



PHOTOSYNTHETIC GAS EXCHANGE AND CHLOROPHYLL IN BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA* L. VERDC.) SUBJECTED TO WATER DEFICIT

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ABSTRACT

The study evaluated the response of two Bambara groundnut (*Vigna subterranea* L. Verdc.) landraces sourced from contrasting environments to periods of water deficit initiated at vegetative stage. The two landraces AHM-753 and Bogor, from Namibia and Indonesia, respectively, were grown in pots and irrigation was withheld 30 days after sowing in stressed group while the control plants were fully watered. The responses were studied by measurements of relative water content (RWC), photosynthetic gas exchange, and SPAD chlorophyll. RWC and gas exchange parameters were found to decrease in stressed plants. Significant ($P < 0.001$) decrease in SPAD chlorophyll concentration was seen only in Bogor landrace. The results showed a possible drought avoidance strategy in Bambara groundnut in response to water deficit. The results pointed a greater tolerance to the imposed drought in the landrace AHM-753 compared to Bogor suggesting that the origin of the landrace and the environment it is adapted to determine its response to water deficit. **Keywords: Bambara groundnut; Chlorophyll; Gas exchange; Photosynthesis, Water deficit**

INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is an important legume crop commonly grown for its seeds by subsistence farmers in many parts of Africa (Linnemann and Azam-Ali, 1993; Basu *et al.*, 2007). It has great economic potentials and towards food security considering its superb nutritional composition and other properties. Bambara groundnut has been referred to as a drought tolerant plant (Berchie *et al.*, 2012; Collinson *et al.*, 1999) and was reported to produce relatively greater seed yield than other legumes under the same conditions (Jorgensen *et al.*, 2010). Mwale *et al.* (2007) observed a reduction in yield among Bambara groundnut landraces after drought imposition, even though the results pointed at the resilience potential of the species to drought. Jorgensen *et al.* (2010) observed differences in the response of two Bambara groundnut landraces and attributed that to the climatic conditions in the area of collection.

Plant growth and productivity are unfavourably affected by nature by means of various biotic and abiotic stress factors. Drought is the main abiotic stress factor limiting crop production, leading to decreases in crop yields after exposure to constant or intermittent periods of drought (Chaves *et al.*, 2003). Water is vital for plant growth and makes up 75 to 95 percent of plant tissues. Plants use water and CO₂ to form sugars and more complex carbohydrates. Water also acts as a carrier of nutrients and a cooling agent and as an intercellular reaction medium and provides an element of support through turgor (Rehman *et al.*, 2005). Water limitation is already one of the major threats in crop production and it is expected to get significantly worse in coming decades (Cominelli *et al.*, 2009). Plants act in response

and acclimatize to drought stress at the cellular, molecular, physiological and biochemical levels (Urano *et al.*, 2010). Limited evidence exists on the degree and mechanism of resistance of Bambara groundnut to drought. In order to improve the yield and to fully exploit the great potentials of Bambara groundnut, a more thorough understanding of the mechanisms of its response to drought need to be achieved. The aim of this

MATERIALS AND METHODS

Two landraces of Bambara groundnut Bogor (from Indonesia) and AHM-753 (Africa) were used. The plants were grown under natural environmental conditions in a shade house, the environmental data were monitored and logged every 30 minutes throughout the study period by a mini weather station (Watchdog 2000 Series, Spectrum Technologies, Plainfield, IL, USA). Photosynthetically active radiation (PAR) ranged between 100-790 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the day, temperature was between 23-36/23-25 °C day/night, while humidity ranged from 52-99%. The plants were grouped into three with four replicates per group. The first group (Control Group) was irrigated to field capacity once every two days. The second and third groups were subjected to water deficit at 30 days after planting by totally withholding irrigation for a period of two weeks (stressed group T2) and four weeks (stressed group T3). At the end of the respective stress periods, irrigation was resumed for two weeks to assess recovery. Leaf water status was estimated by measuring the relative water content (RWC) according to the method described by Barrs and Weatherly (1986).

Gas exchange measurements (photosynthesis, stomatal conductance and transpiration) were done weekly at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, reference CO_2 concentration of 390 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and leaf temperature of 29 °C using Li-6400XT Photosynthesis System (Li-Cor Biosciences, Lincoln, NE, USA). Chlorophyll was measured using a SPAD 502 Plus Chlorophyll Meter (Konica Minolta, Osaka, Japan). Statistical analyses were done using Genstat software version 10.3 (VSN International Ltd). Analysis of variance (ANOVA) was carried to compare the means of values between different groups.

RESULTS

The results of relative water content (RWC) are shown in Table 1. RWC decreased significantly

($P < 0.001$) with time after water deficit treatment in both AHM-753 and Bogor landraces. The decrease in RWC is more pronounced in Bogor compared to AHM-753. The least RWC, 56.4%, was observed in Bogor after four weeks of water deficit. It is worth noting that even among the control groups; there was difference in RWC between the two landraces, being higher in Bogor. By analysis of covariance, it was seen that the effect of drought stress treatment between two and four weeks stress was significant in both cases ($P < 0.001$). The effect of genotype as well as that of the interaction between genotype and stress treatment were also significant ($P < 0.05$). The difference between the two landraces after four weeks of water stress is much pronounced, being lower in Bogor.

Table 1: Relative water content, RWC (%) in well watered (Control), two weeks (T2) and four weeks (T3) drought stressed Bambara groundnut landraces before stress (BS), 2 weeks stress, 4 weeks stress and after resumption of irrigation (Recov).

RWC (%)	BS	2	4	Recov.
AHM751 (Control)	92.93 ± 0.5	91.72 ± 1.5 ^a	92.19 ± 1.8 ^a	91.49 ± 2.0 ^a
AHM751 (T2)	91.94 ± 1.5	87.6 ± 3.5 ^b	*90.89 ± 1.6 ^a	□ Not sampled
AHM751 (T3)	92.99 ± 1.3	□ Not sampled	79.00 ± 5.5 ^b	90.21 ± 1.8 ^a
BOGOR (Control)	93.30 ± 1.7	93.53 ± 2.2 ^a	93.92 ± 0.3 ^a	94.04 ± 1.0 ^a
BOGOR (T2)	93.61 ± 1.2	85.32 ± 4.3 ^b	*88.78 ± 1.6 ^b	□ Not sampled
BOGOR (T3)	93.34 ± 1.3	□ Not sampled	56.40 ± 5.4 ^c	76.49 ± 2.5 ^b

* Values after resumption of irrigation (recovery) for group T2.

□ Not sampled: RWC was not determined in the group at this period in time.

Results are mean ± standard deviation. Values within the same column bearing dissimilar superscripts are significant at $P < 0.001$.

The results of chlorophyll levels as estimated by SPAD chlorophyll meter readings (SCMR) are shown in Figure 1. SCMR values were generally higher in Bogor than in AHM-753 landrace. As can be deduced from the figure, there were no appreciable changes in SCMR up to two weeks of stress. But by four week of stress, the SCMR values

obtained in both landraces were lower compared to the respective control plants. SCMR was significantly ($P < 0.001$) lower in Bogor after four weeks of stress and even during recovery. Decrease in SCMR values in AHM-753 were insignificant ($P > 0.005$) and improved during recovery. The SCMR values in Bogor remained low up to two weeks after stress treatment.

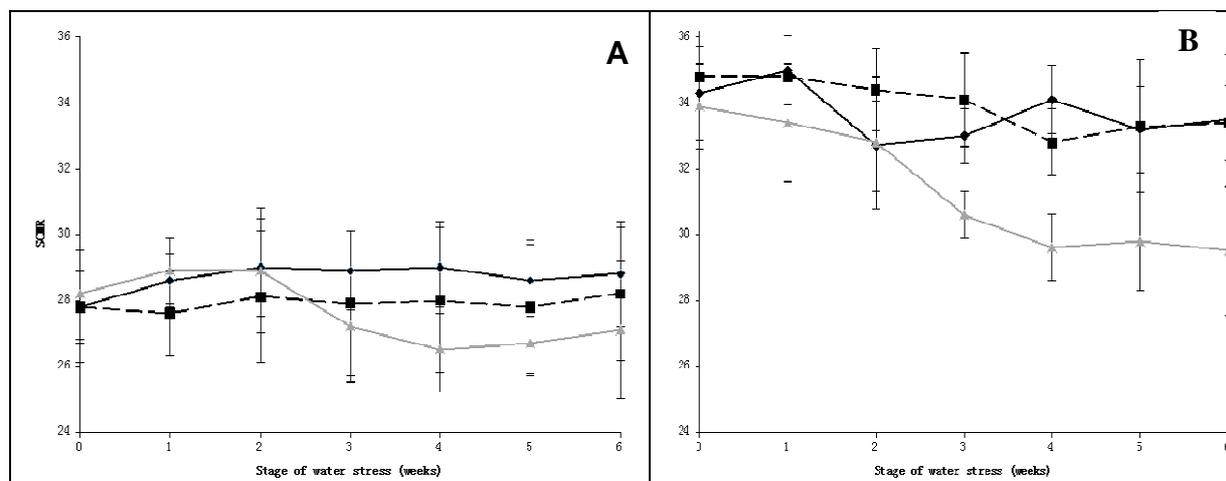


Figure 1: SPAD chlorophyll meter readings (SCMR) in two Bambara groundnut landraces after water deficit treatment and recovery (from week 3 for T2, week 4-6 for T3); (A) AHM-753 landrace and (B) Bogor landrace. Well watered (□), T2 – two weeks stress (□), T3 – four weeks stress (□). Error bars represent standard deviation, n = 4.

Water stress caused in both landraces progressive decreased transpiration, stomatal conductance and photosynthesis (Figure 2). Transpiration (E) was generally higher in Bogor than in AHM-753 landrace. In both landraces, the effects were more pronounced after four weeks of stress. E was significantly ($P < 0.001$) higher in control plants compared to stressed after two weeks of stress. Plants exposed to only two weeks of stress fully recovered in E one week after irrigation was resumed in both landraces. In contrast, E was still low in plants stressed for four weeks even during recovery.

Similarly, stomatal conductance (g_s) decrease was more pronounced in Bogor and it was significantly lower in stressed plants by three weeks of stress. Moreover, there was a decrease with time in g_s even among the control seedlings. Photosynthetic assimilation rate (P_n) has a relatively similar pattern of decrease in both landraces and was significantly lower ($P < 0.05$) in stressed compared to well watered control plants. P_n increased insignificantly during recovery in plants stressed for four weeks in both landraces.

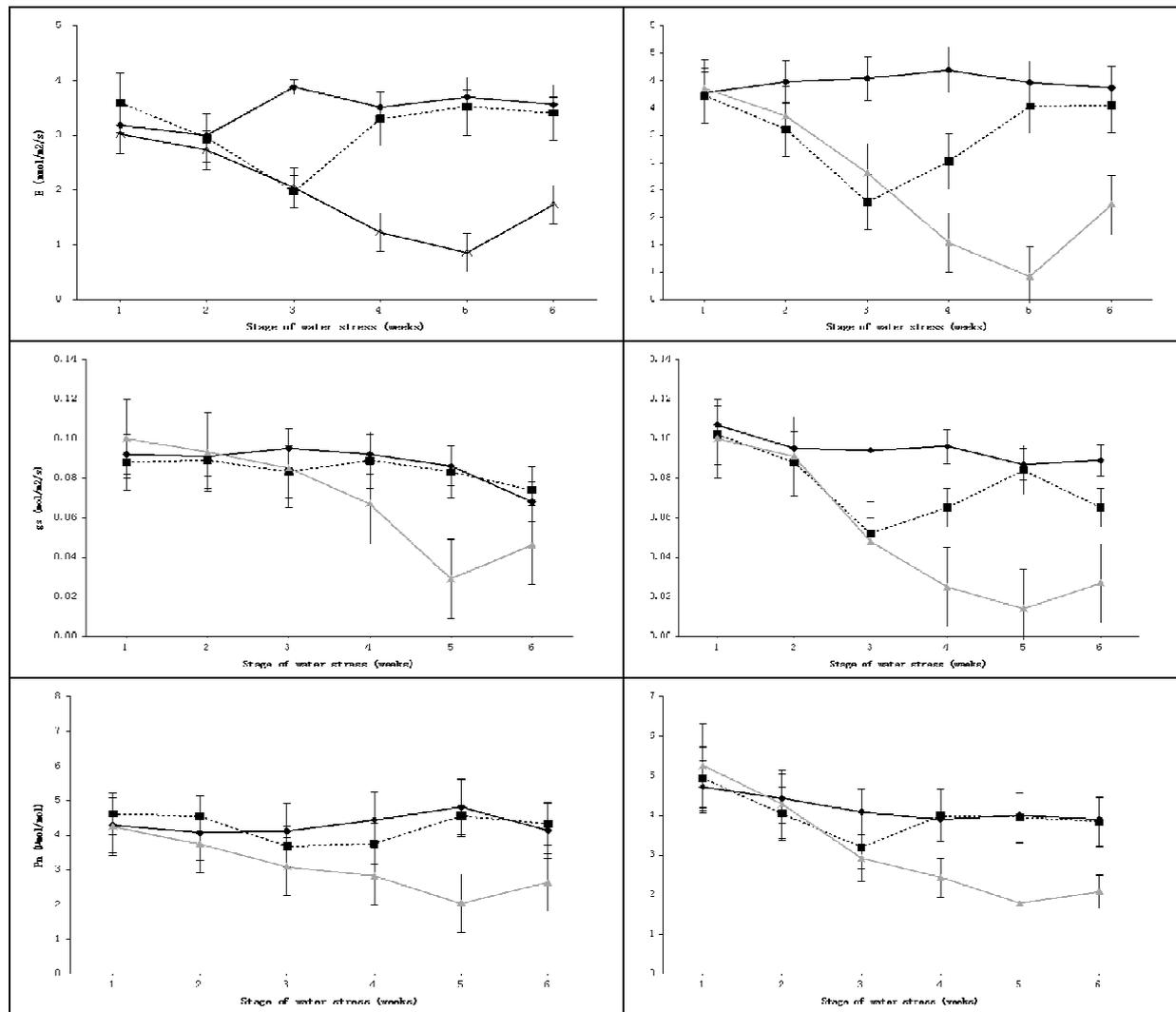


Figure 2: Changes in transpiration (E), stomatal conductance (g_s) and photosynthetic CO_2 assimilation rates (P_n) in two Bambara groundnut landraces after water deficit treatment and recovery (from week 3 for T2, week 4-6 for T3); (A) AHM-753 landrace and (B) Bogor landrace. Well watered (\square), T2 – two weeks stress (\circ), T3 – four weeks stress (\triangle). Error bars represent standard deviation, $n = 4$.

DISCUSSION

RWC is an indicator of plant water status and has a value in assessing plant's tolerance to drought (Kimani *et al.*, 1994; Li *et al.*, 2011) and its maintenance in a period of leaf water reduction is a key factor of yield stability in plants (Teulat *et al.*, 1997). RWC can be used as a reasonable approximation of cell volume (Wilson *et al.*, 1980) and plant metabolism is generally affected by leaf water status, which RWC is a measure of. Bogor

landrace showed more decline in RWC compared to AHM-753. Similarly, comparatively higher transpiration rates and stomatal conductance (g_s) were observed in Bogor. Differences between the two landraces in terms of transpiration rates might be correlated with the differences in leaf area between them. This might have contributed to higher water loss through the stomata leading to lower RWC (Hadley and Smith, 1990).

Decreased RWC leads to stomatal closure (Gindaba *et al.*, 2004) leading to decreased CO₂ assimilation (Li *et al.*, 2011). The decrease in RWC observed in this work is possibly the cause of the observed decrease in g_s in the plants. The observed decrease in transpiration after stress might primarily be a result of stomatal closure as indicated by decreased g_s . Stomatal opening is the primary determinant of transpirational water loss (Song and Assmann, 2010). Even though there was decrease in RWC in both landraces after two weeks of water deficit treatment, the values obtained still indicated an ability of the Bambara groundnut plant to maintain relatively high leaf water status under such conditions.

Several researchers have indicated that stomata respond to ambient humidity, (Aphalo and Jervis, 1991; Sanchez-Blanco *et al.*, 2004) even though it is difficult to separate this response with that caused by temperature (Aphalo and Jervis, 1991) and other factors (Grantz, 1990). Sanchez-Blanco *et al.* (2004) have also shown that the reduction in g_s as a result of low air humidity was very variable. The plants in this work were grown in an environment with high ambient humidity levels (52-99%). This could have affected the stomatal behaviour and might have lessened the severity of the intended water deficit imposition.

A linear relationship was shown to exist between SPAD values and extracted chlorophyll (Campbell *et al.*, 1990; Sibley *et al.*, 1996; Loh *et al.*, 2000). Some studies have indicated that the linearity of the relationship may differ with plant genotype (Sibley *et al.*, 1996) and stage and conditions of growth (Campbell *et al.*, 1990). The accuracy of the SPAD meter values have been reported to decrease at high and low concentrations of chlorophyll (Hawkins *et al.*, 2009). Some works have also shown that the relationship between extracted chlorophyll concentration and SCMR may be nonlinear (Hawkins *et al.*, 2009). The observed decreased chlorophyll as suggested by SCMR might have contributed to the decline in photosynthetic capacity especially at later stages of water deficit.

The decrease observed in SCMR in both Bogor and AHM-753 landraces may be due to decreases in chlorophyll especially in newly expanded leaves as a result of general decrease in metabolic processes due to the decreased tissue water content. Similarly, water deficit usually causes an increase in the activity of chlorophyllase, an enzyme responsible for the breakdown of chlorophyll, resulting in a decrease in the amount of chlorophyll (Nunes *et al.*, 2008). Drought has been known to affect chlorophyll content in many crops thereby reducing photosynthetic capacity (Arunyanark *et al.*, 2008). The ability to maintain chlorophyll concentration under water deficit was suggested as part of the drought resistance mechanism in many crop plants (van der Mescht *et al.*, 1999). Arunyanark *et al.* (2008) have

shown that SCMR can be used as an index for selection of peanut genotypes for drought resistance.

The rate of transpiration in the Bambara groundnut landraces used in this study decreased with severity of water stress, corresponding to the decreased stomatal conductance indicating stomatal closure to preserve tissue water. Stomatal limitation was generally accepted to be the main determinant of reduced photosynthesis in drought (Chaves and Oliveira, 2004; Grassi and Magnani, 2005). This is credited to a reduction in internal CO₂ (C_i) and assimilation which ultimately hinders photosynthesis related metabolic processes. Others reports have argued that the reduced photosynthesis is more due to metabolic impairments (Tezara *et al.*, 1999).

The decrease in photosynthetic assimilation rate observed corresponds with the decrease in stomatal conductance which might be due to decreased tissue water as measured by RWC. Photosynthesis is closely related to dry matter production in most crops, even though it has been argued that instantaneous measurements of photosynthesis is ambiguous and that crop yield is more linked to the net photosynthetic assimilation of CO₂ over the whole season (Zelitch, 1982).

AHM-753 landrace maintained higher leaf water and had also higher photosynthesis after the water deficit treatment in comparison to Bogor and can thus be considered to have more drought tolerance potential than Bogor. This may not be surprising considering the fact that AHM-753 is an African landrace adapted to an environment with low annual mean precipitation (mean annual rainfall in Namibia is 370mm (www.climateinfo.info/namibia)). On the other hand, Bogor an Indonesian landrace is adapted to a much wetter environment (mean annual rainfall 1755mm (www.climateinfo.info/indonesia)).

Changes in the rates of gas exchange parameters are among the indicators of responses and adaptation to stress. Decrease in transpiration and stomatal conductance points to the possibility of a drought avoidance strategy in Bambara groundnut having the ability to maintain relatively high tissue water content under soil water deficit. The results of this work also pointed at the ability of Bambara groundnut to recover well from the effects of water deficit when conditions return to normal. This report has pointed some important physiological responses to water deficit in the seedlings of selected Bambara groundnut landraces.

CONCLUSION

Results of this study have shown a decrease in relative water content, photosynthetic and transpiration rates, stomatal conductance and SPAD chlorophyll in Bambara groundnut in response to imposed water deficit. Differences were observed in the response between the landrace AHM-753 and Bogor indicating that the source of a landrace, i.e. the environment it is adapted to, determines its response to water deficit.

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