OPEN ACCESS

African Journal of Agricultural Research

31 May, 2018
ISSN 1991-637X
DOI: 10.5897/AJAR
www.academicjournals.org

ACADEMIC JOURNALS
expand your knowledge

ABOUT AJAR

The African Journal of Agricultural Research (AJAR) is published weekly (one volume per year) by Academic Journals.

African Journal of Agricultural Research (AJAR) is an open access journal that publishes high-quality solicited and unsolicited articles, in English, in all areas of agriculture including arid soil research and rehabilitation, agricultural genomics, stored products research, tree fruit production, pesticide science, postharvest biology and technology, seed science research, irrigation, agricultural engineering, water resources management, marine sciences, agronomy, animal science, physiology and morphology, aquaculture, crop science, dairy science, entomology, fish and fisheries, forestry, freshwater science, horticulture, poultry science, soil science, systematic biology, veterinary, virology, viticulture, weed biology, agricultural economics and agribusiness. All articles published in AJAR are peer- reviewed.

Contact Us

Editorial Office: ajar@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJAR

Submit manuscript online http://ms.academicjournals.me/

Editors

Editorial Board

African Journal of Agricultural Research

Table of Contents: Volume 13 Number 22, 31 May, 2018

ARTICLES

***In vitro* regeneration of *Treculia africana* Decne. From embryo explants on different nutrients and sucrose conditions** **1111**
Isaac, U. G., Okafor, C. U. and Okezie, C. E. A

Harnessing cultivar performance and stability for deploying superior groundnut plant types in the Lake Albert Crescent Zone of Uganda **1120**
Kakeeto, R., Wambi, W., Barwogeza, M., Auma, L., Odongo, W., Ozuma, G. and Ssekiwoko, F.

Characterization and evaluation of volatile compounds of three grape varieties *(Vitis labrusca*) from the region of Bento Gonçalves – RS **1128**
I. T. S. Viana, H. V. Pereira, M. R. Silva, M. N. S. Guedes, A. G. de Souza, R. Augusti, J. R. L. Freitas, G. H. Bueno and J. O. F. Melo

Can early peroxidase quantification detect graftcompatible in anonaceous rootstocks? **1136**
Daniel Baron, Amanda Cristina Esteves Amaro, Ana Claudia Macedo, Carmen Sílvia Fernandes Boaro and Gisela Ferreira

Nutritive potential of amaranth weed grains **1140**
João Barcellos Xarvier, Douglas Correa de Souza, Leticia Cavalheiro de Souza, Thiago Sampaio Guerra, Luciane Vilela Resende and Joelma Pereira

Plant cover management and nitrogen fertilization in maize crop in a dystrophic red Latosol Brazilian Cerrado (Savannah) **1148**
José Antônio Maior Bono, Bruna Kelle Della Colleta, Marlos Ferreira Dornas, Silvia Rahe Pereira, José Francisco dos Reis Neto and Bianca Obes Corrêa

Effect of splitting nitrogen fertilization on Tifton 85: Yield, nitrogen use efficiency, and nitrogen nutritional status of plants and soil **1154**
Tangriani Simioni Assmann, Etiane Tanise Sonego, Alceu Luiz Assmann, Paulo Fernando Adami and Claucia Cuzzi

Vol. 13(22), pp. 1111-1119, 31 May, 2018
DOI: 10.5897/AJAR2018.13102
Article Number: C3D98B057246
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

In vitro regeneration of *Treculia africana* Decne. from embryo explants on different nutrients and sucrose conditions

Isaac, U. G., Okafor, C. U.* and Okezie, C. E. A

Plant Biotechnology Research Laboratory, Department of Plant Science and Biotechnology, Faculty of Biological Sciences, University of Nigeria Nsukka, Enugu State, Nigeria.

Received 5 March, 2018; Accepted 24 April, 2018

The study is essential in reducing germination time of *Treculia africana* var. *inversa*. The effect of three different concentrations of sucrose namely 2, 3 and 4% were investigated on the *in vitro* regeneration of embryo explants of *T. africana* Decne. on the media of Murashige and Skoog (MS) and Gamborg et al. (B5) respectively without any growth regulator. The experimental design was a 2 × 5 factorial in a completely randomized design with each treatment consisting of ten replicates. Results showed that while both media including control (contains agar only) supported the *in vitro* regeneration of *T. africana* embryo explants, B5 medium was found to be significantly superior (P≤0.05) to MS medium in all the growth parameters studied. B5 medium at 4% sucrose elicited the best response in all the growth parameters determined while control gave the least response. The protocol reported here can be used for large scale propagation of true-to-type *T. africana* plants within a short time for the purpose of improvement through genetic transformation (mutagenesis) and the development of a viable conservation programme.

Key words: *Treculia africana,* Murashige and Skoog (MS) medium and Gamborg et al. (B5) medium, embryo explant.

INTRODUCTION

Treculia africana Decne. (commonly known as African bread fruit, Wild jack fruit, or African boxwood), is an important multipurpose indigenous tree species in West Africa belonging to the family Moraceae (Nutrecul Agroforestry Company (NAC), 2013). It is a monoecious dicotyledonous plant with flowers crowded into compact heads (Ugwoke et al., 2003). *T. africana* is a large, evergreen tree growing in the forest up to 30 m high with a girth of 4-6 m (Agbogidi and Onomeregbor, 2008). It has a dense spreading crown and fluted trunk. There are three varieties of African breadfruit which include: *T. africana* var. *africana*, *T. africana* var. *inversa* and *T. africana* var. *mollis* (Okafor, 1983). Their taxonomic differences are based mainly on the size of the fruit head (infructence) and the hairiness of the branchlets and leaves (NAC, 2013).

T. africana serves as a nutritive food for the local population in its distribution area. Analysis of the hexane

*Corresponding author. E-mail: uche.okafor1287@unn.edu.ng. Tel: +234 7035043578.

extract of the seeds indicates that it contains a stearine solid fat fraction, resembling that of palm kernel oil and an aleine fraction with a composition similar to that of cotton seed oil; carbohydrate content 40-50% starch, 3-8% glucose and a good protein content with its lysine 50% higher than that of soya beans and methionine content 1.65% as in soya beans (NAC, 2013). Therefore, *T. africana* seems to have an important role in those regions that have a shortage of good protein sources due to its high protein content.

Appiah et al*.* (2014) affirmed that despite the dietary and economic importance of African breadfruit, it has remained an underutilized species and its potentials remain under-exploited. The underutilization is due to a number of reasons. Firstly, the long gestation period of ten or more years of the species has not helped in cultivar improvement leading to limited cultivation of the species (Nuga and Ofodile, 2010). Futhermore, high rate of deforestation especially in urban places due to industrial, construction and agricultural purposes call for the need of conservation of this species to avoid genetic erosion (NAC, 2013). Therefore, there is an urgent need for application of a reliable and efficient *in vitro* system that results in efficient differentiation, shoot development, and whole plant regeneration for the improvement of *T. africana* through genetic transformation or mutagenesis (NAC, 2013). In addition, cell and tissue culture techniques have been used to obtain biotic/abiotic tolerant plants employing two *in vitro* culture approaches including selection of mutant cell line (somaclones) and to introduce novel strands of interest (Arzani and Mirodagh, 1999; Arzani, 2008; Arzani and Ashraf, 2016; Kyesmu et al*.,* 2004). Some factors have been identified as affecting *in vitro* regeneration and these include: temperature, light, pH, plant growth regulators and orientation of the explant on the medium (Arzani and Mirodagh, 1999; Srivastava and Johri, 1973). The physical status of the plant and the genotypes also has a role to play in regeneration process (Srivastava and Johri, 1973). The choice of tissue culture media largely depends upon the species and even genotype of the same species to be cultured (Arzani Darvey, 2001) Therefore, there is an urgent need for application of a reliable and efficent *in vitro* system that result in efficient differentiation, shoot development and whole plant regeneretion for the improment of *T. africana* through genetic transformation or mutagenesis (NAC, 2013).

Different studies on plants other than *T. africana* have been done using *in vitro* regeneration methods*. In vitro* culture of embryonic axis of different cultivars of *Phaseolus vulgaris,* Motta-Aldana et al*.* (2010) showed successful regeneration using the MS (Murashige and Skoog, 1962) medium supplemented with 100 mg l^{-1} myoinositol, 1 mg l^{-1} thiamine, 30 g l^{-1} sucrose, BAP (0, 5 and 10 mg l^{-1}) and 8 g 1^{-1} agar. Prabhat et al*.* (2009) reported that *Rauwolfia serpentina* L. an endangered species, was also regenerated using the juvenile leaf explants on the MS medium supplemented with various combinations of growth regulators. In addition, a study on *Artocarpus heterophyllus* (Ashrafuzzaaman et al*.,* 2012*)* showed that regeneration of roots increased comparatively better when MS medium was enriched with 2 mg l^{-1} of BAP (6 benzyladenine). However, few works have been done on *T. africana* zygotic embryos. For example, Okafor et al. (2016) compared the strengths of MS medium on *T. africana* zygotic embryos while Attah and Okezie (2015) compared the efficacy of three basal medium on *T. Africana,* namely: MS, Gamborg (B5) and Hilderbrant basal media. No study have determined the effect of sucrose conditions on zygotic embryos of this plant thus, necessitating the need for this study. This study is aimed at developing a protocol for regeneration of *T. africana* from embryonic axis of seed as a prerequisite for improvement through genetic transformation and to assess the effects of different levels of sucrose on the germination and growth of zygotic embryo of *T. africana* in two different media (MS and B5).

MATERIALS AND METHODS

Site of the experiment

This study was conducted at the South-East Zonal Biotechnology Central Laboratory, University of Nigeria, Nsukka.

Source of explants

T. africana tree (Figure 1) at a farm in Nsukka, in Nsukka Local Government area of Enugu State was used for this study. Fresh fruit heads (after 3 days of fall of the fruit heads) (Figure 2a) was processed to liberate seeds for the experiment. Five hundred seeds of *T. africana* (Figure 2b) were obtained from fresh fruit heads, and were later taxonomically identified in the Herbarium of Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The explants were obtained by excising the seeds (separating the cotyledons since the plant is dicotyledonous in nature). The embryos measured between 0.7 and 0.9 cm.

Preparation and sterilization of explants

Seeds were washed with running tap water and soaked for a minute in 70% ethanol while being stirred. This was followed by placing the seeds in 20% (v/v) sodium hypochlorite with two drops of Tween twenty for 20 min and rinsed in three changes of sterile distilled water. The seed coat along with the two cotyledons was separated from the embryo using sterile forceps and scalpels, and then the embryo was used for the *in vitro* culture on the growth media. Embryo excision and culture was done in the laminar air flow chamber that was previously exposed to ultraviolet radiation for 30 min in order to avoid contamination.

Preparation of stock solutions

In this study, series of stock solution of the media were made. The media comprised macro salts, micro salts, iron compounds and

Figure 1. *Treculia africana* tree.

(a) **(b)**

Figure 2. (a) Fruits and (b) Seeds of *Treculia africana.*

organics (myo-inositol, thiamine-HCl, nicotinic acid, pyridoxine and glycine). Appropriate amount of these inorganic salts and vitamins were measured out using a weighing balance (Sartorius BS 323s). One litre of sterile distilled water was added to dissolve the macro and micro nutrients differently using a magnetic stirrer while 100 ml of sterile distilled water was used to dissolve iron compounds, $CaCl_2.H_2O$, myo-inositol and other vitamins. Macro salts were then considered as stock solution A, iron compounds as stock solution B (stored in an amber bottle to protect it from light) and calcium chloride dehydrate ($CaCl_2.H_2O$) as stock solution C. $CaCl_2.H_2O$ even though a macro salt was dissolved separately to avoid precipitation. Similarly, micro salts were considered stock solution D, vitamins as stock solution E and myo- inositol as stock solution F. They were dissolved using a magnetic stirring bar on a magnetic stirrer
hotplate to ensure homogeneity. They were properly labeled and stored at 4°C.

Media and culture conditions

The media comprising macro and minor elements according to Murashige and Skoog (1962) and then Gamborg et al*.* (1968) supplemented with myo-inositol (100 mg l^{-1}), Thiamine HCL mg l^{-1}l), pyridoxine (5 mg l^{-1}), Nicotinic acid (5 mg l^{-1}) and sucrose were employed as basal media in this experiment. Cultures with only agar and water were maintained as control. 20, 30 and 40 g of sucrose were weighed out in two sets (each set for a media) and each put in 1000-ml bottom conical flask to which 900 ml of sterile distilled water was added to respectively. These were dissolved using a magnetic stirrer. 50 ml of stock solution A, 5 ml each of B, C and D and 1 ml each of E and F were added to each conical flask. The pH of the medium was adjusted to 5.8 with 1 M NaOH. 7 g of Fluka agar was added to each conical flask and made up to 1 L prior to autoclaving by steam sterilization at 103 KN M^{-2} pressure and 121°C for 15 min. Ten mililitres of the media were later dispensed into the test tubes accordingly and left to solidify. The embryos were cultured singly in Pyrex test tubes at 27±2°C under 16-h light/8-h dark photoperiod at a photon flux density of 60 µmol m^{-2} s^{-1} provided by cool white fluorescent tubes. All operations starting from the preparation of explants to establishment of cultures were carried out in a laminar air flow hood chamber previously kept sterile by swabbing with alcohol and exposure to ultraviolet light for 30 min. The cultured embryos were left to grow

for four weeks, after which they were scored for the requisite growth parameters.

Experimental design and statistical analysis

In this study, experiment was carried out in a 2×5 factorial in a Completely Randomized Design (CRD). Experimental design consisted of nine treatments with ten replications in each treatment. SPSS software was used to carry out data analysis. One-way analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (DNMRT) was used to test for significance ($P \leq 0.05$) and compare mean values respectively.

Growth parameters assessed after four weeks in culture

The growth and development of embryos of *T. africana* were monitored at a two-day interval from the day of inoculation. On the 13th day, regenerated plantlets under each treatment were scored for the following parameters: number of adventitious roots, length of roots, length of shoots, leaf area, sprout rate and sprout percentage. The numbers of adventitious roots were determined by counting while the length of shoots and roots were measured with a rope later superimposed on a metre rule. For the leaf area, each of the plantlets was detached and the length and width of the leaves measured with a metre rule. The value obtained was multiplied with a constant, 0.75 (Francis et al., 1969). Sprout rate was calculated as the reciprocal of the number of days on which 50% sprouting was achieved.

RESULTS

Percent sprouting

The embryos (Figure 3) of *T. africana* cultured on both MS and B5 media began to show visible changes by the 2nd day in culture. The white or milkish embryo that had a large size of about 0.7 - 0.9 cm had begun expansion on the 2nd day. By the 3rd day, all embryos turned green. The radicle and plumule (sprouting) emerge from the embryo between the 4th and the 5th day (Figure 4) and by the 6th day, all the embryos had sprouted except some in the control. The plumule and radicle finally resulted into shoot and root respectively. Embryo explants cultured on only 0.7 agar and water alone also showed changes by the 2nd day and maintained a healthy growth till the 28th day of the culture. These visible growths in all the treatments enhanced the comparison of growth parameters among them. There were no changes in the growth parameters between the 3rd and the 4th week in MS (Figure 5a-d) and B5 basal media (Figure 6a-d).

Analysis of variance showed that there was no significant difference in percentage sprouting among the treatment means at $P \geq 0.05$. Maximum sprouting percentage was achieved on 4% sucrose in B5 (91.10 ± 8.90) while the least was control (68.87± 15.55) (Table 1).

Analysis of variance also showed that there was a significant difference among the treatment means ($P \leq 0.05$) in terms of sprout rate. Comparison test using Duncan's New Multiple Range Test (DNMRT) (Table 1) showed that all the treatment means were significantly higher than control in terms of sprout rate. It further shows that seven of the nine treatments had the same and highest sprout rate of 0.25 ± 0.00, followed by MS 0 (0.23 ± 0.02) and then control (0.20 ± 0.00) as the least.

Figure 3. Embryos of *T. africana* on 1st day.

Figure 4. Embryo of *T. africana* on 4th day showing sprouting.

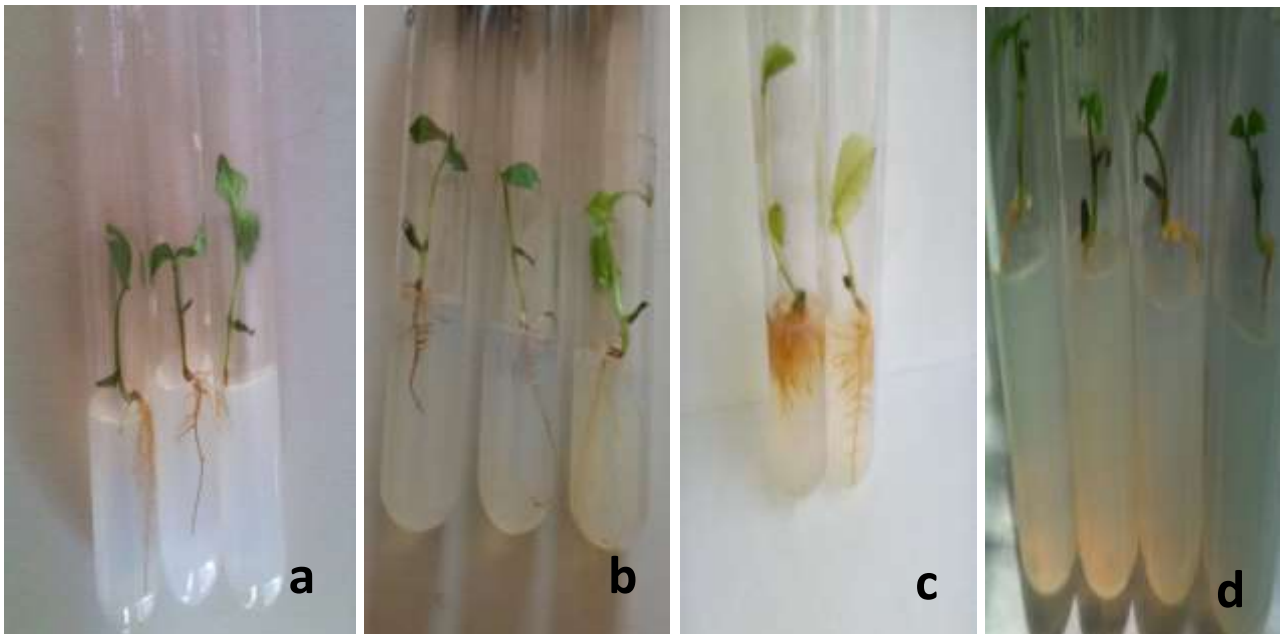

Figure 5. 3 weeks old *Treculia africana* plantlets arising from embryo explants in MS medium containing (a) 2% sucrose (b) 3% sucrose (c) 4% sucrose (d) no sucrose.

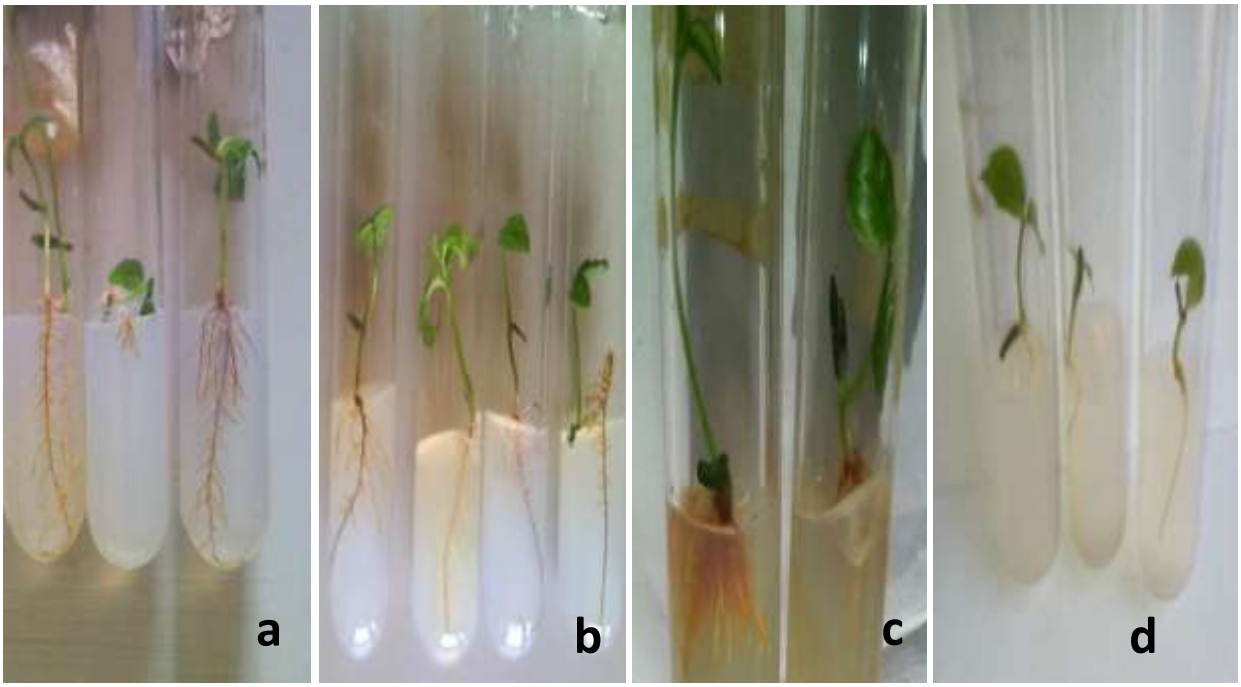

Figure 6. 3 weeks old *Treculia africana* plantlets arising from embryo explants in B5 medium containing (a) 2% sucrose (b) 3% sucrose (c) 4% sucrose (d) no sucrose.

Root and shoot length of plantlets

The highest length of root was recorded at 4% sucrose in B5 (5.02±0.3) and this was significantly different from all others while the least was recorded for control (2.44±0.21) (Table 2). The 4% sucrose in B5 media promoted growth of *T. africana* shoot but did not differ significantly from 3 and 4% sucrose in B5 and MS while

Table 1. Sprout rate of embryo explants of *T. Africana* as affected by sucrose concentrations and basal media.

Basal media	Sucrose concentration %	Sprout rate	Per cent sprouting
MS	0	0.23± 0.02[b]	76.90±11.80
	2	0.25± 0.00[b]	88.90±11.10
	3	0.25± 0.00[b]	88.87±4.43
	4	0.25± 0.00[b]	88.87±4.43
B5	0	0.25± 0.00[b]	82.20±11.10
	2	0.25± 0.00[b]	86.67±13.33
	3	0.25± 0.00[b]	84.48±8.87
	4	0.25± 0.00[b]	91.10±8.90
Control	0	0.20±0.00[a]	68.87±15.55

Values represent Mean± SE. Mean values within a column followed by different letters are significantly different from each other by DNMRT (P≤0.05).

Table 2. Effect of basal media and sucrose concentrations on root and shoot length of *T. africana* plantlets.

Basal media	Sucrose concentration (%)	Root length (cm)	Shoot length (cm)
MS	0	2.54±0.12[a]	1.80±0.10[ab]
	2	2.63±0.15[a]	2.11±0.20[abc]
	3	2.95±0.30[a]	2.87±0.38[bcd]
	4	3.94±0.42[bc]	4.41±0.66[ef]
B5	0	2.54±0.19[a]	1.81±0.25[ab]
	2	3.15±0.19[ab]	3.33±0.35[cde]
	3	4.74±0.53[cd]	3.62±0.25[def]
	4	5.02±0.32[d]	4.77±0.78[f]
Control		2.44±0.21[a]	1.50±0.17[a]

Values represent Mean± SE. Mean values within a column followed by different letters are significantly different from each other by DNMRT (P≤0.05).

differing significantly from others. ANOVA showed that there was a significant difference in basal medium and the interaction between the sucrose levels and basal media in terms of root length and shoot length at p≤0.05.

Number of adventitious root and leaf area of plantlets

Result showed that for both media, the number of adventitious root increased as the sucrose level increased (Table 3). The same table also showed that there was a significant difference among the treatment means in terms of leaf area The 4% sucrose in B5 had the highest effect on number of adventitious root (41.0±2.40) and differed significantly from others, while control (7.20±0.93) recorded the least. Results also showed that for leaf area, 4% sucrose in B5 medium had highest growth (2.94±0.43) but did not differ significantly from 4% sucrose in MS while differing from others. Control also recorded the least. It could be seen from the table that B5 had better effect on leaf area at each level of sucrose concentration (Table 3). Analysis of mean indicated that there were significant differences between the basal media in terms of number of adventitious roots at p≤0.05 and this was due to sugar-media interaction.

DISCUSSION

Effects of sucrose on growth parameters

In this study, increase in growth parameters including number of adventitious root resulted with an increase in sugar concentration until an optimum was reached. For example, for shoot length, it increased from 2.54±0.19 in 0% sucrose to 5.02±0.32 in 4% sucrose both in B5 media. For most observed studies, optimum is usually 4 - 5% sucrose, while levels higher than that led to a decline

Table 3. Effect of basal media and sucrose concentrations on number of adventitious root and leaf area of *T. africana* plantlets.

Basal media	Sucrose concentration (%)	Mean no. of adventitious root	Leaf area (cm^2)
MS	0	9.56±1.07^{ab}	0.47±0.11^{a}
	2	14.40±1.64^{b}	0.81±0.26^{a}
	3	28.10±1.97^{c}	1.25±0.25^{ab}
	4	31.30±3.42^{c}	2.30±0.25^{cd}
B5	0	7.80±0.93^{a}	0.37±0.08^{a}
	2	25.80±2.25^{c}	1.12±0.27^{ab}
	3	30.10±3.00^{c}	1.75±0.44^{bc}
	4	41.00±2.40^{d}	2.94±0.43^{d}
Control		7.20±0.93^{a}	0.39±0.07^{a}

Values represent Mean± SE. Mean values within a column followed by different letters are significantly different from each other by DNMRT (P≤0.05).

in growth parameters and regeneration frequency. This is in line with the results of Manikyam et al. (2014) that used different sucrose levels and different carbon sources on *in vitro* regeneration of *Solanum viarum* (Dunal). They found that as the sucrose concentration increased from 1 - 4%, the mean shoot length also increased from 2.32±0.53 to 4.81±0.37 while at 6%, it declined to 2.23±0.11. Lu et al. (1983) found that culture media with a higher concentration of sucrose improved plant regeneration in *Zea mays*. Gurel and Gulsen (1998) who worked with Almond (*Amygdalus communis* L.) recorded that shoot growth capacity, expressed as the growth rate of the developing shoots showed a steady increase with increasing concentrations, with 5 and 6% sucrose concentrations producing more vigorous shoots than lower concentrations. This may be due to the fact that high sugar levels available in the culture medium may speed up cell division, thus leading to an increase in the volume and weight of tissues cultured, as suggested by other study (Chong and Taper, 1972). In the case of adventitious roots, sucrose was reported to act as morphogenetic trigger in the formation of axillary buds and branching of adventitious root (Vinterhalter and Vinterhalter, 1997).

Effects of basal medium

In this study, two media (MS and B5) were compared. Both of them supported the *in vitro* regeneration of *T. africana* with B5 being superior to MS in all the growth parameters. The differences may be due to different compositions of each media and the quantity of the various salts, since they were all subjected to the same environmental conditions. The differences between the media could also be the result of the quantity of ions in the basal media, since Bhojwani and Razdan (1996) showed that the main difference in the composition of a range of commonly used tissue culture media is based on the quantity of various salts and ions; that the active factor in the medium is the ion of different types rather than the compound. Murashige and Skoog (1962) also noted that nutritional requirement for optimal growth of a tissue *in vitro* may vary with the species and that tissues from different parts of same plant may even have different requirement for maximum growth; therefore, it is believed that no single medium is entirely satisfactory for all plant types, tissues and organs. George (2008) considered two important factors useful in finding out media formulations suitable for different plant species and different culture types, which include: total concentration of nitrogen and the ratio of nitrate and ammonium ions in the medium. B5 medium has a lower quantity of ammonium ions than MS, and therefore it is possible that this contributed to its greater yield, since Gamborg et al. (1968) recorded that ammonium ions depressed the growth of soya beans cells when the concentration exceeded 2 mM. The results of this study agrees with the work on *Jatropha curcas* by Amaefule (2014) who found out that B5 was better than MS media in most growth parameter assessed. However, this may be in contrast with most studies since it is obvious that MS medium is very popular because most plants react favourably to it. The hundred percent germination recorded for most treatments in this study may be attributed to the maturity of the embryo used and this is in line with Warakogda and Subasinghe (2009) who reported that the stage of maturity of *J. curcas* seeds and the basal media had a significant effect on seed germination and subsequent growth of seedlings.

Morphogenesis of *T. africana* embryos

The expansion and greening of the embryo by the second and third day respectively showed the viability of

the embryos and the readiness for sprouting. The change of embryo colour from white to green indicated that photosynthesis has taken place which is a result of transition from semi-autotrophy to full autotrophy that is a feature of *in vitro* systems. Sprouting followed later with the emergence of radicle and plumule. The initiation of radicle either occurred when the osmotic potentials of the cells in the radicle became more negative due to the metabolism of storage reserves or cell walls were more flexible to allow expansion (Hartmann et al*.,* 2007). Growth did not involve callus formation or proliferation of shoots either as a result of the type of explants (which maybe shoot tip, root tip, leaf etc) used, or the absence of growth regulators. In the work on bambara nut, the shortest time for germination (4-5 days) was observed with the embryo axis followed by the seeds without seed coat which germinated in 8-9 days while the seeds with coat have taken between 10 and 14 days to germinate. Compared to the embryonic axis, the time taken by the water to cross the barrier of the integument and to hydrate the cotyledons to initiate the physiological process of germination could explain the delay in germination observed in seeds with or without seed coat (Kone et al., 2015).

Synergisitc effects of sucrose and media

The three various sucrose levels in MS and B5 media respectively supported growth of *T. africana* indicating that energy source and media were essential for growth *in vitro*. For the growth of plant tissues, the carbon source serves as the energy and osmotic agent (Lipavska and Konradova, 2004) for various energy-requiring processes that can occur at the expense of available metabolic substrates for the growth and root initiation (Thorpe, 1983). Control had the least growth, which may be due to lack of basic nutrients, since the zygotic embryo has been deprived of its food storage tissue. On the other hand, significant growth of the control showed it has internally stored carbohydrate and nutrients for initial growth. This is because the study of *T. africana* revealed it contains much carbohydrate, about 40-50% starch aside 3-8% glucose (NAC, 2013). Other *in vitro* works also support the fact that growth of explants especially embryo is possible only on water and agar alone depending on the level of food reserve. Kone et al. (2015) noted that there was a lack of significant difference between basal media and the control containing only agar, suggesting that macro- and microelements were not necessary for germination in Bambara groundnut and, thus, the success of seed germination was mainly related to water availability. The growth observed on all the treatments without plant growth regulators support the finding of George (2008), which states that “matured embryos are hormone autonomous”, meaning that matured embryos possess a high level of endogenous hormones compared to immature embryos which always require growth regulators. Mohammed et al. (1992) recorded that adventitious roots or a single shoot with roots formed on the explants of common and tepary beans cultured on media without plant growth regulators.

Conclusion

This study has described a protocol that will be relevant for the mass propagation of *T. africana* plantlets. High levels of sucrose (3-4%) showed maximum increase in the various growth parameters; therefore, it can be established that high sucrose levels are required for better yield. Also, use of B5 media instead of MS will be most suitable for *in vitro* culture of this plant embryo explants. The plantlets after acclimation would be raised *in situ* (*ex vitro*) to ensure a steady supply of its protein to man and animals; for various pharmaceutical uses and researches. For future research, there is also an urgent need to employ this protocol in producing modified *T.africana* with shorter gestation period to encourage its mass production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Agbogidi OM, Onomeregbor VA (2008). Morphological changes in the seedlings of Treculia Africana grown in crude oil impacted soils. In: Popoola, L. (Ed.). Climate Change and Sustainable Renewable Natural Resources Management. Proceeding of 32nd Annual Conference of the Forestry Association of Nigeria.Umuahia, Abia-State, Nigeria pp. 170-182.

Amaefule CC (2014). In Vitro Plant Regeneration from Mature Embryo Explants of Jatropha curcas L. (Bio- Diesel Plant) on Two Standard Basal Nutrient Media.Unpublished Master’s Project Report, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka 124 p.

Appiah F, Oduro I, Ellis WO (2014). Nutritional composition of breadfruits (Artocarpus spp. and Treculia africana) in Ghana. ISHS Acta Horticulturae 1128: XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): International Symposium on Horticulture in Developing Countries and World Food Production. Doi: 10.17660/ActaHortic.2016.1128.3

Arzani A, Mirodjagh SS (1999). Response of durum wheat cultivars to immature embryo culture, callus induction and in vitro salt stress. Plant Cell, Tissue and Organ Culture 58:67-72.

Arzani A (2008). Improving Salinity Tolerance in Crop Plants: A Biotechnological View. In Vitro Cellular and Developmental Biology-Plant 44:373-383.

Arzani A, Ashraf M (2016). Smart Engineering of Genetic Resources for Enhanced Salinity Tolerance in Crop Plants. Critical Reviews in Plant Sciences 35(3):146-189.

Arzani A, Darvey NL (2001). The effect of colchicine on triticale anther-derived plant: Microspore pre-treatment and haploid plant treatment using a hydroponic recovery system. Euphytica 122:235–241.

Ashrafuzzaaman M, Sukarna K, Dilafroza K, Shamsul HP (2012). In vitro regeneration and multiplication of Jackfruit (*Artocarpus heterophyllus* L.). Research Journal of Biological 2(2):59-65. ISSN: 2049-1727.

Attah EE, Okezie CEA (2015). Comparative growth rate of Treculia africana in three different basal media: Ms, Gamborg and Schenchk and Hilderbrant. Unpublished Master's Project Report, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. 55 p.
Bhojwani SS, Razdan MK (1996). Plant Tissue Culture: Theory and Pratice. A Revised Edition. Elsevier Science, The Netherlands 779 p. ISBN: 0-444-42164-5.
Chong C, Taper CD (1972). Mallus tissue culture: I. Sorbitol (D-glucitol) as a carbon source for callus initiation and growth. Canadian Journal of Botany 50:1399-1404.
Francis CA, Rutger JN, Palmer AFE (1969). A Rapid Method for Plant Leaf Area Estimation in Maize (*Zea mays* L.). Crop Science 9:537-539.
Gamborg OL, Miller R, Ojima K (1968). Nutrient requirement of suspension cultures of soybean root cells. Experimental Cell Research 50:151-158.
George EF (2008). Plant tissue culture procedure. In: George, E. F., Hall, M. A. and Klerk, G. D. (Eds). Plant Propagation by Tissue Culture. Springer Publisher, Netherlands pp. 499-506. ISBN 978-1-4020-5005-3.
Gurel S, Gulsen Y (1998). The Effect of Different Sucrose, Agar and Ph Levels on In vitro Shoot Production Of Almond (*Amygdalus Communis* L). Turkish Journal of Botany 22:363-373.
Hartmann HT, Kester DE, Davies FT, Geneve RL (2007). Plant propagation: Principles and Pratices. Prentice- Hall Incorporated, New Delhi 880 p. ISBN 13: 978-0-13-501449-3.
Kone M, Kone T, Silué N, Soumahoro A, Kouakou HT (2015). In Vitro seeds germination and seedling growth of bambara groundnut (*Vigna subterranea* (L.) Verdc. (Fabaceae). The Scientific World Journal 8 p.
Kyesmu PM, Omaliko, CPE, Maduekwe A (2004). Basic Facts on Plant Tissue Culture: A Guide to Plant Tissue Culture Practice. Dolio-B Press, Abuja, Nigeria 71 p.
Lipavska H, Konradova H (2004). Somatic embryogenesis in conifers: The role of carbohydrate metabolism. *In Vitro* Cellular and Developmental Biology Plant 40(1):23-30.
Lu C, Vasil V, Vasil IK (1983). Improved efficiency of somatic embryogenesis and plant regeneration tissue culture of Zea mays. Theoretical and Applied Genetics 66(3):285-289.
Manikyam DNM, Chandra SP, Challagundla VN (2014). Impact of different carbohydrates and their concentrations on in vitro regeneration of Solanum viarum (Dunal)—An important anticancer medicinal plant. American Journal of Plant Sciences 5:200-204. ISSN: 2158-2742
Mohammed FM, Paul ER, Dermot PC (1992). Plant regeneration from in vitro culture of embryonic axis explants in common and tepary beans. Journal of the American Society for Horticultural Science 117(2):332-336. ISSN: 0003-1062.
Motta-Aldana JR, Serrano-Serrano ML, Hernández-Torres J, Castillo-Villamizar G, Debouck DG, Chacón SMI (2010). Multiple Origins of Lima Bean Landraces in the Americas: Evidence from Chloroplast and Nuclear DNA Polymorphisms. Crop Science 50:1773-1787.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15:473-497.
Nutrecul Agroforestry Company (NAC) (2013). Treculia africana. http://www.Nutreculagroforestry. Com/index-9.html/Assessed on 17th November, 2014.
Nuga OO, Ofodile EAU (2010). Potentials of Treculia africana- an endangered species of southern Nigeria. Journal of Agriculture and Social Research 10(2):21-25.
Okafor CU, Ejiofor AP, Okezie CEA (2016). Comparative growth rates of Treculia Africana decne: Embryo in varied strengths of murashige and skoog basal medium. World Academy of Science, Engineering and Technology, International Journal of Agricultural and Biosystems Engineering 10:539-542.
Okafor JC (1983). Horticulturally promising indigenous wild plant species of the Nigerian forest zone. Acta Horticulture 123:165-176.
Prabhat S, Anand S, Arvind KS, Lalit S, Veena P, Tapan KN (2009). Somatic embryogenesis and in vitro regeneration of an endangered medicinal plant. Life Sciience Journal 6(2):57-62.
Srivastava PS, Johri BM (1973). Morphogenesis in endosperm culture. ZP flanzenphysiology 70:285-304. Thorpe TA (1983). "Carbohydrate utilization and metabolism" In: Bonga, J. M. and Durzan, D. J. (Eds). Cell and Tissue Culture in Forestry. Martinus Nijhoff Publishers, Hague pp. 325-368.
Ugwoke FN, Agbo AE, Ali NC, Attah CP, Ekwueme JI (2003). A Note on African Breadfruit (*Treculia africana* Decne.) Unpublished Report Submitted in Partial Fulfillment of CSC 341, Department of Crop Science, University of Nigeria, Nsukka, Nigeria 18 p.
Vinterhalter D, Vinterhalter BS (1997). Micropropagation of Dracaena sp. In: Bajaj, Y. P. S. (Ed.). Biotechnology in Agriculture and Forestry 40, High-tech. and Micropropagation VI. Springer Publishers, Berlin, Heidelberg pp. 131- 146.

Vol. 13(22), pp. 1120-1127, 31 May, 2018
DOI: 10.5897/AJAR2018.13051
Article Number: 3A3E08357249
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Harnessing cultivar performance and stability for deploying superior groundnut plant types in the Lake Albert Crescent Zone of Uganda

Kakeeto, R., Wambi, W., Barwogeza, M., Auma, L., Odongo, W., Ozuma, G. and Ssekiwoko, F.*

National Agricultural Research Organisation (NARO), Bulindi Zonal Agricultural Research and Development Institute (BuZARDI), P.O. Box 101, Hoima, Uganda.

Received 12, February 2018; Accepted 20 March, 2018

Groundnuts (*Arachis hypogaea* L.) are the second most important legume crops after beans, an important source of protein (23 to 25%), fats/oils (40 to 52%) and carbohydrates (10 to 20 %) and widely grown and consumed in Uganda including the Lake Albert Crescent Zone (LACZ). Due to susceptibility of local varieties to groundnut rosette, National Agricultural Research Organisation (NARO) through the National Semi Arid Resources Research Institute (NaSARRI) developed and released the serenut varieties. Adaptive trials were therefore established in the LACZ, to select the most location specific adapted varieties for promotion in this ecologically diverse zone. Four serenut varieties namely serenut 5, 8, 10 and 14 and a locally grown variety (Red beauty) were planted on three farmers' fields in each of the three sub-ecological areas. Data were collected on total pod dry weight (yield), number of pods and on 100 seed weight. In this study, we show that overall yields of serenut 5, serenut 14, serenut 8 and serenut 10 were highly significantly ($P \leq 0.001$) different for all traits measured across the sub-ecological areas. Best yields were recorded from the humid tropical rain forest sub-ecological area where 1900 kg/ha were obtained for serenut 14, 2366 kg/ha for serenut 10, 1763 kg/ha for serenut 8 and 1795 kg/ha for serenut 5. The yields obtained from these varieties were generally worst in the semi-arid sub-ecological area. These serenut varieties are generally adapted to wider environmental conditions although their performance per se was found to be generally inconsistent. This study has also found that among all the varieties tested, Serenut 5 was the best adapted across all the sub-ecologies. Overall, we therefore recommend farmers in this ecologically diverse zone to grow these groundnut varieties with improved growing practices such as timely planting, timely weeding, earthing up and pest and disease management in order to obtain consistent high yields.

Key words: Groundnut, performance, stability, sub-ecological area.

INTRODUCTION

Groundnut (*Arachis hypogaea* L., 2n = 4x= 40) is the second most important legume crop in Uganda. It is a major food and income source. Groundnut is some nutrient-dense food rich in digestible protein, unsaturated fatty acids (for example, oleic acid), minerals (for example, copper and manganese), vitamins (for example, biotin, niacin, folate, B1), fibre, and polyphenolic antioxidants (for example, p-coumaric acid and

resveratrol) (Ros, 2010; Craft et al., 2010; Settaluri et al., 2012).

In addition, the crop is also a source of income to many small-scale farmers, contributing significantly to poverty alleviation (Kassie et al., 2011; Okello et al., 2014). Given the importance of this crop, production is still constrained by several factors including abiotic and biotic stresses. To overcome some of these production constraints, the National Groundnut Improvement Programme has released several groundnut varieties (Deom and Okello, 2018). However, a significant proportion of the groundnut growers are still using local cultivars because they are considered to be superior to improved varieties (Mugisha et al., 2014). Continued cultivation of local cultivars has resulted in persistently low productivity at farm level (Kaizzi et al., 2012; Okello et al., 2014; Deom and Okello, 2018), often leading to unreliability of the crop's yields and thereby undermining food security at household and national level (Kebede and Tana, 2014; Gadgil et al., 2012).

The other factor contributing to the low groundnut yield levels in the country is the shortage of high yielding and stable varieties which have farmer preferred traits (Mugisha et al., 2014). Groundnut production is also characterised by low input use and production under rain fed conditions (Shiferaw et al., 2010; Mugisha et al., 2011; 2014). Under such circumstances, and in fluctuating environments, it is necessary to develop and/or promote varieties with attributes such as high yield, wider adaptability, biotic and abiotic stress resistance, which are also and low cost management practices. Moreover, the new varieties will only positively impact farmers' incomes, if they are accepted by the community (Khan et al., 2008; Bucheyeki and Mmbaga, 2013). Therefore, the need to develop and promote improved groundnut varieties with farmer-preferred traits in the Lake Albert Crescent Zone (LACZ) is of paramount importance.

New varieties must show high performance for important agronomic traits and their dominance should be consistent over a wide range of production environments (Becker and Leon, 1988). Yield stability among genotypes can become inconsistent due to the wide occurrence of genotype x environment interactions (GE), that is, the ranking of genotypes depending on prevailing conditions at the production environment. GE remains an outstanding challenge to plant breeders and agronomists in making cultivar recommendations to farmers because of the associated consequences especially when selection is based on yield alone (Kang, 1993).

Stability analysis is useful for the identification of stable genotypes and predicting the responses of various genotypes over changing environments. The stable genotypes adjust their phenotypic responses to provide some measure of uniformity in spite of environmental fluctuations (Minde et al., 2017). However, Kempton and Fox (1997) argued that identification of genotypes which can exploit particular environments would be the source of future breeding gains as agricultural environments change. Therefore, stability analysis studies are needed for identification of stable genotypes and in predicting the responses of various genotypes over changing production environments.

Development of new varieties requires full participation of stakeholders (Scoones et al. 2009). On-farm trials have been identified as vital tools for speeding up of breeding processes and enhancing cultivar adoption rates in farming communities (Assefa et al., 2005, Joshi et al., 2007). This is because on-farm trials enable the incorporation of farmers' opinions and ensures testing of technologies under farmers' management conditions (Kaizzi et al., 2006). As a result, increased rates of adoption and reduced variety abandonment have been reported when farmers' knowledge and experiences are acknowledged (Moser and Barrett, 2003, Sibiya et al., 2013).Therefore, an attempt has been made in the present study to;

(1) Evaluate the performance of different groundnut varieties across different locations to know the role of G × E interactions and also to analyse the stability of genotypes for pod yield and its contributing characters, under farmer management conditions,
(2) Identify superior groundnut genotypes for promotion in the LACZ of Uganda.

MATERIALS AND METHODS

The LACZ is ecologically diverse with three major sub-ecologies; namely, the semi-arid rift valley lying sub ecological area (SRV), the humid tropical rain forest sub ecological area (HTR), and the woodland savanna sub ecological area (WS).

In this study, 4 improved groundnut varieties; serenut 5 (S5), serenut 8 (S8), serenut 10 (S10) and serenut 14 (S14) and one locally grown variety, red beauty (Table 1) were planted out on farmers' fields in each of the three sub-ecological areas of LACZ. In each sub ecological area, three farmers were selected to host the trials on whose fields each of the five groundnut varieties were planted on three random 3x5 m plots arranged as randomized complete block design (RCBD) with the three sub-ecological zones considered as blocks and nine replications (three farmers per sub-ecological zone and three plots planted per variety by each farmer).

*Corresponding author. E-mail: fssekiwoko@gmail.com. kakeetor@gmail.com.

Table 1. Characteristics of groundnut varieties used in the study.

Variety	Special attributes	Expected yield (Kg/ha
Serenut 5	Medium to large seed Tolerant to drought Resistant to rosette virus Resistant to leaf spot	2500-3000
Serenut 8	Drought tolerant Uniform mat-type growth Rosette and leafspot resistant, stay green trait	2500-3700
Serenut 10	Drought tolerant Rosette and leafspot resistant stay green trait	2500-3700
Serenut 14	Drought tolerant Rosette and Leafspot resistant stay green trait	2500-3700
Red beauty	Multiline of Red Valencia	1900-2500

Adopted from Okello et al. (2014).

Single groundnut seeds were planted at 15cm between plants and in rows, which were 45 cm apart running perpendicular to the slope. These trials were farmer managed and the trials were run over 3 seasons; September to December 2014 (season 1), March to July 2015 (season 2), and September to December 2015 (season 3). Data were collected on overall yield (total dry weight of unshelled pods), Number of pods per plant and weight of 100 seeds (HSD). All data were analyzed using Genstat version 14 (Payne et al., 2011) and differences between means compared using Fisher's protected least significance difference (LSD) at 5% significance level.

In addition, the relative consistency performance technique (Ketata et al., 1989), was used to show behaviour interpretation of genotypes in different environments. Using Excel software, biplots were generated for each trait by simultaneous use of genotype means and standard deviation of the genotype ranks from different locations to aid interpretation of cultivar performance and stability (Table 1).

RESULTS

Combined analysis for yield and yield components over the three growing seasons are presented in Table 2. There were highly significant ($P \leq 0.001$) differences between genotypes for all traits which explained over 60% of total variation. Highly significant ($P \leq 0.001$) differences due to seasons and ecologies main effects were also observed for all traits measured. The interactions between variety and season were highly significant for all the traits, except for the hundred seed weight. Additionally, the interaction between season x variety and ecology was not significant, except for number of pods per plant (Table 2).

Mean values for yield and associated traits combined over locations are presented in Table 3 and supplementary Tables S1, S2, and S3. The mean values for yield in season 2014B ranged from 1529 to 2268 with an average yield of 1876 kg/ha. Season 2015A yields ranged from 819 to 1624 with an average of 1375 kg/ha, which is 27% lower than season 2014B. Season 2015B yields ranged from 509 to 1416 with an average of 1122 kg/ha, which is 18% and 40% lower than season 2015A and season 2014B, respectively.

Mean values for yield and associated traits combined over seasons are presented in Table 4. The mean values for yield in Humid tropical rain forest sub-ecological area ranged from 1177 to 2366 with an average yield of 1800 kg/ha. The woodland savana sub-ecological area yields ranged from 1120 to 1571 with an average of 1084 kg/ha. The semi-arid rift valley sub-ecological area yields ranged from 524 to 1549 with an average of 1102 kg/ha, which is 40% lower than average yield in the humid tropical rain forest sub-ecological area.

The results for consistency of performance (stability) of genotypes across sub-agro ecologies are presented in Figure 1. The results based on hundred seed weight indicated that serenut 10 was consistently superior while serenut 8 and serenut 5 were consistently and

Table 2. Mean squares for combined analysis of selected agronomic attributes for groundnut varieties tested in different sub-agro ecologies of LACZ over three seasons (2014B, 2015A, and 2015B)

Source of variation	DF	Yield	Explained SS (%)	DF	No. of pods/plant	Explained SS (%)	DF	Hundred Seed weight	Explained SS (%)
Replication	2	11194360	45	2	175.504	31	2	1452.29	69
Ecology (E)	2	10528871***	42	2	162920***	28	2	2554.19***	122
Season (S)	2	8083795***	32	2	249.296***	44	1	2024.01***	48
Variety (V)	4	15922281***	63	4	123.557***	43	4	809.29***	77
E × S	3	10623576***	42	3	305.350***	80	2	228.53NS	11
E × V	7	699756NS	3	6	13.815NS	7	4	57.66NS	5
V × S	5	6375984**	25	4	75.764***	26	2	142.62NS	7
V × E x S	4	982533NS	4	5	15.215*	7	2	68.05NS	3
Error	27	6776440	-	27	5.819	-	18	90.12	-
Total	56	-	-	55	-	-	37	-	-

*Significant at P ≤ 0.05; **Significant at P ≤ 0.01, ***Significant at P ≤ 0.001; NS = Not significant (P > 0.05).

Table 3. Genotypic performance of groundnut varieties combined over sub-agro ecologies for three seasons (2014B, 2015A, and 2015B)

Genotype	2014B			2015A			2015B		
	HSD (g)	No. of Pds/plt	Yield (Kg/ha)	HSD (g)	No. of Pds/plt	Yield (Kg/ha)	HSD (g)	No. of Pds/plt	Yield (Kg/ha)
Serenut 5	-	7.74	2268	38.6	11.6	1624	35.59	7.08	1416
Serenut 8	-	7.14	1667	50	11.2	1496	34.96	6.06	1168
Serenut 10	-	9.24	2041	48.8	4.95	819	33.69	5.67	1122
Serenut 14	-	6.83	1529	51.7	11.38	1560	38.04	6.45	1396
Red-beauty	-	7.74	1876	47.3	9.78	1375	26.09	3.08	509
Mean	-	7.74	1876	47.3	9.78	1375	33.67	5.67	1122
LSD (0.05)	-	4.38	392.1	NS	0.98	526	6.77	1.41	537
CV (%)	-	37.7	26.7	32	21.1	31.6	19.4	24.5	47.6

No. of Pds/plt = No. of pods per plant; HSD = Hundred seed weight.

Table 4. Genotypic performance of groundnut varieties in the three major sub-agro-ecologies combined over three seasons (2014, 2015A, and 2015B)

Genotype	Humid tropical rain forest sub-ecological area			Woodland savana sub-ecological area			Semi-arid rift valley sub-ecological area		
	HSD (g)	No. of Pds/plt	Yield (kg/ha)	HSD (g)	No. of Pds/plt	Yield (Kg/ha)	HSD (g)	No. of Pds/plt	Yield (Kg/ha)
Serenut 5	35	10.72	1795	28.3	9.15	1429	48.5	7.83	1257
Serenut 8	38.66	8.89	1763	38	7.9	1327	40.5	6.17	1077
Serenut 10	39.66	11.46	2366	42.1	7.21	1571	43.8	6.38	1102
Serenut 14	45.33	8.57	1900	34.8	8.09	1120	42.3	8.65	1549
Red-beauty	39.66	5.57	1177	17.7	8.09	-	43.8	2.86	524
Mean	39.66	9.04	1800	32.2	8.09	1084	43.8	6.37	1102
$LSD_{(0.05)}$	6.92	2.76	458	13.91	NS	626.9	NS	3.18	555.9
CV (%)	16.8	30.2	25.3	35.7	82.4	57.2	25.9	46.8	46.4

No. of Pds/plt = No. of pods per plant; HSD = Hundred seed weight; NS = not significant at P>0.05.

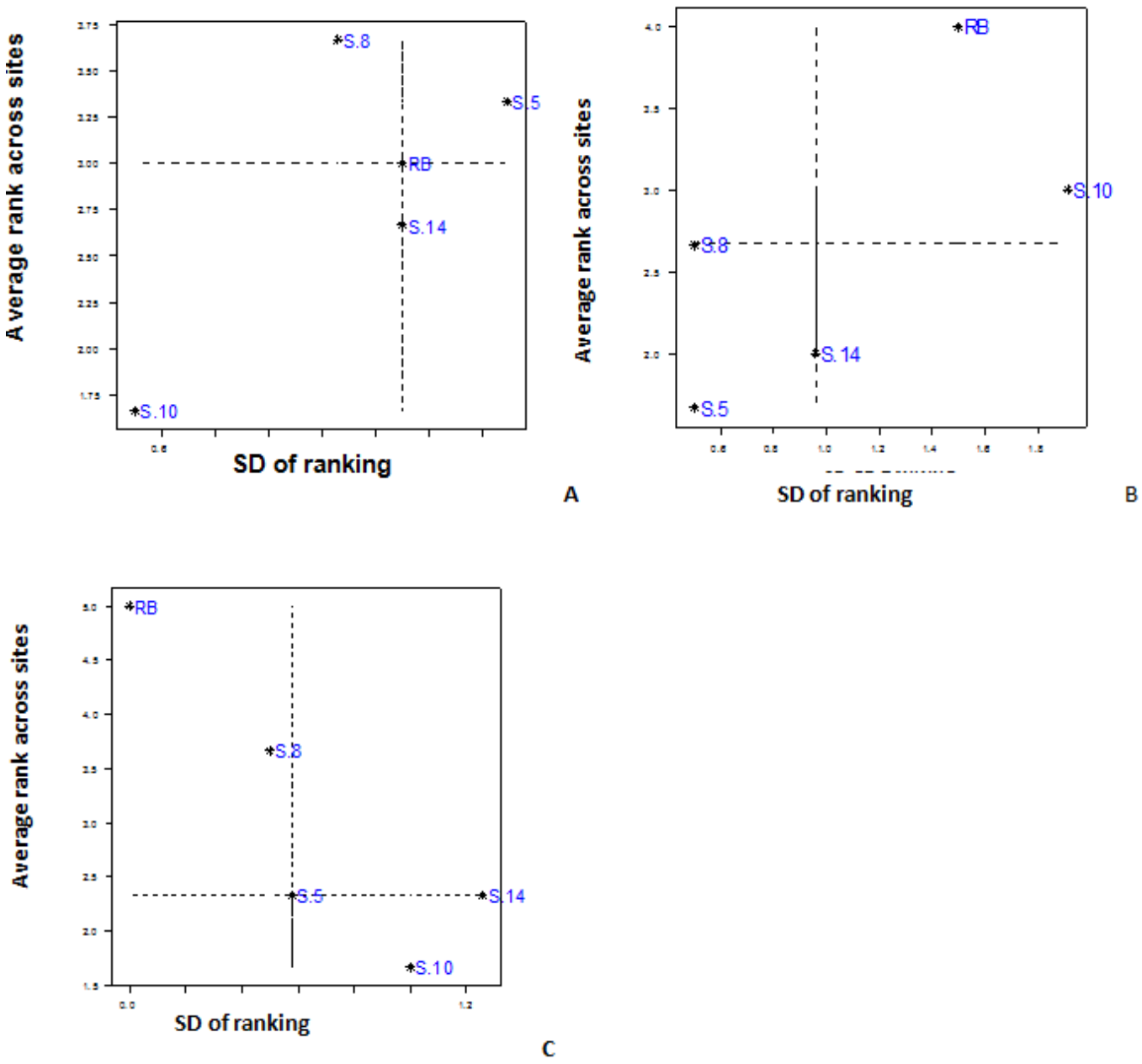

Figure 1. Consistency of genotypic performance based on HSW (A), No. of Pods (B) and Pod Yield (C).

inconsistently inferior, respectively. serenut 14 and Red beauty had average performance across sub-ecologies.

The results based on number of pods/plant indicated that serenut 5 was consistently superior across locations. serenut 10 and red beauty were inconsistently inferior while serenut8 and serenut14 had average performance across sub-ecologies. The results based on overall yield indicated that while serenut10 was inconsistently superior, serenut8 and RED beauty were consistently inferior. Serenut 5 indicated average performance across the sub-ecologies.

Overall, we report that yield and selected yield components classified the genotypes differently. While Serenut 10 was shown to be superior based on hundred seed weight, Serenut 5 was shown to be superior based on number of pods per plant. However, based on overall yield, no genotype was consistently superior. As such, all genotypes were clustered around being inconsistently

superior and consistently inferior in terms of performance.

DISCUSSION

This study showed highly significant differences between groundnut genotypes for all traits measured confirming high level of genetic variation in the varieties used in the study. Moreover, the study also revealed that the interaction between variety and ecological area was not significant suggesting that each of these varieties will perform relatively the same way regardless of the growing environment. However, the same analysis also showed that the sub-agro ecological environments where these genotypes were evaluated were indeed variable. This agrees with results from performance ranking for the groundnut genotypes used in the study.

Based on overall yield, no single genotype/variety was consistently superior in terms of performance. As such, all genotypes were clustered around being inconsistently superior and consistently inferior. The above observation is also in agreement with the fact that groundnut yield traits are variable and their response depends not only on the genetic but also largely influenced by the environmental variation (Mothilal et al., 2010; Songsri et al., 2008; Minde et al., 2017).

Therefore, in spite of the lack of statistical significance for interaction between genotypes and sub-agroecologies, and given that the yield performance was found inconsistent, improving agronomic practices or growing conditions through timely planting, timely weeding, sourcing clean seed, and controlling diseases, among others, would have positive implications for the yield of these groundnut varieties in these locations.

Overall, since there are usually no restrictions to varietal movement by the farmers, it is expected that a change in the locality where a farmer chooses to grow any of those improved varieties within LACZ will not significantly affect the expected yield. This finding is consistent with the legal requirement for release of varieties whose performance is stable across various environments (Halewood et al., 2007). It is also consistent with the purpose for which these varieties were released by NaSARRI (Deom and Okello, 2018). Farmers can therefore freely move and grow these varieties in any area of their choice within LACZ and those areas that share similar agro ecological conditions in Uganda.

The results further showed that the seasons over which the experiment was conducted were variable and this was showed to have an effect on overall yield as the interaction of season with either variety or ecology was significant. This study has therefore showed that seasonal differences rather than agro ecological area characteristics will be one of the other limiting factors to the performance of any of the selected improved groundnut variety in LACZ. However, given the fact that weather events can be very unpredictable (Madzwamuse, 2010), future experiments could consider evaluating these varieties over more locations to be able to determine the best adapted SERENUT varieties in LACZ with acceptable performance regardless of the season of cultivation.

Further still, since the involvement of stakeholders in development of new varieties is key for enhancement of their adoption (Woyengo, 2010; Sibiya et al., 2013), sensory evaluation experiments will also need to be conducted on these ecologically adapted varieties in order to compliment cultivar performance and stability with acceptability of these varieties in the zone.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

This work was funded by the Agricultural Technology Agribusiness Advisory Services (ATAAS) Project in collaboration with Government of Uganda through the National Agricultural Research Organization.

REFERENCES

Assefa T, Abebe G, Fininsa C, Tesso B, Al-Tawaha ARM (2005). Participatory bean breeding with women and small holder farmers in eastern Ethiopia. World Journal of Agricultural Sciences 1:28-35.

Becker H, Leon J (1988). Stability analysis in plant breeding. Plant Breeding 101:1-23.

Bucheyeki TL, Mmbaga TE (2013). On-Farm evaluation of beans varieties for adaptation and adoption in Kigoma region in Tanzania. ISRN Agronomy 5 p.

Craft BD, Kosinska A, Amarowicz R, Pegg RB (2010). Antioxidants properties of extracts obtained from raw, dry-roasted, and oil-roasted US peanuts of commercial importance. Plant Foods for Human Nutrition 65:311-18.

Deom CM, Okello DK (2018). Developing improved varieties of groundnut. In: Sivasankar S, Bergvinson D, Gaur P, Beebe S, Tamo M. (Eds). Achieving sustainable cultivation of grain legumes. Burleigh Dodds Science Publishing, Cambridge, UK. http://dx.doi.org/10.19103/AS.2017.0023.26

Gadgil S, Seshagiri Rao PR, Joshi NV, Sridhar S (1995). Forecasting rain for groundnut farmers-How good is good enough? Current Science 68:301309.

Halewood M, Deupmann P, Sthapit B, Vernooy R, Ceccarelli S (2007). Participatory plant breeding to promote farmers' rights. Biodiversity International, Rome, Italy 7 p

Joshi KA, Musa C, Johansen S, Gyawali D, Harris D, Witcombe J (2007). Highly client-oriented breeding, using local preferences and selection, produces widely adapted rice varieties. Field Crops Research 100:107-116.

Kaizzi CK, Ssali H, Vlek PL (2006). Differential use and benefits of Velvet bean (Mucuna pruriens var. utilis) and N fertilizers in maize production in contrasting agro-ecological zones of E. Uganda. Agricultural Systems 88:44-60.

Kaizzi KC, Byalebeka J, Semalulu O, Alou IN, Zimwanguyizza W, Nansamba A, Odama E, Musinguzi P, Ebanyat P, Hyuha T, Kasharu

AK, Wortmann CS (2012). Optimizing smallholder returns to fertilizer use: Bean, soybean and groundnut. Field Crops Research 127:109-119.
Kang MS (1993). Simultaneous selection for yield and stability in crop performance trials: Consequences for growers. Agronomy Journal 85:754-757.
Kassie M, Shiferaw B, Muricho G (2011). Agricultural technology, crop income, and poverty alleviation in Uganda. World Development 39:1784-1795
Kebede A, Tana T (2014). Genotype by Environment Interaction and Stability of Pod Yield of Elite Breeding Lines of Groundnut (*Arachis hypogaea* L.) in Eastern Ethiopia. Science, Technology and Arts Research Journal 3(2):43-46
Kempton RA, Fox PN (1997). Statistical methods for Plant Variety Evaluation. Chapman and Hall, London. Springer Netherlands XII 192 p.
Ketata HY, Yau SK, Nachit N (1989). Relative consistency performance across environments. International symposium on physiology and breeding of winter cereals for stressed Mediterranean Environments, Montpellier, France pp. 391-400.
Khan ZR, Amudavi DM, Midega CAO, Pickett JA (2008). "Farmers' perceptions of a 'push-pull' technology for control of cereal stem borers and Striga weed in western Kenya," Crop Protection 27(6):976-987.
Madzwamuse M (2010). Climate governance in Africa: Adaptation strategies and institutions. Capetown: Heinrich Böll Stiftung (HBS).
Minde AS, Kamble MS, Pawar RM (2017). Stability analysis for pod yield and its component traits in groundnut (*Arachis hypogaea* L.). Asian Journal of Biological Sciences 12(1):15-20.
Moser CM, Barrett CB (2003). The disappointing adoption dynamics of a yield-increasing, low external-input technology: the case of SRI in Madagascar. Agricultural Systems 76:1085-1100.
Mothilal A, Vindhiyavarman P, Manivannan N (2010). Stability analysis of foliar disease resistant groundnut genotypes (*Arachis hypogaea* L.). Electronic Journal of Plant Breeding 1:1021-1023.
Mugisha J, Lwasa S, Mausch K (2014). Value chain analysis and mapping for groundnuts in Uganda. Socio-economics Discussion paper series number 14.
Mugisha, J, Diiro GM, Ekere W, Langyintuo A, Mwangi W (2011). Characterization of Maize Producing Households in Nakasongola and Soroti Districts in Uganda. DTMA Country Report - Uganda. Nairobi: CIMMYT.
Okello DK, Okello LB, P. Tukamuhabya P, Odongo TL, Adriko J, Deom CM (2014). Groundnut rosette diseases symptoms types distribution and management of diseases in Uganda. African Journal of Plant Science 8:153-163.
Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM (2011). GenStat for windows. 17th edition. VSN International, Hemel Hempstead, UK. Plant Biology 38:1016-1023
Ros E (2010). Health benefits of nut consumption. Nutrients 2:652-682.
Settaluri VS, Kandala CVK, Puppala N, Sundaram J (2012). Peanuts and their nutritional aspects – A review. Food and Nutrition Sciences 3:1644-1650.
Shiferaw B, Muricho G, Okello J, Kebede T, Okecho G (2010). Adoption of Improved Groundnut Varieties in Uganda. ICRISAT.
Sibiya J, Tongoona P, Derera J, Makanda I (2013). Smallholder farmers' perceptions of maize diseases, pests, and other production constraints, their implications for maize breeding and evaluation of local maize cultivars in KwaZulu-Natal, South Africa. African Journal of Agricultural Research 17:1790-1798.
Songsri P, Joygloy S, Kesmala T, Vorasoot N, Akkasaeng C, Patanothai A (2008). Heritability of drought resistance traits and correlation of drought resistance and agronomic traits in peanut. Crop Science 48:2245-2253.
Woyengo VW (2010). Cassava breeding through complementary conventional and participatory approaches in western Kenya. Ph.D Thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa. Available at: https://researchspace.ukzn.ac.za/xmlui/bitstream/handle/10413/8573/Were_Woyengo_Vincent_2011.pdf?sequence=1&isAllowed=y

Supplementary Table S1. Mean squares for season 1 (2014A) analysis of selected agronomic attributes for groundnut cultivars tested over different sub-agro-ecologies of LACZ

Source of Variation	DF	Yield	DF	No. pods/plant
Replication	2	3769427	2	101.484
Ecology	1	7911929**	1	145.045*
Variety	3	1033709*	2	15.486NS
Ecology x variety	2	74895NS	2	0.728NS
Error	3	67972	3	8.509
Total	11		10	

*Significant at P ≤ 0.05; **Significant at P≤0.01, **Significant at P≤ 001; NS = Not significant (P>0.05), DF = degrees of freedom; HSD = hundred seed weight

Supplementary Table S2. Mean squares for season 2 (2015A) analysis of selected agronomic attributes for groundnut cultivars tested over different sub-agro-ecologies of LACZ

Source of Variation	DF	Yield	DF	No. pods/plant	DF	HSD
Replication	2	2607813	2	213.750	2	654.6
Ecology	2	3369932**	2	409.204***	2	1811.1*
Variety	3	1260989*	3	93.272**	3	313.7NS
Ecology x variety	3	158065NS	4	8.987NS	2	99.4NS
Error	5	188410	6	4.266	5	228.7
Total	15		17		14	

*Significant at P ≤ 0.05; **Significant at P ≤ 0.01, **Significant at P ≤ 001; NS=Not significant (P > 0.05), DF = degrees of freedom; HSD = hundred seed weight

Supplementary Table S3. Mean squares for season 3 (2015B) analyses of selected agronomic attributes for groundnut cultivars tested over different sub-agro-ecologies of LACZ

Source of Variation	DF	Yield (kg/ha)	DF	No. pods/plant	DF	HSD
Replication	2	2278886	2	39.945	2	400.27
Ecology	3	1618155***	3	28.367***	3	245.54**
Variety	2	4895470*	2	57.148***	2	734.09*
Ecology x variety	6	94610NS	5	8.711*	4	22.03NS
Error	15	285621	14	1.930	11	42.53
Total	28		26		22	

*Significant at P ≤ 0.05; **Significant at P≤0.01, **Significant at P≤ 001; NS = Not significant (P>0.05), DF = degrees of freedom; HSD = hundred seed weight

Vol. 13(22), pp. 1128-1135, 31 May, 2018
DOI: 10.5897/AJAR2017.12883
Article Number: 914EEBD57255
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Characterization and evaluation of volatile compounds of three grape varieties *(Vitis labrusca*) from the region of Bento Gonçalves – RS

I. T. S. Viana[1], H. V. Pereira[2], M. R. Silva[2], M. N. S. Guedes[3], A. G. de Souza[1], R. Augusti[2], J. R. L. Freitas[2], G. H. Bueno[1] and J. O. F. Melo[1*]

[1]Federal University of São João Del-Rei, Sete Lagoas Campus, Highway MG 424-Km 47, Postal Code 35701970 Sete Lagoas-MG/Brazil.
[2]Federal University of Minas Gerais, Antonio Carlos Avenue, 6627- Pampulha, Postal Code 31270-901 Belo Horizonte-MG/Brazil.
[3]Federal University of Pará, Altamira Campus Highway Emesto Alcyioli-Km 3, Nova Colina Postal Code 68377-630 Altamira-PA/Brazil.

Received 14 November, 2017; Accepted 10 April, 2018

In grape must, the aroma is an indication of adequate conservation status. It is able to indicate losses due to thermal degradation, besides representing a great contribution in the properties of the sensorial quality of the grape juices most appreciated by consumers. This study identified the volatile compounds in different must cultivars of *Vitis labrusca* grapes. The volatile composition was evaluated by the solid phase microextraction method (SPME), using the polydimethylsiloxane/divinylbenzene fiber (PDMS/DVB). The separation and identification of the volatile compounds were carried out through gas chromatography by mass spectrometry (GC-MS). The results showed the identification of forty-four compounds belonging to the following chemical classes: carboxylic acids, alcohol, aldehydes, ketones, furans esters and terpenes. The class of esters was the most numerous compounds identified and is largely responsible for the fresh and fruity aroma of the tested musts. Solid-phase microextraction proved to be a reproducible, sensitive and fast-response technique for the identification of chemical biomarkers in musts of different grape cultivars.

Key words: Aroma, gas chromatography by mass spectrometry (GC-MS), headspace, solid phase microextraction method (SPME).

INTRODUCTION

The production and consumption of whole grape juice in Brazil and in the world increase every year. The grape is the fruit of the vine *(Vitis* sp*.),* a plant of the family *Vitaceae*, and is used in the production of juices, sweets, wines, and raisins *in natura*.

Generally, in the grape juice production are used the species *Vitis vinifera* grapes from both white and purple varieties. In Brazil, the whole grape juice comes from the American group grapes (*Vitis aestivalis, Vitis labrusca, Vitis bourquina*). In Serra Gaúcha, the most widespread cultivars of *V. labrusca* species are Bordeaux, Concord, and Isabel, which are mainly used for the production of grape juice.

One of the decisive attributes in the selection and

acceptance of foods and beverages is the aroma, being perceived by the retronasal and gustatory sense, so that the demand for new flavors has attracted the aromatization industry's attention, for the characterization of volatile compounds that are responsible for these characteristics, which explains the importance they play in the quality of the fruits and their derivatives (Jiang and Song, 2010).

The fruity aroma is formed by volatile compounds of low molecular weight and low polarity, belonging to several chemical classes such as carboxylic acids, alcohols, aldehydes, esters, ethers, lactones and terpenes (mainly mono and sesquiterpenes), as well as amino or sulfur compounds (Bicas et al., 2011).

The volatile aroma compounds are thermolabile substances that can undergo cyclization, rearrangement, and oxidation when exposed to high temperatures. For the separation and identification of these volatile compounds, one of the most commonly used techniques is gas chromatography coupled to mass spectrometry (GC-MS). For the extraction of the volatile compounds (VCs), we have several methods: solid phase extraction (SPE), solid phase microextraction (SPME), liquid-liquid extraction (LLE), packaged sorbent microextraction (PSME) and stir bar sorbent extraction (SBSE) (Huang and Lee, 2012; Xu et al., 2016; Uekane et al., 2017).

Among these methods, the solid phase microextraction (SPME) is widely used for both volatile and semi-volatile fruits and their derivatives, having benefits such as sensitivity, reproducibility, besides being solvent-free. Sample preparation is simple and demands low temperatures (Kataoka et al., 2000; Gutiérrez-Rosales, 2010; Merkle et al., 2015; Mesquita et al., 2017; Rocha et al., 2017).

Volatile compounds are important indicators of the quality and provenance of the food and the varieties used. Thus, the objective of this study was to identify the volatile compounds in musts of *V. labrusca* cultivars, searching for the main markers of each cultivar.

METHODOLOGY

Samples

The samples of *V. labrusca* cultivars grape musts (Bordeaux, Concord, and Isabel) were supplied by Embrapa Grape and Wine from Rio Grande do Sul during the month of August 2016.

Solid phase microextraction (SPME)

For extraction of the volatile compounds, the solid phase microextraction method was used in *headspace* mode (HS-SPME). It has been used for these operations that create the link between the chemical matrix and the analytical instruments, being particularly interesting for Gas Chromatography (GC). The method has high concentration power (adapting to GC detectors sensibility), it is suitable for many types of analytes and facilitates the transport of the extracted material to the chromatograph (Pawliszyn, 1997), based on the retention of the analytes of interest on a stationary phase, which is attached or deposited in a fused silica capillary microfiber. A Polyimethylsiloxane/divinylbezene (PDMS/DVB) 65 µm semi-polar fiber (SUPELCO) was used and conditioned according to the instructions provided by the manufacturer.

Preparation of the samples

For extraction, 2.0 g of samples of each variety were weighed in SPME-specific 20-mL vials, sealed with aluminum and rubber septum (Garcia et al., 2016).

Thereafter, the sample vial was placed in an aluminum block, preheated at 60°C, on a non-shaking heating plate and left for 5 min to establish its balance. After 5 min, the SPME fiber (PDMS/DVB) adapted to a holder was exposed in the vial in *headspace* mode for 15 min, then the fiber was collected and taken for manual insertion into the gaseous chromatograph injector, being exposed for 5 min, for the desorption of volatile organic compounds (VOCs) (Garcia et al., 2016).

The GC-MS analysis parameters

The VOCs identification was done at the Laboratory of Mass Spectrometry of the Department of Chemistry - UFMG, using a gas chromatograph (Trace GC Ultra) coupled to a mass spectrometer (Polaris Q) by Thermo Scientific (San Jose, CA), with an "ion-trap" type analyzer, with a split/splitless injector, in "splitless" mode. Helium gas was used as a carrier gas at a constant flow of 1 mL min^{-1} by electron impact ionization (EI), with energy rate of 70 eV. The chromatographic column used was a TR-1 MS capillary column (100% dimethylpolysiloxane, 60 m long × 0.25 mm of internal diameter × 0.25 µm of a thick film) by Supelco (*Sigma Aldrich*). The chromatographic analysis conditions were: injector temperature at 250°C, 5 min of desorption time, source temperature at 200°C, and interface temperature at 275°C. The column heating was performed with programmed temperature, which started at 40°C, remaining for 2 min in this temperature, and then, it was increasing at a heating rate of 10°C/min reaching 100°C, maintaining the isotherm for 2 min. Next, it was heated at a rate of 15°C/min reaching 180°C, when the isotherm was maintained for 2 min. Then, the heating process was kept at 15°C/min until it reached 245°C,when the isotherm was maintained for 3 min. Data acquisition occurred in the *Full Scan* mode with a range of 50 to 400 m/z (Garcia et al., 2016).

Identification and correlation of detected volatiles

The VOCs spectra were identified according to their fragmentation profile, which was compared to the mass spectra of the *National Institute of Standards and Technology* (NIST) using a similarity level (RSI) higher than 500. In addition, the data were confirmed by

*Corresponding author. Email: onesiomelo@gmail.com

comparison with the compounds already reported in the literature

RESULTS

V. labrusca musts chromatograms (Bordeaux, Concord, and Isabel) are presented in the Appendix Figures 1 to 3, respectively, showing the profile of the cultivars. Volatile organic compounds (VOCs) were identified according to the m/z ratio shown in Table 1. The main chemical classes found were carboxylic acids, alcohols, aldehydes, ketones, esters, furans, terpenes, and sesquiterpenoids, amounting to 41 compounds.

For the three investigated cultivars, octanoic acid was identified in Bordeaux type, in which was also identified the decanoic acid. For Concord type, nonanoic acid was identified.

The common alcohols for the three cultivars evaluated were: 4-penten-2-ol and benzene ethanol. (*Z*) -2-hexen-1-ol was found in Concord and Isabel.

For Bordeaux cultivar musts, cis-2-octane-1-al, ionone and 3,4,5,6,7,8-hexahydro-4,8-dimethyl-1H-naphthalene were identified.

The esters were the most numerous compounds identified, being especially responsible for the fresh and fruity aroma of the juice. The common esters found in all three varieties were ethyl caproate, acetic acid phenyl ester, anthranilic acid methyl ester, capric acid ethyl ester and ethyl-2,4-decadienoate ethyl ester acid (E, Z). The ethyl 2-furancarboxylic acid was detected in the musts of Bordeaux and Isabel cultivars.

DISCUSSION

Among the classes of the compounds identified, the carboxylic acids have an influence on the flavor. They are present in whole grape juice and the aliphatic acids such as octanoic and decanoic acids are produced from fatty acids, influencing negatively the aroma of the juices (Clarke and Bakker, 2004). Carboxylic acids were identified in Merlot and Moscatel wines *(V. viniferas*), in addition to octanoic, nonanoic and decanoic acids using HS-SPME-GCxGC/TOFMS and HS-SPME-1D-G/MS, respectively (Nicolli et al., 2015). In Chardonnay grapes, octanoic acid was detected using GC-MS (Liberatore et al., 2010).

Alcohols can be produced from sugars during processing, causing positive and negative effects to the aroma of the must (Fariña et al., 2015). The 4-penten-2-ol and (E) -2-hexen-1-ol alcohols were identified by HS-SPME using PDMS/DVB, CAR/DVB/PDMS and CW/DVB fibers in Cabernet Sauvignon and Muscat grapes (*V. viníferas*) (Canuti et al., 2009; Sánchez-Palomo et al., 2005). For the Chardonnay and Merlot grapes (*V. viniferas*), the 4-penten-2-ol compound was detected using CAR/DVB /PDMS fiber (Canuti et al., 2009; Welke et al., 2012), and (Z) -2-hexen-1-ol compound was detected in Cabernet Sauvignon grapes (*V. viníferas*) using the PDMS and DVB/CAR/PDMS fibers (Canuti et al., 2009).

Aldehydes and ketones such as cis-2-octaen-1-al, ionone, and 3,4,5,6,7,8-hexahydro-4,8-dimethyl-1H-naphthalene, as well as lactones, contribute to the floral and fruity scent of the must (Webb and Muller, 1972).

The esters contribute to the fresh and fruity aroma (Welke et al., 2012), as the ethyl-2,4-decadienoate ethyl ester acid (*E, Z*), which was detected in Merlot grapes*(V. viníferas)* and in commercial fruits juice (pear, apricot and peach) by using HS-SPME (Welke et al., 2012; Riu-Aumatell et al., 2004). In Chardonnay grapes *(V. viniferas)* 2-furancarboxylic acid ethyl ester was identified (Liberatore et al., 2010).

The terpenes, mainly monoterpenes, contribute to the varietal aroma of the juice because they have low thresholds of perception, favoring floral scents (Welke et al., 2012). The element *cis*-geraniol, which was identified in Moscato Bianco and Moscato Giallo grapes (Berger, 2007; Xin et al., 2013; Closs et al., 2014) was also detected in the Bordeaux cultivar under study. The 3-carene was identified in Merlot grapes (*V. viníferas*) (Welke et al., 2012). The same was detected in Bordeaux and Isabel grape musts. The p-menth-1-en-4-ol was detected in Muscat grapes (*V. viniferas*) (Kang et al., 2010) and was also identified in the three musts of *V. labrusca*. The cis-z-a-bisabolene epoxide was found in Merlot grapes (*V. labrusca*) and the *α*-selinene was identified in Chardonnay wines (*V. labrusca*) (Welke et al., 2012). In commercial fruit juices (pear, apricot and peach), naphthalene-1,2,4,5,8,8-a-hexahydro-4,7-dimethyl-1- (1-methylethyl) were identified (Riu-Aumatell et al., 2004). The sesquiterpenoids identified in Bordeaux grape musts were the cis-*Z*-a-bisabolene epoxide, *trans*-longipinocarveol, naphthalene-1,2,4,5,8,8a-hexahydro-4,7-dimethyl-1- (1- methylethyl), α-selinene, epi-γ-eudesmol and valerianol.

In commercial fruit juices (pear, apricot, and peach), the volatile compound a-damascenone was detected (Riu-Aumatell et al., 2004), being also detected in the three musts of *V. labrusca* evaluated.

Conclusion

The extraction method and the PDMS/DVB fiber used were efficient in identifying forty-one volatile compounds of several chemicals, responsible for the flavor of the grape must. The classes that determined the volatile profile of *V. labrusca* musts studied are carboxylic acids, alcohols, aldehydes, ketones, esters, furans, and terpenoids. Among these forty-one compounds identified, twelve are common volatile compounds to musts of *V. labrusca* cultivars studied, such as: octanoic acid, 4-pent-2-ol, benzene ethanol, ethyl caproate, acetic acid linalool ester, anthranolic acid methyl ester, acetic acid phenyl ethyl ester, capric acid ethyl ester, ethyl 2,4-decadienoate

Table 1. Volatile compounds identified in grape musts of *Vitis labrusca.*

#	Compounds	m/z	Cultivars		
			Bordeaux	Concord	Isabel
CarboxylicAcids					
1	Octanoicacid[a,b,c,d,]	43, 55, 60, 73, 135	X	X	X
2	Nonanoicacid[b,c]	41, 55, 73,24; 115,09; 129,05	ND	X	ND
3	Decanoicacid[a,c,d]	41,27; 55,33; 73,29; 87,22; 129,15; 172	X	ND	ND
Alcohol					
4	4-penten-2-ol	42; 43;86	X	X	X
5	(Z)-2-hexen-1-ol [d,e]	39,24; 43,20; 57,17; 67,13; 82,05; 100	ND	X	X
6	Benzene ethanol (phenylethyl alcohol)	91,25;92,14, 121,27; 65,46; 122	X	X	X
Aldehyde					
7	Cis-2-octane-1-al	41,18; 55,19; 70,08,83,06; 126	X	X	ND
Ketones					
8	Ionones	41; 93; 121; 135; 177; 192	X	X	ND
9	3,4,5,6,7,8-hexahydro-4,8-dimethyl-1H-naphthalen-2-one	97,39; 123,29; 137,23; 179,14; 212,99; 204	X	ND	ND
Furans					
10	5- (Hydroxymethyl) furan-2-carbaldehyde [d]	39,21; 69,39; 97,10; 126	ND	X	X
Esters					
11	Ethylcaproate (ethylhexenoate)	43,25; 55,36; 61,25; 73,25; 88,12; 99,01; 145,09	X	X	X
12	Acid-2-furancarboxylic methylester (2-furoate methyl)[f]	39,24; 95,12; 125,92	X	ND	X
13	*Trans*-2-hexenoate ethyl	39,24; 55,29; 73,32; 99,04;142	ND	ND	X
14	Aceticacidlinalool ester	43; 71; 91; 137; 159; 196	X	ND	ND
15	Methylsalicylic ester	92,3; 120,18; 151,99 e 121,17; 92,31; 120,14; 152,01	X	X	X
16	Acetic acid-phenyl-ethyl ester	65,30; 91,16; 164	X	X	X
17	Benzoicacidphenyl ester	79,44; 103,28; 104,15; 105,11; 226	X	ND	ND
18	Anthranilicacid, methyl ester	92,52; 119,22; 151,07	X	X	X
19	Naphthalene-1,2-dihydro-2,5,8-trimethyl	43,22; 142,35; 157,17; 172,10	ND	X	ND
20	Decenoicacidethyl ester	39,82; 41,27; 55,26; 67,31; 81,33; 152,11	ND	X	ND
21	Capricacid, ethyl ester	55,45; 61,31; 70,24; 73,27; 157,14; 200	X	X	X
22	Ethyl-2-4-decadienoate ethyl ester (E, Z)[d,g]	67,51; 81,34; 97,30; 125,11; 196	X	X	X
23	Anthranilicacid – ethyl ester	92,34; 119,24; 120,22; 137,21; 165,08	X	ND	ND
24	Linolenicacid-ethyl ester	67,35; 79,33; 121,20; 136,18; 178,06; 306	X	ND	ND
Terpenes					
25	(S)-cis-verbenol((1S, 2S, 5S) -4,6,6-trimethylbicyclo [3.1.1] hept-3-en-2-ol)	39;59;95;111;152	X	ND	ND
26	2-ethenyl-6-methyl-5-hepten-1-ol	39; 41; 79; 121; 136; 154	X	ND	ND
27	P-menth-1-en-4-ol[h]	43,38; 71,32; 91,29; 93,20; 111,11; 154	X	X	X
28	Allo-ocimene((4E, 6E) -2,6-dimethylocta-2,4,6-triene)	93,51; 121,15; 136	X	ND	ND
29	Dihydroumbellulone (bicyclo [3,1,0] hexan-2-one, 4-methyl-1- (1-methylethyl) - (1a, 4a, 5a))	39,22; 67,29; 81,25; 97,09; 152	X	ND	ND
30	6-isopropyl-3-methyl-7-oxabicyclo-heptan-2-one	43,23; 55,44; 83,32; 125,14; 168; 182,03	ND	ND	X
31	3-carene (4,7,7-trimethylbicyclo [4.1.0] hept-3-ene)[d]	59,50; 81,50; 93,30; 121,19; 136,10	X	ND	X
32	cis-geraniol (cis-3,7-dimethyl-2,6-octadien-1-ol)[i,j]	41,18; 67,28; 93,16; 121,11; 154	X	ND	ND
Sesquiterpenoids					
33	(Z) -bisaboleneepoxide[d]	41,29; 57,51; 67,33; 97,45; 102,21; 220	X	ND	ND

Table 1. Contd.

34	1,2,4a, 5,8,8a-hexahydro-4,7-dimethyl-1- (1-methylethyl) -naphthaleno[g]	105,58; 133,34; 161,29; 189,19; 204,12	X	ND	ND
35	α-selinene[d]	41;67; 81; 107; 134; 162; 204	X	ND	ND
36	-2epi-γ-Eudesmol	105,50; 133,29; 149,31; 161,20; 189,16; 204,17; 222	X	ND	ND
Others					
37	4-hydroxy-2-butanone-ethyl acetate	42;45;61;70;88	X	X	ND
38	Benzylcyanide	89,34; 90,32; 116,22; 117,09	X	X	X
39	1H-2-indonone, 2,4,5,6,7,7a-hexahydro-3- (1-methylethyl) -7a-methyl	93,32; 121,29; 177,12; 192,07	X	X	X
40	1,2-Dihydro-1,5,8-trimethylnaphthalene	43,22; 142,35; 157,17; 172,10	ND	X	ND
41	A-damascenone (E) -1- (2,6,6-trimethylcyclohexa-1,3-dien-1-il) -but-2-en-1-one [g]	41,82; 69,47; 105,31; 121,21; 190,02; 199	X	X	X

Solid phase microextraction fiber: Polydimethylsiloxane/divinylbenzene (PDM/DVB) with 65 µm. The letters indicate compounds that have already been identified by other authors: [a](Etievant, 1991; Gürbüz et al., 2006); [b](Guio et al., 2010); [c] (Nicolli et al., 2015); [d](Welke et al., 2012); [e](Canuti et al., 2009); [f](Liberatore et al., 2010); [g](Riu-Aumatell et al., 2004); [h](Kang et al., 2010); [i](Xin et al., 2013); [j](Closs et al., 2014). X: Detected, ND: Not detected.

acid ethyl ester (E, Z), p-meth-1-em-4-ol, benzyl cyanide and 1-H-2-indonone-2,4,5,7,7a-hexahydro-3- (1-methylethyl) -7a-methyl - some of them being reported in the literature.

The parameters used in the study were sensitive, fast, easy, and useful for the quantification and identification of volatile compounds according to data reported in the literature and, in the future, it will be possible to determine the aromatic maturity for different grapes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Berger RG, Ferger RG (2007). Flavours and Fragrances. Springer Verlag. https://www.springer.com/br/book/9783540493389

Bicas JL, Molina G, Dionísio AP, Barros FFC, Wagner R, Maróstica Jr MR, Pastore GM (2011). Volatile constituents of exotic fruits from Brazil. Food Research International 44:1843-1855.

Canuti V, Conversano M, Calzi ML, Heymann H, Matthews MA, Ebeler SE (2009). Headspace solid-phase microextraction–gas chromatography–mass spectrometry for profiling free volatile compounds in Cabernet Sauvignon grapes and wines. Journal of Chromatography A 1216(15):3012-3022.

Clarke RJ, Bakker J (2004). Factors influencing sensory perception. In Wine flavour chemistry (pp. 202-204). Blackwell Publishing Oxford, UK.

Closs M, Nicolli KP, Manfroi V, Zini CA (2014). Diferenciação entre espumantes moscatéis provenientes de duas variedades de uva Moscato. Inst. Química. https://www.lume.ufrgs.br/bitstream/handle/10183/114109/Poster_37215.pdf?sequence=2

Etievant PX (1991). Volatile Compounds in Food and Beverages. CRC Press. https://www.crcpress.com/Volatile-Compounds-in-Foods-and-Beverages/Maarse/p/book/9780824783907

Fariña L, Villar V, Ares G, Carrau F, Dellacassa E, Boido E (2015). Volatile composition and aroma profile of Uruguayan Tannat wines. Food Research International 69:244-255.

Garcia YM, Guedes MNS, Rufini JCM, Souza AG, Augusti R, Melo JOF (2016). Volatile compounds identified in Barbados Cherry 'BRS-366 Jaburú'. Scientific Electronic Archives 9(3):67-73.

Guio JCB, Leon DCS, Perez ALM (2010). Compostos voláteis livres e enlaçados glicosídicamente na polpa da uva Caimarona (Pouroumacecropiifolia Mart.). Acta Amazonica 40(1):189-198.

Gürbüz O, Rouseff JM, Rouseff RL (2006). Comparison of aroma volatiles in commercial Merlot and Cabernet Sauvignon wines using gas chromatography− olfactometry and gas chromatography− mass spectrometry. Journal of Agricultural and Food Chemistry 54(11):3990-3996.

Gutiérrez-Rosales F (2010). History and principles of flavor analysis, In: Hui, Y.H. (Ed.), Handbook of fruit and vegetable flavors. John Wiley & Sons pp. 159-175.

Huang Z, Lee HK (2012). Materials-based approaches to minimizing solvent usage in analytical sample preparation. Trends in Analytical Chemistry 39:228-244.

Jiang Y, Song J (2010). Fruits and Fruit Flavor: Classification and biological characterization. In: HUI, Y. H. Handbook of fruit and vegetable flavors. John Wiley & Sons pp. 3-23.

Kang W, Xu Y, Qin L, Wang Y (2010). Effects of Different β-D-Glycosidases on Bound Aroma Compounds in Muscat Grape Determined by HS-SPME and GC-MS. Journal of the Institute of Brewing 116(1):70-77.

Kataoka H, Lord HL, Pawliszyn J (2000). Applications of solid-phase microextraction in food analysis. Journal of Chromatography A 880:35-62.

Liberatore MT, Pati S, Del Nobile MA, La Notte E (2010). Aroma quality improvement of Chardonnay white wine by fermentation and ageing in barrique on lees. Food Research International 43(4):996-1002.

Merkle S, Kleeberg KK, Fritsche J (2015). Recent developments and applications of solid phase microextraction (SPME) in food and environmental analysis - A review. Chromatography 2:293-381.

Mesquita PRR, Nunes EC, Santos FN, Bastos LP, Costa MAPC, Rodrigues FM, Andrade JB (2017). Discrimination of *Eugenia uniflora* L. biotypes based on volatile compounds in leaves using HS-SPME/GC–MS and chemometric analysis. Microchemical Journal 130:79-87.

Nicolli KP, Welke JE, Closs M, Caramão EB, Costa G, Manfroi V, Zini CA (2015). Characterization of the volatile profile of Brazilian moscatel sparkling wines through solid phase microextraction and gas chromatography. Journal of the Brazilian Chemical Society 26(7):1411-1430.

National Institute of Standards and Technology (NIST) Chemistry Web Book. NIST chemistry web book NIST standard reference database number 69, June 2005 Release. Accessed: 10.06.2017.

Pawliszyn J (1997). Solid phase microextraction: Theory and Practice. John Wiley & Sons.

Riu-Aumatell M, Castellari M, López-Tamames E, Galassi S, Buxaderas S (2004). Characterisation of volatile compounds of fruit juices and nectars by HS/SPME and GC/MS. Food Chemistry 87(4):627-637.

Rocha RFJ, Araújo IMS, Freitas SM, Garruti DS (2017). Optimization of headspace solid phase micro-extraction of volatile compounds from papaya fruit assisted by GC–olfactometry. Journal of Food Science and Technology 54(12):4042-4050.

Sánchez-Palomo E, Diaz-Maroto MC, Perez-Coello MS (2005). Rapid determination of volatile compounds in grapes by HS-SPME coupled with GC–MS. Talanta 66(5):1152-1157.

Uekane TM, Nicolotti L, Griglione A, Bizzo HR, Rubiolo P, Bicchi C, Rocha-Leão MHM, Rezende CM (2017). Studies on the volatile fraction composition of three native Amazonian-Brazilian fruits: Murici (*Byrsonimacrassifolia* L., Malpighiaceae), bacuri(*Platoniainsignis* M., Clusiaceae), and sapodilla (*Manilkarasapota* L.,Sapotaceae). Food Chemistry 219:13-22.

Webb AD, Muller CJ (1972). Volatile aroma components of wines and other fermented beverages. In Advances in Applied Microbiology. Academic Press 15:75-146.

Welke JE, Manfroi V, Zanus M, Lazarotto M, Zini CA (2012). Characterization of the volatile profile of Brazilian Merlot wines through comprehensive two dimensional gas chromatography time-of-flight mass spectrometric detection. Journal of Chromatography A 1226:124-139.

Xin H, Wu B, Zhang H, Wang C, Li J, Yang B, Li S (2013). Characterization of volatile compounds in flowers from four groups of sweet osmanthus (*Osmanthus fragrans*) cultivars. Canadian Journal of Plant Science 93(5):923-931.

Xu CH, Chen GS, Xiong ZH, Fan YX, Wang XC, Liu Y (2016). Applications of solid-phase microextraction in food analysis. Trends in Analytical Chemistry 80:12-29.

Appendix

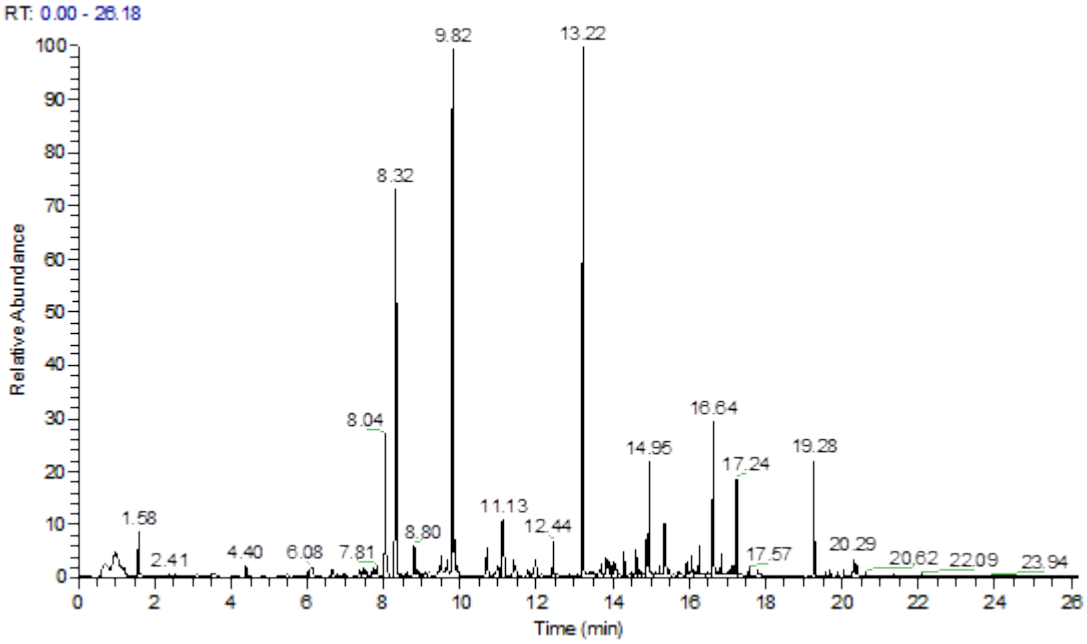

Figure 1. Chromatogram profile of volatile compounds of grape musts belonging to Bordeaux cultivars (*Vitis labrusca*).

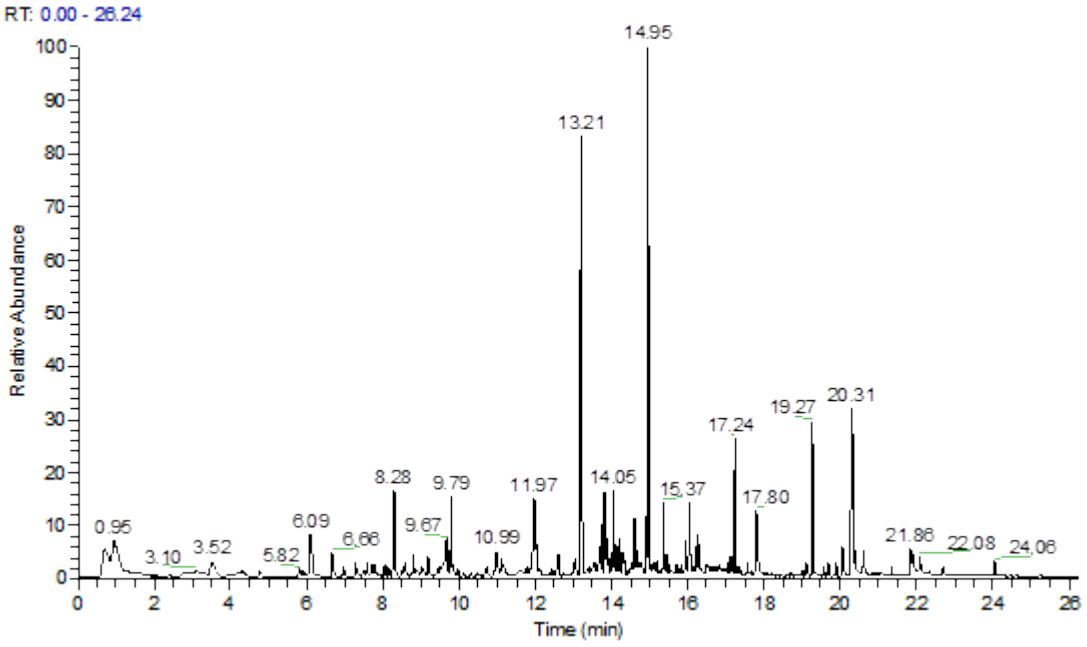

Figure 2. Chromatogram profile of volatile compounds of grape musts belonging to Concord cultivars (*Vitis labrusca*).

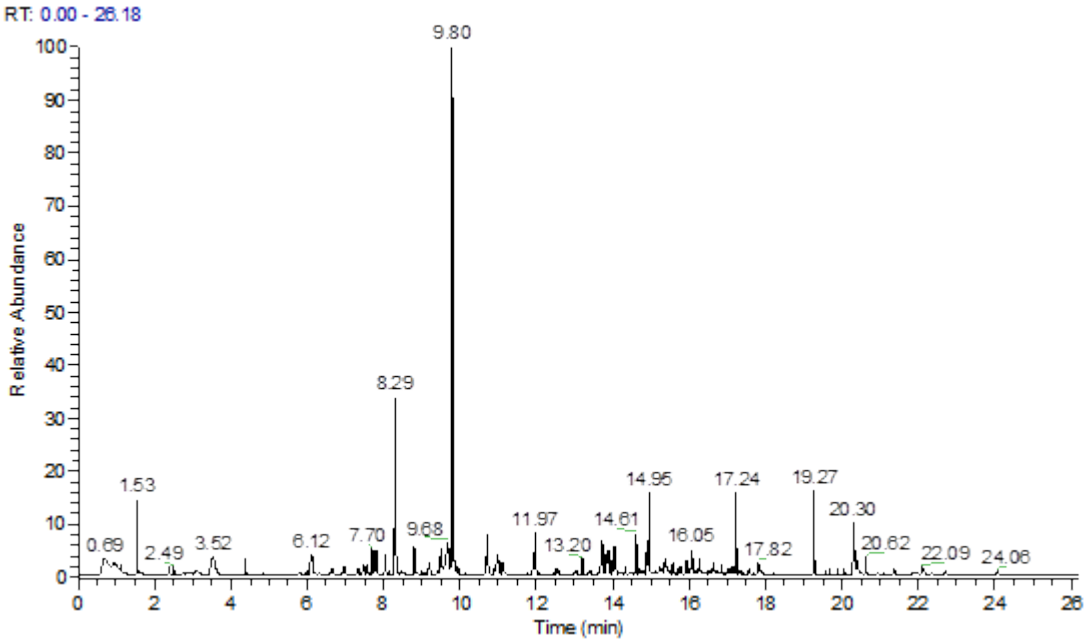

Figure 3. Chromatogram profile of volatile compounds of grape musts belonging to Isabel cultivars (*Vitis labrusca*).

Vol. 13(22), pp. 1136-1139, 31 May, 2018
DOI: 10.5897/AJAR2018.13137
Article Number: 0E6DB0657257
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Can early peroxidase quantification detect graft-compatible in anonaceous rootstocks?

Daniel Baron[1*], Amanda Cristina Esteves Amaro[2], Ana Claudia Macedo[3], Carmen Sílvia Fernandes Boaro[2] and Gisela Ferreira[2]

[1]Center for Natural Sciences (CCN), Plant Physiology and Biochemistry Laboratory,Federal University of São Carlos (UFSCar), Lagoa do Sino campus, CP-094, 18290-000, Buri-SP, Brazil.
[2]Institute of Biosciences (IB), Department of Botany, State University Paulista "Júlio de Mesquita Filho" (UNESP), Campus Botucatu, CP-510, CEP: 18618-970, Botucatu-SP, Brazil.
[3]Department of Horticulture, Faculty of Agronomic Sciences (FCA), Paulista State University (UNESP), Campus Botucatu, José Barbosa de Barros Street, 1780, CP-237, CEP: 18603-970, Botucatu-SP, Brazil.

Received 22 March, 2018; Accepted 26 April, 2018

This study aimed to quantify the class III peroxidase activity in young plants of different species belonging to Annonaceae botany family, with high potential as a rootstock, for early detection of graft-compatible atemoya (*Annona* x *atemoya* Mabb.). The experimental design was randomized block, that evaluated the species *araticum-de-terra-fria* (*Annona emarginata* (Schltdl.) H. Rainer 'variety terra-fria'), *araticum-mirim (Annona emarginata* (Schltdl.) H. Rainer 'variety mirim) and *biribá* (*Annona mucosa* (Schltdl.) H. Rainer), with rootstock potential, and atemoya (scion), which were divided into four blocks, each with four plants. The peroxidase was quantified on the stem before grafting. Statistical analysis showed that only the *biribá* presented different peroxidase activity compared to atemoya plants; and *araticum-mirim* and *araticum-de-terra-fria* were similar to atemoya plants. Thus, the peroxidase activity could not be possible or be used as a tool in the early diagnosis of the graft-compatible in atemoya combinations.

Key words: *Annona emarginata*, *Annona mucosa*, *Annona* x *atemoya*, compatibility, grafting.

INTRODUCTION

The hybrid atemoya is a fruit used worldwide in the food industry and it is propagated by vegetative methods such as grafting, to ensure commercial characteristics (Heenkenda et al., 2009). The rootstocks most often used to graft the atemoya are *araticum-de-terra-fria*, *araticum-mirim*, *biribá* and atemoya itself (Baron et al., 2016).

Grafting is a technique of vegetative propagation, which involves the union of two parts of plants through the

*Corresponding author. Email: danielbaron@ufscar.br.

tissue regeneration, so that the assembly constitutes a new plant (Melnyk and Meyerowitz, 2015; Xu et al., 2016). The mechanism of reestablishment post-grafting is not yet elucidated and several hypotheses have been raised in an attempt to explain them (Melnyk and Meyerowitz, 2015; Xu et al., 2016), besides, predicting the incompatibility of the graft is very important to select graft-compatible and graft-incompatible. The reasons for reestablishing post-grafting are complex and involve many physiological and biochemical processes, such as the peroxidase. Peroxidase is intrinsically linked to the beginning of grafting process, because during the lignification there are specific functions in the lignin biosynthesis (Fernández-Pérez et al., 2015; Mo et al., 2017).

The peroxidase act as a scavenging excess reactive oxygen species induced by wounding (Rogers and Munné-Bosch, 2016), which might play an important role in the graft process. It uses hydrogen peroxide as its electron receptor to oxidize the cinnamic acid and convert the ferulic acid in diferulic, which acts in the bridge of hemicelulose binding the cinnamic acid to the proteins and the carbohydrates of the cellular wall favoring the consolidation (Liu, 2012).

The presence of peroxidase may be used as a marker to predict the compatibility of pecan (*Carya illinoensis*) (Mo et al., 2017); likewise, are found similarly in peroxidase activity between scion and rootstock in interstock of the *Prunus* genus (Telles et al., 2009). Besides, studies claim that the presence of peroxidase isozymes in grafting is an experimental approach to predict the incompatibility reaction (Irisarri et al., 2015).

Therefore, the aim of this study was to quantify the class III peroxidase activity, in young plants of different Annonaceae species, with high potential as rootstock, to investigate the possibility of using this analysis for early prediction of graft- compatible atemoya.

MATERIALS AND METHODS

The experiment was conducted in greenhouse located in the experimental area belonging to Botany Department of *Instituto de Biociências*, *Universidade Estadual Paulista* (Unesp), Botucatu municipality, São Paulo State, Brazil, which has the following geographical coordinates: 48º 24' 35"W, 22º 49'10"S and 850 m of average altitude above sea level.

The seeds of *araticum-de-terra-fria* (*Annona emarginata* (Schltdl.) H. Rainer "variedade terra-fria"), *araticum-mirim* (*Annona emarginata* (Schltdl.) H. Rainer "variedade mirim"), *biribá* (*Annona mucosa* (Bail.) H. Rainer) and atemoya (*Annona* x *atemoya* Mabb.) were disinfected with sodium hypochlorite (10% a.i.) and N-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide (CAPTANA) (CAS 133-06-2) fungicide (1.2 g a.i. kg^{-1} of seeds).

Thereafter, seeds were sown in polystyrene trays filled with vermiculite, until their emergence. The seedlings presented ± 10 cm in length (neck to the stem apex), and were transplanted to plastic bags with a capacity of 5 L, containing a mixture of fertile soil, vermiculite medium texture, coconut fiber and decomposed pine bark (1:1:1:1, v/v). The experimental design was a randomized block with four blocks, each one with four plants. The cultivation was done in a greenhouse and Hoagland and Arnon n°2 was applied, diluted to 50% of its ionic strength, electrical conductivity (EC) of 1.0 miliSiemens cm^{-1} (300 ml per pot) and pH 5.5-6.5, according to Baron et al. (2013).

For biochemical analysis, when the plants were one year old, approximately 5 cm of stem was collected, which was a band cut (bark + wood), 20 cm above the cervical region, in which grafting is usually performed by commercial nurseries. For enzyme extract, 300 mg of the samples were pulverized in liquid nitrogen and homogenized in 4 mL of pre-cooled potassium phosphate buffer (0.1 M, pH 6.8) and 200 mg polyvinylpyrrolidone (PVP). The homogenates were centrifuged at 10,000 x g for 10 min at 4°C, and the resulting supernatants were used for enzyme assay (Kar and Mishra, 1976). The supernatant from the extraction was used to determine the class III peroxidase activity.

The determination of class III peroxidase activity (PRX, EC 1.11.1.7) was performed according to Teisseire and Guy (2000). The reaction system was composed of 30 µL of the enzyme extract, 500 µL of potassium phosphate buffer (50 mM, pH 6.5), 250 µL of pyrogallol (1,2,3-benzenetriol, 20 mM) and 220 µL of hydrogen peroxide (H_2O_2, 5 mM), in a final volume of 1000 µL. The reaction was conducted at room temperature conditions for 5 min. The purpurogallin formation was measured on 430 nm using spectrophotometer (SP-220, Biospectro, Brazil) and its molar extinction coefficient (2.5 mmol L^{-1} cm^{-1}) was used to calculate the specific activity of enzyme (µmol of purpurogallin $min^{-1}mg^{-1}$protein).

The soluble protein content was obtained using the Bradford method (Bradford, 1976). The reaction system was composed of 100 µL of the enzymatic extract and 5000 µL of Bradford-reactive. The reaction was conducted at room temperature for 15 min, and the absorbance reading was measured on 595 nm using spectrophotometer (SP-220, Biospectro, Brazil). Whereas its absorbance readings was performed in 595 nm using spectrophotometer (SP-220, Biospectro, Brazil), utilizing casein as a reference protein.

The statistical package used for the data analysis was SAS 9.2 (SAS Institute Inc., Cary, NC). The Levene test was used to verify the homogeneity of variances of the treatments. Comparisons were made with the hybrid atemoya with each species with potential use for rootstock (*araticum-mirim*, *araticum-de-terra-fria* and *biribá*). Thus, the results were submitted to the Student t test at 5% probability for independent samples (unpaired), comparing their averages two to two.

RESULTS AND DISCUSSION

The Levene test showed that the variances were homogeneous among groups. Thus, it is observed in Figure 1, that only the *biribá* showed different peroxidase activity from atemoya, presenting lower values. On the other hand, *araticum-mirim* and *araticum-de-terra-fria* presented similar peroxide activity to atemoya plants (Figure 1).The *biribá* plants had lower peroxidase activity than atemoya plants. The literature reports that atemoya scion graft onto *biribá rootstock* presents a survival rate lower than the combination of atemoya graft onto *araticum-de-terra-fria* (Santos et al., 2005).

However, Baron et al. (2016) cultivated atemoya scion graft onto biribá rootstock at 60 and 90 days after

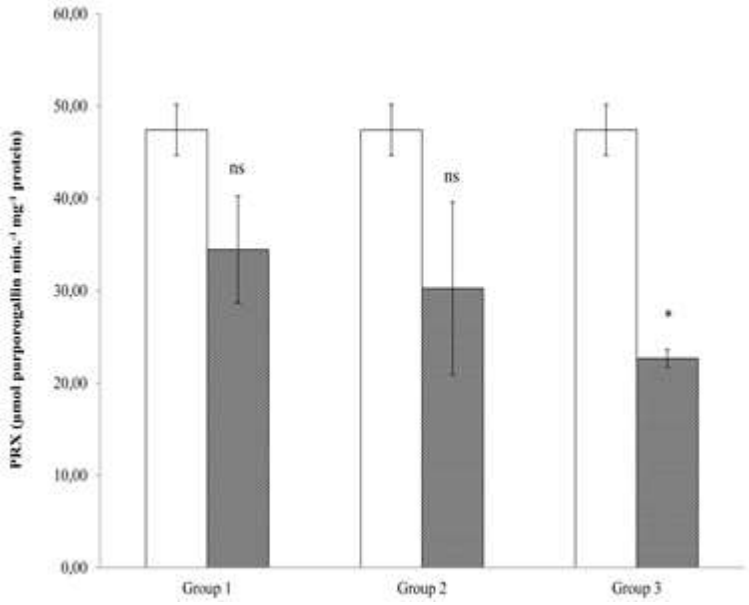

Figure 1. Class III peroxidase activity mean values (PRX, µmol de purpurogallin min^{-1} mg^{-1} protein) of stem in different Annonaceae species. Group 1 [atemoya (not hatch) and *araticum-de-terra-fria* (hatch)]; group 2 [atemoya (not hatch) and *araticum-mirim* (hatch)]; group 3 [atemoya (not hatch) and *biribá* (hatch)]. Columns represent the mean and the error bars represent standard error (n = 4). *Significant at 5% probability ($P \leq 0.05$); and ns denotes not significant ($P > 0.05$).

grafting, that UGP gene expression was similar to that graft-compatible, for example, atemoya graft onto araticum-de-terra-fria and araticum-mirim rootstock. This gene encoded UDP-glucose pyrophosphorylase (UGPase) protein plays an important role in many physiological processes, including carbohydrate metabolism, sucrose and cellulose formation in cell walls (Lerouxel et al., 2006; Janse Van Rensenburg et al., 2018). Despite its important regulatory role, little is known about the expression of this gene associated with graft-compatible (Pina and Errea, 2008). Thus, early increased expression of this gene indicates a rapid union of plant tissues after grafting.

Nevertheless, Rodrigues et al. (2002) reports that rootstocks of peach and plum, for example ‘mirabolano’, presented higher peroxidase activity, indicating that this species, when grafted onto cultivars with lower activity, present graft-incompatibilities. Moreover, in cherry, cultivars with lower peroxidase activity can predict graf-incompatible (Güçlü and Koyuncu, 2012).

Several studies assert that peroxidase and phenolic compounds are involved in tissue lignification (Hiraga et al., 2001; Liu, 2012), which are important in the early stages of the connection between the graft and rootstock (Irisarri et al., 2015), because the cell walls of xylem tissues are dynamic structures composed of poly-saccharides, proteins, minerals and phenolic compounds such as lignin (Herrero et al., 2014).

When the peroxidase activities of *araticum-mirim* and *araticum-de-terra-fria* were evaluated it was found that both did not differ, suggesting graft-compatible species. These results corroborate those previously found in the union of *araticum-de-terra-fria* and atemoya, and are consistent with those obtained by Baron et al. (2016, 2017), who reports that atemoya graft onto *araticum-de-terra-fria* are suitable for seedling formation in commercial orchards.

Araticum-de-terra-fria rootstock compared to *araticum-mirim* rootstock is more agronomical advantageous, because its field duration is larger and do not show signs of "elephant foot" and dwarfism. However, dwarfism in fruit trees is an advantageous feature because it reduces manufacturing costs by increasing the density of the plants in the cultivated area (López-Marín et al., 2017). The atemoya scion grafted onto *araticum-de-terra-fria* is stronger than the one grafted on *araticum-mirim*, while the development is identical between both, however there is need to wait for an additional year for the atemoya produce fruits when grafted on *araticum-de-terra-fria*, compared to *araticum-miri m* (Scaloppi and Martins, 2013).

Rainer (2007) previously reports taxonomic rearrangement, which the sinonimized and ranked both, araticum-de-terra-fria and araticum-mirim, as *Annona*

emarginata (Schltdl.) H. Rainer and, perhaps, justifying the similarity between the activities of peroxidase found in this study for both rootstocks, however, there are morphological and origin center differences between the *araticum-mirim* and *araticum-de-terra-fria* species, such as and lanceolate leaves of *araticum-de-terra-fria*, seeds and fruits morphologies (Couvreur et al., 2012).

CONCLUSION

The peroxidase activity could not be used as a tool in the early diagnosis of the graft-compatible in atemoya combinations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors wish to thank the Foundation for Research Support of the State of São Paulo (FAPESP). Daniel Baron was the recipient of FAPESP Grant n° 2011/00853-8 and FAPESP B.E.P.E. Grant n° 2013/22036-7.

REFERENCES

Baron D, Amaro ACE, Macedo AC, Boaro CSF, Ferreira G (2017). Physiological changes modulated by rootstocks in atemoya (Annona x atemoya Mabb.): gas exchange, growth and ion concentration. Brazilian Journal of Botany 41(1):219-225. https://doi.org/10.1007/s40415-017-0421-0

Baron D, Bravo JP, Maia IG, Pina A, Ferreira G (2016). UGP gene expression and UDP-glucose pyrophosphorylase enzymatic activity in grafting annonaceous plants. Acta Physiologiae Plantarum 38:79. https://doi.org/10.1007/s11738-016-2097-7

Baron D, Ferreira G, Rodrigues JD, Boaro CSF, Macedo AC (2013). Gas exchange, phisiological indexes and ionic accumulation in Annona emarginata (Schltdl.) H. Rainer seedlings in nutrients solution. Revista Brasileira de Fruticultura 35:361-376. http://dx.doi.org/10.1590/S0100-29452013000200005

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72:248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Couvreur TLP, Maas PJM, Meinke S, Johnson DM, KeßLer PJA (2012). Keys to the genera of Annonaceae. Botanical Journal of the Linnean Society 169:74-83. http://doi.org/10.1111/j.1095-8339.2012.01230.x

Fernández-Pérez F, Vivar T, Pomar F, Pedreño MA, Novo-Uzal E (2015). Peroxidase 4 is involved in syringyl lignin formation in Arabidopsis thaliana. Journal of Plant Physiology 175:86-94. https://doi.org/10.1016/j.jplph.2014.11.006

Güçlü SF, Koyuncu F (2012). A Method for Prediction of Graft Incompatibility in Sweet Cherry. otulae Botanicae Horti Agrobotanici Cluj-Napoca 40(1):243-246. http://dx.doi.org/10.15835/nbha4017560

Herrero J, Esteban Carrasco A, Zapata JM (2014). Arabidopsis thaliana peroxidases involved in lignin biosynthesis: in silico promoter analysis and hormonal regulation. Plant Physiology and Biochemistry 80:192-202. https://doi.org/10.1016/j.plaphy.2014.03.027

Hiraga S, Sasaki K, Ito H, Ohashi Y, Matsui H (2001). A Large Family of Class III Plant Peroxidases. Plant & Cell Physiology 42(5):462-468. https://doi.org/10.1093/pcp/pce061

Heenkenda H, Gunathilaka B, Iswara J (2009). Rootstock-scion interactions of selected Annona species. Journal of the National Science Foundation of Sri Lanka 37:71-75. http://doi.org/10.4038/jnsfsr.v37i1.460

Irisarri P, Binczycki P, Errea P, Martens HJ, Pina A (2015). Oxidative stress associated with rootstock–scion interactions in pear/quince combinations during early stages of graft development. Journal of Plant Physiology 176:25-35. ttps://doi.org/10.1016/j.jplph.2014.10.015

Janse Van Rensenburg HC, Van den Ende W (2018). UDP-Glucose: A Potential Signaling Molecule in Plants? Frontiers in Plant Science 8:2230-2236. https://doi.org/10.3389/fpls.2017.02230

Kar M, Mishra D (1976). Catalase, Peroxidase and Polyphenoloxidase Activities during Rice Leaf Senescence. Plant Physiology 57:315-319.

Lerouxel O, Cavalier DM, Liepman AH, Keegstra K (2006). Biosynthesis of plant cell wall polysaccharides — a complex process. Current Opinion in Plant Biology 9:621-630. https://doi.org/10.1016/j.pbi.2006.09.009

Liu CJ (2012). Deciphering the enigma of lignification: precursor transport, oxidation, and the topochemistry of lignin assembly. Molecular Plant 5(2):304-317. https://doi.org/10.1093/mp/ssr121

López-Marín J, Gálveza A, Del Amora FM, Albacete A, Fernández JA, Egea-Gilabert A, Pérez-Alfocea F (2017). Selecting vegetative/generative/dwarfing rootstocks for improving fruit yield and quality in water stressed sweet peppers. Scientia Horticulturae 214:9-17. http://doi.org/10.1016/j.scienta.2016.11.012

Melnyk CW, Meyerowitz EM (2015). Plant grafting. Current Biology 25:183-188.

Mo Z, He H, Su W, Peng F (2017). Analysis of differentially accumulated proteins associated with graft union formation in pecan (Carya illinoensis). Scientia Horticulturae Amsterdam 224:126-134. https://doi.org/10.1016/j.scienta.2017.06.005

Pina A, Errea P (2008). Influence of graft incompatibility on gene expression and enzymatic activity of UDP-glucose pyrophosphorylase. Plant Science 174:502-509. https://doi.org/10.1016/j.plantsci.2008.01.015

Rainer H (2007). Monographic studies in the genus Annona L. (Annonaceae): inclusion of the genus Rollinia ASt.-Hil. Annalen des Naturhistorischen Museums in Wien. Serie B für Botanik und Zoologie 108:191-205.

Rodrigues AC, Diniz ÂC, Fachinello JC, Silva JB, Faria JLC (2002). Peroxidases e fenóis totais em tecidos de porta-enxertos de Prunus sp. nos períodos de crescimento vegetativo e de dormência. Ciencia. Rural 32:559-564. https://doi.org/10.1590/S0103-84782002000400002

Rogers H, Munné-Bosch S (2016). Production and Scavenging of Reactive Oxygen Species and Redox Signaling during Leaf and Flower Senescence: Similar But Different. Plant Physiology 171(3):1560-1568. https://doi.org/10.1104/pp.16.00163

Santos CE, Roberto SR, Martins ABG (2005). Propagação do biribá (Rollinia mucosa) e sua utilização como porta-enxerto de pinha (Annona squamosa). Acta Scientiarum. Agronomy 27:433-436. http://dx.doi.org/10.4025/actasciagron.v27i3.1404

Scaloppi Jr EJ, Martins ABG (2013). A estaquia em espécies de Anonnaceae potenciais como porta-enxertos. In: Ferreira, G., Kavati, R., Boaro, C.S.F., Bortolucci, T., & Leonel, S. editors. Anonáceas: propagação e produção de mudas. FEPAF, Botucatu pp. 59-73.

Teisseire H, Guy V (2000). Copper-induced changes in antioxidant enzymes activities in fronds of duckweed (Lemna minor). Plant Science 153:65-72. https://doi.org/10.1016/S0168-9452(99)00257-5

Telles CA, Biasi LA, Mindêllo Neto UR, Deschamps C (2009). Fenóis totais, peroxidase e suas relações com a compatibilidade de mudas de pessegueiro interenxertadas. Ciência e Agrotecnologia 33:86-91. http://dx.doi.org/10.1590/S1413-70542009000100012

Xu Q, Guo S-R, Li L, An Y-H, Shu S, Sun J (2016). Proteomics analysis of compatibility and incompatibility in grafted cucumber seedlings. Plant Physiology and Biochemistry 105:21-28. https://doi.org/10.1016/j.plaphy.2016.04.001.

Vol. 13(22), pp. 1140-1147, 31 May, 2018
DOI: 10.5897/AJAR2018.13151
Article Number: 161329057259
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Nutritive potential of amaranth weed grains

João Barcellos Xarvier[1*], Douglas Correa de Souza[1], Leticia Cavalheiro de Souza[1], Thiago Sampaio Guerra[1], Luciane Vilela Resende[1] and Joelma Pereira[2]

[1]Departamento de Agricultura, Campus Universitário, Universidade Federal de Lavras (UFLA), 37200-000, Lavras, MG/Brasil.
[2]Departamento de Ciência dos Alimentos, Campus Universitário, Universidade Federal de Lavras (UFLA), 37200-000, Lavras, MG/Brasil.

Received 27 March, 2018; Accepted 11 May, 2018

***Amaranthus* is a species with immense potential; however, information on its nutritional properties is limited, though widely cultivated in some countries. The objective of this work is to characterise the grains of three species of *Amaranthus* sp. aiming at their food potential, comparing two species considered as weed with a commercially grown species. The selected materials were cultivated and submitted to the same culture method and the experiment was performed as a randomized block design with three replicates. Harvested grains were transformed into flour and centesimal composition was determined, as well as macro and micronutrients, starch content and non-nitrogenous. The results were submitted to analysis of variance and compared in Tukey's test. It can be inferred that in the majority of the analyses for centesimal composition, the species *A. hybridus* and *A. viridis* presented higher levels than the *A. retroflexus* (commercial cultivar). *A. hybridus* has the highest amount of N, Mg, B, Mn and Fe, *A. retroflexus* of P, K, S, Cu and Zn whereas *A. viridis* of Ca and Cu. The content of starch in the grains of the species ranged from 32.86 to 36.21%. Regarding the anti-nutritional constituents, the nitrate content present in these three species does not pose a health risk if consumed moderately. Invasive species *A. hybridus* and *A. viridis* present great potential for grain production, whose nutritional properties of flour in most of the analyses performed in this study were superior to the commercial species.**

Key words: *Amaranthus hybridus*, *Amaranthus retroflexus*, *Amaranthus viridis,* organic agriculture, unconventional vegetables.

INTRODUCTION

Amaranthus sp. are relevant in the agricultural environment as invasive species in a majority of crops, being found mainly on soils with good fertility and high content of organic matter, causing damages in several crops. Although considered a weed, studies prove the nutritional and functional versatility of *Amaranthus* sp.

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

(Kinupp and Lorenzi, 2014).

The genus *Amaranthus* is composed of approximately 70 species, among them there is an enormous morphological variety, with annual or short-lived perennial species. The species already cultivated, are used for the production of grains, leaves and for ornamentation of gardens. The most commonly cultivated species for grain production in the Americas and Asia are: *Amaranthus caudatus*, *A. hypochondriacus*, and *A. cruentus*. The grains of these species are rich in nutrients and provide a complete diet of amino acids (Singh, 2017).

Species from this genus are commonly classified as “pseudocereals" and are examples of so-called under-utilised crops that have evolved after centuries of selection and are currently among the 36 most promising crops to feed humans. They contain significant amounts of minerals, nutrients, vitamins and amino acids, besides antidiabetic and antioxidant activities, containing high levels of iron, selenium, phosphorus and low levels of toxic substances (Amaya-Farfán et al., 2005; Ferreira et al., 2007).

Preliminary studies performed in Brazil, e.g. by Samartini (2015), when carrying out the bromatological characterisation and evaluating the antioxidant potential in the leaves of five *Amaranthus* species considered as invasive, with emphasis for *A. spinosus, A. deflexus* and *A. retroflexus* showed higher efficiency in free radical scavenging, thus proving the antioxidant activity of these species. The values obtained by these species can be equated with other crops, such as potato, cauliflower and tomato.

Although studies have already been performed verifying the economic and alimentary potential of the amaranth, few are conducted with such species that are considered as rustic and highly efficient in the production of seeds.

Therefore, scientific studies are important to understand how these species can contribute to the human diet and in the prevention of diseases. Bromatological and phytotechnical studies can encourage the amaranth cultivation on a larger scale and with quality, allowing their commercialisation and hence the consumption of these plants by the population, resulting in economic, social and environmental benefits. In view of the aforementioned, the objective of the present study was to characterize the grains of three *Amaranthus* species, aiming at the food and commercial potential of species considered as invasive non-cultivated.

MATERIALS AND METHODS

The evaluated materials were obtained from the germplasm collection of non-conventional vegetables (2015/2016 harvest). In this collection of germplasm, there are about sixty species of unconventional food plants, with propagation materials available for scientific research, seeking to rescue food species that may contribute to the nutritional enrichment of humanity eating habits.

The work began with seven species of amaranth, with different characteristics, remembering that amaranthus is not cultivated in Brazil. The materials went through a screening process and were previously selected in the field in function of their potential for grain production. Among the seven species, three were selected. Both species present interesting phenotypic characteristics, such as panicle size and high grain yield. Two species considered invasive and of common occurrence in Brazil and another one selected as standard of comparison, being acquired in the local food market as grain (imported from another country), representing a commercial cultivar. The others did not have these characteristics, presenting no commercial interest, such as the presence of spines, small panicles, difficulty in separating the straw from the grain, low rusticity and low productivity.

The materials were identified through exsiccates by EPAMIG (Agricultural research agency of the state of Minas Gerais), being recorded and included in the Epamig Herbarium of Minas Gerais (PAMG) herbarium collection. The records of the *Amaranthus* species are 57999, 58002 and 58003, which refer to the species *A. retroflexus* L., *A. viridis* L. and *A. hybridus* L., respectively. The *A. retroflexus* L. species is the commercial cultivar and the other two are considered as invasive species.

After identification, seeds were sown for on-field evaluation of each species (*A. viridis, A. hybridus* and *A. retroflexus*) when subjected to the same cultivation method. The experiment was performed as a randomized block design (RDB) with three replicates, being each replicate as one block. Each plot had 78 plants with the purpose of selecting for breeding, besides quantifying the yield of each species through a sample within the plot, composing three useful plants per plot. The edge effect was considered in order to prevent the influence of neighbouring plots. Moreover, a physical barrier was also used with maize culture in order to avoid crossing between species, since polyploidy with interspecific hybridizations is common in these species, masking its characteristics (Olusanya, 2017).

The experiment was carried out in two agricultural years, sown in October 2016/2017 and harvested in April 2017/2018 in the experimental area in Lavras, south of the State of Minas Gerais, Brazil, located at 21° 14’ S, 45° 00' W and 918.8 m altitude. The climate of the region is Cwa (mesothermal) with dry winter and rainy summer, according to Köppen classification (Brasil, 1992). The seeds were sown directly in the field in shallow pits, and then thinned in a spacing of 0.5 m x 0.5 m, with a density of 40,000 plants per hectare, without using irrigation.

By means of the soil analysis, the soil corrections were chosen, opting for the organic management of plants, avoiding influence in their bromatological characteristics. A total of 1 t of dolomitic limestone ha^{-1}, 15 t of poultry manure ha^{-1} (before sowing) and 15 t of compound ha^{-1} (after 40 days of sowing). The phytosanitary management was performed from biofertilisers and plant extracts of *Ricinus communis* L. with insecticidal and fungicidal principles.

The grains were harvested at 74, 78 and 137 days after planting in *A. viridis*, *A. hybridus* and *A. retroflexus*, respectively, being harvested manually every 15 days, and the final harvest at 149, 108 and 167 days after planting, accounting for the total yield of each *Amaranthus* species.

The samples were sent to the laboratory and consisted of a grain mixture of each species, making a composite sample. The grains were milled up to the flour point. In the laboratory analyses, the experimental design was completely randomized (CRD), with three replicates for each treatment. The percent composition of the flour was performed: Moisture by the gravimetric method, based on the weight loss of the material subjected to the oven heating at 65ºC until constant weight. The ether extract was determined using the continuous extraction method in Soxhlet apparatus using diethyl ether as solvent. The fixed mineral residue (ash) was determined by

Table 1. Grain yield analysis of three *Amaranthus* species.

Species	First year yield (t ha^{-1})	Second year yield (t ha^{-1})	Standard deviation
A. viridis	1.96^{b}	1.96^{b}	±0.00
A. hybridus	2.56^{a}	2.61^{a}	±1.85
A. retroflexus	0.13^{c}	0.26^{c}	±0.18
CV (%)	30.22	17.55	

Averages followed by the same letter on the column do not differ significantly among themselves by Tukey test (P<0.05).

Table 2. Percent composition of grain flours of different *Amaranthus* species.

Percent composition	*A. retroflexus*	*A. hybridus*	*A. viridis*	CV (%)
Moisture (%)	12.30^{c}	13.34^{b}	14.23^{a}	1.31
Ether extract (%)	5.58^{b}	6.46^{a}	5.58^{b}	4.45
Ash (%)	2.51^{b}	2.79^{b}	3.21^{a}	4.42
Protein (%)	12.79^{a}	13.75^{a}	12.60^{a}	7.47
Crude fibre (%)	2.05^{b}	3.32^{a}	2.45^{b}	9.91
Non-nitrogenous extract (%)	65.85^{a}	61.22^{b}	61.65^{b}	2.21

Averages followed by the same letter on the row do not differ significantly among themselves by Tukey test (P<0.05).

calcination of the sample in muffle at 550°C until clear ash was obtained. The crude protein value was obtained by the Kjeldahl method by determining the nitrogen of food and multiplied by 6.25. The fiber fraction was determined according to the gravimetric method, after digestion in acidic medium (HOROWITZ, 2016), and the carbohydrate fraction was obtained by 100% difference of the sum of the other components, according to the equation: CHF = 100 - (M + EE + P + CF + A), where: CHF is carbohydrate fraction; M is moisture; EE is ether extract; P is protein; CFis crude fiber and A is ash. For analysis of macro and micronutrients, the samples were subjected to the method of analysis of vegetal tissues for fertility evaluation, through wet digestion (Malavolta et al., 1997), determining the percentage of macro and micronutrients.

The starch content of amaranth grain flour was identified by washing through sugar removal, autoclaving, neutralization, deproteinisation and determination by spectrophotometer reading at 510 nm, following the standards of the Instituto Adolfo Lutz (2008).

The anti-nutritional analysis of nitrate was done according to the methodology of Cataldo et al. (1975). The nitrate calculation was made by comparing the results from the standard curve, being expressed in mg NO_3^- kg^{-1} dry sample. The standard curve used in this study was represented by the equation y = 0.0054x + 0.0212 and R^2 = 0.9945.

The results were submitted to analysis of variance and the averages were compared by Tukey test (P<0.05). The experimental accuracy was analysed using the coefficient of variation (CV), and the statistical analysis was performed using the SISVAR® software (Ferreira, 2011).

RESULTS

According to the analysis of variance (Table 1), there was a significant difference for yield among the *Amaranthus* species in the two years of cultivation.

On average, *A. hybridus* showed higher yield in the field, being 92.45% higher than the commercial cultivar. *A. viridis* obtained a yield of 90.05% greater than this same species. *A. retroflexus* (commercial) had the lowest average yield, with 0.195 t ha^{-1}.

The species had a very uneven behaviour in the field when dealing with invasive species (*A. viridis* and *A. hybridus*), which is well understood, since these plants did not undergo a genetic improvement process and were not subjected to crops for grain yield. However, the commercial species had a good uniformity in relation to the emergence of plants and a rapid growth in the field.

Data on the grain composition of *Amaranthus* are presented in Table 2.

A. viridis showed the highest moisture percentage in the grain flour (14.23%), followed by *A. hybridus* (13.34%) and *A. retroflexus* with the lowest percentage (12.30%).

For the ether extract contents, *A. hybridus* obtained the highest value (6.46%), and the other species did not differ statistically among themselves, with a value of 5.58% for both species.

In relation to ash percentage, *A. viridis* showed the highest value (3.21%) and the other two species did not differ statistically among themselves, with 2.79% for *A. hybridus* and 2.51% for *A. retroflexus*.

When evaluating the nutritional quality of macro and micronutrients of the amaranth grain flours, the following results were obtained (Tables 3 and 4).

The *A. retroflexus* had the highest macronutrient

Table 3. Macronutrient content in grain flour of three *Amaranthus* species.

Species	%N	%P	%K	%Ca	%Mg	%S
A. viridis	2.42[b]	0.42[c]	0.52[b]	0.49[a]	0.32[b]	0.14[b]
A. hybridus	2.48[a]	0.46[b]	0.45[c]	0.37[b]	0.35[a]	0.19[a]
A. retroflexus	2.42[b]	0.54[a]	0.76[a]	0.20[c]	0.31[b]	0.18[a]
CV (%)	0.41	2.11	1.73	2.83	3.06	5.88

Averages followed by the same letter on the column do not differ significantly among themselves by Tukey test (P<0.05).

Table 4. Micronutrient content in grain flour of three *Amaranthus* species.

Species	B (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)	Fe (ppm)
A. viridis	7.88[b]	2.77[a]	20.90[b]	26.75[c]	137.65[c]
A. hybridus	8.54[a]	1.91[b]	27.26[a]	29.71[b]	457.52[a]
A. retroflexus	6.56[c]	2.86[a]	15.92[c]	49.95[a]	339.97[b]
CV (%)	0.13	4.93	0.27	0.03	0.02

Averages followed by the same letter on the column do not differ significantly among themselves by Tukey test (P<0.05).

contents, such as phosphorus (0.54%), potassium (0.76%) and sulfur (0.18%). The *A. hybridus* showed the highest contents of nitrogen (2.48%), magnesium (0.35%) and sulfur (0.19%), not statistically differing from the sulfur content of *A. retroflexus*. The *A. viridis* was the species with the lowest overall macronutrient contents, obtaining only the highest calcium content (0.49%) among the three species. Although it is the species with the highest ash content in the percent composition (Table 2), there are other minerals not quantified in this study that influence the total ash content.

The species *A. hybridus* showed the highest micronutrient contents boron (8.54 ppm), manganese (27.26 ppm) and iron (457.52 ppm). The *A. retroflexus* species had the highest contents of copper (2.86 ppm) and zinc (49.95 ppm). For *A. viridis* species, the highest micronutrient content in relation to the other species was copper (2.77 ppm), which did not differ statistically from *A. retroflexus*. Protein contents in amaranth grains did not differ statistically (Table 2).

The *A. hybridrus* had the highest crude fiber content (3.32%), and the other two species did not differ statistically from each other, with 2.45% for *A. viridis* and 2.05% for *A. retroflexus* (Table 2).

The *A. retroflexus* (commercial cultivar) grains have white color as major characteristic, but the other used species are black colored and are recognized as invasive plants in Brazil. It can be suggested that in the majority of the analyses, the species *A. hybridus* and *A. viridis* showed higher contents and dark colored seeds. Therefore, the seed color does not affect the bromatological characteristics of the species.

In relation to the starch content obtained from the spices under study, the following results were obtained (Table 5).

Table 5. Analysis of the starch content in grain flour of three *Amaranthus* species.

Species	% starch
A. viridis	35.72[a]
A. hybridus	32.86[b]
A. retroflexus	36.21[a]
CV (%)	1.77

Averages followed by the same letter on the column do not differ significantly among themselves by Tukey test.

The flour of the species *A. retroflexus* and *A. viridis* showed a higher starch percentage, 36.21% and 35.72%, respectively. The *A. hybridus* contains the lowest starch percentage, 32.86%. However, the *A. hybridus* obtained a grain yield higher than the other two species, being this practical factor very important for large-scale cultivation with the intention to obtain starch.

In relation to the antinutritional nitrate constituent, this study presented the following results for the three amaranth species (Table 6).

The *A. hybridus* was the species with the highest nitrate value among the species (1597.51 mg NO_3^- kg^{-1}), followed by *A. retroflexus* (1452.29 mg NO_3^- kg^{-1}) and *A viridis* (1093.32 mg NO_3^- kg^{-1}).

DISCUSSION

Although the crop management was similar, there was a

Table 6. Analysis of nitrate in the grain flour of three *Amaranthus* species.

Species	mg NO_3^-/kg
A. viridis	1093.32[b]
A. hybridus	1597.51[a]
A. retroflexus	1452.29[ab]
CV (%)	14.54

Averages followed by the same letter on the column do not differ significantly among themselves by Tukey test (P<0.05).

great difference between the species. The *A. retroflexus* underwent great injuries due to the environment, suffering several pest attacks, which certainly hindered its development, showing less adaptability and rusticity for cultivation in the southern Minas Gerais, in Brazil. A larva with a curculioniform shape from the Coleoptera family was observed in all blocks. This pest was difficult to identify because the symptoms resembled to a nutritional deficiency, precluding the control action. In contrast, the other species did not show a single specimen of this larva. The *Diabrotica speciosa* and the *Epicauta atomaria* were common to all species, mainly affecting the leaves, but were not difficult to control.

The amaranth species show very interesting plant characteristics such as rustic plants and resistant to water stress, developing in environments unfavourable to other cereals and legumes. Moreover, it contains great capacity of using water, light and nutrients, mainly due to its deep root system that assures its survival in dry periods. They have the capacity to develop and fruit in environments at high temperatures (35 to 45°C). Species from this genus are also characterized by their wide climatic adaptation. It has fast and vigorous growth, showing high biomass production capacity (Spehar and Trecenti, 2011).

Despite the few studies performed in Brazil, agronomic tests carried out in the northeastern Brazil indicate that it is possible to obtain, in sites without water restriction, average yields of 3 and 1 t ha^{-1} under low humidity conditions (Amaya-Farfan et al., 2005).

Another study performed in Slovenia using the cultivar "G6" (*A. cruentus*) discusses that the amaranth grain yield depends on the environment, weather conditions, species, genotypes and production techniques, thus widely varying from 500 to 2,000 kg of grains ha-1. With suitable varieties and production techniques, yields from 1,500 to 3,000 kg of grains ha^{-1} can be expected. In Europe, there are reports of grain yields between 2,000 and 3,800 kg ha^{-1} (MLAKAR et al., 2010).

Despite the scarcity of scientific knowledge regarding the yield of the species used in this study (Table 1), the yields were similar to those found in the literature for other widely cultivated species. It is noteworthy that, in the present study, the plants were cultivated in the organic system and in the dry farming, without genetic improvement of the species, indicating a good grain yield in relation to the other studies.

In percent composition of grain flours, the lower moisture percentage in the flour represents a trend towards better food conservation. According to the Brazilian legislation that determines that flours, cereal starch and bran should have a maximum moisture content of 15.0 g 100 g^{-1} (Brasil, 2005), thus, the three studied species meet the requirements of this legislation (Table 2).

A study performed by Marcílio et al. (2003) obtained moisture of 9.2% of *A. cruentus*, which is lower than that found in this study, which can be caused by being from different species. The increase in moisture allows a greater interaction between grain sub-structures (germ, bark, fiber and starchy endosperm).

Fujita and Figueroa (2003) evaluated the ether extract content present in cereals and derivatives, such as oat, wheat, triticale and barley showed average values of 5.27, 1.89, 2.07 and 2.26%, respectively. The amounts of oils and fats found in the amaranth composition were higher than the cereals of wheat, triticale and barley. Therefore, the intake period for this food will be lower when in relation to the other cereals, due to the rancidification process. However, these values indicate a higher amaranth potential for oil production.

The oil content in this grain ranges from 6 to 10%, from which 76% are unsaturated, containing interesting linoleic acid contents. They show 7% esquilant, which is superior to that from other vegetables. Another important factor is the presence of tocotrienols, antioxidant substances that resemble vitamin E, which reduce the LDL cholesterol content, have anticancer activities, protect against skin aging and prevent the onset of cardiac and obstructive diseases (Costa and Borges, 2005). When comparing the amaranth oil content with the maize, it is possible to observe lower values in the maize kernels. Studies show values that range according to the cultivar genetics, and Cazares-Sanchez (2015) evaluated oil content in a collection of Mexican maize populations and found values between 3.37 and 4.52%.

Generally, the mineral fraction in ash is composed of macro and micronutrients in large part of their constitution (HOROWITZ, 2016), and these are fundamental for the maintenance of the proper functioning of organism. The qualification and quantification of the minerals present in these amaranth species is suggested by the research because the availability of each mineral can be evaluated (Tables 3 and 4). Recommendations for mineral intake vary according to the demand of each organism, and each element is required at different quantities.

In the literature, different results regarding ash content among some amaranth species, such as 2.28% (± 0.5) in the *A. cruentus* L. flour (Capriles et al., 2006), 2.2 and

1.7% (± 0.1) in the whole and refined flour of the *A. cruentus* L. grain, respectively (Marcílio, 2003). The ash contents found in the flours were consistent with this study, being superior to the studied species (Table 2).

Buratto (2012) studied contents of minerals and proteins in common bean grains and presented values from 8.9 to 161.50 ppm iron and from 11.5 to 69.9 ppm zinc in the grains. These values are closely related to the cultivar genotype. The deficiency of these nutrients in the human diet is considered as challenging, affecting the health of thousands of people worldwide. The values found in the literature for iron are much lower than that found in the three amaranth species under study (Table 4), whereas for zinc, the contents were similar to that of beans.

A study quantified the nutrients present in 100 g of *Amaranthus* grain, comparing with the wheat grain, demonstrating the nutritional superiority of amaranths. Regarding the nutrients found, the potassium content was 101% higher than wheat, with 0.366 g per 100 g of the grain; the calcium content was 528%, with 0.153 g per 100 g of the grain; the phosphorus content was 158%, with 0.455 g per 100 g of the grain; the magnesium content was 211%, with 0.266 g per 100 g of the grain; the iron content was 238%, with 7.59 mg 100 g^{-1} of the grain; the zinc content was 120%, with 3.18 mg 100 g^{-1} of the grain; and copper was 179%, with 0.777 mg 100 g^{-1} of the grain (Costa and Borges, 2005).

Although amaranth grain flour is an important source of nutrients, it is worth mentioning that the concentration of a certain nutrient in food is not necessarily a reliable indicator of the value to be absorbed by the organism. Studies are needed to understand the bioavailability of these nutrients, thus quantifying the nutrient portion that will be available for use by the body in metabolic processes (Chitarra and Chitarra, 2005).

One of the most interesting nutritional properties of *Amaranthus* is the content of biological quality proteins in its grains (approximately 15%), having lysine (representing 5% protein) and other essential amino acids (being 4.4% sulfur-containing amino acids). Another study shows that amaranth has between 12 and 17% proteins, containing well-balanced amino acids, a characteristic not found in other cereals, including a large amount of lysine, ranging between 0.73 and 0.84% of the total protein content. This species also has carbohydrates, fats and minerals. These characteristics are the most limiting factors from the other cultivated grains. Studies show that amaranth has values of proteins, fats and fibers higher than cereals such as wheat, maize, rice and oats (Amaya-Farfan et al., 2005).

Research on cereals show different crude fiber contents for white oats (8.88%), common black oats (8.76%), rye (3.34%), barley (3.89%), triticale (2.50%) and wheat (2.19%) (Guarienti et al., 2001). Based on these data, it can be said that the amaranth has similar contents to other cereals, such as rye, barley, triticale and wheat in relation to the crude fiber percentage present in its food composition (Table 2).

For the non-nitrogenous extract (NNE), the largest value was found in *A. retroflexus* (65.85%), but *A. hybridus* (61.22%) and *A. viridis* (61.65%) did not differ statically (Table 2). The Brazilian food composition table (TACO) (Lima, 2012) does not contemplate this analysis; however, it can be calculated through the difference between carbohydrates and dietary fiber. It is known that NNE constitutes the carbohydrate portion of the food, that is, it provides energy readily available to humans.

Zhu (2017) performed a review on the starch characteristics from different amaranth species and reached the following result; the starch yield, the contents of amylose, total lipid and protein of amaranth starches from different studies in the last five decades were reported to be between 2.0 and 65.2%, 0.0 and 34.3%, 0 and 1.8% and 0.02 and 0.98%, respectively. The species with the highest starch yields are *A. hypochondriacus*, *A. cruentus* and *A. hypochondriacus* x *A. hybridus*.

When a study quantified the starch content of quinoa, amaranth (*A. caudatus*) and wheat, found values between 66.3 and 68.1%, and did not differ statistically among themselves (Srichuwong et al., 2017). Pilat et al. (2016) evaluated the starch content in *A. cruentus* and found the value of 55.53%. The values of starch content found in the literature were higher than in this study (Table 5), this may be an effect of the different species studied and the environmental conditions in which the plants were submitted.

Costa and Borges (2005) concluded that due to the nutritional characteristics of the amaranth, it is possible to substitute other cereals without causing any food deficiency, whether this grain be adopted as basic morning food, through flours, cakes, pancakes etc. Moreover, amaranth can be consumed by people who have allergy to gluten and with high cholesterol rates. The grains of this plant represent a more balanced and energetic diet than other grains, such as maize, wheat or rice, being nutritionally comparable to milk, meat and egg. Due to its agronomical, nutritional and medicinal characteristics, amaranth can minimize food deficiencies in poor or developing regions of the world.

The accumulation of nitrates in raw vegetables, herbs and fruits has a wide range of accumulation. There are a number of factors that influence nitrate accumulation as: Plant species and their genotypes, agronomic factors, environmental conditions prevailing during plant growth (such as light intensity, spectral quality, photoperiod, air temperature and concentration of carbon dioxide), harvesting phase, as well as harvesting time during the day. In addition, post-harvest factors in particular, storage conditions may also cause or inhibit the conversion of nitrates into nitrites (Colla et al., 2018).

Human exposure to nitrate is mainly exogenous, due to

the consumption of raw vegetables (80%). Nitrate is relatively harmless since the fatal dose for adults is considered to be greater than 7.35 g, which is about 100 times higher than the acceptable daily dose of NO_3 as defined by the European Union (3.7 mg/kg body weight per day), equivalent to 222 mg NO_3 per day for individual 60 kg. The EU regulatory commission has set maximum nitrate values for fresh vegetables such as spinach, lettuce and rucula to be between 2,000 and 7,000 mg of NO_3 kg^{-1} fresh matter (Colla et al., 2018). These values are much higher than those found in the grains of the three amaranthus species in this study; these grains were consumed moderately and did not represent an anti-nutritional risk for humans. It should be emphasized that nitrogenous chemical fertilizers can alter the nitrate content of grains from these plants (Colla et al., 2018), and further studies are necessary to establish the food safety of these foods in the conventional system of cultivation.

A diet with nitrate is mainly obtained by the intake of vegetables. Older literature shows nitrate in the diet as a contaminant associated with increased risks of stomach cancer and methemoglobinemia. Consequently, nitrate levels for human intake have always sought to be restricted, being the exposure levels of an acceptable daily intake is 3 to 7 mg kg^{-1}. The average intake of nitrates in the UK is approximately 70 mg/day, although some population groups such as vegetarians can consume three times this value. When assessed clinically, they did not present health-related problems with nitrate (Ashworth and Bescos, 2017).

More recent studies suggest that dietary nitrate can significantly reduce blood pressure and may reduce the incidence of hypertension and mortality from stroke. There is a lack of data demonstrating the actual chronic effect from high nitrate intake in humans. However, due to potential health benefits, some authors recommend that nitrate be considered as a necessary nutrient for health rather than as a contaminant that needs to be restricted. Although nitrate toxicity is low, the oral lethal dose of nitrate to humans has been reported at about 330 mg/kg per day (equivalent to about 23,100 mg for an adult of 70 kg day^{-1}). However, further studies are necessary for the actual understanding of nitrate in the human body (Ashworth and Bescos, 2017).

As final considerations, amaranth plants considered as invasive have a high nutritional and productive potential in relation to other cereals commonly consumed by Brazilians. The present study aimed to demonstrate the possibility of using amaranth as an alternative source for human or even animal feeding.

Borneo and Aguirre (2008) reported that the potential of *Amaranthus* species has been rediscovered along the years, and since then, several studies have been performed emphasizing high protein quality, the presence of unsaturated oil and other valuable components, besides several uses, including high quality roasts, edible films, functional ingredients, among others.

Based on the obtained results, it can be suggested that in the majority of the analyses related to the percent composition, the species *A. hybridus* and *A. viridis* showed contents higher than the commercial cultivar (Table 2).

It was possible to observe that the species showed different amounts of nutrients. The *A. hybridus* has the highest amount of N, Mg, B, Mn and Fe, the *A. retroflexus* of P, K, S, Cu and Zn, and *A. viridis* of Ca and Cu (Tables 3 and 4). This result indicates a food potential of the species considered as weeds, obtaining results as interesting as the commercial cultivar *A. retroflexus*.

Regarding the starch content found in the three species (Table 5), these values were lower than that found in the literature, and this difference could be correlated with the species under study and the environmental conditions to which they were subjected.

In relation to the anti-nutritional constituents, the nitrate content present in these species did not represent a health risk when consumed moderately for the three studied species (Table 6).

Conclusion

The invasive species *A. hybridus* and *A. viridis* showed great potential for grain yield; nutritional properties of flour in most of the analyses performed in this study were superior to the commercial species (*A. retroflexus*).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ashworth A, Bescos R (2017). Dietary nitrate and blood pressure: evolution of a new nutrient? Nutrition Research Reviews 30:1-12. https://doi.org/10.1017/S0954422417000063

Amaya-Farfan J, Marcílio R, Spehar CR (2005). Deveria o Brasil investir em novos grãos para a sua alimentação? A proposta do amaranto (Amaranthus sp.). Segurança alimentar e nutricional 12:47-56. https://doi.org/10.20396/san.v12i1.1838

Brasil (1992). Ministério da Agricultura e da Reforma Agrária. Departamento Nacional de Meteorologia. *Normais climatológicas: 1961-1990*. Brasília, DF. 465 p.

Brasil (2005). Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução RDC nº 263, de 22 de setembro de 2005. *Regulamento Técnico para produtos de cereais, amidos, farinhas e farelos*. Diário Oficial [da] República Federativa do Brasil, Brasília 6 p.

Borneo R, Aguirre A (2008). Chemical composition, cooking quality, and consumer acceptance of pasta made with dried amaranth leaves flour. *LWT* - Food Science and Technology 41:1748-1751. https://doi.org/10.1016/j.lwt.2008.02.011

Buratto JS (2012). *Teores de minerais e proteínas em grãos de feijão e estimativas de parâmetros genéticos.* Dissertation; Universidade

Federal de Lavras. Brasil.
Capriles VD, Coelho KD, Matias ACG, Arêas JAG (2006). Effect of amaranth on nutritional value and sensory acceptability of cookie and sandwich bread. Alim. Nutr. 17:269-274.
Cataldo DA, Haroon M, Schrader LE, Youngs VL (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Communications in Soil Science and Plant Analysis 6:71-80. https://doi.org/10.1080/00103627509366547
Cazares-Sanchez E, Chavez-Servia JL, Salinas-Moreno Y, Castillo-Gonzalez F, Ramirez-Vallejo P (2015). Grain composition variation among native maize (*Zea mays* L.) of Yucatan, Mexico. In Agrociencia 49:15-30.
Chitarra MIF, Chitarra AB (2005). *Pós-colheita de frutas e hortaliças: fisiologia e manuseio*. 785p.
Colla G, Kim H, Kyriacou MC, Rouphael Y (2018). Nitrate in fruits and vegetables. Scientia Horticulturae 237:221-238. https://doi.org/10.1016/j.scienta.2018.04.016
Costa DMA, Borges AS (2005). Avaliação da produção agrícola do Amaranto (*Amaranthus hypochondriacus*). *Holos* 21:97-111.
Ferreira DF (2011). Sisvar: um sistema computacional de análise estatística. Ciência e Agrotecnologia 35:1039-1042.
Ferreira TAPC, Matias ACG, Arêas JAG (2007). Características nutricionais e funcionais do Amaranto (*Amaranthus* spp.). Nutrire: revista da Sociedade Brasileira Alimentação e Nutrição, 32:91-116.
Fujita AH, Figueroa MOR (2003). Composição centesimal e teor de beta-glucanas em cereais e derivados. Ciênc. Tecnol. Aliment 23:116-120.
Guarienti EM, Del Duca LJ, Fontaneli RS, Zanotto DL (2001). Composição química dos principais cereais de inverno do Brasil. Pesquisa Agropecuária Gaúcha 7:7-14.
Horowitz W (2016). Official methods of analysis of the Association of Official Analytical Chemists. 20th ed., 3rd rev. Gaithersburg, Maryland: AOAC 3100 p.
Instituto Adolfo Lutz (2008). *Métodos físico-químicos para análise de alimentos*. São Paulo 1020 p.
Kinupp VF, Lorenzi H (2014). Plantas alimentícias não convencionais (PANC) no Brasil: guia de identificação, aspectos nutricionais e receitas ilustradas. São Paulo: Instituto Plantarum de Estudos da Flora.
Lima DM (2012). Tabela brasileira de composição de alimentos-TACO. NEPAUNICAMP.
Malavolta E, Vitti GC, Oliveira SA (1997). Avaliação do estado nutricional das plantas. Princípios e aplicações. POTAFOS 2:319.
Marcílio R, Farfán JA, Ciacco CF, Spehar CR (2003). Fracionamento do grão de amaranto (*A. cruentus*) brasileiro e suas características composicionais. Ciência e Tecnologia de Alimentos 23:511-516.
Mlakar GS, Turinek M, Jakop M, Bavec M, Bavec F (2010). Grain amaranth as an alternative and perspective crop in temperate climate. Revija za geografijo - Journal for Geography 5(1):135-145.
Olusanya AC (2017). A multi-species assessment of genetic variability in Nigerian Amaranthus accessions: potential for improving intra-and interspecies hybridization breeding. Archives of Agronomy and Soil Science. http://dx.doi.org/10.1080/03650340.2017.1384817
Pilat B, Ogrodowska D, Zadernowski R (2016). Nutrient Content of Puffed Proso Millet (*Panicum miliaceum* L.) and Amaranth (*Amaranthus cruentus* L.) Grains. Czech Journal of Food Science 34(4):362-369.
Samartini CQ (2015). Conteúdo de DNA nuclear, número cromossômico e compostos de interesse nutricional em Amaranthus spp. *Master's thesis, Universidade Federal de Lavras. Lavras (MG).*
Singh AK (2017). Early History of Crop Introductions into India: II. *Amaranthus* (L.) spp. Asian Agri-History 21(4):319-324.
Spehar CR, Trecenti R (2011) Agronomic performance of traditional and innovative species for double and dry season copping in the brazilian savannah high lands. Bioscience Journal 27(1):102-111.
Srichuwong S, Curti D, Austin S, King R, Lamothe L, Gloria-Hernandez H (2017). Physicochemical properties and starch digestibility of whole grain sorghums, millet, quinoa and amaranth flours, as affected by starch and non-starch constituents. Food Chemistry 233:1-10. https://doi.org/10.1016/j.foodchem.2017.04.019
Zhu F (2017). Structure, Physicochemical Properties, and Applications of Amaranth Starch. Critical Reviews in Food Science and Nutrition 57:313-325. https://doi.org/10.1080/10408398.2013.862784

Vol. 13(22), pp. 1148-1153, 31 May, 2018
DOI: 10.5897/AJAR2018.13152
Article Number: 000227B57261
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Agricultural Research

Full Length Research Paper

Plant cover management and nitrogen fertilization in maize crop in a dystrophic red Latosol Brazilian Cerrado (Savannah)

José Antônio Maior Bono[1*], Bruna Kelle Della Colleta[1], Marlos Ferreira Dornas[1], Silvia Rahe Pereira[1], José Francisco dos Reis Neto[1,2] and Bianca Obes Corrêa[1]

[1]Agroindustrial Production and Management, Anhanguera-Uniderp University, Campo Grande, Mato Grosso Do Sul, Brazil.
[2]Environment and Regional Development, Anhanguera-Uniderp University, 79051-320, Campo Grande, Mato Grosso Do Sul, Brazil.

Received 28 March, 2018; Accepted 26 April, 2018

An experiment was conducted in the agricultural year 2014/2015, aiming to evaluate the management of anticipated nitrogen fertilization, applied with a slow-release source in maize crop with two plant covers, in a Dystrophic Red Latosol. Matching the Oxisols in Soil Taxonomy and Ferralsols FAO/United States WRB (Unesco soil classification and the World Reference Base for Soil Resources. *Pennisetum glaucus* (*Pennisetum glaucum* (L.) R. Br.) and *Raphanus sativus* (*Raphanus sativus* L.) were used as green cover, keeping a fallow area. In the areas with vegetal cover, N was applied anticipatedly, during planting and in top-dressing, while in the fallow area it was applied during the planting process and in top-dressing. The treatments were distributed according to a random block design with four replicates. The anticipated N application was made 38 days before planting and top-dressing application 27 days after planting. Leaves were collected for foliar analysis during the tasselling stage of the plants. The harvest and threshing were done manually, and the grains were weighed. Grain yield and leaf N contents were evaluated. The maize crop responded to nitrogen fertilization regardless of the cover used. Nitrogen fertilization using a slow-release N source can be managed in an anticipated stage, without damaging crop yield.

Key words: Soil, slow-release nitrogen, *Zea mays* L.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most cultivated and consumed grains in the world, it is grown on more than 10 million hectares, producing around 50 million tons (Conab, 2017). This culture has a high economic value,

*Corresponding author. E-mail: ayman.elsabagh@agr.kfs.edu.eg.

given its great importance in human and animal nutrition, serving as raw material especially in the pork and poultry production chain, which consumes approximately 70 to 80% of the Brazilian maize production (Duarte et al., 2010). Even though it represents a significant part of the grain harvest in Brazil, its productivity is still threatened by many factors, such as soil fertility, water availability, plant population, seed sowing periods, cultural practices and diseases, pests and weeds (Fancelli and Dourado, 2003).

Nitrogen (N) is an essential chemical element to plants in general, and maize has a high extractive capacity of this nutrient from the soil (Granato et al., 2014; Coelho and França, 2007). During fertilization, N has an important role due to its participation in several processes of the plant metabolism (Andrade et al., 2003). Its presence is crucial in the initial stage of development of the plant, a period in which the absorption is more intense (Basso and Ceretta, 2000). The N supply through nitrogen fertilizers has a high cost because of its low use efficiency, mostly due to the losses to the environment which are usually attributed to very soluble forms, that facilitates the transformations occurring in the soil (Cantarella and Duarte, 2007).

As the risks of crop loss or decrease in production yield in the second crop are relatively large, one of the dilemmas of this cultivation mode is to know which source to use and the amount of N to apply, since water deficiency changes the absorption and the metabolism of N in the plant (Ferreira et al., 2002), diminishing the applied fertilizer´s efficiency. The management of nitrogen fertilization can be difficult in practice, considering that nitrogen is very dynamic in the soil because of its transformation processes, which causes losses by volatilization, leaching and denitrification. In this sense, alternatives have been sought to improve its efficiency (Souza et al., 2001; Kluthcouski et al., 2006; Fernandes and Libardi, 2007).

Studies with slow-release nitrogen fertilizers (Setti et al., 2006) detected an alternative to reduce nitrogen losses. Since it is a protected product, the slow-released N provides a controlled release in the soil, allowing applications of higher doses during planting and even before that, which results in a greater flexibility in the use of N in production systems. Motta et al. (2015) have also verified that the use of stabilized sources with inhibiting polymers of enzyme urease, and the nitrification of ammonium, does not increase grain yield or the agronomic efficiency of N use, when compared to common urea and ammonium nitrate, irrespective of the dose of N applied in the cover. Studies carried out by Research Foundation of state Mato Grosso do Sul (Brasil) have shown good results in the anticipation of the recommended N dose in soils with low loss potential of N by leaching, which allows application of the N doses by instalments (Broch and Ranno, 2008).

The anticipation of N application in non-revolved soils and the cultivation of cover crops provide changes in nutrient cycling, with nitrogen being the most affected, mainly due to the slower decomposition of the vegetal residues left on the soil surface to influence the processes of immobilization, mineralization, leaching, volatilization and denitrification (Sá, 1996; Sallet et al., 1997; Cabezas et al., 2004). The quality of the vegetal residue, mainly its C/N ratio, and the availability of mineral N in the soil solution, can influence the decomposition rate (Ceretta et al., 2002) and the N utilization of these residues by the maize (Cabezas et al., 2004, Ernani et al., 2005).

Grasses have been frequently used as cover plants on cerrado conditions, with emphasis on *Pennisetum americanum*, due to their greater resistance to water deficit, higher biomass production and lower seed cost. In addition, high temperatures and high humidity during summer, result in a rapid decomposition of plant residues with low C/N ratio (Cabezas et al., 2004). Heinz et al. (2013) in their work with *Raphanus sativus,* have confirmed that 5,7 t ha^{-1} of dry matter is the adequate quantity for soil coverage in a no-till maize plantation. Pedrotti et al. (2015) verified that *Raphanus sativus* have influenced the productivity and leaf N content of the maize crop, and that they are related to the elevation of the nitrogen doses.

Therefore, the objective of this work was to evaluate the management of the anticipated nitrogen fertilization, with a slow-release source in a maize crop with two plant covers, in a Dystrophic Red Latosol.

MATERIALS AND METHODS

The trial was conducted in a field on the following geographical coordinates: latitude 19º30'52.71 "S, longitude 54º29'23.17" O. At an altitude of 670 meters, in the agricultural year 2014/15. The predominant climate in the region is Aw, according to Köppen classification, defined as tropical humid with wet summers and dry winters.

The average annual rainfall is 1700 mm and the average annual temperature 27 °C. The experimental area´s soil was classified as Dystrophic Red Latosol, matching the Oxisols in Soil Taxonomy. Physical and chemical characteristics, from the 0 to 20 cm layer, are shown in Table 1. The area had a record of summer soybean cultivation followed by maize from the second harvest. The coverages for the experiment were implemented in March 2014.

Soil preparation in the experimental area was done through subsoiling, followed by a levelling grader and base fertilization with 250 kg of NPK, using the formulated 10-15-15, applied in the groove, and 54 kg P_2O_5 (Single superphosphate) and 90 kg K_2O (potassium chloride) applied before planting. The experimental treatments consisted of two plant coverages (*P. glaucus and R. sativus*) and an uncovered area considered as fallow. Nitrogen fertilization was managed anticipatedly before planting, during planting and as top-dressing, as it can be observed in Table 2.

The experimental design consisted of completely randomized blocks, with 9 treatments and 4 replicates, and each experimental plot consisted of 10 spaced lines of 0.5m with 5m length. The coverages of *P. glaucus* and *R. sativus* were seeded on September 10th, 2014, accounting for 15 kg ha^{-1} each. The anticipated nitrogen fertilization in treatments with *P. glaucus* and *R. sativus* were

Table 1. Results of the chemical and physical soil analysis of the experimental area, from the 0 to 0.2 m layer, according to Embrapa (2011).

Chemical analysis									Physical analysis		
pH		P	K^+	Ca^{++}	Mg^{++}	Al^{3+}	$H^{++}Al^{3+}$	MO	Clay	Silt	Sand total
H_20	$CaCl_2$	mg dm^{-3}		cmolc dm^{-3}				g dm^{-3}	g kg^{-1}		
6.41	5.60	20	76	3.10	0.60	0.0	2.97	18.4	376	238	386

Table 2. Treatment description for the experiment with nitrogen fertilization management with two vegetation covers, in a Dystrophic Red Latosol, in the region of São Gabriel do Oeste - MS.

Vegetation cover	N dosage (kg ha^{-1})		
	Anticipated	Planting	Top-dressing
Pennisetum glaucum	0	0	0
	30	80	70
	30	60	90
Fallow area	0	0	0
	0	90	90
	0	30	150
Raphanus sativus	0	0	0
	30	80	70
	30	60	90

carried out manually on October 10th, 2014, 30 days after the coverage sowing, in the quantities that were mentioned in Table 2. On November 3rd, 2009, the coverage of *P. glaucus and R. sativus*, as well as the fallow area, were desiccated with glyphosate.

The sowing of maize was performed with a seeder, spaced 0.6 m, on November 17th, 2014, with a density of 3 to 4 seeds per linear meter, with a target population of 60,000 plants ha^{-1}. The maize cultivar that was used, is the hybrid Simple Modified 2B604 (Dow AgroSciences). At this stage, the nitrogen fertilization was applied in the planting process. Top-dressing was manually done on December 14th, when maize had 4 to 5 fully expanded leaf pairs.

The productivity and leaf sample evaluations were based on the two centerline portions of 5 m long. Leaf sampling was according to Malavolta (2006). After the leaf samples were gathered, they were stored in paper bags and dried at 65°C in a greenhouse with forced air system for 48 hours. Afterwards, they were shredded, and grinded in a Wille mill, and submitted to sulfuric and distilled digestion in order to obtain the N content by the Kjeldahl method, according to Embrapa (2011).

For the productivity evaluation, the spikes of all the plants on the two central lines of the experimental plot were collected manually on February 26th, 2015, and later destrawed. Afterwards, the grains were also manually threshed. The plants harvested from the two central lines were counted, in order to correct the productivity stand of 60,000 ha^{-1} plants. In order to determine the moisture content, 100 grams of the threshed grains were used, which were oven dried at 105°C.

The temperature and precipitation data were obtained from Cemtec, whose metrological station is 5 km away from the experimental area (Figure 1). The data from leaf N content and grain yield were submitted to variance analysis, followed by a mean test, using the SAS statistical program in PROC GLM procedure.

RESULTS AND DISCUSSION

Table 3 shows the results of average square and value of variance significance, for grain yield and leaf N content. There was a significant outcome for both grain production and leaf N, for grain yield 1% and foliar contents 5%. The coefficient of variation (CV) for this experiment was 7.65 and 13.09% respectively for grain yield and leaf N. Researchers often use the coefficient of variation in order to estimate the precision of the experiments. The coefficient expresses the standard deviation as a percentage of the mean (Clemente and Nuniz, 2002).

In practice, the lower the CV the more homogeneous is the data of the variable. Gomes (2000) considers a CV as low, when it is lower than 10%, average when it is in between 10 a 20%, and high when between 20 and 30%. Therefore, the coefficient of variation for grain yield in this experiment is considered low and leaf N as medium. The average levels of foliar N, for treatments that received nitrogen fertilization, were in average 27.43 g kg^{-1}, whereas the treatments without nitrogen application remained with 24.01 g kg^{-1}. This shows that the maize crop responded to nitrogen fertilization (Table 4).

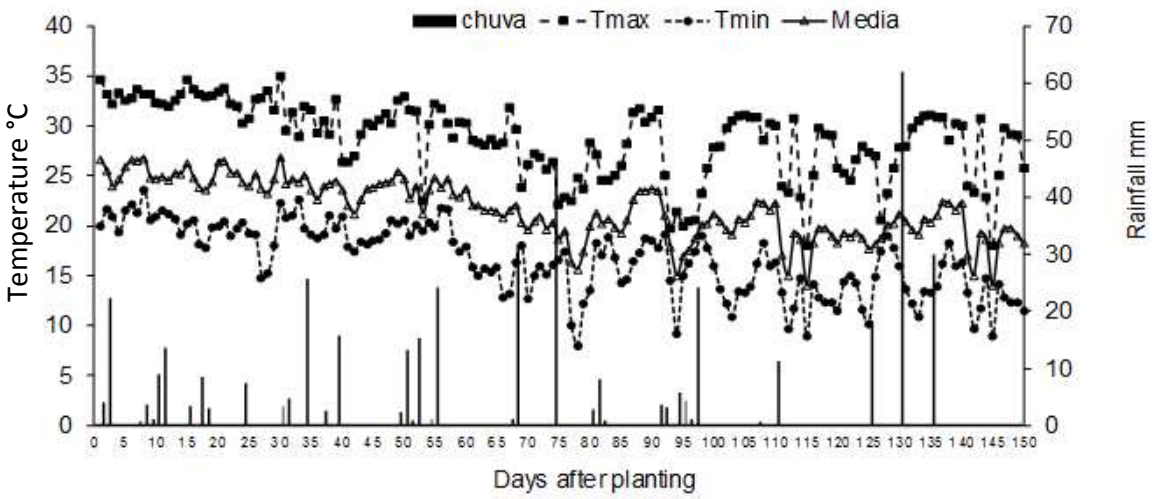

Figure 1. Average, maximum and minimum temperatures and rainfall precipitation during the experimental period. Source: Cemtec (2015).

Table 3. Results of the average square and statistical value of F for the variance analysis of the production of maize grains and foliar contents, in a Dystrophic Red Latosol, in the region of São Gabriel do Oeste - MS.

Sources of variation	GL	Average square		F	
		Production	Foliar N	Production	Foliar N
Treatments	8	2492221.01	39.18775	9.78**	3.09*
Repetition	3	1150686.62	0.915393	4.52*	0.07^{ns}
Residues	24	254786.59	12.697443	-	-
Coef. of variation (%)		7.65	13.09	-	-

** significant 1%; * significant 5% and ns = not significant.

Table 4. Average values of foliar nitrogen in maize crop with different vegetation covers and N fertilization management in a Dystrophic Red Latosol.

Cover	N dosage (kg ha^{-1})			N (g kg^{-1})	
	Anticipated	Planting	Top-dressing		
Pennisetum glaucum	0	0	0	22.08	B
Rafhanus sativus	0	0	0	22.93	AB
Fallow area	0	0	0	27.02	AB
Average				24.01	
Pennisetum glaucum	30	60	90	30.24	AB
Rafhanus sativus	30	60	90	30.66	A
Average				30.45	
Pennisetum glaucum	30	80	70	30.42	AB
Rafhanus sativus	30	80	70	27.20	AB
Average				28.81	
Fallow area	0	90	90	26.18	AB
Fallow area	0	30	150	28.35	AB
Average	-	-	-	27.27	-

Values followed by the same letter do not differ by Tukey test at 5% probability.

Table 5. Average values of maize grain yield in different vegetation covers, and N fertilization management, in a Dystrophic Red Latosol in the region of São Gabriel do Oeste - MS.

Cover	N dosage (kg ha^{-1})			Grain yield (kg ha^{-1})	
	Anticipated	Planting	Top-dressing		
Pennisetum glaucus	0	0	0	5622.5	BC
Raphanus sativus	0	0	0	5552.1	C
Fallow	0	0	0	5685.0	BC
Average	-	-	-	**5619.9**	
Pennisetum glaucus	30	60	90	6649.0	ABC
Raphanus sativus	30	60	90	7436.7	A
Average	-	-	-	**7042.9**	
Pennisetum glaucus	30	80	70	7487.2	A
Raphanus sativus	30	80	70	7286.6	A
Average				**7386.9**	
Fallow	0	90	90	6915.1	A
Fallow	0	30	150	6782.1	AB
Average	**-**	**-**	**-**	**6848.6**	**-**

Values followed by the same letter do not differ by Tukey test at 5% probability.

Malavolta (2006) suggests that foliar N values of 28 to 35 g kg^{-1} are critical, and Fontes (2001) defends that 27.5 g kg^{-1} is a critical value. According to Roscoe and Gitti (2013) these values are adequate. Therefore, we can assess that the average values of the treatments, without nitrogen fertilization, were below the critical level. For the other treatments, we can consider that the values were at the critical level or very close to it.

The treatments with *P. glaucus and R. sativus*, that received nitrogen fertilization in advance (30kg of N), showed no significant differences (p> 0.05) because of its plant coverage, even when compared to the fallow area, in which there was no previous fertilization treatment (Table 4). Although statistically similar, the leaf N contents are higher when a slow-release nitrogen fertilization is performed. A possible cause for the lack of response of foliar N between the cover and fallow areas, is that the dilution or concentration factors may have interfered in the foliar N content values, that is, the nutrient content dilutes as plants grow (Faroni et al., 2009).

As the soil in the fallow treatment was not stirred and the desiccation of the planted crop lasted 54 days, invasive plants grew in the area, which might have caused the absorption of N from the organic matter mineralization. Another fact that could have explained this non-response, would be the N supply by organic matter mineralization in the soil. Souza et al. (2002) consider that every 10 g of O. M./dm^{-3} can provide about 20 kg ha^{-1} of N to plants. In this case, taking into account 18.4 g dm^{-3} of organic matter, the soil would be able to provide 36.8 kg ha^{-1} of N, a greater quantity than what was applied in advance.

The treatments that received nitrogen in advance, during planting and top-dressing, presented an average productivity of 7093 kg ha^{-1}, while the treatments without nitrogen fertilization had an average of 5620 kg ha^{-1} (Table 5). This confirms the response of nitrogen fertilization nitrogen for grain yield, as well as foliar N. The treatments which received anticipated slow-released nitrogen fertilization, showed an average grain yield of 7214.86 kg ha^{-1}, whereas treatments that did not receive an anticipated nitrogen fertilization had an average of 6848.6 kg ha^{-1} (Table 5). It indicates that the maize crop positively responded to the anticipated fertilization. Sá et al. (2007) while working in the state of Paraná in 3 localities, also found the early fertilization of the maize crop effective.

The treatments with *P. glaucus* and *R. sativus* coverage that received the anticipated nitrogen fertilization (30 kg ha^{-1} N), did not show any significant difference because of the plant coverage (Table 5). In these treatments, those that received 80 kg ha^{-1} of N during planting, presented a slightly higher average than the treatments of 60 kg ha^{-1} of N. This was also observed in the fallow treatment, where the 90 kg ha^{-1} dose of N during planting presented higher values than the dose of 30 kg ha^{-1} of N. Borges et al (2015) on a study with *P. americanum* with densities 10, 15 and 20 kg of seed ha^{-1}, proved it to be a good coverage option for maize crop, regardless of the N dose for top-dressing, giving academic support for these data.

It can be affirmed that an anticipated nitrogen fertilization of 30 kg ha^{-1} on green cover, plus 80 kg ha^{-1}

at planting in the form of slow-release N, and 90 kg ha^{-1} at planting in the fallow area, which corresponds to 50% and 61.6% respectively of the total of the 180 kg of N required for the desired crop yield, showed better productivity results. Although they are still not significant in comparison with the treatments that did not receive the anticipated N fertilization.

It can be observed a better use of N by the plants when the slow-released N is applied in their system in advance. A fact that is reflected in their foliar N and grain yield.

Conclusion

Maize crop responded to the nitrogen fertilization regardless of its plant coverage. The vegetation coverages *P. glaucus* and *R. sativus* did not affect the maize grain yield. The nitrogen fertilization with a slow-release N source can be managed in advance, when applied on green coverages such as *P. glaucus* and *R. sativus,* without damaging productivity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Andrade AC, Fonseca DM, Queiroz DS, Salgado LT, Cecon PR (2003). Adubação nitrogenada e potássica em capim-elefante (*Pennisetum purpureum* schum. cv. napier). Ciência e Agrotecnologia, Lavras pp. 1643-1651.

Basso CJ, Ceretta CA (2000). Manejo do nitrogênio no milho em sucessão a plantas de cobertura de solo, sob plantio direto. Revista Brasileira de Ciência do Solo 24(4):905-915.

Borges WLB, Freitas RS, Mateus GP, Eustáquio de Sá M, Alves MC (2015). Produção de soja e milho cultivados sobre diferentes coberturas. Revista Ciência Agronômica 46(1):89-98.

Broch DL, Ranno SK (2008). Fertilidade do solo, adubação e nutrição da cultura do milho. Revista e Produção: Soja e Milho safra 2008/2009 1(5):133-140.

Cabezas WARL, Alves BJR, Caballero SSU, Santana DG (2004). Influência da cultura antecessora e da adubação nitrogenada na produtividade de milho em sistema plantio direto e solo preparado. Ciência Rural 34(4):1005-1013.

Cantarella H, Duarte AP (2004). Manejo da fertilidade do solo para a cultura do milho. In: Galvão JCC, Miranda GV. Tecnologias de produção do milho. Viçosa, UFV pp. 139-182.

CEMTEC (2015. Centro de Monitoramento de Tempo, do Clima e dos Recursos Hídricos de Mato Grosso do Sul (CEMTEC) 2015. Available in: <http://www.cemtec.ms.gov.br>.

Ceretta CA, Basso CJ, Herbes G.M, Poletto N, Silveira MJ (2002). Produção e decomposição de plantas invernais de cobertura do solo e milho, sob diferentes manejos da adubação nitrogenada. Ciência Rural 32(1):49-54.

Clemente AL, Muniz JÁ (2002). Avaliação do coeficiente de variação em experimentos com gramíneas forrageiras. Ciência e Agrotecnologia, 26(1):197-203.

Coelho MA, França GE (2007). Adubação da cultura do milho. Available in: <www.cnpms.embrapa.br>.

CONAB (2017). Companhia Nacional de abastecimento: Levantamento de outubro de 2017 (2017). Available in: http://www.conab.gov.br/OlalaCMS/uploads/arquivos/

Duarte JO, Cruz JC, Garcia JC, Mattoso MJ (2010). Economia da produção e utilização do milho. In: Cultivo do milho. EMBRAPA. Centro Nacional de Pesquisa de Milho e Sorgo, Sistema de produção, 2. Available in: < http://sistemasdeproducao.cnptia.embrapa.br>.

Empresa Brasileira de Pesquisa Agropecuária, Manual de análises químicas de solos, plantas e fertilizantes (EMBRAPA) (2011). (Org) Donagema GC, Calderano SB, Campos DVB, et al. Rio de Janeiro: Embrapa Solos 230 p.

Ernani PR, Sangoi L, Lech VA, Rampazzo C (2005). A forma de aplicação da ureia e dos resíduos vegetais afeta a disponibilidade de nitrogênio. Ciênc. Rural 35(2):360-365.

Faroni CE, Trivelin PCO, Franco HCJ, Vitti AC, Otto R, Cantarella H (2009). Estado nutricional da cultura de cana-de-açúcar (cana-planta) em experimentos com ^{15}N. Revista Brasileira de Ciência do Solo 33(6):1919-1927.

Fernandes FCS, Libardi PL (2007). Percentagem de recuperação de nitrogênio pelo milho, para diferentes doses e parcelamentos do fertilizante nitrogenado. Revista Brasileira de Milho e Sorgo 6(3):285-296.

Fontes PCR (2001). Diagnóstico do estado nutricional das plantas. Viçosa, Ed. UFV 122 p.

Gomes FP (2000). Curso de Estatística Experimental. 14.ed. Piracicaba: DEGASPARI 77 p.

Granato ISC, Bermudes FP, Reis GG, Dovale, JC, Miranda, GV, Fritsche-Neto, R (2014). Index selection of tropical maize genotypes for nitrogen use efficiency. Bragantia 73(1):153-159.

Heinz R, Viegas Neto AL, Garbiate MV, Mota LHS (2013). Desenvolvimento inicial do milho após diferentes manejos de Raphanus sativus. Revista Agrarian 6(21):363-367.

Kluthcouski J, Aidar H, Thung M, Oliveira FRA (2006). Manejo antecipado do nitrogênio nas principais culturas anuais. Piracicaba: POTAFOS 24 p.

Malavolta E (2006). Manual de Nutrição Mineral de Plantas. São Paulo: Editora Agronômica Ceres, 638 p.

Pedrotti MC, Souza LCF, Freitas ME, Torres LD, Tanaka KS, Bottega SP, Rech J, Maquino PA (2015). Milho cultivado com doses de N em cobertura em sucessão a oleaginosas. Revista Agrarian 8(28):115-123.

Roscoe R, Gitti DC (2013) Manejo e Fertilidade do solo para a cultura da soja In: Tecnologia e Produção: soja 2013/2014, Fundação MS 44 p.

Sá JCM (1996). Manejo de nitrogênio na cultura de milho no sistema plantio direto. Passo Fundo: Aldeia Norte 23 p.

Sá JCM, Cardoso EG, Santos JB, Oliveira AF, Ferreira FC, Massinham A, Siuta DJ, Sá MFM (2007). Manejo de fertilizantes nitrogenados em sistemas de produção envolvendo os cultivos de soja/trigo e soja/milho no sistema de plantio direto. Anais... Nitrogênio e Enxofre na Agricultura Brasileira.

Setti JCA, Bono JAM, Cabrera FR, Vidis RY, França J (2006). Viabilidade do uso de fertilizante nitrogenado de liberação lenta na cultura do milho, In: Reunião Brasileira de Fertilidade e Nutrição de Planta, 27, 2006, Bonito. Ferbio2006 A busca das raízes. Bonito - MS: Anais... Sociedade Brasileira de Ciência do Solo e Sociedade Brasileira de Microbiologia.

Souza AC, Carvalho JG, Pinho RGV, Carvalho MLM (2002). Parcelamento e época de aplicação de nitrogênio e seus efeitos em características agronômicas do milho. Ciência e Agrotecnologia 25(2):321-329.

Vol. 13(22), pp. 1154-1162, 31 May, 2018
DOI: 10.5897/AJAR2017.12869
Article Number: 70CF52E57267
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Effect of splitting nitrogen fertilization on Tifton 85: Yield, nitrogen use efficiency, and nitrogen nutritional status of plants and soil

Tangriani Simioni Assmann[1*], Etiane Tanise Sonego[2], Alceu Luiz Assmann[3], Paulo Fernando Adami[1] and Claucia Cuzzi[2]

[1]Course of Agronomy of the Federal Technological University of Paraná (UTFPR), Campus Pato Branco / PR, Brazil.
[2]Postgraduate Program in Agronomy, Federal Technological University of Paraná (UTFPR), Brazil.
[3]Agricultural Research Institute of Paraná, IAPAR, Pato Branco, PR, Brazil.

Received 9 November, 2017; Accepted 21 May, 2018

This two year study aims to verify the necessity of splitting nitrogen (N) fertilization by assessing the effect of different N fertilization strategies (split and not split), on cultivated Tifton 85 (*Cynodon* spp.) accumulation rate and total dry matter (DM) production, as well as quantifying the levels of nitrate (NO_3) in Red Dystrophic Oxisol. Research was carried out at the experimental unit of the Agronomic Institute of Paraná (IAPAR), Pato Branco - PR, in 2011/2012 and repeated in 2012/2013. Experimental treatments were replicated four times using a split plot randomized block design. Main plots consist of accumulated days of evaluation. In the split plot, the N-levels (0, 100, 200 and 300 kg ha^{-1}) were applied following different N-fertilization strategies (applied all at once or split into two or four applications). Tifton 85 total DM production increased as N-levels increased in both years up to 300 kg ha^{-1}. The highest total DM production (16.1 Mg ha^{-1}) was achieved in the second period with 300 kg ha^{-1} of N, regardless of N-management. Low N-recovery and N-use efficiency were observed in the first experimental period due to the previous shortage of N-fertilization. This is probably because the soil-microbe population immobilized the N applied in the first experiment period. In this case, splitting N-fertilization resulted in greater DM accumulation. After N-fertilization in the second experimental period, on the other hand, there was no response to splitting N-doses. The lack of response to the N-fertilization strategies indicates a soil-microbes plant system stabilization, which resulted in smaller N-immobilization, greater N-recovery and greater N-use efficiency by the Tifton. Furthermore, splitting small rates of N (less than 200 kg ha^{-1}) reduces fodder N-concentration in the initial growth phase, which could impair performance of plant growth and reduce plant protein content. Fodder dry mass more than double at the highest nitrogen level, although, there were no effects regarding to the nitrogen management, inferring that both option may be used.

Key words: *Cynodon* spp., forage production, nitrogen management, soil-plant interaction.

INTRODUCTION

Brazilian cattle production based on pastures provides excellent market competitiveness once it reduces costs production. Among pastures species, Bermuda grass are greatly used in Brazilian pastures, mainly for dairy production. Nitrogen fertilizer management is determinant for yield and nutritive value of forages. Due the complex

biological processes involved in the N cycle, nitrogen soil analyses cannot be used directly to assess N nutrition of plants. Therefore, it is necessary to analyze the crop nitrogen content as the percentage of the above-ground biomass N. However, the absolute N also varies during the growth crop period and season of the year.

N fertilization of grasses frequently increases yield, as well as the level of crude proteins (Monteiro, 1995). However, more studies are needed on N-management. The common recommendation to split N-application into two or more applications is based on the assumption that splitting applications can improve N-supply synchronization with a plant's ability to utilize nutrients, reducing potential N-loss. Due to this, there is an idea that the greater the number of N-splits, the smaller is the N loss (Coelho, 2007).

Fertilization must be sufficient to provide optimal plant development and final yield. Critical N-concentration has been defined as the minimum crop N-concentration required for reaching maximum crop growth. Once N-concentration is below this level, plant yield tends to decrease. Early on spring, after winter frost, death biomass may impaired plant regrowth, especially due to N immobilization.

Sartor et al. (2011) reported that the amounts of N absorbed by *Urochloa plantaginea* differed statistically between N-levels (200 and 400 kg ha^{-1}). Treatment with 200 kg ha^{-1} of N, split into two applications, resulted in plant N content below the critical N-dilution curve proposed by Lemaire (1997), limiting its yield potential. This result shows the high N-recovery potential of *U. plantaginea* and the negative effect of N-splitting, when N-availability does not meet plant demand.

Even when there is an ample supply of N, the N-concentration in plants within dense canopies declines as they grow (Greenwood et al., 1986). This phenomenon has usually been interpreted as resulting from plant ageing and is related to plant phenology. The use of the critical N-dilution curve has been proposed as a plant bases approach for assessing crop N nutrition.

N-recovery and agronomic efficiency varies among species; although, forage grasses have a higher capacity with an overall N-recovery of 68 to 75% (Primavesi et al., 2001). This is directly related to its better development and deep root system, which provide better soil cover and higher N-absorption, providing a secure means of recycling the nitrate.

Nitrate is the main form of N available in the soil, resulting from applied N-fertilizer or organic matter mineralization. When nitrate is not absorbed by plants or immobilized by soil microbes, it remains free in soil solution and is subject to leaching into deeper layers, reaching surface waters or groundwater (Dynia et al., 2006).

The objective of the current study was to study the N dynamics (nitrogen nutritional status, accumulation rate and total dry matter (DM) production, soil nitrate levels and from it, calculate the N-recovery and N-use efficiency) under different N management aiming to understand the effect of N-split sidedress application on Tifton 85.

MATERIALS AND METHODS

The study was carried out in the 2011/2012 period (December to May) and repeated in 2012/2013 (September to February), at the experimental unit of the Agronomic Institute of Paraná (IAPAR), in Pato Branco, (located 26º07' S; 52º39' W) on an established 4-year-old Tifton 85 bermudagrass pasture and the fertilization practices were guided by soil chemical analyses and also based on the recommendation of the Brazilian Commission for Chemical and Soil Fertility (CQFS/RS-SC, 2004). Management history prior to this current study includes occasional mechanical mowing and chemical control to suppress weed infestation. Broadcast nitrogen fertilizers were not applied before this study.

Experimental site soil is classified as Red Dystrophic Oxisol with clay texture (EMBRAPA, 2006). At the beginning of the experiment, soil samples (10 sub-samples) were collected from the 0-20 cm depth of soil, which showed the following chemical traits: pH ($CaCl_2$) = 5.50; organic matter (OM) = 50.93 g dm^{-3}; P = 13.25 mg dm^{-3}; K = 0.53 $cmol_{(c)}$ dm^{-3}; Ca = 6.96 $cmol_{(c)}$ dm^{-3}; Mg = 3.79 $cmol_{(c)}$ dm^{-3}; base saturation = 71%; and Cation Exchange Capacity (CEC) of 15.87 $cmol_{(c)}$ dm^{-3}.

The climate of the region is subtropical humid (Cfb) with well-distributed rainfall throughout the year, according to the Köppen classification. Average annual rainfall for the period of 1980 to 2010 was 2,077 mm $year^{-1}$ (IAPAR). Figure 1 shows the meteorological data observed throughout the experimental period.

Experimental treatments were replicated four times using a split plot randomized block design. N-management was applied all at once or split into two or four applications in the main plots. N-levels of 0, 100, 200 and 300 kg ha^{-1} were used in the split plot (15 m^2). Titfon 85 was rubbed and N-fertilization (urea with 45% of N) applied manually. Weather conditions (rainfall and soil moisture) were observed in order to allow the system to maximize the benefit of the N. Soils sampling to evaluate nitrate levels were collected at 0-5, 5-10 and 10-20 cm depths.

Forage DM accumulation (kg ha^{-1} day^{-1}) was evaluated by cutting the pasture in a square area of 0.25 m^2. When the pasture reached a height of 28 cm, plants were manually cut with a cleaver, at a height of 5 cm from ground level to determine the pasture growth rate (Table 1.) For all treatments the pasture was cut four times. Samples were dried in a forced-air oven at 60°C until they reached constant weight and then converted to kg ha^{-1} of DM. Total forage production was obtained by adding the forage production of each period.

In order to assess the N-nutritional status of plants according to treatments in the second experimental period, the data of forage DM accumulation (Mg ha^{-1}) were related to the plant N-concentration. The intersection points between these variables

*Corresponding author. E-mail: tangriani@utfpr.br.

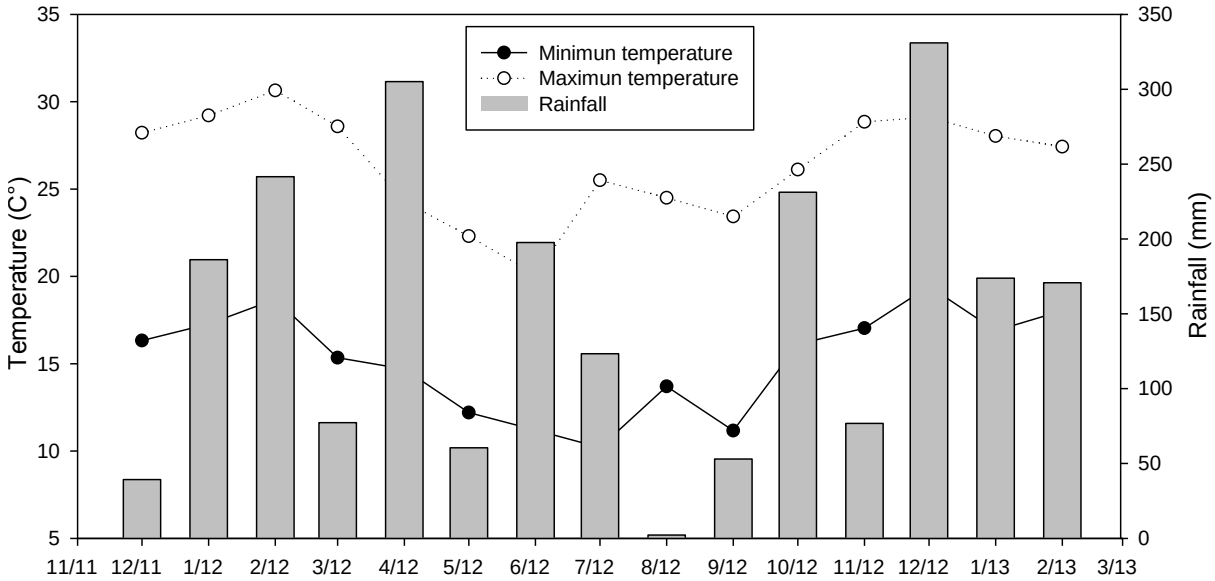

Figure 1. Meteorological data observed throughout the experimental period.
Source: Agronomic Institute of Paraná (IAPAR).

Table 1. Tifton 85 DM evaluation data and accumulative days of evaluation in the experimental periods of 2011/2012 and 2012/2013.

Cut	Day	Accumulated days
Cut to uniform height	12-08-11	0
Jan/12	12-01-12	34
Feb/12	02-11-12	63
Mar/12	03-15-12	97
May/12	05-07-12	149
Cut to uniform height	09-28-12	0
Oct/12	10-30-12	31
Nov/12	11-30-12	61
Jan/13	01-02-13	93
Feb/13	02-18-13	139

were contrasted with the N-dilution curve proposed by Lemaire (1997). N-use efficiency and N-recovery rates were evaluated from pasture DM production and its N-content. N-use efficiency of fertilization on forage yield (kg DM per kg N applied) was calculated assuming that the contribution of soil N was similar among treatments with or without N. Forage N-content multiplied by forage yield enabled the determination of N absorbed by the pasture. Each period was evaluated in this manner, and the sum of the periods resulted in the total N-absorbed.

N-recovery and N-use efficiency of Tifton 85 were calculated using the following equation:

Nitrogen Recovery (%) = [(NUF - NUNF)/AFA] x 100
N-use efficiency (kg DM per kg N) = [(DMY - DMYNF)/AFA]

Where: NUF (kg N ha^{-1}) = N-uptake of forage fertilized in the N treatments; NUNF (kg N ha^{-1}) = N-uptake of forage at 0 kg N ha^{-1}; DMYF (kg ha^{-1}) = yield of forage fertilized with N-treatments; DMYNF (kg ha^{-1}) = yield of forage at 0 kg N ha^{-1}; and AFA (kg N ha^{-1}) = amount of fertilizer applied in the N-treatments.

To determine soil nitrate levels, soil samples were collected in each sub-plot, every ten days, on average, after the N-application, from the following soil depths 0-5, 5-10 and 10-20 cm, by using a shovel and a graduated ruler. Samples were dried in a forced-air oven at 55°C for 72 h, and the concentration of NO_3^- was determined by the Kjeldahl method described by Tedesco et al. (1995). Forage N-determination was also evaluated by the Kjeldahl method.

Results were subjected to analysis of variance assessed by the Bartlett test for homogeneity. When results showed significance at 5 and 1% probability; the means of qualitative factors (N-management and soil sampling depth) were compared using the Tukey test at 5% probability of error. Polynomial regression was applied to the quantitative factors (N-rates). Linear and quadratic models were tested and the selection of a model was based on significance (less than 5%) and the coefficient of determination. For the significant interaction, evaluations were conducted on the increasing N-rates within each situation and, subsequently, on the behavior of each condition within each N-rate. Where the

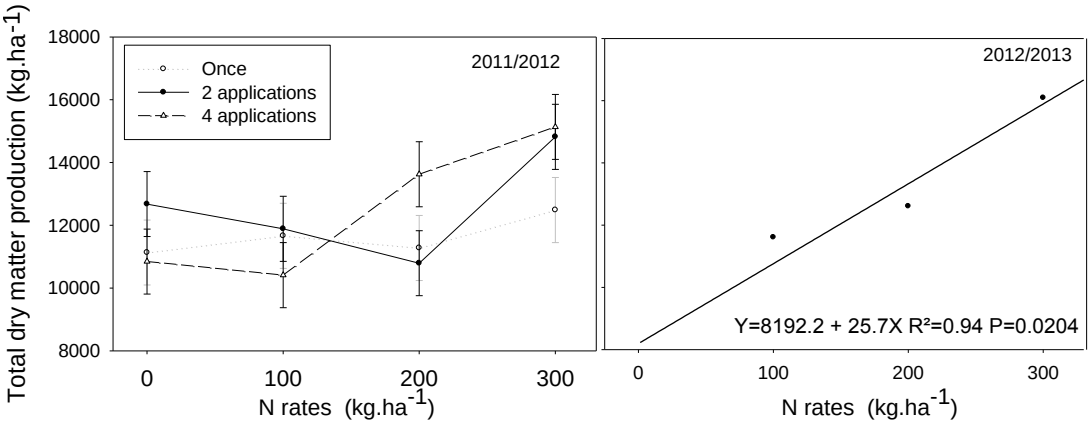

Figure 2. Tifton 85 total DM production (Kg ha^{-1}) in relation to N-management (applied all at once or split into two or four applications) and N- rates (0, 100, 200 and 300 kg ha^{-1}) in 2011/2012 and 2012/2013 respectively (Pato Branco, 2013). For the bars that are not coincident, the means differ by MSD (minimum significant difference) test at 5% probability.

interaction was not significant; factors were analyzed separately.

RESULTS AND DISCUSSION

Total DM production

In the 2011/2012 period, there was a significant interaction (P=0.0158) between N-management and N-rates for total DM production (149 days of evaluation), whereas in the 2012/2013 period, only the effect of the N-rates were present (P=0.0000). In the 2011/2012 period, the highest total DM production was of 14,976 kg ha^{-1}, with 300 kg ha^{-1} of N, when treatments were split into two and four applications. With the same N-rate, N-management applied all at once resulted in a total DM production 17%, or 2,491 kg ha^{-1} lower, when compared to the average value for N-management with two and four applications (Figure 2).

In the 2012/2013 period, only a linear effect of N-rates on total DM production was observed, reaching a value of 16.9 Mg ha^{-1} at 300 kg ha^{-1} of N. This production is 104% higher than the control and for each kg ha^{-1} of N applied, a dry mass yield increase of 26.0 kg ha^{-1} (Figure 2) was observed. Quaresma et al. (2011) noticed increases of 22.7 kg ha^{-1} in the production of Tifton total DM for each kg ha of N applied.

Total DM production reported in this paper was higher than the values reported by Quaresma et al. (2011), which DM yield (period from January to April) ranged from 6.3 and 11.7 Mg ha^{-1} at the treatments without N and with 240 kg ha^{-1} (N-split in four times), respectively. Under similar soil and climate conditions, Cecato et al. (2001) reported Tifton 85 total DM production of 7.5 and 14.3 kg ha^{-1} in response to treatments without N and with 400 kg ha^{-1}, respectively. Thus, Alvim et al. (1999) evaluating Tifton 85 DM yield (23.1 Mg ha^{-1}) along the year, reported pasture nitrogen response to higher levels of N (600 kg ha^{-1}).

The application of N in the first experimental period occurred four years after pasture establishment. During this interval there was no N-fertilization. This probably caused a great shortage in soil N, once the increase of soil microbiota was stimulated by N-fertilization and a great part of N was temporarily immobilized in the soil microbiota.

Probably, in the case of N being applied all at once, the microbial population was not large enough to absorb all of it and part of N may have been lost. Although, when N-fertilization was split, the first N-application stimulated the increase of the microbial population which could have captured the N-fertilization applied in sequence.

The lack of response to the N-fertilization strategies treatment in the second experimental period can be a indicative of a soil-microbes plant system stabilization. The strategy of split N-fertilization into a few applications aims to reduce losses and improve its use efficiency and recovery (Cantarella and Marcelino, 2008). However, low N-recovery by plants can result from a temporary N-immobilization by soil microbiota, with the N becoming available after the death of microorganisms. In the second experimental period, there was probably enough mineral N in the soil for the microbes and the Tifton.

Queiroz et al. (2012) studying nitrogen levels (200 and 400 kg ha^{-1}) and management (split or not and applied along rainy or dried rainfall season) on *Cynodon* spp cv. tifton 85 under irrigation reported that nitrogen fertilization strategy alters the curve of forage distribution throughout the year. The concentration of total or partial nitrogen fertilization in the dry period allows the maintenance of the pasture without big alterations in pasture support capacity. In spite of the lower response to N application in the dry period, the authors estimated that this may not be

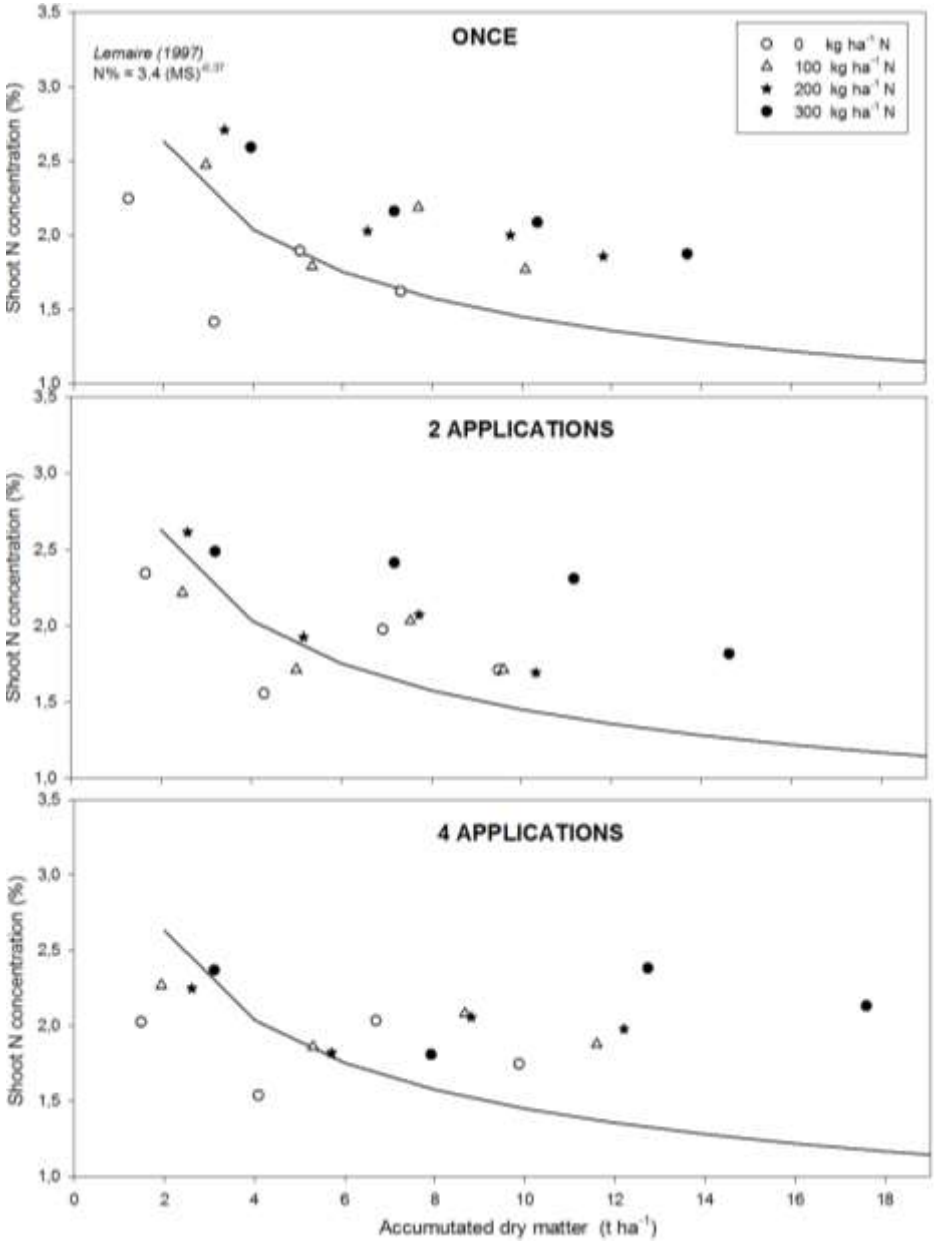

Figure 3. Shoot N-concentration in Tifton 85 in relation to accumulated DM according to N-levels and three forms of N-management (1, 2, and 4 applications) in the 2012/2013 period compared with the N-dilution curve proposed by Lemaire (1997).

a problem, once the N non-absorbed in the dry season will be used in the following rainy period and, since in the irrigation there is control of the water depth applied, there are no risks of N leaching even at high doses.

Sartor et al. (2014) working with Alexandergrass (*Urocloa plantaginea*) with 200 kg ha^{-1} of N split into two applications, reported that the plant's N-nutritional status was below the recommended level according to Lemaire and Gastal (1997). This data showed the high potential of N-recovery by even in its early stage of growth, where 100 kg ha^{-1} of N was not able to supply enough N for the plants.

Nitrogen dilution curves

In the second experimental period (2012/2013), the N-nutritional status of Tifton was assessed, based on the relation between forage DM accumulation (Mg ha^{-1}) and plant N-concentration in comparison with the N-dilution curve proposed by Lemaire (1997).

In the treatment without N-application (Figure 3), the

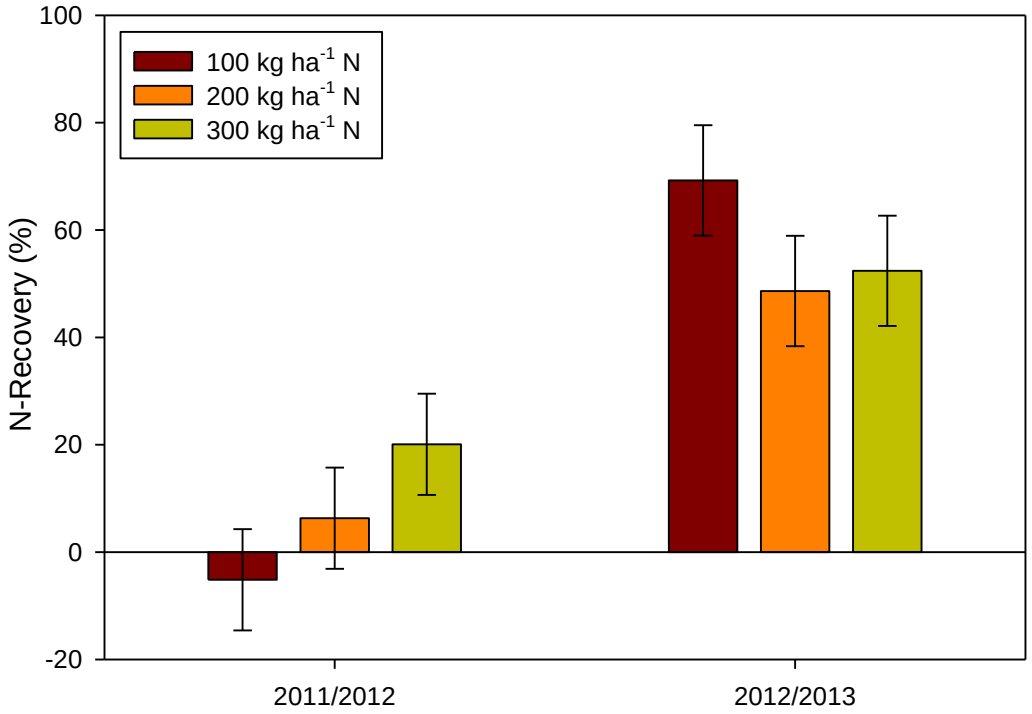

Figure 4. Tifton 85 N-recovery in the 2011/2012 and 2012/2013 periods in relation to N-levels. (Pato Branco, 2013). For the bars that are not coincident in the same period (years), the means differ by MSD (minimum significant difference) test at 5% probability

element concentration in the plant remained below the critical curve proposed by Lemaire (1997) in almost all plant cycle, indicating inadequate supply and N-uptake by the plants in this treatment. The N-dilution phenomenon was poorly observed when the largest N-fertilizer doses were applied (300 kg ha^{-1} N), primarily when this dose was split into two or four applications.

When the 100 and 200 kg ha^{-1} of N-fertilizer doses were split in two or four applications, more points below the curve of sufficiency propose by Lemaire (1997) was observed until 6 Mg ha^{-1} of DM accumulation (61 accumulated days). This is in agreement with the hypostasis that N-split can lead to N shortcoming in early development plants stage, because the split action reduces the initial N availability.

Consequently, the recommendation of splitting small N-doses (below 200 kg ha^{-1}) reduces the production and qualitative potentials of C_4 forages. On the other hand, the application of 300 kg ha^{-1} had caused greater fodder N-concentration with a slight advantage when it was applied all at once. The biggest DM productions were observed (18 Mg ha^{-1}) when the 300 kg ha^{-1} of N were split in two or four applications. However, no significant differences caused by different N-management forms on total production of DM were observed.

After successive N-applications, in special when N-fertilizer was split in four times, the pasture started to show satisfactory N-nutritional indices and according to the plant biomass analysis, there was an N “luxury consumption” showed by N levels beyond what would be necessary for its growth, which may characterize the highest forage quality or accumulation of reserves (Lemaire et al., 1989).

Nitrogen recovery

In both experimental periods, N-recovery was affected by N-rate (Figure 4). In the first experimental period, N-recovery increased as N-rate increased (P=0.0079), whereas, in the second experimental period, N-recovery tended to reduce as N-rate increased (P=0.0020). N-recovery for the treatment with 100 kg ha^{-1} of N, in the 2011/2012 period was negative (-5.2%). At the same N-level, N-recovery increased to 69.2% in the second experimental period. The increase in N-recovery between periods shows the residual effect of the N-rate applied in the 2011/2012 period. Such residual effect might store N in the soil increasing N-availability for the subsequent period (Figure 4).

N-recovery in the first experimental period (2011/2012) was also affected by N-management (P=0.0326). The lower N-recovery (-10.0%) was observed when N-rate was split twice. Meanwhile, N-recovery was, on average, 15.6% higher when N-rate was applied all at once or split into four applications.

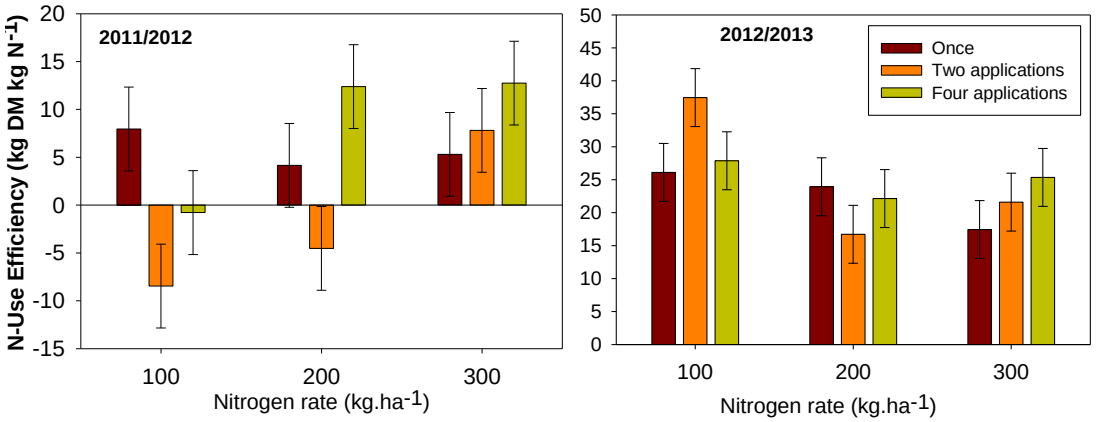

Figure 5. Tifton 85 N-use efficiency in the 2011/2012 and 2012/2013 periods, in function of N-rate and N-fertilization strategy: Applied all at once or split into two or four applications (Pato Branco, 2013). For the bars that are not coincident in the same period (years), the means differ by MSD (minimum significant difference) test at 5% probability.

For a given period, total crop N-uptake is the addition of N derived from synthetic N-fertilizer applied in the same year, and from a variety of other sources (organic soil N, N-fertilizer applied in previous years, N-deposition from the atmosphere, biological N-fixation, applied crop residue and manure) (Yan et al., 2014). In this manner, Sartor et al. (2011) found an N-recovery rate of 110% by plants, that is, the plants absorbed more N than what was applied to the soil.

In addition, low N-recovery rates may not mean high loss of N, since this N may be in other soil compartments. In this manner, the lower N-recovery in the first period (2011/2012) can be a result of high immobilization of applied N-fertilizers as a result of no N-fertilization in the four prior years of the experiment.

When soil microorganisms have greater access to N in pastures due to higher N fertilizations, the additional N is used to produce microbial biomass. This process can result in greater N immobilization and higher N content in litter under high N fertilization (Liu et al., 2011). The results shows that the addition of N fertilization increased N content of Tifton biomass after 128 days indicating that microbes accessed N exogenous to litter. This demonstrates that plant litter deposition in grasslands can result in immobilization of N and limit availability for plant growth (Wedin, 1996).

These results are in accordance with Berg and Staaf (1981), which described a three-phase conceptual model for N release from residues, including (i) initial rapid release of labile nitrogenous compounds, (ii) N concentration and even absolute amount of N increasing in the decomposing material due to microbial immobilization of exogenous N, and (iii) N loss due to mineralization.

The greater values of N-recovery observed in the second evaluation period was a result of N-recovery from the N applied in the first experimental period, when the addition of N-fertilizer probably increased the microbial community, which may have absorbed the added N. The added N was not lost but rather temporarily immobilized and later released by mineralization process.

Nitrogen use efficiency

In the first experimental period (2011/2012), Tifton N-use efficiency was low and even negative when 100 kg ha^{-1} of N were split and when 200 kg ha^{-1} of N were split twice (Figure 5). Splitting small amounts of N-fertilizer probably affected initial plant growth, since there was not enough N to allow for initial plant regrowth and the increasing microbial community. Moreover, under low N-availability, there is competition between the microbial community and plants for this N, which tends to be used by the microbial community since it is more efficient in absorbing nutrients than plants. Furthermore, NUE of smaller N-fertilizer rates applied once were positive, demonstrating the importance of greater N-fertilizer rates in the initial periods. In the first period (2011/2012), splitting N-fertilizer rates was a successful strategy only for greater N-rates (300 kg ha^{-1}). In the second period (2012/2013), in general, NUE increased showing a lower immobilization process compared to the first experimental period.

Soil mineral nitrogen

Soil nitrate levels were affected by N-levels. Higher differences were noticed in the first period of evaluation, when nitrate levels reached 250 mg kg^{-1} (18 days after N-application) at the 0-5 cm soil layer depth for the N-levels of 200 and 300 kg ha^{-1}. Probably, a low microbial population at the time of N-application associated with an immediately low capacity of N-absorption resulted in

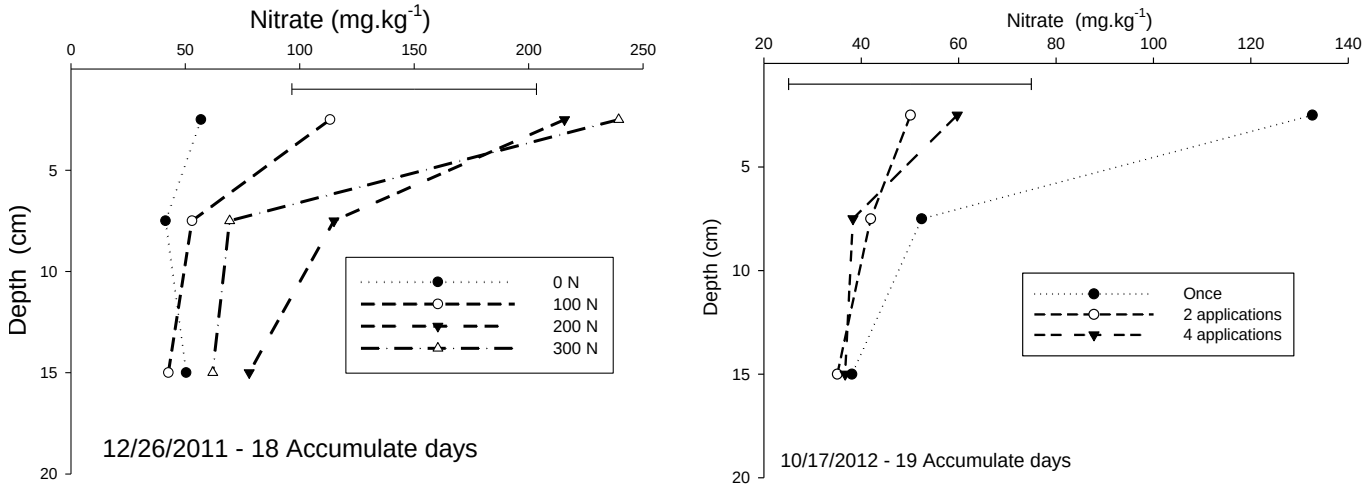

Figure 6. Soil nitrate levels in the 2011/2012 period in relation to N-levels (0, 100, 200 and 300 kg ha^{-1}) and soil layer depth, and in the 2012/2013 period in relation to N-management (applied at once or split into two or four applications) and soil layer depths (Pato Branco, 2012-2013). Bar horizontally compares each depth between nitrogen management by Minimum Significant Difference ($P < 0.05$).

higher nitrate levels in the soil (Figure 6).

When comparing the 2nd period in relation to the 1st one, it is noticed that soil nitrate levels were lower at the 0-5 cm soil layer (Figure 6). This may indicate an increased microorganism population caused by previous N-fertilization. The microorganism population and the Tifton plants were more efficient in absorbing N from the soil. The highest value of nitrate was observed when N-fertilizer was applied all at once in the 0-5 cm soil layer.

In this period, an effect of N-rate over soil nitrate concentration (P=0.0389) was also noticed. For each Kg of N applied, soil nitrate levels increased 0.11 mg kg^{-1} with the highest level (70 mg kg^{-1}) being found at the 0-5 soil layer in the treatment with 300 kg ha^{-1} of N.

The results were similar to the early findings of Costa et al. (2008), which reported an increase of 68% in soil nitrate values with increasing levels of N on Brachiaria. However, based on N-recovery rate and N-use efficiency it is possible to affirm that there was no nitrate leaching. Primavesi et al. (2006) affirm that the application of N doses up to 500 kg ha^{-1} split in up to five times does not present risk of nitrate leaching to the groundwater, even during the rainy period.

Conclusion

Splitting small rates of N (less than 200 kg ha^{-1}) reduces fodder N-concentration in the initial growth phase, which could impair performance of plant growth and biomass accumulation. The result of the study suggest that Tifton dry matter yield increased as N levels were increased up to 300 kg N ha^{-1}, however, there was no significant effect in relation to the nitrogen management. Thus, it is not recommended to split N-application in pasture that historically receives no N or low levels of N fertilization.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Alvim MJ, Xavier DF, Verneque RS, Botrel MA (1999). Resposta do tifton 85 a doses de nitrogênio e intervalos de cortes. Pesq. Agropec. Bras., Brasília 34(12):2345-2352.

Cantarella H, Marcelino R (2008). Fontes alternativas de nitrogênio para a cultura do milho. Informações Agronômicas 122:12-14.

Berg B, Staaf H (1981). Leaching, accumulation and release of nitrogen in decomposing for- est litter. p. 163163ccumulation and release Rosswall (ed.) Terrestrial nitrogen cycles: Processes, ecosystem strategies, and management impacts. Ecological Bulletin Book Series. Munksgaard Publ., Stockholm, Sweden.

Comissão de Química e Fertilidade do Solo RS e SC (CQFS)- RS/SC (2004). Manual de adubação e de calagem para os Estados do Rio Grande do Sul e de Santa Catarina. 10. ed. Porto Alegre 394 p.

Cecato U, Santos GT, Machado MA, Gomes LH, Damaceno JC, Jobim CC, Ribas NP, Mira RT, Cano CCP (2001). Avaliação de cultivares do gênero Cynodon com e sem nitrogênio. Acta Scientiarum Agronomy 23:781-788.

Coelho AM (2007). Manejo da adubação nitrogenada na cultura do milho. Embrapa, Sete Lagoas, MG, circular técnica 96.

Costa KA, Faquin V, Oliveira IP, Rogrigues C, Severiano EC (2008). Doses e fontes de nitrogênio em pastagem de capim-marandu. I-Alterações nas características químicas do solo. R. Bras. Cience. Solo 32:1591-1599.

Dynia JF, Souza MD DE, Boeira, RC (2006). Lixiviação de nitrato em Latossolo cultivado com milho após aplicações sucessivas de lodo de esgoto**.** Pesq. Agropec. Bras 41(5):855-862.

EMBRAPA. Empresa Brasileira de Pesquisa Agropecuária (2006). Sistema Brasileiro de Classificação de Solos. 2.ed Rio de Janeiro: Embrapa solos 306 p.

Greenwood DJ, Neeteson JJ, Draycott A (1986). Quantitative relationships for the dependence of growth rate of arable crops to

their nitrogen content, dry weight and aerial environment. Plant Soil 91:281-301.
Lemaire G, Gastal FN (1997). N uptake and distribution in plant canopies. In: Lemaire G. (Ed.) Diagnosis on the nitrogen status in crops. Berlin: Springer pp. 3-43.
Lemaire G, Gastal F, Salette J (1989). Analysis of the effect of N nutrition on dry matter yield of a sward by reference to potential yield and optimum N content. In Proceedings of the XVI International Grassland Congress pp. 4-11.
Liu K, Sollenberger LE, Silveira ML, Vendramini JMB, Newman YC (2011).Grazing Intensity and Nitrogen Fertilization Affect Litter Responses in "Tifton 85" Bermudagrass Pastures: II. Decomposition and Nitrogen Mineralization. Agronomy Journal 103:163-168.
Monteiro FA (1995). Nutrição mineral e adubação. In: Simpósio sobre manejo da pastagem. Anais Fealq 12:227.
Primavesi O, Correa LA, Primavesi AC, Cantarella H, Armelin MJA, Silva AG, Freitas AR (2001). Adubação com ureia em pastagem de *cynodon dactylon* cv. Coastcross sob manejo rotacionado: eficiencia e perdas**.** São Carlos- Embrapa Pecuária Sudoeste 42 p (Circular Técnica, 30).
Primavesi O, Primavesi AC, Corrêa LA, Silva AG, Cantarella H (2006). Lixiviação de nitrato em pastagem de coastcross adubada com nitrogênio. Revista Brasileira de Zootecnia 35(3):683-690.
Quaresma JPS, Almeida RG, Abreu JG,Cabral LS, Oliveira MA, Carvalho DMG (2011). Produção e composição bromatológica do capim-Tifton 85 (Cynodon spp.) submetido a doses de nitrogênio. Acta Sci - Animal Science 33:145-150.
Queiroz DS, Menezes MAC, Oliveira RA de, Viana MCMM, Silva EA (2012). Nitrogen fertilization strategies for xaraes and tifton 85 grasses irrigated in the dry season. Revista Brasileira de Zootecnia 41(8):1832-1839.
Sartor LR, Assmann TS, Soares AB (2014). Assessment of the nutritional status of grassland: nitrogen nutrition index. Seminário de Ciências Agrárias 35:449-456.
Sartor LR, Assmann TS, Brugnara AS, Adami PF, Assmann AL, Pitta CSR (2011). Nitrogen fertilizer use efficiency, recovery and leaching of an alexandergrass pasture. Rural Brasilian Cience Solo 35:899-906.
Tedesco MJ, Gianello C, Bissani CA, Bohnen H, Volkweiss SJ (1995). Análise de solo, plantas e outros materiais. UFGRS: Departamento de Solos, Porto Alegre 174 p.
Yan X, Ti C, Vitousek P, Chen D, Leip A, Cai Z and Zhu Z (2014). Fertilizer nitrogen recovery effi-ciencies in crop production systems of China with and without consideration of the residual effect of nitrogen. Environmental Research Letters 9:1-9.
Wedin, DA (1996). Nutrient cycling in grasslands: An ecologist's perspective. In: R.E. Joost and C.A. Roberts (ed.) Nutrient cycling in forage systems. PPI/FAR, Columbia, MO. pp. 29-44.

Related Journals: