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RESEARCH ARTICLE

Genetic variability and association studies for drought tolerance in groundnut

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Abstract

The current study was undertaken to estimate variability (phenotypic and genotypic), heritability, genetic advance (GA), correlation and path analysis among traits of 25 groundnut genotypes evaluated under drought or moistures stress and well-watered condition. The results indicated high genetic coefficient of variation (GCV), coupled with high genetic advance (GA), genetic advance as percent of mean (GAM) and heritability for number of mature pods per plant, biomass, pod yield per plant and SPAD chlorophyll meter reading. Seed yield was highly significant and positively correlated with biomass, pod yield, relative water content, SPAD chlorophyll meter reading, harvest index, shelling percentage and days to maturity. Furthermore, path analysis showed that pod yield and SHP had the highest direct and indirect effects on seed yield respectively. Therefore, integration of these traits in drought tolerance or moisture stress tolerance breeding scheme would be advantageous in selecting groundnut genotypes that are more capable in water use efficiency.

Key words: Correlation, genetic variability, groundnut, selection

Introduction

Groundnut (*Arachis hypogaea* L,) is one of the major sources of food and income for smallholder's farmers in Malawi. It is a valuable food security crop that supplies fats and proteins to the predominantly maize–based Malawian diet. Although, groundnut production is a profitable venture for smallholder farmers in Malawi, its total production has remained low (Longwe-Ngwira *et al*., 2012). Groundnut production in Malawi relies on rainfed agriculture, of which drought is a major production constraint (Simtowe *et al*., 2010). Monyo and Gowda (2014) reported that current groundnut yields in Malawi have remained low averaging less than 1 t ha^{-1} compared to the yield of about 4 t ha^{-1} obtained at research stations. The low groundnut yields are attributed to unreliable rainfall, often with midseason and terminal droughts (Simtowe *et al*., 2012). Therefore, breeding for drought tolerance would be an important strategy for alleviating low yield in groundnut.

Selection based on yield under drought conditions has been slow and ineffective because of the complex nature of seed yield, which is influenced by many interrelated traits directly or indirectly (Girdthai *et al*., 2010; Shoba *et al*., 2012; Nigam, 2014). Path coefficient analysis is a helpful tool for making decisions on selection criteria based on the influence of component traits (Vara Prasad and Shivan, 2017). Additionally, success from selection for an economic trait depends on the magnitude of genotypic variability existing within a particular population (John, 2010; Jandong *et al*., 2019; Khan and Khan Amin, 2019). However, the overall genotypic variation needs to be partitioned into heritable and non-heritable portion using genetic parameters such as phenotypic coefficient of variations (PCV), genotypic coefficient of variations (GCV), genetic advance (GA), genetic advance as percent of mean (GAM) and broad sense heritability (BSH). These parameters have mostly been advocated for theoretically, but few attempts have been made to establish genetic control under drought stress condition (Savita *et al.*, 2014; Thakur *et al.*, 2013). Therefore, the current study was undertaken to determine the extent of genotypic variability, correlation coefficients and path coefficients among the traits. This information will guide decision regarding the selection procedures to be employed for identifying superior groundnut genotypes under drought conditions.

Material and Methods

Plant material

The experimental material comprised of 25 F3 lines developed from crossing of 10 parents selected at ICRISAT, Chitedze, Malawi. Genotypes ICGV-SM 99551, ICGV-SM 99555, ICGV-SM 01721, CG 7 bred in Malawi and Pendo bred in Tanzania; were used as drought susceptible female parents with different attributes. Pendo and ICGV – SM 99551 are *Spanish* bunch types and early maturing varieties released and grown in Tanzania. ICGV – SM 99555 is a *Spanish* bunch type, early maturing and rosette resistant variety released in Tanzania (Monyo, 2010). CG 7 is a *Virginia* bunch type that is high yielding, with high oil content and wide adaptability. It has been released in Malawi, Tanzania, Uganda, Zambia and Mozambique (Subrahmanyam *et al*., 2000). ICGV-SM 01721 is a high yielding and rosette resistant *Virginia* bunch type bred in Malawi and released in Tanzania (Monyo, 2010). Akwa, Malimba, Baka, ICGV-SM 02724 and ICGV-SM 94139, which are sources of drought tolerance were used as male parents. Baka is an early maturing and aphid resistant *Spanish* bunch type bred in India and has been released in Malawi, Uganda, Zambia and Mozambique (Deom *et al*., 2006). Akwa is *Valencia* bunch type and early maturing variety released in South Africa (Merwe and Joubert, 1995). ICGV – SM 02724 is a drought tolerant, high yielding and rosette resistant line, which has not been released. Malimba and ICGV – SM 99139 are *Spanish* bunch types bred in Malawi and recommended for low lands (altitude of 200 to 300 masl) and rosette resistance, respectively. Crosses evaluated in this study were developed in 2015/2016 and 2016/2017 seasons at International Crops Research Institute for Semi-Arad Tropics (ICRISAT), Chitedze, Malawi, station using a 5 x 5 NCD II mating design. The F1 seeds of the 25 progenies obtained from the crosses were selfed up to F_3 generations. Selfing was done to multiply seeds for evaluation and to allow segregation among genotypes.

Glasshouse experiment

The pot experiment was conducted in a glass house at the International Crops Research Institute for Semi – Arid Tropics (ICRISAT) Center at Chitedze in Lilongwe, Malawi during the 2016-2017 season. The soil type used for pot experiment were sandy loamy (humisols soils), rich in organic matter collected from Chilende forest, 5 km from ICRISAT Center (latitude: S $13^0$58’46”, longitude: E 33^0 39’24”, altitude of 1103.07 m above sea level). Pots of 32 cm diameter and 25 cm height were filled with dry soil from the bottom to 5 cm below the top to create uniform bulk density. Twenty-five progenies were planted in pots in a randomized complete block design (RCBD) with four replications. Four seeds were planted per pot and seedlings were thinned to two plants per pot, 14 days after planting (DAP). Almost care has been taken to raise the healthy crop.

Soil moisture regimes in a glasshouse

Three moisture regimes were used in the present study which includes well-watered conditions throughout the season, midseason season drought stress imposed at flowering stage and late season drought stress imposed during seed filling. Initially water was maintained at field capacity (FC) from planting to 30 DAP. Stress was induced by withholding water at 30 DAP for midseason drought treatment and was maintained at 1/3 of available water (AW) to 60 DAP, then watering was resumed to FC until harvest. For terminal drought stress treatment, stress was induced at 60 DAP and was maintained at 1/3 AW to 90 DAP then resumed at FC up to harvest as described by Painawadee *et al*., (2009). In the control treatment, water was kept at field capacity (FC) throughout the season until harvest.

Field experiment

The field experiment was carried out at National Agricultural Research Station (NARS), Ngabu in Chikwawa region, southern Malawi (34^0 53’43.04” E, 16^0 27’28.89” S, altitude of 110 masl), located 425 km south of Chitedze ICRISAT Centre. It is characterized by warm and dry conditions. The site has clay loam–vertisol soils with pH 7.12, organic carbon (OC) 1.01%, organic matter (OM) 2.05%, total N 0.30%, phosphorus (P) 8.27 ppm, potassium (K) 1.00 meq/100g, calcium (Ca) 25.55 meq/100g, magnesium (Mg) 5.45 meq/100g and sodium (Na) 0.48 meq/100g. The experiment was carried out from December 2016 to June 2017 in a drought-testing site under natural rain-fed conditions. It was laid out in a randomized complete block design with 4 replications. Seeds were sown in plots of four rows of 5 m length, with inter-row spacing of 70 cm and intra-row spacing of 15 cm. Two seeds were planted per hill and then seedlings were thinned to one plant per hill 14 days after emergence. The variety JL 24 was grown around the trial as a guard row to avoid damage from animals and boarder effects. Recommended agronomic and plant protection measures were performed as suggested by Santos *et al*., (2006).

Data collection

Relative water content (RWC) was determined following laboratory procedures as proposed by Bajji *et al*., (2001). The SPAD chlorophyll meter reading (SCMR) and specific leaf area (SLA) were recorded at 60 DAP after moisture stress imposition as suggested by Nigam (2014). SCMR was measured using a handheld portable SCMR meter (SPAD – 502 Plus, Spectrum Technology, USA) on four leaflets per plant. Determination of SLA was done following the procedures suggested by Wilson *et al*., (1999). Plant height (PH), number of primary branch (NPB) and days to maturity (DM) were recorded during the growth period.

At harvest, pod yield (PY) and shelling percent (SHP) and seed yield (SY) were recorded. Shelling percentage was calculated based on formula suggested by Painawadee *et al*., (2009). Root and above ground samples were oven-dried at 80^0C for 48 hours and dry weights were recorded. The harvest index (HI) was calculated based on the relationship suggested by Nautiyal *et al*., (2002).

Data analysis

Pearson's correlation coefficients (*r*) were calculated using SPSS version 23 software (SPSS, 2012) to determine the relationship between yield and the yield attributing traits. Correlation coefficients were further partitioned into direct and indirect effects on seed yield through path coefficient analysis using the procedures suggested by Dewey and Lu (1959). The phenotypic coefficient of variations (GCV) and genotypic coefficient of variations (GCV) were calculated as suggested by Singh and Chaudhury (1985). The genetic advance (GA) was calculated according to Johnson *et al*., (1955) and the genetic advance as percent of mean (GAM) as suggested by Shukla *et al*., (2006). The PCV and GCV values were categorised as proposed by Sivsubramanian and Menon (1973).

Results and discussion

Genetic variability, heritability and genetic advance of drought tolerance traits

Based on the ANOVA table (not shown) indicated that good amount of genetic variation among the genotypes which justify the selection of the parents. The results for genetic variability among the genotypes for all traits studied are presented in Table 1. The highest GCV was recorded for biomass per plant (160.32), pod yield (96.51), specific leaf area (78.85), seed yield (89.31) and days to maturity (181.61), which indicated existence of extensive genetic variations for these traits among genotypes. Padmaja *et al*., (2013), Mukesh *et al*., (2014), Prabhu *et al*., (2015), Kadam *et al*., (2016), Divyadharsini *et al*., (2017) and Syed Sab *et al*., (2018) reported similar results on pod yield per plant and seed yield per plant. Moderate GCV were exhibited by relative water content (18.93), SPAD chlorophyll meter reading (37.07) and plant height (18.61), whereas root dry weight (3.21), shelling percentage (0.00), number of primary branch (4.28) and harvest index (0.58) displayed very low GCV value. The low GCV exhibited by these traits indicated high influence of the environment in the expression of these traits, which is attributed to their polygenic nature; hence, there is limited scope for selection. Padmaja *et al.,* (2013), Mukesh *et al*., (2014), Savithramma (2016) and Syed Sab *et al*., (2018) reported similar moderate to low GCV for these traits. High PCV was observed for shelling percentage (45.43), plant height (70.87) and number of primary branches per plant (37.15). The high PCV revealed by these traits suggested a greater contribution of environment factor on manifestation of these traits. Padmaja *et al*., (2013), Mukesh *et al*., (2014) and Vasanthi *et al*., (2015) also reported high PCV on these traits. Days to maturity (21.01), specific leaf area (23.13), seed yield (14.97) and pod yield (11.02) exhibited moderate PCV value, while relative water content (6.32), root dry weight (8.12) and SPAD chlorophyll meter reading (3.01) recorded low PCV value, indicating the possible high reliability and repeatability of the data. Similarly, low PCV for these traits were reported in earlier studies conducted by Mukesh *et al*., (2014), Kadam *et al*., (2016) and Syed Sab *et al*., (2018). The PCV for HI were nearer to their corresponding GCV values indicating that, the environment had little influence on both phenotype and genotype expression of these traits. Similar results were also reported by Kalpande *et al*., (2014) who found closer values of PCV and GCV for days to 50% flowering.

Table 1: Estimates of broad sense heritability (BSH), PCV, GCV, GA and GAM for drought tolerant traits at harvest, for F_3 groundnut population

Character	Mean	PCV	GCV	BSH	GA	GAM
Biomass	53.72	2.76	160.32	0.90	119.87	123.15
Pod yield/ plant	16.87	11.02	96.51	0.95	18.04	106.95
Relative water content	77.60	6.32	18.93	0.63	6.17	7.94
Root dry weight	2.13	8.12	3.21	0.28	0.81	38.28
SPAD Chlorophyll meter reading	44.98	3.01	37.07	0.85	25.33	56.32
Specific leaf area	154.57	23.13	78.85	0.77	7.02	4.54
Shelling percentage	69.23	45.43	0.00	0.35	0.00	0.00
Seed yield	11.76	14.97	89.31	0.94	12.29	104.53
Days to maturity	105.31	21.01	181.65	0.99	17.81	16.91
Number of primary branch	4.98	37.15	4.28	0.38	0.24	4.76
Plant height	60.83	70.87	18.61	0.18	0.54	0.89
Harvest index	0.33	0.29	0.58	0.71	4.07	124.36

The broad-sense heritability is the ratio of total genetic variance to total phenotypic variance. High broad sense heritability estimates were observed for days to maturity (0.99), pod yield per plant (0.95), seed yield per plant (0.94), biomass (0.90), SPAD chlorophyll meter reading (0.85), specific leaf area (0.77) and harvest index (0.71). High heritability under these traits indicates the opportunity existing on these traits for improvement under drought conditions. These results agree with the findings reported by Songsri *et al*., (2008), Ashish *et al.*, (2014), Prabhu *et al*., (2015), Divyadharsini *et al*., (2017), and Syed Sab *et al*., (2018).

In addition, previous studies reported that inheritance of drought tolerance traits as predominantly controlled by additive gene action in SLA, SCMR and HI (Songsri *et al*., 2008). In connection to this, heritability estimates were moderate for relative water content (0.63) and low for shelling percentage (0.35), root dry weight (0.28) and plant height (0.18), indicating that genetic improvement through selection under these traits may be ineffective (Panse, 1957).

High GA coupled with high GAM were noted for biomass (119.87&123.15); and high GAM coupled with moderate GA were recorded for pod yield per plant (106.95&18.04), SCMR (56.32&25.33), SY (104.53&12.29) and HI (124.36&4.07). This indicated that genetic control had more influence in the expression of these traits than environmental effects; hence, selection for drought tolerance or for moisture stress tolerance among groundnut genotypes under these traits would be more effective.

Earlier Padmaja *et al*., (2013) reported similar results for SCMR. Furthermore, the low GA coupled with low GAM was observed for RWC, SHP, PH and NPB. This indicated that, the traits were highly influenced by environmental factors; hence, phenotypic selection for improvement of these traits under drought conditions would be ineffective and progress in breeding would be slow. Similar findings were also reported in studies conducted by Misra *et al.,* (2000). They reported low heritability coupled with low genetic advance for shelling percentage and number of primary branches which supports the present findings.

Correlation of yield and drought tolerant traits of the F_3 population

Correlations analysis between yield, yield attributing traits and water efficiency showed that except for plant height, other traits were positively correlated with seed yield (Table 2). Highly significant positive (P<0.01) correlation coefficient of seed yield with pod yield (r=0.992), biomass (r=0.796), relative water content (r=0.736), shelling percentage (r=0.731), days to maturity (r=0.580), SPAD chlorophyll meter reading (r=0.508) and root dry weight (r=0.437) were recorded. Simultaneous selection based on these traits would be effective for improving seed yield under drought conditions. Similar results were reported in previous studies conducted by Sadeghi and Seyyed (2012), Padmaja *et al.,* (2013), Thirumala *et al.,* (2014) and Syed Sab *et al*., (2018). Importance of yield components on selection for yield improvement in groundnut has been reported in previous studies (Vaithiyalingan *et al*., 2010; Shoba *et al*., 2012; Syed Sab *et al*., 2018). Correlation analysis revealed a negative correlation between seed yield and plant height suggesting a limited scope for yield improvement through selection under this trait. These results concurred with previous findings by Girdthai *et al*., (2010), and Sadeghi and Seyyed (2012) who reported negative correlation between seed yield and plant height. Among the water use efficient traits, SCMR, HI and RWC had positive significant correlation with PY, while SLA exhibited low correlation with PY. Songsri *et al*., (2008) also reported low correlation between PY and SLA. Therefore, integration of these traits in drought tolerance breeding scheme would be advantageous in selecting groundnut genotypes that are more efficient in water use.

Estimates of path coefficients for direct and indirect effects on seed yield

The path coefficient analysis for 11 characters with direct and indirect effect on seed yield showed that only pod yield per plant exhibited the highest positive direct effect on seed yield per plant (0.850) followed by positive direct effect on shelling percentage (0.166) (Table 3). Raut *et al*., (2010) and Shoba *et al*., (2012) reported similar results on these traits. The high direct effects exhibited by pod yield and shelling percentage revealed in this study suggested that selection based on these traits would result in genetic gain toward groundnut yield improvement under drought stress. Other positive direct effects on seed yield per plant were observed for BM, RWC, SCMR, DM, PH and HI. However, the magnitude was low, indicating that the direct effects may be confounded with indirect effects.

Therefore, improvement of seed yield under drought conditions based on these traits would be more effective if the indirect effects would be considered. Painawadee *et al*., (2009) and Sadeghi and Seyyed (2012) reported similar results for biomass. Negative direct effect on seed yield were observed for SLA, NB and RDW. Similar results were reported by Lakshmidevamma (2004) for the NB and SLA. The indirect effects of relative water content (0.628), shelling percentage (0.546), days to maturity (0.511), and biomass (0.675) through pod yield were high and positive. These results support the findings of Alam *et al*., (1985) for days to maturity and Lakshmidevamma (2004) for shelling percentage, who concluded that simultaneous selection based on direct and indirect effects of these traits would be of paramount importance for improving yield in groundnut.

Table 2:Correlation coefficient (r) estimates of morphological and physiological traits for water use efficiency (WUE) in F3 population

Trait	Biomass	Pod yield/ plant	Relative water content	Root dry weight	SPAD Chlorophyll meter reading	Specific leaf area	Shelling percentage	Seed yield	Days to maturity	Number of primary branch	Plant height	Harvest index
Biomass	1.000											
Pod yield/ plant	0.794**	1.000										
Relative water content	0.742**	0.739**	1.000									
Root dry weight	0.223	0.443*	-0.023	1.000								
SPAD Chlorophyll meter reading	0.649**	0.511**	0.392	0.276	1.000							
Specific leaf area	0.471*	0.183	0.288	-0.029	0.572**	1.000						
Shelling percentage	0.542**	0.643**	0.485*	0.263	0.307	0.233	1.000					
Seed yield	0.796**	0.992**	0.736**	0.437*	0.508**	0.203	0.731**	1.000				
Days to maturity	0.489*	0.602**	0.439*	0.505*	0.321	0.176	0.240	0.580**	1.000			
Number of primary branch	0.098	0.224	-0.001	0.598**	0.176	0.083	0.087	0.211	0.222	1.000		
Plant height	-0.171	-0.071	0.098	-0.185	-0.256	0.012	0.001	-0.066	-0.065	-0.382	1.000	
Harvest index	-0.019	0.580**	0.236	0.420*	-0.014	-0.278	0.324	0.564**	0.342	0.220	0.112	1.000

**Correlation is significant at the 0.01 level (2- tailed), * Correlation is significant at the 0.05 level (2- tailed).

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Table 3: Direct (diagonal) and indirect (non-diagonal) effects of 11 traits on seed yield in 25 groundnut genotypes

Trait	Biomass	Pod yield/ plant	Relative water content	Root dry weight	SPAD Chlorophyll meter reading	Specific leaf area	Shelling percentage	Days to maturity	Number of primary branch	Plant height	Harvest index	Total effects of correlation with seed yield
Biomass	0.03368	0.67455	0.00035	-0.00182	0.01026	-0.00061	0.09007	0.00746	-0.00001	-0.00045	-0.00060	0.79**
Pod yield/ plant	0.02674	0.84957	0.00035	-0.00361	0.00808	-0.00024	0.10685	0.00918	-0.00001	-0.00019	0.01874	0.99**
Relative water content	0.02499	0.62783	0.00047	0.00019	0.00620	-0.00037	0.08060	0.00669	0.00000	0.00026	0.00763	0.74**
Root dry weight	0.00751	0.37636	-0.00001	-0.00814	0.00436	0.00004	0.04373	0.00770	-0.00004	-0.00049	0.01357	0.44*
SPAD Chlorophyll **meter reading**	0.02186	0.43413	0.00019	-0.00225	0.01581	-0.00074	0.05101	0.00489	-0.00001	-0.00067	-0.00047	0.51**
Specific leaf area	0.01586	0.15507	0.00014	0.00024	0.00904	-0.00129	0.03866	0.00268	-0.00001	0.00003	-0.00898	0.23
Shelling percentage	0.01825	0.54627	0.00023	-0.00214	0.00485	-0.00030	0.16618	0.00367	-0.00001	0.00000	0.01047	0.73**
Days to maturity	0.01647	0.51144	0.00021	-0.00411	0.00507	-0.00023	0.03994	0.01525	-0.00001	-0.00017	0.01105	0.58**
Number of primary branch	0.00329	0.19012	0.00000	-0.00487	0.00279	-0.00011	0.01442	0.00339	-0.00007	-0.00101	0.00712	0.21
Plant height	-0.00575	-0.06011	0.00005	0.00150	-0.00405	-0.00002	0.00014	-0.00099	0.00002	0.00263	0.00361	-0.07
Harvest index	-0.00063	0.49275	0.00011	-0.00342	-0.00023	0.00036	0.05386	0.00522	-0.00001	0.00029	0.03231	0.56**

Conclusion

The study results revealed sufficient variations among the evaluated variables, which also had high heritability, indicating the possibility of improving groundnut yield through breeding for drought tolerance. High genotypic variations for biomass, pod yield per plant and SPAD chlorophyll meter reading coupled with high heritability, GA and GAM (%) confirms considerable existence of genetic variation in the population, and that selection for superior genotypes in early generations is possible. Significant positive correlations of yield with biomass, pod yield per plant, relative water content, SPAD chlorophyll meter reading, harvest index, shelling percentage and days to maturity indicated the ability to improve yield under drought stress through selection based on these attributes. Apart from that, pod yield per plant and shelling percent had direct and indirect effects on seed yield, respectively. Therefore, this information will be helpful to identify the promising groundnut genotypes which could be used for breeding programme and to develop lines that are superior for drought stress.

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