Effect of Water Stress Imposed at Different Growth and Development Stages on Morphological Traits and Yield of Bambara Groundnuts (*Vigna subterranea* L. Verde)

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ABSTRACT

Two greenhouse trials were carried out to evaluate the response pattern of morphological traits of bambara groundnut to short periods of water stress imposed at different developmental stages and also their recuperative ability after rewatering. The treatments consisted of watering plants to 100% Plant Available Water (PAW), withholding water to 30% PAW at vegetative, flowering and pod filling growth stages and rewatering the plants after 21 days of each stress treatment. Water stress reduced the relative leaf expansion rate, leaf number, plant height and shoot: root ratio depending on the stage of development when water stress occurred. When plants were rewatered after each stress treatment, the relative leaf expansion rate of plants stressed at pod filling and flowering stages failed to recover from water stress. Seed yield in all stressed plants was reduced by water stress due to reductions in pods per plant, seeds per pod and seed weight. The highest yield amongst the stressed plants was obtained in plants stressed during the vegetative stage, followed by the flowering and lastly the pod filling stage. Bambara groundnuts reduced growth therefore reducing transpirational area thus reducing water loss under water stress. The results also showed that bambara groundnuts have the ability to recover from water stress after rainfall or irrigation and is therefore capable of producing some yield under water limited conditions.

Key words: Bambara groundnuts, vegetative stage, flowering stage, pod filling stage, water stress

INTRODUCTION

Bambara groundnut is an indigenous African leguminous crop grown primarily for its seeds and has diverse uses. Reports in literature indicate that the mature seeds are a rich source of crude protein (17.5-21.1%), carbohydrate (53-60.8%) and crude fat (7.3-8.5%) (Ominowo et al., 1999). The protein in bambara groundnut has high lysine content (Adu-Dapaah and Sangwan, 2004) and so has a beneficial complementary effect when consumed together with cereals which have low lysine content (Massawe et al., 2005). Bambara groundnut is therefore an ideal food crop but however is still cultivated from local landraces selected over generations. Experimental results and growers experience have indicated that bambara groundnut is able to produce pod yields where many other crops may fail altogether (Collinson et al., 1996). Although, there is a growing awareness of the potential of bambara groundnut to contribute to increased food production in
Africa, a major problem associated with its production is the very low yields often obtained by farmers (Sesay et al., 1999; Hampson et al., 2000). This is because the semi-arid regions where it's usually grown are susceptible to pronounced variability not only in amount of rainfall but also in the distribution and intensity within and between seasons (Usman and Reason, 2004). Water deficit elicits several morphological responses in crop plants (Jones, 2004). Most of these responses are adaptive mechanisms to withstand water deficit or drought and to ensure both survival and reproduction under conditions of water deficit stress. There are three main aspects of plant morphological behaviour in relation to drought: the modulation of root growth (Jackson et al., 2000), the modulation of leaf size and changes in leaf orientation (Chaves et al., 2003). A fundamental problem with these adaptive responses is that most are aimed at reducing water use and consequently affect plant function and productivity through reduction in photosynthesis (Ribaut, 2006). There is hardly any report in literature on morphological responses of bambara groundnut to short periods of water stress imposed at different growth and development stages or on the recuperative ability of the species from drought stress. Information on the response pattern of morphological traits to drought imposed at different growth stages might provide a basis for development of strategies to stabilize yields of bambara groundnut in semi-arid environments. Therefore, the objective of this study was to evaluate the effect of water stress imposed at different developmental stages on morphological traits and yield of bambara groundnuts.

MATERIALS AND METHODS

Experimental site and research design: Two greenhouse trials were carried out between October 2009 and May 2010 at the University of Botswana. The experiment was arranged in a completely randomised design with four replications. In trial 1, which started October 2009, Control-plants were well watered throughout, the experimental period. Bambara groundnuts were stressed for 21 days (25 days after sowing) during the vegetative stage, flowering stage (46 days after sowing) and pod filling stage (80 days after sowing). In trial 2, which started in February 2010. Control-plants were still watered throughout the experimental period. Bambara groundnuts were stressed for 21 days (25 days after sowing), flowering stage (46 days after sowing) and pod filling stage (60 days after sowing).

Crop management: The landrace Uniswa red was used for the experiment. Four seeds were sown per pot at 4 cm depth and seedlings were thinned to one per pot at emergence. Black plastic pots, measuring 225 mm in diameter and 450 mm in height, were each filled with a 17 kg mixture of normal field soil and sand in 5:3 volume ratios. A basal fertiliser (NPK, 2:3:2) was incorporated into the soil at a rate equivalent to 265 kg ha⁻¹. Plants (pots) were spaced 30 cm apart on benches to preclude competition effects among plants. The greenhouse temperature was maintained at 25-28°C in trial 1, but in trial 2, temperature was not controlled.

Variables determined: Prior to the start of the study, the upper plant available water limit was determined by weighing soil from 5 pots two days after they were watered. The plant available water for each pot for any other day was calculated according to Rosenthal et al. (1987).

For each water stress treatment, watering was withheld until the pots reached a stress level of 30% Plant available water. It took the pots about 10 days to reach 30% of Plant available water from beginning of stressing and this stress level was maintained for 15 days. During the study period each pot was weighed daily at 09:00 h and water was added if necessary to maintain the
stress level. Except for the periods of stress, the watering for all treatments was the same as that for the control plants. All measurements taken on plants were done before, during and after each stress treatment. During drought recovery, measurements were taken only from leaves existing before rewatering. Relative Leaf Area Expansion Rate (RLER) was measured during each stress treatment and during recovery from each stress treatment. RLER was determined non-destructively by measuring the length and width of terminal leaflet of the third most recently unfolded leaf and this was done 3 days apart. The actual leaf area was determined using the landrace independent formula of Cornellissen (2005). RLER was calculated according to the formula of Ober and Luterbacher (2002).

**Leaf number:** The total number of leaves (three fully expanded leaflets) was determined by averaging the number for each of the 5 plants per treatment and was recorded twice weekly (every 3 and 4 days) from thinning until maturity.

**Plant height:** Plant height (cm) was determined using a meter ruler by averaging the distance from soil level to the top of each of the five plants.

**Shoot: root ratio:** At maturity both control and stressed plants were removed from the pots. Plants were separated into two parts, root and shoot and oven dried at 80°C for 72 h and weighed to determine total root and shoot dry weights. The shoot: root ratio was calculated.

**Pod yield:** At maturity, the pods were harvested and the average number of pods per plant was determined. The pods were said to be mature when the parenchymatous layer surrounding the embryo had disappeared and there were brown patches in the pod (Doku and Karikari, 1970). The pods were then oven dried at 80°C for 48 h and pods were shelled. The average seed number per plant, 100 seed weight and yield (kg ha⁻¹) per treatment was then determined.

**Statistical analysis:** The data collected was subjected to Analysis of Variance (ANOVA) using the Statistical Analysis System (SAS). Treatment means were compared using the Least Significance Difference (LSD) at p = 0.05.

**RESULTS**

Depending on the stage of bambara plant development or time when stress occurred, water stress reduced the RLER of the plants by 70.3-69.7% (Fig. 1a) and 78.7-99.6% (Fig. 1b) with the pod filling stage having the highest reduction and the vegetative stage having the lowest reduction. The RLER for the control fell sharply to 0 after 19 days in trial 1 (Fig. 1a) and after 23 days in trial 2 (Fig. 1b). After reaching zero the rate remained stagnant up to the last day of observations (Fig. 1a, b). The RLER for the stressed treatments decreased steadily reaching zero after 15, 11 and 8 days in trial 1 (Fig. 1a) after water stressing at the vegetative, flowering and pod filling stages, respectively. In trial 2 it took 15 days for RLER to reach zero after water stressing at the vegetative and flowering stages and 4 days after water stressing at the pod filling stage (Fig. 1b). After rewatering the RLER for all stressed treatments substantially increased. In both trial 1 and trial 2, the RLER for the vegetative stage was not significantly lower than the baseline RLER for the control plants and so recovered from water stress. The RLER for the flowering and pod filling stages was however, significantly lower (p<0.05) than the baseline RLER for the control plants and
so plants which were stressed during the flowering and pod filling stages failed to fully recover from water stress. The pod filling stage had the lowest recovery of 33.5% (Fig. 1a) and 13.5% (Fig. 1b), while the vegetative stage had the highest recovery of 94.5% (Fig. 1a) and 93.6% (Fig. 1b). After recovery, the RLER decreased to zero after 15 days (Fig. 1a) and 16 days (Fig. 1b) for the vegetative stage and 13 days (Fig. 1a) and 14 days (Fig. 1b) for the flowering and pod filling stages and remained constant up to the last day of observations.

Water stress significantly (p<0.05) reduced leaf number of bambara groundnut when the plants were stressed for 21 days during the vegetative, flowering and pod filling stages, respectively compared to unstressed control plants in both trial 1 and 2 (Fig. 2a, b). Varying the time of sowing significantly reduced (p<0.05) leaf number per plant of plants in trial 2 at all stages of growth and development as they were sown end of January as compared to plants in trial 1 which were sown end of October. The number of maturity days was also lower in trial 2 (95 days) as compared to trial 1 (117 days) and pod filling started earlier at 60 days in trial 2 than 80 days for trial 1 (Fig. 2a, b).

The maximum leaf number per plant was significantly higher (p<0.05) in plants grown in trial 1 (101 days) (Fig. 2a) compared to plants grown in trial 2 (72 days) (Fig. 2b) both of them being for the control plants. Leaf number reduction was significantly (p<0.05) high when water stress occurred during the vegetative stage of plant development (Fig. 2a, b). However, plants water
Fig. 2: (a) Effect of water stress at different stages of bambara groundnut plant growth and development on leaf number in trial 1. (b) Effect of water stress at different stage of bambara groundnut plant growth and development on leaf number in trial 2.

stressed for 21 days at the vegetative and flowering stages abscessed leaves at a lower rate compared to plants stressed during the pod filling stages (Fig. 2a, 2b). Once the water stress was removed after rewatering at 46, 67 and 101 days for the vegetative, flowering and pod filling stages, respectively in trial 1 and at 46, 67 and 81 days for the vegetative, flowering and pod filling stages respectively, the number of leaves for the stressed plants at different stages of development was still significantly (p<0.05) lower than for the unstressed control plants, showing failure of fully recovery (Fig. 2a, 2b). Plants stressed during the pod filling stage had a 0% recovery in leaf number after rewatering as compared to the control plants and all the treatments abscessed leaves at the end of the growing season (Fig. 2a, b).
Fig. 3: (a) Effect of water stress at different stages of bambara groundnut plant growth and development on plant height in trial 1. (b) Effect of water stress at different stages of bambara groundnut plant growth and development on plant height in trial 2.

Water stress at different stages of bambara groundnut plant growth and development significantly reduced (p<0.05) plant height compared to non-stressed control plants (Fig. 3a, b). However, the plant height for plants which were grown in trial 2 (Fig. 3b) was significantly lower (p<0.05) than plants which were grown in trial 1 (Fig. 3a). The bambara groundnut plants which were stressed during the pod filling stage were not significantly reduced in plant height as compared to the control plants (Fig. 3a, b). After rewatering, the plants which were stressed during the vegetative and flowering stages significantly failed (p<0.05) to equal the plant height of the control plants and so failed to recover from water stress. Plants which were stressed during the vegetative stage, reached a height which was not significantly different (p<0.05) from that of plants which were stressed during the flowering stage after recovering from water stress in trial 1 (Fig. 3a) but reached a height significantly different from that of plants stressed at the flowering stage in trial 2 (Fig. 3b).

Plants which were stressed during the vegetative stage had a higher rate of increase in plant height (0.44 cm day$^{-1}$) (Fig. 3a) and (0.4 cm day$^{-1}$) (Fig. 3b) compared to that of the plants which were stressed during the flowering stage (0.15 cm day$^{-1}$) (Fig. 3a) and (0.09 cm day$^{-1}$) (Fig. 3b).
Table 1: Effect of water stress on shoot: root ratio of bambara groundnuts in trial 1 and 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot/root ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
</tr>
<tr>
<td>Control</td>
<td>3.17</td>
</tr>
<tr>
<td>Stressed during the vegetative stage</td>
<td>2.88</td>
</tr>
<tr>
<td>Stressed during the flowering stage</td>
<td>2.96</td>
</tr>
<tr>
<td>Stressed during the pod filling stage</td>
<td>3.00</td>
</tr>
<tr>
<td>LSD</td>
<td>0.156</td>
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</tbody>
</table>

Table 2: Effect of water stress on number on pods and seeds per plant, 100 seed weight and yield of bambara groundnuts in trial 1 and 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pods/plant</th>
<th>Seeds/plants</th>
<th>100 seed weight (g)</th>
<th>Yield (kg ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
<td>Trial 1</td>
<td>Trial 2</td>
</tr>
<tr>
<td>Control</td>
<td>42.0</td>
<td>36.0</td>
<td>40.0</td>
<td>34.0</td>
</tr>
<tr>
<td>Stressed during the vegetative stage</td>
<td>33.0</td>
<td>29.0</td>
<td>32.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Stressed during the flowering stage</td>
<td>29.0</td>
<td>8.0</td>
<td>21.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Stressed during the pod filling stage</td>
<td>37.0</td>
<td>31.0</td>
<td>25.0</td>
<td>19.0</td>
</tr>
<tr>
<td>LSD</td>
<td>2.8</td>
<td>2.9</td>
<td>2.5</td>
<td>4.2</td>
</tr>
</tbody>
</table>

The shoot: root ratio was significantly reduced (p<0.05) by water stress imposed at vegetative, flowering and pod filling stages compared to the non stressed control plants (Table 1). The shoot: root ratio for plants in trial 2 (Table 1) was significantly lower (p<0.05) than plants in trial 1 (Table 1). The plants which were stressed during the pod filling stage had the lowest decrease in shoot: root ratio (5.36%) (Table 1) and (4.33%) (Table 1), while the highest decrease was obtained in plants which were stressed during the vegetative stage (9.15%) (Table 1) and (21.4%) (Table 1). There was however, no significant difference (p<0.05) on shoot: root ratio of plants which were stressed at the vegetative stage and those stressed during the flowering stage (Table 1).

Water stress at the vegetative, flowering and pod filling stages of growth and development of bambara groundnut plants significantly reduced (p<0.05) number of pods/plant, number of seeds/plant and 100 seed weight (Table 2). The number of pods and seeds per plant was significantly lower (p<0.05) in trial 2 compared to trial 1 at all stages of development (Table 2). The lowest pod and seed number per plant was obtained in plants water stressed during the flowering stage (Table 2). Plants which were stressed during the pod filling stage had the lowest decrease or percentage loss in number of pods (Table 2) but had the highest percentage loss in 100 seed weight compared to plants water stressed at vegetative and flowering stages (Table 2). The plants which were stressed at the flowering stage had the lowest pod and seed number per plant in trial 2 (Table 2) but had the highest 100 seed weight when compared to other stressed treatments (Table 2). There was however, no significant difference (p<0.05) between the 100 seed weight of plants stressed at flowering and the control plants in trial 2 (Table 2).

Water stress on bambara groundnuts for 21 days at the vegetative, flowering and pod filling stages of development significantly (p<0.05) reduced seed yield (kg ha\(^{-1}\)) compared to non-stressed plants (Table 2). Yield (kg ha\(^{-1}\)) for plants in trial 2 was significantly lower (p<0.05) than yield for plants in trial 1 at all stages of bambara groundnut growth and development (Table 2). The seed yield loss due to water stress ranged between 45-75% in trial 1 and between 50-82% in trial 2 (Table 2) depending with the stage of plant growth and development when water stress occurred.
The highest yield reduction occurred on water stressed plants at the pod filling stage was more in trial 2 (82%) than trial 1 (75%) (Table 2). Plants water stressed at the vegetative stage of growth and development had the lowest seed yield loss compared to non-stressed plants (Table 2).

DISCUSSION

Water stress reduced RLER in all stressed plants compared to non-stressed plants. The non-stressed plants had a high RLER, this was attributed to the first phase of leaf development where both cell division rate and the RLER are maximal. The reduction in RLER in stressed plants was attributed to turgor reduction which is the earliest biophysical effect of water stress. The resulting smaller leaf area transpires less water and this reduction in leaf area can therefore be considered a first line of defence against drought. This reduction in leaf area under water stress is similar to other studies on bambara groundnut (Collinson et al., 1997; Mwale et al., 2007a; European Union PP-5 INCO-DC, 2002). After rewatering, all stressed leaves resumed growth almost immediately and RLER increased probably due to resumption of leaf cell division, culminating in leaf expansion to maximum attainable size. The observation that leaf expansion resumed rapidly after rewatering is consistent with studies which have shown that cell expansion can be halted during brief episodes of water deficit and resume vigorously after rewatering (Munns et al., 2000; Alves and Setter, 2004). The plants water stressed during the vegetative stage had a higher peak RLER after rewatering than those stressed at flowering and pod filling stages. This can be attributed to young plants having a higher potential to recover after water stress.

Water stress reduced the number of leaves per plant in all stressed bambara groundnut plants. These results are consistent with the findings of Collinson et al. (1996) and Mwale et al. (2007b) also on bambara groundnuts. However reduction in leaf numbers was more in plants grown in the second trial than the first trial. The decrease in leaf production in the second trial may have been caused by declining temperatures which usually occurs later in the season in southern Africa since temperatures were not controlled in the greenhouse used in the second trial (Sesay et al., 2008). Reduction in leaf number may have been a result of reduction and termination of new leaf production and also leaf abscission which was more evident in bambara groundnut plants which were water stressed during the pod filling stage. Water deficit stimulates leaf abscission as drought stress has been reported to induce production of ethylene in a variety of species (Apelbaum and Yang, 1981; Kaeperska et al., 1989). The resulting decrease in leaf area is one of the mechanisms of moderating water loss from the crop canopy and averting excessive drought induced injury to the plant. This however may result in decreases in total dry matter production and yield decreases because of reduction in photosynthetically active leaf area. After rewatering, plants stressed during the vegetative and flowering stages increased leaf numbers. This is an important trait for bambara groundnuts as plants are capable of developing a large leaf area very quickly, therefore are better suited to take advantage of occasional wet summers. In plants stressed during the pod filling stage, leaf senescence could not be stopped by rewatering and so plants failed to recover.

Water stress reduced plant height in plants stressed during the vegetative and flowering stages. This was attributed to reduction of stem and leaf expansion. Water deficit did not affect plant height during the pod filling stage because the plants had ceased growing vegetatively by this time. After rewatering, the plants stressed during the vegetative and flowering stage increased in plant height. This may be attributed to resumption of stem cell division and elongation plus leaf expansion. Water deficit reduced shoot: root ratio of all stressed treatments, probably because water deficit modulates root length and density by allocating more carbon to the roots for new growth. A
greater soil volume can therefore be exploited; an important adaptation in drought spells. These results are in agreement with the results of Collinson et al. (1996) on bambara groundnuts under water stress but are in contrast with the results of European Union FP-5 INCO-DC, (2002) which stated that there was no decrease in shoot: root ratio in the landrace uniswa red under water stress. Plants which were stressed during the pod filling stage had lower shoot: root ratio as compared to the non-stressed control plants even though water was withheld well after the plants had stopped growing vegetatively. The irreversible leaf senescence caused by water stress may have reduced shoot dry matter. There was also a significant difference in shoot: root ratio between plants in trial 1 and trial 2 and declining temperatures which occurred later in the season might have reduced leaf production and leaf size, thus dry matter production resulting in lower shoot: root ratio for plants in trial 2. Water stress reduced the number of pods and seeds per plant, 100 seed weight and seed yield (kg ha\(^{-1}\)) in all stressed treatments as compared to the non-stressed control plants. The reduction in seed yield agrees with previous findings on legumes under water stress such as black beans (Nielson and Nelson, 1998); faba beans and bambara groundnuts (Mwale et al., 2007a, b; European Union FP-5 INCO-DC, 2002) and cereals like oats (Sandha and Horton, 1977) and maize (Kamara et al., 2003).

Plants stressed during flowering stage had the lowest pod and seed number per plant. Water stress during this period may have resulted in death of pegs before pod initiation. After rewatering, the plants resumed flowering reaching physiological maturity with small pods without mature seeds. Plants stressed during pod filling stage had a higher number of seeds per plant compared to other stressed treatments. This may be because water was withheld when most of the pegs had formed pods and so they managed to form seeds. The number of seeds per plant was however, less than the control probably due to stress induced abortion of newly formed seeds. The plants stressed during the vegetative stage also had a reduced pod and seed number per plant. This is because water stress during this stage reduced plant growth therefore may have delayed and reduced appearance of nodes and so resulting in plants with fewer inflorescence, fewer pod and seed numbers per plant after rewatering. Water stress reduced 100 seed weight and seed yield (kg ha\(^{-1}\)) in all stressed treatments.

The decrease in RLER and leaf number resulted in a decrease in total bambara plant leaf area which decreased the photosynthetically active leaf area and therefore resulted in decreased photosynthesis and photosynthates production which resulted in low seed yield. Generally, plants which were grown in trial 2 produced significantly lower pod and seed number per plant, 100 seed weight and seed yield (kg ha\(^{-1}\)). This might have been caused by reduction in the dry matter production which might have been a consequence of the effect of sowing date on leaf production, canopy development and the substantial reduction in the reproductive period as sowing was delayed (Harris and Azam-ali, 1993; Collinson et al., 2000; Sesay et al., 2008). The reduction of the reproductive period has a major impact on the productivity of bambara groundnut since pod filling is dependent more on partitioning of assimilates from current photosynthesis than from remobilization of stored assimilates from vegetative organs (Brink, 1999; Sesay et al., 2004).

CONCLUSION

The current study shows appreciable differences among stages of growth in respect to their response to drought. It has also shown that the production of yield by bambara groundnuts under water stress may be linked to maintenance of relatively low shoot: root ratio biomass under water stress and also small leaf area which restricts transpirational water loss. It can also be linked to
bambara groundnut’s ability to recover leaf area after receiving water after stress. Water stress experienced by bambara groundnut plants has cumulative effects ultimately manifested by reduction in yield. The various amounts of bambara groundnut yield (kg ha⁻¹) obtained on different treatments showed that bambara groundnut is capable of producing worthwhile yield even if it has been affected by stress at any stage of growth. The author recommends that where possible, adequate water must be available to bambara groundnuts at all developmental stages in order to obtain an optimum yield.

REFERENCES