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Genotypic Variability for Tolerance to drought Stress of Bambara Groundnut (*Vigna subterranea* (L) Verda)

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ABSTRACT

Three land races of *Vigna subterranean* (L.) Verds: {Pink eye (PeBg), white eyed (WeBg) and freckled (FeBg)} sourced from Department of Crop Science, Kogi State University, Ayangba in Nigeria were evaluated for agronomic, phenotypic and genotypic parameters. The experiment was in Factorial Design for four replicates and three varieties and four watering regimes. Differences based on drought tolerance for agronomic, phenotypic and genotypic parameters were estimated under different watering regimes (R_1- 1 day (control), R_2- 4 days, R_3- 7 days and R_4- 10 days). There were significant differences between all the water regimes applied. The watering regime (R_3) at seven days interval showed the highest mean values for the characters examined, while prolonged drought of 10 days watering intervals recorded the least. Significant potentials exist for the improvement of the crop productivity by selecting plants that are better equipped genetically to cope with drought. Phenotypic coefficients of variation (PCV) for pod length (17.0cm) and 100 seed weight (338.41g) were higher compared to other parameters studied. Genotypic coefficient of variation (GCV) revealed similar trends as the phenotypic variation for all characters. High values for heritability and genetic gain were recorded for pod length (Ho=50.55% and GG=1.43) and seed length (Ho=50.0% and GG=1.57), indicating that selection based on these characters may be effective for improved yield under drought conditions. There was positive and significant correlation between number of pods/plant, pod length, weight of pod/plant, weight of 100seed and grain yield per plant. Based on the present study, the white eyed variety (WVs) is the most adapted for drought prone areas.

Keywords: Drought, Water Regime, PCV, GCV, H_O in the broadsense

INTRODUCTION

The exact area of origin for Bambara groundnut *(Vigna subterranea* (L.) Verdc) is not ascertained (Karikari, 2000). The crop has been cultivated in tropical region of sub-Sahara Africa and in Madagascar for many centuries. The distribution of wild Bambara groundnut is now known to extend from Jos Plateau and Yola in Nigeria to Garona in Cameroon and probably beyond. Begemann (1998) confirmed the hypothesis that the center of origin of bambara is the region of northern Cameroon and northeast Nigeria. The crop is presently grown throughout Nigeria with the exception of the riverine and swampy areas of the south. It is grown by subsistence farmers on small scale and often intercropped with sorghum and cotton among others (Linnemman, 1994).

Bambara groundnuts are adapted to both poor and fertile soil of the African continent. The plant produces seeds that are high in protein (14-24%) and carbohydrate (60%) content (Remison,2005). The plant exhibits considerable tolerance and grows in soil with varied nutrient fertility including low and high nitrate levels. It produces yields in poor soil where very few other crops can survive (Linnemman, 1994). The root growth of the bambara groundnut shows significant response to drought stress (Lewitt, 1990). The effect of drought stress on bambara growth and seed yield depends on the degree of stress and stage of growth at which stress occurred. Drought stress during vegetative growth reduces growth and yield, thus limiting water uptake and implicating the whole plant metabolism (Bohnert *et al*., 1995). Earl (2002) stated that drought stress during reproductive growth reduces the flowering period and number of flowers, consequently number of pods and seeds per plant due to floral abortion. Earl (2002), stated that drought stress in general results in decreasing groundnut yield. Consequently, drought tolerance in bambara groundnut can be obtained by applying understanding to drought physico – morphological related traits. Breeding and molecular works have been successful in identifying traits controlling plant responses to drought stress (Fukai and Copper, 1995). According to Evan, (1996), drought stress during early pod formation causes the greatest reduction in number of pods and seeds at harvest.

Empirical evidence and limited research indicated that bambara groundnut is a promising crop, which has been largely neglected by National and International Agricultural Researches (Collinson *et al*, 2002) as a result it is one of the underutilized crops in legumes. Previous research work on the philosophy and agronomy of bambara groundnut has been carried out on a short term trial and error bases, mainly owing to the limited fund available for research on underutilized crop species (Karikari, 2000). The development of genetic material should be pursued in conjunction with the search for optimal management practices for commercial and subsistence production (Mensah and Eruotor, 1993). Thus, this research highlights the, genotypic variability for tolerance to drought stress of bambara groundnut cultivated in the Edo North Province of Nigeria is carried out.

MATERIALS AND METHOD

The experiment was carried out at the Teaching and Research farm, Ambrose All University Ekpoma (Lat 6^0 42′N. Long 6^0 68′E). Three varieties of bambara groundnuts (purple eyed, white eyed and freckled) obtained from Department of Crop Science, Kogi State University, Ayangba in Nigeria were evaluated using 3x4 factorial design. Bambara groundnuts were sown in polyethylene bags (50litres) as potted plants arranged 30cm by 30cm between and within rows in a land divided into 36 plots. Each plot contains 6 potted plants leading to a total of 216 plants. Two seeds were planted in each polyethylene bag and thinned to one per bag after emergence. The bambara groundnut was planted as a late crop (5^{th} October to December 2015) in a screened house. The planted crops were watered at intervals of one day, four days, seven days and ten days respectively. The sets of plants which were watered on alternate days served as check for comparison.

Hand weeding was carried out weeks after planting. To enhance high yield, 3.04kg of N.P.K (20:10:10) soaked in 10 litres of water for three days, corresponding to 400kg/ hectare was applied whereas, insecticide (Karate EC) was applied at the rate of 30ml per litre of water at 6 weeks and 12 weeks to control insect pests as part of cultural practices.

Data such as plant height, leaves/plant, leaf area, branches/plant, days to 50% flowering and maturity, pod length, pod/plant, seed/ pod, seed/plant, seed length, weight of pod and100 seeds and grain yield/plant were taken following the methods of Mensah and Eruotor (1993)

Data Analysis: Data collected were analyzed using analysis of variance (ANOVA) at 5% level of probability and the means separated using Least Significant Differences (LSD) as well.

Coefficient of Variation (CV): The coefficient of variation for treatment means were calculated using the formula by Steele and Torrie (1980).

$$\partial^2/\mu \times 100$$

Where μ = Mean

∂^2 = Standard deviation

Genetic Analysis: The genetic analysis was based on the data collected above. The means squares at treatment levels were taken as the phenotypic variation (which is the portion of phenotypic variance caused by variation in genes) the mean square at the error level was subtracted from their corresponding phenotypic variance to give the genotypic variance and calculated after Allard (1999) as follows:

Heritability (Ho): Heritability in the broad sense was calculated using the formula:

$$Ho = \partial^2 g/\partial^2 ph \times 100$$

Where Ho = Heritability

$\partial^2 g$ = Genotypic Variance
$\partial^2 ph$ = Phenotypic Variance

Genetic Advance: This was calculated by using the formula:

$\partial^2 g/\sqrt{(\partial^2 ph)} \times K$

Where GA = Genetic Advance
K = 2.06 (10% Selective index)
$\partial^2 g$ = Genotypic Variance μ
$\sqrt{(\partial^2 ph)}$ = Square root of Phenotypic variance

Genetic Gain (GG): The Genetic Gain was calculated in terms of the Genetic Advance (GA) expressed as a percentage of the population mean:

$GG = GA/\mu \times 100$

Where GG = Genetic Gain
GA = Genetic Advance
μ = Population mean

RESULTS AND DISCUSSION

Agronomic studies

The variation for percentage emergence, plant height, leaves/plant and leaf area were evaluated for four water regimes applied (R_1 = daily watered, R_2 = every 4 days, R_3 = every 7 days and R_4 = every 10 days (Table 1). The percentage seedling emergence for the three varieties of bambara groundnut under investigation showed that no variety recorded less than 80% emergence, which showed that all the varieties were highly viable. Seeds, if well preserved, are normally healthy with high germination and percentage emergence.

R_3 watering regime recorded the highest mean value of 15.7cm for plant height, 19.08cm for number of leaves/ plant and 354cm^2 leaf area/plant. There were significant differences between all the water regimes applied in terms of plant height whereas there were no significant differences between R_4, R_1 and R_2 for number of leaves/plant. R_3 showed significant increase from other watering regimes for leaves/ plant. Leaf area showed no significant difference between R_4 and R_3 and also between R_3 and R_2. High soil moisture enhanced the growth of bambara groundnuts although it was observed that watering regimes of seven days interval (R_3) showed the highest mean values for plant height, however extended drought to ten days resulted in reduced values of leaves/plant and leaf area/plant. Thus, bambara groundnuts were confirmed to be drought tolerant and drought resistant as previously reported by Ndubisi (1982) and Okeleye *et al*. (1999). These authors reported that heavy rainfall favours vegetative growth as observed in bambara groundnuts in southern Nigeria. Hence soil moisture content affects the yield of bambara. However in the present study, high vegetative growth was associated with low seed yield. Rainfall favoured vegetative growth of bambara groundnut in Southern Nigeria (Ndubisi, 1982; Okeleye *et al*., 1999).

Plant height, leaves/plant and leaf area/plant depended on water regimes as well as the variety under study. Variety (V2) white eyed recorded the highest mean value of 15.83cm for plant height, 19.19 for number of leaves/plant and least mean value of 323cm^2 for leaf area/plant.

Water regimes R_2 and R_4 differed significantly from each other and others for plant height. Only R_2 was significantly different for leaf area/plant. Pods/plant and weight of pod showed no significant difference between R_1 and R_2 and also between R_3 and R_4. R_1 significantly varied from others for length of pod and seeds/plant respectively. Different watering regimes affected the number of pods/plant. R_1 was significantly different from other water regimes for weight of 100 seeds and yield/plant. Significant difference between freckled, white eyed and purple eyed were observed in plant height. There were no significant differences between purple eyed among the three varieties studied. Number of leaves/ plant of purple eyed freckled were similar, but significantly different from white eyed. There were variations in the number of pods/plant when compared with other watering regimes. Different watering regimes affected the number of pods/plant. The number of pods/plant was not influenced by the number of days to flowering and this contradicted the earlier report by Okeleye *et al*. (1999) who reported that number of days to 5% flowering has significant influence on pods/plant among the varieties studied. Generally, late marturing varieties accumulated more vegetative matter and hence less seeds. Differences in varieties studies were not significant for pod length; however, white eyed had the highest mean value of 1.41 for pod length.

Freckled seed coat variety of bambara groundnut was significantly different from the other two varieties for length of seeds. The effects of watering regimes R_1 and R_2 were similar on length of pod as no significant difference was recorded. Hence length of seeds did not depend on pod length and soil moisture content had no influence on seed length (Table 2). Weight of pods/plant was significantly different among the varieties studied. Freckled eyed variety was different from other varieties. Thus, weight of pods was dependent on the varieties not water regime applied. There was no significant difference for water regime. Seasonal variations had no effect on pod weight whereas 100 seeds weight was significant for the different soil moisture occasioned by the different watering regimes. Freckled seed coat was significantly different from other varieties in terms of 100 seeds weight. The significant difference recorded by freckled eyed may be due to the weight of pod since weight of pod determined the weight of seed. It can therefore be concluded that V_1 and V_2 were similar in their performance in yield while V_3 recorded lower yield factors. The highest yield was recorded for variety (V2) and the yield was dependent on the pod weight and seed length.

Table 1: Agronomic characters of bambara groundnut using different watering regimes

Watering Regime		Plant height (cm)	Leaf area /plant (cm^2)	Pods /plant	Pod length (cm)	Seed Length (cm)	Weight pod(g)	100 seeds weight (g)	Yield plant (g)
R_1	1 day	15.68^b	18.75^b	342^b	11.18^a	1.40^a	1.81^a	90.81^b	10.06^a
R_2	4 days	15.47^c	18.67^b	360^b	10.83^b	1.51^b	1.44^a	1.81^a	90.75^b
R_3	7 days	15.75^b	19.08^a	345^c	10.83^b	1.49^b	1.26^c	1.82^a	90.95^b
R_4	10 days	15.83^a	18.75^b	306^c	10.75^b	1.50^b	1.34^b	1.81^a	90.84^b

Figures within a column with the same alphabet are not significantly different ($P \geq 0.05$) from each other.

Correlation coefficient showed that seed length was positively correlated with pod weight (P<0.01). Also pod length positively correlated with weight of pod; 100 seed weight positively correlated with pod length, seed length (P<0.05) and weight of pod (P>0.01) thus improving seed weight can also lead to improving pod length, seed length and weight of pod and vice versa (Table 3). It has been reported that tiny and malformed seeds contributed to a reduction in pod and seed weights (Okeleye *et al*., 1999). Similar observation has also been reported that weight of pod is positively correlated to weight of seeds by Backiyarani *et al*. (2000) in *Vigna unguiculata*. According to Karikari (1972) simple correlation analysis indicated that number of stems per plant and 100 seed weight were positively correlated with grain yield and these characters are useful for selection in bambara groundnut. Goli *et al*. (1997) found that characters most strongly correlated with grain yield were number of leaves and pods per plant and 100 seed weight. The current study is therefore in agreement with these earlier reports.

Table 2: Correlation (r) matrix for different phenotypic characters of bambara groundnut with respect to yield parameters

	No. of pods per plant	Pod length (cm)	Seed length (cm)	Weight of pod/ pant (g)	Weight of 100 seeds (g)
Pods/ plant	1				
Pod length	0.005	1			
Seed length	0.153	0.444**	1		
Weight of pod	0.118	0.368*	0.257	1	
100 seed weight	0.107	0.339*	0.297	0.962**	1
Yield/ plant (g)	0.926**	**0.707	0.597**	0.814**	0.348*

*****Difference significantly at 5% of probability. ** Difference significantly at 1% of probability.

Phenotypic and genotypic parameters

The phenotypic coefficient of variation was low (0.04 – 9.25) for plant height, leaves/plant, leaf area, pods/plant, seed length and weight of pod, and height (17 – 338.41) for pod length, 100 seeds weight and grain yield/ plant. Genotypic coefficient of variation revealed similar pattern of variation as shown by the phenotypic coefficient of variation for all characters. It was low (0.020.65) for plant height, leaves/plant, seed length and weight of pod and high for leaf area, pod and high for leaf area, pod length and 100 seeds weight (Table 3)

Leaf area, pod length, seed length, and 100 seeds weight recorded higher heritability estimates (97.28, 50.55, 50.00 and 99.81) respectively. Weight of pod, seed length and leaves/ plant recorded genetic gain estimates (2.92, 1.57, 2.35), indicating that these characters were controlled by additive genes and can therefore be improved upon genetically. Thus selection for improvement can be effective for these characters. Low heritability values for plant height indicated that this character was under the control of non additive gene and will be difficult to improve since it is environmentally controlled. Similar observations of high and low heritability values have been reported by Tanimu *et al*. (1990) for bambara groundnut and other related legumes.

Table 3: Agronomic characters of bambara groundnut for three different varieties

Varieties		Plant Height (cm)	No. of leaves/ plant	Leaf area/ plant (cm)	No. of pods/ plant	Pod length (cm)	Seed length (cm)	Weight of pod (g)	100 seeds weight (g)	Yield/ plant (g)
V_1	Purple eyed(PeBg)	15.75[a]	18.56[b]	361[a]	10.81[a]	1.48[a]	1.37[a]	1.72[a]	91.12[a]	20.40[a]
V_2	White eyed (WeBg)	15.83[a]	19.19[a]	323[b]	11.13[a]	1.57[a]	1.41[a]	2.22[a]	101.48[a]	25.07[a]
V_3	Freckled (FeBg)	15.49[a]	18.69[b]	342[b]	10.69[a]	1.48[a]	1.30[b]	1.51[b]	79.92[b]	12.90[b]

Heritability (Ho), which is a ratio of the genotypic and phenotypic variance, portrays the level of inheritance from one generation to the other. Thus characters with high heritability values are easier to improve than those with low values. Genetic gain is an expression of the extent improvement can be made on the characters under study. A combine study of heritability and genetic gain has been suggested to be more reliable in predicting the effect of selection (Johnson *et al*, 1995). High values for heritability and genetic gain were recorded for pod length, seed length, weight of pod and 100 seeds weight and thus suggesting that selection based on these characters may be effective. Characters with high estimate of high heritability and genetic gain are under the control of additive genes. Similar results of high heritability and genetic gain estimates on the phenotypic performance have been reported by Backiyarani *et al* (2000). Thus, improvement for pod length, seed length, weight of pod and 100 seeds weight may be possible and effective in bambara groundnut under water stress. Johnson *et al* (1995) observed that genetic gain would be low when there is non-additive gene effect and high genetic gain when there is additive gene effect. Therefore, selection based on characters such as pod length, seed length, weight of pod, grain yield and 100 seeds weight with high heritability and genetic gain are avenue for successful crop breeding programme in bambara groundnut under stress.

Exploration of variability in response to drought would permit us not only to identify some tolerant varieties, but also to determine useful criteria for genetic and phenotypic improvement of drought tolerance. Drought is a common abiotic stress condition and hence economically important crops with high levels of drought tolerance are of great value. Under field conditions, drought severity, timing and duration vary from year to year in cultivars, which are successful in one year, might fail in another year. The unpredictable and variable forms in which drought stress manifested itself complicates the selection of superior plant material as well as the breeding programmes. Significant potentials exist for the improvement of crop productivity by selecting plants that are better equipped to cope with unfavourable environmental conditions, such as drought. One way by which we can improve crop performance is to select phenotypes that have improved yield during water deficit conditions. The ability of some plants to maintain higher yield under drought than others is of great importance. Average losses of some major crop plants due to environmental stresses may amount to 50 - 80% of their genetically determined productivity (Lewitt, 1990). The highest proportion of yield losses can be directly attributed to drought. The drought related responses in plants are of a complex nature and results from genomic re-organization and alteration in gene and phenotypic expression. Drought tolerance showed a highly complex trait influenced by multiple genes.

Consequently, bambara groundnut is one of the most drought tolerant legumes and its rich and apparent genetic diversity for stress tolerance makes it an excellent crop model. Nonetheless, direct selection of bambara groundnut for drought tolerant varieties using phenotypes is slow and difficult. This is because there are other contributing factors that affect drought tolerance in crops besides their genetic make-up. Drought tolerance in bambara groundnut was expressed in developmentally specific pattern, such as early vegetative stage, flowering and pollination stage, and post -flowering stage. Based on the present study, the level of tolerance to drought of the three varieties studied is $V_2 \geq V_1 > V_3$

Table 4: genetic parameters of various characters among three varieties of bambara groundnut

Characters	$\bar{X}$±SE	PCV	GCV	Ho%	GA	GG
Plant	15.69±0.25	0.22	0.02	9.99	0.19	1.2
Leaves/plant	18.81±0.69	1.51	0.4	21.49	0.55	2.29
Leaf area	18.34±069	9.25	9.0	97.28	2.0	0.32
Pods/plant	10.88±0.8	3.21	0.65	20.25	0.42	0.08
Pod length	1.5±1.47	17.0	8.7	50.55	1.04	1.43
Seed length	1.35±0.07	0.04	0.02	50.0	1.03	1.57
Weight of pod	0.31±0.003	0.89	0.389	39.00	2.06	2.35
100 seeds weight	90.34±0.37	338.41	337.87	99.81	2.06	0.05
Yield/plant	19.46±0.41	96.6	85.4	88.40	17.92	2.14

$\bar{X}$ = Mean
S.E = Standard error of the agronomic characters
PCV = Phenotypic coefficient of variation
GCV = Genotypic coefficient of variation
Ho% = Heritability expressed in percentage
G.A = Genetic Advance
G.G = Genetic Gain

Significant differences among freckled, white and purple eyed variations were observed in plant height. There were no significant differences between purple and white eyed. Number of leaves/plant of purple eyed and freckled were similar, but significantly different from white eyed. The number of pods/plant was not influenced by number of days to 50% flowering and this contradicts the earlier report by Okeleye *et al* (1999) who reported that number of days 50% to flowering has significant effect on pod/plant. Pod length varied insignificantly among the varieties. Length of seeds did not depend on pod length.

Genotypic coefficients of variation were low (0.02-0.65) for plant height, leaves/plant pods/plant, seed length and weight of pod and high for leaf area, pod length and 100 seeds weight. Leaf area, pod length, seeds length and 100 seeds weight recorded higher heritability estimates (97.28, 50.55, 50.00 and 99.81). Weight of pod, seed length and leaves/plant recorded genetic gain estimates (2.92, 1.57, and 2.35). Characters with estimates of high heritability and genetic gain are under the control of additive genes (Johnson *et al* (1995). Thus selection for improvement can be effective for these characters. Similar observations of high and low heritability values have been reported by Taminu *et al* (1990) and Backiyarani *et al* (2000) for bambara groundnut and other related legumes. Selection based on characters such as pod length, seed length, weight of pod, grain yield and 100 seed weight with high heritability and genetic gain is a clear cut for bambara improvement.

Exploration of variability in response to drought would permit not only to identify some tolerant varieties, but also to determine useful criteria for genetic improvement of drought tolerance in bambara groundnut. Significant potentials exist for the improvement of crop productivity by selecting plants that are better equipped to cope with unfavorable environmental conditions, such as drought. Bambara groundnut is one of the most drought tolerant legumes and its rich and apparent genetic diversity for stress tolerance makes it an excellent crop model.

CONCLUSIONS

Generally, bambara groundnut tolerates a high degree of low soil moisture content, however, extended drought during the growth stage eventually leads to low yield. The characters, which exhibited high heritability and genetic gain and could therefore be improved for drought prone areas are weight of pod, seed length and 100 seeds weight. Among the three varieties studied, V_2 (white eyed) and V_1 (purple eyed) will be better utilized in drought prone areas by farmers.

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