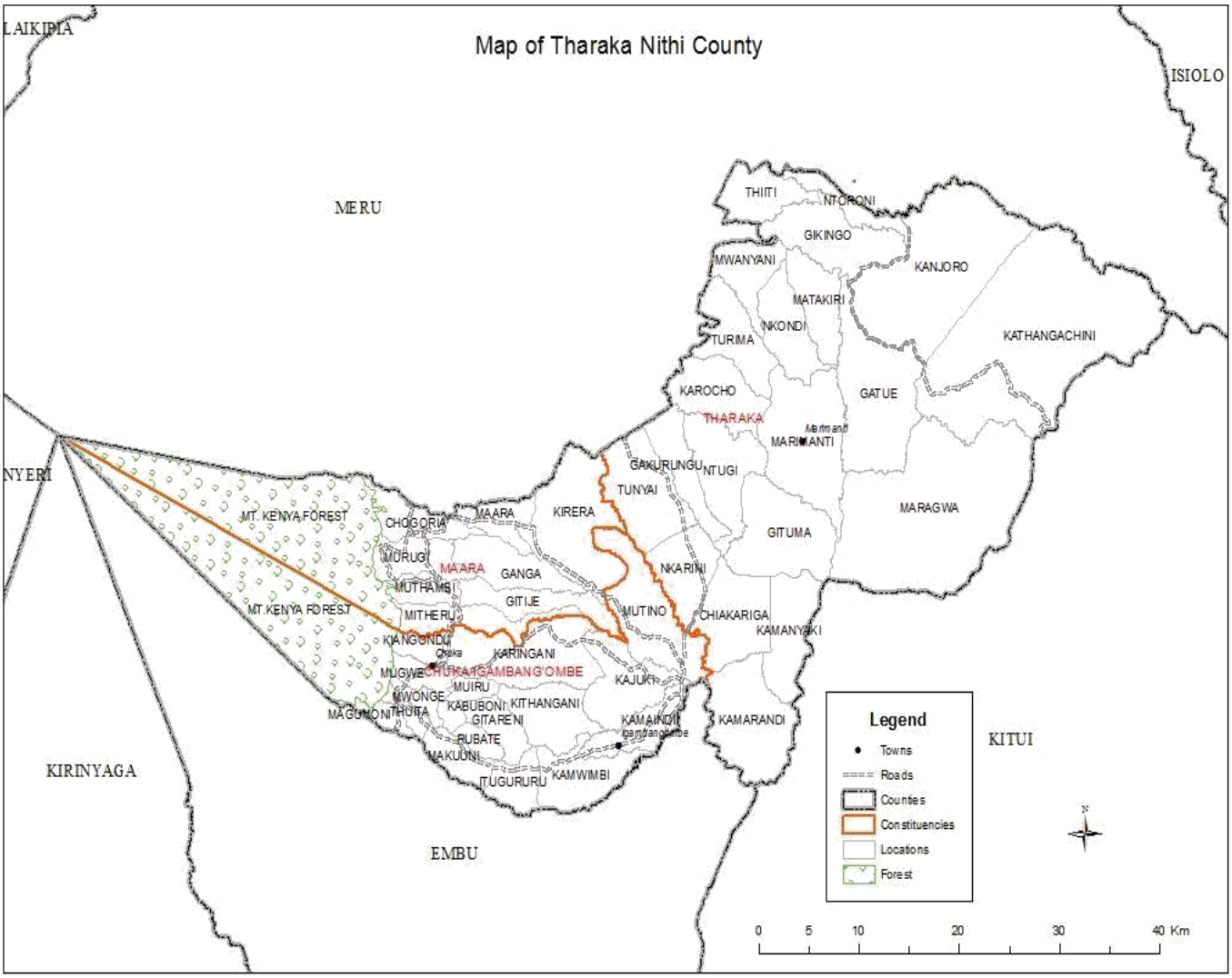

Figure 1. Map of Tharaka Nithi County.

Data analysis

The data were analyzed using descriptive statistics and inferential statistics. Cross tabulation and chi-square statistics was used to test for significance of relationship between land owned and fodder production.

RESULTS AND DISCUSSION

Respondents' characteristics

The respondent characteristics assessed by the study were gender, farm size, land under pasture and under fodder. The results indicate that majority of the respondents (51%) were female while 49% were male. The respondents were from three ASAL divisions where 22% were from Nkondi, 57% were from Igambang'ombe and 21% were from Marimanti.

Farm utilization

Land utilization by farmers is summarized in Table 1. The majority of farmers did not disclose information about their land size and usage and were classified as non responsive. The land sizes ranged from 0.5 to 24 acres. However, the majority of the respondents (68%) owned between 1 and 6 acres. These farm sizes are characteristic of smallholder farms in Kenya (Musalia et al., 2007), whereas there were no farmers who did not spare land for crop production, about 19% did not have land under pasture. The land spared for pasture ranged between 0.25 and 13 acres with the majority of farmers (31%) sparing between ≤1 and 3 acres. Most farmers (34%) allocated between 1 to 3 acres for crops. About 5% of the farmers did not plant any fodder crops as compared to 23% and 1% who planted fodder on ≤1 acre and between 1 to 3 acres, respectively. This implies that

Table 1. Land use in the arid and semi-arid areas of Tharaka Nithi.

Land sizes (acres)	Land under pasture		Land under fodder		Land under crops	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
< 1	10	13.4	17	23	1	1.4
1 to 3	13	17.6	1	1.3	25	33.7
4 to 6	2	2.7	0	0	5	6.8
7 to 9	1	1.4	0	0	0	0
10	2	2.7	0	0	0	0
Non response	46	62.2	56	75.7	43	58.1
Total	74	100.0	74	100.0	74	100.0

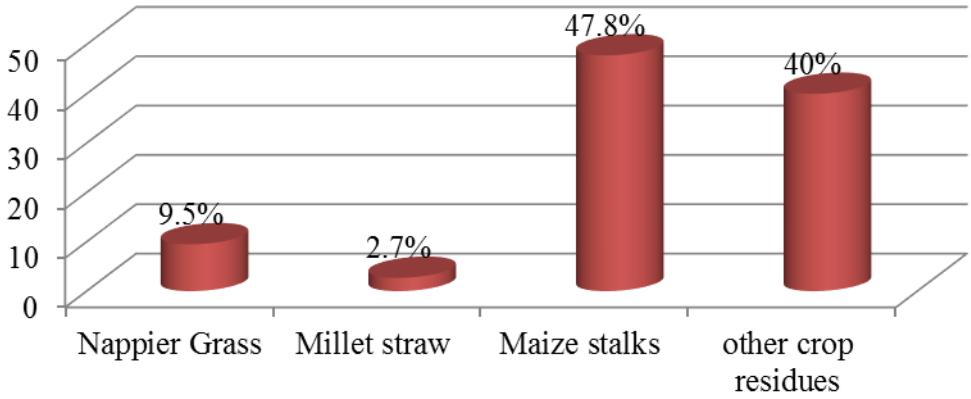

Figure 2. Type of forage fed to animals in arid and semi arid areas of Tharaka Nithi.

the land spared for pasture and amount of fodder grown was too little compared to the acreage spared for crops. Thus, farmers tended to utilize most of the land for crops compared to forage production. This is a typical crop-livestock production system where all farmers in this study planted crops and spared very little land for pastures and fodder production.

Fodder production

The main type of fodder produced by farmers in the study area was Napier grass (*Pennisetum purpureum*), which was cultivated by 10% of the respondents. Napier grass is the major fodder used by smallholder farmers in Kenya (Orodho, 1990). It is estimated to form about 40% of the total dry matter intake in the diet of dairy cattle. However, the cultivation of small amounts of Napier grass in the study area can be attributed to low rainfall and small pieces of land (Glover and Birch, 1962). Apparently, the bulk of feeds for the animals came from the crop residues (Figure 2). About 3% of the respondents used millet straw to feed livestock, 48% used maize stalk and 40% used other crop residues such as beans, pigeon peas (*Cajanus cajan*) and sorghum residues etc. This observation is typical in the crop-livestock farming system where crop residues form a significant portion of the ration due to lack of land that can be spared for pasture and fodder crops (McDowell, 1987). The findings agree with the study of Orodho (1990) who also observed that the smaller the farms the more the contribution of crop residues to the diet of animals. On average crop residues are estimated to provide from 35 to 45% of the total livestock feeds in addition to grazing on the fallow land (Orodho, 1990), which, can go up to 80% in very critical times (Sandford, 1989). These findings are in agreement with Shah et al. (2011) who reported that feed and fodder production and its utilization depend on the cropping pattern, climate, socio-economic condition and livestock type. Apart from the limited land for planting fodder, the study further revealed that one required KES 3000 (32.6 US$) to get cuttings (Napier grass) for planting in 1 acre piece of land. This amount was considered high by most farmers and most probably explains why the production of fodder is too low in the region. Further, the results indicate that 98% confirmed that their animals got enough feeds during the wet season as illustrated in Figure 3. This implies that availability of more feeds during the rainy season can be conserved for use during the dry period. Inadequate supply of feeds during the dry season

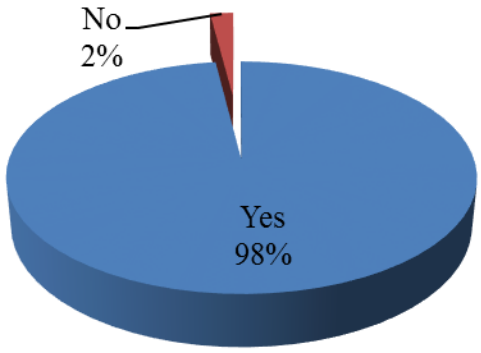

Figure 3. Adequacy of feed during the wet season in arid and semi arid areas of Tharaka Nithi.

Table 2. Chi square test results.

Test	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	59.699[a]	40	0.023
Likelihood Ratio	41.291	40	0.414
Linear-by-linear association	.110	1	0.740
No. of Valid Cases	22		

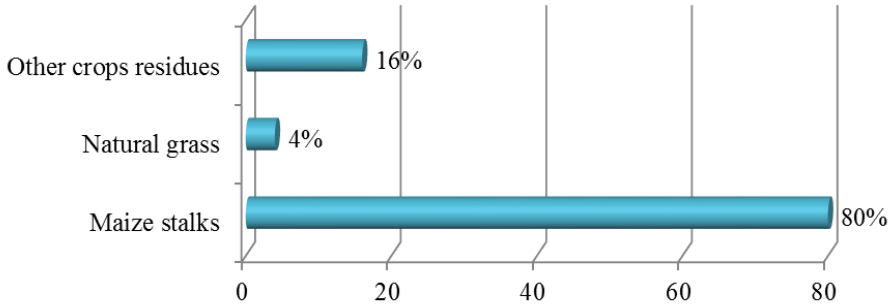

Figure 4. Type of fodder conserved in arid and semi arid areas of Tharaka Nithi.

was expressed by 93%. A chi square test was run to established whether the size of the land influenced the cultivation of fodder. As shown in Table 2, the test yielded a chi square value; χ =59.699 with a high association between land sizes and fodder production ($p < 0.05$). Similar to these findings, Musalia et al. (2007) also observed that farmers with large farms spared more proportion of land for pasture and fodder production. In Contrast, Franzel and Wambugu (2007) found that fodder trees require little or no cash investment or land taken away from producing food or other crops but still most farmers did not grow them. It is estimated that a 250 metre hedgerow of fodder trees like Calliandra calothyrsus, can supplement one dairy cow in a lactation period (Paterson et al., 1998).

Fodder conservation

The study further investigated the kind of fodder that was being conserved, methods used and technologies that were applied in the conservation. Regarding the kind of fodder that was being conserved, 80% of the respondents indicated that they were conserving maize stalks, 16% were conserving other crop residues such as sorghum straw, beans residue and millet straws, while 4% were conserving natural grasses (Figure 4). These findings revealed that maize stalk was the major livestock feed that was conserved by the local community, though other crop residues, and natural grasses were conserved. The higher percentage of maize stalk conservation was due to the fact that the communities practiced mixed

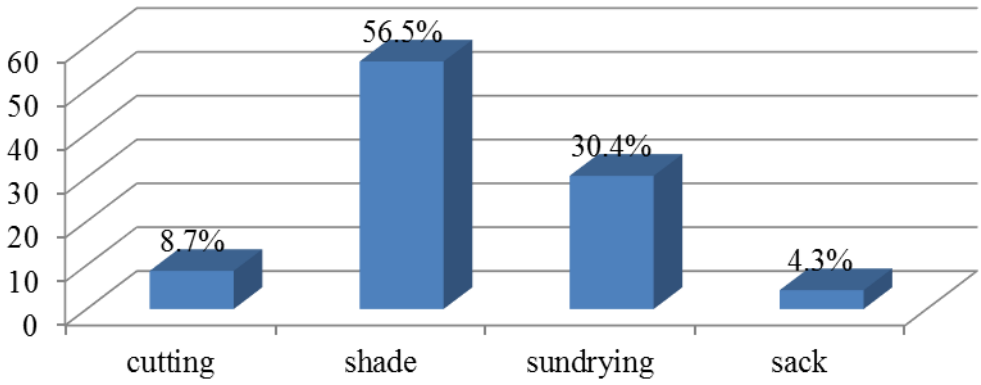

Figure 5. Method used in conservation of fodder in arid and semi arid areas of Tharaka Nithi.

farming whereby maize was considered to be a major staple diet and the residues were used as livestock feeds. These results accord with the work done by Nderi et al. (2014), that mixed farming was common in Igambang'ombe division. Conservation of conventional fodder such as Napier grass was poor probably due to low scale of production which was fed while green thus leaving none for conservation.

Figure 5 also revealed that 57% of the respondents conserved fodder under a shade particularly on tree branches (Figure 6) and makeshift sheds (Figure 7), 30% of them dried fodder under direct sunlight before storing in sacks (4.3%), and only 8.7% of them chopped fodder into small pieces before storage. From these findings, it is clear that the majority of farmers employed traditional methods to conserve fodder. This can be attributed to little knowledge on modern farming methods among the residents which could be due to limited agricultural extension services. This situation, therefore, calls for farmers training on the modern conservation technologies.

Fodder marketing

An assessment of fodder marketing revealed that only 1% of the respondents had produced fodder for sale. This is a clear demonstration that the farmers were not producing adequate fodder despite the potential. Other farmers indicated that they were not producing enough to cater for their livestock needs and sale. Although selling fodder may not be a primary objective of livestock farmers in the County, Ekodere et al. (2014) observed that sustainable fodder production has a significant impact not only on income but also on livestock asset and food security. Furthermore, during periods of drought, lack of fodder is often a major cause of livestock mortality in the ASAL regions such as parts of the study area. As shown in Table 3, 1% of the respondents generated an income KES 500 (5.4 US$), while 1 % generated KES 10,000 (108.7 US$) in one season. However, the majority of the respondents (97%) did not sell any amount of fodder.

Factors influencing price formation and variability

When asked to indicate the factors that influenced price fluctuation and determination, 12% felt that the price changed with seasons while 12% stated that the changes were as a result of demand and supply (Table 4). The outcomes showed that the prices were highly volatile, probably because of the forces of demand and supply. The findings were in accordance with that observed by the Economic Research Service (1999) where the degree of variability in commodity prices is traditionally believed to depend heavily on stock levels and on the nature and frequency of unexpected shifts in demand and supply. Contrary to these findings, Wright (2010) found that fodder prices were also determined by certain quality aspects like good lustre, taste, cleanliness, softness, and moisture contents of fodder other than the market forces. The results also indicate that majority of the respondents (76%) did not know the factors that affected the fodder prices because they had not been involved in the sale of fodder.

Types of livestock kept

An evaluation of the types of cattle kept in the ASAL areas of Tharaka Nithi was also undertaken as presented in Table 5. Results indicate that dairy cattle were mostly kept in Igambang'ombe (54.8%) but they were not popular in Marimanti (0%). There was a similar trend for the local cattle whereby 88% were found in Igambang'ombe and only 1.4% was kept in Marimanti. Generally, it emerged that majority of the farmers reared local breeds as it is practiced by most smallholder farmers (Musalia et al., 2007). However, dairy cattle farming are practiced in the transition agro-ecological zones of the upper parts of Igambang'ombe and Nkondi

Figure 6. Storing hay on a tree in the arid and semi arid areas of Tharaka Nithi.

Figure 7. Storing crop residues in a constructed structure in the arid and semi arid areas of Tharaka Nithi.

Table 3. Income from sales of fodder (Kenya shillings) in Tharaka Nithi County.

Income	Frequency	Percent
500.00	1	1.4
10000.00	1	1.4
Never sold	72	97.3
Total	74	100.0

Table 4. Factors influencing price formation and variability of fodder.

Factor	Frequency	Percent
Seasonal	9	12.2
Demand and supply	9	12.2
Total	18	24.3
Don't Know	56	75.7
Total	74	100.0

where climate is more favorable but at a relatively lower scale. The introduction of modern farming technologies like irrigated fodder crops can spur livestock production in ASAL where land is plenty, thus enhancing feed security and improving rural livelihood.

Nkondi division reported the highest milk yield of 2.9 L per cow per day (Table 6). This production could be due to the favorable agro-ecological location of Nkondi division and influenced by the lucrative dairy farming enterprises in Meru central region which is an immediate neighbour. The level of milk production in the area is low and animals do not require high inputs in terms of feeds which can be supplied by fodder trees planted as hedgerows (Paterson et al., 1998).

Table 5. Types of cattle kept in the arid and semi-arid lands of Tharaka Nithi county.

Division		Female dairy cattle	Male dairy cattle	Total dairy cattle	Female local cattle	Male local cattle	Total local cattle
Nkondi	Frequency	10.00	4.00	14.00	9.00	13.00	22.00
	%	50.0	36.4	45.2	7.0	18.3	10.5
Igambang'ombe	Frequency	10.00	7.00	17.00	119.00	55.00	184.00
	%	50.0	63.6	54.8	93.0	77.5	88.0
Marimanti	Frequency	0	0	0	0.00	3.00	3.00
	%	0	0	0	0.0	4.2	1.4
Overall	Frequency	20.00	11.00	31.00	128.00	71.00	209.00
	%	100.0	100.0	100.0	100.0	100.0	100.0

N= 74.

Table 6. Milk yield per cow in the arid and semi arid areas of Tharaka Nithi (litres/day).

Division	Average milk yield
Nkondi	2.8571
Igambang'ombe	2.6207
Total	2.6667

Table 7. Goat production in the arid and semi-arid lands of Tharaka Nithi County

Division		Females	Males	Total
Nkondi	Frequency	28	8	36
	%	7.3	7.1	6.3
Igambang'ombe	Frequency	344	103	522
	%	90.3	92.0	91.9
Marimanti	Frequency	9	1	10
	%	2.4	0.9	1.8
Overall	Frequency	381	112	568
	%	100.0	100.0	100.0

The results on goat rearing in the Arid and Semi-Arid Lands of Tharaka Nithi County are presented in Table 7. Among the divisions, Igambang'ombe had the highest population of goats (91.9%) with Nkondi and Marimanti having very low goat populations. The high number of goats in Igambang'ombe can be attributed to availability of the browse forage compared to Marimanti where some parts are bare, thus having low ability to support livestock growth.

The results further indicate that Igambang'ombe Division was leading in sheep production (96.6%), followed by Nkondi (3.4%) which showed a noteworthy difference (Table 8). This trend is similar to what was observed in goat rearing which implies that Igambang'ombe division is favorable for small stock production probably due to availability of feeds and the possibility of farmers relying on animals as an income earner. Sheep were absent in Marimanti, which is drier compared to the other two divisions.

Conclusion

Very few farmers grew pasture and fodder for livestock feeding. The cost of planting materials was high and together with the small pieces of land may have resulted

Table 8. Sheep rearing in the arid and semi-arid lands of Tharaka Nithi.

Division		Females	Males	Total
Nkondi	Frequency	5	0	5
	%	4.8	0	3.4
Igambang'ombe	Frequency	99	44	144
	%	95.2	100.0	96.6
Total	Frequency	104	44	149
	%	100.0	100.0	100.0

in low production of fodder in the region. The main type of feed for animals was crop residues from the crop-livestock production system that favoured crop cultivation to fodder production. The area produced enough feeds for animals during the wet season with scarcity in the dry season. Farmers did not have the technology for conserving excess forage in the wet season. Fodder production, marketing and conservation were very low despite the high potential for its production and demand. In order to improve the productivity of livestock in the ASAL part of the County there is need to improve linkages between fodder suppliers and farmers to stimulate the production and marketing of fodder. Farmers should also adopt modern fodder conservation technologies to reap the benefits of seasonal price fluctuations and maximize the scarcity during drought.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Evaluation of the nutritional value of soaked-boiled-fermented Java plum (*Syzygium cumini)* seed meal for poultry

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Chemical analysis, apparent metabolizable energy (ME_n), and one feeding trial were conducted to evaluate the nutritional value of Java plum seeds (JPS) that had been subjected to a combination of soaking, boiling, and fermentation (SBF). Five broiler starter diets were formulated with the processed Java plum seed meal (JPSM) comprising 0, 80, 160, 240, and 320 g/kg of the diet. The JPS before and after processing contained 910±5.30 and 888±6.10 g DM; 44.2±0.940 and 48.1±1.02 g CP; 886±9.90 and 888±6.54 g NFE; and 13.2± 0.165 and 13.3±0.154 MJ calculated metabolizable energy; 24.4±1.33 and 9.17±0.940 g tannins per kg, respectively. The ME_n value of the processed JPSM was 14.7±0.973 MJ/kg. Feed intake (FI), weight gain (WG), and feed efficiency (FCR) of broiler chicks decreased (R^2 > 0.850) with increasing JPSM in the diet. At 80 and 320 g/kg inclusion, FI, WG, and FCR were depressed by 16.0 and 34.1%, 20.2 and 42.5%, and 4.90 and 12.5%, respectively. Liver, heart, and pancreas weights relative to body weight were not significantly (P > 0.05) affected. However, caecum, gizzard, and intestine weights increased (R^2 > 0.800), while the heart weight decreased (R^2 = 0.772) with increasing JPSM in the diet. At 80 and 320 g/kg JPSM inclusion, weights of caecum, intestine, and gizzard increased by 48.5 and 68.2%, 18.8 and 43.5%, and 9.55 and 19.2%, respectively. Inclusion of JPSM in chick diets adversely (P < 0.05) affected nitrogen retention (NR), nitrogen digestibility (ND), dry matter digestibility (DMD), and excreta water content (EWC). At 320 g/kg JPSM inclusion, NR, ND, DMD, and EWC were depressed by 30.8, 12.6, 0.42, and 2.45%, respectively. No mortality was recorded at 320 g/kg JPSM inclusion. The SBF did not improve the nutritional value of JPS for poultry production.

Key words: Anti-nutrients, broiler performance, nutrient utilization, organ weights, processing.

INTRODUCTION

The Java plum seeds (JPS) are produced by Java plum (JP) tree, belonging to Myrtaceae plant family (Kurt,

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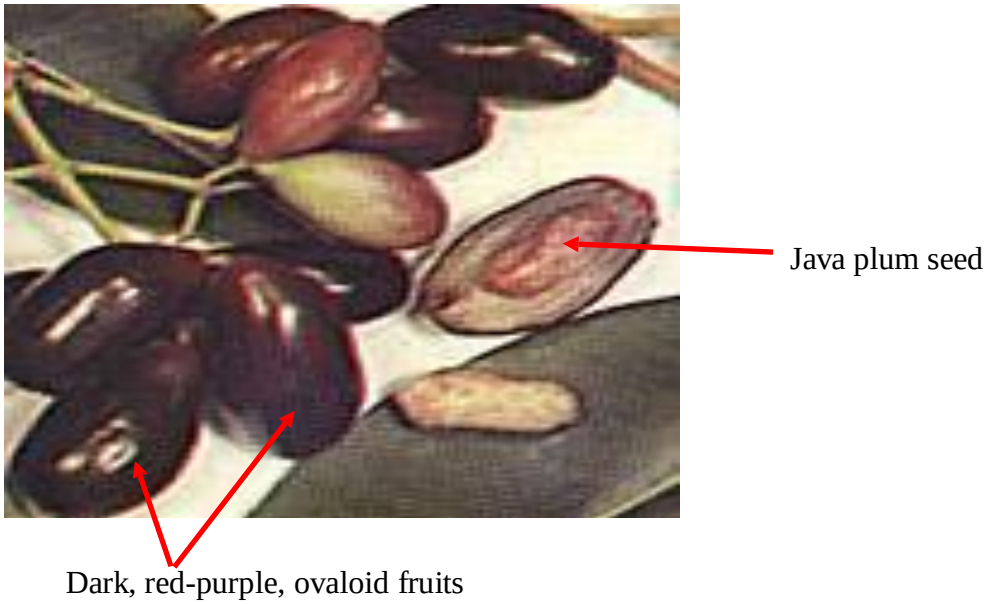

Figure 1. The structure of Java plum fruit and seeds.

2005). The JPS are enclosed in a dark, red-purple, ovaloid fruit (Figure 1). The seeds are mainly dispersed by birds and mammals (Whitinger, 2004). In Uganda, the seeds are dispersed mainly by birds, which eat the fruit pulp and discard the seeds at variable distances from the source (Ndyomugyenyi, 2008). The seedlings from fallen seeds grow naturally under mother trees and thorny bushes, if available in the area, are good cover for the young seedlings (Chhotu et al., 2003). The seeds and leaves of JP are popular livestock feeds in some areas of India (Pankaj, 2003). The JP tree is utilized by humans as food and medicine, and the ripe JP fruit is eaten as a preserve (Okuto and Ouma, 2009; Hutchinson, 2003). The JP fruit pulp is very juicy with a sweet to stringent flavour in poorer varieties and is used to make jelly, jam, squash, wine, and vinegar (Pankaj, 2003). Pods are often fermented to make beer (Chhotu et al., 2003). The seeds were reported to possess anti-inflammatory, anti-arthritic, anti-pyretic, carminative, and astringent properties (Duane et al., 2004; Hutchinson, 2003).

In Uganda, the JPS are unused feed resource and are readily available for livestock feeding. Currently, the JP fruits are mainly eaten by children who climb trees for fun and collect the fruits, which they enjoy eating. However, the JPS left after using the pulp are of little importance and are always discarded as waste (Ndyomugyenyi et al., 2008). The JPS can be widely produced in Uganda, because JP trees thrive very well in a variety of soils including loam, marl, and sandy soils (Morton, 1987). The seeds are a potential energy source, because they are rich in carbohydrates (Pankaj, 2003). Compared to maize with a starch component of 68% (Ewing, 1997), JPS contain 41% starch (Morton, 1987). However, JPS have an advantage of being less costly and less competed for than maize. If the treated JPS meals would replace a larger proportion of maize meal, not only feed costs could reduce, but also competition between humans and livestock for maize.

Despite the availability of JPS, little work has been conducted to include the seeds in poultry diets. An attempt to include Java plum seed meal (JPSM; when JPS were boiled for 50 minutes) in broiler chick diets caused retarded growth of the chicks due to the presence of anti-nutrients (Ndyomugyenyi et al., 2008). Therefore, the ability to include JPS in poultry diets could depend on the processing techniques that eliminate anti-nutrients from the seeds. Although some anti-nutrients in JPS were identified (Ndyomugyenyi, 2008), little effort has been made to eliminate them. In addition, little work has been done to include the adequately processed JPSM in poultry diets. Therefore, this study was conducted to evaluate the nutritional value of soaked-boiled-fermented Java plum seed meal in broiler chick diets.

MATERIALS AND METHODS

Source, processing and chemical analysis of JPS

The JPS were obtained from Wakiso district (00°24′N 32°29′E), Uganda. The seeds were sun-dried and stored in gunny bags on wooden stands until used. The sun-dried seeds were soaked in water at room temperature for 12 h, drained and rinsed once with fresh water, boiled in water at 100°C for 2 h, cooled under shade for 12 h, mixed with fresh water (1 kg of seeds in 65 ml of water), placed in gunny bags, well covered, allowed to ferment for one

Table 1. Composition of broiler starter diets used in the feeding trial (g/kg air-dry basis) (UNGA Farm Care (East Africa) Limited with technical assistance from Frank Wright Limited, part of BASSF Group).

Diets	1	2	3	4	5
Processed Java plum seeds	0.00	80.0	160	240	320
Maize	550	470	390	310	230
Fishmeal (55 g/kg CP)	100	100	100	100	100
Soybean (full fat; roasted)	310	310	310	310	310
DL-Methionine	5.00	5.00	5.00	5.00	5.00
Lake shells	5.00	5.00	5.00	5.00	5.00
Bone ash	20.0	20.0	20.0	20.0	20.0
Salt	5.00	5.00	5.00	5.00	5.00
Vitamin-trace mineral premix[1]	5.00	5.00	5.00	5.00	5.00
Total (10^3 g)	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
Composition of diets (g/kg unless otherwise stated)					
Dry matter	883	885	886	888	889
Metabolizable energy (MJ/kg)	13.4	13.4	13.3	13.2	13.2
Crude protein	216	212	209	205	202
Lysine	13.3	13.1	13.0	12.8	12.6
Methionine	9.48	9.32	9.16	9.00	8.84
Methionine + Cysteine	12.5	12.2	11.9	11.7	11.4
Crude fat	88.0	85.8	83.2	80.7	78.1
Crude fibre	30.0	31.0	31.6	32.6	33.7
Calcium	12.1	12.4	12.6	12.9	13.2
Phosphorus	8.38	8.17	7.97	7.76	7.56
Condensed tannins	0.00	0.734	1.47	2.20	2.93

[1]Premix provided per kg diet: Vitamin A 15,000 I. U., Vitamin D_3 3,000 I. U., Vitamin E 15 I.U., B_{12} 0.013 mg, Vitamin K 4 mg, Riboflavin 10 mg, Folic acid 2 mg, Nicotinic acid 44 mg, Pantothenic acid 13 mg, Biotin 0.064 mg, Vitamin B_1 2.2 mg, Vitamin B6 5.5 mg, Choline Chloride 350 mg, Copper 6.25 mg, Iodine 1.5 mg, Zinc 62.5 mg, Manganese 62.5 mg, Selenium 0.1 mg, BHT (Antioxidant) 100 mg, Zinc Bacitracin 10 mg.

week and then sun-dried. Proximate and mineral compositions were determined using procedures of AOAC (1990). Tannins were determined using modified Vanillin assay method (Price et al., 1978).

Determination of metabolizable energy (ME) of JPS

Metabolizable energy (ME), calculated from chemical composition

The ME of raw and processed JPBM was estimated using the following formula developed by ARC (1977): ME (kcal/kg) = 4.31 x g.dCP + 9.28 x g.dEE + 4.14 x g.dNFE. Digestibility coefficient (d) estimates of 90% for CP, 90% for EE, and 80% for NFE were assumed. In the calculation of ME, it was also assumed feedstuffs did not contain anti-nutritional factors. According to Moughan et al. (2000), in feedstuffs that do not have anti-nutritional factors, digestibility coefficients are numerically the same.

Apparent metabolizable energy (ME_n)

The ME_n of processed JPSM was determined using a modified conventional 4-day total collection procedure of Bourdillon et al. (1990). The ME_n value was corrected to zero nitrogen balance using a factor of 8.22 times the nitrogen retained in the body (Hill and Anderson, 1958). The ME_n per gram feed dry matter = EI - EO - 8.22 N, where EI = Feed intake × Gross energy of feed; EO = Faecal output × Gross energy of faecal; 8.22 = Combustible energy value of uric acid per gram of nitrogen; N = Nitrogen per gram feed - Nitrogen per gram faecal.

Growth assays

One feeding trial that lasted three weeks was conducted to assess the responses of 150 broiler chicks fed varying levels of the soaked, boiled, and fermented (SBF) JPSM. Day-old, Ross strain broiler chicks were randomly distributed into fifteen weld-meshed cages each measuring 1.0 m^2. Five diets were formulated with processed JPSM at dietary levels of 0, 80, 160, 240, and 320 g/kg. Energy supplement was maize while protein supplements were fish meal and full fat roasted soybean meal. The control diet was formulated to meet the nutritional requirements as recommended by NRC (1984). Heat was provided using charcoal via clay pots and 24 h lighting was ensured using kerosene lanterns. The composition of the diets is shown in Table 1.

Determination of nutrient utilization parameters

The excreta (3 samples per treatment) were collected at the

Table 2. Composition of raw and processed JPSM (g/kg DM).

Composition	Raw JPSM	Processed JPSM	Maize
Dry matter	910±5.30	888±6.10	864±4.70
Crude protein	44.2±0.940	48.1±1.02	99.6±3.32
Ether extract	4.00±0.110	4.34±0.0910	40.5±1.24
Crude fibre	34.4±1.20	37.9±1.40	22.6±1.21
Ash	21.7±0.600	8.81±0.200	15.1±0.5
Nitrogen free extract	886±9.90	888±6.54	708±4.52
Sodium	4.30±0.0310	3.30±0.0220	-
Calcium	4.81±0.0420	4.21±0.0250	0.50±0.01
Phosphorus	0.88±0.0110	0.45±0.0130	3.20±0.21
Potassium	8.95±0.930	4.38±0.720	-
Condensed tannins[1]	24.4±1.33	9.17±0.940	-
Calculated metabolizable energy, MJ/kg	13.2± 0.165	13.3±0.154	14.4±0.07
Apparent metabolizable energy, MJ/kg	-	14.7±0.973	14·5±0.046*

[1]Catechin Equivalent. *Cilliers et al. (1994).

end of the feeding trial. The samples were stored in a freezer at 10°C to prevent decomposition or fermentation. The frozen excreta were thawed at room temperature, pooled and homogenized in a blender. The samples of the test feed and fresh excreta were taken for the determination of nitrogen and dry matter using standard procedure of AOAC (1990). The nutrient utilization parameters were calculated using the following formulae:

Nitrogen retention (g) = Nitrogen in the feed - Nitrogen in the excreta

Nitrogen digestibility (g/kg) = Nitrogen in the feed - Nitrogen in the excreta/Nitrogen in the feed × 1000

Dry matter digestibility (g/kg) = Dry matter of the feed – Dry matter of the excreta/Dry matter of the feed × 1000

Excreta water content (g/kg) = Weight of fresh excreta - Oven weight of excreta/Weight of fresh excreta × 1000

Data collection

Body weights of chicks were taken at the start of experiment and at the end of each week for three weeks. All the feed provided was weighed and feed intake (FI) was determined weekly for each replicate. The weekly body weight gain (WG) and FI measurements were used to compute feed efficiency (FCR). Mortality was recorded as it occurred. At the end of the experiment, three chicks from each replicate group were slaughtered to determine organ weights relative to body weight. Cervical dislocation was used to quickly separate the spinal cord from the brain, hence providing a fast and painless death of the birds.

Experimental design and statistical analysis

A Completely Randomized Design was used with three replicates. Each replicate contained ten broiler chicks. Data obtained were analyzed using General Linear Model (GLM) procedures of Statistical Analysis System (SAS, 2001) and regression analysis. Means were separated using Least Significant Difference (LSD) at 5% significant level.

RESULTS AND DISCUSSION

Nutrient composition of JPSM

The nutrient composition of raw and processed JPSM is shown in Table 2. The composition of maize is also included for comparison purposes. The dry matter (DM) and calculated ME of raw and processed JPS were comparable to those of maize. The ME_n of the processed JPS was also comparable to that of maize (Cilliers et al., 1994). However, NFE of raw and processed JPS was higher than that of maize. The NFE of raw and processed JPSM was also higher than the 752 g/kg reported by Ndyomugyenyi et al. (2008). Processing increased CP and NFE contents of JPSM by 8.11 and 0.230%, respectively. The CP of raw JPSM was lower than the 63 to 85 g/kg reported by Morton (1987).

Despite the ME_n of the processed JPSM being lower than that of common energy sources such as cassava meal (14.9 MJ/kg) and wheat (15.1 MJ/kg) (Ewing, 1997), it is still within an acceptable range for use as energy feedstuff. Additionally, the seeds are readily available; face little competition between humans and livestock. Condensed tannins reduced by 62.4% after processing JPS indicating that processing by soaking-boiling-fermentation was not effective in removing tannins from the seeds.

Growth assays

FI, WG, and FCR of broiler chicks decreased (R^2 > 0.850) with increasing JPSM in the diets (Figure 2). At 80 and 320 g/kg inclusion, FI, WG and FCR were depressed by 16.0 and 34.1%, 20.2 and 42.5%, and 4.90 and 12.5%, respectively. Liver, heart, and pancreas

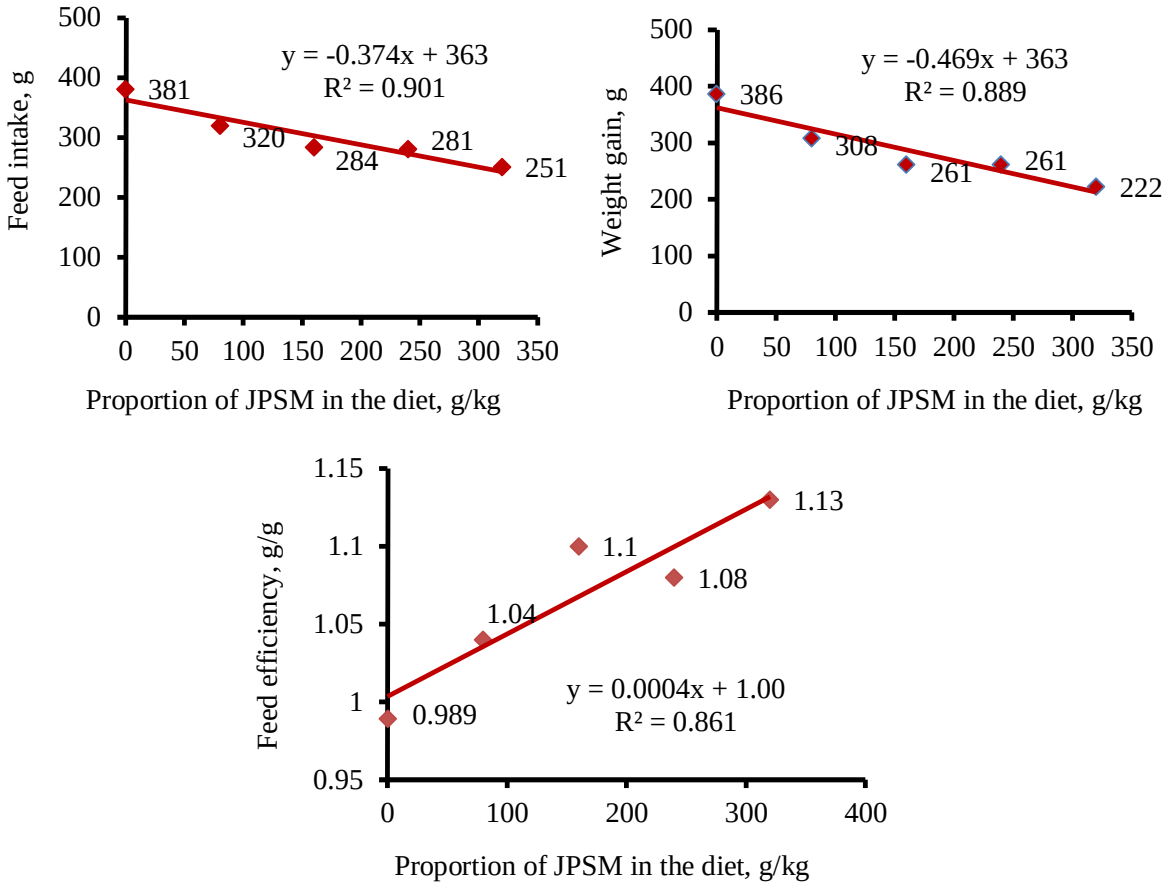

Figure 2. Performance of broiler chicks (0 to 3 weeks) fed graded levels of processed JPSM.

Table 3. Effect of feeding graded levels of processed JPSM on organ weights, nutrient utilization and mortality of broiler chicks (0-3 weeks of age).

Organ weights, g/kg	Processed JPSM inclusion levels (g/kg)						
	0	80	160	240	320	LSD	P
Liver	35.7	32.1	34.6	32.7	33.0	3.65	0.235
Heart	9.18^{a}	9.60^{a}	9.19^{a}	7.93^{ab}	6.81^{b}	1.99	0.0560
Pancreas	7.67^{a}	6.66^{ab}	5.07^{b}	6.12^{ab}	7.64^{a}	1.88	0.0549
Nutrient utilization, g/kg unless otherwise stated							
Nitrogen retention, g	2.21^{a}	2.20^{a}	1.96^{b}	1.91^{b}	1.53^{c}	1.82	<0.0001
Nitrogen digestibility	602^{a}	614^{a}	576^{a}	576^{a}	526^{b}	37.8	0.0039
DM digestibility	711^{a}	690^{abc}	688^{bc}	678^{c}	708^{ab}	20.7	0.0244
Excreta water content	652^{a}	636^{ab}	627^{bc}	615^{c}	636^{ab}	18.9	0.0173
Mortality and cost/kg gain							
Mortality, %	3.33	6.67	6.67	3.33	0.00	-	-
Cost per kg gain (10^{3}), Ugx[1]	2.06	2.18	2.31	2.28	2.39	-	-

^{abcd}Means with different superscripts are significantly different ($P < 0.05$). [1]JPS are locally available; will be obtained at low cost or no cost; Ugx (Uganda shillings)

weights relative to body weight were not significantly ($P > 0.05$) affected by JPSM inclusion (Table 3). However, caecum, gizzard, and intestine weights increased ($R^2 > 0.800$), while the heart weight decreased ($R^2 = 0.772$) with increasing JPSM in the diets (Figure 3). At 80 and 320 g/kg JPSM inclusion,

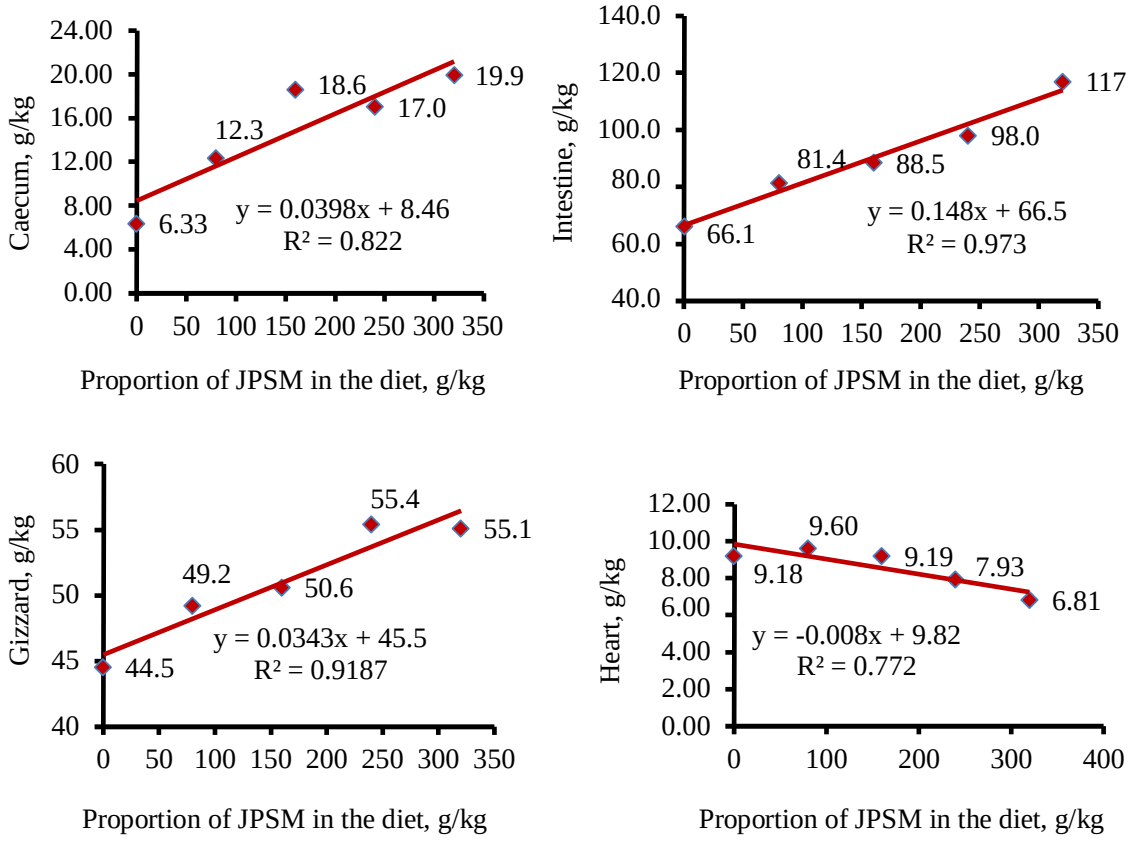

Figure 3. Organ weights of three-week broiler chicks fed graded levels of processed J PSM.

weights of caecum, intestine, and gizzard increased by 48.5 and 68.2%, 18.8 and 43.5%, and 9.55 and 19.2%, respectively. Inclusion of JPSM in chick diets adversely ($P < 0.05$) affected nitrogen retention (NR), nitrogen digestibility (ND), dry matter digestibility (DMD), and excreta water content (EWC) (Table 3). At 320 g/kg JPSM inclusion, NR, ND, DMD, and EWC were depressed by 30.8, 12.6, 0.42, and 2.45%, respectively. No mortality was recorded at 320 g/kg JPSM inclusion. The cost per kg gain of birds increased with increasing JPSM in the diets. The cost increased by 5.5 and 13.8% at 80 and 320 g/kg inclusion, respectively.

The decrease in WG with increasing level of SBF JPSM in the starter diets could be attributed to low FI (Figure 4) and poor nutrient utilization by the birds (Table 3). The low FI was probably due to astringency of JPSM. Tannins were reported to be responsible for the astringent taste and low FI of feedstuffs (Hagerman, 2002; Brown, 2001; Reed, 1995; Van Soest, 1994). According to Hagerman (2002), tannins reduce FI by decreasing palatability and negatively affecting digestion. In the current study, 37.6% tannins remained in JPS after processing (Table 2). Tannins in JPSM could have also caused poor nutrient utilization, hence growth depression of chicks. Tannins form complexes with carbohydrates (Mahmood et al., 2006) and combine with proteins (Teguia and Beynen, 2005; Van Soest, 1994) in the digestive tract thereby negatively affecting their digestibility. Studies on the effect of sorghum tannins on broiler performance (Kyarisiima, 2002; Okot and Mujabi, 2001) also showed that tannins were responsible for growth depression. However, growth depression in the present study could not entirely be attributed to tannins, because tannin content in the chick diets ranged from 0.734 to 2.93 g/kg catechin equivalent (Table 1). Brown (2001) reported that levels of over 5.0 g/kg tannins in poultry diets cause growth depression. Other anti-nutrients reported in JPS such as saponins, alkaloids, phytic acid, oxalates, and triterpenes (Zdunczy et al., 1997) could have also played a role in depressing FI and growth of the chicks. Saponins were reported to significantly affect growth, FI and reproduction of animals (Francis et al., 2002). Saponins also impair digestion of protein and uptake of vitamins and minerals in the gut (Francis et al., 2002). Phytic acid is known to affect protein and lipid utilization (Kumar et al., 2010), because it inhibits enzymes (such as pepsin, amylases, and trypsin) needed to digest food (Coulibaly et al., 2011; Ramakrishna et al., 2006). Oxalates combine with proteins to form complexes that

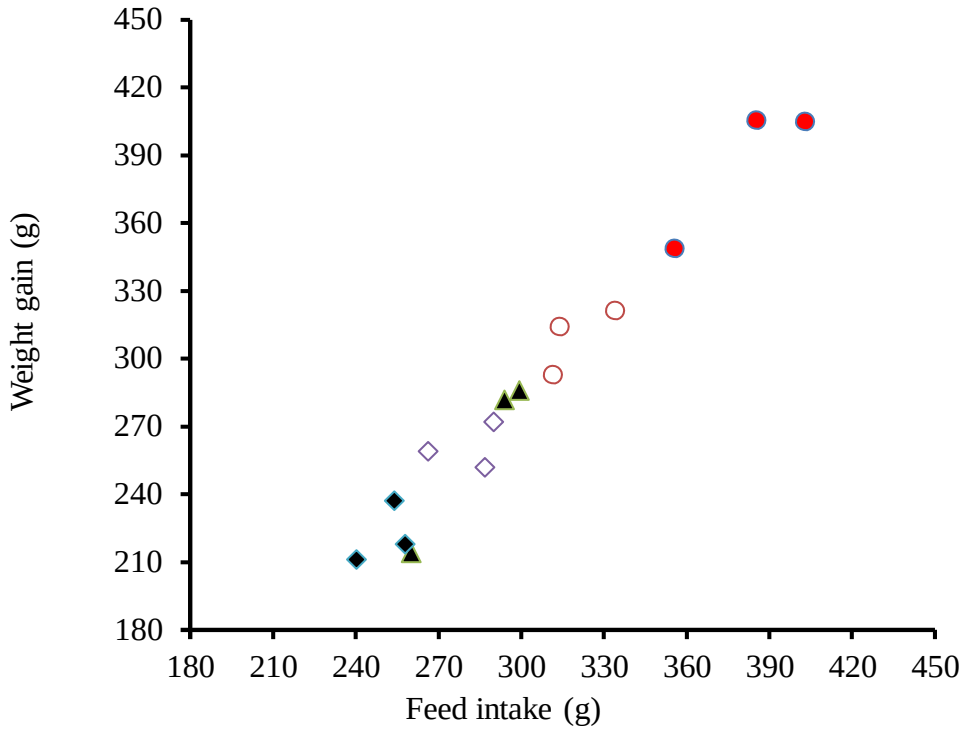

Figure 4. Weight gain versus feed intake of chicks fed on control (●), 80 (○), 160(▲), 240 (◇) and 320 g/kg (◆) processed JPS diets.

inhibit peptic digestion (Akande et al., 2010). The FCR of chicks decreased with increasing levels JPSM meal in the diet probably, because of anti-nutritional factors, such as alkaloids and tannins in meal and the effects of continued consumption of these anti-nutritional factors.

No mortality of chicks was recorded at the highest level of JPSM inclusion (320 g/kg) suggesting that lethal effects of JPSM (Ndyomugyenyi et al., 2008) were minimized by SBF treatment. The cost per kg gain of birds increased with increasing JPSM in the diets, because the seeds were obtained from peri-urban areas at a cost (harvesting and transport costs). However, the seeds are readily available in rural areas and will eventually be obtained at low or no cost. The liver, heart and pancreas weights relative to body weight were not significantly affected suggesting healthy chicks. Gizzard weight increased with JPSM inclusion probably, because of JPSM texture which facilitated the increased rate of contraction of the gizzard. The increase in gizzard weight was also reported when whole maize was used for poultry feeding (Engberg et al., 2004; Gabriel et al., 2007; Lu et al., 2011; Roche, 1981). Increment in caeca weight at higher levels of JPSM could be due to stress exerted on these organs as they attempted to extract nutrients from nutrient-impoverished diets due to the presence of anti-nutrients. The avian caecum is a multi-purpose organ whose functioning can be efficient and very important to a bird's physiology especially during stress periods (Clench and Mathias, 1995). Clench and Mathias (1995) reported that caecal lengths and masses increased when birds were fed on poorer and more fibrous diets. The reason for increment in intestine weight at higher levels of JPSM could not be readily established in the present study.

Conclusions

Including soaked-boiled-fermented Java plum seed meal in diets depressed the performance of broiler chicks. Soaking-boiling-fermentation treatment is not an effective method to improve the nutritional value of Java plum seeds for poultry. Maize remains a better energy source in poultry diets.

Conflict of Interests

The authors have not declared any conflict of interests.

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