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Heat and water stress tolerance of bambara groundnut (*Vigna subterranea* L. Verdc.) landraces

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Abstract

The experiments were conducted to evaluate the performance of Bambara groundnut landraces to heat and water stress. Mottled brown, black, mottled white and white landraces were subjected to watering regimes (watering once in a week till maturity and watering once in a week till the 30th day after planting. The drought study was a 4 × 2 factorial experiment laid in a complete randomized design with three replicates. Data collected included canopy spread, plant height, number of stem and leaves, first flower appearance and days to senescence on different sampling occasions (20, 45 and 60 days after sowing). Heat tolerance of landraces was determined using cell membrane thermostability test. The experiment revealed that the crop has different canopy forms which were significantly different among the various landraces. Watering up to 30 days, black landrace produced shorter plants whiles white landrace produced taller plants as compared to their respective heights when they were watered to maturity. The number of stems and leaves was significantly influenced by the watering regime. Watering till maturity decreased days to flowering and also increased days to senescence compared to plants for which watering was withheld after 30 days of growth. The cell membrane thermostability test revealed that mottled brown landrace was able to sustain significantly less injury as compared to the other landraces. The results of this study reinforces the knowledge that Bambara seed landraces can survive under harsh climate conditions.

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Introduction

Bambara groundnut (*Vigna subterranea*) is an underutilized African legume cultivated throughout sub-Saharan Africa. It is mainly produced as a subsistence crop, usually by resource poor women farmers on soils that are not fertile to support the growth of staple crops (Abu and Buah, 2011). Bambara groundnut has several production advantages, in that it can produce appreciable yield with little rainfall (Agyeman *et al*., 2021; Ekanem, 2023). Nutritionally, the crop is superior to other legumes and is the preferred food crop of many local people (Ofori *et al*., 2001). The seed of Bambara groundnut contains carbohydrate (55.6-67.1%) and amylose content of its starch ranges between 15.7 to 35.3%, while dietary fibre content can be up to 10.3% (Halimi *et al*., 2019; Marcel *et al*., 2014).

Bambara groundnut is considered to be drought resistant (Gulzar and Minnaar, 2017). Local farmers claim that in years when groundnut fails due to low rainfall, Bambara groundnut produces good returns (Agyeman *et al*., 2023). It is probably the most drought–resistant of the grain legumes and may be found growing successfully under climatic conditions where the annual rainfall is below 500 mm and optimum between 900 - 1000 mm per year. According to Ocran *et al.* (1998); Messiaen (1992) this amount of rainfall was reported to be adequate to enable Bambara groundnut accomplish it vegetative cycle and produce satisfactory yields. Besides, water stress, the Bambara groundnut has been found to be resistant to high temperatures which is not suitable for other leguminous crops e.g cowpea (Yamaguchi, 1983). The Bambara groundnut ability to nutritionally solve malnutrition in rural areas has been reported (Swanevelder, 1998). But it's growth and yield performance in less favorable environments (heat and water stress conditions) has not been assessed.

Seed colour is a useful criterion for the initial selection of landraces for improved seed quality. This is especially true for landraces that typically exhibit large variations in seed coat colour. In recent times, seed colour of landraces have been found to have an association with seed quality and vigour in some crops (Zulu and Modi, 2010; Sinefu, 2011). But little is known about the contribution of landraces colour to stress tolerance in Bambara groundnut.

With the potential risk of drought and heat associated with climate change, drought and heat tolerance are likely to become even more important in African agriculture. Studying the effect of these abiotic factors on the growth and development of crops has become necessary to explore the potential and to assess the possibilities of transferring selections to other environments especially with the threat of climate change to agricultural productivity in sub-Saharan Africa.

Bambara groundnut being a neglected and underutilised legume, holds much potential for planting in semi-arid and marginal production conditions (Mabhaudhi and Modi, 2013).

Currently, there are no released improved bambara groundnut cultivars in Ghana. The crop is sown using landraces, of which little is known regarding their heat and water tolerance.

Variations may exist among landraces with respect to drought and heat tolerance**.** It is therefore necessary to identify landraces, which could be sown in areas of high temperature and where rainfall is low (Northern Ghana). The objectives of this study were to evaluate the growth and developmental performances of four bambara landraces under water stress and to determine the heat tolerance of bambara groundnut landraces using the cell membrane thermostability (CMT) test.

Materials and methods

Experiment one

Effect of landraces and water stress on growth and developmental performance of Bambara groundnut.

Experimental site

The experiment was conducted in the experimental field of the Department of Horticulture of the University for

Development Studies, Nyankpala Campus. Nyankpala is located in the Northern Guinea Savanna Ecological Zone. The site is located on longitude 0°98W and latitude 9 °41N and at an altitudse of 183 m above sea level. The area experiences a mono modal annual rainfall of 1000 mm to 1200 mm from April to November (SARI, 2008).

Experimental design

The experimental treatments were laid out in a 2 x 4 factorial experiment laid in a Complete Randomized Design with eight treatments and three replications. The factors were landraces (Black, mottled-brown, mottled-white and white) and drought treatments (Water 1: irrigate with one litre of water once a week till the 30th day after planting) and (Water 2: irrigated with one litre of water till maturity).

Preparation

Containers were arranged in an open field at the experimental site of the Department of Horticulture, University for Development Studies, Nyankpala Campus. A plastic container which is 0.14 m in height and weighs 60.54 g with a volume of 6000 m^3 was used. The containers were perforated to allow excess water to drain off and filled with 3 kg top soil taken from the field at a depth up to 20 cm.

Sowing culture

Four landraces were used for the experiment. Seeds of the four landraces were taken from local farmers in Nyankpala. Two seed were planted per pot and were thinned out to one plant after two weeks of germination. All the plants were watered up to 30 DAS with one liter volume of water a week. After which irrigation was ceased for the drought treatment plant.

Weed control

Weeding was done by hand picking at 45 and 80 days after planting.

Loosening of soil

At 30 days intervals the soil was loosed to allow proper aeration in each container.

Data collection

The following parameters were taken.

Number of leaves per plant

The number of leaves of test the plant was counted at 20, 45, 60, 90, 105 and 120 days after sowing (DAS) and the average was recorded.

First flower appearance: The date of flower appearance of each landrace was taken and recorded.

Plant height

The height (cm) from the ground level to the highest point of the plant was taken with a meter rule. Data on plant height was taken at 20, 45, 60, 90, 105, 120 DAS and the average was calculated.

Plant canopy spread

The horizontal distance from one end of each of the canopies of the plant to the other ends was measured with meter rule and an average taken for the plant canopy spread.

Number of stems

The number of stems of the plants were counted at 20, 45, 60, 90, 105, and 120 days after planting and their average was taken.

Experiment two

Determination of heat tolerance in Bambara landraces using the Cell Membrane Thermostability (CMT) test. This experiment was conducted at the University for Development Studies, Spanish Laboratory Complex, Nyankpala Campus, located in the Northern Region of Ghana.

Procedure

The procedure adopted for the determination of CMT is similar to the one described by Martineau et al. (1979). Recently fully expanded leaves of the four landraces were picked at 45 days after planting. To eliminate any soil particles, the leaves were first properly washed under tap water, and then with distilled water. Using a cork borer, ten leaf discs were cut from each leaf sample in paired adjacent pairs (control and heat treatment), avoiding the midrib, and this was done for other leaves. 10 cut disc (control) and 10 cut (heat-treated) leaf discs were then placed into two separate test-tubes and washed thoroughly with at least four changes of distilled water. This removes exogenous electrolytes adhering to leaf tissue surfaces and released from cut cells at the periphery of the disc. After the final wash, the tubes were drained of excess water.

Sufficient water remained on the disc tube interior to maintain a high humidity. The heat treatment tubes were then wrapped in saran plastic and incubated at 50 °C in a thermostatically-controlled water bath while the control tubes were kept at 25 °C. Heat treated tubes were promptly cooled at 25 °C after the elevated temperature treatment, and both the control and the treatment tubes were filled with 15 ml distilled water and incubated overnight for 18 h at 10 °C to allow diffusion of electrolytes from the disc. The tubes were then transferred to a water bath at 25 °C, thereafter the content was mixed thoroughly for 5 sec on a vortex mixer, and an initial conductance measurement was made using a conductivity meter (model 4071; Jenway, Dunmow, UK).

After this both the control and heat treatment tubes were covered with saran plastic wrap and autoclaved at 121 °C for 15 min to kill all cells and release all electrolytes. All tubes were cooled to 25 °C, the contents were mixed thoroughly, and final conductance measurements were taken.

The relative injury (RI) induced as a result of the initial 50 °C temperature treatment was then calculated as follows: $RI (\%) = \{1 - ([1 - (S_1/S_2)] / [1 - (C_1/C_2)]\} \times 100$.

Where, S and C refer to the conductance value for the heat treatment and control tubes, respectively and subscript 1 and 2 refer to the initial and final conductance reading, respectively.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using the GENSTAT statistical package and differences between treatment means were determined using the standard error of difference (SED).

Results

The study (experiment 1) focused on the evaluation of the growth and developmental performances of four Bambara groundnut landraces to water stress whiles (experiment 2) attempted to determine heat tolerant landraces using the cell membrane thermostability test.

Result of experiment one

Effect of land races and watering regime on plant height

Fig. 1 shows the significance of the landrace × watering regime interaction for plant height. The current study discovered that the two watering regimes have no effect on the height of mottled brown and mottled white landraces. Drought affected the black Bambara groundnut, and shorter plants were formed under water stress (watering for up to 30 days) compared to irrigation till maturity (120 DAS). Watering for up to 30 days treated plants, on the other hand, produced taller plants for white landraces than the other water regime.

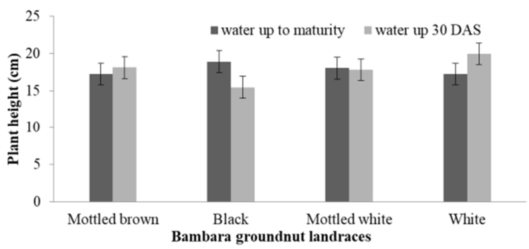

Fig. 1. Effect of watering regime and landrace on plant height. Bars represent two standard errors of the difference

In addition, compared to other landraces, the black Bambara groundnut landrace developed shorter plants when subjected to water stress (watering for up to 30 days).

Main effect of Bambara groundnut landraces on canopy spread

All the interactions and the main effects did not show significant differences except the main effect of landrace (Table 1). Black Bambara groundnut had the highest canopy spread (18.61 cm) whiles mottled brown had the least values as presented in Table 1.

Table 1. Means of the canopy spread on the various Bambara groundnut landrace

Landrace	Canopy spread (cm)
Mottled brown	16.41
Black	18.61
Mottled white	14.88
White	17.51
Rep	8
SED	1.343
F pro	0.50
Df	48

Effect of watering regime on number of stems and leaves

The main effect of watering regime was significant for the number of leaves and stems (Table 2).

Table 2. The effect of watering regime on the number of stem and leaves

	Number of stems	Number of leaves
Water till maturity	21.47	65.1
Water to 30 days	18.50	56.3
Rep	18	18
SED	1.499	4.42
F pro	0.007	0.007
Df	48	48

Plants that were watered till maturity had the highest number of leaves and stems (120 DAS) and this is true for all the landraces.

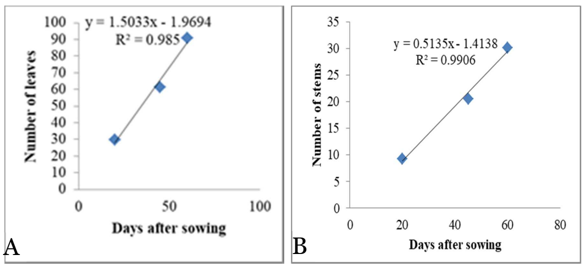

Fig. 2. The effect of days after sowing on the number of leaves (A) and stems (B) of Bambara groundnut

Effect of days after sowing (DAS) on number of stem and leaves

Except for the watering regimes and DAS main effects which exhibited a significant difference in the number of stems and leaves as presented in the Fig. 2, none of the interaction effect or the landrace effect were significant. As the number of days increases, both the number of stems and leaves also increased correspondingly. Days after sowing have a strong correlation with the number of stem (R^2 = 0.9906) and number of leaves (R^2 = 0.985).

Table 3. Effect of watering regime on first flower appearance and senescence

	Days to first flower appearance	Days to senescence
Water to 30 days	76.08	89.50
Water till maturity	73.50	114.20
Rep	12.00	12.00
Sed	0.391	1.790
Fpr	<.001	<.001
Df	16.00	16.00

Effect of watering regime on flower appearance and days to senescence

The main effect of landraces main and the interaction effects were not significant for days to senescence. However, the main effect of watering regime was significant (< 0.001) for the days to flowering and (Table 3). The table below shows that flower appearance was greatly affected by drought, that is, the plants that were watered till maturity flowered early and were able to stay alive for a longer period than those that were not watered up 30 days.

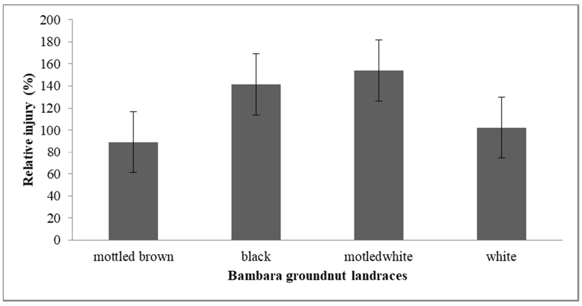

Fig. 3. The effect of relative injury on four Bambara groundnut landraces

Bars represent two standard errors of the mean

Result of experiment two

Relative injury

Relative injury ranged from 89.3 % (mottled brown) to 154.3 % (mottled white). The variability in relative injury within each landrace was high (CV = 27.8), making it difficult to detect any difference between the four landraces in response to heat stress. Though, the mottled white appeared to have high relative injury as compared to black and white landraces. It is evident from this experiment that mottled brown as compared to the mottled white landraces has lower susceptibility to heat (Fig. 3).

Discussion

The study focused on evaluating the growth performance of four Bambara landraces to water stress (experiment one). The mean height of the plants ranged from 15.46 cm to 19.97 cm and this contradict an earlier report made by Wongwichaiwat *et al.* (2023) that bambara groundnut grows to a height of between 34.86 cm and 44.92 cm at harvest. The reduction in plant height in the case of black Bambara groundnut with increase in water stress agrees with results of Siddique *et al.* (2000) where less watering resulted in shorter plants in wheat. Growth involves cell division, cell enlargement and differentiation and these processes are sensitive to water deficit because of their dependence upon turgor (Jones and Lazenby, 1988). The inhibition of cell expansion is usually followed by a reduction in cell wall synthesis (Salisbury and Ross, 1992). This may have affected plant height of the Bambara groundnut landraces. This study has shown that white Bambara groundnut was generally taller under water stress (water till 30 days) than the plants that were watered till maturity. This implies that white bambara can probably withstand higher level of water stress.

From the current study the differences in canopy spread may be due to their genetic differences, cultivar or an adaptive mechanism in response to heat and drought. In a similar study, Bambara groundnut did not show any significant differences in their canopy size but the authors made reference to a landrace (Burkina) having a bunch canopy (Berchie *et al.,* 2012) under heat conditions. Different growth forms (bunch, spreading and intermediate) exist for Bambara groundnut. From the current study, the black landrace exhibited the highest canopy spread and may likely be a spreading type (Doku, 1996). Also, from the current study, the broad canopy exhibited by the black landrace makes it a better inter crop with other landraces (Ouedraogo *et al.*, 2008).

The number of stems influenced the number of leaves of Bambara groundnut. The landraces exhibit a trifoliate leave system and a greater number of stems leads to higher the number of leaves.

The number of leaves was greatly affected by watering regime (i.e., water till maturity) giving the highest number of leaves (120 DAS). More water to the plants led to the turgidity of the leaves which might have resulted in more interception of light for carbohydrate production for more stem and leaves production. This result is consistent with the findings of Collinson *et al* (1996) who also found out that more watering led to the production of more leaves and stem in Bambara groundnut. Also, number of leaves per plant increased with an increased number of days. Obviously, this was due to growth and development which led to cell division and elongation in the plants as the days were increasing. Crops with more leaves could have the capability of capturing and utilizing more solar energy (Martin *et al.,* 2006), which can improve photosynthetic capacity. However, where the arrangement of such large number of leaves is not done well, there could be shading among the leaves, which can also retard photosynthetic capacity (Salisbury and Ross, 1985).

Days to flower appearance were greatly affected by the different watering regimes with flowering starting as far as 72 days for plants that were watered to maturity. Gao *et al.* (2020) indicated that flowering in bambara groundnut occurs within 30 - 45 days after sowing. The high day temperature during growing season (25 - 30^{o}C) might have resulted in the late flowering of the crop in this experiment. This assertion is in agreement with that of Nielsen and Hall (1985) and Patel and Hall (1990) who reported that the combination of high temperatures, drought and long days can slow down or inhibit floral bud development, resulting in few flowers production in cowpea. This experiment revealed that the plants that were watered till maturity flowered early as compared to the plants that were watered up to 30 days. This may be due continuous metabolic activities which might have been enhanced by continuous watering which probably led to early flowering. Days to senescence were delayed in plants that were watered up to maturity and this may be due to more carbohydrate production as a result of photosynthesis. Plants that were watered till maturity had more leaves and were able to grow well for a longer period thereby enabling it to capture and utilize sunlight for its growth and development. On the other hand, senescence was hastened in plants that were not watered till maturity as their leaves dried out and wilted.

Cell membrane thermostability test (Relative injury)

The electrolyte leakage test is one of the most convenient methods of screening crops for heat tolerance (Sullivan, 1972). In the current studies it is evident that electrolyte leakage under a temperature of 50 ˚C was high for all the four landraces and hence giving a high relative injury.

However, comparing the four landraces used in this experiment it is evident that mottled brown sustained less injury as compared to white bambara groundnut. This implies that mottled brown landrace has a strong cell membrane to withstand high temperature and could therefore be considered as a drought tolerant landrace.

Conclusion

The results of the study revealed that the growth of bambara groundnut landraces differ in response to different watering regimes. Plants that were watered till maturity performed better in terms of growth and development. Black bambara groundnut landrace having the spreading canopy form was the first to reach senescence even under irrigation till maturity. White bambara groundnut under irrigation performed well in terms of vegetative growth, followed by mottled brown and mottled white landraces. Cell membrane thermostability test revealed that mottle brown has relatively lower injury under heat as compared to mottled white and hence it could be considered a heat tolerant landrace. We recommend mottled brown landrace for areas that are experiencing high temperature while white landrace for drier areas.

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