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Full Length Research Paper

Vegetation cover affects rhizobia-tree legume symbiosis in the semi-arid region of Brazil

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Biological nitrogen fixation (BNF) is the main form of introducing nitrogen into systems with low fertilizer input, which prevail in the semi-arid region of Brazil. BNF was evaluated in leucena and sabiá grown in samples of a soil collected under different vegetation cover: native vegetation; capoeira (area abandoned for the restoration); agricultural use; sabiá grove; and leucena grove. Nodulation was abundant in soil under all the types of cover. Leucena grown in soil under capoeira exhibited the highest dry biomass of nodules, while the same cover provided the lowest dry biomass of nodules for sabiá. Growth and shoot biomass were higher in plants grown in soil under caatinga. BNF was high in leucena and sabiá, reaching rates higher than 97% in plants grown in soil under capoeira. The highest amounts of fixed nitrogen (N) were found in leucena plants grown in soil under caatinga. It is the first estimate of the potential to fixed N for sabiá and leucena in soils of the semi-arid region, providing an initial estimate of the amounts of N that can be fixed in the field by these tree legumes in the Brazilian semi-arid.

Key words: Indigenous rhizobia, isotope, *Leucaena leucocephala* (Lam.) de Wit, *Mimosa caesalpiniifolia* Benth, N-15 natural abundance method, tropical dry forest.

INTRODUCTION

Shifting cultivation, which is predominant in semi-arid tropical regions, causes a reduction in native vegetation cover due to successive cycles of deforestation, burning, farming and/or overgrazing, and abandonment of the area (Galindo et al., 2008; Menezes et al., 2012; Sousa et al., 2012). These practices may lead to the degradation of natural resources and the reduction of soil fertility, caused by erosion, nutrient export by the harvesting of agricultural products and forage intake by animals (Nunes et al., 2012). Biological nitrogen fixation (BNF) has a key role in maintaining N stocks in these systems characterized by low fertilizer input (Freitas et al., 2015).

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The understanding of the diverse aspects that involve the symbiotic process between rhizobia and tree legumes (occurrence of native rhizobia, nodulation and efficiency of the symbiotic process) in soils of these regions is important to establish an adequate management of BNF.

There are too many limited studies that relate the various aspects of the rhizobia-legume symbiosis with the different types of vegetation cover found in these regions. The first factor to consider is that symbiosis is not established in the absence of native populations of bacteria capable of nodulating certain legume species. Microsymbiont (rhizobia) populations are generally abundant in soils of regions where legume species are native to (Bala et al., 2003). However, in several situations, non-native legumes used in agroforestry systems can also present effective symbiosis (Martins et al., 2015). Due to several edaphoclimatic factors not yet understood, it is also possible that symbiosis is not efficient even in the presence of compatible rhizobia populations (Faye et al., 2007; Silva et al., 2017). Both the growth of free-living rhizobia in soils as well as their ability to nodulate plants and fix nitrogen are sensitive to environmental conditions and may be dependent on soil quality. Different types of vegetation cover or management affect the diversity of rhizobia (Guimarães et al., 2012), and may favor more or less efficient populations differently (Calheiros et al., 2013; Santos et al., 2017).

Sabiá (*Mimosa caesalpiniifolia* Benth.) and leucena (*Leucaena leucocephala* (Lam.) de Wit) are tree legumes widely used in the recovery of degraded areas and in agroforestry systems in the semi-arid region of Brazil (Silva et al., 2016). These species present characteristics of rusticity, rapid growth, high biomass production and especially the ability to establish symbiosis with rhizobia (Chaer et al., 2011). Soils of the Brazilian semi-arid region harbor populations of bacteria that nodulate *M. caesalpiniifolia* and *L. leucocephala* and apparently vary in symbiotic efficiency, thus affecting nodulation and plant growth (Silva et al., 2016). However, there are no estimates of N rates fixed by leucena and sabiá in soils of the semi-arid region. Other tree legumes can obtain more than 50% of their nitrogen requirements through BNF in this region (Freitas et al., 2010).

The aim of this study was to estimate the efficiency of the rhizobial populations that nodulate sabiá and leucena in a soil of the Brazilian semi-arid region under different vegetation cover by determining symbiotic parameters and estimating the contribution of symbiosis to plant nitrogen nutrition.

MATERIALS AND METHODS

Seedlings of leucena (*L. leucocephala* (Lam.) de Wit) and sabiá (*M. caesalpiniifolia* Benth.) were grown in pots containing samples (2.0 kg) of a Luvissolo Crômico (Embrapa, 2011) Luvisols or Aridisols in the FAO and American classifications, respectively located in the city of Serra Talhada (7°59'7" S and 38°17'34" W, 443 m altitude, average annual rainfall of 686 mm and average annual temperature of 23.8°C), Brazil. The soil samples were collected in the surface layer (0.0-0.2 m) in areas located under the same soil patch, but with different vegetation cover: (1) native vegetation (preserved caatinga, the Brazilian dry forest, with no history of clear cutting for agricultural use); (2) capoeira (area abandoned for restoration of natural fertility after consecutive cycles of intercropped corn, *Zea mays* (L.), and cowpea, *Vigna unguiculata* (L.) Walp.); (3) agricultural use (intercropping of corn and cowpea); (4) sabiá grove installed 20 years ago; and (5) leucena grove installed 20 years ago. In each area, we obtained composite samples from 5 simple samples collected at randomly marked points in the field. The composite soil samples were air-dried, broken down, homogenized and passed through a 4 mm mesh sieve. In each composite sample, subsamples were collected to determine pH (water); P (Mehlich-1); exchangeable K^+, Na^+, Ca^{2+} and Mg^{2+}, total organic carbon (TOC); and sand, silt and clay ratios (Table 1) (Embrapa, 2011).

A greenhouse experiment was conducted using a completely randomized design with three replicates in a 2 × 5 factorial arrangement (two tree legume species and five types of vegetation cover). Extra pots containing soil samples from the 5 areas with different vegetation cover were grown with *Bauhinia cheilantha* (Bong.) Steud. and *Senna spectabilis* (DC.) H.S. Irwin & Barneby, which are non-nodulating tree legumes (Allen and Allen, 1981) from the Caesalpinioideae sub-family, used as reference plants to estimate the contribution of BNF in leucena and sabiá.

Prior to sowing, the seeds of the legumes were subjected to thermal shock, with water at 80°C for 15 min, followed by immersion in water at room temperature for 12 h to break dormancy. The seeds were then disinfested with 95% ethyl alcohol for one minute, immersed in 1% sodium hypochlorite for 2 min and washed 10 times with distilled and sterile water. At the time of sowing, four seeds were sown in each pot with 2 kg of soil, and after seven days, one plant was left per pot.

Plants were harvested 100 days after planting. The plants were then separated into shoots and roots from which all nodules were collected. All material was dried in an oven at 65°C for 72 h and weighed to determine dry biomass and the number of nodules was counted. All samples of the plant shoots were ground to fine powder. A subsample was placed in a capsule and loaded into a Thermo Quest-Finnigan Delta Plus isotope ratio mass spectrometer (Finnigan-MAT; CA, USA) interfaced with an Elemental Analyzer (Carlo Erba model 1110; Milan, Italy) at the Laboratory of Isotope Ecology (CENA-USP, Brazil) to obtain the nitrogen isotope ratio and the total nitrogen content of these samples. Stable isotope ratios of nitrogen were measured according to the internationally recognized standards. Internal reference materials (atropine, yeast and soil standard no. 502 - 308 from LECO Corporation) were included in every analytical run. The concentrations of ^{15}N were expressed in δ units in relation to the international standard (atmospheric N_2), based on the equation

$$\delta = \left(\frac{\text{Rsample}}{\text{Rstandard}} - 1\right) x\ 1000$$

where R_{sample} and $R_{standard}$ are the ratio ^{15}N:^{14}N of the sample and the standard (air), respectively.

Estimates of the percentage of nitrogen derived from the atmosphere (%Ndfa) were done whenever the $\delta^{15}N$ signals of leucena and sabiá were significantly different from the mean signal of the reference species (Högberg, 1997). The equation proposed by Shearer and Kohl (1986) was used to calculate %Ndfa:

$$\%\text{Ndfa} = [\left(\frac{\delta 15\text{N(reference)} - \delta 15\text{N(fixing}}{\delta 15\text{N(reference) B}}\right) x 100]$$

where $\delta^{15}N_{(reference)}$ is the mean value of $\delta^{15}N$ of the reference plants

Table 1. Chemical and physical attributes of soil samples of Luvissolo Crômico under different types of vegetation cover.

Attribute	Vegetation Cover				
	Native Vegetation (caatinga)[1]	Capoeira[2]	Intercrop[3]	Sabiá grove[4]	Leucena grove[5]
pH in water (1:2.5)	7.22	7.17	7.03	6.36	6.34
P - Mehlich -1 (mg dm^{-3})	51	54	52	55	61
K^+ - Mehlich - 1 (cmolc dm^{-3})	0.61	0.56	0.71	0.57	0.7
Na^+ - Mehlich - 1 (cmolc dm^{-3})	0.42	0.36	0.41	0.37	0.41
Ca^{2+} - 1 mol L^{-1} ($cmol_c$ dm^{-3})	4.02	2.78	2.68	3.81	3.68
Mg^{2+} - 1 mol L^{-1} ($cmol_c$ dm^{-3})	2.76	2.41	2.46	3.12	2.46
Al^{3+} - 1 mol L^{-1} ($cmol_c$ dm^{-3})	0	0	0	0	0
TOC (dag kg^{-1})	9.61	6.41	5.67	10.8	9.77
Sand (dag kg^{-1})	38	48	54	47	44
Silt (dag kg^{-1})	26	20	18	27	26
Clay (dag kg^{-1})	36	32	28	26	30

(1) Native vegetation (preserved caatinga, with no history of clearcutting for agricultural use); (2) capoeira (area abandoned for the restoration of natural fertility after cultivation with consecutive cycles of intercropped corn, *Zea mays* L., and cowpea, *Vigna unguiculata* (L.) Walp.); (3) area under agricultural use (intercropping of corn and cowpea); (4) area with sabiá grove installed 20 years ago; and (5) area with leucena grove installed 20 years ago.

(*B. cheilantha* and *S. spectabilis*) grown in pots containing soil collected under each of the 5 types of vegetation cover, $\delta^{15}N_{(fixing)}$ is the mean value of $\delta^{15}N$ of each legume for each vegetation cover and B is the $\delta^{15}N$ value of the species when grown with atmospheric N_2 as the sole N source (no soil N). The B value of -1.24‰ determined by Reis et al. (2010) for *M. caesalpiniifolia* Benth was used in the present calculations.

N content in each nodulating legume was determined by multiplying the content of the element by the corresponding biomass. The amount of N fixed in plant shoots was estimated by multiplying the value of %Ndfa by the N content of each fixing species.

Data from soil analysis were tested for normality and variance of homogeneity and then submitted to analysis of variance, considering subsample values as replicates and a completely randomized design. For the pot experiment, biomass and nodule data were submitted to analysis of variance, considering a completely randomized design, and comparing the averages by the Tukey test at 5%. Foliar $\delta^{15}N$ values of each nodulating species, in each area, were compared to those of all reference plants of the same area using the T test.

RESULTS AND DISCUSSION

The seedlings of leucena and sabiá produced the highest shoot biomass and accumulated the most N when grown in soil samples of the area with little anthropic influence, covered with native vegetation (caatinga) (Table 2).

Microsymbiont populations capable of nodulating both tree legume species are naturally established in the soil, regardless of vegetation cover (Table 3). However, different responses to the type of vegetation cover were observed for leucena and sabiá in terms of natural nodulation, although all the seedlings exhibited symbiotic nodules. The number of nodules (average of 84 nodules $plant^{-1}$) did not significantly differ between the sabiá seedlings grown in soil under the different types of vegetation cover ($p \leq 0.05$). The smallest biomasses of nodules (40 mg) were found in plants grown in soil under intercropping and capoeira, and the largest biomass (300 mg) was formed in plants grown in soil under leucena grove.

Leucena nodulation was favored when the soil was covered with sabiá and impaired in plants grown in soil under corn and cowpea intercropping (Table 3). The largest nodules were formed by plants grown in soil under capoeira, but did not differ ($p \leq 0.05$) in size compared to the plants grown in soil under native vegetation (caatinga).

The mean $\delta^{15}N$ values in the leaves of the reference plants (*B. cheilanta* and *S. spectabilis*) were generally high and did not differ between plants grown in soils under different ($p \leq 0.05$) vegetation cover (Table 4). Regardless of the species and the vegetation cover of the soil in which they were grown, all legume plants were isotopically impoverished in relation to the reference plants in at least 3.3‰ (in sabiá seedlings grown in soil under caatinga). Thus, BNF contributed to nitrogen nutrition of both species when grown in soil samples under the five types of vegetation cover, and in most cases accounted for high proportions of plant nitrogen (up to 50%), reaching up to more than 90%. Between the species evaluated in this study, BNF was less important for nitrogen nutrition of *M. caesalpiniifolia*, with an overall average of N derived from the atmosphere of 57% in contrast to 76% in *L. leucocephala*. The maximum proportion of fixed nitrogen in *M. caesalpiniifolia* was approximately 97% in plants grown in soil under capoeira and only 38% in soil under corn and cowpea intercropping (Table 4).

The highest amounts of N were fixed in the symbiosis of leucena (average of 140 mg pot^{-1}), which was

Table 2. Shoot dry biomass, N content and accumulation in the biomass of two tree legumes grown in samples of Luvissolo Crômico under different vegetation cover.

Species	Vegetation Cover				
	Native vegetation (caatinga)	Capoeira	Intercrop	Sabiá grove	Leucena grove
Shoot dry biomass (g)					
Leucaena leucocephala	11.65[a]	8.12[c]	2.58[d]	5.58[c]	6.5[bd]
Mimosa caesalpiniifolia	8.98[a]	4.23[b]	4.17[b]	6.48[ab]	4.75[b]
Total nitrogen content (%)					
Leucaena leucocephala	2.47[a]	2.65[a]	2.56[a]	1.48[b]	2.49[a]
Mimosa caesalpiniifolia	1.97[a]	1.47[b]	1.39[b]	2.23[a]	1.87[ab]
Accumulation of total N in shoots (mg pot^{-1})					
Leucaena leucocephala	296[a]	221[ab]	72[d]	87[cd]	166[bc]
Mimosa caesalpiniifolia	182[a]	65[bc]	62[c]	149[ab]	93[bc]

Means followed by the same letter in the row for the different vegetation cover did not differ by the Tukey test ($p<0.05$).

Table 3. Number and dry biomass of nodules of two tree legumes grown in samples of Luvissolo Crômico under different vegetation cover.

Species	Vegetation Cover				
	Native vegetation (caatinga)	Capoeira	Intercrop (corn + cowpea)	Sabiá grove	Leucena grove
Number of nodules					
Leucaena leucocephala	44[b]	58[ab]	31[b]	115[a]	68[ab]
Mimosa caesalpiniifolia	127[a]	34[a]	85[a]	142[a]	35[a]
Dry biomass of nodules (mg)					
Leucaena leucocephala	650[ab]	730[a]	270[c]	500[bc]	460[bc]
Mimosa caesalpiniifolia	300[a]	40[b]	100[b]	180[ab]	190[ab]
Specific mass of nodules (mg nodule^{-1})					
Leucaena leucocephala	14.7[a]	12.5[a]	8.7[ab]	4.3[b]	6.7[b]
Mimosa caesalpiniifolia	2.3[ab]	1.1[b]	1.1[b]	1.2[b]	5.4[a]

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

approximately more than twice the amount of N fixed by sabiá. Leucena plants grown in soil under caatinga and capoeira were the ones with the highest amounts of fixed N (245 and 218 mg pot^{-1}, respectively). For sabiá, the vegetation cover did not influence the amount of N fixed in plant shoots.

Nodulation and/or BNF efficiency may be constrained by various plant-related conditions, by the microsymbiont, and by soil and climate conditions affecting symbiosis. Rhizobia populations capable of nodulating legumes are generally abundant in soils of regions where the species are native to (Bala et al., 2003). The nodulation of sabiá, a native species of the Caatinga Biome, confirms this assumption and has already been observed in other studies with soils of the region (Reis et al., 2010; Silva et al., 2016). Although leucena is an exotic species native to Central America, its nodulation evidences the presence of microsymbiont populations in the soils of the semi-arid region of Brazil. It is possible that the ability of this legume to establish symbiosis with a wide range of rhizobia species belonging to the *Rhizobium* (Pereyra et al., 2015), *Mesorhizobium* (Rangel et al., 2016), *Sinorhizobium* (Xu et al., 2013), *Bradyrhizobium* (Wang et al., 2006), *Allorhizobium* and *Cupriavidus* (Florentino et al., 2009) genera promotes a greater chance of nodulation in different soil and climatic conditions.

The presence of compatible rhizobia populations and the formation of nodules do not necessarily mean that

Table 4. Natural abundance of $\delta^{15}N$ (‰), nitrogen derived from the atmosphere (%Ndfa) and fixed nitrogen of tree legumes grown in samples of Luvissolo Crômico under different vegetation cover.

Species	Vegetation Cover				
	Native vegetation (caatinga)	Capoeira	Intercrop	Sabiá grove	Leucena grove
$\delta^{15}N$ (‰)					
Reference plants	7.26^{a}	6.32^{a}	5.87^{a}	5.59^{a}	6.63^{a}
Leucaena leucocephala	0.25*	-1.08*	1.37 *	3.37 *	-0.61*
Mimosa caesalpiniifolia	3.98*	-1.94*	3.24 *	2.14 *	1.93*
Ndfa (%)					
Leucaena leucocephala	83^{a}	99^{a}	64^{ab}	42^{b}	92^{a}
Mimosa caesalpiniifolia	39^{b}	97^{a}	38^{b}	51^{b}	27^{b}
Fixed N (mg pot^{-1})					
Leucaena leucocephala	245^{a}	218^{ab}	51^{c}	37^{c}	153^{b}
Mimosa caesalpiniifolia	75^{a}	63^{a}	27^{a}	86^{a}	51^{a}

Means followed by the same letter, lowercase in the row for the different vegetation cover did not differ by the Tukey test ($p<0.05$).

symbiosis will be efficient (Faye et al., 2007; Silva et al., 2017). Both the growth of the free-living rhizobia in soils as well as their ability to nodulate plants and fix nitrogen are sensitive to environmental conditions and may be dependent on soil attributes, such as acidity, aluminium toxicity (Rufini et al., 2011), salinity (Medeiros et al., 2008), phosphorus (Sulieman and Tran, 2015; Silva et al., 2017), and molybdenum availability (Matoso and Kusdra, 2014), high temperatures (Ferrari et al., 1967) and water availability (Zilli et al., 2013). As the samples were collected in adjacent areas with similar chemical and physical attributes (Table 1), differences in nodulation (Table 4) possibly occurred because the different types of vegetation cover or management affect the diversity of rhizobia (Guimarães et al., 2012) and may favor more or less efficient populations differently. Furthermore, the effect of vegetation cover on the microsymbiont populations varies according to the specificities of macrosymbiont (Silva et al., 2016). Leucena was more sensitive to the different vegetation cover, presenting almost three times the number of nodules when grown in soil covered with sabiá grove compared to soil under caatinga (Table 3). Thus, inoculation with previously selected bacteria species could be a strategic method in sites with low and ineffective compatible rhizobia population.

The number of nodules did not explain the proportions of nitrogen fixed by the plants. The lowest fixation rates in leucena plants grown in soils covered with sabiá grove (Table 2) contrast with the highest number of nodules (Table 3). In sabiá, the vegetation cover that provided the highest fixation rate (Table 4) was not the same that presented the highest nodulation (Table 2), indicating that most of the nodules were not effective. Small and non-functional nodules represent a photoassimilate drain (Atkins, 1984). Therefore, the number of nodules may be a variable that inaccurately explains the efficiency of symbiosis.

The results of the isotopic signals allowed accuracy in the estimates of biological nitrogen fixation (Högberg, 1997). The signals of the reference plants were high in every vegetation cover evaluated in this study and differed by more than three $\delta^{15}N$ units from the fixing plants. The differences were more pronounced in *M. caesalpiniifolia* with $\delta^{15}N$ values ranging from 2.63‰ (intercropping) to 8.26‰ (capoeira). This pattern of nitrogen isotopic composition was also found by Freitas et al. (2015) in tree plants of the Caatinga region. In these conditions, the effect of the isotopic discrimination associated with the biological nitrogen fixation process is very small, that is, the impact of using B=0 or B=-1.24‰ in estimates of %Ndfa is only of a few percentage units. Thus, for greater simplicity, we chose to present only the results using B=-1.24‰ (Table 3), which are more conservative, possibly slightly underestimating the amounts of fixed nitrogen.

The high contributions of biological nitrogen fixation to the tree legume seedlings contrasts with several estimates presented in the literature for legumes such as soybean (Oberson et al., 2007), cowpea (Adjei-Nsiah et al., 2008), peanut and faba bean (Herridge et al., 2008). It is known that biological fixation is an important process in acquiring nitrogen by the native tree species of the caatinga under field conditions (Teixeira et al., 2010; Sousa et al. 2012). For example, in natural fragments of caatinga, adult plants of *M. tenuiflora*, a species occurring in dry areas from Brazil to Mexico (Queiroz 2009), can derive more than 80% of their nitrogen nutrition from atmosphere (Freitas et al., 2010), in association with beta proteobacterial rhizobial symbionts (Bontemps et al., 2010; Reis et al. 2010). However, absence of fixation in this species could not be explained by absence of microsymbionts but likely to low symbiosis efficiency due to relatively high N and low P availability (Silva et al., 2017).

There is no data in the literature on the fixation rates of sabiá. Data on fixation by exotic species grown in the Brazilian semi-arid region are very scarce. In agroforestry systems, *Gliricidia sepium*, a no native species, can also fix considerable amounts of N (Ndda > 50%) and contribute to 40 kg ha^{-1} of leaves to the system (Martins et al., 2015).

Conclusions

L. leucocephala and *M. caesalpiniifolia* have the capacity to abundantly nodulate in a Luvissolo Crômico of the Brazilian semi-arid region, but this capacity depends on the type of vegetation cover. Both species exhibited high biological nitrogen fixation capacity (values above 50% in most plants), which in some cases reached 99% of nitrogen derived from the atmosphere. However, fixation is less important for *M. caesalpiniifolia.*

These information are important because it is the first estimate of the potential to fix N for sabiá and leucena in soils of the semi-arid region. In spite of being a study in pots, it provides an initial estimate of the amounts of N that can be fixed in the field by these tree legumes. It is undoubtedly different under field conditions, with all possible limitations, especially water availability, which can reduce the symbiotic potential and biomass production. However, high contribution of the fixed nitrogen to both legumes is an indicator of the great potential of these legumes to fix atmospheric N in agroecosystems in the semi-arid region of Brazil.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Stakeholders' perception of weaver ant's effects on mango fruits quality in Benin

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Weaver ant (*Oecophylla longinonda*) used in biological control of pest, is said to improve the organoleptic quality of protected fruits. This study aims at bringing out stakeholders' perception of weaver ants effect on mango quality. A survey was performed in Parakou (Benin), with stakeholders to assess their perception of mango quality and their opinion about weaver ant's effect on mango quality. Then, the taste and the appearance (performed on unwashed and washed fruits) of three categories of mango: Control mango CM (Mango without ants), ants mango without scale insect (AM) and ants mango with scale insects (AMS) were evaluated by 25 panelists. Survey showed that maturity, appearance, size, non-infestation and firmness were the main criteria used by stakeholders to assess mango quality. Differences between protected and non-protected mango were based mainly on taste, appearance and non-infestation (68.8%; 48.4%; 31.3% of respondents, respectively). Most respondents (88.6%), who used taste to differentiate protected mango from non-protected mango, declared that the former is sweeter than the latter. Some respondents (35.5%) negatively pointed out the presence of scale insects and / or ants marks on the peel of protected mango. All respondents stated that protected mango is non-infested by pest. Similarly, sensory test showed that AMS scored the highest grade (4.5) followed by AM (3.9) and CM (2.8) for the taste (for washed fruits), registered the highest score (3.8) ahead AM (3.2) and CM (2.7). This investigation showed that weaver ants improve mango, taste and appearance. Mango quality changes due to the presence of weaver ants should be investigated.

Key words: *Oecophylla longinoda*, biological control, survey, criteria, Parakou, organoleptic quality.

INTRODUCTION

Mango [*Mangifera indica* L (Sapindales: Anarcardiaceae)] is the eighth most produced fruit in the world (UNCTAD, 2016), with a global production raising from more than 43 million tons in 2013 to nearly 46 million tons in 2016 (FAOSTAT, 2018). In Benin, mango is widely consumed and has a nutritional, social and economic importance especially in central and northern rural parts which are the main production areas in the country (Vayssières et al., 2008, 2012).

As for most of crops, mango production has some constraints. Pest attack by fruit flies [*Bactrocera invadens* (Hendel) (Diptera: Tephritidae) and *Ceratitis cosyra*

(Walker) (Diptera:Tephritidae)] is one of the major problems faced by producers in developing countries with limited resources including Benin (Sinzogan et al., 2008; Adandonon et al., 2009). The use of weaver ants [*Oecophyla longinoda* (Latreille) (*Hymenoptera: formicidae*)] as biological control agent, is one of the pest management methods developed in mango orchard (Ouédraogo, 2011; Vayssières et al., 2009; Sinzogan et al., 2008). Biological control by the use of weaver ants in different horticultural systems (cashew, citrus, mango, etc.) has shown its efficiency and economic benefit in many countries (Australia, China, Ghana, Guinea, and Benin) (Peng et al., 1997; Van Mele and Cuc, 2000; Sinzogan et al., 2008; Van Mele et al., 2009; Offenberg and Wiwatwitaya, 2009). There are two species of weaver ants in the world [*Oecophylla smaragdina* (Fabricius) (*Hymenoptera: formicidae*)] living in Asia and [*Oecophylla longinoda* (Latreille) (*Hymenoptera: formicidae*)] native to Africa,) (Hölldobler and Wilson, 1977; Offenberg and Wiwatwitaya, 2009). Weaver ants are dominant and predatory ants, living in colonies (Déguénon, 2009). Its presence on a plant prevents pests attack (fruit flies, various insects, rodents, bats, etc.) of this plant and its fruits (Van Mele et al., 2009; Adandonon et al., 2009). Even though, weaver ants prey on most insects, they guard scale insects (*Pseudococcidae*) as though they were dairy cattle (Hölldobler and Wilson, 1997). Indeed, weaver ants gather and feed on the honeydew (sugary secretion) that scales insects produce (Ledoux, 1949; Van Mele and Cuc, 2007; Van Mele and Cuc, 2008; Dwomoh et al., 2009). These scale insects live on different parts of the host tree (leaf, fruit, bark).

According to different stakeholders, weaver ants are supposed to improve the quality, especially the organoleptic quality of protected fruit (Barzman et al., 1996; Sinzogan et al., 2008; Van Mele et al*.,* 2009; Olotu et al., 2013). Indeed, many statements in relation to a probable quality improvement of fruit protected by the ants are declared by stakeholders. So, the use of weaver ants could present a comparative advantage from efficiency and economic benefit point of view. This study aims at bringing out Benin stakeholders' view of weaver ants effect on mango fruits compared to their sensory quality.

MATERIALS AND METHODS

To fulfil the study aims, the main quality criteria, used by the stakeholders when choosing a mango, were recorded. Then, their knowledge of weaver ants and its effect on mango quality were assessed. Finally, a sensory test was carried out on protected and non-protected mango to compare results of the stakeholders' view.

Study areas and material

The survey was performed in Parakou municipality, Department of Borgou, Central Region of Benin (Figure 1). The sensory test was performed using mango cultivar "Gouverneur" which presents a commercial importance in Benin. The mango samples used for sensory test come from a mango orchard at Korobourou (9°371 N / 002°6708 E), municipality of Parakou. The orchard of Korobourou is one of the orchards where biological control of mango pest by the use of weaver ants (*O. longinoda*) is done. This orchard is a homogeneous mango orchard with cashew trees nearby.

Survey

Face to face interviews were carried out with stakeholders (18 farmers, 21 women mango-pickers and 51 consumers) in April (mango middle ripening season in Benin) 2012. The survey aimed at identifying various criteria used by stakeholders to appreciate the quality of the mango and assessing their knowledge of weaver ants. Moreover, the survey focused on ants' effect on mango quality and the difference between mango from ant trees (protected mango) and mango from trees without ants (non-protected mango). Respondents were randomly selected from mango orchards (farmers and mango-pickers) and from the city (consumers).
The data collected from this survey were used to determine the parameters to be evaluated for the sensory test.

Sensory test on mango fruits

The sensory test was performed on mango samples, "gouverneurs" cultivar under laboratory conditions. Samples of about 40 fruits per category were harvested from two groups of trees (control and ants trees) in the experimental site of the orchard. For the control treatment, mango trees were not colonized by weaver ants; for the ant treatment, mango trees were colonized by weaver ants at a high level that may assure pest control, but ants' density data were not registered. The trunk of control trees was covered with a band of grease at 50 cm above the ground to prevent ants from climbing these trees. Also, the control trees were pruned so that their longer branches do not touch the branches of other trees around; grasses under and around them were regularly cleaned off. On a tree colonized by ants, we can see some fruits carrying scale insects (Figure 2). During our on-farm research activities we did not encounter scale insect on mango trees which are not colonised by weaver ants. For this reason, during the sensory test, for non-protected mango fruit called control mango, we consider only one type of fruit; but for protected mango fruit called ants mango, we consider two types of fruits: ants mango without scale insects and ants mango with scale insects.

So, for the sensory test, tree categories of mango fruits (control mango, ants mango without scale insects and ants mango with scale insects) have been analysed. Control mango fruits (CM) were picked from control trees, whereas ant mango without scale insects (AM) and ant mango with scale insects (AMS) were picked from ant trees. Mango fruits (40 fruits per category) were randomly selected from the four zones (north, south, east, and west) of trees. All the mango fruits harvested had similar degree of maturity, approximately similar size and were at physiological maturity stage. After harvest, mango fruits were transported to the laboratory. Fruits

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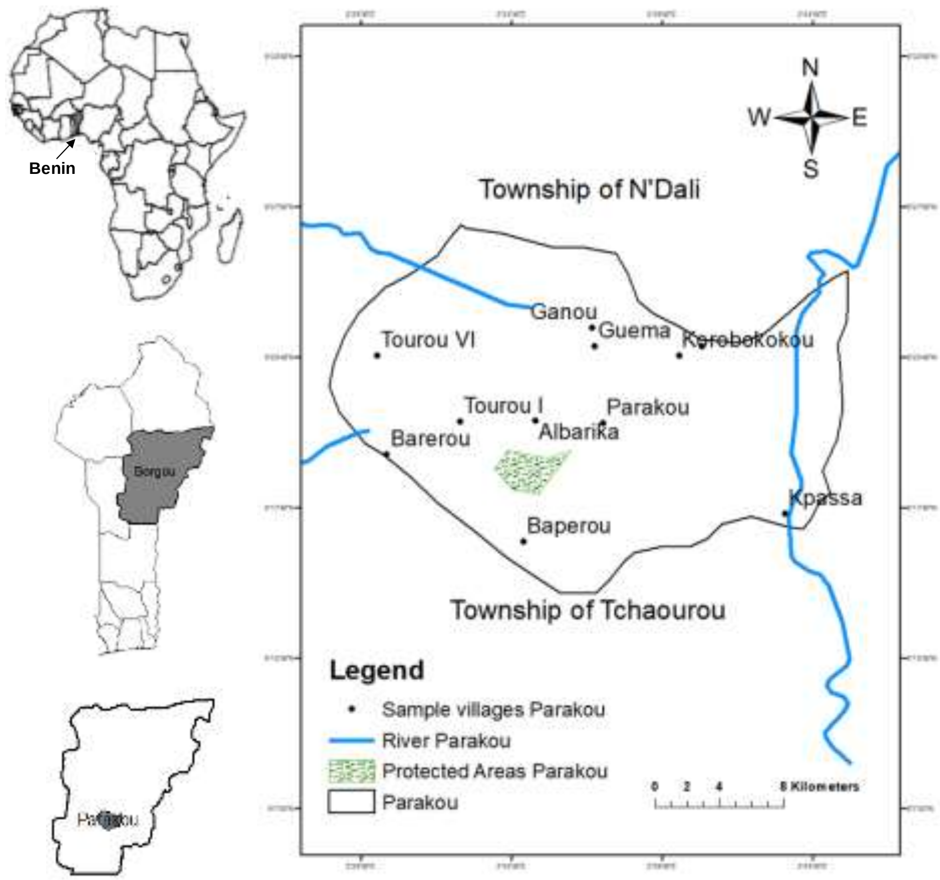

Figure 1. An overview of the study area.

Figure 2. Mango with scale insects and weaver ants patrolling on it for honeydew collect.

Table 1. Score performed by criteria used for assessing mango quality.

Stakeholders	Appearance	Maturity	No-infestation	Size	Firmness
Farmers (n=18)	16	16	16	16	15
Pickers (n=21)	5	17	11	14	11
Consumers (n=51)	22	43	21	15	31
Total (n=90)	43	76	48	45	57

Each value represents the number of stakeholders who mention the criteria.

were allowed to ripe fully (apparent maturity stage) at room temperature (25-28°C) before the test. The taste and appearance of the three categories of mango (Control Mango, Ants Mango, Ants Mango with Scale insects) were evaluated using 25 panellists. The choice of these two parameters (appearance and taste) was based on the survey resuts. Appearance was evaluated on unwashed and washed mango since pickers used to wash mango before exposing it for sale. Unwashed and coded mangoes are presented to the panellists for appreciation. After their appreciation, the same fruits were carefully washed, and re-presented to the panellists for new appreciation. For taste appreciation, a coded slice of each mango sample was presented to the panellists. Sensory quality assessment was realised using hedonic test with quantitative scaling approach value of 1 to 5. For each parameter analysed, each panellist provided a grade ranging from 1 to 5 to each sample; the highest note being attributed to the sample of best quality. At the end of the test, the average score obtained by each sample was calculated for each parameter evaluated.

Statistical analysis

Data collected from the survey were analysed using R system. Chi-square test was performed on contingency tables to see if the perceptions depend or not on the categories of respondents. When in contingency table, more than 20% of cells contain census data less than 5 or 1; the Fisher exact test was used instead of Chi-square test. Test of comparison of proportions was also performed to see if there is significant difference in the respondents' opinions. Sensory test data were analyzed with non-parametric Wilcoxon tests (because of lack of normality and variance homogeneity in the data).

RESULTS

Criteria used by stakeholders to assess mango quality

Different criteria were used by the stakeholders to appreciate mango: maturity, firmness, size, appearance, non-infestation by fruit flies and aroma (to a less extent). Table 1 presents score performed by each criterion according to different stakeholders. The quality criteria used varied according to stakeholders (Figure 3A). Consumers used the maturity as main mango quality criteria. But pickers, on the contrary, rely on the size to choose a mango. But the perceptions on each criteria did not vary among categories informants (P-value>0.05). Indeed, more the mango is fully ripe (Figure 3B), firm (Figure 3C) and big (Figure 3D), more it is of best quality whatsoever is the respondent. Considering all groups of respondents (consumers, pickers and famers), maturity is the first criterion of choice (84.4%). The firmness was the second criterion of choice (63.3%), followed by the non-infestation, size and appearance with 53.3; 50 and 47.8% of the interviewers, respectively.

As each criterion scored different number of stakeholders, proportion of stakeholders (about opinions on each criterion) is calculated based on the score of each criterion. As far as maturity is concerned, 82.9% of respondents (using this criterion as quality criterion) whether they are farmers, pickers or consumers preferred fully ripe mango fruits. When it comes to the firmness, preference was given to firm mango by 80.7% of concerned respondents, while 17.5% of them preferred soft mango. Regarding the size, almost all stakeholders using this criterion prefer a very big or a big mango fruit. Finally all respondents who mentioned the non-infestation prefer mangoes which are not contaminated by pest larva. To summarize, a mango fully ripe, firm, big and exempted of pests is globally preferred by stakeholders. In this paragraph, for each criterion, percentages relate to number of stakeholders using the criterion.

Stakeholders' perception of weaver ants and their effect on mango fruits

The knowledge of weaver ants did not depend on category of respondents (Fisher's Exact Test P-value = 0.8209). Farmers, pickers as well as consumers were aware of the existence of weaver ants. The proportion (94.4%) of respondents who knew about the occurrence of weaver ants is significantly higher than the proportion (5.6%) of respondents who did not know about the existence of weaver ants (P-value < 2.2e 16). In the further course of this paragraph, all percentages were calculated based on number of respondents knowing the existence of weaver ants (total of 85 respondents constituted of 18 farmers, 20 pickers and 47 consumers). When it comes to the perception of weaver ants effect, 75.3% of stakeholders (who knew about the occurrence of weaver ants) admit differences between mango fruits harvested from trees colonized by ants (protected mango) and those harvested from non-colonized trees (non-protected mango). Only 4.7% of them did not point

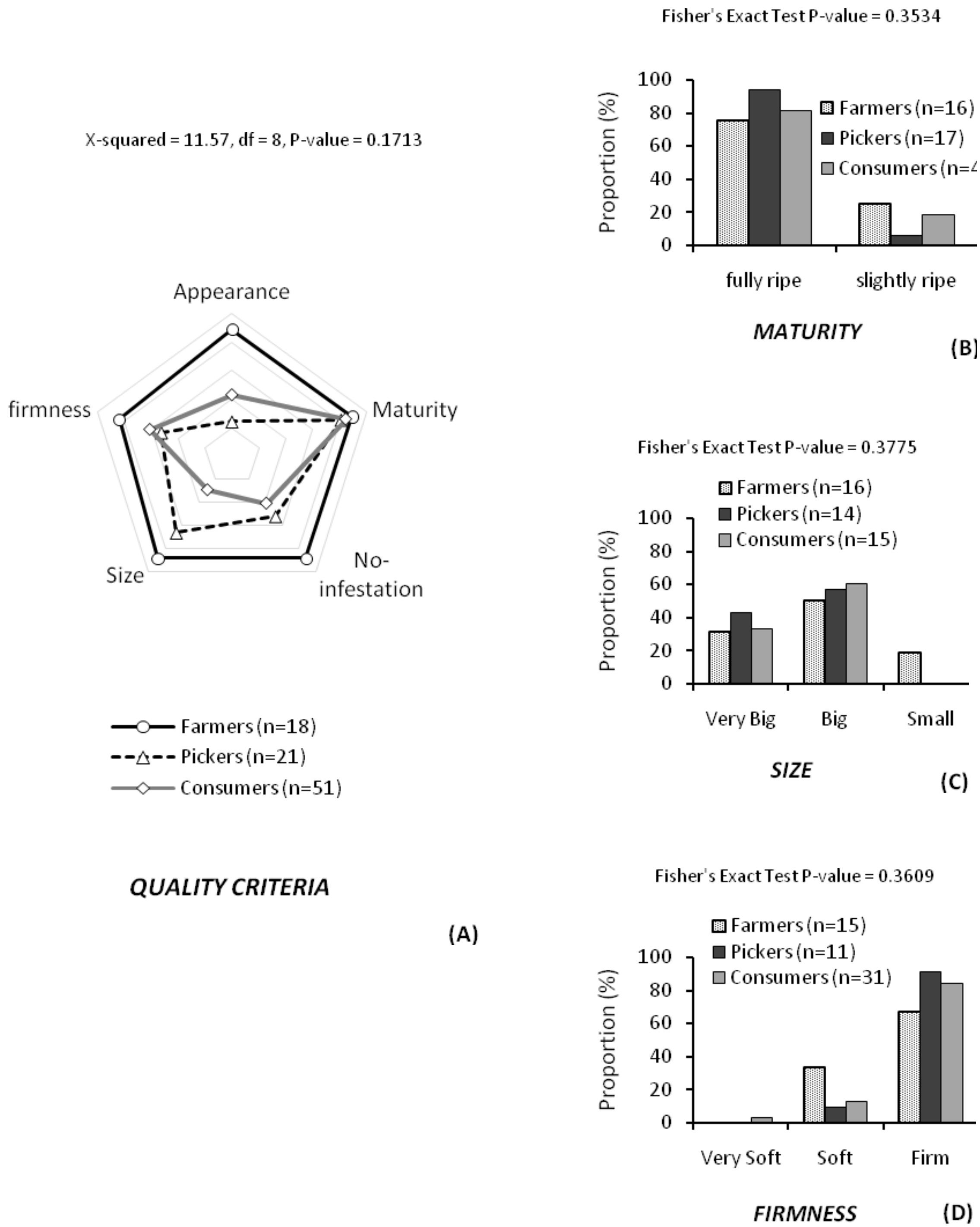

Figure 3. Main quality criteria used by stakeholdersfor mango choice. (A) Importance of criteria according to stakeholders; (B) Stakeholders opinion on mango maturity; (C) Stakeholders opinion on mango size; (D) Stakeholders opinion on mango firmness.

out any difference between the two categories of mango whereas the remaining 20% (constituted only of consumers) were without opinion. Statistic test (Chi-squared = 70.3, df = 2, P-value = 5.348e-16) showed significantly high difference between the proportion of respondents who admitted difference between PM and NPM and the proportion of respondents who did not. Perception of weaver ants' effect on mango fruits (Figure 4) varied according to category of stakeholders (Fisher's Exact Test P-value = 7.291e-05). Whereas all pickers

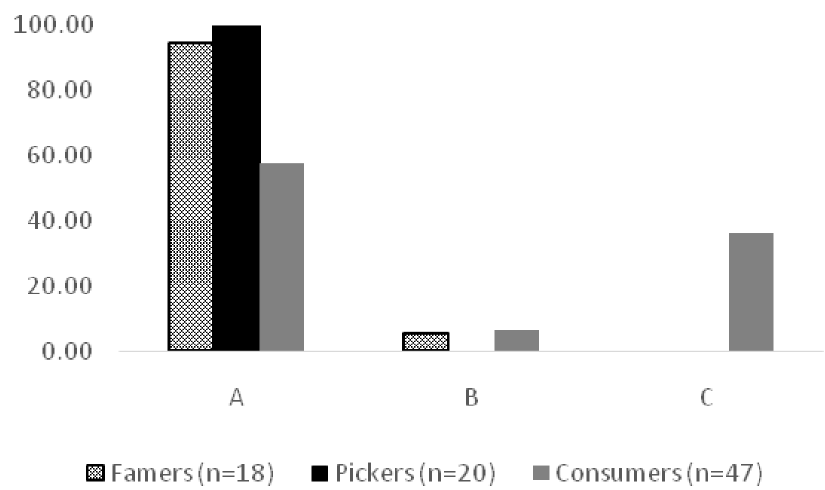

Figure 4. Perception of weaver ants' effect on mango fruits according to different Stakeholders. A: Proportion of different stakeholders admitting difference between protected and non-protected mango; B: Proportion of different stakeholders founding no difference between protected and non-protected mango; C: Proportion of Stakeholders without opinion.

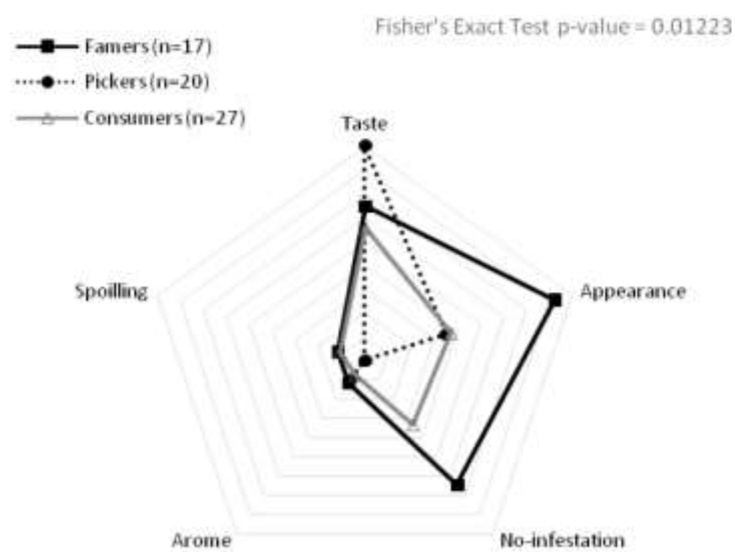

Figure 5. Criteria of difference between protected and non-protected mango.

and farmers admitted that there are differences between protected and non-protected mango, only 57.5% of consumers admitted differences between the two categories of fruits.

Criteria making difference between protected mango and non-protected mango

According to the interviewers, the differences between protected mango (PM) and non-protected mango (NPM) concern mainly the taste, appearance and non-infestation by fruit flies (Figure 5). For non-infestation, respondents mean absence of fruit flies larva inside mango pulp. So in the present study, the term non-infestation refers to absence of fruit flies attack. The criteria of difference perceived varied from one group of stakeholders to another (Fisher's Exact Test p-value = 0.01223). The appearance, taste and non-infestation were the main criteria making the difference between PM and NPM

Table 2. Repartition of stakeholders according to criteria of difference between PM and NPM.

Stakeholders (*)	Taste	Appearance	No-infestation	Aroma	Spoilling
Farmers (n=17)	11	14	11	2	2
Pickers (n=20)	18	7	0	2	0
Consumers (n=27)	15	10	9	2	3
Total (n=64)	44	31	20	6	5

(*) This table concerns only respondents who admitted that there is a difference between PM and NPM.

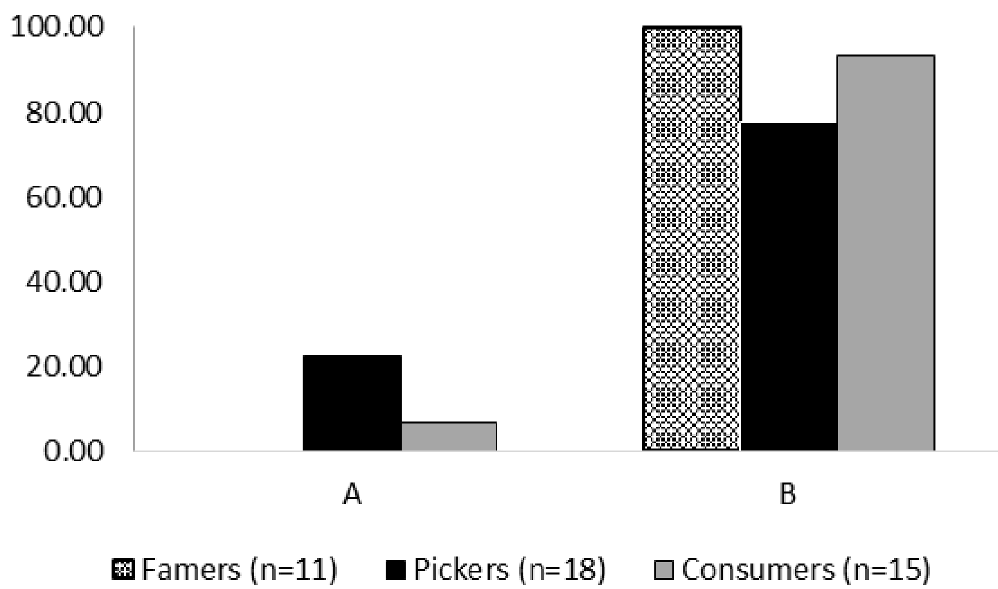

Figure 6a. PM and NPM comparison according to stakeholders using taste as difference criterion
A: Proportion of stakeholders saying protected mango (PM) is less sweet than non-protected mango (NPM). B: Proportion of stakeholders saying protected mango (PM) is sweeter than non-protected mango (NPM)

mentioned by farmers while the taste was the main difference of criterion for pickers. As for consumers, taste was the first criterion of difference followed by appearance and non-infestation. Few respondents mentioned aroma and spoiling as difference criterion.

Globally each respondent mentioned one or several difference criteria. Table 2 presents the numbers of stakeholders admitting difference between PM and NPM according to criteria of difference they mentioned. Considering all stakeholders, taste is the most important criterion of difference between PM and NPM followed by appearance, non-infestation, aroma and spoiling (Chi-squared = 52.396, df = 4, p-value = 1.14e-10). Taste is considered as criterion of difference by 68.8% of respondents who admit difference between PM and NPM; appearance by 48.4%, non-infestation by 31.3%; aroma by 9.4% and spoiling by 7.8% of the respondents. All these percentages relate to number of respondents admitting difference between PM and NPM (64 respondents among which 17 are farmers; 20, pickers and 27, consumers). As taste and appearance were the two most important criteria of difference between PM and NPM, they were used for the sensory test.

Comparison between protected mango and non-protected mango

For the comparison, only respondents who admitted difference between protected and non-protected mango were considered. These respondents mentioned one or several difference criteria. So percentage concerning a criterion is related to number of respondents using that criterion (Table 2). About 88.6% of stakeholders who considered taste as criterion of difference say that protected mango fruits are sweeter than non-protected ones (Figure 6a). Figure 6b shows the opinion of respondents who considered appearance as criterion of

Table 3. Sensory characteristic of mango according to panellists.

Treatment	Appearance		Taste
	Unwashed mango	Washed mango	
Control mango	3.9 ± 0.4[a]	2.7 ± 0.2[b]	2.8 ± 0.2[c]
Ants mango	3.2 ± 0.4[b]	3.2 ± 0.1[a]	3.9 ± 0.2[b]
Ants mango scale	2.6 ± 0.5[b]	3.8 ± 0.4[a]	4.5 ± 0.1[a]
Wilcoxon statistic	Chi-square = 37.5; df = 2; P < 0.0001	Chi-square = 35.9; df = 2; P < 0.0001	Chi-square = 83.5; df = 2; P < 0.0001

For each parameter (in the same column), means with same letter are not significantly different at 5%.

difference. According to 64.5% of these respondents, protected mango fruits have on their peel some marks which non protected mango fruits do not have. Moreover, some of these respondents (35.5%) negatively pointed out the presence of scale insects on the peel of protected mango. On the other hand, all respondents who considered fruit fly infestation as difference of criterion declared that protected mango fruits are not generally infested by fruits flies because they found no or less larvae inside these categories of mango fruits. As for the aroma, only six respondents mentioned it as criterion of difference; and four (04) of them found that protected mangoes have better aroma than non-protected ones whereas the other two said the opposite, claiming that weaver ants leave an unpleasant smell on protected mango fruits. Finally, all respondents (5/5) who evoked spoiling as difference of criterion declared that non-protected mangoes spoil faster than protected mangoes.

Sensory quality of protected and non-protected mango fruits

The organoleptic test realized on control mango (CM), ants mango (AM) and ants mango with scale insects (AMS) showed significant differences among treatments for the appearance (of washed and unwashed mango) and taste (Table 3). Concerning appearance, the panelists preferred control mango to ants mango when the fruits were unwashed. After washing, the opposite trend was observed, with AMS being the most preferred. Similarly, the panelists attributed the highest score to AMS for the taste. They asserted that AMS followed by AM are sweeter than CM.

DISCUSSION

Stakeholders' perception of mango quality and *O. longinoda* effect on mango fruits

This study shows that maturity, firmness, size and non-infestation by pests are the main criteria all stakeholders used to assess mango quality. It occurred that the perceptions of mango quality did not depend on the categories of stakeholders (Figure 3A). So whether they are farmers, pickers or consumers, all respondents perceived mango quality in same way. This similarity of perception may be due to the fact that generally, farmers and pickers are also mango consumers.

Even though the stakeholders perceived mango quality same way, they did not appreciate *O. longinonda* effect on mango quality accordingly. According to the farmers and pickers there is difference between protected mango (PM) and non-protected mango (NPM). As they are directly involved in harvesting and always in contact with orchard, they are used to check the two categories of mango before picking. Most of the people having no opinion about the question of difference between PM and NPM were consumers. This is probably due to the fact that they have no enough contact with mango orchards. Proportion of respondents who admitted difference between PM and NPM was significantly higher than the proportion of respondents who did not. The difference between PM and NPM was differently perceived by the respondents. Farmers' main difference criteria concerned appearance and non-infestation while pickers' were essentially taste and appearance (especially scale insects presence). Consumers use mostly these three criteria. According to the respondents, weaver ants leave some marks and / or small black spots on mango. Previous studies reported two types of marks which are produced by weaver ants on mango fruits: ant marks which are visible and caused by the deposition of formic acid when the ants catch prey (Peng and Christian, 2009), and anal spots which are produced by the ants as territorial pheromones and cues of interspecific competition (Hölldobler and Wilson, 1978; Offenberg et al., 2004; Offenberg, 2007). Farmers are more concerned with appearance and non-infestation certainly because these parameters may affect their income. For example, weaver ant marks (due to formic acid deposit) which affect PM appearance leads to their downgrade in certain countries such as Australia (Peng and Christian, 2009). Non-infestation might reduce post-harvest lost, enhancing then farmers' gain. Pickers are more concerned with taste and appearance difference probably because these criteria may impact their sales as consumers prefer sweetest fruits. Globally, taste, appearance and no infestation are the most difference criteria between protected and non-protected mango used by all categories of respondents (consumers, pickers, famers).

According to respondents, PM is sweeter than NPM and PM are not infested by fruit flies. In Guinea, 57% of producers reported that mangoes protected by *O. longinoda* are sweeter than those unprotected (Van et al., 2009). But appearance of PM is unpleasant to pickers and consumers because of ants' marks and particularly the presence of scale insects. Although this unpleasant appearance, pickers prefer PM with scale insects saying there are the sweetest and are ready to wash them before exposing for sale. They even declare that once washed mangoes with scale insect have better appearance. This quality improvement of fruits protected by *O. longinoda* has been already reported. Sixty percent (60%) of Benin producers (involved in the experimental use of weaver ants for crops protection) believe that *O. longinoda* improves the quality of protected crops in terms of appearance, flavor, and texture (Sinzogan et al., 2008). Also better quality (taste, color) of citrus protected by *O. smaragdina* has been mentioned by the producers of the Mekong Delta in Vietnam (Barzman et al., 1996).

Weaver ant's protection and sensory quality improvement

Sensory test showed a significant difference between tastes of the three category of mango evaluated (Table 3). This result confirmed the better taste of protected mango mentioned by stakeholders during the survey. Likely the respondents' view on protected mango appearance, the sensory test for the appearance of unwashed mango attributed the lowest score to protected mango with scale insects. But once the mango fruits have been washed and the appearance test being repeated, the highest score went to PM with scale insects. So, the appearance test performed on washed mango showed that really weaver ants improve the appearance of mango fruit. But the improvement of the appearance was perceptible only after washing. This may explain pickers' behaviour who despite the unpleasant opinion of the consumers about PM appearance, prefer to harvest PM especially those with scale insects and wash them before exposing for sale. Similar to this result, a better shininess of citrus protected by *O. smaragdina* has also been reported in South Asia (Barzman et al., 1996). Indeed, many statements in relation with possible quality improvement of fruits protected by the ants have been reported by several scientists (Van Mele et al., 2009; Sinzogan et al., 2008; Barzman et al., 1996). Crops quality improvement associated with the use of weaver ants as biological control agent had been assessed through this sensory test for the first time. The concordance between the survey and the sensory test results opens pathway for further exploration to make out other unknown properties of weaver ants. Yet, besides their efficiency in biological control of crops pests, recent studies brought out some properties of weaver ants such as ability to ameliorate pollination by deterring less effective pollinators (Gonzálvez et al., 2013) or ability to slow global warming by boosting CO_2 absorption (Dorn, 2014).

Conclusion

This study highlights the ability of African weaver ants (*O. longinoda*) to improve mango quality. For the first time in Benin, the characteristics of mango fruit as desired by stakeholders have been established. The ideal mango fruit according to the respondents is a mango that is fully ripe, firm, big and exempted of pests attacks. The investigation revealed that African weaver ants (*O. longinoda*) improve mango quality especially taste, appearance and non-infestation. The sensory test confirmed the survey results. This advantage of weaver ants could be used to enhance their acceptability by farmers who are sceptical of adopting them.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Prediction of genetic gain using selection indices in maize lines

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Maize is an important cereal crop in scientific research area and the world economy, where Brazil is one of the leading producers. High production and productivity are partly due to plant breeding programs, where the selection of superior genotypes is based on indexes. This strategy has been very efficient o obtaining superior genotypes since there are simultaneous gains for various agronomic and economic characters. The aim of the research was to measure genetic gain through inbred selection based on the selection index, as well to compare the efficiency of the different index in order to verify which one is more recommended to a phenotypic selection of maize inbreeds. To achieve this, 256 inbreeds were experimentally evaluated in 12 environments. The characters evaluated were grain yield, fecundity, lodging and breaking plant, plant height, ear height, relative position of the ear, male and female flowering and the flowering interval. Selection intensities of 10 and 20% were applied in the direct selection in seven study indexes. The analysis showed that the direct selection of the characters was not effective for the selection of superior maize genotypes. and the Smith and Hazel's index and Williams's index got higher gains yield for the genotypes evaluated.

Key words: direct selection, obtaining hybrids, pure lines, *Zea may* L.

INTRODUCTION

Maize is an important food crop in the world economy and in scientific research. It is grown worldwide and serves as a food source for human beings and animals as well as a raw material for industry (Embrapa, 2015).

According to Conab (2018), the grain production of the Brazilian 2017-2018 crop is estimated at around 87 million of tons, ranking the country in third place for world maize production, ranked only behind the USA and China. The current mean Brazilian productivity is higher than 5,000 kg ha^{-1}, compared to around 3,500 kg ha^{-1} for 10 years ago. This increase has been driven by research in various areas, principally with hybrids. Plant genetic improvement is the most valuable strategy for a sustainable and ecologically balanced increase in production and productivity, combined with the best cultural practices, involving suitable management, fertilizer application and irrigation (Borém and Miranda, 2005). Among the main objectives of research institutions working with genetic improvement in maize is the development and recommendation of lines which are

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good parentes with specific characteristics between male or female in a certain environment and which result in lower costs for hybrid seed production. The selection of superior genotypes in breeding programs are based on index selections and not for each character separately. This strategy has been very efficient since that there are simultaneous gains for various important agronomic and economic characters. Selection indices function like an additional characters which is a result of the combination of various characteristics from which selection responses are desired (Santos et al., 2007), which allows the improvement of various characters simultaneously, independently of the existence or not of a correlation between them (Smith, 1936; Hazel, 1943; Williams, 1962; Cruz and Regazzi, 2001; Vilarinho et al., 2003).

The best selection index depends on the relative importance of the characters that have been considered, the type of material which is being selected and the objectives of the breeding program and, therefore, there can be changes over time. Thus, the most suitable index may be changed, and it is not possible to define the best general index. However, independently of the index under consideration, it is an objective method, which can determine the relative merit of a series of genotypes and thereby provide a basis for differentiating them (Carena, 2009). Therefore, the selection of inbred lines is based on an index can increase the efficiency of the selection process, permitting the selection of genotypes with agronomic standards closer to ideal genotypes (ideotypes) within a shorter space of time (Lande and Thompson, 1990). The aim of the present research was to compare the gains from selection in maize inbred lines considering different selection strategies and observing which one is the most efficient.

MATERIALS AND METHODS

The S1 maize lines were obtained from a crossing between the L14-04B and L08-05F lines, both of which were developed by the Department of Genetics of ESALQ (Escola Superior Luiz de Queiroz) of the University of São Paulo (USP). A random sample of 256 plants was taken from the F2 generation, sown in rows. The manual crossing by the SIB scheme (*Self in Brothers*) were made in each row to increase the quantity of available seeds. This resulted in obtaining 256 S1 progeny, which were evaluated at up to four sites per year between 2002/2003 and 2006/2007 in Piracicaba county in São Paulo state, creating 12 environments.

The experimental design was a simple 16 x 16 lattice, with the plots composed of 4 m of rows with 0.80 m between rows and 0.20 m between plants. Fifty seeds were distributed per plot and plants thinned out approximately 30 days after sowing, leaving approximately 20 plants per each plot. This sowing density corresponds to a mean population of 62,500 plants per hectare. The following data were collected for all plot plants evaluating male and female flowering, on days when 50% of the plants had produced the male and female inflorescences respectively; the stand, considering the number of plants per plot at harvest; lodging, considering the number of plants per plot with an angle of inclination greater than 30° compared to the vertical; breakage, considering the number of plants per plot broken off below the main ear; number of ears per plot; grain weight per plot and percentage grain humidity. Data on plant height and ear height, was measured at distance in centimeters from the soil to the flag leaf and up to the insertion of the first ear respectively, were collected for five plants per plot (Moro, 2011).

The statistical-genetic analyses were made on the following characters: grain production (PG) in t ha^{-1}, lodging and breakage (ACQ) in percentage of lodged plants, fecundity (PROL) in number of ears per plant, interval between flowering (IF) in days, male flowering (FM) in days, female flowering (FF) in days, relative position of the ear (PRE), plant height (AP) in cm and ear height (AE) in cm. The statistical-genetic analyses were carried out using the "PROC GLM" procedure of the SAS software (SAS Institute, 2012). First of all, an individual analysis of variance was made for each environment, according to the mathematical model for lattice experiments:

$$Yijk = m + Li + Rj + bk(j) + eijk,$$

were *Yijk* is the observed value of lineage i in block k, within the repeat j; *m* is the overall mean of the experiment; L_i is the effect of strain i, with i ranging from 1 to 256; R_j is the effect of repetition j, with j being 1 or 2; $b_{k(j}$ is the effect of block k, hierarchical within the repetition j and e_{ijk} is the experimental error. After, the adjusted means obtained from the individual analysis, joint analysis of variance of the experiments were made and based on the mean squares of the joint analysis of variance the following the model $Yijk = m + Li + Rj + (LR)ij + eijk$ were Y_{ijk} is the observed value of lineage i in block k, within the environment j; *m* is the overall mean; L_i is the effect of the lineage i with i ranging from 1 to 256; R_j is the effect of the environment j; $(LR)_{ij}$ is the effect of the interaction of the lineage i with the environment j and e_{ijk} is the average effective error (Cochram and Cox, 1976).

The variance components for each character were estimated by the phenotypic variance for mean values ($\hat{\sigma}_F^2 = QM_L/A*R$), genotypic variance ($\hat{\sigma}_g^2 = (QM_L - QM_{GxE}/A*R$), variance of the genotype × environment interaction ($\hat{\sigma}^2_{GxE} = QM_{GxE}/A*R$), environmental variance ($\hat{\sigma}_E^2 = QM_e$) and coefficient of the herdability in the broad sense for mean values ($h^2 = \hat{\sigma}_g^2/\hat{\sigma}_F^2$) were QM_L is the middle square of the lineages; A is the number of environments; R is the number of repetitions; QM_{GxA} is the middle square of the genotype x environment interaction and the QM_e is the middle square of the residue.

The correlation and covariance analyses were made to be used in calculating some selection indices. After obtaining the means, different selection indices for the lines were constructed using all the characters considered. The selection indices proposed by Cruz (2006); Elston (1963); Smith (1936); Hazel, (1943); Williams (1962); Pesek and Baker (1969); Mulamba and Mock (1978) and Subandi et al. (1973), were calculated. The selection indices and gains were calculated using the Genes Program (Cruz, 2006) and when the weighting coefficients and population parameters were not obtained directly, the values in Table 1 were used. Different selection indices were obtained for each line and after ranking, selection indices of 10 and 20% were applied, selecting the 26 and the 52 best lines respectively. The direct and indirect selection for each character was simulated by estimating the gains with different types of selection (Cruz and Regazzi, 1994).

RESULTS AND DISCUSSION

Significant differences ($p < 0.01$) were observed in the analysis of variance between lines and lines × environments for all the characters analyzed (Table 2). These differences demonstrate the genetic variability,

Table 1. Weighting and ki values to calculate the b coefficient, established for each selection index.

Selection Index	PG	ACQ	PROL	IF	FM	FF	PRE	AP	AE
Cruz (2006)	10%	-10%	8%	-7%	-7%	-7%	-7%	-8%	-7%
Elston (1963)	4.0	3.0	0.95	1.3	71	72	0.8	240	145
Mulamba and Mock (1978)	1	1	1	1	1	1	1	1	1
Pesek and Backer (1969)	10%	-10%	8%	-7%	-7%	-7%	-7%	-8%	-7%
Smith (1936) and Hazel (1943)	1.0	-1.0	0.8	-0.7	-0.7	-0.7	-0.7	-0.8	-0.7
Subandi et al (1973)	1.0	-1.0	0.8	-0.7	-0.7	-0.7	-0.7	-0.8	-0.7
Williams (1962)	1.0	-1.0	0.8	-0.7	-0.7	-0.7	-0.7	-0.8	-0.7

Table 2. Summary of analysis of variance

F V	GL	PG	ACQ	PROL	IF	FM	FF	PRE	AP	AE
A	11	1115.265 **	505.691 **	15.807 **	195.674 **	1590.314 **	1322.312 **	0.410 **	124183.478 **	68906.016 **
P	255	25.256 **	12.695 **	0.350 **	14.530 **	90.954 **	124.47 **	0.010 **	1928.633 **	959.429 **
QM P x A	2805	1.392 **	2.472 **	0.032 **	1.683 **	3.202 **	3.971 **	0.001 **	132.305 **	75.687 **
Effective error	2700	0.590	1.986	0.024	1.379	2.163	2.620	0.001	96.089	56.528
CV (%)	-	19.377	43.479	17.097	116.038	2.055	2.220	4.910	5.202	7.494
Mean	-	4.630	2.516	0.935	1.210	67.694	68.585	0.527	190.899	105.317

PG = production in tons per ha; ACQ = lodging and breakage in percentage of plants; PROL = fecundity in number of ears per plant; IF = interval between flowering in days; FM = male flowering in days; FF = female flowering in days; PRE = relative position of the ear; AP = plant height in cm; AE = ear height in cm; FV = Sources of Variation; GL = degrees of freedom; QM = mean square; A = Environment; P = Progeny; CV = Coefficient of Variation; ** Significant at the 1% level of probability by the F test, respectively.

essential for selection and difference in the development of lines in the experimental environments, indicating that the relative behavior of the lines was inconsistent between the different years. This same variability was observed by Garbuglio et al. (2007) when they evaluated 7 populations of maize lines in the S_1 generation. The coefficients of variation for each character considered are within the ideal limits for this type of genotypes and characters (Hallauer and Miranda, 1988), making the data very reliable.

The principal function of heritability is its predictive role, which expresses the reliability of the phenotypic value in relation to the estimate of the genotypic value (Falconer, 1978). Thus, we can see that the values of the coefficient of heritability are satisfactory and above 80%, also including PG and ACQ which are the two main characters for plant breeders and farmers (Table 3). Values for genetic gain in percentage and unit of the characters for direct and indirect selection are shown in Table 4. Despite the high gains in direct selection, it should be remembered that these values were only obtained for the character in question and when the indirect response to the selection of each characteristic is analyzed, values which adversely affect the selection are apparent. An example is the direct selection for PG which shows a high gain for the character, but the ACQ also increases which is undesirable for breeding programs. These data show that the gains obtained from direct and indirect selections did not produce satisfactory combined results for the 9 characters evaluated. Similar findings obtained by Martins et al. (2006), concluded that direct and indirect selections were not efficient in distributing expected gains when the aim was to do a selection for a group of characters with

Table 3. Estimates of the components of variance.

Parameter	PG	ACQ	PROL	IF	FM	FF	PRE1	AP	AE
σ2G	0.994	0.426	0.013	0.535	3.656	5.021	0.36	74.847	36.823
σ2GxE	0.401	0.243	0.004	0.152	0.519	0.675	0.1	18.108	9.58
σ2F	1.052	0.529	0.015	0.605	3.79	5.186	0.397	80.36	39.976
σ2E	0.59	1.986	0.024	1.379	2.163	2.62	0.706	96.089	56.528
h2	0.945	0.805	0.909	0.884	0.965	0.968	0.905	0.931	0.921

PG = production in tons per ha; ACQ = lodging and breakage in percentage of plants; PROL = fecundity in number of ears per plant; IF = interval between flowering in days; FM = male flowering in days; FF = female flowering in days; PRE = relative position of the ear; AP = plant height in cm; AE = ear height in cm; G = genotypic variance; σ^2G_{xA} = variance of genotype x environment interaction; σ^2F = phenotypic variance; σ^2E = environmental variance; h = heritability coefficient; 1 The parameters: σ^2G σ^2G_{xA}, σ^2Fe σ^2E were multiplied by 1000 due to the low values.

negative and positive gains. Costa et al. (2004) also observed lower gains with direct and indirect selection in soybean lines when compared to other selection methods. Coimbra et al. (1999) achieved satisfactory gains with this type of selection in dry beans and Oliveira et al. (2008) with passion fruit since all the characters under study showed a favorable correlation. According to Falconer and Mackay (1996), indirect selection requires a very favorable correlation between the variable and the study variables as well as high heritability for the character to be selected.

The estimated genetic gains calculated using the index selection method with a selection intensity of 10 and 20% are shown in Table 5. Arnhold and Silva (2009) were positive results for the simultaneous selection of 3 characteristics in sweet corn genotypes were see using the index of Cruz (2006) and gains by Vasconcelos et al. (2010) were higher compared to other selection indices. According to Oliveira et al. (2008), even with high gains using direct selection, the distance genotype-ideotype index was more promising for the selection of superior genotypes. Rocha et al. (2012) oberved that simultaneous selection of 4 characters in *Jatropha curcas,* showed that this index produced a higher total gain and resulted in a more balanced change in the character means. However, despite positive results, the index used by Cruz (2006) did not show the best gains for the characters analyzed.

The index used by Elston gave the best gain for the IF character when compared to other indices. This character shows a high percentage gain due to its low absolute value and to the fact that this index apportions the same weight to all the characters, which may result in an undesirable distribution for line selection. However, Elston's selection index was unable to distribute gains in accordance with the aims established the selection of a single progeny of passion fruit. Martins et al. (2006) observed that the construction structure of the index, which establishes minimum values for selection, showed a tendency to increase characters associated with production in Eucalyptus. Oliveira et al. (2008) observed desired gains using the same index for the primary characters directly correlated with production in passion fruit.

Cruz et al. (1993) and Costa et al. (2004) achieved positive results superior gains with the index proposed by Mulamba and Mock (1978), using the indices of Mulamba and Mock in soybeans and maize respectively. Vilarinho et al. (2003) found that this index gave the best estimates of gains in sweet corn S1 and S2 progenies. Santos et al. (2007) also obtained satisfactory gains in maize with the selection index for families of half-siblings. The use of Mulamba and Mock's index allowed Rangel et al. (2011) to forecast simultaneous gains for the two main characteristics (production and expansion) associated with improvement in sweet corn. The index of Pesek and Backer (1969) showed the highest gain for the 10% selection intensity for the ACQ character compared to other indices. Bárbaro et al. (2007) did not obtain satisfactory results using this index for selecting a soybean genotype. Gonçalves et al., (2007) also observed similar results to Mulamba and Mock's index when selecting superior genotypes of yellow passion fruit. Smith and Hazel's index showed a satisfactory gain for the primary characters PG and ACQ and a superior gain for PG compared to other characters. The results were also satisfactory for the other characters, except for AP and AE, where the gain was positive. This result disagreed with that observed by Gonçalves et al., (2007), who found that Mulamba and Mock's index showed a superior gain, compared to the index of Smith (1936) and Hazel (1943). Paula et al. (2002) showed that Smith and Hazel's index is promising for the improvement of multiple characteristics and better than other selection criteria. Working with 166 families of half-siblings of sweet corn, Granate et al. (2002) obtained higher predicted gains with this same selection index. According to Ferreira et al. (2005), when this index was used for simultaneous selection with weightings obtained after various attempts and it allowed more suitable predicted gains to be estimated for the improvement of *C. canephora*.

Table 4. Estimates of genetic gains in units of the character and percentage using the direct (values diagonally in bold type) and indirect selection methods.

I S		PG	ACQ	PROL	IF	FM	FF	PRE1	AP	AE
	PG	**55.84**	11.71	19.24	-37.66	-4.81	-3.91	1.01	3.65	4.63
		-2.59	-0.29	-0.18	(-0.46)	(-3.25)	(-2.68)	-0.06	-6.98	-4.88
	ACQ	-7.09	**-65.76**	-4.48	-5.14	0.09	0	-2.1	-0.43	-2.6
		(-0.33)	**(-1.66)**	(-0.04)	(-0.06)	-0.06	0	(-0.12)	(-0.82)	(-2.74)
	PROL	39.92	3.59	**33.23**	-28.9	-3.67	-2.9	0.8	2.82	3.69
		-1.85	-0.09	**-0.31**	(-0.35)	(-2.48)	(-1.99)	-0.04	-5.38	-3.88
	IF	11.28	10.03	7.03	**-80.93**	-1.63	-1.88	-0.18	0.56	0.63
		-0.52	-0.25	-0.07	**(-0.98)**	(-1.11)	(-1.29)	(-0.01)	-1.07	-0.67
	FM	39.41	1.03	11.36	-35.32	**-6.47**	-4.86	-1.51	2.17	0.67
		-1.83	-0.03	-0.11	(-0.43)	**(-4.38)**	(-3.33)	(-0.08)	-4.14	-0.71
	FF	46.68	12.43	16.78	-56.33	-6.27	**-5.07**	-0.55	3.64	3.05
		-2.16	-0.31	-0.16	(-0.68)	(-4.25)	**(-3.48)**	(-0.03)	-6.94	-3.21
	PRE	-6.37	-25.43	-8.25	-12.88	-0.87	-0.8	**-9.37**	0.04	-9.41
	(-0.29)		(-0.64)	(-0.08)	(-0.16)	(-0.59)	(-0.55)	**(-0.05)**	-0.07	(-9.92)
	AP	-20.07	-8.66	-11.77	8.87	1.06	0.75	-0.48	**-9.4**	-9.67
	(-0.93)		(-0.22)	(-0.11)	-0.11	-0.72	-0.51	(-0.03)	**(-17.94)**	(-10.19)
	AE	-22.45	-23.31	-13.1	-1.23	0.26	0.2	-6.31	-6.97	**-12.89**
	(-1.04)		(-0.59)	(-0.12)	(-0.02)	-0.18	-0.14	(-0.04)	(-13.31)	**(-13.58)**
	PG	41.63	7.95	16.46	-36.62	-3.7	-3.02	1.11	2.9	**4**
		(-1.93)	(-0.2)	-0.15	(-0.45)	(-2.50)	(-2.07)	(-0.06)	(-5.54)	**(-4.22)**
	ACQ	-4.06	-55.2	-2.27	11.05	-0.01	0.08	-2.23	-0.16	**-2.39**
		(-0.19)	(-1.39)	(-0.02)	-0.13	(-0.01)	-0.05	(-0.12)	(-0.30)	**(-2.52)**
	PROL	29.96	5.34	25.37	-25.59	-2.57	-2.04	0.75	1.35	**2.14**
		-1.39	-0.13	-0.24	(-0.31)	(-1.74)	(-1.40)	-0.04	-2.57	**-2.25**
	IF	14.99	5.09	7.61	-75.44	-1.96	-2.08	-0.08	0.46	**0.43**
		-0.69	-0.13	-0.07	(-0.92)	(-1.33)	(-1.42)	0	-0.88	**-0.45**
20%	FM	31.6	2.2	10.6	-27.09	-5.18	-3.74	-0.54	1.64	**1.18**
		-1.46	-0.06	-0.1	(-0.33)	(-3.51)	(-2.59)	(-0.03)	-3.13	**-1.25**
	FF	31.65	1.31	13.05	-51.97	-4.74	-4.02	-0.47	1.96	**1.54**
		-1.47	-0.03	-0.12	(-0.63)	(-3.21)	(-2.76)	(-0.03)	-3.75	**-1.62**
	PRE	-7.92	-16.79	-5.83	-2.3	-0.32	-0.14	-7.37	-0.16	**-7.54**
		(-0.37)	(-0.42)	(-0.06)	(-0.03)	(-0.22)	(-0.10)	(-0.04)	(-0.30)	**(-7.94)**
	AP	-16.64	-4.53	-8.72	18.01	0.74	0.71	-0.63	-7.63	**-8.11**
		(-0.77)	(-0.11)	(-0.08)	-0.22	-0.5	-0.49	(-0.03)	(-14.56)	**(-8.54)**
	AE	-12.21	-10.35	-7.52	-4.84	-0.33	-0.23	-5.45	-5.35	**-10.52**
		(-0.57)	(-0.26)	(-0.07)	(-0.06)	(-0.23)	(-0.16)	(-0.03)	(-10.22)	**(-11.07)**

PG = production in tons per ha; ACQ = lodging and breakage in percentage of plants; PROL = fecundity in number of ears per plant; IF = interval between flowering in days; FM = male flowering in days; FF = female flowering in days; PRE = relative position of the ear; AP = plant height in cm; AE = ear height in cm; IS = Selection intensity; Values in bold type for gain in the direct selection of the character in question; Values in brackets: genetic gains in units of the character; 1 Gains in unit of the character multiplied by 10 due to low values.

The index of Williams (1962) showed superior gains for the primary characters, PG and ACQ at selection intensities of 10 and 20%. The gains for IF, FM and FF were satisfactory when compared to the other indices. As previously mentioned, the undesirable gain for AP and AE did not adversely affect the selection due to the mean of the original population. Granate et al. (2002) found that estimates of simultaneous predicted gains for 2 characters of interest could not be obtained using the selection index of Williams (1962).

Although Subandi's index showed the greatest gain for PROL, this did not result in satisfactory gains for the group of characters under study. However, research by Bhering et al. (2011), demonstrated that this index showed higher genetic gains for selection of *Jatropha curcas.* Moraes (2006) also found suitable values for gains for simultaneous selection of six characteristics in *Pinus.*

Table 5. Estimates of genetic gains in units of the character and percentage using the index selection method.

IS	Selection Index	PG	ACQ	PROL	IF	FM	FF	PRE1	AP	AE
	Cruz (2006)	32.53	-10.56	17.74	-25.04	-0.49	-0.77	1.20	0.24	1.38
	Elston (1963)	22.61	-25.96	15.37	-58.25	-3.89	-3.43	-1.13	0.32	-0.94
	Mulamba and Mock (1978)	25.75	-21.12	14.91	-50.80	-4.29	-3.51	-4.52	-1.48	-5.92
10%	Pesek Backer (1969)	25.22	-54.88	11.12	-14.95	-2.85	-2.46	-2.40	2.00	-0.52
	Smith (1936) and Hazel (1943)	46.76	-28.65	16.84	-41.46	-4.91	-4.14	-0.50	4.01	3.45
	Subandi et al (1973)	29.34	-6.06	21.47	-18.47	-3.08	-3.45	-3.68	-3.29	-0.04
	Williams (1962)	43.65	-34.16	14.87	-39.25	-4.12	-3.67	-0.32	3.33	2.98
	Cruz (2006)	11.73	-8.57	10.64	-23.67	-1.89	-1.75	0.34	0.44	0.68
		(0.54)	(-0.22)	(0.10)	(-0.29)	(-1.28)	(-0.12)	(0.66)	(0.47)	(1.45)
	Elston (1963)	14.32	-19.85	18.81	-49.62	-1.86	-2.05	0.36	1.59	1.91
		(0.66)	(-0.50)	(0.18)	(-0.60)	(-1.26)	(-1.41)	(0.20)	(3.04)	(2.01)
	Mulamba and Mock (1978)	19.96	-11.79	11.76	-50.47	-3.31	-2.87	-3.64	-1.23	-4.79
		(0.92)	(-0.30)	(0.11)	(-0.61)	(-2.24)	(-1.97)	(-2.00)	(-2.34)	(-5.04)
20%	Pesek and Backer (1969)	15.62	-47.97	6.52	-14.45	-1.93	-1.56	-1.26	1.58	0.27
		(0.72)	(-1.21)	(0.06)	(-0.18)	(-1.31)	(-1.07)	(-0.69)	(3.02)	(0.29)
	Smith (1936) and Hazel (1943)	33.06	-28.71	13.61	-26.82	-3.45	-2.77	-0.18	3.00	2.78
		(1.53)	(-0.72)	(0.13)	(-0.33)	(-2.33)	(-1.90)	(-0.10)	(5.73)	(2.93)
	Subandi et al. (1973)	30.83	-4.69	18.19	-16.87	-2.97	-2.69	-2.69	0.57	-2.11
		(1.43)	(0.12)	(0.17)	(-0.20)	(2.01)	(2.19)	(1.21)	(-0.62)	(2.00)
	Williams (1962)	30.47	-33.37	12.66	-21.17	-3.02	-2.46	-0.36	2.38	1.99
		(1.41)	(-0.84)	(0.12)	(-0.26)	(-2.04)	(-1.69)	(-0.20)	(4.55)	(2.09)

The selection gains were different for the different indices studied, demonstrating the need for constant evaluation of the best index in accordance with the objectives of the breeding program and of the population to be improved. Not all the indices showed appropriate gains for all the characters for this population of lines. The best gains for both selection intensities were obtained using the indices of Smith (1936), Hazel (1943) and Williams (1962).

On comparing the genotypes selected for each index at 10 and 20% intensities, it was possible to observe that some genotypes were selected in all the selection indices and, therefore, were promising to keep in breeding program. The total of 256 genotypes, 25 of them (10%) were selected in at least 5 of the 7 indices presented. This demonstrates that these genotypes have a potential to continue in the breeding program of line development for obtaining superior hybrids and therefore, merit more attention within the program.

Conclusion

The direct selection of characters was not effective in the selection of superior genotypes for the characters under study. The use of selection indices in the study population was effective within the improvement program since it allowed the simultaneous selection of characters and the indices of Smith (1936), Hazel (1943) and Williams (1962) resulted in better gains for the genotypes studied.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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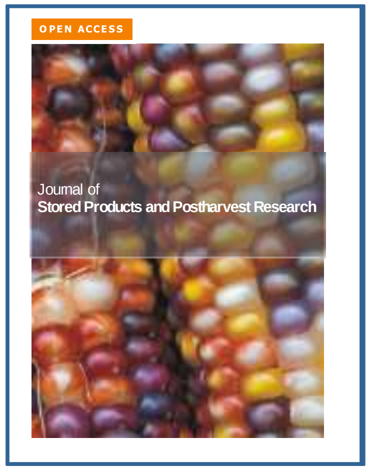

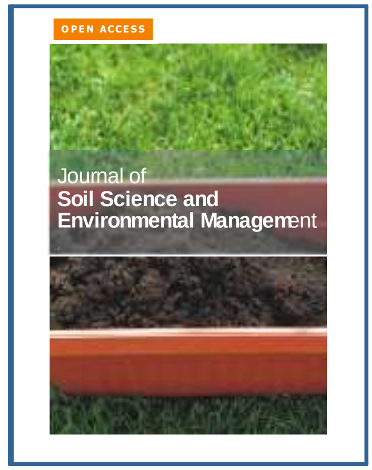

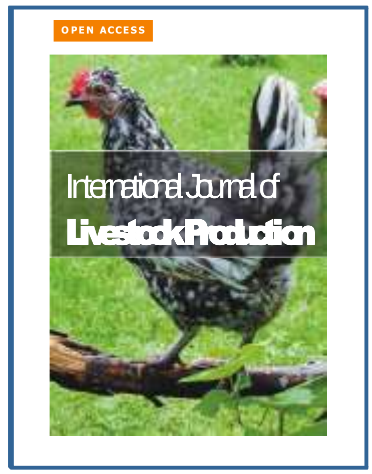

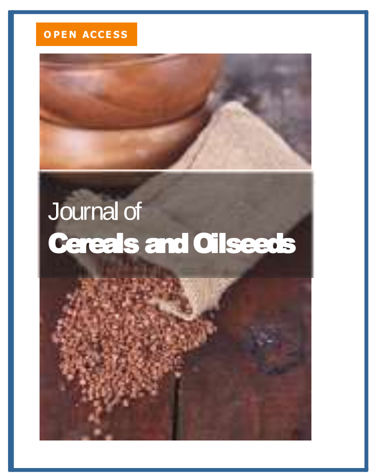

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