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Full Length Research Paper

Growth and senescence of *Urochloa brizantha* under Brazilian Cerrado conditions

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The growth and senescence of *Urochloa brizantha* cv. Marandu were analyzed under the Brazilian Cerrado conditions. The seeds were sowed in November at the beginning of the rainy season. Samples of tillers were collected. The culms, inflorescences, along with green and dead leaves were removed to obtain leaf area and dry matter measurements. The maximum leaf area index (LAI = 5.80 m^2 m^{-2}) was achieved at 97 days after emergence (DAE), the maximum above ground total dry matter (TDM = 22.8 Mg ha^{-1}) at 151 days, and the maximum crop growth rate (CGR = 0.260 Mg ha^{-1}) at 70 days. Leaf senescence began at 55 DAE, with less than 0.001 Mg ha^{-1} of dead leaves dry matter (DLDM), and reached 1.753 Mg ha^{-1} at 151 DAE. Between 117 and 151 DAE, in the final cycle of the plants, there was lodging of the canopy by wind. This lodging had favored the sprouting of new tillers and a sudden increase in net assimilation rate (NAR). The LAI values did not show such expressive increase during the same period, suggesting that NAR may contribute to crop growth more than LAI due to increased light penetration after the occurrence of the lodging. In this paper an equation that estimates the net photosynthetic rate from the net assimilatory rate was presented.

Key words: Leaf area, dry matter, growth rate, net assimilation rate, pasture grass.

INTRODUCTION

The Cerrado is the second largest biome available for food and fiber production in Brazil, after the Amazon rainforest, covering an area of approximately 200 million hectare, about 23% of the Brazilian territory. The climate in this region has two well-defined seasons, a dry season (from April to September), with virtually no precipitation, and a rainy season, (from October to March). The soils are generally acidic, rich in aluminum and poor in nutrients and organic matter (Goedert et al., 1980; Fageria and Barbosa Filho, 2008). The vegetation

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includes a heterogeneous mix of grassland, bushes, and twisted trees, characteristic of the region (Ribeiro et al*.,* 1983; Gottsberger and Silberbauer-Gottsberger, 2006). In the Cerrado, predominantly located in the mid-western region of Brazil, the cultivated pastures cover about 50 million hectare. *Urochloa* sp. is a grass species that presents an exceptional adaptation to the edaphoclimatic conditions of this biome (Valle et al*.,* 2000; Costa et al., 2016).

Urochloa grass (*Urochloa* sp.) is a species introduced from Africa that has expanded throughout Brazil, especially in the Cerrado region. *Urochloa* grass has enabled the use of vast areas of pastures, making possible the expansion of cattle and contributing to making Brazil a large producer and exporter of meat (ABIEC, 2014; Guard and Guard, 2014). The grass growth is rapid during the rainy season, but gradually decreasing in the dry season. Ultimately, water limitation paralyzes the plant growth inducing the death of the aerial parts, resulting in great herd weight loss, and consequently, economic loss to the producer.

Although *Urochloa* occupies wide areas of cultivated pastures in Brazil, scientific studies on this species have not been carried out systematically. Besides being used as cattle food, the residues of grass promote soil amelioration due to their higher content of lignin. The input of lignin increases the level of carboxylic and humic acids in the substrate and favors the structure and stability of soil aggregates (Primavesi, 1982; Fassbender and Bornemisza, 1994; Oliveira et al., 2015; DiDonato et al., 2016). Besides, the increase in lignin content turns the soil less susceptible to compaction and erosion (Stone et al*.*, 2004), and improves the microbiological soil conditions, such as higher mycorrhizal fungi (Soares et al., 2010).

Among the issues that deserve attention and further studies lies the follow-up of *Urochloa* growth in the Cerrado environment, as well as its senescence and residue production. The technique of growth analysis is employed to follow up plant growth, and, for higher plants, is based on the conversion of light energy into chemical energy, because an average of ca. 96% of the dry matter accumulated by plants during their growth period results from photosynthesis (Hunt, 1990; Walker, 1992). Nevertheless, in most studies focusing on plant growth analysis researchers do not calculate the crop senescence rate (CSR). However, information on CSR is important because it allows crop physiologists and agronomists to create patterns for each type of culture, thus guiding the producer in order to predict the duration of each growth phase and therefore maximizing yield. Also, this helps estimate the production of *Urochloa* residue at the end of its life cycle, the contribution of this biomass residue production to soil amelioration, and the quantity of carbon accumulated in the residue.

The plant growth analysis, although a classic technique (Blackman, 1919) is still very useful in determining the partitions of photosynthates among the various plant organs, providing subsidy for those working on grain or forage production by helping in the selection of the best genotypes. Additionally, the plant growth analysis requires few instrumental resources, therefore being a low-cost technique, a very appropriate tool for researchers based on developing countries.

Depending on the planting system, it might be desirable to have a faster or a slower development pace of the plants at a specific time. For instance, this is what happens in pasture recuperation/renewal systems intercropped with annual crops (that is, crop-grass mixture, locally known as Barreirão System). In this system, from the beginning of the annual crop's development until harvest it is desirable that they face the least grass competition as possible. From this point on, the grass should have a good development so that the cattle can start grazing in a short time (Oliveira et al., 1996; Portes et al., 2000). Besides, Avci and Bilir (2013) reported that there could be many genetical and environmental factors on plant growth. They reported large morphological variation among and within clones of *Lavandula hybrida* and *L. officinalis*.

The aim of this study was to quantify, using the technique of growth analysis, *Urochloa brizantha* cv. Marandu growth, a C4 species, under Brazilian Cerrado conditions, as well as its senescence during the growth cycle.

MATERIALS AND METHODS

A field experiment was conducted in a degraded pasture area planted with *Urochloa decumbens* at the Federal University of Goias in Goiania (16°41'S and 49°17' W, 730 m altitude). The chemical characteristics of the soil before the experiment were: Organic matter - 23 g dm^{-3}; pH 5.2 Mehlich I extractable P - 2.3 mg kg^{-1}; K - 50 mg kg^{-1}; Ca - 5.7 cmolc mg kg^{-1}; Mg - 0.5 cmolc kg^{-1}; cation exchange capacity - 9.4 cmolc kg^{-1}. *U. brizantha* cv. Marandu seeds were sowed in a clayey Oxisol fertilized with 2500 kg ha^{-1} lime, 300 kg ha^{-1} NPK (4-30-16), 30 kg ha^{-1} micronutrients (FTE BR 12), and 20 kg ha^{-1} zinc sulfate.

Initially, the soil was tilled with a disc harrow to grind the *Urochloa* residues and to destroy termite mounds. After that, the soil was tilled with a moldboard plow to incorporate the *Urochloa* residues at 0.40 m depth to make crop regrowth and the remaining seed germination more difficult.

Urochloa seeds were mixed with fertilizers and distributed mechanically in the planting furrow at a 10 cm depth along a soil band of approximately 120 m length and 10 m width, divided into four plots of 4 m × 10 m, where the samples were collected. In each plot, two linear meter was marked in the planting row, excluding the border and the useful areas to count the number of tillers (NT). The rainfall and monthly average temperature data were monitored daily during the experiment Figure 1.

Plant samples were collected at 39, 47, 55, 70, 82, 97, 117 and 151 days after emergence (DAE) for growth analysis. Each sample consisted of two plants collected in each plot and placed in PVC tubes (1 m length × 0.10 m diameter) with their roots immersed in

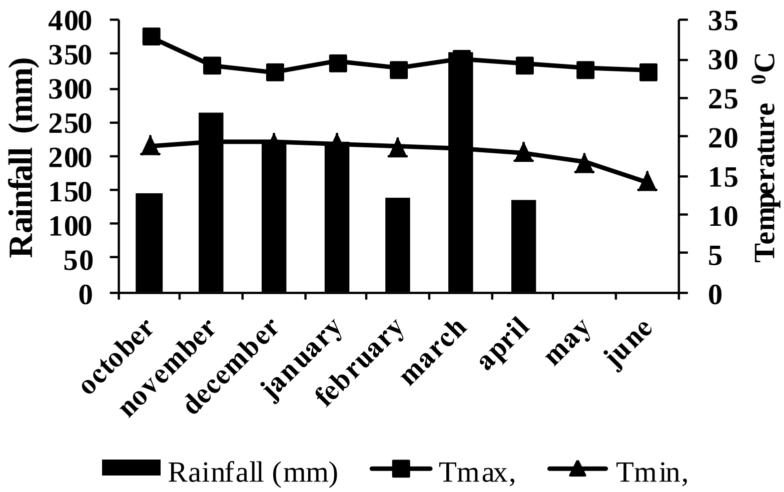

Figure 1. Rainfall (mm) and average temperature (°C), during the period of experiment, recorded at the Weather Station of Embrapa Rice and Beans, in Goiania-GO, Brazil.

water to avoid wilting. Then they were transported to the laboratory, where the roots were removed and the leaves were separated from the stems and inflorescences. The tillers were counted (tiller sample^{-1}) and the leaf area (LA), green leaf dry matter (GLDM), stem dry matter (SDM), inflorescence dry matter (IDM), dead leaf dry matter (DLDM), and total dry matter (TDM) were measured.

The number of tillers per square meter (NT m^{-2}) was calculated based on the number of tillers per linear meter (NT m^{-1}), counted within a 2 m line previously marked in the planting row, excluding the border and the useful areas, and dividing the value by the space between the rows (m):

NT m^{-2} = [(NT m^{-1}) × (space between rows^{-1})]

The LA, in cm^2, of each sample was measured using an LI-COR electronic leaf area meter (LI 3100, Lambda Instruments-Corportion, Lincoln, Nebraska). After that, the leaf area index (LAI) was estimated using the following equation:

LAI = [(NT m^{-1} × LA sample^{-1}) × (space between rows × NT sample^{-1} × 10.000)$^{-1}$] or LAI = [(NT m^{-2} × LA sample^{-1}) × (NT sample^{-1} × 10.000)$^{-1}$]

Where LAI is the leaf area index in m^2 of leaves per m^2 of ground surface and 10.000 transform cm^2 to m^2.

The green leaves, stems, inflorescences, and dead leaves were placed in paper bags and air-dried at 65ºC for 24 h, until constant weight. Based on the collected data estimates were made for GLDM, SDM, IDM, and DLDM using the following equation:

DM = [(NT m^{-1} × DM sample^{-1}) × (space between rows × NT sample^{-1})$^{-1}$]

Where DM is the dry matter per m^2; NT m^{-1} is the number of tillers per linear meter; DM sample^{-1} is the dry matter per sample collected and NT sample^{-1} is the number of tillers per sample collected. To calculate TDM, the results of GLDM, SDM, and IDM were added up.

Mathematical equations were fitted to the values obtained for LAI, GLDM, SDM, IDM, DLDM and TDM, as a function of time (DAE), and the significance tests for the regression were carried out using the computer program "Ajuste" (Zullo and Arruda, 1987).

Using TDM and LAI fitted equations, the physiological indicators of the growth, crop growth rate (CGR), net assimilation rate (NAR), and leaf area ratio (LAR), in instant values, were calculated using the growth analysis program "Anacres" (Portes and Castro Jr, 1991). The equations used to calculate these variables were the following: CGR = dTDM/dT; LAR = LAI/dTDM; NAR = dTDM/dT/LAI, where d is the derivative of the equation TDM and T = DAE (Hunt, 1982; Portes and Castro Jr, 1991).

In order to estimate photosynthesis rate in µmol CO_2 m^{-2} s^{-1} from net assimilate rate (g of dry matter m^{-2}day^{-1}) the following equation was used:

$$\mu mol CO_2 m^{-2} s^{-1} = g\ of\ dry\ matter \times \left(C/_{100}\right) \times \left(44/_{12}\right) \times (1/44) \times \left(1/_{86400}\right) \times 10^6$$

Where: C/100 = % of carbon (C) in the dry matter; 44/12 = transform C in CO_2; 1/44 = transform g of CO_2 in mol of CO_2 (that is, one mol of CO_2 equals 44 g); 1/86400 = transform hours in seconds and 10^6 transform mol of CO_2 in µmol. The percentage of carbon in the dry matter is approximately 45% (Walker, 1992).

Instantaneous CSR (Crop Senescence Rate) was calculated using DLDM data and the following equation: CSR = dDLDM/dT, where dDLDM/dT is the derived DLDM fitted equation in relation to time (DAE).

RESULTS AND DISCUSSION

Table 1 presents the cubic exponential polynomial equation [$y = k\ Exp\ (ax + bx^2 + cx^3)$] with better fit to the data of LAI, GLDM, SDM, and TDM (*y* axis) with time (x = day after emergence, DAE). For DLDM the best equation was the quadratic polynomial $(y = a + bx + cx^2)$, whereas IDM the best model was a linear equation

Table 1. Mathematical models fitted to NT, LAI, GLDM, SDM, IDM, DLDM, and TDM data as a function of time (x = DAE), and the respective correlation coefficients (r) and F test.

Variable	Mathematical models	r	*p*
NT	$y = 1.186\,Exp\,(0.145x - 1.516 * 10^{-3} * x^2 + 5.050 * 10^{-6} x^3)$ NT m^{-1}	0.95	<0.01
LAI	$y = 9.830 * 10^{-4}\,Exp\,(0.25x - 2.356 * 10^{-3} x^2 + 7.104 * 10^{-6} x^3)$ ($m^2\,m^{-2}$)	0.98	<0.01
GLDM	$y = 2.52 * 10^{-4}\,Exp\,(0.269x - 2.480 * 10^{-3} x^2 + 7.471 * 10^{-6} x^3)$ (g m^{-2})	0.99	<0.01
SDM	$y = 1.576 * 10^{-5}\,Exp\,(0.334x - 2.865 * 10^{-3} x^2 + 8.356 * 10^{-6} x^3)$ (g m^{-2})	0.99	<0.01
IDM	$y = -7.42 * 10^{-1} + 1.374 * 10^{-2} x$ (g m^{-2})	0.96	<0.01
DLDM	$y = 821.630 - 25.694x + 0.210x^2$ (g m^{-2})	0.99	<0.01
TDM	$y = 2.91 * 10^{-2}\,Exp\,(0.27x - 2.311 * 10^{-3} x^2 + 6.739 * 10^{-6} x^3)$ (g m^{-2})	0.99	<0.01

NT = number of tillers per linear meter, LAI = Leaf area index, GLDM = Green leaf dry matter, SDM = Stem dry matter, IDM = Inflorescence dry matter, DLDM = Dead leaf dry matter, TDM = Total dry matter, DAE = Days after emergence.

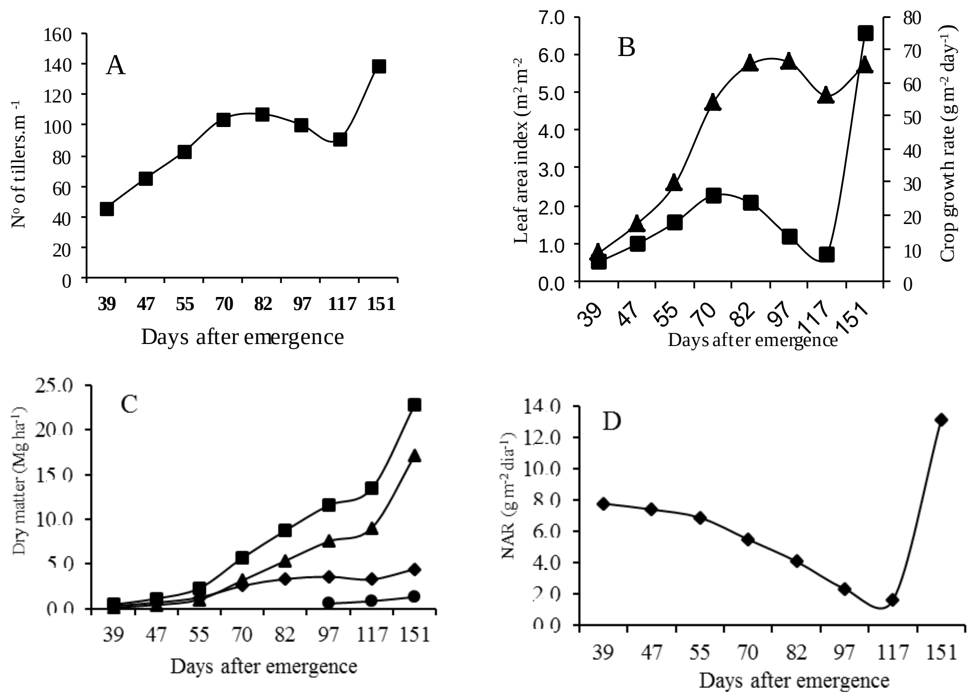

Figure 2. A: Number of tillers per square meter (NT m^{-2}); B: Leaf area index (LAI =▲), Crop growth rate (CGR = ■); C: Total dry matter (TDM = ■), Stem dry matter (SDM = ▲), Green leaf dry matter (GLDM = ◆), Inflorescence dry matter (IDM = ●); D: Net assimilation rate (NAR); of *U. brizantha* cv. Marandu cultivated under Brazilian Cerrado conditions.

$(y = a + bx)$. The physiological indicators of the growth NAR, LAR, and CGR were derived from the equation fitted to LAI and TDM data, whereas CSR was derived from the equation fitted to DLDM data, as previously described.

In this research, the experiment was divided in two parts, the first from emergence up to 117 DAE, when the plants were in full bloom. The second part from 117 up to 151 DAE, when there was lodging of the plant canopies by wind, allowing the solar radiation to reach the inferior strata with the sprouting of new tillers.

At the beginning of sampling (39 DAE) *Urochloa* already presented higher NT m^{-1} (approximately 42.25), with constant increment until 82 DAE, when the crop reached the value of 107.35 tillers m^{-1} (Figure 2A); after lodging there was an increase to 138.33 tillers m^{-1}. The LAI increased exponentially until 5.8 m^{-2} m^{-2} at 97 DAE (Table 1 and Figure 2B). Decreased after this date (4.1 m^{-2}) but increased again due sprouting of new tillers after lodging, reaching in this situation 5.7 at 151 DAE. Optimum LAI (4.7) occurred when maximum CGR (0.260 Mg ha^{-2} day^{-1}) was reached by plants at 70 DAE. For other side, maximum CGR was observed at 70 DAE (0.26 Mg ha^{-1} day^{-1}) in the inflexion point of the equation of TDM in function of DAE (Table 1, Figure 2B and C), but after the lodging it reached 0.751 Mg ha^{-2} day^{-1}.

It was observed at 39 up to 55 DAE (Figure 2C) an initial allocation of photoassimilates for leaf production (GLDM = 0.324 Mg ha^{-1}) over stem production (SDM = 0.151 Mg ha^{-1}). However, after 70 DAE this pattern inverted when the grass started to invest more in stem production, reaching a maximum value of 17.11 Mg ha^{-1} at 151 DAE. For animal production, this investment on stems is a disadvantage since the animals prefer to feed on leaves rather than others parts of the plant. In this same date GLDM reaching 4.45 Mg ha^{-1} and TDM 22.8 Mg ha^{-1} (Figure 2C).

The inflorescence dry matter started to be registered at 97 DAE coinciding with the period when *Urochloa* reached maximum LAI of 5.80 (Figure 2B and C), suggesting preferential investment in the formation of reproductive organs after 82 DAE. The IDM continued showing a linear increase until 151 DAE. The decrease of LAI after 97 DAE might be explained by leaf self-shading, which reduces light penetration cross the crop canopy profile. Light reduction might lead to an unbalance between photosynthesis and respiration with a lower gain of carbon compared to the loss of carbon in the respiratory process triggering the senescence of leaves. Corroborating this idea, it was shown recently that there is a convergence of the two most important factors expected to trigger the leaf senescence, which is the absence of light and the presence of ethylene. Leaf senescence might also involve the transcription of a gene related to phytochrome (PIFs), and ethylene production (EIN3) (Jeong et al., 2016).

Under favorable environmental conditions, LAI increases with plant age, consequently increasing CGR (Silva and Pedreira, 1997). Nevertheless, it is important to emphasize that besides LAI, NAR also plays an important role in CGR performance. The comparative analysis of Figure 2B and D suggest that the increase observed in LAI between 39 and 70 DAE, simultaneously with an almost constant NAR, favored the increase in CGR (Figure 2B), which reached a maximum of 0.260 Mg ha^{-1} day^{-1} at 70 DAE. However, after 70 DAE, when CGR was maximum and LAI was considered optimum, CGR started to decrease and reached a very low value (0.079 Mg ha^{-1} day^{-1}) at 117 DAE, the same occurring to NAR.

When the crop reached optimum LAI, the stratum in the bottom of the canopy were progressively shadowed leading to a less effective CO_2 exchange and causing an inflection in the growth curve (Table 1 and Figure 2C).

Thus, high LAI causes low NAR (Figure 2D) due to self-shadowing (Portes and Castro Jr, 1991; Silva and Pedreira, 1997; Portes and Melo, 2014). But after the lodging of grass plants, in the second part of the experiment, TAL started to grow again resulting in an increase in the TDM, since this variable increased continually up to a maximum of 22.60 Mg ha^{-1} at 151 DAE (Figure 2C).

The NAR values shown in Figure 2D are 7.8, 7.40, 6.90, 5.50, 4.10, 2.40, 1.60 and 13.10 g m^{-2} day^{-1} observed at 39, 47, 55, 70, 82, 97, 117 and 151 DAE respectively. Used in the equation presented in material and methods, which transforms NAR (g m^{-2} day^{-1}) in photosynthetic rates (μmol CO_2 m^{-2} s^{-1}), the values of photosynthesis obtained would be: 3.80, 3.57, 3.32, 2.65, 1.97, 1.14, 0.78 and 6.33 μmol CO_2 m^{-2} s^{-1}. These values are very low when compared to net photosynthesis rates measured by IRGA equipment (infrared gas analysis). For example, in full sunlight conditions, a rate up to 42 μmol of CO_2 m^{-2} s^{-1} was found for *U. brizantha* (Dias-Filho, 2002). However, the values calculated for the net assimilate rates are averages of a 24 h-period (e.g. considering the periods of day and night). Thus, during the night there is a considerable loss of CO_2 by respiration, when the gain of CO_2 by photosynthesis is zero. Then, considering the self-shading during the day and the loss of CO_2 during the night, the values found here are acceptable. The amount of essential nutrients from the soil (that is, ash) that make up the organic matter ranges from 1 to 5% (Walker, 1992) and because it is a relatively small amount it was disregarded in the study.

The lodging of plant canopies by wind allowed the solar radiation to reach the inferior strata favoring a sudden increase in NAR (Figure 2D), resulting in an increase in TDM as well as in its components; the culms and the leaf dry matter (Figure 2C). Consequently, there was also an increase in CGR, which reached a maximum of 0.751 Mg ha^{-1} day^{-1} in the same period (Figure 2B). The LAI values did not show such an expressive increase in the same period, suggesting that NAR may contribute to crop growth more than LAI due to increasing light penetration after the lodging (Figure 2C).

As CGR estimate does not take into account the dry matter already present in the plant, the relative growth rate (RGR) better expresses the evolution of crop growth. The RGR of *Urochloa* decreased continually from 39 DAE until it reached values close to zero at 117 DAE, following the decrease of LAR (Figure 3A and B).

As found for other crops (Gomide and Gomide, 1999), the RGR decreased with the grass age (Figure 3A) and increased after 117 DAE due to the sprouting of news culms after the lodging of plants. The same occurred for NAR (Figure 2D), which presented high values in the

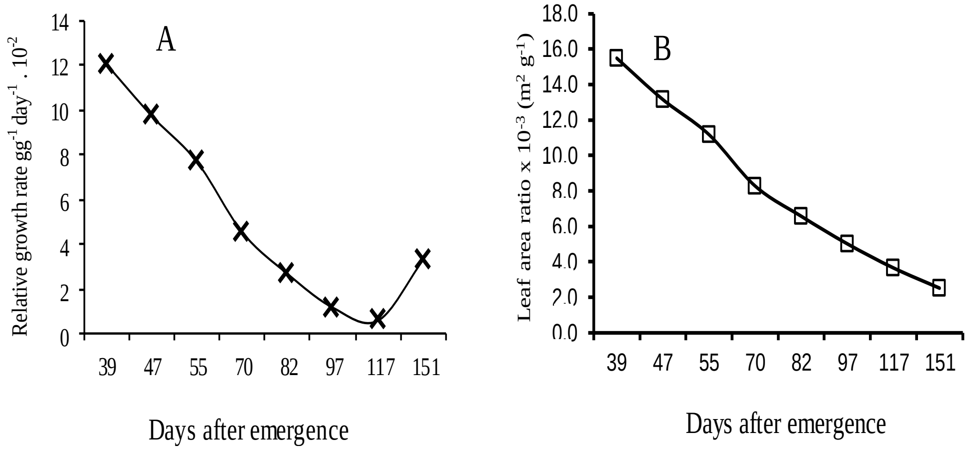

Figure 3. A: Relative growth rate (RGR); B: Leaf area ratio (LAR) of *U. brizantha* cv. Marandu cultivated under Brazilian Cerrado conditions.

initial period due to higher leaf formation and low self-shadowing. This is an evidence that there is an initial investment in broader LAR (Figure 3B) and, consequently, higher NAR (Figure 2C).

As the plant grows, LAR declines due to self-shadowing, enhancing the interference of the upper leaves in the inferior ones (Benincasa, 1988). In the specific case of *Urochloa*, LAR decreased significantly until 151 DAE (Figure 3B). The reduction of LAR, associated to leaf senescence promoted by self-shadowing as well as by internal factors, contributed to the low values of RGR at 117 DAE.

It was expected that any effects on RGR might be interpreted as effects either on NAR or LAR, or on both (Portes and Castro Jr, 1991). Even when NAR values were almost constant between 39 and 70 DAE (Figure 2D), RGR continued to decrease. The continuous decreasing on RGR demonstrates a strong influence of LAR on RGR. So even when there is an increase in dry matter per leaf (efficient NAR) during this period, the useful leaf area for photosynthesis (LAR) is small due to self-shadowing, which explains the continuous decrease in LAR. Thus, the non-shadowed leaves are efficient in their role as “sources”, maintaining CGR. The influence of NAR over RGR was only perceptible after 70 DAE, when the decrease of NAR favored the decrease in RGR even more. It is important to observe that RGR increased again along with NAR as a consequence of the lodging between 117 and 151 DAE (Figure 3A).

The carbohydrate allocation process occurring on the leaves as a result of the photosynthesis is vital for the plant correct functioning as well as for its growth. The carbohydrate allocation also performs a critical role in grasses since there is a continuous removal of the “unit” of production, the leaves (Silva and Pedreira, 1997). The preference to allocate photoassimilates varies according to the crop phase. In general, during the initial growth phase, the leaves have a higher strength to drain photoassimilates compared to the stems or roots, but the inflorescences predominate as a drain during the reproductive phase (Snyder and Carlson, 1984). During the exponential phase of growth, the stems have priority to receive photoassimilates, whereas the roots are the strongest drain when this stage ceases (Snyder and Carlson, 1984).

Besides the exponential phase, another factor that interferes in the allocation process is grazing. The meristematic tissues need the energy derived from photosynthesis to support continuous growth and to regenerate the parts of the plant that are removed. This can be explained since the photoassimilate flux towards the roots after leaf removal is minimum or non-existent and a flux in the opposite direction prevails (Silva and Pedreira, 1997).

The allocation and partitioning of photosynthate and tillers formation are highly regulated by the hormonal balance in plants. In rice and wheat auxin inhibits axillary buds growth and consequently the tillers differently of the cytokinin which stimulate the lateral bud outgrowth as a result of apical dominance inhibition induced by auxin (Chatfield et al., 2000, Liu et al., 2011). In field conditions, it is expected that cattle grazing eliminates the aerial part of young plants favoring the action of cytokinins, which is highly produced in roots overcoming the apical dominance, and increasing the tillers growth (Koa et al., 2014).

Urochloa DLDM production (Figure 4) started prematurely at 55 DAE, even before the crop reached optimum LAI (Figure 2B), which occurred at 70 DAE. A premature DLDM production reinforces the idea that, in this period, leaf senescence was promoted by internal factors, since theoretically self-shadowing just starts

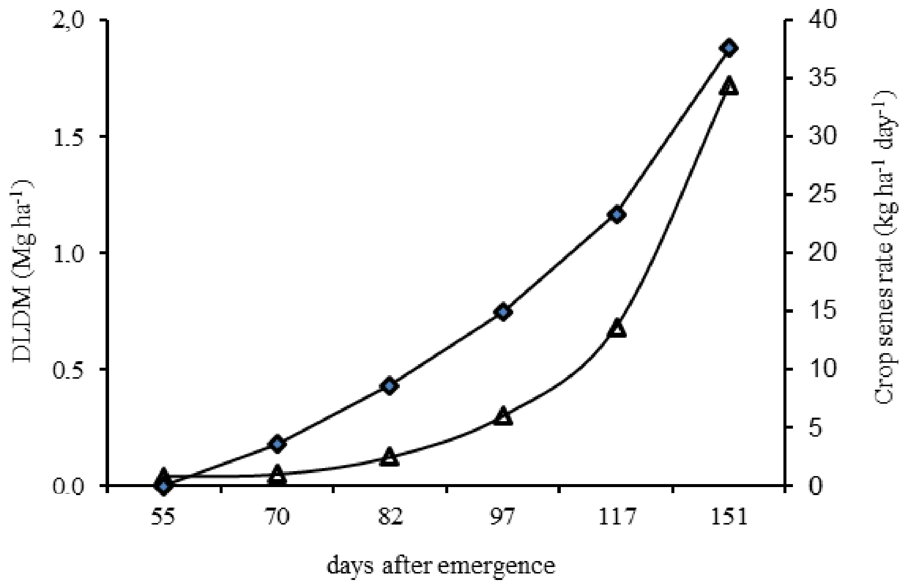

Figure 4. Dead leaf dry matter (DLDM = ◊), Crop senescence rate (CSR = ∆) of *U. brizantha* cv. Marandu cultivated under Brazilian Cerrado conditions.

when the crop reaches optimum LAI. According to Corsi and Nascimento Jr (1994), leaf senescence might occur due to factors such as shadowing, water stress, insects attacks, diseases, grazing intensity, nutritional insufficiency such as nitrogen and phosphorus, and, evidently, genetic control, a characteristic of each species.

The DLDM values also started to increase sharply at 82 DAE (Figure 4). These results emphasize the importance of adequate grazing management, because while the animals feed on the leaves, the grass produces new sprouts.

An initial production of 0.042 Mg ha^{-1} of dead leaves was registered, but it increased significantly between 117 and 151 DAE due to the lodging, reaching a maximum value of 1.719 Mg ha^{-1} at 151 DAE (Figure 4). Consequently, CSR (Figure 4) followed the values of DLDM, progressively increasing from zero value at 55 DAE up to a maximum of 0.038 Mg ha^{-1}day^{-1} at 151 DAE, when DLDM was also maximum (Figure 4). Nevertheless, when GLDM production is compared to DLDM (Figure 2C and 4), it is possible to conclude that even with the death of older leaves the production of green leaves was predominant. This suggests a continuous photosynthetic activity and, therefore, almost constant NAR until 70 DAE (Figure 2D), which would be capable of maintaining CGR in the same period (Figure 2B).

Conclusion

The maximum leaf area index (5.80 m^{2} m^{-2}) was achieved at 82 days after emergence (DAE), maximum above ground total dry matter (22.6 Mg ha^{-1}) at 151 days, and maximum crop growth rate (0.260 Mg ha^{-1}) at 70 days. Leaf senescence began at 55 DAE, with less than 0.001 Mg ha^{-1} of dead leaves dry mass and reached 1.719 Mg ha^{-1} at 151 DAE. Between 117 and 151 DAE, at the end of plant cycle, the wind caused lodging of plants and this favored a sudden increase in net assimilate rate (NAR), resulting in an increase in the crop growth rate (CGR) and total dry matter (TDM). After 82 DAE, with a maximum LAI, *Urochloa* starts investing at forming the reproductive structures (inflorescences), thus characterizing the beginning of the crop reproductive phase. The premature registering of dead leaf dry matter (DLDM) and, consequently, the Crop Senescence Rate (CSR) at 55 DAE, even before the crop reaches optimum LAI, reinforces the idea that during this phase senescence is promoted mainly by internal factors. In this paper it was adapted and presented, by the first time in the literature, an equation that estimates the net photosynthetic rate in μmol CO_2 m^{-2} s^{-1} from net assimilation rate data in g m^{-2} day^{-1}.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of row spatial arrangements on agromorphological responses of maize (*Zea mays* L.) and cowpea [*Vigna unguiculata* (L.) Walp] in an intercropping system in Southern Cote d'Ivoire

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Field trials were conducted at the University Nangui Abrogoua, Abidjan, southern Cote d'Ivoire during the 2015 and 2016 short rainy season to study the performance of maize (*Zea mays* L.) and cowpea [*Vigna unguiculata* (L.) Walp] intercrop as influenced by row arrangement. A randomized complete block design (RCBD) with five treatments (SM; SC; MC; 1M1C and 2M4C) and three replications was used. SM was sole maize, SC was sole cowpea, MC was intra-line pattern, 1M:1C was inter-line pattern and 2M4C was strip-intercropping maize-cowpea. The results revealed that there were significant effects of cropping patterns on growth and yield components of maize and cowpea crops. Among the cropping systems studied during both years growing seasons 2015 and 2016, the highest yield advantage for cowpea and maize (land equivalent ratio = 1.62; 1.10) was obtained from intercropping pattern 2M:C4. The observed Land equivalent ratio (LER) values correspond to 38.27 and 9.90 % of lands saved which could be used for other agricultural purposes. With this cropping pattern, land equivalent coefficient (LEC) values (0.62 and 0.30) indicated that, greatest productivity per unit area was achieved by growing the two crops together. In this cropping pattern, both crops were highly complementary and most suitable in mixture as confirmed by competitive ratio (CR) values of 0.72.

Key words: Crop row arrangement, yield component, maize, cowpea, system productivity.

INTRODUCTION

During the recent decades, food requirements have increased sharply, while the availability of cultivated land has decreased considerably. Thus, the increase in yield per unit area remains the main way of increasing agricultural production. Due to this high pressure of food, agriculture is now intensive with high inputs in irrigation, seeds and chemicals. This has caused serious environmental problems (Zhang et al., 2004), including groundwater pollution by soil nitrates (Ju et al., 2006), the emission of gases into the air (Zhang et al., 2012) and soil acidification (Blumenberg et al., 2014). To ensure food security and environmental quality, it is essential to search best management practices which involve suitable cropping systems that can efficiently utilize solar and soil resources with minimum nutrient inputs.

In cropping systems, there are often two or more crop species grown in the same field for a certain period of time, even though the crops are not necessarily sown or harvested simultaneously. In practice, most intercropping systems involve only two crops, as inclusion of more crops results in higher labor costs (Wu and Wu, 2014).

Traditionally, small farmers used intercropping to increase the density of their products and to ensure the stability of their output. The success of intercropping systems is due to an enhanced temporal and spatial complementarity of resource capture, for which both above-ground and belowground parts of crops play an important role (Midega et al., 2014).

Cereal crops intercropping with legumes are a popular option in intercropping. Even though the two crops compete for soil N as they both need it for the growth, the competition drives legumes to fix atmospheric N_2 in symbiosis with Rhizobium (Caviglia et al., 2011). This actually results in complementary utilization of N by the crops, which is of particular importance in soils where inorganic N is limited or over-fertilized.

Numerous studies have reported that intercropping can increase crop yield (Zhang et al., 2007) because of efficient utilization of nutrients (Zhang and Li, 2003) and light (Zuo and Zhang, 2008), and enhanced positive interactions between crops (Betencourt et al., 2012). However, most of these studies were focused on effects of different intercrop species (Zhang and Li, 2003). Despite the importance of intercropping, very few reports are found in the literature concerning the effect of the pattern of intercropping system on the growth parameters and productivity of the component species. The available data refer mainly to plant resources availability, planting date (Maurice et al., 2010) and plant density (Ewansiha et al., 2015). Thus, rare studies have been undergone to investigate effects of ratio of rows between crops within a specific intercropping system.

Row arrangement is one of the important management tools that could be explored to minimize competitive pressure created by a component crop in an intercropping system (Maluleke et al., 2004).

The management of mixed crops in the traditional agricultural systems of Cote d'Ivoire is unpredictable and without sufficient attempt to model crops for an effective interception of essential resources. There is neither report in literature about optimum row ratio of maize intercropping with cowpea, nor explanation of the processes behind. Therefore, the objective of this study was to investigate the effects of row arrangement on the growth and yield of the maize and cowpea crops, in intercropping trials at University Nangui Abrogoua. Information on these subjects is essential for a better understanding of the system that will help management decisions.

MATERIALS AND METHODS

Description of the study site

The experiment was conducted during the 2015 and 2016 short rainy season at the research farm of the Natural Sciences Unit of Formation and Research (UFR SN), University Nangui Abrogoua, (5°23 N latitude, 4°11 W longitude and 100 m above the sea) in the southern of Cote d'Ivoire. The site is characterized by two rainy seasons and dry seasons.

The first and second growing seasons (herewith referred to as long and short rainy season, respectively) last typically from March to July and October to November, respectively. A short dry spell occurs from August to September. The major dry season starts in December and lasts through end of February or beginning of March. The total rainfall registered was 2161.86 and 1433.34 mm and the average annual temperature was 26.7 and 27°C in 2015 and 2016, respectively (Figure 1).

Plant material, experimental design and treatments

Maize cultivar "EV8728" (early-maturing, 90 to100 days) and cowpea cultivar "Touba" (early maturing, erected) were used in this study. Both seeds were obtained from the National Center of Agronomic Research (CNRA), Korhogo Regional Office (Cote d'Ivoire). Experiments were carried out from September to December 2015 and 2016 and were arranged each year in randomized complete blocks design (RCBD) with three replications which included five treatments with different planting patterns. A constant 75 x 50 cm inter and intra-row spacing, respectively, was maintained in both cropping systems. Experimental plots used for this study were 15 m^2 (3 × 5 m) sizes each.

The treatments were: maize and cowpea in sole culture (SM, SC) population (49231 plants/ha), maize and cowpea in intra-row pattern M:C (maize holes alternating with those of cowpea) population (24615 plants/ha), maize and cowpea in single-line pattern 1M:1C (a line of maize alternating with a cowpea line) population (24615 plants/ha) and strip-intercropping maize-cowpea 2M:4C (2 rows of maize alternating with 4 rows of cowpea) population (19692 maize plants/ha and 29538 cowpea plants/ha). Before the start of the study in September 2015, soil was sampled randomly in the field by using a soil auger to determine basic

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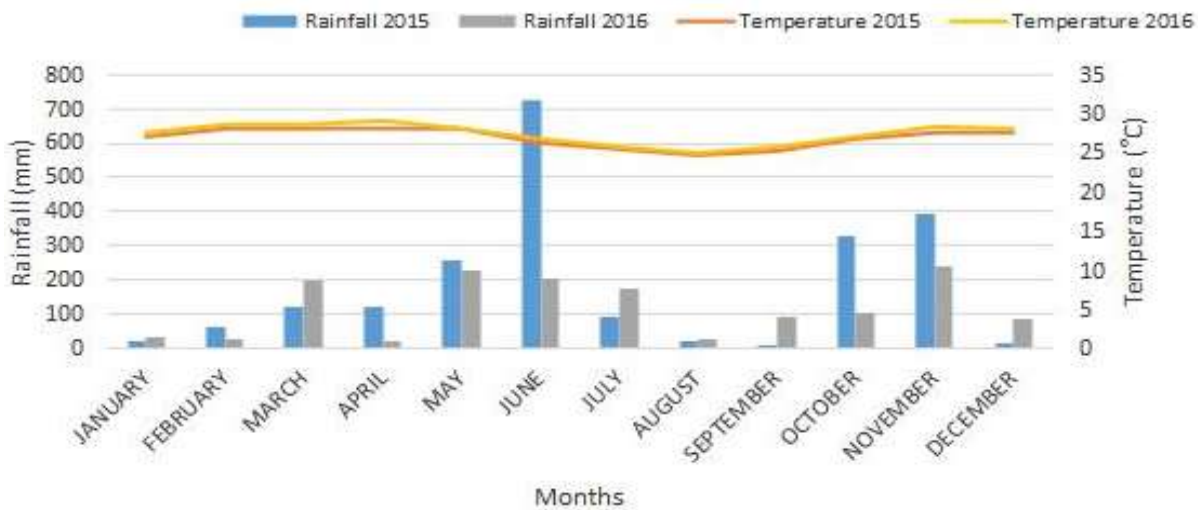

Figure 1. Monthly precipitation (bar) and air temperature (curve) of the experimental site in 2015 and 2016 (Source: www.tutiempo.net)

physical and chemical properties of soil.

Agronomic practices

Three maize seeds were sown per hole and later thinned to one plant per stand at two weeks after planting (WAP). Cowpea seeds were sown at six weeks after maize was sown. Four seeds of cowpea were sown and later thinned to two plants per stand at two WAP. Cowpea was harvested when the pods turned brown and seeds were at the hard-dough stage, which indicated that moisture content is between 14 and 16% (Dugje et al., 2009). Maize was harvested when the signs of senescence appeared at cob maturity (Ijoyah and Jimba, 2012).

Data collection and analysis

Measurement of plant growth parameters and yield and yield components

To determine the response of maize and cowpea crop in row arrangement intercropping, data were collected on growth parameters, yield and yield components, vis., plant height (PH), stem diameter (SD), leaf area (LA), total above-ground biomass (TB), 100-seeds weight (100 SW) and total grain yields (TGY).

Leaf area of cowpea (LAC) was calculated by the formula: $0.662 \times L^{0.952} \times l^{1.052}$ (Dagba, 1974). Leaf area of maize (LAm) was calculated by the formula: L x l x 0.75 (Bonhomme et al., 1982).

Assessment of the advantages of maize/cowpea intercropping system

Land equivalent ratio: Land equivalent ratio (LER) is the most common index adopted in intercropping to measure the land productivity. The LER is a standardized index that is defined as the relative area required by sole crops to produce the same yield as intercrops (Mead and Willey, 1980). The LER is the ratio of land required by pure (sole) crop to produce the same yield, where intercrop was determined according to the following formula:

LER = Ym-ic/Ym-sc + Yn-ic/Yn-sc
Ym-ic = intercropped yield of maize;
Ym-sc = sole yield of maize;
Yn-ic = intercropped yield of cowpea;
Yn-sc = sole yield of cowpea;

If LER is greater than 1.00, there is a yield advantage by intercropping; otherwise there is no yield advantage. The data on land equivalent coefficient (LEC) and percentage land saved were determined as described by Ijoyah et al. (2013) and Workayehu (2014) using the formulae below:

LEC = LERm × LERc

Where LERm is the partial LER of maize and LERc is the partial LER of cowpea

Land saved (%) =100 − (1/LER×100)

The competitive ratio (CR) as described by Willey and Rao (1980) was determined using the formula: CR = Lm/Lc, where Lm: Partial LER for maize; Lc: Partial LER for cowpea. Data collected were subjected to analysis of variance (ANOVA) using the software Statistica version 7.1. Differences between treatment means were separated using the Newman-Keuls test procedure at 95% confidence interval.

RESULTS

Physico-chemical characteristics of the soil of the experiment site

Physico-chemical analysis of the soil samples collected

Table 1. Physical and chemical properties of the experimental soil.

Components	values
pH	4.83
N(%)	0.37
NO_3 (méq/100g)	0.05
C (%)	1.91
K (%)	0.12
Na (%)	0.02
Ca (%)	0.02
P (%)	0.16
Mg (méq/100g)	0.26
CEC (méq/100g)	1.43

at 0 to 30 cm soil depth indicated that, the soil reaction was acidic with pH values of 4.83. In both trials Total nitrogen (N) content, organic carbon (C), potassium (K) and available phosphorus (P) were high. Cation exchange capacity (CEC) and exchangeable bases (Na, Ca and Mg) were low (Table 1).

Effect of cropping systems on the growth of maize and cowpea plants

The growth of maize and cowpea plants evaluated in terms of plant height, stem diameter and leaf area is recorded in Table 2. The analysis of the results showed that plants height of maize in sole culture during the 2015 and 2016 rainy short seasons was significantly lower than those of the plants in intercropping systems with the cowpea.

The tallest plants were observed in cropping system M: C (intra row). With cowpea, the results obtained in 2015 revealed that the plant height was increased in 1M: 1C maize: cowpea intercrops (37.46 cm) but was reduced in MC (32.50 cm) and 2M: 4C (32.33 cm) cropping patterns. In year 2016, no statistical difference was noted in the cowpea plant height under monocropping and the cropping systems 1M: 1C and 2M: 4C. The highest cowpea plant height was obtained with M: C cropping system.

During the year 2015, monocropped maize gave the same stem diameter (8.25 cm) compared to those obtained with M: C maize: cowpea intercrop (8.30 cm) and 1M: 1C maize: cowpea intercrop (8.72 cm). These values were significantly higher than the stem diameter of maize plant obtained in 2M: 4C maize: cowpea intercrops (7.44 cm). Compared to maize monoculture achieved during the year 2016, an increasing of plant stem diameter was recorded with M: C (20.53 cm); 1M: 1C (19.9 cm) and 2M: 4C (19.54 cm) cropping systems.

In both years, measurements of cowpea plant stem diameter revealed that values obtained with 1M: 1C maize: cowpea intercrops (9.09 and 6.36 cm) were statistical higher than that observed with the other cropping systems.

In contrary to 2015 where a decrease was reported in maize leaf area with the cropping systems M: C, 1M: 1C and 2M: 4C, an increase of the leaf area in these cropping systems was observed during the 2016 short rainy season, comparing to the monocropped maize. No statistical difference was noticed between these cropping systems. In cowpea, increasing of leaf area was obtained with the M: C and 1M: 1C cropping systems during the 2015 and 2016 short rainy season.

Effect of cropping systems on the yield and yield components of maize and cowpea

Yield and yield components of maize and cowpea as a sole crop and in intercrop at Abidjan, in 2015 and 2016 cropping seasons are given in Table 3. Compared to the tested cropping systems, lowest total above-ground biomass was recorded with monocropped maize (58.80 g) during 2015 growing season. In 2016, highest total above-ground biomass was obtained with M: C and 2M: 4C cropping patterns and the lowest were observed with 1M: 1C cropping pattern.

Total above-ground biomass obtained in 2015 with monocropped cowpea (23.08 g) was not statistically different to those recorded with the cropping systems M: C (24.77 g) and 2M: 4C (20.88 g). But these values were lower than that observed with 1M: 1C maize-cowpea (29.00 g). The cropping pattern 1M:1C (28.54 g) followed by 2M:4C (20.00 g) produced during 2016 short rainy season, a total above-ground biomass significantly superior than those of cowpea monoculture (19.06 g) and MC maize-cowpea intercropped (16.57 g).

Concerning 100 seeds weight, no significant difference was observed between monocropped maize (15.87 g) and the maize-cowpea cropping systems MC (14.35 g) and 2M:4C (15.71 g) in 2015. But these values were lowest than that of 1M: 1C cropping pattern (16.02 g). During this same year, an increase in one hundred seeds weight was obtained with MC (14.26 g) and 1M:1C (14.29 g) cropping systems compared to sole cowpea (13.21 g) and 2M:4C maize-cowpea intercropped (13.24 g). The cropping patterns MC (20.57 g) and 2M: 4C (20.56 g) increased maize to one hundred seeds weight during 2016 rainy season. On the other hand, no statistical difference was observed between the cropping systems tested and the cowpea monoculture.

During the 2015 and 2016 growing seasons, all the cropping systems studied, decreased the grain yield of maize and cowpea in pure culture. In 2016, planting maize and cowpea in M: C, 1M: 1C or 2M: 4C arrangement, depressed grain yield of maize by 55.30,

Table 2. Effect of cropping pattern on maize and cowpea plant height, stem diameter and leaf area during the short rainy seasons of 2015 and 2016 in Abidjan.

Cropping systems	Plant height (cm)				Stem diameter (cm)				Leaf area (cm)			
	2015		2016		2015		2016		2015		2016	
	Maize	Cowpea	Maize	Cowpea	Maize	Cowpea	Maize	Cowpea	Maize	Cowpea	Maize	Cowpea
Sole	118.75± 8.54[c]	35± 2.59[b]	102.84 ± 14.10[d]	28.95 ± 3.53[b]	8.25 ± 1.25[a]	7.64 ± 0.72[b]	16.20 ± 1.53[b]	5.37 ± 1.00[b]	283.44 ± 14.44[a]	58.41 ± 10.50[b]	318.76 ± 17.016[b]	59.93 ± 7.95[b]
M : C	140.0 ± 11.46[a]	32.50 ± 2.16[c]	131.27± 6.93[a]	32.88± 4.3[a]	8.30 ± 0.00[a]	5.226 ± 0.85[c]	20.53 ± 2.70[a]	4.897 ± 0.78[c]	219.23 ± 14.99[b]	69.41 ± 10.62[a]	447.97 ± 23.93[a]	70.67 ± 11.24[a]
1M : 1C	127.66 ± 0.5[b]	37.46± 1.11[a]	115.6± 6.51[c]	28.73 ± 3.59[b]	8.72 ± 0.91[a]	9.09 ± 0.2[a]	19.94 ± 2.1[a]	6.36 ± 1.26[a]	269.73 ± 9.48[a]	69.41 ± 5.54[a]	391.04 ± 21.3[a]	69.79 ± 4.07[a]
2M : 4C	132.66 ± 12.43[b]	32.333 ± 0.5[c]	124.32± 10.25[b]	30.02 ± 4.59[b]	7.44 ± 0.5[b]	7.92 ± 1.84[b]	19.54 ± 2.05[a]	5.39 ± 0.71[b]	189.47 ± 14.63[c]	60.01 ± 8.68[b]	416.487 ± 26.4[a]	61.696 ± 10.61[b]

In the same column, the figures followed by the same letters are statistically identical (Newman-Keuls, 5%). M: C (intra row); 1M: 1C (inter row); 2M: 4C (2 rows of maize alternating with 4 rows of cowpea).

Table 3. Effect of cropping pattern on maize and cowpea total above-ground biomass, 100-seeds weight and total grain yields during the short rainy seasons of 2015 and 2016 in Abidjan.

Cropping systems	Total above-ground biomass (g)				100-seeds weight (g)				Total grain yields (kg/ha)			
	2015		2016		2015		2016		2015		2016	
	Maize	Cowpea	Maize	Cowpea	Maize	Cowpea	Maize	Cowpea	Maize	Cowpea	Maize	Cowpea
Sole	59.92 ± 2.79[d]	23.08± 2.02[b]	57.67 ± 4.69[b]	19.06 ± 1.6[c]	15.87± 1.1[b]	13.21± 1.94[b]	16.38 ± 0.78[b]	12.26 ± 7.05[a]	180.4 ± 14.44[a]	217.69 ± 10.50[a]	500.87 ± 17.016[a]	458.57± 7.95[a]
M : C	86.45 ± 0.87[c]	24.77± 2.10[b]	74.5± 12.19[a]	16.57± 0.91[c]	14.35 ± 1.33[b]	14.26± 1.39[a]	20.57± 2.39[a]	11.76± 0.95[a]	139.40 ± 0.1[b]	172.15 ± 3.00[b]	223.88± 10.26[b]	228.07± 37[d]
1M : 1C	96.04 ± 2.5[b]	29.00± 4.8[a]	48.79± 5.73[c]	28.54± 1.26[a]	16.02± 1.06[a]	14.29± 1.32[a]	15.5± 0.83[b]	12.77 ± 1.05[a]	85.21 ± 3.00[c]	164.3 ± 17.95[b]	181.89± 17.03[c]	261.01± 16.36[c]
2M : 4C	106.39 ± 5.20[a]	20.88 ± 10.53[b]	72.94± 5.62[a]	20.00± 2.7[b]	15.71 ± 0.61[b]	13.24 ± 2.83[b]	20.56± 3.65[a]	11.53± 1.14[a]	182.18 ± 6.00[a]	134.35 ± 7.65[c]	231.22± 10.6[b]	292.78 ± 32.13[b]

In the same column, the figures followed by the same letters are statistically identical (Newman-Keuls, 5%). M: C (intra row); 1M: 1C (inter row); 2M: 4C (2 rows of maize alternating with 4 rows of cowpea).

Table 4. Land productivity potential in maize-cowpea intercropping during the 2015 and 2016 short rainy seasons in Abidjan.

Cropping systems	Total grain yields (kg/ha)		LERm		LERc		LER		LEC		CR		Land saved (%)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Sole maize	180.4	500.87	-	-	-	-	-	-	-	-	-	-	-	-
Sole cowpea	217.69	458.57	-	-	-	-	-	-	-	-	-	-	-	-
Maize M:C	139.40	223.88	0.77(49%)	0.47(48.45%)	-	-	-	-	-	-	-	-	-	-
Maize1M:1C	85.21	181.89	0.47(38.52%)	0.36(38.71%)	-	-	-	-	-	-	-	-	-	-
Maize 2M:4C	182.18	231.22	1.00(61.73%)	0.46(41.81%)	-	-	-	-	-	-	-	-	-	-
Cowpea M:C	172.15	228.07	-	-	0.80(50.95%)	0.50(51.54%)	-	-	-	-	-	-	-	-
Cowpea 1M:1C	164.3	261.01	-	-	0.75(61.47%)	0.57(61.63%)	-	-	-	-	-	-	-	-
Cowpea 2M:4C	134.35	292.78	-	-	0.62(38.2 %)	0.64(58.18%)	-	-	-	-	-	-	-	-
M:C	-	-	-	-	-	-	1.57	0.97	0.61	0.23	0.96	0.94	36.30	-3.1
1M:1C	-	-	-	-	-	-	1.22	0.93	0.35	0.20	0.62	0.20	18.03	-7.5
2M:4C	-	-	-	-	-	-	1.62	1.10	0.62	0.30	1.61	0.72	38.27	9.90

63.68 and 53.83%, respectively, and cowpea yield by 50.26, 43.08 and 36.15%, respectively.

Productivity of Maize-Cowpea Intercropping

The results of the effect of intercropping systems on land productivity potential are presented in Table 4. Analysis of these results has shown that in year 2015, the cropping systems (maize-cowpea) namely, MC, 1M1C and 2M: 4C gave respectively LER values of 1.57, 1.22 and 1.62. With these LER values, 36.30, 18.03 and 38.27% of lands were saved, which could be used for other agricultural purposes.

In year 2016, a reduction of the LER was observed with the values of 0.97, 0.93 and 1.10, respectively with MC, 1M1C and 2M: 4C cropping systems. Among these cropping systems, land was only saved (9.90%) with 2M: 4C intercropped maize-cowpea.

For year 2015, LEC values of 0.61, 0.35 and 0.62 were obtained in MC, 1M1C and 2M: 4C cropping systems, respectively. These values of LEC decreased in year 2016 to 0.23, 0.20 and 0.30, respectively for MC, 1M1C and 2M: 4C cropping systems

The CR between the two crops when inter-cropped during 2015 short rainy season were 0.96, 0.62 and 1.61 in MC, 1M: 1C and 2M: C cropping patterns, respectively. In year 2016, these values were 0.94, 0.20 and 0.72.

DISCUSSION

Intercropping is one of the most common practices used in sustainable agricultural system which has an important role in increasing the productivity and stability of yield, in order to improve resource utilization and environmental factors (Qin et al., 2013).

In this study, growth, yield components and system productivity of maize and cowpea were estimated in a cropping patterns consisted of MC (intra row), 1M: 1C (inter row) and 2M: 4C (strip intercropping).

Effect of cropping systems on the growth of maize and cowpea plants

In our experimental conditions, growth parameters evaluated in the different cropping systems during the cropping season 2015 were higher than those for 2016. On the other hand, yield parameters recorded during the cropping season 2016 were higher than those in 2015.

These interactions might have been due to the differences in rainfall both in the amount and distribution between the two years, where there was a high amount of rainfall from October to November in 2015 compared to 2016. The rainfall differences may have caused cross ranking of maize and cowpea populations and cropping patterns between the two years. Moreover, the year-to year variation in temperature may have influenced cropping systems performance in the two different years. The differences observed in 1000-seed weight between the two cropping seasons could be attributed to the role of environment during seed development (Tang, 1982).

During the 2015 and 2016 short rainy seasons in Abidjan, the tallest mean plant height was observed from intercropped compared to monocropping maize and cowpea. This justifies the assertion that as the intra and inter row competition increases; so does the height of the plant linearly due to competition of natural resources. The height of maize plant under intercropping system was more than that in the sole maize due to competition of associated crops for intercepted light intensity, therefore leads to an increase in maize plant (Hamd Alla et al., 2014).

In line with this study, Lulie et al. (2016) reported that intercropping increased the plant height of maize as compared to monocropping. In contrary to our findings, Lemlem (2003) found that intercropping maize with cowpea reduced maize plant height as determined by environmental factors and competition between the two crops.

To our knowledge, leaf area (LA) is an important agronomic parameter which reflects crop growth and has great influence on crop yields (Fageria et al., 2006). The results on leaf area of maize plant in cropping systems compared to monoculture indicated a decrease and an increase, respectively during the cropping seasons 2015 and 2016. In cowpea, increasing of leaf area was obtained with the cropping systems during the 2015 and 2016 short rainy season. These results revealed that LA was affected by intercropping. Similar results were reported in intercropping Sorghum and Bambara groundnut (Karikari, 2000).

During the year 2015, intercropping decreases the leaf area of the maize crop; this is because, this could be influenced by high competition of the component crops and shading effect of maize over cowpea that leads to decreased photosynthetic capacity of the crops (Ali et al., 2003). Such a severe impact of intercropping on LA could be one of the major factors for the low yield, recorded by the cowpea component. The increasing LA of cowpea plant when intercrop with maize could be explained by the fact that, plants with different heights make more use of light when intercropped than when monocropped. The spatial advantage was due to the differences in the height of the cowpea and maize.

Effect of cropping systems on the yield and yield components of maize and cowpea

Above-ground, total biomass of maize varied depending on the cropping system. Generally, the resulted obtained in this study revealed that total above-ground biomass in cropping system was higher than that observed in sole culture. Contrary to our results, a decrease in total plant biomass of maize under maize/cowpea intercropping had been reported by Egbe et al. (2010).

One hundred seeds weight varied depend on the cropping patterns. Highest one hundred seeds weight was obtained with 1M: 1C cropping pattern in 2015. Singh et al. (2000) reported that 100 grain weight of maize was also increased by intercropping with legumes. On the other hand, there were no significant differences in the weight of one hundred seeds weight between treatments. This is in agreement with Chakma et al. (2011) who observed no significant difference in weight of 100 seed weight in a popcorn-mungbean/cowpea intercropping system.

All the cropping systems tested during this study decreased the grain yield of maize and cowpea in pure culture. The maximum grain yield was obtained from sole cropping system of maize and cowpea while the lower grain yield was maintained for intercropped maize-cowpea. This suggests lower intra-specific competition of sole maize and sole cowpea for natural resources (light, water and nutrients) compared to maize intercropped with cowpea.

Yield reduction in an intercropping could be due to a more extensive root system; particularly a larger mass of fine roots of maize which compete more for soil nutrients. Kheroar and Patra (2014), in line with this finding, reported that yield of intercrops were reduced by intercropping with maize that was caused due to receipt of lower amount of solar radiation.

The reduction in seed yield by intercropping could be due to interspecific competition and depressive effect of maize, as C4 species on cowpea as C3 crop. Crops with C4 photosynthetic pathways such as maize have been known to be dominant when intercropped with C3 crops like cowpea.

System productivity

The productivity of intercropping maize with cowpea in the present study was assessed using LER and related attributes described in previous sections. According to Workayehu (2014), when LER < 1 there is obvious disadvantage of the intercropping and the available resources are used more efficiently by the sole crop than may be used by the intercrop. In addition, Mariotti Ariotti et al. (2006) and Kitonyo et al. (2013) stressed that when LER = 1 there is no advantage or disadvantage of the intercropping in respect to sole crop but when LER > 1, an intercropping warrants an advantage in terms of the improved use of available resources for plant growth and development.

During the cropping season 2015, the total LER of the different cropping systems were greater than 1 which shows an advantage of intercropping maize with cowpea compared with growing each crop. In other words, these results signify that it is advantageous having both crops in mixture than growing them separately. This could be due to greater efficiency of resource utilization in intercropping.

The highest yield advantage for cowpea and maize (LER=1.62) was obtained from intercropping pattern 2M: C4. The observed LER values correspond to 36.30, 18.03 and 38.27% of lands saved, could be used for other agricultural purposes. Similar to the results obtained in 2015 growing season, Ijoyah et al. (2013) found that 28.6 and 22.5% of lands were saved in two separate growing seasons of intercrops suggesting that these saved lands could be used for other production activities. According to Matusso et al. (2014), one of the most important reasons for intercropping is to ensure that an increased and diverse productivity per unit area is obtained compared to sole cropping.

In year 2016, a reduction of the LER was observed with the MC, 1M1C cropping systems with values < 1. LER values less than 1.0 indicate that there was an intercropping disadvantage at the MC and 1M1Ccroppins patterns, presumably due to high intra and inter specific competitions in the maize/cowpea intercropping. Thus, the available resources are used more efficiently by the sole crop than may be used by these intercropping systems. The LER reduction observed with the MC, 1M1C cropping systems would be linked to insufficient rainfall during the year 2016. However, the lower LER obtained in 2016 could also be explained by the findings of Ofori and Stem (1986) who reported that light is the most important factor determining LER of maize and soybean intercropping and LER declines, when legume becomes severely shaded.

In 2015, LEC values of 0.61, 0.35 and 0.62 were obtained in MC, 1M1C and 2M: 4C cropping systems, respectively. These values of LEC decreased in year 2016 to 0.23, 0.20 and 0.30, respectively for the MC, 1M1C and 2M: 4C cropping systems. LEC values which exceeded 0.25 indicate yield advantage of the intercropping systems MC, 1M1C and 2M: 4C during the same year.

But in 2016, yield advantage was only obtained with the 2M4C cropping system. Thus, the LER and LEC values obtained during both years 2015 and 2016, indicate that greatest productivity per unit area was achieved by growing the two crops together in 2M: 4C cropping system. In this cropping pattern, both crops were highly complementary and most suitable in mixture.

Willey and Rao (1980) suggested CR instead of “aggressivity” to indicate the degree that one species competes with the other in an intercrop system. The CR represents the ratio of individual LERs of the two intercropped components. The CR between the two crops when intercropped during 2015 short rainy season were 0.96, 0.62 and 1.61 in MC, 1M: 1C and 2M: C cropping patterns, respectively. In year 2016, these values were 0.94, 0.20 and 0.72, respectively. Excepted the cropping pattern 2M: 4C in 2015, CRs in this study were less than 1 in all intercrop treatments in both years. This index measures the existence of a yield advantage, such that if the competitive ratio is less than 1, then there is an advantage in intercropping (Reddy and Willey, 1981). Thus in this study, all intercropping patterns were advantageous over sole cropping.

Conclusion

For farmers who have limited sources, income and stability yield of agricultural systems is very important. Several crops grown together, fail to produce a product, could be compensated by other crop, and thereby reduce risk. Risk of agronomy, failure in multi cropping systems is lower than pure cropping systems. The results reported in this study showed that the optimal intercropping system was strip intercropping of 2 maize rows with 4 cowpea rows (2M: 4C), which had positive effects on yield and environment.

In general, even though yields of the intercrop components were lower than their sole crop counterparts, the intercrop components were more productive than the sole crop components as evidenced by the LERs and the land saved is obtained with the 2M: 4C cropping system. It can be concluded that in Abidjan a location within the Forest agro-ecological zone of Cote d’Ivoire, for higher yield, maize should be introduced with cowpea using cropping pattern 2M: 4C. It is however suggested that further investigation could be conducted across different agro ecological zones of Cote d’Ivoire.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of seeds pretreatment and storage on improvement of the germination and emergence of *Anthyllis cytisoïdes* L.

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***Anthyllis cytisoïdes* L. is a species of shrubby legume that is a member of the Fabaceae family and grows in zones located along the coast, near the Mediterranean Sea. This shrubby forage species is considered a good aliment source for some ruminants. Pretreatment seeds germination and their viability after storage for long periods of time were tested. Pretreatment conditions were as follows: (T1) seeds were soaked in running water for 48 h; (T2) seeds were lightly scarred; (T3) seeds were strongly scarred; (T4) seeds were soaked in running water for 24 h; (T5) seeds were soaked in hot water for 10 min and (T6) seed coats were removed. Each of these pretreatments was compared with the control (seeds without any pretreatment). As for viability after long storage periods, seeds from lots collected in four different years (2006, 2008, 2009 and 2010) were used. Results show that T6 was the best seed pretreatment, with an average germination rate of 69% followed by T5 pretreatment with an average germination rate of 23%. The other pretreatments had very low germination rates. The viability of seeds from 2008, 2009 and 2010 were 18.0, 20.5 and 19.7%, respectively. On the other hand, seeds from 2006 had a very low germination rate (12.7%). These results provide useful information on crop multiplication in nurseries and transplantation of *A. cytisoïdes* L. in the field.**

Key words: *Anthyllis cytisoïdes* L., germination, scarification, seed pretreatment, viability.

INTRODUCTION

Anthyllis cytisoïdes L. is a shrubby species belonging to Fabaceae family that can grow to a height of 1.5 m. This species can be found in Mediterranean regions, especially along the coast, North of Morocco, and on its way towards extinction, and is becoming increasingly rare. Most of the natural regeneration of the species occurs

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along roads (Al Hoceima city [lat. 35°15′05″N, long. 3°56′14″W] to Nador city [lat. 35°10'7.02"N, long. 2°55'39.3"W]; Al Hoceima to Taza [lat. 34°12'38.02"N, long. 3°59'52.97"W]; Oujda [lat. 34°40′53″N, long. 1°54′30″W] to Taza city) or along rivers. In this region, planting of other species like *Atriplex nummularia* promotes regeneration of *A. cytisoïdes* L. because the former prevents rain water from running off slopes. The water retained by *A. nummularia* induces germination of *A. cytisoïdes* L. seeds after their imbibition. Moreover, *A. cytisoïdes* L. can grow in different types of soil, preferably in plowed fields and/or in fallow land (Robledo et al., 1990). *Anthyllis* is a great pastoral interest and is highly nutritious for livestock (Selmi and Ferchichi, 2015). It is also of great ecological importance and plays a special role in improving soil fertility (Boza et al., 1988; Moreno Rios et al., 1985). Legumes are interesting to research on because of their adaptation to arid and semi-arid environments, their capacity to grow in poor soil, their quality as forage crops for livestock, and their ease of multiplication and natural dispersion of seeds. Because soil erosion and degradation processes currently affect much of the Mediterranean coastline, there is an urgent need to take action regarding the conservation of natural resources, regeneration and improving the fragile ecosystems in arid and semi-arid regions. To accomplish this, it would be important to rehabilitate *A. cytisoïdes* L., which is currently in danger of disappearing because of its extensive use in eastern Morocco. Rehabilitation of certain indigenous legumes, including *A. cytisoïdes* L. and *Retama sphaerocarpa* Boiss., has been the focus of several recent research studies. Many authors mentioned that legumes often have impermeable integuments that induce physiological dormancy and make germination of their seeds very difficult (Jayasuriya et al., 2013). For this reason, it is important to identify and investigate the endogenous and exogenous factors that influence the normal emergence of stored legume seeds. Seed lots with high initial viability have a higher germination capacity than seed lots with low initial viability. *A. cytisoïdes* L. seeds have an anatomical structure typical of legumes, which is characterized by a hard envelope that prevents the imbibition of the seed by water, awakens the embryo and triggers germination and emergence. Under these conditions, germination is still weak, slow and heterogeneous. This implies that natural and artificial decortications of the seed envelope could improve imbibition and germination (Musara et al., 2015; Valente et al., 2016). To test this, experiments were carried out to determine which factors can improve the rate of germination and emergence of this species. It is important to note that germination and emergence are two crucial steps for native and domestic species (Giusti and Grau, 1983).

The main objective of this study was to verify the hypothesis that pretreatment of seeds would have significant effects on the germination of *A. cytisoïdes* L. and that seeds lose their viability with age.

MATERIALS AND METHODS

Date and location of seed collection

The used plant material was seeds of the species, *A. cytisoïdes* L., which is an indehiscent legume containing a single seed. The seeds have dimensions of 0.2 × 0.3 mm and the average dry weight is 1 to 4 mg. They are naturally disseminated mainly by wind, water and sometimes animals. The seeds were collected in July 2006, 2008, 2009 and 2010 in the area of Guenfouda (a province of Jerada) [lat. 34°18'22.24"N, long. 2°10'45.89"W]. Seeds were stored at room temperature in the laboratory at the Institute National de la Recherche Agronomique, (INRA) of Oujda-Morocco, in plastic bottles.

Pretreatment of seeds

The seeds of *A. cytisoïdes* L. are covered by hard and cutinized envelopes, which completely prevent the imbibition of water and, sometimes, even prevent gas exchange. However, without imbibition and gas exchange, initiation of embryonic growth and germination is impossible. The physical dormancy of this kind of seed is often observed in several leguminous species.

Therefore, to break dormancy, seeds of *A.* cytisoïdes L. were pretreated as follows: (T1) seeds were soaked in running water for 48 h; (T2) light scarification was made with abrasive paper; (T3) strong scarification was made with abrasive paper; (T4) seeds were soaked in running water for 48 h; (T5) seeds were soaked in hot water for 10 min; (T6) seeds were totally decorticated. These pretreated seeds were compared to controls seeds lacking any pretreatment (T0).

Seed scarification

Physical pretreatment methods that facilitate transition from dormancy consist of piercing, or splitting the integument in order to make it more permeable without damaging the embryo or the endosperm of the grain (Bonner, 1984). To test the effectiveness of mechanical scarification, seeds were placed between two sheets of abrasive paper (10 cm in diameter) and rubbed several times upstream and moving forward under the pressure of hands for light scarification in experiment T2. This method was used for T3 with increased friction until the yellow layer appeared. After mechanical scarification of the pericarp, seeds were placed in pots filled with potting soil.

Soaking in running water

Some pretreatments involve soaking seeds in water. These treatments make it possible to combine the effects of softening integuments and leaching of chemical inhibitors from the seeds (Vázquez et al., 2014). seeds were soaked in water for 24 and 48 h in a beaker filled with running water. Then, they were used in experiments T1 and T4, respectively.

Soaking in hot water

Pretreatments in hot water were tested for seeds. Seeds were immersed in boiling water at 100°C for 10 min, removed from the heat source and cooled slowly for about 1 h. This pre-treatment is

Table 1. Effects of pretreatments and seeds age on *A. cytisoïdes* L. germination.

Source	Sum of type III squares	Ddl	Average of squares	F	Signification$^{\gamma}$
Corrected model	70803,336 $^{\delta}$	27	2622,346	53,588	0.000000 (*)
Ordered at origin	43966,864	1	43966,864	898,462	0.000000 (*)
Pretreatment	67004,686	6	11167,448	228,206	0.000000 (*)
Age	1274,307	3	424,769	8,680	0.000032 (*)
Pretreatment x age	2524,343	18	140,241	2,866	0.000372 (*)
Error	5480,800	112	48,936		
Total	120251,000	140			
Total (corrected)	76284,136	139			

Each pot contained 20 seeds of *A. cytisoids* L. and each pretreatment condition was repeated five times. $^{\delta}R_2$ = 0.928 ($R_{2adjusted}$ = 0.911). $^{\gamma}\alpha$ = 0.05; *Significant.

usually used to reduce the mechanical restriction of the cover layer of the seed, make it softer and help in germination (Hajlaoui et al., 2016). The seeds that had imbibed water and swelled were only subsequently planted in pots filled with potting soil.

Decorticating seeds

Seed extraction is easily accomplished by spreading seeds on a platform, a straw mat or other suitable support and beating them with a flail or a thin pole. If the quantities of decorticated seeds extracted are high, it is possible to adapt mechanical methods used in agriculture (Habit et al., 1981). Extraction of the seeds was carried out by threshing and using abrasive paper to take off the coat and release the seed (Appendix 1). The threshing of 100 seeds by hand took about 30 min, and separation was done manually.

Seed viability

The effects of seed viability on the germination rate were evaluated and tested using four seed lots collected in the years, 2006, 2008, 2009 and 2010.

Experimental procedures

Before seeding, 140 pots (2 L) were filled with soil (compost) rich in organic matter. Each pot contained 20 seeds and each pretreatment condition was repeated five times. The pots were irrigated and after 24 h, pretreated grains were seeded into the pots. During the emergence period, the pots were irrigated every 2 days. The pots were placed in a glasshouse located at the INRA-Oujda for optimal germination and growth conditions. The duration of the experiments was 35 days, from January 30th until March 5th, 2015. Observations were recorded daily. The appearance of a cotyledon was the criterion for seed emergence (Appendix 2).

Statistical analysis

The experimental design was a complete random block with two factors. The first factor was the pretreatment condition, which included seven variations, and the second factor was age of the seeds, with four age groups. The pretreatment and viability data were analyzed using an ANOVA (analysis of variance) via IBM SPSS Statistics 21 software. The univariate general linear model was used. Comparisons of pretreatment averages and seed age were made by Tukey's multiple comparison test.

RESULTS

Effects of pretreatments

Effects of decortication

Regardless of seed age, results shown in Table 1 revealed a significant effect of the pretreatment and seeds age on the rate of emergence. The pretreatment T6 had a significant higher value of germination relative to all other pretreatments, with emergence reaching 69% at the end of the experiment (Table 2). In terms of the time to emergence, the seeds germinated quickly, within 15 to 20 days, and accounted for 60 to 70% of the germination rate (data not shown).

Effects of boiling in water

The T5 pretreatment had a significant effect on seed emergence of Anthyllis cytisoïdes L. In that case, the germination rate was 23%, which is higher than the control (Table 2). Figure 1 shows that seeds from the 2009 lot with T5 pretreatment had a germination rate of 40%. This result is also supported in Figure 2 where it shows that T5 pretreatment had the second highest effect on germination rate after T6 pretreatment. This was observed from 17 days after seedling.

Effects of seed scarification

The results from either the light or strong scarification pretreatment showed only a very small increase relative to the control. The low mean germination rates in T3 and T4 pretreatment groups were 12.75 and 6.25%,

Table 2. Comparison among the averages of each pretreatment (germination rate, %), processed by test of Tukey.

Seeds pretreatment	N^{δ}	Subset of homogeneous averages (germination rate, %)			
		1	2	3	4
T0	20	4.00a			
T1	20	4.50a			
T2	20	4.55a			
T4	20	6.25a	6.25b		
T3	20		12.75b		
T5	20			23.00c	
T6	20				69.00d
Signification$^{\gamma}$		0.949 (n.s)	0.059 (n.s)	1.000	1.000 (n.s)

(T1) Seeds were soaked in running water for 48 h; (T2) seeds were lightly scarred; (T3) seeds were strongly scarred; (T4) seeds were soaked in running water for 24 h; (T5) seeds were soaked in hot water for 10 min and (T6) seed coats were removed. Each pot contained 20 seeds of *A. cytisoids* L. and each pretreatment condition was repeated five times. The error term is medium square (Error) = 48.9; $^{\delta}$Number of samples of the harmonic means = 20; $^{\gamma}\alpha = 0.05$; (n.s): not significant.

Table 3. Comparison among the averages of the seeds viability for each lots (germination rate, %), processed by test of Tukey.

Seeds viability	N^{δ}	Subset of homogeneous averages (germination rate, %)	
		1	2
Seeds of 2006	35	12.74a	
Seeds of 2008	35		18.00b
Seeds of 2010	35		19.57b
Seeds of 2009	35		20.57b
Significance$^{\gamma}$		1.000	0.419 (n.s)

Seeds from lots collected in four different years: 2006, 2008, 2009 and 2010 were used. Each pot contained 20 seeds of *A. cytisoids* L. and each pretreatment condition was repeated five times. The error term is medium square (Error) = 48.9; $^{\delta}$Number of samples of the harmonic means = 35; $^{\gamma}\alpha = 0.05$; n.s = not significant.

respectively (Table 2).

Effects of soaking seeds in regular running water

A Tukey's test (Table 2) demonstrated that there is no significant effect *(P<0.05)* on the means germination rate among the pretreatment groups T2, T1 and T0: the values were 4.55, 4.50 and 4.00%, respectively. The low levels for T0, T1 and T2 groups were observed in seeds from the 2006, 2008, 2009 and 2010 lots (Figures 1 and 2).

Seed viability following multi-year storage

A comparison of the averages (Table 3) showed that the loss in viability was very low and the three seed lots showed no significant differences. The average germination rates were 18.00, 19.57 and 20.57% for seeds from the 2008, 2010 and 2009 lots, respectively. Only seeds from the 2006 lot showed significantly lower percentages of germination as compared to the other lots (12%).

DISCUSSION

The significant effect detected in the T6 pretreatment on the rate of emergence can be explained by the fact that once the mechanical and chemical dormancy was broken by decortication, the imbibition of water and gas exchange allowed the resumption of embryonic growth, germination and emergence of the seeds. As observed, the seeds envelopes constitutes the main barrier to uniform and rapid emergence. In its natural environment, decortication can occur in the mouth of animals as the seeds are crushed by teeth and released. This may explain the high dispersal capacity and abundance of this

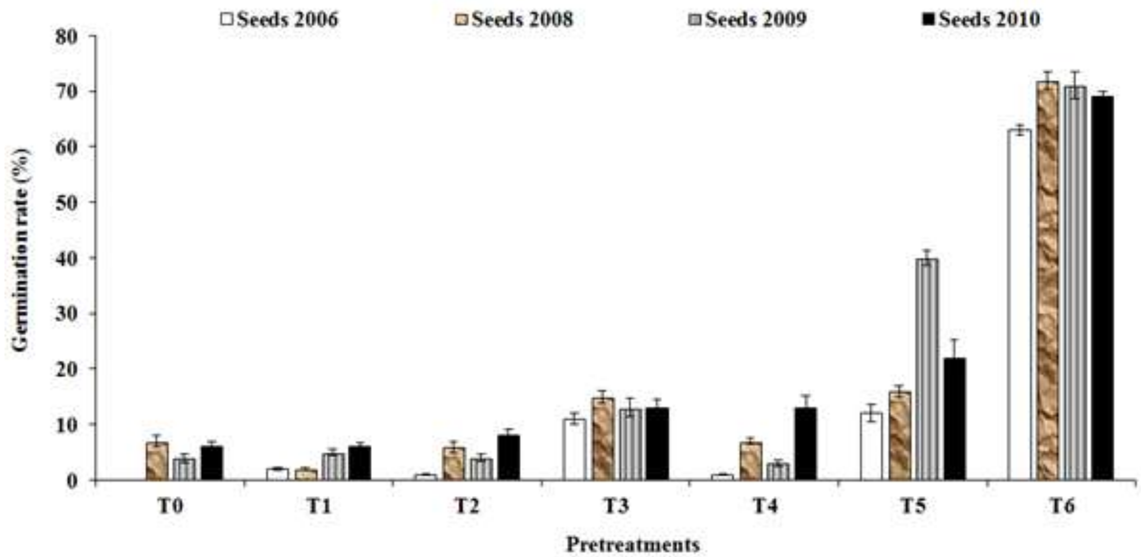

Figure 1. Effects of pretreatments and storage age of the seeds on *A. cytisoides* L. germination rate. (T1) Seeds were soaked in running water for 48h; (T2) seeds were lightly scarred; (T3) seeds were strongly scarred; (T4) seeds were soaked in running water for 24 h; (T5) seeds were soaked in hot water for 10 min and (T6) seed coats were removed. Each pot contained 20 seeds of *A. cytisoids* L. and each pretreatment condition was repeated five times. Seeds from lots collected in four different years: 2006, 2008, 2009 and 2010 were used.

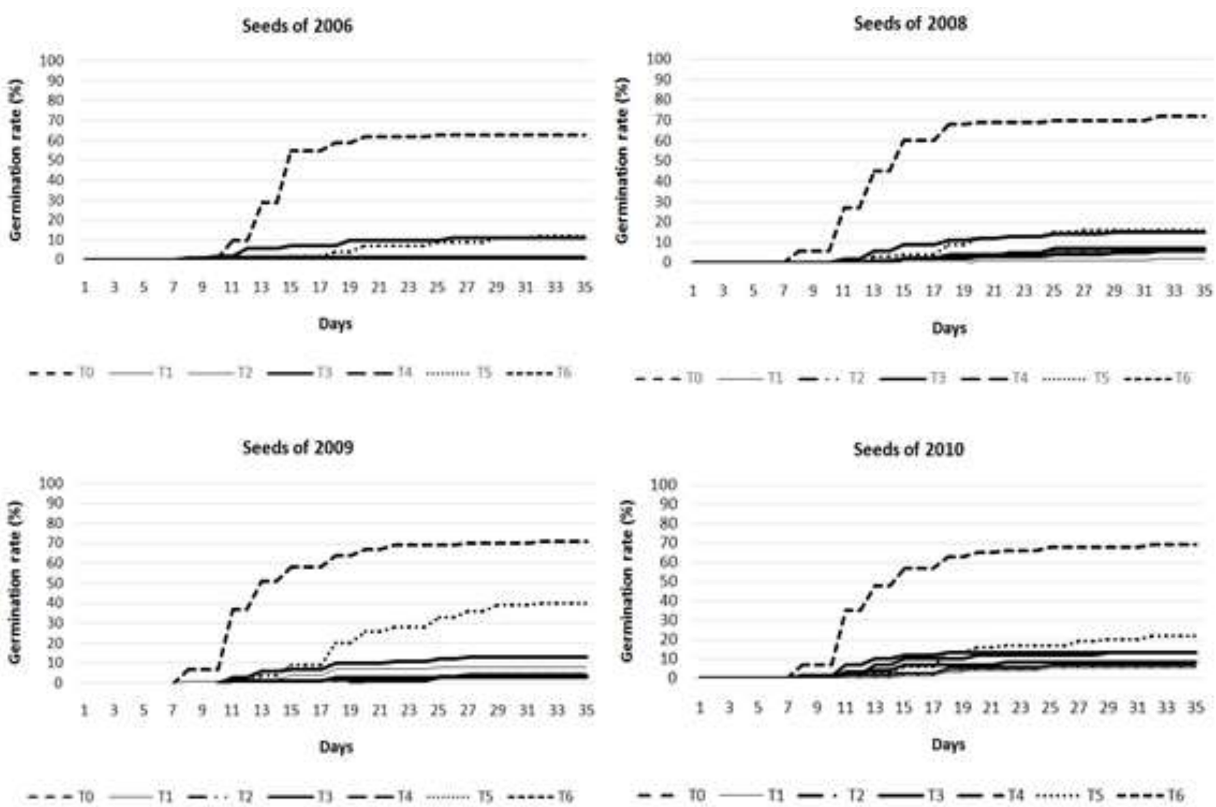

Figure 2. Daily emergence rate for different pretreatments and for different seeds lots. (T1) Seeds were soaked in running water for 48 h; (T2) seeds were lightly scarred; (T3) seeds were strongly scarred; (T4) seeds were soaked in running water for 24 h; (T5) seeds were soaked in hot water for 10 min and (T6) seed coats were removed. Each pot contained 20 seeds of *A. cytisoids* L. and each pretreatment condition was repeated five times. Seeds from lots collected in four different years: 2006, 2008, 2009 and 2010 were used.

species in areas where it is consumed by livestock (Appendix 3). In addition, the strong emergence observed in pretreatment T5 can be explained by the effect of boiling water on chemical dormancy, which is caused by chemical inhibitors in the seed husk (Sulisetijono et al., 2016). Soaking in hot water may destroy these inhibitors. It may also open the seed coat (Mavengahama and Lewu, 2012) and weaken the thick, solid envelope that impose mechanical dormancy to prevent embryonic growth. Gordon and Rowe (1982) mentioned that energetic treatments such as soaking seeds in boiling water often relieve physical dormancy, allowing liquids to penetrate through the envelopes of seeds subjected to mechanical dormancy and kill the embryos. Regarding the results noted for the effect of seed scarification, the low germination rates in T3 and T4 pretreatment could be due to the presence of inhibitors which prevent seeds emergence (Ye and Zhao, 2016). However, results reported by Moffett (1952) on germination of *Anthyllis* cytisoides L. indicated that scarification was an efficient pretreatment method for increasing the germination percentage close to its capacity. These results were also supported by Maatougui (2013) and Hardegree and Emmerich (1991), who reported that scarification allows more rapid germination. This effect was demonstrated in most cases of legumes by Peinetti et al. (1993) and Janzen (1981). On the other hand, our experiments demonstrated that there is no significant effect of soaking seeds in running water on the mean germination rate. Germination inhibitors appear to be resistant to pretreatment in running water. However, Matias et al. (1973) observed that soaking seeds for 48 h in running water at room temperature ensures more uniform germination than non-soaking seeds.

Regarding seeds viability following multiyear storage, seeds from 2006 lot showed significantly lower germination rate as compared to seeds from 2008, 2009 and 2010 lots. Ellis and Roberts (1981) showed that Anthyllis seeds can maintain their initial viability for up to 10 years under normal conditions. For some seeds, the aging process and deterioration depends more on the storage conditions than the seed "age". The time elapsed since maturation and harvest is an inadequate estimate of aging, which gives a sense of the rate of viability loss and their progression towards irreversible deterioration, and therefore death.

This research demonstrates that seeds of *A. cytisoïdes* L. have an integument-dormancy. Removal of the seed coat significantly improved the rate of seed germination and emergence for this species. Under these conditions, germination and emergence was very quick and uniform. In terms of viability, seeds of *A. cytisoïdes* L. could maintain their original viability for a long period of time, more than 10 years. The lowest rate of germination was observed with the oldest seeds harvested in 2006 (12.74%). In this case, the seeds remain viable and can exceed a strike rate of 60% if they are totally decorticated. This study provides useful information on the preservation and conservation of this native species, which is especially useful now that *A. cytisoïdes* L. is becoming rarer. This study is of interest to multiple species in these parts of Morocco for pasture rehabilitation, erosion reduction and diminishing the impact of climate change. For these reasons, it is recommended that future research be done for a better understanding of both sexual and asexual propagation, morphology and anatomy of the seeds and the ecology of this rare species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Appendix 1. A) Seeds before treatments; B) envelopes after scarification; C) seeds decorticated.

Appendix 2. Emergence of *A. cytisoïdes* L.

Appendix 3. *A. cytisoids* L. in the spontaneous state in region of Al Hoceima, Morocco (2015).

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Sample size for evaluation of eggplant and gilo seedlings

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In horticultural nurseries, it is important to determine the number of plants to be evaluated to obtain reliable inferences about seedling growth. The objective of this study was to determine the sample size required to estimate the mean of quality traits of eggplant and gilo seedlings. The seedlings of both vegetables were produced in expanded polystyrene trays using (8×16) 128 cells each and 40 cm^3 high. At 45 days after sowing, the following traits were evaluated in each seedling of the two crops: leaf number; total leaf area; fresh matter mass of shoot, roots and the total of fresh matter mass of shoot and roots, and Dickson quality index. Measures of central tendency and variability were calculated; the normality of the sample data was tested, and the sample size was calculated. The sample size required is different among the different traits of eggplant and gilo seedlings, and the same trait is different between the two crops. The sample size to evaluate seedlings for an estimation error of 10% of the estimated mean and at a confidence level of 95% is 32 and 26 for eggplant and gilo, respectively.

Key words: *Solanum melongena*, *Solanum gilo*, sampling, experimental precision.

INTRODUCTION

In the production of good quality vegetables such as eggplant (*Solanum melongena*) and gilo (*Solanum gilo* Raddi) of the Solanaceae family, the formation of seedlings is one of the most important stages for the crop cycle, directly influencing the final performance of plants, nutritional and productive aspects, existing a direct relationship between healthy seedlings and productive plants in the field (Campanharo et al., 2006). Well-

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formed seedlings can ensure successful implementation and, consequently, vegetable plant productivity. On the other hand, seedlings with compromised development may result in damage to crop growth, increasing its cycle and leading to production losses (Guimarães et al., 2002), and consequently profit losses.

Burin et al. (2014) mention that because of limitations of financial resources (time and labor), it is common to measure samples that must represent adequately the population. To do this, it is necessary to establish an adequate sample size, which provides an estimate of the mean of the trait with an appropriate level of precision, thus, it is important to measure as many traits as possible to maximize the information on the crop.

There are several studies in literature that approach sample size for crops such as rice (Sari et al., 2016), corn (Wartha et al., 2016), soybean (Antúnez et al., 2016); fruits such as apple (Toebe et al., 2014), mombin (Silva et al., 2016), papaya (Schmildt et al., 2017), peach (Pazolini et al., 2016); and vegetables such as carrot (Silva et al., 2009), lettuce (Santos et al., 2010), green bean (Haesbaert et al., 2011) and tomato (Lucio et al., 2012).

Concerning the evaluation of seedlings, there are studies on the sample size for seedlings of *Pinus* (Silveira et al., 2009), *Cabralea canjerana* (Filho et al., 2012), and pecan walnut (Filho et al., 2014b). However, no study was found in literature on the sample size for the evaluation of eggplant and gilo seedlings.

Zar (2010) stated that the larger the sample size, the greater the precision of the experiment, with a reduction in the sample mean variance although the demand for resources is also high. On the other hand, a reduced sample size may decrease experimental precision. Bussab and Morettin (2012) points out that the sample size is directly proportional to the variability of the data and the desired reliability in the estimation which is inversely proportional to the estimation error.

Determining sample size is important in experimentation because if this sample size is excessive, unnecessary time and resources will be expended. However, taking samples smaller than necessary, inaccurate estimates will be obtained, which may even invalidate the work (Coelho et al., 2011).

The objective of this study was to determine the sample size (number of seedlings) required to estimate the mean of quality traits of eggplant and gilo seedlings.

MATERIALS AND METHODS

The evaluations were carried out using seedlings of eggplant (*Solanum melongena*) cultivar Embú and gilo (*Solanum gilo* Raddi) cultivar Grande Rio. The seedlings were produced in a protected environment at the horticulture sector of the Federal Institute of Espírito Santo - Campus Itapina, Colatina, in the Northwest region of Espírito Santo. The region is characterized by an Aw tropical dry climate, according to the Koppen classification, 70 m altitude, 19°30' South latitude and 40º20' West longitude. The evaluations took place at seedling transplant stage, 45 days after sowing (DAS).

The seedlings of both vegetables were produced in expanded polystyrene trays (8×16) with 128 cells, and 40 cm^3 high. The cells were filled with Bioplant® substrate, and 3 seeds were sown per cell. After emergence, thinning of the plants left only one seedling per cell. The seedlings were irrigated three times a day from the emergence to the end of the experimental period.

In August 2016, when the seedlings had at least 4 leaves, all the 128 seedlings of eggplant and gilo were evaluated for the following traits: Leaf number (LN), total leaf area (TLA) in cm^2, using digital images in HP Deskjet F4480 scanner and processed by the ImageJ® Software, public domain (Schindelin et al., 2015); shoot fresh matter mass (SFMM) in g; root fresh matter mass (RFMM) in g; total fresh matter mass (TFMM) in g, and Dickson quality index (DQI).

The DQI was determined as a function of shoot height (H), shoot diameter (SD), shoot dry matter mass (SDMM), root dry matter mass (RDMM), and total dry matter mass (TDMM), using the equation (Dickson et al., 1960):

$$\text{DQI} = \frac{\text{TDMM}(g)}{\frac{H(\text{cm})}{\text{SD}(\text{mm})} + \frac{\text{SDMM}(g)}{\text{RDMM}(g)}}$$

The data collected for each crop were then analyzed separately using the descriptive statistics: Minimum and maximum values, arithmetic mean, standard deviation, coefficient of variation, and Shapiro-Wilk normality test. These statistics were obtained to characterize the database and verify its adequacy for the study of sample size via deterministic method or the need to use a simulation method (Ferreira, 2009).

To determine the sample size by simulations in each trait, this study used the interval estimation via bootstrap using percentile interval (Martinez and Neto, 2001; Ferreira, 2009). A total of 128 sample sizes were set for each trait of each crop; the initial sample size used was one seedling, and the others were obtained by adding one seedling to the previous quantity until it got to 128 seedlings.

For each sample size set of each trait of eggplant and gilo, 4000 simulations were performed by resampling, with replacement (Martinez and Neto, 2001). For each simulated sample, the mean were estimated. Thus, for each sample size of each trait of eggplant and gilo seedlings, 4000 mean estimates were obtained (Ferreira, 2009). The 95% confidence interval (95% CI) was then calculated by the difference between the 97.5% percentile and the 2.5% percentile for each sample size, and these results were plotted graphically.

Next, the sample size (number of seedlings) was calculated for the estimation of the mean of each trait of each crop. For this calculation, the initial size (one seedling) was taken and the sample size was considered as the number of seedlings from which the means remained within the limit of the 95% confidence interval (Haesbaert et al., 2017).

The sample size also was calculated by Chebyshev's inequality using the mean and standard deviation calculated by the 4000 simulations. The statistical analyses were performed using the R (R Development Core Team, 2016) program and graphs were created with Microsoft Office Excel® application (Levine et al., 2012).

RESULTS AND DISCUSSION

Table 1 shows the results of the eggplant seedling evaluation. The mean values obtained for the traits are as

Table 1. Minimum, maximum, mean, standard deviation (SD), coefficient of variation (CV%) and Shapiro-Wilk normality test (p value) for six traits measured in 128 seedlings of eggplant (*Solanum melongena*).

Traits [(1)]	Minimum	Maximum	Mean	SD	CV%	p Value[(2)]
LN	3.0000	8.0000	5.2500	0.9307	17.73	<0.01
TLA	10.3400	27.5000	17.4977	3.5489	20.28	0.4942
SFMM	0.3390	0.8540	0.5838	0.1177	20.17	0.4584
RFMM	0.2190	0.8060	0.4677	0.1087	23.25	0.0459
TFMM	0.5590	1.6600	1.0515	0.2061	19.60	0.5284
IQD	0.0079	0.0415	0.0243	0.0069	28.58	0.4160

[(1)] LN, Leaf number, in units; TLA, Total leaf area in cm^2; SFMM, Shoot fresh matter mass in g; RFMM, Root fresh matter mass in g; TFMM, Total fresh matter mass in g; DQI, Dickson quality index. [(2)] p Values ≥ 0.05 indicate normal distribution of data.

follows; 0.5838 g for shoot fresh matter mass (SFMM), 1.0515 g for total fresh matter mass (TFMM), 0.0243 for the Dickson index (DQI) and 17.4977 cm^2 of total leaf area (TLA) per seedling (Table 1); these traits showed sample data normally distributed according to the Shapiro-Wilk test.

The leaf number (LN) and root fresh matter mass (RFMM) data were not normally distributed. Thus, the sample size was calculated using the bootstrap percentile simulation method, considering that this procedure requires no assumptions about the probability distribution of the estimator (Ferreira, 2009).

LN and TFMM showed coefficients of variation (CV) between 10 and 20%, which is considered the mean experimental precision. TLA, SFMM, RFMM and DQI showed values between 20 and 30%, which is considered of low experimental precision (Storck et al., 2011). The traits of low experimental precision will require samples of larger size for the same confidence and error assumed (Ferreira, 2009).

The amplitude of 95% confidence interval for mean gradually decreased with increasing sample size (number of seedlings) for all traits, which is consistent with those of other studies (Burin et al., 2014; Schmildt et al., 2017), and reveals an increase in precision in the estimation of the mean of each trait of eggplant seedlings (Figure 1). The mean bootstrap estimated for each sample size is invariant (Martinez and Neto, 2001), which allows the graphical analysis to determine the sample size of each trait for different sample errors assumed around the mean.

Table 2 shows the sample sizes of each trait evaluated in eggplant for different errors assumed around the mean. In this study, the minimum sample size required is different among the different traits, for each sampled error, and this is in agreement with the findings of other studies on the production of seedlings of other agricultural crops (Silveira et al., 2009; Cargnelutti Filho et al., 2012, 2014b). The smallest sample size required was found for LN, with 50 seedlings, and the largest sample size was found for DQI, with 127 seedlings, with 5% error around the mean.

In situations where a 10% error around the mean is allowed, a sample of 32 seedlings is sufficient to size all the traits mentioned in this study. Using the Chebyshev inequality, the sample size is 171 seedlings. Literature reviews have indicated that there are no studies on sample size of eggplant or other horticultural seedlings, nor studies evaluating the quality of seedlings by DQI. The evaluation of the sample sizes is important to obtain reliable inferences about seedling growth.

Silveira et al. (2009) investigated sample size in *Pinus taeda* seedlings and concluded that, with a 10% error around the mean, 25 seedlings are required in the sample. Evaluating sample size in *Cabralea canjerana* seedlings, Cargnelutti Filho et al. (2012) found that with 10% error around the mean, the sample size was 18 seedlings, which is lower than that found in this study for eggplant seedlings.

The results of descriptive statistics and normality test in the evaluation of different traits of gilo seedlings are presented in Table 3. The mean values obtained for the traits are as follows: 5.4766 units for LN; 16.6341 cm^2 for TLA; 0.5050 g for SFMM; 0.4003 g for RFMM; 0.9053 g for TFMM; and 0.0386 for DQI, with different variabilities measured by the CV. Only RFMM and DQI had sample data normally distributed by the Shapiro-Wilk test. Different variabilities among different traits were also reported in sample size studies of other horticultural crops such as lettuce (Santos et al., 2010) and tomato (Lucio et al., 2012).

The analysis of gilo data showed some differences compared with eggplant, since all traits of the gilo seedlings, except for RFMM that had lower variability than those of eggplant seedlings. The traits LN, TLA, SFMM and TFMM of gilo had CV values between 10 and 20%, which is considered of medium experimental precision (Storck et al., 2011), whereas for eggplant seedlings, only LN and TFMM had been included in this classification. These results indicate that for gilo seedlings, RFMM will have the largest sample size.

Considering that not all traits evaluated in gilo seedlings had sample data normally distributed, the sample size was determined by the bootstrap percentile method (Figure 2), considering that this procedure requires no assumptions about the probability distribution of the data

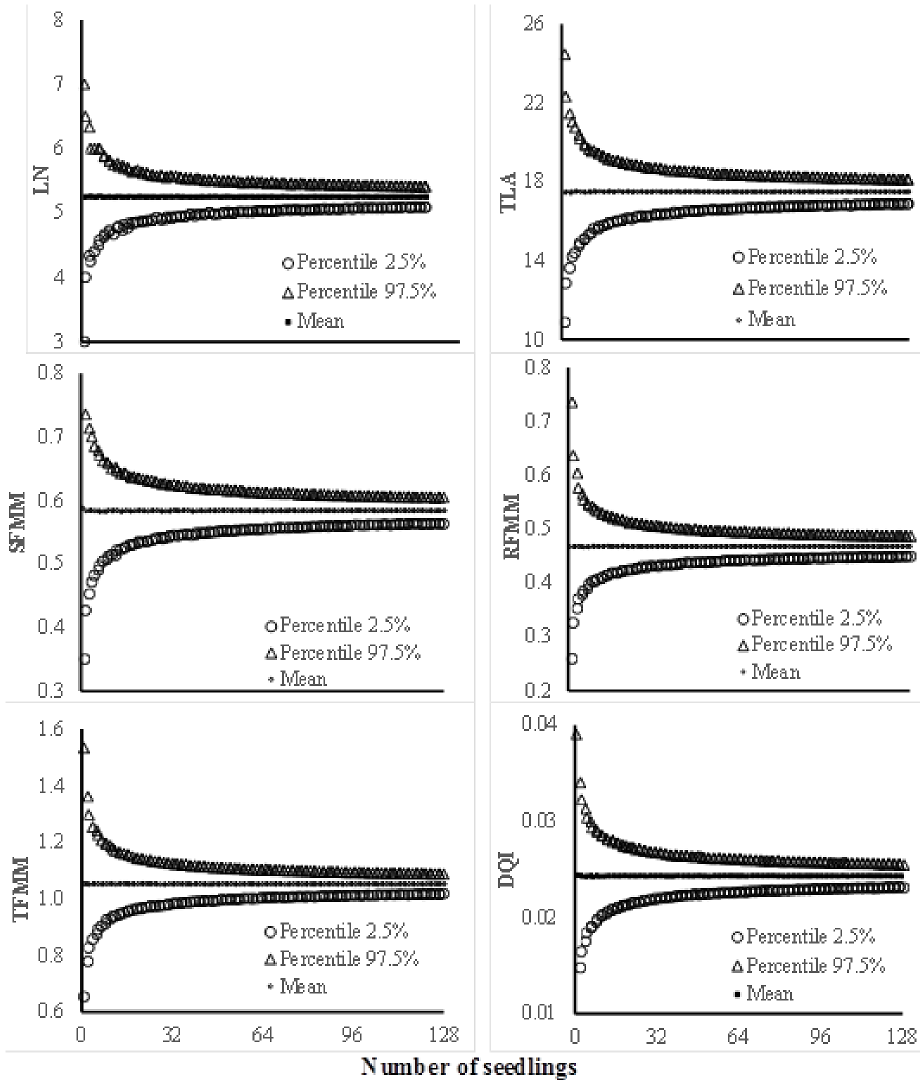

Figure 1. Percentile 2.5%, mean and percentile 97.5% of the 4000 means of seedling traits obtained by resampling in 128 different sample sizes (1, 2, 3, ... 128 seedlings) of eggplant (*Solanum melongena*) cv. Embú. LN, Leaf number, in units; TLA, Total leaf area in cm^2; SFMM, Shoot fresh matter mass in g; RFMM, Root fresh matter mass in g; TFMM, Total fresh matter mass, in g; DQI, Dickson quality index.

Table 2. Number of seedlings required to estimate the mean of six traits of eggplant (*S. melongena*), cv. Embú, for amplitudes of 95% confidence interval for mean.

Traits(1)	Amplitudes of 95% confidence interval				
	Bootstrap				Chebyshev
	5%	10%	15%	20%	10%
LN	50	13	6	4	69
TLA	65	17	8	5	82
SFMM	64	16	8	4	81
RFMM	85	22	10	6	109
TFMM	61	16	7	4	73
DQI	127	32	15	8	171

(1) LN, Leaf number, in units; TLA, Total leaf area in cm^2; SFMM, Shoot fresh matter mass in g; RFMM, Root fresh matter mass in g; TFMM, Total fresh matter mass in g; DQI, Dickson quality index.

Table 3. Minimum, maximum, mean, standard deviation (SD), coefficient of variation (CV%), Shapiro-Wilk normality test (p value) for six traits measured in 128 seedlings of gilo (*Solanum gilo* Raddi) cv. Grande Rio.

Traits [(1)]	Minimum	Maximum	Means	SD	CV%	p Value[(2)]
LN	3.0000	8.0000	5.4766	0.7732	14.12	<0.01
TLA	5.8900	22.3200	16.6341	2.9991	18.03	<0.01
SFMM	0.1330	0.6840	0.5050	0.0868	17.19	<0.01
RFMM	0.0850	0.6750	0.4003	0.1015	25.35	0.8794
TFMM	0.2180	1.324	0.9053	0.1689	18.65	0.0104
DQI	0.0101	0.0584	0.0386	0.0086	22.42	0.5677

[(1)] LN, leaf number in units; TLA, Total leaf area in cm^2; SFMM, Shoot fresh matter mass in g; RFM, Root fresh matter mass in g; TFMM, Total fresh matter mass in g; DQI, Dickson quality index). [(2)] p Values ≥ 0.05 indicate normal distribution of data.

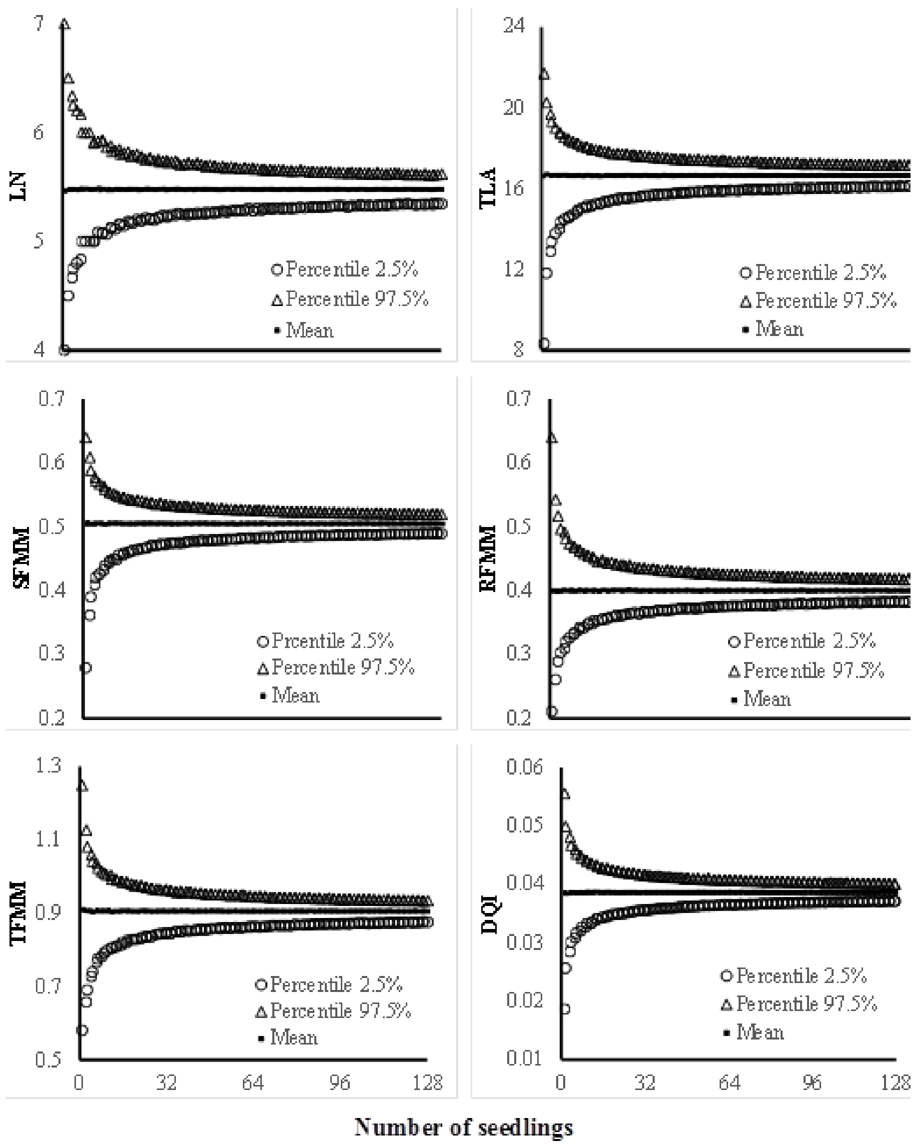

Figure 2. Percentile 2.5%, mean and percentile 97.5% of the 4000 means of seedling traits obtained by resampling in 128 different sample sizes (1, 2, 3, ... 128 seedlings) of gilo (*Solanum gilo* Raddi) cv. Rio Grande. LN, Leaf number, in units; TLA, Total leaf area in cm^2; SFMM, Shoot fresh matter mass in g; RFMM, Root fresh matter mass in g; TFMM, Total fresh matter mass in g; DQI, Dickson quality index.

Table 4. Number of seedlings required to estimate the mean of six traits of gilo seedlings (*S. gilo* Raddi) cv. Grande Rio, for amplitudes of 95% confidence interval.

Traits (1)	Amplitudes of 95% confidence interval				
	bootstrap				Chebyshev
	5%	**10%**	**15%**	**20%**	10%
LN	32	8	4	2	40
TLA	51	13	6	4	65
SFMM	47	12	6	3	58
RFMM	101	26	12	7	129
TFMM	55	14	7	4	70
DQI	78	20	9	5	99

(1) LN, Leaf number, in units; TLA, Total leaf area in cm^2; SFMM, Shoot fresh mass in g; RFMM, Root fresh mass in g; TFMM, Total fresh matter mass in g; DQI, Dickson quality index.

(Ferreira, 2009)

The sample size differed among the different traits of gilo seedlings (Table 4) and, as in eggplant, the smaller sample size is required for LN. With a 10% error around the mean, only 8 seedlings are needed in the sample. This finding has important implications for nursery producers, because most farmers evaluate seedling quality based on LN, and in this way, evaluations will be done with a lower number of seedlings. In addition, it is a non-destructive method, fast and easy to implement.

However, for gilo seedlings, the largest sample size was found for RFMM, which, although it is as important as LN, it is not performed by most of the producers, and the two traits are not significantly correlated ($r = -0.0130$, $p = 0.8843$, $H0: \rho = 0$), indicating that classifying seedlings by LN may not be the best strategy. This study showed that, with a 10% error around the mean, 26 seedlings of gilo are required to characterize RFMM. Using the Chebyshev inequality, the sample size is 129 seedlings.

In this study, the results show that eggplant and gilo, both belonging to the same family, have different sample size requirement for the same traits, and this is in line with the findings of Coelho et al. (2011) for sample size of mature fruits of *Passiflora edulis* and Schmildt et al. (2017) for fruits of *Passiflora foetida*.

Conclusions

The sample size requirement is different among the different traits for eggplant and gilo seedlings, and it is also different for the same trait between the two crops. The sample size for seedling evaluation, for an estimation error of 10% of the mean estimate, at 95% confidence level, is 32 for eggplant and 26 for gilo seedlings.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Agronomic practices and causes of decline in trifoliate yam (*Dioscorea dumentorum Kunth Pax*) production in Enugu State, Nigeria

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Trifoliate yam is a species of yam with limited documented research and information on its production and importance in spite of its high yielding quality, nutritional, medicinal and industrial uses. This study was therefore carried out to describe agronomic practices, production trend and causes of decline in trifoliate yam production in Enugu state, Nigeria. Three agricultural zones, six blocks, eighteen circles and 108 respondents purposively selected from the state constituted sample for the study. An interview schedule was used to collect data while percentage, mean score and standard deviation were used for data analysis. Findings of the study reveal that the respondents had no extension contact but sourced information on trifoliate yam from neighbours, friends or relatives (90.7%). Hence they produced trifoliate yam using indigenous methods and varieties (73.1%). Production trend shows that mean size of land allocated to trifoliate yam production was relatively steady, mean cost of input and income were increasing while mean output was decreasing within the years under consideration (before 2001 to 2014). Poor finance ($\bar{x}$=1.8) and drudgery ($\bar{x}$=1.72) were some of the causes of decline in trifoliate yam production in the area. The study recommended that more research and public enlightenment campaign on the importance of trifoliate yam should be carried out by research institutes and extension organisations respectively in order to attract interest of people in growing, consumption and industrial utilization of the crop. This will prevent the crop from going extinct but contribute to food security and sustainable development.

Key words: Agronomic practices, causes, decline, trifoliate yam.

INTRODUCTION

Trifoliate yam *(Dioscorea dumetorum)* from the family Dioscoreaceae (the Yam family) contains approximately 622 to 652 species. The type species is *Dioscorea sativa* (Hortipedia, 2013). According to Onwueme and Sinha (1999), trifoliate yam originated from Africa. It is found in West Africa, primarily in Eastern Nigeria. Trifoliate yam is known by various names such as three-leaved yam, bitter yam and cluster yam. It is also known as 'ji una' or 'ji ona' in Ojoto and many Igbo speaking areas in the South-Eastern Nigeria, where it is regarded as food for adult

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(Egbuonu et al., 2014).

Trifoliate yam differs from other yams by having trifoliate leaves. It also contains a bitter toxic alkaloid called dihydroscorine, which can be removed by soaking and boiling in water. Nutritionally, the tuber is superior to the commonly consumed yams (like white and yellow yams) having high protein and mineral content (Martin et al., 1983). It has a mean protein content of 9.6% (dry weight basis) compared to 8.2% for water yam (*D. alata*) and 7.0% for White yam (*D. rotundata*) (Mbome-Lape and Treche, 1994). The proteins are more balanced than those of white yam and it is rich in vitamins and minerals. Trifoliate yams are highly yielding compared to other yam species.

Bitter yam is good for diabetic patients. It can also be used as a vegetable, but not pounded into 'fufu'. Owing to its soft texture, it is favoured by old people with poor teeth. The wild forms are regarded as famine food. It is becoming a preferred yam in Cameroon. The tubers of trifoliate yam, when properly processed can be used in the production of yam flakes; instant flour for the bakery sector or starch in diverse pharmaceutical preparations (Ukpabi, 2010).

However, there is limited information on the use of flours from trifoliate yam in food industries and at household levels in Nigeria (Abiodun and Akinoso, 2014). This could probably be due to lack of information on the usefulness and importance of the flours for functional food products (Abiodun and Akinoso, 2014). Trifoliate yam is also one of the numerous tropical tubers that are yet to be exploited and is fast being driven into extinction despite being good source of phyto-proteins, carbohydrates, vitamins and minerals for human nutrition (Medova et al., 2005). A research in Enugu North Agricultural Zone of Enugu state by Iwuchukwu and Onwubuya (2012) showed that average yield (kg/ha) of *D. dumetorum* for 2006 to 2008 were 22,337, 19,563 and 17,800 kg/ha respectively These data obviously suggest decrease in *D. dumetorum* production in the area.

Onuegbu et al. (2011) reported that so far the yam has no industrial application. Moreover, the crop is yet to attract adequate research interest in tapping the potentials and its culinary uses are relegated to the background (Degras, 1993). Consequently, yam farmers are moving into less laborious and more economical crops like garden-egg, maize, cucumber, cocoyam production among others. Very few farmers are growing bitter yam in recent times. In yam markets, little or no bitter yam is displayed or sold.

The above stated scenario necessitated the need to investigate the activities of trifoliate yam farmers with a view to ascertain the possible causes of decline in trifoliate yam production in Enugu State. Specifically, the study identified and described trifoliate yam farmers sources of information, extension contact, purpose and season of production, agronomic practices, trend in production as well as causes of decline in trifoliate yam production in the area.

METHODOLOGY

The study was carried out in Enugu state, Nigeria. Enugu State is one of the states in the Eastern part of Nigeria. Trifoliate yam farmers in the state constituted the population for the study. The multi-stage random sampling technique employed in selecting the respondents is as follows:

Stage one: From the six agricultural zones in the state, three zones namely Udi, Enugu Ezike and Nsukka, were purposely selected because of their involvement in the trifoliate yam production.
Stage two: Two blocks where trifoliate yam were mostly produced were purposively selected from each of the zones giving a total of six blocks for the study.
Stage three: From each of the selected blocks three cells or circles where trifoliate yam farmers can be mostly found were selected giving a total of eighteen cells for the study.
Stage four: from each of the circles, six trifoliate yam farmers were selected giving a total of 108 respondents for the study.

Data for the study were collected from the respondents through the use of structured interview schedule. It contained relevant questions based on each objectives of the study. Respondents were asked to indicate their number of extension contacts in the last one year and their source(s) of information on trifoliate yam production. They were also requested to indicate the agronomic practices they employed in trifoliate yam production for examples, variety grown, cropping pattern, method of land preparation, fertilizer application, weeding, harvesting and types of tool used. In order to assess the trend in production of trifoliate yam, respondents were requested to estimate the size of land they allocated to trifoliate yam production (in hectare), cost of input (in Naira), output (in kg) and income (in Naira) realized from the product before 2001, 2001 to 2007 and 2008 to 2014. Causes of decline in trifoliate yam production were captured using a modified Likert-type scale of three points as follows "to a large extent, to a little extent and to no extent", with nominal values of "2, 1 and 0" assigned to them respectively with a mean of 1. Respondents were requested to rate the extent to which they perceive possible causes provided and others they enumerated as causes of decline in trifoliate yam in the area on this scale and mean scores were computed. Variables with mean scores greater than or equal to 1.0 were regarded as major causes, while variables with mean scores less than 1.0 were regarded as minor causes of decline in trifoliate yam production. Data were analyzed with percentage, mean scores and standard deviation.

RESULTS AND DISCUSSION

Personal contact with extension agents

In the present investigation, it has been found that the majority (80.6%) of the respondents did not have any personal contact with agricultural extension agents on agricultural matters in 2013 while 14.8% had only one contact with extension agents in 2013 (Table 1). The mean number of personal contact with extension agents in 2013 was 0.25. Thus the respondent's contact with extension agent was almost inexistent. This is probably because of lack or shortage of extension staff. In line with

Table 1. Distribution of respondents according to their social characteristics.

Social characteristics	Frequency	Percentage	Mean (M)
Contact with extension agents in 2013			
None	87	80.6	
Once	16	14.8	0.25
Twice	4	3.7	
Three times	1	0.9	
Source of information on trifoliate yam *production			
Neighbours/friends/relatives	98	90.7	
Radio	23	21.3	
Extension agents	3	2.8	
Television	3	2.8	
Newspaper	2	1.9	

Source: Field survey, 2014. *Multiple responses.

this Ogbeh (2016) reported that extension workers have almost disappeared in Nigeria as the country presently has an average of one extension worker to about 3,000 farmers. This high farmer- extension ratio may make it extremely impossible for the extension workers to have the required contacts with the clients. Extension visits help to identify the needs of farmers/rural people, equip them with current innovations in agriculture and provide solutions to their problems directly or indirectly through researchers. When the visit is lacking farmers, agriculture and economy suffer.

Sources of information on trifoliate yam production

Table 1 reveals that majority (90.7%) of the respondents' sourced information on trifoliate yam production from their neighbours/friends/relatives while about 21% got information on trifoliate yam production from the radio. Based on finding on their extension contact, it may be said that little or no extension contact resulted to very few people receiving information from the extension worker and other good information sources that extension worker may have linked them to. Hence, these farmers relied on information from informal sources for the production of trifoliate yam. Thus reliability of information from these sources is questionable and these informants may not boast of current scientifically proven innovations on trifoliate yam that they can communicate to these farmers for improved output, productivity and income. Consequently, farmers may be demoralized to consolidate the effort or invest in trifoliate yam enterprise.

Purpose and season of production and sources of planting material

Purpose of production

According to the data obtained, it has been found that greater proportion (67.6%) of the respondents produced trifoliate yam for consumption while 52.8% produced it for commercial purposes (Table 2). Normally, farmers in rural communities of developing countries produce crops and rear animals mainly for subsistence with little of the output commercialized. In corroboration to this finding Verter and Bečvářová (2015) asserted that yams as staple food crops do not only serve as integral vehicle for food security, but also as a source of income and a further source for employer of labour in yam producing areas in Nigeria.

Season of production

Entries in Table 2 also show that majority (70.4%) of the respondents grew trifoliate yam during rainy season, 16.7% cultivated during dry season while 6.5% of the respondents grew trifoliate yam in both seasons. The finding suggests that trifoliate yam is a crop that is grown mainly during rainy season in the area. There could be scarcity of trifoliate yam during dry season which may result to high market price of this commodity during this season. Farmers can explore this opportunity by producing during dry season for higher income.

Sources of planting material (trifoliate yam seed)

Data in Table 2 also show that majority (89.8%) of the respondents obtained planting materials (trifoliate yam seeds) from their farms while 16.7% sourced their planting materials from the market. When there is no subsidy on agriculture, cost of agricultural inputs like seeds, fertilizers, pesticides are likely to be high and unaffordable for farmers especially peasant farmers. They may resort to keeping some of their previous harvest as planting materials for the next planting season. Recycling the species of trifoliate yam especially when it is not improved type may subject these farmers to

Table 2. Percentage distribution according to purpose and season of production and sources of planting material.

Purpose and season of production	Frequency	Percentage
Purpose of production*		
Consumption	73	67.6
Income	57	52.8
Hobby	1	0.9
Reduce erosion	1	0.9
Soil/nutrient conservation	1	0.9
No response	5	4.6
Season of production		
Rainy season (main)	76	70.4
Dry season (off)	18	16.7
Both main and off season	7	6.5
No response	7	6.5
Sources of planting material*		
Individual	97	89.8
Market	18	16 7
No response	7	6.5

Source: Field survey, 2014. *Multiple responses.

cultivation of low quality and poor-yielding varieties of trifoliate yam. Although the importance of seed provisioning in food security and nutrition, agricultural development and rural livelihoods, and agrobiodiversity and germplasm conservation is well accepted by policy makers, practitioners and researchers but the role of farmer seed networks is less understood (Coomes et al., 2016). The authors further stated that the networks need to be strengthened and fed by innovations from research in order to maximize the primary and secondary products as well as productivity from trifoliate yam.

Agronomic practices of trifoliate yam farmers

Planting operations

Methods of land clearing: Entries in Table 3 show that majority (71.3%) of the respondents used hand tools such as hoe and cutlass while 14.8% used herbicides in clearing the land before cultivation. Land clearing is an operation usually carried out before the conventional tillage in a farm land. There are several operations that are involved in land clearing depending on the type of vegetation. Soil condition, topography, the extent of clearing required and the purpose for which the clearing is done (Ugbobor, 2013) are some of the critical factors to put into consideration before clearing the land for agriculture. Also, land clearing should be done in such a way that desirable attributes of the land such as nutrient and moisture availability, erosion resistance and accessibility are not lost. Further, the findings show that agronomic activities of trifoliate yam were done manually which is typical of agriculture in developing world where manual labour is the main source of farm power and farmers produce at subsistent level because of limitations of human power.

Production site

Table 3 shows that 75% of the respondents cultivated their trifoliate yam in farms far away from the place of residence while 49.1% cultivated within their residence. Since majority of the respondents cultivated the trifoliate yam in farms far away from their residence, production cost accruing from cost of transporting farmers, trifoliate yam and other agricultural inputs to and from home, farm and even market may be high. This could lead to poor management of the farm in terms of timely and proper execution of routine activities like weeding, pests and diseases control in the farm.

Varieties of trifoliate yam grown

Entries in Table 3 also show that majority (73.1%) of the respondents cultivated indigenous variety of trifoliate yam while 19.4% cultivated both indigenous and improved variety of trifoliate yam. Indigenous variety grown by these respondents may have some inherent good characteristics but cannot equate the improved type that can be said to be epitome of good qualities for trifoliate yam. Consequently, researchers, policy makers, and foundations are working hard to improve the seed provisioning to farmers in developing countries in order to

Table 3. Distribution of respondents according to their planting operations.

Planting operations	Frequency	Percentage
Method of land clearing used		
Manual	77	71.3
chemical (herbicide)	16	14.8
None	13	12.0
Chemical (herbicide) and manual	2	1.9
Site of production of trifoliate yam*		
Farm away from residence	81	75
Within the residence	53	49.1
No response	6	8.46
Garden	1	1.41
Varieties grown		
Indigenous	79	73.1
Both indigenous and improved	21	19.4
No response	5	4.6
Improved	1	0.9
***Planting/cropping system**		
Trifoliate yam grown in a mixture with other crops	94	87.0
Trifoliate yam grown as a sole or only crop in the farm	12	13.4
No response	4	4.4
Staking		
No	4	3.7
Yes	104	96.3

Source: Field survey, 2014. *Multiple responses.

increase agricultural productivity, nutrition and rural well-being (Coomes et al., 2016). The Alliance for a Green Revolution in Africa (AGRA) has also placed particular emphasis on strengthening the seed sector and promoting the commercialization, distribution and adoption of improved crop varieties (AGRA, 2013). Many development donors have also projects, aimed at improving farmers access to adapted and certified seed, as well as supporting the informal seed sector (FANRPAN, 2010; Gill et al., 2013). Trifoliate yam farmers are likely to tell success stories about their enterprises if they benefit from these activities or programmes by way of growing improved trifoliate yam seed varieties.

Planting/cropping system

Data in Table 3 show that majority (87%) of the respondents practiced mixed cropping while13.2% grew trifoliate yam as a sole crop in the farm. Mixed/multiple cropping involves growing two or more crops on the same piece of land and at the same time. When the right crop combination is made, it leads to an improvement in the fertility of the soil and increase in crop yield because the products and waste from one crop help in the growth of the other crop and vice-versa. Most importantly, farmers especially small scale farmers resort to multiple cropping in order to guard against crop failure in such a manner that when a crop fails another one may not fail.

Staking

Entries in Table 3 show that majority (96.3%) of the respondents staked trifoliate yam while the remaining 3.7% did not stake their trifoliate yam. Staking is a laborious activity in yam production. It is believed that staked yams grow better than those that are not staked. Staking provides each plant the ability to grow without bending to the point where it breaks the plant and stops growth. Having plant grown upward after it is staked allows the plant to get the necessary sunlight it needs to continue the growth and can be used as a method to keep the aisles of each row of plants clear and decent (County and Henson, 2013) . Unfortunately, stakes are costly and not easy to be found nowadays. They consequently, constitute major cost of trifoliate yam production in areas where they are used thereby increasing drudgery and draining income accruable from the enterprise.

Types and time of fertilizer application

Entries in Table 4 reveal that 58.3% of the respondents

Table 4. Distribution of the respondents according to their fertilizer application, weed management practices and method/s of harvesting.

Fertilizer and weed management	Frequency	Percentage	Mean (M)
Type of fertilizer used			
Organic	63	58.3	
Both organic and inorganic	34	31.5	
Inorganic	3	2.8	
None	8	7.4	
Period of fertilizer application			
No response	8	7.41	
Before planting	56	51.9	
After planting	5	4.6	
Before and after planting	36	33.3	
After germination	3	2.8	
Methods of weed control employed*			
Hand tools	83	76.9	
None	11	10.2	
Hand picking	13	12.0	
Chemical method	6	5.6	
Hand tools and hand picking	2	1.6	
Number of weeding done per growing period			
Twice	51	47.2	
Three times	25	23.2	
None	21	19.4	2
Once	7	6.5	
Four times	4	3.7	
Harvesting			
Manual	104	96.3	
No response	4	3.7	

Source: Field survey, 2014. *Multiple responses.

used organic fertilizer in trifoliate yam production while 31.5% used both organic and inorganic fertilizers. More than half of the respondents grew the trifoliate yam organically, which is ideal, given the associated problems of inorganic fertilizer like environmental pollution and health hazards. However, worldwide experiences in agricultural development have provided much evidence that inorganic fertilizer application is the most efficient measure for sustainably increasing crop production and ensuring food security while sustained yield growth will almost be impossible without inorganic fertilizer supply (Wang et al., 2012). Given the positive features of these types of fertilizer, farmers should strike a balance in using them so as to get maximum benefits from both and without any harm from any of them.

The fertilizer that was applied to trifoliate yam was before cultivation (after land clearing) (51.9%) while 33.3% applied it before and after planting. This shows that although majority of the respondents applied fertilizer for trifoliate yam production, greater proportion of them applied it once before the crop was planted. From the foregoing, it can be inferred that fertilizer use appeared to be currently common in Nigeria and not as low as conventional wisdom suggests (Liverpool-Tassie et al., 2015; Sheaham and Barrett, 2014) but the rate of fertilizer use among Nigerian farmers may be considerably low probably due to scarcity and expensive nature of the products (both organic and inorganic fertilizers) in recent time.

Method and frequency of weed control

Table 4 also reveals that majority (76.9%) of the respondents used hand tools in controlling weed while10.2% picked the weeds in their farm. Also, 47.2% of these farmers weeded their trifoliate yam farms twice while 23.2% weeded three times in a growing season. Average number of weeding per growing season was two, suggesting that production of trifoliate yam is relatively labour intensive. Similarly, manual methods of controlling weeds by majority of the farmers are stressful but purposeful with precision and may not lead to acid concentration on the farm/production site as in the case

Table 5. Production trend of trifoliate yam.

Production trend	Before 2001 Mean (M)	2001 - 2007 Mean (M)	2008 - 2014 Mean (M)
Size of land allocated to trifoliate yam (in hectares)	0.21	0.22	0.21
Cost of production (₦)	650.47	1,777.78	2,756.48
Quantity of trifoliate yam produced (kg)	634.95	456.44	445.23
Income from trifoliate yam (₦)	3,611.11	6,004.63	8,208.33

Source: Field survey, 2014.

of chemical like herbicide used in controlling weeds. According to Stachler (2012), cultural and mechanical weed control practices must be utilized in conjunction with herbicides, otherwise herbicide-resistant biotypes will increase. Cultural practices are those practices that maximize crop growth (biomass production) such as proper fertilization, narrow row spacing, high crop stand densities, and many others. According to the author, mechanical weed control must be used wisely to effectively control weeds and reduce soil erosion.

Method/s of harvesting

Majority (96.3%) of the respondents harvested their produce manually (Table 4). This may be because the farmers did not have access to basic farm equipment and implements such as the harvesters that will ease harvesting operations probably due to lack of fund or other constraints

Production trend of Trifoliate yam (*Dioscorea dumentorum*)

Entries in Table 5 reveal production trend of trifoliate yam. The table shows that a mean of 0.21 hectares of land was allocated to the production of trifoliate yam before 2001 while 0.22 and 0.21 ha were allocated to it in 2001-2007 and 2008 - 2014 respectively by the respondents. The table further reveals that the mean cost of production of trifoliate yam before 2001 was ₦650.47 whereas ₦1.777.78 and 2,756.48 were spent between 2001-2007 and 2008 – 2014, respectively on trifoliate yam production by the respondents.

Table 5 also shows that the respondents produced about 634.95 kg of trifoliate yam before 2001 while 456.44 and 445.23 kg of trifoliate yam were produced between 2001 - 2007 and 2008 - 2014 respectively. The mean income earned by the respondents from trifoliate yam production before 2001 was ₦3,611.11 while ₦6,004.63 and 8,208.33 were earned by the respondents in 2001-2008 and 2007 – 2014, respectively (Table 5).

These findings show that the mean cost of input used for growing this species of yam has been increasing before 2014. The mean size of land allocated to its' production may be said to be relatively steady from 2001 to 2014. It further shows that the quantity of trifoliate yam produced before 2001 and 2014 has been decreasing whereas the income generated/earned from trifoliate yam over the years has been increasing tremendously. This increase in income irrespective of decrease in output may be as a result of increase in price of the commodity as a result of its scarcity which may be termed inflation. This condition is not ideal because actually farmers are investing more and harvesting less. Factors that directly/positively affect farm level technical efficiency and yam output like farmers' education, family labour, extension contact and experience of farmers as enumerated by Etim et al. (2013) need to be investigated and enhanced to maximum level in order to maximize trifoliate yam output and yield. If not, when this negative trend continues there may be money to buy trifoliate yam but no trifoliate yam to be bought. This may further lead to species extinction and aggravation of food insecurity.

Causes of decline in trifoliate yam production

The major causes of decline in trifoliate yam production as shown in Table 6 are lack/poor finance (M=1.8); drudgery associated with trifoliate yam production (M=1.72) and lack of awareness of the nutritional, economic and health values of trifoliate yam (M=1.71). Likewise Verter and Bečvářová (2015), identified lack of finance, inadequate farm inputs, storage facilities and high cost of labour as primary constraints to yam production in Nigeria. Other major causes include lack of interest on the part of the youths in agricultural production (M=1.55); lack of good agricultural education (M=1.48); change in taste by the younger generation in terms of food/trifoliate yam production (M=1.46); destruction caused by stray animals (M=1.44); problems of basic social amenities (M=1.44); unpredictable climate (M=1.37); notion/belief that trifoliate yam can kill or cause madness when consumed (M=1.33); poor tools and farm machines (M=1.29); poor transport system (M=1.27); insufficient land for large scale production (M=1.19); lack of agricultural inputs (M=1.19); land degradation/infertile land/soil (M=1.16); lack/poor storage and processing

Table 6. Mean distribution of respondents according to perceived causes of decline in trifoliate yam production.

Causes of decline in trifoliate yam production	Mean (M)	Std. deviation
Problems of good storage and processing facilities	1.06*	0.62
Poor extension activities	1.01*	0.74
Poor marketing system	1.01*	0.79
Pest and diseases attack	0.78	0.69
Unpredictable climate	1.37*	0.65
Lack of agricultural inputs (e.g fertilizer)	1.19*	0.83
Insufficient land for large scale production	1.20*	0.78
Lack of seed yam	0.72	0.67
High cost of seed yam	0.69	0.72
Drudgery associated with trifoliate yam production	1.72*	0.56
Problems of land tenure system	1.04*	0.85
Problems of basic social amenities	1.44*	0.71
Poor financing	1.80*	0.45
Poor transport system	1.27*	0.68
Lack of good agricultural education	1.48*	0.63
Poor tools and farm machines	1.29*	0.74
Unstable policies and programs of government	0.92	0.83
Erosion	0.84	0.74
Land degradation/infertile land/soil	1.16*	0.76
Cultural barriers associated with trifoliate yam	0.32	0.53
Lack of awareness about the nutritional, economic and health values of trifoliate yam among farmers and consumers	1.71*	0.47
Change in taste by the younger generation in terms of food/trifoliate yam consumption	1.46*	0.70
Lack of interest on the part of the youths in agricultural production	1.55*	0.54
Quest for ‘white collar’ jobs	0.98	0.83
Notion/belief that trifoliate yam kills or can cause madness when consumed.	1.33*	0.74
Destruction caused by stray animals	1.44*	0.66

Source: Field survey, 2014. *Causes of decline.

facilities (M=1.06); problems of land tenure system (M=1.04); poor extension activities (M=1.01) and poor marketing system (M=1.0). It is a known fact that developing countries are faced with numerous problems such as land tenure, lack of credit/finance, poor extension education and contact, lack/poor infrastructural facilities among others which militate against the development of agriculture. Adebowale et al. (2013) also observed that despite the nutritional advantages of trifoliate yam, it is highly underutilized in Nigeria mainly due to tuber hardening which begins a few hours after harvest thus becoming hardened and hard to chew even after long hours of cooking, making their consumption almost impossible.

Minor causes of decline in trifoliate yam production as shown in Table 6 include quest for ‘white- collar jobs’ (M=0.98); unstable agricultural policies and programs of government (M=0.92); erosion (M=0.84); high cost of seed yam (M=0.69) among others. Thus there are agreements in the responses of these respondents on the causes of decline in trifoliate yam production as can be proved by the standard deviation of less than one in all the variables in the table. This signifies relevance of the data for policy. In view of the constraints/causes of decline, there is an urgent need for the Nigerian government to provide conducive environment by subsidising farm inputs and providing affordable loans to the smallholder yam farmers for sustainable production (Verter and Bečvářová, 2015).

Conclusion

It can be deduced from the study that trifoliate yam farmers had no agricultural extension contact but relied on informal sources for information. The farmers produced trifoliate yam during rainy season for consumption using traditional agronomic practices/methods. Moreover, poor finance, drudgery, lack of awareness of the nutritional, economic and health values of trifoliate yam were the major causes of decreased trifoliate yam production.

RECOMMENDATIONS

1. Government should encourage and sponsor more research on trifoliate yam especially in area of inventing improved varieties. These varieties and other necessary agro-inputs should be made available to trifoliate yam farmers as incentives or subsidies in order to consolidate their interests and boost the production of trifoliate yam.
2. Agricultural extension agencies should carry out adequate periodic public enlightenment campaigns on the nutritional, economic as well as health benefits of trifoliate yam. This will arouse the interest of farmers and consumers of trifoliate yam and may lead to commensurate and constant demand of this product thereby motivating farmers to produce more for consumption and income. In these ways this yam species will not get extinct but contribute towards food security and agricultural /economic growth and development.
3. Infrastructural facilities (good roads, market and agricultural machines) as well as incentives and subsidies (like loan, seed yam, fertilizer, agrochemical) should be provided to farmers by the government through extension information and activities. This is to ensure that these provisions are equitably distributed among the target beneficiaries and ultimately motivate, boost and consolidate the efforts of these farmers in trifoliate yam production and agriculture.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Influence of boll sampling method and water stress on fiber quality of irrigated cotton (*Gossypium hirsutum* L.)

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The quality of cotton fibers in Brazil has been studied. This was done by studying the fiber samples obtained from bolls removed from the middle third of the plants. These fiber samples are referred to as "standard sample". This way to collect fiber data requires lots of labor, and may disguise the results obtained in experimental appraisals, due to human errors in gathering boll. Besides, cotton yield and quality is influenced by water availability, especially during abiotic tests with water deficit, in which, fiber quality samples may be affected by boll position. Thus, the objective of this study was to evaluate the influence of sampling method on the technological characteristics of cotton fibers, in irrigated and water stress tests at different stages of the crop cycle. Two methods were made to collect cotton fiber. The first method was the standard sample and the second way was gathering sample of bolls in randomized position, through all experimental plot (called randomized sample). The results show that the analysis performed by standard sample tend to overestimate the values of the fiber quality parameters, differing from the results obtained with the randomized sample that is representative of all plot. It was observed that the variability of cotton fiber quality affected by water stress treatments were best represented using bolls obtained by randomized method. Consequently, in the case of experiments with water stress, the most representative method to collect cotton fiber, is through a sampling of all the plant, and not only of the middle third.

Key words: Boll position, cotton fiber, HVI, standard sample, water stress.

INTRODUCTION

Cotton profitability relies on both yield and quality of cotton fiber, and also depends on the interaction of several factors, such as crop management, environment factors and genetics.

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Table 1. Soil chemical characteristics at depth of 0-40 cm, in experimental area of Apodi, RN.

Year	pH	OM	P	Na^+	K^+	Ca^{2+}	Mg^{2+}	H + Al	CEC	BS
	water	(g kg^{-1})	(mg kg^{-1})	......................($cmol_c$ dm^{-3}).........................						
2014	6.20	16.4	10.7	0.4	1.6	34.8	10.0	23.1	69.9	46.8

(OM) - organic matter; CEC - cation exchange capacity, BS – Base Sum.

Beltrão and Azevedo (2008) affirmed that cotton fiber is mainly conditioned by hereditary factors, although some technological characteristics are decisively influenced by environmental factors and crop management. Thus, the cultivars selection in breeding programs is an important activity, in which crop behavior, conditioned by environmental features must be taken into account during evaluation.

Whilst cotton fiber yield is easily quantified, fiber quality is a complex parameter (Bradow et al., 1997). Measurements of fiber quality are complicated due to natural and environmental variations in fiber structure and maturity. These variations may occur both in bale, plant, boll or seed. Thus, this is essential for breeding programs to make progress in quality improvements, and the results obtain from the research are reliable. Uniformity of the sample collected for analysis represents the real condition of the fiber quality of any experimental area or plot (Bradow et al., 1997).

Fiber quality analysis in Brazil are done using a "standard sample” from each plot, in which bolls are harvested from the middle third of the plants, and, in this case not representative of all experimental plot, considering that plants may suffer any stress, after or during boll formation. Therefore, this methodology can generate erroneous estimates of the cotton fiber yield or characteristics of the quality fiber measure by High Volume Instruments (HVI) (Belot and Dutra, 2015; Kelly at al., 2015).

The standard sample consists of harvesting 20 bolls of the middle third of the plants, which may mask research results. According to Belot and Dutra (2015), comparing the boll of top and lower position with the middle third of the plants, great discrepancies in some characteristics such as micronaire, maturity and percentage of fibers were found. These differences occur probably due to complexes interactions among soil properties, soil water and nutrients availability and plant populations (Bradow et al., 2000). So, the use of standard methodologies in research, harvesting only in specific positions, do not allow safety evaluation of the cotton fiber quality produced in field (Belot and Dutra, 2015).

This problem can be further aggravated in the case of tests with abiotic stresses, such as water stress, since this may occur at different stages of the crop cycle, disturbing the cotton fiber formation with consequent changes in quality. These changes depend on the fruit positions at the time of the water deficit. Thus, ideally, samples should be taken to represent all the fruiting points of the plant, and not only those of the middle third.

The objective of the present study was to evaluate the influence of the sampling methodology on the characteristics of cotton fiber quality to cultivars under irrigation system, with and without water stress, in the Brazil's semi-arid region.

MATERIALS AND METHODS

The experiment was carried out from June to November 2015, at the Experimental Farm of the Agricultural Research Company of Rio Grande do Norte (EMPARN), located in Apodi, RN. Experimental area have central geographical coordinates of 5°37 '19 "S and 37°49' 06" W, with altitude range of 128 to 132 m.

The climate of the region is characterized as hot and semi-arid tropical, with predominance of BSw'h' type, according to Köppen's climatic classification. The soil of the experimental area was classified as eutrophic Cambisol (Santos et al., 2006), clay-sandy texture, with 49% sand, 45% clay and 6% silt. It was used no-tillage system with a 3 row mechanized sowing machine, and no thinning was required. Fertilization was performed according to the technical recommendations for the crop, based on soil fertility analysis (Table 1).

The experiment was carried out in a randomized complete block design with split plot arrangement, and with water deficit periods in main plot. Cotton cultivars were in subplots, and sampling methods in sub-subplots with four replications. In the plots, treatments consisted of 6 periods of water deficit, named:

1. Initial (IN)
2. Floral bud (FB)
3. Early bloom (EB)
4. Peak bloom (PB)
5. Open bolls (OB) and
7. Control without water deficit (IR).

Cotton cultivars were:

1. BRS 286
2. BRS 335
3. BRS 336
4. BRS 372
5. BRS 368RF
6. BRS369RF
7. BRS370RF and
8. BRS 371RF

Sub-subplots sampling methods: the standard sample (SS) and plot sample (PS). The standard sample consisted of 20 bolls harvested in the middle third of plants, while the plot sample consisted of 100 g of cotton fiber, randomly collected from the all experimental plot, harvested in different plant positions.

Each experimental unit consisted of 4 spaced rows of 0.8 and 6.0

Table 2. Water deficit period in each treatment.

Treatment	Start of water suppression	Net irrigation depth (mm)
Initial (IN)	After stand establishment	650
Floral Bud (FB)	Beginning with the first flower bud at least in 10% of the plants	634
Early Bloom (EB)	Opening of first flower at least in 10% of the plants	577
Peak Bloom (PB)	Boll loading. At least 10% of plants heavily fruited where first bolls were completely full	584
Open Boll (OB)*	Opening of the first bolls in 10% of the plants	621
Without Water Stress (IR)	Without deficit irrigation during all crop cycle.	700

*After treatment, cotton plants did not receive water anymore, since crop cycle was in conclusion.

Table 3. Agronomic data and irrigation parameters during cotton crop cycle.

Parameters	Period
Planting date	30/06/2015
Line space	0.8 m
Planting density	8-12 plants m^{-1}
Fertilization at planting	150 kg ha^{-1} of P_2O_5 and 30 kg of N (MAP* form)
Topdressing	150 kg of N ha^{-1} (Urea)
Last irrigation	21/10/2015 (106 DAE)
Harvest date	17/11/2015
Crop cycle duration	131 days
Total rainfall in season	0.0 mm

*MAP – Monoammonium phosphate.

m length, totaling a gross area of 19.2 m^2, with the two central rows as useful area (8 m^2), excluding at least 1.0 m from each border. Water deficit applied consisted of a 15 days period, without irrigation during programmed phenological stages (Table 2). After each deficit period, plants returned to normal frequency of irrigation, calculated considering the crop evapotranspiration. Total depth irrigation for each treatment is presented in Table 2.

A fixed conventional sprinkler system was used for irrigations, with sprinkler spacing of 12 x 15 m, application intensity of 9 mm h^{-1} and Christiansen uniformity coefficient (CUC) of 85%. Irrigations were made at each 3 days, with irrigation depth determined by crop evapotranspiration (ETc) (Allen et al., 2006). Agronomic and irrigation data are presented in Table 3.

Phytosanitary treatments were carried out, when the first symptoms of pests and diseases appeared, as well as the control of weeds. The time of harvest were evaluated with the lint percentage and inherent characteristics of fiber quality as length (UHM), uniformity (UNF), short fiber index (SFI), strength (STR) , elongation (ELG), micronaire index (MIC), reflectance (Rd) and yellowing degree (+b). The quality characteristics of the fibers were evaluated in the Fibers and Yarns Laboratory of Embrapa Cotton, through the High Volume Instruments (HVI) equipment.

Evaluated variables data were submitted to analysis of variance by the F test at 1, and 5% of probability. For statistical analysis, R software (R Development Core Team, 2011) was used. When a significant effect was verified in the variance analysis, data obtained in different treatments were compared through the Tukey test at 1 and 5% of probability.

RESULTS AND DISCUSSION

Results of the variance analysis to lint percentage (%Lint), and to fiber quality characteristics as length (UHM), uniformity (UNF), short fiber index (SFI), strength (STR), elongation (ELG), micronaire index (MIC) maturity (MAT), reflectance (Rd) and yellowing degree (+b) are shown in Table 4.

To accomplish the influence of the sampling methodology on cotton fiber quality, the discussion focused on the sample factor and its interaction with water deficit and cultivars. Observing the results presented in Table 4, it can be noted that the sampling method had no influence on the data of UNF and SFI. For the interaction between the factors, considering the water deficit versus sampling, the interaction was not significant for the STR and ELG parameters, while the cultivar versus sampling interaction was significant only for the UHM data. These results proved that there is variation in cotton fiber quality within the same plant, depending on the boll position, as discussed by several authors, such as Bradow and Davidonis (2000), Bauer et al. (2009) and Feng et al. (2011).

Thus, if the bolls are harvested bolls just in the middle third of the plants, the result of fiber quality analysis can be masked, not representing the real condition of the plot, agreeing with the study of Belot and Dutra (2015). Additionally, for experiments or field appraisal, in which cotton plants endured abiotic stresses, water deficit is the best way to estimate cotton fiber quality in harvesting the whole plant.

Table 4. Mean squares for lint percentage and fiber quality characteristics evaluated as a function of water deficit, cultivars and sampling method, Apodi, 2015.

Source of variation	GL	Mean squares									
		% Lint	UHM	UNF	SFI	STR	ELG	MIC	MAT	Rd	+b
Block	3	3.37*	1.41	0.39**	0.24	2.28	0.32	0.36	0.0002	0.96	0.32
Déficit (D)	5	10.86**	30.2**	54.83	18.7**	50.17**	0.52*	7.03**	0.04*	27.44**	9.57**
Residue a	15	0.91	1.03	0.66**	0.29	2.90	0.17	0.35	0.0002	3.64	0.34
Cultivar (C)	7	166.6**	113.91**	19.03	6.75**	108.41**	23.12**	3.11**	0.003**	13.33**	8.21**
C × D	35	3.29**	1.4	1.64	0.79**	4.68	0.33*	0.68**	0.0004**	3.16	0.55*
Residue b	126	1.57	1.42	1.29	0.28	4.02	0.18	0.19	0.0001	2.58	0.34
Sampling (S)	1	4.49*	41.81**	1.26	0.58	56.51**	0.82*	8.28**	0.006**	86.29**	24.9**
D x S	5	4.14**	7.87**	2.98*	0.91*	2.91	0.24	0.78**	0.0005**	9.02**	1.42**
C x S	7	1.31	2.13*	0.94	0.50	2.73	0.11	0.16	0.0001	2.75	0.19
D x C x S	35	1.28*	0.86	1.77*	0.47	3.76	0.28	0.13	0.00008	2.13	0.26
Residue c	144	0.79	0.79	1.22	0.32	3.02	0.20	0.09	0.0001	2.67	0.28

** and *Significant at 1 and 5% of probability, respectively.

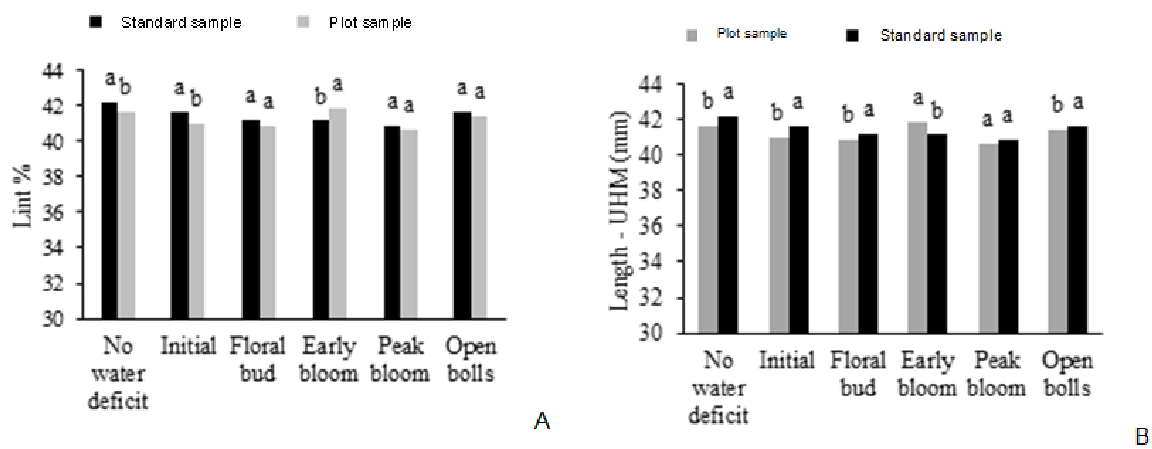

Figure 1. Lint percentage (A) and fiber length (B) as a function of water stress and sampling methods for cotton fiber quality.

Environmental variation that occurs within the plant canopy, between plants and between plots, causes great variability in fiber quality characteristics, not only at boll level, but also among plants and plots (Bauer et al., 2009; Bradow and Davidonis, 2000; Feng et al., 2011). In this way, the more uniform and representative the conditions of the plant and the plot as a whole is with the sampling, the more representative the results of the fiber quality analysis will be.

The cotton fruits generally develop rapidly up to 16 days after the anthesis (DAA), reaching their maximum size approximately 24 DAA, being mature and open between 40 and 60 days after the anthesis (Kim, 2015). When water stress is applied in diverse phases of the crop phenological cycle, the stress will affect the bolls differently, depending on the stage of the fiber formation. Thus, for the determination of the fiber quality in tests of water stress, the most indicated is to gather boll samples representing all plant positions, in order to avoid results with mistaken estimates. Figures 1 to 5 show the values of the cotton quality parameters of the evaluated fibers. It is observed that the lint percentage (Figure 1A), determined from the SS, was underestimated just when water deficit occurred at the early flowering, being overestimated in the other treatments when the analysis was performed based the SS gathering. This is due to the fact that in the standard sampling method, only the middle third of the plants are selected, excluding the bolls of top and bottom, and so, inadequate for assessments in water stress treatments, on which lint percentage may be affected depending on the phenological phase of the harmful stress.

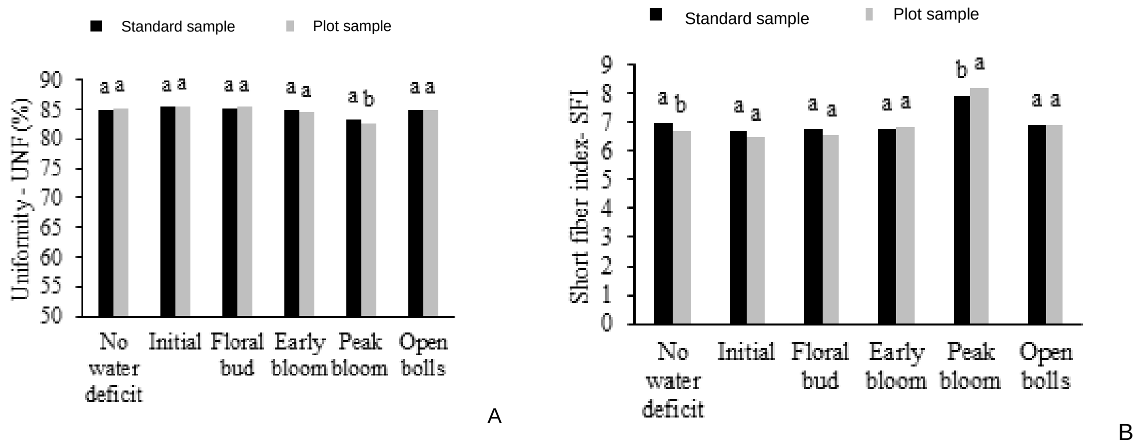

Figure 2. Fiber uniformity (A) and short fibers index (B) of cotton as a function of water stress and sampling methods to fiber quality collected for analysis

Belot and Dutra (2015) found that the lint percentage is higher in the middle third of the plants, when compared with the bottom position, and lower in relation to those in the upper position. Thus, for treatment with early flowering period of water stress application, the bolls of the middle third were the most affected, and the values of lint percentage were underestimated when compared to the whole plant data in this phase.

Several authors such as Wen et al. (2013), Brito et al. (2011), De Tar (2008) and Pettigrew (2004) have shown that cotton is influenced both in yield and lint percentage, as fiber quality when submitted to irrigation with water deficit. Beltrão and Azevêdo (2008) stated that cotton fiber is mainly conditioned by hereditary factors, although some technological characteristics are decisively influenced by environmental factors as temperature, luminosity or water availability, and also depend on the crop management. Hence, the sampling performed in an unrepresentative manner of the applied treatments can lead to errors in the interpretation of the results.

Similar overestimation behavior can be observed for the fiber length parameter (Figure 1B). Results showed that, except to data collected through SS in the treatment with water deficit during early flowering, the remaining data were overestimated for this sampling method. Possibly, in this phase where the water stress was applied, bolls in the middle third were being formed and, therefore, they had a greater influence of the applied treatments, which demonstrates the importance of the data collection of the whole plant for fiber analysis, especially when crop was submitted to water stress. The period of fiber formation, according to Abidi et al. (2010), occurs within 3 weeks after the anthesis, thus periods of water stress in this phase can compromise the length of the fibers formed in these bolls.

Considering the fiber length overestimation in the other treatments to data acquired by SS method, the result is due to the fact that, according to Kelly et al. (2015), the fiber length values vary according to boll position in the plant, being larger in the middle and bottom thirds, and lower in the upper positions. Consequently, when merely collecting the samples of the middle third, the values tend to display overestimation in relation to samples of the whole plant that are more representative.

It is also observed in Figure 1B more accentuated overestimation to irrigated treatments or for treatments with water deficit in the initial phase (IN) and floral bud (FB) stages, because water stress did not occur or was less severe in the fruit formation phase. Less accentuated overestimation was observed to treatments where stress occurred in the filling and opening boll periods, since stress happened in the phase of fruits formation, and probably affecting fruits that were being formed in others parts of the plant, like the top positions (Figure 2).

Fiber uniformity is presented in Figure 2A. This characteristic was less influenced by standard sampling method, with changes observed only when water stress was applied in the peak bloom (boll filling stage). Another characteristic that affected SS method was the short fiber index (Figure 2B), this was influenced by the sampling method that is well irrigated in the control treatment, and to application of water stress in the peak bloom (boll filling phase), showing values overestimated and under-estimated, respectively. The fiber analysis results were affected by sampling method, since it is completely irrigated cotton, that is the best condition, the SS sampling method conferred an overestimation of values, due to the collection being performed in the middle third of the plants.

In contrast, the most sensitive phase to water stress is during cotton boll filling stage (peak bloom), in which water deficit was imposed (Cock et al., 1993; Gwathmey et al., 2011; Snowden et al., 2014). So, the sample

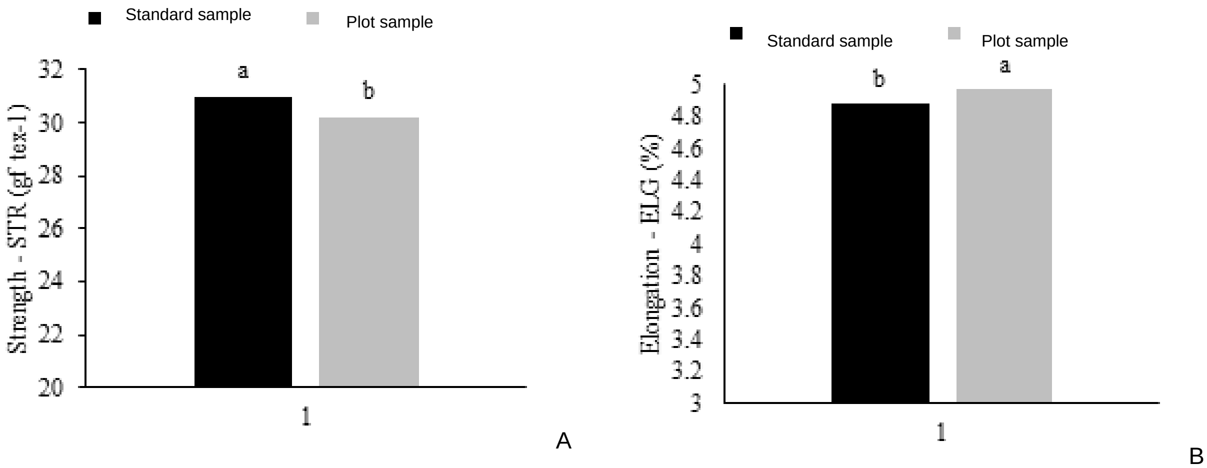

Figure 3. Strength (A) and elongation (B) of cotton fibers as a function of sampling method to take data for fiber quality analysis.

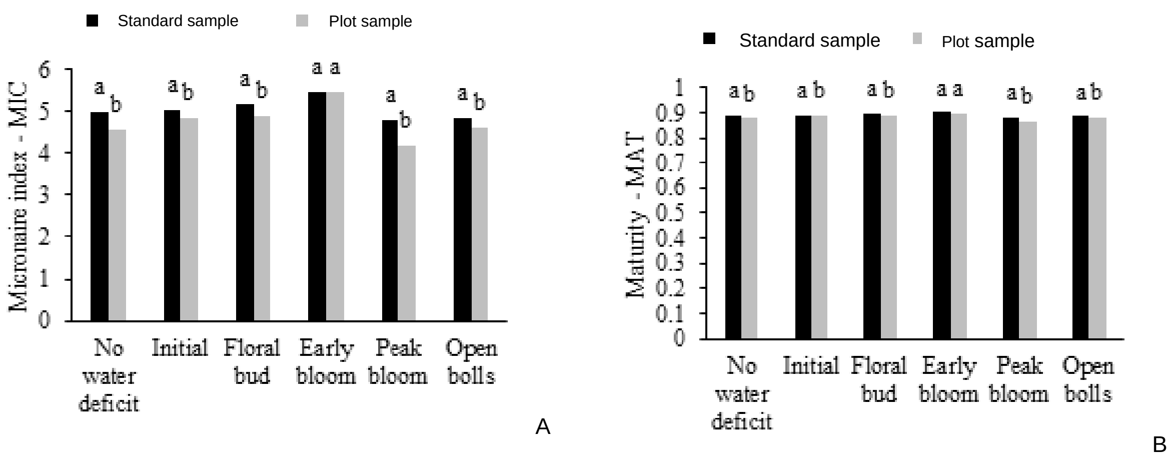

Figure 4. Micronaire index (A) and maturity (B) of cotton fibers as a function of water stress and sampling methods to take data for fiber quality analysis.

collection by SS method in this phase resulted in an underestimation of the results, probably because the sampling may occurred in the area of the plant with the most affected bolls by water stress (Figure 3). Independent of the water stress treatment was applied, SS method affected characteristics as strength (Figure 3A) and elongation (Figure 3B), with values over-estimated and underestimated, respectively.

One of the most important cotton fiber parameter of quality to be evaluated is micronaire index (Figure 4A). Results showed overestimation for all evaluated treatments when used with SS method to sampling fiber. But, there were no statistically different results with water stress beginning from early flowering, if compared with the two methods of sampling.

Several authors such as Cordão Sobrinho et al. (2015) and Zonta et al. (2015) have reported micronaire index values above 5.0 in experiments with irrigated cotton in the semi-arid region, considered coarse fiber, and above the tolerable value by the textile industry (Fonseca and Santana, 2002). According to the results founded in this study, this high value of micronaire index can be associated with the SS method to collect fiber samples, in which mainly first-rank bolls are collected, where fiber bring the highest values of micronaire (Belot and Dutra, 2015).

For the control treatment and using the method of sampling to whole plant, it was observed that the average of micronaire values was 4.5. This value is considered, acceptable by the textile industry, that range from 3.8 to4.5, and so, not resulting in discount in the fiber price.

Maturity (Figure 4B) also followed the same general behavior of the other characteristics, being overestimated when samples were determined by SS method. According

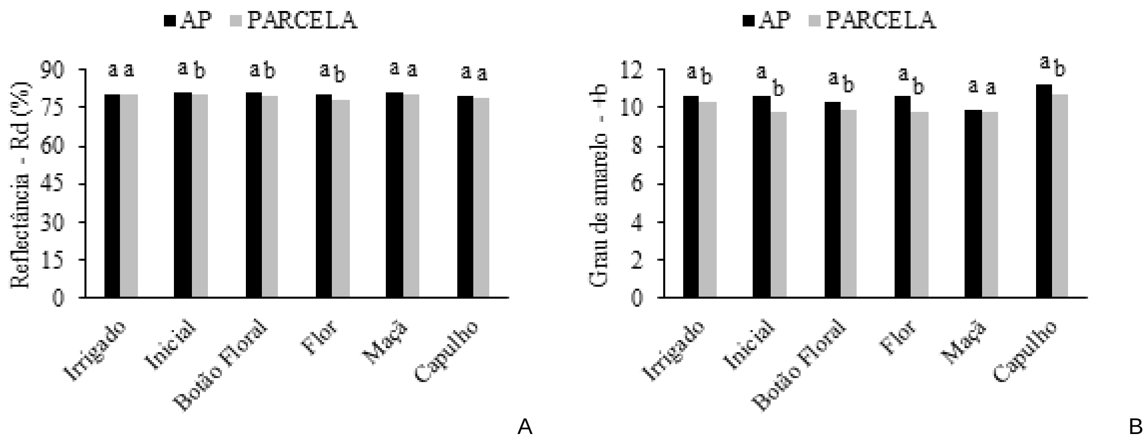

Figure 5. Reflectance (A) and yellowing degree (B) of cotton fibers as a function of water stress and sampling methods of take data for fiber quality analysis.

to Kelly et al. (2015), the fiber maturity decrease towards from bottom to top of the plants, this explains the overestimation of this parameter when bolls were obtained for the middle third of the plant (SS) in relation to the whole plant sampling. Fiber maturity is an important parameter for the textile industry, because its variability has negative impact on the final product, especially during dyeing, since the immature fibers have a lower ink absorption capacity, making the fabric not uniform (Kelly et al., 2015; Kim, 2015). This statement demonstrates the importance of the correct determination of this parameter.

For the characteristics related to fiber color, reflectance (Figure 5A) and yellowing degree (Figure 5B), the values also followed the tendency to be overestimated when determined from the SS method, and when compared to the values determined by sampling the whole plant. Discoloration of a cotton sample may be an indication of problems such as exposure of the fiber to conditions that lead to reduced strength of fiber such as long exposure to the climate under field conditions, hence the importance of its precise determination (El Mogahzy and Chewning, 2001).

Conclusions

Results showed that fiber analysis performed from samples collected from the middle third of the plants (Standard Sample) tend to overestimate fiber quality parameters when compared to the results of data obtained from fiber samples of the whole cotton plant. These result are worsen with the occurrence of water stress in different phases of the cotton phenological cycle, because standard sample harvested bolls just to the middle third positions of the plant, and so, can dissemble the influence of water stress on cotton quality properties. At the time of water stress, bolls being formed may be affected and further, when in the middle third position of plants, their fibers can be measured, influencing the results obtained. On the other hand, if bolls in formation are in the middle third position during the water stress, the effect of water stress will not be observed afterwards using standard sampling, which also changes the results. Thus, for the determination of the cotton fiber quality in water stress tests, the most indicated method to collect samples is to gather boll samples representing the whole plant, using all positions, not only the fruitful positions of the middle third of the plants, in order to avoid results with mistaken estimates.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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