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Full Length Research Paper

Phenotypic characterization of physic nut populations

João Paulo de Morais Oliveira[1]*, Jéssica Ferreira Silva[2], Bruna Santos de Oliveira[1], Priscilla Gomes de Freitas Santos[1], Patrícia Souza da Silveira[1] and Fábio Santos Matos[1]

[1]Graduation Program on Plants Production, Ipameri Campus, State University of Goiás (UEG), Zip Code: 75780-000, Goiás, Brazil.
[2]Agronomy School, Federal University of Goiás (UFG), Zip Code: 74690-900, Goiânia, Goiás, Brazil.

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This study aimed to identify phenotypic plasticity in populations of Jatropha curcas. The experiment was conducted with a completely randomized design with eleven treatments and four replications. Morphophysiological variables were analyzed in the agricultural year, 2014-2015. Positive correlations were only observed between crown diameter and seed production, and canopy diameter and stomatal density in adaxial epidermis, indicating that canopy diameter can be used as a descriptor in plant breeding programs. Cluster analysis confirmed the existence of diversity among populations of J. curcas, with the formation of two groups, demonstrating the narrow genetic basis of Jatropha found in different regions of Brazil. The analysis of phenotypic plasticity demonstrated that morphological variables had a higher coefficient of plasticity in relation to physiological and productive variables. The morphological and physiological variables can be used in *J. curcas* breeding programs to study diversity and phenotypic plasticity.

Key words: Biofuel, genetic diversity, *Jatropha curcas*, oleaginous.

INTRODUCTION

The world's dependence on energy sources has compromised natural resources, owing to the increase in gases that enhance the greenhouse effect in the atmosphere as a result of human activities such as burning of fossil fuels and land use. The search for sources of clean and renewable energy becomes necessary to minimize climate change. In this context, biofuels represent a sustainable alternative for partial or total replacement of fossil fuels.

Brazil features a high capacity for biofuel production, owing to its vast territory, climatic diversity, a number of potentially suitable species, and manpower with technical expertise in the field of agricultural science (Santos et al., 2014). The main raw materials used for production of biodiesel in Brazil are soybeans, beef tallow and cotton, with contributions of 69.24, 26.18 and 3.07%, respectively, and other materials accounting for only 1.51% of the production (ANP, 2015). Therefore, the need exists for diversifying the sources of raw material with the introduction of promising species. *Jatropha curcas*, popularly known as physic nut, stands out as a plant with great potential for oil extraction as well as high physical and chemical quality for biodiesel production (Laviola et al., 2012).

*Corresponding author. E-mail: joaopaulo.ueg@gmail.com.

Table 1. Description of the place of collection of *J. curcas* seeds in different regions of Brazil.

City collection	Geographic coordinates	Populations
Ariquemes	09° 54' 48″ S, 63° 02' 27" W, 142 m	RO
Barra dos Bugres	15° 04' 21" S, 57° 10' 52" W, 171 m	MT
Formoso do Araguaia	11° 47' 48" S, 49° 31' 44" W, 240 m	TO
Jales	20° 16' 08" S, 50° 32' 45" W, 478 m	SP
Jataí	17º 52' 53" S, 51° 42' 52" W, 696 m	GO
João Pinheiro	17° 44' 45" S, 46° 10' 44" W, 765 m	MG
Natal	05° 47′ 42″ S, 35° 12′ 32″ W, 30 m	RN
Novo Repartimento	04° 19' 50" S, 49° 47' 47" W, 0 m	PA
Petrolina	09° 23' 55" S, 40° 30' 03" W, 376 m	PE
São Luís	02° 31' 47" S, 44° 18' 10" W, 24 m	MA
Serra da Ibiapaba	03° 52′ 47″ S, 40° 57′ 50″ W, 954 m	CE

J. curcas is a perennial, monoecious species belonging to the family Euphorbiaceae and has been used by rural communities for various ends, such as soil conservation and as source of decomposed organic matter rich in nitrogen, phosphorus and potassium. The latex extracted from the stem and branches presents healing effect, being used for pharmaceutical purposes (Openshaw, 2000). Its high cultivation potential has attracted the attention of researchers and promoted rapid expansion of the species in the world. The planted area of *J. curcas* corresponded to 900,000 ha in 2008, rising to 4.7 million ha in 2010 and expected to reach 12.8 million ha in 2016 (Contran et al., 2013).

Although, *Jatropha* is distributed in different regions of Brazil, the absence of cultivars with improved production stability has limited the expansion of the species. Thus, research aiming to develop improved *J. curcas* cultivars has been intensified; nevertheless, breeding programs are still rare when compared with other oleaginous species (Laviola et al., 2012). Several genetic diversity studies report that the genetic basis of *Jatropha* is narrow, probably because of a common ancestry (Kanchanaketu et al., 2012; Reis et al., 2015).

The large edaphoclimatic diversity existing in Brazil, with marked variation of abiotic factors between regions, may favor the selection over generations of genotypes with greater ability to display phenotypic plasticity. Breeding programs of *J. curcas* have aimed to solve the problematic environment-genotype interaction. Expansion of the commercial exploitation of this culture depends on the clarification of basic agronomic aspects not yet available for the species, as well as on the development of superior materials with uniform maturation and production stability.

In this perspective, it is observed that the species, *J. curcas* lacks superior materials and basic elucidations to ensure production stability. The degree of improvement of *Jatropha* is still incipient, and raises concern among investigators that research is in demand as regards genetic improvement, interaction of genotype x environment, phenotypic plasticity and management practices. Thus, this study aimed to identify the diversity and phenotypic plasticity in populations of *J. curcas*.

MATERIALS AND METHODS

The experimental area of the present study has Aw climate according to Köppen classification, with annual rainfall of 1,447 mm, average temperature of 21.9°C, and average air relative humidity ranging from 58 to 81%. The region has two distinct seasons: a rain season from October to April, and drought from May to September. The soil of the experimental area is classified as dystrophic red-yellow latosol (EMBRAPA, 2006).

Seeds of *J. curcas* from different geographical regions of Brazil were planted in November 2011 with spacing of 4 × 2 m (Table 1). The work was conducted following a randomized complete block design with eleven treatments and four replications, between August 2014 and August 2015. The treatments were defined as *J. curcas* populations.

To obtain the stomatal density, two replicas of adaxial and abaxial leaf surfaces, depicting the middle third region of hydrated leaves, were made with colorless enamel. The count was carried out using an optical microscope equipped with a camera lucida, following the recommendations of Jadrná et al. (2009).

To obtain the specific leaf area (SLA), six leaf disks of 1.2 cm diameter were taken from fully expanded leaves and subsequently dried at 70°C for 72 h for determination of the dry weight. The SLA was obtained via equation proposed by Radford (1967). The leaf area was determined following equation proposed by Severino et al. (2006). The amount of inflorescences was established by counting their number as they emerged in the plant. The number of female, male, hermaphrodite and asexual flowers was obtained by the respective number of flowers counted in each inflorescence upon their opening in the plant, according to Pereira et al. (2011).

For determination of photosynthetic pigments, leaf discs with 1.2 cm of diameter were removed and placed in glasses containing dimethyl sulfoxide (DMSO). Next, extraction was performed in water bath at 65°C for one hour. Samples were taken for spectrophotometric reading at 480, 649 and 665 nm. The content of chlorophyll *a* (Cl *a*), chlorophyll *b* (Cl *b*), carotenoids (Car) and ratio of chlorophyll *a* to *b* were determined following the equations proposed by Wellburn (1994).

The number of branches was obtained by counting all the ramifications from the base of the main stem. Plant height was determined using a measuring tape graduated in meters, covering the stem length up to the apex of the main branch. The stem diameter was established with a digital caliper at the height of sample collection. The crown diameter was measured between the two lateral limits of the plant.

The length, width and diameter of the seeds were determined using a digital caliper, using 25 random seeds as samples. The weight of 100 seeds was obtained using a precision scale (0.001 g). The yield per plant was measured by weighing the seeds. Subsequently, analysis of the oil content of the seeds was performed by nuclear magnetic resonance (NMR). Oil productivity was determined by total seed productivity (kg ha^{-1}) divided by the average density of *Jatropha* oil (0.910), expressed in L h^{-1}.

The differences in plasticity index associated with morphological, physiological and productive variables were analyzed by Scott-Knott test ($p < 0.05$). The phenotypic plasticity index, ranging from 0 to 1, was calculated based on the relative distance (DR) between the values of treatments (RDPI), according to Valladares et al. (2006).

The genotypic correlation test between variables as well as the cluster analysis by Mahalanobis distances (D2), using the unweighted average link method (UPGMA), was accomplished using the GENES software (Cruz, 2013). To perform the principal component analysis, created if a correlation matrix and the selection criteria of the axes followed the Broken Stick model in multiple regression analysis to assess productivity, the forward stepwise model (Sokal and Rolf, 1969) was used and both analyses were carried out using the R software (R CORE TEAM, 2015).

RESULTS AND DISCUSSION

The results revealed differences between *J. curcas* populations, corroborating those found by Reis et al. (2015) when evaluating the genetic diversity in different accessions of this species, based on agronomic descriptors. In addition, this paper reports the existence of phenotypic plasticity in populations of *J. curcas*.

The descriptive analysis of variables is shown in Table 2. The number of inflorescences and female, male, hermaphrodite and asexual flowers did not follow a normal distribution according to the Shapiro-Wilk test. Variables showing normal standard with greater variations were: oil production (PO), seed productivity (PROD), height of 1^{st} branch (FBH) and specific leaf area (SLA). The PO found in the seeds presented variation from 55.67 to 508.40 L ha^{-1}, with an average of 242.21 L ha^{-1}. The FBH ranged from 4 to 12 cm, with an average of 6.7 cm. The AFE had an average of 71.20 m^2 kg^{-1}, varying between 37 and 109 m^2 kg^{-1}. The obtained TOS was 33.20%, ranging from 28.40 to 35.80%.

Only variables that presented significance by analysis of variance were subjected to genotypic correlation test (Table 3). Positive correlations were observed between crown diameter and seed production (0.99), crown diameter and stomatal density in adaxial (0.86), crown diameter and diameter of the stem (0.82), productivity and stomatal density the adaxial (0.80), seed weight and seed length (0.80), and seed weight and seed diameter (0.79). In multiple regression analysis, shown in Table 4, it was observed that the model explained 75% of the variance of *J. curcas* productivity.

In this regression model, the crown diameter, number of inflorescences, stomatal density in adaxial epidermis and seed length were the variables with greatest contribution to the productivity of *J. curcas*.

Genotypic correlation and multiple regression analysis indicate that the crown diameter, stomata density in the adaxial epidermis and length of the seeds can be used as a descriptor in plant breeding programs in order to increase the yield of seeds (Laviola et al. 2011). To assess the genetic diversity of plants *J. curcas*, Reis et al. (2015) concluded that the morphophysiological descriptors are of paramount importance to the improvement of the species.

The correlation between canopy diameter and stomatal density in adaxial epidermis is associated with self-shadowing in plants with large crown diameter and leaf area index. The high shading creates local conditions (lower incidence of direct radiation, lower temperature, high humidity and high boundary layer) is appropriate for the influx of CO_2 and transpiration. Under these conditions, the leaves are larger and have high stomatal density in the adaxial epidermis to maximize gas exchange and thereby increase productivity (Castro et al., 2009).

In *J. curcas*, inflorescences are located at the apex of the branches and are correlated with the seed yield, interfering decisively with the amount of fruit (Drummond et al., 2010). According to Rocha et al. (2012), crown diameter, number of inflorescences, stomatal density in adaxial epidermis and seed length are quantitative attributes that suffer strong environmental influence, being able to generate differential genotypes over the years.

In principal component analysis (Figure 1), it is observed that only the first two components were necessary to explain 70% of the variation of the data. PC1 explains 50% of the variation; the variables contributing the most to the ordination of *Jatropha* populations were crown diameter; number of inflorescences, female, male and hermaphrodite flowers; and seed and oil yield. The populations of GO, RO, RN, MT and CE showed the highest values for these variables. PC2 explains 20% of the variation in the data, and only the variable 'total chlorophyll' contributed significantly to the formation of the second axis; the populations of GO, RO, MT and MG showed the highest values for this variable.

However, the principal component analysis failed to identify the formation of different groups among the populations of *J. curcas* based on physiological, morphological and productive variables. This proves that the materials under study, although derived from different geographic regions of Brazil, present intra- and inter-population variations. Contradictory results were obtained by One et al. (2014), studying the phenotypic and genotypic diversity in *Jatropha*, while Osorio et al. (2014) found great phenotypic variations in *J. curcas* in Central

Table 2. Descriptive analysis of the variables- number of branches (NR), plant height (PH), stem diameter (DCL), canopy diameter (DCP), the first branch height (FBH), leaf area (LA) , number of inflorescences per plant (NI), feminine flower (FF), masculine (FM), hermaphrodites (FH), asexual (FA) diameter seed (DS), the length of the seed (CS), width of the seed (LS), weight of 100 seeds (PS), productivity (PROD), oil content in the seeds (TOS), oil yield (PO), stomatal density in adaxial epidermis (EAD) and abaxial (EAB), leaf concentration of carotenoid total (CAR) ratio of chlorophyll a and b (Cl a / Cl b), total chlorophylls [Cl (a + b)] and specific leaf area (SLA), analyzed in populations *J. curcas* found in naturally different geographic regions of Brazil.

Variables	DV	CV (%)	Minimum	Maximum	Average	p (normal)
NR	7.50	16.9	11.00	50.00	34.90	0.66 ^{ns}
PH (m)	0.30	9.9	2.20	3.50	3.00	0.61 ^{ns}
DCL (cm)	16.70	10.60	95.00	160.00	10.60	0.44 ^{ns}
DCP (m)	0.40	18.2	1.10	3.10	2.30	0.62 ^{ns}
FBH (cm)	1.90	25.70	4.00	12.00	6.70	0.06 ^{ns}
LA (cm^2)	30.00	17.70	105.60	221.50	159.00	0.20 ^{ns}
NI	12.60	91.80	1.00	45.00	13.70	0.05 *
FF	4.60	106.30	1.00	20.00	4.40	0.03 *
FM	42.40	73.40	29.00	145.00	58.00	0.01 **
FH	1.40	104.20	1.00	4.00	1.30	0.03 *
FA	2.00	113.00	1.00	7.00	1.70	0.02 *
DS (mm)	0.20	1.80	8.00	8.90	8.50	0.25 ^{ns}
CS (mm)	0.40	2.00	16.70	18.80	17.70	0.93 ^{ns}
LS (mm)	0.30	2.30	10.00	10.90	10.50	0.11 ^{ns}
PS (g)	4.30	2.30	69.20	82.40	75.30	0.16 ^{ns}
PROD (kg ha^{-1})	290.00	43.70	170.00	1370.00	660.00	0.07 ^{ns}
TOS (%)	1.50	4.20	28.40	35.80	33.20	0.09 ^{ns}
PO (L ha^{-1})	106.96	44.15	55.67	508.40	242.21	0.24 ^{ns}
EAD (mm^{-2})	35.00	14.10	149.00	277.00	202.20	0.22 ^{ns}
EAB (mm^{-2})	93.00	10.60	521.00	871.00	680.40	0.94 ^{ns}
CAR (g kg^{-1})	0.40	20.60	1.00	2.70	1.80	0.60 ^{ns}
Cl*a*/Cl*b*	0.70	10.90	1.90	5.50	2.80	0.41 ^{ns}
Cl *a*+*b* (g kg^{-1})	2.40	19.30	6.50	18.80	11.40	0.20 ^{ns}
AFE (m^2 kg^{-1})	17.20	22.00	37.00	109.00	71.20	0.20 ^{ns}

Standard deviation (DV), coefficient of variation (CV), minimum and maximum, mean and normality test p (normal); ^{ns} = not significant, ** significant at 1% and *significant at 5% probability level.

America as compared to Africa, Asia and South America.

Cluster analysis (Figure 2) based on the Mahalanobis distance ranked the populations of *J. curcas* in two groups, observing similarity between the groups generated at the point 60 of the connection distance. Group one, which included most *J. curcas* materials, included the states of MT, CE, PE, TO, MA, PA, GO and SP. Group two comprised the states of RO, MG and RN.

Cluster analysis confirmed the existence of genetic diversity among populations of *J. curcas*, showing that the genetic basis of *Jatropha* found in different regions of Brazil is narrow, possibly because the analyzed materials were derived from few populations or exchange of seeds occurred after introduction in Brazil (Kanchanaketu et al., 2012). According to Reis et al. (2015), the introduction of materials derived from other countries is necessary to generate greater diversity.

The analysis of phenotypic plasticity (Table 5) showed that the morphological variables had greater plasticity coefficient (0.37) in relation to physiological (0.09) and productive (0.10) variables. Among the morphological and productive variables with higher values of phenotypic plasticity were: asexual (0.67), hermaphrodite (0.59), female (0.53) and male (0.52) flowers, number of inflorescences per plant (0.48), oil yield (0.32) and seed (0.31). The physiological variables had reduced phenotypic plasticity index for the studied characteristics. According to Fuzeto and Lomônaco (2000), populations occupying heterogeneous environments have large plastic potential in their external characteristics (phenotype) without genotypic changes being necessary. The detected variations confirm the performance of plasticity as generating mechanism of phenotypic variability and point out the latter's importance in adaptive and evolutionary processes of species, as the produced changes facilitate the exploration of new niches, resulting in increased environmental tolerance (Via, 1993).

The morphological plasticity seen in populations of *J.*

Table 3. Estimates of genotypic correlation between morphophysiological characters- stem diameter (DCL), crown diameter (PCD), of the first branch point (FBH), seed diameter (DS), length of the seed (CS), width of the seed (LS), weight of 100 seeds (PS), total productivity (PROD), oil content in the seeds (TOS), stomatal density in adaxial epidermis (EAD) and abaxial (EAB), ratio chlorophyll a and b (Cl*a*/Cl *b*) and oil yield (PO) in different populations of *J. curcas* found naturally in different regions of Brazil.

Genotypic correlation												
Var.	**DCP**	**FBH**	**DS**	**CS**	**LS**	**PS**	**PROD**	**TOS**	**EAD**	**EAB**	**Cl*a*/Cl*b***	**PO**
DCL	0.82*	-0.38	-0.24	0.01	0.07	-0.37	0.70	-0.31	0.16	0.49	0.07	-0.43
DCP		0.40	-0.15	-0.22	0.12	-0.48	0.99**	0.23	0.86*	-0.35	0.60	0.28
FBH			0.16	-0.11	0.42	-0.10	0.40	-0.03	0.18	0.68	0.23	-0.10
DS				0.32	-0.20	0.79*	-0.15	0.01	-0.12	-0.03	0.34	0.04
CS					0.50	0.80*	-0.40	0.48	-0.29	-0.29	0.34	0.51
LS						0.16	0.02	-0.01	0.02	-0.72	-0.05	-0.08
PS							-0.63	0.06	-0.62	0.04	-0.03	0.15
PROD								0.35	0.80*	-0.17	0.68	0.37
TOS									0.20	-0.54	0.38	0.64
EAD										-0.18	0.54	-0.60
EAB											-0.43	-0.51
Cl*a*/Cl*b*												-0.44

**Significant at 1% probability (p <0.01); *significant at 5% probability (p <0.05).

Table 4. Multiple regression model to assess the total productivity by criteria stepwse using variables- diameter crown (DCP), inflorescence number (NI), stomatal density in adaxial epidermis (EAD), length of seed (CS), average oil content in the seeds (TOS), weight of 100 seeds (PS); leaf area (LA); total chlorophyll [Cl (a + b)]; stem diameter (DCL) and plant height (PH).

Production	**Model explanation**	**F**	**P**			
	R^2= 0.75	**F(10.33) = 13.94**	**p<0.001**			
	Beta	**Std.Err.**	**B**	**Std.Err**	**t(33)**	**p-level**
Intercept			-3.34	0.83	-4.03	0.001
DCP (cm)	0.40	0.12	0.17	0.05	3.30	0.002**
NI	0.23	0.11	0.01	0.00	2.13	0.040*
EAD (mm^{-2})	0.32	0.09	0.01	0.00	3.46	0.002**
CS (mm)	0.42	0.13	0.16	0.05	3.31	0.002**
TOS (%)	0.14	0.08	0.02	0.01	1.69	0.100
PS (g)	-0.22	0.11	0.01	0.01	-1.93	0.062
LA (cm^2)	-0.15	0.09	-0.00	0.00	-1.59	0.121
Cl *a*+*b* (g kg^{-1})	0.12	0.09	0.01	0.01	1.37	0.179
DCL (cm)	0.11	0.09	0.00	0.00	1.31	0.197
PH (m)	0.14	0.11	0.09	0.07	1.27	0.211

**Significant at 1% probability (p <0.01); *significant at 5% probability.

curcas occurred mainly due to environmental factors, suffering genetic influences. According to Gilbert (2016), inflorescences as well as male, female and hermaphrodite flowers are structures sensitive to changes in the external environment. Productive plasticity was also observed in the present study, where interactions between seed and oil production and the environment could be verified. Therefore, *J. curcas* exhibits high plasticity, which may vary from higher to lower intensity according to cultivation conditions. These results corroborate those obtained by Drummond et al. (2010) when evaluating the agronomic performance of *J. curcas* genotypes, finding wide variations in the study variables.

Conclusion

J. curcas populations present phenotypic and genotypic variability, and morphological and physiological variables

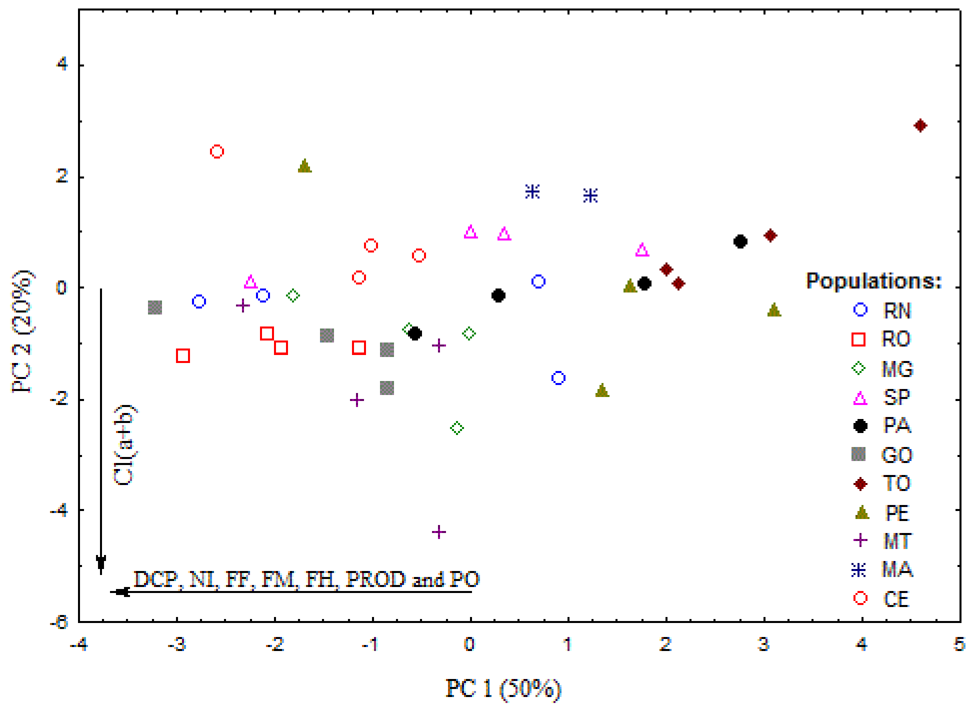

Figure 1. Principal component analysis (PC) for morphophysiological diversity of populations of *J. curcas*. Arrows indicate the directions in which each variable increases in relation to the axis, selecting those above 70%. The shapes and colors of the icons represent the populations of *J. curcas* found naturally in different regions geographical of Brazil.

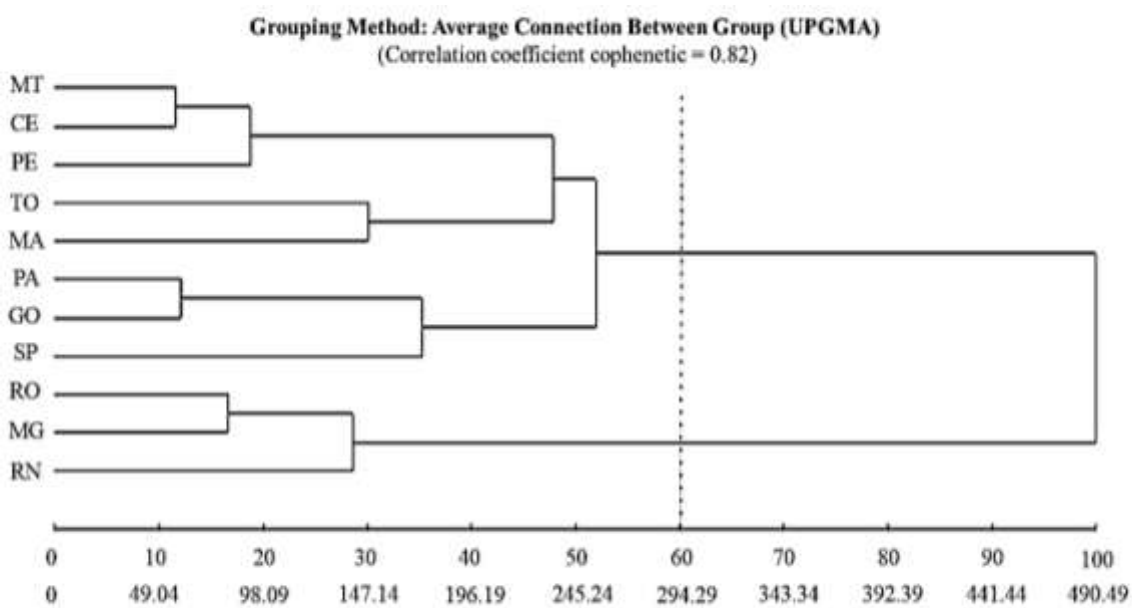

Figure 2. Dendrogram based on the Mahalanobis distance by UPGMA method of 24 variables in populations *J. curcas* from different geographical regions of Brazil.

may be used in breeding programs. Plasticity analysis confirmed the performance of morphological, physiological and productive variables as generating mechanism of phenotypic variability.

Table 5. Relative distance plasticity index for morphological, physiological and productive characteristics in different populations of *J. curcas*.

Morphological characteristics	Plasticity index
Abaxial stomatal density	0.06^{d}
Adaxial stomatal density	0.13^{d}
Specific leaf area	0.13^{d}
Leaf area	0.11^{d}
Inflorescence	0.48^{b}
Female flowers	0.53^{b}
Male flowers	0.52^{b}
Hermaphrodite flowers	0.59^{a}
Asexual flowers	0.67^{a}
Average	0.37^{A}
Physiological characteristics	
Number of branches	0.08^{d}
Plant height	0.05^{d}
Height of the first branch	0.13^{d}
Stem diameter	0.09^{d}
Crown diameter	0.08^{d}
Carotenoids	0.10^{d}
Chlorophyll ratio *a* and *b*	0.07^{d}
Total chlorophylls	0.09^{d}
Average	0.09^{B}
Characteristics productive	
Length seeds	0.01^{d}
Width seeds	0.01^{d}
Diameter of seeds	0.01^{d}
Weight of 100 seeds	0.02^{d}
Seed productive total	0.31^{c}
Average oil content in seeds	0.02^{d}
Oil Productivity	0.32^{c}
Average	0.10^{B}

Means followed by the same letter in the column do not differ by the Scott-Knott test ($p < 0.05$).

Canopy diametercontributes to increased productivity in *J. curcas*, and can be used as a descriptor for breeding programs of the species.

Abbreviation

CAR, Carotenoids; **Cl *a*,** chlorophyll *a*; **Cl *b*,** chlorophyll *b*; **CS,** the length of the seed; **DCL,** stem diameter; **DCP,** canopy diameter; **DMSO,** dimethyl sulfoxide; **DS,** diameter seed; **EAB,** stomatal density in abaxial; **EAD,** stomatal density in adaxial epidermis; **FA,** asexual; **FBH,** the first branch height; **FF,** feminine flower; **FH,** hermaphrodites flower; **FM,** masculine flower; **LA,** leaf area; **LS,** width of the seed; **NI,** number of inflorescences per plant; **NR,** number of branches; **PCD,** crown diameter; **PH,** plant height; **PO,** oil yield; **PROD,** productivity; **PS,** weight of 100 seeds; **SLA,** specific leaf area; **TOS,** oil content in the seeds.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Methods for breaking seed dormancy of ryegrass during storage periods

Andréa Bicca Noguez Martins[1]*, Aline Klug Radke[1], Manoela Andrade Monteiro[1], Letícia Winke Dias[1], Lilian Vanusa Madruga de Tunes[1], Geri Eduardo Meneghelo[1], Fernanda Da Motta Xavier[1], André Pich Brunes[1], Caroline Jácome Costa[2] and Andréa Mittelmann[2]

[1]Faculty of Agronomy Eliseu Maciel, Post Graduate Science and Seed Technology, Federal University of Pelotas, Capão do Leão Campus, P. O. Box 354, ZIP Code 960001-970, Pelotas, RS, Brazil.
[2]Research Embrapa Temperate, Highway BR-392, Km 78, P. O. Box 403, Zip Code 96010-971, Pelotas, RS, Brazil.

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The presence of dormant seeds makes it difficult to evaluate physiological quality and requires the use of appropriate methods in order to break seed dormancy. The objective of this study was to evaluate the effectiveness of different methods to break the dormancy of ryegrass seeds stored under environment conditions in different periods. Ryegrass seeds, BRS Ponteio cultivar, produced in two locations were used and evaluated after 60, 90, 120, 150 and 180 days of storage under environment conditions. Seeds were submitted to the following methods to break dormancy: sowing at 20 to 30°C without applying any method to break dormancy (control); pre-cooling (5°C) for 3 days + KNO_3 followed by sowing at 15 to 25°C; pre-drying (45°C) for 96 h followed by sowing at 20-30°C; immersing the seeds in sodium hypochlorite (NaClO) at 0.5% for 24 h, followed by drying at 45°C for 6 h and sowing at 15 to 25°C. At 60 days after harvest, the most effective method was immersion of seeds in a NaClO solution followed by drying at 45°C. From 90 days after harvest, all methods were equally effective to break seed dormancy, except pre-drying (45°C) for 96 h, which negatively affected the physiological quality of seeds. There is a difference in the effectiveness of the methods employed to break the dormancy of ryegrass seeds depending on the type of post-harvest storage.

Key words: *Lolium multiflorum*, post-harvest storage, pre-cooling.

INTRODUCTION

Ryegrass (*Lolium multiflorum* Lam.) has a widespread occurrence in southern Brazil, especially in Rio Grande do Sul (RS) state, where it is used as forage for livestock and soil cover in agricultural areas during the winter

*Corresponding author. E-mail: amartinsfv@hotmail.com.

period. This forage species can be considered important for the agricultural context in southern Brazil due to its growing cycle complementarity with natural pastures, high nutritional value, ease of establishment and excellent capacity of natural reseeding (Costa et al., 2013).

However, Lacerda et al. (2010) suggested that approximately 80% of Brazilian pasture areas are degraded. The low germination rate of seeds causes such problem and may be related to a phenomenon called dormancy, i.e., seeds do not germinate even when exposed to favorable environmental conditions. This phenomenon is one of the main strategies used by plant species to increase their survival rates and establish young plants. It thus provides an additional period for its natural dispersion (Taiz and Zeiger, 2013). The dormancy mechanism has peculiarities according to species, making the generalization about its causes complex. It may occur independently or in combination as in most grasses (Previero et al., 1998).

However, the presence of dormancy in forage species seeds is fundamental, allowing them to survive the unfavorable period of the summer and germinate only in the fall, when environmental conditions are suitable for the development of this culture.

As mentioned by Baldi et al. (2012), dormancy often is not a problem for the users of seeds since they tend to break naturally between the sowing and the harvesting of the culture. However, it is a partially problem for seed production, dormancy soon after harvest makes the evaluation of the physiological quality and the implementation of measures related to quality control of production difficult, which requires appropriate methods to break the dormancy of seeds. Furthermore, it is known that, for many forage species, the effectiveness of methods designed to break dormancy varies depending on the age of the seeds (Costa et al., 2011) and that the storage itself is enough to promote dormancy breaking.

In this context, studies on breaking the dormancy of seeds have been conducted. However, studies are scarce when it comes to forage species. Popinigis (1985) indicated several methods to break the dormancy of grass seeds. Among the main methods, there are rupture of caryopsis, treatment with potassium nitrate (KNO_3), exposure to light, use of alternating temperatures, pre-cooling and treatment with hormones (gibberellin, cytokinin and ethylene).

The objective of this study was to evaluate the effectiveness of different methods to break the dormancy of ryegrass seeds stored under environment conditions in different periods.

MATERIALS AND METHODS

The experiments were conducted at the Temperate Embrapa Seed Analysis Laboratory in Capão do Leão/RS and at the teaching laboratory of seed analysis "Flavio Rocha" of the Faculty of Agronomy "Eliseu Maciel" of the Federal University of Pelotas between January and June 2014. Annual ryegrass seeds (*Lolium multiflorum* Lam.), BRS Ponteio cultivar, produced in Pedras Altas/RS and Capão do Leão/RS, were used. Seeds were collected, processed and stored under non-controlled environment conditions and evaluated at 60, 90, 120, 150 and 180 days after harvest. The following methods were used to break the dormancy of seeds:

Method 1: Sowing at 20 to 30°C without applying any method intended to break seed dormancy: four replications using 100 seeds were sown on two sheets of blotting paper moistened with distilled water in an amount equal to 2.5 times the dry mass and kept under alternating temperatures of 20 to 30°C. The seeds were evaluated 14 days after the test installation and the percentage of germination was recorded.

Method 2: Pre-cooling (5°C) for 3 days + KNO_3 followed by sowing at 15-25°C: four replications of 100 seeds were sown on two sheets of blotting paper moistened with a potassium nitrate (KNO_3) solution at 0.2% in an amount equivalent to 2.5 times the dry mass and stored at 5°C for three days. After this period, seeds were transferred to a Biochemical Oxygen Demand (BOD) chamber with alternating temperatures of 15-25°C and a photoperiod of 8 hours. The percentage of germination was evaluated 14 days after the installation of the germination test.

Method 3: Pre-drying (45°C) for 96 h followed by sowing at 20-30°C: the methodology was similar to that described for the Method 1, except that the seeds were subjected to a pre-drying at 45°C for 96 h prior to the installation of the germination test.

Method 4: Immersion of seeds in sodium hypochlorite (NaClO) at 0.5% for 24 hours, followed by drying at 45°C for 6 h and sowing at 15-25°C. The methodology was similar to that described for Method 1, with the difference that seeds were soaked in sodium hypochlorite (NaClO) at 0.5% for 24 h followed by washing in water. Seeds were dried at 45°C for 6 h.

The experiments were arranged completely randomized design with four replications in a 5 (storage period) x 4 (treatment) factorial design. Treatments consisted of a combination of five storage periods after harvest and four methods for breaking seed dormancy. The data were transformed into arcsin $(x/100)^{1/2}$ and subjected to analysis of variance. The averages were compared by Tukey test ($p<0.05$).

RESULTS AND DISCUSSION

It was observed that, at 60 days after seed harvest, the most efficient method for breaking seed dormancy was immersion in sodium hypochlorite (NaClO) at 0.5% for 24 h, followed by drying (45°C) for 6 h and sowing at 15-25°C (Tables 1 and 2). In this case, seeds produced in Pedras Altas/RS that have not undergone any method to break seed dormancy had 49% of germination, which increased to 69% after the procedure to break seed dormancy (Table 1). For seeds produced in Capão do Leão/RS, this method increased the germination of seeds from 87% to 99% (Table 2). Using rice seeds, this method was also the most efficient after pre-drying at 45°C for 96 h to break seed dormancy of the cultivar

Table 1. Germination (%) of ryegrass seeds, BRS Ponteio cultivar, produced in Pedras Altas/RS, subjected to different methods to break seed dormancy at 60, 90, 120, 150 and 180 days after harvest.

Methods for breaking seed dormancy*	Days after harvest				
	60	90	120	150	180
Method 1	49^{b}	51^{a}	57^{a}	48^{ab}	64^{a}
Method 2	48^{b}	53^{a}	59^{a}	60^{a}	57^{ab}
Method 3	16^{c}	18^{b}	49^{a}	45^{b}	44^{b}
Method 4	69^{a}	45^{a}	49^{a}	48^{ab}	54^{ab}
CV (%)	10.39				

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

Table 2. Germination (%) of ryegrass seeds, BRS Ponteio cultivar, produced in Capão do Leão/RS, subjected to different methods to break seed dormancy at 60, 90, 120, 150 and 180 days after harvest.

Methods for breaking seed dormancy*	Days after harvest				
	60	90	120	150	180
Method 1	87^{ab}	69^{a}	97^{a}	98^{a}	98^{a}
Method 2	71^{b}	72^{a}	94^{a}	100^{a}	96^{a}
Method 3	31^{c}	29^{b}	45^{b}	49^{c}	60^{b}
Method 4	99^{a}	58^{a}	72^{b}	78^{b}	76^{b}
CV (%)	14.04				

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

IRGA 425 and the cultivar SCS 114 Andosan (Baldi et al., 2012). According to these authors, the advantage of this method is the possibility of using mechanical counters for the sowing of seeds after the procedure because seeds are dried. These results are in agreement with those obtained by Vieira et al. (1994), who studied the effects on rice seeds by soaking them into a sodium hypochlorite solution for different periods (24, 36 and 48 h). They concluded that this method was not effective in breaking seed dormancy.

Unlike the results found for rice seeds, the pre-drying at 45°C for 96 h (Method 3) was detrimental to the physiological quality of seeds because it reduced the germination throughout the entire experiment. It was not recommended as a method to break the dormancy of ryegrass seeds. Studies conducted with forage species reveal that the optimum temperature for germination is specific for each cultivar and that seeds do not germinate at temperatures above 45°C (Seepaul et al., 2011).

Importantly, many methods employed to break seed dormancy immediately after harvest are not completely effective for several forage seeds, resulting in a high percentage of dormant seeds after the germination test, as noted by Eichelberger et al. (2001) for ryegrass seeds and confirmed by the results obtained in this study. Thus, after 90 days of storage, although all methods were equally effective to break seed dormancy (except for pre-drying at 45°C for 96 h, which damaged the physiological quality of seeds in all storage periods), it was observed that the expression of the maximum germination potential of the seeds was not achieved. It remained even below the values observed at 60 days after harvest (Tables 1 and 2).

The storage of ryegrass seeds produced in Capão do Leão/RS for 120 days seems to have been enough to promote a natural break of dormancy since, after this period, seeds not subjected to any method to break dormancy showed a 97% germination rate (Table 2). This period is very close to that observed for natural dormancy breaking of ryegrass seeds and reported by Costa et al. (2013). Research shows that, in freshly harvested seeds, the water ingress in tissues hinders the absorption of oxygen and that the storage of dry seeds for a certain time promotes the diffusion of oxygen into the interior, determining a reduction in the amount of germination inhibitors and favoring breaking seed dormancy (Duclos et al., 2013).

From 120 days of storage, the pre-drying of the seeds (45°C) for 96 h and the immersion in NaClO solution followed by drying were harmful to the physiological quality of seeds produced in Capão do Leão/RS. It reduced the germination to 45 and 72% respectively after 120 days of storage, to 49 and 78% respectively after 150 days of storage, and to 60 and 76% respectively after 180 days of storage (Table 2). As the physiological maturation process of ryegrass seeds is uneven, it is likely that seeds that were already at an advanced stage at harvest suffered a greater deteriorating stress over the storage period, being affected by the immersion in NaClO (Method 4). Lima et al. (2012) used the immersion of coffee seeds in an aqueous solution of sodium hypochlorite at concentrations of 3, 4, and 5% of active chlorine, resulting in an acceleration of germination.

This behavior has been highlighted by Eichelberger et al. (2001) for ryegrass seeds subjected to pre-cooling. The authors state that, because of dormancy, the germination test conducted soon after harvest may underestimate the quality of seeds even after the adoption of pre-cooling as a method to break seed dormancy. The findings of this study corroborate the data found by Amaro et al. (2012). They concluded that, upon evaluating dormancy breaking methods in basil (*Ocimum basilicum* L.) and, after seed storage, this same method may cause stress to the seeds, confusing the results of the germination test. These results were observed for the seeds produced in Pedras Altas/RS, whose germination after 120 days of storage was not affected by any method intended to break seed dormancy (Table 1).

After 150 and 180 days of storage, the only method that negatively affected the germination was pre-drying (45°C) for 96 hours (Table 1). It is known that dormancy and seed quality in general are greatly affected by environmental conditions during the process of seed formation. This may explain the different behavior of seeds produced in Pedras Altas/RS and Capão do Leão/RS regarding the effectiveness of the methods used to break seed dormancy and the quality of obtained seeds.

The officially recommended method to break the dormancy of ryegrass seeds (pre-cooling at 5°C for 3 days) was not effective in any of the evaluated periods for seeds produced in the two locations. This resulted in a germination rate statistically similar to the germination of seeds that did not undergo any treatment to break seed dormancy in all situations (Tables 1 and 2).

Conclusions

There is a difference in the effectiveness of the methods employed to break the dormancy of ryegrass seeds depending on the type of post-harvest storage. The immersion in NaClO solution at 0.5% for 24 h followed by drying (45°C) for 6 h was effective in breaking the dormancy of seeds stored for up to 88 days after harvest. The method pre-drying (45°C) for 96 h was detrimental to the physiological quality of seeds. It was not recommended as a method to break seed dormancy.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Soil physico-chemical properties and land suitability evaluation for maize (*Zea mays*), beans (*Phaseolus vulgaris*) and Irish potatoes (*Solanum tuberosum*) in tephra soils of the western slopes of mount Kupe (Cameroon)

Roger Kogge Enang*, Bernard Palmer Kfuban Yerima and Georges Kogge Kome

Soil Science Department, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon.

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Soils of the western slopes of Kupe Mountain in Cameroon were characterized to understand their proper land use and sustainable management. Evaluation of land suitability for maize (*Zea mays*), beans (*Phaseolus vulgaris*) and Irish potatoes (*Solanum tuberosum*) were assessed, considering the socio-economic importance of these crops in Cameroon. Three sites, representative of the study area were identified following a toposequence; Nyassosso (900 m), Mbule (700 m) and Tombel (500 m) with soils, respectively, classified as: Hyperdystri – humic Leptosol (loami – andic), Skeleti – Umbric Andosol (colluvi – loamic) and Dystri – skeletic Andosol (Colluvi – loamic). Soil properties indicated that colour varies from black and brownish black at the surface to reddish brown in subsurface horizons with some horizons showing varied colours. The soils were friable and granular in structure, had low bulk density ranging from 0.38 to 1.08 g/cm^3, and had a loamy texture. Chemically, pH$_{(H2O)}$ ranged from strongly acidic to slightly acidic (4.8 - 6.2), organic carbon ranged from low to very high (0.88 - 7.26%), electrical conductivity was very low (0.02 - 0.29 dS/m), cation exchange capacity ranged from medium to high (20.5 to 38.08 cmol(+)kg^{-1}), total nitrogen from very low to very high (0.09 - 1.7%), available phosphorous from very low to low (7.13 - 19.76 ppm), Ca^{2+} from very low to high (2.08 - 10.64 cmol(+)kg^{-1}), Mg^{2+} from very low to medium (0.08 to 2.56 cmol(+)kg^{-1}), K^+ from very low to medium (0.01 - 0.31 cmol(+)kg^{-1}) and Na^+ was very low (0.01 - 0.05 cmol(+)kg^{-1}). Amorphous iron and aluminum ranged between 0.06 - 1.35 and 0.16 - 1.01%, respectively. Land suitability evaluation indicated that soils of Nyassosso were marginally suitable for rain-fed cultivation of Irish potatoes, beans and maize. Soils of Mbule were marginally suitable for rain-fed cultivation of Irish potatoes and maize but not suitable for beans, while soils of Tombel were marginally suitable for rain-fed cultivation of Irish potatoes and temporarily non suitable for rain-fed cultivation of maize and beans.

Key words: Mount Kupe, soil properties, suitability classes, climatic index, land index.

INTRODUCTION

Food security is at the top of the list of Millennium Development Goals (MDGs) with the goal of eradicating poverty and hunger, especially in sub-Saharan Africa where the situation remains a great challenge due to rapid population growth (Bremner, 2012). In Cameroon and most sub-Saharan African countries, per capital food production continues to decline due to insufficient food production (Sanchez, 2005). Optimal food production is further constrained by the serious degraded nature of most African soils (Nkonya et al., 2016). Volcanic tephra soils are among the most productive soils in the world since they accumulate large amounts of organic carbon (OC) and nitrogen (N) (Shoji and Takahashi, 2002). In the Mount Kupe area of Cameroon, these soils have been extensively used for cultivation of African oil palm (*Elaeis guineensis*), cocoa (*Theobroma cacao*), tubers such as cassava (*Manihot esculenta*) and taro (*Colocasia esculenta)*, and plantains (*Musa* spp.) and banana (*Musa* spp.). In order to meet the high demand for food in this locality due to increase in population, and to improve on the local nutritional standards and promote crop diversification, there is a need to evaluate the suitability of these soils for other crops. Furthermore, because of the pressures that an increasing population and economic growth have put on limited land resources, land suitability evaluation is recommended since it can assist in the efficient use of land resources at a regional level (Gong et al., 2012). Land evaluation, using a scientific procedure is essential to identify the potential and constraints of a given land for defined use (agriculture for our case) in terms of its fitness and ensure its sustainable use (Nahusenay and Kibebew, 2015). The objective of this study was to qualitatively assess the physical and chemical land suitability for rain-fed cultivation of maize (*Zea mays*), beans (*Phaseolus vulgaris*) and Irish potato (*Solanum tuberosum*), given their importance at the local and national scale. Furthermore, there is great necessity for understanding of soils of this area through proper characterization in order to propose management strategies that will make the farming systems of the area sustainable. Crop yield is generally dependent on the fertility status of the soil, and this soil quality combines several soil properties (physical, chemical and biological), all of which affect directly or indirectly nutrient dynamics and availability (Akinrinde, 2004; FAO, 2006a). These soil properties alongside environmental or climatic conditions are the determinants of sustainable crop production through proper soil fertility management, and for the special case of farmers, the most important properties of soils are their physical condition and chemical fertility (FAO, 2006a).

Evaluation of land suitability for the production of maize, beans and Irish potatoes has been done in the western highlands of Cameroon, notably around mount Bambouto, where the climate is colder (Tsozué et al., 2015) and soils vary among, oxisols, inceptisols, entisols and andosols (Tematio et al., 2004). Mount Kupe is one of the few areas in Africa where virgin forests exist. Humidity at mount Kupe and the surrounding areas isvery high, usually above 80%. The need for land suitability evaluation of these three crops: maize, beans and Irish potatoes comes from the fact that these crops are highly consumed in Cameroon and their demand keeps increasing with the increasing population.

MATERIALS AND METHODS

Study area

Mount Kupe is located at latitude 4°48' N and longitude 9°42' E, approximately 100 km North of Mount Cameroon (Figure 1). It has a very heterogeneous terrain with steep slopes, long shrunken ridges, rocky outcrops, bare cliffs and small peaks. It also has flat contrasting areas between the peaks at an altitude of 1,600 m. It was formed as a result of geological fractures and is 2,064 m high, limited by structural depressions (Gartlan, 1989). Volcanic activity occurred in these depressions and several cones are visible on the lower flanks of the mountain.

The soils of Mount Kupe are young and relatively fertile. The mid-slopes and lower slopes have deep and fertile soils (Gartlan, 1989). Micro-aggregated cambisols are more common and more developed than entisols. There is no evidence of peat formation at high altitudes and the soils are usually well drained.

The climate is the mountain Cameroon type, typical of central Africa precisely with two seasons: the rainy season from March to October accounting for about 80% of annual precipitation (the wettest months are from July to September accounting for 50% of the precipitation) and the dry season for the rest of the year.

The drainage system consists of several permanent streams emanating from mount Kupe which acts as a watershed for collecting and supplying the surrounding villages with water (Gartlan, 1989).

Soil description and sampling

Three sites, representative of the study area were identified following a toposequence: Nyassosso (at an altitude of 900 m at the foot of mount Kupe, located at Lat. 4° 49' 32.8" N, Long. 9° 41' 9.1" E), Mbule (at an altitude of 705 m, located at Lat. 4° 48' 0.7" N, Long. 9° 39' 45.4" E) and Tombel (at an altitude of about 475 m, located at Lat. 4° 43' 31.3" N, Long. 9° 41' 20.5" E). At each of the sampling sites, a representative soil profile in virgin land was dug. The profiles were described following the FAO guidelines (FAO, 2006b). Soil samples were collected per horizon, stored in

*Corresponding author. E-mail: enangrog@yahoo.com. Tel: +237 675 794 329.

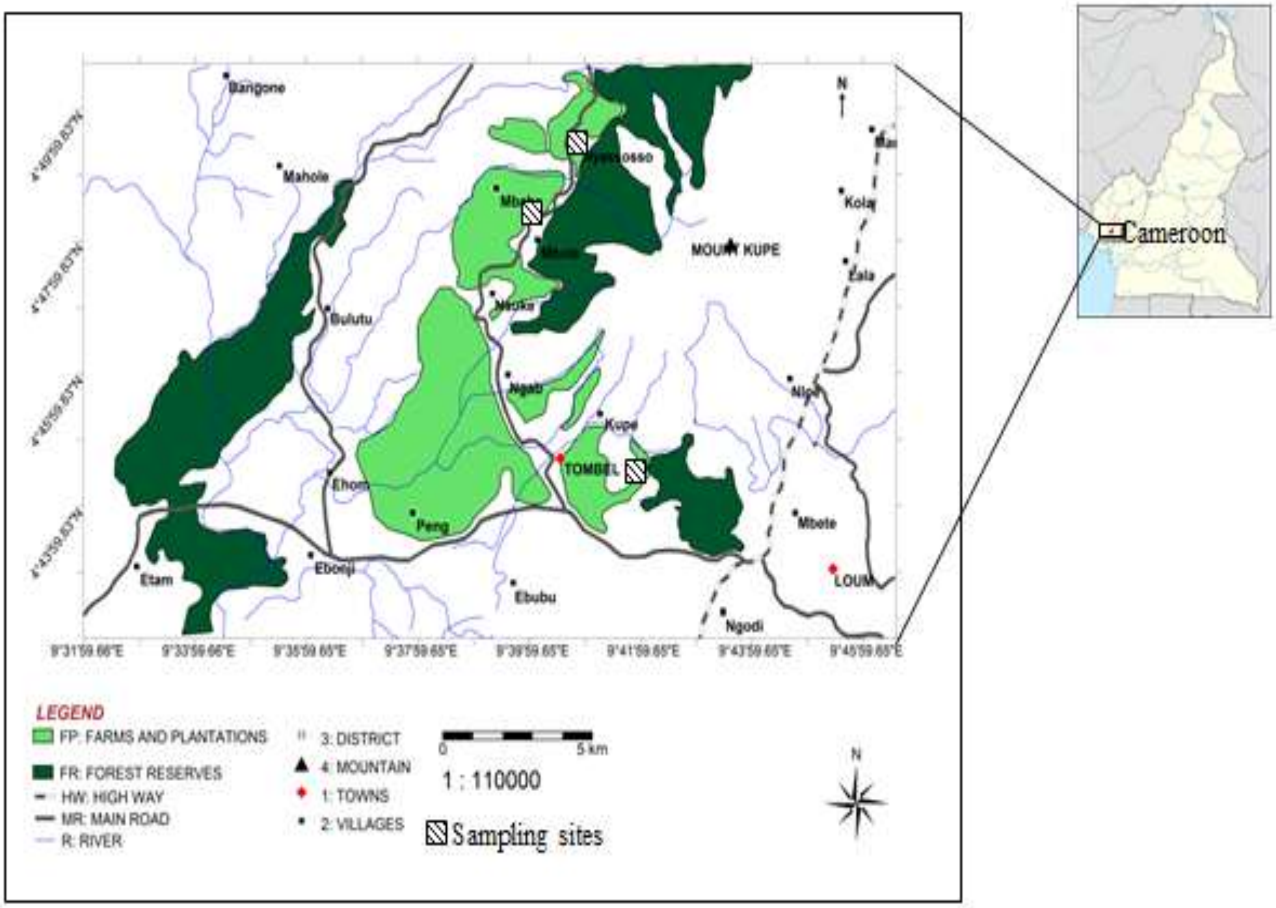

Figure 1. Location of Mount Kupe, the surrounding areas and sampling sites, at Cameroon.

polythene bags, and characterized for classification of the soils. Undisturbed samples for bulk density were collected in duplicates for quality control using Kopecky rings of 100 cm^3 volume. At each site, 16 representative surface samples were randomly collected at a depth of 0 to 30 cm, homogenized and characterized for evaluation of land suitability. A general description and soil classification of the sites harboring the three profiles studied are summarized in Table 1.

Laboratory analyses

Chemical properties were determined following procedures described by Pauwels et al. (1992). Soil OC content was determined by the Walkley and Black wet combustion method, while bulk density was determined as the oven dry (105°C) mass of each undisturbed core sample per volume. A 1:2 soil-H_2O and 1:2 soil-KCl solutions were used for $pH_{(H2O)}$ and $pH_{(KCl)}$ determinations, respectively. Total N and available phosphorous (P) were determined by the Kjeldahl wet digestion and the Bray II methods, respectively. Exchangeable bases were determined following the Schollenberger method using a 1 M ammonium acetate solution buffered at pH 7. The concentrations of exchangeable sodium (Na^+) and potassium (K^+) in the extract were obtained by flame photometry, and for calcium (Ca^{2+}) and magnesium (Mg^{2+}) by complexometry using a 0.002 M Na_2-EDTA solution. Cation exchange capacity (CEC) was determined by a direct continuation of the Schollenberger's method using a 1 N KCl solution for displacement of ammonium ions. Exchangeable acidity (Al^{3+} + H^+) was determined after displacement with a 1 N unbuffered KCl solution. Amorphous iron (Fe) and aluminum (Al) were determined by acid ammonium oxalate extraction (Pauwels et al., 1992). The hydrometer method was used for particle size distribution following procedures described by Bouyoucos (1962) after dispersion of the soil with a 2.5 N sodium hexametaphosphate solution to ensure proper dispersion of the soils.

Determination of the growing period in the study area

After obtaining the daily precipitation for 31 years, the average monthly precipitation over a 31-year period was determined. The potential evapotranspiration (PET) (Figure 2) was estimated using the radiation method (Jensen et al., 1990) defined as follows:

$$PET = c\ x\ (W\ x\ R_s)$$

where: c is a coefficient depending on the relative humidity and wind speed, representing an adjustment factor as presented in the Penman (1948) equation; W represents a weighting for the effect of radiation on PET at a particular temperature and altitude. Rs is the total solar radiation, a function of sunshine hours and extra-

Table 1. Description of study sites and soil classification of representative soil profiles.

Site characteristics	Nyassosso series	Mbule series	Tombel series
Land use/vegetation	Protected forest, apparently early exploitation of timber. Marantaceae, shrubs, *Carapa grandiflora*, *Cephaelis mannii*, *Dictonalepsis vestita*, *Ficus mucuso*, *Garcinia smaethmannii*, *Dorstenia*, *Dracaena*, *Haemanthus* and *Selaginella*.	Natural evergreen forest Marantaceae vegetation, twigs/twines, *Garcinia smaethmannii*, *Dorstenia*, *Dracaena*.	Evergreen forest, apparently early exploitation of timber. Marantaceae vegetation, twigs/twines, *Garcinia smaethmannii*, *Dorstenia*, *Dracaena*.
Physiography	Foot slope, 2 - 5 % (gently sloping), and presence of many pyroclastic rock out-crops (15 – 40 %) about 5 – 20 m apart, mountainous, occurrence of many streams signaling fault and fracture lines. Good internal and external drainage. Signs of slight geologic erosion.	Strongly sloping landscape. Middle slope, 10-15 %, absence of rock outcrops, unstable landscape susceptible to landslides. Very good internal and external drainage. Signs of geologic erosion.	Sloping landscape, (5-10 %), Sub mountain evergreen forest, few rock outcrops, pumice and scoria, all of quaternary origin. Good internal and external drainage, geologic erosion.
Relief/elevation	Highland 900 m	Highland 705 m	Graben 475 m
Parent material	Volcanic tephra (coarse tuff and scoria)	Volcanic tephra deposits (pumice, coarse tuff, scoria)	Volcanic tephra (pumice, scoria, coarse tuff)
Soil classification	Hyperdystri – humic Leptosol (loami – andic)	Skeleti – Umbric Andosol (colluvi – loamic)	Dystri – skeletic Andosol (Colluvi – loamic)
Soil temperature regime	Hyperthermic	Hyperthermic	Hyperthermic
Soil moisture regime	Udic	Udic	Udic

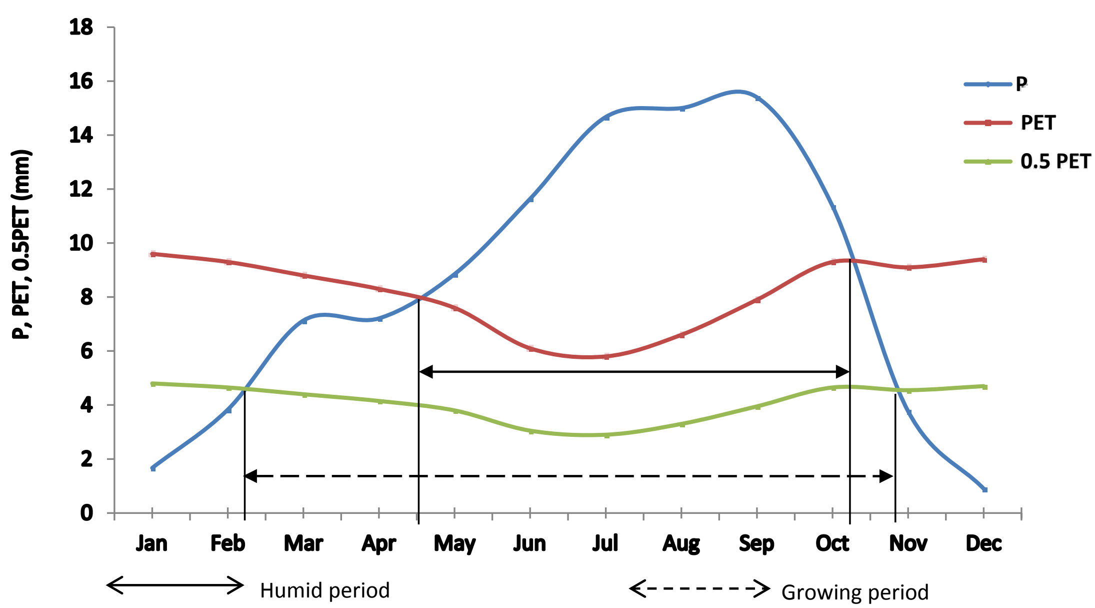

Figure 2. Climatic diagram of the study area showing the growing period. P = precipitation, PET = potential evapotranspiration.

Table 2. Qualitative land suitability classes for the different land indices.

Land index	Definition	Symbol
90-100	Highly suitable with no limitations	S1- 0
85-90	Highly suitable with slight limitations	S1- 0/1
75-85	Highly suitable with slight limitations	S1-1
60-75	Highly suitable to moderately suitable	S1-1/S2
50-60	Moderately suitable	S2
40-50	Moderately suitable to marginally suitable	S2/S3
25-40	Marginally suitable	S3
15-25	Marginally suitable to not suitable	S3/N
0-15	Not suitable	N

Adapted from Beernaert and Bitondo (1993).

Table 3. Classification of critical fertility levels in soils for organic carbon (OC), total nitrogen (N), available phosphorous (P), cation exchange capacity (CEC), base saturation (BS) exchangeable cations and soil reaction.

Soil properties (< 2 mm fraction)	Critical fertility level				
	Very low	Low	Medium	High	Very high
OC (%)	< 0.4	0.4-1.0	1.0-1.8	1.8-3.0	> 3.0
Total N (%)	< 0.05	0.05 - 0.125	0.125 - 0.225	0.225 - 0.30	> 0.30
C/N	< 10 = good, 10 - 14 = medium and > 14 = poor				
Ca^{2+} ($cmol_c kg^{-1}$)	< 2	2 - 5	5 - 10	10 - 20	> 20
Mg^{2+} ($cmol_c kg^{-1}$)	< 0.5	0.5 - 1.5	1.5 - 3.0	3 - 8	> 8
K^+($cmol_c kg^{-1}$)	< 0.1	0.1 - 0.3	0.3 - 0.6	0.6 -1.2	> 1.2
Na^+ ($cmol_c kg^{-1}$)	< 0.1	0.1 - 0.3	0.3 - 0.7	0.7 - 2.0	> 2.0
P ($mgkg^{-1}$)	< 7	7 - 16	16 - 46	> 46	-
$pH_{(H2O)}$	≤5.5 (strongly acidic)	5.6 - 6.0 (moderately acidic)	6.1 - 6.5 (slightly acidic)	7.4 - 7.8 (slightly alkaline)	7.9 - 8.4 (soderately alkaline)
CEC ($cmol(+)kg^{-1}$)	< 6	6 - 12	12 - 25	25 - 40	> 40
BS (%)	0 - 20	21 - 40	41 - 60	61 - 80	81 - 100
EC (dS/m)	< 2 (non - saline)	2 - 4 (slightly saline)	4 - 8 (moderately saline)	8 - 16 (highly saline)	> 16 (extremely saline)

Adapted from Cass (1998), Hazelton and Murphy (2007), and Euroconsult (1989).

terrestrial radiation.

Land use, crop requirements and evaluation of land suitability

The land use envisaged is rain fed cultivation of maize, beans and Irish potatoes by small scale farmers at minimal management level. Land suitability assessment was done using tables for different crop requirements in land evaluation proposed by Sys et al. (1993). The evaluation method enabled the identification of both soil and climatic parameters limiting the growth and production of the selected crops in the study area. A parametric method was used to classify the suitability of the soils as highly suitable (S1), moderately suitable (S2), marginally suitable (S3) and not suitable (N). In this parametric method, land and climatic characteristics are defined using different ratings. These characteristics were determined using the Storie method (a parametric method) as described by Sys et al. (1993).

$$I = A x \frac{B}{100} x \frac{C}{100} x \frac{D}{100} x \frac{E}{100} x \ldots$$

where, I is the specified index and A, B, C, D, E, etc, are different ratings given for each property.

Climatic characteristics considered in this study were rainfall, temperature and relative humidity, while land characteristics considered were topography (slope), wetness (flooding and drainage), soil physical characteristics (texture, coarse fragment volume % and soil depth), soil fertility characteristics (CEC, base saturation- BS, OC, soil reaction - pH), salinity (electrical conductivity - EC) and alkalinity (exchangeable sodium percentage-ESP).

By determining the land index and making use of guidelines outlined by Sys et al. (1991), the qualitative land suitability classes (Table 2) and the limiting factors for plant growth in different soil series for each crop were determined. Critical values of soil nutrients and soil fertility parameters are shown in Table 3.

RESULTS AND DISCUSSION

Morphological properties

Soil colour

In profile 1, colour varies from dark reddish brown (5YR 3/3) in the A horizon to dull reddish brown (5 YR 5/4, 5 YR 4/4, 5 YR 4/4) in the ABw, Bw and Bw/Cr horizons, respectively. Colour in profile 2 is brownish black (5YR 2/2) in the A horizon, dark reddish brown (5YR 3/2) in the Bw horizon, brownish black (7.5 YR 3/2) in the 1C horizon, dark brown (7.5 YR 3/3) in the 2C and 3C horizons, a brownish black (5YR 2/1) matrix with very dark reddish brown (2.5YR 2/3) inclusions in the 4C horizon and very dark reddish brown (7.5 YR 2/3) in the 5C horizon (Table 4).

Profile 3 colour varies from brownish black (5 YR 3/1) in the A horizon, through dark reddish brown (5 YR 3/3) in the Bw/Cr horizon to Black (5 YR 1.7/1) in the Cr horizon. The presence of different colours in this profile reflects the varied parent materials observed (Brady, 1990), dominated by tuff and scoria with some colluvial material mostly of granitic nature.

Soil structure, consistence and texture

All the three soil profiles have dominantly granular structure (Table 4) which reflects the parent materials from which these soils were developed. This structure has great influence on soil stability. Field observations indicated that landslides were very common in the study area due to the occurrence of K-cycles described by Fitz Patrick (Yerima and Van Ranst, 2005a). These soils are of low stability and are inappropriate for infrastructural works.

The surface horizons of these soils are friable when moist and slightly sticky to sticky and slightly plastic to plastic when wet, while in subsurface horizons it is firm when moist and dominantly non sticky and non-plastic when wet.

These morphological properties are consistent with the soil texture which is dominated by the sand fraction, followed by clay which gives the soils a loamy texture.

Physical properties

The three soils have bulk density values varying from 0.38 to 1.08 g/cm^3 with an average value of 0.71 g/cm^3 (Table 4). These values are typical of volcanic ash soils which generally have a bulk density less than 0.9 g/cm3 (Olafur, 2008). In profiles 1 and 2 (Nyassosso and Mbule series), variations of bulk density with depth are erratic. These profiles are located in areas with frequent deposition of colluvial materials associated with the unstable geomorphic surfaces, especially for profile 2 located on a strongly sloping landscape (Table 1). In profile 3 (Tombel series), bulk density has a regular depth function. Tombel is a graben with a greater stability as compared to the other two series.

Apparently the stability of the geomorphic surface would enable the development of a soil with a higher profile differentiation. Particle size analysis indicates that, the sand fraction is dominant in all the three soils, followed by the clay fraction. In all the three soils, sand content > clay content > silt content.

Chemical properties

Soil reaction (pH)

All three soils are acidic in nature (Table 5). $pH_{(H2O)}$ values range between 5.1 and 6.2, while $pH_{(KCl)}$ values ranged from 3.6 to 4.9 for profile samples. For surface samples, $pH_{(H2O)}$ values varied from 4.8 to 6.2, while $pH_{(KCl)}$ values range between 3.5 and 4.8. Therefore, the soils can be considered with moderate acidity, typical of mineral soils in humid regions (Brady, 1974). The acidity of these soils is associated with the high rainfall, coupled with the porous nature of the soils, resulting in the leaching of bases. For all the profiles, pH increases with depth, which is consistent with the leaching of bases.

Organic carbon

Organic carbon contents are relatively high in all soils (Table 5). Distribution of OC within profiles especially for profiles 1 and 2, are erratic. Alternation of organic matter with volcanic ash layers as indicated by bulk density values explains the erratic distribution of OC with depth. Colluvial deposits and landslides frequently observed at these sites are responsible for the occurrence of buried horizons which explain the high organic matter contents in subsurface horizons as compared to that of surface horizons.

The prevalence of earth movements at these sites apparently hinder a progressive evolution of soils in these environments, resulting in erratic distributions of soil properties with depth. Erratic OC depth functions have been reported in soils in similar environments (Kubotera and Yamada, 1995). The mineralization of the OC is retarded by the presence of allophane in these soils, which forms more stable allophane-humus complexes (Yerima and Van Ranst, 2005a). In profile 3 however, distribution of OC with depth follows a regular decreasing pattern with depth. These soils are located in a graben where the stability of the geomorphic surfaces enables a more progressive and regular development of soil.

Total nitrogen

Total nitrogen (TN) in these soils ranged from low

Table 4. Morphological and physical characteristics of representative soil profiles in the study area.

Horizon	Depth (cm)	Colour (moist)	Sructure	Consistence		Boundary	Porosity (%)	BD (g/cm³)	Sand (%)	Silt (%)	Clay (%)	Coarse fraction > 2 mm (%)	Textural Class
				Moist	Wet								
Nyassosso series: Hyperdystri – humic Leptosol (loami – andic)													
Oi	0-10	-	-	-	-	-	-	-	-	-	-	-	-
A	10-20	5YR 3/3	FI, SB→GR	FR	SST and PL	C and S	75.1	0.66	77.3	5.8	16.9	17	SL
ABw	20-40	5YR 5/4	FI, SB→GR	FR	SST and SPL	C and S	69.8	0.8	78.6	4.3	17.1	19	SL
Bw	40-80/90	5YR 4/4	SB→GR	FR	SST and SPL	D and B	59.3	1.08	78.2	6.4	15.4	16	SL
Bw/Cr	80/90-115	5YR 4/4	SB	FR	NST and NPL	D and S	59.6	1.07	74.0	9.7	16.3	23	SL
Mbule series: Skeleti – Umbric Andosol (colluvi – loamic)													
Oi	0-5	-	-	-	-	-	-	-	-	-	-	-	-
A	5-20	5YR 2/2	FI GR	FR	SST and SPL	C and S	80.75	0.51	71.8	2.3	25.9	19	SCL
Bw	20-65	5YR 3/2	FI, CO GR	FR	SST and SPL	C and S	72.83	0.72	69.8	9.7	20.5	25	SCL
1C	65-78	7.5 YR 3/2	CO GR	FI	NST and NPL	A	77.74	0.59	74	5.3	20.7	31	SCL
2C	78-98	7.5 YR 3/3	CO GR	FR	NST and NPL	A	74.72	0.67	71.9	4.7	23.4	29	SCL
3C	98-140	7.5 YR 3/3	CO GR	FR	NST and NPL	A	76.23	0.63	69.5	11.9	18.6	42	SL
4C	140-155/140-165	5YR 2/1 (matrix), 2.5YR 2/3(inclusions)	CO GR	FR	SST and NPL	C and W	76.98	0.61	76.8	2	21.3	39	SCL
5C	155/165-200	7.5 YR 2/3	CO GR	FR	SST and NPL	C and W	75.85	0.64	76.7	6.7	16.7	44	SL
Tombel series: Dystri – skeletic Andosol (Colluvi – loamic)													
Oi	0-10	-	-	-	-	-	-	-	-	-	-	-	-
A	10-22	5 YR 3/1	FI GR	FR	NST and NPL	C and S	85.66	0.38	65.6	2.2	32.2	33	SCL
Bw/Cr	22-86	5 YR 3/3	CO GR	FI	NST and NPL	C and S	72.45	0.73	75.5	4.3	20.2	41	SCL
Cr	86-155	5 YR 1.7 / 1	SB	VFI	NST and NPL	D and I	67.17	0.87	81.9	6.5	11.6	59	LS

SB = Sub angular blocky, GR = Granular, SB→GR = Sub angular blocky parting to Granular, FI = Fine/thin (1-2 mm), ME = Medium (2-5 mm), CO = Coarse/thick (5-10 mm), WE = Weak, MS = Moderate to Strong, FR = Friable, FI = Firm, VFI = Very firm, ST = Sticky, SST = Slightly sticky, PL = Plastic, NST = Non-sticky, NPL = Non-plastic, C = Clear (2-5 cm), G = Gradual (5-15 cm), D = Diffuse (>15 cm), S = Smooth, W = Wavy, I = Irregular, A = Abrupt. NB: FI= fine (for structure) and firm (for consistence). Source: FAO (2006b).

Table 5. Chemical characteristics of the representative soil profiles in the study area.

Horizon/depth (cm)	pH H_2O	pH KCl	OC (%)	Total N (%)	C/N	P Bray II (mg kg^{-1})	Exch. bases (cmol(+)kg^{-1})				∑bases	Exch. Al^{3+}	CEC pH7 (cmol(+)kg^{-1})	BS (%)	ESP (%)	EC (mS/cm)	Ammonium oxalate extractable	
							K^+	Na^+	Mg^{2+}	Ca^{2+}							Al_2O_3	Fe_2O_3
Nyassosso series: Hyperdystri – humic Leptosol (loamy – andic)																		
Oi (0-10)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A (10-20)	5.1	4	4.44	0.67	6.63	9.41	0.21	0.03	0.72	2.32	3.28	0.6	26.24	13	0.11	0.2	0.26	0.67
ABw (20-40)	5.2	3.6	2.25	0.36	6.25	8.84	0.25	0.03	0.08	2	2.36	0.08	23.68	10	0.13	0.04	0.26	0.06
Bw (40-80/40-90)	5.5	3.7	2.73	0.23	11.87	11.12	0.02	0.01	0.16	2.24	2.43	0.52	23.04	11	0.04	0.02	0.19	0.89
Bw/Cr(80-115/90-115)	5.5	3.8	2.34	0.16	14.63	10.55	0.01	0.01	0.64	2.8	3.46	0.56	22.4	15	0.04	0.02	0.16	0.14
Mbule series: Skeleti – Umbric Andosol (colluvi – loamic)																		
Oi (0-5)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A (5-20)	5.2	4.2	5.38	0.91	5.91	11.12	0.05	0.02	0.56	9.44	10.07	0.1	30.72	33	0.07	0.29	0.97	0.44
Bw (20-65)	5.7	4.3	2.07	0.15	13.80	7.13	0.31	0.05	2.56	5.12	8.04	-	27.2	30	0.18	0.02	0.37	1.12
1C (65-78)	5.9	4.5	0.88	0.13	6.77	9.98	0.2	0.03	1.04	6.24	7.51	-	23.68	32	0.13	0.03	0.32	0.44
2C (78-98)	6.2	4.6	1.88	0.15	12.53	11.12	0.25	0.04	2.4	8.8	11.49	-	22.08	52	0.18	0.03	0.99	0.21
3C (98-140)	6.2	4.4	3.26	0.41	7.95	10.55	0.03	0.01	1.36	10.64	12.04	-	35.2	34	0.03	0.04	1.01	1.19
4C (140-155/140-165)	6.2	4.6	1.87	0.12	15.58	13.39	0.25	0.03	2.32	8.08	10.68	-	37.12	29	0.08	0.03	0.44	1.35
5C (155/165-200)	6.2	4.6	1.76	0.09	19.56	9.98	0.24	0.03	0.88	6.08	7.23	-	30.08	24	0.10	0.03	0.26	0.97
Tombel series: Dystri – skeletic Andosol (Colluvi – loamic)																		
Oi (0-10)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A (10-22)	5.6	4.7	7.26	1.70	4.27	15.47	0.02	0.01	1.52	6.24	7.79		38.08	20	0.03	0.12	0.41	0.89
Bw/Cr (22-86)	5.8	4.9	4.31	0.50	8.62	17.22	0.08	0.01	1.28	7.92	9.29		22.08	42	0.05	0.03	0.30	0.74
Cr (86-155)	5.9	4.9	2.33	0.21	11.10	19.76	0.12	0.04	1.33	7.98	9.47		20.5	46	0.20	0.02	0.32	0.82
Mean values of chemical properties for surface samples in the study area																		
Depth (cm)	**Nyassosso series: Hyperdystri – humic Leptosol (loamy – andic)**																	
0 – 30	5.3	3.9	4.86	0.46	10.57	9.03	0.24	0.03	0.29	2.11	2.67	0.25	24.53	10.88	0.12	0.09	0.26	0.26
Mbule series: Skeleti – Umbric Andosol (colluvi – loamic)																		
0 – 30	5.7	4.4	4.67	0.34	13.74	8.13	0.25	0.04	2.06	6.20	8.55	-	28.08	30.45	0.14	0.09	0.52	0.95
Tombel series: Dystri – skeletic Andosol (colluvi – loamic)																		
0 – 30	5.8	4.8	6.35	0.69	9.20	16.94	0.07	0.01	1.32	7.65	9.05	-	32.61	27.75	0.03	0.04	0.32	0.76

(0.09%) to very high (1.7%) with most samples having high TN concentration (Table 5). High N concentration is associated with high organic matter contents at surface horizons (Whitbread, 1995). The N concentrations in the profiles decrease with depth.

Available phosphorous

Available P values are low, ranging between 7.13 and 19.76 mg kg^{-1} (Table 5). These low P concentrations are associated with the acidic nature of the soils: all the three soils studied are acidic with pH values < 6.5 (4.8 to 6.2). At these pH values, Fe, Al and manganese (Mn) are highly soluble. Because the ionic form in which P exists in this pH range is the phosphate ($H_2PO_4^-$) (Harrison, 2007), those cations will react with the phosphate ions to form hydroxy-phosphate which is insoluble, becoming unavailable for plant uptake.

Amorphous iron and aluminium

Amorphous Fe and Al are important soil parameters which can inform on the structural stability of soils, especially aggregate stability (Stefanou and Papazafeiriou, 2013). They can contribute to the fragic nature of some soil horizons (Steele et al., 1969), inform on the rate of weathering (Degörski, 2011), and can also be used as a criteria for soil classification (FAO, 2014). Generally, Fe and Al oxides contribute to the formation of compacted soil horizons (hardpans, duripans, hard settings) which are recognized as genetic soil horizons (Soil Survey Staff, 1999). Fe oxides and Al oxides are not by themselves cohesive or coagulating agents of soil particles, though they play a significant role in cementing some soils (Stefanou and Papazafeiriou, 2013).

For the three soils studied, concentrations of amorphous Fe ranged from 0.06 to 1.35%, while concentrations of amorphous Al range between 0.16 and 1.01% (Table 5). These concentrations are high enough to have an effect on the structural stability of soils. According to Stefanou and Papazafeiriou (2013), a concentration of about 1% amorphous Fe may cause a the occurrence of progressive weathering of parent materials, but the weathering is not advanced enough to qualify the soils as old penetration resistance of about 7 MPa, while a concentration of about 1 % amorphous Al may cause a root penetration resistance of about 34 MPa. The above concentration ranges of amorphous Al and Fe indicated (Degörski, 2011). The soils have vitric materials indicated by the (Al+1/2Fe)o values which are greater than 0.4% (Olafur, 2008). The presence of these vitric materials is in conformity with the high sand contents (Table 4).

Electrical conductivity

Electrical conductivit (EC) is low in all soils. Values for EC ranged from 0.02 to 0.29 mS/cm (Table 5). According to Richards (1954), the EC of these soils belongs to “Class A”. This range of EC reflects osmotic potentials of 0 to 70 KPa and, further indicates that with respect to crop salt tolerance, the crops are sensitive (Richards, 1954). In all profiles, EC values decrease with depth. Generally, EC is related to soil texture. EC values are generally higher for fine-textured soils than for coarse-textured soils (Sudduth et al., 2004). Field descriptions and observations of the soils indicated that surface horizons were more fine-textured as compared to subsurface horizons. This explains why EC values decrease with depth.

Cation exchange capacity

Cation exchange capacity is high in all soils (Table 5). CEC ranged between 20.5 and 38.08 cmol(+)kg^{-1} soil. The CEC values are higher in surface horizons and decrease with depth. The high CEC values may be associated with the high organic matter contents in these soils as organic matter is a source of negative charge in soils. In similar soils around mount Cameroon and in the Mungo (close to Tombel), higher values of CEC (35 to 55 cmol(+)kg^{-1}, with organic matter contents between 6 and 10%) have been reported (Yerima and Van Ranst, 2005b).

Exchangeable bases

Exchangeable bases (Ca^{2+}, Mg^{2+}, K^+, Na^+) in all soil profiles showed the following trend: Ca^{2+} > Mg^{2+} > K^+ and Na^+. This is a general observation in most soils (Kim, 1998). Equally, Na^+ is more labile than the other elements (Yerima and Van Ranst, 2005a). Quantitatively, the concentrations of these cations vary as follows: Ca^{2+} (between 2.0 and 10.64 cmol(+)kg^{-1} soil), Mg^{2+} (between 0.08 and 2.56 cmol(+)kg^{-1} soil), K^+ (between 0.01 and 0.31 cmol(+)kg^{-1} soil) and Na^+ (between 0.01 and 0.05 meq/100 g soil). These concentrations are quite low (especially for Na^+ and K^+) not only because of the porous nature of the soils that are prone to base leaching but equally to the prevalence of pH values of less than 6, where these cations are deficient (Kim, 1998). Harrison (2007) also reported a decrease of soil concentrations of Ca^{2+}, Mg^{2+}, N and sulphur (s) with increasing soil acidity, and unavailability of P under low and high pH values (below 6 and above 8, respectively). Nonetheless, Ca^{2+} and Mg^{2+} are in moderate concentrations with respect to critical fertility levels and indicate the presence of considerable amounts of weatherable minerals.

Evaluation of land suitability

Climatic and land suitability indices for maize, beans and Irish potatoes are presented in Tables 6 and 7. The soils of Nyassosso are marginally suitable for rain-fed

Table 6. Evaluation of climatic characteristics for beans, maize and Irish potatoes in the three sites.

Climatic characteristic	NYASSOSSO						MBULE						TOMBEL					
	Maize		Beans		Irish potato		Maize		Beans		Irish potato		Maize		Beans		Irish potato	
	V	Par V	V	Par V	V	Par V	V	Par V	V	Par V	V	Par V	V	Par V	V	Par V	V	Par V
Precipitation of growing cycle (mm)	1061	90.36	712	67	1060	100	1061	90.36	712	67	1061	100	1061	90.36	712	67	1061	100
Precipitation of the 1st month (mm)	221	85.14	-	-	221	100	221	85.14	-	-	221	100	221	85.14	-	-	221	100
Precipitation of the 2nd month (mm)	216	98.44	-	-	216	100	216	98.44	-	-	216	100	216	98.44	-	-	216	100
Precipitation of the 3rd month (mm)	274	90.25	-	-	274	100	274	90.25	-	-	274	100	274	90.25	-	-	274	100
Precipitation of the 4th month (mm)	349	73.91	-	-	349	95	349	73.91	-	-	349	95	349	73.91	-	-	349	95
Mean temp. growing cycle (°C)	21.6	86	21.6	77.5	21.6	91	23.6	98	23.6	86	23.6	86.3	25.6	99.5	25.6	73.3	25.6	73.3
Mean min. temp. growing cycle (°C)	21.3	85.75	21.3	91.75	-	-	23.3	93.8	23.3	86.75	-	-	25.3	65.42	25.5	57.5	-	-
Relative humidity of developmental stage (%)	86	88	86	78.3	-	-	86	88	86	78.3	-	-	86	88	86	78.3	-	-
Average daylength of growing cycle (h)	-	-	-	-	14.3	97.16	-	-	-	-	14.3	97.16	-	-	-	-	14.3	97.16
Relative humidity of maturity stage (%)	87	80	86	-	-	-	87	80	86	-	-	-	87	80	86	-	-	-
Climatic Index (CI),		50.7		55.68		83.99		55.46		45.12		79.66		38.68		30.17		67.66
Climatic Rating (CR)		62.3		66.78		92.26		66.58		57.23		88.36		51.41		43.82		77.56

V= Value, Par V= parametric value.

Table 7. Evaluation of land and soil characteristics for beans, maize and Irish potatoes for each of the three sites.

Landscape and Soil characteristics	*Nyassosso* : Epidystri – Andic Cambisol (humi – loamic)						*Mbule :* Epidystri – Skeletic Andosol (Colluvi – loamic)						*Tombel: Dystri – Skeletic Andosol (Colluvi – loamic)*					
	Maize		Beans		Irish potato		Maize		Beans		Irish potato		Maize		Beans		Irish potato	
	V	Par V	V	Par V	V	Par V	V	Par V	V	Par V	V	Par V	V	Par V	V	Par V	V	Par V
Topography (t)																		
Slope (%) (l)	2-5	95	2-5	95	2-5	95	10-15	72.5	10-15	72.5	10-15	72.5	5-10	90	5-10	90	5-10	90

Table 7. Contd.

Wetness (w)																		
Flooding	None	100	None	100	None	100	None	100	None	100	None	100	None	100	None	100	None	100
Drainage	Good	100	Good	100	Good	100	Good	100	Good	100	Good	100	Good	100	Good	100	Good	100
Physical soil characteristics (s)																		
Texture/structure	SL	72.5	SL	72.5	SL	90	SCL	90	SCL	90	SCL	100	SCL	90	SCL	90	SCL	100
Coarse fragments (volume %)	15	95	15	95	15	95	27	76.25	27	76.25	27	76.25	35	60	35	60	35	60
Soil depth (cm)	>100	100	>100	100	>100	100	>100	100	>100	100	>100	100	>100	100	>100	100	>100	100
Soil fertility (f)																		
CEC (meq/100g)	24.53	100	24.53	100	24.53	100	28.08	100	28.08	100	28.08	100	32.61	100	32.61	100	32.61	100
BS (%)	10.88	50.9	10.88	50.9	10.88	67.7	30.45	77.4	30.45	77.4	30.45	81.8	27.75	72.9	27.75	72.9	27.75	79.8
pH-H_2O	5.3	50.0	5.3	46.6	5.3	66.3	5.7	87.5	5.7	76.6	5.7	87.5	5.8	90.0	5.8	85.5	5.8	90.0
OC (%)	4.86	100	4.86	100	4.86	100	4.67	100	4.67	100	4.67	100	6.35	100	6.35	100	6.35	100
Salinity and sodicity (n)																		
ESP (%)	0.12	100	0.12	100	0.12	100	0.14	100	0.14	100	0.14	100	0.03	100	0.03	100	0.03	100
EC (mmhos/cm)	0.09	100	0.09	100	0.09	100	0.09	100	0.09	100	0.09	100	0.03	100	0.03	100	0.03	100
Land index	20.4		20.4		49.7		25.6		21.8		40.0		18.2		15.5		33.4	
Suitability class	S3/N,f		S3/N,f		S2/S3,f		S3		S3/N,f		S2/S3		N,s,f		N,s,f		S3	

V= Value, Par V= parametric value.

cultivation of Irish potatoes, but not suitable for the cultivation of maize and beans due to fertility constraints, notably low base saturation and low pH (Table 7). At these low pH values, Al becomes soluble, thus occupying exchange sites. These bases can however be corrected through liming and fertilization (especially organic amendments). The land indices of 49.7, 20.4 and 20.4 for Irish potatoes, beans and maize, respectively indicate that soils of Nyassosso are most suitable for the cultivation of Irish potatoes, followed by beans and maize. Soils in the Mbule series are marginally suitable for maize and Irish potatoes cultivation but not suitable for the cultivation of beans due to fertility constraints (low BS and low pH). The land indices of 40.0, 25.6 and 21.8 for Irish potatoes, maize and beans, respectively indicate that soils are more suitable for Irish potatoes cultivation, followed by maize, and then beans. In the Tombel series, land indices of 33.4, 18.2 and 15.5 for Irish potatoes, maize, and beans, respectively, indicate that soils in Tombel are marginally suitable for rain-fed cultivation of Irish potatoes but currently not suitable for maize and beans.

In Tombel, the main constraint is the high amount of gravel in the soils which will certainly have an impact on root development associated with low water and nutrient retention, high rate of organic matter mineralization, high base leaching, and low stability, especially for maize. This limitation can however be managed by selecting the appropriate sites where surface horizons have low gravel contents. Nyassosso and Mbule will thus be more appropriate.

From Nyassosso (900 m above sea level) to Mbule (700 m above sea level) and Tombel (500 m above sea level), there was a progressive decrease in land indices for Irish potatoes.

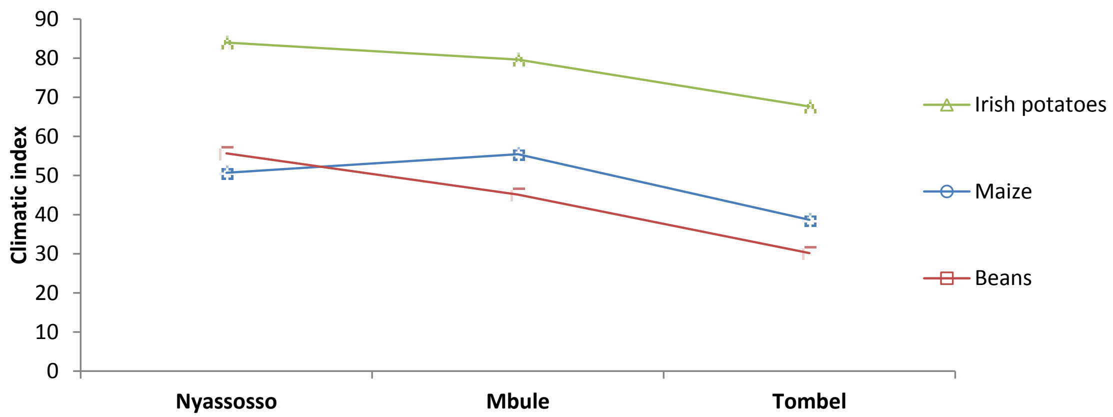

Figure 3. Variation of climatic index along a toposequence for rain-fed cultivation of Irish potatoes, maize and beans.

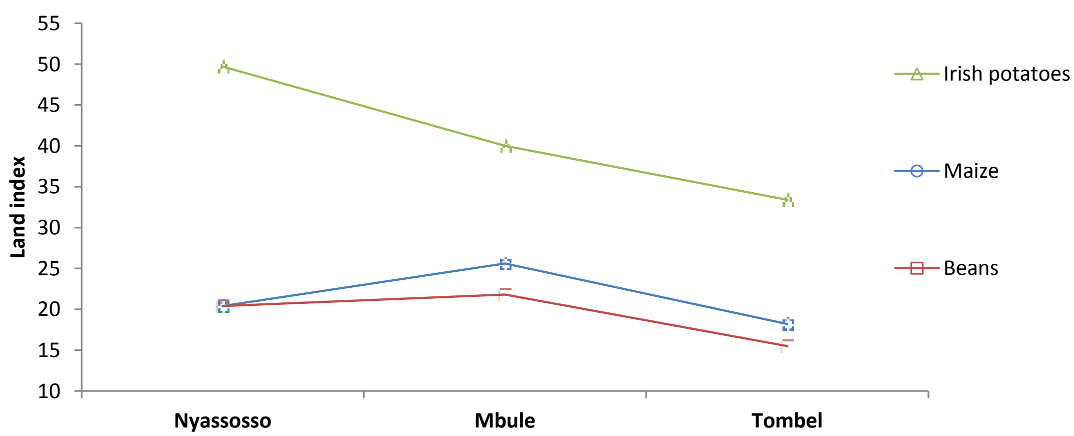

Figure 4. Variation of land index along a toposequence for rain-fed cultivation of Irish potatoes, maize and beans.

Therefore, a better performance of Irish potatoes will be observed at higher altitude and/or lower temperature. For maize and beans however, land indices indicated that best crops performance will be observed in the Mbule series.

Figures 3 and 4 show that performance of maize, beans, and Irish potatoes vary along a toposequence. Climatic indices (Table 6) indicated that best performance of these crops will be observed at higher altitudes and lower temperatures rather than at lower altitudes where the temperatures are higher. Cultivation of these crops has given good results on the western highlands of Cameroon, precisely on the Bambouto Mountains with varied soil types and lower temperatures (Tsozué et al., 2015).

Conclusions

Although, soils of the western slopes of Kupe mountain show soil properties that are conducive for crop production (high organic matter content and low bulk density), the coarse-textured nature of these soils, and the high humidity (higher elevations) and higher temperature (lower elevation) prevailing in the areas may increase the mineralization of organic matter leading to the fast release of nutrient elements which are quickly leached due to the high rainfall; especially, in Tombel, where temperature is higher. The soils in the study area are only marginally suitable for the rain-fed cultivation of corn, beans and Irish potatoes due to climatic limitations, soil acidity and stoniness (coarse fragments). Nevertheless, management practices such as the use of organ manures, liming and chemical fertilizers can readily mitigate the limitations.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Agronomic performance and path analysis of roundup ready and conventional soybean from two-way crosses in the northwestern of São Paulo, Brazil

Wallace de Sousa Leite*, Sandra Helena Unêda-Trevisoli, Eder Licieri Groli, Cleber Vinicius Giaretta Azevedo, Bruno Henrique Pedroso Val, Eduardo Henrique Bizari, Fabiana Mota da Silva and Antonio Orlando Di Mauro

Department of Crop Production, São Paulo State University "Júlio Mesquita Filho", FCAV/UNESP, Via Prof. Paulo Donato Castellani, s/n, ZIP 14884-900, Jaboticabal - SP, Brazil.

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The aim of this study was to compare the agronomic performance of RR soybean genotypes with conventional soybean genotypes derived from two-way crosses and evaluate through path analysis the influence of important traits for culture on the grain yield (GY) in the Northwestern of São Paulo, Brazil. It was used the randomized block design with three replications. Among the analyzed RR genotypes, three genotypes has high GY, with average values over 4575.5 kg ha^{-1}, while among the conventional, ten genotypes, and the check Conquista showed superiority for GY, with average values over 3511.4 Kg ha^{-1}. In general, the most productive RR soybean genotypes showed higher values when compared with conventional genotypes with higher yield. However, conventional soybean showed a higher number of superior genotypes with similar behavior when compared to the RR soybean. For the group of RR soybean genotypes, all agronomic traits, except one hundred seed weight (HSW), correlated positively with GY. For the group of conventional soybean genotypes, there was no significant correlation between GY and all agronomic traits analyzed. The genotypic correlation and path analysis indicate the plant height at flowering (PHF) and plant height at maturity (PHM) as the most favorable and direct effect on GY.

Key words: *Glycine max*, breeding, genetic correlations, productive performance, direct effect, RR soybeans.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill] is considered one of the most important crops in Brazil due to be the most widely grown in different regions, moving significantly the economy and generating jobs and rents. Brazilian soybean production in crop year 2015/16 was 95.43 millions of tons of grain, consolidating the country as the

*Corresponding author. E-mail: leitewallace@hotmail.com.

second largest producer of the world. In São Paulo state,in the crop year 2015/2016, the area cultivated with soybean was 857,600 hectares, with an average productivity of grains of 3316 kg ha^{-1} and production of 2.84 millions of tons of grains (Conab, 2016). It is noteworthy that the high level of soybean production has great contribution of breeding with the development of transgenic cultivars.

The area sown with Roundup Ready (RR) cultivars in Brazil reached 93.5% of the total area, corresponding to 29.4 million hectares (Céleres, 2015). According to Lima et al. (2008) and Matsuo et al. (2009), the adoption of RR cultivars is based on the ease of management of areas infested with weeds, allowing the efficient check of weeds in post-emergence stage.

Since the implementation of RR soybeans in commercial crops, there have been some questions about the efficiency of production of these cultivars when compared to conventional cultivars. In the literature, some of the studies already carried out, comparing both, have demonstrated differences in agronomic performance (Fonseca et al., 2013; Lima et al., 2008; Lima et al., 2015). The soybean agronomic performance is greatly influenced by the interaction genotype x environment, which makes difficult the identification of adapted and stable genotypes for the different growing regions (Batista et al., 2015; Branquinho et al., 2014). So, to recommend new cultivars, regional studies aimed at minimizing the environmental effects of developing cultivars are always needed (Azevedo et al., 2015; Gomez et al., 2014).

In soybeans, as in other crops, grain yield is a complex character, as it is the result of the expression and association of several factors that act indirectly and interact with each other. Knowledge of associations between traits, estimated by genetic correlations, is of great importance in plant breeding because it provides information that helps the breeder in the selection process (Malik et al., 2007; Nogueira et al., 2012). For low-heritability and/or difficult-to-assess traits, the indirect selection can be adopted, by using another trait that has high heritability and/or is easy to assess, provided that this another trait is highly correlated with that which is of difficult direct selection (Almeida et al., 2010).

The breakdown of the correlation coefficients in direct and indirect effects of agronomic traits, which act on the grain yield by path analysis, is of great importance because it allows a more precise identification of the key traits that determine productivity (Akram et al., 2011; Alcântara Neto et al., 2011).

In order to supply the demand of soybean genotypes with high productivity of grains, and specific to the northwestern region of São Paulo, the soybean breeding program of the FCAV/UNESP –Jaboticabal/SP - Brasil, it is focused in the conduction of final competition tests for the identification of genotypes with optimal agronomic performance.

Given the dearth of information and research that contrast the agronomic performance of RR soybeans with conventional soybeans, comparative studies between genotypes of both groups become important. Therefore, the aim of this study was to compare the agronomic performance of RR soybean genotypes with Conventional soybean genotypes deriving from two-way crosses and evaluate through path analysis the influence of important traits for the culture on the grain yield in the Northwestern of São Paulo, Brazil.

MATERIALS AND METHODS

Two experiments were conducted in the agricultural year 2014/15 in the Teaching, Research and Extension Farm (FEPE) of the São Paulo State University "Julio de Mesquit a Filho" UNESP- FCAV-Jaboticabal Campus, latitude 21° 15' 22" S and longitude 48° 18' 58" W, with an approximate altitude of 595 m. The soil of the experimental area is classified as eutrophic Red Latosol (Oxisol) (Santos et al., 2013), with gently rolling relief. The climate has hot and humid summers and dry winters with average annual temperature of 22.2°C and average annual rainfall of 1,451 mm (Vianna et al., 2013). Sowing was mechanically held on November 18, 2014, for the two experiments.

The experiments were conducted in randomized blocks experimental design (RBD), experiment I (27 RR genotypes and two checks) and experiment II (23 conventional genotypes and two checks) with three replications. Each experimental plot consisted of four rows of 5 m long, spaced 0.5 m between rows. The two central rows were considered as useful area, despising 0.5 m from each end, totaling 4 m².

Prior to sowing, there was plowing and harrowing of the soil. The fertilization was performed according to the requirements of the culture, after previous soil analysis, by applying 350 kg ha^{-1} of the formula 00-20-20. Seeds were inoculated with inoculant Gelfix 5 (*Rhizobium*). The experimental plots were maintained throughout the crop cycle, with strict check of pests, diseases and weeds, as recommended for the soybean crop. All the above mentioned cultivation traits were made in accordance with the recommendations of Embrapa Soja (Embrapa, 2013).

The genotypes used were developed from two-way crosses between carriers of the RR gene and the conventional. The RR generators were cultivars of commercial soybeans and the conventional generators were soybean lines with good agronomic performance for traits related to grain production, belonging to the genetic breeding program of the Paulista State University - UNESP / FCAV - Jaboticabal-SP, namely: C4 (M 8336 RR x JAB.00-01-21/4M1D), C5 (M 7578 RR x JAB.00-05-5/4A2D), C6 (M 7908 RR x JAB.00-05-5/4A2D), C7 (M 8221 RR x JAB.00-05-5/4A2D), C8 (M 7211 RR x JAB.00-05-1/5C3B), C9 (M 7639 RR x JAB.00-05-1/5C3B), C10 (M 8211 RR x JAB.00-05-1/5C3B), C11 (M 8336 RR x JAB.00-05-1/5C3B), C12 (M 8360 RR x JAB.00-05-1/5C3B), C13 (M 7211 RR x JAB.00-05-8/2D3C), C14 (M 7636 RR x JAB.00-05-8/2D3C), C15 (M 7908 RR x JAB.00-05-8/2D3C), C16 (M 8336 RR x JAB.00-05-8/2D3C), C17 (M 8230 RR x JAB.00-06-2/3I3D), C18 (M 8236 RR x JAB.00-06-2/3I3D) and C20 (M 8360 RR x JAB.00-02-3/6A4D).

The progenies of these crosses were conducted by the pedigree method until the F5 generation, where they were separated into two groups: the first containing the RR genotypes and the second, the conventional genotypes. The characterization and identification of RR genotypes was performed in the laboratory and they were detected by PCR (polymerase chain reaction) technique, which distinguishes soybean genotypes by the presence of molecular markers, derived from specific sequences of the transgenic DNA, which is amplified. The RR genotypes were identified by using specific primers that pair in the sequence *Forward* 5'TGATGTGATATCTCCACTGACG 3' and *Reverse* 5'TGTATCCCTTGAGCCATGTTGT 3', which codes the region EPSPS (Marcelino et al., 2007).

Thus, the present study evaluated F_6 generation genotypes, in two separate experiments, after the process of selection of superior genotypes of each identified group. The first experiment was composed of 27 soybean genotypes carriers of RR gene and two checks (BMX Forca RR and BRS Valiosa RR). The second experiment was composed of 23 conventional genotypes and two conventional cultivars as checks (CD-216 and Conquista).

Genotypes were assessed as they reached the ideal phenological stages for their assessments. The following agronomic traits were evaluated: number of days to maturity (NDM), in days; plant height at flowering (PHF) in cm; plant height at maturity (PHM) in cm; insertion height of the first pod (HFP) in cm; lodging (Lg,), visual notes, where 1 indicates all upright plants and 5 all lodged plants; agronomic value (AV), visual notes, where 1 indicates plants with undesirable agronomic traits and 5 plants with optimal agronomic traits; one hundred seed weight (HSW), in grams; grain yield (GY), in kg ha^{-1} corrected to 13% moisture.

For data transformation when necessary, authors used the Box and Cox (1964) methodology. Data from each experiment (I and II) were submitted to analysis of variance using the GLM procedure of SAS ® 9.3 *software* (2011). Then, they were submitted to averages cluster analysis (Scott-Knott, at 5% level probability), phenotypic correlations, genotypic correlation and path analysis, using the computer *software* Genes (Cruz, 2013). Path analysis was obtained according to the method proposed by Wright (1921). The GY was chosen as the main variable, that is, the consequences of the correlations in direct and indirect effects of the other traits were estimated on the GY. Before the path analysis, a diagnosis of multicollinearity was performed (Montgomery and Peck, 1981), in order to identify potential problems in the analysis.

RESULTS AND DISCUSSION

The analysis of variance enabled verifying significant differences by the F test at 1% probability, between RR genotypes for all traits. The coefficients of variation ranged from 2.6% (NDM) to 19.3% (GY) (Table 1), and were within suitable values. Conventional genotypes also showed significant differences at 1% probability for all traits, except for the GY trait (5% significance probability). The coefficients of variation ranged from 1.4% (NDM) to 21.6% (GY) (Table 2), also considered suitable, due to the nature of the traits. Among the analyzed traits, GY had the highest coefficient of variation. This can be explained by the fact of being a quantitative character controlled by many genes and that tends to be highly influenced by the environment (Costa et al., 2008).

For the group of RR genotypes, as well as for conventional genotypes, results were significant by Scott-Knott test at 5% probability for all traits. For GY traits, there were average values from 2039.7 to 4994.8 kg ha^{-1} for the RR genotype (Table 1). Genotypes JAB.09-05/8, JAB.09-17/7 and JAB.09-08/3 stood out for GY, with average values over 4575.5 kg ha^{-1}. In addition to these, statistically superior to the others, 14 other genotypes had higher GY than the national average of 2870 kg ha^{-1} (Conab, 2016). For conventional genotypes, the GY traitr showed average values between 2101.7 and 4502.6 kg ha^{-1} (Table 2). Ten genotypes, as well as the check Conquista, presented superiority to the trait, with average values over 3511.4 Kg ha^{-1}. In general, the best RR genotypes showed GY superior to checks, while the best conventional genotypes were statistically equal to the check conquista.

Despite the conventional genotypes had presented higher number of statistically superior genotypes, from the overall average of the experiments (3359.0 kg ha^{-1} RR genotypes and 3390.0 kg ha^{-1} conventional genotypes), it can be seen that the presence of favorable alleles for the GY trait is more important to achieve high productivities than the presence or absence of the RR gene.

Lima et al. (2015), when comparing conventional soybean cultivars with essentially derived RR cultivars, observed no differences among them, because the conventional cultivar SOY M-6101, had 31.9% higher yield compared with the essentially derived M-SOY 7211 RR, whereas the essentially derived cultivar BRS Valuable RR had yield 28.5% higher than the conventional cultivar BRS/MG46 Conquista. Fonseca et al. (2013) concluded that for PHM, HFP and GY, the RR genotypes had higher values than the conventional genotypes, whereas Lima et al. (2008) did not identify difference for productive performance between conventional and RR soybean lines.

The best RR genotypes (Table 1) for the trait NDM were JAB.09-04/7 RR, JAB.09-04/8 RR, JAB.09-06/7 RR, JAB.09-07/1 RR and the cultivar BMX Forca RR, with cycle between 110.2 and 115.3 days, consequently, classified in maturity group from 5.4 and 5.9, respectively, according to Alliprandini et al. (2009), being characterized as early genotypes (Rocha et al., 2012). Despite these genotypes have presented the best values for NDM, they did not show satisfactory values for GY, and the best values for GY were presented by the late cycle genotypes. For conventional genotypes, the cycle ranged from 98.0 (CD-216) to 125.7 days (JAB.09-11/5, JAB.09-14/2 e JAB.09-18/8), being classified in maturity group from 4.2 and 6.9, respectively, according to Alliprandini et al. (2009), highlighting the JAB.09-15/6 genotype, which in addition to reduced NDM, presented satisfactory results for GY and the other traits.

In general, RR genotypes showed more precociousness when compared with conventional genotypes. The precocity is considered a desirable feature especially for cultivation in sugarcane renewal areas or to meet off-season areas in regions where the second crop is traditional. It is noteworthy that the NDM may vary according to the growing region because it is influenced by latitude, due to the sensitivity of soybean to the photoperiod (Rocha et al., 2012).

The RR genotypes with lower NDM also had the lowest values for PHF and PHM, whereas conventional

Table 1. Mean values for eight traits on 27 F_6 genotypes of RR soybean deriving from two-way crosses and two checks in the season 2014/15.

Genotype	NDM	PHF	PHM	HFP	Lg[1]	AV[1]	HSW	GY
JAB.09-04/4 RR	126.4[a]	60.9[d]	68.2[c]	9.7[a]	1.8[a]	3.4[a]	17.9[a]	2860.8[c]
JAB.09-04/7 RR	110.2[c]	56.0[e]	57.0[d]	6.8[c]	1.0[b]	3.5[a]	14.5[c]	2716.4[c]
JAB.09-04/8 RR	110.2[c]	57.9[d]	58.7[d]	8.0[b]	1.2[b]	2.2[b]	15.1[c]	2755.4[c]
JAB.09-05/8 RR	122.0[b]	64.2[c]	89.1[a]	10.8[a]	1.8[a]	4.3[a]	13.6[d]	4994.8[a]
JAB.09-06/1 RR	120.3[b]	65.3[c]	68.2[c]	8.6[b]	1.2[b]	3.8[a]	19.0[a]	3831.7[b]
JAB.09-06/2 RR	118.0[b]	64.1[c]	68.3[c]	10.7[a]	1.2[b]	4.2[a]	17.3[b]	3956.5[b]
JAB.09-06/4 RR	121.7[b]	65.2[c]	67.2[c]	9.2[a]	1.5[a]	3.7[a]	14.9[c]	4050.8[b]
JAB.09-06/7 RR	110.3[c]	58.6[d]	60.1[d]	4.6[d]	1.0[b]	2.6[b]	16.5[b]	3286.9[c]
JAB.09-07/1 RR	111.8[c]	52.9[e]	53.4[d]	6.4[c]	1.2[b]	3.1[b]	17.3[b]	2039.7[c]
JAB.09-13/1 RR	118.4[b]	64.0[c]	63.7[d]	8.9[a]	1.8[a]	2.9[b]	15.2[c]	3341.8[c]
JAB.09-17/4 RR	131.4[a]	63.3[c]	68.1[c]	10.4[a]	1.5[a]	4.9[a]	11.6[e]	4297.3[b]
JAB.09-17/7 RR	126.0[a]	75.2[a]	79.3[b]	9.7[a]	3.0[a]	3.5[a]	13.9[d]	4575.7[a]
JAB.09-18/2 RR	125.3[a]	63.7[c]	63.7[d]	9.1[a]	1.5[a]	3.8[a]	14.8[c]	3704.2[b]
JAB.09-18/3 RR	126.7[a]	60.4[d]	67.3[c]	10.6[a]	1.5[a]	4.2[a]	13.7[d]	2815.5[c]
JAB.09-18/8 RR	126.4[a]	60.7[d]	69.8[c]	11.1[a]	1.0[b]	4.2[a]	13.5[d]	3972.8[b]
JAB.09-20/3 RR	120.0[b]	56.0[e]	62.3[d]	9.1[a]	2.0[a]	3.2[b]	14.3[c]	3199.6[c]
JAB.09-20/8 RR	123.3[b]	62.8[c]	63.3[d]	9.7[a]	1.5[a]	3.3[a]	13.7[d]	2956.7[c]
JAB.09-06/1 RR	118.9[b]	61.4[d]	59.1[d]	8.0[b]	1.0[b]	3.4[a]	16.0[c]	2171.9[c]
JAB.09-06/5 RR	120.3[b]	62.9[c]	62.2[d]	8.3[b]	1.2[b]	3.5[a]	15.3[c]	2815.1[c]
JAB.09-06/6 RR	119.0[b]	59.1[d]	59.2[d]	8.3[b]	1.5[a]	3.0[b]	15.5[c]	3342.7[c]
JAB.09-08/3 RR	124.9[a]	67.0[c]	74.4[b]	9.5[a]	1.5[a]	4.2[a]	14.7[c]	4887.9[a]
JAB.09-09/3 RR	121.7[b]	60.1[d]	67.9[c]	10.3[a]	1.8[a]	3.0[b]	14.6[c]	2765.5[c]
JAB.09-09/4 RR	119.3[b]	59.4[d]	63.4[d]	8.0[b]	2.5[a]	2.5[b]	14.7[c]	2553.9[c]
JAB.09-09/5 RR	121.9[b]	67.0[c]	72.3[b]	8.9[a]	1.8[a]	3.2[a]	14.6[c]	4166.0[b]
JAB.09-09/6 RR	120.7[b]	63.0[c]	64.6[c]	7.6[b]	2.0[a]	2.8[b]	14.7[c]	3284.2[c]
JAB.09-09/7 RR	123.2[b]	63.9[c]	66.6[c]	9.6[a]	2.2[a]	3.5[a]	14.9[c]	3655.5[b]
JAB.09-09/8 RR	123.0[b]	64.9[c]	65.8[c]	9.1[a]	1.8[a]	2.8[b]	14.9[c]	3402.6[c]
BMX Força RR	115.3[c]	52.8[e]	70.5[c]	7.6[b]	1.7[a]	2.8[b]	15.7[c]	2269.3[c]
BRS Valiosa RR	125.9[a]	69.3[b]	69.4[c]	10.0[a]	2.8[a]	3.7[a]	15.6[c]	2235.0[c]
Mean	120.9	62.1	66.6	9.0	1.6	3.4	15.1	3359.0
CV (%)	2.6	6.0	8.9	14.3	12.4	9.9	6.2	19.3

Mean followed by the same letter do not differ by the Scott-Knott test ($P \leq 0.05$). [1] Transformation 1/X^0.5; CV: coefficient of variation; NDM: number of days to maturity; PHF: plant height at flowering (cm); PHM: plant height at maturity (cm); HFP: insertion height of the first pod (cm); Lg: lodging; AV: agronomic value; HSW: one hundred seed weight (grams); GY: grain yield (kg ha^{-1}).

genotypes with higher values for NDM also showed superiority for PHF and PHM. According to Dallastra et al. (2014), genotypes with reduced NDM (more precocious) may also have reduced PHF and PHM due to reduction in vegetative and reproductive stages of plant.

For the PHF trait, the JAB.09-17/7 genotype RR was higher than others (Table 1). Then, other 14 RR genotypes had intermediate values for PHF, ranging from 62.8 (JAB.09-20/8) to 67.0 cm (JAB.09-08/3 and JAB.09-09/5). For conventional genotypes (Table 2), JAB.09-14/2 was superior to all others for the PHF trait. Then, other six genotypes showed intermediate values for PHF, ranging from 73.0 (JAB.09-20/2) to 77.4 cm (JAB.09-11/5).

For PHM, values ranged from 53.4 (JAB.09-07/1 RR) to 89.1 cm (JAB.09-05/8 RR) for RR genotypes (Table 1). As for conventional genotypes (Table 2), values ranged from 63.6 (JAB.09-15/6 and JAB.09-18/8) to 98.2 cm (JAB.09-05/7). In general, RR genotypes with higher PHM had higher values of GY. According to Amorim et al. (2011), it must be considered a minimum PHM of 50 cm for soybean, whereas Carvalho et al. (2010) claim that plants with PHM higher than 100 cm tend to lodging and hinder the efficiency of harvesters at harvest. Thus, both the RR and the conventional genotypes had satisfactory values for PHF and PHM, being close or equal to the recommended value for the trait.

For the trait HFP, RR genotypes (Table 1) showed average values ranging from 4.6 (JAB.09-06/7 RR) to

Table 2. Mean values for eight traits in 23 F_6 genotypes of conventional soybeans deriving from two-way crosses and two checks in the season 2014/15.

Genotype	NDM	PHF	PHM	HFP	Lg[1]	AV	HSW	GY
JAB.09-05/7	120.9[c]	66.8[c]	98.2[a]	9.1[b]	2.1[a]	3.5[c]	12.7[c]	4326.2[a]
JAB.09-10/2	123.4[b]	74.5[b]	73.2[c]	10.9[a]	1.4[b]	4.0[b]	14.3[b]	4492.7[a]
JAB.09-11/5	125.7[a]	77.4[b]	80.9[b]	10.5[a]	4.0[a]	2.7[d]	14.7[b]	3716.9[a]
JAB.09-11/7	124.4[a]	66.9[c]	73.5[c]	9.9[a]	1.9[a]	4.0[b]	14.8[b]	3345.6[b]
JAB.09-13/2	119.4[c]	65.7[c]	68.8[c]	8.3[b]	1.9[a]	3.5[c]	15.3[b]	4484.6[a]
JAB.09-14/2	125.7[a]	85.0[a]	86.8[b]	9.4[b]	3.0[a]	3.5[c]	12.7[c]	3777.7[a]
JAB.09-15/1	117.9[d]	68.2[c]	70.6[c]	11.1[a]	1.6[b]	3.2[c]	15.6[b]	2693.6[b]
JAB.09-15/2	116.4[d]	68.5[c]	72.9[c]	8.4[b]	1.1[b]	3.2[c]	13.6[c]	2688.7[b]
JAB.09-15/6	115.8[d]	65.0[c]	63.6[d]	9.0[b]	1.3[b]	3.3[c]	17.5[a]	3727.0[a]
JAB.09-15/7	117.2[d]	61.7[c]	65.4[d]	8.1[b]	1.3[b]	2.9[d]	17.3[a]	3196.3[b]
JAB.09-18/5	122.3[b]	58.5[c]	62.6[d]	7.9[b]	1.6[b]	3.3[c]	17.4[a]	4502.6[a]
JAB.09-20/1	121.3[b]	77.3[b]	79.9[b]	9.1[b]	1.3[b]	3.7[b]	16.1[a]	3511.4[a]
JAB.09-20/2	122.4[b]	73.0[b]	77.9[b]	12.9[a]	1.6[b]	3.5[c]	17.9[a]	3106.0[b]
JAB.09-06/2	120.3[c]	69.1[c]	72.8[c]	8.5[b]	1.3[b]	3.3[c]	16.5[a]	2579.2[b]
JAB.09-07/1	123.0[b]	54.5[c]	71.3[c]	9.4[b]	2.5[a]	3.2[c]	13.6[c]	2498.2[b]
JAB.09-12/8	123.8[a]	73.4[b]	85.7[b]	9.8[a]	2.8[a]	3.8[b]	14.4[b]	2792.7[b]
JAB.09-14/3	122.3[b]	68.3[c]	73.3[c]	9.7[a]	2.0[a]	3.3[c]	14.9[b]	2389.6[b]
JAB.09-16/1	122.7[b]	69.5[c]	73.9[c]	10.4[a]	1.3[b]	3.9[b]	15.5[b]	4369.3[a]
JAB.09-16/3	119.4[c]	62.3[c]	68.8[c]	8.8[b]	2.1[a]	2.5[d]	14.5[b]	3170.7[b]
JAB.09-16/4	123.7[a]	73.8[b]	77.0[b]	10.1[a]	2.5[a]	3.2[c]	15.4[b]	3529.8[a]
JAB.09-16/5	122.0[b]	64.8[c]	66.0[d]	7.9[b]	1.8[a]	3.0[d]	14.7[b]	3257.2[b]
JAB.09-17/7	124.0[a]	69.8[c]	74.3[c]	10.2[a]	2.7[a]	3.3[c]	14.2[b]	3313.3[b]
JAB.09-18/8	125.7[a]	62.3[c]	63.6[d]	8.3[b]	2.5[a]	2.9[d]	14.3[b]	2839.7[b]
CD-216	98.3[e]	64.1[c]	70.2[c]	9.4[b]	1.1[b]	4.3[b]	15.6[b]	2101.7[b]
Conquista	125.3[a]	64.7[c]	66.0[d]	10.7[a]	1.3[b]	4.8[a]	16.5[a]	4260.8[a]
Mean	121.3	68.6	73.6	9.5	2.0	3.4	15.8	3390.0
CV (%)	1.4	8.5	8.6	15.8	13.3	11.3	7.3	21.6

Mean followed by the same letter do not differ by the Scott-Knott test ($P \leq 0.05$). [1] Transformation 1/X^0.5; CV: coefficient of variation; NDM: number of days to maturity; PHF: plant height at flowering (cm); PHM: plant height at maturity (cm); HFP: insertion height of the first pod (cm); Lg: lodging; AV: agronomic value; HSW: one hundred seed weight (grams); GY: grain yield (kg ha^{-1}).

11.1 cm (JAB.09-18/8 RR), while conventional genotypes (Table 2) showed HFP between 7.9 (JAB.09-18/5 and JAB.09-16/5) and 12.9 cm (JAB.09-20/2). Almeida et al. (2011) point out that the selection of plants with HFP less than 10 cm and PHM greater than 80 cm may cause losses in mechanical harvesting, whereas Rocha et al. (2012) state that the satisfactory HFP is around 15.0 cm, although most modern harvesters can make good harvest with plants showing first pods at 10.0 cm. Therefore, the RR genotypes with better values for GY also showed satisfactory values for HFP, whereas for conventional genotypes, the most productive showed high variation for this trait.

For the trait Lodging, 9 RR genotypes (Table 1) and 12 conventional genotypes (Table 2) showed the best values for the trait, with notes ranging from 1.0 to 1.2 and 1.1 to 1.6, respectively. Conventional genotypes showed higher values for lodging compared with the RR genotypes. This can be explained by the fact that conventional genotypes have shown higher PHM, because according to Carvalho et al. (2010), plants with high PHM values tend to lodging with ease. In the present study, this fact was confirmed, because genotypes with greater PHF and PHM values also showed high lodging, indicating relationship between these traits. The trait lodging should always be taken into consideration during the process of selection of superior genotypes, as plants with reduced lodging can minimize losses during the mechanical harvesting process.

Regarding trait AV, the best RR genotypes (Table 1) showed mean values ranging between 3.4 and 4.9, and the genotypes with higher GY were included in this range. However, conventional genotypes (Table 2) with higher GY did not necessarily present a high AV. Thus, the GY was not affected directly by the AV due to this being measured taking into account a set of visual traits of interest of the plant, such as: architecture, pod

Table 3. Estimates of the phenotypic (pr) and genotypic (gr) correlation coefficients between agronomic traits in RR soybean genotypes (above the diagonal) and conventional soybean genotypes (below the diagonal).

Trait		NDM	PHF	PHM	HFP	Lg	AV	HSW	GY
NDM	pr	1	0.563**	0.573**	0.773**	-0.487**	-0.644**	-0.439*	0.484*
	gr	1	0.671**	0.630**	0.851**	0.430*	0.715**	-0.493**	0.538**
PHF	pr	0.357	1	0.698**	0.441*	0.473*	0.332	-0.170	0.705**
	gr	0.456*	1	0.761**	0.462*	0.620**	0.464*	-0.194	0.854**
PHM	pr	0.327	0.608**	1	0.647**	0.442*	0.521**	-0.301	0.806**
	gr	0.408*	0.655**	1	0.689**	0.507**	0.688**	-0.330	0.962**
HFP	pr	0.299	0.461*	0.350	1	0.265	0.642**	-0.398*	0.477*
	gr	0.343	0.552**	0.368	1	0.320	0.753**	-0.455*	0.531**
Lg	pr	0.678**	0.298	0.392	0.166	1	-0.159	-0.304	0.244
	gr	0.757**	0.393	0.435*	0.211	1	-0.083	-0.353	0.397
AV	pr	0.195	0.344	0.331	0.334	-0.313	1	-0.287	0.570**
	gr	0.267**	0.347	0.361	0.393	-0.261	1	-0.397*	0.618**
HSW	pr	-0.376	-0.175	-0.495*	0.085	-0.489*	-0.037	1	-0.301
	gr	-0.434*	-0.201	-0.571**	0.104	-0.552**	-0.046	1	-0.416*
GY	pr	0.100	0.163	0.133	-0.022	-0.066	0.327	0.045	1
	gr	0.194	0.071	0.094	-0.066	-0.007	0.320	-0.015	1

** and *: Significant at 1 and 5% probability, respectively by t-test. NDM: number of days to maturity; PHF: plant height at flowering; PHM: plant height at maturity; HFP: insertion height of the first pod; Lg: lodging; AV: agronomic value; HSW: one hundred seed weight; GY: grain yield.

dehiscence, plant vigor and health, among others. It can also be seen that for both RR and for conventional genotypes, there was a positive relationship between AV, PHF, PHM and GY. Therefore, genotypes with these characteristics tend to have better performance, confirming the results obtained by Ferreira Junior et al. (2015).

For the trait HSW, RR genotypes (Table 1) showed mean values of 11.6 (JAB.09-17/4 RR) and 19.0 grams (JAB.09-06/1 RR), whereas the conventional genotypes (Table 2) had mean values of 12.7 (JAB.09-05/7 and JAB.09-14/2) and 17.9 g (JAB.09-20/2). RR genotypes with superiority for GY had lower average values for HSW. According to Dallastra et al. (2014), this may be due to the fact that more productive plants tend to produce smaller seeds due to the greater amount of seeds produced per plant.

Estimates of phenotypic and genotypic correlation coefficients for the two groups of genotypes are shown in (Table 3). In the study of correlations, three aspects should be considered: the direction, the significance and the magnitude.

Positive correlations indicate the tendency of a variable increase when the other increases, negative correlations indicate a tendency of a variable increase while the other decreases (Nogueira et al., 2012). Positive correlations show possible occurrence of pleiotropism, in which the same gene affects the expression of more than one trait (Falconer, 1987).

In general, the genotypic correlations showed higher values than their corresponding phenotypic correlations. When genotypic correlations are higher than their corresponding phenotypic correlations, this indicates that the phenotype is hardly influenced by the environment, that is, the associations found mainly occur due to genetic causes (Almeida et al., 2010; Nogueira et al., 2012).

For the group of soybean RR genotypes, all agronomic traits, except HSW, were positively correlated with GY, both for phenotypic and for genotypic coefficient (Table 3). These results agree with those obtained by Akram et al. (2011) and Haghi et al. (2012), which found negative and no significant correlation between HSW and GY.

Positive, significant ($p<0.01$) and high magnitude genotypic correlations were observed between the trait GY and the traits NDM (0.538), PHF (0.854), PHM (0.962), HFP (0.531) and AV (0.618) (Table 3), indicating that the selection of plants in later cycle, combined with greater height at flowering and maturity, high first pod insertion and high agronomic value index will result in more productive plants, according to the results of Malik et al. (2007) and Akram et al. (2011).

Positive genotypic correlation was observed (p <0.01) between the trait NDM and the traits PHF (0.671), PHM (0.530), HFP (0.851) and AV (0.715) (Table 3). These results corroborate those obtained by Nogueira et al. (2012). However, they differ from the results obtained by Almeida et al. (2010).

Genotypic correlations were positive (p <0.01) between PHM and the traits PHF (0.761), HFP (0.689), Lg (0.507) and AV (0.688) (Table 3), revealing that the selection of tall plants simultaneously promotes the selection of plants with the other related traits with high values. Haghi et al. (2012) found, for the trait PHM, positive correlation with HFP (0.750), which can be attributed to a larger vegetative growth of plants.

For conventional genotypes there was no significant correlation in the phenotypic and genotypic coefficient between GY and all analyzed agronomic traits (Table 3). These results agree with those obtained by Ramteke et al. (2010) and Haghi et al. (2012), who observed for GY negative and non-significant correlation with the traits PHM, HFP and HSW. In this case, the selection should be applied directly to the GY, because no other trait is efficient to be adopted as criteria of indirect selection. For this group of genotypes, positive and high magnitude genotype correlation (p<0.01) were observed between PHF and PHM (0.655) and HFP (0.552).

Considering the two groups of soybean genotypes (RR and Conventional), the trait HSW correlated negatively with all other characteristics, except HFP for conventional genotypes (Table 3). Similar results were obtained by Ramteke et al. (2010). Diverging from the results obtained in this study, Almeida et al. (2010) observed a positive and significant correlation between the trait HSW (p<0.05) and the traits HFP, NDM and GY.

The information obtained from the correlation coefficients can be detailed through path analysis, which has proven useful in providing additional information that describes the relationships of cause and effect between the dependent variable (principal) and the independent variables (explanatories). For the path analysis, weak and moderate multicollinearity was observed, indicating that the ratio between the largest and smallest eigenvalues of matrix of correlations were near and below 100 and therefore do not affect the result of the analysis (Table 4).

In both genotype groups, the genotypic direct effects outweighed the phenotypic direct effects (Table 4), agreeing with the estimates of correlations (Table 3). Thus, the genotypic direct effects are intrinsically more useful than the phenotypic effects to decide selection strategies. Therefore, in this case, the genotypic correlations explain the true association between the traits analyzed and GY.

Among the RR genotypes, the traits that most influenced the GY were PHF, PHM and AV, as they showed the highest values of favorable genotypic direct effects (Table 4). Thus, the traits PHF and PHM can be considered as key traits in breeding soybean to increase the yield, as they exert great contribution to the determination of GY. According to Akram et al. (2011), Leite et al. (2016) and Malik et al. (2007), the PHM can be used as criteria of indirect selection for GY due to the fact that it was the most important trait to determine the GY. While Alcantara Neto et al. (2011) observed negative direct effect of PHM on GY.

The traits NDM, PHF, HFP and AV had the highest positive indirect effects via the PHM on the GY in the genotypic coefficient for the RR genotypes (Table 4). These indirect effects have high contribution in the correlations of these variables with GY because the direct effects were negative and/or lower than genotypic correlations, except for PHF, agreeing with the results obtained by Malik et al. (2007).

The direct and indirect genotypic effects of HSW on GY were negative and of low magnitude for the RR genotypes (Table 4), showing that it is a variable with little cause and effect relationship on GY. The trait Lg had a negative genotypic direct effect on GY, indicating a favorable condition, because the most productive genotypes showed no lodging problems. The positive genotypic correlation between Lg and GY was due to the indirect effects of Lg on GY via PHF and PHM.

In the group of conventional soybean genotypes, for the phenotypic and genotypic coefficient, all traits, except AV and HSW, had no relevant direct and indirect effects on GY (Table 4). Similar results were obtained by Haghi et al. (2012), who observed, for most traits, negative and low magnitude indirect and direct effects on GY. The trait AV had a greater influence on GY followed by HSW because these traits had a favorable positive direct effect on GY in the genotypic coefficient. As for the traits NDM, PHF, PHM, HFP and Lg, the direct and indirect effects on GY were of low magnitude.

As for the studied genotypes, there was influence on the magnitude of phenotypic and genotypic correlations between GY and most agronomic traits. The traits PHF and PHM had a favorable direct effect and can be used for indirect selection for GY in soybean.

Conclusions

The most productive RR genotypes have higher values as compared with the highest yield conventional genotypes. The presence or absence of the RR gene is not a determining factor to increase the productive performance. It was possible the identification of appro-priate genotypes with great agronomic performance to the continuity of the soybean breeding program, and in the future they can be indicated for cultivation in the

Table 4. Breakdown of phenotypic (pr) and genotypic (gr) correlations in components of direct and indirect effect involving the main dependent variable (GY) and the independent explanatory variables (NDM, PHF, PHM, HFP, Lg, AV, HSW) for conventional and RR soybean genotypes.

Variables	Effects	RR genotypes		Conventional genotypes	
		pr	gr	pr	gr
NDM	Direct in GY	-0.1227	-0.1209	0.0526	0.0434
	Indirect via PHF	0.2096	0.3710	0.0295	-0.0153
	Indirect via PHM	0.3363	0.4264	0.0333	0.0258
	Indirect via HFP	-0.1421	0.0075	-0.0845	-0.1401
	Indirect via Lg	-0.0355	-0.1589	0.0775	0.2556
	Indirect via AV	0.1804	-0.0994	0.0770	0.1498
	Indirect via HSW	0.0585	0.1359	-0.0847	-0.1247
	Total	0.4846	0.5389	0.1008	0.1945
PHF	Direct in GY	0.3717	0.5525	0.0825	-0.0335
	Indirect via NDM	-0.0691	-0.0812	0.0188	0.0198
	Indirect via PHM	0.4099	0.5153	0.0618	0.0413
	Indirect via HFP	-0.0812	0.0041	-0.1300	-0.2253
	Indirect via Lg	-0.0425	-0.2293	0.0341	0.1327
	Indirect via AV	0.0944	-0.0645	0.1354	0.1946
	Indirect via HSW	0.0226	0.0535	-0.0395	-0.0578
	Total	0.7058	0.8542	0.1632	0.0718
PHM	Direct in GY	0.5870	0.6768	0.1016	0.0631
	Indirect via NDM	-0.0703	-0.0762	0.0172	0.0177
	Indirect via PHF	0.2595	0.4207	0.0502	-0.0219
	Indirect via HFP	-0.1190	0.0061	-0.0988	-0.1502
	Indirect via Lg	-0.0397	-0.1874	0.0448	0.1468
	Indirect via AV	0.1482	-0.0956	0.1303	0.2027
	Indirect via HSW	0.0402	0.0910	-0.1116	-0.1640
	Total	0.806	0.9625	0.1338	0.0943
HFP	Direct in GY	-0.1838	0.0089	-0.2821	-0.4081
	Indirect via NDM	-0.0948	-0.1030	0.0157	0.0149
	Indirect via PHF	0.1642	0.2553	0.0380	-0.0185
	Indirect via PHM	0.3802	0.4665	0.0356	0.0232
	Indirect via Lg	-0.0238	-0.1184	0.0190	0.0713
	Indirect via AV	0.1827	-0.1046	0.1318	0.2207
	Indirect via HSW	0.0530	0.1255	0.0193	0.0299
	Total	0.4778	0.5319	-0.0224	-0.0665
Lg	Direct in GY	-0.0898	-0.3694	0.1142	0.3373
	Indirect via NDM	-0.0485	-0.0520	0.0357	0.0329
	Indirect via PHF	0.1760	0.3429	0.0246	-0.0132
	Indirect via PHM	0.2600	0.3435	0.399	0.0274
	Indirect via HFP	-0.0488	0.0028	-0.0469	-0.0863
	Indirect via AV	-0.0452	0.0115	-0.1234	-0.1465
	Indirect via HSW	0.0405	0.0975	-0.1102	-0.1587
	Total	0.2442	0.3073	-0.0662	-0.0072
AV	Direct in GY	0.2842	-0.1389	0.3936	0.5603
	Indirect via NDM	-0.0778	-0.0865	0.0102	0.0116
	Indirect via PHF	0.1234	0.2568	0.0283	-0.0116
	Indirect via PHM	0.3061	0.4661	0.0336	0.0228
	Indirect via HFP	-0.1181	0.0067	-0.0944	-0.1607
	Indirect via Lg	0.0143	0.0307	-0.0358	-0.0882
	Indirect via HSW	0.0383	0.1095	-0.0084	-0.0132
	Total	0.5705	0.6180	0.3273	0.3209

Table 4. Contd.

HSW	Direct in GY	-0.1332	-0.2756	0.2253	0.2872
	Indirect via NDM	0.0539	0.0596	-0.0197	-0.0188
	Indirect via PHF	-0.0632	-0.1074	-0.0144	0.0067
	Indirect via PHM	-0.1772	-0.2235	-0.0503	-0.0360
	Indirect via HFP	0.0732	-0.0040	-0.0242	-0.0425
	Indirect via Lg	0.0273	0.1307	-0.0559	-0.1864
	Indirect via AV	-0.0817	0.0552	-0.0146	-0.0258
	Total	-0.3010	-0.4168	0.0458	-0.0159
Determination coefficient (R^2)		0.7685	0.9785	0.1702	0.2119
Residual effect		0.4810	0.1465	0.9108	0.8877

GY: grain yield; NDM: number of days to maturity; PHF: plant height at flowering; PHM: plant height at maturity; HFP: insertion height of the first pod; Lg: lodging; AV: agronomic value; HSW: one hundred seed weight.

region.

The traits plant height at flowering and plant height at maturity can be considered as key traits in soybean breeding to increase grain yield, as they have direct favorable effect on determining the yield. Conventional genotypes have no significant correlation between grain yield and all agronomic traits analyzed.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of phosphorus fertilization on yield and quality of onion bulbs

José Novo Júnior[1], Rayanne Maria Paula Ribeiro[1], Aridênia Peixoto Chaves[1], Valdívia de Fátima Lima Sousa[1], Leilson Costa Grangeiro[2]*, Maria Zuleide de Negreiros[2], Saulo de Tárcio Pereira Marrocos[3] and Gardênia Silvana Oliveira Rodrigues[4]

[1]Post-Graduation Program in Agronomy, Universidade Federal Rural do Semi-Árido, City of Mossoró, State of Rio Grande do Norte, Brazil.
[2]Department of Plant Sciences, Universidade Federal Rural do Semi-Árido, city of Mossoró, State of Rio Grande do Norte, Brazil.
[3]Instituto Federal de Educação, Ciência e Tecnologia do Amapá, Campus Porto Grande, city of Macapá, State of Amapá, Brazil.
[4]University Mater Christi, city of Mossoró, State of Rio Grande do Norte, Brazil.

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Obtaining information about the onion responses to different doses of phosphorus can contribute to optimizing the use of fertilizers and consequently make the activity more profitable with less impact on the environment. Therefore, this study aimed to evaluate the effects of different phosphorus doses on yield and quality of onion bulbs. The design of the experiment was in a randomized block design with four replications. The treatments were seven phosphorus doses 0.00; 33.75; 67.50; 101.25; 135.00 and 168.75 kg ha^{-1} P_2O_5. The application of 168.75 kg ha^{-1} of P_2O_5 provided the maximum yield commercial of bulbs, gross income, net income, rate of return and profitability rate in onion crops. However, there was no effect on the quality of the bulbs.

Key words: *Allium cepa* L., plant nutrition, fertigation, phosphate fertilizer, pungency.

INTRODUCTION

Onion (*Allium cepa* L.) is one of the most important vegetable crops grown in Brazil, particularly in the south and northeast regions because of its socioeconomic importance, (Trani et al., 2014). In 2015, approximately 56,200 hectares of land were cultivated with onion in Brazil, with an output of more than 1.5 million tons and average yield of 26.9 t ha^{-1} (IBGE, 2015). Fertilizer application is one of the most important factors in onion

*Corresponding author. E-mail: leilson@ufersa.edu.br.

Table 1. Chemical characteristics of the soil to a depth 0-20 cm, sampled prior to the experiment.

Parameter	Values
pH (water)	7.8
$P_{(mehlich)}$ (mg dm^{-3})	10.60
K ($Cmol_c$ dm^{-3})	0.28
Ca ($Cmol_c$ dm^{-3})	1.50
Mg ($Cmol_c$ dm^{-3})	0.12
Al ($Cmol_c$ dm^{-3})	0.00
M.O. (g kg^{-1})	3.80

M.O. = organic matter.

production because it directly affects growth, development and yields (Kurtz et al., 2013). Phosphorus (P) deficiency is one of the biggest constraints to crop production in many tropical soils, due to low native content and high P immobilization within the soil (Fairhust et al., 1999). A characteristic of the onion plant with regard to P is its inefficient extraction of this nutrient from the soil because the root hairs are mostly shorter than the length of phosphate diffusion. This requires a special care from growers with respect to the levels of this nutrient in the soil as well as the sources and forms of application of phosphate-based fertilizers (Brewester, 1994). The response of onion to phosphorus fertilization depends on the genotype used, P level in the soil, P source, soil and weather conditions (Grant et al., 2005).

Onion response to the application of up to 90 kg ha^{-1} P_2O_5 was reported by Costa et al., (2009), achieving a yield of 33.4 t ha^{-1}. Fonseca et al., (2011) obtained higher yields with an application of 120 kg ha^{-1} P_2O_5. Resende et al., (2016) observed better yields for onion cultivars: Franciscana IPA-10 and Vale Ouro IPA-11 with a dose of 132 kg ha^{-1} P_2O_5, comparable to economic dose of 130 kg ha^{-1} P_2O_5. Fertigation enables the application of soluble fertilizers and other chemicals along with irrigation water, uniformly and more efficiently. Work done with vegetables show that they respond very well to fertigation. As in potato (Badr et al., 2011), capsicum (Brahma et al., 2010) cucumber (Moujabber et al., 2002) tomato (Shedeed et al., 2009) and some leafy vegetables (Ueta et al., 2009). In onion, Rumpel et al. (2004) obtained higher marketable onion yields when the 50 kg ha^{-1} N rate was applied through drip fertigation (41% increase) and highest after applying 150 kg ha^{-1} N through fertigation (79% increase) as compared to the control (without fertigation and irrigation). Dingre et al. (2012) showed that drip fertigation resulted into 12 to 74% increase in the productivity of onion seed as compared to conventional method. Rajput and Patel (2006) recorded the highest onion yield in daily fertigation followed by alternate day fertigation. Lowest yield was recorded in monthly fertigation frequency. The study therefore sought to assess the effect of phosphate fertilization through fertigation on bulb yield and quality of onion under semiarid conditions.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted from April to September 2013 at the experimental field of the Department of Plant Sciences, Federal Rural University of Semi-Arid, Mossoro-RN, Brazil (Latitude 5°12' 26" south, and longitude 37°19’ 04" west and altitude of 18 m). The soil at the experimental site was classified as Red Yellow Argisol. The chemical characteristics of the soil are found in Table 1. The total rainfall was 610.01 mm and average temperature was 25.4°C during the period of the experiment.

Treatments and experimental design

Six treatments doses of phosphorus (0.00; 33.75; 67.50; 101.25; 135.00 and 168.75 kg ha^{-1} P_2O_5) were evaluated in a randomized complete block design with four replications. Each experimental unit consisted of a bed of 3.0 × 0.8 m, containing eight rows of plants spaced 0.10 × 0.10 m. The six central rows were harvested as useful plants without the border plants.

Planting and agronomic practices

The soil was prepared by one round of ploughing and harrowing. This was followed by construction of the beds and phosphorus fertilizer application in the form of triple superphosphate (41% P_2O_5) at half the dosage recommended for each treatment. The onion cultivar, Vale Ouro IPA-11, was used for the experiment. The seeds of the onion cultivar were nursed on seed beds measuring 1.0m wide and 0.2m high at the rate of 10 g m^{-2} in grooves of 0.01 m deep and 0.1m spacing between grooves dug across the bed length. Transplanting was done 53 days after sowing, when the seedlings were 15 to 20 cm high. Drip irrigation was used for watering the beds. Each bed was supplied with two hoses spaced at 0.4 m with self-compensating drippers of average flow rate of 1.40 l h^{-1} spaced at 0.2m apart. The onion was irrigated daily, based on crop evapotranspiration estimated by multiplying the reference evapotranspiration by the crop coefficient, at various stages of growth and development. Fertigation began 10 days after transplanting (DAT) and continued up to 70 DAT during which nitrogen was applied as urea and ammonium sulfate at the rate of 135.0 kg ha^{-1} N and potassium was applied as potassium chloride at the rate of 135 kg ha^{-1} K_2O. The other half of phosphorus treatment dosage was also applied through fertigation as phosphoric acid. The distribution of fertilizers was performed according to Table 2.

Mancozeb was sprayed at 7 days interval at the rate of 2.5 g l^{-1} to control disease such as stain purple. Clorfernapir and deltamethrin were applied alternately at 14 days interval at the rate of 0.5 ml l^{-1} and 0.3 ml l^{-1} respectively to control thrips and mites. Weed was controlled as and when necessary by hoeing. More than 70% of the onion was harvested at 153 DAP. The remaining (less than 30%) was harvested 7 days later. The delay was to enable supplementary healing process.

Data collection

Data were collected on yield of commercial bulbs (total weight of bulbs with diameter> 35 mm); yield of non-commercial bulbs (total

Table 2. Percentage distribution of nitrogen, phosphorus and potassium throughout the cycle Onion.

DAT*	N (%)	P (%)	K (%)
10 - 20	9.0	0.0	9.0
21 - 30	15.0	0.0	15.0
31 – 40	25.0	30.0	20.0
41 – 50	35.0	45.0	30.0
51 – 60	10.0	20.0	20.0
61 - 70	6.0	5.0	6.0

*DAT: Days after transplanting.

weight of bulbs with diameter < 35 mm); total yield of bulbs (sum of commercial and non-commercial yield of bulbs); bulb shape ratio (longitudinal diameter of bulb divided by transverse diameter); Phosphorus content in leaf (g kg^{-1}) was collected the highest sheet of 15 plants of the harvest area of the plot at 45 days after transplanting. Ten onion bulbs from each plot were analyzed for quality characteristics. The bulbs were ground in a food processor and then filtered in a funnel with filter paper to extract the juice. Soluble solids were determined by direct reading in digital refractometer; Titratable acidity (percent of pyruvic acid) was determined in a sample of 20 ml of the bulb juice to which three droplets of 1% phenolphthalein were added. Subsequently, titration was performed to the endpoint with a NaOH (0.1N) solution, which was previously standardized.

Then, the ratio of soluble solids to titratable acidity (SS/TA) was determined. The onion pungency (µmol of pyruvic acid g^{-1}) was determined by quantifying the pyruvic acid concentration, which was estimated by using the 2.4-dinitrophenylhydrazine (DNPH) reagent, according to the method described by Schwimmer and Weston (1961). The pH value was determined by using a digital pH meter, according to the method recommended by IAL (2008). Gross income was calculated considering the estimated commercial yield of onion and the price per kilo paid to producer at the time of harvest (US$ 0.45 per kilo). Net income was determined by the difference between the gross income and production costs. The rate of return was determined by the ratio of gross income to the production costs in each treatment, corresponding to the value obtained for each dollar spent in production costs. Profitability was the result from the ratio of net income to gross income expressed in percentage.

Statistical analysis

Data were subjected to analysis of variance using the Sisvar software (Ferreira, 2011). Whenever the means were significant, regression analysis was carried out and the models were chosen based on the significance level, by adopting 1% probability, and on the coefficients of determination (R^2).

RESULTS AND DISCUSSION

Plant dry mass and leaf phosphorus content

Plant dry mass and leaf phosphorus content were significantly influenced ($p< 0.05$) by phosphorus doses. The means of plant dry mass (PDM), in reason of the different phosphorus doses were adjusted to the quadratic regression model, with a maximum estimate of 12.26 g $plant^{-1}$ with the dose of 139.5 kg ha^{-1} P_2O_5. Regarding the treatment without P application (9.07 g $plant^{-1}$) there was an increase of 26.02% in the PDM. However, at the dose of 168.75 kg ha^{-1} P_2O_5, reduction of PDM was of 1.06%, compared to the maximum dose (Figure 1A). The PDM increase is due to the P participation in the plant processes such as photosynthesis, cellular division, absorption and transportation of ions. It also acts on the roots growth, especially on secondary roots, which are more efficient in absorbing water and nutrients (Fageria, 2009; Hawkesford et al., 2012).

The phosphorus content in leaf in regard to the P doses was adjusted to the quadratic regression model. In the treatment without P application, the P content was 5.36 g kg^{-1}; however, with P supply, leaf content increased up to the dose of 70.88 kg ha^{-1} P_2O_5 (6.34 g kg^{-1}). From this point there were reductions in the leaf P contents, and the minimum estimated content was 4.97 g kg^{-1} for the dose of 168.75 kg ha^{-1} P_2O_5, which was below that obtained in the control treatment, i.e., without P application (Figure 1B). All P levels found, irrespective of the applied dosage, fell into the range considered appropriate for onion crop, according to Trani et al. (2014), which is 2 to 5 g kg^{-1}.

Yield and quality of bulbs

The commercial yield (CY) and total yield (TY) increased linearly with the addition of phosphate fertilization. The maximum estimates for CY (48.87 t ha^{-1}) and TY (49.03 t ha^{-1}) were obtained with the application of 168.75 kg ha^{-1} P_2O_5 (Figure 1C and D). This dose was 25% higher than that recommended by Cavalcanti, (2008) for soils with P contents ranging from 6 to 12 mg dm^{-3}. It was also higher than the values found in other experiments with onion plants in Brazil, that is, 71 kg ha^{-1} P_2O_5 found by Costa et al. (2009), 60 kg ha^{-1} P_2O_5 by Wamser et al. (2011) and 130 kg ha^{-1} P_2O_5 by Resende et al. (2016). These results show the ability of Onion to respond to phosphorus application and undergird reports by different authors that the element contributes markedly to better productivity on the culture, especially in the production of bulbs size.

In some studies, onion bulbs responded significantly to phosphorus application at doses of 50 kg ha^{-1} P_2O_5 (Dudhat et al., 2010), 42 kg ha^{-1} P_2O_5 (Simon et al., 2014), 20 and 60 kg ha^{-1} P_2O_5 (Agumas et al., 2014). The onion yield of 49.0 t ha^{-1} produced by the cultivar Vale Ouro IPA-11 in this study was higher than the average national yield of 26.9 t ha^{-1} in Brazil (IBGE, 2015). The climate and phytosanitary conditions during the growing period favored the good crop performance. Onion bulb quality characteristics (bulb shape ratio, soluble solids, titratable acidity, soluble solids to titratable acidity ratio, pungency and hydrogen potential) were not significantly ($P>0.05$) affected by P fertilization (Table 3).

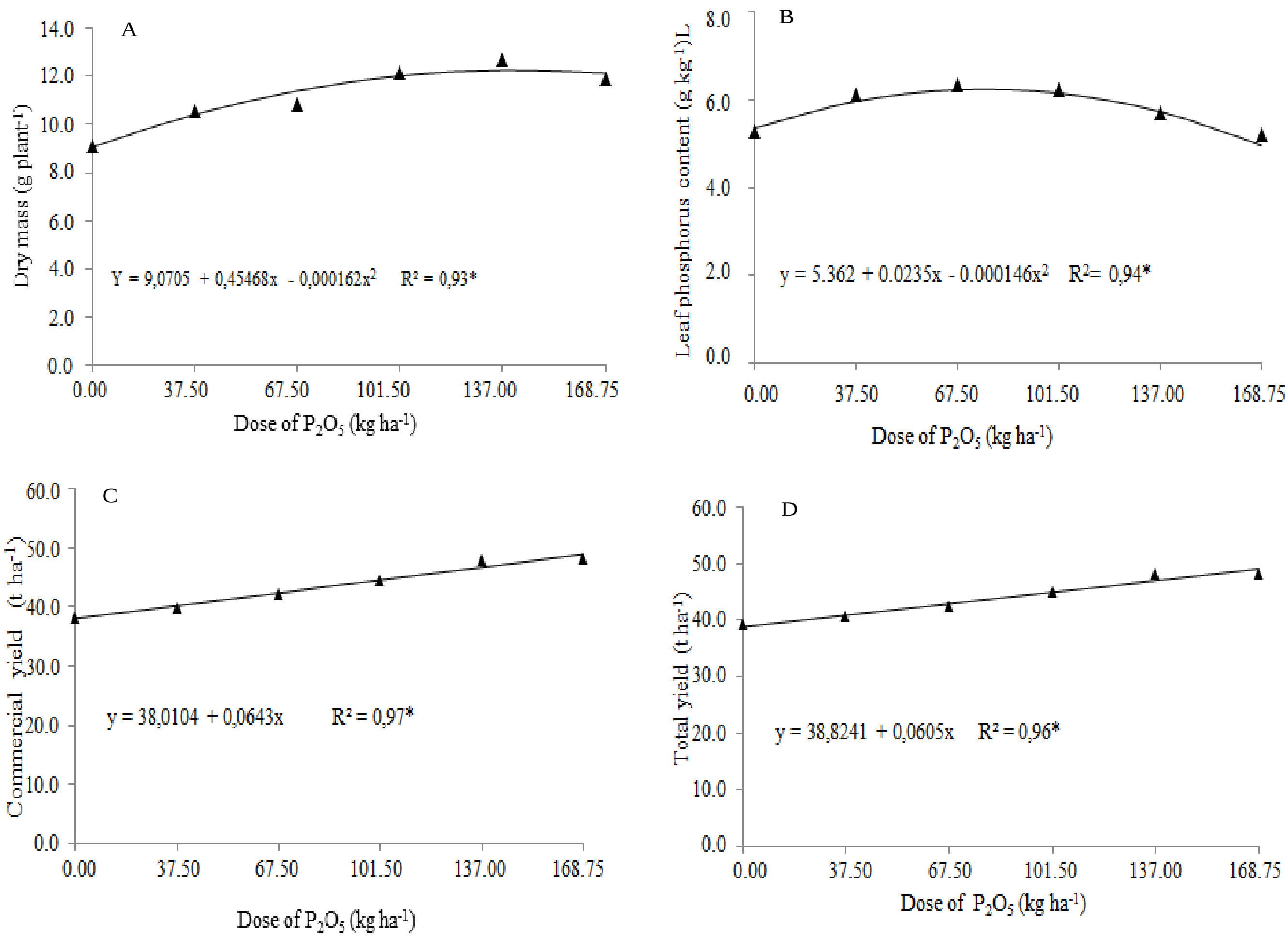

Figure 1. Plant dry mass (A), leaf phosphorus content (B), commercial yield (C) and total yield (D) in reason of phosphorus doses applied to onion cv. IPA-11.

Table 3. Quality characteristics of onion cultivar IPA-11 as influenced by phosphorus.

Doses (kg ha^{-1} of P_2O_5)	BSR	SS (%)	TA (% pyruvic acid)	SS/TA	PG (μmol of pyruvic acid g^{-1})	pH
0.00	0.83	10.35	3.93	2.65	6.72	5.46
33.75	0.83	10.43	4.29	2.43	7.55	5.51
67.50	0.84	10.55	3.93	2.74	7.60	5.47
101.25	0.85	10.60	4.42	2.41	7.02	5.48
135.00	0.82	10.05	4.29	2.35	6.48	5.49
168.75	0.87	10.28	4.29	2.40	7.53	5.48
Means	0.84	10.37	4.19	2.38	7.15	5.48
CV (%)	4.91	10.00	7.05	26.06	16.63	1.00

The results corroborate those found by Álvarez-Hernández et al. (2011), Tekalign et al. (2012) and Agumas et al. (2014). The onion cultivar Vale Ouro IPA-11 produced bulbs with quality characteristics that met the requirement s of the Brazilian consumer.

Economic analysis

The net income was positive for all treatments, and the dosage of phosphorus that provided the maximum commercial yield was also responsible for the highest

Table 4. Commercial yield (CY), production costs (PC), gross income (GI), net income (NI), rate of return (RR) and profit ratio (PR) as a function of phosphorus dosages applied to onion cv. IPA-11.

Doses of P_2O_5	CY	PC	GI	NI	RR	PR
(kg ha^{-1})	(t ha^{-1})		(US$ ha^{-1})			(%)
0	38.01	4,680.90	17,104.68	12,423.78	3.65	72.63
33.75	40.19	4,738.31	18,085.50	13,347.19	3.82	73.80
67.50	42.35	4,795.71	19,058.94	14,263.23	3.97	74.84
101.25	44.56	4,853.12	20,051.96	15,198.84	4.13	75.80
135.00	46.70	4,910.53	21,013.16	16,102.63	4.28	76.63
168.75	48.87	4,967.93	21,991.73	17,023.80	4.43	77.41

gross income, net income, rate of return, and profit ratio, that is, of US$ 21,991.73; US$ 17,023.80; US$ 4.43 and 77.41, respectively (Table 4). Without the application of phosphorus resulted in less GI, NL, RR and PR, with values of US$ 17,104.68; US$ 12,423.78; US$ 3.65 and 72.63%, respectively, and commercial yield of 38.01 t ha^{-1}. As can be seen, even without phosphorus addition, net income was high, a reflection of the high commercial yield achieved and the price paid for onion during harvest. This result corroborates the results by Resende et al. (2016), who found yield of 69.1 t ha^{-1} of onion bulbs without phosphate fertilization.

Conclusion

The application of 168.75 kg ha^{-1} of P_2O_5 provided the maximum yield commercial of bulbs, gross income, net income, rate of return and profitability rate in onion crops. However, there was no effect on the quality of the bulbs.

Conflict of Interests

The authors have not declared any conflict of interests.

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Fremont mandarin: Fruit with a long shelf life for the fresh fruit market

Camilla de Andrade Pacheco[1]*, Evandro Henrique Schinor[2], Mariângela Cristofani-Yaly[3], Marcos Antônio Machado[3] and Fernando Alves de Azevedo[3]

[1]Centre of Agricultural Sciences, State University of Londrina Celso Garcia Cid Highway, Km 380, P. O. Box 6001, 86051-990, Londrina, Paraná State, Brazil.
[2]Centre of Agricultural Sciences, Federal University of São Carlos Anhanguera Highway Km 174,13600-970, Araras, São Paulo, Brazil.
[3]Centre APTA Citrus Sylvio Moreira/IAC Anhanguera Highway Km 158, P. O. BOX 01, Cordeirópolis, São Paulo, Brazil.

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The goals of the present study were to identify resistant mandarin varieties with potential for the fresh fruit market that could enable an extension of the harvesting season and to develop appropriate methods to maintain fruit quality for longer storage periods. Fremont mandarin fruits were harvested at optimum maturity and stored with and without wax coating under different storage conditions: ambient (21 ± 2°C and 60 ± 5% relative humidity (R.H.)) and under refrigeration (8 ± 2°C and 90 ± 5% R.H.). Fruit physicochemical characteristics and acceptability and purchase intention, which were assessed through sensory analysis, were evaluated weekly. Mass loss was higher for fruits stored at ambient temperature. The soluble solids concentrations of fruits stored under refrigeration remained similar to the values measured at harvest. Fruits stored under refrigeration exhibited slightly higher gloss and color than fruits stored at ambient temperature. Wax-coated fruits exhibited lower mass loss and higher gloss and color than uncoated fruits. Similar results were observed for the sensory analysis: wax-coated fruits stored under refrigeration were better rated by panelists until the final evaluation (day 35 after the beginning of storage). Purchase intention was higher for fruits stored under refrigeration than at ambient temperature. It is concluded that uncoated fruits stored at ambient temperature exhibit a tendency to lose quality, which confirms the need for refrigeration and wax coating for post-harvest preservation of fresh citrus fruits. However, the physicochemical and sensory qualities of Fremont mandarins stored under refrigeration for 30 to 35 days are accepted by consumers.

Key words: *Citrus* spp., preservation, refrigerated storage, sensory analysis, post-harvest.

INTRODUCTION

China is the largest global producer of mandarins, with a production of approximately 15 million tons (mi t), more than 50% of which is aimed at the internal market, followed by Spain (2 mi t), who is responsible for most of the global export production, and Turkey, with a production of 942 thousand tons. Brazil is the fourth largest global producer of mandarins, with 938 thousand tons produced in 2013 (Food and Agricultural

Organization (FAO), 2015). In spite of Brazil's prominence, the existing range of mandarin varieties is still very limited. More than 80% of the mandarin orchards in Brazil are based on Ponkan mandarins (*Citrus reticulata* Blanco) and Murcott tangor [*C. reticulata* × *C. sinensis* (L.) Osbeck]. New research is being developed to identify varieties that are easy to peel, that have a homogeneous and attractive color, and that may result in an extended harvesting season, thereby constituting an additional source of income for small citrus producers (Pimentel et al., 2014). This is the case for Fremont mandarin, a hybrid resulting from the crossing between Clementine (*C. clementina* Hort. ex Tanaka) and Ponkan (*C. reticulata* Blanco) mandarins. Fremont is a promising variety because it exhibits precocious maturation in mid-season, a yellow-red fruit peel, a fruit shape and peel texture that are very similar to clementines (Saunt, 1990), resistance to *Alternaria alternata*, the fungus responsible for Alternaria brown spot (Pacheco et al., 2012), and is productive under Brazilian conditions (Pio et al., 2006).

The quality of citrus fruits is extremely important for their commercialization in the fresh fruit market. Quality is basically evaluated through an analysis of the fruit physicochemical characteristics (size, color, texture, acidity, soluble solids, SS/TA ratio) and sensory properties (product analysis using the sense organs). Many of these characteristics are specific to a particular cultivar or are influenced by climate conditions. However, some of them, such as appearance, flavor and texture, may be improved through pre- or post-harvest techniques (Malgarim et al., 2007).

The permanent supply of fresh fruit to the market is increasingly important, making it necessary to extend the fruit offer by extending the harvesting season. The mandarin harvesting season is relatively short and may be extended using technological operations. If well conducted, these will minimize post-harvest losses as well as maintain fruit quality and extend the period of commercialization. These fruits therefore reach the internal and/or external market when the prices are more profitable (Nascimento et al., 2011). Sensory analysis, which indicates how the fruits are perceived by the human senses, complements physicochemical analysis and is essential to assess, analyze and interpret the reactions produced in response to food characteristics. This type of analysis is used for quality control by many companies because it can be used to evaluate the degree of product acceptance by consumers and because it indicates what changes need to be made to increase product acceptance, which increases consumers' preference for specific products in an increasingly demanding market (Manzocco and Lagazio, 2009).

The Brazilian citrus industry lacks new varieties for fresh consumption and often new varieties are not accepted by the producer. Consequently, the goal of the present study was to evaluate the physical, chemical and sensory quality of new variety, Fremont mandarin, which were uncoated or coated with commercial wax, at different storage times and under different storage conditions. It´s also with the objective of minimize losses and maintain fruit quality, and verify their acceptability and purchase intent by Brazilian consumers.

MATERIALS AND METHODS

Post-harvest assay (storage)

The experiment was conducted at the Laboratory of Citrus Quality and Post-harvest of the Citrus Center Sylvio Moreira of Agronomic Institute (IAC), Cordeirópolis, São Paulo (SP), Brazil. Ripe fruits of the Fremont IAC 548 variety (*Citrus clementina* x *C. reticulata* Blanco) were used. The fruits were collected in June 2012 from three-year-old plants grafted onto Rangpur lime rootstock and planted at a 6 m x 3 m spacing (Figure 1).

The fruits were surface sterilized with 5% sodium hypochlorite, dried, and subjected to physicochemical analyses. The following parameters were evaluated: (i) fresh mass (g), measured by direct weighing using a digital scale with a precision of 5 g; (ii) longitudinal (Ø L) and transverse (Ø T) diameter, in centimeters, measured directly using a digital caliper; (iii) juice yield, determined by squeezing the fruit using a juice extractor OIC OTTO 1800 and calculated as the juice mass/fruit mass ratio, expressed as a percentage (%); (iv) fruit mass loss, calculated as the difference between the initial fruit mass and the fruit mass on each sampling day, expressed as a percentage (%); (v) fruit peel gloss, measured at two equidistant points on the equatorial region of the fruits, using a Glossmeter ETB6-F1; (vi) firmness, measured by applying pressure at two equidistant points on the equatorial region of the fruits, using a digital penetrometer (Turoni); and (vii) acidity (%), soluble solids - SS (°Brix), and the soluble solids/acid ratio; fruit peel color, determined through determination of L* (lightness), a* (intensity of red color, ranging from green to red), and b* (intensity of yellow color, ranging from blue to yellow), using a digital colorimeter (Minolta CR-400) (Papadakis et al., 2000). *a** and *b** were used to calculate the hue angle ($°h=\tan^{-1}(b^*/a^*)$) and chroma ($c^*=\sqrt{(a^*)^2+(b^*)^2}$), which is a measure of the color strength.

The collected fruits were subjected to the following storage treatments: fruits with or without wax coating stored under refrigeration (8 ± 2°C and 90 ± 5% R.H.), and fruits with or without wax coating stored at room temperature (21 ± 2°C and 60 ± 5% R.H.), i.e., the control. Wax coating was performed using commercial wax combined with fungicides to avoid the development of typical post-harvest pathogens (UE Wax, 16% citrosol + 4.0 mL Imazalil + 1.0 L UE Polyethylene + starch), according to the procedures used in processing units. Physicochemical analyses (described previously) were performed weekly until 42 days after the beginning of storage. A completely randomized experimental design with split-plots was used, replicated three times. The factors tested were storage time (six

*Corresponding author. E-mail: camillaap@uel.br.

Figure 1. General view of characteristic Fremont mandarin fruits and plants (Cordeirópolis, SP, July/2012).

Table 1. Physicochemical analyses of Fremont mandarin fruits, Mogi Mirim, SP. (2011/2012 harvest).

Variety	Mass (g)	Ø L (cm)	Ø T (cm)	Juice yield (%)	Acidity (g 100 mL^{-1})	Soluble solids (°Brix)	SS/TA ratio	Peel color (h°)	Pulp color (h°)
Fremont	125.5	6.7	5.7	44.8	0.97	11.16	11.5	51.11	39.08

*Values are the average of 20 fruits harvested in June 2012. Ø L = fruit longitudinal diameter; Ø T = fruit transverse diameter.

weeks), storage conditions (ambient and refrigerated), and wax coating (coated and uncoated). The plots consisted of 10 fruits for the mass loss assay, and 28 fruits per box for the storage assay. Regression analyses were performed, and models were selected for each variable based on the significance of the parameters and the R^2 value.

Sensory analyses

Sensory analyses were performed weekly to determine the acceptance and purchase intention of Fremont mandarins. The evaluations were performed by a team composed of 10 panelists who are employees of the Citrus Center Sylvio Moreira and who consume mandarins weekly; their ages range between 25 and 60 years old. The samples were identified using four number codes and were presented to the panelists on disposable plates in the presence of drinking water to cleanse the palate between samplings. The panelists evaluated the appearance of the fruit (general appearance, peel color, peel texture, firmness, ease of peeling) and internal characteristics (consistency, aroma, flavor). A nine-point hedonic scale, varying from "dislike extremely" (1) to "like extremely" (9) (Behrens et al., 1999), and a structured five-point purchase intention scale, varying from "certainly would buy" (5) to "certainly would not buy" (1), were used. Data were collected using individual sheets. The present study received ethics approval from the Human Research Ethics Committee from the Federal University of São Carlos (UFSCar) according to protocol N° 2305.0.000135-11. All panelists signed an informed consent form before participating in the study (Resolution 196/96 - National Health Council). Data were subjected to analysis of variance and means were compared by Tukey test ($p < 0.05$).

RESULTS

Physicochemical characterization of Fremont mandarins in post-harvest assay (storage)

The Fremont mandarin fruits used in the post-harvest assay were collected in June, that is, at their optimum maturity point (Table 1). These fruits presented a SS/TA ratio higher than 11, an average weight of 125.5 g, a juice yield of approximately 45%, a soluble solid concentration (SS) of 11.16 °Brix, a titratable acidity (TA) of approximately 1.0%, a longitudinal diameter of 6.7 cm, and a transverse diameter of 5.7 cm. However, the fruit peel and pulp presented different colors (51.11 h° and 39.98 h°, respectively). Changes in the evaluated parameters (mass loss, acidity, soluble solids, SS/TA ratio, gloss, texture and hue angle) were observed over storage time. Mass loss, soluble solids concentrations, the SS/TA ratio and texture increased during storage. In contrast, titratable acidity, gloss and peel color presented a slight decrease over time (Figures 2, 3, 4 and 5). The

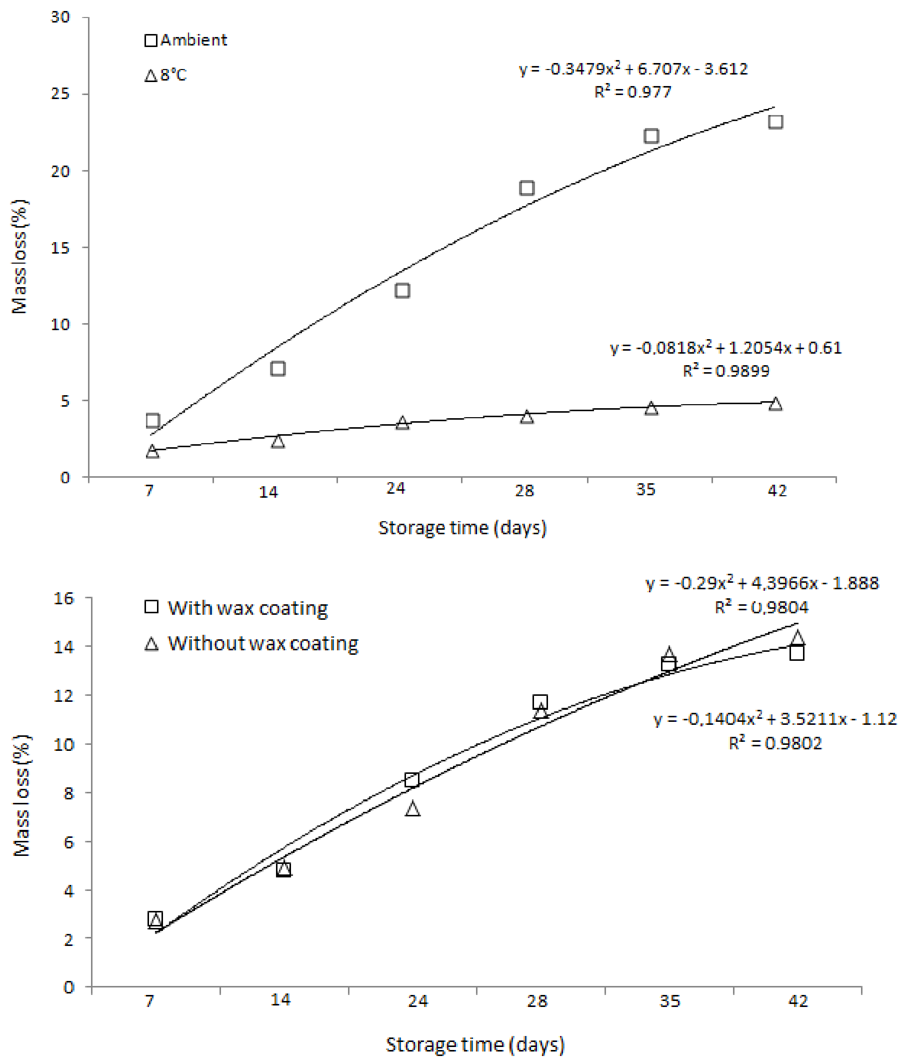

Figure 2. Variation in mass loss (%) of Fremont mandarin fruits stored under ambient conditions (21 ± 2°C and 60 ± 5% R.H.) or under refrigeration (8 ± 2°C and 90 ± 5% R.H.), with or without wax coating, for 42 days.

fruits stored under ambient conditions presented a higher mass loss, soluble solids concentration and SS/TA ratio, and a lower gloss and hue angle compared with the refrigerated fruits. In addition, the refrigerated fruits presented a relatively lower mass loss, and the soluble solids concentration, soluble solids/acid ratio, gloss and hue angle (h°) were similar to the values observed at the beginning of storage. The wax coating only affected the soluble solids/acid ratio, gloss and color, that is, the wax-coated fruits presented a higher soluble solids/acid ratio, gloss and hue angle.

Sensory quality of Fremont mandarins with different storage times

The fruits stored under refrigeration presented higher acceptability for all evaluated characteristics (appearance, firmness, ease of peeling, and flavor) (Table 2). In general, the fruits stored under refrigeration presented a better appearance than the fruits stored under ambient conditions, independent of wax coating. The appearance of fruits stored under refrigeration was rated between "like very much" and "like slightly" throughout storage, whereas the acceptance of the appearance of fruits stored under ambient conditions decreased with storage, ranging from "like moderately" to "dislike slightly", by day 35 of storage.

Fruit firmness decreased by day 14 for all treatments. However, this firmness loss was lower for the fruits stored under refrigeration than for the fruits stored under ambient conditions, which exhibited a pronounced decrease in firmness by day 28 of storage, independent of wax coating. The wax-coated fruits presented a lower

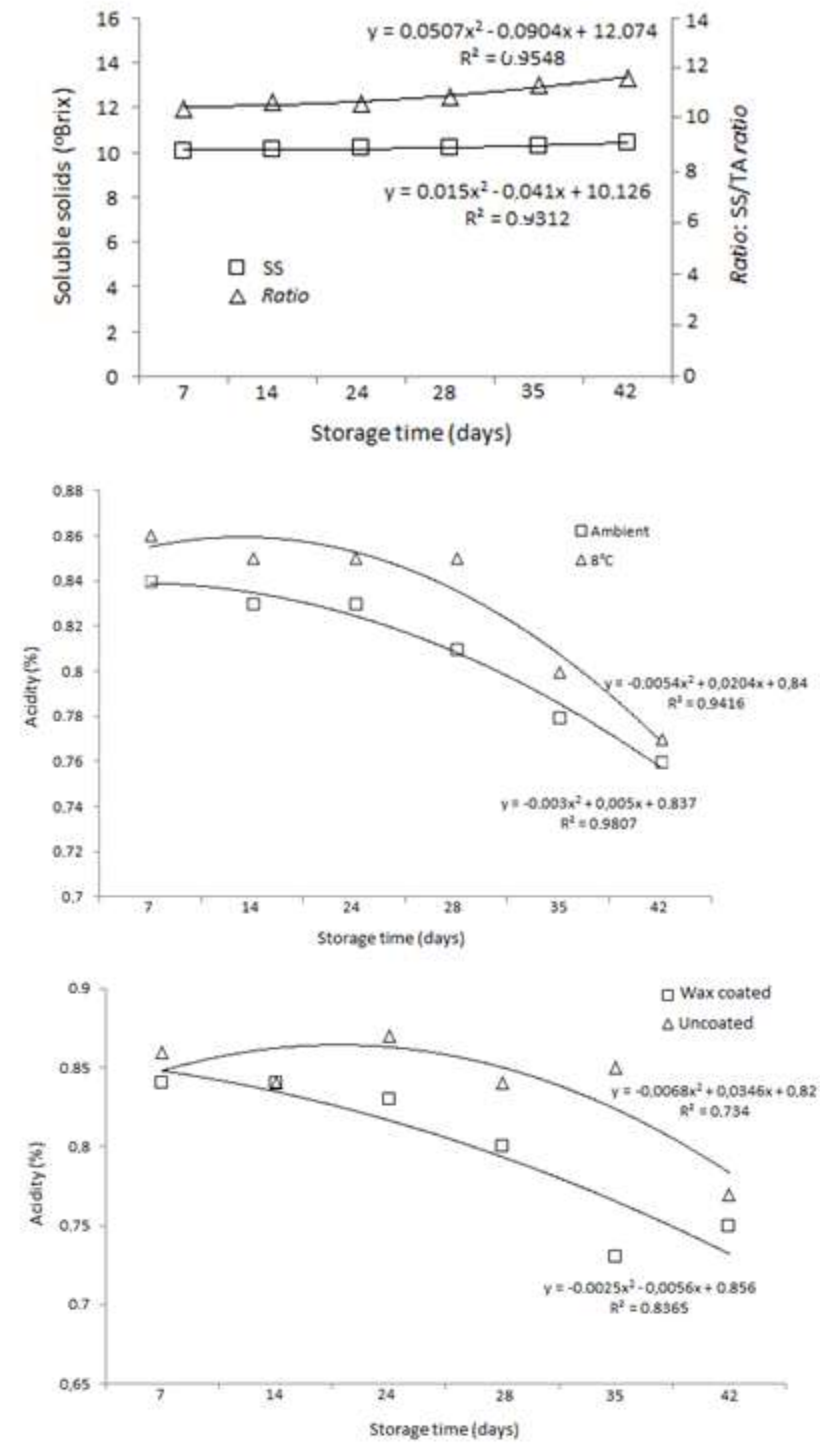

Figure 3. Variation in soluble solids concentration (°Brix), acidity (%), and the SS/TA ratio in Fremont mandarin fruits stored under ambient conditions (21 ± 2°C and 60 ± 5% R.H.) or under refrigeration (8 ± 2°C and 90 ± 5% R.H.), with or without wax coating, for 42 days.

ease of peeling from week 4 (day 28) when stored under ambient conditions and an increased ease of peeling from day 14 when stored under refrigeration.

The wax-coated fruits presented a change in flavor in

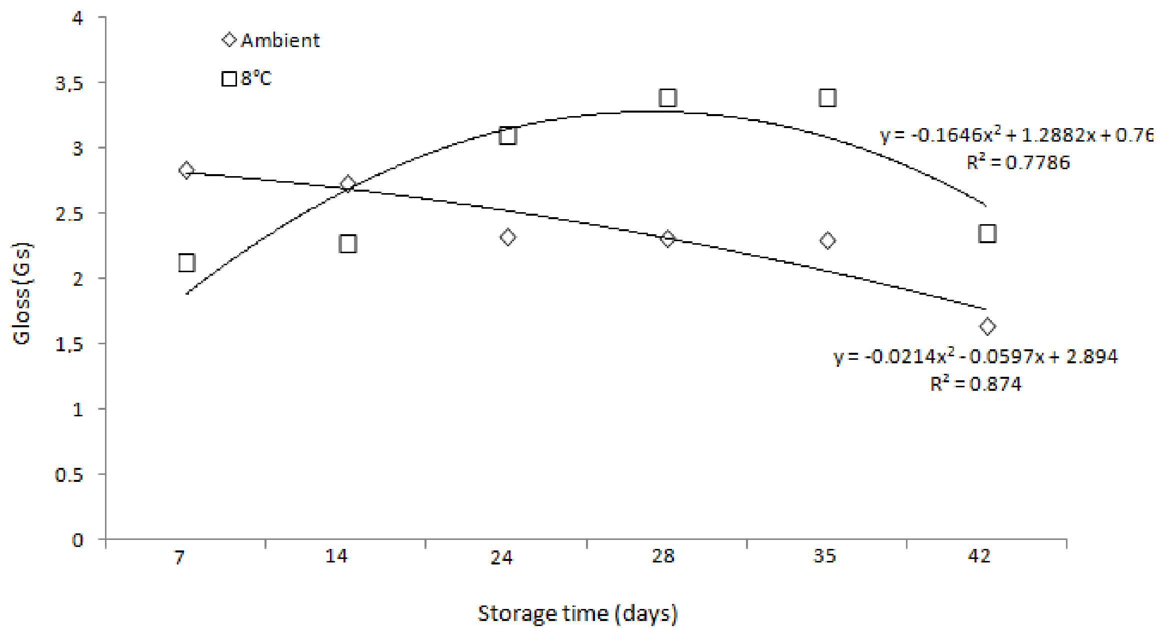

Figure 4. Variation in the gloss (Gs) of Fremont mandarin fruits stored under ambient conditions (21 ± 2°C and 60 ± 5% R.H.) or under refrigeration (8 ± 2°C and 90 ± 5% R.H.), for 42 days.

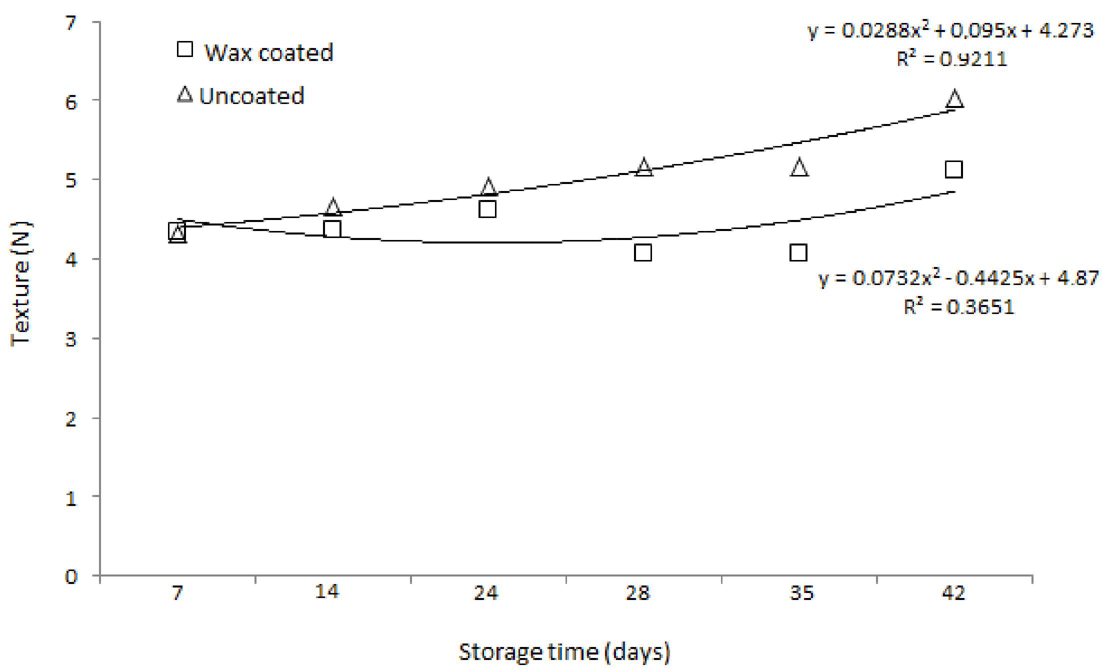

Figure 5. Variation in the texture (N) in Fremont mandarin fruits stored under ambient conditions (21 ± 2°C and 60 ± 5% R.H.) or under refrigeration (8 ± 2°C and 90 ± 5% R.H.), with or without wax coating, for 42 days.

the first week of storage but later recovered a pleasant flavor (Table 2). The fruits stored under refrigeration presented a better flavor than the ones stored under ambient conditions; the wax-coated fruits maintained their flavor characteristics until day 35 and subsequently became unsuitable for consumption. Uncoated fruits stored under refrigeration presented an improved flavor in the third week of storage (day 24) and only began

Table 2. Sensory characteristics of Fremont mandarin fruits, with and without wax coating, stored under different temperatures and analyzed weekly until day 35 (Cordeirópolis, SP, 2012).

Sensory characteristics	Storage time (days)				
	7	14	24	28	35
Appearance**					
FASW***	5.3^{bAB}*	5.3^{bAB}	6.4^{aA}	4.8^{bB}	4.3^{bB}
FAS****	5.8^{bA}	5.8^{bA}	4.7^{bAB}	3.8^{cB}	4.1^{bB}
FSSW*****	7.3^{aA}	7.3^{aA}	7.5^{aA}	6.8^{aA}	6.8^{aA}
FSS******	6.2^{abA}	6.2^{abA}	6.7^{aA}	6.4^{aA}	6.3^{aA}
Firmness**					
FASW	6.9^{bA}	5.6^{bBC}	5.8^{bAB}	4.5^{bC}	4.4^{bC}
FAS	6.7^{bA}	6.0^{bAB}	4.8^{cBC}	4.0^{bC}	4.3^{bC}
FSSW	7.9^{aA}	7.4^{aA}	7.1^{aA}	7.1^{aA}	6.9^{aA}
FSS	8.2^{aA}	7.1^{aAB}	6.9^{aB}	6.9^{aB}	6.7^{aB}
Ease of peeling**					
FASW	4.9^{bAB}	4.3^{cB}	6.0^{aA}	4.7^{bB}	4.7^{bB}
FAS	4.3^{cA}	4.9^{bcA}	4.6^{bA}	4.2^{bA}	4.9^{bA}
FSSW	5.1^{bB}	6.6^{aA}	5.9^{aAB}	6.1^{aAB}	6.1^{aAB}
FSS	6.2^{aA}	5.6^{bA}	6.1^{aA}	6.1^{aA}	6.0^{aA}
Flavor**					
FASW	4.8^{cB}	4.9^{bB}	5.8^{bA}	4.9^{cB}	4.6^{cB}
FAS	7.3^{aA}	7.1^{aA}	6.9^{abB}	5.5^{bC}	5.2^{bC}
FSSW	6.7^{bA}	7.3^{aA}	6.7^{abA}	6.9^{abA}	6.5^{aA}
FSS	6.9^{abA}	7.0^{aA}	7.4^{aA}	7.5^{aA}	6.4^{aB}

*Values are averages. Values followed by the same lower case letter within a column and upper case letter within a line are not significantly different by Tukey test ($p \leq 0.05$). **Intensity of the characteristics evaluated: 9-point hedonic scale. ***Fremont fruit stored under ambient conditions with wax coating. ****Fremont fruit stored under ambient conditions without wax coating. *****Fremont fruit stored at 8°C with wax coating. ******Fremont fruit stored at 8°C without wax coating.

presenting a decreased flavor in the fifth week (day 35). However, the flavor of the fruits stored under ambient conditions, with or without wax coating, started changing in the fourth week of storage (day 28), receiving an average rating of approximately 4 ("dislike slightly"). It should be highlighted that wax-coated fruits stored under ambient conditions were rated lower than the uncoated fruits stored under ambient conditions. However, the purchase intention was higher for the fruits stored under refrigeration compared with those stored under ambient temperature. The refrigerated fruits were rated by the panelists as "might or might not buy it" (a rating of 3) only between days 28 and 35 of storage, whereas doubts regarding the purchase intentions of the panelists for fruits stored under ambient conditions were observed from day 24 of storage, with ratings between 3 and 2 ("might or might not buy it" and "probably would not buy it", respectively) until the end of the evaluations (Figure 6).

DISCUSSION

The Fremont mandarin fruits used in the present study presented 0.97% acidity, 11.16 °Brix and a SS/TA ratio of 11.50, that is, a very good sugar-acid balance, which is appropriate for commercialization and consumption (CEAGESP, 2011). An acidity of approximately 1% is important for the industrial use of citrus fruits, allowing flexibility of sugar addition for the production of ready-to-drink beverages and making deterioration by microorganisms more difficult (Franco, 2005). The optimum SS/TA ratio range for industrial processing of citrus fruits is reported to be between 8.8 and 15.4, according to Sartori et al. (2002), and 12 and 13, according to Couto and Canniatti-Brazaca (2010).

Ponkan, the main mandarin variety commercialized in Brazil, presents 138 g average weight, 43% juice yield, 10.8 °Brix, 0.85% acidity and a 12.7 SS/TA ratio (Pio et al., 2005). Fremont mandarins present similar characteristics to Ponkan mandarins, except for the smaller fruits, with a higher longitudinal/transverse diameter ratio, which is characteristic of oblong fruits (Table 1). The juice yield is higher for Fremont (44.8%) compared with Ponkan mandarins (43%). Fremont mandarins are juicier, present a higher total soluble solids concentration, a low-acid flavor, and pleasant taste and

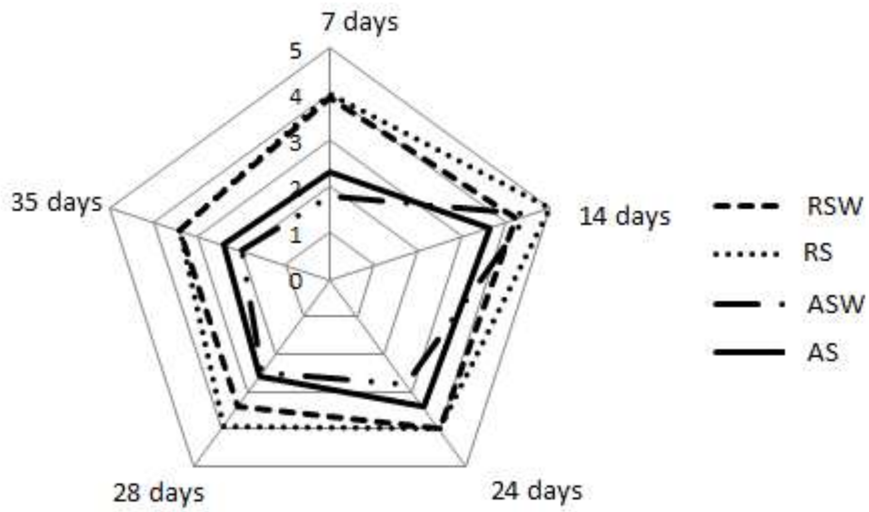

Figure 6. Panelists' purchase intention with respect to Fremont mandarin fruits with different storage treatments and times: RSW = refrigerated storage with wax coating; RS= refrigerated storage without wax coating; ASW = storage under ambient conditions with wax coating; AS = storage under ambient conditions without wax coating (Cordeirópolis, SP, June 2012).

do not cause gastric discomfort (Frata; Valim; Monteiro, 2006). Another positive aspect of Fremont mandarins, from a consumer point of view, is the peel color, which is bright orange (Hue angle of 51.11), whereas Ponkan mandarins present a yellow orange color (Hue angle between 62.6 and 76.9; Silva et al., 2014). Peel color is considered a very important quality parameter for the fresh fruit market, being one of the factors determining fruit acquisition by consumers, which may please both the fresh fruit market and the juice processing industry (Santos et al., 2010). In general, mandarin varieties and hybrids with orange color are more highly accepted by consumers (Pio et al., 2001).

The Fremont variety is recommended for the fresh fruit market and, because it presents different fruits, can reach very interesting prices for producers. Due to the quality of its pulp, the juice of Fremont mandarins may also be featured in agroindustry as a different type of mandarin juice aimed at the internal market, although the fresh fruit market pays better for this type of product (ASSOCITRUS, 2012). Fruit mass loss was more pronounced for storage under ambient conditions (Figure 2). This results from higher water loss due to transpiration, caused by differences in water vapor pressure between the fruit and the ambient air (Sousa et al., 2000). A lower temperature under refrigeration lowers fruit metabolism, resulting in lower mass loss (Jeronimo and Kanesiro, 2000). This is extremely important for commercialization because high mass loss may result in withering and loss of fruit consistency, resulting in decreased quality.

Small variations in SS concentrations were observed (Figure 3). Mass loss during storage contributes to an increase in sugar concentration. These small changes in SS are common because the sugar content of citrus fruits changes when the harvest is performed during or following the maturation stage (Chitarra and Chitarra, 2005). In contrast, acidity decreased during storage (Figure 3). Acidity decreases following harvest and during storage in most fruits because the concentration of organic acids tends to decrease due to their use as a respiration substrate and source of carbon skeletons for the synthesis of new compounds (Kays, 1991). This observation was previously reported by Malgarim et al. (2008) for Nova tangelo fruits (*C. reticulata* Blanco × *C. paradisi* Macfad.).

In contrast, increases in the SS/TA ratio were observed during storage. This effect was also reported by Lima et al. (1999), who studied the quality of Ponkan mandarins stored under ambient temperature. The SS/TA ratio tends to increase during fruit maturation due to a decrease in acids and an increase in sugars. The SS/TA ratio varied between 11 and 13, which is in agreement with the maturity index for citrus fruits. Kluge et al. (2007) reported a SS/TA ratio between 10.79 and 14.87 for Murcott tangor and between 9.24 and 11.13 for Valencia oranges, under refrigerated storage. The higher ratings given to fruits stored under refrigeration compared with ambient conditions, independent of wax coating, are associated with the sugar/acid balance. One characteristic stands out relative to the others. In the case of fruits, the characteristic that is most taken into consideration is the fruit sugar concentration (ABNT, 1993; Anzaldúa-Morales, 1994).

The pressure applied to the wax-coated fruits was practically constant during storage, with a penetration force varying between 4.5 N and 5.9 N from the beginning to the end of storage (Figure 4). This result supports the use of wax coating for maintenance of fruit turgidity because it decreases the loss of water from the fruit to the external medium by changing the air surrounding the fruit (Hagenmaier and Baker, 1994). Wax coating of Tahiti acid limes also delayed the occurrence of changes in the fruit cell walls; these changes resulted in a wilted appearance and difficulty in penetrating the fruit surface with a penetrometer (Jomori et al., 2003).

The firmness of the fruits stored under refrigeration was better rated by the panelists than that of the fruits stored under ambient conditions, independent of wax coating. Consumers consider fruit texture an indicator of quality, that is, product freshness, and this factor contributes to the purchase decision (Surmacka-Szczesniak, 2002). Fruit peel gloss slightly decreased with storage under ambient conditions, and the fruits became duller (Figure 5). Peel color and gloss are the quality attributes that most influence the purchase decision, and these factors depend on storage conditions (Gvozdenović et al., 2000). The fruit peel gloss of Navelina oranges has been reported to decrease during storage, especially for uncoated fruits (Malgarim et al., 2007). This explains the better acceptance of fruits stored under refrigeration compared with those stored under ambient conditions, independent of wax coating, because visual appearance is generally the first contact consumers have with a product, and they show a preference for color and appearance. All products possess expected colors and appearances, which are associated with personal reactions of acceptance, indifference or rejection by panelists (Anzaldúa-Morales, 1994).

The purchase intention of panelists was highest for uncoated fruits stored under refrigeration and lowest for wax-coated fruits stored under ambient conditions (Figure 6). The maintenance of fruit quality obtained with storage under refrigeration was the main reason for the higher purchase intention of the panelists. The use of refrigeration for storage of citrus fruits is a good alternative for the maintenance of fruit quality and a decrease in post-harvest losses (Hagenmaier and Baker, 1993). It is therefore concluded that fruit quality results from a set of physical, chemical and/or sensory characteristics, which should be preserved during storage, and that a safe storage period should be defined to avoid changes in fruit structures as much as possible.

Conclusion

Fremont mandarins present physicochemical and sensory characteristics that are accepted by Brazilian consumers and may be stored under refrigeration (8 ± 2°C and 90 ± 5% R.H.) for 30 to 35 days without losing these characteristics. Purchase intention was higher for fruits stored under refrigeration than at ambient temperature. It is concluded that uncoated fruits stored at ambient temperature exhibit a tendency to lose quality, which confirms the need for refrigeration and wax coating for post-harvest preservation of fresh citrus fruits.

Conflict of Interests

The authors have not declared any conflict of interests.

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Evaluation and selection of promising sunflower germplasm under early winter planting conditions

Karim Houmanat[1,2], Mohamed EL Fechtali[1], Hamid Mazouz[2] and Abdelghani Nabloussi[1]*

[1]Research Unit of Plant Breeding and Plant Genetic Resources Conservation, INRA, CRRA of Meknes, P. O. Box 578, Meknes, Morocco.
[2]Laboratory of Biotechnology and Molecular Biology, Faculty of Sciences, University Moulay Ismail, Meknès, Morocco.

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Agronomic potential of traditional sunflower spring varieties is low because their flowering and grain filling are often exposed to mid and end-season drought. To overcome this, new breeding strategy consisted of selecting varieties tolerant to winter cold in order to shift to autumn or early winter planting. Nowadays, 'Ichraq' is the only registered autumn variety in Morocco. The objective of this research was to evaluate various genotypes selected in different environments under autumn planting conditions. This germplasm was planted early during winter in 2014 at 'Annoceur', a mountain site known for its pronounced winter cold. Morphological, physiological, agronomic and technological parameters were considered for the germplasm assessment. Analysis of variance showed significant differences between genotypes for most of these parameters. Plantlet initial vigor average was 3.5 varying from 1 for genotype M32 to 5 for AN8. Leaf area average was 162 cm² varying from 25 to 375 cm² for genotypes M17 and AN34, respectively. Total chlorophyll content average was 43 mg/g, varying from 28 to 79 mg/g for genotypes K7 and M29, respectively. Number of days from sowing to flowering varied from 162 for genotype AN21 to 180 for genotypes M27 and M29. Mean seed yield per plant was 49 g, with a large variation from 8 to 110 g for M18 and K8, respectively. Mean seed oil content was 36%, ranging from 22% for M8 to 47% for K4. Plants exhibiting more performance than 'Ichraq' were selected to develop new sunflower germplasm suitable for autumn or early winter sowing.

Key words: Sunflower, genetic variability, autumn sowing, cold tolerance, physiological and agronomical traits.

INTRODUCTION

Agricultural sector continues to dominate Morocco's economic activity. The rural population accounts for 40% of the total population. Thus, agriculture is an effective engine of economic growth and guaranteed food security. To upgrade and boost domestic agriculture, different strategies have been implemented during the Moroccan contemporary history. The latter being the Green Morocco Plan implemented since 2008. Owing to its

*Corresponding author. E-mail: abdelghani.nabloussi@gmail.com. Tel: 212 6 65 88 47 33.

importance in the cropping system and the food security challenge in vegetable oils, the oilseed is considered among the priority sectors. Since 2001, the year of oilseed sector reform, and until 2013, the year of signature of a sector program contract, sunflower has been the major annual oilseed crop grown in Morocco, with an average area of about 50000 ha and an average seed yield below 1 t/ha. Indeed, national seed oil production covers barely 2 to 3% of the overall needs of the edible oil in the country estimated at over 410.000 t. The gap is covered by importation which has negative repercussion on the economy and food security of the country (Nabloussi et al., 2015).

In Mediterranean area, sunflower traditionally sown in spring has limited productivity as it does not benefit from fall and/or winter precipitation, and it is often exposed to drought and high temperatures of mid and late cropping cycle. Such constraints coincide with periods of flowering and seed filling that are critical for determining seed productivity and seed oil content (Ouattar et al., 1992). Its cultivation is often secondary and is considered as catch crop, following early droughts or floods that affect autumn crops growing, mainly cereals. However, several studies have shown the benefits of early planting (autumn or early winter) in improving the seed yield and oil content in Morocco (Boujghagh, 1993; Gosset and Vear, 1995; Aboudrare et al., 2000), Spain (Gimeno et al., 1989) and France (Allinne et al., 2009). Early sowing, two to three months earlier than conventional sowing, induced a significant drop in temperature at planting and during early stages of vegetative growth (Allinne et al., 2009). Low temperature has an effect on maximum biological processes. It initiates a number of physiological alterations which make the plant to be more cold tolerant (Browse et al., 2001). Among the frequent metabolic changes, photosynthesis is the leading physiological process studied under low temperature conditions in various species including sunflower (Allinne et al., 2009), rice (Zhi-Hong et al., 2005), maize (Fryer et al., 1995) and Arabidopsis (Uemura et al., 1995). Photosynthetic variations are particularly characterized by changes in photochemical efficiency (Jompuk et al., 2005), in photoinhibition (Verheul et al., 1995) and reaction to photooxidation (Wise et al., 1995). The characterization and evaluation of sunflower genotypes adapted to low temperature conditions, during early vegetative growth stages, requires analyzing the impact of such conditions on the physiological processes associated with initial seedling vigor and plant cold tolerance. Agronomic, morphological, physiological, technological and biochemical attributes could be taken as valuable criteria to identify and select genotypes adapted to winter cold conditions. Early sowing of sunflower in Moroccan environment was envisaged such as mid-season and terminal drought avoidance strategy. However, this implies early stages of crop development are exposed to winter cold which could be harmful to sunflower as it is known as a spring crop sensitive to low temperatures, especially during early growth stages. Therefore, breeding new varieties tolerant to low temperatures, and thus adapted to autumn or early winter planting, is necessary to adopt this strategy (Allinne et al., 2009).

Nowadays, “Ichraq” is the only autumn variety registered in the Moroccan Official Catalogue (Nabloussi et al., 2008). It is a late maturing, drought and winter cold tolerant and combines good seed yield and high seed oil content. Current research continues to develop new sunflower populations, resistant (or tolerant) to winter cold and agronomically performant, which would be the basis for selection of new improved varieties better than the variety “Ichraq”. Thus, the present work aimed to evaluate new sunflower genetic materials for agro-morphological, physiological and technological traits under early winter planting conditions.

MATERIALS AND METHODS

Plant material

The plant material used in this study consisted of 46 sunflower genotypes including ‘Ichraq’, the first and only one autumn variety, considered as check, and 45 individual selected plants derived from ‘Ichraq’. As this latter is a population variety (Nabloussi et al., 2008), there was opportunity to select individual plants (PS) in order to release new germplasm that will be more performant than ‘Ichraq’. The 45 PS were selected under various environmental conditions, in early winter planting, for their vigor, habit and agro-morphological performances. Most of the 45 genotypes selected, being more performant than ‘Ichraq’, were phenotypically different from each other. The main selection criteria were earliness, reduced number of leaves per plant, leaves hairiness, high seed oil content and absence of diseases.

Methods

The 46 genotypes were planted on 2 January 2014 at the INRA experimental station located at ‘Annoceur’, mountainous area known for its rough winter cold. It is located 50 km from Fez city, in the north of Morocco, at an altitude of 1350 m (33°41'05.2"N 4°51'19.9"W).

During the cropping cycle, the minimum temperature was -5°C, registered on January and February whilst the maximum temperature was 37°C, recorded on May. On the other hand, cumulative rainfall was around 245 mm, and the rainiest month was January with about 119 mm, ensuring good emergence and early seedling establishment. Figure 1 shows maximum and minimum average monthly temperatures and rainfall, characterizing the experiment environment. Daily data were gathered from the meteorological station located in ‘Annoceur’ experimental station.

The experiment was conducted under rainfed conditions following a randomized complete blocks with two replications. Each genotype was grown in a single 5 m-length row, and inter and intra row spacing were 60 and 30 cm, respectively. Initial N-P-K fertilization was 80-80-30 units, respectively, followed by cover N fertilization with two inputs of 40 units, one at stem elongation stage and the other at flowering stage. No phytosanitary treatment was applied.

Morphological, phenological, physiological, agronomic and technological parameters were studied. During plant vegetative

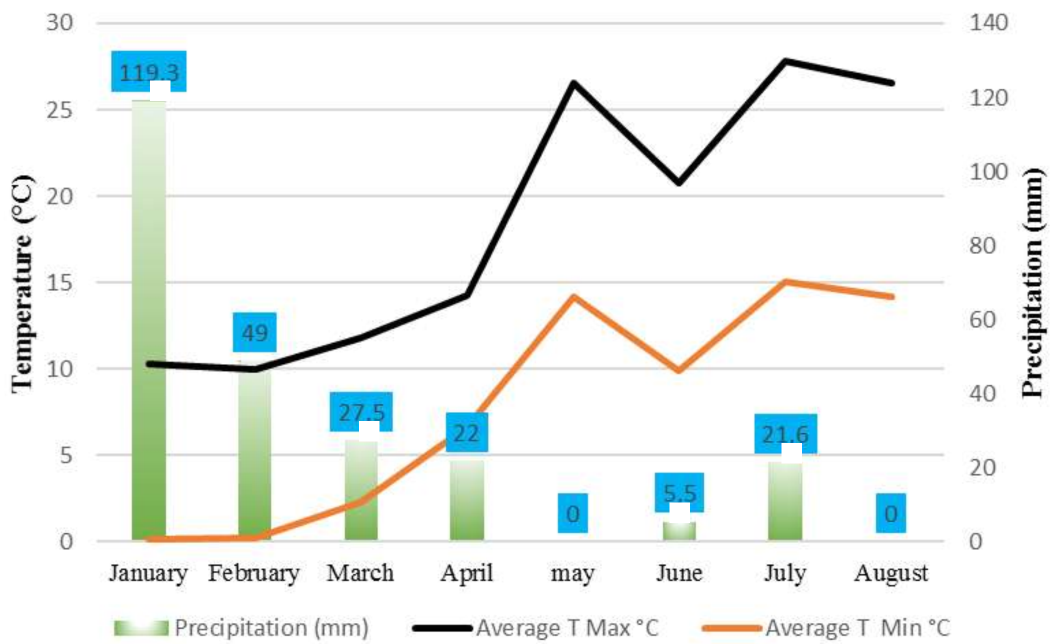

Figure 1. Maximum and minimum average monthly temperatures and precipitation recorded in the experimental station 'Annoceur' during 2014.

growth, plant height (cm) was measured from the ground to the top of the plant at the maturity, and growth rate (cm/d) was determined at stem level, using a graduated ruler. Collar diameter (mm) was measured using a caliper. Initial vigor of young seedlings was evaluated at two leaves stage according to a grading scale from 1 (weakest vigour) to 5 (strongest vigour). Number of leaves per plant and number of branches per plant were determined at full flowering. Leaf area (LA, cm^2) was also measured at full flowering using the following formula (Kiani, 2007): LA = Length x Width x 0.7. Flowering time of each genotype was determined by counting the number of days between planting date and the date when 50% of plants of this genotype have flowered. Chlorophyll content (mg/g) was calculated according to the method of Billore and Mall (1975). The optical density (OD) of all the supernatant obtained was measured in a spectrophotometer at 645 and 663 nm. The concentrations of chlorophyll pigments are given by the following formulas:

CHL A = 12.7 (OD 663) – 2.69 (DO 645)

CHL B = 22.9 (OD 645) – 4.56 (DO 663)

At maturity, head diameter and head aborted diameter, expressed in cm, were measured using graduate ruler. After harvest, seed yield per plant (g) was determined by weighting total plant seeds, using a precision balance. Also, its components (number of seeds per propeller and 1000 seeds weight) were determined. Seed oil content was determined using NMR method (Oxford 4000). This method of quantitative determination is reliable, fast and easy to implement. It analyzes directly on whole seed, without preparation or destruction of biological material.

From each genotype and each block, four plants were taken randomly to be used for all the measurements considered in this study both in field and laboratory. Descriptive analysis of gathered data, analysis of variance and analysis of correlation were performed using different procedures of SAS program. Duncan's new multiple range test was applied to compare genotypes means. This enabled us to distinguish which genotypes were significantly different from others and, thus, to select those interesting genotypes according to an established threshold. Selection threshold was fixed on the basis of nature of parameters studied and their relation with abiotic stresses (cold and drought) and agronomic performance, overall average of each parameter and check performance. Furthermore, Agglomerative Hierarchical Clustering (AHC), created by XLSTAT statistical software, was performed to group homogeneous genotypes.

RESULTS AND DISCUSSION

Morphological parameters

Analysis of variance showed there were significant differences ($P < 0.001$) between the 46 genotypes for all studied parameters (Table 1). Initial vigor of young seedlings varied from 1 for genotype M32 to 5 for genotype AN8, with an average of about 3.5, higher than 'Ichraq' vigor (3). In many studies, seedling and plantlet initial vigor was found as a good selection criterion correlated with adaptation and performance of genotypes under environmental abiotic stresses (Foolad and Lin, 2001). In the present work, all genotypes having an initial vigor of 4 or 5 were selected for further evaluation and germplasm improvement. For growth rate, the overall mean was 2.31 cm/d, with a minimum of 0.53 cm/d, registered for genotype AN21 and a maximum of 3.92 cm/d for genotype M34, slightly higher than that of the check, which was 3.15 cm/d (Table 1). Genotypes having growth rate higher than that of the check will be selected. The average plant height was 147 cm, with a

Table 1. Descriptive analysis (average, minimum and maximum), analysis of variance (significance level of differences) and selection threshold for agromorphological, physiological and technological parameters of 45 sunflower genotypes and the check variety evaluated under early winter planting conditions at 'Annoceur' during 2014.

Parameter	Genotype	Average	Minimum		Maximum		Value from the check 'Ichraq'	Threshold for selection
			Value	Genotype	Value	Genotype		
IV(1)	*** (2)	3.49	1	M32	5	AN8	3	4-5
GR	***	2.31	0.53	AN21	3.92	M34	3.15	>3.15
PH	***	146.77	75	M18	200	K20	166.66	<145
NLP	***	27.46	17	M4	38	K3	25.33	<25
LA	**	162.23	24.5	M17	374.85	AN34	161.7	<162
CD	***	20.08	11.11	M18	30.82	M7	23.11	>20
CHLT	ns	43.21	28.2	K7	79.1	M29	54.6	>54
CHLA	***	4.04	0.86	K9	11.31	K4	1.86	>1.86
CHLB	***	6.77	1.74	K8	19.89	K4	2.77	>2.77
DSF	***	170.48	162	AN21	180	M29, M27	170	<170
NBP	***	0.93	0		6	M30	0	0
THD	***	12.79	6	M22, M18	23	K9	16.33	>16
AHD	ns	2.48	0		7	K3, K9	2	<2
PAD	ns	21.2	0		62.5	M32	13.96	<13.96
NSP	***	18.39	8	M22	27	K20, K8	20	>20
HSY	***	49.36	8.184	M18	110.3	K8	62.14	>62
TSW	***	47.52	12.4	M18	83.6	K8	56.86	>56
SOC	***	36.43	21.83	M8	46.85	K4	38.52	>38

IV: Initial vigor, GR: growth rate, PH: plant height, NLP: number of leaves per plant, LA: leaf area, CD: collar diameter, CHLT: total chlorophyll content, CHLA: chlorophyll a content, CHLB: chlorophyll b content, DSF: days from sowing to flowering, NBP: Number of branches per plant, THD: total head diameter, AHD: aborted head diameter, PAD: percentage of aborted diameter, NSP: number of seeds per propeller, HSY: head seed yield, TSW: 1000 seeds weight, SOC: seed oil content. *, ** and *** significant at 0.05, 0.01 and 0.001 levels, respectively. ns not significant.

variation from 75 to 200 cm for M18 and K20, respectively. Plant height of the check was about 167 cm. It was reported that very high plants were often susceptible to lodging and late drought (Sposaro et al., 2008). Thus, plants with a height less than the observed average (< 145 cm) could be interesting for selection. Number of leaves per plant varied from 17 for M4 to 38 for K3, with an average of 27.5 leaves per plant. The check had 25 leaves per plant. The average leaf area was 162 cm², which is equal to the check value. The genotypes M17 and AN34 exhibited the extreme values: 24.5 and 374.85 cm², respectively. Specific leaf area was found to be genetically associated with cold tolerance in sunflower (Allinne et al., 2009). Furthermore, elevated number of leaves per plant and high leaf area are correlated with high plant transpiration (Romero-Aranda et al., 2001). Thus, this study aimed to select those plants having less than 25 leaves and a leaf area less than 162 cm². Regarding collar diameter, genotype M7 exhibited the strongest value which was about 31 mm, whilst genotype M18 showed the lowest value which was 11 mm. The overall mean value was 20 mm and the check value was 23 mm. Like initial vigor, collar diameter is an indicator of good adaptation under stressed environments (Liua et al., 2012a). Therefore, all the genotypes exhibiting a collar diameter more than the observed average (20 mm) could be selected for further evaluation. Among the 46 studied genotypes, 27, including 'Ichraq', the check variety, had no branching, while 19 were branched, with a number of branches per plant varying from one to six. Genotype M30 was the most branched, having six branches per plant. The overall average was 0.93. Sunflower branching is an indicator of plants susceptibility to cold conditions (Alba et al., 2010). The plants selected for further evaluation and new germplasm constitution should have no branching.

Physiological parameters

Analysis of variance revealed significant effect of genotype on flowering earliness, chlorophyll a content and chlorophyll b content ($P < 0.001$), and non-significant effect on total chlorophyll content (Table 1). However, a large variation was observed, ranging from 28 mg/g for genotype K7 to 79 mg/g for genotype M29. The average total chlorophyll content was 43.21 mg/g, while the content concerning the check variety was 54.6 mg/g (Table 1). Genotypes maintaining high chlorophyll content under abiotic stresses, like drought or cold, exhibit tolerance to such stresses (Yang et al., 2015). In sunflower, chlorophyll content is genetically associated with cold tolerance and, thus, could be suggested as selection criterion for cold tolerance in breeding programs (Allinne et al., 2009). All genotypes having total chlorophyll content higher than that of the check will be selected. Regarding chlorophyll a and b contents, the genotype K4 exhibited the highest values for both, 11.3 and 19.8 mg/g, respectively. The lowest contents were 0.86 mg/g, registered in genotype K9, and 1.74 mg/g, registered in genotype K8, for chlorophyll a and b, respectively. The check variety had 1.86 and 2.77 mg/g for these parameters, respectively. Vegetative period before flowering was too long, with an average duration exceeding 170 days from sowing date to flowering date. It ranged from 162 days for genotype AN21 to 180 days for genotypes M27 and M29. The check variety has bloomed in 170 days after sowing. Flowering earliness is a desired character in environments under terminal drought stress (Ribot et al., 2012). Thus, genotypes having a sowing-flowering period shorter than that of the check will be selected.

Agronomic and technological parameters

Analysis of variance showed there were significant differences ($P<0.001$) between the studied genotypes for all agronomic and technological parameters, excepted aborted head diameter (AHD) and percentage of aborted diameter (PAD) (Table 1). However, one could observe some variation among genotypes for these parameters (Table 1). Most of the evaluated genotypes had no AHD, and among the few ones having AHD, genotypes K3 and K9 exhibited the largest value (7 cm). The overall average AHD was about 2.5 cm. The overall average PAD was about 21%, ranging from 0%, for most of the genotypes, to more than 62%, for genotype M32. All genotypes exhibiting some AHD should be discarded from the selected population as aborted sunflower head is an indicator of plant susceptibility to cold (Hladni et al., 2010). Average total head diameter (THD) was 12.8 cm, ranging from 6 cm, for genotypes M22 and M18, to 23 cm, for genotype K9. The check variety 'Ichraq' had a THD of 16 cm, an AHD of 2 cm and a PAD of 14%. A large range was observed for number of seeds per propeller, from 8 in genotype M22 to 27 in genotypes K8 and K20. The check variety had a number of 20 seeds per propeller.

Regarding seed yield per head, the overall mean was slightly higher than 49 g, and a large range was found, from 8 g in genotype M18 to 110 g in genotype K8, which is much higher than head seed yield of the check (62 g). Thousand seed weight (TSW) ranged from 12.4 g in genotype M18 to 83.6 g in genotype K8, and the average was 47.52 g. TSW of the check was about 57 g. Total head diameter, number of seeds per propeller, single head seed yield and TSW are components of seed yield which are correlated with this latter, and thus could be considered as selection criteria for seed yield breeding (Yasin and Singh, 2010). In this study, all those genotypes showing values higher than those of the check were selected. Finally, seed oil content (SOC) fluctuated from 21.80% in genotype M8 to 46.85% in genotype K4, and had a mean value of 36.43%. The check 'Ichraq' had a SOC of 38.52%, which was slightly higher than the overall average. Genotypes with SOC exceeding that of the check were selected. Table 2 shows the pools of genotypes selected, according to described threshold for each of studied parameters.

Pearce (1999) subdivided the plants into three categories according to their tolerance to cold and ability to adapt to low temperatures. Plants are susceptible to low temperatures and suffer damage as early as 12°C, plants are tolerant to low positive temperatures and plants capable of acclimatizing to survive under temperatures below zero degree. Xin and Browse (2000) showed that there are many physiological mechanisms that allow plants to better withstand severe stress (temperatures below zero) after a long time at low temperature (acclimatization). Many studies have shown low temperature had direct effects on cells (Pearce, 1999), on seed germination (Durr et al., 2001), on photochemical reactions of photosynthesis and carbon fixation (Liua et al., 2012b). Likewise, cold causes reduction of cell water content (Kacperska, 2004).

These findings have shown there was a genetic diversity among the sunflower genotypes evaluated for most of the studied parameters. In all cases, these genotypes were compared with the check variety 'Ichraq'. This study allowed us to identify and select genotypes more interesting than the check for morphological, physiological, agronomic and technological parameters under winter early planting conditions. Globally, taking into account all these parameters, the genotypes AN8, AN27, AN23, AN21, AN24, K30, K20, K10, K8, K7 and K4 seemed to be performant and promising. After confirming their performance in further seasons, they could be useful for intercrossing to develop a new variety more performant and more tolerant to winter cold than 'Ichraq', the only one autumn variety ever registered in Morocco.

On the other hand, molecular analysis to better understand the genetic control of agronomic, morphological and physiological traits associated with cold tolerance would be of a great interest for autumn sunflower breeding programs. Allinne et al. (2009) studied the genetic control of physiological traits associated to cold tolerance in sunflower under early sowing conditions and found that several putative genomic regions were involved in the variation of such physiological traits. Some SSR markers are associated with major QTLs for cold tolerance, such as ORS331_2 for cell membrane stability, ORS1040 for chlorophyll content and ORS1144 and ORS1146 for osmotic potential.

Table 2. Pools of sunflower genotypes selected for their performance based on morphological, physiological, agronomic and technological parameters under early winter planting conditions.

Parameters	Sunflower genotype pools
IV	AN8, AN6, AN3, M45, M43, M30, M26, M17, K8, K7, K6, K5, K4, A35, A34, A33, A32, A27, A23, A21, A13, A11.
GR	M34, M32, M30, K30, K20, K10, K9, K8, K5.
PH	AN8,AN3, M43, M41, M37, M34, M32, M29, M26, M27, M22, M19, M18, M17, M13, M8, M7, M6, M5, M4, AN21.
NLP	AN8, AN3, M41, M37, M34, M32, M29, M27, M22, M19, M18, M17, M8, M6, M4, K8, AN33, AN27, AN21.
LA	AN6, AN3, M45, M37, M34, M32, M29, M26, M22, M19, M18, M17, M8, M7, M6, M4, K30, K20, K9, K8, K4, K3, AN32, AN31, AN27, AN24, AN23, AN21, AN13, AN11, AN9.
CD	AN8, AN3, M45, M41, M37, M34, M30, M26, M22, M13, M7, M5, K30, K20, K10, K9, K8, K7, K6, K5, K4, K3, AN35, AN34, AN32, AN31, AN27, AN24, AN23, AN21, AN13, AN11.
CHLT	M29, M27, M22, M8, M6, K30, K3, AN31.
CHLA	All genotypes except M45, M34, M30, K9, K8, AN35, AN31, AN23, AN9.
CHLB	All genotypes except M30, K8, AN35, AN34, AN31, AN23.
DSF	AN8, AN6, M22, M18, M7, M4, K30, K7, K4, AN35, AN34, AN33, AN27, AN31, AN24, AN23, AN21, AN13, AN9.
NBP	AN8, AN6, AN3, AN45, M43, M41, M37, M13, M7, K30, K20, K10, K9, K8, K7, K3, K4, K5, K6, AN35, AN32, AN27, AN24, AN9, AN11, AN13.
THD	AN8, M45, M37, K20, K10, K9, K8, K7, K5, K4, K3, AN31, AN24, AN27.
AHD	AN3, M43, M34, M32, M27, M29, M30, M18, M7, K20, K10, K9, K8, K7, K5, K3, AN34, AN32, AN27, AN24, AN23, AN13, AN9.
PAD	AN3, M43, M37, M34, M32, M27, M29, M30, M18, M7, K20, K10, K9, K8, K7, K5, K3, AN34, AN32, AN31, AN27, AN24, AN23, AN13, AN9.
NSP	AN8, AN3, M45, M43, M41, M37, M34, M32, M30, M13, M8, M7, M6, M5, K30, K20, K10, K9, K8, K7, K6, K5, K4, K3, AN34, AN31, AN27, AN24, AN23, AN11, AN9.
HSY	AN8, AN6, AN3, M45, M41, M37, M32, M30, M8, M7, K30, K20, K10, K8, K7, K4, K3, AN35, AN34, AN27, AN24, AN23, AN21, AN11.
TSW	AN8, AN6, AN3, M45, M43, M41, M37, M34, M32, M30, M18, M7, K30, K20, K10, K8, K7, K5, K4, K3, AN35, AN34, AN33, AN27, AN24, AN23, AN21, AN13.
SOC	AN8, AN6, AN3, M41, M22, M19, M17, M6, M5, M4, K30, K20, K10, K9, K8, K7, K6, K5, K4, K3, AN34, AN33, AN32, AN31, AN27, AN24, AN23, AN21, AN13, AN11, AN9.

IV: Initial vigor, GR: growth rate, PH: plant height, NLP: number of leaves per plant, LA: leaf area, CD: collar diameter, CHLT: total chlorophyll content, CHLA: chlorophyll a content, CHLB: chlorophyll b content, DSF: days from sowing to flowering, NBP: Number of branches per plant, THD: total head diameter, AHD: aborted head diameter, PAD: percentage of aborted diameter, NSP: number of seeds per propeller, HSY: head seed yield, TSW: 1000 seeds weight, SOC: seed oil content.

Genotypes hierarchical grouping

Agglomerative hierarchical clustering (AHC) allowed obtaining the dendrogram showed in Figure 2. It presents AHC results as similarity index (Pearson correlation) in groups of homogeneous genotypes on the basis of all studied parameters. Three distinct groups with a very high level of similarity could be identified. The first group contains three genotypes, M17, M19 and M22, which are similar for the parameters PH, NLP, LA and SOC. The second group is formed by only two genotypes, K10 and AN34, which are comparable for CD, AHD, PAD, NSP, HSY, TSW and SOC. The third group contains the rest of the studied genotypes and is divided into three homogeneous subgroups on the basis of all the parameters, with a similarity index of about 0.98. The first homogeneous subgroup constitutes 31 genotypes, including the control variety 'Ichraq'. The second subgroup contains only two genotypes, M29 and M27, comparable for PH, NLP, CHLT, AHD and PAD. The last subgroup contains the genotypes M4, M5, M18, M13, M6, M32 and M26, with a similarity index of about 0.99, comparable for PH, NLP, LSA and NGP.

Previous studies has also shown sunflower genotypes hierarchical clustering and usefulness of genotypes grouped in the same pool, according to agronomic, phenological and technological character, for breeding programs in various countries in the world. Among these studies, Poletine et al. (2012) evaluated 16 sunflower genotypes in Brazil, throughout analysis of seven agronomic and morphological characters, and could classify them into five distinct groups. Another work carried out at Tunisia classified 73 sunflower lines and 7 hybrids (checks) into 5 groups and 8 sub-groups, based on morphological and phenological parameters, with a similarity coefficient of 90% (Khoufi et al., 2013).

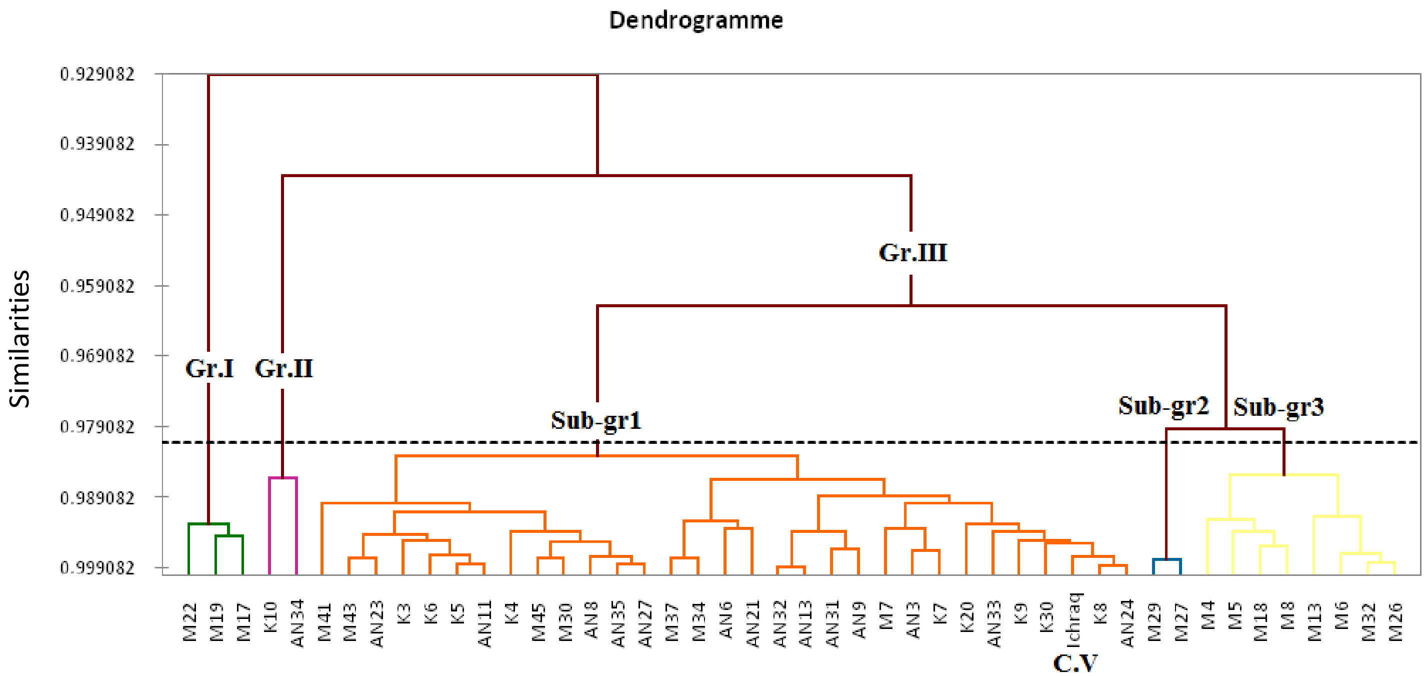

Figure 2. Dendrogram with groups and sub-groups of homogeneous individuals based on all parameters studied. C.V: control variety 'Ichraq'.

Likewise, Ruždik et al. (2015), based on agronomic parameters, had classified 20 sunflower varieties into four groups in their study in Republic of Macedonia. Classifying thirteen sunflower genotypes in Libya by UPGMA procedure, on morphological basis, Mahmoud et al. (2012) had found two major groups. Similar results were described by Kholghi et al. (2011) in Iran and Punitha et al. (2010) in Coimbatore. All these homogeneous groups and subgroups carried out in these studies were useful for corresponding local sunflower breeding programs.

In this study, the homogeneous groups and subgroups could be taken as genetic pools to be used in autumn sunflower breeding program. Each group should be improved and multiplied apart before any crossing or intercrossing with the other groups.

Relationship between characters

Table 3 shows correlation coefficients between the different parameters studied along with their significance level. There are significant correlations between plant seed yield (HSY) and other related parameters. Such correlations are either positive, with SOC, TSW, NSP, THD, CD, LA, PH and IV, or negative, with PAD, DSF, NBP and AHD. These results indicate that non-branched and early flowering plants with reduced aborted head diameter could be more productive than branched and late plants. However, the strongest correlations were observed between HSY with TSW (0.87, P<0.001) and NSP (0.73, P<0.001), indicating that TSW and NSP are the most important seed yield components, and thus could be taken as selection criteria to improve sunflower productivity under autumn early planting conditions. On the other hand, seed oil content (SOC) is significant and positively correlated with IV, PH, CD, THD, HSY and TSW, and significant and negatively correlated with DSF and NBP. Similar results on the positive correlation between SOC and TSW was also found by Joksimovic et al. (2004), Mijić et al. (2009), Kaya et al. (2009) and Anandhan et al. (2010). The highest correlations were observed between SOC and DSF (-0.47, P<0.001) and TSW (0.44, P<0.001), indicating that early flowering genotypes with large seeds could be characterized by high seed oil content. Interestingly, one could observe the significant and positive correlation between HSY and SOC (0.35, P<0.001). This will allow simultaneous breeding and selecting for both characters. Early flowering, large seeds and high seeds number per head could be valuable selection indices to bred high seed oil yielding germplasm under autumn sunflower planting conditions.

In conclusion, a large variability was observed between the evaluated genotypes for most of the traits studied. Genotypes having expressed more performance than the check 'Ichraq' in terms of seed yield, oil content and flowering earliness should be confirmed in further studies under autumn planting conditions in other additional environments. They could be considered as valuable genetic stock for autumn sunflower breeding program. The clustering homogeneous groups should be taken as genetic pools to be separately multiplied and thereafter intercrossed with each other to develop a new cold

Table 3. Correlation between characters measured in 45 sunflower genotypes at 'Annoceur' during 2014.

PSICH	IV[1]	GR	PH	NLP	LA	CD	CHLT	CHLA	CHLB	DSF	NBP	THD	AHD	PAD	NSP	HSY	TSW	SOC
IV		-0.09ns	0.39***	0.26***	0.24**	0.28***	-0.28***	0.43***	0.43***	-0.36***	-0.19*	0.28***	-0.02ns	-0.15*	0.25**	0.30***	0.31***	0.28***
GR		1	0.55***	0.34***	0.17ns	0.25**	-0.03ns	-.07ns	-.05ns	0.21**	-0.13ns	0.36***	-0.07ns	-0.19ns	0.27***	0.12ns	0.01ns	-0.08ns
PH			1	0.66***	0.42***	0.67***	-0.10ns	0.18*	0.20**	-0.38***	0.49***	0.72***	-0.07ns	-0.35***	0.52***	0.55***	0.46***	0.41***
NLP				1	0.29**	0.52***	-0.02ns	0.22**	0.25**	0.20*	-0.32***	0.55***	0.04ns	-0.18*	0.39***	0.24**	0.14ns	0.23**
LA					1	0.35***	-0.20*	0.01ns	0.00ns	-0.09ns	-0.31**	0.38***	0.01ns	-0.17ns	0.33***	0.36***	0.29**	0.19*
CD						1	-0.01ns	0.17*	0.18*	-0.15ns	-0.39***	0.69***	-0.03ns	-0.29***	0.62***	0.87***	0.40***	0.29***
CHLT							1	-0.18*	-0.20*	0.04ns	0.15*	-0.11ns	0.059ns	0.15ns	-0.11ns	-.03ns	0.02ns	0.09ns
CHLA								1	0.98***	0.00ns	-0.27***	0.08ns	-0.04ns	-0.06ns	0.00ns	0.03ns	0.06ns	0.17ns
CHLB									1	-0.01ns	-0.29***	0.09ns	-0.01ns	-0.07ns	0.01ns	0.02ns	0.05ns	0.20*
DSF										1	0.26***	-0.24**	0.02ns	0.13ns	-0.13ns	-.37***	-.49***	-0.47***
NBP											1	-0.47***	0.01ns	0.22**	-0.37***	-.31***	-0.21**	-0.30***
THD												1	-0.07ns	-0.72***	0.63***	0.58***	0.40***	0.31***
AHD													1	0.89***	-0.10ns	-0.18*	-0.17*	-0.08ns
PAD														1	-0.35***	-.38***	-.30***	-0.21**
NSP															1	0.73***	0.35***	0.10ns
HSY																1	0.87***	0.35***
TSW																	1	0.44***
SOC																		1

IV: initial vigor, GR: growth rate, PH: plant height, NLP: number of leaves per plant, LA: leaf area, CD: collar diameter, CHLT: total chlorophyll content, CHLA: chlorophyll a content, CHLB: chlorophyll b content, DSF: days from sowing to flowering, NBP: Number of branches per plant, THD: total head diameter, AHD: aborted head diameter, PAD: percentage of aborted diameter, NSP: number of seeds per propeller, HSY: head seed yield, TSW: 1000 seeds weight, SOC: seed oil content. *, ** and *** significant at 0.05, 0.01 and 0.001 probability levels, respectively. ns not significant.

tolerant germplasm with better agronomic and technological performance than the check variety 'Ichraq'. Specific molecular markers such as SSR should be used to confirm these homogeneous groups and to be considered thereafter in autumn sunflower breeding programs.

To bred autumn sunflower for both high seed yield and high seed oil content, the most relevant selection criteria would be early flowering, large seed size and high seeds number per head.

Conflict of Interests

The authors declare that there was no financial/relevant interest that may have influenced the study.

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Periodicity of crop coefficient and soil water depletion fraction in a climatological water balance

Bruno César Gurski[1]*, Jorge Luiz Moretti de Souza[1], Daniela Jerszurki[2], Robson André Armindo[1] and Adão Wagner Pêgo Evangelista[3]

[1]Department of Soil and Agricultural Engineering, Federal University of Paraná, 1540, Rua dos Funcionários street, 80035-050, Curitiba, Brazil.
[2]Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede Boqer Campus, 84990, Israel.
[3]Department of Agronomy, Federal University of Goiás, Avenida Esperança, s/n., Campus Samambaia, 74690-900, Goiânia, Brazil.

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In comparison to a measured field water balance (FWB), we aimed to evaluate the impact of using different functions to daily estimate the crop coefficient (Kc) and soil water depletion fraction (p) in a climatological water balance (CWB), and verify that the grouping of output variables provides improved results. The FWB was conducted in Telêmaco Borba, Southern Brazil. The data were collected at weekly intervals in 2009, in an area of loblolly pine with 6 years-old. The CWB considers different equations to estimate daily Kc and p values. The output components of the CWB were estimated daily, then weekly and monthly grouped for comparison with the FWB. Linear correlation analysis, index "d" of concordance, index "c" of performance, mean error, mean absolute error and root mean square error were performed in order to compare the water balances, based on the soil-water storage variation (ΔS) and actual evapotranspiration (ETa). The use of a Kc measured weekly improved the CWB, providing high correlation and small errors in relation to a measured water balance, independent of the comparison scale. On the other hand, the use of a Kc that considers climate variables (Kc_k) had the worst levels of accuracy and precision, and the biggest mistakes in all analyzes and all tested variables. There was no significant improvement with the daily variation of p, both grouping weeks as in months. The proposed equations do not represent any gain in the CWB, in comparison with the use of a constant p value over time. The estimate of the CWB and its subsequent grouping in months for comparison provided greater degree of accuracy and precision for the variables analyzed, but caused the biggest mistakes. Therefore, the calculation of CWB should be performed with the highest periodicity possible, and grouping the CWB output variables should only be performed for comparison.

Key words: *Pinus taeda*, crop evapotranspiration, actual evapotranspiration, soil water storage, field water balance.

INTRODUCTION

The field water balance (FWB) is the accounting of inputs and outputs of water at any given volume control over a specific time interval. It can be calculated by means of crop evapotranspiration (ETc) measurement instruments as lysimeters and evapotranspirometers, or by measuring the soil moisture. The monitoring of soil water storage, combined to the understanding about crop needs have been considered important tools to the agroforestry activities planning, improvement of soil water use efficiency by irrigation practices and agroclimatic zoning (Yan et al., 2012; Khazaei and Hosseini, 2015).

The study of water demand in soils under plantation of exotic woods, such as *Pinus taeda*, assists in the development of alternatives to the rational use of water, which implies in no compromising of the environmental balance and promoting the development of silvicultural activity (Rigatto et al., 2005).

Currently, the FWB is more used in scientific researches and their measures are commonly used to verify mathematical models, which are developed to simulate and perform estimations. Thus, many researchers have sought to develop indirect methods to estimate it from climatic variables, in parts because complete field measurements are time consuming, costly and experimentally difficult depending of the size of the area to be monitored (Zhang et al., 2004; Praveena et al., 2012; Yan et al., 2012).

In this context, the estimated climatological water balance (CWB) is required. However, some input components of CWB do not represents the real conditions of the crop in the field, especially for perennial crops, such as forest species, in relation to the variations in time. Due to the lack of specialized studies and local complexity measurements, the water components are usually estimated empirically and are considered constant over time, such as the crop coefficient (Kc) and soil water depletion fraction (p) (Allen et al., 1998). Using a constant value for these variables can significantly affect the output components, such as crop evapotranspiration (ETc); soil water storage (S); actual evapotranspiration (ETa). Since consistent, the highest frequency of the input data in the CWB generally improve their sensitivity to small variations over time (Khazaei and Hosseini, 2015), making it more reliable for the silvicultural planning.

In comparison to a measured field water balance, we aimed to evaluate the impact of using different equations to daily estimate the crop coefficient (Kc) and soil water depletion fraction (p) in a climatological water balance, and verify that the grouping of output variables provides improved results.

MATERIALS AND METHODS

A field water balance (FWB) was conducted in Telêmaco Borba, state of Paraná, Southern Brazil, 24°13′19”S, 50°32′33”W, 700 m altitude (Figure 1). The region has a climate type transitional wet subtropical to temperate (“Cfa/Cfb”) with an average temperature in the coldest month below 16°C including frost events, and an average temperature in the warmest month above 22°C (Alvares et al., 2013).

This experiment served as a witness, being considered the actual values for comparison with the models proposed in this manuscript. The data were collected at weekly intervals in 2009, in an area of 12.5 ha of 6-years old *Pinus taeda* planting, with standard spacing of 2.0 × 3.0 m (1,667 trees/ha) in a clay Oxisol with undulated relief. For details of the methodology (Souza et al., 2016).

The output components (ΔS - soil-water storage variation and ETa - actual evapotranspiration) of the FWB, were compared with a climatological water balance (CWB). ETa in the FWB was calculated as follows:

$$ETa = -\Delta S + P - D + U \quad (1)$$

Where: *ETa* is actual evapotranspiration (mm week^{-1}); ΔS is soil-water storage variation at the root zone (mm week^{-1}); *P* is precipitation (mm week^{-1}); *D* is downward drainage out of the root zone (mm week^{-1}); *U* is upward capillary flow across root zone (mm week^{-1}).

Soil water storage (S) was calculated preliminarily, with the ΔS obtained from the difference between previous (S_j) and current water storage (S_{j+1}):

$$S_j = \theta_1 \cdot z_1 + \sum_{i=1}^{n} \frac{(\theta_i + \theta_{i+1}) \cdot z_i}{2} \quad (2)$$

Where: S_j is soil water storage in *j*-th week year (mm); θ_i is volumetric moisture in *i*-th soil depth (cm^3/cm^3); z_i is soil depth (m); *j* is weeks over year that samples were taken ; *I* is sample collection depths (m).

A daily climatological water balance (CWB) was estimated according to Thornthwaite and Mather (1945) methodology. The daily data series of precipitation (P) used in the simulations were the same used in FWB (Souza et al., 2016). Reference evapotranspiration (ETo) was estimated by Penman-Monteith method (Allen et al., 1998). Daily climatological data were provided by an automatic weather station. Soil water storage (S) was estimated from cosine equation (Rijtema and Aboukhaled, 1975). The initial value of S for 2009 was recorded on December 31, 2008, being equal to 52.5 mm, and was considered an average of total available water (TAW) equal to 174 mm, with no variation in the effective root depth (z = 0.80 m).

Different methodologies and functions to estimate the Kc were used to calculate the CWB (Figure 2). A basic value that did not change over time was used (Kc_A), this value was proposed by Allen et al. (1998) to conifers species. In addition, measured values were used, obtained in the FWB cited, which were grouped weekly and monthly (Kc_m and Kc_{month}, respectively). Finally, we tested an equation, proposed by Allen et al. (1998), that uses climate variables to estimate the daily Kc_k:

$$Kc(DAP)_k = Kc_A + [0.04 \cdot (u_2 - 2) - 0.004 \cdot (RH_{min} - 45)] \cdot \left(\frac{h}{3}\right)^{0.3} \quad (3)$$

Where: Kc_k is crop coefficient (dimensionless); Kc_A is crop coefficient recommended by Allen et al. (1998) (dimensionless); u_2 is daily average wind speed at 2 m height (m s^{-1}); RH_{min} is minimum daily average relative humidity (%); *h* is average plant height (m).

*Corresponding author. E-mail: brunogurski@ufpr.br.

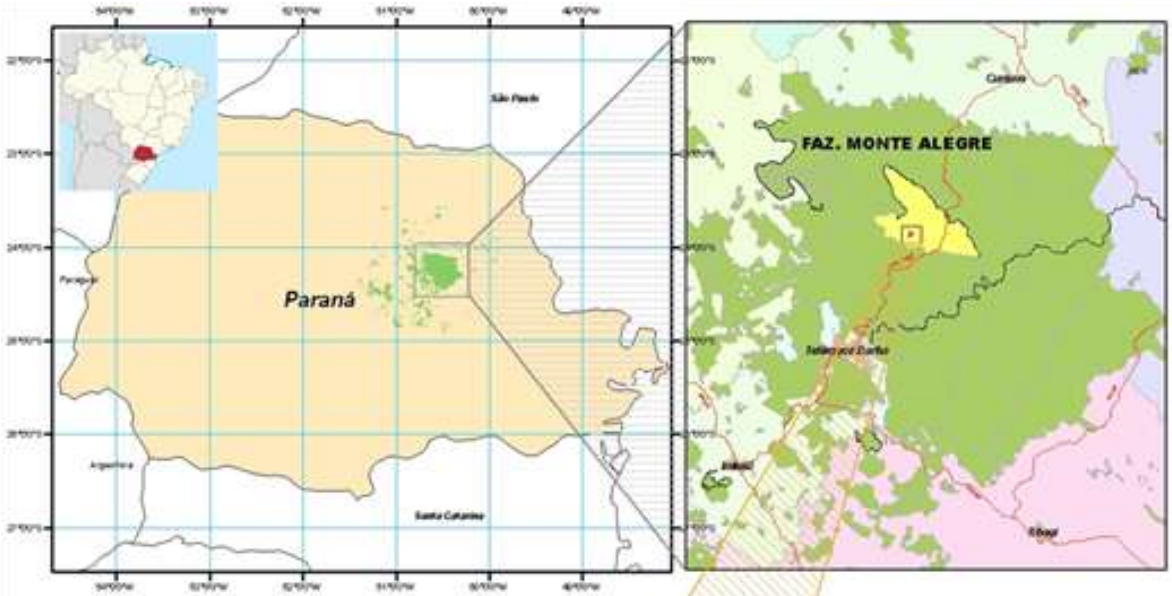

Figure 1. Location of the study area in Southern Brazil.

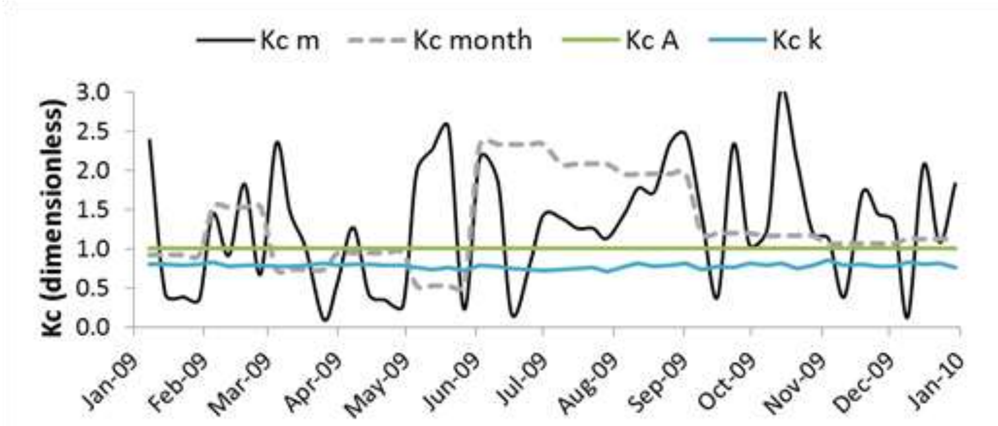

Figure 2. Different crop coefficients (Kc's) used in 2009 for pine, as follows: Kc proposed by Allen et al. (1998) (Kc_A); Kc measured in a soil water balance (Kc_m); Kc measured, but grouped monthly (Kc_{month}); Kc obtained by climatic variables (Kc_k).

Three scenarios of p estimation were tested as follow (Figure 3): Constant over time (p_A), value recommended by Allen et al. (1998); Doorenbos and Kassan (1979) (p_{DK}); and an equation proposed by Allen et al. (1998) (p_{Ai}):

$$p_{DK_i} = 0.0025 \cdot ETc_i^2 - 0.0869 \cdot ETc_i + 1 \text{ for } 0 \le ETc \le 17 \text{ mm day}^{-1} \quad (4)$$

$$p_{Ai} = p_A + 0.04 \cdot (5 - ETc_i) \text{ for } p_A \le 0.8 \quad (5)$$

Where: ETc_i = crop evapotranspiration in the i-th day (mm day^{-1}); p_A = *soil* water depletion fraction recommended by Allen et al. (1998) (dimensionless).

The output components were calculated daily in the CWB (ΔS_{CWB} and ETa_{CWB}), and then grouped in weeks and months for comparison with the FWB (ΔS_{FWB} and ETa_{FWB}). The evaluation was performed using the coefficient of determination (R^2), index "d" Willmott et al. (1985), index "c" of Camargo and Sentelhas (1997): c > 0.85 = great accuracy; c from 0.85 to 0.76 = very good; c from 0.75 to 0.66 = good; c from 0.65 to 0.61 = average; c from 0.60 to 0.51 = tolerable; c from 0.50 to 0.41 = bad; and c ≤ 0.40 = very bad. Mean error (ME) were also used; mean absolute error (MAE) and root mean square error (RMSE). It is important to note that the comparisons were made with the ΔS instead S, because the differences between methodology of FWB and CWB, and the ΔS_{CWB} and ETa_{CWB} results were demonstrated in mm $month^{-1}$ and mm day^{-1}, respectively, to facilitate discussion and comparison to

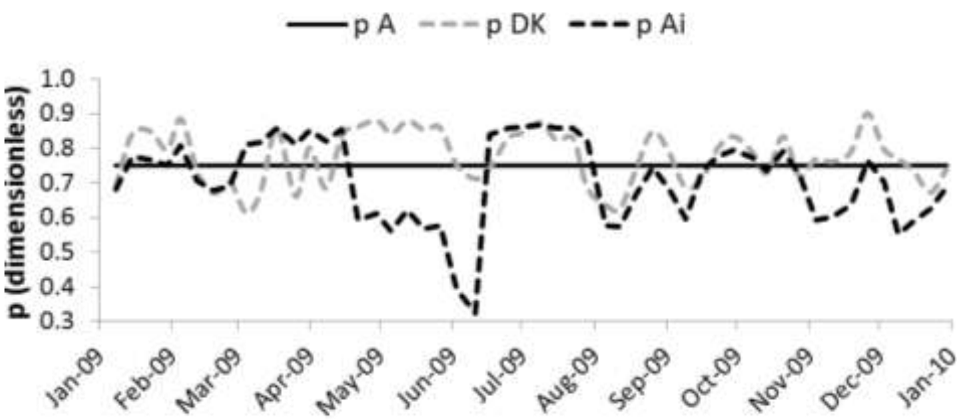

Figure 3. Different soil water depletion fraction used in 2009 for pine, as follows: Constant over time (p_A); value recommended by Doorenbos and Kassan (1979) (p_{DK}); and depending on the daily crop evapotranspiration (p_{Ai}).

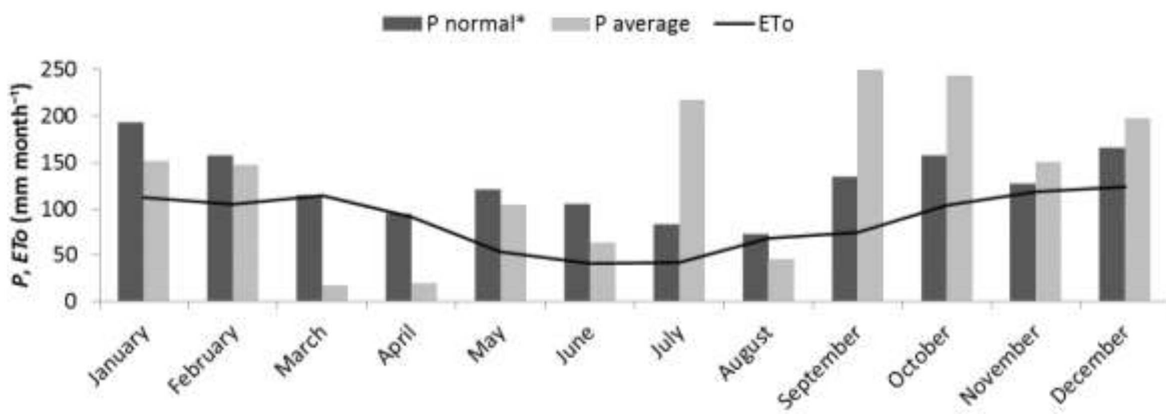

Figure 4. Monthly normal average precipitation (P_{normal}), precipitation ($P_{average}$) and reference evapotranspiration (ETo), in Telêmaco Borba, Southern Brazil, in 2009. *Standard climatological series observed between 1947 and 2005 for Telêmaco Borba, Southern Brazil.

the literature:

$$ME = \frac{1}{n} \cdot \sum_{i=1}^{n} (E_i - O_i) \quad (6)$$

$$MAE = \frac{1}{n} \cdot \sum_{i=1}^{n} (\mid E_i - O_i \mid) \quad (7)$$

$$RMSE = \sqrt{\frac{1}{n} \cdot \sum_{i=1}^{n} (E_i - O_i)^2} \quad (8)$$

Where: *ME* = mean error; *MAE* = mean absolut error; *RMSE* = root mean square error; *n* = number of observations (dimensionless); E_i = estimated value in the i-th day; O_i = observed value in the i-th day.

RESULTS

As expected, the ETo in 2009 showed typical trend throughout the year, with the lowest and highest values in winter and summer, respectively. Despite, $P_{average}$ was atypical regarding P_{normal} of Telêmaco Borba region (Figure 4).

The annual $P_{average}$ was higher than P_{normal} presenting a total value of 1,608.1 and 1,490.0 mm, respectively, with poor distribution of precipitation throughout 2009 and significant accumulation from September to December. Historically characterized as a month of low precipitation, July presented mean $P_{average}$ 38% higher than P_{normal}. It is important to note that 2009 may be considered as an

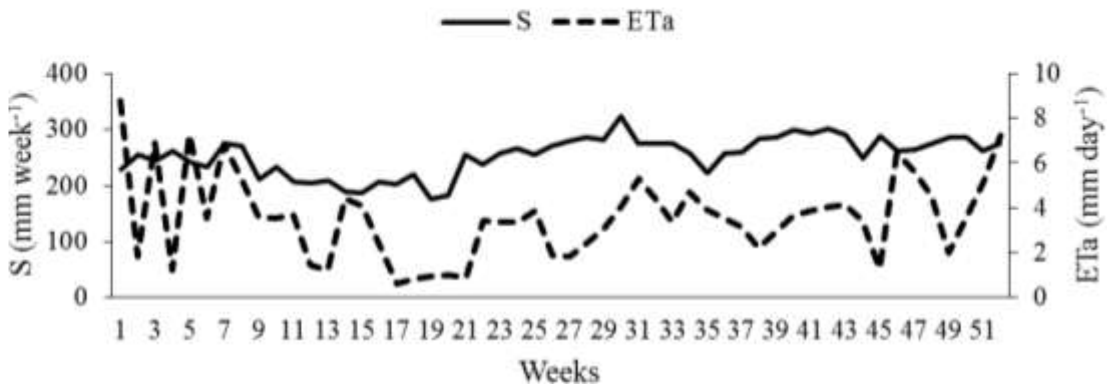

Figure 5. Soi water storage (S) and actual evapotranspiration (ETa) in the field water balance for pine, in Telêmaco Borba, in 2009.

atypical year, especially in relation to observed precipitation (Figure 4), in which there has been much lower values (March and April) or higher values (July, September and October) in relation to P_{normal}. The major occurrence of inaccuracies was related to the atypical series of $P_{average}$, when the precipitation was much higher or lower than the historical data.

There were larger differences in ΔS_{CWB} compared to ΔS_{FWB} when the mean $P_{average}$ was higher, and ETa_{CWB} in relation to ETa_{FWB} when $P_{average}$ was lower (Figure 5). The poorly adjustment of the ΔS_{CWB} occurred in July and September, when $P_{average}$ overcame P_{normal} by 38 and 52%, respectively. However, there were also minor differences when the situation was the opposite.

Regarding the ETa_{CWB} the greatest errors occurred when the mean $P_{average}$ was below P_{normal}, especially in March and April. The ETa_{CWB} had its highest values in the same periods when the largest precipitation occurred (Figures 4 and 5). This relation is similar to obtained by Silva et al. (2009) with corn in Piracicaba, Southestern Brazil.

There was no significant improvement, in both the S and ETa, with the daily variation of p, both grouping weeks as in months. The use of the proposed equations does not represent any gain in the CWB, in comparison with the use of a constant p value over time (Tables 1 and 2).

The use of Kc_m represented the highest degree of accuracy and precision, and minor errors, both to S and ETa,, independent of the comparison scale. On the other hand, Kc_k had the worst levels of accuracy and precision, and the biggest mistakes in all analyze and all tested variables. The use of the equation proposed to daily estimate Kc was inadequate and did not contribute to the CWB, on the contrary, because the equation showed worse results than even Kc_A, which is constant over time.

The estimate of the CWB and its subsequent grouping in months for comparison provided greater degree of accuracy and precision for the variables analyzed, but caused the biggest mistakes.

DISCUSSION

There is definitely influence of precipitation on S and ETa. Zhang et al. (2004) report that the *S* directly influences *ETa*, in the extent that the soil water deficits reduces the ETa. On the other hand, Praveena et al. (2012) found that the excess water leads to increase in *ETa.* Farré and Faci (2006) found that the factors that most influence ETa are the S and P. The reason is due to a higher evaporation in the surface layers up to 0.40 m deep (Cruz et al., 2005). When ETa_{CWB} was very low (March, April and May) there was small P, and low variations within the months came from deeper layers (0.60 and 0.80 m), which have a higher water retention capacity, contributing to the root water uptake (Souza et al., 2013).

According to Souza et al. (2013) when long periods without precipitation occurs, there is the process of soil water drying, with variation of the humidity, especially within the first 0.20 m deep. In this condition, a large evaporative demand by atmosphere cannot be attended by soil, because the amount of water available on the surface is restricted, and the water conductivity begins to influence evaporation. At this stage, the evaporation rate is controlled by the vapor transfer mechanisms and adsorption on the soil solid matrix.

Many authors attested the influence of p in crop productivity (Doorenbos and Kassan, 1979; Tao et al., 2003; Steduto et al., 2009), however, there was no improvement in the adjustment of the component values of the CWB to the FWB, even varying p daily (p_{DK} and p_{Ai}) in relation to the constant value (p_A) over time. It may be that $P_{average}$ allowed high S throughout the year. As a consequence, the soil was constantly in the wet zone (in other words, when $S \geq TAW (1 - p)$), and ETa and crop evapotranspiration (ETc) have been showed almost the same values under this condition.

Bruno et al. (2007) using a Kc obtained by lisymeter and then grouped in four phenological phases, to estimate the CWB for coffee in Piracicaba,

Table 1. Comparison of soil-water storage variation (ΔS) obtained in a field (FWB) and climatological (CWB) water balances, grouped weekly and monthly, with different crop coefficients (Kc) and soil water depletion fraction (p) for pine in Telêmaco Borba, in 2009.

Analyses	Weekly			Monthly		
Kc; p	Kc_k; p_{DK}	Kc_k; p_{Ai}	Kc_k; p_A	Kc_k; p_{DK}	Kc_k; p_{Ai}	Kc_k; p_A
R^2	0.35	0.35	0.36	0.21	0.21	0.21
"*d*"*	0.74	0.74	0.74	0.65	0.65	0.65
"*c*"*	0.44	0.44	0.44	0.30	0.30	0.30
Performance	Bad	Bad	Bad	Terrible	Terrible	Terrible
ME*	1.17	1.17	1.17	10.12	10.12	10.12
MAE*	16.35	16.35	16.06	37.23	37.26	37.91
RMSE*	20.00	20.00	19.84	47.72	47.72	47.70
Kc; p	Kc_{month}; p_{DK}	Kc_{month}; p_{Ai}	Kc_{month}; p_A	Kc_{month}; p_{DK}	Kc_{month}; p_{Ai}	Kc_{month}; p_A
R^2	0.37	0.37	0.36	0.89	0.89	0.90
"*d*"	0.77	0.77	0.76	0.94	0.94	0.95
"*c*"	0.47	0.47	0.46	0.89	0.89	0.90
Performance	Bad	Bad	Bad	Great	Great	Great
ME	1.39	1.42	1.42	9.84	9.84	9.84
MAE	18.06	17.98	18.07	20.79	19.83	19.07
RMSE	4.21	4.20	4.21	26.96	26.97	24.93
Kc; p	Kc_m; p_{DK}	Kc_m; p_{Ai}	Kc_m; p_A	Kc_m; p_{DK}	Kc_m; p_{Ai}	Kc_m; p_A
R^2	0.33	0.34	0.34	0.85	0.85	0.84
"*d*"	0.74	0.75	0.75	0.93	0.93	0.93
"*c*"	0.43	0.44	0.44	0.86	0.86	0.86
Performance	Bad	Bad	Bad	Great	Great	Great
ME	1.37	1.37	1.37	10.12	10.12	10.12
MAE	18.04	17.56	17.26	21.03	21.68	21.96
RMSE	0.30	0.34	0.34	27.44	28.31	27.40
Kc; p			Kc_A; p_A			Kc_A; p_A
R^2	-	-	0.38	-	-	0.33
"*d*"	-	-	0.76	-	-	0.73
"*c*"	-	-	0.47	-	-	0.42
Performance	-	-	Bad	-	-	Bad
ME	-	-	1.27	-	-	10.08
MAE	-	-	16.43	-	-	32.59
RMSE	-	-	0.38	-	-	42.90

*"d", Index of Willmott et al. (1985); "c", index of Camargo and Sentelhas (1997); ME, mean error; MAE, mean absolut error; RMSE, root mean square error.

Southeastern Brazil, with 14-days intervals, obtained, on average, R^2 = 0.75 for ΔS_{CWB} and R^2 = 0.84 for ETa_{CWB}. For pine, we found for Kc_m and Kc_{month}, which were the best in accuracy, R^2 = 0.85 and 0.89 for ΔS_{CWB}, and R^2 = 0.76 and 0.64 for ETa_{CWB}, respectively, when monthly compared. These results demonstrate that the use of a variable Kc, unlike Kc_A, significantly improves the ETa, including the pine, but because it is a perennial crop, it did not show significant difference between Kc_m and Kc_{month} for ΔS_{CWB}. It may be that in plants with smaller cycles and higher phenological changes, this difference existed for ΔS.

Probably, the ΔS_{CWB} have been influenced by the litter of Pinus taeda, due to its low density and high potential for water retention. The litter forms a layer of dissipative energy, reducing evaporation losses from soil to the atmosphere, but has the disadvantage of intercepting and storing water from precipitation, which is subsequently lost to the atmosphere before infiltrates in the soil profile. According to Silva et al. (2006), the evaporated water in the soil-plant system correlates significantly with water initially stored in the litter. The authors found that 1,000; 4,000 and 8,000 kg/ha of corn straw with 412, 255 and 260% humidity in relation to its volume, respectively, have lost large amounts of stored water, reaching 0, 41 and 53%, respectively. Water storage in the litter is another source of error in the CWB, because all the water from precipitation (less interception) was considered as

Table 2. Comparison of actual evapotranspiration (ETa) obtained in a field (FWB) and climatological (CWB) water balances, grouped weekly and monthly, with different crop coefficients (Kc) and soil water depletion fraction (p) for pine in Telêmaco Borba, in 2009.

Analyses	Weekly			Monthly		
Kc; p	Kc_k; p_{DK}	Kc_k; p_{Ai}	Kc_k; p_A	Kc_k; p_{DK}	Kc_k; p_{Ai}	Kc_k; p_A
R^2	0.09	0.09	0.11	0.28	0.29	0.32
"*d*"*	0.48	0.48	0.50	0.56	0.56	0.57
"*c*"*	0.14	0.14	0.16	0.30	0.30	0.33
Performance	Very Bad	Very Bad	Very Bad	Terrible	Terrible	Terrible
ME*	-1.25	-1.25	-1.27	−1.19	−1.20	−1.22
MAE*	1.77	1.77	1.75	1.32	1.32	1.32
RMSE*	2.08	2.08	2.07	1.55	1.55	1.55
Kc; p	Kc_{month}; p_{DK}	Kc_{month}; p_{Ai}	Kc_{month}; p_A	Kc_{month}; p_{DK}	Kc_{month}; p_{Ai}	Kc_{month}; p_A
R^2	0.29	0.29	0.28	0.65	0.64	0.65
"*d*"	0.72	0.72	0.72	0.89	0.88	0.88
"*c*"	0.39	0.39	0.38	0.72	0.71	0.71
Performance	Very Bad	Very Bad	Very Bad	Good	Good	Good
ME	-0.12	-0.10	-0.13	−0.10	−0.09	−0.11
MAE	1.23	1.25	1.25	0.62	0.64	0.66
RMSE	1.10	1.11	1.11	0.76	0.81	0.84
Kc; p	Kc_m; p_{DK}	Kc_m; p_{Ai}	Kc_m; p_A	Kc_m; p_{DK}	Kc_m; p_{Ai}	Kc_m; p_A
R^2	0.36	0.39	0.40	0.76	0.77	0.73
"*d*"	0.78	0.80	0.80	0.93	0.93	0.91
"*c*"	0.47	0.50	0.50	0.81	0.82	0.78
Performance	Bad	Bad	Tolerable	Very good	Very good	Very good
ME	-0.17	-0.14	-0.16	−0.1557	−0.13	−0.15
MAE	1.01	1.01	1.10	0.50	0.50	0.57
RMSE	0.30	0.30	0.30	0.61	0.63	0.75
Kc; p			Kc_A; p_A			Kc_A; p_A
R^2	-	-	0.19	-	-	0.43
"*d*"	-	-	0.57	-	-	0.69
"*c*"	-	-	0.25	-	-	0.45
Performance	-	-	Very Bad	-	-	Bad
ME	-	-	-0.91	-	-	−0.86
MAE	-	-	1.50	-	-	1.03
RMSE	-	-	1.22	-	-	1.25

*"d", Index of Willmott et al. (1985); "c", index of Camargo and Sentelhas (1997); ME, mean error; MAE, mean absolut error; RMSE, root mean square error.

input in the system. The same does not occur in accounting for FWB. In addition, other factors are influenced by litter, such as entering solar radiation in the system, temperature, runoff, ETc, among others.

The precision and accuracy increased, when comparing values grouped together, because the average decreases the variability in the data. However, this analysis is performed to identify trends or cyclicity in series data, and not to statistical inference (Morettin and Toloi, 1981). So that when the data were grouped monthly to compare this generated higher R^2, "d" and "c", but also the biggest mistakes.

Overall, the CWB and FWB are subject to other sources of error, such as the frequency of calculations that always influences the results (Bruno et al., 2007). In FWB performed, the frequency was weekly, that is, data relating to the sum or mean of values obtained along the week, and it is not possible to determine exactly the time of data sampling. In the calculation of both CWB and FWB some simplifications were needed. It was considered a homogeneous experimental area, without input or output of water from the system via surface and subsurface drainage. However, it is known that there is spatial variability of soil physical parameters (Yan et al., 2012).

Conclusions

The use of a crop coefficient (Kc) measured weekly improved the climatological water balance (CWB), providing high correlation and small errors in relation to a measured water balance, independent of the comparison scale. On the other hand, the use of a Kc that considers climate variables (Kc_k) had the worst levels of accuracy and precision, and the biggest mistakes in all analyzes and all tested variables.

There was no significant improvement with the daily variation of soil-water depletion fraction (p), both grouping weeks as in months. The proposed equations do not represent any gain in the CWB, in comparison with the use of a constant p value over time.

The estimate of the CWB and its subsequent grouping in months for comparison provided greater degree of accuracy and precision for the variables analyzed, but caused the biggest mistakes. Therefore, the calculation of CWB should be performed with the highest periodicity possible, and grouping the CWB output variables should only be performed for comparison.

Conflicts of interests

The authors have not declared any conflict of interests.

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Technical efficiency among irrigated and non-irrigated olive orchards in Tunisia

Hajime Kamiyama[1]*, Kenichi Kashiwagi[2] and Mohamed Kefi[2]

[1]Alliance for Research on North Africa (ARENA), University of Tsukuba, 1-1-1, Tennodai, Tsukuba, Ibaraki 305-8572, Japan.
[2]Laboratory of Natural Water Treatment, Water Research and Technologies Centre of Borj-Cedria (CERTE), Tunisia.

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Tunisia olive production fluctuates yearly because it is highly dependent on annual precipitation, and growers need to enhance productivity and efficiency by introducing irrigation. Investigating how irrigation affects the technical efficiency of olive production may contribute to improvement in productivity. This study employs the Data Envelopment Analysis (DEA) and Stochastic Frontier Analysis (SFA) methods to estimate non-parametric and parametric frontiers for a sample of Tunisian olive orchards. It identifies factors which determine variations in technical and scale efficiencies among orchards. The DEA results show that average output-oriented technical efficiency under constant returns to scale (CRS) and variable returns to scale (VRS) is 8.9 and 17.8%, respectively. The SFA results show that average technical efficiency of the half-normal model with constant returns to scale is estimated at 81.2%, indicating Tunisian olive growers can raise output by an average of 18.8% by improving technology and using fewer inputs. Average technical efficiency in irrigated orchards under the DEA approach was higher than in irrigated ones while irrigated orchards under the SFA approach was less technically efficient than non-irrigated ones. However, the test results of mean difference indicate that average VRS technical and scale efficiencies in irrigated orchards under the DEA approach were not significantly higher than in non-irrigated ones. On the other hand, technical rather than scale inefficiency is the major source of overall inefficiency in irrigated orchards because room for improvement in technical efficiency was larger than in scale efficiency. These results suggest that Tunisian olive growers should raise output and efficiency by introducing more advanced technologies for improving the performance of irrigation systems.

Key words: Olive orchards, technical efficiency, scale efficiency, irrigation, Tunisia.

INTRODUCTION

In Tunisia olives are vital to the domestic economy and financial resources, especially in impoverished rural areas, accounting for 15.7% of Tunisia's agricultural production in 2013 (NIS, 2016). Also, promoting exports

*Corresponding author. E-mail: kamiyama.hajime.gn@u.tsukuba.ac.jp. Tel: +81298533992.

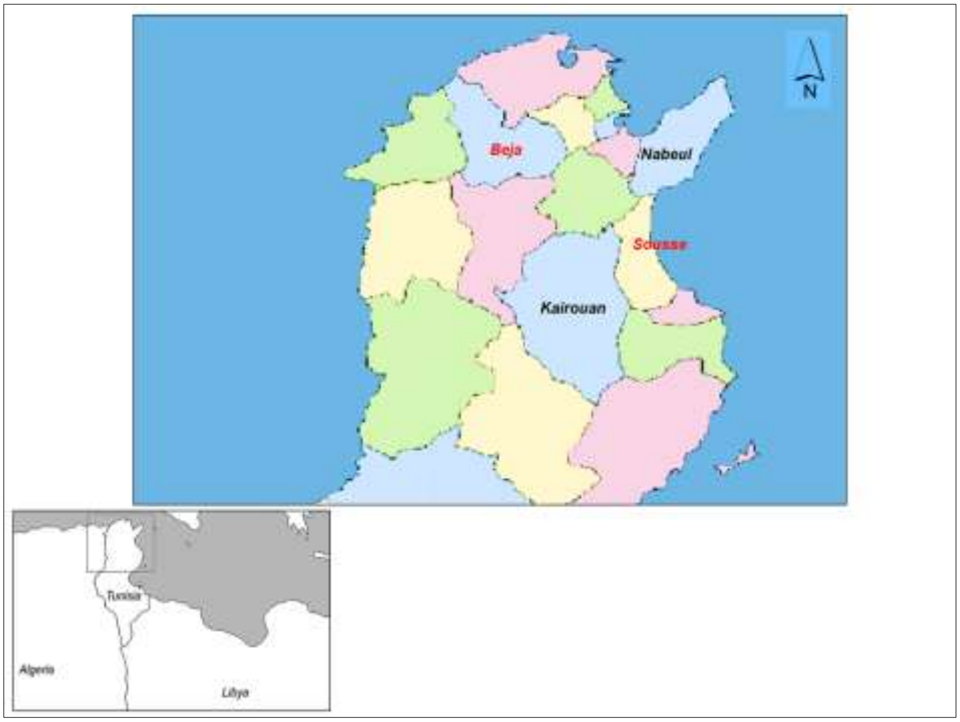

Figure 1. Map of study areas.

of olive oil is central to Tunisia's national development strategy following the Euro-Mediterranean Partnership eliminated export quotas, tariffs and trade barriers on agricultural commodities. According to Food and Agriculture Organization of the United Nations (FAO) statistics, the export of olive oil occupies 19.7% of the country's agricultural exports in 2013. Therefore, enhancing productivity and technical efficiency has become a primary challenge for Tunisia's olive growers. Tunisian olive production fluctuates yearly because it is highly dependent on annual precipitation. To stabilize and increase production, the Tunisian government encourages olive growers to introduce irrigation and to increase the proportion of their orchards under irrigation. Given the country's limited water resources, investigating how irrigation affects the technical efficiency of olive production may contribute to improvement in productivity.

Limited studies have investigated productivity in olive-growing farms. Tzouvelekas et al. (1999) and Tzouvelekas et al. (2001) estimated technical efficiency among Greek olive growers, and Artukoglu et al. (2010) carried out the same for Turkey. Lachaal et al. (2005), Chemak (2012) and Kashiwagi et al. (2013) analysed the productivity and technical efficiency of Tunisian olive orchards. Most of the previous studies have investigated technical efficiency, while the effects of unconventional inputs (e.g., irrigation) and conventional ones (e.g., land, labour and capital) as factors to improve technical efficiency have not been well examined in the previous studies.

The objective of this study is to investigate how the introduction of irrigation affects the productivity and technical and scale efficiencies of Tunisian olive production by estimating non-parametric and parametric frontiers for a sample of olive orchards, located in four governorates in Tunisia; to quantify the technical efficiency of irrigated and non-irrigated orchards using Data Envelopment Analysis (DEA) and Stochastic Frontier Analysis (SFA) methods; to identify factors which determine variations in technical and scale efficiencies among orchards; and to compare the results from the DEA and SFA approaches to estimate non-parametric and parametric frontiers for a sample of Tunisian olive orchards. Our study contributes to the literature on the productivity and technical efficiency of olive orchards in Tunisia, the effect of irrigation on productivity and production efficiency and the methods to estimate production function frontiers for olive orchards.

MATERIALS AND METHODS

Study area and sample size

Our survey was conducted in Beja, Nabeul, Sousse and Kairouan Governorates during the period from March to December 2011 (Figure 1). Beja Governorate in North-western Tunisia covers 3,740 km^2 and has a population of 303,032. Nabeul Governorate in north-eastern Tunisia covers 2,788 km^2 and has a population of 787,920. Sousse Governorate in eastern Tunisia is 2,669 km^2 with a

Table 1. Frequency distribution by governorate and by hectares under olive cultivation.

Governorates	Number of orchards (Total: 118)			
	≤ 5 ha	5 ha < area ≤ 10 ha	> 10 ha	Percentage
Sousse	13	10	3	22.0
Nabeul	14	2	1	14.4
Kairouan	13	18	9	33.9
Beja	10	3	22	29.7
Percentage	42.3	28.0	29.7	100

Table 2. Statistics for olive orchards of the study area.

Variable	Mean	Minimum	Maximum	Coefficient of variation
Y: Olive production (TND)	27,887.54	600.0	920.0	3.21
L: Labour costs (TND)	4,476.30	120.0	29,200.0	1.05
A: Total area of production (ha)	15.50	0.4	230.0	1.86
M_i: Intermediate costs (TND)	3,194.13	70.0	133,960.0	4.01
D_I : Dummy of irrigation (1: irrigated, 0: non-irrigated)	0.61	0.0	1.0	0.78
D_S: Regional dummy (1: Sousse governorate, 0: otherwise)	0.22	0.0	1.0	1.88
D_K: Regional dummy (1: Kairouan governorate, 0: otherwise)	0.33	0.0	1.0	1.40
D_B: Regional dummy (1: Beja governorate, 0: otherwise)	0.29	0.0	1.0	1.54
MAC: Stock of capital inputs (TND)	1,945.24	36.0	10,486.9	1.24
AGE: Owner's age (year)	56.77	30.0	87.0	0.22
EDU: Level of education of owner (1: illiterate, 2: qur'anic education, 3: primary, 4: secondary, 5: university)	3.17	1.0	5.0	0.38
EMP: Share of employed labour to total labour (%)	81.05	0.0	100.0	0.33
IRR: Share of irrigated area to total (%)	49.75	0.0	100.0	0.89
D_{OF}: Dummy of off-orchard (1: engage in off- orchard, 0: otherwise)	0.32	0.0	1.0	1.45

population of 674,971. Kairouan Governorate in central Tunisia comprises 6,712 km^2 and 570,559 people. We selected olive orchards randomly directed questionnaires to their growers. Of the 167 responses collected, we have chosen 118 from 17 administrative divisions within four governorates. Table 1 shows the frequency distribution of the 118 orchards by governorate and by hectares under olive cultivation. As indicated in Table 1, 42.3% of orchards (50 orchards) are 5 ha or smaller, 28.0% (33 orchards) are 5 to 10 ha and 29.7% (35 orchards) exceed 10 ha. Summary statistics are presented in Table 2.

We defined one output and three input variables to estimate efficiency scores in the DEA approach and Cobb-Douglas stochastic frontier production in the SFA approach. The output variable (Y_i) is olive production denominated in Tunisian dinars. The three input variables are labour costs in Tunisian dinars (L_i), production area in hectares (A_i) and intermediate costs in Tunisian dinars (M_i). The SFA approach includes four dummy variables in terms of irrigation and region. The irrigation dummy (D_{Ii}) has a value of 1 if the orchard had introduced irrigation and 0 otherwise. The Sousse dummy (D_{Si}), Kairouan dummy and Beja dummy (D_{Bi}) take 1 for orchards located in those respective governorates and 0 otherwise.

Model: DEA approach

The DEA approach measures relative efficiency. Proposed by Farrell (1957) and expanded by others, DEA mathematical programming allows researchers to construct a non-parametric piecewise frontier that includes input and output data. These data indicate which costs are minimized to calculate efficiency scores for each observation. As it is used here, the technique creates a frontier set of efficient olive orchards and compares it with inefficient orchards to produce efficiency indices. Olive orchards are given between 0 and 1, with completely efficient orchards scoring 1.

The DEA approach shows how one decision-making unit (DMU) (an olive orchard) manages relative to others in the sample and provides a benchmark for best practice technology from the experience of the sampled orchards. The DEA can estimate efficiency under the constant returns to scale (CRS) and variable returns to scale (VRS) hypothesis. The CRS assumption is appropriate only if all surveyed olive orchards operate at optimal scale. Obviously, many factors may cause olive orchards to operate below their optimal scale, and the CRS specification obscures the measures of technical and scale efficiencies in those cases. The DEA uses sample data to derive the efficiency frontier against which each orchard is assessed. No explicit functional form for production need to be specified. Instead, the production frontier includes piecewise linear segments that assign efficiency scores for each orchard. Here, an output-oriented model is used because an increase in olive production is the main objective in our study.

The DEA considers the technological phases of production function. This is unitized to estimate the cost and revenue frontiers, convenient for decomposing production efficiency into technical and scale efficiencies without requiring estimates of input and output prices. As to *N* DMUs in the olive-growing industry, all sample inputs and outputs are characterized by K and M, respectively. The efficiency of each orchard is then calculated by

$$e_s = \frac{u' y_{is}}{v' x_{is}} \quad (1)$$

Where x_{is} is the amount of the ith input of the sth orchard; y_{is} is the amount of the ith output of the sth orchard; v' is a vector of input weights [K×1]; and u' is a vector of output weights [M×1]. The resulting efficiency score (e_s, the ratio of all outputs over all inputs) is maximized to select optimal weights subject to:

$$\frac{u' y_{js}}{v' x_{js}} \le 1, \quad \text{for } j = 1,2,\ldots, N; \; u \ge 0; \; v \ge 0 \quad (2)$$

Where the first inequality ensures the efficiency ratios to be at least one and the second inequality requires that the weights are positive.

First, derived efficiency measures are based on maximizing the ratio of all outputs over all inputs. To avoid an infinite number of solutions, the constraint, $u' y_{is} = 1$ is imposed to provide the multiplier form of the output-oriented linear programming problem. And, this program of the output-oriented CRS DEA model can be converted into the dual problem:

Maximize θ_s subject to $Y\lambda \ge \theta_s y_{js}$;

$$x_{js} - X\lambda \ge 0$$

$$\lambda \ge 0 \quad (3)$$

Where θ_s is a scalar of the sth olive orchard, and λ is an N×1 vector of constant; and X is a K×N inputs matrix, and Y is an M×N outputs matrix. The first constraint shows that production of the ith output by observation s cannot exceed any linear combination of output by all orchards in the sample. The second constraint includes the use of inputs by observation s. $1/\theta_s$ is the overall technical efficiency score for the sth orchard that satisfies: $1/\theta_s < 1$, where a value of 1 indicates the point on the frontier. $1/\theta_s = 1$ indicates that the orchard is technically efficient.

Second, when not all orchards operate at an optimal scale, the CRS DEA model is extended to the VRS DEA model. Technical efficiency is given by $1/\phi_s$ for the sth olive orchard. If calculated technical efficiency in the CRS DEA model differs from that in the VRS DEA model, the orchard has scale inefficiency. This means that scale efficiency (σ_s) is calculated by θ_s/ϕ_s. Hence, the use of VRS specification permits calculation of technical efficiency devoid of the scale effect. That is, it decomposes technical efficiency into pure technical efficiency. The CRS linear programing problem can be modified into the VRS linear one by adding the convexity constraint:

Maximize ϕ_s subject to $Y\lambda \ge \phi_s y_{js}$

$$x_{js} - X\lambda \ge 0$$

$$\lambda \ge 0$$

$$\sum_{j=1}^{N} \lambda_j = 1 \quad (4)$$

Where ϕ_s is a scalar of the sth olive orchard. This approach constructs convex hull intersecting planes that envelop data points more tightly than under CRS. For instance, scale efficiency equals 1 if and only if the technology exhibits CRS specification. However, scale inefficiency may exist because of either increasing returns to scale (IRS) or decreasing returns to scale (DRS). In order to obtain these results, the solution to the VRS linear programming problem (4) must be restricted with the sum of the λ from 1 to N.

Data Envelopment Analysis Program (DEAP) Software Version 2.1 is used for measuring the technical and scale efficiencies in the DEA.

Model: SFA approach

A commonly used technique to parametrically estimate production function and to measure firm-level technical efficiency is the stochastic frontier method (Aigner et al., 1977; Meeusen and van den Broeck, 1977). This approach is frequently applied for agricultural farms to estimate inefficiency effects to capture the stochastic nature of agricultural production. A general form of stochastic frontier production function can be expressed as follows:

$$\ln Y_i = F(X_i ; \beta) + v_i - u_i , \quad (5)$$

Where Y_i is the output of the ith orchard; X_i is the vector of input quantities used by ith orchard; β is a vector of unknown parameters to be estimated. The term v_i represents random disturbance terms, assumed to be an independent and identically distributed N (0, σ_v^2). The term u_i is a non-negative variable representing inefficiency in production relative to the stochastic frontier. It is assumed to be independently and identically distributed, that is, $u_i \sim$ iid N (0, σ_u^2), which could be half-normal at 0 mean, truncated half-normal (at mean μ) and exponential (Aigner et al., 1977; Meeusen and van den Broeck, 1977; Stevenson, 1980; Jondrow et al., 1982; Greene, 1990) introduces a gamma distribution model.

For the estimation of stochastic frontier model, we employ Cobb-Douglas form of production function. Following Okikie et al. (2004), the effects of inputs on productivity in different conditions are explicitly incorporated in the production function using fixed-effects method. The binary dummy of irrigation is explicitly inserted in order to capture the impact of irrigation on productivity. Also, three regional dummies are used for controlling the difference in general environmental conditions in each particular country of Tunisia. The stochastic frontier production function is specified:

$$\ln Y_i = \beta_0 + \beta_l \ln L_i + \beta_a \ln A_i + \beta_m \ln M_i + \lambda_I D_{Ii} + \lambda_S D_{Si} + \lambda_K D_{Ki} + \lambda_B D_{Bi} + v_i - u_i \quad (6)$$

Where β_0 is the intercept term; β_i (i = l, a, m) and λ_i (i = I, S, K, B) denote unknown parameters to be estimated corresponding to three intermediate input variables (costs of fertilizer, pesticides, water and transport) and four dummy variables in terms of irrigation and region (Sousse, Kairouan and Beja), respectively.

Following Battese and Coelli (1995), the technical inefficiency effects, u_i could be further expressed as a linear function of explanatory variables, reflecting orchard-specific characteristics. The inefficiency component of the stochastic frontier can be specified:

Table 3. Frequency distribution and scores of efficiencies.

Percentage	p	VRS TE	SE
<10	80	59	3
11-20	31	34	3
21-30	4	8	6
31-40	2	3	7
41-50	0	6	6
51-60	0	0	6
61-70	0	0	7
71-80	0	0	12
81-90	0	0	20
91-100	1	8	48
Mean	0.089	0.178	0.743
Median	0.059	0.100	0.875
Standard deviation	0.109	0.248	0.275
Minimum	0.004	0.004	0.025
Maximum	1.000	1.000	1.000
Orchards	1	8	3
	IRS	**CRS**	**DRS**
Return to scale (Number of orchards)	74	15	29
Mean VRS TE	0.197	0.099	0.180
Mean SE	0.764	1.000	0.554

CRS TE: Constant returns to scale technical efficiency. VRS TE: Variable returns to scale technical efficiency (pure technical efficiency). SE: Scale efficiency. Orchards: Number of perfectly efficient orchards. IRS: Orchards operating under increasing returns to scale. CRS: Orchards operating under constant returns to scale. DRS: Orchards operating under decreasing returns to scale.

$$u_i = \delta_0 + \sum_{h=1}^{6} \delta_h Z_{hi} + w_i, \quad (7)$$

Where δ_0 is the intercept term; δ_h (h = 1,…,6) is a vector of unknown parameters to be estimated. z_{hi} is a vector of explanatory variables associated with technical inefficiency in production. w_i is a random variable. From Equation (7), the inefficiency effects to be estimated are defined follows:

$$u_i = \delta_0 + \delta_1 MAC_i + \delta_2 AGE_i + \delta_3 EDU_i + \delta_4 EMP_i + \delta_5 IRR_i + \delta_6 D_{OFi} + w_i \quad (8)$$

Where MAC_i is the stock of productive capital inputs (including tractors, cultivators and sprayer); AGE_i is the owner's age; EDU_i is the discrete variable that represents the owner's education on a scale of 1 to 5 (1: illiterate, 2: qur'anic education, 3: primary, 4: secondary, 5: university). EMP_i is the share of employed labour to total labour. IRR_i is the share of irrigated area to total (IRR_i has a value of 0 if the orchard had not introduced irrigation). D_{OFi} is the off-orchard dummy, which equals 1 if the orchard engages in non-agricultural activities and 0 otherwise. The parameters of both the stochastic frontier and the inefficiency effects can be estimated consistently by maximum likelihood (ML) procedures.

RESULTS AND DISCUSSION

DEA approach

The CRS and VRS DEA models are estimated using the DEAP for efficiency measurement. Table 3 indicates the estimated frequency distribution and scores of technical and scale efficiencies. The average scores for CRS and VRS technical efficiencies are 0.089 and 0.178, respectively. One orchard was identified as fully technically efficient under CRS specification and eight under VRS specification, respectively. These results mean that olive orchards in Tunisia could increase production on average by 91.1% (0.911 = 1 - 0.089) and 82.2% (0.822 = 1 - 0.178), respectively, to reach full technical efficiency.

The average score of scale efficiency is 0.743. Three orchards were identified as fully scale efficient. Also, number of olive orchards operating under IRS, CRS and DRS as a percentage of the total is 62.7, 12.7 and 24.6%, respectively. This reflects that 87.3% (103 orchards) of the olive orchards in the sample can enhance overall efficiency by improving scale of production. Specifically, the majority of olive orchards can achieve it through increasing scale of production. However, improvement in scale of production under IRS may have less effect on overall efficiency than under

Table 4. Efficiency scores by governorate.

Model	Mean	Minimum	Maximum
CRS TE			
Sousse	0.109	0.008	0.331
Nabeul	0.088	0.043	0.177
Kairouan	0.108	0.004	1.000
Beja	0.053	0.010	0.384
VRS TE			
Sousse	0.335	0.068	1.000
Nabeul	0.315	0.072	1.000
Kairouan	0.168	0.004	1.000
Beja	0.120	0.011	1.000
SE			
Sousse	0.799	0.122	1.000
Nabeul	0.534	0.043	0.989
Kairouan	0.807	0.110	1.000
Beja	0.728	0.025	0.999

Table 5. Efficiencies of irrigated and non-irrigated orchards.

Model	CRS TE	VRS TE	SE	Number of samples
Irrigation	0.108(0.127)	0.207(0.263)	0.762(0.275)	74
Non-irrigation	0.057(0.058)	0.129(0.213)	0.710(0.277)	44
Mean difference	0.051	0.078	0.052	
t-statistics	2.520	1.675	0.990	

Standard deviations are in parentheses.

DRS because the average score of scale efficiency is close to 1 compared with that for orchards operating under DRS. On the other hand, the average score of VRS technical efficiency for orchards operating under CRS is 0.099, which is lower score than that under IRS and DRS. This result shows that even the fully scale-efficient orchards need to enhance overall efficiency by improving their technical efficiency. Consequently, substantial inefficiencies occur in the surveyed area and technical rather than scale inefficiency is the major source of overall inefficiency because room for improvement in technical efficiency is larger than in scale efficiency.

Table 4 shows the efficiency scores by governorate. Olive orchards in Sousse are the highest average technical efficiency scores under VRS specification. On the other hand, olive orchards in Beja are the lowest. Specifically, orchards operating in Beja and Keirouan require to substantially improve their technical efficiency. Also, olive orchards in Kairouan are the highest average scale efficiency scores, while olive orchards in Nabeul are the lowest. One orchard in Sousse and two orchards in Kairouan are operating at optimal scale.

Most noteworthy about the average score for VRS technical efficiency is that orchards in the surveyed areas are producing only 17.8% of the maximum output levels for the existing inputs. This result shows that substantial improvement is needed to increase technical efficiency.

Table 5 includes the effect of irrigation systems on average efficiency scores and test of mean differences in the average level of efficiency. In the irrigated orchards of each governorate, average technical efficiency scores under CRS and VRS specifications were 10.8 and 20.7%, respectively. These empirical findings reveal that average efficiency in irrigated orchards exceeds that in non-irrigated ones. Also, most of the fully technically efficient orchards under CRS and VRS specifications and scale-efficient orchards had introduced irrigation. Three orchards are identified as fully scale-efficient. The statistical tests confirm that mean difference of efficiencies between irrigated and non-irrigated orchards is statistically significant for only the average score for CRS technical efficiency, while there is no significant difference in the average level of VRS technical and scale efficiencies. These results imply that irrigated orchards are not necessarily more technically and scale efficient than non-irrigated ones.

We assess effects on technical and scale inefficiencies

Table 6. Tobit regressions.

Variable	CRS TE	VRS TE
Intercept	1.016***(0.072)	0.476***(0.169)
MAC	0.000(0.000)	0.000(0.000)
AGE	-0.001*(0.000)	0.002(0.001)
EDU	-0.006(0.009)	-0.005(0.021)
EMP	0.030(0.040)	0.288***(0.094)
IRR	-0.058**(0.023)	-0.108**(0.054)
D_{OF}	0.015(0.021)	0.123**(0.049)
Number of samples	118	118
Sigma	0.103	0.240
Log-likelihood	96.506	-12.535
BIC	-154.847	63.236

*, **, and ***Significance at the 10, 5 and 1% levels, respectively. Standard errors are in parentheses.

using Tobit regression. As technical and scale efficiencies have the propensity to be censored at unity, we employ a standard Tobit model with upper censoring. Consequently, no inefficiency scores were less than 0. We estimate parameters in Tobit regressions using ML procedures. The technical and scale inefficiencies effectsto be estimated are defined as follows:

$$ineff = \beta_0 + \beta_1 MAC_i + \beta_2 AGE_i + \beta_3 EDU_i + \beta_4 EMP + \beta_5 IRR_i + \beta_6 D_{OFi} + w \quad (9)$$

Where β_0 is the intercept term; β_i (i = 1,2…,6) is an unknown parameter to be estimated; and w represents the error term. The technical and scale inefficiencies effect is estimated using STATA 11 package. The results are indicated in Table 6.

Under CRS specification, only the estimated coefficients of *AGE* and *IRR* significantly affect technical efficiency. The estimated coefficient of *AGE* is negative and statistically significant at the 1% level: Older farmers are more efficient than younger ones. The estimated coefficient of *IRR* is negative and significant at the 5% level, suggesting that growers enjoy greater technical efficiencies as hectares under irrigation increase.

On the other hand, technical efficiency under VRS specification is significantly affected by the estimated coefficients of *EMP*, *IRR* and D_{OF}. The estimated coefficient of *EMP* is positive and significant at the 1% level: the larger the share of employed labour in total labour, the less technically efficient is the orchards. This result implies that increasing the proportion of family members among total labour can boost technical efficiency. Our result disagrees with finding by Tzouvelekas et al. (2001), but, as noted by those authors, an agency problem may exist due to informational asymmetry between the parties. The estimated coefficient of *IRR* is negative and significant at the 5% level. The estimated coefficient of D_{OF} is positive and significant at the 5% level: involvement in non-agricultural activities reduces technical efficiency.

SFA approach

Maximum likelihood estimates of parameters in the model of Cobb-Douglas stochastic frontier production are obtained using STATA 11 package. Table 7 displays parameter estimates and standard errors of the estimators. Although two models are estimated corresponding to the distributional assumptions of half normal and exponential for the one-sided error term (u_i), a model estimated with the assumption of truncated normal distribution for the one-sided term could not achieve convergence; therefore, it is not reported. The information criteria are used for choosing the two models because the half normal distribution is not nested in the exponential distribution for the one-sided error term. Since the value of the criterion of the half normal model is marginally less than of exponential model, the Bayesian information criterion (BIC) favours half normal model. On the other hand, the constant returns to scale (CRS) hypothesis cannot be rejected by the F-test. Alternatively, the SFA model under CRS specification is estimated, assuming the half normal distribution of the one-sided error term. Since models estimated with the assumption of exponential and truncated normal distribution for the one-sided error term could not achieve convergence, both models are not reported. Therefore, results of the SFA model under variable returns to scale (VRS) and CRS specifications, assuming half normal distribution for the one-sided error term, are worth discussing.

The results of model under CRS and VRS

Table 7. Parameter estimates of stochastic frontier and inefficiency effects models.

Variable	CRS specification		VRS specification			
	Normal-half normal		Normal-half normal		Normal-exponential	
	Coefficient		Coefficient		Coefficient	
Stochastic frontier model						
Intercept	4.406(0.849)	***	5.399(0.964)	***	5.084(0.955)	***
ln*L*	0.383(0.124)	***	0.1836(0.131)		0.209(0.130)	
ln*A*			0.625(0.167)	***	0.621(0.168)	***
ln*M*	0.176(0.087)	**	0.273(0.094)	***	0.269(0.094)	***
Implicit ln*A*	0.441					
D_I	0.507(0.248)	**	0.766(0.288)	***	0.721(0.282)	**
D_S	-0.355(0.260)		-0.291(0.309)		-0.267(0.312)	
D_K	-0.111(0.306)		-0.254(0.382)		-0.267(0.372)	
D_B	-1.013(0.246)	***	-1.303(0.348)	***	-1.312(0.351)	***
Inefficiency effects model						
Intercept	12.316(5.771)	**	3.043(1.722)	*	3.174(2.442)	
MAC	-0.00004(0.0002)		0.0002(0.0001)	*	0.0002(0.0002)	*
AGE	-0.224(0.095)	**	-0.519(0.022)	**	-0.072(0.032)	**
EDU	-1.334(0.562)	**	-0.458(0.222)	**	-0.631(0.324)	*
EMP	-0.856(1.932)		-0.352(0.985)		-0.320(1.455)	
IRR	0.808(1.297)		1.419(0.723)	*	1.787(0.987)	*
D_{OF}	2.730(1.355)	**	0.493(0.483)		0.642(0.676)	
Variance parameters						
$\ln\sigma_v^2$	(0.146)	***	-0.852(0.288)	***	-0.790(0.249)	***
Log-likelihood	-135.051		-149.581		-149.625	
No. of observations	118		118		118	
BIC	341.661		375.493		375.582	

*, **, ***Significance at the 10, 5 and 1% levels, respectively. Standard errors are in parentheses.

specifications show signs of estimated parameters are as expected. Estimated coefficients for intermediate inputs are positive and statistically significant in both models. The coefficient of labour is significant at model under CRS specification. In both models, land remains most important input in olive production. This significance of land input is similar to the results of Tzouvelekas et al. (1999), Tzouvelekas et al. (2001) and Karaginnis and Tzouvelekas (2001) for Greek olive orchards. The estimate of the irrigation dummy is positive and statistically significant at 1% level in both models. This result suggests irrigation significantly increases production. Also, this result is consistent with that of Lambarraa et al. (2007), which found a positive impact of irrigation in deterministic part of the production function for olive orchards in Spain. In this study, signs for regional dummies suggest that production is low in Beja compared with production in Nabeul.

In the inefficiency effects model, the estimated coefficients of *AGE* and *EDU* are negative and statistically significant in both models. These results suggest older and better-educated growers are more technically efficient producers, and low education restricts development of the sector. The positive effect of increasing age and education on technical efficiency is also confirmed by Karagiannis and Tzouvelekas (2001) for Greek olive orchards and by Lambarra et al. (2007) for Spanish olive orchards. The estimated coefficient for the stock of capital inputs (*MAC*) is positive and statistically significant in the model with VRS specification, while that of the model under CRS specification is not significant. These results suggest that mechanization is not a significant factor for upgrading efficiency. This result is not surprising and is similar to that of Tzouvelekas et al. (2001) for Greek olive orchards. The positive coefficient for the off-orchard dummy (D_{OF}) in the model under CRS specification indicates that involvement in non-agricultural activities impairs technical efficiency. This result is consistent with Ali and Flinn (1987) which suggested off-orchard employment compete with on-orchard work.

Regarding the effect of the share of irrigated area (*IRR*) on efficiency, the estimated coefficient is positive and statistically significant at models under VRS specification but it is not significant at model under CRS specification. At least, these results imply that efficiency does not

Table 8. Frequency distribution and scores of technical efficiency.

Technical efficiency (TE)	CRS TE	VRS TE
TE < 0.2	1(0.8)	11(9.3)
0.2 < TE < 0.4	8(6.8)	16(13.6)
0.4 < TE < 0.6	10(8.5)	41(34.7)
0.6 < TE < 0.8	20(16.9)	47(39.8)
TE > 0.8	79(66.9)	3(2.5)
Mean	0.812	0.534
Median	0.914	0.570
Standard deviation	0.217	0.200
Minimum	0.138	0.051
Maximum	0.999	0.868

Table 9. Technical efficiency scores by governorate.

Governorates	Mean	Minimum	Maximum
CRS TE			
Sousse	0.806	0.201	0.990
Nabeul	0.843	0.342	0.997
Kairouan	0.719	0.138	0.973
Beja	0.909	0.353	0.999
VRS TE			
Sousse	0.513	0.066	0.809
Nabeul	0.569	0.150	0.794
Kairouan	0.455	0.051	0.862
Beja	0.624	0.138	0.868

improve as the area under irrigation increases. This result is different from the finding by Lachaal et al. (2004), but alongside the positive sign for the irrigation dummy implies that irrigation contributes significantly to higher production. Its effect is significant for growth and olive production, but irrigation should be scheduled during suitable periods (Ahmed et al., 2007). However, we cannot confirm the positive effect of irrigation on technical efficiency and inefficiencies remain among some irrigated orchards. In addition, within the context of introducing technological innovations and efficient use of inputs, Tzouvelekas et al. (1999) point out that increased machinery inputs somewhat affect olive production in Greece if and only if they increased land productivity (that is, mechanised irrigation). Also, as shown by Chebil et al. (2014), the irrigation water use in Tunisian wheat farms is inefficient and the substantial decrease in water use could be attained by using the existing irrigation technology. Results for stock of capital inputs (*MAC*) and share of irrigated area (*IRR*) imply a need to introduce the modern irrigation technology that will raise land productivity. In fact, the use of innovative systems such as drip irrigation can increase yields and consequently incomes (Cetin et al., 2004).

The estimated frequency distribution of technical efficiency appears in Table 8. The average level of technical efficiency is 0.534 and 0.812 for models under VRS and CRS specifications, respectively. In the model under CRS, the level of technical efficiency ranges from a minimum of 0.138 to a maximum of 0.999. This estimated result of efficiency score is similar to other studies for Tunisian olive orchards. The average level of technical efficiency was estimated at 0.835 in Mehdia region (Lachaal et al., 2004), and samples from Sfax region was 0.82 (Lachaal et al., 2005). The estimated results of our study are based on the sample from Sousse, Nabeul, Kairouan and Beja. It suggests that the orchards can increase their production by 18.8% on average, given present state of technology and input levels.

Table 9 presents frequency distribution of efficiency scores by region. Both models under VRS and CRS specifications suggest that the average level of estimated technical efficiency is the highest at Beja, while it is the lowest at Kairouan. In Table 10, results of comparison of efficiency scores between irrigated and non-irrigated orchards and tests of mean differences in the average level of efficiency are presented. The statistical tests suggest that mean difference of technical efficiency between irrigated and non-irrigated orchards is statistically significant for both half normal and exponential models. These results imply that irrigated orchards are less efficient than non-irrigated ones. These

Table 10. Technical efficiency of irrigated and non-irrigated orchards.

Parameter	CRS TE	VRS TE	Number of samples
Irrigation	0.761(0.243)	0.473(0.204)	74
Non-irrigation	0.899(0.127)	0.637(0.146)	44
Mean difference	0.138	0.164	
t-statistics	3.490	4.666	

Standard deviations are in parentheses.

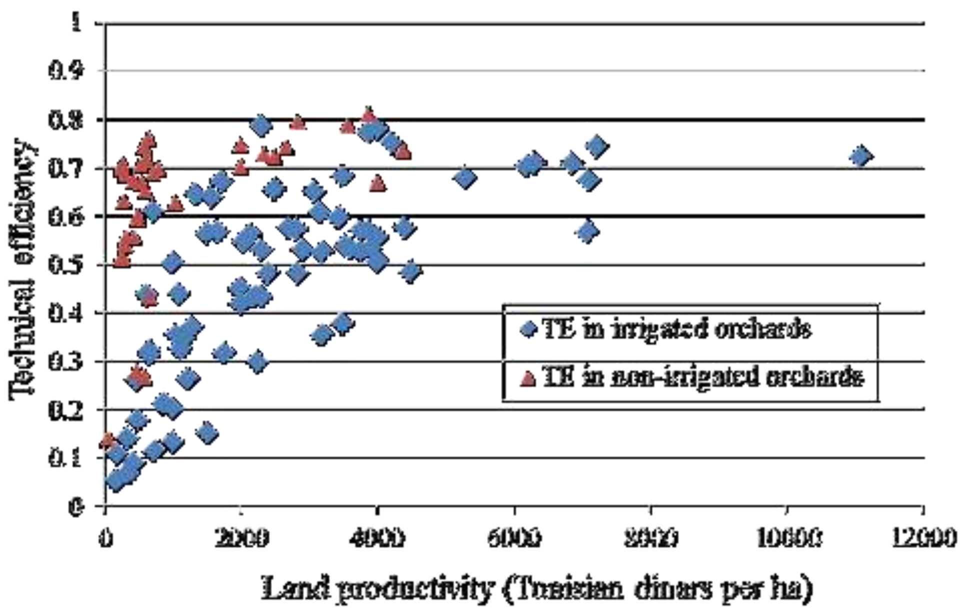

Figure 2. Relation between land productivity and technical efficiency under CRS specification.

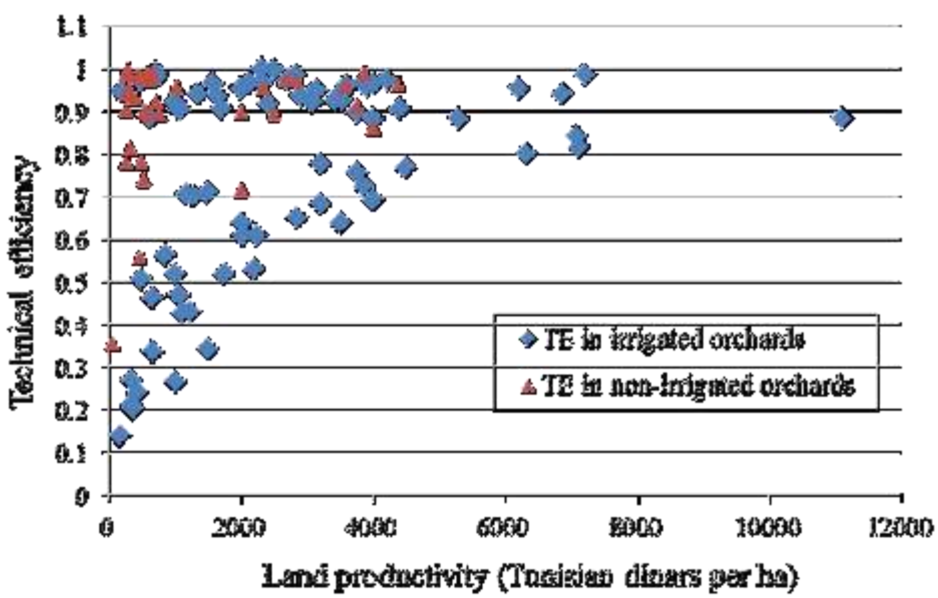

Figure 3. Relation between land productivity and technical efficiency under VRS specification.

Table 11. Spearman's rank-order correlation matrix.

	DEA CRS	DEA VRS	SFA CRS	SFA VRS
DEA CRS	1.000			
DEA VRS	0.855***	1.000		
SFA CRS	0.107	0.055	1.000	
SFA VRS	0.384***	0.299***	0.568***	1.000

***Significance at the 1% levels.

results also show the possibility of enhancing efficiency to upgrade the productivity and competitiveness. Indeed, improper use of irrigation systems could damage production efficiency. Growers need to schedule appropriately and have suitable knowledge of field conditions.

Figures 2 and 3 shows the relation between land productivity and technical efficiency for models under VRS and CRS specifications. It is noteworthy that production per hectare is higher in irrigated orchards in both models. However, technical efficiency varies among irrigated orchards and they are less efficient than non-irrigated ones. These results imply that introducing irrigation could potentially increase the level of production, but current production technology is somewhat distant from the best practices frontier.

Comparison between DEA and SFA results

This section reports the results from the DEA and SFA approaches that quantify olive orchards' technical and scale efficiencies and identify factors which determine variations in efficiency among orchards.

First, the SFA model produces a higher average technical efficiency score than the DEA model. Specifically, the average level of technical efficiency under the DEA approach was much lower than that under the SFA approach. One explanation for this discrepancy might be a difference between deterministic and stochastic frontier estimations. On the other hand, the DEA approach is more sensitive to data noise, measurement errors and uncontrollable factors than the SFA approach. Therefore, it should be noted that efficiency scores obtained in the SFA approach cannot simply be compared with that obtained in the DEA approach. However, we can identify correlation between two approaches calculating the Spearman's correlation coefficient based on rankings of individual orchard efficiency score under CRS and VRS specifications. Table 11 reports the correlation matrix. Efficiency scores in the DEA approach and efficiency ones in the SFA approach under VRS specification are lowly positively correlated with each other and significantly different from 0 at the 1% level, while no correlation exists between the DEA and SFA approaches under CRS specification. This result may imply that the sampled olive orchards operate under variable returns to scale or under increasing returns to scale rather than under constant returns to scale.

Second, results showing the effects of technical inefficiency in the DEA and SFA approaches were similar under CRS specification. Specifically, our econometric estimations show that nog-agricultural activities negatively affect technical efficiency, but the growers' age and education positively affects it.

Third, estimated results for technical inefficiency differ between the two approaches. Although an increase in the share of irrigated area to total exhibits a significant positive effect on technical efficiency in the DEA approach, the opposite results were obtained in the SFA approach. In addition, average technical efficiency in irrigated orchards under the DEA approach was higher than in non-irrigated ones, while irrigated orchards under the SFA approach were less efficient than non-irrigated ones. However, the test results of mean difference show that there is no significant difference in the average level of VRS technical and scale efficiencies. This result implies a need to ascertain the efficient scale of production.

Unlike the SFA approach, the DEA approach estimates scale efficiency. However, Table 5 indicates that average scale efficiency in irrigated orchards under the DEA approach is not significantly higher than in non-irrigated ones. Also, it indicates that technical rather than scale inefficiency is the major source of overall inefficiency in irrigated orchards because room for improvement in technical efficiency is larger than in scale efficiency. Furthermore, Table 6 shows that technical efficiency under the DEA approach significantly rises as the area under irrigation increases, but the opposite results are obtained in the SFA approach. A difference between deterministic and stochastic frontier estimations might explain the discrepancy. As a consequence, irrigated orchards have not produced the maximum achievable output, but irrigated orchards enjoyed higher production than non-irrigated ones by introducing irrigation. Therefore, it can at least be stated that enhancing technical efficiency requires not only introducing irrigation but also improving current production technologies and irrigation scheduling. These results suggest that Tunisian olive growers should raise output and efficiency by introducing more advanced technologies such as drip irrigation and sprinklers for improving the performance of irrigation systems.

Conclusion

Our study has investigated how irrigation affects productivity and technical efficiency by estimating non-parametric and parametric frontiers. It quantified the technical and scale efficiencies of irrigated and non-irrigated orchards using the DEA and SFA approaches and identified factors which determine variations in technical and scale efficiencies among orchards.

The SFA approach exhibited a higher average technical efficiency score than the DEA approach. A difference between deterministic and stochastic frontier estimations might explain the divergence. Also, estimations of technical efficiency under the VRS DEA generally mirrored those of the SFA under CRS specification with the exception of results for mechanization, the share of employed labour in total labour and the share of irrigated area to total.

Average technical efficiency in irrigated orchards under the DEA approach was higher than in irrigated ones, while irrigated orchards under the SFA approach was less technically efficient than non-irrigated ones and the technical efficiency of irrigated orchards under the SFA approach varied across orchards. Also, average scale efficiency in irrigated orchards under the DEA approach was higher than in non-irrigated ones. However, the test results of mean difference indicated that average VRS technical and scale efficiencies in irrigated orchards under the DEA approach were not significantly higher than in non-irrigated ones. On the other hand, technical rather than scale inefficiency was the major source of overall inefficiency in irrigated orchards because room for improvement in technical efficiency was larger than in scale efficiency.

Consequently, irrigated orchards had not produced the maximum achievable output during the period of this study, but irrigated orchards enjoyed higher production than non-irrigated ones by introducing irrigation and thereby it could at least be stated that enhancing technical efficiency requires improving current production technologies and irrigation scheduling as well as introducing irrigation. These results suggest the need for technical and financial assistance from government and international donors to improve the performance of irrigation systems and to rectify inefficiencies in current production technologies.

Conflict of interests

The authors have not declared any conflict of interests.

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Reproduction of *Meloidogyne javanica* in crambe plant and influence on crop yield and oil content

Carolina Amaral Tavares-Silva[1,2]*, Claudia Regina Dias-Arieira[1], Luiz Antônio Baccarin[3], Tiago Roque Benetoli da Silva[4] and Thais Santo Dadazio[5]

[1]Universidade Estadual de Maringá (UEM), Programa de Pós-Graduação em Agronomia, UEM/CCA/PGA, Av. Colombo, 5790, 87020-900, Maringá, PR, Brazil.
[2]Universidade Paranaense, Departamento de Engenharia Agronômica, Umuarama, Paraná, Brazil.
[3]Universidade Estadual de Maringá (UEM), *Campus* Umuarama, Paraná, Brazil. Departamento de Ciências Agronômicas. Estrada da Paca, São Cristóvão, 87501-970, Umuarama, Paraná, Brazil.
[4]Universidade Estadual de Maringá (UEM), Programa de Pós-Graduação em Ciências Agrárias, UEM/CCA/PAG, Estrada da Paca, São Cristóvão, 87501-970, Umuarama, Paraná, Brazil.
[5]Universidade Estadual Paulista, Faculdade de Ciências Agronômicas, Programa de Pós Graduação em Proteção de Plantas, Botucatu, São Paulo, Brazil.

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The crambe is an oilseed plant of the Brassicaceae family, which has the potential to grow in the winter, in succession to soybean crop, and whose seeds have been used for extraction of oil for biofuel production. However, in areas with nematode infestation, special care is needed in the selection of the species to be used in crop succession. Thus, the present study aimed to assess the susceptibility of crambe to plant-parasitic nematode, *Meloidogyne javanica* and its interference in vegetative growth parameters: crop yield and seed oil content. Therefore, crambe (*Crambe abyssinica* Hochst) seedlings were inoculated with a suspension containing 0, 1300, 2600 and 5200 eggs and second-stage juveniles (J2) of *M. javanica*, in experiment 1 (January to March, 2012), and 0, 1000, 2000 and 4000 eggs and J2, in experiment 2 (May to July, 2012). The tomato plant was inoculated to assess the viability of the inoculum. The assessments were done at two different times: 60 days after inoculation, assessment of the nematode reproduction factor, plant height and fresh and dry mass of the aerial part, was done with four repetitions. At the end of the crop cycle, 90 days after inoculation, seed yield and oil content were assessed in the remaining four replications. When grown in a period of higher temperatures, the crambe showed susceptibility to root-knot nematodes, with a negative impact on plant yield. However, this did not occur when the plant was grown in a more favorable season. In both experiments, seed oil content was not affected by the presence of the nematode.

Key words: *Crambe abyssinica*, oil, susceptibility, nematodes.

INTRODUCTION

The crambe is a cruciferous winter oilseed, belonging to the Brassicaceae family (Machado et al., 2008), originating in the Mediterranean and with short cycle, which ranges from 90 to 100 days (Oplinger et al., 1991). The plant is 70-90 cm tall, blooms 35 days after seeding, and the seeds are rounded and covered with a gray integument. Its main characteristic is the high concentration of oil, with levels ranging from 35 to 60% and used in biodiesel production (Oliveira et al., 2011).

Being a very robust plant, the crambe can grow in very adverse climate conditions, tolerating the frosts typical of the south of the country and temperatures as low as minus 6°C (Pilau et al., 2011), and show good yield in the dry season (Oplinger et al., 1991). Because it is resistant to drought and low temperature periods, crambe has been an alternative to sowing the winter crop in no-tillage system, with subsequent planting of soybeans (Pitol et al., 2010; Oliveira et al., 2011). However, there is little information on the management and potential of this plant, including its use as an alternative control importance of nematodes to soybeans, as *Meloidogyne javanica* (Treub) Chitwood and *Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans Steckhoven.

The soybean, *Glycine max* (L.) Merrill is currently the most important crop in the world. According to data of USDA (2015), soybean yield in the 2015/16 harvest was 106.58 million of tons, in a total of 33.79 million hectares cultivated.

Despite the high yield, damage caused by phytonematodes are very common, and the losses caused by them have become a matter of growing concern among producers and researchers (Asmus, 2004; Kubo et al., 2004; Machado et al., 2006). The main nematodes associated with soybean crops include *Meloidogyne incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood, that form root galls, *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekh which cause root lesions, as well as the cyst nematode, *Heterodera glycines* Ichinohe, and the reniform nematode, *Rotylenchulu sreniformis* Linford & Oliveira (Dias-Arieira and Chiamolera, 2011).

The nematode galls are important plant pathogens that affect agricultural production worldwide (Al-Raddad, 1995). This is favored by the high reproductive capacity of the nematodes, which leads to the rapid growth of populations in the field (Ferraz, 1985).

Due to the limited number of varieties resistant to nematodes, adapted to the different farming regions and the lack of nematicides registered for the crop, alternative methods to control the pest are constantly sought. Therefore, the use of non-host plants in rotation or succession of cultures with soybean is one of the main management strategies. With this practice, the populations of nematodes can be kept below the economic damage threshold, without any risk to the environment (Carneiro et al., 2007). The main crops to be used in succession to soybean are corn, oats, wheat, and, to a lesser extent, sunflower (EMBRAPA, 2004).

Despite the aggressiveness of phytonematodes, which attack crops of commercial interest, there is limited information on the reproduction of *M. javanica* and its interference in vegetative and productive parameters of the crambe. In view of the above considerations, the present study aimed at assessing the susceptibility of the crop to *M. javanica*, the interference of this nematode on the vegetative parameters of the crop and on the seeds and oil content.

MATERIALS AND METHODS

The experiments were conducted in greenhouse, at Universidade Estadual de Maringá, Campus Regional de Umuarama, Umuarama, Paraná, Brazil, in completely randomized designs, with four treatments (levels of initial inoculum) and eight replications. Period of the experiments: January to March 2012 (experiment 1) and repeated from May to July 2012 (Experiment 2).

The average temperatures recorded during the experimental periods were minimum of 21.2 and maximum of 31.9°C in experiment 1, and minimum of 12.9 and maximum of 21.7°C in experiment 2, according to data obtained from SIMEPAR (2013).

Initially, crambe seeds of the variety MS Brilhante were sown in trays containing the substrate Plantmax®. Seedlings were transplanted 15 days after germination to vases with capacity of 2 L, with two seedlings per vases. The substrate used was soil classified as Oxisol Dystrophic with a sandy texture (USDA, 1998), which was previously autoclaved for two hours at constant temperature of 120°C.

Two days after transplantation, the crambe was inoculated with suspension of *M. javanica*. In Experiment 1, the initial populations (Pi) were 0, 1300, 2600 and 5200 eggs and second-stage juveniles (J2), and in experiment 2, they were 0, 1000, 2000 and 4000 eggs and J2. The tomato plant of Santa Clara variety was inoculated with 5200 and 4000 eggs, in the respective experiments, to demonstrate the viability of the inoculum.

The inoculum was obtained from a pure population of nematodes, kept in the tomato plant, and extraction began in the roots, which were ground with a sodium hypochlorite solution in a blender, according to the method proposed by Hussey and Barker (1973) and adapted by Boneti and Ferraz (1981). The suspensions were calibrated using Peter's chamber, under optical microscope. Inoculation was performed by adding 4 mL of the respective suspensions to four equidistant holes, opened in the soil, around the plant.

After 60 days of cultivation, four plants of each treatment were randomly evaluated on vegetative parameters: plant height, dry mass of the aerial part, the latter obtained by drying in forced circulation at 65°C for three days. Regarding the nematological parameters, the number of galls and eggs per root was determined, which was considered the final population (Pf). The eggs were extracted according to the cited methodology and assessed in optical microscope in Peter's chamber.

The nematode reproduction factor (RF) in the crambe was calculated using the formula RF = Pf/Pi, as proposed by Oostenbrink (1966), in which plants are considered susceptible when RF≥1, resistant when RF<1 and immune when RF=0.

At the end of the crop cycle, the remaining plants were assessed for the number of seeds per plant, seed weight and oil content. The extraction of oil from the seeds was performed in laboratory using

*Corresponding author. E-mail: karoltavares@yahoo.com.br.

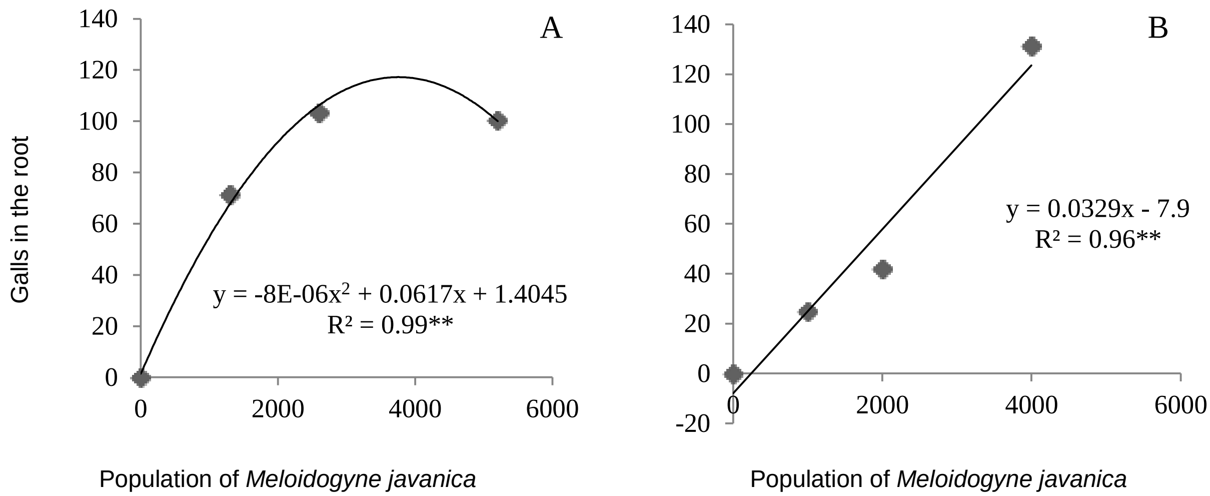

Figure 1. Number of galls in crambe roots depending on the population of *Meloidogyne javanica*. A = Experiment 1 and B = Experiment 2. ** = significant regression at 1% of probability.

methodology proposed by Silva et al. (2015).

The data were subjected to analysis of variance and the means to regression analysis, with a significance level of 5%, for the data concerning the inoculum levels used in the crambe. For the comparison of data with the susceptibility pattern (tomato plant), the means were compared by Tukey test at 5% probability, using the SISVAR 5.3 software (Ferreira, 2010).

RESULTS AND DISCUSSION

In Experiment 1, the crambe was susceptible to *M. javanica*, with reproduction factors equal to 3.3, 2.6 and 1.5, when inoculated with 1300, 2600 and 5000 eggs, respectively. In the second experiment, RF values were equal or close to 1, with means of 1.0, 0.7 and 0.8 for plants inoculated with 1000, 2000 and 4000 eggs, respectively. In both experiments, the reproduction values of the nematode in the crambe were significantly lower than those obtained in the tomato plant, used as control treatment, whose RF values were 14.9 and 53.8 in Experiments 1 and 2, respectively.

The difference in the reproductive activity of *M. javanica* in the two years can be explained by the experimental period. In Experiment 1, plant growth occurred from January to March, being affected by the high temperatures, resulting in physiological stress that made the plants susceptible to the establishment of the pathogen in the roots, while Experiment 2 was conducted in May and June, period of milder temperatures, which is more appropriate for the crambe crop (Knights, 2002; Pitol et al., 2010; Silva et al., 2013).

The climate factors have also directly impacted the reproductive activity of root-knot nematodes, because the genus *Meloidogyne* spp. usually has high activity in the soil and roots under high temperatures, and its ideal temperature for reproduction is 25°C. However, species such as *M. incognita* and *M. javanica* cannot survive at temperatures lower than 10°C (Ritzinger et al., 2010; Campos et al., 2011). Campos et al. (2011) reported that the reproduction of *M. javanica* in soybean plants increased at a temperature of 28°C, both in resistant and susceptible varieties.

The number of galls and eggs was found to be directly proportional to the population levels of *M. javanica* (Figures 1 and 2). In experiment 1, the relationship between the number of galls and eggs in crambe roots and the population of phytonematodes was best fitted by the quadratic equation (Figures 1A and 2A). Both parameters showed decrease when the population of nematodes was higher than 4000 eggs (Figure 1A). This was possibly due to food restriction, as it has been observed in other pathosystems involving *Meloidogyne* spp., in which increase in nematode population reduced the number of galls, giving rise to the hypothesis that the parasite had difficulty establishing feeding sites, due to the competition generated by the penetration of a large number of J2 in the roots (Schochow et al., 2004; Fabry et al., 2009).

In Experiment 2 (Figures 3B and 4B), the number of galls and eggs showed linear increase in relation to the initial population of *M. javanica*. The data obtained corroborate the findings of Souza et al. (2011), that classified the crambe as susceptible to this nematode, and its reproduction was directly proportional to the increase in inoculums levels of the parasite.

Asmus and Andrade (2001) also reported crambe susceptibility to *M. javanica*. However, the authors observed that the reproduction of the phytonematode was less intense than in other hosts, such as canola and quinoa. Despite the multiplication of *M. javanica* in the

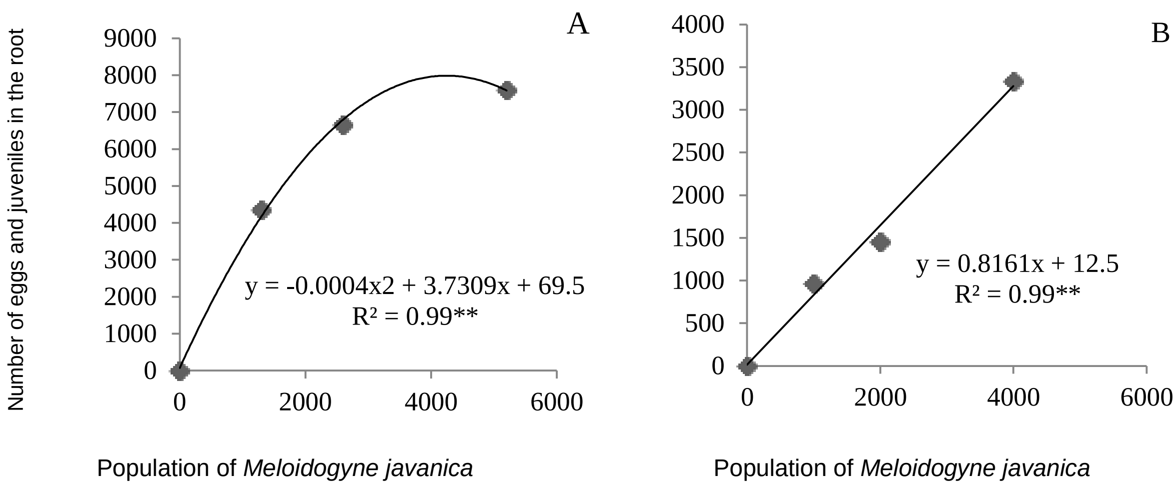

Figure 2. Regression of the number of eggs and juveniles in crambe roots depending on the population of *Meloidogyne javanica*. A = Experiment 1 and B = Experiment 2. ** = significant regression at 1% probability.

Table 1. Means related to the fresh mass of the aerial part (FMAP), dry mass of the aerial part (DMAP) and height of crambe plants depending on the different concentrations of *Meloidogyne javanica*.

Experiment 1				Experiment 2			
Treatment	Height	FMAP	DMAP	Treatment	Height	FMAP	DMAP
	cm	------------g----------			cm	-----------g---------	
0	46.8	1.9	0.8	0	59.2	3.8	1.6
1300	46.7	1.9	0.6	1000	56.7	6.7	2.5
2600	42.5	1.2	0.5	2000	51.7	3.3	1.6
5200	44.3	1.5	0.6	4000	58.5	4.6	1.8
CV(%)	17.3	26.7	30.3	CV(%)	19.2	35.2	32.0
L.R.	ns	ns	ns	L.R.	ns	ns	ns
Q.R.	ns	ns	ns	Q.R.	ns	ns	ns

L.R. = Linear regression; Q.R.= quadratic regression; ns = not significant at 5% probability.

crambe, RF indexes are still lower than those of other cultures commonly used in crop succession, such as sunflower, RF = 29.15 (Rosa et al., 2013) and black oat, RF=6.85 (Asmus et al., 2005).

The crambe is a commercially new culture, and, thus, further studies are needed on this species. Nevertheless, some authors have investigated the susceptibility of other brassicas to phytonematodes, and their results corroborate our experiment showing the susceptibility of *Brassica napus* Linnaeus (rapeseed) and *Brassica campestris* L. (turnip rape) to *Meloidogyne chitwoodi* Golden, O'Bannon, Santo and Finley 1 and 2, and *M. hapla* Chitwood (Mojtahedi et al., 1991), canola to *M. hapla* and *M. incognita* (Bernard and Montgomery-Dee, 1993), and *Sinapis alba* L. (mustard) to *M. enterolobii* Yang and Eisenback (*M. mayaguensis* Rammah and Hirschmann) (Brito et al., 2007), *Raphanus sativus* L. var. *oleiferus* Metzg. (forage turnip) to *M. javanica* (Rosa et al., 2013) and *Brassica oleracea* to *M. javanica*e and *M. incognita* (Dias-Arieira et al., 2012). In turn, studies conducted by Charchar et al. (2007) indicated that black mustard (*Brassica nigra* (L.) Koch.) was considered a bad host for a mixed population of *M. incognita* and *M. javanica*.

Analysis of vegetative parameters of the crambe (Table 1) showed that the presence of the nematodes did not affect plant growth. In comparison with data related to plant height found in the literature and cited by Oliveira et al. (2011), experiment 2 showed that, the development of the aerial part was nearly ideal for the culture, confirming that the period of the experiment was more favorable.

Despite the susceptibility of the crop, RF values close to 1 indicate that the crambe could be an option for use in crop succession systems, particularly because it ensures economic benefits to the producer. Also, since it is a species of Brassicaceae, release of glucosinolates may

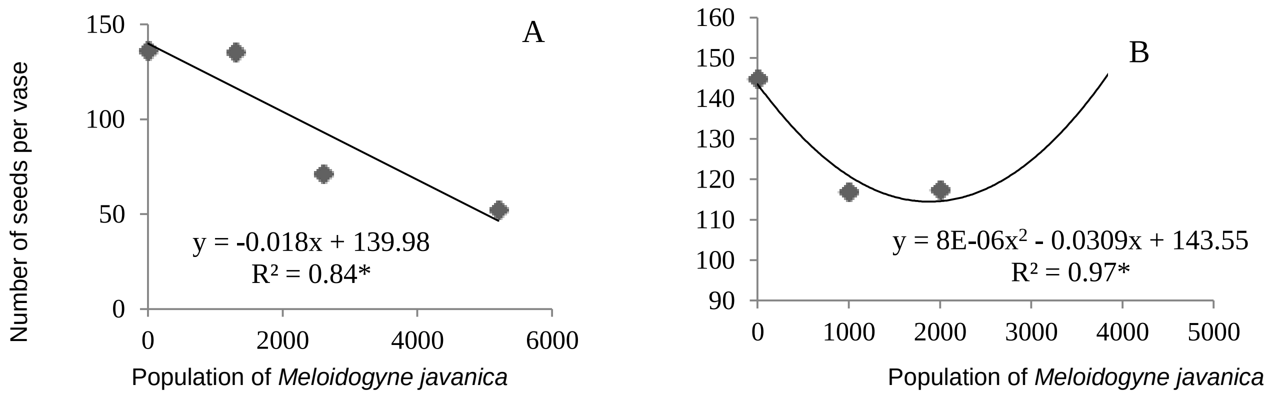

Figure 3. Regression to the number of crambe seeds per vase depending on the population of *Meloidogyne javanica*. A = Experiment 1 and B = Experiment 2. * = significant regression at 5% probability CV (%): A = 25.7 and B = 19.4.

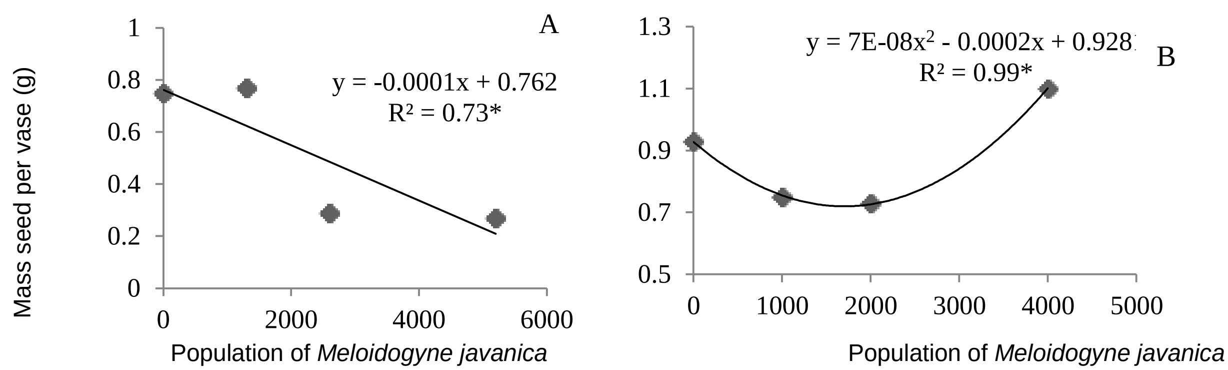

Figure 4. Regression of the mass of seeds (g) of crambe per vase, depending on the population of *Meloidogyne javanica*. A = Experiment 1 and B = Experiment 2 * = significant regression at 5% probability. CV(%): A = 29 and B = 22.1.

occur in the process of waste decomposition, as already observed for several species of the same family (Mojtahedi et al., 1991; Potter et al., 1999; Mazzola et al., 2001; McCully et al., 2008; Rizzardi et al., 2008; Reardon et al., 2013). However, Mojtahedi et al. (1991) observed that the suppressive effect of rapeseed on the nematodes was only possible when crop residues were incorporated to the soil.

In experiment 1, the increase in the population of *M. javanica* promoted the proportional decrease in seed production (Figure 3A). The same was observed in experiment 2 (Figure 3B). However, this decrease occurred in populations of up to 2000 eggs. The increase in population levels led to increase in the production of crambe seeds. Similar results were observed for the parameter mass of seeds per vases (Figure 4).

Although increase in inoculum levels of the nematode affected the number of seeds and mass of seeds, a difference was observed in the fitting of the regression equation in experiment 1, the reductions were linear and in Experiment 2, there was reduction up to the level of 2000 eggs per plant.

Some factors can explain these results, and the temperature during the experimental period is possibly the most important. According to Falasca et al. (2010), the ideal temperatures for the vegetative period of the crambe are between 15 and 25°C. But in experiment 1, the experiment was conducted from January to March, during which temperatures were above the ideal for the crop, ranging from 21.2 to 31.9°C. Thus, the decrease in seed production cannot be associated only with increase in nematode population, but also to adverse climate conditions, as observed in previous studies that reported that the crambe had lower growth rates under higher temperatures, resulting in lower grain production (Silva et al., 2013).

On the other hand, in experiment 2 carried out in the months of May and July, the climate conditions were more favorable to plant development, with temperatures between 12.9 and 21.7°C, which are indicated for crambe crop (Knights, 2002; Pitol et al., 2010; Silva et al., 2013). Therefore, the decrease in seed production, and,

Table 2. Means related to oil content in seeds depending on the population of *Meloidogyne javanica.*

Experiment 1		Experiment 2	
Treatment	**Oil content (%)**	**Treatment**	**Oil content (%)**
0	30.4	0	30.0
1300	30.2	1000	30.2
2600	30.6	2000	30.4
5200	30.7	4000	30.5
CV (%)	1.7	CV (%)	1.2
L.R.	ns	L.R.	ns
Q.R	ns	Q.R	Ns

L.R.= Linear regression; Q.R.= quadratic regression; ns = not significant.

consequently, in seed mass, can be directly associated with the increase in the population of *M. javanica* up to the level of 2.000 eggs per plant, in which the plant production recovered. The increase in seed production may have occurred because of the competition between nematodes for feeding sites, as already discussed.

Despite the decrease in seed production (Table 2), oil content was not significance, in the two experiments, showing that the population levels of *M. javanica* did not influence oil content in crambe seeds. The average oil production of the analyzed plants was 30.5 and 30.3% in experiments 1 and 2, respectively. According to Laghetti et al. (1995), the crambe has an oil content of approximately 38% in the mass, higher than the one found in the present study, which was about 30%.

Being a rustic species, the crambe did not show changes in oil content in the seeds, even under unfavorable climate and soil conditions, as those found in experiment 1. Silva et al. (2013) also observed the oil content in crambe seeds was not affected when the plants were grown in different climate conditions. Thus, it can be inferred that even in conditions of higher population levels of *M. javanica* in the soil, oil content in the seeds will not be affected.

The reproduction of *M. javanica* was lower at the season most favorable for crambe vegetative development, indicating that winter cultivation can be recommended, since the pathogen will have decreased reproductive activity. Also, the crop will not be affected, which demonstrates that crambe is a cost-effective crop. However, further research in this field is needed to confirm this hypothesis.

Conclusion

The crambe was susceptible to root-knot nematodes. However, parasitism did not affect the vegetative growth of the crop. Regarding the production of crambe seeds, it was affected by the nematode population, in association with climate factors, such as high temperatures. The population of nematodes in the plants did not affect the seed oil content.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Urban crop production in southeast Nigeria: Potentials and constraints

Anthonia Asadu[1]*, Innocent Enwelu[1], Philip Ifejika[2] and Edwin Igbokwe[1]

[1]Department of Agricultural Extension, University of Nigeria, Nsukka, Nigeria.
[2]National Institute for Freshwater Fisheries Research P. M.B. 6006, New Bussa, Niger State, Nigeria.

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Urban farming (UF) offers potential benefits to urban areas and has captured the attention of residents. Based on a survey and interview of 210 urban farmers, this article explores the potential benefits, and constraints to urban farming, in southeast Nigeria. The farmers indicated that the potential benefits included: food source, income source and efficient utilization of space among others. Major food produced were staples like cassava and maize, while vegetables were *Telfaria,* okra *and Amaranthus* spp. The farmers also reported that insecurity of land, lack of access to credit facilities; theft and destruction of crops by stray animals were among the constraints faced by the urban farmers. It is, therefore, necessary that urban farming be legalized to be part of urban policy in order to realize the full potentials

Key words: Urban farming, southeast Nigeria, potential benefits, constraints.

INTRODUCTION

United Nations (2012) estimates that in 2050, the global population will reach 9.6 billion, with the majority of that growth taking place in urban areas of less developed regions. Sub-Saharan Africa in particular, constitutes a great portion of that growth as the urban population expands faster than any other region and is projected to double between 2010 and 2030 (FAO, 2012). The rapid expansion of urban population puts direct pressure on food sources and agricultural production, thus, there exists a serious challenge in supplying enough nutrition and safe food among such rapid urbanization.

Despite many technological and mechanical improvements like improved seed varieties and modern processing methods in food production, hunger and malnutrition remain central issues as poverty continues to be prevalent in many cities around the world (Magnusson et al., 2014). It is estimated that 40% of urban inhabitants are living on less than US$1 a day, while 70% are living on US$2 a day (FAO, 2012). Similarly, impoverished urban households are estimated to spend 60to 80% of incomes on food, making them more vulnerable to food price volatility (Cohen and Garret, 2010). Rapid

*Corresponding author. E-mail: toniaasadu@yahoo.com. Tel: +2348166979076.

urbanization has produced a large group of urban poor, proliferating widespread issues like food insecurity and malnutrition in the developing world. The global food price crisis and the protests across the world pointed to the vulnerability of the urban poor (Anderson, 2014). The fact that the global urban population surpassed the rural one for the first time in the history of the planet (Satterthwaite et al., 2010) underscores the possibility that also future food security concerns would take on an increasingly urban slant.

As Africa urbanizes, urban poverty increases, there is a corresponding growth in urban food insecurity (White and Hamm, 2014). Consequently, the most pressing need of any urban agglomeration is the question of food security and ensuring the right to food. Urban populations depend on the reliable and stable availability of food products, as well as affordable and convenient access to them. High levels of urban income poverty paired with rising food prices, however, often make the formal urban food supply systems unaffordable and inaccessible to the urban poor.

With cities in Africa growing rapidly, farming in the urban area is expected to play a greater role in feeding urban population. Urban agriculture (UA) has been widely upheld as a solution to the food crisis facing increasingly metropolitan populations (Stewart et al., 2013). According to them, UA have a role to play in addressing urban food insecurity problems, which are bound to become increasingly important with the secular trend towards alleviating poverty in urban areas. Urban agriculture generates significant livelihood opportunities, not only for urban farmers, but also for trades, input suppliers and other service providers along the value chain for domestic produce (Lagerkvist, 2014).

Globally, between 15 and 20% of consumed food is produced by UA (Corbould, 2013). Urban production varies between countries, with a greater proportion taking place in low-income countries. Vietnam has the highest participation in local production of urban food. In Hanoi, 80% of fresh vegetables are produced within city borders (Corbould, 2013). Over 70% of urban growers in the city of Tamale, Ghana, state their main occupation as vegetable growing, primarily for market and less for their own consumption (Abudakari and Mahunu, 2007). Gallaher et al. (2013) demonstrated how sack gardening improved social capital, especially if carried out collectively, they stated that it enabled a measure of resistance to food insecurity by poor women in the Kibera slums of Nairobi.

Well-managed vegetation cover from urban horticulture has a positive impact on urban environments. Bernholt et al. (2009) studied the effect of species richness and diversity in homes and commercial gardens in Niger. The highest diversity was found in large, well maintained urban gardens with production of mainly vegetables and fruits for market. Maintaining green open spaces and enhancing vegetative cover in the city are important adaptive and mitigation measures for climate change (De Zeeuw et al., 2010). According to them, urban farming may prevent building on risk-prone land. By maintaining such areas like agro-forestry spaces, not only are the impacts of climate change due to flooding, landslides and other disasters reduced but, also, urban diversity and living conditions are improved (De Zeeuw et al., 2010). It also contribute immensely to the quality of life in towns and cities (FAO, 2001), the recreation benefits includes landscape maintenance like parks. Evaporation from the canopy lowers temperature and the vegetation filters dust from the air (WOCAT, 2007).

Van Averbeke (2007) mentioned other potentials of urban farming as ability to build social network and self-worth through farming. Besides, enjoyment and mental well-being provided by agricultural activity and the chance to use knowledge and skills are also potential benefits. Women gain pride and a sense of self-worth when their produce is consumed by her family. Their involvement in productive activities may also help them to gain dignity, hope and self-respect and enhance their self-reliance (Bradiford et al., 2009).

Renewed interest in UA amongst scholars and policy makers is a positive development since local and international environments have changed greatly since the 1980s and 1990s, when most of the initial research on the concept was conducted (Crush et al., 2010). As a result, many cities of the world have developed different strategies on UA. For example, the city of Johannesburg identifies UA as its main intervention to address food security within the city. However, in some African countries like Nigeria, urban planning and development approaches do not consider food production as an objective; thus, food production capacity may become severely constrained as urbanization proceeds. In the absence of friendly land use policy and plan that encourage urban farming, city farmers are subjected to harassment and subsequent eviction from government lands. Some urban farmers gain access to land for urban farming only as customary tenants on private land, and are only allowed to cultivate annual crops (Wakuru and Drescher, 2009).

Analyzing the extent to which UA may help shield urban dwellers from some of these food problems becomes therefore a tropical policy question. However, Zezza and Tasciotti (2010) stated that important, nature and food security implication of UA is however hindered by lack of good quality and reliable data. Magnusson et al. (2014) also noted that the current scientific literature regarding UA has its shortcoming as most studies are single-city studies. Reliable data are therefore necessary to put forward urban farming practices and its potential benefits to city planners. This will help the municipal authorities and urban planners in integrating urban farming into the urban system in a more viable and sustainable way.

In Nigeria, urban crop productions are persistent features of cities. It often occurs informally and opportunistically in the in-between spaces of towns and cities. However, despite the glaring facts on the presence of UA in Nigeria, especially in big cities like Abuja, Lagos, Kano and Ibadan, policy makers and government have deliberately neglected this veritable sector and have not made concerted effort to acknowledge it and channel attention to it. The most striking feature of urban production is that it is integrated into the urban economic and ecological system. Urban crop production is not a relic of the past that will fade away nor brought to the city by rural immigrants that will lose their rural habits over time. The findings on the potentials of urban farming need to be made available to the urban planners and policy makers in the planning of urban areas.

Literature search on urban farming in Nigeria reveal that most have been on single city or in a state. Olaniyi (2012) carried out a study on the attitudinal disposition of urban dwellers towards participation in urban agriculture in Oyo State, Nigeria. Salau and Attah (2012) also reported on the socio-economic analysis of urban agriculture in Nassarawa State, while Egbuna (2008) looked at urban agriculture as a strategy for poverty alleviation in Abuja. Chah et al. (2010) did an assessment of contribution of urban crop agriculture in Enugu Metropolis, Nigeria. Other empirical studies of Nigerian urban agriculture have concentrated majorly on the resource use efficiency in UA. This present study looked at the potential benefits of and constraint to urban crop cultivation in southeast, Nigeria. The findings of this study will expose issue for policy consideration in the whole states of the southeast in particular and Nigeria in general.

Countries have their own unique mechanisms of defining what constitutes urban or rural, and these mechanisms determine the definition of urban and rural areas. For the purpose of this study, delineations used by the National Population Commission were employed. This reflects the definition used to administer government programs. The definition of urban crop production here refers to production of any crop whether for sale or for own-consumption within the administrative boundary of an urban area.

The main purpose of this study was to show the potential benefits and challenges of urban crop production in southeast Nigeria. Specifically the study:

1. Identified major crops grown,
2. Ascertained the potential benefits of urban crop production and
3. Determined constraints to urban crop production.

METHODOLOGY

The study adopted a survey design and was carried out in southeast Nigeria. This zone is made up of five states *viz* Abia, Anambra, Ebonyi, Enugu and Imo States. The population of the study comprised all urban farmers involved in crop production in the area. Out of the five states, three (Ebonyi, Enugu and Imo) were selected through simple random sampling technique. Each state has three senatorial zones. Two senatorial zones were randomly selected from each state, giving a total of six zones. In each zone, a major urban centre was selected to give a total of six urban centres. Five urban (political) wards were purposively selected from each urban centre based on their active involvement in urban crop production. Therefore, a total of 30 urban wards were used for the study. In each ward, snow-ball technique was used to select seven urban households actively involved in crop production. This gave a sample size of 210 respondents. Data were collected using interview schedule, focus group discussion (FGD) and observations, and analyzed using descriptive statistics (percentage and mean). The instrument was validated by three senior lecturers in the Department of Agricultural Extension, University of Nigeria, Nsukka. Reliability was achieved using test-retest method, with a coefficient of 0.91.

To ascertain the potential benefits of urban crop production, the respondents were presented with a list of benefits collated from the result of focus group discussion held. The farmers were requested to rate them on a 4-point Likert-type scale indicating the extent he/she considered an item or a variable in the list, as a benefit derived from cultivating in urban area. The scale was: to a great extent (3); to an extent (2); to a little extent (1) and to no extent (0). The values were added to get 6, which was divided by 4 to get a mean of 1.5. Any item/variable with mean ≥ 1.5 was regarded as a major benefit.

To determine the quantity of major crops produced, the following measurements were used for the different crops. One wheel barrow of cassava was measured as 100 kg; a bag of maize, tomatoes and garden egg as 50 kg each. A bundle of *Telfaria* and *Amaranthus* was measured to be 2 kg each. The average quantity of each crop was determined by the total quantity (kg) produced divided by the number of producing households.

To determine constraints to urban crop production, a list of constraints from the FGD was given to the respondents to indicate how serious a constraint affected his/her farming activities in the urban area. The response options were assigned the following values: very serious = 2; serious = 1 and not serious = 0. The values were added to give 3 which was later divided by the number of options (3) to get a mean of 1.0. Any constraint with a mean of 1.0 and above was regarded as a serious constraint while constraints with mean less than 1.0 were minor constraints. The statistical product and service solutions (SPSS) was used for the analysis.

RESULTS AND DISCUSSION

Major crops grown

Entries in Table 1 reveal that majority (89.5, 87.1, 79.5 and 71.9%) of the respondents grew maize, *Telfaria,* cassava and *Amaranthus.* Other crops grown by the respondents included yam (50%), okra (51.4%), cocoyam (25.2%), sweet potato (about 15%) and tomatoes (12.9%). The results also show that more farmers cultivated yams (67.1%) in Ebonyi State than Enugu and Imo States. The findings show that short-duration crops were produced. Fruit trees like orange, plantain and banana were not grown by many of the respondents. This may be due to insecurity of land used for cultivation in urban areas. A study in Cotonou (Benin) showed that high-value labour-demanding crops were predominant on land with secure tenure, whereas more extensive

Table 1. Percentage distribution of respondents according to crops grown.

Crops	Enugu (%)	Imo (%)	Ebonyi (%)	All (%)
Yam	35.7	4.7	67.1	50.0
Maize	82.9	97.1	88.6	89.5
Cocoyam	11.4	34.3	30.0	25.2
Cassava	70.0	91.4	77.1	79.5
Rice	0.0	0.0	14.6	4.8
Cowpea	8.6	2.9	11.4	7.6
Sweet potato	12.9	4.3	27.1	14.8
Plantain	8.8	20.0	11.4	13.3
Banana	5.7	14.3	12.9	11.0
Orange	4.6	2.4	2.8	2.3
Carrot	5.7	0.0	0.0	1.9
Cabbage	4.3	1.4	1.4	2.4
Lettuce	4.3	1.4	2.9	2.9
Tomatoes	12.9	5.7	20.0	12.9
Okra	40.0	57.1	57.1	51.4
Telfaria	94.3	87.1	80.0	87.1
Amaranthus	75.7	72.9	67.1	71.9
Garden egg	21.4	22.9	14.3	19.5

production of staple crops such as cassava and maize took place on plots with lower tenure security (Brock and Foeken, 2006). In Nairobi, Kenya, *Solanum* and *Amaranthus* were the most widely grown crops. Majority of urban farmers in Oyo State, Nigeria, are, also, growing mainly vegetables (Olaniyi, 2012). Similarly, Salau and Attah (2012) reported that urban farmers in Nasarawa State, Nigeria grow mainly vegetables, maize and sweet potato.

The result, also, revealed that indigenous vegetables were grown more than the exotic ones. Examples of indigenous/local vegetables are *Telfaria* and *Amaranthus*, while the exotic ones are lettuce and cabbage. Most of the respondents reported that they preferred local vegetables because of the taste and easy access to planting materials. Neergard et al. (2009) asserted that choice of crops in urban cultivation reflects local preferences and availability of seeds. Besides, the greater drought tolerance of traditional vegetables as compared to exotic species means that they can be cropped for longer periods of low rainfall (Oluoch et al., 2009). However, Ruma (2009) reported that 98% of urban farmers in Katsina metropolis, in northern Nigeria, cultivate mainly exotic vegetables like lettuce, cabbage and carrots.

Average quantity of crops produced and sold

The average quantity of cassava and maize produced in 2014 were 582.7 and 612.5 kg, respectively, while about 99 kg of cassava and 189 kg of maize were sold (Table 2). The quantities of garden egg, *Telfaria* and *Amaranthus* produced on average were 488.2, 84.6 and 69.9 kg, respectively while about 406 kg of garden egg, 24.4 kg of *Telfaria* and 9.6 kg of *Amaranthus* were sold. The finding suggests that most of the crops produced were consumed at home. The households that produced garden egg sold most of their produce, indicating that the crop is grown mainly for commercial purpose. Other crops like maize, cassava, *Telfaria, Amaranthus* and tomatoes were mainly consumed at home. This confirms the assertion that a relatively large number of the households consume a large part of their crop produced in urban areas (Foeken et al., 2004). This means that urban crop cultivation contribute to household food security and urban food supply. Therefore, interventions like government or non-governmental programmes developed to improve urban agriculture should be encouraged. In Nakuru, Kenya, the average quantity of maize, beans, cowpeas produced in 2005 were 224, 75 and 67 kg, respectively (Foeken, 2006). The average output of farmer per production cycle of garden egg in Uyo metropolis, Nigeria, is 890.82 kg (Okon et al., 2012).

Potential benefits of urban farming

The respondents stated that UF was important in various ways. Entries in Table 3 show that urban farming is important as food source ($\bar{x}$ = 1.86) and an income source ($\bar{x}$ = 1.74). Other benefits of urban agriculture such as improving urban diet ($\bar{x}$ = 1.73), improving food security among urban households ($\bar{x}$ = 1.75), efficient

Table 2. Average quantity of major crops (kg) produced and sold (per producing household).

Major crops	Average quantity (kg) produced	Average quantity sold (kg)
Maize	612.5	189.2
Cassava	582.7	99.1
Telfaria	84.6	24.4
Garden egg	488.2	406.2
Amaranthus	69.9	9.6
Tomatoes	14.6	--

Table 3. Mean distribution of potential benefits of urban farming.

Potential benefits of UF	Mean ($\bar{x}$)	SD
UF is a food source	1.86*	0.366
UF is an income source	1.74*	0.429
Creation of employment	1.43	0.718
Reducing seasonal gap in fresh food	1.54*	0.662
Creation of green zone	1.20	0.720
Production of floriculture	1.13	0.720
Improving urban microclimate	1.06	0.739
Improving urban diet	1.73*	0.499
Recycling solid and liquid waste in cities	1.09	0.791
Ensuring cleanliness in urban areas	1.16	0.797
Improving food security among urban households	1.75*	0.488
Improving urban economy	1.58*	0.551
Efficient utilization of spaces	1.60*	0.635
Helps farmers to build social network	1.20	0.748
Creates chance to use knowledge and skills	1.51*	0.667
Helps to regain dignity and hope for practioners	1.23	0.779
Enhances self-worth and self-reliance	1.37	0.771
Improves the mental well-being of urban farmers	1.39	0.774
Improves physical exercise	1.37	0.637
Efficient use of household labour	1.58*	0.673
Helps to arouse youths' interest in farming	1.24	0.761

*Major benefits

utilization of spaces ($\bar{x}$ = 1.60), efficient use of household labour ($\bar{x}$ = 1.58), improving urban economy ($\bar{x}$ = 1.58), creates chance to use knowledge and skill ($\bar{x}$ = 1.51). Improving microclimate ($\bar{x}$ = 1.06), helping farmers to build social network ($\bar{x}$ = 1.20) enhances self-worth and self-reliance among practitioners ($\bar{x}$ = 1.37), which are regarded as minor benefits of urban farming.

That UF improves food security among urban household is not surprising. Urban farming contributes to food availability in cities and also to the diet of urban consumers (Hoestra, 2010). This is particularly important for fresh foods (vegetables, eggs and poultry) which can be produced for home consumption or sold on the street and markets. This implies that urban farming helps to improve food security among urban farming households. Salau and Attah (2012) reported that additional household income is a major benefit of urban agriculture in Nassarawa State, Nigeria. Hovorka et al. (2009) noted that UF has important positive effects on poverty alleviation, local economic development, food security, nutrition and health of the urban poor. In Nairobi, Mugambi (2002) indicated that farming households are better off in terms of energy consumption when compared with non-farming households. Foeken (2006) also reported better height and weight growth among children of urban farmers than non-urban farmers.

That improving urban microclimate, recycling solid and

Table 4. Mean distribution of constraints to urban crop production.

Constraints	Mean	SD
Inadequate land for cultivation	1.86	0.363
Theft of crops	1.52	0.669
Insecurity of land	1.68	0.544
Lack of access to credit facilities	1.66	0.552
Lack of input for crop cultivation	1.39	0.739
Lack of safe water for irrigation	1.12	0.842
Lack of capital to invest	1.57	0.612
Inadequate information on urban farming	1.09	0.753
Lack of farmer organization	1.00	0.834
Inadequate labour to hire	1.00	0.863
No market to sell surplus produce	0.70	0.842
Lack of modern storage facilities	1.38	0.710
Inadequate processing equipment	1.32	0.744
Sewage bursts can destroy crops	1.00	0.835
Harassment by municipal authorities	1.22	0.796
Pest and diseases infestation	1.41	0.634
Poor soil condition	1.20	0.790
Inadequate rain which affects crop yield	1.17	0.782
Variation in climate	1.00	0.787
Inadequate extension service	1.10	0.848
Destruction of crops by stray animals	1.47	0.503

liquid wastes in cities, and improving physical exercise were not regarded as major benefit of urban farming was surprising. This is because several studies have shown that urban agriculture helps in improving urban microclimate and physical exercise. Boland (2002) asserts that recycling of organic waste can be an effective and sustainable way of improving soil fertility in urban areas.

Bryld (2003) also reported that urban farming has a number of benefits for the local microclimate. Firstly, evaporation from the crops lowers temperatures in cities. Again, the vegetation filters dust from the air, thereby improving air quality. Improved air quality can also contribute to decreasing respiratory diseases (Baumgartner and Belevi, 2001). According to Pasquini (2006), UF offers wide-range of benefits including improved waste recycling, and health benefits due to increased physical activity.

Another aspect of UF perceived to be important was its contribution to urban greening. Nel et al. (2009) indicate that greening the environment is important. So, continuous cropping on the same plot contributes to urban greening. Hence, environmental policy concerns the maintenance of green spaces in city areas as part of urban greening and management of air quality.

The standard deviation values of the mean of potential benefits of UF were less than one. This implies that the respondents' opinion on the potential benefits of urban farming did not differ much. The major contributions had lower standard deviations as revealed in Table 3. These included UF as a source of food ($\bar{x}$ = 1.86; SD = 0.366); UF as income source ($\bar{x}$ = 1.74; SD = 0.429) and improving food security among urban households ($\bar{x}$ = 1.75; SD = 0.488).

Constraints to crop cultivation

Table 4 presents constraints to crop cultivation as indicated by the urban farmers. Almost all the constraints listed were perceived as major constraints by the respondents. These include, inadequate land for cultivation ($\bar{x}$ = 1.86), insecurity of land ($\bar{x}$ = 1.68), lack of access to credit facilities (($\bar{x}$ = 1. 66), theft of crops ($\bar{x}$ = 1.52) and lack of capital to invest ($\bar{x}$ = 1.57). Other constraints included pest and disease infestation ($\bar{x}$ = 1.41), lack of farmer organization (M = 1.00), lack of safe water for irrigation ($\bar{x}$ = 1.12), lack of modern storage facilities ($\bar{x}$ = 1.38) harassment by municipal authorities ($\bar{x}$ = 1.22) inadequate extension service ($\bar{x}$ = 1.10) and destruction of crops by stray animals ($\bar{x}$ = 1.47).

Inadequate land for cultivation was the most critical problem. This is not surprising because land is a scarce resource in urban areas. Many farm sites are too small for the farmers to invest and expand their crop production. Related to the problem of land, is the frequency of harassment by government officials and plot owners.

According to Baumgartner and Belevi (2001), availability and access to land have been the crucial elements for engagement in UF. This finding is consistent with that of Egbuna (2008), who reported that land access and tenure security including harassment by environmental authorities are major problems faced by urban farmers in Abuja, Nigeria. However, urban farmers in Katsina urban, Nigeria, do not complain of any harassment by local authority (Ruma, 2009).

Lack of information was another problem faced by urban farmers. This may reflect inadequate extension assistance. Urban farmers need to apply production or farming techniques appropriate to their urban situation. Ruma (2009) noted that urban farmers, who cannot access the services of extension, apply chemicals using their own instinct. These have negative consequences on the environment and health of farmers. Salau and Attah (2012) reported poor extension service as constraints to urban farming in Nasarawa State, Nigeria.

Theft of crops was also perceived as a serious problem to urban farmers. Visser (2004) had earlier reported that theft is a big impediment to urban farming. Crops and equipment get stolen if they are not properly guarded. The provision of security again places extra cost on the farmers. In-depth interview during the FGD revealed that thieves steal produce in the farm. This forces people to harvest crops before they are fully matured. This finding is in line with most studies that had earlier indicated that theft of crops was a major constraint to urban agriculture (Foeken and Owuor, 2000; Egbuna, 2008; Chah et al., 2010).

Destruction of crops by animals was also a problem to the farmers. This may be attributed to the fact that some farmers allow their animals to roam about and scavenge for food. These animals are usually allowed to go about during dry season when it is difficult to get food for them. They can enter peoples' farm and destroy crops. They usually disturb those who engage in dry season production.

Some problems mentioned are not specific to urban setting, example, poor soil, lack of inputs, pest and diseases and inadequate rainfall. However, constraints that are typically urban include, theft of crops, harassment by government officials, insecurity of land and sewage burst which can destroy crops.

Conclusion

Crop farming is thriving in cities of southeast Nigeria. The importance of urban farming cannot be overlooked. Farmers are engaged in producing various crops which provide them with food to meet their health and growth requirements. Furthermore, urban farming improves household's security in terms of uncertainty by having access to more stable food sources.

However, challenges facing production systems have also been identified. Specifically, they include inadequate land for cultivation, theft of crops, lack of access to credit facilities, inadequate information from extension, to mention a few.

Land access remains a major factor in urban production systems. The uncertainty of rights is an obstacle for long-term farming strategies. Land is becoming largely expensive and unavailable, leaving it to the rich who use it for capital development and not agriculture.

Based on these findings, there is need to use intensification methods where more is produced from less land. Extension personnel will then play a role in providing the urban farmers with the required technical assistance.

The city authorities should also designate areas for crop cultivation. In this case, farmers should be given permanent ownership to avoid harassment by the government. This will make room for urban farming to be properly legislated to maximize its potentials.

Conflict of Interests

The authors have not declared any conflict of interests.

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Effects of chemical and bio-fertilizers on yield, yield components and grain quality of maize (*Zea mays* L.)

Hashim A. Fadlalla, Hatim A. A. Abukhlaif and Somaya S. Mohamed*

Environment, Natural Resources and Desertification Research Institute, National Centre for Research, Sudan.

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A field experiment was conducted in two consecutive seasons (2009 and 2010) to assess the effect of two microbial fertilizers, chicken manure, urea and their possible combinations on the performance of a maize variety (Hudeiba 45). The bio fertilizers used were *Azospirillum brasilense* (A), *Azotobacter* sp (B) and chicken manure (CM) at a rate of 7t/ha). Urea (46% N) was applied at rates of 120kg/ha (N) and 40kg/ha (T). The combinations used were *Azospirillum brasilense* with urea (A+T), *Azotobacter* sp with urea (B+T), A+CM and B+CM in addition to control. The field experiment was laid out in a Randomized Complete Block Design with four replicates. Four seeds were sown in holes with 20 cm between holes in 4×4 m^2 plots with 5 ridges 70 cm apart. Sowing was carried out in the last week of October in 2009 and the first week of November in 2010. The fertilizers were applied on one side of the ridge and plants were irrigated at seven days intervals. Data were collected on cob length, cob diameter, number of rows/cob, number of seeds/row, 100-grain weight, grain yield/plant and grain yield/ha. Data were collected for yield components at physiological maturity and for grain yield after harvesting. The moisture, ash, fat, crude fiber, crude protein, tannins and carbohydrates contents of the grains were determined. Results showed that biofertilizers have significantly ($p < 0.05$) increased crop productivity. CM increased the grain yield (t/h) by 20 and 30% over the control in 2009 and 2010, respectively. Application of chicken manure solely or in combinations with *Azosprillum barasilence* or *Azotobacter* sp have also increased grain yield and its components. The combinations of N_2 fixer inoculants with manure and/or urea improved grain quality of maize under field conditions.

Key words: *Azosprillum barasilence*, *Azotobacter* sp, maize, chicken manure, grain yield.

INTRODUCTION

Maize (*Zea mays* L.) or corn is a cereal crop that grown over a wide environmental range .It is considered as a multi-purpose crops that have a lot of uses such as human diet and animal and poultry feed. It can be used as grain, green fodder or silage (Haque, 2003).In developing countries including Sudan maize is a major source of income to many farmers (Tagne et al., 2008). Moreover, the possibility of blending maize with wheat for

*Corresponding author. E-mail: somasirmo@gmail.com. Tel: + 249-912765151.

Table 1. Some physical and chemical properties of Shambat soil.

Parameter	Particle size distribution (%)			Total K (ppm)	Total P %	Total N%	EC ds/m	pH (paste)
	Clay	Silt	Sand					
value	69.74	9.00	21.24	0.140	0.076	0.069	1.87	7.8

bread making has also increased the demand of maize in Sudan (Ali et al., 2009). Therefore, farmers are encouraged to incorporate the crop into the farming systems under both irrigated and rain fed agriculture. Nitrogen (N) is generally deficient in Sudan's soils as in most other semi-arid regions. In such regions nitrogen is usually added to the soil in large quantities. Therefore, intensive farming practices that aim at producing higher yield require extensive use of nitrogen fertilization which are costly and create environmental pollutions (Baser et al., 2012). Biofertilizers which are eco-friendly play an important role for supplementing the essential plant nutrients for sustainable agriculture and economy (Mugilan et al., 2011). Moreover, microbial fertilizers can clean the environment, enhance the productive capacity of land and reducing the amount of chemical fertilizer consumption (Hossein and Farshad, 2013) and improve plant growth and health (Raaijmakers et al., 2002). Enhancement of cereals yields by inoculation with non-symbiotic nitrogen fixing bacteria was recorded by many researchers (Salantur et al., 2006). *Azospirillum* species have been potentially studied to the greatest extent and appeared to have significant potential for commercial production (Kumaresan and Reetha, 2011). *Azotobacter* species besides playing a role in nitrogen fixation, it has the capacity to synthesize and secrete considerable amounts of biological active substances like vitamins, gibberellins and auxins (Suhag, 2016). Chicken manure is an excellent source of nutrients and can be incorporated into most of the fertilization programs. Ayeni and Adetunji (2010) reported that, the mean increase in grain yield of maize due to application of poultry manure alone varied between 34 and 68% compared to 11-57% gained by application of NPK fertilizers. Corn is considered as one of the most important cereal crops over the world that needs large quantities of chemicals. In Sudan few reports on the importance of biofertilizers inoculation in increasing maize production and improving the physical and chemical quality of maize grains are available. Therefore, the objective of this study is to estimate the effects of microbial inoculation, chicken manure and urea fertilization on maize grain yield, components and quality.

MATERIALS AND METHODS

Site

The field experiment was conducted at the experimental farm of the college of Agricultural Studies, Sudan University of Science and Technology, Shambat (Latitudes 15° 40´ N, Longitude 32° 32´ E and 380 m above the sea level) for two consecutive years (2009 and 2010). Shambat climate is tropical, usually hot and humid in summer and cold and dry in winter. The temperature reached a maximum value (45.9°C) in June and a minimum value (22°C) in January. The temperature usually drops during July to October due to the incidence of the rainy season. The soil of Shambat was analyzed to determine the physical and chemical characteristics according to Richard (1954). Nitrogen was measured using Kjeldahl method as described by Anderson and Ingram (1993). Data are shown in Table 1.

Source of inoculants and biological materials

A variety of maize (Hudeiba 45) was obtained from the Agricultural Research Corporation, Sudan. The effects of chemical and biofertilizers on the performance of maize were studied. *Azospirillum brasilence* and *Azotobacter* sp inoculants were obtained from Biofertlization Department, Environment and Natural Resources Research Institute, National Centre for Research, Sudan. Chicken manure was obtained from the Animal Research Centre, Sudan.

Land preparation

The land was disc ploughed, harrowed, leveled and then ridged. The area was divided into 4×4 m² plots with 70 cm apart five ridges. Four seeds were sown per hole and 20cm distance between holes was maintained. Sowing was carried out in the last week of October in 2009 and the first week of November in 2010. Seeds inoculation was performed by coating the seeds with the charcoal- based inoculants. Gum Arabic was used as an adhesive. Chicken manure was applied at a rate of 7 t/ha, distributed on the ridged-plot and mixed with soil. The plots were then irrigated weekly for two weeks before sowing date. Plants were irrigated immediately after sowing then every seven days interval. Plants were thinned to one plant/hole after a week from sowing. Weeding was carried out manually when needed. The nitrogen content and mineral contents of chicken manure were determined by Kjeldahl method and Atomic Absorption Spectrophotometer, respectively.

Experimental design and data collection

The field experiment was laid out in a Randomized Complete Block Design with four replicates .The treatments used were *Azospirillum brasilense* (A), *Azotobacter* sp (B), chicken manure (CM) at a rate of 7t/ha and nitrogen (N) in the form of urea (46% N).Urea was applied at two rates: 120kg/ha (N) and 40 kg/ha (T) , *Azospirillum brasilense* + urea (A+T), *Azotobacter* sp + urea (B+T), A+CM and B+CM in addition to the control. Data were recorded using a sample of six plants taken randomly from the outer two ridges in each plot. The data were collected at physiological maturity for yield components and after harvesting for grain yield. The data were collected to measure cob length, cob diameter, number of rows/cob, number of seeds/row, 100-seed weight, grain yield/plant and grain yield(t/ha). For the chemical composition, a sample of

Table 2. Effects of bio and chemical fertilizers on yield and yield components of maize at Shambat in 2009.

Treatments	Cob length (cm)	Cob diameter (mm)	No. of rows/cob	No. of grains/row	100-grain weight (g)	Grain yield/plant (g)	Grain yield (t/ha)
Control	11.23[c]	30.21[d]	12.25[c]	26.50[d]	9.88[c]	24.13[d]	2.00[c]
N	11.58[c]	32.27[c]	12.75[bc]	30.00[bc]	11.83[ab]	27.25[bc]	2.20[b]
A	11.00[c]	29.90[d]	12.75[bc]	28.75[cd]	10.81[bc]	24.40[cd]	1.98[c]
B	11.49[c]	31.55[c]	13.00[bc]	29.00[cd]	10.14[c]	24.12[d]	1.96[c]
CM	13.67[a]	35.09[a]	14.50[a]	33.75[a]	12.56[a]	32.75[a]	2.40[a]
A+CM	11.59[c]	32.60[bc]	13.00[bc]	30.50[bc]	12.44[a]	29.25[b]	2.32[ab]
B+CM	12.75[b]	33.51[b]	13.25[bc]	32.50[ab]	12.11[a]	32.39[a]	2.40[a]
A+T	11.57[c]	32.55[bc]	12.50[bc]	30.25[bc]	11.61[ab]	27.75[b]	2.25[b]
B+T	11.58[c]	32.00[c]	13.50[ab]	30.00[bc]	11.49[ab]	27.97[b]	2.26[ab]
Mean	11.83[a]	32.19[a]	13.06[a]	30.14[a]	11.43[a]	27.78[a]	2.19[a]
C.V%	5.02	2.37	5.29	6.04	7.35	7.52	4.81

Within each column, means have the same letter(s) are not significantly different according to DMRT at 5% level of significance.

Table 3. Effects of bio and chemical fertilizers on yield and yield components of maize at Shambat in 2010.

Treatments	Cob length (cm)	Cob diameter (mm)	No. of rows/ cub	No. of grains/row	100-grain weight (g)	Grain yield/plant (g)	Grain yield (t/ha)
Control	12.50[a]	29.75[c]	12.75[a]	26.00[cd]	12.41[a]	29.73[d]e	2.48[cd]
N	12.79[a]	34.75[a]	12.50[a]	28.50[abc]	13.43[a]	31.00[cd]	2.58[bc]
A	11.25[a]	29.00[c]	12.00[a]	24.25[d]	12.49[a]	25.15e	2.10[d]
B	12.39[a]	30.75[bc]	13.25[a]	27.25[bcd]	12.40[a]	28.87[d]e	2.40[cd]
CM	12.68[a]	35.50[a]	13.50[a]	30.75[a]	13.30[a]	38.75[a]	3.20[a]
A+CM	12.18[a]	33.75[ab]	12.25[a]	29.75[ab]	12.34[a]	35.75[abc]	2.97[ab]
B+CM	12.29[a]	34.50[a]	12.75[a]	30.50[ab]	12.45[a]	37.89[ab]	3.06[a]
A+T	12.04[a]	35.00[a]	12.25[a]	28.25[abc]	12.26[a]	29.95[cd]e	2.48[cd]
B+T	12.42[a]	34.28[a]	12.50[a]	29.25[abc]	12.60[a]	32.39[bcd]	2.56[bc]
Mean	12.28[a]	33.03[a]	12.64[a]	28.28[a]	12.63[a]	32.16[a]	2.65[a]
C.V%	6.36	7.00	7.91	8.40	6.49	12.43	11.09

Within each column, means have the same letter(s) are not significantly different according to DMRT at 5% level of significance.

seeds (50g) was taken from grain yield of each plot, mechanically ground and 5 g was taken to determine the moisture, ash, fat, crude fiber, crude protein and carbohydrates contents according to the methods of AOAC (1984). Tannins content was estimated quantitatively using modified vanillin HCl method (Price et al., 1978).

Statistical analysis

The data were subjected to standard statistical analysis following the procedures described by Gomez and Gomez (1984). Means were separated for significance using Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Grain yield components

The effect of various studied treatments on yield and its components was presented in Tables 2 and 3 for the years 2009 and 2010, respectively. Significant increments of the studied parameters were obtained mostly due to the application of chicken manure in the two years, compared to the control. Moreover, positive association effects due to the combined application of chicken manure with *Azospirillum brasilence* and/or *Azotobacter* sp were observed in some studied parameters. It is generally assumed that grain yield was influenced mainly by yield attributing components like length and diameter of cob, number of grains per cob and per row, grain weight, weight of 100 grains and filled and unfilled ratio.

Cob length and cob diameter are parameters that influence the number of grains per cob, grain size and subsequently grain yield. Application of CM increased cob length by 21% over the control in 2009 while in 2010

Table 4. Effects of bio and chemical fertilizers on biochemical characters of maize at Shambat in 2009.

Treatment	Moisture (%)	Dry (g)	Ash (g)	Fat (%)	Fiber (%)	Protein (%)	Tannin (%)	C (%)
Control	5.67[a]	94.33[a]	1.58[a]	3.38[a]	3.54[a]	6.13[a]	0.07[a]	79.71[ab]
N	5.70[a]	94.30[a]	1.63[a]	3.48[a]	3.99[a]	8.10[a]	0.08[a]	77.12[c]
A	5.72[a]	94.28[a]	1.70[a]	3.05[a]	3.14[a]	6.34[a]	0.09[a]	80.04[ab]
B	5.55[a]	94.45[a]	1.73[a]	3.13[a]	4.11[a]	7.00[a]	0.08[a]	78.49[abc]
CM	5.85[a]	94.15[a]	1.63[a]	3.53[a]	3.40[a]	6.71[a]	0.09[a]	78.89[abc]
A+CM	5.75[a]	94.25[a]	1.63[a]	3.10[a]	3.06[a]	6.13[a]	0.08[a]	80.34[a]
B+CM	5.50[a]	94.50[a]	1.80[a]	3.68[a]	3.18[a]	6.57[a]	0.09[a]	79.28[ab]
A+T	5.77[a]	94.23[a]	1.53[a]	3.93[a]	2.85[a]	6.35[a]	0.06[a]	79.58[ab]
B+T	5.62[a]	94.38[a]	1.85[a]	3.50[a]	3.62[a]	7.00[a]	0.06[a]	78.41[bc]
Mean	5.68[a]	94.32[b]	1.67[b]	3.42[b]	3.43[a]	6.70[b]	0.08[a]	79.10[a]
C.V%	4.07	0.25	11.34	19.73	20.79	13.99	47.97	1.60

* Within each column, means have the same letter(s) are not significantly different according to DMRT at 5% level of significance.

the treatments did not show significant increase in cob length. Comparison of individual treatments means indicated that maximum cob diameters were recorded for CM in both 2009 and 2010. Therefore, application of chicken manure was found to increase cob length and cob diameter. Such results could be attributed to the more availability and supply of nutrients which might have increased the production and enhanced the transfer of assimilates (Anees et al., 2016). Similarly, application of CM significantly ($p < 0.05$) increased the number of rows per cob. This result might be attributed to the stimulation effects of chicken manure on cell division and/or cell expansion or cell elongation (Yu et al*.,* 1999). Previously, Farhad et al. (2009) reported that number of rows per cob in maize was significantly ($p < 0.05$) affected by the application of different levels of poultry manure and the maximum values were recorded when applied at a rate of 12 t/ha. Number of grains/row is an important yield determining factor in maize. CM increased the number of grains/row by 27% and 18% compared to the control in 2009 and 2010, respectively. The combined application of chicken manure and the biofertilizres significantly ($p < 0.05$) increased the number of grains/row compared to the sole application of each biofertilizer. A + CM increased the number of grains/row by 12% compared to the sole application of A in 2009. Also, B+CM increased the number of grains/row by 22% compared to the sole application of B in 2010. Unexpectedly, the combined application of CM and biofertilizers showed non significant increase in number of grains/row compared to the sole application of CM. It has been previously reported that application of biofertilizer had significant effects on number of grains per row (Tarang et al*.,* 2013).

The results also indicated that maximum 100- grain weight (12.56) was recorded for CM application compared to the other treatments. Moreover, the combined effects of the application of chicken manure with the biofertilizers were superior to the use of biofertilizers alone in 2009. The percentages of increment were 15.07 and 19.42% for A+CM and B+CM, respectively, as compared to the sole application of A and B in 2009. This improvement in 100- grain weight could be attributed to the energy source provided to the microbes via organic manure thereby enhancing biological activities and availability of nitrogen. Similar observation and conclusions were also reported by Abdullahi et al. (2014). As shown in Tables 4 and 5, application of CM solely or in combination with A and B significantly ($p < 0.05$) increased grain yield/plant and grain yield (t/ha) in both years. CM increased the grain yield (t/h) by 20 and 30% over the control in 2009 and 2010, respectively. Several studies have revealed the positive effects of biofertilizers in combination with organic amendments to increase plant nutrients availability, up take and an increase in crop yield (Abdullahi et al., 2013). In 2009, the integrated application of A and B with 40kgN/ha produced grain yield/plant and grain yield/ha comparable to those obtained by the application of the recommended dose of Urea (120kgN/ha). This promoting effect of *Azospirillum brasilense* and *Azotobacter* sp could be attributed to their ability to produce biologically active substances, provide significant amount of available nitrogen through biological nitrogen fixation, improve photosynthesis performance and promote root growth which in turn enhance nutrients and water uptake there by resulting in crop improvement. Similar results were recorded previously by Naserirad et al. (2011) and Tarang et al. (2013).

Grain quality

The measured parameters of grain quality showed different responses towards the different treatments

Table 5. Effects of bio and chemical fertilizers on biochemical characters of maize at Shambat in 2010.

Treatments	Moisture (%)	Dry (g)	Ash (g)	Fat (%)	Fiber (%)	Protein (%)	Tannin (%)	C (%)
Control	6.97^{a}	93.03^{a}	1.99^{a}	3.85^{ab}	2.85^{a}	9.19^{b}	0.11^{a}	75.15^{cd}
N	7.15^{a}	92.85^{a}	1.67^{bc}	3.50^{bc}	2.42^{a}	8.56^{b}	0.08^{a}	76.70^{abc}
A	7.18^{a}	92.82^{a}	1.85^{ab}	3.60^{bc}	2.70^{a}	7.88^{b}	0.14^{a}	76.73^{abc}
B	7.27^{a}	92.73^{a}	1.62^{bc}	3.85^{ab}	2.89^{a}	8.32^{b}	0.08^{a}	76.05^{bc}
CM	6.59^{a}	93.41^{a}	1.62^{bc}	3.72^{b}	2.48^{a}	12.13^{a}	0.08^{a}	73.46^{d}
A+CM	6.68^{a}	93.32^{a}	1.58^{bc}	3.90^{ab}	3.04^{a}	8.31^{b}	0.08^{a}	76.50^{abc}
B+CM	6.55^{a}	93.45^{a}	1.85^{ab}	2.52^{bc}	2.79^{a}	7.88^{b}	0.10^{a}	77.40^{ab}
A+T	6.87^{a}	93.13^{a}	1.63^{bc}	4.25^{a}	2.65^{a}	8.46^{b}	0.12^{a}	76.14^{bc}
B+T	6.73^{a}	93.27^{a}	1.56^{c}	3.20^{c}	2.37^{a}	7.88^{b}	0.04^{a}	78.26^{a}
Mean	6.89^{a}	93.11^{b}	1.71^{b}	3.71^{a}	2.69^{b}	8.73^{b}	0.09^{b}	76.27^{a}
C.V%	9.52	0.70	11.63	5.59	14.40	12.38	76.81	1.75

* Within each column, means have the same letter(s) are not significantly different according to DMRT at 5% level of significance.

(Tables 4 and 5). In both years, microbial fertilizers, chicken manure and urea did not significantly (P<0.05) affect moisture, ash and dry matter contents of maize seeds. However, the highest values of moisture content (5.85 and 7.28%) were observed in 2009 when CM was applied and in 2010 when *Azotobacter* inoculum was used. The highest percentages of dry matter contents were recorded in 2009 (94.50%) and (93.45%) in 2010 under the application of B+CM. The highest values of fat contents were recorded in both years under the combined application of A and T. The percentages of increment in fat content were 16.27 and 10.38% over the control in 2009 and 2010, respectively. Fiber content is an important constituent for human food and animal feed. It is generally affected by environmental conditions, varietal characteristics and fertilizer treatments (Elsheikh and Mohameszein, 1998). The highest fiber contents were 4.11% recorded for B with an overall mean of 3.43 and CV 20.79% in 2009 and 3.04% for A+CM with an overall mean of 2.69% and CV 14.40% in 2010. Protein is the most important biochemical character. The highest percentage of protein content (12.13%) was recorded under the application of CM in both years. Such superior effect was achieved due to the increase of nitrogen supply which has paramount effect in the synthesis of protein (Anees et al., 2016). Tannins content is usually affected by many factors such as genotype, time of harvest and temperature .Tannins have been reported to possess anti carcinogenic and antimicrobial properties as well as anti-oxidant activities. The highest value of tannins content (0.14%) was recorded for A in 2010 and the lowest value (0.04%) was recorded for B+T in both years.

The highest values of carbohydrates were 80.34% recorded for A+CM in 2009 and 78.26% for B+T in 2010. These results indicated that carbohydrate content has increased due to the combined effects of the biofertilizers with either chicken manure or urea. These findings are previously reported by Mahfouze and Sharafeldin (2007) who recorded an increase of the total carbohydrate content by the application of biofertilizers combined with 50% nitrogen fertilizer. However, the combined analysis of variance for yield and its components across 2009 and 2010 (Table 6) indicated highly significant differences between the years (environment) and treatments and non-significant for Y×T interactions for the grain yield and its components except for the number of rows/cob .The combined analysis (Table 7) for grain quality indicated significant difference across the years for moisture, dry matter, fiber, protein and carbohydrate contents. Moreover, significant differences between the treatments and Y×T interaction for protein and carbohydrate contents were recorded. The significant differences in maize productivity observed in the two years indicate the strong environmental impact on the treatments. Our findings were in accordance with the previous reports which highlighted the positive effect of chicken manure in improving soil physical properties which enhances root development, water and nutrients uptake and consequently improve maize yield and grain quality (Brady and Weil, 2005; Habashy and Hemeid, 2011) The promoting effect of *Azospillium brasilense* and *Azotobacter* sp besides being nitrogen fixers could be attributed to the biologically active substances produced by these organisms such as auxin, cytokinin and amino acids.

Conclusion

The result of this study revealed that, maize grain yield and components are affected positively by the application of chicken manure and the combined application of nitrogen fixing bacteria with chicken manure or urea.

Table 6. Means some of squires from combined analysis of variance for yield and yield components of maize across 2009 and 2010

Characters	Source of variation			
	Year (Y) d.f=1	Treatment (T) Df=8	Y xT d.f=8	Pooled error df=48
Cob length/cm	3.68**	2.49**	1.14ns	0.51
Cob diameter/mm	12.84ns	30.96**	3.76ns	2.90
No of rows per cob	3.13ns	2.12ns	0.56ns	1.00
No of grains per row	62.35**	32.78**	3.19ns	5.10
100 grains wt(g)	25.87**	2.87**	1.56ns	0.74
Yield/ plant (g)	346.24**	116.88**	7.08ns	6.57
Yield ton/ha	3.70**	0.53**	0.10ns	0.04

* =highly significant at 1% level of probability, ** =significant at 5% level of probability, ns= is non- significance.

Table 7. Means some of squires from combined analysis of variance for grain quality of maize across 2009 and 2010.

Characters	Source of variation			
	Year (Y) d.f=1	Treatment (T) Df=8	Y xT d.f=8	Pooled error df=48
Moisture content	26.16**	0.15ns	0.18ns	0.34
Dry matter content	26.16**	0.15ns	0.18ns	0.34
Ash content	0.02ns	0.06ns	0.07ns	0.05
Fat content	1.56ns	0.40ns	0.29ns	0.26
Crude fiber content	9.77**	0.36ns	0.57ns	0.36
Crude protein content	74.24**	4.31**	4.41**	1.16
Tannin acid content	0.001ns	0.003ns	0.002ns	0.001
Carbohydrate content	144.13**	5.01**	6.37**	2.07

* =highly significant at 1% level of probability, ** =significant at 5% level of probability, ns= is non- significance.

Furthermore, considerable effects on the biochemical attributes used to measure the grain quality were also recorded. Environmental conditions had a clear impact on maize productivity.

Conflict of Interests

The authors have not declared any conflict of interests.

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Dynamics of biomass of pearl millet and Paiaguas palisadegrass in different forage systems and sowing periods in yield of soybean

Raoni Ribeiro Guedes Fonseca Costa[1], Kátia Aparecida de Pinho Costa[2]*, Renato Lara de Assis[2], Charles Barbosa Santos[2], Eduardo da Costa Severiano[2], Ana Flávia de Souza Rocha[1], Itamar Pereira de Oliveira[2], Pedro Henrique Campos Pinho Costa[2], Wender Ferreira de Souza[2] and Millena de Moura Aquino[2]

[1]State University of Goiás (Universidade Estadual de Goiás - UEG, Campus Quirinópolis), Quirinópolis, GO, Brazil.
[2]Goiás Federal Institute (Instituto Federal Goiano – IF Goiano, Campus Rio Verde), Rio Verde-Goiás, GO, Brazil.

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There is increasing global concern with environmental food production and sustainability to maintain high carbon stocks in soil biomass. The biomass produced in crop-livestock integration system increases soil organic matter, acts in nutrient cycling, improves the physical, chemical and biological characteristics of soil and increases grain production. Moreover, this soil management system mitigates greenhouse effect and preserves the environment. However, in the Savannah of Central Brazil region, an efficient mulching production of biomass is one of the factors limit sustainability of direct seeding of soybean, mainly due to accelerated decomposition of crop residues. Thus, this study aimed to evaluate biomass dynamics of pearl millet and Paiaguas palisadegrass in different forage systems and sowing periods on soybean yield. The experiment followed a randomized block design with a 5 x 2 factorial arrangement and three replications, under five forage systems (monocropped pearl millet, monocropped Paiaguas palisadegrass, pearl millet intercropped in rows with Paiaguas palisadegrass, pearl millet intercropped between rows of Paiaguas palisadegrass and pearl millet oversown and intercropped with Paiaguas palisadegrass) at two sowing periods (February and March). The results showed that Paiaguas palisadegrass in a monocropped system generated the highest biomass production and lowest carbon/nitrogen ratio. The highest carbon/nitrogen ratio and cumulative biomass loss occurred with millet in monocropped and intercropped with oversown Paiaguas palisadegrass in which biomass production was reduced by plant competition. Paiaguas forage systems in palisadegrass monocropped and intercropping on and between rows supported higher yield of soybean. The second sowing periods resulted in higher production of remaining biomass and grain yield, in all forage systems. Intercropping in crop-livestock integration systems showed a promising cultivation technique to maintain a sustainable stock of soil carbon.

Key words: Carbon/nitrogen ratio, half-lives, [*Pennisetum glaucum* (L.) R. Br.], *Urochloa brizantha* cv. BRS Paiaguas.

INTRODUCTION

There is a growing global concern about climate changes on the planet, result mainly from rising levels of carbon dioxide (CO_2) and other greenhouse gases, such as methane (CH_4) and nitrous oxide (N_2O). However, some systems of land management in different biomes of Brazil, such as the no-tillage system (NTS), the crop-livestock integration system (CLIS) under no-tillage, the adoption of reforestation and management of rangelands, may significantly alter the carbon inventory and other greenhouse gas emission from the soil to the atmosphere and therefore be important in relation to the mitigation of global warming (Carvalho et al., 2010).

In this sense, the consortium in integrated crop-livestock system is a soil management system mitigating greenhouse gas emissions and preserving environmental sustainability due to an increased efficiency in storing carbon in the soil (sequestration of atmospheric carbon). When carried out correctly it can be efficient in soil carbon storage and modulating soil Carbon to Nitrogen ratio, by operating through the root plant system. It also increasesbiomass production to better cover the ground for establishingtillage system to increase sequential crop productivity (Chioderoli et al., 2010) without interfering with the productivity of the intercropped annual crop (Ribeiro et al., 2015; Costa et al., 2016).

So, the production of biomass in no-tillage systems increases diversification when using intercropping, which minimizes the risk of crop losses and provides options for the adoption of crop succession and rotation (Horvathy Neto et al., 2012). In addition, biomass improves the physical, chemical, and biological conditions of the soil, aiding in weed control and in the stabilization of production as well as in the recovery and maintenance of soil quality. One of the ways to achieve these results is intercropping by means of an integrated crop-livestock system (Boer et al., 2008).

However, the correct choice of plant species for the production of biomass on the soil surface is extremely important for the success of a no-tillage system because the climatic factors characteristic of each region and soil type must be considered (Costa et al., 2015). In the region, the climate is characterized by a dry winter, high temperatures throughout the year, and a long dry season. These climatic conditions hinder the establishment of cover crops, mainly by hindering the production of biomass in the crop area, and constitute some of the greatest obstacles to maintaining a no-tillage system (Pacheco et al., 2011).

Therefore, the provision of an efficient biomass soil cover is one of the factors that limit the sustainability of no-tillage systems in the Brazilian savannah region, mainly due to the fast decomposition of residues. This situation reinforces the need for producing plant residues with a slower decomposition rate to maintain the residue on the ground for a longer duration, especially in the off-season (Costa et al., 2015).

Several crops have been tested and utilized for the purpose of producing vegetation cover to ground and for adoption of no-tillage system. Among the most promising highlight millet crop, for presenting resistance to water stress, good biomass production and high carbon/nitrogen ratio, which provides greater persistence of straw on the soil surface (Soratto et al., 2012). However, the grasses of the genus *Urochloa*, are also widely used, with promising results, and may or may not be under intercropping (Costa et al., 2016). Forage plants are efficient in organic matter supply, determine the improvement in physical structure and soil chemistry, favors the conservation of soil moisture and increases the biodiversity, a fact clearly observed in areas of crop-livestock integration (Krutzmann et al., 2013).

Although known the benefits of intercropping systems and tillage system, more appropriate is scarce information about the vegetation cover, especially the millet intercropped with *Urochloa* spp. in off-season conditions and with regard to the recommendations of construction and operation of grain production. Thus, this study aimed to evaluate the dynamics of biomass of millet [*Pennisetum glaucum* (L.) R. Br.] and Paiaguas palisadegrass (*Urochloa brizantha* cv. BRS Paiaguas) in different forage systems and sowing periods in yield of soybean in crop-livestock integration.

MATERIALS AND METHODS

The experiment was conducted in the field (17°48’ S; 50°55’ W; and 748 m altitude), in the municipality of Rio Verde, Goiás, Brazil, during the 2014 off-season in a Latossolo Vermelho Distroférrico (Embrapa, 2013), an Oxisol. Soil samples were collected before planting to determine the physical and chemical characteristics of the 0-20 cm soil layer. The results were as follows: 600, 140 and 260 g kg^{-1} of clay, silt and sand, respectively; pH in $CaCl_2$: 6.02; Ca: 3.50 $cmol_c$ dm^{-3}; Mg: 1.43 $cmol_c$ dm^{-3}; Al: 0.05 $cmol_c$ dm^{-3}; Al+H: 5.90 $cmol_c$ dm^{-3}; K: 0.35 $cmol_c$ dm^{-3}; CEC: 11.18 $cmol_c$ dm^{-3}; V: 47.22%; P (Mehlich): 2.29 mg dm^{-3}; Cu: 3.50 mg dm^{-3}; Zn: 5.10 mg dm^{-3}; Fe: 34.1 mg dm^{-3}; O.M.: 37.06 g kg^{-1}.

The experiment followed a randomized block design with a 5 x 2 factorial arrangement and three replications, comprising five forage systems (monocropped pearl millet, monocropped Paiaguas palisadegrass, pearl millet intercropped in rows with Paiaguas palisadegrass, pearl millet intercropped between rows of Paiaguas palisadegrass and pearl millet oversown and intercropped with Paiaguas palisadegrass) and two sowing periods (February and March). The ADR 8010 pearl millet hybrid, which is medium-sized and dual purpose (grain production and forage) was used.

Before implementing the experiment, the area was cultivated with saccharine sorghum and *Urochloa brizantha* cv. Marandu, which, after desiccation, led to an increase in organic matter (OM) in the soil. The area was prepared by desiccating the weeds using 3.5 L

*Corresponding author. E-mail: katia.costa@ifgoiano.edu.br.

ha^{-1} glyphosate (480 g L^{-1} acid equivalent), with a spray volume of 150 L ha^{-1}. At 30 days after desiccation, harrowing was conducted with a disc harrow to eliminate weeds not controlled by the herbicide, followed by subsoiling and level harrowing. Soil acidity was corrected with calcitic lime with 100% TRNP (total relative neutralizing power), with the application of 675 kg ha^{-1} at 30 days before sowing.

One week before implementing the experiment, level harrowing was conducted again, and the field was sown in furrows using a seeder with 0.50 m between rows spacing. The furrows for sowing Paiaguas palisadegrass in between rows and for oversowing pearl millet were manually dug to a 3 cm depth using hoes. Sowing was carried out on February 12 and March 4 for the first and second periods, respectively, using 240 kg ha^{-1} of P_2O_5 (single superphosphate form) and 20 kg ha^{-1} of FTE BR 12 fertilizer.

Monocropped and intercropped pearl millet was sown at a 3 cm depth. Paiaguas palisadegrass was sown in the rows at a 6 cm depth and in between rows at 0.25 m from the pearl millet rows, and in the oversown system, it was sown 15 days after sowing pearl millet in the between rows at 0.25 m. For pearl millet, 12 kg of seeds ha^{-1} were used, seeking to obtain a final population between 250 and 300 thousand plants ha^{-1}, and 5 kg of pure viable seeds per hectare were used for the forage species. The plots comprised eight 3.0 m long rows in all forage systems. The usable area was obtained by only considering the four central rows and eliminating 0.5 m from the end of each row. At 30 and 50 days after sowing (DAS), 60 kg ha^{-1} nitrogen and 40 kg ha^{-1} K_2O (in urea and potassium chloride forms, respectively) were applied by casting.

Manual weeding was conducted weekly until 50 DAS for post-emergence weed control. The fall armyworm (*Spodoptera frugiperda*) was controlled using two applications of chlorpyrifos (1 L ha^{-1}) and teflubenzuron (50 ml ha^{-1}), performed at 40 and 50 DAS, and two applications (37 and 44 DAS) of azoxystrobin + cyproconazole (0.5 L ha^{-1}). Pearl millet grains were manually harvested at 115 and 118 DAS for the first and second sowing periods, respectively, when the plants were at the physiological maturity stage. The remainder of the plants (stems and leaves) were left at the site for the evaluation of biomass.

After the pearl millet was harvested, the dry weight production of the Paiaguas palisadegrass was evaluated in the off-season crop (simulating grazing) over successive cuttings, in which 1 m^2 samples were collected by randomly placing a quadrat within each plot and cutting at a 0.20 m height from the ground to maintain an adequate residue height to promote grass regrowth. The first cutting was performed at the time of the pearl millet harvest, on 06/04/2014 and 06/24/2014 for the first and second periods, respectively. The second cutting occurred 79 days after the first, on 08/22/2014 (first period), and 72 days after the first, on 09/04/14 (second period). After both cuttings were performed, standard cutting of all plants at the experimental site was carried out, at the same height as for the evaluated plants, and the resulting residue was removed from the site. Next, the Paiaguas palisadegrass was left to rest for regrowth, to allow it desiccate it to form biomass for soybean planting during the next crop season.

Desiccation was conducted on 10/31/2014 through application of the herbicide glyphosate at a dose of 4.5 L ha^{-1} (588 g/L) and spray volume of 150 L ha^{-1}. To quantify biomass production, biomass samples were collected one day before planting of the soybean crop by randomly placing a $1m^2$ quadrat within each plot. The plant material inside the quadrat was cut, using a height of 0.05 m from the soil surface as a reference. The cut material was weighed, and the samples were dried to constant weight in a forced-air oven at 55°C, and the amounts were extrapolated to kg ha^{-1}.

Intacta RR 2 PRO soybean cultivar M 7110 plants were sown under a no-tillage system above the biomass of the forage systems. At sowing, the soybeans were inoculated with strains of *Bradyrhizobium japonicum* (SEMIA 5079 – CPAC 15 and SEMIA 5080 – CPAC 7), with a minimum guarantee of 7.2 x 10^9 CFU/g (Biomax Premium Turfa commercial brand) in the following proportion: 60 g / 50 kg of seeds. Sowing was carried out on 11/20/2014 using a seeder, with the application of 120 kg ha^{-1} P_2O_5, 30 kg ha^{-1} K_2O, 2 kg ha^{-1} boron, 0.4 kg ha^{-1} molybdenum and 6 kg ha^{-1} zinc, in the form of single superphosphate, potassium chloride, boric acid, molybdenum sulfate and zinc sulfate, respectively.

Weeds were controlled using the herbicide Transorb (3.5 L ha^{-1}), with a spray volume of 150 L ha^{-1}, on 12/17/2014. One application of the insecticide chlorpyrifos (L ha^{-1}) was performed to control the soybean caterpillar (*Anticarsia gemmatalis*), and one preventive application of the fungicide azoxystrobin + cyproconazole (0.3 L ha^{-1}) was performed on 01/13/2015. To quantify biomass production, straw samples were collected by randomly placing a $1m^2$ - square within each plot. The plant material was cut, using a height of 0.05 m from the soil surface as a reference. The cut material was weighed, and the samples were dried to constant weight in a forced-air oven at 55°C, and the amounts were extrapolated to kg ha^{-1}.

After this management procedure (cutting), the fresh biomass from each plot was placed in nylon litter bags for decomposition (Thomas and Asakawa, 1993). The bags consisted of a 2 mm mesh and measured 15 x 20 cm. Four bags containing residues of the studied species in an amount proportional to the dry biomass produced per hectare were deposited in direct contact with the soil. At 30, 60, 90 and 120 days after cutting, one litter bag was removed from each plot to evaluate the remaining biomass and determine the decomposition time during the 120 day period (soybean harvest).

After cleaning the material in the laboratory to remove adhered soil, it was dried in an oven at 55°C for 72 h to obtain the dry biomass. Next, samples of the plant material were ground to determine the concentration of the following macronutrients according to the method proposed by Malavolta et al. (1997): nitrogen (N) and carbon (C).

Assessments of agronomic characteristics of soybean were held on 03.17.2015 to 117 DAS. They were evaluated in the final stand of soybean plants (plant number count sequenced in 3 samples of 1m linear, the usable area of the plot); plant height and height of insertion of the first and final pod (measures of distance between the ground surface and the apical end and between the soil surface to the insertion of the first and last pod on the main stem, respectively); number of pods per plant (counting all pods with grain in 10 plants in the useful area of the plot, calculating the average number of pods per plant).

To estimate the weight of 100 seeds and grain yield in kg ha^{-1} were collected in the useful area of each parcel, a sample of all plants contained in three core lines with a meter. These plants, after dried in the sun, were mechanically weighted and threshed by a stationary threshing. The grains obtained were weighed on a precision scale (0.01 g). Then was pulled out a grain sample to determine the moisture carried out with the aid of a digital moisture determiner and later held the mass of the correction of the output of 13% moisture, turning it into kg ha^{-1}. During the experiment, daily rainfall and the mean monthly temperature were monitored.

Data of agronomic characteristics and soybean yield, biomass production and C / N ratio subjected to analysis of variance and the means were compared using Tukey's test, with a significance level of 5%. Statistical analyses were performed using SISVAR 4.6 statistical software (Ferreira, 2011).

To describe the decomposition of plant residues, the data were fit to an exponential mathematical model, using the SigmaPlot application. For regression equation comparisons, the procedure described in Snedecor and Cochran (1989) was used after data linearization.

To calculate the half-life ($t_{1/2}$), that is, the time required for 50% of the remaining biomass to be decomposed, the Paul and Clark (1989) equation was used: $t_{1/2}=0.693/k$, where $t_{1/2}$ is the half-life of the dry biomass, and k is the decay constant of the dry biomass.

Table 1. Biomass production (soybean sowing) and remaining biomass at 30, 60, 90, and 120 days of pearl millet and Paiaguas palisadegrass in monocropped and intercropped under different forage systems and sowing periods.

Forage system	Sowing period	
	First	Second
	Soybean sowing (kg ha^{-1})	
Monocropped pearl millet	1.466^{Bb}	1.629^{Ba}
Monocropped Paiaguas palisadegrass	2.766^{Ab}	3.810^{Aa}
Row pearl millet x Paiaguas palisadegrass	1.422^{Bb}	1.792^{Ba}
Between rows pearl millet x Paiaguas palisadegrass	1.370^{Bb}	1.623^{Ba}
Oversown pearl millet x Paiaguas palisadegrass	560^{Cb}	713^{Ca}
CV (%)	.... 21.71	
	30 days (kg ha^{-1})	
Monocropped pearl millet	739^{Bb}	821^{Ba}
Monocropped Paiaguas palisadegrass	1.304^{Ab}	1.898^{Aa}
Row pearl millet x Paiaguas palisadegrass	692^{Bb}	983^{Ba}
Between rows pearl millet x Paiaguas palisadegrass	435^{Cb}	794^{Ba}
Oversown pearl millet x Paiaguas palisadegrass	368^{Cb}	445^{Ca}
CV (%)	.... 25.73	
	60 days (kg ha^{-1})	
Monocropped pearl millet	610^{Bb}	740^{Ba}
Monocropped Paiaguas palisadegrass	1.151^{Bb}	1.616^{Aa}
Row pearl millet x Paiaguas palisadegrass	593^{BCb}	821^{Ba}
Between rows pearl millet x Paiaguas palisadegrass	399^{Cb}	676^{Ba}
Oversown pearl millet x Paiaguas palisadegrass	335^{Cb}	405^{Ca}
CV (%)	.... 24.15	
	90 days (kg ha^{-1})	
Monocropped pearl millet	609^{Bb}	685^{Ba}
Monocropped Paiaguas palisadegrass	1.064^{Ab}	1.536^{Aa}
Row pearl millet x Paiaguas palisadegrass	501^{Bb}	660^{Ba}
Between rows pearl millet x Paiaguas palisadegrass	370^{Cb}	564^{Ba}
Oversown pearl millet x Paiaguas palisadegrass	278^{Cb}	373^{Ca}
CV (%)	.... 26.07	
	120 days (kg ha^{-1})	
Monocropped pearl millet	604^{Bb}	695^{Ba}
Monocropped Paiaguas palisadegrass	1.012^{Ab}	1.347^{Aa}
Row pearl millet x Paiaguas palisadegrass	494^{BCb}	589^{Ba}
Between rows pearl millet x Paiaguas palisadegrass	346^{Cb}	499^{Ba}
Oversown pearl millet x Paiaguas palisadegrass	215^{Ca}	298^{Ca}
CV (%)	.... 30.01	

Means followed by different uppercase letters in the columns (forage systems) and lowercase letters in the rows (sowing periods) differ from each other according to Tukey's test at the 5% probability level.

RESULTS AND DISCUSSION

Production and decomposition of biomass

The production of biomass was affected (P<0.05) by the sowing period and forage system. However, there was no effect (P>0.05) of the interaction between forage system and sowing period (Table 1).

For the first and second sowing periods, the highest biomass was obtained in the Paiaguas palisadegrass monocropped, which showed biomass production satisfactory for use in the no-tillage system. After the pearl millet harvest, two cuttings of the Paiaguas palisadegrass plants were performed in the forage systems, and new tillers sprouted at the beginning of the rainy season (September). This resulted in the production of 3.810 and 2.766 kg ha^{-1} of biomass for the first and second periods, respectively, thus forming the soil cover for soybean planting (Table 1). These results indicate that Paiaguas palisadegrass has favorable features for use in integrated crop-livestock systems, such as forage production during the dry season, with good regrowth. Franchini et al. (2009) reported that the higher total soil cover can bring benefits, such as nutrient recycling and improvements in soil physical and biological conditions.

Similar results were obtained from biomass by Costa et al. (2015), who evaluated the production of straw forage species (pearl millet, sorghum and Xaraes palisadegrass) verified biomass of Xaraes palisadegrass of 3607 and 3867 kg ha^{-1}, for the harvest of 2009/2010 and 2010 / 2011 respectively. The lowest biomass was obtained when the Paiaguas palisadegrass was intercropped with pearl millet under oversowing (Table 1), thus showing that this type of sowing system affects grass development. This result occurred because the Paiaguas palisadegrass was established 15 days after pearl millet sowing, increasing the shading by the pearl millet plants of the Paiaguas palisadegrass during its early germination stages (Costa et al., 2016). Thus, there was a decrease in forage production, directly affecting the production of biomass in the no-tillage system. A similar result was obtained by Gazola et al. (2013), who showed that shading by corn plants affected dry biomass production by *Urochloa brizantha* cv. Marandu and *Urochloa ruziziensis* due to the delayed emergence of the *Urochloa* plants. Seidel et al. (2014) evaluated dry biomass production by MG-4 palisadegrass and observed a decrease of 81.70 and 62.56% when the grass was sown in row and between rows, respectively, 25 days after corn sowing. Notably the Paiaguas palisadegrass among many *Urochloa* cultivars, can be one of the most suitable in crop-livestock integration systems to settle with less water availability and produce during the off-season (Costa et al., 2016). Thus, it can be considered excellent choice as producing crop biomass, aimed at soybean sowing in no-tillage system in the Southwest region of Goiás, Brazil.

In addition to these favorable features, the results obtained by Machado and Vale (2011) showed that in the three years of evaluation, Paiaguas palisadegrass (B 6 lineage) was not only the most productive option but also the one showing the highest desiccation efficiency, when compared with the genotypes Marandu, MG-4, Xaraes, Piata, and Arapoty. This feature is important because at least 21 days are needed after herbicide is applied to the forage before soybeans can be sown. Regarding to the sowing date (Table 1) in all forage systems the second sowing periods showed higher production of biomass and biomass remaining at 30, 60, 90 and 120 days. This result may be correlated with the uneven distribution of rainfall in February, with frequent dry spells observed at the beginning of emergence, which impaired the initial plant development forage systems (Costa et al., 2016), which was influenced at final production of biomass.

Table 1 shows that for the remaining biomass at 30, 60, 90, and 120 days, the forage systems containing pearl millet in monocropped and intercropped with Paiaguas palisadegrass in row and between rows showed similar results but differed ($P<0.05$) from those containing oversown and intercropped pearl millet and monocropped Paiaguas palisadegrass. The cespitose growth habit observed in both Paiaguas palisadegrass and pearl millet most likely contributed to a similar decomposition rate in these systems, as it enabled not only a better biomass distribution on the soil but also a similar biomass production, thus resulting in less contact of the biomass with the soil. Given the above, it can be concluded that these sowing methods did not affect the remaining biomass when compared with that of the monocropped pearl millet because the latter showed a lower biomass loss due to its greater amount of lignified material. This again shows the importance of intercropping for biomass production within no-tillage systems in the Cerrado region.

Assessing the consortium corn with *Urochloa brizantha* cv. Xaraes and *Urochloa ruziziensis* in the Mato Grosso do Sul State, Costa et al. (2014) found that between 90 and 120 days after handling, decomposition of the remaining biomass showed decreasing effect, and the temperature and rainfall favored the rapid decomposition of the straw on the soil surface in Brazilian savannah region. Similar results were obtained in this study for the two sowing dates.

Regarding the sowing periods, lower remaining biomass amounts were observed for the second sowing period at 30 and 60 days for Paiaguas palisadegrass in monocropped and intercropped in row and between rows as well as at 90 days for the Paiaguas palisadegrass in monocropped and intercropped in between rows. Moreover, at 120 days, only the Paiaguas palisadegrass in monocropped showed a lower amount of remaining biomass (Table 1). This result can be explained by the longer forage rest duration during the first period (79 days) compared with the second period (72 days), thus resulting in better use of the September rains and allowing better regrowth. With the exception of pearl millet in monocropped and oversown as well as intercropped with Paiaguas palisadegrass, the first sowing period provided higher biomass production. This result can be related to the uneven distribution of rainfall from May to August, when low rainfall was observed, hindering plant development when the sowing was conducted in March.

When evaluating the cumulative percentage loss (Table 2) for the first sowing period, all the assessments showed the lowest loss for pearl millet oversown and intercropped

Table 2. Cumulative loss biomass of pearl millet and Paiaguas palisadegrass in monocropped and intercropped under different forage systems and sowing periods.

Forage system	Sowing period	
	First	Second
	Cumulative loss (%) - 30 days	
Monocropped pearl millet	49.60^{Aa}	49.33^{Aa}
Monocropped Paiaguas palisadegrass	51.80^{Aa}	53.14^{Aa}
Row pearl millet x Paiaguas palisadegrass	50.14^{Aa}	51.69^{Aa}
Between rows pearl millet x Paiaguas palisadegrass	51.08^{Aa}	55.27^{Aa}
Oversown pearl millet x Paiaguas palisadegrass	42.39^{Ba}	48.44^{Aa}
CV (%)	.... 7.57.....................	
	Cumulative loss (%) - 60 days	
Monocropped pearl millet	52.50^{Aa}	51.94^{Aa}
Monocropped Paiaguas palisadegrass	58.47^{Aa}	58.33^{Aa}
Row pearl millet x Paiaguas palisadegrass	54.08^{Aa}	57.77^{Aa}
Between rows pearl millet x Paiaguas palisadegrass	57.42^{Aa}	58.94^{Aa}
Oversown pearl millet x Paiaguas palisadegrass	46.50^{Ba}	53.03^{Aa}
CV (%)	.... 6.38	
	Cumulative loss (%) - 90 days	
Monocropped pearl millet	57.83^{Aa}	55.28^{Aa}
Monocropped Paiaguas palisadegrass	60.64^{Aa}	61.22^{Aa}
Row pearl millet x Paiaguas palisadegrass	63.25^{Aa}	62.47^{Aa}
Between rows pearl millet x Paiaguas palisadegrass	64.59^{Aa}	61.99^{Aa}
Oversown pearl millet x Paiaguas palisadegrass	50.16^{Ba}	56.05^{Aa}
CV (%)	.... 5.41	
	Cumulative loss (%) - 120 days	
Monocropped pearl millet	60.94^{BCa}	58.41^{Aa}
Monocropped Paiaguas palisadegrass	64.80^{Aba}	63.19^{Aa}
Row pearl millet x Paiaguas palisadegrass	69.30^{Aa}	65.03^{Aa}
Between rows pearl millet x Paiaguas palisadegrass	68.58^{Aa}	64.55^{Aa}
Oversown pearl millet x Paiaguas palisadegrass	54.91^{Ca}	59.55^{Aa}
CV (%)	.... 4.55	

Means followed by different uppercase letters in the columns (forage systems) and lowercase letters in the rows (sowing periods) differ from each other according to Tukey's test at the 5% probability level.

with Paiaguas palisadegrass, which differed from the losses in the other forage systems. However, for the second period, the values were similar, with no significant effect (P>0.05) of forage system on the loss. The same result was observed when the sowing periods were compared. The greatest percentage decrease in biomass occurred at 30 days after management due to the initial decomposition of the less lignified leaves and other materials, which were easily decomposed (Wolf and Wagner, 2005). Thus, the presence of Paiaguas palisadegrass in the intercropped systems contributed to a more rapid decomposition of the biomass because of the high leaf production of this grass. Conversely, the smallest percentage loss values were found in the monocropped pearl millet and pearl millet oversown and intercropped with Paiaguas palisadegrass, which mostly contributed greater amounts of lignified material to the biomass due to the predominance of pearl millet.

In general, the forage plant biomass durability is evaluated using the carbon/nitrogen ratio of the plant, with higher decomposition below a ratio of 25:1 (Costa et al., 2015). Thus, the higher carbon/nitrogen ratio found in pearl millet is responsible for the lower biomass decrease found in these systems.

Studies evaluating the cumulative biomass loss for the no-tillage system are still scarce for intercropped systems of pearl millet with *Urochloa* species. However, for the corn crop, some studies have already evaluated

Table 3. Carbon/nitrogen ratio of pearl millet and Paiaguas palisadegrass in monocropped and intercropped under different forage systems and sowing periods.

Forage system	Sowing period	
	First	**Second**
	C/N ratio - soybean sowing	
Monocropped pearl millet	49.01^{Ab}	53.61^{Aa}
Monocropped Paiaguas palisadegrass	36.65^{Ba}	34.21^{Ba}
Row pearl millet x Paiaguas palisadegrass	35.72^{Ba}	35.66^{Ba}
Between rows pearl millet x Paiaguas palisadegrass	32.42^{Ba}	31.18^{Ba}
Oversown pearl millet x Paiaguas palisadegrass	35.30^{Ba}	38.92^{Ba}
CV (%)	.... 6.21	
	C/N ratio - 30 days	
Monocropped pearl millet	45.48^{Ab}	52.25^{Aa}
Monocropped Paiaguas palisadegrass	29.41^{Ca}	30.46^{Ca}
Row pearl millet x Paiaguas palisadegrass	28.27^{Ca}	29.78^{Ca}
Between rows pearl millet x Paiaguas palisadegrass	31.41^{BCa}	29.35^{Ca}
Oversown pearl millet x Paiaguas palisadegrass	37.75^{Ba}	38.12^{Ba}
CV (%)	.... 8.30	
	C/N ratio - 60 days	
Monocropped pearl millet	42.53^{Ab}	50.39^{Aa}
Monocropped Paiaguas palisadegrass	33.70^{Ba}	34.50^{Ca}
Row pearl millet x Paiaguas palisadegrass	30.82^{Ca}	32.17^{Ca}
Between rows pearl millet x Paiaguas palisadegrass	33.35^{Ba}	31.39^{Ca}
Oversown pearl millet x Paiaguas palisadegrass	38.13^{ABa}	38.99^{Ba}
CV (%)	.... 5.85	
	C/N ratio - 90 days	
Monocropped pearl millet	46.31^{Aa}	46.11^{Aa}
Monocropped Paiaguas palisadegrass	37.41^{Ba}	37.75^{Ba}
Row pearl millet x Paiaguas palisadegrass	33.46^{Ca}	33.93^{Ca}
Between rows pearl millet x Paiaguas palisadegrass	34.56^{Ca}	33.87^{Ca}
Oversown pearl millet x Paiaguas palisadegrass	39.10^{Ba}	40.34^{Ba}
CV (%)	.... 5.10	
Monocropped Perola millet	C/N ratio - 120 days	
Monocropped Paiaguas palisadegrass	46.36^{Aa}	49.17^{Aa}
Row pearl millet x Paiaguas palisadegrass	39.94^{Ba}	30.89^{Ca}
Between rows pearl millet x Paiaguas palisadegrass	35.94^{Ba}	36.33^{Ba}
Oversown pearl millet x Paiaguas palisadegrass	36.85^{Ba}	35.03^{Ba}
CV (%)	41.11^{ABa}	41.20^{Ba}
	.... 5.50	

Means followed by different uppercase letters in the columns (forage systems) and lowercase letters in the rows (sowing periods) differ from each other according to Tukey's test at the 5% probability level.

intercropping with species of *Urochloa*, and Costa et al. (2014) found that the cumulative loss percentage of the remaining biomass was 15 to 60% at 120 days. Kliemann et al. (2006) observed a relative loss of 56% by 150 days, and Santos et al. (2014a) found that one half of the dry cumulative biomass had decomposed by 115 days.

Compared with the other forage systems, the monocropped pearl millet showed a higher carbon/ nitrogen ratio ($P<0.05$) for both sowing periods at soybean planting at 30 and 90 days. At 60 and 120 days, the carbon/nitrogen ratio of the pearl millet was similar ($P<0.05$) to that in the intercropping with oversown Paiaguas palisadegrass (Table 3) because of the greater competition in this system.

Boer et al. (2008) found a carbon/nitrogen ratio of 34:1 for the ADR500 pearl millet at the full flowering stage, a ratio lower than that found in the present study. The decomposition rate is directly related to the carbon/ nitrogen ratio of the residue on the soil. This relationship indicates the potential of these cover plants for soil cover maintenance due to the greater soil residue persistence, especially in the off-season.

The intercropping of pearl millet with Paiaguas palisadegrass in various forage systems provided lower carbon/nitrogen ratio values because of the higher production by Paiaguas palisadegrass of leaves, which rapidly decompose, influencing the end result of the carbon/nitrogen ratio. Pacheco et al. (2011) found that the lower carbon/nitrogen ratio (34:1) found in *Urochloa ruziziensis* compared with pearl millet (61:1) at 200 days after sowing was caused by the higher proportion of leaves to stems of the former in response to high regrowth rates after the early summer rains.

A downward trend was observed in the carbon/nitrogen ratio when comparing the soybean sowing assessments through 120 days. This tendency was due to decreases in the biomass C concentrations arising from an increase in the microbial population during the early stages of decomposition because these organisms use the N available in the soil to metabolize C, thus immobilizing it. As the decomposition continues, the increase in soil microbiota leads to a higher C consumption in the biomass and to a decrease in the carbon/nitrogen ratio, with greater mineralization being observed when this ratio is near 20:1.

Note that a lower carbon/nitrogen ratio can shorten the duration of biomass retention on the soil surface, especially under the cerrado climate conditions, characterized by high temperatures and humidity (Kliemann et al., 2006). However, a lower carbon/nitrogen ratio at the end of the off-season provides a faster mobilization of nutrients to the soil after its desiccation, thus favoring the annual crops in rotation (Pacheco et al., 2011). Nevertheless, in all the forage systems of the present study, the carbon/nitrogen ratio was above 30:1, a reference value for the carbon/nitrogen ratio considered by Trinsoutrot et al. (2000) to be high enough to characterize residue as suitable for use in a no-tillage system.

Certain studies indicate that intercropping may favor an increase in dry biomass, which is essential for the no-tillage system, providing an increase in the carbon/ nitrogen ratio and contributing to a reduction in the biomass decomposition rate (Kliemann et al., 2006; Seidel et al., 2014). In these studies, higher carbon/ nitrogen ratios were observed for the intercropping of corn with the grasses *Urochloa brizantha* cv. Marandu and *Urochloa brizantha* cv. MG-4, and such ratios were conditioned by the higher amount of stem and lignified material in corn compared with pearl millet in the present study, which favored the intercropping system.

Table 4 shows the regression equations for the decomposition of remaining biomass and the coefficients Po and k for the regression equation $P=Po^{-kt}$ and R^2 as well as the coefficients of determination (R^2) for the biomass decomposition of the different forage systems, which were determined in both sowing periods from 0 to 120 days after management. The half-life was lower in both sowing period treatments for pearl millet intercropped with Paiaguas palisadegrass in row and between rows, when compared with the other forage systems. These results are due to the lower carbon/ nitrogen ratio (Table 3) of these combined materials, caused by a higher leaf production by Paiaguas palisadegrass and consequent increase in the rate of biomass decomposition.

The highest half-life values were observed in the pearl millet oversown and intercropped with Paiaguas palisadegrass (first period) and in monocropped pearl millet (second period) (Table 4). These results are explained by the relatively large amount of lignified material in pearl millet, which thus stays on the ground for a relatively long time, and its short development cycle. Floss (2000) reported that residues decompose more slowly as their lignin contents and carbon/nitrogen ratios increase.

The half-lives of pearl millet were also evaluated by Boer et al. (2008), who found a value of 105 days when the millet was managed at full flowering, and by Assis et al. (2013), who found a half-life of 187 days for ADR 500 pearl millet. The differences among the half-lives observed by these authors and detected in the present study are due, among other factors, to the genotype examined: ADR 500 is a variety used specifically for the production of soil biomass cover, whereas ADR 8010 has a dual purpose, being used for both grain and biomass production. In addition, the pearl millet was desiccated in these previous studies during the flowering stage, when there was a greater stem diameter and consequently a greater amount of lignified material in the biomass, which slowed its decomposition and increased its half-life.

The relatively high biomass loss during the first days for both sowing periods. This high loss can be explained by the large amount of leaves, which are easily decomposed, increasing the microbial activity and the decomposition of the easily degraded soluble components, such as sugars, starches, and proteins that are quickly used by decay-causing organisms (Wolf and Wagner, 2005).

The monocropped Paiaguas palisadegrass showed a higher production of remaining biomass for both sowing periods, as well as faster initial decomposition caused by the high leaf: stem ratio of this forage plant (Machado and Valle, 2011). These results show the importance of the production of biomass with a greater resistance to decomposition for maintaining the soil cover during the off-season.

The decomposition rate of the remaining biomass

Table 4. Coefficients the regression equation $P=Po^{-kt}$ and R^2 and half-lives for biomass decomposition in the various forage systems from 0 to 120 days after management for both sowing periods.

First sowing period				
Forage system	**Coefficients the regression equation**			
	Po	**k**	**R^2**	**half-lives (days)**
Monocropped pearl millet	1459.08	0.0094	0.79*	74
Monocropped Paiaguas palisadegrass	3441.83	0.0106	0.82*	65
Row pearl millet x Paiaguas palisadegrass	1676.89	0.0113	0.92**	61
Between rows pearl millet x Paiaguas palisadegrass	1492.94	0.0122	0.87*	57
Oversown pearl millet x Paiaguas palisadegrass	513.46	0.0074	0.92*	94
	Second sowing periods			
Monocropped pearl millet	1301.11	0.0087	0.75*	80
Monocropped Paiaguas palisadegrass	2475.20	0.0108	0.78*	64
Row pearl millet x Paiaguas palisadegrass	1288.31	0.0112	0.83*	61
Between rows pearl millet x Paiaguas palisadegrass	863.35	0.0110	0.77*	63
Oversown pearl millet x Paiaguas palisadegrass	637.29	0.0089	0.78*	78

Significant at 1 and 5% probability and (ns) not significant.

determines the persistence of the soil cover, which is influenced by the lignin content and carbon/nitrogen ratio of the residue. Grasses generally yield relatively large amounts of biomass that is characterized by a high carbon/nitrogen ratio, which helps increase the persistence of the soil cover (Noce et al., 2008). Kliemann et al. (2006) found that compared with monocropped corn, intercropping corn with Marandu grass contributed to a lower biomass loss at 150 days after sowing. For all the forage systems, the second sowing period provided the highest remaining biomass production.

The results obtained with Paiaguas palisadegrass showed that this grass provides a promising option for the production of high-quality biomass in the off-season. In the Brazilian savannah, efficient mulching with biomass is one of the factors needed for the sustainability of no-tillage systems, mainly because of fast residue decomposition. Under these conditions, the use of Paiaguas palisadegrass, which showed a relatively slow decomposition rate, represents a strategy to increase the soil cover efficiency, especially in the planting prior period of soybeans in the summer season.

Agronomic characteristics and yield of soybean

The height of the soybean plants was not influenced (P>0.05) by the interaction of forage systems and sowing period. However, there was significant effect (P<0.05) for forage systems in the first planting date. The greatest heights of plants were obtained in monocropped Paiaguas palisadegrass e intercropped in rows and between rows (Table 5). These results are correlated with higher biomass produced in these systems, associated with a high carbon/nitrogen ratio (Tables 1 and 3), increasing the durability of the dry matter in soil (Table 2), which may have contributed to a slow decomposition and greater accumulation and availability of nutrients for the soybean crop. Among the characteristics observed, stay on soil also brings physical benefits (airier soil) and biological (increased microbial activity for decomposition) that promote the best establishment of soybean plants as recommended (Barbosa et al., 2011).

Notably, the biomass importance of tropical grasses to keep soil moisture, especially in the Central Brazilian savannah conditions (Torres et al., 2006), resulting in sustainability and efficiency, storing carbon in the soil. In this study, the vegetative stage of soybean development in January was dry spell of 20 days. In this sense, the forage systems pearl millet monoculture and intercropped with Paiaguas palisadegrass in oversowing, soybean development was hampered, influencing in a smaller plant height (Table 5) due to lower production of biomass (Table 1) in these systems.

However, when evaluated the second sowing periods, there was no significant effect (P>0.05) in plant height between forage systems (Table 1), averaging the height of 84.43 cm. The same occurred in comparison to sowing period where forage systems did not influence the plant heights. The effect of vegetation on the agronomic characteristics of soybean were also evaluated in some studies as the Santos et al. (2014b) found that soybean plants heights ranging from 85 to 90 cm in different covers of perennial pastures under no-tillage system, being similar to those obtained in this study.

However, Barbosa et al. (2011) found higher height values of 108.27 and 108.75 cm soybean plants, the straws of pearl millet and Marandu grass in exclusive crop, respectively. Borges et al. (2015) evaluated

Table 5. Plant height, insert the first and final pod and number of pod per soybean plant under monocropped pearl millet and intercropped with Paiaguas palisadegrass under different forage systems and sowing periods.

Forage system	Sowing period	
	First	**Second**
	Plant height (cm)	
Monocropped pearl millet	76.21^{Ba}	82.99^{Aa}
Monocropped Paiaguas palisadegrass	85.55^{Aa}	86.55^{Aa}
Row pearl millet x Paiaguas palisadegrass	93.88^{Aa}	88.55^{Aa}
Between rows pearl millet x Paiaguas palisadegrass	91.33^{Aa}	86.44^{Aa}
Oversown pearl millet x Paiaguas palisadegrass	75.33^{Ba}	78.10^{Aa}
CV (%)	.... 5.21.......................	
	First pod insertion (cm)	
Monocropped pearl millet	15.21^{Ca}	16.32^{Ba}
Monocropped Paiaguas palisadegrass	20.33^{ABa}	19.22^{Aa}
Row pearl millet x Paiaguas palisadegrass	16.44^{Ba}	17.88^{Ba}
Between rows pearl millet x Paiaguas palisadegrass	17.66^{Ba}	16.99^{Ba}
Oversown pearl millet x Paiaguas palisadegrass	15.10^{Ca}	16.44^{Ba}
CV (%)	.... 9.8.......................	
	Final pod insertion (cm)	
Monocropped pearl millet	74.44^{Ca}	76.33^{Ba}
Monocropped Paiaguas palisadegrass	85.55^{ABa}	89.10^{Aa}
Row pearl millet x Paiaguas palisadegrass	92.77^{Aa}	86.64^{Aa}
Between rows pearl millet x Paiaguas palisadegrass	91.42^{Aa}	86.44^{Aa}
Oversown pearl millet x Paiaguas palisadegrass	75.10^{Ca}	77.99^{Ba}
CV (%)	.... 5.23	
	Number of pod per plant	
Monocropped pearl millet	25.20^{Ba}	24.22^{Ba}
Monocropped Paiaguas palisadegrass	31.44^{Aa}	32.89^{Aa}
Row pearl millet x Paiaguas palisadegrass	28.22^{Aa}	29.42^{Aa}
Between rows pearl millet x Paiaguas palisadegrass	29.55^{Aa}	29.99^{Aa}
Oversown pearl millet x Paiaguas palisadegrass	24.88^{Ba}	25.33^{Ba}
CV (%)	.... 18.80.....................	

Means followed by different uppercase letters in the columns (forage systems) and lowercase letters in the rows (sowing periods) differ from each other according to Tukey's test at the 5% probability level.

agronomic traits soybean under different vegetable toppings, found lower values when compared to this study, plant height ranging from 47 to 52 cm on the biomass of *Pennisetum americanum* and 47 to 51 cm on biomass of *Urochloa ruziziensis*.

The forage systems affected (P<0.05) at the time of insertion of the first and end soybean pod in both periods (Table 5). In the first date, the greatest heights were obtained studying Paiaguas palisadegrass in monocropped and intercropping system on row and between rows, differing pearl millet monocropped and intercropped oversown. Probably the highest yields of biomass accumulated in these systems (Table 1) influenced the date of insertion of the first and final soybean behavior. Thus it is evident the importance of intercropping systems through crop-livestock integration, to increase the height of insertion of string beans, which is directly related to grain yield. In the second sowing, for the first pod height, only the Paiaguas palisadegrass monocropped system differentiated from other systems with greater height. And for the insertion height of the final pod, pearl millet monocropped and intercropped with Paiaguas palisadegrass in oversown, showed lower height.

The first pod insertion height is an important agronomic trait, because it is related to the efficiency of mechanical harvesting of grain operations, it is recommended that this variable present at least 13 cm to reduce the losses during harvest (Medina et al., 1997). Thus, considering the mean height of insertion of the values of the first pod observed in this study, regardless of the forage systems, it appears that there is no limitation to the mechanical harvesting of soybean, with the lowest average of this characteristic was observed system pearl millet monocropped and intercropped with Paiaguas palisadegrass in oversown the first date, with an average value of 15.15 cm. Results similar to these forage systems were obtained by Barbosa et al. (2011) found that insertion height of the first pod of 15.81 and 15.31 cm on the exclusive biomass pearl millet and Marandu palisade grass respectively. Assessing the sowing periods of the first insertion height and last pod, it appears in Table 5 that the times did not influence (P>0.05) at the time all forage systems, showing similar results. The total number of pods per plant was influenced (P>0.05) by the forage systems, however, there was no significant effect on the sowing dates (Table 5). The highest values were observed in Paiaguas palisadegrass systems in monoculture and intercropping on the row and between rows sowing periods. Again, these results show that these systems favored for best soybean development due to increased biomass production (Table 1) which resulted in a higher moisture and lower soil temperature.

Rather, the pearl millet system in monocropped and intercropped with Paiaguas palisadegrass in oversown resulted in lower soybean development, which can be correlated with the lower ground cover due to the lower production of biomass and to pass a water stress period in the vegetative phase, hindered the development of soybean. It is noteworthy that the number of pods per plant is considered the main component that controls the soybean crop production.

The number of pods observed in this study was higher than that found by Lima et al. (2009) with values of 10.8 pod per plant of pearl millet biomass and lower than that observed by Barbosa et al. (2011) with 68.65 and 66.75 values for pearl millet covers and Marandu palisadegrass, respectively.

It is also possible to verify that there is a positive correlation between the number of pods per plant with the other characteristics discussed previously, where the larger plant height, insertion height of the first and last pods provided in a larger number of pods per plant. Probably a larger rod provided greater area for tillering and string soybeans production. It is noteworthy that the highest values were observed in all of these characteristics are also related to higher biomass of Paiaguas palisadegrass systems in monoculture and intercropping on rows and between rows (Table 1), which indicates the efficiency in the adoption of these systems for suitable biomass production for the no till system.

The population density of the soybean plants final stand was influenced (P>0.05) by forage systems (Table 6). It is observed that the intercropped with pearl millet with Paiaguas palisadegrass in rows showed higher final stand with 31.92 plants m^{-1}, and 26.51% more plants than the over seeded system, which presented the lowest final stand for both seasons. However, there was no seeding date effect (P>0.05) in all forage systems for this characteristic.

Probably the biggest biomass production (Table 1), and the lowest decomposition, may have favored the early development of seedlings and also their survival until the end of the cycle. Among the beneficial factors of this accumulation and retention of biomass in the soil are: increased moisture retention, greater control of soil temperature range (Torres et al., 2006), control weeds, and (Nunes et al., 2010) the release of nutrients to the decomposition of biomass for soybeans also providing for greater productivity (Boer et al., 2008).

Lemos et al. (2003) observed that the largest soybean production occurred in conditions of greater production of biomass. Thus the Paiaguas palisadegrass monocropped and intercropping on the rows and between rows, because they showed a higher biomass production (Table 1) may have favored the population density of soybean plants of the final stand. As for the weight of 100 grains, there was no significant effect (P>0.05) for forage systems and sowing period (Table 6). Many studies also found no effect of different vegetation cover on the weight of 100 seeds, as shown in the research of Lima et al. (2009); Santos et al. (2014b) and Borges et al. (2015). The results obtained for grain yield were influenced (P<0.05) by forage systems and sowing period (Table 6). The Paiaguas palisadegrass system in monocropped and intercropped in rows between rows, provided the best results in both evaluation periods. This result shows the importance of *Urochloa* for biomass as ground cover, for soybean yield in no-tillage system.

Among *Urochloa* cultivars is worth mentioning that Paiaguas palisadegrass is one of the most indicated in the crop-livestock integration system to establish with lower water availability and produce well during the period of low rainfall and may be considered an excellent option as producing crops biomass (Machado and Assis, 2010), aiming at the implementation of no-tillage system in the Central West of Brazil (Costa et al., 2016), a fact clearly observed in grain yield data. According to Lopes et al. (2009) the addition of plant residues in the soil in areas under use of livestock farming system integration by direct seeding is very important for maintaining and increasing the levels of organic matter in the soil, which plays a key role in maintaining sustainability of production over time and also in carbon stock, resulting in more sustainable soy production. The soybean yield values obtained in this study were similar to those observed by Borges et al. (2015) who found significant effects on the type of coverage of grain production, where millet coverage provided grain production ranging from 4218 to 4613 kg ha^{-1} and *Urochloa ruziziensis* 4396 to 4679 kg ha^{-1}.

Regarding the sowing period, in all forage systems the second season periods had higher grain yield (Table 6).

Table 6. Population density, mass 100 grains, soybean yield under straw pearl millet and Paiaguas palisadegrass monocropped and intercropped in various forage systems and sowing period.

Forage system	Sowing period	
	First	Second
	Population density (plant m^{-1})	
Monocropped pearl millet	17.00^{Ba}	18.00^{Ba}
Monocropped Paiaguas palisadegrass	19.66^{Aa}	20.16^{ABa}
Row pearl millet × Paiaguas palisadegrass	20.66^{Aa}	20.66^{Aa}
Between rows pearl millet × Paiaguas palisadegrass	19.33^{Aa}	18.00^{Ba}
Oversown pearl millet x Paiaguas palisadegrass	15.66^{Ba}	16.33^{Ba}
CV (%)	.... 14.09....................	
	Mass 100 grains (g)	
Monocropped pearl millet	15.76^{Aa}	17.27^{Aa}
Monocropped Paiaguas palisadegrass	17.73^{Aa}	17.88^{Aa}
Row pearl millet × Paiaguas palisadegrass	17.00^{Aa}	16.78^{Aa}
Between rows pearl millet × Paiaguas palisadegrass	17.43^{Aa}	16.93^{Aa}
Oversown pearl millet x Paiaguas palisadegrass	14.29^{Aa}	15.62^{Aa}
CV (%)	.... 6.96	
	Grain yeld (kg ha^{-1})	
Monocropped pearl millet	4080.16^{Bb}	4422.59^{Ba}
Monocropped Paiaguas palisadegrass	4975.98^{Ab}	5284.80^{Aa}
Row pearl millet × Paiaguas palisadegrass	4724.14^{Ab}	5300.56^{Aa}
Between rows pearl millet × Paiaguas palisadegrass	4841.24^{Ab}	5193.60^{Aa}
Oversown pearl millet × Paiaguas palisadegrass	3408.20^{Bb}	3990.89^{Ba}
CV (%)	.... 13.93......................	

Means followed by different uppercase letters in the columns (forage systems) and lowercase letters in the rows (sowing periods) differ from each other according to Tukey's test at the 5% probability level.

This result may be correlated with increased biomass production in this period (Table 1), which reflected positively on soybean productivity.

Conclusions

The Paiaguas palisadegrass in monocropped system presented higher production of remaining biomass and in return presented lower carbon/nitrogen ratio. The higher carbon/nitrogen ratio and cumulative loss were obtained from pearl millet in monocropped and intercropped with Paiaguas palisadegrass in oversown, which biomass production had reduced by the effect of plant competition. For all systems the Paiaguas forage systems in palisadegrass monocropped and intercropping on the rows and between rows provided higher yield of soybean. The second sowing periods resulted in higher production of remaining biomass and grain yield, in all forage systems. Intercropped systems through in crop-livestock integration showed a promising cultivation technique to maintain the stock of carbon in the soil with sustainability.

Conflict of Interests

The authors have not declared any conflict of interests.

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Abundance and distribution of Striga (*Striga hermonthica* (Del.) Benth.) infestation in selected sorghum (*Sorghum bicolour* L. Moench) growing areas of Tigray Region, Ethiopia

Atsbha Gebreslasie[1]*, Taye Tessema[2], Ibrahim Hamza[3] and Demeke Nigussie[4]

[1]Abergelle Agricultural Research Center-TARI, Mekelle Ethiopia.
[2]National Project Coordinator of Integrated Striga Control Project -EIAR, P. O. Box 2003, Addis-Ababa Ethiopia.
[3]Ambo University, P. O. Box 19, Ambo Ethiopia.
[4]Ethiopian Institute of Agricultural Research Office-EIAR, P. O. Box 2003, Addis-Ababa Ethiopia.

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A survey was conducted in 2014 to determine the abundance and distribution of *Striga hermonthica* in selected woredas of Tigray. *S. hermonthica* was commonly distributed across all study sites, its prevalence varied among sites (Kebelies). The highest levels of Striga infestation was observed at Hadinet (400), Dabano (390), Nebar Hadinet (351), Giera (299), Hadas Lemlem (278), Selam (267), and Zuriya Dansha (261). Whereas, the lowest levels of Striga infestation was registered at Genetie (33), Gergellie (54), Kara Adishabo (59), Rawyan (81), Mentebteb (81), and Adi Keyh (84) shoots of striga shoots per m^2, respectively. Abundance and distribution map of *S. hermonthica* was developed for the study area. On the other hand, the highest density of Striga shoots per plant of sorghum was recorded at Kulugizie Lemlem (38), Nebar Hadinet (27), Zuriya Dansha (27), Hadinet (19), Selam (17), Dabano (17), and Giera (16) respectively. Whereas, the lowest density of Striga shoots per plant of sorghum was registered at Genetie (4), Mentebteb (4), Rawyan (5), May Shek (7), Kara Adishabo (7) and Mitsa Werki (7). The highest level of Striga infestation was recorded at sites which had got the highest population density of sorghum, sites with low organic matter and available soil phosphorous content and sandy textured soils. Management practices channeled towards improving these limitations have been suggested for controlling of *S. hermonthica* in the region.

Key words: Abundance and distribution, Striga, sorghum, Tigray region.

INTRODUCTION

The agricultural sector is the largest contributor to the economies and livelihoods of many African countries and on it accounts for 35% of the continent's GDP, 40% of export earnings and 70% of employment (Nyage et al., 2011). Although its share of GDP has been declining steadily over the past decade, agriculture continues to be

*Corresponding author. E-mail: atsbha0087@gmail.com.

the backbone of the Ethiopian economy, contributing 42.7% to GDP, about 80% of employment and 70% of export earnings (UNDP, 2014).

Crops are the major source of food for humans and constitute 93% of the world's diet (Stubbs et al., 1986). Of the crops, cereals contribute two thirds of the food, of which wheat, maize and rice together account for 87% of all grain production world wide and 43% of all food calories (FAO, 2007). In Ethiopia cereals occupy about 79% of the area and account for 86% of the production from the major crops (CSA, 2014).

Sorghum [*Sorghum bicolor* L. Moench] is one of the world's major cereal crops. It is the fifth most important crop globally and feeds around 500 million people (Shapiro and Wortmann, 2006). It is most widely grown in the semi arid tropics frequently subjected to drought and where water availability is limited. About 100 countries grow sorghum. Of these, 66 cultivate over 1000 ha. Asian and African countries like India and Nigeria have the largest area devoted to sorghum cultivation. Those in West Asia (like Israel and Jordan) and Europe (Italy and France) reaped the highest yields (FAO, 2007).

Ethiopia is the fifth major producer and consumer of sorghum in Africa and eighth in the world. In Ethiopia, sorghum is the major crop next to teff grown all over the country across high, intermediate and low altitude areas and it is the second in total productivity next to maize. Sorghum occupies 0.7 to 1.1 million hectares with 1 to 1.6 million tons of production annually. It contributes about 15-20% of total cereals production in the country. Sorghum production in Ethiopia is showing an increasing trend in the past 15 years (Sinafikeh, 2008).

Sorghum is the dominant crop in Tigray (Northern Ethiopia) where it accounts for 14.5% of the total cultivated area. In the region the average annual coverage of sorghum is 255,000 ha (Shapiro and Wortmann, 2006). Though the productivity of sorghum has increased in the last few years, the overall national productivity of sorghum is low (2.106 t/ha) compared to the average production of 2.3 t/ha of developed countries (CSA, 2013). The low productivity of sorghum can partially be attributed to the parasitic weed *Striga hermonthica.* (Gebreyesus et al., 2011).

Striga is a Latin word which stands for 'witch'. It is known as witch weed because it causes stunted growth and early discoloration of crop leaves before its emergence (Fischer, 2006). There are many *Striga* species which are economically important. Of the most economically important Striga species worldwide are purple witch weed (*S. hermonthica* (Del.) Benth.) and Asiatic witch weed (*Striga asiatica* (L) Kuntze). Among these *S. hermonthica* is the most damaging parasitic weed in the study area. Therefore *S. hermonthica* has been studied in this survey and will henceforth be referred to as *Striga*.

S. hermonthica is a plant which grows up to 80 cm with hairy, hard quadrangle shaped and fibrous stem, narrow leaf, and spike-shaped raceme inflorescence bearing up to 60 flowers for the terminal and 10 ~ 20 for the latera linflorescence with bright pink, rose-red, white, or yellow color (Musselman, 1980). The weed is dependent on its host during parts of its life cycle, that is, germination, flowering and reproduction. The root system of Striga is vestigial, where the germinated seed radicle produces haustorium instead of characteristic angiosperm root in order to interact with the host. The seeds are very tiny and range between 0.15 ~ 0.3 mm in diameter (Andrews, 1947). However, many biological aspects of striga including photosynthesis, respiration, transpiration, water relations, cause of heavy crop yield reductions, morphology, and analoging of the haustorium in relation to its function are not fully understood (Haussmann et al., 2000).

S. hermonthica has a wide host range, however it is the most ubiquitous parasitic weeds of staple crops namely maize, sorghum, pearl millet (*Pennisetum glaucum*), upland rice, tobacco, and sugarcane (Musselman, 1980; Gebisa Ejeta, 2007). Its seed germinate only after being exposed to favorable moisture and temperature conditions for several days (preconditioning). If the condition does not favor germination of seeds remain dormant for several months (Cardoso et al*.,* 2010). Striga seed can remain viable for up to 20 years (Berner et al*.,* 1996).

S. hermonthica is believed to be originated around the border of Sudan and Ethiopia (currently referred to as Nuba) where it causes severe losses in most cultivated crops, impacting the livelihoods of over 100 million African people. Though, it is endemic in the African savanna currently, *Striga* constrained the production of sorghum globally (Parker and Riches, 1993).

The annual yield loss and geographic distribution of striga infestation is steadily increasing, particularly, in Sub-Saharan Africa. Most of the available research findings show that the average yield loss of sorghum due to striga exceeds 50% and in severe cases complete crop failure can occur, forcing farmers to abandon cereal production (Abunyewa and Padi, 2003).The situation in many locations is getting worse because continuous cultivation of susceptible crops and the limited application of agricultural inputs (Mando, 1997).

Based on the reports of Hadas (2010), *S. hermonthica* mainly disseminated across farms through floods from nearby farms, farm tools, and/or via winds. Similarly the statement of Berner and his colleagues (1996) revealed that the seeds of striga are mostly introduced by contaminated host crop seeds and by cattle.

S. hermonthica is a major biotic constraint in sorghum growing parts of Ethiopia in general and in the Tigray region in particular. However, the current prevalence and distribution of *Striga* is not accurately known and clearly indicated on map. Therefore this study has been designed to determine the prevalence and distribution of *S. hermonthica* infestation across locations of Tigray region.

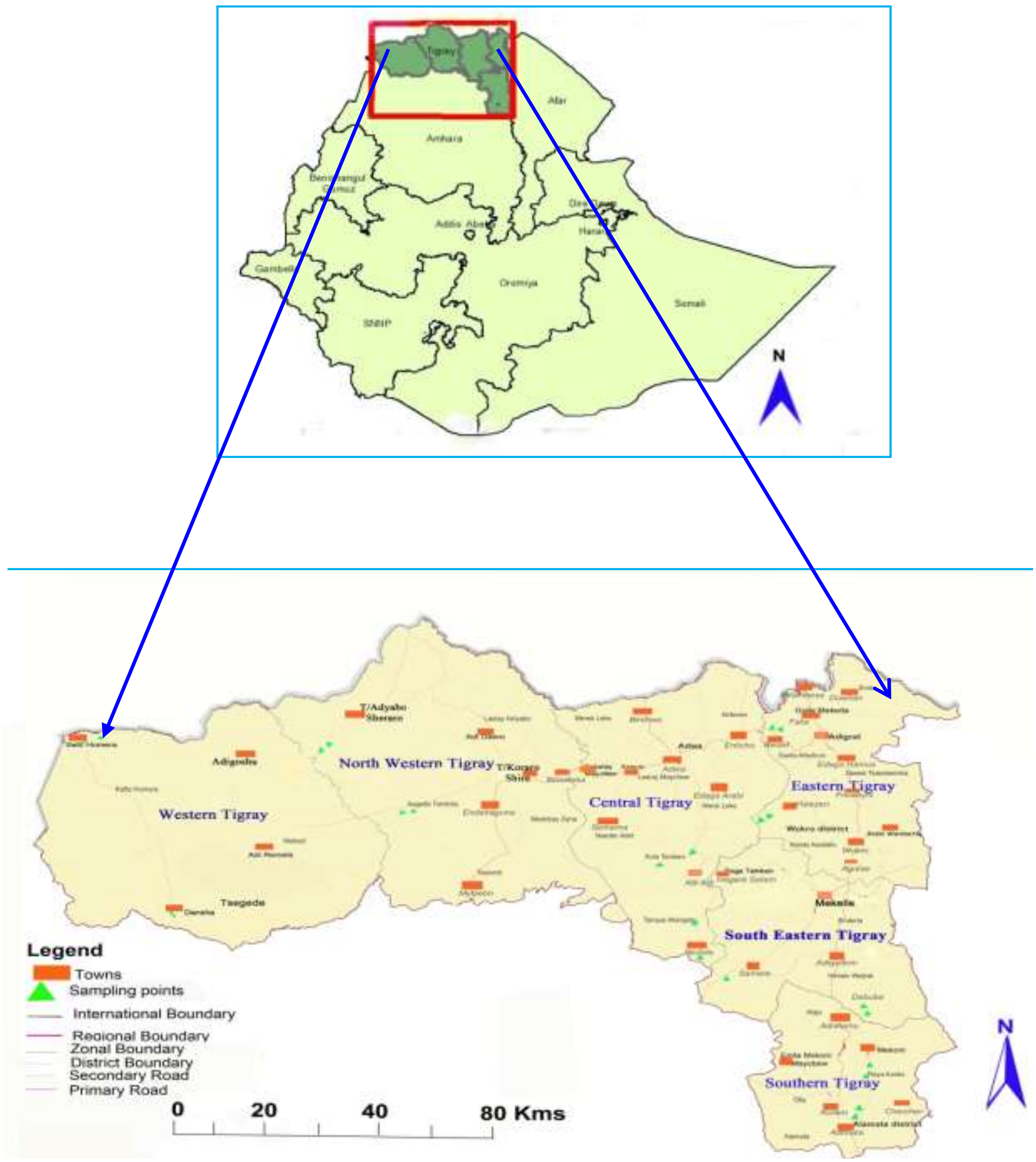

Figure 1. Map of the study area.

MATERIALS AND METHODS

The study was conducted in 2014 in the regional state of Tigray, northern Ethiopia (Figure 1). It covers a total area of 53,000 square kilometers. Geographically, it lies between 12º15'N and 14º57'N latitudes and 36º27'E and 39º59'E longitudes. There are three regionally recognized seasons in Tigray. The first is the main rainy monsoon season which lasts from June to September (locally called Kiremti), the second is the dry season from October to February (called Kewee), and the third is pre-monsoon hot season from March to May (called Hagay) (Fasil Reda, 2010).

An informal survey was made before the commencement of the actual research survey activities (ILCA, 1990). Following the informal survey a total of 12 major sorghum growing districts were selected from the whole of Tigray in consultation with Tigray Bureau of Agriculture and Rural Development experts. Two districts (woredas) and sites (Kebeles) were selected purposively from each zone and districts respectively. Thus, a total of five sorghum growing fields per site were selected with a systematic random sampling method. Field surveys were conducted from mid September to the end of October 2014 when *S. hermonthica* was easily visible above ground. The history of the field such as the field management practices, preceding crops, and the type of cultivar that farmers grow was noted during the research survey. Taking this into consideration the survey was conducted at fields sown with local cultivars of sorghum for at least three years.

Sampling was made following community made pathways and road transect survey method (Wittenberg et al*.,* 2004) was employed for sampling of striga and sorghum between farms within site. Two inverted 'M' patterned 50 m long transects, on average 1 km apart from each other was determined by systematic random sampling method. Finally, 10 evenly spaced sampling points per m^2 were established. Hence, a total of 1200 sampling points (one m^2 each) were delineated from 120 sampling sites and finally *S. hermonthica* and sorghum counts were made. Data collected from a total of ten sampling points (one m^2 each) per field were summarized and finally the abundance of *S. hermonthica* and the plant density of sorghum were determined (Booth et al., 2003).

The number of striga shoots per sorghum plant was determined once striga abundance and the population of sorghum m^{-2} were determined. To arrive at the latter figure, the density of striga and the population of sorghum in the whole sampling area had to be computed. Finally, the number of striga shoots per sorghum plant was determined by dividing average number of striga by the density of sorghum per m^2 (Booth et al., 2003).

During the biophysical survey about 120 waypoints were identified and the coordinates of each waypoint was recorded using hand held GPS. The data on level of infestation, number of sorghum plants and number of striga shoots per sorghum plant at a sampling point was recorded on a spread sheet along with the coordinates of each study site. Finally an accurate map showing the distribution of *S. hermonthica* and number of striga shoots per sorghum plant was developed.

RESULTS AND DISCUSSION

Abundance and distribution of *S. hermonthica*

Based on the results of the research survey, striga was distributed throughout the study areas (120 sites) (Figure 2). However, the levels of striga infestation differ among sites.

Accordingly the highest number of striga was recorded in most sites relatively. On the other hand, there are a few sites which have got less infestation level of striga per m^2. Striga are mostly introduced and disseminated by contaminated crop seeds, floods, farm tools, cattle and via wind (Berner et al., 1996; Hadas, 2010). These could be the reason for the occurrence and distribution of Striga at different level across locations.

The analysis of variance result (Table 1) revealed that the average level of striga infestation was 190 striga shoots per m^2. The highest striga infestation was observed at Hadinet (400), Dabano (391), Nebar Hadinet (351), and Giera (299) striga per m^2 respectively. In contrast, the lowest striga infestation was observed at Genetie (33), Gergellie (54), and Kara Adishabo (59) striga per m^2 respectively.

The highest sorghum plant population was recorded almost in all kebeles with the exception of Nebar Hadinet (14 plant stand m^{-2}) (Table 2). The plant population recorded at Hadinet (26), Dabano (24), Giera (19), Hadas Lemlem (25) and Selam (15) was by far greater than the population at Genetie (9), Gergellie (7) and Kara Adishabo (9) sorghum per m^2 respectively. In most cases, the recorded figure was by far greater than the nationally recommended optimum population density of sorghum per unit area of land (8-9 sorghum m^{-2}) (EIAR, 2007).

Consequently the highest level of striga infestation was also recorded in the sites with the higher plant population. There are many reasons for the high numbers of striga shoots registered; most probably due to the extensive root systems produced by higher density of sorghum. Esilaba et al*.* (2000) reported that the emergence of striga is positively associated with increased root surface area due to extensive roots systems of sorghum and the subsequent release of germination stimulants.

The results of soil laboratory analysis revealed that the lowest amount of organic matter content was recorded at almost all study sites with exception of Genetie, Gergellie and Kara Adishabo compared than the minimum required amount (2-4%).

The soil laboratory analysis result (Table 3) showed that the amount of organic matter content at Hadinet, Dabano, Nebar Hadinet, Giera, Hadas Lemlem, Selam, and Zuriya Dansha was 0.58, 0.79, 0.89, 0.90, 0.90%, 0.99 and 1.01%, respectively. Whereas, the amount of soil organic matter recorded at Genetie, Gergellie, and Kara Adishabo was 4.2, 3.32 and 3.06% respectively. Therefore the highest level of striga infestation across most study sites occurred in soils low in organic matter and available phosphorous. This result is in lined with the findings of Samaké et al. (2005) who stated that striga infestation is strongly associated with decline of soil fertility, thus the problem is aggravated as a result of the decline of soil fertility.

Prevalence of *S. hermonthica* around individual sorghum plant

As the average level of striga infestation per m^2 varied, the numbers and distributions of striga around the individual stands of sorghum were different across sites (Figure 3). The result of the survey indicated that the highest density of striga per plant of sorghum was recorded at the study sites of Kulugizie Lemlem (38), Nebar Hadinet (27), Zuriya Dansha (27), Hadinet (19), Selam (17), Dabano (17), Giera (16), and Edaga Hibret (15) respectively (Table 4). Whereas, the lowest density of striga emerged around plants of sorghum was registered at Adi Keyh (8), Gergellie (8), Mitsa Werki (7), May Shek (7), Kara Adishabo (7), Rawyan (5), Mentebteb (4), and Genetie (4). This could be due to the fact that density of striga per plants of sorghum depends on the type of cultivars of sorghum, the variability of locations and mean population of sorghum. The germination and

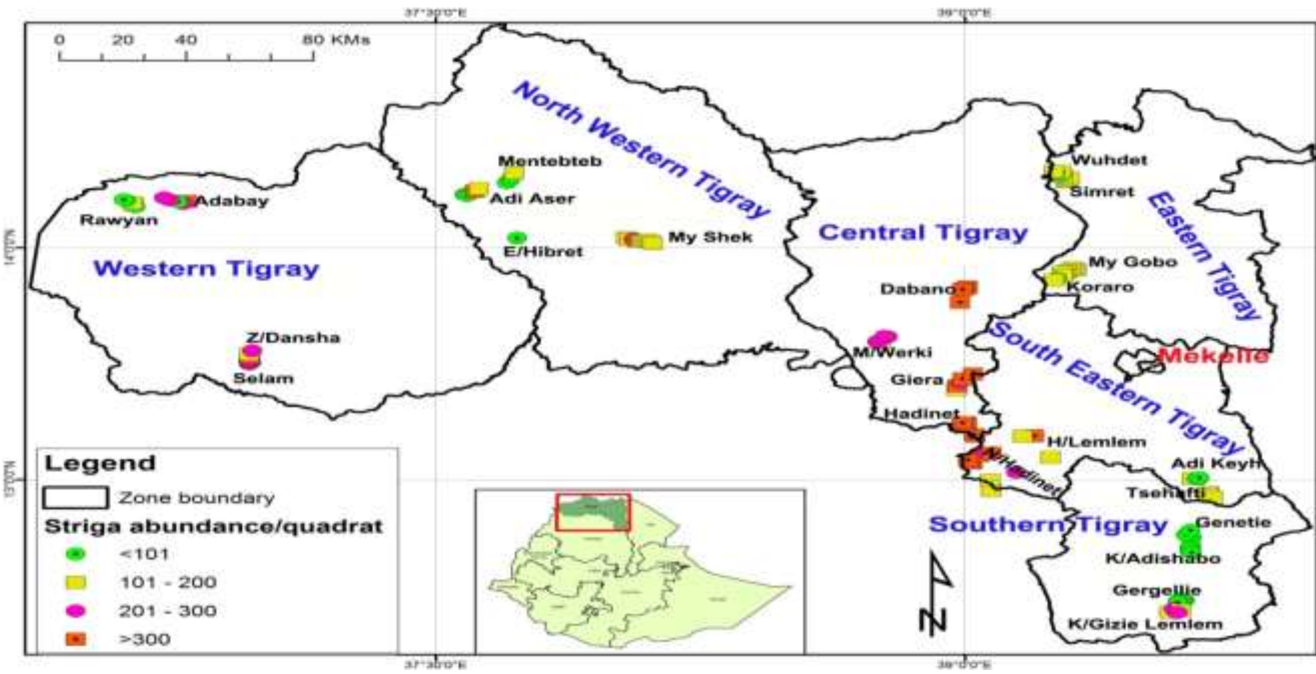

Figure 2. Distribution of *S. hermonthica* across the study sites.

Table 1. Mean abundance of *Striga hermonthica* (Del.) Benth. shoots (no./m^2) in different locations of Tigray Region (N=120) (2014).

Locations (Kebeles)	N	Mean (no./m^2)	Mean Rank	Std. Deviation	Std. Error	95% Confidence Interval	
						Lower Bound	Upper Bound
Nebar Hadinet	5	351	3	57.8	25.9	279.6	423.2
H/ Lemlem	5	278	5	64.3	28.7	198.4	358.0
Hadinet	5	400	1	16.1	7.2	380.4	420.4
Giera	5	299	4	69.3	31.0	213.4	385.4
M/Werki	5	219	10	34.5	15.4	176.6	262.2
Dabano	5	391	2	50.1	22.4	328.4	452.8
Selam	5	268	6	19.9	8.9	242.9	292.3
Z/Dansha	5	261	7	75.6	33.8	167.5	355.3
Adabay	5	221	9	86.7	38.8	113.1	328.5
Rawyan	5	81	21	48.9	21.9	20.0	141.6
Adiaser	5	155	13	120.5	53.9	5.8	305.0
Mentebteb	5	82	20	21.9	9.8	54.4	108.8
E-Hibret	5	252	8	98.2	43.9	130.1	373.9
May Shek	5	110	18	7.1	3.2	101.2	118.8
Mygobo	5	191	12	4.1	1.8	186.1	196.3
Koraro	5	145	15	29.6	13.3	108.0	181.6
Simret	5	152	14	38.4	17.2	104.2	199.4
Wuhdet	5	134	16	36.5	16.3	88.5	179.1
Genetie	5	33	24	3.7	1.7	28.4	37.6
K/Adishabo	5	59	22	1.3	0.6	57.2	60.4
Gergellie	5	54	23	2.9	1.3	50.5	57.9
K/ Lemlem	5	209	11	21.6	9.7	181.9	235.7
Tsehafti	5	129	17	24.4	10.9	98.7	159.3
Adi Keyh	5	85	19	34.1	15.2	42.3	126.9
Total	120	190		114.3	10.4	169.3	210.6

Table 2. Mean density of sorghum (no./m^2) in different locations of Tigray Region (N=120).

Locations (Kebeles)	N	Mean (no./m^2)	Mean Rank	Std. Deviation	Std. Error	95% Confidence Interval	
						Lower Bound	Upper Bound
Nebar Hadinet	5	14	14	3.5	1.6	9.6	18.4
Hadas Lemlem	5	25	3	6.6	3.0	16.4	32.8
Hadinet	5	26	2	15.0	6.7	7.3	44.7
Giera	5	19	6	2.7	1.2	15.9	22.5
M/Werki	5	36	1	15.2	6.8	16.7	54.5
Dabano	5	24	4	3.4	1.5	19.4	27.8
Selam	5	15	12	0.5	0.2	14.7	16.1
Z/Dansha	5	11	20	2.8	1.2	7.4	14.2
Adabay	5	18	7	6.5	2.9	10.2	26.2
Rawyan	5	16	10	7.6	3.4	6.5	25.5
Adiaser	5	16	11	4.5	2.0	10.0	21.2
Mentebteb	5	19	5	2.6	1.2	16.0	22.4
E-Hibret	5	17	9	3.6	1.6	12.7	21.7
May Shek	5	18	8	7.5	3.4	8.5	27.1
Mygobo	5	14	15	0.5	0.2	12.9	14.3
Koraro	5	14	17	1.1	0.5	12.2	15.0
Simret	5	14	13	2.3	1.0	11.1	16.9
Wuhdet	5	14	16	2.6	1.2	10.4	16.8
Genetie	5	9	21.5	1.6	0.7	6.8	10.8
K/Adishabo	5	9	21.5	1.3	0.6	7.2	10.4
Gergellie	5	7	23	1.5	0.7	5.4	9.0
K/Lemlem	5	6	24	1.9	0.8	3.7	8.3
Tsehafti	5	11	18	1.9	0.9	8.8	13.6
Adi Keyh	5	11	19	1.7	0.8	8.8	13.2
Total	120	16	14	8.3	0.8	14.4	17.4

The appearance of zero between lower and upper bound indicates no statistical difference in striga infestation at 95% confidence interval, the appearance of no zero value between lower and upper bound indicates statistical difference in striga infestation among sites.

Table 3. Major soil chemical properties of the study sites.

Woreda	kebele	Basic chemical properties of the soil			
		pH	% Organic Matter	% of Total Nitrogen	Available Phosphorous (ppm)
S/Samre	N/Hadinet	7.16	0.89	0.08	6.34
	Hadas Lemlem	6.33	0.90	0.04	2.8
T/Abergelle	Hadinet	6.42	0.58	0.04	2.12
	Giera	7.33	0.90	0.11	2.8
K/Tembien	Mitsa Werki	6.08	1.23	0.11	3.16
	Dabano	7.53	0.79	0.05	3.02
W/Tsegedie	Selam	6.41	0.99	0.05	1.88
	Z/Dansha	6.36	1.01	0.05	2.04
K/Humera	Adabay	7.87	1.03	0.05	1.82
	Rawyan	7.49	2.61	0.04	3.62
T/Adyabo	Adiaser	6.01	1.90	0.05	2.94
	Mentebteb	7.09	2.40	0.06	2.42

Table 3. Contd.

A/Tsimbilla	E-Hibret	6.02	1.02	0.05	1.88
	May Shek	5.58	2.08	0.05	2.12
Hawzien	May Gobo	6.79	1.84	0.04	2.18
	Koraro	6.73	2.04	0.06	1.74
G/Afoshum	Simret	6.52	1.96	0.10	3.4
	Wuhdet	6.53	2.05	0.04	4
Raya Azebo	Genetie	7.41	4.20	0.04	7.84
	K/Adishabo	7.21	3.06	0.12	8.16
G/Raya Alemata	Gergellie	7.47	3.32	0.14	8.52
	K/Lemlem	7.31	1.45	0.07	7.62
Hintallo Wajirat	Tsehafti	7.12	2.06	0.07	3.54
	Adi Keyh	7.82	2.26	0.09	12.08

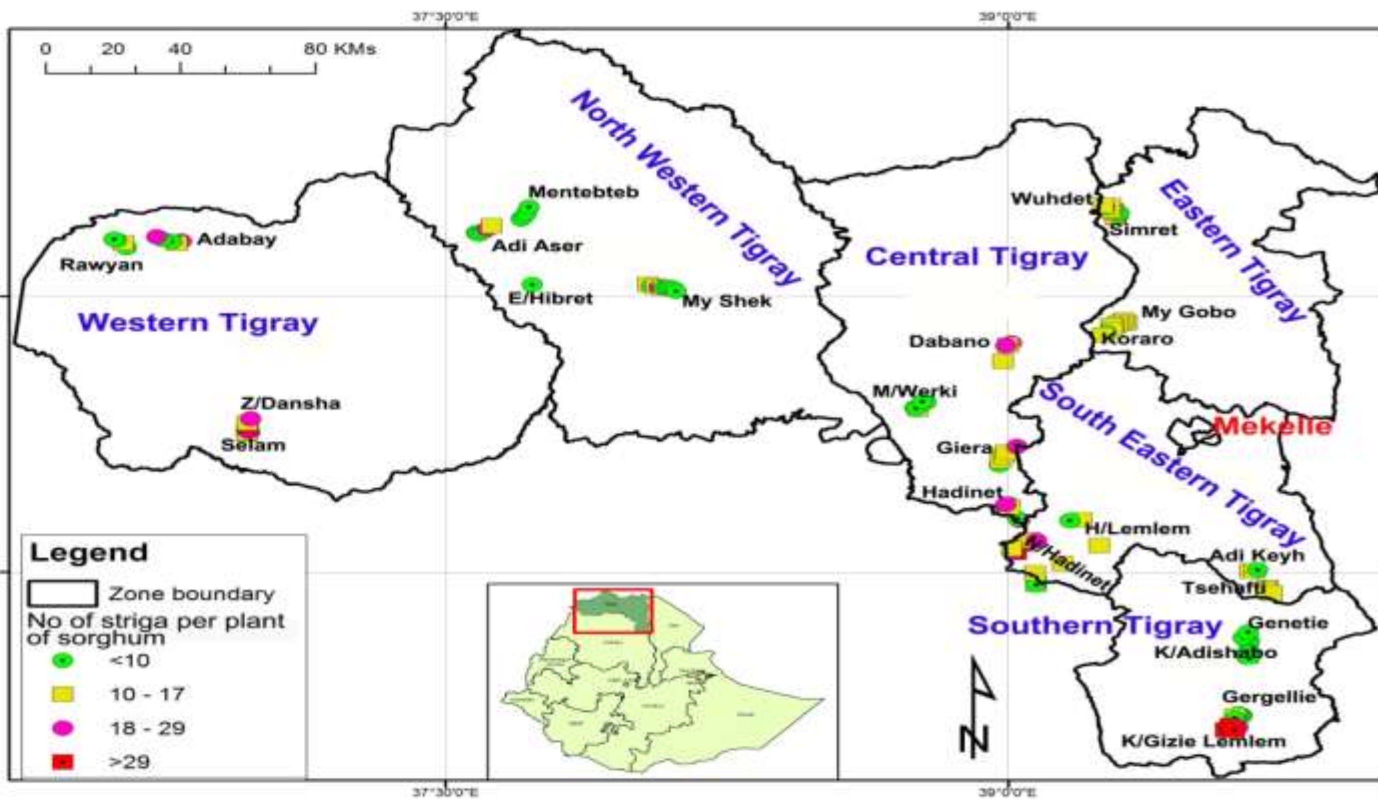

Figure 3. Distribution of *S. hermonthica* per plant of sorghum in different locations of Tigray Region.

survival of striga has been associated with the host and the factors that affect the host. It is expected that, cultivars used by farmers have their own unique level of susceptibility to striga.

Conclusion

S. hermonthica was distributed over all the surveyed areas with altitudes ranging from 621-2245 m above sea

Table 4. Striga shoots per plant of sorghum in different sites of Tigray (N=120)/2014.

Locations (Kebeles)	N	Mean	Mean Rank	Std. Deviation	Std. Error	95% Confidence Interval	
						Lower Bound	Upper Bound
Nebar Hadinet	5	27	3	9.5	4.3	15.3	39.0
H/Lemlem	5	12	11	5.0	2.2	5.7	18.2
Hadinet	5	19	4	8.1	3.6	8.9	29.0
Giera	5	16	7	4.8	2.2	10.1	22.1
M/Werki	5	7	19	2.3	1.0	4.1	9.8
Dabano	5	17	6	3.0	1.3	13.0	20.4
Selam	5	17	5	1.2	0.6	15.7	18.8
Z/Dansha	5	27	3	15.4	6.9	8.0	46.3
Adabay	5	14	9	8.2	3.7	3.9	24.3
Rawyan	5	5	22	3.7	1.7	0.7	10.0
Adiaser	5	9	16	5.1	2.3	3.2	15.9
Mentebteb	5	4	23	1.5	0.7	2.5	6.2
E-Hibret	5	15	8	6.5	2.9	7.0	23.2
May Shek	5	7	21	2.0	0.9	4.3	9.2
Mygobo	5	14	10	0.8	0.4	12.9	14.9
Koraro	5	11	14	2.3	1.0	8.0	13.7
Simret	5	11	13	2.9	1.3	7.3	14.5
Wuhdet	5	10	15	2.9	1.3	6.6	13.7
Genetie	5	4	24	1.2	0.5	2.4	5.4
K/Adishabo	5	7	21	1.0	0.4	5.5	8.0
Gergellie	5	8	18	2.2	1.0	5.0	10.4
K/ Lemlem	5	38	1	14.1	6.3	20.8	56.0
Tsehafti	5	11	12	1.4	0.6	9.5	13.0
Adi Keyh	5	8	17	3.4	1.5	3.7	12.2
Total	120	13		9.7	0.9	11.6	15.1

level. However, its abundance was not even across the sites. The highest striga infestation was observed at Hadinet, Dabano, Nebar Hadinet, Giera, Hadas Lemlem, Selam Zuriya Dansha and Edaga Hibret. Conversely the lowest density of striga infestation was recorded at Genetie, Gergellie, Kara Adishabo, Rawyan, and Mentebteb followed by Adi Keyh, May Shek and Tsehafti.

Striga shoot count per sorghum plant was found to be different across locations. Accordingly the highest relative count of striga shoot per sorghum plant was registered at Kulugizie Lemlem, Nebar Hadinet and Zuriya Dansha. Conversely, the lowest density of striga was recorded from Kara Adishabo, Rawyan, Mentebteb and Genetie.

Finally, the highest level of striga was recorded at sites which had got the highest population density of sorghum, less organic matter content and available soil phosphorous content. Therefore management practices should be channeled towards using proper planting of sorghum, improving organic matter content of the soil, available phosphorous and soil pH so that to control *S. hermonthica* in the region. The abundance and distribution was limited in scope and geographic coverage. Therefore, detailed and broader studies should be carried out in the future covering areas away from the road sides. This will allow for specific conclusion on abundance and distribution of *S. hermonthica.*

Conflict of Interests

The authors have not declared any conflict of interest.

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Kinetics drying of *Spirulina platensis*

Pâmella de Carvalho Melo, Ivano Alessandro Devilla, Cristiane Fernandes Lisboa*, Mateus Morais Santos and Pedro Henrique Tolentino Duarte

Universidade Estadual de Goiás, Brazil.

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Spirulina platensis **is a blue-green multicellular photosynthetic cyanobacterium and it is used in many countries as human and animal feed. The aim of this work is to determine and set kinetics drying for *S. platensis* at different temperatures (30, 40, 50 and 60°C). A completely randomized design was adopted and the treatments were the drying temperatures. *S. platensis* was furnished by the company, Brasil Vital, located in Anápolis – Goiás. The initial water content from the product was determined according to analytical norm from Association of Official Analytical Chemists (AOAC). The product was subjected to drying in a heater at temperatures of 30, 40, 50 and 60°C. The samples were placed on stainless steel removable trays with background screen; they consisted of three replications. The temperature and relative humidity from the air in the environment were monitored with a thermo-hygrometer. During the process of drying, the trays with samples were periodically weighed to obtain a constant weight. Afterwards, the math models were set on the experimental drying data, using Statistica 12.0 software. The criteria for the selection of the estimative statistics were: R^2 close to 100%, P < 10% and SE close to zero. The effective diffusion coefficient was obtained from the mathematical model of liquid diffusion. One can conclude that the necessary time for *S. platensis* to reach constant weight was 7.00, 4.58, 3.83, and 3.25 h, at temperatures of 30, 40, 50, and 60°C, respectively. The recommended model used to predict the drying phenomenon for *S. platensis* was the Midilli Mathematical model at temperatures of 30, 40, and 50°C, and the model Approach of Diffusion at temperature of 60°C. The diffusion coefficient increased with increased temperature; its values were from 3.343×10^{-8} to 14.881×10^{-8} m^2 s^{-1} at temperature ranging from 30 to 60°C. It activates energy for liquid diffusion at 39.52 $kJ.mol^{-1}$.**

Key words: Post-harvest, water content, microalgae.

INTRODUCTION

Microalgae are microorganisms that grow in a liquid environment. They multiply fast and are capable of performing oxygen photosynthesis, producing a biomass rich in compounds that are biologically active (Mendonça et al., 2014).

Spirulina platensis is a blue-green multicellular photosynthetic cyanobacterium. It has high protein content within its biomass (50 to 70%), essential fatty acid γ-linolenic among many other compounds (Bezerra, 2010). It is used in many countries in aquaculture, as human and animal feed, for pigment extraction, biofuel production, and as pollutant removal (Adiba et al., 2011).

In post-harvest phase, drying is the most applied process to ensure quality and stability of the product, taking into account that as water decreases within the material, it reduces the biologic activity and the chemical and physical changes that occur during storage (Ullmann et al., 2010). Air is used in the drying process as a means of heat conduction and transfers excess water from the feed to the atmosphere. Low humidity in the product allows its storage for long periods, besides its monetary valuation. However, if drying is not well done, the product might decay during storage (Domenico and Conrad, 2015).

Studying of the drying process is of great importance to make one know the phenomenon of energy and mass transfer within the product and the drying environment, which are fundamental to elaborate projects, operation and simulation of drying systems and dryers (Corrêa et al., 2010).

The drying curves vary according to product, species, variety, environmental conditions and post-harvest preparation methods, among other factors (Resende et al., 2010). In order to perform a simulation process, it is necessary to apply a mathematic model that best describes the drying situation of a given product. Besides performing the drying forecast, it is possible to analyze, though models, other variables such as temperature, relative humidity, etc. (Domenico and Conrad, 2015). Therefore, it is of undeniable importance to set different mathematic models for the experimental data of drying, and also, that new studies take place to know the most adequate model for a given product (Radünz et al., 2011).

Amongst the applied theoretical models in the drying process, diffusion method is the one which is intensively investigated. For a diffuse model to be used in the description of the kinetics of drying of a product, the diffusion equation must be resolved. The solution for the diffusion equation, in various situations of interest, requires the need to establish a hypothesis for the physical description (Silva et al., 2013). Besides the mathematical settings of the drying curves, the values from the effective diffusivity and energy activation are also fundamental for the project and the construction of drying equipment (Celma et al., 2009).

Taking into account the importance of theoretical study and the limitations of the information regarding the phenomena that occur during drying, mainly, of products classified as “new foods”, this study aims to determine and set the kinetics of drying for the microalgae, *S. platensis* at different temperatures (30, 40, 50 and 60°C).

MATERIALS AND METHODS

The experiment was conducted at Laboratory of Drying and Storage of Vegetable Products of Campus Anápolis of Exact and Technological Sciences Henrique Santillo, State University of Goiás located in Anápolis - Goias.

The microalgae, *S. platensis* was furnished by the company Brazil Vital, located in Anápolis – Goias. The geographical coordinate of the county is at latitude 19’ 43” south and longitude 48° 57’ 12” west, in the State of Goias. The company furnished filtered samples. Afterwards, the samples were pressed into cylindrical pellets of 0.002 m thickness.

The treatment was the drying temperatures (30, 40, 50 and 60°C), consisting of three replications. Temperatures above 60°C were not chosen, for studies show that temperatures above the aforementioned have a negative effect on the nutritional composition of *S. platensis* (Bennamouna et al., 2015).

The initial water content of *S. platensis* was determined according to the analytical norm AOAC (1995) of 105°C up to constant mass, with three replications

The product was subjected to drying in a hothouse with forced air circulation at temperatures of 30, 40, 50 and 60 ± 1°C. The samples were placed on removable stainless steel trays with background screen for air flow. The temperature and air relative humidity were monitored through a digital thermo-hygrometer set in the lab. During the drying process, the trays with samples were periodically weighted up to constant mass. A semi-analytical scale was used with precision of ±0.01 g.

Equation 1 was used to estimate the humidity reasons from the *S. platensis* during drying at different temperatures.

$$RX = \frac{X - X_e}{X_i - X_e} \qquad (1)$$

where RX is the water ratio within product, dimensionless; X is the water content within product, decimal b.s.; Xi is the initial water content, decimal b.s.; and Xe is the equilibrium water content, decimal b.s.

The mathematical models (Table 1) were set to experimental drying data using Software Statistica 12.0. The statistical estimators of the models were the coefficient of the adjusted determination (R^2), relative error (P) and estimated average error (SE). The values for P and SE were estimated according to Equations 2 and 3.

$$P = \frac{100}{n} \sum_{i=1}^{n} \frac{|Y - Y_0|}{Y} \qquad (2)$$

$$SE = \sqrt{\frac{\sum_{i=1}^{n} (Y - Y_0)^2}{GLR}} \qquad (3)$$

where Y is the experimental value; Y_0 is the estimated value by the model; n is the number of experimental observations; and GLR is the grade number of liberty of the model.

The selection criterion for statistical estimators was R^2 close to 100%, P < 10%, and SE close to zero (Madamba et al., 1996). The effective diffusion coefficient was obtained through setting of mathematic model of liquid diffusion, depicted by Equation 4, to the experimental data of drying of *S. platensis*. The equation is the analytical solution for the second law of Fick, taking into account the cylindrical geometric shape, disregarding volumetric shrinkage of it (Crank, 1975).

*Corresponding author. E-mail: cflisboa.engenharia@hotmail.com.

Table 1. Mathematical models used to predict drying phenomenon of farm products (Kucuk et al., 2014).

Model designation	Model	
RX = a exp (-k t) + (1 - a) exp (-k b t)	Diffusion Approach	(2)
RX = a exp (- k t) + (1 - a) exp (- k a t)	Two Exponential Terms	(3)
RX = a exp(-k t)	Henderson and Pabis	(4)
RX = a exp(-k t) + b exp(-k_0 t) + c exp(-k_1 t)	Henderson and Pabis Modified	(5)
RX = a exp(- kt)+ c	Logarithmic	(6)
RX= a exp(-k t^n)+ b t	Midilli	(7)
RX = exp(- k t)	Newton	(8)
RX = exp(- k t^n)	Page	(9)
RX = exp ((-a-(a^2 + 4 b t)^{0,5})/ 2 b	Thompson	(10)
RX = a exp(-k t) + (1 - a) exp(-k_1 t)	Verma	(11)
RX = 1+ a t + b t^2	Wang and Singh	(12)

RX: Water content ration within product, dimensionless; t: drying time, h; k, ko, k_1: drying constants, h^{-1}; e a, b, c, n: coefficients of the models.
Source: Kucuk et al. (2014)

$$RU = \frac{U - U_e}{U_i - U_e} = 4\sum_{n=1}^{\infty} \frac{1}{\mu_n^2} \exp\left(-\frac{\mu_n^2 \,.\, D_{ef} \,.\, t}{R_P^2}\right) \quad (4)$$

where D_{ef} is the effective diffusion coefficient, m^2 s^{-1}; μ_n is the equation roots of Bessel at order zero; R_p is the radius of the cylindrical particle, m; t is the time, h; and n is the number of terms.

Eight terms have been used, from which was observed that the value of D_{ef} did not vary. The analytical solution for Equation 5 presents itself in a form of infinite series and, thus, the finite number of terms (n) at the truncation might determines the precision of the results. To evaluate temperature influence in the effective diffusion coefficient, the equation of Arrhenius was used (Equation 5):

$$D_{ef} = D_0 \left(-\frac{E_a}{R\,T_a}\right) \quad (5)$$

where D_o is the pre-exponential factor, m^2 s^{-1}; Ea is the energy activation, kJ mol^{-1}; R is the gas universal constant, 8.314 kJ $kmol^{-1}$ K^{-1}; and Ta is the absolute temperature, K.

Arrhenius' equation coefficients were linearized resulting in Equation 6, with logarithm application as follows:

$$LnD_{ef} = LnD_0 - \frac{E_a}{R} \cdot \frac{1}{T_a} \quad (6)$$

RESULTS AND DISCUSSION

In the process of determining the drying curves for the microalgae *S. platensis*, the initial water content was 83.40±0.2% b.u. (498.77±0.2% b.s), up to the final contents of 6.74, 5.00, 4.76, and 3.58% b.u, for temperatures of 30, 40, 50 and 60°C, respectively.

In Figure 1, the experimental drying values are displayed for the *S. platensis*, performed under various temperature conditions studied.

One can observe in Figure 1, that the necessary time for *S. platensis* to reach constant mass (hygroscopic equilibrium) was 7.00, 4.58, 3.83, and 3.25 h (420, 275, 230, and 195 min) at temperatures of 30, 40, 50, and 60°C, respectively. Reduction in water content was sharper at the beginning of the drying process at temperatures of 40, 50 and 60°C. While for the temperature at 30°C, the water content reduction was slow, increasing drying time.

Sarbartly et al. (2010) obtained drying time of approximately 90 min for the algae, *Eucheuma spinosum in natura* at 60°C, until sample's water content became 30% (b.u.). Faria (2012) observed that necessary time amount for the algae *Kappaphycus alvarezii* to reach water content of 30% (b.u.) was 360, 170 and 100 min at 40, 60 and 90°C temperatures, respectively, taking into account that the drying time can be influenced by room temperature, air relative humidity, species and solely by product type.

As expected, it has been observed that drying rate increased with temperature increase, resulting in an expressive difference amongst all studied temperatures. This behavior is explained by the difference of temperature gradient established between external temperature and inner temperature of the sample. This gradient is the one that rules drying speed in the first decreasing drying rate period.

In Tables 2 and 3, the applied statistical parameters can be found, to compare amongst eleven drying analyzed models, in the various drying conditions for *S. platensis*.

For the four temperatures applied for *S. platensis* drying, it has been observed that in all mathematical models set to experimental data, presented determination coefficient (R^2) close to 1.0 (Tables 2 and 3). According to Madamba et al. (1996), this coefficient alone does not represent a good criterion for the selection of non-linear

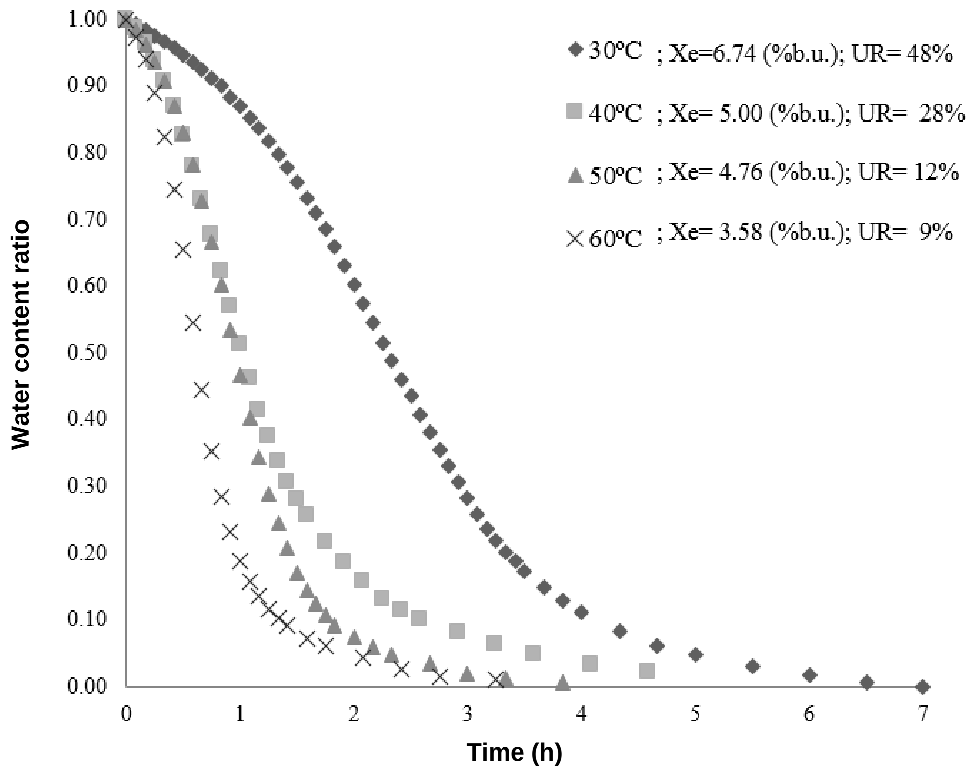

Figure 1. Experimental drying spots for *Spirulina platensis*, at temperatures 30, 40, 50 and 60°C.

Table 2. Determination coefficients (R^2, %), relative error (P,%), average estimated error (SE, decimal) for analyzed models, to the drying of the *Spirulina platensis* at temperatures 30 and 40 ºC.

Model	30°C			40°C		
	R^2 (%)	P	SE	R^2 (%)	P	SE
Diffusion proximity	99.35	22.071	0.039	98.46	20.559	0.064
Two exponential terms	99.14	29.339	0.045	99.89	9.672	0.017
Henderson and Pabis	96.38	66.368	0.091	98.91	13.494	0.053
Henderson and Pabis Modified	99.48	19.222	0.036	99.93	4.961	0.013
Logarithmic	98.00	54.815	0.066	99.08	15.737	0.050
Midilli	99.96	6.331	0.009	99.85	8.327	0.019
Newton	94.07	89.794	0.114	97.88	23.745	0.073
Page	99.92	7.260	0.013	99.76	15.407	0.025
Thompson	94.07	89.786	0.115	97.88	23.739	0.074
Verma	99.35	22.071	0.039	99.89	8.965	0.017
Wang and Singh	97.20	52.786	0.079	98.79	24.522	0.054

models; therefore, the values for estimated average error (SE) and relative average error (P) were disregarded. Many researchers used these statistical parameters to choose the best mathematical model for a given kind of product (Faria et al., 2012; Costa et al., 2015; Corrêa Filho et al., 2015; Martins et al., 2015).

The models which presented the values for statistical parameters according to the selection criterion used were: temperature at 30°C, the mathematic models of Midilli (R^2 = 99.96%; P = 6.331 and SE = 0.009) and Page (R^2 = 99.92%; P= 7.260% and SE=0.013), temperature of 40°C, the models Two Exponential Terms (R^2= 99.89%; P= 9.672% and SE=0.017), Henderson and Pabis modified (R^2 = 99.93%; P=4.961% and SE=0.013),

Table 3. Determination coefficients (R^2, %), relative errors (P, %), estimated average errors (SE, decimal) for analyzed models, for *Spirulina platensis* drying at temperatures 50 and 60°C.

Model	50 ºC			60 ºC		
	R^2 (%)	P	SE	R^2 (%)	P	SE
Diffusion proximity	99.64	12.307	0.033	99.78	9.109	0.026
Two Exponential Terms	99.50	14.573	0.038	99.74	8.520	0.028
Henderson e Pabis	97.31	51.138	0.087	98.06	26.079	0.080
Henderson and Pabis Modified	99.69	11.775	0.032	98.46	18.569	0.071
Logarithmic	97.88	42.773	0.076	98.24	20.906	0.076
Midilli	99.94	9.743	0.013	99.81	12.112	0.024
Newton	95.64	71.737	0.109	96.85	35.707	0.098
Page	99.91	17.126	0.016	99.74	14.824	0.027
Thompson	95.64	71.739	0.111	96.85	35.710	0.101
Verma	99.64	12.307	0.033	97.52	24.744	0.089
Wang and Singh	97.58	73.434	0.079	97.05	29.602	0.077

Midilli (R^2 = 99.85%; P = 8.327 and SE = 0.019) and Verma (R^2= 99.89%; P=8.965% e SE=0.017), temperature at 50°C model of Midilli (R^2 = 99.94%; P = 9.743 and SE = 0.013) was the only one which fit the selection criterion, and temperature of 60°C, mathematical model diffusion proximity (R^2 = 99.78%; P = 9.109%, and SE = 0.026) and Two Exponential Terms (R^2=99.74%; P=8.520% and SE=0.028).

The mathematical model of Midilli is one of the most sensible, presenting fewer coefficient numbers, and making its application and use simpler, for drying simulations (Kashaninejad et al., 2007). However, the mathematical model diffusion proximity is also intensively used, because it holds only three coefficients, which also makes its application simpler. Due to the simplicity of these models, besides being fit for the selection criteria, the model of Midilli was selected for temperatures of 30, 40 and 50°C and diffusion proximity for temperature at 60°C.

In studies with other products, such as gorse (Radünz et al., 2011), the leaves of wolf apple lobo (Prates et al., 2012), basil leaves (Reis et al., 2012), Brazilian peppertree leaves (Goneli et al., 2014b), leaves of Cordia Verbenacea (Goneli et al., 2014a), the model of Midilli were also the one which best fit the experimental drying data. And in other types of products, the model diffusion proximity was also selected for it presented kinetic of drying (Faria et al., 2012).

In Table 4, the mathematical coefficient models are depicted chosen by the selection criterion from the statistical estimators in the modeling of the drying curves for the *Spirulina platensis* at temperatures of 30, 40, 50 and 60°C.

In analyzing the results, one can observe that in the mathematical model of Midilli and the model of diffusion proximity, the drying constant “k” had an increase in its value with the increment of the drying temperature, displaying the influence from coefficient k in relation to the drying temperature. According to Madamba et al. (1996), the drying constant “k” can be used as proximity to characterize the temperature effect and it is linked to the effective diffusivity in the drying process during the decrease period and to liquid diffusion that controls the process. In Equation 7, the set for the drying constant “k” in relation to the drying temperatures at 30, 40, and 50°C was displayed.

$$K = -0.7838 + 0.0325T \quad (7)$$

R^2= 83.67%

Where K is the drying constant; T is the drying temperature, °C.

In Figure 2, the drying curves are depicted for *S. platensis* with experimental and estimated data by the chosen mathematical model: Midilli for temperatures at 30, 40 and 50°C, and the diffusion proximity model for the temperature at 60°C for time function (h).

In Figure 2, the good adjustment of the model of Midilli for temperatures at 30, 40 and 50°C can be observed and the diffusion proximity model for the temperature at 60°C, once they fit properly the experimental data, reinforcing the applicability of the models for the forecast of the drying data of the *S. platensis*.

Table 5 presented the values of effective diffusion coefficient for the *S. platensis* at studied temperatures, using the radius of the cylindrical particle of 0.001 m.

It is noticeable that with a rise in temperature, the values of the diffusion coefficient increased significantly, as well as displaying the results reported. During the drying of the *S. platensis*, the diffusion coefficient presented magnitude between 3.343×10^{-8} and 14.881×10^{-8} m^2s^{-1}, for temperature range of 30 up to 60°C.

Goneli (2008) explains that when an increase in temperature occurs, the level of vibration in the water

Table 4. Coefficients from the mathematical models chosen by the selection criterion of the statistical estimators set from the drying curve of the *Spirulina platensis*, at studied temperatures.

Model	Coefficients						
	a	b	c	n	k	k_0	k_1
Temperature at 30ºC							
Midilli	1.015	0.007	-	1.542	0.681	-	-
Page	-	-	-	2.083	0.125	-	-
Temperature at 40ºC							
Two exponential terms	2.066	-	-	-	1.244	-	-
Henderson and Pabis modified	24.392	0.143	0.433	-	1.868	-23.555	1.951
Midilli	1.015	0.007	-	1.542	0.681	-	-
Verma	1.866	-	-	-	1.196	-	2.814
Temperature at 50ºC							
Midilli	0.988	0.005	-	2.061	0.757	-	-
Temperature at 60ºC							
Diffusion proximity	59.615	1.021	-	-	3.072	-	-
Two exponential terms	2.181	-	-	-	2.259	-	-

H, k, ko, k_1: Drying constants h^{-1}; e a, b, c, n: coefficients of the models.

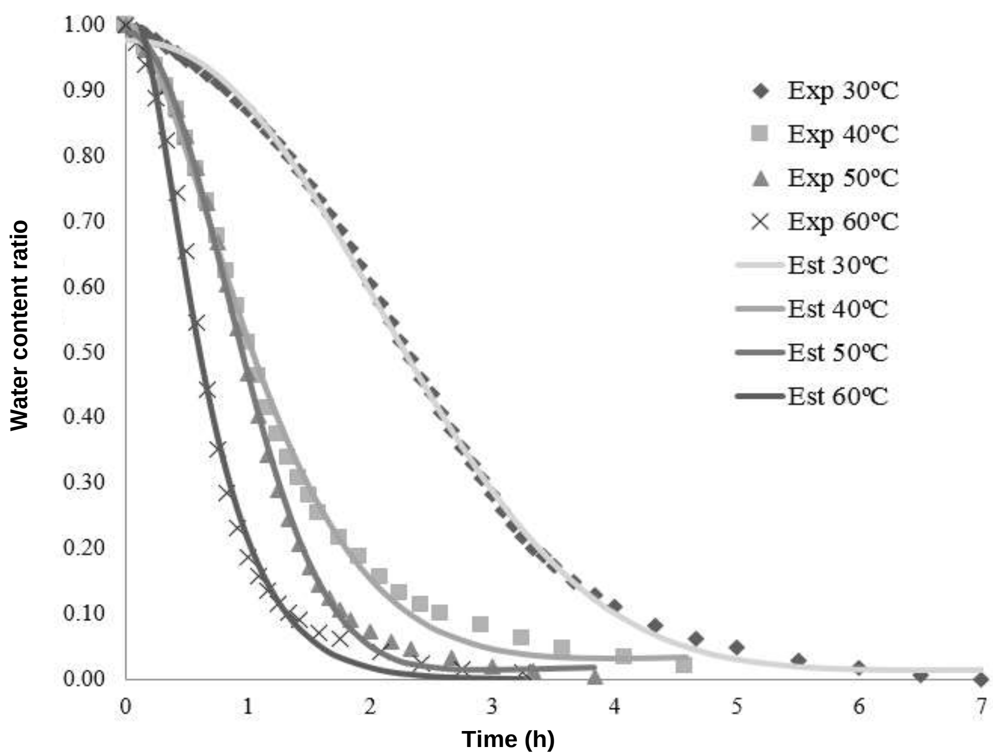

Figure 2. Estimated drying curves (Est) and experimental (Exp) of the *Spirulina platensis*, for temperatures at 30, 40, 50 and 60°C.

molecules also intensifies and its viscosity decreases, which is a measure of fluid resistance to ullage. The variations within this state imply changes in water diffusion into the capillaries of farm products that, alongside with a more intense vibration of water molecules, contribute to a faster diffusion. Therefore, one

Table 5. Effective diffusion coefficient for the *Spirulina platensis* at studied temperatures.

Temperature (ºC)	D_{ef} (×10^{-8}) m²s^{-1}
30	3.343
40	7.838
50	9.608
60	14.881

Table 6. Effective diffusion coefficients for drying various farm products.

References	D_{ef} (m² s^{-1})	Product
Silva et al. (2016)	4.07 × 10^{-9} and 21.42 × 10^{-9}	Cabacinha pepper
Martins et al. (2015)	0.66 × 10^{-11} and 12.07 × 10^{-11}	Fish stupefying leaves
Rodovalho et al. (2015)	2.67 × 10^{-12} and 3.33 × 10^{-12}	Goat pepper seeds
Reis et al. (2015)	1.65 × 10^{-10} and 5.01 × 10^{-10}	Little beak pepper
Goneli et al. (2014a)	1.13 × 10^{-11} and 9.49 × 10^{-11}	Cordia Verbenacea leaves
Goneli et al. (2014b)	0.15 × 10^{-11} and 1.58 × 10^{-11}	Brazilian peppertree leaves

can state that there has been a greater diffusion at 60°C.

According to Rizvi (1995), the effective diffusion coefficient is dependent on the temperature of air used for drying, besides the variety and composition of materials, amongst others; this justifies its increase, with temperature increments of the air used for drying.

In Table 6, the results of the effective diffusion coefficients for drying various farm products are displayed.

Comparing Tables 5 and 6, it is noticeable that the effective diffusion coefficients from the *S. platensis* gathered from the studied temperatures were superior values to the products: cabacinha pepper, fish stupefying leaves, goat pepper seeds, little beak pepper, Cordia Verbenacea leaves and Brazilian peppertree leaves. This can be explained by the chemical constitution of *S. platensis*, which presents weak water link with nutrients, making a higher level of vibration in the water molecules possible, resulting in a reduction in viscosity of the product.

In Equation 8, the linear setting of effective diffusion coefficients is displayed for *S. platensis* in relation to drying temperatures at 30, 40, 50 and 60°C.

$$Def = -7.4546 \times 10^{-8} + 3.638 \times 10^{-9} T \quad (8)$$

R^2 = 96.94%

where D_{ef} is effective diffusion coefficient, $m^2\ s^{-1}$; T is drying temperature, °C.

In Figure 3, the calculated results from D_{ef} are diagrammed, also in the form "ln D_{ef}", described in the mutual function of absolute temperature (1/Ta). The inclination of the curve in Arrhenius' representation delivers the relation Ea/R, whereas its intersection with the Y-axis indicates the value from D_o.

In Figure 3, one can observe that decreasing linearity points out variation uniformity of the drying rate in the studied temperature range.

Equation 9 portrays the coefficient of Arrheinus' equation set for the effective diffusion coefficients of the *S. platensis*, calculated according to Equation 9.

$$D_{ef} = 0.339325.\exp(39{,}520.6/R.Ta) \quad (9)$$

The activation energy (Ea) for liquid diffusion of the *S. platensis*, calculated as the slope of the obtained line, was 39.52 kJ.mol^{-1}. For Zogzas et al. (1996), the activation energy for farm products ranges from 12.7 to 110 kJ mol^{-1}, and the energy found in this study is according to the value range proposed by those authors.

In Table 7, the results of the activation energy for drying various farm products are displayed. Observing the energy activation values for various products and compared to the gathered value 39.52 kJ mol^{-1} of *S. plantesis,* an activation energy close to adzuki beans and crambe is noticeable. In comparing the chemical constitution, the grains have nutrients existing within the *S. platensis*, gathering values close to the activation energy.

It is highlighted that in the drying processes, the lesser the activation energy, the greater will be water diffusion within the product (Goneli et al., 2014a; Jangam et al., 2010). In other words, the energy needed will be smaller, so that during physical transformation, in this case, occurs the transformation of liquid water into vapor (Corrêa et al., 2010). The activation energy is a barrier that should be broken so that the diffusion process is able

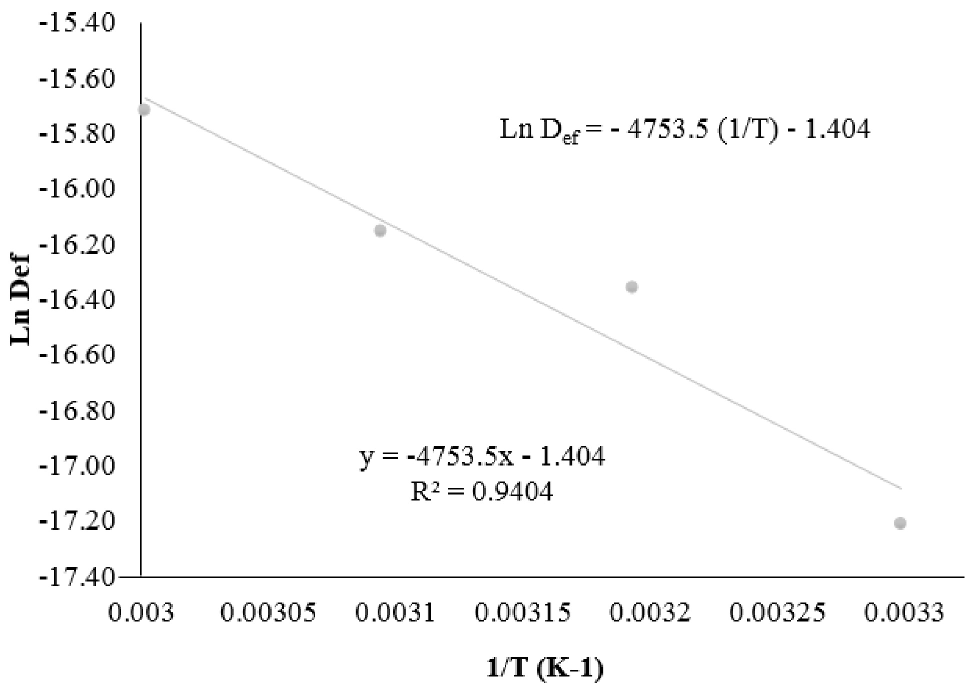

Figure 3. Representation for the effective diffusion coefficient, in relation to air temperature, during the drying of the *Spirulina platensis*.

Table 7. Energy for drying various farm products.

References	E_a (kJ mol^{-1})	Product
Baptestini et al. (2015)	33.10	Soursop foam
Goneli et al. (2014a)	62.89	Cordia Verbenacea leaves
Silva et al. (2014)	34.51	Pigeon pea seeds
Ferreira et al. (2012)	24.512	Fermented grape pomace
Costa et al. (2011),	37.07	crambe
Sousa et al. (2011)	24.78	Turnip feed
Resende et al. (2010)	38.94	Adzuki beans
Martinazzo et al. (2007)	63.47	Lemon grass leaves

to be unleashed in the product (Goneli et al., 2014a; Jangam et al., 2010).

Conclusion

From the results gathered in the study of drying for the *S. platensis* and under the conditions, this work was conducted and one can conclude that:

(1) The amount of time needed for *S. platensis* to reach its constant mass (hygroscopic equilibrium) was 7.00, 4.58, 3.83 and 3.25 h at temperatures at 30, 40, 50 and 60°C, respectively;
(2) The Mathematical model of Midilli at temperatures of 30, 40 and 50°C and the diffusion proximity model at temperature of 60°C are recommended to predict the drying phenomenon of the *S. platensis* for the temperatures studied;
(3) The coefficient of diffusion increased with temperature raise, presenting values ranging from 3.343×10^{-8} to 14.881×10^{-8} $m^2 s^{-1}$, for temperature range of 30 to 60°C;
(4) The relation between the diffusion coefficient and drying temperature can be described by Arrhenius' equation, which presents activation energy for liquid diffusion of the *S. platensis* of 39.52 kJ.mol^{-1}.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Morphophysiological changes in young plants of *Jatropha curcas* L. (Euphorbiaceae) subjected to water stress and recovery

Priscila Souza de Oliveira[1]*, Leandro Dias da Silva[1], Tessio Araújo de Santana[1], Bruno Galvêas Laviola[2], Arlicélio Queiroz Paiva[1], Marcelo Schramm Mielke[1] and Fábio Pinto Gomes[1]

[1]Department of Biological Sciences, State University of Santa Cruz, 45662-900, Ilhéus, Bahia, Brazil.
[2]Brazilian Agricultural Research Corporation (EMBRAPA) - Agroenergy, 70770-901, Brasília, Distrito Federal, Brazil.

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To investigate drought-induced changes in morphophysiological characteristics, seedlings of two genotypes of *Jatropha curcas* (CNPAE 183 and CNPAE 191) were grown under two watering regimes: irrigated (-33.1 to 13.5 to kPa) and water deficit (-409.5 to 49.5 to kPa) for 55 days, followed by six days of rehydration (*DAR*). Withholding water led to a significant reduction (p<0.05) of leaf water potential (Ψ_W) and an increase in relative water content (*RWC*). The values of net photosynthetic rate (P_N), stomatal conductance to water vapor (g_S) and transpiration (*E*) were significantly (p<0.05) reduced 21 and 34 days after starting treatment (*DAST*) in plants of genotypes CNPAE 183 and CNPAE 191, respectively. After 6 DAR, only CNPAE 191 achieved a recovery of P_N and *E*. Moreover, significantly (p<0.05) lower g_S was measured in recovering plants of both genotypes, as compared to the controls. Drought stress led to reductions of 57 and 65% in whole-plant hydraulic conductance (K_L) in genotypes CNPAE 183 and CNPAE 191, respectively. Full recovery of K_L was observed after 6 DAR. The average water consumption was 18% lower in plants subjected to water shortage, as compared to irrigated plants. However, drought-induced reduction in growth led to lower biomass water use efficiency ($WUE_{biomass}$) in plants subjected to water deficit. The effect of water stress was more intense in CNPAE 183 than in CNPAE 191, regarding the growth variables (leaf area, height and diameter), dry mass and root volume. Moreover, a delay in the effect of water stress in genotype CNPAE 191 was also observed, which suggests a higher tolerance of this genotype as compared to CNPAE 183. Altogether, the results showed strong drought-induced stomatal limitation of carbon assimilation and growth in *J. curcas*. Slight genotypic differences were detected, CNPAE 191 being less sensitive to the imposed experimental conditions than CNPAE 183.

Key words: Hydraulic conductivity, root volume, tolerance, leaf gas exchange, water relations, water status.

INTRODUCTION

Jatropha curcas L. *(*Euphorbiaceae) is an oilseed species, which, despite showing a strong capacity for survival and recovery from water stressed conditions (King et al., 2009; Wang et al., 2011; Verma et al., 2012), has shown negative responses to water deficit in reduced growth and biomass production (Fini et al., 2013; Sapeta et al., 2013). The attractive characteristics of *J. curcas* include its expected lifespan of 50 years and its broad climatic tolerance, covering zones with annual precipitation between 250 and 1200 mm (Achten et al., 2008). Moreover, production of *J. curcas* oil does not entail competition with food crops, because its oil is non-edible (Tiwari et al., 2007; Islam et al., 2014) and this is one of the effective ways to overcome the problems associated with energy crisis and environmental issues (Ong et al., 2013). Its seeds may contain 11.7 to 42.1% oil depending on soil type and environmental conditions (Kaushik and Bahrdway, 2013), which makes it very promising for the production of biodiesel (Kheira et al., 2009).

Water is one of the main limiting factors for plant production worldwide. Due to the high economic and ecological costs of irrigation and the need for plant production in increasingly arid environments, the production and use of cultivars adapted to drought is of great importance. One of the more studied mechanisms through which plants can increase water use efficiency (WUE) is the stomatal regulation of water loss by transpiration. However, drought tolerant species with such characteristics tend to exhibit reduced growth rates, due to stomatal limitations to the uptake of CO_2 for photosynthesis (Verma et al., 2012).

The use of deficit irrigation has achieved promising results for *Cocos nucifera* (Arecaceae) (Azevedo et al., 2006) and *Citrus latifolia* (Rutaceae) (Sampaio et al., 2010) as a tool for increasing WUE. Through deficit irrigation regimes, three strategies could be used to increase WUE: (1) increase the capacity of water absorption; (2) increase the transpiration efficiency by drought-induced signaling and (3) modify the pattern of allocation of assimilates in favor of the economically viable structure (Condon et al., 2004), that is, augmenting the harvest index.

Regardless of some results showing a delay in the growth of *J. curcas* subjected to water deficit, Fini et al. (2013) concluded that the species can survive dry periods (20% of field capacity) of moderate duration (18 days). However, the effects on the economic profitability of these crops are still unknown. Thus, studies dealing with drought effects on growth and development of different genotypes are likely to be essential components in the success of breeding programs.

This experiment aimed to evaluate the initial growth and estimate the effects of water stress and post-drought recovery on the water relations of two genotypes of *J. curcas* (CNPAE 183 and CNPAE 191). The main hypothesis is that the effects of water stress on the morphophysiological characteristics of *J. curcas* vary with time and intensity of stress and are genotype dependent.

MATERIALS AND METHODS

Plant material and growth conditions

The experiment was conducted under greenhouse conditions from June to September 2012, in the campus of the State University of Santa Cruz, Ilhéus, BA (14°47'00"S, 39°02'00" W). According to the Köppen climate classification, the local climate is type Af, with annual average temperatures of 22to 25°C (Koppen, 1900).

J. curcas seeds of two genotypes were used, the CNPAE 183 and the CNPAE 191, from Jaíba/MG and São Francisco of Assis/RS, respectively. The selection of these genotypes was based on the differences obtained from preliminary data from EMBRAPA Agroenergia. The CNPAE 183 is a non-toxic genotype with a low yield (500 g of seed plant^{1}), average height of 2.4 m, and is from a tropical region. The CNPAE 191 has a high productivity (1030 g seed plant^{-1}), 3 m of height, is toxic to animals and is from a region of subtropical climate. The *J. curcas* seeds were germinated in pots containing 65 kg of substrate soil : sand (2:1), to match the loamy sand textural class. Forty days after germination, the pots were covered with aluminum foil to prevent loss of water by evaporation, thereby accounting for the water lost only by leaf transpiration. Then, 22 plants were subjected to a controlled irrigation treatment (60% of field capacity) for 55 days, which led to substrate water deficit (-49.5 to -409.2 kPa) and the other 22 plants to field capacity (-13 1 to -33.1 kPa), followed by rehydration for 6 days.

Water consumption in the two treatments was measured by means of periodic weighing of pots, using load cells CSA/ZL - 100 (MK Control Instruments, Brazil) coupled with an automatic data collector. The determination of the soil water content was carried out weekly by the gravimetric method, and simultaneously the soil matric potential for each treatment was determined using a soil water retention curve. The photosynthetically active radiation (PAR) was monitored using quantum sensors S-LIA-M003. The temperature and relative humidity were recorded using Hobo H8 Pro Series data loggers (Onset, USA). A summary of the microclimatic conditions during the experiment is shown in Table 1.

Water relations

Leaf water potential (Ψ_W) was measured in three randomly selected leaves per genotype per treatment, using a pressure chamber model 1000 (PMS Instrument Company, USA). The measurements were made between 2 and 4 am ($\Psi_{Wpredawn}$) on 35, 50 and 55 DAST and 6 DAR.

Whole plant hydraulic conductivity (K_L) was estimated on the peak of water stress (55 DAST) using the formula $K_L = g_S$ VPD/($\Psi_{Wpredawn}$) – $\Psi_{Wmidday}$, where g_S is the stomatal conductance to water vapor (see below), VPD is the vapor pressure deficit between the atmosphere and the leaf and $\Psi_{Wmidday}$ is the water potential measured at midday (Hubbard et al., 1999).

Leaf relative water content (RWC) was measured at 55 DAST and 6 DAR. Measurements were performed between 6 to 7 am. For this purpose, five discs were removed from mature leaves, immediately weighed to obtain fresh mass (Mf) and placed under water in the dark for 12 h until full rehydration. The discs were

*Corresponding author. E-mail: priscilagronoma@gmail.com. Tel: +557336805285. Fax: +557336805226.

Table 1. Photosynthetically active radiation (PAR - mol photons m^{-2} day^{-1}), air temperature (Tair - ° C) and relative humidity (RH-%) over the trial period.

Variable	Mean	Maximum	Minimum
PAR	14.22 (0.44)	20.35	8.8
Tair	25.20 (0.20)	28.40	21.9
RH	76.80 (1.13)	97.90	57.0

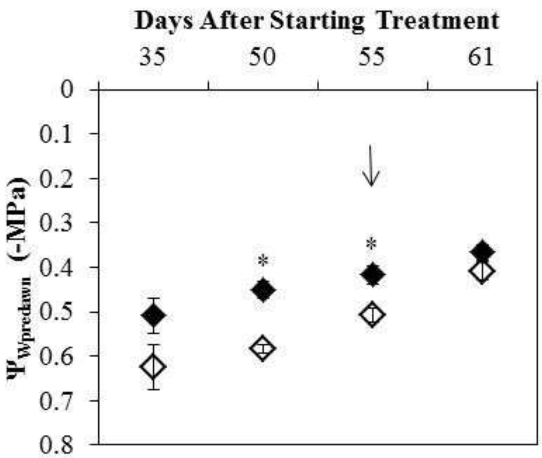

Figure 1. Leaf water potential (Ψ_w) of two genotypes of *J. curcas* plants exposed to water stress and after recovery. Control plants (filled symbols) and plants under water stress (open symbols). The arrow indicates the start of water replacement. Data refer to mean values (n = 6). * Significant by F test (p <0.05).

weighed again to obtain the turgid mass (Mt) and placed in a forced ventilation oven at 75°C until constant dry mass (Md). From these variables, the RWC was calculated as [RWC = ((Mf-Md) / (Mt-Md)) x100] (Nauš et al., 2016).

Leaf gas exchange

Leaf gas exchange variables (net photosynthesis (P_N), transpiration rate (E), stomatal conductance to water vapor (g_S) and the internal CO_2 concentration (C_I)) were measured at 21, 28, 34, 41, 49, 55 DAST and 6 DAR in fully mature leaves positioned on the stem opposite to those sampled for leaf water potential, between 7 and 10 h in all individuals of each treatment (De Santana et al., 2015). A portable gas exchange system (LI-6400, LI-Cor®, Nebraska / USA) was used. During the measurements, the LI-6400 was set to hold constant photosynthetically active radiation (PAR) at 1000 μmol photons m^{-2} s^{-1} and reference CO_2 concentration at 380 μmol mol^{-1}.

Water use efficiencies and water consumption of the plant

Instantaneous and intrinsic water use efficiencies were estimated as the ratios of P_N and E and of P_N and g_S, respectively. Water use efficiency of biomass was calculated as the ratio of the total biomass of the last harvest and water consumed during the experiment, which was measured by sequentially weighting the pots using load cells placed beneath each pot.

Growth

The height, stem diameter, total leaf number and leaf area of all plants were evaluated weekly. Individual leaf area (LA) was estimated from sum of measurements of the length of the midrib (L) and maximum width (W) of each leaf, which were used in the equation $LA = (LW)^{0.9660}$ suggested by Pompelli et al. (2012). The results were summed to obtain the total leaf area.

Root volume of all plants was measured by displacement of water equivalent units (1 mL = 1 cm^3). The length of root systems was also quantified at the end of the experiment by taking the average of the three largest roots. The plants were then collected for the determination of total dry matter of root, stem and leaves, after complete dehydration in a forced ventilation oven (65 ± 5°C).

Statistical analysis and experimental design

The experiment was arranged in a completely randomized factorial (2x2) design, formed by two watering regimes and two genotypes of *J. curcas*, with six replicates. The data were subjected to a factorial analysis of variance and means were compared by F test (comparison between genotypes and water regimes) with a significance criterion of 0.05.

RESULTS

Water relations

Significant reduction ($p<0.05$) of $\Psi_{Wpredawn}$ was observed from 50 DAST in plants of both genotypes, growing under water stress (Figure 1). Full recovery of water status was observed 6 DAR. Hydraulic conductivity (K_L) was significantly ($P<0.05$) lower in stressed plants of CNPAE 183 and CNPAE 191. However, after rehydration there was no significant ($p<0.05$) difference of K_L between the control and stressed plants of either genotype (Figure 2).

Significant differences ($p<0.05$) between treatments (but not between genotypes) were observed 55 DAST for RWC. RWC was shown to increase in water stressed plants of both genotypes (Figure 3A). After rehydration, RWC decreased in water stressed plants of both genotypes as compared to their control but this decrease was only significant ($p<0.05$) in CNPAE 191 (Figure 3B).

Leaf gas exchange

Net photosynthetic rate (P_N), stomatal conductance to water vapor (g_S) and transpiration rate (E) were significantly ($p<0.05$) reduced in both genotypes by the water stress treatment. Moreover, this reduction was more delayed but greater in magnitude in CNPAE 191 than in CNPAE 183 (Figure 4). Water deficit led to P_N, g_S and E reductions of 34, 67 and 48% from 21 DAST on for genotype CNPAE 183 and of 49, 74 and 67% from 34 DAST on for CNPAE 191, respectively. After the six-day

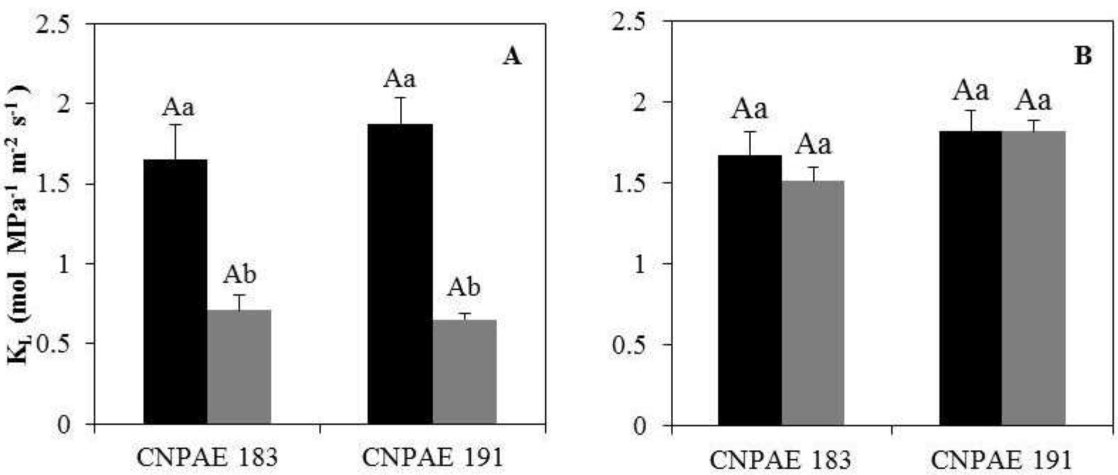

Figure 2. Hydraulic conductivity (K_L - mol H_2O MPa $m^{-1}s^{-1}$) of two genotypes of *J. curcas* (CNPAE 183 and 191) under irrigation (black bars) and water stress (gray bars) after 55 days of starting treatment (A) and after six days rehydration (B). Capital letters indicate comparison between genotypes and lowercase letters between water regimes, by the F test ($p<0.05$). Data refer to mean values of 3 repetitions and the bars indicate the standard error of the mean.

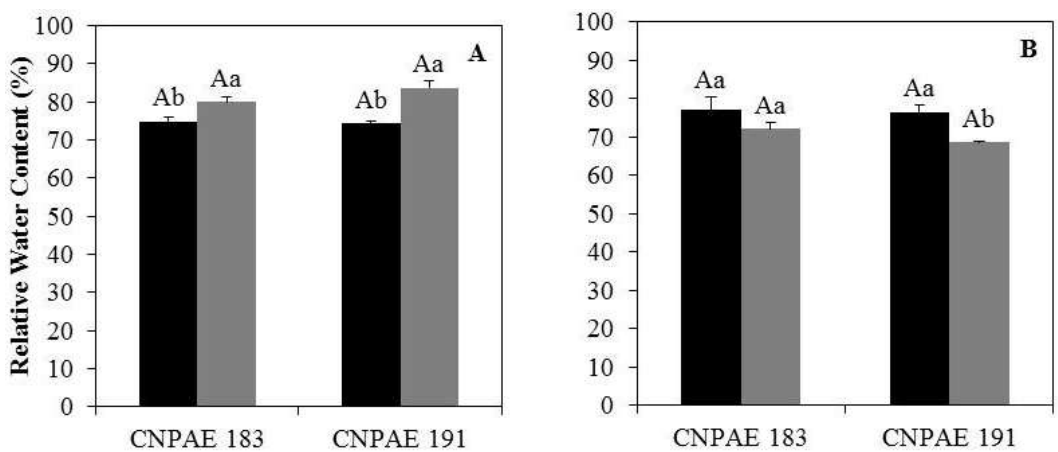

Figure 3. Relative water content (RWC) measured at peak stress to 55 days after starting treatment (A) and six days after rehydration (B) in two genotypes of *J. curcas*. Control plants (black bars) and plants under water stress (gray bars). Capital letters indicate comparison of genotypes and lowercase letters between water regimes, by the F test ($p<0.05$). The points represent the mean values of 3 replicates and the bars indicate the standard error of the mean.

rehydration period, significant differences ($p<0.05$) between treatments for g_S were still observed in both genotypes (Figure 4C and D).

There were no significant differences ($P<0.05$) between genotypes for the intrinsic (P_N/g_S) and instantaneous (P_N/E) water use efficiencies. While no significant differences were observed for P_N/E in CNPAE 183 throughout the experimental period (Figure 5A), a significant ($p<0.05$) increase (30%) of P_N/E was observed from 34 DAST in water stressed plants of CNPAE 191 (Figure 5B). The trend of P_N/g_S was similar for both genotypes, with a significant increase ($p<0.05$) from 34 DAST, which peaked at 85 and 96% increases relative to their controls at 55 DAST in CNPAE 183 and 191, respectively (Figure 5C and D). However, both measures of WUE dropped upon rewatering, but reached values comparable to control only in CNPAE 191 at 6 DAR (Figure 5).

Growth

There were significant differences between genotypes and water regimes for the height, number of leaves and

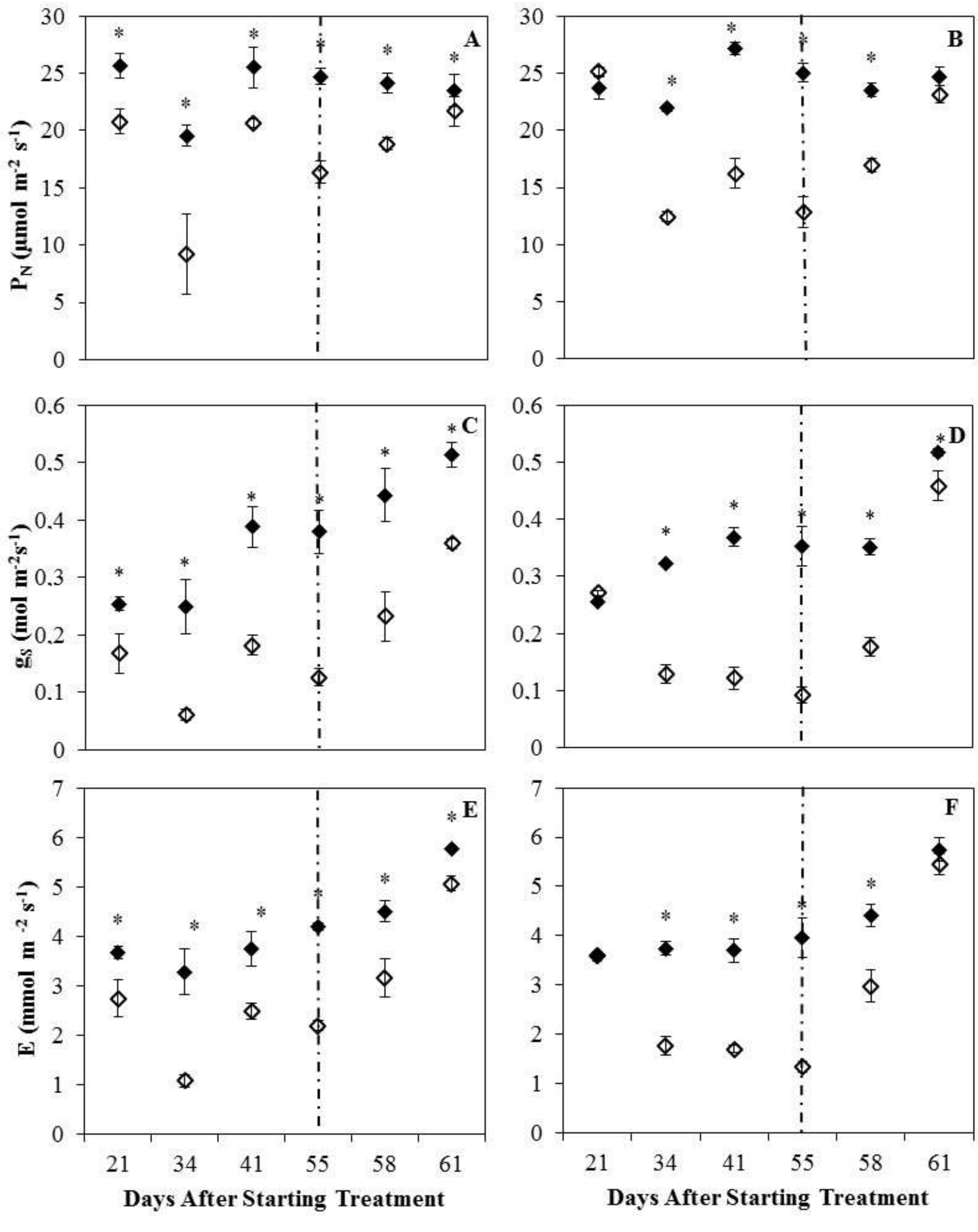

Figure 4. (A and B) Net photosynthetic rate (P_N), (C and D) stomatal conductance (g_S), (Eand F) transpiration (E) of two genotypes of *J. curcas*, CNPAE 183 (A, C and E) and CNPAE 191 (B, D and F), during 55 days of water stress and six days after rehydration. Control plants (filled diamonds) and plants under water stress (open diamonds). Asterisks (*) indicate significant differences between water regimes (F test, $p < 0.05$). Pointed-line indicate the beginning of rehydration. Points represent mean values of 3 to 5 replicates and bars indicate standard error of the mean.

leaf area. However, the diameter was affected significantly only for the treatment of water stress (Figure 6). Although, smaller than CNPAE 183 (69 vs. 85 cm in height), CNPAE 191 exhibited a delay in the effects of water stress as indicated by measurements of growth variables. Significant reductions ($p < 0.05$) in height were

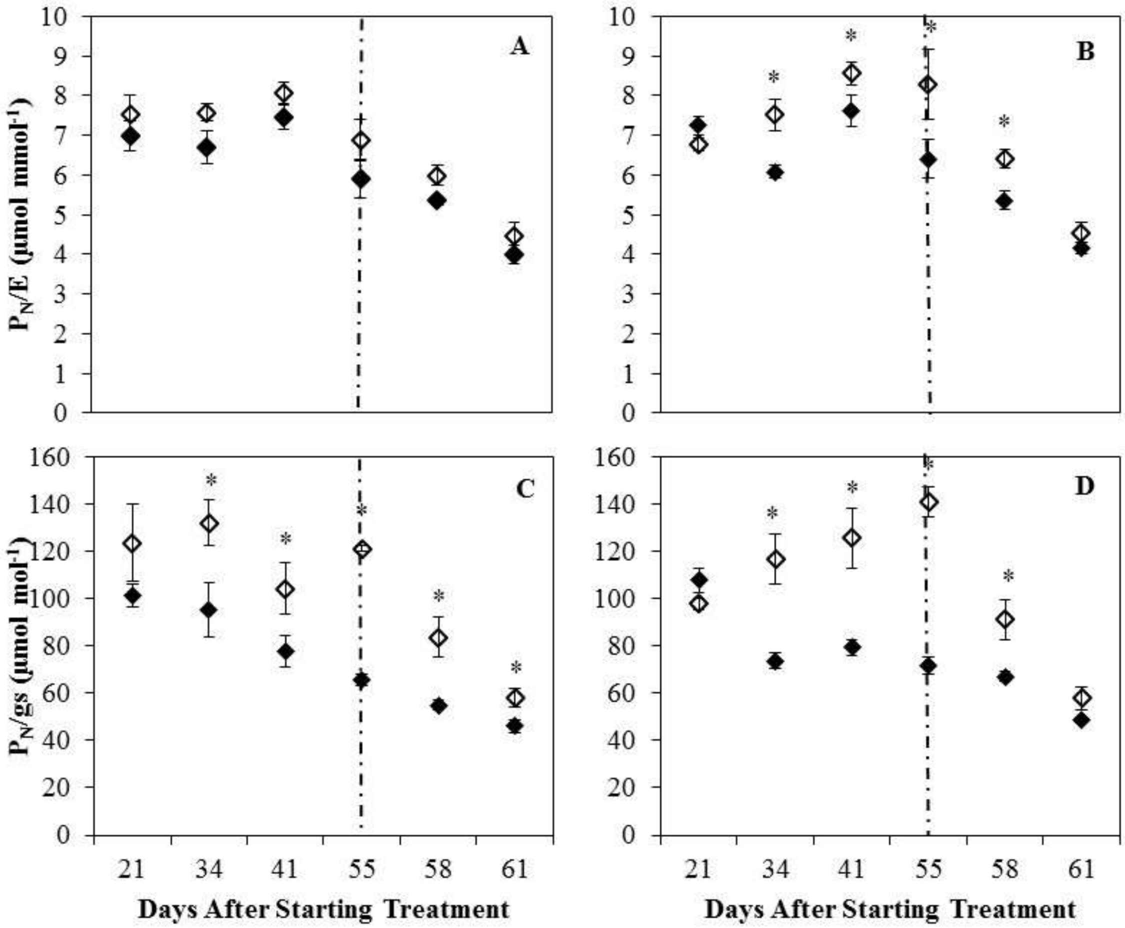

Figure 5. Instantaneous (P_N/E) and intrinsic (P_N/g_S) water use efficiency in two genotypes of *J. curcas,* CNPAE 183 (A and C) and CNPAE 191 (B and D) under control treatment (filled diamonds) and water stress (open diamonds). Asterisks (*) indicate significant differences between water regimes (F test, P<0.05). Pointed-line indicates the beginning of rehydration. Points represent mean values of 5 to 6 replicates and bars indicate standard error of the mean.

observed at 61 and 54 DAST in CNPAE 191 (10%) and CNPAE 183 (30%), respectively (Figure 6A and B). The reduction in diameter was 10% for both genotypes. However, this effect was observed from 41 DAST in CNPAE 183 and from 47 DAST in CNPAE 191 (Figure 6C and D). The number of leaves decreased (24%) significantly (p <0.05) only for CNPAE 183 from 54 DAST (Figure 6E and F).

Significant differences (p<0.05) between genotypes under irrigated conditions were also observed for mean leaf area (0.67 m² in CNPAE 183 and 0.45 m² in CNPAE 191). The effect of water deficit occurred earlier (41 DAST) and was more pronounced (45% reduction in relation to control) in CNPAE 183. In CNPAE 191, a significant difference (p<0.05) between the water regimes for leaf area was observed from 47 DAST, with a reduction of 25% relative to the control (Figure 7).

Water stress led to reductions of leaf (LDM) and total (TDM) dry mass in both genotypes, and of root (RDM) and shoot (SDM) dry mass only in CNPAE 183 (Figure 8). The total biomass yield at the end of the experiment was affected by water stress in plants of CNPAE 183, with observed reductions of 29, 50, 78 and 55% of RDM SDM, LDM and TDM, respectively. In CNPAE 191, the observed reductions in dry weights water stressed plants relative to control plants were 70% for LDM and 49% for TDM (Figure 8).

There were no significant differences between genotypes for water consumption. However, water deficit did lead to a reduction in water consumption by the plants (Figure 9A) and, consequently, a reduction in the production of biomass. There was a 20 and 15% reduction in water consumption for genotypes CNPAE 183 and 191, respectively. Nevertheless, water deficit led to a significant (p<0.05) reduction in the biomass water use efficiency in both genotypes (Figure 9B).

The root volume in well-watered plants of CNPAE 191 was significantly (p<0.05) lower than in CNPAE 183.

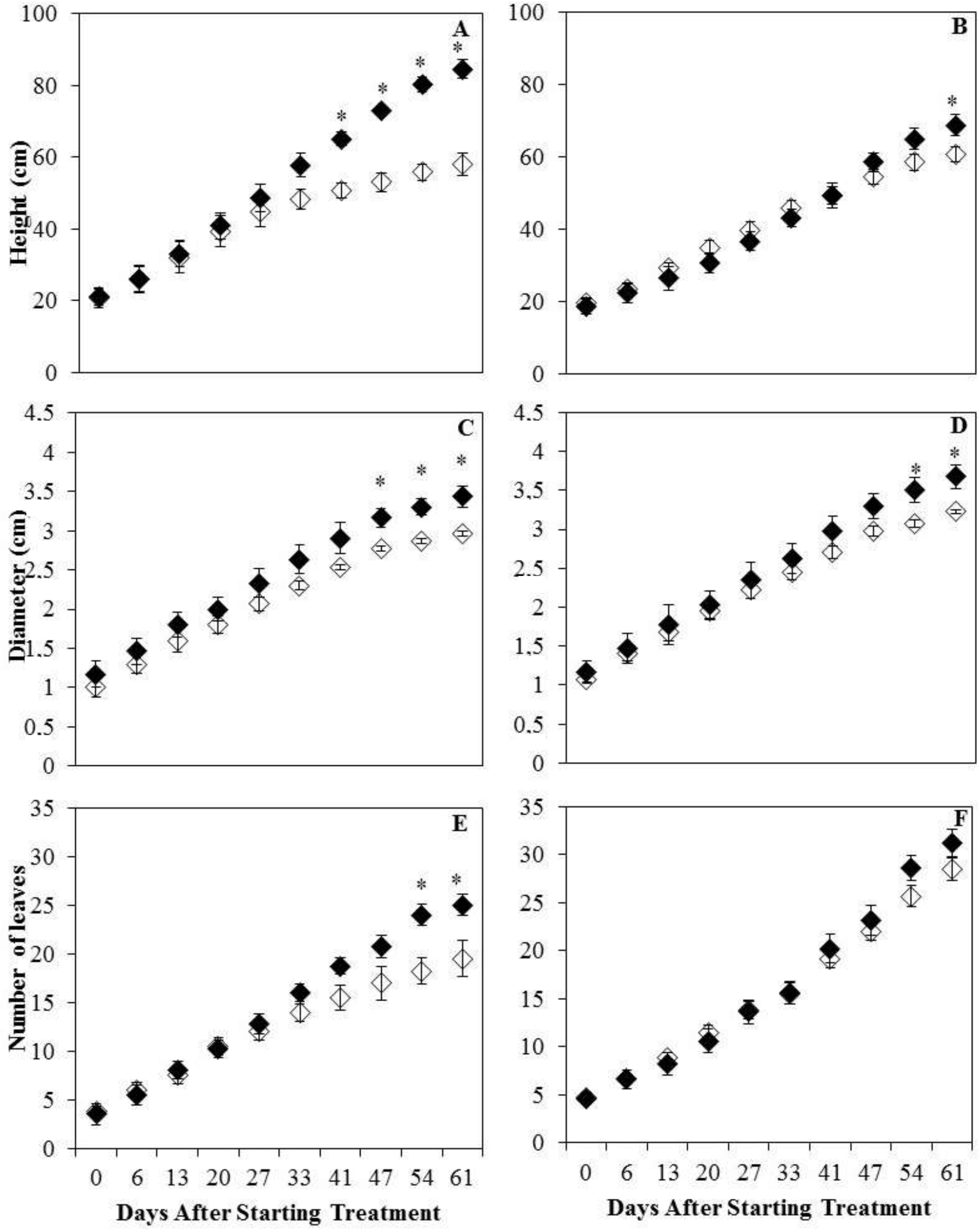

Figure 6. Height, diameter and number of leaves of seedlings of *J. curcas,* genotypes CNPAE 183 (A, C e E) and CNPAE 191(B, D e F), submitted to 55 days of water stress and six days of rehydration. Control plants (filled diamonds) and plants under water stress (open diamonds). Asterisks (*) indicate significant differences between water regimes (F test, p<0.05). Pointed-line indicate the beginning of rehydration. Points represent mean values of 4 to 5 replicates and bars indicate standard error of the mean.

However, water stress induced significant reduction (25% lower than control) of root volume in CNPAE183 and significant increase of root length in CNPAE 191 (Figure 10). For CNPAE 183, there was no difference between

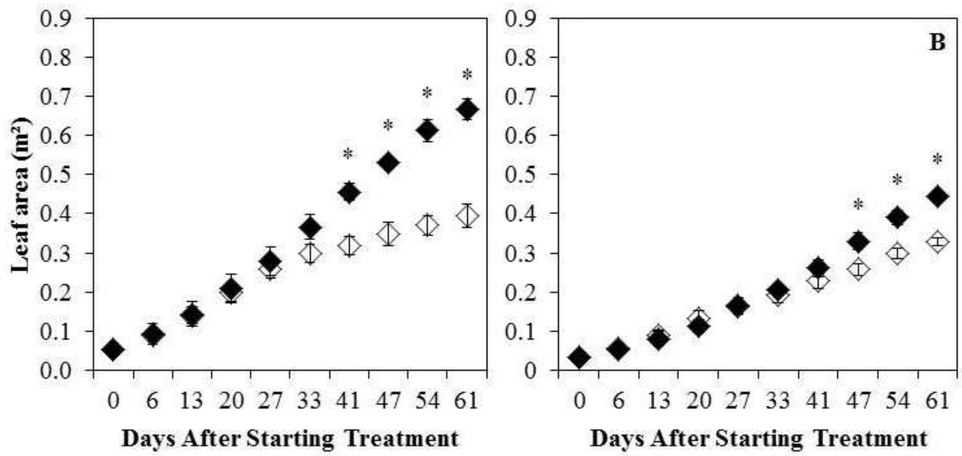

Figure 7. Leaf area (m^2) of seedlings of *J. curcas* genotypes CNPAE 183 (A) and CNPAE 191(B) submitted to 55 days of water stress and six days of rehydration. Control plants (filled diamonds) and plants under water stress (open diamonds). Asterisks (*) indicate significant differences between water regimes (F test, P<0.05). Points represent mean values of 4 to 5 replicates and bars indicate standard error of the mean.

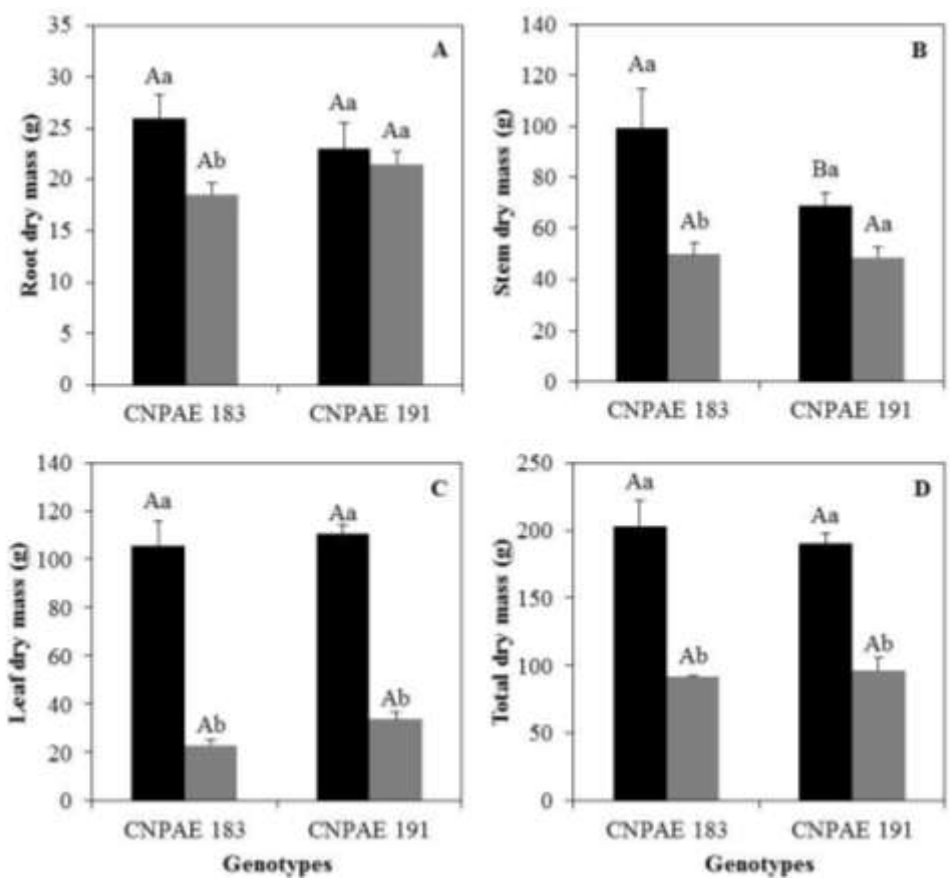

Figura 8. Root dry mass (A), stem dry mass (B), leaf dry mass (C) and total dry mass (D) of two genotypes of *J. curcas* (102 days). Control plants (black bars) and plants under water stress (gray bars). Capital letters indicate comparison of genotypes and lowercase letters between water regimes (F test, p<0.05). Points represent mean values of 4 to 5 replicates and bars indicate the standard error of the mean.

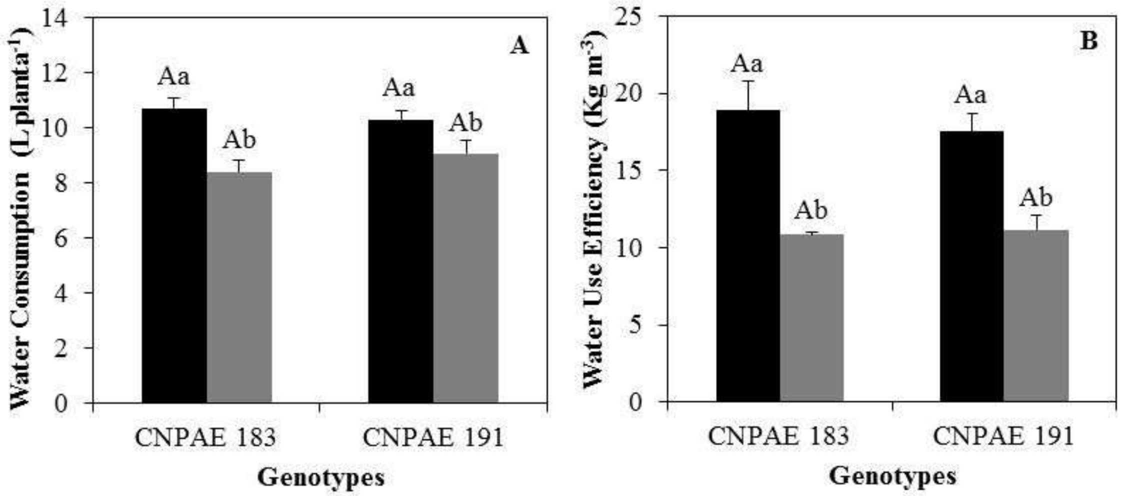

Figure 9. Total water consumption (A) and the biomass water use efficiency (B) calculated at the end of the experimental period. Control plants (black bars) and plants under water stress (gray bars). Capital letters indicate comparison between genotypes and lowercase letters indicate comparison between water regimes (F test, $p<0.05$). Columns represent mean values of 3 replicates and bars indicate standard error of the mean.

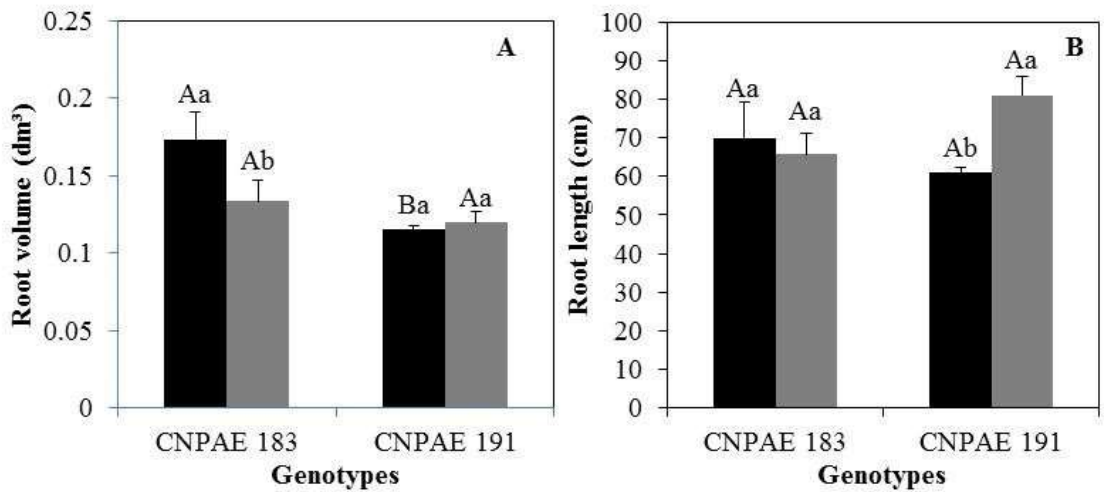

Figure 10. Root volume and root length of genotypes CNPAE 183 and CNPAE 191 of *J. curcas*. Control plants (black bars) and plants under water stress (gray bars). Capital letters indicate comparison of genotypes and lowercase letters between water regimes (F test, $p<0.05$). Points represent mean values of 4-5 replicates and bars indicate standard error of the mean.

roots lengths of water-stressed and control plants.

DISCUSSION

Although, *J. curcas* has been described as being adapted to arid conditions (King et al., 2009; Verma et al., 2012), it is indisputable that under adequate water availability, this species will show higher productivity. However, plants use various strategies to survive the water restriction periods, such as roots and leaves morphological changes, osmotic adjustment, increased abscisic acid content (ABA). The capacity Ψ_W reduction (osmotic adjustment) is also a common mechanism to avoid dryness in *J. curcas*, maintaining cell function through high RWC and stomatal closure (Fini et al., 2013; Tiwari et al., 2013; Fang and Xiong, 2014; Silva et al., 2015).

The continuation of drought stress caused no major decrease in Ψ_W, because as stomata close, there is also a decrease in leaf water loss (Figures 1 and 4), confirming the findings of Fini et al. (2013). These authors also noted that Ψ_W from plants that were drought stressed matched that of control plants after rehydration, thus demonstrating the species' ability to fully recover after a period of water stress. Silva et al. (2010), by imposing water stress on plants of *J. curcas* observed a reduced Ψ_W in stressed plants as compared to the control, but the relative water content (RWC) was only affected by water stress when the substrate water content was lowered to 10% of field capacity. This led to an increase of RWC as compared to the control. The increase in RWC in this work shows that conservation of the water content in plant tissues can also be considered a strategy of this species to survive periods of water deficit in soil. Results obtained by Sapeta et al. (2013) are similar to those found in this work, with an increase in RWC at the peak of water stress and recovery after 7 days of rehydration. According to Silva et al. (2012) osmotic adjustment is responsible for maintaining a high RWC in *J. curcas* tissues.

The coordination of hydraulic conductance with several physiological traits has been demonstrated to be linked to water and carbon balances (Brodribb and Jordan, 2008; Brodribb et al., 2010; Martinez-Vilalta et al., 2014; Pivovaroff et al., 2014). Water stress strongly affects the leaf hydraulic system, causing a decline in water potential (Brodribb and Holbrook, 2006). Genotypic differences in *J. curcas* have been detected recently regarding the trade-offs between K_L and growth under moderate water deficit (Santana et al., 2015). The authors demonstrated that three genotypes showed similar reductions in biomass accumulation (about 37%), although one of them (CNPAE 126) showed lower reduction in K_L (62% as compared to 88% in the other two genotypes). In the present work, a similar effect of water deficit on biomass accumulation (50% of reduction as compared to their controls) was observed for both genotypes (Figure 8D), although a greater reduction of K_L (down to 67% of the control value) was observed in CNPAE 191 than in CNPAE 183 (58%) (Figure 2A).

In a study by Díaz-Lopez et al. (2012), *J. curcas* showed higher P_N/g_S after 27 days under deficit irrigation (75% field capacity). This effect was corroborated in our study by 34 DAST for both genotypes. After six days of rehydration, the stressed plants of genotype CNPAE 191 matched the control, which shows once again that these plants decreased g_S and E to prevent dehydration under water deficit. Similar results were found by Fini et al. (2013), where the decrease of irrigation resulted in a gain of P_N/g_S and P_N/E, and after 12 days of rehydration plants fully recovered. This shows that *J. curcas* is able to use physiological mechanisms to survive periods of water deficit, resuming normal physiological function upon return to well-watered conditions.

Water stress reduces photosynthetic rate due to decreased stomatal conductance (Figure 4), through stomatal closure. However, with closed stomata, gas exchange and CO_2 assimilation by C3 photosynthesis is negligible (Chaves et al., 2009). This may account for a delay in the growth of the plant.

Verma et al. (2012) found similar patterns in gas exchange when they subjected plants to water stress at 50% of field capacity (FC). Sousa et al. (2012), also using a drought treatment of 50% of FC, found reductions of around 70% of carbon assimilation as compared to the control. Variation in the results of such studies can be attributed to the soil and climatic conditions of each region, pot size, the vapor pressure deficit and temperature, as well as nutritional status and genetic factors that may influence the physiological characteristics of the plant.

After six days of rehydration P_N and E recovered to the control values in genotype CNPAE 191. However, g_S shown by rehydrated plants remained significantly different from that of control plants. Similar results show that the recovery of g_S is slower than P_N, because this recovery is linked to the gradual decrease in the concentration of abscisic acid (ABA), and the time required for this to occur depends on the plant species and degree of stress (Pompelli et al., 2010). On the other hand, Silva et al. (2015) recently showed a more rapid restoration (5 days) of g_S than of to P_N (10 days), suggesting the need for recovery of g_S to facilitate the restoration of P_N.

Pompelli et al. (2010) reported values of P_N lower than 5 µmol CO_2 m^{-2} s^{-1}, when soil water content had reached 5%. However, plants recovered in at least four days, reaching values of P_N and g_S higher than the control plants, confirming the data presented here, which allows us to infer that these plants controlled dehydration by reducing g_S. However, the decrease of g_S caused by the closure of the stomata for water conservation entails an unavoidable decrease in CO_2 up take, thereby limiting plant productivity.

Water stress causes negative effects on cell expansion and photosynthesis, which causes a reduction in plant growth (Zhu, 2002), as observed in this study. Drought-induced reduction of growth in *J. curcas* has been considered a bottleneck concerning the potential use of this species as a bioenergy crop in arid and semiarid environments worldwide, where soil water potential may become very low during long time of drought (Fini et al., 2013). Reductions of 50% in the number of leaves for genotypes *J. curcas* from Brazil and Tanzania and a 90% for genotypes from Suriname, after 18 days without irrigation have been demonstrated (Fini et al., 2013). In this study, drought led to reductions of 22 and 9% in the number of leaves for genotypes CNPAE 183 and CNPAE 191, respectively. However there was no shedding leaf, but a reduction in the emission of new leaves.

Sapeta et al. (2013) demonstrated significant reductions in height and number of leaves of *J. curcas* from the 7th day of severe water stress imposition. In this study, the lowest number of leaves on the water stressed plants of genotype CNPAE 183 was due to reduced initiation of new leaves, as a mechanism to decrease the surface area for transpiration. However, this mechanism may cause losses in crop yield, because with a reduced leaf area, there is a decrease in light interception, thus decreasing overall photosynthetic capacity. Maes et al. (2009), in subjecting *J. curcas* to water deficit (40% FC), found reductions in leaf area of approximately 57% as compared to control. Verma et al. (2012), after 50 days of imposing water stress at 75 and 25% of field capacity reported 11 and 55% reductions in leaf area of *J. curcas*.

The similarity between the RDW of control and stressed plants for CNPAE 191 explains the smaller effect of water stress on the biometric characteristics (height, diameter, number of leaves and leaf area), making a good indicator of drought tolerance (Figure 8). The stressed plants of genotype CNPAE 191, however, as a mechanism to prevent dehydration, increased the length of their roots, to explore a larger volume of soil (Figure 10), which according to Hammer et al. (2009) are characteristics associated with drought resistance. With the reduction in leaf area and maintenance of RDW, there is greater hydration of plant tissue (measured as RWC), favoring the continued growth and development of the plant (Silva et al., 2010). As experimental water stress was not imposed for a long time, the recovery of gs was faster, favoring CO_2 entry into the cell, which is an interesting quality for genetic improvement, as it allows to increase the effective use of water (EUW), influencing the plant stress tolerance and avoiding reduced productivity (Blum, 2009).

Conclusions

Moderate and rapidly-imposed water deficit, as imposed here, negatively affect the gas exchange and biomass water use efficiency of *J. curcas*, despite reductions in water consumption and increased photosynthetic water use efficiency. Maintenance of high RWC and Ψw under water deficit, as well as a lack of genotypic variation in that characteristic indicate that both *J. curcas* genotypes are water savers. The genotype CNPAE 191 should be considered for further investigation concerning the tradeoffs between RWC and root traits, in the search for genetic material suitable for cultivation in areas subject to short periods of soil water deficit.

Abbreviations

CI, Internal **CO2** concentration; **DAR**, days after rehydration; **DAST**, days after starting treatment; **DW**, dry weight; **E**, transpiration rate; **FC**, field capacity; **gS**, stomatal conductance to water vapour; **KL**, hydraulic conductivity; **LA**, leaf area; **PAR**, photosynthetically active radiation; **PN**, net photosynthesis; **PN/gS**, intrinsic water use efficiency; **PN/E**, instantaneous water use efficiency; **RWC**, relative water content; **Ψw**, leaf water potential; **WS**, water stress conditions; **WUE**, water use efficiency.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

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