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African Journal of Agricultural Research

*Full Length Research Paper*

# Effects of fungicide application and different nitrogen fertilizer levels on yield components of three varieties of common bean *Phaseolus vulgaris* L.

**Sanyang S. F.[1*], Tankou C.[2], Yaouba A.[1] and Kinge T. R.[3]**

[1]Department of Crop Protection, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon.
[2]Department of Crop Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon.
[3]Department of Biological Sciences, Faculty of Science, The University of Bamenda, P. O. Box 39, Bambilli, Bamenda, Cameroon.

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**In Cameroon, common bean is produced and highly consumed as a source of protein and means of generating income by small farm holders. However, diseases like angular leaf spot caused by *Phaeoisariopsis griseola* (Sacc), poor agronomic practices and low soil fertility are negatively impacting the production of the crop. A field experiment was conducted under natural conditions in the University of Dschang during the 2017 main cropping season. The experiment was laid out in a RCBD in a split-split plot arrangement with three replications: Fungicide application (sprayed and unsprayed); Fertilizer level: F1 (Control), F2 (10 t/ha *Tithonia*), F3 (3.5 t/ha poultry manure) and F4 (0.4 t/ha 14.24.14 NPK fertilizer). Bean varieties that occupied each experimental unit were V1 (GLP-190 S), V2 (PH201) and V3 (PNG). There was a significant difference (P<0.05) between sprayed and unsprayed plots with respect to disease severity. The highest number of pods was obtained from the *Tithonia* treatment (F2) while the lowest was gotten from the mineral fertilizer treatment (F4). As concerns the interactions, fertilization and variety, spray and variety, there was a significant difference (P<0.05) among the various components. In all varieties, sprayed plots had more pods, seed weight, 100-seed weight compared to unsprayed plots. From the study, it shows that fungicide treatment reduced disease severity and the different nitrogen fertilizers greatly improved yield components of the crop.**

**Key words:** Angular leaf spot, common bean, fungicide spray, nitrogen fertilizers, Western Highlands, yield components.

## INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a major grain legume crop grown worldwide for its nutritional value (Amin et al., 2014), supplying about 20% of the protein intake per person worldwide (CIAT, 2001). The FAO (2014) reports that half of the world's common bean production occurs in low income food deficit countries

*Corresponding author. E-mail: sangsylsylvester@gmail.com. Tel: +237-675428886.

where this staple crop contributes to food security. The other half is produced in countries like in US, where common bean is an important economic crop. In Cameroon, common beans is mainly cultivated and marketed in the Western Highlands of Cameroon (Tatchago, 1987; Siri et al., 2017). This region contributes more than 90% of the national bean production (Anonymous, 2010). It is grown for its high nutritional value and potential as a source of income for the smallholder farmer. This makes haricot beans an ideal crop for simultaneously achieving three developmental goals: reducing poverty, hunger and improving human health (Akibode and Maredia, 2011). Bean is a good source of protein, folic acid, dietary fibre and complex carbohydrates (Jones, 1999). Even though the crop is very important, the national average yield of common bean in Cameroon is very low, around 0.5 t/ha (Pamo et al., 2005) compared to the potential of the crop which is estimated at 1.5 t/ha for promising varieties. The low national yield has been attributed to various constraints (Wachenje, 2002). Among these are diseases, insects and low soil fertility. Common bean diseases such as Angular Leaf Spot (ALS) caused by *Phaeoisariopsis griseola* (Sacc.) Ferraris is one of the most widely distributed and damaging disease of crop, causing yield losses as high as 80% (Mahuku et al., 2004; Buruchara et al., 2010). When weather conditions are favourable for its development, ALS is a very destructive disease with crop losses resulting mainly from premature defoliation (Mwamgombe et al., 1994). The disease affects foliage and pods throughout the growing season and is particularly destructive in areas where warm and moist conditions are accompanied by abundant inoculums from infected plant residues and contaminated seeds. ALS incidence and severity increased in many areas where beans are cultivated (Stenglein et al., 2003). Yield losses due to ALS have been estimated at 50% in USA, 70% in Brazil and 80% in Columbia and Cameroon (Tiomo, 1994; Jesus et al., 2001). Different management options such as use of resistant varieties, use of disease free seeds, fungicide application and crop rotation are known to be the ideal way to manage this disease (Mekonen, 2017). However, in Cameroon, resistant varieties are insufficient due to the fact that the pathogen has been developing virulent resistant genotypes (Mahuku et al., 2009; Wagara et al., 2011). Different fungicides applications and use of nitrogen fertilizers remain a better option in controlling disease spread as well as improving yields. Nitrogen is the most important element limiting crop production in the tropics.

Previous surveys estimated that over 60% of the bean production areas in Central, Southern, and Eastern Africa was affected by N Deficiency (Thung and Rao, 1999). This caused yield losses of up to 40% compared to the N-fertilized areas (Thung and Rao, 1999). Beans are legumes that can fix atmospheric nitrogen ($N_2$) into the soil in symbiosis with soil rhizobia. However, common bean is considered to be a poor fixer of atmospheric N when compared with other crop legumes (Piha and Munns, 1987). It generally responds poorly to inoculation of rhizobia in the field conditions (Buttery et al., 1987) and rarely derives more than 50% of their N from the atmosphere (Wortmann, 2001). Various organic and inorganic fertilizer sources have been used to improve soil fertility as well as improving quality of yields of beans and other vegetables (Mohammad et al., 2016). Application of inorganic fertilizer is a faster way to maintain the productivity of crop because the nutrients are releasing nutrients like NPK which is easily available to plants. On the other hand, organic fertilizers (cow manure, poultry manure, and green manure) have been shown to help preserve natural resources and reduce degradation of ecosystem (Mäder et al., 2002; Francis and Daniel, 2004). Application of chemical fertilizers containing N, P, and K not only increase crop yields but also improve nutritional quality of crop yields, such as protein, oil, starch, essential amino acids and vitamins in pulses, oil seeds, tubers and vegetables (Wang et al., 2008). The present work was carried out with the objective of improving the production system of beans through the contributions of fungicide spray against angular leaf spot disease and the use of appropriate nutrient sources (nitrogen fertilizers).

## MATERIALS AND METHODS

### Description of the study area

The field experiment for the management of common bean angular leaf spot through fungicides and nitrogen fertilizers was conducted at the Faculty of Agronomy and Agricultural Sciences (FASA) experimental farm, University of Dschang (UDs), West Region, during the main cropping season (March - July) of 2017. Dschang is located at 5° 26'N, 10° 04'E at an altitude of 1400 m above sea level. The precipitation of Dschang varies between 1800 and 2000 mm annually while temperatures range from 21 to 25°C. Its relative humidity is generally above 60%. Dschang receives an average insulation of 2000 h a year. The soil type of the experimental site is sandy loam with a pH value of 6.1 (Soil Science Laboratory, FASA, UDs, 2017).

### Experimental materials used

Three common varieties, namely GLP 190-S (MIDENO), PH201 (Meringue) and PNG (Koussi) were used for the experiment. These varieties are widely used in these localities because of their growth habit, taste and productivity. The seeds were obtained from IRAD Dschang Cameroon. The contact fungicide Agreb (Agreb 80WP) with the active ingredient Maneb, the poultry manure and the mineral fertilizer (14.24.14) were obtained from the local market. The Mexican sunflower leaves/shoots were harvested around the experimental farms of FASA.

### Experimental design and treatments

The experiment was laid out in a randomized complete block design (RCBD) in a split-split plot arrangement with three

replications. The main plots were the fungicide treatments while the sub-plots were the fertilizer treatment and the sub-sub plots were for the bean varieties. There were 12 plots, each consisting of 5 rows. Plant to plant distance and row to row distance was maintained at 35 and 20 cm, respectively. Each main plot had a length of 24.5 m and width of`19.7 m. There were four levels of fertilizer application as thus: F1- control (unfertilized), F2- *Tithonia*, F3- Poultry manure and F4-NPK (14.24.14) formed main plots. Each subplot has a length of 9.8 m and a width of 1.5 m. The size of each experimental unit (sub-subplot) was 2.6 m × 1.5 m (3.9 $m^2$) having five rows each containing 26 plants. A distance of 1 and 1.1 m were left between plots and blocks, respectively. This gave a total experimental plot surface area of 482.7 $m^2$. Two seeds per hole were sown at the recommended planting depth of 6 cm. All agronomic practices such as cultivation, weeding and mulching were done manually when the crops were at vegetative stage (three weeks after sowing) and mid reproductive stage (Fontem et al., 2007; Mboussi et al., 2012).

#### Soil sampling and laboratory analysis

Prior to planting of seeds, soil samples were collected at a depth of 0 to 30 cm from representative spots of the entire experimental field by using diagonal sampling method (Turuko and Amin, 2014). The composite soil sample was air dried and made fine by using mortar and pestle. The sample was later taken to the laboratory of Soil Science of FASA, UDs where the physical and chemical properties of the soil were analyzed.

#### Fungicide application

Each main plot was divided into two halves spray (Sp) and unsprayed (Unsp). Sprayed plots were separated from unsprayed plots by a bean-free zone of 1.1 m. The contact foliar fungicide Agreb was applied using the manual Solo Knapsack sprayer with a single flat fan nozzle that delivers about 600 L/ha at a maximum pressure of 3 kg/$cm^2$ (Fontem et al., 2007). The first treatment was applied at first foliar symptoms (35 days after planting, DAP) and subsequent sprayings were done during flowering (45-55 DAP) and at podding stage (60-65 DAP). Plants were sprayed in the early hours of the day when the wind speed was low.

#### Angular leaf spot disease assessment

Bean crops were inoculated by naturally occurring inocula of angular leaf spot pathogen in the field. Percentage disease severity was scored at eight days interval on five randomly selected plants in the centre rows of each experimental unit. This was done using the standard disease grading scales of 1 to 9, where 1 = no visible disease symptoms; 3 = plants with 5 to 10% leaf area having lesions; 5 = plants with 20% leaf are having lesions and sporulation; 7 = plants with up to 60% leaf are having lesions, associated with chlorosis necrotic tissue, and 9 = plants with 90% leaf area having lesions, associated with early leaf fall and death (VanSchoonhoven and Pastor-Corrales, 1991). Five ratings of disease severity were collected starting from the 35 DAP.

#### Microscopic confirmation of the pathogen

Representative samples of all diseased leaves of plants (two leaves) of each variety showing symptoms of angular leaf spot, were collected, placed between two clean papers, labeled and taken to the laboratory of the Catholic University of Cameroon (CATUC) Bamenda in order to confirm field identifications through microscopic observation. Each sample having suspected disease symptom(s) was cut into smaller pieces of 2 cm from the edge of the diseased leaf and surface sterilized for) min in 10% sodium hypochlorite solution and rinsed in sterilized water. The sterilized pieces were placed on 2.5% Potato Dextrose Agar (PDA) medium. The plates were kept at room temperature in the laboratory for 4 to 7 days. Fungal growths on each plate were sub-cultured to a new plate and the plates were kept in an incubator at 27 to 29°C. The growth of fungus was observed daily for its typical characteristics. Later pure culture of the isolated fungus was identified morphologically using a compound microscope and observed at magnification of 10 and 40 ×.

#### Harvesting and yield assessment

Mature pods (more than 90% ripe) of each cultivar per sub-subplot were harvested from the two central rows excluding one border row on both sides to minimize border effects. These were used to assess number of pods, and number of seeds per experimental unit and weight of 100-seeds. Marketable yields were measured as weight of clean dry seed per subplot and expressed in tons per hectare. Amount of shriveled or discoloured seeds were counted from randomly selected hundred seeds and converted to percentage (Hirpa and Selvaraj, 2016). Yield loss and gain were determined using the formula of Nkalubo et al. (2007) as thus:

$$\% \text{ Yield loss or reduction} = \frac{PPY - IPY}{PPY} x100$$

$$\% \text{ Yield gain} = \frac{PPY - IPY}{IPY} x100$$

where PPY = Protected or sprayed plot yield and IPY = Unprotected or unsprayed plot yield.

#### Data analysis

Data on disease severity, fungicide and fertilizer treatment and yield components (number of pods, number of seeds per plant, weight of seeds) were subjected to an analysis of variance (ANOVA) using an MSTAT (or GENSTAT) statistical package. Means were separated using Fisher's least significance difference at P=0.05.

## RESULTS

### Disease severity and fungicide application

There was a high significant difference between sprayed and unsprayed plots with respect to disease severity (P< 0.05). Unsprayed plots were thrice more infected with ALS than sprayed plots at all periods of recording for the three varieties of common bean.

The severities of ALS infection increased with time from 35 DAP onward. The mean severity of ALS infection was least at period 1 or 35 DAP (0.90%) and highest period 5 (48.35%). Variety 1 (GLP-190-S) had the highest disease severity for both sprayed and unsprayed plots, while variety 3 (V3 or PNG) was least severed. There was no significant difference (P>0.05) among the severities in terms of fertilization and severity. Severity was highest for poultry manure (F3) and inorganic fertilizers (22.3 and

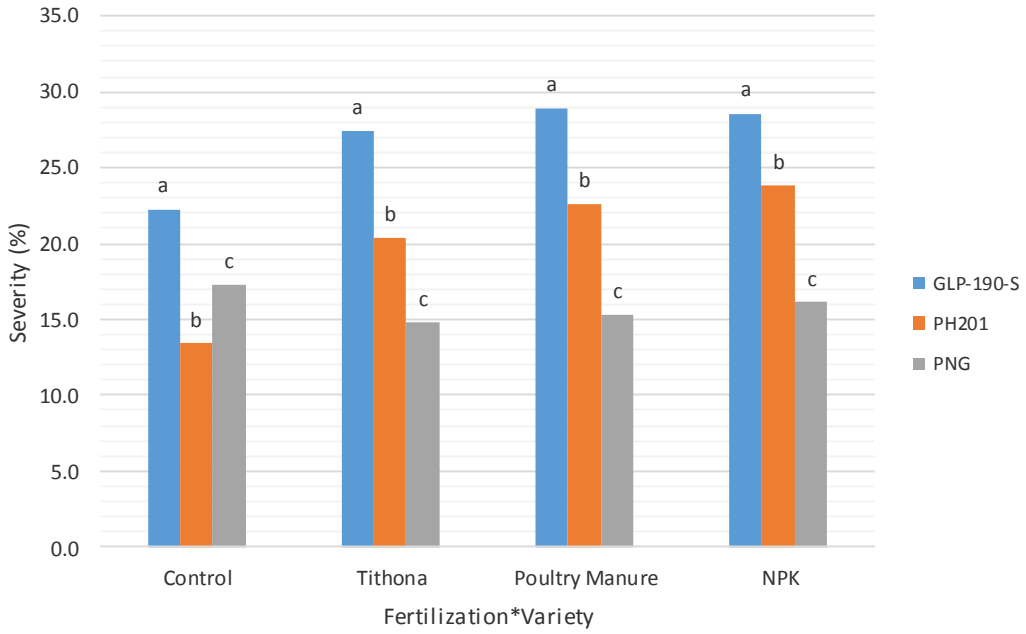


**Figure 1.** Percentage disease severity for sprayed and unsprayed plots at different fertilization levels for three varieties of common bean.

22.8%, respectively) while the control or unfertilized (F1) had the least (17.7%). This indicated that the disease response to fungicide treatment varied from cultivar to cultivar. There was no significant difference (P>0.05) between the interaction fertilization and period for all varieties (Figure 1).

## Yield components at different fertilization levels

There was a high significant difference (P< 0.05) among the different yields components (number of pods per subplot, pods per plant, weight of seed per variety, mean seed weight and 100-seed weight) at the four levels of fertilizer application.

## Number of pods

*Tithonia* (F2) applied at 10 t/ha had the highest number of pods (260.2) while mineral fertilizer (F4) recorded the least (230.2). Thus the number of pods and number of pods per plant was significantly affected by all treatments at P<0.05.

## Seed weight, mean seed-weight and 100-seed weight at different fertilization level

F2 had the highest seed weight (376.4 g) and mean seed weight of 18.8 g, while F1 (control) had the least seed weight of 320.3 g and a mean seed weight of 16.0 g. For 100-seed weight F1 had the lowest (35.0 g) while F4 (inorganic fertilizer) was the highest. Thus all fertilizer treatment significantly increased the pods, weight of seed per plant in comparison with the control.

## Shriveled seed at different fertilization level

The percentage of shriveled seed was least at F1 (6.7%) and highest at F2 (9.3%) (Figure 2).

## Variety

There was a significantly different among the different yield components at (P< 0.05) for the three varieties. For number of pods and pods per plant, PNG (V3) had the highest number of pods and mean pod per plant (299.9 and 15.1 respectively), while GLP 190-S (V1) had the lowest (183.4). In terms of weight, meringue (V2) was the highest (396.4 g) and mean of 19.8 g, while GLP – 190-S (MIDENO) was the lowest (250.4 g) and a mean of 12.5 g. For 100-seed weight, GLP – 190-S (V1) had the highest weight (50.2g) while PNG (V3) was the least (27.0 g). PNG (V3) had the highest percentage of shriveled seeds (11.5%) as compared to GLP – 190-S (V1) with a percentage of 4.3%. For this interaction, there was a high significant difference (P<0.05) among the yield components at various levels of fertilization. Variety 3 (PNG) had the highest number of pods (329.2) at

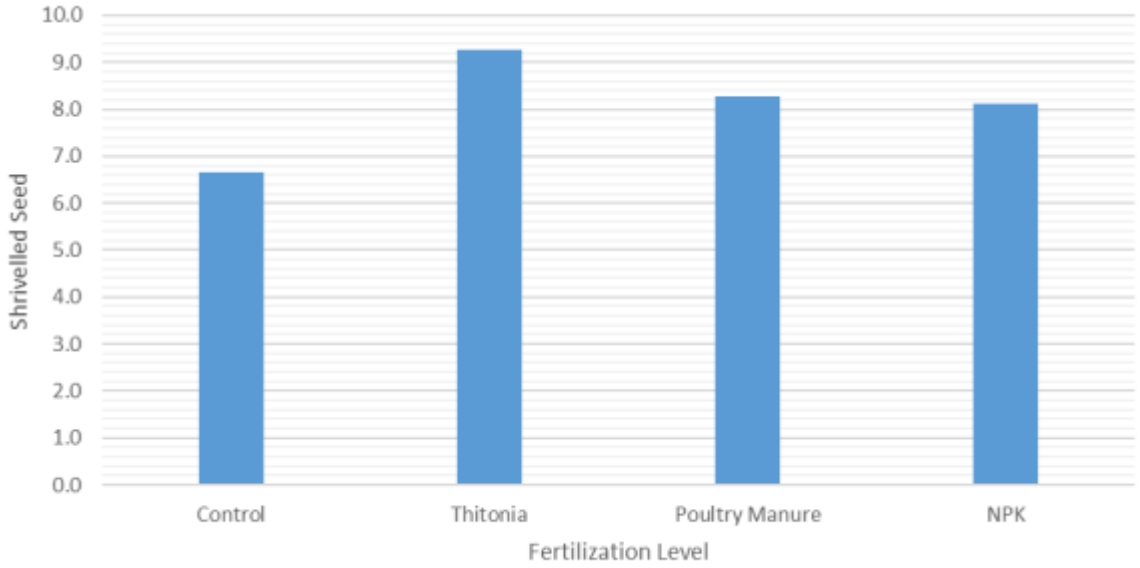


**Figure 2.** Percentage of shriveled seeds at different fertilization level.

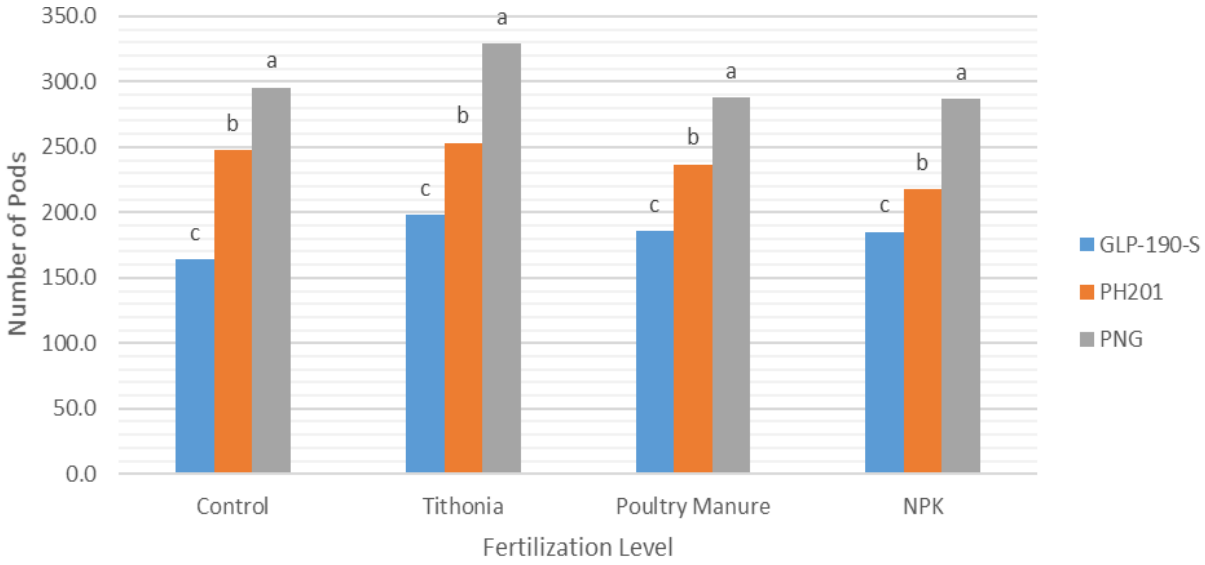


**Figure 3.** Interaction of fertilization and variety with respect to number of pods.

fertilization level 2 (*Tithonia*) while the least was obtained from GLP 190-S (164.3) at fertilization level 1 (control). Thus *Tithonia* (F2) had the highest number of pods per subplot/plant for all varieties, while control or no fertilizer (F1) had the lowest. The same trend applies to seed weight. Highest seed weight was at fertilization level 2 (*Tithonia*) for all varieties of bean, while control (F1) had the least. PNG (V3) had the highest seed weight at all levels of fertilization. Highest seed weight was obtained from PNG (V3) (436.0 g) at fertilization level 2 (*Tithonia*), while the lowest was from GLP 190-S (V1) at fertilization level 1 (F1) (Figures 3 and 4).

### 100-Seed weight

The highest weight (50.2 g) was obtained at fertilization level 4 (inorganic or mineral fertilizer) in the variety GLP 190-S (V1), while PNG (V3) had the least (27.0 g).

With respect to shriveled seeds, PNG (V3) had the highest mean percentage of shriveled seeds at all levels of fertilization, while GLP-190-S (V1) had the lowest. V3

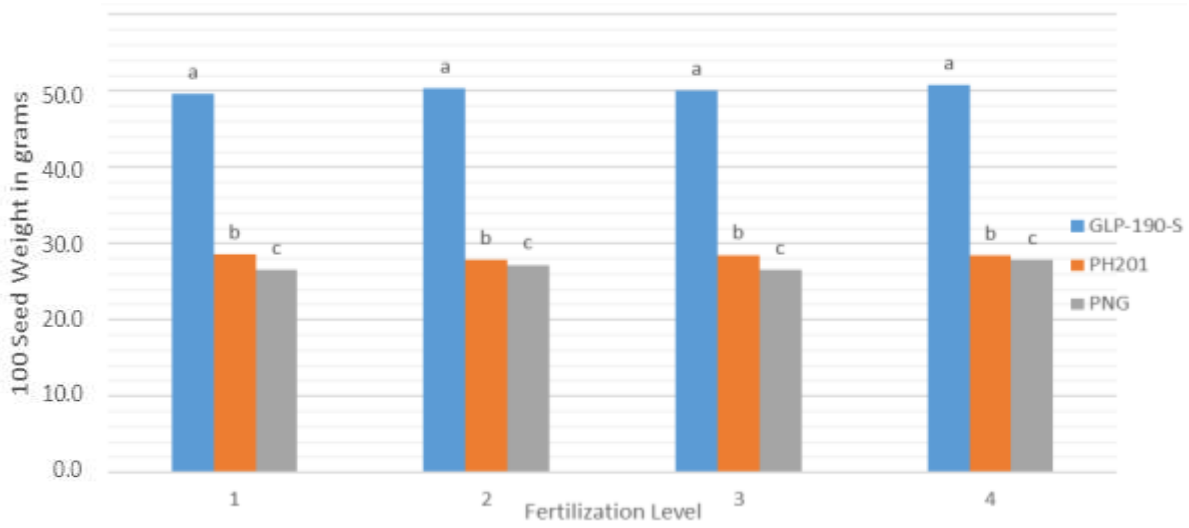


**Figure 4.** Interaction between varieties with respect to 100- seed weight.

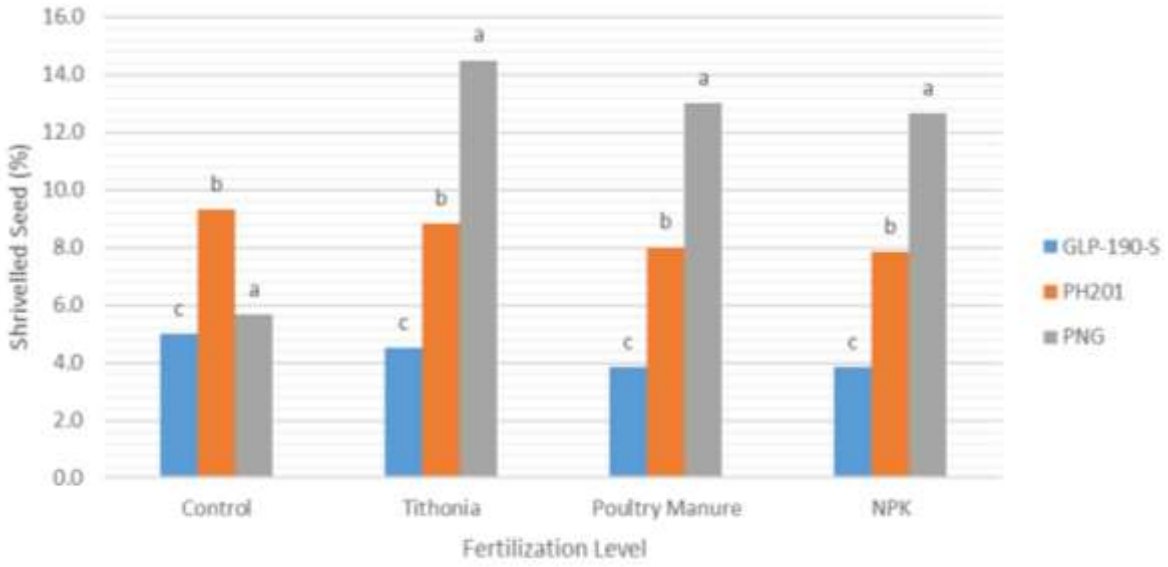


**Figure 5.** Interaction between fertilization and varieties with respect to shrivelled seeds(%).

or PNG at fertilization level 2 (*Tithonia*) had the highest number of shriveled seeds (14.5). GLP-190-S had the lowest percentage of shriveled seeds at fertilization levels 3 (poultry) and 4 (mineral fertilizer) (3.8%) (Figure 5).

### Variety and fungicide treatment

In all aspects of yield components, sprayed plots had the highest values compared to unsprayed plots. Number of pods in sprayed plots (258.3) was higher than unsprayed plots (223.2). The same applies to number of pods per plant, weight of seeds, mean seed weight and 100-seed weight. Unsprayed plots had a greater percentage of shriveled seeds (11.1%).

### Interaction of fertilization and spray

Number of pods per subplot, per plant, weight of seed and mean seed weight were comparatively higher in sprayed plots than in unsprayed plots at all levels of fertilization. Fertilization level 2 (*Tithonia*) had the highest number of pods (281.7) in sprayed plots while in unsprayed plots, the number of pods (217.3) was observed at fertilization levels 1, 3 and 4 in that

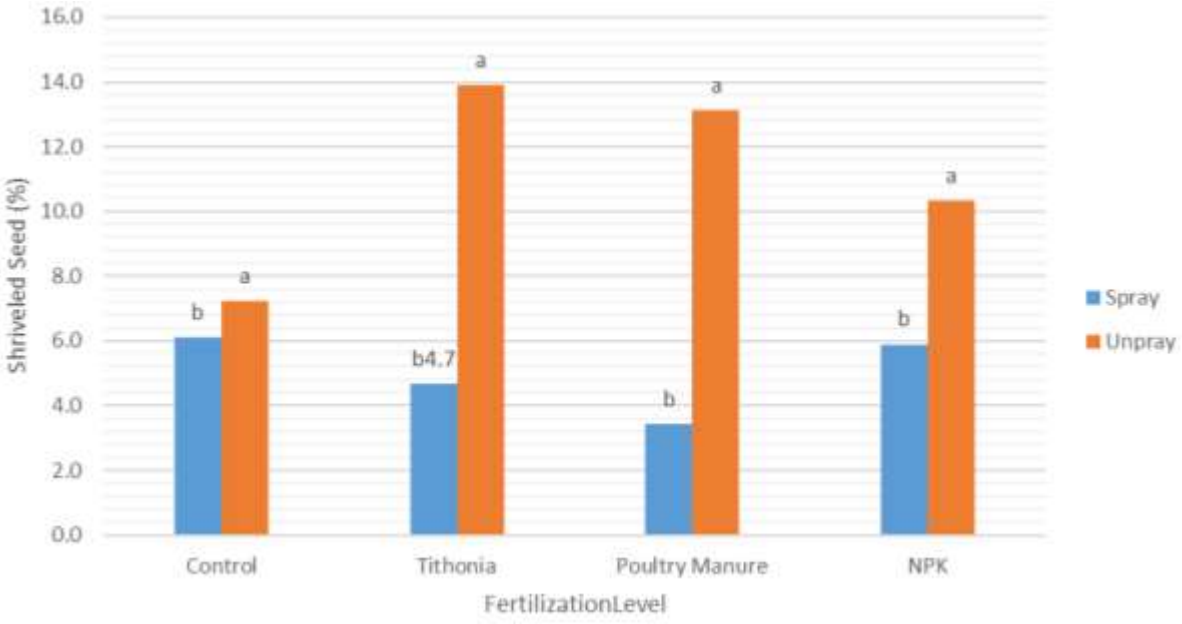


**Figure 6.** Interaction between fertilization and fungicide application with respect to percentage shriveled seed.

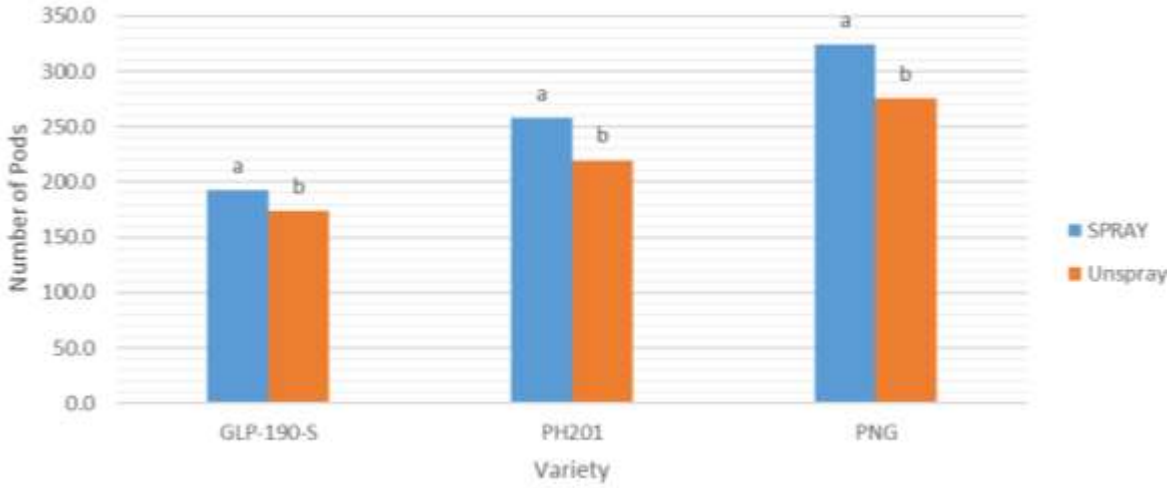


**Figure 7.** Interaction between fungicide treatment and varieties with respect to number of pods.

descending order. With respect to seed weight, the organic fertilizer-*Tithonia* (F2) had the greatest seed weight (448.6 g) in sprayed plots compared to the lowest (262.7 g) at fertilization level 4 (inorganic fertilizer). In terms of 100-seed weight, sprayed plots at fertilization level 4 had the highest number of seed weight (40.2 g) while fertilization level 3 (poultry fertilizer) had the lowest weight (30.7 g). The least percentage of shriveled seeds (3.4%) was observed at fertilization level 3 (poultry fertilizer) in the sprayed plots, while the highest percentage (13.9%) was seen at fertilization level 2 (*Tithonia*) in unsprayed plots. Thus, there was significant difference ($P \leq 0.05$) in percentage of shriveled seeds for this interaction (Figure 6).

## Spray and variety

In all varieties, sprayed plots had more pods per subplots, pods per plant, weight of seeds, mean seed weight and 100-seed weight. PNG (V3) had the highest pods/subplot (323.9 g) for the sprayed plots while in unsprayed plots, GLP-190-S had the lowest number of pods (173.9 g) (Figure 7). In terms of pods/per plant, GLP-190-S had the lowest for both sprayed and unsprayed plots (9.7 and 8.8 g), respectively. On the

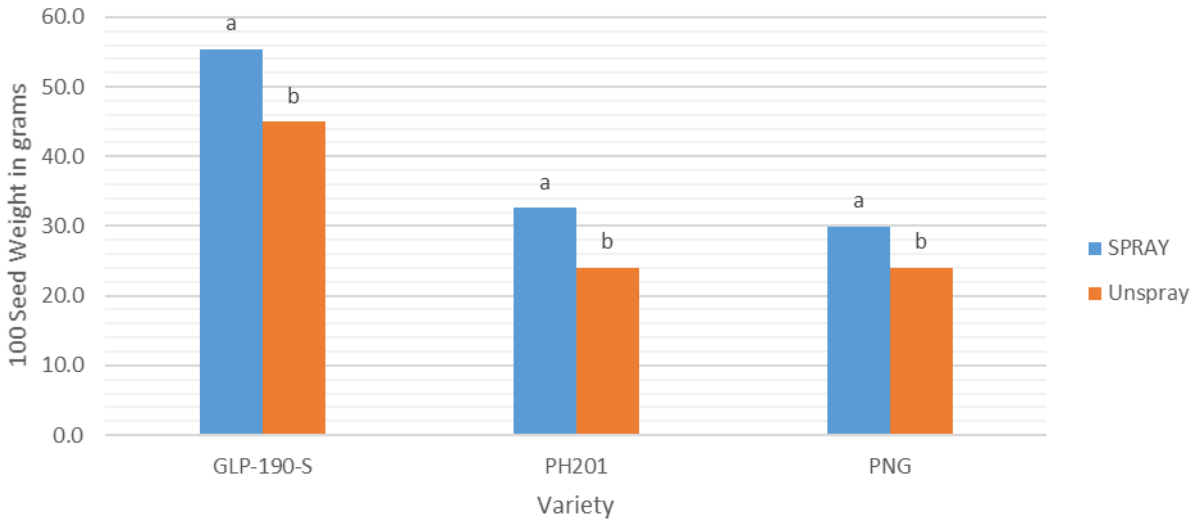


**Figure 8.** Interaction between fungicide treatment and varieties with respect to 100-seed weight.

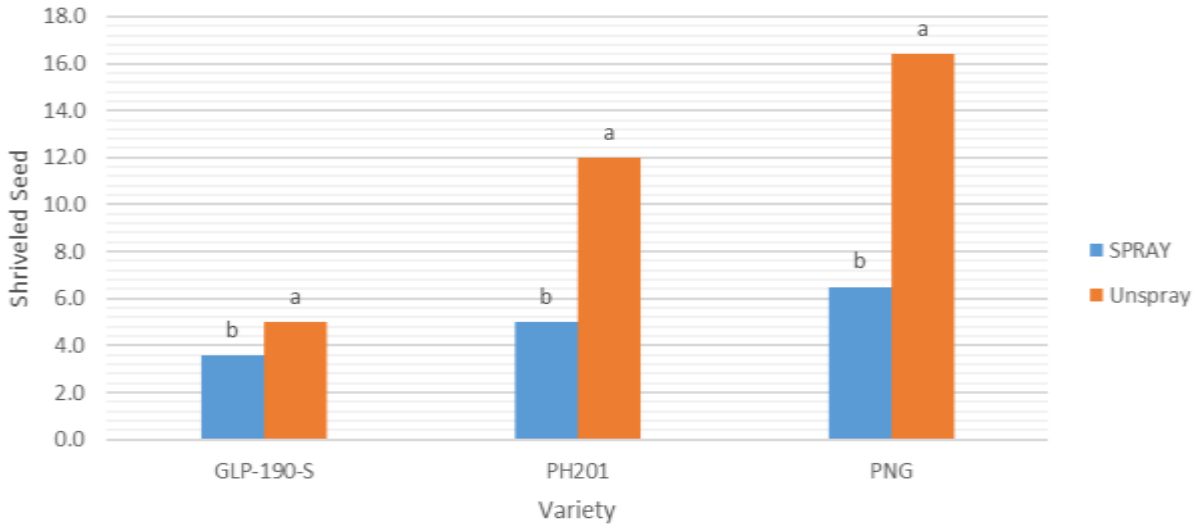


**Figure 9.** Interaction between fungicide treatment and varieties with respect to percentage shriveled seeds.

other hand, PNG had the highest (16.3 and 13.3 g, respectively). Meringue (V2) had the greatest seed weight (469.9g) and mean seed weight for the sprayed plots. The least was observed in GLP-190-S (288.4 and 14.4 g). In the unsprayed plots, GLP-190-S had the lowest seed weight (212.4 g) and mean weight (14.4 g) compared to the other two varieties. For 100-seed weight GLP-190-S had the highest (55.4 g) compared to the lowest in PNG (30.0) in sprayed plots. In the unsprayed plots GLP-190-S also had the highest 100 seed weight (45.0 g) compared to Meringue and PNG (Figure 8).

The percentage of shriveled seeds in sprayed plots was far lower than those in unsprayed plots for all varieties of bean. The variety, GLP-190-S had the lowest percentage of shriveled seeds in both sprayed and unsprayed plots (3.6 and 5.0%, respectively). PNG (V3) had the highest percentage of shriveled seeds for both sprayed and unsprayed plots (6.5 and 16.4%) (Figure 9).

## Percentage yield loss and gain for the three varieties

The three varieties showed differences in yield loss and gain for both treated and untreated plots. PNG (V3) had the highest yield loss and gain of 25.6 and 52.7%, respectively. On the other hand, PH201 (V2) had the lowest yield (18.2%) for unsprayed plots while the yield gain for the same variety almost tripled in value (50.0%).

Thus yield losses were highly reduced by the fungicide chemical Agreb compared to the untreated control.

## DISCUSSION

### Fungicide application and severity

The pathogen, *P. griseola*, the causal pathogen of angular leaf spot (ALS) is known to have greatly affected yields and yield components of common bean in different parts of the world, especially Cameroon. In the present study, ALS was found to naturally infect the three varieties of common bean though with varying degrees of severity for both sprayed and unsprayed plots. The symptoms observed macroscopically on natural infected leaves, petioles and stems confirm the disease as angular leaf spot (ALS). This is in line with similar observations made by Stenglein et al. (2006) and Djeugap et al. (2014). There was considerable reduction of ALS severity in all sprayed plots treated with the fungicide Agreb (Agreb®80WP) compared to unsprayed plots. From the findings, the climbing cultivar (PNG) was least susceptible, followed by the cultivar PH201 (Meringue) while the dwarf cultivar (GLP 190.S) was the most susceptible. These results are similar to those of Nibod (2003) and Fontem et al. (2007) who showed that the climbing cultivar (PNG) in Cameroon is less susceptible to ALS compared to dwarf cultivars. Increase in yield components (number of pods, seed weights, and 100-seed weights) in the sprayed plots was largely due to fungicide treatment. There was a significant difference between sprayed and unsprayed plots with respect to shriveled seeds in the different varieties. Paparu et al. (2014) observed that the use of the fungicide ORIUS to control ALS and rust in common bean resulted in significant yield increments. Amin et al. (2014) working on anthracnose disease of common bean concluded that plots which received various fungicide treatments significantly reduced disease severity. It has been suggested that fungicide sprays particularly permitted the crop to reach physiological maturity without being under severe anthracnose infection. The climbing cultivar (PNG) was more productive in terms of yield components, followed by PH201 and the least was observed in GLP.190-S. Ngwa (1998) also reported that the dwarf cultivar GLP 190-S had a low production potential compared to climbing cultivars. Similar findings associated with severity and yields have been reported for diseases of beans and several crop plants like, Anthracnose disease of common beans (Nkalubo et al., 2007), *Sclerotinia* stem rot of Canola (Del Rio et al., 2007). CIAT (2001), categorized bean cultivars into three groups based on their response to ALS and other diseases. Thus, from the present findings, cultivar PNG with rating severity of less than 4 was considered as resistant while PH201 (Meringue) and GLP. 190.S were intermediate. The resistance of PNG to ALS is in confirmation with other research findings carried out with this variety and others under different ecological conditions (Fontem et al., 2007; Ngueguim et al., 2011; Sanglard et al., 2013). This resistance to ALS has been largely attributed to the genetic makeup (genotypes) of this variety and other local ones. According to Djeugap et al. (2014) the identification and characterization of the genes of interest can be a good base for the amelioration of the genotypes of different varieties of common bean in Cameroon. There was a progressive increase in disease severity from the initial date (35 DAP) of notification of disease symptoms to full bloom appearance during flowering and maturity stage. This can be attributed to inoculum density and environmental conditions. Since infection is natural, there was inoculum built up and spread among crops as days pass by and rate of humidity increased. This was particularly noticed in the unsprayed plots of all three varieties. The highest mean disease severity (48.4%) was recorded at period five (67 DAP) compared to the least (0.90%) at period one (35 DAP). At a more than 50% flowering environmental conditions like increase in rain fall and temperatures favour infection and disease development. According to Celetti et al. (2006), high ALS severity and occurrence is often experienced under conditions of warm temperature (24°C) but it also occurs within a temperature of 16 to 28°C, if accompanied by high relative humidity (95-100%) alternating with windy conditions.

Disease severity was not affected by the fertilization level, despite the fact that unsprayed plots were significantly infected compared to sprayed plots at all levels of fertilization. From the present study, severity was highest for poultry manure (F3) (22.9%) as against (17.7%) for control (F1). These results can be explained in that poultry manure is rich in organic matter such as potassium and phosphorus and this can serve as a good source of inoculum for the pathogen causing ALS. This can easily infect the crops especially unsprayed plots. Tarla (2008) suggested that increase in late blight of huckleberry in plots treated with poultry manure was due to the fact that the poultry manure contains more nitrogen than the control.

### Nutrient application and yield components

It was generally observed that application of organic and inorganic fertilizers have a positive effect on the yield components of common bean though to a varying degree for the three varieties. Fertilization level 2 (*Tithonia*) had the highest number of pods and highest seed weight for all varieties, while control or no fertilizer (F1) had the lowest. Highest seed weight was obtained from PNG at fertilization level 2 (F2), while the lowest value was from GLP.190.S (V1) at fertilization level 1 (F1). However, the highest 100-seed weight was obtained at fertilization level

4 (F4) in the variety GLP.190.S (V1), while PNG (V3) had the least. Thus there was a significant difference in the weight of 100 bean grains among the varieties tested. The mass of 100 bean grains is an agronomic characteristic that depends on the variety and is measured in grams. GLP-190S with the highest 100-seed weight has large grains while PNG with small grains had the least weight. According to Mboussi et al. (2012), this characteristic which is variety specific can also be influenced by environmental factors like diseases (web blight, ALS) and drought that can reduce the grain mass. From the findings, it showed that the application of increasing doses of both organic and inorganic fertilizer produced significant effects for all yield components. These results corroborate with the results presented by Zucareli et al. (2006) and Pela et al. (2009) who reported significant increases in yield components of common bean with increasing rates of fertilizer application. In the present study, the physicochemical properties of the soil were moderate in organic matter (OM), nitrogen, potassium, calcium, magnesium, phosphorus and also moderate soil pH, thus favouring the growth and productivity of common bean. Both organic and inorganic fertilizers contain the essential elements nitrogen, phosphorus and potassium (NPK) which are very important in promoting the growth and yield of plants. However, their composition (percentage) and their effects on growth of the plant differ significantly, as evident from the present study. Here *Tithonia* (Mexican flower) as organic fertilizer produced the highest pod number and seed weight for all varieties compared to the other nutrients. This can be partially attributed to the fact that *Tithonia* contains a higher amount of organic matter which tends to reduce bulk density. Organic matter is known to reduce soil compaction. Also, increased organic matter and associated improvement in soil texture should have enhanced infiltration of rain water leading to improved retention and availability of water in the soil. Atayese and Liasu (2001) found that soil under *Tithonia* and siam weed have higher pH propensity, moisture content, N, P, K, Ca, Na, mycorrhizal fungi spores and earthworm casts density and lower bulk density compared with bare soil. *Tithonia* is known to be rich in N, P, and Ca (Liasu and Achakzai, 2007). These qualities greatly favour the growth and development of common beans. Just like *Tithonia*, poultry manure (F3) greatly improved the various yield components compared to mineral fertilizer (F4) and control (F1). Poultry manure is also known to be an excellent organic fertilizer as it contains high N, P, K and other essential nutrients (Farhad et al., 2009). It has been reported to supply P more readily to plants than other organic sources (Garg and Bahla, 2008). Uwah et al. (2012) reported that poultry manure increased soil pH, organic matter content, available P, exchangeable cation and micro nutrients, reduced exchangeable Al and Fe contents and bulk density. The positive response of common bean to chicken manure application could be due to the reduction of soil pH by the manure that makes the nutrient such as phosphorus more available to the plants. Some researchers have concluded that increasing levels of phosphorus fertilizer application have been known to improve the number of pods and seeds per plant (Turuko and Amin, 2014). Phosphorus is known to promote the formation of nodes and pods in legumes (Buttery et al., 1987). In addition to increasing the soil fertility, poultry manure amends the soil by adding organic matter to the soil. Although the rate of organic matter decomposition in poultry manure varies with temperature, drainage, rainfall and other environmental factors like organic matter of the soil, poultry manure greatly improves water holding, soil aeration, soil structure, nutrient retention and microbial activities. Yields of beans can be reduced as much as 60 to 75% in soils that are unable to release sufficient P levels during the growing season. Balbhim et al. (2015) found out that organic fertilizer increased growth, yield pods in cluster bean followed by chemical fertilizer as compared with the control. Organic fertilizer like cow dung has been known to increase pod weight, pod dry weight, and total yield of French bean (Olfati et al., 2012). All the aforementioned results agreed with the present results which indicated that all treatments increased the productivity of common bean over control (F1).

Although organic manures (OM) have to mineralize to release their nutrients, which may lead to poor response in the season of application, *Tithonia* is classified as a high-quality OM (Jama et al., 2000) hence it decomposes very fast and is able to release N at rates that match the crop demand. In addition, OM such as *Tithonia* has been found to reduce P-fixation therefore increasing P availability in soils (Nziguheba et al., 2000). When the soil is fertile, the plant spacing is optimum and the climatic conditions are ideal, the number of pods per plant and the number of grains per pod will be expression of the genetic potential of the variety. A deficiency in soil nutrients or water deficit during flowering may reduce the number of pods per plant, but if these deficiencies occurred at grain filling stage, it may result in reduction of the mass of 100 grains, but number of grains per pods will be constant as it is variety dependent (Mboussie et al., 2012). The varieties with the higher number per pod did not necessarily have the higher number of grains per pod.

## Conclusion

The study indicated that the use of the fungicide Agreb greatly reduced ALS disease severity and improved yields of the sprayed crops. The least severity was recorded in V3 (PNG), while the highest severity was recorded in V1 (GLP-190-S). The research work also revealed that fertilization, whether organic or inorganic, was found to enhance yield components of the three

varieties of common bean. Highest number of pods and seeds were obtained from crops that received *Tithonia* treatment (F2) while the lowest number of pods was obtained from mineral fertilizer treatment (F4). In terms of weight, PH201 (V2) had the highest seed weight while GLP-190-S (V1) had the lowest. For 100 seed weight V1 had the highest weight while V3 was the least.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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*Full Length Research Paper*

# Exploring the induction of doubled haploids in cassava through gynogenesis

**Julius Karubanga Baguma[1,2]*, Robert Kawuki[1], Ssetumba B. Mukasa[2], Mary Buttibwa[1], Polycarp Nalela[1], Maurine Eyokia[1], Brenda Oshaba[1], Zaida Lentini[3] and Yona Baguma[1]**

[1]National Crops Resources Research Institute, (NaCRRI), Kampala, Uganda.
[2]School of Agricultural Sciences, Makerere University, Kampala, Uganda.
[3]School of Natural Sciences, ICESI University, Cali, Colombia.

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**Production and use of double haploids is important for attainment of systematic genetic gains, and indeed several plant breeding programmes have benefited from it. Gynogenesis, the *in vitro* culture of unfertilized ovules and/or embryos has specifically been exploited in several economically important crops but not cassava. In this study, we examined possibilities of generating doubled haploids (DH) in cassava through gynogenesis, by bagging female flowers of selected varieties to prevent pollination. A total of 2,466 flowers across 32 elite cassava varieties were bagged for a period of one-to-three days. Early embryo rescue and ovule culture were done at 7 to 42 days after anthesis. Consequently, 517 fruits (21%) were harvested and dissected to obtain 97 seeds from which 47 unique embryos and 18 callus lines were obtained *in vitro*. Only six of the rescued embryos (12.8%) regenerated into plantlets. Upon undertaking ploidy analysis and single nucleotide polymorphism (SNP) genotyping, it was established that all samples analyzed (regenerated plants and calli) were diploid. SNP genotyping revealed increased homozygosity (up to 85.7%), but no doubled haploids were noticed. The knowledge generated is a significant contribution towards understanding cassava flowering biology and thus a foundation to on-going efforts towards developing protocols for generation of cassava DH.**

**Key words:** Anthesis, embryo rescue, gynogenesis, homozygosity, ploidy.

## INTRODUCTION

Parthenogenesis (a form of “apomixis”) refers to the development of a haploid embryo from a reduced egg nucleus (*n*) in an asexual embryo sac without fertilization by the sperm nucleus (Palmer and Keller, 2005; Acquaah, 2007). This has been investigated in various crops since the 1940s (Freitas and Nassar, 2013). It occurs naturally or spontaneously and is widely distributed in plants including a few species of agricultural importance. In addition to parthenogenesis, other methods of producing embryos without fertilization have been observed, notably: apospory, diplospory, adventitious embryony, androgenesis and semigamy (Acquaah, 2007).

The regeneration of haploid embryos and plants through unpollinated female gametophytes has been described as gynogenesis (Chen et al., 2011). *In vitro*

*Corresponding author. E-mail: bagumakj@gmail.com. Tel: +256 772 995 207.

culture of unfertilized ovules has long been recognized as an important tool to produce haploid and homozygous double haploid (DH) plants for genetic studies and plant breeding programmes (Godbole and Murthy, 2012). In this case megaspores or female gametophytes of plants have been successfully triggered to undergo sporophytic development leading to plant regeneration. Production of haploid plants through gynogenesis by culturing unfertilized ovaries was first described in barley (Chen et al., 2011). Similarly, successful gynogenesis has been reported in many plant species, e.g., Egyptian henbane (*Hyoscyamus muticus*) (Chand and Basu, 1998), onion (*Allium cepa)* (Alan et al., 2007; Fayos et al., 2015), sweet potato (*Ipomoea batatas*), tulip (*Tulipa generiana*), maize (*Zea mays*) (Leblanc et al., 2009), sugar beet (*Beta vulgaris*), cucumber (*Cucumis sativus*) (Tantasawat et al., 2015), and wheat *(Triticum durum)* (Berzonsky et al., 2003).

Indeed, haploids of 21 angiosperm species have been obtained from *in vitro* unfertilized ovules or ovary culture since 1976; in most cases using explants at uninucleate to mature embryo sac stages (Wu et al., 2004). However, the success of the method and its efficiency is greatly influenced by several biotic and abiotic factors. The variety of donor plants, combined with growth conditions, is among the crucial factors (Murovec and Bohanec, 2012). In recent advances involving tissue culture and embryology, there has been successful induction of haploid plants by the culture of unpollinated ovaries or ovules (Yang and Zhou, 1982; Chen et al., 2011). It is on this premise that this study was initiated to explore the effect of no pollination on fruit and seed set and its potential in inducing cassava haploids (Hs) and DHs.

Reports from studies so far carried out on cassava indicate there is evidence that apomixis occurs in different varieties, though, at low rates (Nassar et al., 1998). Some other studies have suggested that the apomixis genes have also been successfully transferred to cassava through hybridizing with the wild species, *Manihot glaziovii* (Nassar and Collevati, 2013). Use of molecular markers on a few varieties has revealed that apomixis is controlled by genes which occur naturally in the cassava gene pool, though at a low frequency (Freitas and Nassar, 2013).

Therefore, the purpose of this study was to explore the effect of no pollination on fruit and seed set and the possibility of inducing haploid and/or doubled haploid cassava plants through gynogenesis.

## MATERIALS AND METHODS

### Parental varieties and nurseries

A total of 32 elite cassava varieties were selected for this study based on their profuse flowering behaviour. These included 52TME14, NAROCASS1, NASE12, NASE13, NASE14, NASE15, NASE16, NASE18, NASE19, NASE3, NASE4, TME204, UG120004, UG120014, UG120035, UG120078, UG120106, UG120109, UG120114, UG120125, UG120149, UG120156, UG120174, UG120192, UG120211, UG120219, UG120223, UG120243, UG120261, UG130005, UG130048 and UG130107. Some of these cassava varieties have been officially released by National Agriculture Research Organisation (NARO) while others are still undergoing evaluation. Pedigree information of these varieties can be accessed at www.cassavabase.org. Due to the differences in variety responses observed in several DH induction and apomixes studies, a diverse selection of varieties was preferred to increase chances of haploidy induction and recovery. Each of the donor plants was established in a crossing nursery at National Crops Resources Research Institute (NaCRRI), Namulonge and Rwebitaba Zonal Agricultural Research and Development (ZARD) in the first growing season of 2014. Each variety was represented by about 100 plants.

### Experimental lay out and pollination control

In each test, variety plants were purposely selected, and on each plant some inflorescences were selected for the test treatment and others for the control. The inflorescences for the test treatment were bagged to prevent pollination while the ones for control were left for natural open pollination. On each selected plant some inflorescences were bagged at one to three days before anthesis while others were left un-bagged for open pollination to occur. The bags were left on the inflorescences for three to seven days after anthesis (DAA) to ensure that no pollination occurred. At intervals of three to seven days from the day of bagging the numbers of surviving fruits per inflorescence, plant and variety were recorded up to harvest time between 7 and 42 DAA, after which fruit harvest and embryo rescue were done. This was done at two to seven levels of flowering in the selected varieties. In a revised design, the test and control inflorescences were selected and fruits harvested at 7 to 14 DAA, ovules excised and cultured. Nine rounds of bagging and not bagging were carried out, and this involved up to 2,644 bagged and 1,990 un-bagged flowers. In all the rounds, only plants with good and mature inflorescences (before anthesis) were purposely selected.

### *In vitro* embryo rescue and culturing

Surviving fruits of bagged inflorescences were harvested for *in vitro* embryo rescue or ovule culture. Initially embryo rescue was undertaken at 42 DAA for the fruits in the first six rounds and then adjusted to 7 to 14 DAA. For comparison purposes, a few fruits from open-pollinated flowers were harvested. All fruits were taken through surface sterilisation by being washed in soapy tap water (2-3 times), rinsed and immersed in 70% alcohol for 1 min. Thereafter, the fruits were soaked (while being shaken) twice in 2% sodium hypochlorite (NaOCl) containing 2 to 3 drops of Tween 20 added as a surfactant for 20 min (10 min each soaking). The fruits were then rinsed three times with sterile distilled water in a laminar flow hood.

The fruits at 42 DAA were dissected and embryos excised. The embryos were cultured *in vitro* on modified Murashige and Skoog (MS) basal medium (1962) (M6 or $^{1}/_{2}$ MSREm) in glass jars with radicles pushed down into the medium. The M6 medium contained half MS basal salts, supplemented with 1.0 mg/L gibberellic acid (GA3), 2% sucrose and 0.2% gelrite or agar as a gelling agent (as described by Huabing et al. (2014). Meanwhile, for the fruits at 7 to 14 DAA, ovules were excised and cultured *in vitro* on modified MS (MS2 or MS3) induction medium supplemented with 2 mg/L 2,4 D, 0.5 mg/L kinetine, 1 mg/L GA3, 0.2 mg/L benzylaminopurine (BAP), MS vitamins, 8% sucrose and 0.3% gelrite or agar in petri dishes (90 × 14.2 mm). In all cases the pH of media was adjusted to 5.8 before autoclaving at 121°C for 15 min.

The embryos at 42 DAA were incubated at 28 ± 1°C under a 12/12 h (day/night) photoperiod with light supplied by white

fluorescent tubes (25 µmol $m^{-2}s^{-1}$). The immature embryos were left in the growth room for four weeks for regeneration into plantlets. The resulting plantlets were sub-cultured and transferred to basic MS medium containing 4.3 g/L of solid medium of MS salts supplemented with 2% sucrose, 1.0 ml/L MS vitamins and 0.3% phytagel with pH adjusted to 5.8 by adding a base in glass jars. Meanwhile, the dishes containing ovules were cultured in darkness (improvised by enclosing the dishes in aluminium foils) at 28°C for one month and then taken through callus formation and plantlet regeneration process.

#### Histological analyses

Ovules excised from fruits at 7, 14, 21 and 28 DAA were fixed in glacial acetic acid and 96% ethanol (in ratio of 1:3) in falcon tubes and kept in darkness at 4°C for at least 3 h. The ovules were processed using a tissue processor (Leica TP 1020), embedded in Paraffin wax (Histowax), and then sectioned using a rotary microtome (Leica RM 2235; section thickness: 5 µm). They were then stained with Schiff's reagent and counterstained with Naphthol Blue black, NBB (5% w/v). Stained sections were mounted using Depex to make permanent slides. Examination of slides for embryological analyses was performed under an inverted light microscope (Nikon, Eclipse TS100-F) and images taken using a camera head (Nikon DS-L3).

#### Ploidy level and homozygosity analyses

Flow cytometry was performed using Partec GmbH ploidy analyzer (Otto-Hahn-Str. 32, D-48161 Münster) to determine the ploidy level of plantlets generated from rescued and cultured embryos, and some calluses from cultured ovules following the method described by Dolezel et al. (1995). In brief, approximately 25 mg of sample plus control were chopped with a sharp razor blade in a petri dish containing 0.5 ml of cold OTTO1 buffer (0.1M citric acid monohydrate and 0.5% v/v of Tween-20). The homogenate was filtered through a 50 µm nylon filter into a cuvette. In each case, the diploid parental cassava lines were used as internal controls. The samples were incubated for about 5 min before 1 ml of OTTO II buffer (0.4 M anhydrous $Na_2HPO_4$, 4 µg/ml of 4, 6´-diamidino-2-phenylindole (DAPI), and 1 µl/ml β-metcaptoethanol) was added. The flow cytometer was adjusted so that the peak representing 2n or 2C DNA in a diploid at G1 phase of the control was localized at channel 100. The ploidy level of the sample was determined by comparing the relative position of the sample's G1 peak and that of the control. A total of 22 samples together with six controls were analysed.

For the homozygosity analysis, twenty eight samples of genomic DNA were extracted from young fresh leaves of regenerated plantlets (07 samples) generated from rescued and cultured embryos, selected calluses (15 samples) derived from cultured ovules and the mother (parental) lines as controls (06 samples) using the QIAGEN (DNeasy) plant kit, following the manufacturer's instructions. DNA concentration was determined using a NANODROP 2000 (Thermo SCIENTIFIC, USA) and then analyzed on 0.8% agarose gel stained with ethidium bromide. The DNA samples were shipped to the Laboratory of the Government Chemist (LGC) Genomics Ltd., UK for SNP genotyping to ascertain homozygosity. A panel of 34 heterozygous and polymorphic SNPs developed and validated for cassava (Ferguson et al., 2012) was used to assay the 28 genomic DNA samples.

#### Data analysis

Different kinds of data sets were generated both in the field and laboratories. These included number of flowers bagged, fruit set and survival at different levels of flowering, number of seeds or ovules and embryos excised, number of plantlets and calli regenerated, ploidy and homozygosity levels of generated calli and plantlets. From this, mean number of flowers, fruits, ovules, seeds, and embryos, for each round of experiment were computed. Further still, the data were subjected to analysis of variance (ANOVA) at the significant level of 5% ($P \leq 0.05$) using Genstat statistical software, edition 12 and R-Studio Statistical Programming software (R Core Team, 2017). Additionally, ploidy level comparisons between progeny samples and controls (mother plants) was done by using channel mean and mean ratio of sample to diploid parental lines. The channel and/or peak means of diploid cassava were used to compute ratios that were used to discriminate the ploidy levels of the samples. In this analysis, channel mean for a diploid was set at 100, ploidy level was computed by multiplying the mean ratio of target sample to diploid mother by diploid number of mother used as a control; 2x=2n=36. When channel mean is 50 (or mean ratio is 0.5) it is expected that the target sample is a haploid (Dolezel et al., 1995; Ochatt, 2006). Computation of percentage homozygosity was done by summing up the number of homozygous loci in each progeny sample followed by division with the sum of heterozygous loci in the corresponding mother sample.

## RESULTS

### Fruit and seed set from non-pollinated and open-pollinated flowers

A total of 2,466 flowers were bagged from which only 517 fruits were harvested representing a survival rate of 21% (Table 1). On the other hand, 1,990 flowers were not bagged (open-pollinated) out of which 895 fruits were surviving at the time of harvest, that is, 7 to 42 DAA representing a survival rate of 45%. In rounds 1 to 6 fruits were harvested at 42 DAA for embryo rescue. Due to high fruit abortion rates of the non-pollinated flowers, harvest period was reduced to 7 to 14 DAA for ovule culture in round 7 to 9. Only fruits from non-pollinated (NP) flowers were harvested for embryo rescue or ovule culture.

By 42 DAA, only 100 fruits out 1,891 bagged flowers (5%) had survived compared to 895 out of 1,798 (42.7%) fruits from open-pollinated (OP) flowers in rounds 1 to 6. Highest fruit survival rates from bagged flowers were registered in rounds 7 to 9 in which fruit harvest was done at 7 to 14 days after anthesis. On the whole, there were significant differences in the mean number of fruit-set between non-pollinated and open-pollinated flowers ($P<0.001$) (Table 1)

Although the results in Table 2 show more flower production between second and fourth branching levels, there is no consistent trend in fruit set in the non-pollinated flowers. The percentage fruit set varied across levels of branching, with the lowest and highest percentages noted at second and seventh levels, respectively. Meanwhile in the open-pollinated flowers, there was a notable declining trend with increase in the branching level, except at seventh level. Overall percentage fruit and seed set was lower in the non-

**Table 1.** Fruit set following bagging to prevent pollination of cassava flowers.

| Round | Number of flowers | | Number of fruits set | | Percentage fruit set | |
|---|---|---|---|---|---|---|
| | NP | OP | NP | OP | NP | OP |
| 1 | 201 | 61 | 43 | 22 | 21.4 | 36.1 |
| 2 | 93 | 68 | 4 | 31 | 4.3 | 45.6 |
| 3 | 1419 | 1490 | 464 | 797 | 32.7 | 53.5 |
| 4 | 100 | 98 | 0 | 32 | 0.0 | 32.7 |
| 5 | 61 | 64 | 6 | 12 | 9.8 | 18.8 |
| 6 | 17 | 17 | 0 | 1 | 0.0 | 5.9 |
| 7[1] | 173 | 86 | 0 | 0 | 0.0 | 0.0 |
| 8[1] | 72 | - | 0 | - | 0.0 | - |
| 9[1] | 330 | 106 | 0 | 0 | 0.0 | 0.0 |
| Total | 2,466 | 1,990 | 517 | 895 | - | - |
| Mean | 274.0 | 248.8 | 57.4 | 112.3 | 7.6 | 24.1 |

[1]Rounds in which fruits were harvested for ovule culture at 7-14 DAA. Most fruits harvested at 42 DAA had no developed seeds.

**Table 2.** Comparison of fruit set in non-pollinated and open-pollinated flowers at different branching levels.

| Branching level | Number of flowers | | Number of fruits set | | Percentage fruit set | |
|---|---|---|---|---|---|---|
| | NP | OP | NP | OP | NP | OP |
| 2 | 708 | 597 | 258 | 326 | 36.4 | 54.6 |
| 3 | 854 | 771 | 124 | 342 | 14.5 | 44.4 |
| 4 | 441 | 383 | 76 | 170 | 17.2 | 44.4 |
| 5 | 226 | 184 | 36 | 50 | 15.9 | 27.2 |
| 6 | 69 | 49 | 6 | 7 | 8.7 | 14.3 |
| 7 | 45 | 6 | 0 | 0 | 0 | 0 |
| Unknown | 123 | - | 17 | - | 13.8 | - |
| Total | 2,466 | 1,990 | 517 | 895 | | |
| Mean | 352.30 | 331.70 | 73.9 | 179.2 | 15.2 | 30.8 |

Data sets generated from 32 cassava varieties.

pollinated (bagged) flowers than in the open-pollinated (un-bagged) ones. However, in both cases, the mean number of fruit survival reduced with progress of the experiment (Figure 1). There was significant difference in both length and width of fruits from non-pollinated (NP) flowers and open-pollinated (OP) flowers ($P<0.001$).

The number of fruits that survived by harvest time varied significantly ($P < 0.001$) among the varieties (Table 3). At 42 DAA, a total of 47 unique embryos were rescued, out of which only seven unique plantlets were regenerated. NASE 19 had the highest number of rescued embryos and regenerated plantlets. From the ovules excised for early ovule culture to induce gynogenesis, only 18 of them developed calluses from the embryo sac region.

Histological examinations done on a few selected ovules revealed accelerated degeneration of embryo sac after 14 DAA in ovules of unpollinated flowers. This is noticed by the presence of many empty ovules with disorganizing tissues. However, in the open-pollinated flowers there was evidence of normal embryo development (Figure 2).

## Ploidy and homozygosity analysis results

Ploidy analysis by flow cytometry revealed that all of the 22 samples (obtained from six out of 32 varieties) were diploids just as the controls (Table 4). They all produced one peak in G1 phase (Figure 3). For homozygosity analysis, comparisons were limited to heterozygous alleles in the mother and progeny samples. It was observed increased by homozygosity at varying degrees. Among plantlets the lowest was 45.5% in NASE19 (sample P25) and the highest being 85.7% in the plantlet of UG141658 (sample P22). This variety was not in the original selection of this study. Meanwhile, in most calli no increase in homozygosity was noted (0%), that is, the

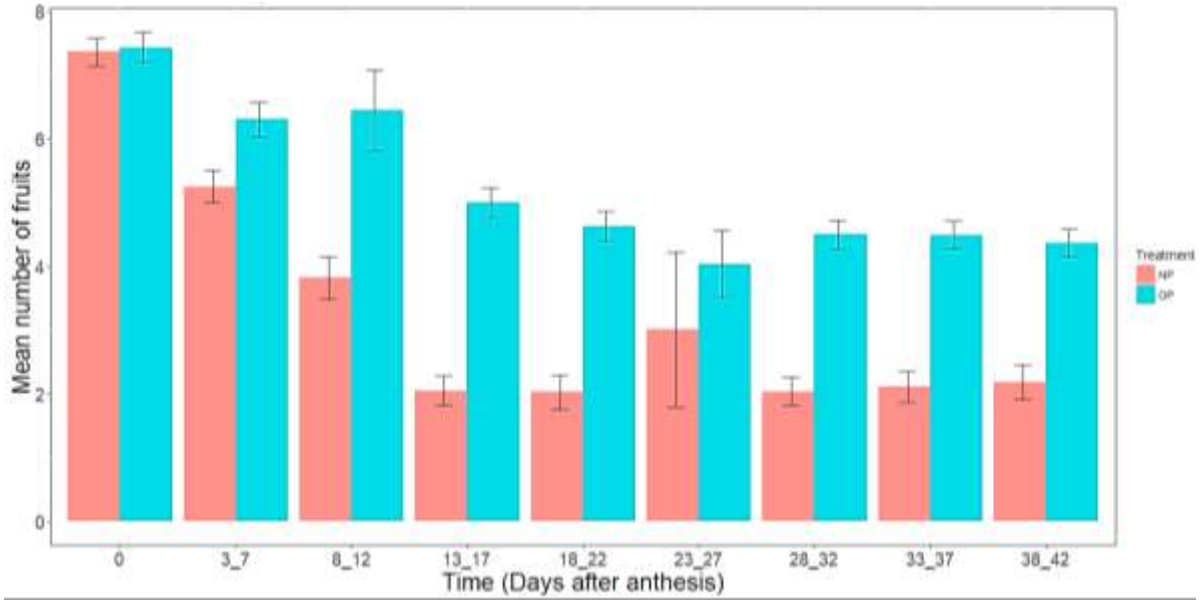


**Figure 1.** Mean number of surviving fruits during the experiment. Red bars = fruits harvested from non-pollinated flowers (NP); Blue bars = fruits of open-pollinated flowers (OP). The vertical bars represent the error deviation.

**Table 3.** Number of cassava fruits, seeds, ovules, embryos rescued, calli and plantlets generated from different cassava varieties following non-pollination and open pollination.

| Variety | Number of flowers | | Number of fruits set | | Number of seeds | Number of embryos | Number of calli | Number of plantlets |
|---|---|---|---|---|---|---|---|---|
| | NP | OP | NP | OP | NP | NP | NP | NP |
| 52TME14 | 49 | - | 7 | - | 0 | 0 | - | 0 |
| NAROCASS 1 | 31 | 11 | 0 | 0 | - | - | - | - |
| NASE 12 | 45 | 6 | 0 | 0 | - | - | - | - |
| NASE 13 | 23 | 19 | 0 | 0 | - | - | - | - |
| NASE 14 | 323 | 172 | 2 | 31 | 6 | 0 | 6 | 0 |
| NASE 15 | 74 | - | 10 | - | 0 | - | - | - |
| NASE 16 | 81 | 80 | 0 | 25 | - | - | - | - |
| NASE 18 | 60 | 35 | 9 | 12 | 0 | - | - | - |
| NASE 19 | 57 | 37 | 21 | 16 | 20 | 11 | - | 3 |
| NASE 3 | 207 | 106 | 4 | 11 | 12 | 0 | 12 | 0 |
| NASE 4 | 23 | - | 0 | - | - | - | - | - |

**Table 3.** Contd.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| TME 204 | 74 | 34 | 0 | 3 | - | - | - | - |
| UG120004 | 76 | 82 | 44 | 55 | 13 | 13 | - | 0 |
| UG120014 | 72 | 66 | 47 | 43 | 6 | 0 | 0 | - |
| UG120035 | 86 | 69 | 15 | 47 | 0 | - | - | - |
| UG120078 | 48 | 58 | 27 | 48 | 2 | 0 | - | - |
| UG120106 | 70 | 88 | 28 | 46 | 5 | 3 | - | 1 |
| UG120109 | 112 | 120 | 28 | 11 | 2 | 0 | - | - |
| UG120114 | 110 | 101 | 26 | 59 | 13 | 6 | 0 | 2 |
| UG120125 | 121 | 138 | 63 | 86 | 13 | 10 | 0 | 0 |
| UG120149 | 81 | 60 | 54 | 3 | 0 | - | - | - |
| UG120156 | 51 | 48 | 13 | 24 | 0 | - | - | - |
| UG120174 | 43 | 62 | 9 | 32 | 0 | - | - | - |
| UG120192 | 131 | 104 | 32 | 69 | 0 | - | - | - |
| UG120211 | 75 | 90 | 7 | 65 | 3 | 3 | - | 0 |
| UG120219 | 76 | 95 | 3 | 44 | 0 | - | - | - |
| UG120223 | 41 | 38 | 2 | 16 | 0 | - | - | - |
| UG120243 | 72 | 87 | 27 | 47 | 2 | 1 | 0 | 0 |
| UG120261 | 61 | 87 | 9 | 66 | 0 | - | - | - |
| UG130005 | 36 | 40 | 2 | 11 | 0 | - | - | - |
| UG130048 | 16 | 16 | 10 | 15 | 0 | - | - | - |
| UG130107 | 41 | 41 | 18 | 10 | 0 | - | - | - |
| Total | 2466 | 1990 | 517 | 895 | 97 | 47 | 18 | 6 |
| Mean | 77.1 | 68.6 | 16.2 | 30.9 | 3.7 | 3.6 | 3.0 | 0.6 |

Only varieties that had surviving fruits at harvest time are presented. For comparison purposes some inflorescences were left for natural open-pollination and only fruit counts were recorded at the time of harvest. The embryos and plantlets were obtained from fruits harvested at 42 DAA. Ovules were excised from fruits harvested at 7-14 DAA and these have so far developed calluses. Only calluses that developed from the embryo sac regions were recorded.

level of heterozygosity was same as that of the mother samples, the highest was 10.5% in NASE3 (sample P15).

## DISCUSSION

### Fruit and seed set

Nine rounds of bagging to prevent pollination were carried out across 32 test varieties in order to induce parthenogenic fruit and seed set. Since cassava has unisexual and protogynous flowers, detection of seed production in absence of pollination is easy as only the precaution required is to avoid pollen contamination (Leblanc and Mazzucato, 1980). It is therefore rational to conclude that fruits and seeds obtained from un-pollinated flowers in this study are apomictic. The noted drastic reduction in fruit retention and seed set following non-pollination is primarily attributed to the abscission of fruits and failure of endosperm development that is required to support embryo growth. The reductions observed in open-pollinated fruits, point to inherent obstacles to pollination. The improvement in the percentage fruit survival observed in rounds 7 to 9 is due to reduced fruit abscission since fruit harvest (for early ovule culture) was done between 7 and 14 DAA.

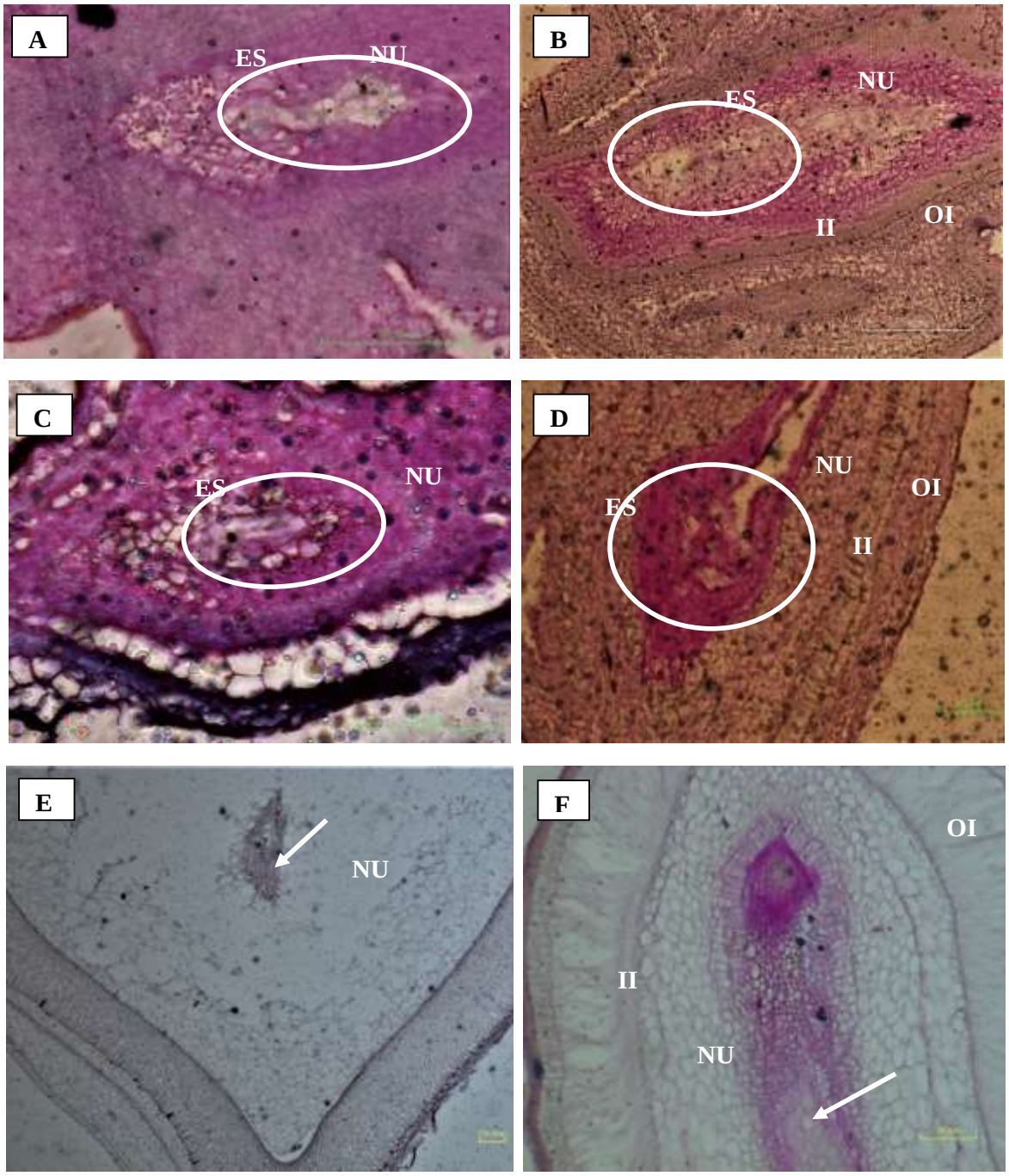


**Figure 2.** Comparison of developmental stages in non-pollinated and self-pollinated cassava flowers: (A) ovule at 7 DAA in a non-pollinated flower showing a degenerating egg apparatus in embryo sac; (B) ovule at 7 DAP in a self-pollinated flower showing cell proliferation in embryo sac; (C) ovule at 21 DAA in a non-pollinated flower showing a disorganizing embryo sac; (D) ovule at 21 DAP in a self-pollinated flower showing an organizing embryo sac; E) ovule at 28 DAA in a non-pollinated flower showing degenerated embryo sac (white arrow); F) ovule at 28 DAP in a self-pollinated flower showing embryo and surrounding tissues developing (white arrow).ES=embryosac; NU=nucellus; OI=outer integument; II=inner integument.

**Ploidy and homozygosity level of regenerated plantlets**

The diploids revealed by flow cytometry analysis of the regenerated plantlets were either as a result of embryo formation from unreduced embryo-sacs (diplospory) or embryos formed from embryo-sacs that arose from somatic cells in the ovule (apospory) or from unreduced

**Table 4.** Ploidy and homozygosity levels in callus and plantlets obtained from fruits of non-pollinated cassava flowers.

| Sample ID | Variety | Source of DNA | Channel mean | %CV | Mean ratio of sample to diploid mother | Ploidy level | No. of heterozygous loci | | No. of homozygous loci in progeny | Percentage homozygosity in progeny |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Mother sample | Progeny sample | | |
| P1 | NASE 3 | Callus | 101.49 | 7.64 | 1.016 | 2x | 21 | 21 | 0 | 0.0 |
| P2 | NASE 14 | Callus | 97.7 | 4.86 | 1.000 | 2x | 11 | 10 | 1 | 9.1 |
| P4 | NASE 3 | Callus | 98.81 | 5.31 | 0.989 | 2x | 21 | 21 | 0 | 0.0 |
| P5 | NASE 3 | Callus | 100.17 | 5.24 | 1.003 | 2x | 21 | 19 | 2 | 9.5 |
| P6 | NASE 3 | Callus | 95.97 | 5.47 | 0.961 | 2x | 20 | 20 | 0 | 0.0 |
| P7 | NASE 3 | Callus | 100.63 | 4.72 | 1.007 | 2x | 20 | 19 | 1 | 5.0 |
| P8 | NASE 3 | Callus | 100.39 | 5.73 | 1.005 | 2x | 21 | 21 | 0 | 0.0 |
| P9 | NASE 3 | Callus | 101.78 | 4.67 | 1.019 | 2x | 21 | 21 | 0 | 0.0 |
| P10 | NASE 3 | Callus | 99.11 | 4.29 | 0.992 | 2x | 21 | 21 | 0 | 0.0 |
| P11 | NASE 3 | Callus | 100.29 | 5.23 | 1.004 | 2x | 21 | 21 | 0 | 0.0 |
| P12 | NASE 3 | Callus | 99.02 | 5.81 | 0.991 | 2x | 17 | 16 | 1 | 5.9 |
| P13 | NASE 3 | Callus | 101.01 | 4.21 | 1.011 | 2x | 21 | 21 | 0 | 0.0 |
| P15 | NASE 3 | Callus | 97.87 | 5.87 | 0.980 | 2x | 19 | 17 | 2 | 10.5 |
| P17 | NASE 3 | Callus | 94.53 | 5.55 | 0.946 | 2x | 21 | 21 | 0 | 0.0 |
| P18 | NASE 3 | Callus | 100.04 | 4.75 | 1.001 | 2x | 21 | 21 | 0 | 0.0 |
| P21 | UG120106 | Leaf lobes | 99.85 | 5.26 | 1.000 | 2x | 17 | 7 | 10 | 58.8 |
| P22 | UG141658 | Leaf lobes | 97.06 | 8.50 | 1.003 | 2x | 14 | 2 | 12 | 85.7 |
| P24 | NASE 19 | Leaf lobes | 96.04 | 4.95 | 0.980 | 2x | 11 | 5 | 6 | 54.5 |
| P25 | NASE 19 | Leaf lobes | 98.77 | 6.33 | 1.008 | 2x | 11 | 6 | 5 | 45.5 |
| P26 | NASE 19 | Leaf lobes | 97.52 | 6.41 | 0.992 | 2x | 11 | 5 | 6 | 54.5 |
| P27 | UG120114 | Leaf lobes | 97.53 | 5.38 | 0.992 | 2x | 13 | 4 | 9 | 69.2 |
| P28 | UG120114 | Leaf lobes | 98.21 | 4.84 | 0.999 | 2x | 13 | 6 | 7 | 53.8 |
| Mean | - | - | - | - | - | - | 17.6 | 14.8 | 2.8 | 21.0 |

Channel mean for a diploid was set at 100; ploidy level was computed by multiplying the mean ratio of target sample to diploid mother by diploid number of mother used as a control; 2x=2n=36. Percentage homozygosity was computed as (number of homozygous loci in progeny/number of corresponding heterozygous loci in mother sample) x 100. Genotyping was done using 34 SNPs. CV=coefficient of variation, % = percentage.

egg cells (parthenogenesis) that underwent chromosome doubling. Freitas and Nassar (2013) also reported apospory in cassava using molecular techniques. This therefore justifies the need to do high throughput genotyping so as to ascertain the exact origin of the embryos.

Apospory is by far the most common mechanism of embryo formation in higher plants (Bashaw, 1980; Barcaccia and Albertini, 2013; Lone and Lone, 2013) and has been reported in *Beta, Brachiaria, Cenchrus, Chloris, Compositae, Eriochloa, Heteropogon, Hieracium, Hyparrhenia, Hypericum, Panicum, Paspalum, Pennisetum, Ranunculus, Sorghum, Themeda,* and *Urochloa* (Barcaccia and Albertini, 2013). Diplospory has been reported in *Tripsacum, Eragrostis*, and *Taraxacum* where apomictic female gametes (2n) undergo embryogenesis autonomously (Kandemir and Saygili, 2015). In a previous review by Asker (1979) and later on by Barcaccia and Albertini (2013) it was affirmed that maternal offspring from

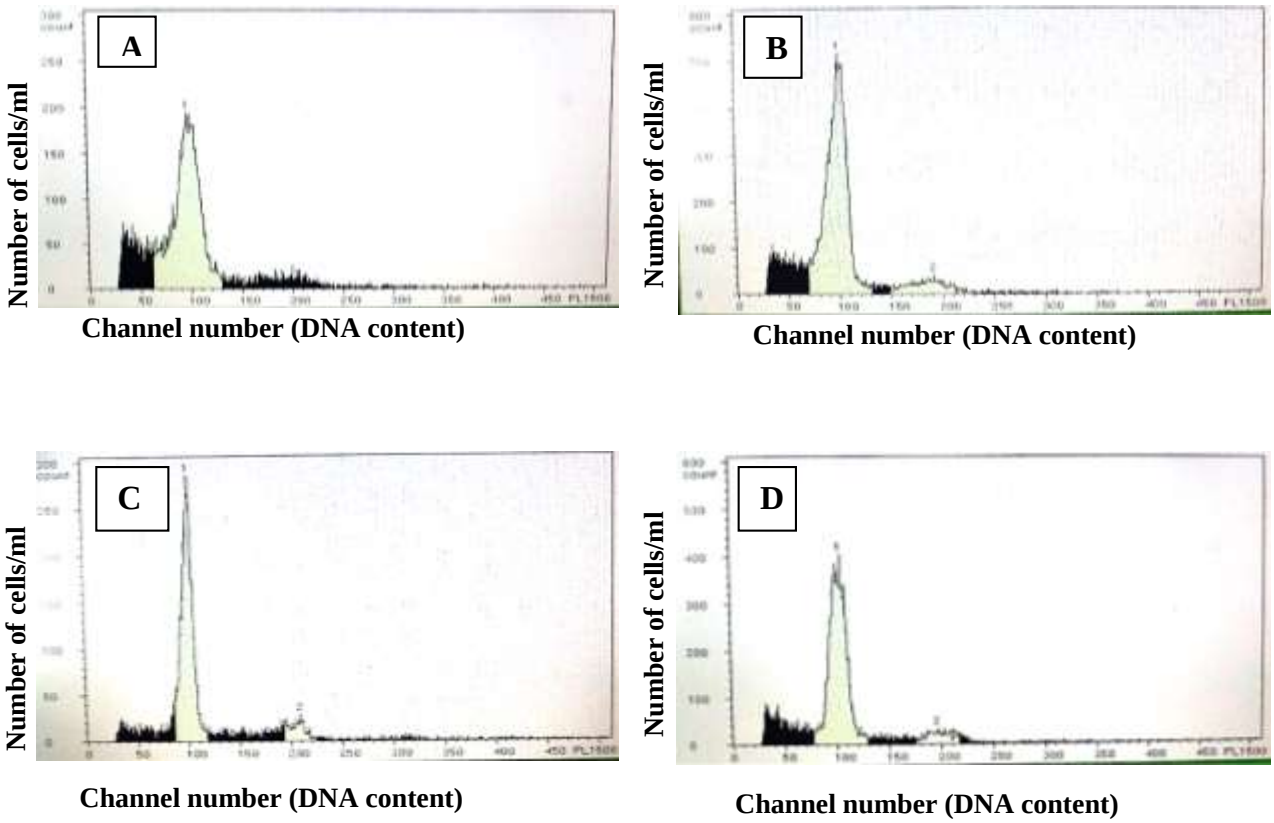


**Figure 3.** Distribution of DNA content of nuclei isolated from some regenerated plantlets and callus: (A) diploid mother sample of variety UG141658 (*2n=36*) used as a standard; (B) plantlet generated from non-pollinated flowers of variety UG141658 mixed with UG141658 nuclei of mother sample as a standard; (C) NASE 3 diploid mother sample used as another standard and (D) Callus generated from non-pollinated flowers of NASE 3. A peak at channel 50 would represent *1n* or *1C* DNA content in a haploid. In all cases the peaks indicated diploids in which peak 1 represents *G1* nuclei with *2C* DNA and peak 2 represents *G2* DNA.

asexually produced seeds usually result from parthenogenetic development of unreduced egg cells, however, such offspring may also be formed by nucellar embryony, or from parthenogenetic development of reduced egg cells followed by chromosome doubling. Various environmental factors are known to influence the balance between apomictic and sexual reproduction, such as light and temperature regimes. However, these were not measured in this study.

According to the theory of "wound-hormone or necrohormone" (Asker and Jerling, 1992) and programmed cell death (Bell, 1996), an egg cell can be stimulated to develop into an apomictic embryo by necrohormones released by surrounding dying cells or tissues. So, it can be concluded that the rescued embryos in this study developed through a similar stimulation due to dead or degenerated embryo sacs since no endosperm tissue was formed to support their survival.

The diploidy observed in the calli suggest that they originated from the somatic cells of the ovule integuments and/or nucellus tissue, despite the fact that care was taken to isolate callus that emerged only from the embryo sac regions of the ovules. The calluses with *4C* DNA content must have contained cells at the *G2* phase of the cell cycle. On the other hand, the observed deviations in peak positions could be attributed to instrument instability as well as due to variation in sample preparation and the intrinsic differences in DNA content. This is a finding that is consistent with previous studies (Dolezel et al., 1995; Doležel and Bartoš, 2005).

Further still, the diploid nature and increased homozygosity revealed by SNP genotyping in some samples confirmed doubled chromosome numbers, since all the loci had paired alleles. It is likely that automictic parthenogenesis (automixis) occurred. In Mogie (1986) and Lone and Lone (2013), automixis is defined as a process in which a new individual is formed from a product or products of a single meiotically dividing cell. In this case, the diploid chromosome number may have been spontaneously restored by a mutation process which involved fusion of two haploid nuclei, or formation of a restitution nucleus or endomitosis as described in Asker and Jerling (1992).

### Plant regeneration from young ovules

No embryo and/or plantlet was regenerated from the calli derived from young ovules. This failure could be attributed to the general slow response of cassava or the effect of several media components on the regeneration (not measured in this study) or probably the calluses initially isolated were not embryogenic. In normal routine tissue culture it takes one to four months to regenerate plantlets from calluses. This is dependent on the explant used (Chen et al., 2011; Mishra and Goswami, 2014). However, plantlets of gynogenic origin regenerate directly from an embryo or an embryoid and rarely from callus (Chand and Basu, 1998; Mishra and Goswami, 2014). It is known that i*n vitro* parthenogenesis where egg cells are triggered to form a sporophyte, and *in vitro* apogamy where the other cells of the embryo sac are induced to form the embryo are the two main origins of gynogenic haploids (Chand and Basu, 1998; Lone and Lone, 2013). Thus, the diploid calli as revealed by results of flow cytometry analyses in this study is an implication that the calluses isolated may not have been embryogenic and/or they originated from somatic cells within the ovules.

Gynogenesis efficiency in plants is highly dependent on the variety used and the quality of the donor material. Cassava seems to be not exceptional. The variety factor proved to be one of the most important factors affecting *in vitro* gynogenesis in squash in which the percentage of gynogenic ovules ranged from 0 to 48.8% (Chen et al., 2011). This is true with the results of this study in which out of 32 varieties used, none has regenerated plantlets from the several callus lines obtained. Other varieties need to be sought for future studies. Though the stage of ovule development at time of inoculation has not been studied extensively, its influence on embryo formation and/or plant regeneration from callus cannot be ruled out. The review in Chen et al. (2011) indicates that the most responsive ovules are those with nearly mature or fully mature embryo sacs.

## Conclusion

Results from this study reveal the possibility of embryo formation in cassava when pollination is prevented. Spontaneous diploidization and increased homozygosity in cassava embryos following no pollination also provide further evidence of parthenogenic fruit and seed set in cassava. Besides, it was observed that the rescue of embryos at an advanced period after anthesis, rather than ovule culture at an earlier period is a better strategy, since efforts of plant regeneration from callus were futile. The knowledge generated is a significant contribution towards understanding flowering biology of cassava and thus contributing to on-going efforts towards developing protocols for generation of cassava DH. All in all, this study opens up more opportunities to explore gynogenesis and/or other techniques of DH breeding in cassava. The allocation of more resources into this kind of work is crucial for a rapid breakthrough.

## CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

## ACKNOWLEDGEMENTS

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# Gas exchange in upland cotton cultivars under water deficit strategies

**Whéllyson Pereira Araújo[1], José Rodrigues Pereira[2*], João Henrique Zonta[2], Hugo Orlando Carvallo Guerra[1], Mailson Araújo Cordão[1] and Marcos Eric Barbosa Brito[3]**

[1]Agricultural Engineering, Campina Grande Federal University, Paraíba, Brazil.
[2]Irrigation, Embrapa Algodão, Rua Oswaldo Cruz, 1.143-Centenário, CEP 58.428-095, Campina Grande, Paraíba, Brazil.
[3]Agricultural Engineering, Sergipe Federal University (UFS), São Cristóvão - SE, 49100-000, Brazil.

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**Aiming to evaluate the gas exchange of upland cotton cultivars cultivated in the Brazilian semiarid, subject to water deficit periods on the phenological stages, an experiment was carried out at the Campina Grande Federal University, Pombal county campus, Paraíba State, Brazil, between June and December 2015. Treatments were formed from a split-plot arrangement, in which the plots were 6 water deficit periods (P) (P1 = No deficit, P2 = Deficit in the initial growth stage, P3 = Deficit in the flower bud stage, P4 = Deficit in the flower stage, P5 = Deficit in the boll stage and P6 = Deficit in the open boll stage) and the subplots, 2 upland cotton cultivars (C) (C1 = Brazil Seeds 286 and C2 = BRS 336), in randomized block design, with 4 replicates. Water deficits reduced the gas exchange of the upland cotton plants, mainly stomatal conductance, transpiration and photosynthesis. The cotton cultivars BRS 286 and BRS 336 presented similar behavior in the different water deficits applied on different phenological stages. Cotton was less tolerant to water deficits in the boll formation stage and more tolerant in the initial growth and flower bud stages.**

**Key words:** *Gossypium hirsutum* L. r. *latifolium* H., water stress, physiology.

## INTRODUCTION

Cotton is one of the most important socioeconomic products for Brazil. Besides being the most important natural source of fibers, it gives the country a privileged place in the international scene, as it is one of the five largest producers in the world, along with China, India, the United States and Pakistan (Abrapa, 2018).

Because of its $C_3$ metabolism, upland cotton highly demands light, but is considered inefficient on its absorption once it shows leaf senescence regarding to its phenology (Beltrão et al., 2011). In this sense, cotton crop in the Brazilian semiarid zones has a favorable factor, as, according to Silva et al. (2010), the duration of the mean solar day that is approximately 12 h, since the region is near to the Equator line.

In the semiarid region of the Northeast of Brazil, cotton is frequently subjected to soil water deficit in different

*Corresponding author. E-mail: jose.r.pereira@embrapa.br; Tel: +558331824373.

durations and intensities, mainly because of the scarcity or lack of rainfall in this region. Ashraf (2010) considers water deficit to be one of the main environmental constraints, which contributes to the decrease in crop yield and food security around the world.

The occurrence of water deficit is visible at any phenological stage of the plant and it may vary according to the severity and duration of the stress (Farooq et al., 2009), which includes changes in the photosynthetic rate, transpiration rate and in the stomatal conductance (Furlan et al., 2012).

Studies about water relations in plants and the interactions caused by water deficit on physiological processes are of fundamental importance, as well as the knowledge on the variation of the water consumed by a crop in its different stages of development. Such information allows the description of the physiological behavior and its consequences (Peixoto et al., 2006).

Several physiological indexes are related to the use of water by plants. Among them, photosynthesis and stomatal conductance stand out, as osmotic adjustment, such as stomatal closure, allows plants to escape dehydration and loss of turgor through the maintenance of the water content in the cells (Roza, 2010).

One of the ways to verify if the crop is under suitable conditions of cultivation is related to plant gas exchange, as, according to Taiz and Zeiger (2013), the plant under stress tends to reduce its cellular water potential, closing the stomata and reducing the formation of photoassimilates.

As gas exchanges are directly linked to the availability of water (Taiz and Zeiger, 2013), irrigation is needed to meet the water needs of the crop for a successful production; on the other hand, techniques that allow the maintenance of soil water can also be used, as they allow the plants to complete their production cycle (Guimarães et al., 1996).

However, the use of irrigation, while presenting great advantages to the agricultural production system, can cause environmental problems and increase the production cost, which is why it is important to increase the efficient use of water in irrigated crops, especially in arid and semiarid regions, where water availability is limited.

It is also known that some crops have economically viable yields even under soil water deficit, while others are sensitive to relatively low levels of scarcity. This difference is due to factors related to the root system, in particular to factors that influence growth, such as the physical characteristics of the soil, the genetic characteristics of plants and the management of irrigation systems (Reichardt and Timm, 2004).

When subjected to water deficit, plants present different responses and some are tolerant, even if they have to modify their morphophysiological and biochemical characteristics, while others, considered not tolerant, develop stress symptoms (Chakraborty et al., 2015).

Therefore, the efficiency of water use for irrigated production systems need to be optimized, especially in the cotton crop, as it is a species of great economic and social importance, so, it is important to identify adequate strategies to optimize the use of water.

Based on these reports, the objective of this study was to evaluate the gas exchange of upland cotton cultivars cultivated in the Brazilian semiarid, subjected to water deficit periods on the phenological stages, in order to relate the rational use of water to sustainable crop production in the semiarid region of Paraíba State, Brazil.

## MATERIALS AND METHODS

The experiment was conducted under field conditions between June and December 2015 in the experimental area of the Center for Agricultural Science and Technology, of the Campina Grande Federal University, Campus of Pombal county, Paraíba State, Brazil, located in the following geographic coordinates; 06°47'52''S, 37°48'10''W and 175 m above mean sea level.

The predominant climate of the region is hot semiarid (the BSh type), according to Köppen climate classification. The soil of the experimental area was classified as Fluvic Neo-soil (Santos et al., 2013), loamy sand texture (80% sand, 5.96% clay and 14.51% silt) and water tension curve of 15.49% (at 0.1 atm – Field Capacity - FC), 4.63% (at 15.0 atm – Permanent Wilting Point - PWP) with available water content (AWC) of 6.63% at the depth of 0 to 40 cm.

Fertilization was carried out according to the technical recommendations for the crop (Cavalcanti, 2008), based on the analysis of soil fertility (Table 1), in the foundation, by the application of 30 kg $ha^{-1}$ of N, 40 kg $ha^{-1}$ of $P_2O_5$ and 10 kg $ha^{-1}$ of $K_2O$ and in 2 covers, with the application of 30 kg $ha^{-1}$ of N and 5 kg $ha^{-1}$ of $K_2O$. Liming was not needed.

Upland cotton cultivars were planted in single rows, spaced 1.0 m between rows × 0.10 m among plants.

The water used in the irrigation presented $C_2S_1$ salinity (low alkali and medium salinity hazard, an electric conductivity - EC of 0.315 $dSm^{-1}$) and low sodium adsorption ratio (SAR = 1.78). Such water can be used for irrigation whenever there is a moderate degree of leaching and special care in the preparation of the soil.

Water was applied by a localized irrigation system, with drip tapes and emitters spaced 0.10 m apart. Each treatment consisted of a lateral line, spaced from the other lines by 1 m, with 6 m of length, each.

Subsequently, after installation of the irrigation system and beginning of the experiment, a water distribution test was carried out in the field. Through this, the mean precipitation applied was determined as 8.86 mm $h^{-1}$ and application efficiency (Ae) as 91%, according to Bernardo et al. (2008).

Irrigations were carried out daily, always in the morning, based on the availability of soil water (AWC) to plants. The replacement water volume was calculated considering the water lost by the crop evapotranspiration, which is represented as the difference between the soil water content (SWC) in the field capacity (FC) and the current mean SWC measured in the depths of 0.10, 0.20, 0.30 and 0.40 m, which were measured before irrigations. The current SWC was determined by the time-domain reflectometry (TDR) method, using a Delta-T-PR2 probe introduced through access pipes installed in each treatment.

With the data of the current SWC, using an Excel spreadsheet, in which the daily values of the current SWC and the AWC to plants were recorded, the depth for the replacement of water and the time of irrigation were calculated for the treatments, which were the basis for the determination of the Net and Gross Irrigation Depth

**Table 1**. Chemical characteristics of the soil of the experimental area at different depths. Pombal county, Paraíba state, Brazil. 2015.

| Depth (cm) | pH (water) | OM (%) | P (mg 100 $g^{-1}$) | $Na^+$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ |
|---|---|---|---|---|---|---|---|
| 0-20 | 6.79 | 1.16 | 51.5 | 0.14 | 0.42 | 4.28 | 1.40 |
| 20-40 | 6.94 | 0.78 | 49.0 | 0.15 | 0.27 | 4.03 | 1.89 |

pH = hidrogenionic potential; OM = organic matter.
Source: Irrigation and Salinity Laboratory, UFCG, Campina Grande county, Paraíba State, Brazil.

**Table 2.** Detail of the deficit treatments. Pombal county, Paraíba state, Brazil. 2015.

| Treatment | Period of application of the deficit | Beginning of the deficit | Ending of the deficit | Total irrigation depth applied (La - mm) |
|---|---|---|---|---|
| No deficit (P1) | - | - | - | 732.41 |
| Deficit in the initial growth stage (P2) | 22/Jul to 04/Aug | 29 DAG | 43 DAG | 686.65 |
| Deficit in the flower bud stage (P3) | 03/Aug to 16/Aug | 40 DAG | 54 DAG | 608.39 |
| Deficit in the flower stage (P4) | 18/Aug to 31/Aug | 54 DAG | 68 DAG | 603.53 |
| Deficit in the boll stage (P5) | 26/Aug to 08/Sep | 62 DAG | 76 DAG | 610.85 |
| Deficit in the open boll stage (P6) | 03/Oct to 16/Oct | 100 DAG | 114 DAG | 649.67 |

(P1), …,(P6) = treatments designation; DAG = days after germination.

(NID and GID), according to Mantovani et al. (2009).

Treatments were formed from a split-plot arrangement, in which the plots were 6 water deficit periods (P) (P1 = No deficit, P2 = Deficit in the initial growth stage, P3 = Deficit in the flower bud stage, P4 = Deficit in the flower stage, P5 = Deficit in the boll stage and P6 = Deficit in the open boll stage) and, the subplots, 2 upland cotton cultivars (C) (C1 = Brazil Seeds 286 and C2 = BRS 336), in randomized block design, with 4 replicates, amounting to 48 experimental subplots.

Each period of water deficit consisted of 14 days without irrigation in the predetermined phenological stage, according to Table 2. After this period, the plants had normal irrigation until the end of the cycle. The total irrigation depth applied for each treatment is also presented in Table 2. The necessary phytosanitary treatments were carried out when the first injuries and symptoms of pests and diseases appeared, as well as crop treatments for weed control.

The gas exchanges evaluations were performed at 29, 40, 54, 62 and 100 days after germination (DAG) from measuring stomatal conductance (gs) (mol $H_2O$ $m^{-2}$ $s^{-1}$), transpiration (E) (mmol $H_2O$ $m^{-2}$ $s^{-1}$), net photosynthesis (A) (µmol $CO_2$ $m^{-2}$ $s^{-1}$) and internal $CO_2$ concentration (Ci) (µmol $CO_2$ $mol^{-1}$). With these data, the instantaneous water-use efficiency (iWUE) (A/E) [(µmol $CO_2$ $m^{-2}$ $s^{-1}$) / (mmol $H_2O$ $m^{-2}$ $s^{-1}$)$^{-1}$] and the instantaneous carboxylation efficiency (iCE) (A/Ci) [(µmol $CO_2$ $m^{-2}$ $s^{-1}$) / (µmol $CO_2$ $mol^{-1}$)$^{-1}$] were estimated, following Konrad et al. (2005) and Magalhães Filho et al. (2008). These evaluations were performed with a plant gas exchange (model LCpro – SD, ADC Bioscientific, UK), containing an infrared gas analyzer (IRGA). The readings were performed on the third fully expanded leaf, conducted under natural conditions of air temperature, $CO_2$ concentration and using an artificial radiation source of 1200 µmol $m^{-2}$ $s^{-1}$.

The obtained data were subjected to analysis of variance through the F-test and the means of the factor levels, both qualitative, were compared by the Tukey test at 5% of probability using the statistical program SISVAR (Ferreira, 2011).

## RESULTS AND DISCUSSION

Because the higher concentration of cotton roots is in the 0.0 to 0.40 m depth layer, according to Amaral and Silva (2008), the soil moisture profiles were evaluated in this layer, during 126 days, in all treatments of water deficit periods (P1, …, P6) (Figure 1), comparing them to the water content in the FC and PWP averages of soil of experimental area.

It can be observed that soil moisture in all treatments of each water deficit period was very

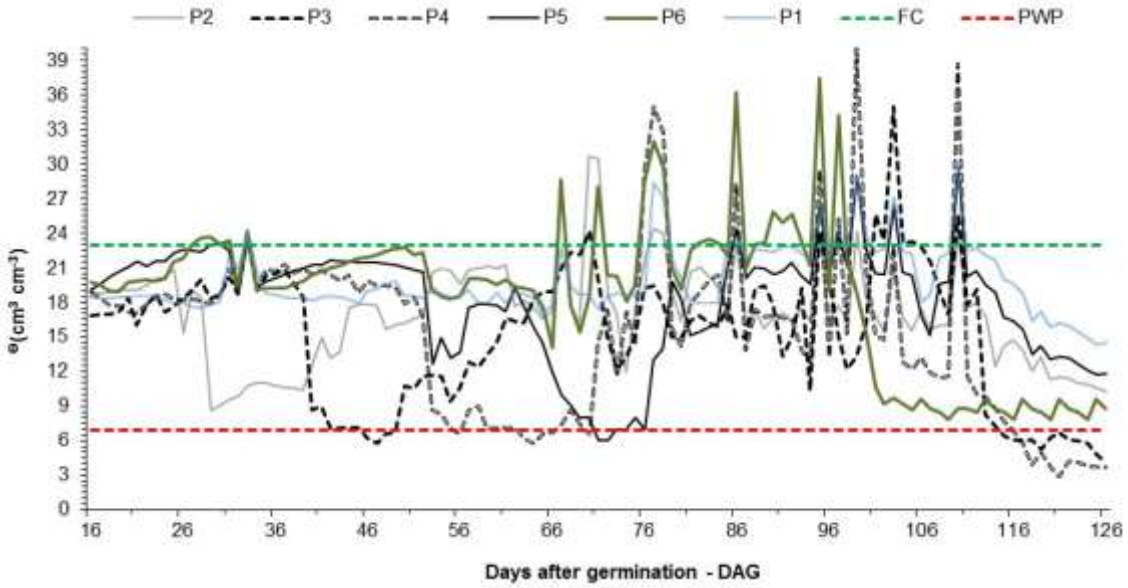


**Figure 1.** Variation of soil water content on the different water deficit treatments along experimental period. Pombal county, Paraíba state, Brazil. 2015.

close to the PWP, which increased during the period of application of the deficit and remained in approximately 50% of the AWC after this application. The deficit treatment applied in the open boll stage presented the same behavior of the irrigated treatment until a little before the application of the deficit period (Figure 1).

According to Sun et al. (2015), tolerance to water stress depends on the plant growth stage and, when water deficit occurs at critical stages, such as the reproductive stage, plant growth and development may be affected. Thus, it is very likely that the metabolic and physiological functions of the plants have been severely affected in this study.

Based on the analysis of variance, a significant difference could be seen for water deficit periods (P) in *gs* and E (except for 54 DAG, for E), A (at 29, 40, 54, 62 and 100 DAG), Ci (only at 29 and 40 DAG) and iCE (at 29 and 62 DAG). No statistical significance was observed for cultivar (C) and interaction (P × C) (Table 3).

In the comparison of means (Table 4) of *gs*, at 29, 40, 54, 62 and 100 DAG in the water deficit periods, the lowest mean values were found at 29 DAG in which the lowest value was observed in plants under P2 (0.16 mol $H_2O$ $m^{-2}$ $s^{-1}$); at 40 DAG, under P3 (0.15 mol $H_2O$ $m^{-2}$ $s^{-1}$); and at 54, 62 and 100 DAG under P4, P5 and P6, with mean values of 0.20, 0.12 and 0.13 mol $H_2O$ $m^{-2}$ $s^{-1}$, respectively.

In addition, there was a decrease in *gs* of 46.67% (P2), 48.28% (P3), 28.58% (P4), 60.00% (P5) and 38.10% (P6), in comparison with plants that were not subjected to stress (P1) (Table 4). These results corroborate Rocha and Távora (2013), who have found a significant effect of water deficit on cowpea and stated that water restriction for 15 days in the vegetative stage reduced the plant *gs*.

According to Taiz and Zeiger (2013), *gs* is dependent on stomatal opening, which, among other factors, depends on the soil water availability. In this study, the decrease in *gs* (opening) was therefore due to the water stress to which the cotton plants were exposed and it is normal to expect lower values after the deficit period.

However, when determining the difference between the value observed in the plants under stress in the period with the values of of the plants that were not subjected to stress, the greatest decrease can be observed in plants subjected to stress at P5 (60%), which corresponds to the boll period (Table 4), when there is a high demand for water for fruit growth and this cause the plant to adapt by closing the stomata more effectively.

Influence of water deficit on gs has also been observed by Vasconcelos et al. (2018) who have studied cotton under water deficit after the second week of water suppression, however, as in this work, it was possible to observe the recovery of the plants after stress, which indicates their tolerance to stress.

It is probable that in the water deficit periods, the photosynthetic apparatus of the plants used strategies to minimize the effects of the deficit, following the same trend. According to Echer (2014), the stoma begins to close as a reaction to the decrease in leaf water potential, which decreases the rate of water loss.

As regard as the cultivar influence, the mean values of *gs* were 0.27, 0.26, 0.26, 0.26 and 0.19 mol. $H_2O$ $m^{-2}s^{-1}$ for cultivar BRS 286 and BRS 336, respectively (Table 4). Stomatal conductance determined under field conditions

**Table 3.** Summary of the analysis of variance for gas exchange variables at different evaluation ages of two upland cotton cultivars under different water deficit strategies in the phenological stages (Pombal county, Paraíba State, Brazil. 2015).

| Variable | DAG | DF | MS (Deficit periods (P)) | DF | MS (Cultivar (C)) | DF | MS (P x C) | CV1 % | CV2 % | General mean |
|---|---|---|---|---|---|---|---|---|---|---|
| *gs* | | 5 | 0.0263$^{**}$ | 1 | 0.0001$^{ns}$ | 5 | 0.0015$^{ns}$ | 19.23 | 15.42 | 0.27 |
| *E* | | 5 | 1.2008$^{*}$ | 1 | 0.0013$^{ns}$ | 5 | 0.0172$^{ns}$ | 10.34 | 9.59 | 2.70 |
| *A* | | 5 | 114.7063$^{**}$ | 1 | 11.4270$^{ns}$ | 5 | 9.8188$^{ns}$ | 9.36 | 8.82 | 21.23 |
| *iWUE* | | 5 | 1.9625$^{ns}$ | 1 | 0.9240$^{ns}$ | 5 | 1.5026$^{ns}$ | 14.54 | 9.44 | 7.59 |
| *Ci* | 29 | 5 | 4644.98$^{**}$ | 1 | 675.00$^{ns}$ | 5 | 640.90$^{ns}$ | 10.06 | 13.51 | 195.20 |
| *iCE* | | 5 | 0.0012$^{*}$ | 1 | 0.0010$^{ns}$ | 5 | 0.0006$^{ns}$ | 17.26 | 16.06 | 0.10 |
| *gs* | | 5 | 0.0268$^{**}$ | 1 | 0.0065$^{ns}$ | 5 | 0.0032$^{ns}$ | 20.57 | 16.09 | 0.26 |
| *E* | | 5 | 2.4424$^{*}$ | 1 | 0.4800$^{ns}$ | 5 | 0.0493$^{ns}$ | 23.71 | 16.23 | 3.18 |
| *A* | | 5 | 75.5322$^{**}$ | 1 | 42.3752$^{ns}$ | 5 | 5.9201$^{ns}$ | 16.98 | 13.58 | 22.09 |
| *iWUE* | | 5 | 1.2570$^{ns}$ | 1 | 1.8881$^{ns}$ | 5 | 1.5025$^{ns}$ | 31.97 | 19.10 | 7.25 |
| *Ci* | 40 | 5 | 4650.18$^{**}$ | 1 | 63.02$^{ns}$ | 5 | 126.02$^{ns}$ | 8,32 | 9.47 | 202.93 |
| *iCE* | | 5 | 0.0010$^{ns}$ | 1 | 0.0006$^{ns}$ | 5 | 0.0003$^{ns}$ | 21.93 | 21.84 | 0.11 |
| *gs* | | 5 | 0.0139$^{**}$ | 1 | 0.000002$^{ns}$ | 5 | 0.0006$^{ns}$ | 18.01 | 12.97 | 0.25 |
| *E* | | 5 | 0.9737$^{ns}$ | 1 | 0.0143$^{ns}$ | 5 | 0.0100$^{ns}$ | 26.68 | 9.17 | 3.35 |
| *A* | | 5 | 25.9298$^{**}$ | 1 | 0.1376$^{ns}$ | 5 | 3.3243$^{ns}$ | 8.84 | 8.51 | 22.27 |
| *iWUE* | | 5 | 0.7836$^{ns}$ | 1 | 1.0354$^{ns}$ | 5 | 0.1635$^{ns}$ | 21.21 | 11.75 | 6.94 |
| *Ci* | 54 | 5 | 1813.48$^{ns}$ | 1 | 667.52$^{ns}$ | 5 | 721.12$^{ns}$ | 18.03 | 9.16 | 181.56 |
| *iCE* | | 5 | 0.0004$^{ns}$ | 1 | 0.00005$^{ns}$ | 5 | 0.0003$^{ns}$ | 20.63 | 14.61 | 0.12 |
| *gs* | | 5 | 0.0411$^{**}$ | 1 | 0.0001$^{ns}$ | 5 | 0.0008$^{ns}$ | 23.76 | 15.64 | 0.26 |
| *E* | | 5 | 4.8588$^{**}$ | 1 | 0.0645$^{ns}$ | 5 | 0.1973$^{ns}$ | 21.61 | 15.58 | 3.81 |
| *A* | | 5 | 258.2148$^{**}$ | 1 | 17.5087$^{ns}$ | 5 | 24.7644$^{ns}$ | 15.22 | 18.27 | 21.08 |
| *iWUE* | | 5 | 3.3935$^{ns}$ | 1 | 0.0792$^{ns}$ | 5 | 0.9592$^{ns}$ | 22.41 | 18.87 | 5.59 |
| *Ci* | 62 | 5 | 2102.23$^{ns}$ | 1 | 2.52$^{ns}$ | 5 | 910.37$^{ns}$ | 17.85 | 15.74 | 179.43 |
| *iCE* | | 5 | 0.0062$^{**}$ | 1 | 0.0006$^{ns}$ | 5 | 0.0020$^{ns}$ | 22.45 | 31.30 | 0.12 |
| *gs* | | 5 | 0.0064$^{**}$ | 1 | 0.00003$^{ns}$ | 5 | 0.0001$^{ns}$ | 9.91 | 7.10 | 0.19 |
| *E* | | 5 | 0.8774$^{**}$ | 1 | 0.0760$^{ns}$ | 5 | 0.4822$^{ns}$ | 9.96 | 6.17 | 3.99 |
| *A* | | 5 | 15.9181$^{**}$ | 1 | 7.8246$^{ns}$ | 5 | 0.7661$^{ns}$ | 10.07 | 7.06 | 14.60 |
| *iWUE* | | 5 | 0.2357$^{ns}$ | 1 | 0.1354$^{ns}$ | 5 | 0.3589$^{ns}$ | 13.04 | 8.50 | 3.68 |
| *Ci* | 100 | 5 | 678.78$^{ns}$ | 1 | 126.75$^{ns}$ | 5 | 1855.90$^{ns}$ | 10.83 | 10.53 | 195.58 |
| *iCE* | | 5 | 0.0003$^{ns}$ | 1 | 0.0005$^{ns}$ | 5 | 0.0004$^{ns}$ | 16.09 | 16.24 | 0.07 |

$^{ns}$, $^{**}$, $^{*}$: not significant and significant at $p \leq 0.01$ and $p \leq 0.05$; respectively (F-Test). DAG = days after germination; MS = Mean Squares; CV = coefficient of variation

**Table 4.** Mean values of stomatal conductance at different evaluation ages of two upland cotton cultivars under different water deficit strategies in the phenological stages (Pombal county, Paraíba State, Brazil. 2015).

| Factor (Deficit periods) | Stomatal conductance (*gs*) | | | | |
|---|---|---|---|---|---|
| | 29 DAG | 40 DAG | 54 DAG | 62 DAG | 100 DAG |
| P1 | 0.30[a] | 0.29[a] | 0.28[a] | 0.30[a] | 0.21[a] |
| P2 | 0.16[b] | 0.28[a] | 0.29[a] | 0.31[a] | 0.20[a] |
| P3 | 0.30[a] | 0.15[b] | 0.21[b] | 0.27[a] | 0.21[a] |
| P4 | 0.28[a] | 0.28[a] | 0.20[b] | 0.26[a] | 0.20[a] |
| P5 | 0.31[a] | 0.26[a] | 0.29[a] | 0.12[b] | 0.19[a] |
| P6 | 0.29[a] | 0.31[a] | 0.27[a] | 0.29[a] | 0.13[b] |
| General mean | 0.27 | 0.26 | 0.26 | 0.26 | 0.19 |
| (Cultivars) | | | | | |
| BRS 286 | 0.27[a] | 0.27[a] | 0.26[a] | 0.26[a] | 0.19[a] |
| BRS 336 | 0.27[a] | 0.25[a] | 0.26[a] | 0.25[a] | 0.19[a] |
| General mean | 0.27 | 0.26 | 0.26 | 0.26 | 0.19 |

Same letters in the column indicate no significant difference among among each factor level (Tukey, $p<0.05$).
DAG = days after germination.

**Table 5**. Mean values of transpiration at different evaluation ages of two upland cotton cultivars under different water deficit strategies in the phenological stages (Pombal county, Paraíba State, Brazil. 2015).

| Factor (deficit periods) | Transpiration (*E*) | | | | |
|---|---|---|---|---|---|
| | 29 DAG | 40 DAG | 54 DAG | 62 DAG | 100 DAG |
| P1 | 2.87[a] | 3.46[a] | 3.66[a] | 4.28[a] | 4.25[a] |
| P2 | 2.00[b] | 3.14[ab] | 3.69[a] | 4.34[a] | 3.77[ab] |
| P3 | 2.96[a] | 2.08[b] | 3.19[a] | 3.82[a] | 4.18[a] |
| P4 | 2.89[a] | 3.52[a] | 2.76[a] | 4.12[a] | 4.17[a] |
| P5 | 3.04[a] | 3.35[a] | 3.49[a] | 2.27[b] | 4.14[a] |
| P6 | 2.95[a] | 3.49[a] | 3.31[a] | 4.04[a] | 3.41[b] |
| General mean | 2.79 | 3.18 | 3.35 | 3.82 | 3.99 |
| (Cultivars) | | | | | |
| BRS 286 | 2.79[a] | 3.28[a] | 3.33[a] | 3.85[a] | 4.03[a] |
| BRS 336 | 2.78[a] | 3.08[a] | 3.36[a] | 3.78[a] | 3.95[a] |
| General mean | 2.79 | 3.18 | 3.35 | 3.82 | 3.99 |

Same letters in the column indicate no significant difference among each factor level (Tukey, $p<0.05$). DAG = days after germination.

is difficult to predict for many cultivars because of the environmental variations that occur during an evaluation procedure that may affect *gs* throughout the period (Echer, 2014).

Opposite results have been found by Soares (2016), who has studied the tolerance of colored cotton genotypes to saline stress in the different phenological stages and by Graciano et al. (2016), who have studied the gas exchange of peanut cultivars under soil water deficit, which, with the restriction of available soil water, had a significant decrease in gs in all cultivars studied.

Because of the partial stomatal closure, decreased *E* could be observed when water deficit was applied at 29 DAG in which the lowest value was observed in plants under P2 (2.00 mmol $H_2O$ $m^{-2}$ $s^{-1}$), at 40 DAG in P3 (2.08 mmol $H_2O$ $m^{-2}$ $s^{-1}$) and at 62 and 100 DAG under P5 and P6, with mean values of 2.27 and 3.41 mmol $H_2O$ $m^{-2}$ $s^{-1}$, respectively (Table 5).

There was a decrease in *E* of 30.31% (P2), 39.88% (P3), 23.77% (P4), 46.96% (P5) and 19.78% (P6) when compared to plants that did not undergo stress (P1) too (Table 5), following, in part, the results observed for gs, since soil water deficit induces stomatal resistance, decreasing the loss of water by transpiration, which may be related to the possible decrease in water potential as a consequence of the water deficit.

**Table 6.** Mean values of net photosynthesis at different evaluation ages of two upland cotton cultivars under different water deficit strategies in the phenological stages (Pombal county, Paraíba State, Brazil. 2015).

| Factor (deficit periods) | Net photosynthesis (*A*) | | | | |
|---|---|---|---|---|---|
| | 29 DAG | 40 DAG | 54 DAG | 62 DAG | 100 DAG |
| P1 | 23.48[a] | 23.80[a] | 23.44[a] | 25.26[a] | 16.02[a] |
| P2 | 13.62[b] | 22.52[a] | 22.68[a] | 23.32[a] | 14.75[a] |
| P3 | 22.39[a] | 15.93[b] | 20.69[ab] | 22.35[a] | 15.36[a] |
| P4 | 21.65[a] | 23.25[a] | 19.43[b] | 21.06[a] | 14.49[a] |
| P5 | 23.23[a] | 22.90[a] | 23.60[a] | 9.90[b] | 15.04[a] |
| P6 | 23.00[a] | 24.14[a] | 23.80[a] | 24.57[a] | 11.93[b] |
| General mean | 21.23 | 22.09 | 22.28 | 21.08 | 14.60 |
| (Cultivars) | | | | | |
| BRS 286 | 21.72[a] | 23.03[a] | 22.33[a] | 21.68[a] | 15.00[a] |
| BRS 336 | 20.74[a] | 21.15[a] | 22.22[a] | 20.47[a] | 14.19[a] |
| General mean | 21.23 | 22.09 | 22.28 | 21.08 | 14.60 |

Same letters in the column indicate no significant difference among each factor level (Tukey, $p<0.05$). DAG = days after germination.

This corroborates Rocha and Távora (2013), who have stated that water restriction for 15 days in the vegetative stage decreased transpiration to levels significantly below those normally found in irrigated plants and this decrease, although significant, allowed the maintenance of the transpiration process and a recovery in the transpiration of cotton plants could be observed after the deficit period and return of irrigations. Corroborating with this research, Soares (2016) and Graciano et al. (2016) have also verified decreases in E as a function of treatments. The decrease in E may have been caused by the lack of water in the root zone of the plant, as well as by the low capacity of osmotic adjustment of the crop and the decrease in the total water potential, caused by the decrease in soil moisture.

Cruz (2006) has found significant decreases in leaf transpiration in maize genotypes subjected to water restriction. In those, relative transpiration decreased with soil water restriction and become practically zero, with 20% of available water in the soil (Bergonci and Pereira, 2002). Nable et al. (1999) have found decreases in E rates in sorghum and sugarcane plants as the fraction of available soil water decreased. Possibly, these decreases in cotton transpiration may be influenced by other factors, such as reduced leaf area (shedding) from the applied water deficit. Thereby, Bezerra et al. (2003) reported that osmotic stress reduces the availability of water to plants and may affect their gas exchange.

The variation in the mean values of the E rate among the cultivars was minimal throughout the evaluations, with mean value of 2.79, 3.18, 3.35, 3.82 and 3.99 m mol $H_2O$ $m^{-2}$ $s^{-1}$ for the cultivars BRS 286 and BRS 336 (Table 5).

With these results, decrease could be observed in plant water flow, possibly because of the water deficit to which the cotton plant was subjected, which decreased the plant metabolism as the stomatal control of the E is a mechanism used by many species to restrict the loss of water and overcome periods of drought (Silva et al., 2003) and it probably seems to indicate cotton tolerance to avoid excessive loss of water. Much of the water absorbed by the cotton plant is used to cool it, to keep the leaf temperature at the optimum limit with dissipation as evaporation, thus favoring enzymatic activity (Echer, 2014).

Because of the decrease observed in *gs* and *E*, *A* was significantly compromised when the cotton plants were subjected to all water deficit periods, with mean values of 13.62, 15.93, 19.43, 9.90 and 11.93 µmol $CO_2$ $m^{-2}$ $s^{-1}$, with decreases of 41.99, 33.06, 17.10, 60.80 and 25.53% in *A* at 29, 40, 54, 62 and 100 DAG, respectively, when compared to plants that were not subjected to stress (P1) (Table 6).

Therefore, there was a trend of greater sensitivity to water stress in all the different periods (cotton stages), as it reduced gs and E, as discussed previously, probably because of a decrease in the performance of the photosynthetic apparatus of the plants in relation to plants without water deficit (P1) (Table 6), as well as possibly because of the influence of the low water potential caused by the water deficit.

This confirms the results of Marenco and Lopes (2009), who stated that photosynthesis is directly affected by factors such as light intensity, temperature, $CO_2$ concentration, leaf nitrogen content and soil moisture.

A decline in the photosynthesis of cotton plants has also been verified by Meloni et al. (2003), Brito (2015) and Soares (2016) in those cultivated under irrigation with saline water. Probably, the soil water deficit caused a decrease in the photosynthesis of the cotton, as observed in this work.

Loka et al. (2011) stated that water deficit reduces the photosynthetic rate from a combination of stomatal and non-stomatal limitations. The stoma begins to close as a reaction to the decrease in leaf water potential, decreasing the rate of water loss, but also decreasing $CO_2$ entry and photosynthesis in the plant, which may have occurred here in treatments with water deficit.

The decrease in A may have been due to the closure of the stomata, which restricts the influx of $CO_2$ in the mesophyll cells. Stomata can respond quickly depending on the air or soil moisture (Turner et al., 1985).

The non-existence of oxygen, possibly because of stomatal closure, may induce a decrease in respiration, thus compromising the energy level, since active absorption needs energy resulting from the oxidative respiration and requires oxygen available in the soil (Souza et al., 1997).

Souza et al. (2000), studying the physiology and productivity of sesame in soils with water deficiency, have found decreases in photosynthesis after two days without irrigation, with a decrease of 88%. The same authors have found decreases in photosynthesis and respiration of 87 and 60%, respectively, with the application of water stress.

Although stomatal closure during the reduction of soil moisture coincides with the decrease in leaf water potential, several experiments have also shown decreases in gs, even though leaf water potential is kept constant (Davies et al., 1986; Gollan et al., 1986).

There is also evidence that dehydration, in addition to affecting photosynthesis from $CO_2$ flow restriction, has detrimental effects directly on the photosynthetic apparatus (Kaiser, 1987). Austin et al. (1982) and Johnson et al. (1987) have observed significant differences in photosynthesis among different wheat species.

According to Hsiao (1993), the difference in transpiration between plants results from differences in the efficiency of stomatal control, which has implications for the efficiency of water use, as well as stomatal control of transpiration; on the other hand, it imposes diffusion limitations for $CO_2$ that may lead to a decrease in the photosynthetic rate.

Researchers as Neves et al. (2009) and Silva et al. (2011) mentioned that the decrease in the photosynthesis rate is caused by partial stomatal closure associated with the osmotic effect and the ionic toxicity on the metabolism of the plants subjected to salinity conditions.

In this research, this decrease in the photosynthesis rate was probably caused by the water deficit to which the plants were subjected. James et al. (2002), states that both stomatal conductance and transpiration are reduced by the decrease in root water potential or by the transport of abscisic acid to the leaves.

Regarding the cultivar factor, photosynthesis was not affected in all periods of application of the water deficit (29, 40, 54, 62 and 100 DAG) with mean values of 21.23, 22.09, 22.28, 21.08 and 14.60 μmol $CO_2$ $m^{-2}$ $s^{-1}$ for the cultivars BRS 286 and BRS 336, respectively (Table 6), which are satisfactory values as cotton is a $C_3$ plant, with photosynthetic rates ranging between 10 and 20 μmol $CO_2$ $m^{-2}$ $s^{-1}$ (Taiz and Zeiger, 2013).

Possibly, the water stress imposed on cotton increased the leaf-to-air vapor pressure deficit ($VPD_{leaf-air}$), which can cause the water molecules to exit the stomatal cells into the external air, which is known as peristomatic evaporation (Maier-Maercker, 1983), promoting stomatal closure, especially in the treatments that were subjected to water deficit, minimizing the water exiting the cells.

Some researchers report in their work the negative effects of $VPD_{leaf-air}$ on gas exchange, which provides stomatal closure (Erismann et al., 2006; Costa and Marenco, 2007), thus reducing gs, E and A of plants, as observed in this work.

The mean values of *iWUE* were 7.59, 7.28, 6.95, 5.59 and 3.68 [(μmol $CO_2$ $m^{-2}$ $s^{-1}$) / (mmol $H_2O$ $m^{-2}$ $s^{-1}$)$^{-1}$] at 29, 40, 54, 62 and 100 DAG, respectively, which shows a decrease in *iWUE* as the water deficit was applied (Table 7).

As the *iWUE* is the result of the ratio of photosynthesis to transpiration, this fact is explained by the decrease in photosynthesis ($CO_2$ assimilation rate) in this study after 54 DAG (Table 6) from the decrease in water restriction. Lower absolute values can be observed for *iWUE* in the periods when the cotton plants underwent water restriction.

This decrease in *iWUE* observed in the treatments may be associated with a change in leaf transpiration and $CO_2$ assimilation rates (photosynthesis), possibly because of the low soil water availability, which induces the plant to the leaf osmotic adjustment, resulting in stomatal resistance and consequently reducing leaf transpiration and $CO_2$ assimilation rate, directly affecting *iWUE* as stated by Willadino and Camara (2004). Contradicting results have been found by Soares (2016) and Graciano et al. (2016), who have found a significant effect of the treatments studied on the iWUE. Larcher (2006) stated that the best ratio between photosynthesis and water consumption probably occurs when the stomata are partially closed, which can be demonstrated from the moment the water deficit starts when the two diffusion processes are readily decreased, which results in higher photosynthesis/transpiration ratio (A/E).

Therefore, the increase in iWUE of the cultivars evaluated in this work may have been favored by stomatal closure, observed from the results of gs, E and A. This result indicates that the cultivar that can keep a high A/E ratio under soil water deficit presents a higher tolerance to this condition.

In agreement with the results of gs, E and A, *Ci* presented the same trend observed for those variables in the treatments under water deficit, that is, the value of *Ci* decreased at 29 and 40 DAG, with mean value of 153.00 and 171.50 μmol $CO_2$ $m^{-2}$ $s^{-1}$, respectively (Table 8).

**Table 7.** Mean values of instantaneous water-use efficiency at different evaluation ages of two upland cotton cultivars under different water deficit strategies in the phenological stages (Pombal county, Paraíba State, Brazil. 2015).

| Factor (deficit periods) | Instantaneous water-use efficiency (*iWUE*) | | | | |
|---|---|---|---|---|---|
| | 29 DAG | 40 DAG | 54 DAG | 62 DAG | 100 DAG |
| P1 | 8.19[a] | 7.36[a] | 6.79[a] | 5.97[a] | 3.79[a] |
| P2 | 6.71[a] | 7.59[a] | 6.65[a] | 5.74[a] | 3.95[a] |
| P3 | 7.56[a] | 7.58[a] | 6.74[a] | 5.81[a] | 3.70[a] |
| P4 | 7.50[a] | 6.60[a] | 7.26[a] | 5.23[a] | 3.48[a] |
| P5 | 7.72[a] | 7.01[a] | 6.81[a] | 4.48[a] | 3.64[a] |
| P6 | 7.86[a] | 7.42[a] | 7.41[a] | 6.32[a] | 3.54[a] |
| General mean | 7.59 | 7.28 | 6.95 | 5.59 | 3.68 |
| (Cultivars) | | | | | |
| BRS 286 | 7.73[a] | 7.45[a] | 7.09[a] | 5.63[a] | 3.73[a] |
| BRS 336 | 7.45[a] | 7.10[a] | 6.80[a] | 5.55[a] | 3.63[a] |
| General mean | 7.59 | 7.28 | 6.95 | 5.59 | 3.68 |

Same letters in the column indicate no significant difference among each factor level (Tukey, $p<0.05$). DAG = days after germination.

**Table 8.** Mean values of internal $CO_2$ concentration at different evaluation ages of two upland cotton cultivars under different water deficit strategies in the phenological stages (Pombal county, Paraíba State, Brazil. 2015).

| Factor (deficit periods) | Internal $CO_2$ concentration (*Ci*) | | | | |
|---|---|---|---|---|---|
| | 29 DAG | 40 DAG | 54 DAG | 62 DAG | 100 DAG |
| P1 | 199.25[a] | 233.75[a] | 189.20[a] | 177.37[a] | 202.00[a] |
| P2 | 153.00[b] | 177.00[b] | 177.64[a] | 203.00[a] | 200.75[a] |
| P3 | 215.87[a] | 171.50[b] | 196.67[a] | 180.50[a] | 205.12[a] |
| P4 | 190.62[a] | 215.75[a] | 153.80[a] | 172.87[a] | 197.25[a] |
| P5 | 220.62[a] | 214.12[a] | 188.18[a] | 154.37[a] | 183.00[a] |
| P6 | 191.87[a] | 205.50[a] | 183.89[a] | 188.50[a] | 185.37[a] |
| General mean | 195.20 | 202.94 | 181.56 | 179.43 | 195.58 |
| (Cultivars) | | | | | |
| BRS 286 | 191.45[a] | 204.08[a] | 177.83[a] | 179.20[a] | 193.95[a] |
| BRS 336 | 198.95[a] | 201.79[a] | 185.29[a] | 179.66[a] | 197.20[a] |
| General mean | 195.20 | 202.94 | 181.56 | 179.43 | 195.58 |

Same letters in the column indicate no significant difference among each factor level (Tukey, $p<0.05$). DAG = days after germination.

The deficit periods decreased *Ci* in 23.21% (P2), 26.63% (P3), 18.71% (P4), 12.96% (P5) and 8.23% (P6) at 29, 40, 54, 62 and 100 DAG, respectively, compared to the period without deficit (P1) (Table 8), probably because of the carbon flux for the synthesis of organic compounds, which were not being metabolized by the photosynthetic apparatus given the water stress condition to which the cotton plants were exposed at different water deficit periods.

The decreases recorded in Ci reflect the observed decreases in the rate of carbon dioxide assimilation, which is justified by the fact that, during the gas exchange process, the absorption of $CO_2$ converges in the loss of water and, conversely, the decrease in this water loss restricts the carbon dioxide assimilation and consequently converges to a lower internal $CO_2$ concentration (Shi Mazaki et al., 2007).

In addition, according to Jadoski et al. (2005), the Ci in the leaf mesophyll is reduced by the stomatal closure with a consequent decrease in the rate of carbon dioxide assimilation, which, in this work, was observed in all water deficit periods. On the other hand, Raschke (1979) and Dai et al. (1992) stated that the increase in the rate of $CO_2$ assimilation causes a decrease in evaluation ages, exerting a strong negative retroactive effect and, consequently, causing a decrease in the rate of $CO_2$

assimilation.

However, the above auhors reported that the decrease in Ci stimulates greater stomatal opening, thus allowing greater Ci for the substomatal cavity. It should be noted that, in this study, there was an increase in the rate of $CO_2$ assimilation in all deficit periods (29, 40, 54, 62 and 100 DAG) when compared to the period without deficit (P1), but only after irrigation return (Table 8). Larcher (2006) stated that values considered high for leaf Ci indicate that $CO_2$ is not being used for the synthesis of sugars by the photosynthetic process with the accumulation of this gas, which indicates that some non-stomatal factor is interfering in this process.

The increase in Ci can be attributed to the decrease in gs with the application of deficit periods, which is a common response in plants subjected to water stress. For Pereira et al. (2012), this type of behavior evidenced the occurrence of not only damage to the photosynthetic apparatus in the carboxylation stage but also an increase in the photorespiration process, since Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is the one that catalyzes the first step of this pathway.

Machado et al. (1999) stated that the increase in *Ci* can be related to the decrease in the activity of enzymes involved in the $CO_2$ fixation process. On the other hand Grassi and Magnani (2005) attributed this increase to non-stomatal factors, such as decrease in RuBisCO activity and concentration, photoinhibition, electron transfer rate and decreased photochemical efficiency of PSII, which may impair photosynthesis.

When evaluating the Ci of the cotton cultivars studied, mean values of 195.20, 202.94, 181.56, 179.43 and 195.58 µmol $CO_2$ $mol^{-1}$ were observed at 29, 40, 54, 62 and 100 DAG for the cultivars BRS 286 and BRS 336, respectively (Table 8). These results are similar to those found by Ferraz (2012), who has studied the cultivars BRS Rubi, BRS Topázio and BRS Safira, under field conditions, with mean values ranging from 182.9 to 223.7 µmol $CO_2$ $mol^{-1}$, however these values are higher than those found by Soares (2016) for these same cultivars, who has obtained mean values ranging from 154.69 to 172.39 µmol $CO_2$ $mol^{-1}$.

On the other hand, Freire et al. (2014), studying yellow passionfruit plants under saline stress, have recorded *Ci* of 259.70 and 229.47 µmol $CO_2$ $mol^{-1}$ in plants that did not undergo saline stress, this serves as evidence for the negative effects of stress on the plant carbon metabolism. For Larcher (2006), high concentrations of *Ci* in the substomatal cavity of leaves mean that $CO_2$ is not being used by photosynthesis, which indicates that some non-stomatal factor is interfering in this metabolic process.

The *iCE* is a way of studying the non-stomatal factors that interfere with the photosynthetic rate, since this parameter has a close relation with Ci and with the rate of $CO_2$ assimilation (Konrad et al. 2005; Machado et al. 2010). At 29 and 62 DAG, the effect of the periods of water deficit can be observed on *iCE* with mean values of 0.12 and 0.06 ((µmol $CO_2$ $m^{-2}$ $s^{-1}$) / (µmol $CO_2$ $mol^{-1}$)$^{-1}$) and decreases in *iCE* of 33.33% and 57.14%, compared to plants that did not undergo water deficit (P1) (Table 9).

Probably, the decrease in *iCE* may be related to the higher *Ci* in plants, also under water deficit in the boll formation stage, when there is a high water demand for fruit growth, which causes the plant to adapt and close the stomata more effectively. This decrease is probably a reflection of the low $CO_2$ assimilation in relation to the $CO_2$ found in the substomatal cavity in these plants, because, if *Ci* increases and there is a decrease in $CO_2$ consumption in chloroplasts from the decreased photosynthetic activity, the A/*Ci* ratio will also decrease (Suassuna, 2013).

A decrease in iCE can be observed at 40, 54 and 100 DAG in the periods of water deficit with mean values of 0.11, 0.12 and 0.07 [(µmol $CO_2$ $m^{-2}$ $s^{-1}$) / (µmol $CO_2$ $mol^{-1}$)$^{-1}$], as well as in the treatments that again had irrigation from the possible recovery and not in the treatments of flower bud (P3), flower (P5) and open boll (P6), as the deficit was not enough to cause differences in this variable. In the treatments that had a significant effect, the lowest mean values were observed in the treatments with water deficit (Table 9). Even so, *iCE* has a close relation with the intracellular concentration of $CO_2$ and the rate of carbon dioxide assimilation (Machado et al., 2005).

For the cultivar factor, in the periods of water deficit (29, 40, 54, 62 and 100 DAG), the mean values were 0.11, 0.11, 0.12, 0.12 and 0.07 ((µmol $CO_2$ $m^{-2}$ $s^{-1}$) / (µmol $CO_2$ $mol^{-1}$)$^{-1}$) for the cultivars BRS 286 and BRS 336, respectively (Table 9).

Opposite results have been found by Soares (2016), who has studied the tolerance of colored cotton genotypes to saline stress in the different phenological stages, in which there was a significant decrease in *iCE* in all cultivars studied with the restriction of available soil water. Considering that the cotton plants were subjected to different conditions of water deficit according to their phenological stages, when gas exchange were also measured, it is assumed that the deficit caused by the water restriction reached the water status of the plant at the stomatal level.

Nevertheless, according to Marenco and Lopes (2009), in situations of moderate or severe water deficit, stomatal resistance may occur from the increase in the diffusion of the acid towards the guard cells, whereas the stomata tend to remain open in mild stress. Padilha et al. (2016) stated that the abscisic acid induces stomatal closure, as long as its synthesis is stimulated by water scarcity. In these circumstances, stomatal closure is related to the decrease in leaf water potential (Pereira, 2012).

Therefore, it is believed that the cotton plants, in the different evaluation periods, reached cellular turgescence with potential to cause stomatal changes. Thus, when the gas exchange in plants under water deficit exhibits

**Table 9.** Mean values of instantaneous carboxylation efficiency at different evaluation ages of two upland cotton cultivars under different water deficit strategies in the phenological stages (Pombal county, Paraíba State, Brazil. 2015).

| Factor (deficit periods) | Instantaneous carboxylation efficiency (*iCE*) | | | | |
|---|---|---|---|---|---|
| | 29 DAG | 40 DAG | 54 DAG | 62 DAG | 100 DAG |
| P1 | 0.12[a] | 0.10[a] | 0.12[a] | 0.14[a] | 0.07[a] |
| P2 | 0.08b | 0.12[a] | 0.13[a] | 0.12[a] | 0.07[a] |
| P3 | 0.10[a]b | 0.09[a] | 0.11[a] | 0.13[a] | 0.07[a] |
| P4 | 0.11[a]b | 0.11[a] | 0.12[a] | 0.12[a] | 0.07[a] |
| P5 | 0.10[a]b | 0.10[a] | 0.13[a] | 0.06b | 0.08[a] |
| P6 | 0.12[a] | 0.11[a] | 0.12[a] | 0.13[a] | 0.06[a] |
| General mean | 0.11 | 0.11 | 0.12 | 0.12 | 0.07 |
| (Cultivars) | | | | | |
| BRS 286 | 0.11[a] | 0.11[a] | 0.12[a] | 0.12[a] | 0.07[a] |
| BRS 336 | 0.10[a] | 0.10[a] | 0.12[a] | 0.11[a] | 0.07[a] |
| General mean | 0.11 | 0.11 | 0.12 | 0.12 | 0.07 |

Same letters in the column indicate no significant difference among each factor level (Tukey, $p<0.05$). DAG = days after germination.

different behavior than that without water restriction, the effects of soil water deficit probably interfere with the photosynthetic processes of the plants.

The results found for Ci followed the same trend of gs and E (except at 54 DAG) as the stomatal movement is the mechanism that regulates the gas exchange and increases in gs, which means a greater influx of $CO_2$ for the leaf mesophyll, resulting in higher rates of carbon dioxide assimilation (Shi Mazaki et al., 2007). The treatments (deficit periods) that resulted in an increase in gs, Ci and E, consequently had greater photosynthesis, which denotes close connection, since gs allows a greater entry of $CO_2$, directly influencing the photosynthetic performance (Pereira, 2012).

The decreases in the variables of gas exchange studied in this work probably occurred because of the decrease of energy in the root water potential and/or the transport of the abscisic acid to the leaves, which reflect an increase in stomatal resistance and a decrease in carbon concentration in the substomatal cavity. This is explained by the direct relation between gas exchange (implied $CO_2$ absorption) and water loss, in which stomatal closure results in decreased E and, consequently, lower Ci (Shi Mazaki et al., 2007), which probably induces a decrease in A, iWUE and iCE.

These decreases may also be associated with a decrease in amylaceous reserves in cotton leaves under conditions of water deficit that can be explained considering that soil water deficit may lead to decreases in assimilate synthesis and consequently starch reserves are rapidly used for plant metabolism, which is why the contents of soluble sugars are stable when cotton plants are induced to water deficit (Souza and Silva, 1983; Souza et al., 2000), thus corroborating Souza et al. (2000), who stated that the physiological behavior of sesame was influenced by soil water deficit.

Depending on the duration of the soil water deficit, the cotton plant underwent physiological changes in all the periods subjected to the deficit and although the photosynthetic activity is changed at fourteen days of soil water deficit, the plants can recover after its suspension and the return of irrigation, depending on the duration of irrigation. The decrease in the photosynthetic activity of the cotton plant that occurred when subjected to water deficit may be due to its stomatal closure efficiency to reduce cotton gas exchange and transpiration.

## Conclusion

Water deficits reduced the gas exchange of the upland cotton plants, mainly stomatal conductance, transpiration and photosynthesis; the cotton cultivars BRS 286 and BRS 336 presented similar behavior in the different water deficits applied on different phenological stages; cotton was less tolerant to water deficits in the boll formation stage; and, cotton was more tolerant to water deficit in the initial growth and flower bud stages.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Fiber quality in upland cotton cultivars under water deficit strategies

**Whéllyson Pereira Araújo[1], José Rodrigues Pereira[2]*, João Henrique Zonta[2], Hugo Orlando Carvallo Guerra[1], Mailson Araújo Cordão[1] and Robson Felipe de Lima[1]**

[1]Agricultural Engineering, Campina Grande Federal University, Paraíba, Brazil.
[2]Irrigation, Embrapa Algodão, Rua Oswaldo Cruz, 1.143-Centenário, CEP 58.428-095, Campina Grande, Paraíba, Brazil.

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**An evaluation was made of upland cotton cultivar fiber quality, when subjected to water deficit periods, on the phenological stages. The experiment was carried out at the Campina Grande Federal University, Pombal county Campus, Paraíba State, Brazil, between June and December 2015. Treatments were formed using a split-plot arrangement, in which the plots were 6 water deficit periods (P) (P1 = No deficit; P2 = Deficit in the initial growth stage; P3 = Deficit in the flower bud stage; P4 = Deficit in the flower stage; P5 = Deficit in the boll stage; and P6 = Deficit in the open boll stage) and the subplots, 2 upland cotton cultivars (C) (C1 = Brazil Seeds 286 and C2 = BRS 336), in randomized block design, with 4 replicates. The water deficits applied affected cultivars fiber quality, except maturity, reflectance and yellowness. The treatment without water deficit promoted the best fiber values, except of short fiber index, elongation and micronaire. Tested upland cotton cultivars were more tolerant to water deficit in the initial growth and boll stages. In general, BRS 336 was more tolerant to water deficits than BRS 286.**

**Key words:** *Gossypium hirsutum* L. r. *latifolium* H., stress, cotton lint industry technological characteristics.

## INTRODUCTION

Upland cotton (*Gossypium hirsutum* L. r. *latifolium* H.) is a dicotyledon of high economic and social importance that is cultivated in more than 100 countries of the world and its fiber, its main product, dresses almost half of humanity (43%). It is the only plant that economically produces fiber (41% on average in relation to the cotton seed dry weight - CSDW), oil (14 to 28% in relation to the CSDW) and protein (mean of 26% in relation to the CSDW), with high biological value (Beltrão, 2006).

In the semiarid region, irrigated cotton crops can be an excellent opportunity for the cotton sector, since the climatic characteristics of this region are able to produce fibers of excellent quality (Brito et al., 2011). However, according to Zonta et al. (2015a) research should seek to improve the irrigation management of cotton for high quality fibers.

In the semiarid region of the Brazilian Northeast, this plant has always stood out as one of the main subsistence crops (Sousa, 1994) and although it is considered a relatively drought-tolerant crop, its yield can

*Correspondent author. E-mail: jose.r.pereira@embrapa.br. Tel: +558331824373.

be considerably reduced when water deficits occur during its development cycle (Bezerra et al., 2003).

Cotton requires, for its growth and development, an adequate amount of water defined according to the soil and climate. Lack of water at critical times of the cycle compromises crop growth, development and yield (Hussein et al., 2011; Luo et al., 2013). Beltrão et al. (2001) and Almeida et al. (2016) stated that water scarcity affects the growth of cotton and it most critically affects the phenological stages of flowering and formation and development of fruits.

Yazar et al. (2002) stated that final yield can be affected when water stress occurs during the cotton growing season, since it depends on the production and retention of open bolls, which can decrease with water stress. According to Pettigrew (2004), when water stress occurs in the early development stages, fibers are the most affected because they involve several physiological mechanisms of cell expansion.

In the initial stage of fiber elongation, up to 15 days after anthesis, water stress inhibits fiber elongation, length and uniformity (Lokhande and Reddy, 2014). Beltrão et al. (2008) stated that water deficit in the period of fiber elongation causes a decrease in its length.

Because of the presence of the genotype and environment interaction in cotton, a single cultivar cannot adapt to all cultivation regions of Brazil and it is important to identify the most appropriate cultivars for each region. Therefore, the success of a good agronomic performance of upland cotton will depend on the correct choice of the cultivar to be planted, as well as the environment and the cultural management (Araújo et al., 2013).

For Zonta et al. (2017), the value of the cotton fiber and the economic return obtained with the cotton crop depends on both yield and fiber quality, which depend on the interaction of several factors, such as management, environment and plant genetics. Santana et al. (2008) stated that the technological characteristics of cotton fiber, although conditioned by hereditary factors, are influenced by environmental factors and depend on the conditions of cultivation.

The objective of this study was to evaluate the fiber technological characteristics of upland cotton cultivars cultivated in the semiarid region of the Brazilian Northeast, subject to water deficit periods, on the phenological stages, in order to relate the rational use of water for sustainable crop production in the semiarid region of Paraíba State, Brazil, identifying the most appropriate management.

## MATERIALS AND METHODS

The experiment was conducted under field conditions between June and December 2015 in the experimental area of the Center for Agricultural Science and Technology, of the Campina Grande Federal University, Pombal County Campus, Paraíba State, Brazil, located in the following geographic coordinates: 06° 47' 52" S, 37° 48' 10" W and 175 m above mean sea level. The predominant climate of the region is hot semiarid (the BSh type), according to Köppen climate classification. The soil of the experimental area was classified as Fluvic Neo-soil (Santos et al., 2013), loamy sand texture (80% sand, 5.96% clay and 14.51% silt) and water tension curve of 15.49% (at 0.1 atm. Field Capacity - FC), 4.63% (at 15.0 atm. Permanent Wilting Point - PWP) with available water content (AWC) of 6.63% at the depth of 0 to 40 cm.

Fertilization was carried out according to the technical recommendations for the crop (Cavalcanti, 2008), based on the analysis of soil fertility (Table 1), in the foundation, by the application of 30 kg $ha^{-1}$ of N, 40 kg $ha^{-1}$ of $P_2O_5$ and 10 kg $ha^{-1}$ of $K_2O$ and in 2 covers, with the application of 30 kg $ha^{-1}$ of N and 5 kg $ha^{-1}$ of $K_2O$. Liming was not needed.

Upland cotton cultivars were planted in single rows, spaced 1.0 m between rows × 0.10 m among plants.

The water used in the irrigation presented $C_2S_1$ salinity (low alkali and medium salinity hazard, an electric conductivity - EC of 0.315 $dSm^{-1}$) and low sodium adsorption ratio (SAR = 1.78). Such water can be used for irrigation whenever there is a moderate degree of leaching and special care in the preparation of the soil.

Water was applied by a localized irrigation system, with drip tapes and emitters spaced 0.10 m apart. Each treatment consisted of a lateral line, spaced from the other lines by 1 m, with 6 m of length, each.

Subsequently, after installation of the irrigation system and beginning of the experiment, a water distribution test was carried out in the field. Through this, the mean precipitation applied was determined as 8.86 mm $h^{-1}$ and application efficiency (Ae) as 91%, according to Bernardo et al. (2008).

Irrigations were carried out daily, always in the morning, based on the availability of soil water (AWC) to plants. The replacement water volume was calculated considering the water lost by the crop evapotranspiration, which is represented as the difference between the soil water content in the field capacity (FC) and the current mean soil water content (SWC) measured in the depths of 0.10, 0.20, 0.30 and 0.40 m, which were measured before irrigations.

The current SWC was determined by the time-domain reflectometry (TDR) method, using a Delta-T-PR2 probe introduced through access pipes installed in each treatment.

With the data of the current SWC, using an Excel spreadsheet, in which the daily values of the SWC and the AWC to plants were recorded, the depth for the replacement of water and the time of irrigation were calculated for the treatments, which were the basis for the determination of the Net and Gross Irrigation Depth (NID and GID), according to Mantovani et al. (2009).

Treatments were formed from a split-plot arrangement, in which the plots were 6 water deficit periods (P) (P1 = No deficit; P2 = Deficit in the initial growth stage; P3 = Deficit in the flower bud stage; P4 = Deficit in the flower stage; P5 = Deficit in the boll stage; and P6 = Deficit in the open boll stage) and the subplots, 2 upland cotton cultivars (C) (C1 = Brazil Seeds 286 and C2 = BRS 336), in randomized block design, with 4 replicates, amounting to 48 experimental subplots (Figure 1).

Each period of water deficit consisted of 14 days without irrigation in the predetermined phenological stage shown in Table 2. After this period, the plants had normal irrigation until the end of the cycle. The total irrigation depth applied for each treatment is also shown in Table 2.

The necessary phytosanitary treatments were carried out when the first injuries and symptoms of pests and diseases appeared, as well as crop treatments for weed control.

For each cotton cultivar, the technological characteristics of the fiber were determined in a standard sample of 20 open bolls, collected in the middle third of the plants in the useful area of each subplot, before harvest, using a High Volume Instrument (HVI) of the Laboratory of Fibers and Yarns of Embrapa Cotton in Campina Grande county, Paraíba State, Brazil.

The fiber technological characteristics evaluated were Length

**Table 1.** Chemical characteristics of the soil of the experimental area at different depths (Pombal county, Paraíba State, Brazil, 2015).

| Depth (cm) | pH Water | OM (%) | P (mg 100g$^{-1}$) | $Na^+$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ |
|---|---|---|---|---|---|---|---|
| 0-20 | 6.79 | 1.16 | 51.5 | 0.14 | 0.42 | 4.28 | 1.40 |
| 20-40 | 6.94 | 0.78 | 49.0 | 0.15 | 0.27 | 4.03 | 1.89 |

pH = Hidrogenionic potential; OM = organic matter.
Source: Irrigation and Salinity Laboratory, UFCG, Campina Grande County, Paraíba State, Brazil.

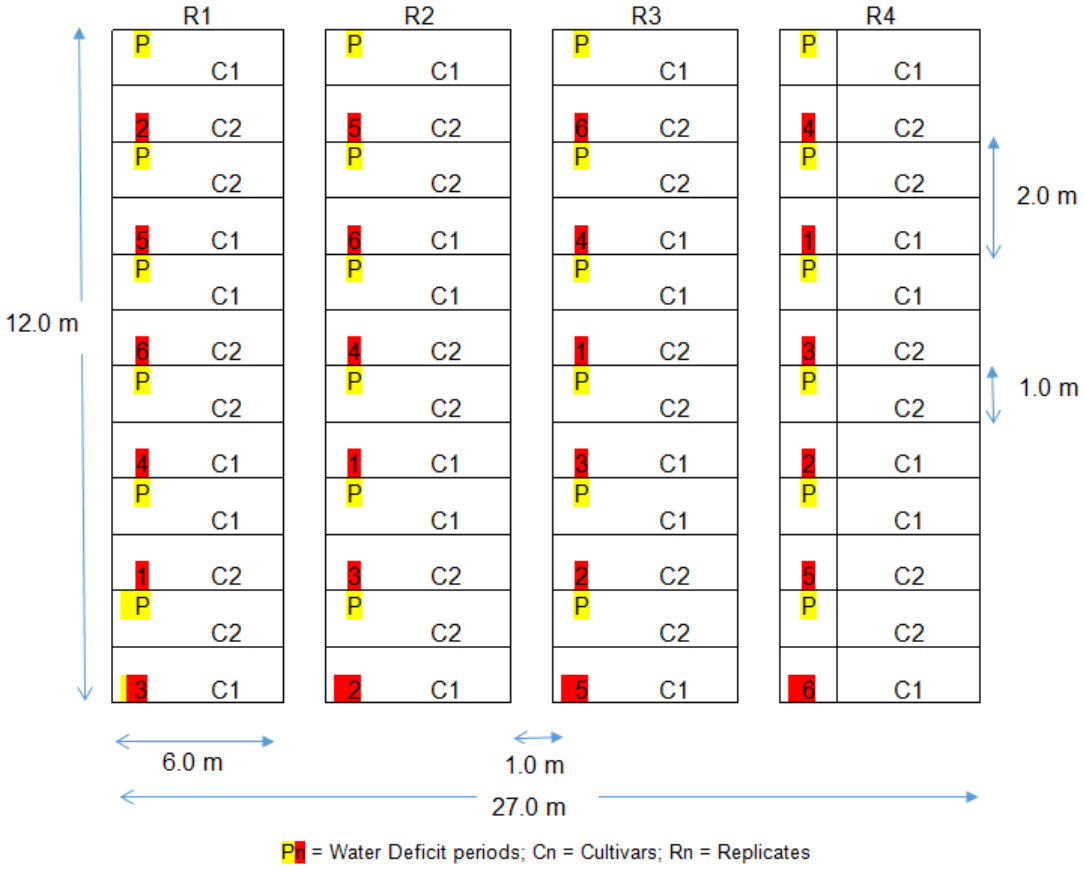


**Figure 1.** Design of the experimental area.
Source: UFCG, Pombal County Campus, Paraíba State, Brazil (2015).

**Table 2.** Detail of the deficit treatments (Pombal County, Paraíba State, Brazil, 2015).

| Treatment | Period of application of the deficit | Beginning of the deficit | Ending of the deficit | Total irrigation depth applied (La - mm) |
|---|---|---|---|---|
| No deficit (P1) | - | - | - | 732.41 |
| Deficit in the initial growth stage (P2) | 22/Jul to 04/Aug | 29 DAG | 43 DAG | 686.65 |
| Deficit in the flower bud stage (P3) | 03/Aug to 16/Aug | 40 DAG | 54 DAG | 608.39 |
| Deficit in the flower stage (P4) | 18/Aug to 31/Aug | 54 DAG | 68 DAG | 603.53 |
| Deficit in the boll stage (P5) | 26/Aug to 08/Sep | 62 DAG | 76 DAG | 610.85 |
| Deficit in the open boll stage (P6) | 03/Oct to 16/Oct | 100 DAG | 114 DAG | 649.67 |

(P1), …, (P6) = Treatments designation; DAG = days after germination.

(UHM_mm), Uniformity (UNF_%), Short Fiber Index (SFI_%), Resistance (STR_gf $tex^{-1}$), Elongation (ELG_%), Micronaire index (MIC_ µg $inch^{-1}$), Maturity (MAT_%), Reflectance (Rd_%), Yellowness (+b_dimensioless) and Count Strength Product (CSP_dimensionless).

The obtained data were subjected to analysis of variance through the F-test and the means of the factor levels or treatments, both qualitative, were compared by the Tukey test at 5% of probability using the statistical program SISVAR (Ferreira, 2011).

## RESULTS AND DISCUSSION

Because the higher concentration of cotton roots is in the 0.0 to 0.40 m depth layer, according to Amaral and Silva (2008), the soil moisture profiles were evaluated in this layer, during 126 days, in all treatments of water deficit periods (P1, …, P6) (Figure 2), comparing them to the water content in the FC and PWP averages of soil of the experimental area.

It can be observed that soil moisture in all treatments of each water deficit period was very close to the PWP, which increased during the period of application of the deficit and remained in approximately 50% of the AWC after this application. The deficit treatment applied in the open boll stage presented the same behavior of the irrigated treatment until a little before the application of the deficit period (Figure 2).

According to Sun et al. (2015), tolerance to water stress depends on the plant growth stage and when water deficit occurs at critical stages, such as the reproductive stage, plant growth and development may be affected. Thus, it is very likely that the metabolic and physiological functions of the plants have been severely affected in this study.

Significant effect of the deficit periods (P) for almost all studied fiber variables at 1% probability level, except for maturity, reflectance and yellowness, was detected. Regarding cultivar (C), there was a significant effect for all the variables of fiber at 1% of probability. In relation to the interaction (P × C), there was no significant effect for any of the variables studied, which means that the effect of the periods of water deficit tested did not depend on the cultivars studied and vice and versa (Table 3).

Other research studies corroborate all the evaluated variables, such as Zonta et al. (2015b), who have studied the effect of irrigation on yield and fiber quality in upland cotton cultivars; Zonta et al. (2017), who have studied the influence of sampling on the analysis of the fiber quality of irrigated and water stressed cotton; and Almeida et al. (2016), who have studied the effect of periods of water deficit in different phenological stages for cotton fiber quality, who have also found significant differences between treatments and cultivars studied for fiber variables at 1% probability level.

In relation to UHM, the lowest value was found in the P6 water deficit period, which differs from all other deficit treatments; and these, are not differentiated among each other (Figure 3A). In this variable, the cultivars also differed between each other, with emphasis on cultivar BRS 336, classified as very long fiber cotton, with a mean of 32.31 mm, which is higher than value to cultivar BRS 286 with 29.04 mm, classified as long fiber cotton (Figure 3B), according to the industrial classification (Santana et al., 2008).

Both cultivars presented mean values within the cultivar standard, which is from 29.1 to 31.3% for BRS 286 and from 32.0 to 34.0% for BRS 336, according to Silva Filho et al. (2008) and Morello et al. (2011), respectively.

The P6 water deficit decreased the UHM when compared to the other treatments and accentuated water deficits produce inferior fibers when compared to the other deficit treatments in the phenological stages (Figure 3A). This behavior has also been observed by other authors, such as Wen et al. (2013), who have stated that many fiber quality characteristics are directly influenced by the water deficits applied to the soil in different phenological stages.

In addition, according to Abidi et al. (2010), when the fiber growth period occurs within 3 weeks after anthesis, it may compromise UHM formed in these open bolls. Kim (2015) stated that the open bolls generally develop rapidly up to 16 days after anthesis and reach their maximum size approximately 24 days after it; the open bolls reach maturity between 40 and 60 days after anthesis.

The values of UHM vary according to the position of the open boll in the plant, being it higher in the middle and lower third and lower in the upper positions on the plant. Thus, when only samples from the middle third are harvested, the values tend to be overestimated in relation to the representative sample collection of the whole plant, which may have occurred in this assay (Kelly et al., 2015).

The results were similar to those found by Almeida et al. (2016) who, when studying fiber quality under water deficit in all phenological stages, except for the open boll stage, have found no significant effect of the water deficit on UHM.

The results obtained also confirm those of Pettigrew (2004), who stated that the occurrence of water stress soon after flowering and during the fiber elongation stage reduced UHM because of the direct connection with the physiological mechanisms of cell expansion. Similarly, Beltrão et al. (2008) have observed that the occurrence of water deficit in the fiber elongation period significantly decreased UHM.

Similarly to UHM, the lowest value of UNF (Figure 4A) was found in the P6 water deficit, which differs from the other deficit treatments, and these, do not differ among each other. The cultivars also differed between each other, with emphasis on BRS 336 (86.56%), classified as very uniform fiber cotton, with a higher mean than BRS 286 (85.06%), classified as uniform fiber cotton (Figure 4B), according to the industrial classification (Santana et al., 2008).

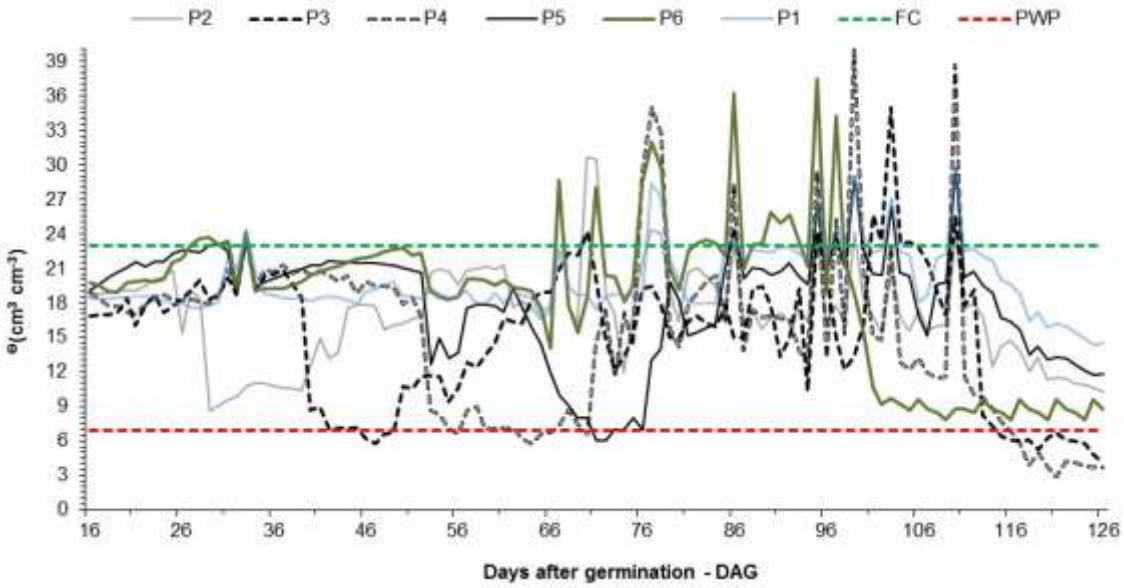


**Figure 2.** Variation of soil water content on the different water deficit treatments along experimental period.
Source: UFCG, Pombal County Campus, Paraíba State, Brazil (2015).

**Table 3.** Summary of the analysis of variance of fiber technological characteristics variables of two upland cotton cultivars under water deficit strategies in phenological stages (Pombal county, Paraíba State, Brazil, 2015).

| SV | DF | UHM | UNF | SFI | STR | ELG | MIC | MAT | Rd | +b | CSP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean squares | | | | | | | | | |
| Block | 3 | 0.32 | 1.96 | 0.13 | 1.27 | 0.0047 | 0.01 | 0.000017 | 0.87 | 0.07 | 36258.57 |
| Deficit periods (P) | 5 | 26.28** | 17.76** | 5.02** | 20.60** | 1.0103** | 0.58** | 0.000158$^{ns}$ | 2.51$^{ns}$ | 0.58$^{ns}$ | 1272342.47** |
| Error 1 | 15 | 2.20 | 0.98 | 0.29 | 3.15 | 0.2315 | 0.07 | 0.000055 | 0.78 | 0.42 | 72402.59 |
| Cultivar (C) | 1 | 128.38** | 27.00** | 8.41** | 127.72** | 17.2800** | 0.46** | 0.002408** | 7.36** | 17.16** | 2697534.18** |
| (P × C) | 5 | 0.33$^{ns}$ | 0.53$^{ns}$ | 0.13$^{ns}$ | 0.78$^{ns}$ | 0.1115$^{ns}$ | 0.06$^{ns}$ | 0.000033$^{ns}$ | 0.11$^{ns}$ | 0.30$^{ns}$ | 7875.73$^{ns}$ |
| Error 2 | 18 | 1.48 | 1.11 | 0.61 | 2.90 | 0.1756 | 0.04 | 0.000040 | 0.34 | 0.30 | 59672.81 |
| Total | 47 | | | | | | | | | | |
| General Mean | | 30.67 | 85.81 | 6.51 | 33.67 | 4.44 | 5.01 | 0.89 | 83.67 | 9.70 | 3039.89 |
| CV 1 (%) | | 4.85 | 1.16 | 8.38 | 5.27 | 10.82 | 5.38 | 0.83 | 1.06 | 6.72 | 8.85 |
| CV 2 (%) | | 3.97 | 1.23 | 12.06 | 5.06 | 9.43 | 4.20 | 0.71 | 0.70 | 5.72 | 8.04 |

$^{ns}$, $^{**}$, $^{*}$: not significant and significant at $p \leq 0.01$ and $p \leq 0.05$, respectively (F-Test). MS = Mean squares; CV = coefficient of variation.

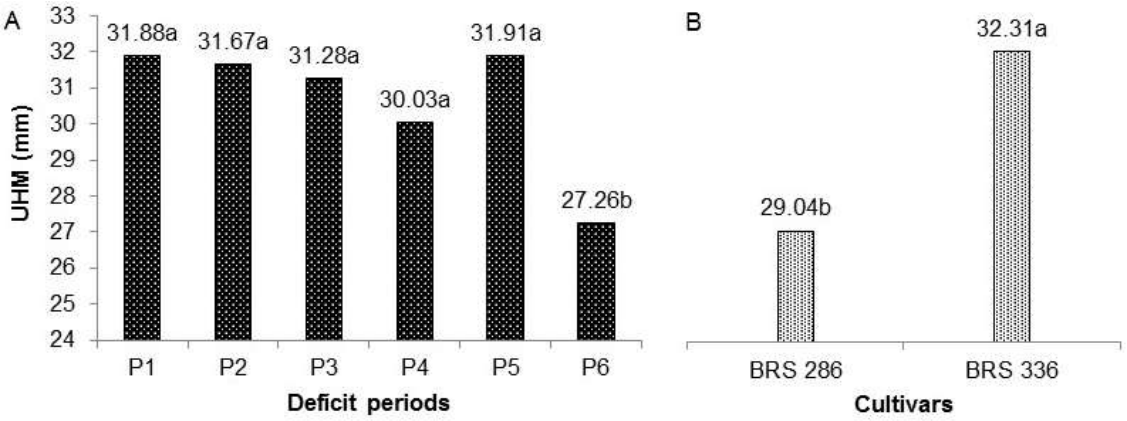


**Figure 3.** Means of fiber length of two upland cotton cultivars under water deficit strategies in phenological stages (A. Deficit periods; B. Cultivars). Same letters in the factors (A and B) indicate no significant difference among means (Tukey, p<0.05).
Source: UFCG, Pombal County Campus, Paraíba State, Brazil (2015).

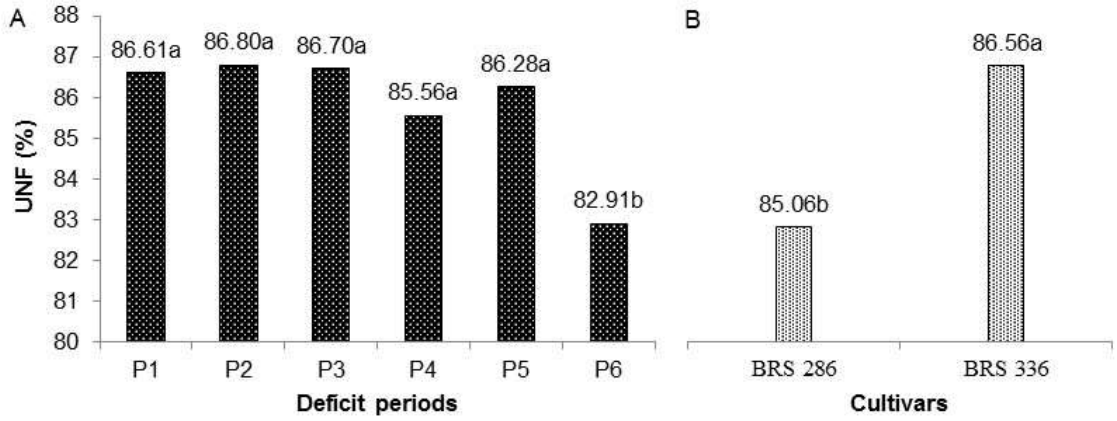


**Figure 4.** Means of fiber uniformity of two upland cotton cultivars under water deficit strategies in phenological stages (A. Deficit periods; B. Cultivars). Same letters in the factors (A and B) indicate no significant difference among means (Tukey, p<0.05).
Source: UFCG, Pombal County Campus, Paraíba State, Brazil (2015).

Both cultivars presented mean values within the cultivar standard, from 83.5 to 85.5% for BRS 286 and from 82.6 to 86.3% for BRS 336 (Figure 4B), according to Silva Filho et al. (2008) and Morello et al. (2011), respectively.

The other water deficit treatments, both for UHM and UNF, probably recovered, in this research, from the irrigation return at the end of their deficit periods. The same did not occurred in P6, when, after the deficit period, irrigation was definitively suspended as it was the end of the cotton cycle. Thus, only P6 was affected, which reduced the cotton UHM and UNF produced on it.

Wen et al. (2013) stated same results when stating that environmental conditions such as water deficit may decrease the elongation rate or shorten the elongation period of the fibers, thus decreasing their UHM and UNF.

Considering the variable of SFI, the highest index (worst index) was obtained when the water deficit was applied in the open boll stage (P6), which is significantly higher to those obtained in the other treatments, and these, do not differ among each other (Figure 5A). Bradow and Davidonis (2000) that, although UHM is a primarily genetic trait, the SFI depends, in addition to genotype, on the cultivation conditions, among them water availability; the result in this work was similar to these authors only for cultivar factor.

SFI was also affected by the cultivars, especially BRS 336 (shorter, but better index) with mean of 6.09%, lower than the 6.92% value of BRS 286 (Figure 5B); however both were classified as short fiber cotton, according to the industrial classification (Santana et al., 2008). Only

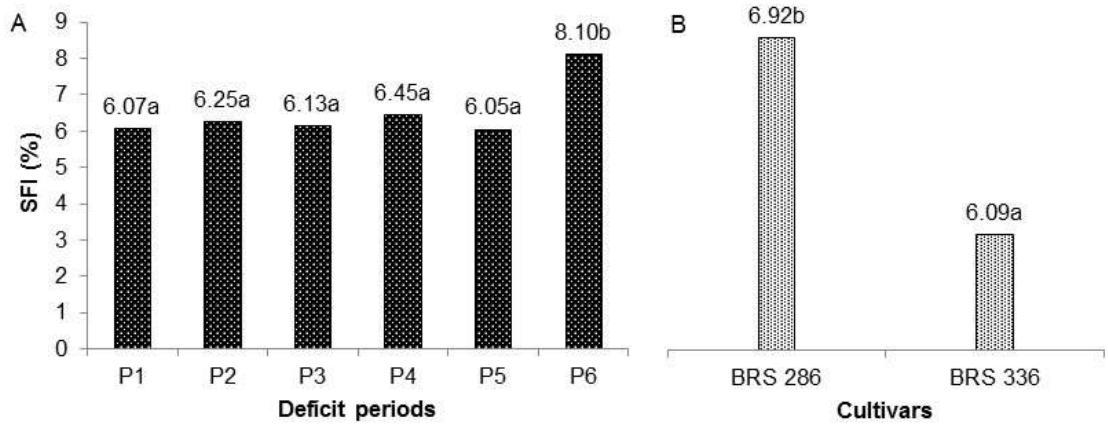


**Figure 5.** Means of short fiber index of two upland cotton cultivars under water deficit strategies in phenological stages (A. Deficit periods; B. Cultivars). Same letters in the factors (A and B) indicate no significant difference among means (Tukey, p<0.05).
Source: UFCG, Pombal County Campus, Paraíba State, Brazil (2015).

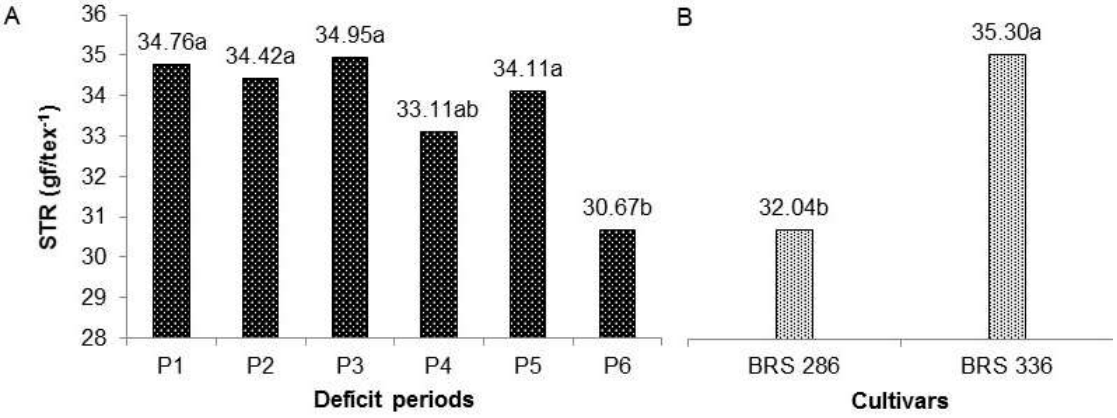


**Figure 6.** Means of fiber resistance of two upland cotton cultivars under water deficit strategies in phenological stages (A. Deficit periods; B. Cultivars). Same letters in the factors (A and B) indicate no significant difference among means (Tukey, p<0.05).
Source: UFCG, Pombal County Campus, Paraíba State, Brazil (2015).

cultivar BRS 336 showed a mean value within the variety standard, which is 4.6 to 7.3%, according to Morello et al. (2011) (Figure 5B).

The results found in this study were satisfactory because, according to Cordão Sobrinho et al. (2015), the lower the SFI, the better the performance in the yarn manufacturing process and the greater the market interest in the product.

For STR, the lowest value was found in the open boll stage (P6) as well, except for the value of the treatment applied in the flower stage (P4) that did not differ from either P6 or the other treatments (Figure 6A).

Mean STR for BRS 286 was 32.04 and for BRS 336 was 35.30 gf $tex^{-1}$ (which is more resistant than BRS 286) (Figure 6B), are classified as strong and very strong resistance, respectively, according to the industrial classification (Santana et al., 2008).

Both cultivars presented mean values above the cultivar standard, which is from 27.8 to 31.5 for cultivar BRS 286 and from 31.0 to 34.2% gf $tex^{-1}$ for BRS 336, according to Silva Filho et al. (2008) and Morello et al. (2011), respectively.

The cultivars studied in this study presented mean STR that fit the characteristics desirable by the industry. According to Cordão Sobrinho et al. (2015) and Zhao et al. (2012), the higher the STR, the greater its commercial value, quality gain and yield in the textile market.

BRS 336 had greater STR than BRS 286, which is interesting, as according to Zhao et al. (2012), UHM and STR are significant determinants of fiber quality. Bradow and Davidonis (2000) have concluded that STR has a negative correlation with yield.

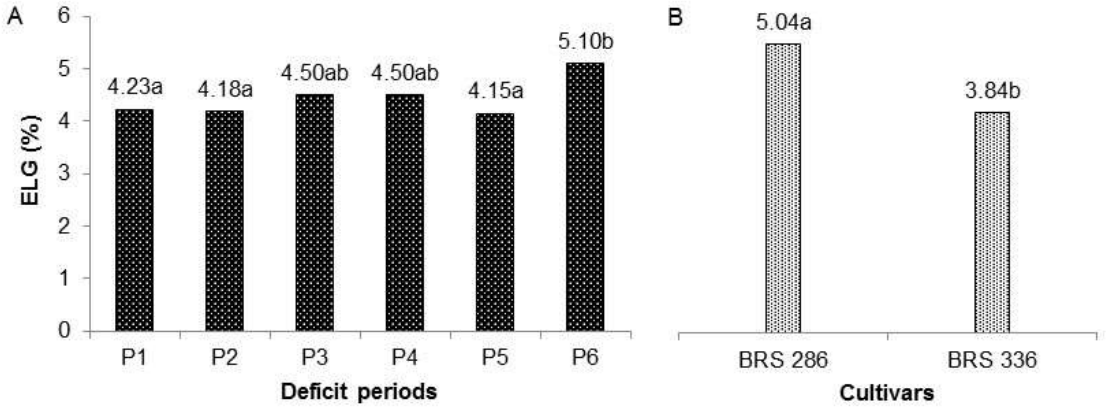


**Figure 7.** Means of fiber Elongation of two upland cotton cultivars under water deficit strategies in phenological stages (A. Deficit periods; B. Cultivars). Same letters in the factors (A and B) indicate no significant difference among means (Tukey, p<0.05).
Source: UFCG, Pombal County Campus, Paraíba State, Brazil (2015).

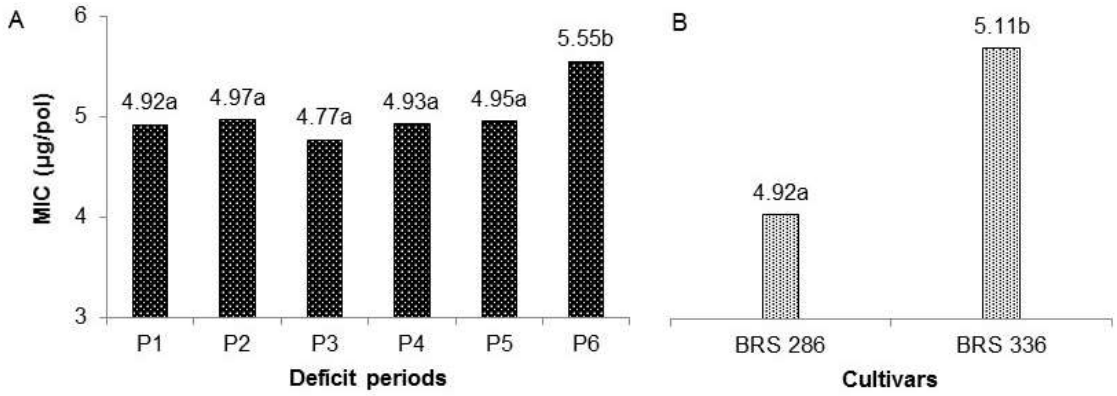


**Figure 8.** Means of fiber micronaire index of two upland cotton cultivars under water deficit strategies in phenological stages (A. Deficit periods; B. Cultivars). Same letters in the factors (A and B) indicate no significant difference among means (Tukey, p<0.05).
Source: UFCG, Pombal County Campus, Paraíba State, Brazil (2015).

As for ELG, the best values were found in the treatments without deficit (P1) and with water deficits applied in the initial growth (P2) and boll (P5) stages, which did not differ from the other treatments, except for P6, or between each other. Water deficits applied in these two stages (P2 and P5) decreased ELG (Figure 7A), as, according to Cordão Sobrinho et al. (2015), the lower the ELG, the greater the resistance of the yarn.

On the other hand, the water deficit applied in the stages of flower bud (P3), flower (P4) and open boll (P6) negatively affected the ELG. According to Freire (2015), the process of fiber formation occurs from the fertilization of the flower, thus, water deficit at this stage can negatively affect fiber quality.

For the cultivars, the mean obtained for ELG was 5.04% for BRS 286 and 3.84% for BRS 336 (Figure 7B), which classify them as low and very low elongation, respectively, according to the industrial classification (Santana et al., 2008).

Both cultivars presented mean values below the cultivar standard, which is from 7.5 to 9.5% for BRS 286 and from 4.6 to 7.1% for BRS 336, according to Silva Filho et al. (2008) and Morello et al. (2011), respectively.

Regarding the MIC, the highest (and worst) can be observed in the water deficit applied in the open boll stage (P6), which is significantly higher than in the other deficit treatments, and these, did not differ among each other (Figure 8A). The studied cultivars behaved differently with water deficits in different phenological stages, with a mean value of 4.92 and 5.11 µg $pol^{-1}$ for BRS 286 and BRS 336, respectively (Figure 8B).

Both cultivars presented mean values above the

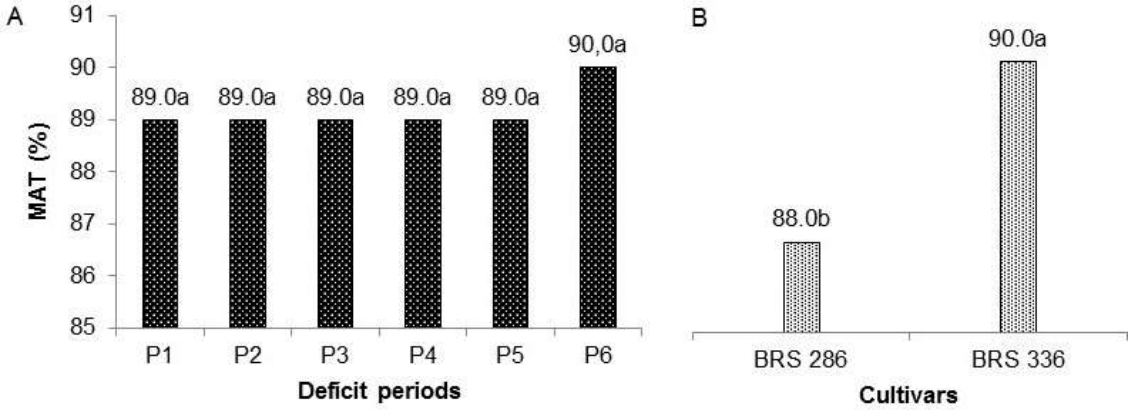


**Figure 9.** Means of fiber maturity of upland cotton cultivars under water deficit strategies in phenological stages (A. Deficit periods; B. Cultivars). Same letters in the factors (A and B) indicate no significant difference among means (Tukey, $p<0.05$).
Source: UFCG, Pombal County Campus, Paraíba State, Brazil (2015).

cultivar standard, which is from 3.9 to 4.5 for BRS 286 and from 4.0 to 4.9 µg pol$^{-1}$ for BRS 336, according to Silva Filho et al. (2008) and Morello et al. (2011), respectively.

Regarding the industrial classification, the fibers of BRS 286 and BRS 336 are classified as medium and thick for the MIC, respectively, according to Santana et al. (2008) and Kljun et al. (2014).

According to Ge (2007), cotton fibers with MIC greater than 5.0 µg pol$^{-1}$ are very thick fibers and subject to more irregularities and imperfections because of fiber reduction in the cross section of the yarn. Fibers with micronaire up to 3.80 µg pol$^{-1}$ are classified as thin and are recommended for the manufacture of fine yarns, with higher commercial value in the textile industry. Lower micronaire values (<3.5) suggest that the fiber is immature and may cause neps and, consequently, low dye affinity (Kljun et al., 2014).

According to Cordão Sobrinho et al. (2015), it is important to keep a constant micronaire value, since a flow of thick fibers can cause loss of yarn resistance and decreased efficiency in the process, while thin fibers increase the number of neps in carding and ruptures in the process, besides negatively affecting the dyeing. Zhao et al. (2013) claim that fiber micronaire and maturity are important commercial quality parameters that guide management in fiber production.

According to Zonta et al. (2017), the results found in this study have a high micronaire index and may be associated with the collection of the fiber samples, in which mainly first position open bolls are collected.

Several authors such as Cordão Sobrinho et al. (2015) and Zonta et al. (2015a) have reported micronaire values above 5.0 µg pol$^{-1}$ in experiments with irrigated cotton, considered thick and above the market tolerance. Similarly, Belot and Dutra (2015), presented higher values for this characteristic, which micronaire were close to or above 5.0 µg pol$^{-1}$.

The treatment periods of water deficit did not affect the MAT, with a mean value of 89% (Figure 9A). BRS 336 had a mean value of 90%, which is significantly higher than the BRS 286 value, which was 88% (Figure 9B), and they are classified as high and very high maturity, according to Santana et al. (2008). The same authors stated that the mean values found in the treatment periods of water deficits are classified as very mature fiber cotton.

MAT is a very important characteristic for the textile industry, since its variability has a negative impact on the final product, mainly in the dyeing, as immature fibers have lower absorptive capacity, making the fabric uneven (Kelly et al., 2015; Kim, 2015).

In relation to Rd and +b, which are related to the color of the fiber, both had the same behavior as MAT and were not affected by the water deficit periods, with mean value of 83.66% (Rd) (Figure 10A) and 9.70 (+b) (Figure 11A).

BRS 336 had a reflectance of 84.06%, which is significantly higher than that for BRS 286 (83.27%) (Figure 10B). Both cultivars presented mean values above the cultivar standard, which is from 75 to 80% for cultivar BRS 286 and from 68.4 to 82.8% for BRS 336, according to Silva Filho et al. (2008) and Morello et al. (2011), respectively. Regarding industrial classification, Rd was classified as white cotton fiber for both cultivars (Santana et al. 2008).

These results, according to Cordão Sobrinho et al. (2015), are satisfactory, since the higher the Rd, the lower its graying and, consequently, the greater the interest of the cotton and textile industry chain as it adds a higher value to the product. Santana et al. (2001) have found similar results in a test assessing intrinsic

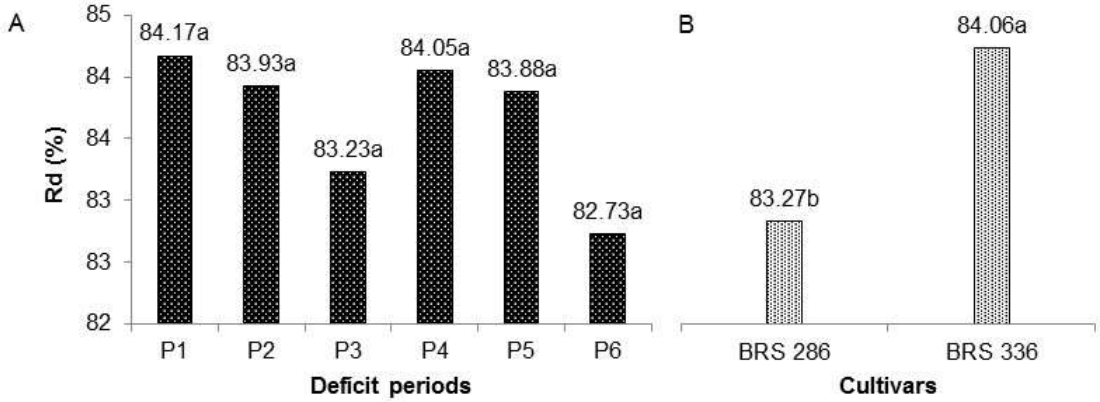


**Figure 10**. Means of fiber reflectance of two upland cotton cultivars under water deficit strategies in phenological stages (A. Deficit periods; B. Cultivars). Same letters in the factors (A and B) indicate no significant difference among means (Tukey, p<0.05).
Source: UFCG, Pombal County Campus, Paraíba State, Brazil (2015).

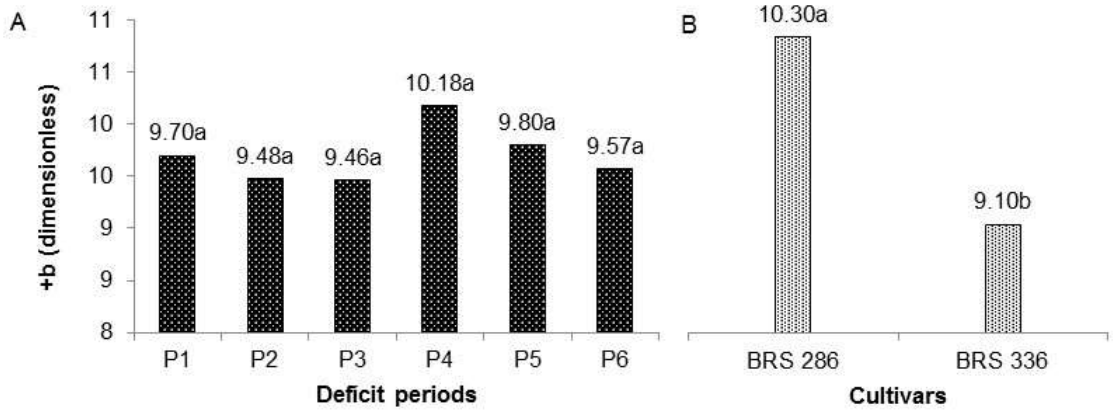


**Figure 11.** Means of fiber yellowness of two upland cotton cultivars under water deficit strategies in phenological stages (A. Deficit periods; B. Cultivars). Same letters in the factors (A and B) indicate no significant difference among means (Tukey, p<0.05).
Source: UFCG, Pombal County Campus, Paraíba State, Brazil (2015).

characteristics of cotton fiber in Northeast Brazil.

Regarding +b, both cultivars presented mean values above the variety standard, which is from 7.0 to 9.0 for BRS 286 and from 4.9 to 8.6 for BRS 336, according to Silva Filho et al. (2008) and Morello et al. (2011), respectively. Yellowness also differed among cultivars, in which BRS 286 had the highest yellowness (10.60) and BRS 336 the lowest yellowness (9.23) (Figure 11B), both classified as white (Santana et al., 2008).

According to Bradow and Davidonis (2000), fiber color is directly linked to environmental factors during the growing season, which in this work was the water stress in different phenological stages. Zonta et al. (2015b) state that factors such as the application of defoliants and desiccants, pest attack, among others, can also influence fiber color.

According to Cordão Sobrinho et al. (2015), knowledge about fiber color is important, as it cannot always be seen with the naked eye and only in ultraviolet light and, if yellowness in the mixture is not controlled, problems such as differences in shades after dyeing can happen in the yarn and fabric.

Regarding CSP, it was lower when the water deficit was applied in the open boll stage (P6), which differs significantly from the other deficit treatments, and these, did not differ among each other (Figure 12A).

The mean value obtained for CSP by BRS 286 was 2802.83 and for BRS 336 was 3276.95 (best index), both classified as very high (Santana et al., 2008). Regarding cultivar standard, the values found in this study (Figure 12B) were higher than those found by Silva Filho et al. (2008) and Morello et al. (2011), respectively.

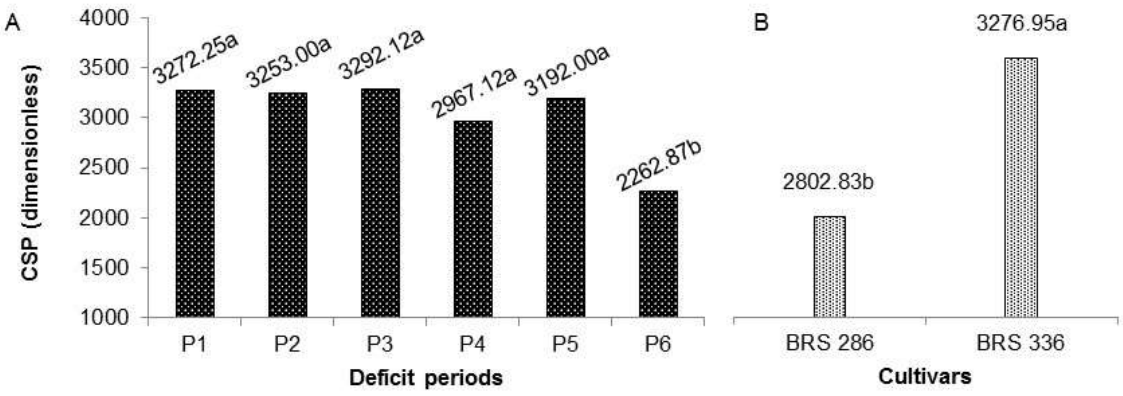


**Figure 12.** Means of fiber Count Strength Product of upland cotton cultivars under water deficit strategies in phenological stages (A. Deficit periods; B. Cultivars). Same letters in the factors (A and B) indicate no significant difference among means (Tukey, $p<0.05$).
Source: UFCG, Pombal County Campus, Paraíba State, Brazil (2015).

These results are satisfactory as, according to Cordão Sobrinho et al. (2015), the values found for CSP (greater than the variety mean) reflect the characteristic of yarn resistance that depends especially on individual fibers.

In summary, treatment without deficit (P1, irrigated throughout the cycle) promoted the best values in all analyzed variables, which indicates that treatments with deficit periods applied in different phenological stages of upland cotton cultivars BRS 286 and BRS 336 made possible fiber standards currently demanded by the market and cotton industry, except when the water deficit was applied in the flower bud (P3), flower (P4) and open boll (P6) stages, which negatively affected the cotton fiber quality.

BRS 336 presented the best results for almost all fiber characteristics evaluated, except micronaire. On the other hand, despite presenting lower values, BRS 286 presented values within the cultivar standard and thus it also meets most technological characteristics required for fibers by the modern textile industry. Finally, the cultivars tested have fiber quality in accordance with the cultivar and commercial standards of medium (BRS 286) and long (BRS 336) fibers.

The results obtained in this work demonstrate that the two cultivars evaluated have potential for irrigated cultivation in the semiarid region, provided that the correct management of irrigation and other cultural treatments is carried out. The differences found between the cultivars were expected as, according to Bradow and Davidonis (2000), there are always differences in fiber quality characteristics among different genotypes.

Santana et al. (2008) stated that the most important fiber characteristics in the current and modern textile processes are micronaire and resistance. It is important to note that even for micronaire, it was within an acceptable quality range by the brazilian textile industry (Zonta et al., 2015b).

Authors such as Bradow and Davidonis (2000), Bauer et al. (2009) and Feng et al. (2011) mentioned in their work that variation in fiber components can occur within a single plant and, when working with standard samples, as in this research, in which the middle third of the plants are collected, the result of these fiber analysis can be masked, not representing the actual condition of the plot or block, especially when working with experiments in which abiotic stresses, such as water stress, are applied.

The same authors also mentioned that the environmental variation that occurs within the plant canopy, between plants, or between plots, causes the fiber characteristics to present great variability as to open boll, plant and plot. In this way, the more uniform and representative is the sampling about the conditions of the plant and plot as a whole, the more representative the results of the fiber analysis (Zonta et al., 2017).

In order not to underestimate or overestimate the results, Zonta et al. (2017) indicate, for the determination of fiber quality in water stress tests, the collection of samples representing all open boll positions of the plant, in order to avoid erroneous estimates of the results. The same authors stated that when water stress is applied at different stages of the phenological crop cycle, this stress will affect the open bolls at different stages of growth and maturation, influencing them differently, which highlights a fact that may have happened in this study.

For cotton, several authors such as Wen et al. (2013), Brito et al. (2011), De Tar (2008) and Pettigrew (2004) have shown that fiber yield, percentage and quality in the crop are influenced when subjected to water deficit irrigation. Santana et al. (2008) and Zonta et al. (2017) stated that, although they are conditioned by hereditary factors, the technological characteristics of the cotton fiber undergo decisive influence of environmental factors (temperature, luminosity, water availability) and depend on the conditions of cultivation.

## Conclusion

The water deficits applied in the different phenological stages of the upland cotton cultivars affected fiber quality, except maturity, reflectance and yellowness; treatment without water deficit promoted the best fiber values, except of short fiber index, elongation and micronaire; tested upland cotton cultivars was more tolerant to water deficit in the initial growth and boll stages; in general, BRS 336 was more tolerant to water deficits than BRS 286.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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