Vol. 11(9), pp. 234-253, October-December 2019
DOI: 10.5897/JPBCS2018.0781
Article Number: 83BB7E262372
ISSN 2006-9758
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http://www.academicjournals.org/JPBCS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Heritability studies of drought tolerance in groundnuts using the North Carolina design II fashion and variance component method

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Received 10 October, 2018; Accepted 4 February, 2019

Drought is the most important abiotic limitation to groundnut yields across the world, and the Northern Regions of Ghana. The study estimated the heritability and genetic variability of selected parents of groundnut for drought tolerance traits to aid in their effective selection and utilization. The North Carolina II mating design was adopted while the variance component method was used to estimate heritabilities in the narrow and broad sense as well. Chlorophyll content (greenness of leaves) was recorded at 60 and 80 DAP. The objective was to measure the chlorophyll content and hence the drought tolerance performance of the entries. Mean squares caused by differences among crosses was partitioned into difference due to male parents and female parents, which was attributed to general combining ability (GCA), as well as difference due to male x female interaction, which was attributed to specific combining ability (SCA). Narrow Sense Heritability from the variance components for different traits varied under both water regimes, ranging from 12.2% to 95.7%. The most heritable traits were: dry biomass weight (95.7%), days to 50% flowering (91.0%), seed yield (90.0%), plant height at harvest (76.0%), SCMR 60 DAP (71.7%), days to maturity (67.0%) and SCMR 80 DAP (66.0%). Pod yield (12.3%) and harvest index (12.2%) exhibited low narrow sense heritabilities. Additive gene effects largely controlled the inheritance of pod, seed and biomass yields. Positive association between most yield and yield components as well as higher heritabilities shows that selection for higher yield and maturity is conceivable in improving groundnuts.

Key words: Abiotic, constraints, chlorophyll content, drought, genetic, groundnut, heritability, North Carolina II mating design, tolerance, yield.

INTRODUCTION

For groundnuts to escape natural risks and vulnerabilities including drought, diseases and pests, there is the need to develop varieties that combine early maturity, drought tolerance and higher yield. These cultivars are also needed in various groundnut growing areas to fit into a smart cropping scheme that ensures that possibly, two crops are grown per each year.

In regions such as Upper East, Upper West and Northern Ghana, where agriculture is chiefly rainfed and drought is most importantly a major constraint to groundnut production, it is imperative to undertake improvement of the crop for drought tolerance.

According to studies (Nageswara et al., 1985; Wright et al., 1994; Ndunguru et al., 1995), drought that occurs at the end of the production season in most agro-climatic and semi-arid groundnut production environments is the most predominant type. Breeding for tolerance to end-of-season drought, therefore, may improve productivity in drought-susceptible environments - such as in the Northern Ghana - as well as decrease aflatoxin contamination (Oppong-Sekyere et al., 2018b).

Nigam and Aruna (2008) indicated that it is now possible to estimate with ease, surrogates of Transpiration Efficiency (TE), a trait that is linked with drought tolerance, specific leaf area (SLA) and soil plant analytical development (SPAD) chlorophyll meter readings (SCMR). In this regard, breeding and selection schemes in crops, such as groundnut, integrate transpiration efficiency through the surrogates with all the possibilities.

SCMR is a term that gives an indication of the light-transmittance characteristics of the leaf, and it is dependent on the chlorophyll content of the leaf (Richardson et al., 2002). SCMR is low cost, easy to operate, reliable, fairly stable and a non-invasive surrogate of transpiration efficiency (Sheshshayee et al., 2006). According to Sheshshayee et al. (2006), transpiration efficiency is highly correlated with specific leaf area (SLA) and SCMR. Upadhyaya et al. (2005), Lal et al. (2006) and Sheshshayee et al. (2006) indicate that, Specific Leaf Area and SCMR have shown significant genetic variation in groundnut. Moreover, positive correlation between Transpiration Efficiency and SCMR has been reported (Bindu al., 2003; Sheshshayee et al., 2006).

Studies by Nageswara et al. (2001) and Upadyaya (2005) found a significant but negative correlation between SCMR and Specific Leaf Area and proposed the chlorophyll meter (SCMR) as a rapid and reliable measure that is capable of identifying cultivars with high water use efficiency in groundnut. Upadhyaya et al. (2005) reported of Soil Plant Analytical Development (SPAD) and Chlorophyll Meter Readings (SCMR) to be more stable than Specific Leaf Area. SCMR was also found to correlate with pod yield in groundnut (Reddy et al., 2003a, b).

Studies by Songsri et al. (2008) in assessing groundnut performance under both well-watered and long-term drought conditions confirmed that Harvest Index correlated with Specific Leaf Area and SCMR.

Combining ability is a term that is very useful in the design of any plant breeding programme. Combing Ability (CA), as it applies in crosses, is explained as the ability of parents or cultivars to combine among each other during the process of hybridization so that favourable and promising genes or characters are transferred to their progenies (Panhwar et al., 2008). It is particularly valuable in testing procedures that are used to study and compare the performance of lines in hybrid combinations. The two main types of combining ability; *Specific Combining Ability* (SCA), is defined as the deviation in the performance of hybrids from the expected productivity in relation to the average performance of lines involved in the hybrid combinations; whereas *General Combining Ability* (GCA) is defined as the average performance of a line in a series of crosses (Griffing, 1956; Falconer and Mackey, 1996).

General combining ability occurs as a result of genes which are largely additive in their effects whereas specific combining ability is due to the genes with dominance or epistatic effects (Sprague and Tatum, 1942). Several researchers have studied the effects of GCA and SCA in different crops. Rawlings and Thompson (1962) estimated GCA and SCA of inbred parents using line by tester analysis.

Information on combining ability is very important, most especially in the development of new cultivars through the process of hybridization; also, estimates of heritability from segregating populations become valuable in understanding and appreciating the genetics of hybridization and inbreeding (Ali and Wynne, 1994). Breeders are therefore afforded the very important information regarding selection and utilization of superior characters and individuals from a population, which subsequently lead to crop improvement. Heritability is the proportion of phenotypic variance in a population that is due to genetic variation between individuals. It is also the degree to which the characteristic of the parent are repeated in its progeny. The two major types of heritability are *Heritability in the Broad Sense* and *Heritability in the Narrow Sense*. According to Fernandez and Miller (1985), heritability in the narrow sense is important, in that, the effectiveness of selection depends on the additive portion of genetic variance in relation to total variance. The parent-offspring regression method is generally used to calculate heritability estimates of quantitative characters in both cross- and self-fertilizing crops (Fernandez and Miller, 1985). Examples of parent-offspring combinations in self-fertilizing crops that are commonly used include; F1/F2, F2/F3, and F3/F4 (Smith and Kinman, 1965). Therefore, knowledge of the combining abilities of lines (Chinese, Sinkara, Ndogba and Chaco-pag – all landraces) and an understanding of the mechanisms underlying the inheritance of the target traits is thus required.

The main goal of the study was to estimate the

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Table 1. Source and phenotypic characteristics of Groundnut parental population.

S/N	Genotype	*Sub-species	Source	Days to maturity, days	Phenotypic characteristics and other trait			
					Drought characteristics	**Early leaf spot disease**	**Late leaf spot disease**	***Oil content and other traits**
1	Chinese	*Hypogaea (Spanish)*	Landrace, Ghana	85-90	Tolerant	Susceptible	Susceptible	*Oil Content: 35% Early maturing Use: Soup and Confectionery
2	Sinkara	*Hypogaea (Spanish)*	Landrace, Ghana	100-115 (120)	Tolerant	Resistant	Resistant	*Oil Content: 45% Seed colour: Red Yield Potential: 2.2t/ha
3	Ndogba	*Fastigiata*	Landrace, Ghana	85-90	Moderately Tolerant	Moderately Susceptible	Moderately Susceptible	Seed colour: Tan red
4	Chaco – pag	*Fastigiata*	Landrace, Ghana	100-115	Tolerant	Moderately Resistant	Moderately Resistant	Seed colour: Red

*Sub-Species, *Oil Content and Other Traits: are from CSIR-CRI and SARI published data; CSIR-Council for Scientific and Industrial Research, SARI – Savanna Agriculture Research Institute, Ghana, CRI – Crops Research Institute, 'Landrace'- Farmers' popular and locally adapted variety.

heritability of some selected parents of groundnut for drought tolerance and agronomic traits to aid in their effective selection and utilization in a future groundnut breeding programme. It also sought to assess the two parents, P_1, P_2, their F_1, BC_1 and BC_2 generations for genotypic variations based on molecular analysis in laboratory trials in order to ascertain their genetic and phenotypic diversity.

MATERIALS AND METHODS

Experimental site

The hybridization activities (crosses) involving F1s and Backcrosses (BC) for the two parental populations (P1 and P2) were carried out in the screen house of the CSIR-Savanna Agricultural Research Institute (SARI), Nyankpala, Tamale, beginning from 2nd August, 2016. The field work for this phase, comprising the field assessment of parental lines (P1and P2) and their F1s, F2s and BC generations was begun on 1st January, 2017 and undertaken at the experimental fields of the CSIR-SARI and the Department of Ecological Agriculture, Bolgatanga Polytechnic (in November, 2017).

Genetic material and hybridization techniques

The genetic material that formed the parental lines included one farmers' preferred variety, Chinese (landrace) - an early maturing and drought tolerant variety selected by farmers from a PRA study (Oppong-Sekyere et al., 2018a), and three (3) other landraces, Sinkara, Ndogba and Chaco-pag, selected from germplasm screening (Table 1) (Oppong-Sekyere et al., 2018b). Ndogba and Chaco-pag varieties constituted the female parental lines while the Chinese and Sinkara varieties formed the male parental lines. Each of the two male parental lines were crossed to each of the female parents in a 2 × 2 North Carolina mating design II to produce four (4) sets of F1 generations for drought tolerance combination (representing populations 1 and 2), in a fashion as follows; Chinese × Ndogba, Chinese × Chaco-pag (for Population 1), Sinkara × Ndogba, Sinkara × Chaco-pag (for populations 2).

The resulting F1s from the crosses between the parents of the two populations were then backcrossed to the individual male parents to form BC1 and BC2 respectively, for each population. About six crosses were made on each individual female to increase hybrid seeds. At harvest, all F1 plants were examined carefully for several morphological traits including plant height, leaf color, pod and seed characters, and compared with both parents to confirm their hybridity. The F1 crosses were harvested during the first week of December, 2016. The F1s from each population were selfed to get F2 populations. Harvesting of F2s was done in September, 2017. Seeds of F1s, F2s, parents 1 and 2 and BC1 and BC2 for populations 1 and 2 were saved for subsequent genetic studies.

Field activities and crop management practices

After planting the groundnut genotypes, all cultural practices including filling-in, fertilizer application (DAP [Diammonium phosphate $(NH_4)_2HPO_4$] 150 kg/ha) (Jogloy et al., 2011), weed control and earthen-up were carried out as recommended. Weeding was done by hoeing between rows and hand pulling weeds on top of plots and within rows to reduce damage to developing "pegs". Earthen-up was done alongside all the weeding regimes.

Evaluation of groundnut populations

Observations were recorded on ten (10) plants selected at random among parents (P1 and P2), F1, F2 and BC populations. All recommended agronomic and plant protection measures were observed during the conduct of the experiment.

Evaluation of populations 1 and 2 with their set of F_1, F_2, BC_1, BC_2, P_1 and P_2 was carried out in pots using CRD with three (3) replications to determine heritability and other components of variation for the different groundnut traits. Each pot contained three (3) plants.

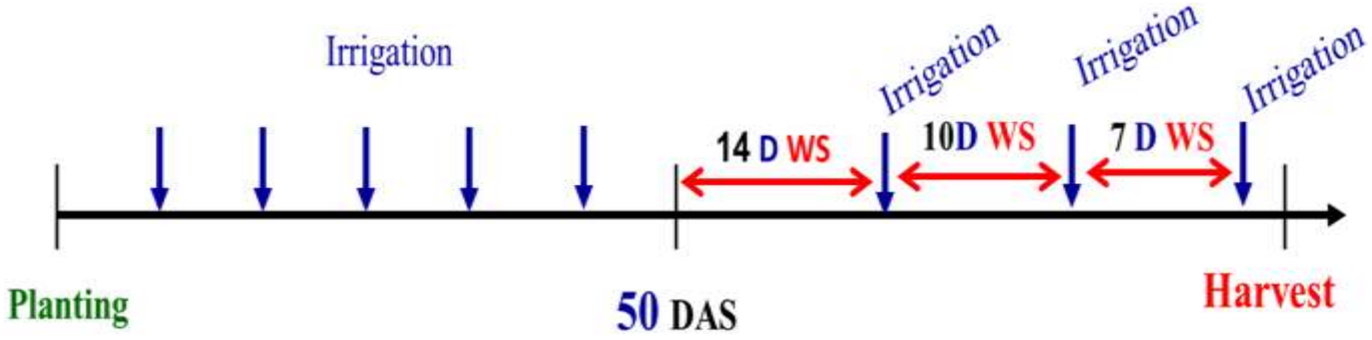

Figure 1. Drought stress imposition and irrigation frequencies (Adapted from; Mamadou, Coulibaly Adama, PhD. Thesis, 2013; http://ugspace.ug.edu.gh). *D: Days, *WS: Water-Stressed, *DAS: Days after Sowing, 14 D: 14 days, 10 D: 10 days, 7 D: 7 days.

Correlations and evaluation of populations for drought tolerance

Selected drought-tolerant F1 crosses (hybrids) together with the male and female parents were put under field experiment with regular water (well-watered, WW) and less water (water-stressed, WS) conditions to assess the drought effect.

Procedure

The selected crosses and their parents were evaluated at the experimental fields of the Department of Ecological Agriculture, Bolgatanga Polytechnic, Upper East Region. The treatments were arranged in a randomized complete block design (RCBD) with three replications. Recommended agronomic and plant protection measures were adopted during the experiment.

Drought-tolerant entries were planted in an α-lattice design and replicated three times in the two environments (well-watered and water-stressed conditions). Two-row plots of ten (10) seeds each were hand planted. Harvesting was done about 90 days after planting. Observations were recorded on plants selected at random among parents (P1 and P2), and F1, F2 and BC populations.

Irrigation management for well-watered and water-stressed environments (water regimes)

After sowing, the well-watered plots were irrigated fully two times a day until harvest stage. For the water-stressed environment, the crops were irrigated twice a week up to when 50% plants flowered (30 DAP). After that, the plants were irrigated twice a day until pod filling time. The plants were exposed gradually to end-of-season drought from the pod-filling (50 DAP) until maturity. At 50 DAP, which corresponded with peg penetration and pod filling, drought stress was imposed for 14 days and irrigation was resumed at the 15th day. Then drought stress was imposed for 10 days, followed by irrigation. After that, drought stress was imposed for 7 days followed by irrigation up to harvest (http://ugspace.ug.edu.gh) (Figure 1).

Data collection and other parameters measured for drought tolerance

Parameters measured for populations 1 and 2 and their combinations, as regards P1 and P2, F1s, F2s, BC1 and BC2 include days to 50% emergence, days to 50% flowering, days to maturity, plant height at harvest, SPAD Chlorophyll Meter Reading (SCMR) at 60 and 80 DAP, fresh and dry biomass (haulm) weights (g), number of pods (pod yield), number of seeds (seed yield), pod weight (g), seed weight (g), harvest index (HI) and drought (stress) tolerance index (DTI). Drought tolerance index (defined as the ratio of trait value measured under water-stressed conditions over value recorded under well-watered conditions) was computed for HI, fresh and dry biomass weights, pod yield and SCMR 60 and 80DAP. DTI value greater than 1; indicate drought tolerance, and DTI less than 1; not drought tolerant (Table 2). Combined analysis of variance (ANOVA) and correlation performance among the groundnuts under well-watered (WW) and water-stressed (WS) (drought) conditions were evaluated for significant difference of the tested progenies. Mean squares and mean squares of traits from the combined ANOVA for parental lines and F1s, F2s and BCs under well-watered (WW) and water-stressed (WS) conditions were also estimated for Table 5b.

The SPAD chlorophyll meter reading (SCMR)

Procedure

The chlorophyll content was recorded at 60 and 80 DAP (using CCM-200*plus*Chlorophyll Content Meter, *OPTI-SCIENCES)*.

Five plants from each plot were sampled at random, and the second fully expanded leaf from the top of the main stem was used for SCMR assessment during the morning period (0900±1200 h) as proposed by Nageswara et al. (2001). The chlorophyll content was recorded on each of the four leaflets of the tetrafoliate leaf. An average SCMR for each plot was derived from 20 single observations (four leaflets × 5 plants per plot) (Arunyanark et al., 2008). Care was taken to ensure that the SPAD meter sensor fully covered the leaf lamina in order to avoid interference from veins and midribs during the SCMRs (Nageswara et al., 2001).

Estimation of heritability: The variance component method

The variance component method of estimating heritability uses the statistical procedure of analysis of variance (ANOVA). Variance estimates depend on the types of populations in the experiment.

Total variance of a quantitative trait at F_2 may be mathematically expressed as follows:

$$V_P = V_G + V_E + V_{GE}$$

Table 2. ANOVA for North Carolina II Mating Design.

Source of variation	Degree of freedom	Mean square	Expected mean square
Sets	s-1		
Replications	S (r-1)		
Males	S (m-1)	M1	VE + rVfm + rfVm
Females	S (f-1)	M2	VE+ rVfm + rmVf
Male x Female	S (m-1)(f-1)	M3	VE + rVfm
Error	S (mf-1)(r-1)	M4	VE
Total	Smfr-1		

Source: (Kearsey and Pooni, 1996; Acquaah, 2012).

Where V_P = total phenotypic variance of the segregating population, V_G = genetic variance, V_E = environmental variance, and V_{GE} = variance associated with the genetic and environmental interaction.

The genetic component of variance may be further partitioned into three components as follows:

$V_G = V_A + V_D + V_I$

Where V_A = additive variance (variance from additive gene effects), V_D = dominance variance (variance from dominance gene action), and V_I = interaction (variance from interaction between genes, epistatic). Additive genetic variance (or simply additive variance) is the variance of breeding values and is the primary cause of resemblance between relatives. Hence, V_A is the primary determinant of the observable genetic properties of the population, and of the response of the population to selection. Further, V_A is the only component that the researcher can most readily estimate from observations made on the population.

The total phenotypic variance may then be rewritten as:

$V_P = V_A + V_D + V_I + V_E + V_{GE}$

Heritability estimate using F2 and backcross populations is as follows:

$V_{F2} = V_A + V_D + V_E$

$V_{B1} + V_{B2} = V_A + 2V_D + 2V_E$

$V_E = V_{P1} + V_{P2} + V_{F1}/3$

$H = (V_A + V_D)/(V_A + V_D + V_E) = V_G/V_P$
$h^2 = (V_A)/(V_A + V_D + V_E) = V_A/V_P$

(i). $h^2 = V_A/V_P$

(ii). $H^2 = V_G/V_P$

$V_E = [V_{P1} + V_{P2} + V_{F1}]/3$
$V_A = 2V_{F2} - (V_{B1} + V_{B2})$

$V_D = [(V_{B1} + V_{B2}) - F_2 - (V_{P1} + V_{P2} + F_1)]/3$

Broad sense heritability (H^2)

Heritability estimated using the total genetic variance (V_G), called broad sense heritability is expressed mathematically as:

$$H^2 = \frac{V_G}{V_P}$$

Narrow sense heritability (h^2)

Because the additive component of genetic variance determines the response to selection, where the narrow sense heritability estimate is more useful to plant breeders than the broad sense estimate. It is estimated as:

$$h^2 = \frac{V_A}{V_P}$$

Estimate of GCV and PCV

$$\text{GCV} = \sqrt{\frac{V_G}{X}}\ x\ 100$$

$$\text{PCV} = \sqrt{\frac{V_P}{X}}\ x\ 100$$

North Carolina Design II

Each member of a group of parents used as males in this case was mated to each member of the group of parents used as females. This design employs the factorial mating scheme (Table 2 and Figure 2). The design is used to evaluate inbred lines for combining ability; and was adopted in the current study because it is most adapted to plants that have multiple flowers so that each plant can be used repeatedly as both male and female, as typical of groundnuts. The North Carolina II mating design allows *Blocking*, which permits all mating involving a single group of males to a single group of females to be kept intact as a unit (Acquaah, 2012). It also allows for the measurement of both GCA and SCA. The design is a two-way ANOVA in which the variation may be partitioned into difference between males (m) and females (f) and their interaction (Hill et al., 1998; Athanase et al., 2013).

The North Carolina II mating design has mf set of crosses in which ‘m’ is male and ‘f’ is female plant. Due to male and female variance, it provides additive effects. It also provides dominance variance if male × female variance exist (Acquaah, 2012; Sarfaraz et al., 2014). NCII design is influenced by maternal effects (Hill et al., 1998). It is an intermediate design which involves F2 plants in crossing. Variance is divided in three fractions due to males and females and due to male × female cross (Kearsey and Pooni, 1996; Acquaah, 2012). The convention is as follows;

COVPHS=1/4 VA

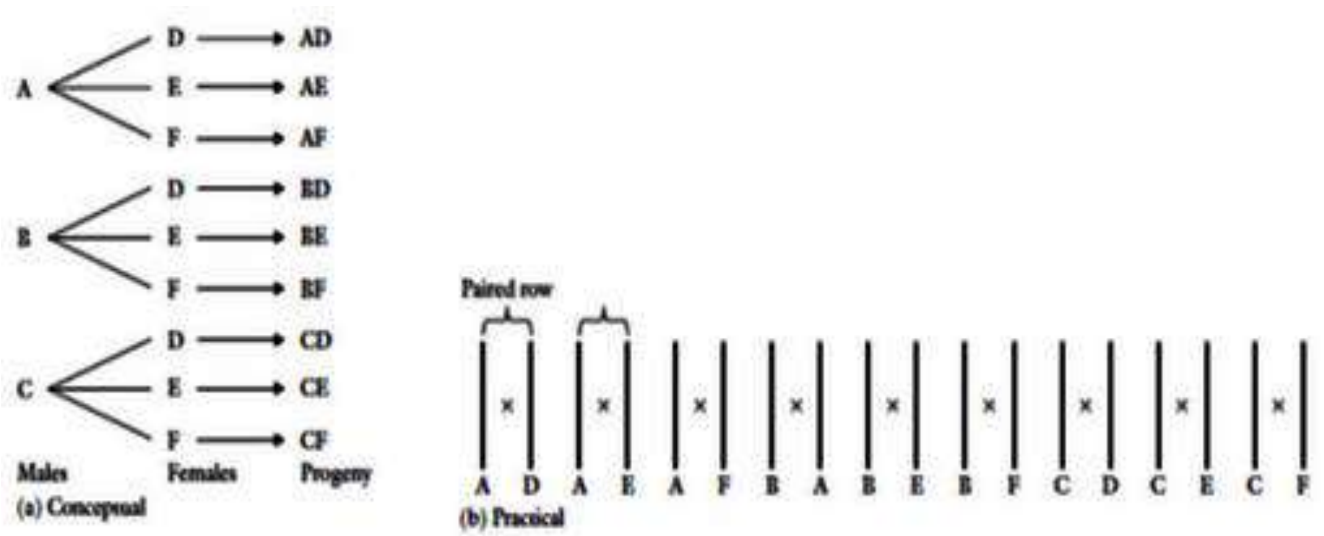

Figure 2. NC II Design (factorial design with paired rows).
Source: Kearsey and Pooni, 1996; Acquaah, 2012.

COVMHS=1/4 VA

V female × male = COVFS – COVHSm - COVHSf

= 1/4 VD

*Where; COVPHS = Covariance of Paternal Half-Sibs, COVMHS = Covariance of Maternal Half-Sibs, VA = Additive Variance, V = Variance, COVFS = Covariance of Full-Sibs, COVHSm = Covariance of Half-Sib Males, COVHSf = Covariance of Half-Sib Female, VD = Dominance Variance.

Crossing Block Layout for Hybridization Activities

P_1 X P_2
Resistant ⇩ Susceptible
F_1

Design: North Carolina mating Design II

Ndogba/Chinese
Chaco-pag/Chinese } **Population 1:** (Two sets of F_1 generations)

Ndogba/Sinkara
Chaco-pag /Sinkara } **Population 2:** (Two sets of F_1 generations)

4 sets of F_1 and F_2 generations and their back crosses (Table 3).

Data analysis

GenStat pc software 17.0 was used to carry out the analysis where the variance component could be obtained. Combined analysis of variance (ANOVA) of the two water regimes data was performed to determine the association and effect of the two water regimes (drought) on the groundnut performance. Least square difference (LSD) at $P \leq 0.05$ was used to compare means. Mean squares caused by difference among crosses was partitioned into difference due to male parents and female parents, which was attributed to general combining ability (GCA), and difference due to male x female interaction, which was attributed to specific combining ability (SCA).

RESULTS

Field evaluation of groundnut populations (phenotyping)

Results of the mean performance of parental lines (P1 and P2) (Table 3) and F1, F2, BC1 and their BC2 populations for physiological and yield traits (Table 4) indicate that, generally, it took about seven (7 days) for the groundnuts to emerge after planting and about 26 days to achieve 50% flowering. Average plant height of the groundnut at the time of flowering was 15 cm while an average height of 89 cm was achieved at maturity, before harvesting. Average maturity period recorded by the groundnut was 89.17 days after planting (Table 4).

Agronomic, chlorophyll content and drought-tolerance performances (DTI) of groundnut entries under well-irrigated (WW) and less-watered (WS) conditions

Among the males (Table 5), Sinkara scored the highest values for pod yield (WW:37.14; WS:39.11), seed yield (89.32; 93.82), fresh biomass weight (659.56; 512.54) and dry biomass weight (349.05; 331.76) for well-watered (WW) and water (WS) conditions respectively. The

Table 3. Crossing block layout.

Female	**Male**	
	Chinese	**Sinkara**
Ndogba	X	X
Chaco-pag	X	X

Table 4. Mean performance for growth characteristics of parental lines, F1s, F2s and their Backcrosses.

Groundnut population	**Source**	**Growth habit**	**Days to 50% emergence, (days)**	**Days to 50% flowering, (days)**	**Avg. plant height at flowering, (cm)**	**Avg. plant height at harvesting, (cm)**	**Days to maturity, days**
Males							
Chinese	Landrace, Ghana	Erect/Bunch	6	21	10.3	53.3	87
Sinkara	Landrace, Ghana	Erect/Bunch	8	27	11.0	47.7	89
Females							
Ndogba	Landrace, Ghana	Semi-Erect/Bunch	7	22	19.6	32.0	89
Chaco-pag	Landrace, Ghana	Erect/Bunch	7	25	16.6	50.7	90
F1s							
Chinese x Ndogba	Cross	Erect/Bunch	7	24	19.0	46.7	90
Chinese x Chaco-pag	Cross	Erect/Bunch	7	27	13.6	56.3	90
Sinkara x Ndogba	Cross	Erect/Bunch	7	27	18.3	71.7	87
Sinkara x Chaco-pag	Cross	Erect/Bunch	7	28	10.3	40.7	90
F2s							
Chinese x Ndogba	Cross	Erect/Bunch	7	24	19.0	36.3	89
Chinese x Chaco-pag	Cross	Erect/Bunch	7	27	19.3	43.0	90
Sinkara x Ndogba	Cross	Erect/Bunch	7	27	19.0	46.0	89
Sinkara x Chaco-pag	Cross	Erect/Bunch	8	28	9.0	44.0	90
BCs							
Chinese x Ndogba	Cross	Erect/Bunch	7	25	15.6	58.7	94
Chinese x Chaco-pag	Cross	Erect/Bunch	6	27	10.6	49.0	93
Sinkara x Ndogba	Cross	Erect/Bunch	8	27	16.3	63.7	94
Sinkara x Chaco-pag	Cross	Erect/Bunch	8	29	15.13	48.7	92
Mean	-	-	**7.08**	**25.58**	**15.42**	**47.37**	**89.17**
Range	-	-	**6.0-8.0**	**21.0-28.0**	**9.0-19.6**	**32.0-71.7**	**87.0-80.0**
CV%	-	-	**7.30**	**9.20**	**21.50**	**21.50**	**1.20**
S.d. (*S*)			**0.3**	**5.5**	**17.9**	**104.9**	**1.2**

highest value of 0.28 for harvest index was recorded by Sinkara against 0.24 for the second male, Chinese (Table 5a).

Among the females (Table 5a), Ndogba recorded the highest in the following; pod yield (33.33), seed yield (72.11), fresh biomass weight (561.32), dry biomass weight (299.42) and harvest index (0.25) respectively under well-watered conditions, whereas Chaco-pag (31.73), Chaco-pag (77.65), Ndogba (419.19), Chaco-pag (270.46) and Ndogba (0.32) scored highest under water-stressed conditions in the same traits (Table 5a).

Among the F1s, and under well-watered conditions (Table 5a), the crosses Chinese x Chaco-pag (35.57), Chinese x Chaco-pag (73.55), Chinese x Chaco-pag (587.20), Chinese x Ndogba (298.46), and Sinkara x Chaco-pag (0.42) exhibited high values respectively

Table 5. Mean yield performance of parental lines, F1s, F2s and back crosses under well-watered and water-stressed conditions.

Groundnut populations	No. of pods (Pod yield)		Pod weight (g)		No. of Seeds (Seed yield)		Seed weight (g)		Fresh biomass weight (g)		Dry biomass weight (g)		Harvest index (HI) for WW	Harvest index (HI) for WS
	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
Males														
Chinese	23.33	**26.98**	254.67	**294.51**	67.72	**59.81**	209.92	**196.52**	497.45	**414.23**	278.51	**227.90**	**0.24**	**0.26**
Sinkara	37.14	**39.11**	462.10	**471.12**	89.32	**93.82**	391.73	**376.22**	659.56	**512.54**	349.05	**331.76**	**0.23**	**0.28**
Females														
Ndogba	33.33	**29.31**	326.37	**298.49**	72.11	**68.99**	291.65	**283.51**	561.22	**419.19**	299.42	**215.41**	**0.24**	**0.32**
Chaco-pag	30.00	**31.73**	311.54	**331.11**	71.59	**77.65**	298.03	**303.56**	549.13	**399.16**	284.71	**270.46**	**0.25**	**0.29**
F1s														
Chinese x Ndogba	31.23	**35.60**	319.22	**331.74**	69.01	**61.87**	249.75	**198.78**	560.12	**448.87**	298.45	**237.77**	**0.23**	**0.26**
Chinese x Chaco-pag	35.57	**29.99**	338.73	**312.51**	73.55	**66.79**	301.71	**279.94**	587.20	**403.07**	297.07	**283.08**	**0.25**	**0.24**
Sinkara x Ndogba	25.89	**33.81**	270.16	**301.71**	58.90	**64.75**	249.28	**237.66**	459.40	**287.96**	239.31	**223.51**	**0.25**	**0.29**
Sinkara x Chaco-pag	29.91	**37.22**	317.01	**389.30**	68.78	**59.46**	291.51	**310.25**	258.10	**198.97**	165.14	**181.16**	**0.42**	**0.33**
F2s														
Ndogba x Chinese	73.21	**66.78**	512.67	**418.92**	103.07	**99.76**	489.79	**492.23**	865.91	**687.90**	427.34	**410.71**	**0.24**	**0.25**
Ndogba x Sinkara	76.48	**67.91**	690.89	**499.98**	112.08	**109.71**	610.87	**567.10**	941.22	**596.69**	593.61	**401.49**	**0.19**	**0.27**
Chaco-pag x Chinese	78.46	**69.26**	759.91	**678.86**	116.49	**110.92**	689.88	**659.23**	968.42	**602.77**	491.70	**447.76**	**0.24**	**0.25**
Chaco-pag x Sinkara	89.73	**77.11**	849.40	**751.28**	129.21	**147.20**	818.18	**843.42**	989.37	**747.47**	518.66	**501.41**	**0.25**	**0.29**
BCs														
Chinese x Ndogba	39.16	**36.88**	432.09	**361.77**	98.12	**79.51**	401.16	**431.81**	667.12	**358.28**	338.03	**299.89**	**0.29**	**0.27**
Chinese x Chaco-pag	32.26	**36.42**	312.21	**340.83**	86.41	**92.33**	289.99	**293.46**	566.02	**346.72**	282.19	**198.96**	**0.31**	**0.46**
Sinkara x Ndogba	38.97	**44.79**	469.19	**497.96**	96.77	**97.94**	421.12	**412.13**	659.91	**535.33**	376.93	**373.04**	**0.26**	**0.26**
Sinkara x Chaco-pag	34.10	**56.31**	311.23	**469.30**	78.61	**69.79**	277.67	**256.66**	672.92	**491.58**	331.69	**319.29**	**0.24**	**0.22**
Mean	44.30	45.00	433.59	421.84	86.98	85.02	392.64	383.91	653.94	465.67	348.24	307.73	0.26	0.28
Range	23.38	77.11	254.67	751.28	58.90	147.20	209.92	843.42	258.10	747.47	165.14	501.41	0.19	0.46
LSD	13.940		116.501		16.212		128.250		125.989		74.772		0.038	
CV%	48.80	37.10	42.20	32.10	23.60	28.50	44.80	46.90	30.60	31.10	31.90	31.10	19.50	19.30
S.d. (*S*)	467.7	277.8	33627.4	18434.9	419.4	586.9	30876.0	32221.0	39937.7	20953.6	12228.1	2918.1	0.254	0.00299
Com'd S.d.(*S*)	360.8		25228.6		487.9		30550.5		38610.5		10801.2		0.285	

LSD = Least Significant Difference, CV% = Coefficient of Variations (Percentage), S.d. (*S*): Sample Standard deviation, Com'd S.d.: Combined Sample Standard deviation.

for all the measured traits. Nonetheless, the crosses, Sinkara x Chaco-pag (37.22), Chinese x Chaco-pag (66.79), Chinese x Ndogba (448.89), Chinese x Chaco-pag (283.08) and Sinkara x Chaco-pag (0.33) showed highest values for the same traits under water-stressed conditions (Table 5a).

Among the groundnut crosses in F2 population (Table 5a), Chaco-pag x Sinkara scored highest

Table 5b. Range, Mean, LSD, CV (%), Chlorophyll content at 60 and 80 DAP, and drought tolerance indices (DTI) of Parents and F1s, F2s and Back cross populations under well-watered (WW) and end-of-season drought (water-stressed, WS) conditions for five traits.

Groundnut populations	SCMR 60DAP		DTI	SCMR 80DAP		DTI	No. of Pods (Pod Yield), g		DTI	Fresh Biomass Weight (g)		DTI	Dry Biomass Weight (g)		DTI	Harvest Index (HI)		DTI
	WW	WS		WW	WS		WW	WS		WW	WS		WW	WS		WW	WS	
Males																		
Chinese	23.95	4.99	0.21	25.63	33.28	1.30	23.33	26.98	1.16	497.45	414.23	0.83	278.51	227.90	0.82	0.24	0.26	1.08
Sinkara	29.53	6.28	0.21	28.11	37.58	1.34	37.14	39.11	1.05	659.56	512.54	0.78	349.05	331.76	0.95	0.23	0.28	1.22
Females																		
Ndogba	20.01	31.31	1.56	42.54	29.09	0.68	33.33	29.31	0.88	561.22	419.19	0.75	299.42	215.41	0.72	0.24	0.32	1.33
Chaco-pag	20.64	5.31	0.26	23.63	37.49	1.59	30.00	31.73	1.06	549.13	399.16	0.73	284.71	270.46	0.95	0.25	0.29	1.16
F1s																		
Chinese x Ndogba	15.44	22.11	1.43	24.24	29.59	1.22	31.23	35.60	1.17	560.12	448.87	0.80	298.45	237.77	0.80	0.23	0.26	1.13
Chinese x Chaco-pag	22.33	46.46	2.08	32.14	36.59	1.14	35.57	29.99	0.84	587.20	403.07	0.69	297.07	283.08	0.95	0.25	0.24	0.96
Sinkara x Ndogba	19.21	17.74	0.92	26.73	29.93	1.12	25.89	33.81	1.31	459.40	287.96	0.63	239.31	223.51	0.93	0.25	0.29	1.16
Sinkara x Chaco-pag	19.26	11.83	0.61	23.93	25.24	1.05	29.91	37.22	1.24	258.10	198.97	0.77	165.14	181.16	1.10	0.42	0.33	0.79
F2s																		
Ndogba x Chinese	17.48	8.86	0.51	20.54	37.66	1.83	73.21	66.78	0.91	865.91	687.90	0.79	427.34	410.71	0.96	0.24	0.25	1.04
Ndogba x Sinkara	21.99	26.25	1.19	17.19	28.84	1.68	76.48	67.91	0.89	941.22	596.69	0.63	593.61	401.49	0.68	0.19	0.27	1.42
Chaco-pag x Chinese	22.26	28.41	1.28	26.54	37.81	1.42	78.46	69.26	0.88	968.42	602.77	0.62	491.70	447.76	0.91	0.24	0.25	1.04
Chaco-pag x Sinkara	17.93	34.28	1.91	25.43	33.06	1.30	89.73	77.11	0.86	989.37	747.47	0.76	518.66	501.41	0.97	0.25	0.29	1.16
BCs																		
Chinese x Ndogba	20.34	31.14	1.53	25.39	27.38	1.08	39.16	36.88	0.94	667.12	358.28	0.54	338.03	299.89	0.89	0.29	0.27	0.93
Chinese x Chaco-pag	17.70	30.86	1.74	26.18	15.28	0.58	32.26	36.42	1.13	566.02	346.72	0.61	282.19	198.96	0.71	0.31	0.46	1.48
Sinkara x Ndogba	21.11	41.84	1.98	29.05	24.96	0.86	38.97	44.79	1.15	659.91	535.33	0.81	376.93	373.04	0.99	0.26	0.26	1.00
Sinkara x Chaco-pag	19.26	40.04	2.08	20.34	25.78	1.27	34.10	56.31	1.65	672.92	491.58	0.73	331.69	319.29	0.96	0.24	0.22	0.92
Mean	20.50	24.20	1.22	26.10	30.60	1.22	44.30	45.00	1.07	653.94	465.67	0.72	348.24	307.73	0.89	0.26	0.28	1.11
Range	15.40	46.50	1.87	17.20	37.80	1.25	23.33	77.11	0.81	258.10	747.47	0.29	165.14	501.41	0.42	0.19	0.46	0.69
LSD	7.175			4.296			13.940			125.989			74.772			0.038		
CV%	15.80	56.60	55.1	21.60	20.40	27.5	48.80	37.10	20.1	30.60	31.10	12.0	31.90	31.10	12.1	19.50	19.30	16.8
S	10.4	187.70		31.9	38.8		467.70	277.8		39937.7	20953.6		12228.1	2918.1		0.254	0.003	
Comb'd *S*	99.003		0.452	39.44		0.109	30550.50		0.045	38610.87		0.0072	10801.99		0.013	0.285		0.003

DTI: Drought tolerance index, SCMR60DAP: SPAD Chlorophyll Meter Reading at 60DAP, SCMR80DAP: SPAD Chlorophyll Meter Reading at 80DAP, HI: Harvest Index, *(*S*): Sample standard deviation, Comb'd *S:* Combined standard deviation.

values for the traits; pod yield (WW: 89.73, WS: 77.11), Seed yield (WW: 129.21, WS: 147.20), fresh biomass weight (WW: 989.37: WS: 747.47), dry biomass weight (WS: 501.41, 593.61 for Ndogba x Sinkara), and harvest index (WW: 0.25, WS: 0.29) under well-watered environment. Under well-watered condition, the seed yield was highest for Chaco-pag x Chinese (116.49) and Ndogba x Sinkara (593.61) respectively (Table 5a).

The Backcrosses (BCs) (Table 5a) scored the following values among well-watered and water-stressed conditions respectively; Chinese x Ndogba (39.16); Sinkara x Chaco-pag (56.31), Chinese x Ndogba (98.12); Sinkara x Ndogba (97.94), Sinkara x Chaco-pag (672.92); Sinkara x Ndogba (535.33). Also, Sinkara x Ndogba (WW: 37.93; WS: 373.04) and Chinese x Chaco-pag (WW; 0.31; WS: 0.46) were scored for the considered traits (Table 5a).

Across the two water regimes (WS and WW) (Table 5a), the F2 populations recorded highest (70.27) average pod yield for WS environment as against 79.47 for well-watered conditions for average pod yield. The F1s scored the lowest for average pod yield at 34.16 (WS) as against 30.24 (WW) by the Parent 1 respectively (Table 5a).

Average seed yield was highest for the F2 populations for WS at 116.90 and WW: 115.21 respectively. The F1s (WS: 63.22) and (WW: 67.56) scored the lowest in both environments respectively (Table 5a).

Average fresh biomass weight for F2 populations was recorded for WS as 658.71 and 941.23 for WW respectively. The F1 populations had the lowest values of 334.72 (WS) and 446.21 (WW) respectively (Table 5a). A similar trend was observed in average dry biomass weight as follows; WS: 440.34 for F2s, WW: 507.83 also for F2s against the lowest biomass values for F1s at WS: 231.38 and WW: 241.99 in respective cases (Table 5a).

Harvest Index in the current study for the crosses (Table 5a) was highest (0.31) for P2 populations for water-stressed conditions as opposed to 0.29 for F1s under well-watered conditions. Under both water regimes (WS and WW), F2 populations scored lowest figures of 0.26 and 0.23, respectively (Table 5a).

SPAD Chlorophyll Meter Reading at 60DAP values (Table 5b) ranged from 15.40 to 46.50 with the highest value recorded for the male parent Sinkara (29.53) for WW condition and the cross; Chinese x Chaco-pag (46.50) for water-stressed (WS) condition. SPAD Chlorophyll Meter Reading at 80DAP values also ranged from 17.20 to 42.54, with the female, Ndogba scoring the highest value of 42.54 for WW condition and the cross; Chaco-pag x Chinese recording the highest value of 37.81 for the water-stressed (WS) condition (Table 5b). Generally, the SCMR80DAP recorded greater values than SCMR60DAS for almost all the populations. The highest harvest index (HI) values were recorded by the crosses Chinese x Chaco-pag (0.46) and Sinkara x Chaco-pag (0.42) for the water-stressed and well-watered conditions respectively (Table 5b).

The crosses; Chinese x Chaco-pag and Sinkara x Chaco-pag scored equal and the highest drought tolerance indices (DTI) of 2.08 for SCMR60DAP whereas DTI for SCMR80DAP was highest with a value of 1.68 for the cross, Ndogba x Sinkara (Table 5b). DTI for pod yield of 1.65 was scored by the cross, Sinkara x Chaco-pag whereas DTI for fresh biomass weight was recorded by the parent Chinese with a value of 0.83. Dry biomass weight had a DTI of 1.10, scored by the cross, Sinkara x Chaco-pag (Table 5b). The highest DTI for Harvest Index was recorded by the cross; Chinese x Chaco-pag with a score of 1.48 among the groundnuts (Table 5b).

Generally, SCMR60 and SCMR80DAP recorded the highest drought tolerance indices (DTI) of 1.22 and 1.22, respectively among the measured traits, with fresh biomass weight scoring the lowest (0.72) (Table 5b).

Phenotypic and genotypic coefficient of variation estimates

Generally, phenotypic coefficient of variation (PCV) estimates in the current study was greater than estimates for genotypic coefficients of variation (GCV) for all the traits studied (Table 6), though a similar trend could be observed between the two. GCV values ranged from 0.45 to 45.82%, and PCV values ranged from 1.31 to 45.86% (Table 6).

Fresh biomass weight recorded high GCV (45.82%) and PCV (45.86%) respectively. Seed weight and seed yield scored GCV (41.18%); PCV (41.22%), and GCV (25.41%); PCV (25.63%), respectively. Pod weight recorded GCV and PCV of 32.58 and 32.63% whereas pod yield scored similar figures of 30.23 and 30.59%, respectively for GCV and PCV estimates. Height at 50% flowering and height at harvest recorded GCV and PCV values respectively of 31.70%; 33.15% and 35.23%; 35.85% respectively. The traits, days to 50% flowering and days to maturity recorded low GCV and PCV estimates (Table 6).

Narrow sense heritability estimates

Estimates from the Narrow sense heritability from the variance components for different traits under the current study ranged from 12.2 to 95.7% (Table 6). Very high heritability estimate figures were obtained for dry biomass weight (95.7%), days to 50% flowering (91.0%), seed yield (90.0%), plant height at harvesting (76.0%) and SCMR60DAP (71.70%), whereas moderate estimates were found for days to maturity (67.0%), SCMR80DAP (66.0%), plant height at flowering (62.5%), seed weight (60.0%), fresh biomass weight (59.1%) and pod weight (56.00%). Pod yield (12.30%) and harvest index (12.20%) exhibited low heritability estimates, but rather scored very high values for broad sense heritability (98.0%), and (69.50%) respectively (Table 6).

Drought tolerance

Based on the evaluation of populations 1 and 2, individual

Table 6. Components of variation for different groundnut traits

Trait	Mean	MSg	MSe	σ^2_p	σ^2_g	σ^2_e	GCV (%)	PCV (%)	h^2_n	H^2_b	GA	LSD
Days to 50% to emergence	7.083	0.487	0.278	-	-	0.47	-	-	-	-	0.0910	-
Days to 50% flowering	25.583	5.780	4.611	1.10	-1.20	2.30	-	4.10	0.910	0.545	-11.1912	-
Days to maturity	89.167	10.797	1.380	1.37	0.16	1.21	0.45	1.31	0.670	0.120	7.2858	-
Plant height at flowering	15.417	22.366	7.978	26.12	23.88	2.24	31.70	33.15	0.625	0.914	48.6159	-
Plant height at harvesting	47.367	103.461	105.341	288.38	278.46	10.24	35.23	35.85	0.760	0.970	1108.3366	-
Pod yield	44.624	2525.122	40.191	186.35	181.92	4.43	30.23	30.59	0.123	0.980	-405.1983	13.940
Pod weight	427.712	133912.917	9127.248	19475.09	19421.42	53.67	32.58	32.63	0.560	0.997	3641449.991	116.501
Seed yield	86.001	2909.765	129.114	485.93	477.52	8.41	25.41	25.63	0.900	0.983	-6341.3865	16.212
Seed weight	388.272	184715.463	7711.237	25609.15	25558.82	50.33	41.18	41.22	0.600	0.998	-5836228.995	128.250
Fresh biomass weight	559.806	174693.435	18450.512	65904.7	65795.42	109.28	45.82	45.86	0.591	0.998	-3024909.87	125.989
Dry biomass weight	327.982	62900.278	3082.824	8537.48	8490.12	47.36	28.09	28.17	0.957	0.994	-499250.359	74.772
Harvest Index (HI)	0.271	0.0024	0.0029	-0.082	-0.114	0.032	-	-	0.122	0.695	0.0001	0.038
SCMR60DAP	22.40	106.94	97.94	206.05	194.84	11.21	62.37	64.14	0.717	0.946	727.17	7.175
SCMR80DAP	28.30	64.21	35.81	69.880	63.82	6.06	28.19	29.50	0.660	0.913	635.503	4.296

MSg = Mean sum of squares due to genotypes, MSe = Mean sum of squares due to error, σ^2p=Phenotypic variance, σ^2g=Genotypic variance, σ^2e=Environmental variance, PCV=Phenotypic coefficient of variation, GCV=Genotypic coefficient of variation, h^2_n = Heritability in the narrow sense, H^2_b =Heritability in broad sense, GA=Genetic advance, CV(%) = Coefficient of variation (percentage), LSD = Least Significant Difference.

Table 7. Selected drought-tolerant genotypes.

S/N	Male parent	Female parent	Selected drought-tolerant (F1) hybrids (crosses)
1	Chinese	Ndogba	Chaco-pag x Sinkara
2	Sinkara	Chaco-pag	Chinese x Ndogba
3	-	-	Chaco-pag x Chinese

accessions (F1 hybrids) that showed drought tolerance from the segregating F2 populations were selected as follows (Table 7).

Correlations among groundnut populations across water regimes

Among the male and female parents (Table 8), strong, significant (F ≤ 0.05) and positive correlation was recorded between pod yield and pod weight (r = 0.9392), seed yield (r = 0.8884), seed weight (r = 0.9316), and dry biomass weight (r = 0.7218) (Table 8). In a similar manner, pod weight strongly, positively and significantly (F ≤ 0.05) correlated with seed yield (r =0.9309), seed weight (r = 0.9050) and dry biomass weight (r = 0.7835). Seed yield associated strongly, positively and significantly (F ≤ 0.05) with seed weight (r = 0.9351), and dry biomass weight (r = 0.8343) (Table 8).

Seed weight was positively and significantly (F ≤ 0.05) correlated with dry biomass weight at r = 0.7579. Fresh biomass weight scored a positive and strong association with dry biomass (r = 0.8254) but a significant (F ≤ 0.05) and negative correlation with harvest index (HI) (-0.7364) in the

Table 8. Correlations among parents (Males: Chinese, Sinkara and Females Ndogba, Chaco-pag) across water regimes.

Variable	Pod yield	Pod weight	Seed yield	Seed weight	Fresh biomass	Dry biomass
Pod yield	-					
Pod weight	0.9392*	-				
Seed yield	0.8884*	0.9309*	-			
Seed weight	0.9316*	0.9050*	0.9351*	-		
Fresh biomass	0.4942	0.5483	0.5224	0.4548	-	
Dry biomass	0.7218*	0.7835*	0.8343*	0.7579*	0.8254	-
Harvest Index	0.0039	-0.0965	-0.0412	0.0042	-0.7364*	-0.5743

*Significant at $P \leq 0.05$.

Table 9. Combined correlation among groundnut populations across water regimes (WW and WS).

Variable	Pod yield	Pod weight	Seed yield	Seed weight	Fresh biomass	Dry biomass
Pod yield	-					
Pod weight	0.9197*	-				
Seed yield	0.8504*	0.8847*	-			
Seed weight	0.9040*	0.9403*	0.9402*	-		
Fresh biomass	0.7587*	0.7485*	0.7514*	0.7224*	-	
Dry biomass	0.8731*	0.8757*	0.8610*	0.8668*	0.9019*	-
Harvest Index	-0.2726	-0.2524	-0.0966	-0.1781	-0.5394*	-0.5420

*Significant at $P \leq 0.05$.

current study (Table 8).

A combined correlation analysis (Table 9) among the groundnut populations across water regimes (WW and WS) in the current study produced significant ($P \geq 0.05$) association among many of the measured traits (Table 9).

Pod yield recorded a significant ($F \leq 0.05$) and positive association with pod weight (r = 0.9197), seed yield (r = 0.8504), seed weight (r = 0.9040), fresh biomass (r = 0.7587) and dry biomass (0.8731). Pod weight revealed a positive and significant ($F \leq 0.05$) with seed yield (r = 0.8847), seed weight (r = 0.9403), fresh biomass (r = 0.7485) and dry biomass (r = 0.8757). A positive and significant relationship was observed between seed yield and seed weight (r = 0.9402), fresh biomass (r = 0.7514) and dry biomass (0.8610). Similarly, there was an association between seed weight and fresh biomass (0.7224) as well as dry biomass (0.8668). Among the groundnut populations across the water regimes, fresh biomass correlated positively and significantly with dry biomass (0.9019) but negatively and significantly with harvest index at r = -0.5394 (Table 9).

Mean squares of traits from ANOVA and combined ANOVA across water regimes

Mean squares of traits from the ANOVA for physiological traits and pod yield, and biomass are presented in Table 10. Results indicate that the parents and F1, F2 and BC populations differed significantly ($P \leq 0.05$) for all the physiological traits except for SPAD chlorophyll meter reading at 60DAP, SCMR80DAP and harvest index (HI) (Table 10). Combined ANOVA (Table 10) showed large and significant ($P \leq 0.05$) difference between all genotypes for all traits except for SCMR60DAP and harvest index (Table 10).

Under the combined analysis of variance (Table 11), the two water regimes (well-watered, and water-stressed) differed differently ($P \leq 0.05$) in SCMR80DAP and fresh biomass but non-significantly ($P \geq 0.05$) in SCMR60DAP, pod yield, dry biomass and harvest index (Table 11). The parents (male and female) showed significant ($P \leq 0.05$) difference in SCMR80DAP, pod yield, fresh biomass and dry biomass but no significant ($P \geq 0.05$) difference was observed for SCMR60DAP and harvest index (Table 11). Based on the combined ANOVA, no significant ($P \geq 0.05$) interaction effect was shown between the water regimes and the parents for all the traits except SCMR60DAP (Table 11).

Genotypic variation

Leaf samples of the various groundnut generations such as F_1, F_2, BC_1, BC_2, and their parents, P_1 and P_2 for the two populations were collected for molecular analysis to assess genotypic variations.

Table 10. Mean squares of traits from ANOVA for parental lines and F1, F2 and BC populations Mean Squares.

Source of variation	Df	50% DPF	SCMR60DAP	SCMR80DAP	Pod yield	Fresh biomass Wt.	Dry biomass Wt.	Harvest index (HI)
Parents	4	8.438	106.94	64.21	2525.12*	174693.44*	62900.28*	0.0024179
Error	27	4.025	97.94	35.81	40.19	18450.51	3082.82	0.00291111
Total	31	5.286	99.10	39.47	360.83	38610.89	10801.21	0.0028500

*Significant at P ≤ 0.05, 50% DPF: 50% Days to Plant Flowering, SCMR60DAP: SPAD Chlorophyll Meter Reading at 60DAP, SCMR80DAP: SPAD Chlorophyll Meter Reading at 80DAP, PY: Pod Yield, HI: Harvest index.

Table 11. Mean squares of traits from the Combined ANOVA for parental lines and F1s, F2s and BCs under Well-Watered (WW) and Water-Stressed (WS) conditions Mean Square.

Source of variation	Df	50%DPF	SCMR60DAP	SCMR80DAP	PY	Fresh Biomass	Dry Biomass	HI
Model	9	8.438	168.234*	71.88*	1157.28*	112723.91*	29704.17*	0.002064
Water regime	1	-	3.032	142.98*	3.3859	223093.84*	11842.83	0.006428
Parents	4	8.438	106.938	64.21*	2525.12*	174693.44*	62900.28*	0.0024171
Water Regime X Parents	4	-	244.144*	57.08	77.915	8043.234	651.484	0.0009172
Residual	22	4.025	70.815	26.21	35.0037	8291.93	3068.17	0.0031682
Total	31	5.286	99.098	39.47	360.83	38610.89	10801.206	0.0028475

*Significant at P ≤ 0.05, 50% DPF: 50% Days to Plant Flowering, SCMR60DAP: SPAD Chlorophyll Meter Reading at 60DAP, SCMR80DAP: SPAD Chlorophyll Meter Reading at 80DAP, PY: Pod Yield, HI: Harvest index.

Procedure

DNA samples were extracted from germinating tissues of the various groundnut crosses using the protocol; DNA Extraction – Qiagen Dneasy Kit (www.qiagen.com), in genetic study. Accession number, genotype and entry for the molecular work (PCR study) has are as indicated in Table 12. Eight primers were used to reveal polymorphisms at the molecular level to assess genetic diversity and varietal identification; GM1949, TC7E04, IPAHM103, TC2D06, S11, pPGSseq17F6, Ah2TC7H11 and GM1954 (Appendix Table 1).

DISCUSSION

Components of variation

GCV values ranged from 0.45 to 45.82%, while PCV values ranged from 1.31 to 45.86%. Phenotypic coefficient of variation (PCV) provides a measure of the total relative variation that exists in a particular trait (Roychowdhury and Tah, 2011). Genotypic coefficient of variation (GCV) gives an estimate of the amount of variation present in a particular character (Narasimhulu et al., 2012). Phenotypic coefficient of variation (PCV) estimates in the current study was generally greater than estimates for GCV for all the traits studied. This observation implies that there existed generally greater total relative (comparative) variation or diversity among the groundnuts studied.

Fresh biomass weight recorded high GCV (45.82%) and PCV (45.86%) respectively. Seed weight and seed yield scored GCV (41.18%); PCV (41.22%), and GCV (25.41%); PCV (25.63%) respectively. Pod weight recorded GCV and PCV of 32.58 and 32.63% whereas pod yield scored similar figures of 30.23 and 30.59%, respectively for GCV and PCV estimates. Height at 50% plant flowering and height at harvesting recorded GCV

Table 12. Accession number, genotype/population and entry of groundnut genotypes based on genotypic variation.

DNA wel position	Genotype (population)	Entry
1	Chaco-pag	Female parent
2	Chinese	Male parent
3	Ndogba	Female parent
4	Sinkara	Male parent
5	Chaco-pag x Chinese	F1
6	Chaco-pag x Chinese	BC
7	Chaco-pag x Sinkara	F1
8	Chaco-pag x Sinkara	BC
9	Ndogba x Chinese	F1
10	Ndogba x Chinese	BC
11	Ndogba x Sinkara	F1
12	Ndogba x Sinkara	BC

and PCV values respectively of 31.70, 33.15 35.23 and 35.85%, respectively. Studies by Sumathi et al. (2010), Roychowdhury and Tah (2011) and Narasimhulu et al. (2012) have revealed similar results in which PCV estimates proved to be higher than GCV estimates for most traits studied, which indicates the effect of environment on the expression of characters.Narrow sense heritability estimates from the variance components for different traits ranged from 12.2 to 95.7%. Very high heritability estimate figures were obtained for dry biomass weight (95.7%), days to 50% flowering (91.0%), seed yield (90.0%), plant height at harvesting (76.0%) and SCMR60DAP (71.70%), whereas moderate estimates were obtained for days to plant maturity (67.0%), SCMR80DAP (66.0%), plant height at flowering (62.5%), seed weight (60.0%), fresh biomass weight (59.1%) and pod weight (56.00%). Pod yield (12.30%) and harvest index (12.20%) exhibited low heritability estimates.

In the current study, heritability estimate for Narrow sense heritability from the variance components were very high for the traits; dry biomass weight (95.7%), days to 50% flowering (91.0%), seed yield (90.0%), plant height at harvesting (76.0%) and SCMR60DAP (71.70%), whereas moderate estimates were found for days to plant maturity (67.0%), SCMR80DAP (66.0%), plant height at flowering (62.5%), seed weight (60.0%), fresh biomass weight (59.1%) and pod weight (56.00%). This generally indicates that these characters are governed by additive gene action; hence, heterosis breeding will be useful. These characters can be improved through selection in a future groundnut breeding programme. Heritability in the narrow sense is useful for plant breeding in selection of elite types from segregating populations. Thus, crosses are made in a definite fashion in order to determine estimates of the variances and hence, heritabilities. When heritability in the narrow sense is high, it indicates characters are governed by additive gene action; therefore, heterosis breeding will be beneficial.

Even though pod yield (12.30%) and harvest index (12.20%) exhibited low narrow sense heritability (h^2) estimates, they recorded very high broad sense heritabilities (98.0%), (69.5%) respectfully. Therefore, selection for improvement of pod yield and harvest index traits may be useful in a groundnut breeding programme. If heritability in the broad sense (H^2) is high, it means characters are least influenced by the environment, hence, selection for improvement of such characters may be useful.

Genetic Advance (GA) was observed in the current study to have recorded very high values for most traits studied. Genetic variability therefore exists among the current selected and studied groundnuts. Genetic advance (GA) explains the improvement in the mean genotypic value of selected plants over the parental population. It is the measure of genetic gain under selection. The greater the amount of genetic variability in the base populations, the higher the genetic advance. The GA is high with characters having high heritability. Moreover, the higher the selection intensity, the better the results. Low GA indicates the character is highly influenced by environmental effects, thus, genetic improvement through selection will be difficult. Where GA is high, the character is governed by additive genes and selection will therefore be beneficial for such traits (Roychowdhury and Tah, 2011; Songsri, et al., 2008; Ali and Wynne, 1994).

Markers (Appendix Table 1) used in the current study were highly informative for linkage analysis; genetic diversity and varietal identification in the groundnut genotypes (populations) studied. There was considerably high but varying levels of polymorphism revealed by these SSR markers for drought tolerance in groundnuts. More than fifty percent of the primers used in the current study indicated polymorphism among the groundnuts. Tang et al. (2007) obtained high level of polymorphic information for similar SSR primers studied in groundnuts. While primers GM1954, Ah2TC7H11 and pPGSseq17F6 revealed greater diversity at the gene level among the male and female parents as well as their F1s and backcross populations, primers IPAHM103, TC7E04 and GM1949 showed relatively low genetic diversity.The female parents showed greater polymorphism as revealed by the primer GM1949 whereas the male parents proved polymorphic at the gene level according to the primers GM1949 and Ah2TC7H11. The F1s showed considerably great diversity and polymorphism as revealed by the primer IPAHM103. However, primers GM1949, S11 and Ah2TC7H11 showed considerably high variation among the backcross populations.

According to Dwivedi et al. (2001), Mace et al. (2006) and Shoba et al. (2010), different levels of polymorphism exist in cultivated groundnut. He and Prakash (2001), and Selvaraj et al. (2009) have reported low level of genetic diversity in the groundnut gene pool in comparison with other crops. However, simple sequence repeat (SSR) markers have been able to detect a relatively higher level of variation (Mace et al., 2006), as they found up to 56% diversity in cultivated groundnut with SSR markers. This trend was also observed by Shoba et al. (2010) who reported values ranging from 0.54 to 1.00 genetic dissimilarities in groundnut.

Groundnut varieties that showed diversity for drought tolerance at the phenotypic level were found to have shown similar diversity at the molecular level as revealed by the primers. There was clear association between marker data and drought tolerance among the groundnut populations. Therefore, the eight primers used in the current study will be very useful in further molecular studies/characterization in commercially cultivated groundnut. Drought-tolerant and higher yielding varieties found in this study can be crossed to drought-susceptible but potentially higher yielding and foliar disease tolerant groundnut varieties in a future breeding programme.

Heritability studies and drought tolerance in groundnut populations

Performance of the males indicated that, 'Sinkara', a farmer preferred variety scored the highest values for pod yield, seed yield and fresh and dry biomass weights and harvest index under both water regimes. Chinese also performed significantly well in terms of pod and seed yields, biomass yields and harvest index. Among the females, Ndogba performed better in terms of the traits; pod yield, seed yield, fresh and dry biomass weights and harvest index respectively under the two water environments, though Chaco-pag also showed significantly high performance. Performance of the groundnut crosses in F2 population showed that the crosses, Chaco-pag x Sinkara, Chaco-pag x Chinese, Ndogba x Sinkara scored significantly higher values for pod yield, seed yield, fresh and dry biomass weights and harvest index under the two water conditions. All the back-crosses; Chinese x Ndogba, Sinkara x Chaco-pag, Sinkara x Ndogba and Chinese x Chaco-pag scored significantly higher values for pod and seed yields and biomass weights.

Across the two water regimes (WS and WW), the F2 populations recorded highest values for WS condition (70.27) as against 79.47 for well-watered conditions for average pod yield. The F1s scored the lowest for average pod yield at 34.16 (WS) as against 30.24 (WW) by the Parent 1 respectively.

F2 populations recorded highest average seed yield, fresh and dry biomass weights, among the groundnuts under the two water regimes. However, the F1 population scored the lowest in both environments. Harvest index in the current study for the crosses was highest for P2 populations for water-stressed conditions as opposed to F1s under well-watered conditions. Under WS and WW conditions, F2 populations scored the lowest harvest index (HI) figures of 0.26 and 0.23 respectively.

Pod yield, fresh and dry biomass, pod and seed weights generally decreased under drought stressed environment whereas SCMR60 and SCMR80DAP increased. Earlier studies under several environmental conditions by Nigam and Arum (2008), Songsri et al. (2009), and Girdthai et al. (2010) corroborates these results. Drought tolerance index (DTI) was useful in explaining how some genotypes had higher pod yield, seed yield, biomass and harvest index under drought-stressed conditions. The crosses, Chinese x Chaco-pag, Sinkara x Chaco-pag, Ndogba x Sinkara showed high promise and could therefore, pass as promising drought-tolerant progenies. Studies by Nigam et al. (2001) and Surihan et al. (2005) on inheritance of drought-tolerance indicated a principal role of additive gene effects in specific leaf area and harvest index. Painawadee et al. (2009) stated that loss of moisture from plant cells could affect the concentration of chlorophyll. Groundnut accessions that recorded high SCMR possess more photosynthetic machinery per unit leaf area and thus have the capability for better assimilation under drought-stress conditions (Songsri et al., 2009). The estimates of phenotypic coefficient of variation (PCV) were greater than genotypic coefficients of variation (GCV) for all the physiological traits. The traits pod yield, biomass and harvest index showed moderate PCV estimates. High values of GCV indicate that these traits can be easily improved by selection (Reddy et al., 2013). Narrow sense heritability estimates varied under both well-watered and drought-stressed conditions.

The heritability estimates for pod yield (12.3%) and fresh (59.1%) and dry biomass (95.7%) were low and moderately high respectively. Heritability values for Harvest index (12.2%) and SCMR60 (71.7%) and SCMR80DAP (66.0%) proved very high and moderate respectively. Days to fifty percent (50%) plant flowering showed very high (91.0%) heritability estimate, which is contrary to results found by Songsri et al. (2008) who found moderate figures for end-of-season drought stress for all the physiological traits except for pod yield. Girdthai et al. (2012), in a similar study found high values for broad sense heritability, results that are in agreement with those found in the current study where broad sense heritability estimates were very high for pod yield (98.0%), pod weight (99.7%), seed yield (98.3%), seed weight (99.8%), fresh (99.8%) and dry biomass (99.4%), harvest index (69.5%) and SCMR60DAP (94.6%) and SCMR80DAP (91.3%).

Selection for higher yield among drought tolerance traits is conceivable among the studied groundnut populations because of higher heritabilities. Tsaur et al. (1989) reported high heritability for pod and seed yield, among other traits studied. Holbrook et al. (1989) reported high heritability estimates for maturity in their research study involving F1 and F2 plants and some late-maturing groundnut lines.

Highly significant and positive association between pod yield and harvest index was found in both water regimes. Simultaneous improvement of these traits should be possible. Opportunity for indirect selection of such traits (pod yield and harvest index) is also achievable.

Warunyuwat and Tongsri (1990) reported highly significant correlations between pod and seed yield, pod yield and number of mature seeds per plant, and seed yield and number of mature seeds per plant, whereas shelling percentage had varying correlation with pod and seed traits in different generations.

Wuma et al. (2009) reported moderate correlation figures between HI and biomass in a research study under early drought and irrigated condition. Similar findings were found by Ravi et al. (2012) for SCMR and harvest index. Whether through direct or indirect selection of these significant associations among yield and yield-related components or traits, when properly harnessed, would aid or simplify the breeder’s work in any crop improvement programme.

In times past, breeders focused their attention on earliness as a drought-escape mechanism, especially when dealing with end-of-season drought because that was easily predictable. Currently, climate variability has made this increasingly difficult to achieve. Rainfall has become very unpredictable, floods and intermittent drought spells have become recurrent. This makes the drought-escape approach insufficient because it is hard to predict the end-of-season drought. Notwithstanding, drought-escape mechanisms are still valuable. Early-maturity and drought-tolerant crosses identified in this study could be exploited in a bid to developing new and promising varieties, based on their evaluation across different environments. Genetic variability for drought-tolerance among groundnut accessions, through conventional breeding, can be identified and the genetic variation that is identified can be incorporated through different mating designs into cultivars with promising agronomic characteristics. Relationships between farmers and seed companies and/or research institutions as well as Extension Officers under the Ministry of Food and Agriculture (MoFA) have to necessarily be reinforced and sustained in order to implement a viable groundnut breeding programme in Ghana. Farmers' confidence in groundnut production should be restored by development of new improved early, drought or disease-tolerant groundnut varieties. To achieve success through traditional breeding, several selection and breeding cycles are essential. This is because, conventional plant breeding is a very time-consuming and cost and labour-intensive venture. When transferring desired genes from one plant to other through the use of conventional plant breeding procedures, a number of undesired genes are also transferred. The limited success regarding the improvement of crops to drought-tolerance is because drought tolerance is controlled by multiple genes with additive effects; with a strong interaction existing thereof between the genes for drought-tolerance and those involved in yield potential. There is therefore the need to adopt more efficient and workable methods for genetically modifying crops for enhanced drought-tolerance. Marker-assisted selection (MAS) has currently made it conceivable to evaluate several thousands of genomic regions of a crop under water-stressed regimes (Ashraf, 2010). Quantitative trait loci (QTLs) for drought tolerance have been reported in previous research, which can be exploited to introgress drought-tolerant related traits such as transpiration, TE, SLA, SCMR into elite early maturing variety (Ravi et al., 2010). Based on farmers' perceptions about early-maturity and drought-tolerance, breeding interventions could be targeted on preferred and ideal varieties that can combine earliness, drought and disease-tolerance and also high yielding. Marker assisted backcrossing could be employed in the development and or improvement of ideal varieties in a more efficiently manner.

In terms of climate change variability and crop breeding, breeding interventions in the near future, should target drought-tolerance and high temperatures. Thus, a better understanding of the interactions as well as the relationships that exist between biotic and abiotic stresses should be established in developing a workable and sustainable breeding programme. Conclusively, the results from the genetic analysis in the current study show that it is feasible to select for both earliness and drought-tolerance in early generations. Information generated from this study can be used to develop new groundnut varieties that combine both traits. Marker assisted selection procedures could help enhance this process based on the availability of QTLs and genes for the traits and markers developed in that regard. Additive gene effects largely controlled the inheritance of pod yield, seed yield, biomass weight, and harvest index. Based on the positive association between most yield and yield components as well as heritability estimates, these traits could be used to improve yield of groundnut. Estimates of days to 50% plant flowering and days to plant maturity give a positive indication as good criterion for earliness selection. High heritability estimates observed by most traits assessed in the current study indicate that breeding progress should be conceivable. SCMR is a very useful selection approach and criterion for drought-tolerance in groundnut due to high heritability and ease of data collection. Groundnut lines with the capability to maintain high chlorophyll content and high biomass under water-deficit (drought) situations could as well show better tolerance to drought.

Conclusion

There was the influence of additive gene action on the governance and expression of the inheritance of traits such as pod yield, seed yield, seed weight, biomass weight and maturity index. Very high, high and moderate narrow sense, and in most cases, broad sense heritabilities among some traits such as seed weight and yield and fresh and dry biomass yields coupled with their positive and significant correlation and relationship with pod yield, signifies that these traits could be good criteria for yield selection in improvement programmes to groundnut in Ghana. High heritability estimate for days to maturity in association with yield parameters could present a good criterion for earliness selection due to its strong and positive correlation with days to emergence and flowering.

The variety Sinkara was identified as the best male parent for pod yield (WW: 37.14, WS: 39.11), seed yield (89.32; 93.82), seed weight (391.73; 376.22), fresh biomass weight (659.56; 512.52) and dry biomass (349.05; 331.76), under both water regimes. The variety Chinese was the best male parent for days to emergence (6 days), days to 50% flowering (21 DAP) and day to maturity (87 DAP).

Ndogba variety was the best female parent for pod yield under well-watered environment (WW): (33.33), seed yield (72.11), fresh biomass weight (561.22; 419.19) and dry biomass weight (299.42); whereas Chaco-pag variety performed best under water-stressed (WS) environment respectively at WS: 31.73 for pod yield, 77.63 for seed yield, WW: 298.03, WS: 303.56 for seed weight and 270.46 for dry biomass weight.

Female variety, Ndogba performed best in terms of days to emergence (7 DAP), days to 50% flowering (22 DAP) and days to maturity (89 DAP).

Many of the physiological characters measured in the groundnut population recorded high heritability estimates, an indication that significant progress can be made in future breeding programme through selection. SCMR60DAP was highest for the male parent, Sinkara (WW: 29.53; WS: 6.28). SCMR80DAP was again highest for Sinkara (WW: 28.11; WS: 37.58) with the males recording the highest drought tolerance index of 1.34. Among the female parents, Ndogba scored highest SCMR60DAP at 42.54, whereas the female parent Chaco-pag scored 37.49. Drought tolerance index (DTI) among the female parents was 1.56. Among the F1s, the cross, Chinese x Chaco-pag recorded the highest DTI (2.08) for SCMR60DAP. DTI for SCMR80DAP was highest for the cross, Chinese x Ndogba (1.22).

The highest DTI for pod yield (1.24), fresh biomass (0.80), dry biomass (1.10) and harvest index (1.16) was scored by the crosses, Sinkara x Chaco-pag, Chinese x Chaco-pag, Sinkara x Chaco-pag and Sinkara x Ndogba, respectively. Among the F2s, the crosses; Chaco-pag x Sinkara (1.91), Ndogba x Chinese (1.83), Ndogba x Chinese (0.91) and Ndogba x Sinkara (1.42) recorded highest drought tolerance indices respectively. Back Cross population showed highest DTI for the crosses Sinkara x Chaco-pag (2.08), Sinkara x Chaco-pag (1.27), Sinkara x Chaco-pag (1.65) and Chinese x Chaco-pag (1.48), respectively.

As per the results of the study, harvest index (HI) and SPAD chlorophyll meter reading observations can easily and conveniently be recorded at both well-irrigated and water-stressed environmental conditions. Groundnut breeders are therefore afforded the flexibility of collecting these observations and parameters in larger number of segregating populations and breeding lines, hence, making it easier to incorporate these physiological characters associated with drought tolerance in breeding and selection programmes in groundnut. Due to high heritability and ease of collecting data, SPAD chlorophyll meter reading could be very useful as a selection criterion for drought tolerance in groundnuts. Groundnut genotypes that show potential and ability to maintain significantly high chlorophyll content and high fresh and dry biomass under water-stressed or limited environments and conditions could also possibly show better tolerance to drought.

High heritability estimates recorded by harvest index (HI) together with strong, significant and positive relationship with pod yield, seed yield and biomass under both well-watered and water-stressed conditions suggest that harvest index (HI) could also be considered as a selection criterion capable of guaranteeing improvement and progress for pod yield in a future breeding programme in Ghana.

The SSR markers used in this study detected relatively high levels of polymorphism and were successful in distinguishing groundnut genotypes with various levels of drought-tolerance. In this study, it was shown that moderate levels of genetic variation could be detected effectively in cultivated groundnut using SSR markers. The grouping of the genotypes at molecular level indicated a clear distinction between parents, F1s and their backcross populations among groundnut with differential levels of drought tolerance. This molecular study has provided useful information toward parental selections and specific SSR markers that can be used for varietal identification.

The assessment of genetic diversity of drought-tolerant groundnut genotypes present in the working germplasm collection would help groundnut breeders to formulate crosses by choosing parent with different genetic backgrounds and will assist in the development of gene-mapping populations with greater marker polymorphism.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Appendix 1. Groundnut SSR Primers used for the study of genetic diversity and varietal identification in groundnuts

No.	SSR Marker id (Name)	Forward Sequence (5'- 3')	Reverse Sequence (5'- 3')	Annealing T^o (Melting Temperature - 5)
1	GM1949	GCACCAATAGAAAATGCCAAA	CAGCAACAGCAACAATTCTGA	52
2	TC7E04	GAAGGACCCCATCTATTCAAA	TCCGATTTCTCTCTCTCTCTCTC	56
3	IPAHM103	GCATTCACCACCATAGTCCA	TCCTCTGACTTTCCTCCATCA	56
4	TC2D06	AGGGGGAGTCAAAGGAAAGA	TCACGATCCCTTCTCCTTCA	52
5	S11	TTACATGCCTTACGCTGCTG	TGAGCAAAGCATCCATGAAG	52
6	pPGSseq17F6	CGTCGGATTTATCTGCCAGT	AGTAGGGGCAAGGGTTGATG	56
7	Ah2TC7H11	CCAGTTTAGCATGTGTGGTTCA	CACGACGTTGTAAAACGACTTAGCGACAAAGG ATGGTGAG	56
8	GM1954	GAGGAGTGTGAGGTTCTGACG	TGGTTCATTGCATTTGCATAC	56