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Full Length Research Paper

Estimates of genetic parameters and genotype by environment interactions for sugar yield and its components in sugarcane genotypes in Western Kenya

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This study was conducted to estimate broad sense heritability, genetic advance, GE interactions and correlations among quality traits in sugarcane clones in western Kenya. Thirteen sugarcane promising clones and one check cultivar were evaluated plant and ratoon crops in three locations under rain fed conditions using the randomised complete block design with three replications. Analysis of variance showed significant differences in hand refractometer brix, sucrose content (Pol% cane), juice purity, fire content, sugar yield and brix yield. Sucrose content, fibre content, sugar yield and brix yield exhibited significant genotype × location (GL) interactions. The genotype mean squares exceeded the GE interactions for all the quality traits suggesting that more emphasis should be placed on testing clones in many locations than on testing ratoon crops within locations. High genetic coefficient of variation (GCV) was detected for cane yield (8.12%), brix yield (6.39%), sugar yield (5.69%) and sucrose content (3.69%). Broad sense heritability was high for sucrose content (0.712) and moderate for cane yield (0.515), fibre content (0.474), juice purity (0.445) and refractometer brix (0.380). Cane yield (10.3%), brix yield (6.7%), sucrose content (5.5%) and sugar yield (5.4%) showed highest expected genetic advance. The results indicated that these traits may respond positively to selection and present opportunities for improvement through breeding. High genetic correlation (r_g=0.998) between refractometer brix and sucrose content suggest that selection for refractometer brix can be effective in identifying varieties with high sucrose content.

Key words: *Saccharum* spp. heritability, genetic advance, sucrose content, selection, sugar.

INTRODUCTION

Genetic improvement of varieties plays a pivotal role in the development of sugar industries in almost all sugarcane growing countries. Improved cane yields, sucrose content and disease and pest resistance and maintaining acceptable fibre levels for milling are usually the main breeding objectives in most sugarcane breeding programmes (Jackson, 2005). Studies on exploitation of sugarcane as a sustainable energy source are on the increase (Corcodel and Roussel, 2010; Hoang et al., 2015; Priya et al., 2018). An improvement in sucrose

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content in sugarcane has high economic value as it increases sugar yield with very little increase in marginal costs through harvesting, cane transport or milling (Jackson, 2005). Thus gains in sucrose content are economically more beneficial than corresponding increases in cane yield thus increased sucrose content is a key objective of sugarcane breeding programmes.

Breaux (1984) reported that the record increase in sugar recovery in Louisiana was attributed to wide acceptance of high sucrose varieties that were introduced in 1973. The high sucrose content of the varieties was a result of continuous breeding and selection effort since the 1920s (Breaux, 1984; Legendre, 1992). Significant increase in the sucrose content of both experimental clones and cultivars grown in a number of locations throughout the sugarcane-growing region of Louisiana since 1928 was reported by Irvinne and Richards (1983). The average sucrose % cane of experimental varieties rose from 5.54% in 1928 to 13.56% in 1978; while the average sucrose % cane for all adopted cultivars increased from 7.25% in 1928 to 12.7% in 1981. In addition the study showed a strong correlation between the sucrose content of experimental varieties and actual mill recovery (r = 0.79) and sucrose content of commercial varieties and mill recovery (r = 0.80) during the same period. However, further improvement in sucrose content through breeding may be difficult as the sucrose content of parent varieties reaches an apparent plateau (Legendre, 1992; Inman-Bamber, 2014).

The effectiveness of selection for sugar yield and its components depends largely on the genetic variability present in the breeding population and the heritability of the traits. It is necessary to identify traits with high genetic variation. The easiest way to estimate variance components is to test a large number of genotypes for two or more years and at two or more locations (Mayo, 1980). Components of juice quality are largely determined by the genotype but can be significantly influenced by the environment (Tena et al., 2016; Singh et al., 2019). Sucrose content and purity are conventional indicators of maturity commonly used as selection criteria and they are widely investigated in sugarcane breeding programmes (Mariotti et al., 2001). The sugarcane industry in Kenya is largely dependent on a few varieties that have low sucrose content and sugar yield (Jamoza, 2011). In recent years the industry has emphasized development and adoption of high yielding sugar rich varieties. This study aimed to estimate (i) broad sense heritability (ii) potential genetic advance and (iii) correlations among juice quality traits in promising Kenyan sugarcane clones.

MATERIALS AND METHODS

Experimental sites and genotypes

The test genotypes, experimental sites and methodology applied to obtain estimates were as described by Jamoza et al. (2014). In brief, 13 clones (KEN01-24. KEN01-26, KEN01-41, KEN01-279, KEN01-345, KEN01-592, KEN01-819, KEN01-848, KEN01-1009, KEN01-1104, KEN01-1108, KEN01-1139 and KEN01-1294) representing eight crosses involving 15 parents and one commercial variety (N14) were chosen for study and evaluated in plant and first ratoon crops between August 2007 and June 2010. They were grown at three locations in western Kenya namely: Kibos (34° 48'E, 0° 04'N) 1,184 m above sea level on clay loam soil with long term mean annual rainfall of 1,490 mm. The temperatures range from 15.3 to 30°C; Mumias (34° 30'E, 0° 21'N) at 1,314 m above sea level, receives 2,194 mm annual rainfall with a temperature range of 16.4 to 30.9°C and has free draining loam soils; and Nzoia (34° 40'E, 0° 35'N) situated at 1,445 m above sea level, receives average annual rainfall of 1,650 mm with a temperature range of 13 to 32°C and has sandy clay loam soils. The randomised complete block design with three replications was used at each location. The following cane quality data were collected in both crops.

Field brix

Hand held refractometer (0-32^{o}) was used to determine brix of 5 millable stalks taken randomly from each plot in the field at harvest.

Sugarcane analysis

At harvest millable stalks in each plot were cut at ground level, well topped and hand stripped to remove the trash and green leaves. Twelve millable stalks were randomly taken from each plot, bundled, tied, labelled and transported to the laboratory for juice and fibre analysis. Juice was extracted from six stalk samples using a simple three roller cane press (Milligan et al., 1990a). The juice was filtered through Whatman filter paper No.1 and 100 ml portions of the filtrate used to determine brix (percent soluble solids w/w) using a bench refractometer as described in the Laboratory Manual for South African Sugar Factories (Anon, 1985). For the determination of Pol % juice approximately 300 ml samples of the extracted juice were placed in a beaker and clarified using 3 g of sub lead acetate. The mixture was then filtered using Whatman filter paper No. 91. Polarimetric readings of the clarified juice were obtained using a digital automated sucromat while sucrose content (Pol% cane) was calculated from the values of Pol % juice and fibre content (BSES, 1970).

The other six stalks from the harvested sample for each cane variety were used to determine fibre content following the procedure described by Clayton (1971). Six pieces were cut from different (top, middle, and bottom) portions of the stalks in order to obtain a subsample equivalent to one whole stalk. The pieces were further cut into smaller pieces (approx. 3 cm) then shredded in a laboratory hammer mill (shredder). The shredded samples were well mixed and then 200 g subsamples were placed in pre weighed fibre bags and washed alternately in cold and hot water to remove all sugars (mainly sucrose, fructose and glucose). The samples thus processed were dried in an air oven at 105°C for 24 h to constant weight. The fibre content was calculated directly from the 200 g fresh weight and dry weight as:

$$Fibre\ content\ \% = \frac{weight\ of\ dried\ sample}{weight\ of\ fresh\ sample} * 100$$

Purity of juice was computed as:

$$Purity\ \% = \frac{Pol\ \%\ cane}{Brix\ \%\ cane} * 100$$

Other derived quality characters were computed as follows:

Table 1. Mean squares for quality traits combined over the plant and first ratoon crops and three locations (Mumias, Nzoia and Kibos) in 2007-2010.

Source	DF	Hand refractometer Brix (°)	Sucrose content (Pol % cane)	Juice purity (%)	Fibre content (%)	Estimated sugar yield (tha^{-1})	Brix yield (tha^{-1})
Location (L)	2	11.706**	48.628**	553.605**	108.297**	244.957**	279.526**
Rep (location)	6	1.482**	11.864**	37.885**	2.419*	142.943**	264.371**
Crop-year (Y)	1	0.6**	2.177	889.842**	1.232	356.215**	854.386**
Genotype (G)	13	3.608**	6.142**	16.481**	2.874**	36.701**	92.687**
G x L	26	1.136	1.283*	5.932	1.511*	25.993*	55.397*
G x Y	13	0.794	1.386*	5.108	0.76	15.747	34.77
L x Y	2	50.549**	0.791	148.655**	1.767	242.565*	858.954**
G x L x Y	26	0.41	0.905	3.652	0.666	8.17	15.289
Error	162	0.486	0.715	6.842	0.889	14.209	31.359
Mean	-	20.851	13.367	87.056	16.224	13.556	21.142
CV%	-	3.344	6.328	3.0	5.812	27.808	26.487
R^2	-	0.746	0.738	0.734	0.702	0.62	0.618

*, ** = significant at p≤0.05 and p≤0.01, respectively.

Estimated sugar yield tha^{-1} = Sucrose content (Pol % cane) × cane yield (tha^{-1}) × 100

Brix yield tha^{-1} = Brix % cane × cane yield (tha^{-1}) × 100

Statistical data analysis

All the data were submitted to analysis of variance and covariance, estimation of genetic, genotype by environment interaction and error variance components, broad sense heritability, genetic advance and correlations as described by Jamoza et al. (2014). The genotypes were assumed to be fixed while genotype by environment interactions and environments were random (Chang, 1996; Brown and Glaz, 2001).

RESULTS

Combined analysis of variance for quality components over three locations and two crops

Mean squares for the traits are shown in Table 1. Genotypes exhibited significant ($p \leq 0.01$) differences for hand refractometer brix, sucrose content, juice purity, fire content, estimated sugar yield and brix yield. Locations played a significant ($p \leq 0.01$) role in the phenotypic expression of all the quality traits. However, years or crops significantly ($p \leq 0.01$) influenced genotypes only for hand refractometer brix, juice purity, estimated sugar yield and brix yield. Significant ($p \leq 0.01$) genotype × location (GL) interactions were detected for sucrose content, fibre content, estimated sugar yield and brix yield. Location × crop-year (LY) effects were significant for hand refractometer brix, juice purity, estimated sugar yield and brix yield. Genotype × crop-year interactions (GY) were only significant ($p \leq 0.05$) for sucrose content. However, genotype × location × crop-year (GLY) interactions were not significant ($p \leq 0.01$) for any of the traits studied. For all the traits mean squares for genotypes were larger than GL (1.4 - 4.5 times), GY (2.3 - 4.5 times), GLY (4.3 - 8.8 times) and error (2.4 - 4.8 times) mean squares.

Genetic variability, heritability and genetic advance

Genetic variabilities (GCV) for sucrose content, juice purity, fibre content and sugar yield were higher in first ratoon than plant crop (Table 2). Differences between GCV and phenotypic coefficient of variation (PCV) for all traits were large indicating the influence of environmental factors in the traits. Heritability for the traits ranged from 0.376 for juice quality and sugar yield to 0.685 for refractometer Brix in the plant crop and from 0.321 to 0.81 in the ratoon crop. Expected genetic gains for all the traits in both crop years were less than 10% with sucrose content, sugar yield and Brix yield recording 3.2, 7.9 and 8.1% respectively. Genetic gain for sucrose content and fibre content were much

Table 2. Variance components and heritability for sugar yield and related traits in 14 sugarcane genotypes evaluated in Kibos, Mumias and Nzoia for plant crop (PC) (2007-2009) and first ratoon crop (FR) (2009-2010).

Trait	σ^2_g		σ^2_{gl}		σ^2_e		GCV		PCV		h^2		GA%	
	PC	FR	PC	FR	PC	FR	PC	FR	PC	FR	PC	FR	PC	FR
Refractometer Brix (^{o})	0.212	0.106	0.173	0.019	0.359	0.611	2.211	1.557	2.673	2.031	0.685	0.588	3.2	2.1
Sucrose content (Pol% cane)	0.119	0.474	0.192	0.119	0.609	0.646	2.603	5.114	3.774	5.684	0.476	0.810	3.2	8.1
Juice purity (%)	0.405	1.038	0.000	0.000	6.060	5.826	0.747	1.145	1.219	1.460	0.376	0.616	0.8	1.6
Fibre content (%)	0.107	0.225	0.273	0.000	0.681	0.971	2.024	2.911	3.237	3.541	0.391	0.676	2.2	4.2
Sugar yield (t ha^{-1})	1.161	0.880	1.775	0.000	11.986	16.765	7.308	7.587	11.911	13.392	0.376	0.321	7.9	7.6
Brix yield (t ha^{-1})	2.613	3.704	1.642	0.571	26.871	37.097	7.033	5.818	10.786	8.559	0.425	0.462	8.1	7.0

σ^2_g, σ^2_{gl}, σ^2_e, = genotypic, genotype × location interaction, environmental variances; GCV, PCV = genetic, phenotypic coefficients of variation, h^2 = broad sense heritability and GA% = expected genetic advance as percentage of the phenotypic mean of the trait

Table 3. Combined variance components and heritability for sugar yield and related traits in 14 sugarcane genotypes evaluated in Kibos, Mumias and Nzoia in plant and first ratoon crops (2007-2010).

Trait	σ^2_g	σ^2_{gl}	σ^2_{gy}	σ^2_{gly}	σ^2_e	GCV%	PCV%	h^2	GA%
Hand refractometer Brix(^{o})	0.076	0.000	0.054	0.584	0.486	1.323	2.147	0.380	1.4
Sucrose content (Pol % cane)	0.244	0.064	0.000	0.061	0.715	3.694	4.376	0.712	5.5
Juice purity (%)	0.407	0.000	0.000	0.769	6.842	0.733	1.099	0.445	0.9
Fibre content	0.076	0.108	0.000	0.000	0.861	1.696	2.463	0.474	2.1
Estimated sugar yield (tha^{-1})	0.595	0.665	0.000	2.599	14.209	5.690	10.534	0.292	5.4
Brix yield (tha^{-1})	1.827	0.000	0.000	9.481	31.361	6.393	10.733	0.366	6.7

σ^2_g, σ^2_{gl}, σ^2_{gy}, σ^2_{gly}, σ^2e = genotypic, genotype × location, genotype × crop-year, genotype × location × crop-year interaction, environmental variances; GCV, PCV = genetic, phenotypic coefficients of variation, h^2 = broad sense heritability and GA% = expected genetic advance as percentage of the phenotypic mean of the trait

higher in the ratoon than the plant crop. This was probably due to the higher genetic variance for the two traits in the ratoon crop. Error variance components for sugar yield and Brix yield were 19 and 10 fold the respective genotypic components. GCV values for all the cane quality characters were less than 10% in both crops. Most of the quality traits had moderate to high heritability (>0.5) but sugar yield had low heritability (0.321). Genetic parameters from the combined analysis are shown in Table 3. For all traits, error variances were higher than the genetic components. G×Y variances were negligible in all the traits except refractometer Brix. The magnitude of GCV relative to PCV ranged from 54% for sugar yield to 84.4% for sucrose content. Heritability was highest (0.712) for sucrose content and lowest (0.292) for sugar yield. Brix yield (6.7%), sucrose content (5.5%) and sugar yield (5.4%) had highest genetic gains.

Genetic and phenotypic correlations coefficients between sugar yield and its attributes

High genetic and phenotypic correlations were detected between hand refractometer brix and Pol% cane (r_g = 0.998, r_p = 0.966) and between Pol% cane and juice purity (r_g = 0.624, r_p = 0.523) (Table 4). Correlations between fibre content and juice quality traits were low.

Table 4. Genotypic (upper row) and phenotypic (lower row) correlations for cane quality traits over crops and locations (2007 – 2010)

Trait	Sucrose content (Pol% cane)	Juice purity (%)	Fibre content (%)
Hand refractometer Brix (^{o})	0.998*^{g} 0.966*^{p}	0.66* 0.17*	0.295 0.015
Sucrose content (Pol % cane)		0.624* 0.523*	Not estimable
Juice purity (%)			0.11 -0.132*

* = significant if |r|> at least twice its standard error (Holland, 2006); ^{g} = genotypic correlation, ^{P} = phenotypic correlation.

The association between Pol% cane and fibre content could not be estimated.

DISCUSSION

Combined analysis of variance and genotype × environment interactions

The significant differences among the clones for all the traits indicate existence of genetic variation in the material. This suggests that opportunities for further improvement through selection do exist. Significant GL interactions for sucrose content (Pol% cane), fibre content, estimated sugar yield and brix yield indicated that the test environments discriminated the sugarcane clones differently. Similarly, significant GY effects for sucrose content indicated the inconsistent nature of this trait from one crop-year to another. However, no significant GL, GY and GLY interactions were detected for hand refractometer brix and juice purity suggesting that performance of clones in these traits was stable over the locations and crop-years. This suggests that the variance components for these traits could be estimated from one location and one crop-year data. Chang (1996) obtained similar results for juice purity in Taiwan.

Interactions of genotypes with environments (GEI) complicate the identification of superior genotypes by plant breeders during selection and cultivar recommendations. GEI have been reported to be a major problem in breeding programmes as they reduce progress from selection (Comstock and Moll, 1963; Mirzawan et al., 1993; Kimbeng et al., 2009). In de Sousa-Vierra and Milligan (2005) reported significant GL and GY interactions for Pol% cane. In a recent study, Shikanda et al. (2017) reported significant GL interactions for Brix in selected Kenyan clones. Similar results have been reported in other programmes (Tena et al., 2016; Singh et al., 2019). The results of our study suggest that more emphasis should be placed on testing clones in many locations than on testing ratoon crops within locations for reliable selection (Khan et al., 2004).

Variability, heritability and genetic advance

High GCV values suggest good prospects for improvement in the traits by selection. However, Burton (1952) suggested that the use of GCV together with heritability estimates gave a better understanding of heritable variation present in a population. The magnitude of heritability of a trait indicates the effectiveness of selection based on phenotypic observation of the trait (Hanson, 1963). Most quality characters had moderate heritability (>0.4) except sugar yield. Thus improvement of these traits through selection would be somewhat difficult but more effective than selecting for sugar yield *per se*. Butterfield and Nuss (2002) reported that effective selection of superior clones depended not only on heritability but also on genetic advance (GA). In this study, moderate GA values were associated with moderate heritability and GCV. The low heritability coupled with low GCV implies large influence of environmental and genotype × environment interaction effects on some traits and limited scope for their improvement. This explains the low expected genetic gain for cane quality traits. Singh (1993) observed that selection for traits with low heritability may be practically difficult. However, Cesnick and Vencovsky (1974) obtained moderate heritability for brix (0.52) and Pol% juice (0.54) and considered that breeding progress for these traits was still possible. The results of our study suggest that brix yield, sugar yield and sucrose content may respond positively to selection and offer opportunities for improvement in the breeding programme. The expected GA for sugar yield, sucrose content, brix yield and cane yield indicate considerable potential for improvement through breeding. Milligan et al. (1990b) and Singh et al. (2019) reported similar observations but with higher GA values.

Genotypic and phenotypic correlations among traits

The strong positive correlation between hand refractometer brix and sucrose content indicated that the

former was a reliable indicator of sugar content in cane. Similar results were obtained by Kang et al. (1983), Milligan et al. (1990b) and Chang (1996). Sucrose content and purity of juice are tedious and costly to measure as they are determined in the laboratory while brix can easily be measured in the field with a hand refractometer and punch. Brix measures total soluble solids in cane juice and a high fraction of these solids contain sucrose thus brix is a useful correlated trait for selection. This study suggests that it is possible to identify varieties with high sucrose content and purity by selecting for high hand refractometer brix.

Conclusion

The study has demonstrated availability of genetic variability among the genotypes for the cane quality traits studied implying that genetic improvement through selection is possible. The presence of GL effects and absence of GY and GLY interactions suggests that sugarcane clones should be evaluated in more locations rather than years/seasons for effective selection. The high expected genetic gains for sucrose content, sugar yield and Brix yield indicates that selection and genetic improvement for these traits would be effective. Refractometer Brix is a reliable correlated trait when selecting for sucrose content.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Anonymous (1985). Laboratory Manual for South Africa Factories including the official methods. 3rd Ed. Published by SASTA.

Breaux RD (1984). Breeding to enhance sucrose content of sugarcane varieties in Louisiana. Field Crops Research 9:59-67.

Bressiani JA, Da Silva JA, Vencovsky R, Sordi RA, Burnquist WL (2006). Combining high yields of cane and sucrose in sugarcane through recurrent selection. Journal of American Society of Cane Technologists 26:26-37.

Brown JS, Glaz B (2001). Analysis of resource allocation in final stage sugarcane clonal selection. Crop Science 41:57-62.

Butterfield MK, Nuss KJ (2002). Prospects for new varieties in the medium to long term, the effects of current and future breeding strategy on variety characteristics. South African Sugar Industry Agronomists Association, Kwa-Shukela pp. 41-48.

Bureau of Sugarcane Experiment Stations (BSES) (1970). Laboratory manual for Queensland sugar mills, Fifth Edition. Watson, Ferguson and Company, Brisbane P 250.

Burton GW (1952). Quantitative inheritance in grasses. 6th Grassland Congress 1:277-283.

Cesnick R, Venkovsky R (1974). Expected response to selection, heritability, genetic correlations and response to selection of some characters in sugarcane. Proceedings of International. Society of Sugar Cane Technologists 15:96-100.

Chang YS (1996). Estimating heritability of and correlations among brix, purity and sugar content in sugarcane using balanced multiple location and year data. Report of Taiwan Sugar Research Institute 151:1-10.

Clayton JL (1971). System of cane sugar factory control, 3rd Ed. Queensland Society of Sugar Cane Technologists, Brisbane P 87.

Comstock RE, Moll RH (1963). Genotype-environment interactions. *In:* Hanson WD, Robinson HF (Ed) Statistical Genetics and Plant Breeding. National Academy of Science, National. Research Council Publication No.982. Washington D.C.

Corcodel L, Roussel C (2010). Energy content: a new approach to cane evaluation. Proceedings of International. Society of Sugar Cane Technologists 27:1-7.

De Sousa-Vierra O, Milligan SB (2005). Interrelationships of cane yield components and their utility in sugarcane family selection: Path coefficient analysis. Interciencia 30:92-96.

Hoang NV, Furtado A, Botha FC, Simmons BA, Henry RJ (2015). Potential for Genetic Improvement of Sugarcane as a Source of Biomass for Biofuels. Frontiers in Bioengineering and Biotechnology 3:182.https://doi: 10.3389/fbioe.2015.00182.

Hanson WD (1963). Heritability. In: W.D. Hanson and H.F. Robinson (ed) Statistical Genetics and Plant Breeding. National Academy of Science, National Research Council Pub1ication No.982. Washington D.C

Holland JB (2006). Estimating genotypic correlations and their standard errors using multivariate restricted maximum likelihood estimation with SAS Proc Mixed. Crop Science 46:642-654.

Inman-Bamber G (2014). Sugarcane yields and yield-limiting processes. In: Morre, P.H., Botha, F.C. (Eds.), Sugarcane: Physiology, Biochemistry, and Functional Biology. Wiley-Blackwell, pp. 579-600. https://doi.org/10.1002/9781118771280.ch21.

Irvinne JE, Richards CA (1983). Production statistics as evidence of technological improvement. The Louisiana sugar cane industry as a case study. Sugar Cane (May/June) 1:13-18.

Jackson PA (2005). Breeding for improved sugar content in sugarcane. Field Crops Research 92:277-290.

Jamoza JE (2011). KESREF releases new sugarcane varieties. KESREF Digest 5(4):3-4.

Jamoza JE, Owuoch JO, Kiplagat O, Opile W (2014). Broad-sense heritability estimation and correlation among sugarcane (*Saccharum* spp. hybrids) yield and some agronomic traits estimated in western Kenya. International Journal of Agricultural Policy and Research 2(1):016-025.

Kang MS, Miller JD, Tai PYT (1983). Genetic and phenotypic path analyses and heritability in sugarcane. Crop Science 23:643-647.

Kimbeng CA, Zhou MM, da Silva JA (2009). Genotype × environment interactions and resource allocation in sugarcane yield trials in the Rio Grande Valley Region of Texas. Journal of American Society of Cane Technologists 29:11-24.

Legendre BL (1992). Potential for increasing sucrose content of sugarcane varieties in Louisiana through breeding. Proceedings of International Society of Sugar Cane Technologists 21:367-377.

Khan IA, Khatari A, Siddiqui MA, Nizamani GS, Raza S (2004). Performance of promising sugarcane clones for yield and quality in different ecological zones of Sindhi. Pakistan Journal of Botany 36(1):83-92.

Mariotti JA, Cuenya MI, García de Salas MB (2001). The performance of families in breeding for quality traits in sugarcane. Proceedings of International Society of Sugar Cane Technologists 24:495-499.

Mayo O (1980). The Theory of Plant Breeding. Clarendon Pres-Oxford. New York.

Milligan SB, Gravois KA, Bischoff KP, Martin FA (1990a). Crop effects on broad-sense heritability and genetic variances of sugarcane yield components. Crop Science 30:344-349.

Milligan SB, Gravois KA, Bischoff KP, Martin FA (1990b). Crop effects on genetic relationships among sugarcane traits. Crop Science 30:927-931.

Mirzawan PDN, Cooper M, Hogarth, DM (1993). The impact of genotype × environment interactions for sugar yield on the use of indirect selection in southern Queensland. Australian Journal of Experimental Agriculture 33:629-638.

Priya MS, Reddy KHP, Kumar MH, Rajarajeswari V, Naidu GM (2018). Studies on genetic parameters for diversified uses in sugarcane (Saccharum spp). International Journal of Current Microbiology and Applied Sciences 7(8):4138-4142.

https://doi.org/10.20546/ijcmas.2018.708.431.
Shikanda E, Jamoza J, Kiplagat O (2017). Genotypic evaluation of sugarcane (*Saccharum* spp. hybrids) clones for sucrose content in western Kenya. Journal of Plant Breeding and Crop Science 9(3):30-36.
Singh BD (1993). Plant Breeding. 5th ed. Kalyani Publishers, Rajender Nagar, Ludhiana, India pp. 102-104.
Singh G, Mishra KM, Sangera GS (2019). Variability and character association for commercial cane sugar and its components in early maturing sugarcane clones. Agricultural Research Journal 56(2):321-3214.
Tena E, Mekbib F, Ayana A (2016). Heritability and correlation among sugarcane (*Saccharum* spp.) yield and some agronomic and sugar quality traits in Ethiopia. American Journal of Plant Sciences 7:1453-1477.

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Genetic dissimilarity and growth of coffee in Cerrado

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Genetic dissimilarity can be used to identify promising genotypes for cultivation in specific conditions. Thus, the objective of this work is to study the genetic dissimilarity among 35 genotypes of *Coffea arabica* in the Cerrado, under irrigation, using phenological data and multivariate statistics. Plant height, stem diameter, canopy diameter, number of orthotropic branch nodes, length of orthotropic branch internodes, length of primary plagiotropic branches, and average plagiotropic branch internode length were evaluated at 6, 12, 18 and 24 months after planting. Data were analyzed using Hierarchical Agglomerative Cluster Analysis and Principal Component Analysis. Three clusters were formed for each evaluation (6, 12, 18 and 24 months). At 6 months, the most distant group consisted of Yellow Catucaí 2SL, Araponga MG 1, Sacramento MG 1, 23 II, Yellow Catucaí 20/15 pit 479, Sarchimor MG 8840, IBC-Palma 2, and New Acauã genotypes. At 12 months, the most distant group consisted of Yellow Catucaí 2SL, Asa Branca, Sacramento MG 1, and Sarchimor MG 8840. At 18 months, the most distant group consisted of Yellow Catucaí 2SL, Tupi IAC 1669-33, 23 II, Red Obatã IAC 1969-20, Sacramento MG 1, and Sarchimor MG 8840. At 24 months, Yellow Catucaí 2SL was distinct from the other 34 genotypes. Phenological variables strongly contributed to genetic dissimilarity (>75%) and there was a positive correlation for most variables.

Key words: Environment, *Coffea arabica* L., phenology, multivariate analysis, genetic, dissimilarity.

INTRODUCTION

Coffee production has contributed significantly to economic and social development in Brazil and is of great importance to Brazilian agribusiness. Brazil has been the world's largest producer and exporter of coffee for over 150 years (Paiva et al., 2010). National productivity in 2018 was 1903.2 kg per hectare. In 2019, 1509.6 kg per hectare was estimated (CONAB, 2019).

The Brazilian coffee industry has undergone significant changes as crop has moved into the Cerrado areas, particularly in its production system (Oliveira et al., 2010). The Cerrado produces excellent quality coffee due to its two well-defined seasons: rainy summer and dry winter (Fernandes et al., 2012). In addition, controlled water stress can be used to standardize the flowering and ripening of fruits in the Cerrado (Guerra et al., 2005).

Brazil has 131 registered cultivars of *Coffea arabica* L. However, not all are able to adapt to different growing conditions and reach their productive potential. Botelho et al. (2010) point out that genotype with superior behavior in a certain environment may not behave satisfactorily under other conditions. Thus, it is necessary to improve and select genotypes to ensure that they express desired

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traits. The development of new cultivars is achieved through genetic improvement processes (Paiva et al*.,* 2010), which require genetic variability in the population (Ivoglo et al*.,* 2008). Evaluating progenies in several locations is an important step in the final phase of a plant breeding program. With this information, the interaction between genotype and environment can be determined. Additionally, when interactions exist, subsidies can be provided to encourage cultivation at specific sites (Pinto et al*.,* 2012). By characterizing genetic divergence, efficiency in the selection of parents in breeding programs can be increased (Silva et al*.,* 2013). In this process, hundreds to thousands of individuals are evaluated to identify superior and divergent genotypes for certain characteristics in order to design by recombination (Silva et al*.,* 2016). The dissimilarity analysis is used to quantify genetic variability and the relative contribution of the variables to the genetic dissimilarity, allowing for the identification of promising combinations (Torres et al*.,* 2015).

The genetic dissimilarity allows one to identify promising genotypes for breeding programs and to recommend for cultivation. Giles et al. (2019) verified genetic divergence among 34 genotypes of *Coffea* sp. and conclude that phenotypic variations occurred predominantly due to genetic causes. Thus, the objective was to study the genetic dissimilarity among 35 genotypes of *C. arabica* in the Cerrado, under drip irrigation, using phenological data and multivariate statistics.

MATERIALS AND METHODS

The experiment was conducted at the Ceres Campus of the Federal Institute Goiano, GO. The Ceres Campus is located in the center of Goiás in the São Patrício Valley (UTM: E = 649,582.00 m and N= 8,302,194.00 m), and is characterized by having flat relief, very deep eutroferric red nitosol, clay texture, and an altitude of 556 m. The climate, according to the classification of Köppen, is Aw type (tropical climate with wet and dry seasons- Tropical Seasonal, dry winter), with an average annual temperature of 25.4ºC (average minimum: 19.3°C; average maximum: 31.5°C). The annual precipitation is approximately 1700 mm.

The experiment was conducted on April 8, 2015 in a randomized complete block design. The experiment consisted of 35 treatments, 31 cultivars and 4 progenies (Table 1) with four replication and 10 plants that were placed 3.50 × 0.75 m apart. The eight central plants were considered for analysis. During the experiment, recommended management practices as fertilization, phytosanitary management and irrigation for the crop were followed. In the dry season, drip irrigation occurred on Mondays, Wednesdays, and Fridays to account for the need for the crop (Kc) and evapotranspiration in a class A tank. Fertilization was performed based on soil analysis results and recommendations of the 5th approximation of the Soil Fertility Commission of the State of Minas Gerais (Guimarães et al*.,* 1999). At 6, 12, 18 and 24 months after planting, stem diameter (DST), canopy diameter (DCA), plant height (HEI), number of orthotropic branch nodes (NOBN), average length (cm) of orthotropic branch internodes (ALOBI), total number of nodes at the 2nd plagiotropic branch pair (TNPB), total length (cm) at the 2nd plagiotropic branch pair (TLPB), and average length (cm) of plagiotropic branch internodes (ALPBI) were measured.

Data were analyzed using Analysis of Variance, F, and the Scott-Knott test at 0.05 of means for phenological parameters. Hierarchical Agglomerative Cluster (HAC) was used to examine dissimilarity by measuring average Euclidian distance. Additionally, Ward's agglomeration method was used to obtain dendrograms and the Pearson's method (n) for Principal Component Analysis (PCA) was used to obtain the correlation matrix and distance Biplot. Statistical analyses of genetic data were performed using the software XLSTAT 2014.5.03. The number of groups in the dendrogram was determined by the automatic truncation function, which attempts to create homogeneous groups (XLSTAT-MX, 2005).

RESULTS AND DISCUSSION

Coffee genotypes showed differences in phonological variables at 6, 12, 18, and 24 months after planting (Tables 1 to 4). Evaluations that occurred at 6 and 18 months after planting coincided with the end of the dry season, while evaluations that occurred 12 and 24 months after planting coincided with the end of the wet season. Temporal variability was observed in genotype behavior, as the growth of each material to diverse edaphoclimatic conditions differed among evaluations.

Meireles et al*.* (2009) state that various phenological phases of *C. arabica* are affected by environmental conditions, especially by photoperiodic variation and meteorological conditions (rainfall distribution and air temperature). In this experiment, the evaluations at 6 and 18 months after planting, in month October, of season rainy beginning and the photoperiod increasing, peaking in December. At 12 and 24 months after planting, month of April, the end of the rainy season and the photoperiod with short days, with minimum in June. Genetic diversity was observed between genotypes in the adaptability and interaction of the genotypes with the environment, so multivariate techniques were used to evaluate genetic divergence.

The 35 genotypes were clustered into three groups at each evaluation using Hierarchical agglomerative cluster analysis (Figures 1 to 4). Differences in genotypes were observed among groups for each evaluation. At 6 months, the most distant group consisted of Yellow Catucaí 2SL, Araponga MG 1, Sacramento MG 1, 23 II, Yellow Catucaí 20/15 pit 479, Sarchimor MG 8840, IBC-Palma 2, and New Acauã. At 12 months, the most distant group consisted of Yellow Catucaí 2SL, Asa Branca, Sacramento MG 1, and Sarchimor MG 8840. At 18 months, the most distant group consisted of Yellow Catucaí 2SL, Tupi IAC 1669-33, 23 II, Red Obatã IAC 1969-20, Sacramento MG 1, and Sarchimor MG 8840. At 24 months, Yellow Catucai 2SL was distinct from the other genotypes.

Genotype divergence in each group within and among evaluations may be associated with the interaction of the genotypes with the environment, as the environment may increase or decrease the genotype expression.

Table 1. Phenological variables of coffee trees six months after the plantation was cultivated and irrigated in the Cerrado of Goiás.

Genotype	DST** (mm)	DCA (cm)	HEI (cm)	NOBN	ALOBI (cm)	TNPB	TLPB (cm)	ALPBI (cm)
Oeiras MG 6851	12.2^{c}*	44.6^{c}	65.9^{c}	12.2^{c}	5.5^{b}	15.8^{b}	60.2^{c}	3.8^{c}
Catiguá MG 1	13.5^{b}	46.2^{c}	66.4^{c}	12.4^{b}	5.5^{b}	17.9^{b}	64.8^{c}	3.5^{d}
Sacramento MG 1	14.8^{a}	66.4^{a}	76.9^{b}	14.4^{a}	5.3^{c}	20.4^{a}	83.3^{a}	4.1^{b}
Catiguá MG 2	13.4^{b}	56.3^{b}	64.9^{c}	13.1^{a}	5.0^{d}	18.2^{b}	72.9^{b}	4.0^{b}
Araponga MG 1	14.8^{a}	62.2^{a}	69.8^{c}	13.6^{a}	5.2^{c}	21.7^{a}	87.1^{a}	4.0^{b}
Paraíso MG 419-1	12.7^{c}	46.7^{c}	63.0^{d}	12.8^{b}	5.0^{d}	18.7^{b}	66.8^{c}	3.6^{d}
Pau Brasil MG 1	13.8^{b}	52.0^{c}	65.2^{c}	13.6^{a}	4.8^{d}	19.7^{a}	76.5^{b}	3.9^{c}
Catiguá MG 3	13.2^{b}	49.9^{c}	62.5^{d}	12.8^{b}	4.9^{d}	17.3^{b}	67.1^{c}	3.8^{c}
Topázio MG 1190	14.3^{a}	48.7^{c}	66.7^{c}	12.9^{b}	5.2^{c}	19.0^{a}	67.2^{c}	3.5^{d}
'23 II'	15.4^{a}	61.3^{a}	76.6^{b}	13.4^{a}	5.8^{b}	19.3^{a}	85.4^{a}	4.5^{a}
IPR 104	15.1^{a}	51.7^{c}	64.2^{c}	12.3^{b}	5.3^{c}	18.6^{b}	70.2^{c}	3.8^{c}
Sarchimor MG8840	15.2^{a}	57.8^{b}	69.9^{c}	11.6^{c}	6.1^{b}	17.9^{b}	78.9^{b}	4.4^{a}
Red Catucaí 20/1 pit 476	12.9^{c}	45.6^{c}	63.3^{d}	12.0^{c}	5.3^{c}	17.1^{b}	60.8^{c}	3.5^{d}
Tupi IAC 1669-33	13.9^{a}	56.4^{b}	56.8^{e}	12.8^{b}	4.5^{d}	20.3^{a}	74.7^{b}	3.7^{c}
Red Obatã IAC 1669-20	14.8^{a}	56.3^{b}	64.8^{c}	12.1^{c}	5.4^{c}	18.7^{b}	74.4^{b}	4.0^{b}
Yellow Obatã IAC 4932	13.7^{b}	47.5^{c}	64.5^{c}	13.3^{a}	4.9^{d}	17.4^{b}	66.2^{c}	3.8^{c}
Red Catuaí IAC 15	12.9^{c}	45.8^{c}	67.4^{c}	13.7^{a}	4.9^{d}	18.3^{b}	64.4^{c}	3.5^{d}
Yellow Catuaí IAC 062	13.7^{b}	48.9^{c}	71.1^{c}	14.1^{a}	5.1^{c}	20.8^{a}	74.0^{b}	3.6^{d}
IPR 98	14.6^{a}	55.8^{b}	61.8^{d}	12.3^{b}	5.1^{c}	20.2^{a}	70.3^{c}	3.5^{d}
IPR 99	14.7^{a}	50.4^{c}	69.4^{c}	12.7^{b}	5.5^{b}	18.3^{b}	72.5^{b}	4.0^{b}
IPR 100	13.7^{b}	46.7^{c}	66.3^{c}	12.8^{b}	5.2^{c}	19.8^{a}	72.3^{b}	3.7^{c}
IPR 103	13.5^{b}	48.3^{c}	68.1^{c}	13.0^{a}	5.3^{c}	18.3^{b}	70.0^{c}	3.8^{c}
Yellow Catucaí 2SL	14.5^{a}	55.8^{b}	97.4^{a}	14.1^{a}	6.9^{a}	17.8^{b}	82.9^{a}	4.7^{a}
Yellow Catucaí 24/137	12.8^{c}	50.1^{c}	67.3^{c}	12.4^{b}	5.5^{b}	17.8^{b}	67.1^{c}	3.8^{c}
Yellow Catucaí 20/15 pit 479	14.0^{a}	53.8^{b}	79.2^{b}	13.9^{a}	5.7^{b}	19.9^{a}	79.7^{b}	4.0^{b}
Red Catucaí 785/15	13.7^{b}	51.1^{c}	69.3^{c}	14.4^{a}	4.8^{d}	19.4^{a}	65.2^{c}	3.4^{d}
Acauã 2 and 8	14.0^{a}	45.6^{c}	57.9^{e}	11.5^{c}	5.1^{c}	17.8^{b}	59.9^{c}	3.4^{d}
Late Sabiá or Sabiá 398	14.9^{a}	50.3^{c}	66.4^{c}	13.4^{a}	5.0^{d}	19.5^{a}	74.2^{b}	3.8^{c}
Asa Branca	12.8^{c}	47.4^{c}	66.5^{c}	11.8^{c}	5.8^{b}	16.2^{b}	68.4^{c}	4.2^{b}
IBC - Palma 2	13.5^{b}	58.9^{b}	67.2^{c}	13.7^{a}	4.9^{d}	20.7^{a}	76.4^{b}	3.7^{c}
Acauã	14.5^{a}	52.8^{b}	63.9^{c}	13.0^{a}	4.9^{d}	20.2^{a}	70.8^{c}	3.5^{d}
New Acauã	14.5^{a}	56.4^{b}	67.4^{c}	13.3^{a}	5.2^{c}	20.9^{a}	78.1^{b}	3.7^{c}
'H-419-3-3-7-16-4-1'	13.9^{a}	53.1^{b}	65.3^{c}	12.6^{b}	5.2^{c}	18.6^{b}	68.9^{c}	3.7^{c}
'Paraíso H 419-10-6-2-12-1'	11.4^{c}	44.6^{c}	55.1^{e}	11.8^{c}	4.7^{d}	17.8^{b}	64.2^{c}	3.6^{d}
'Paraíso H 419-10-6-2-10-1'	12.6^{c}	45.1^{c}	62.3^{d}	11.5^{c}	5.5^{b}	18.2^{b}	65.8^{c}	3.6^{d}

*Averages followed by the same letter in the column do not differ by Scott-Knott test at 5% probability of error. **Stem diameter (DST), canopy diameter (DCA), plant height (HEI), number of orthotropic branch nodes (NOBN), average length (cm) of orthotropic branch internodes (ALOBI), total number of nodes at the 2nd plagiotropic branch pair (TNPB), total length (cm) at the 2nd plagiotropic branch pair (TLPB), and average length (cm) of plagiotropic branch internodes (ALPBI).

Fernandes et al. (2012) reported that coffee tree growth was highest in the hottest and rainy months, which would be October to April in this experiment, period in which we obtained better results of growth of the studied genotypes. In addition, longer days occur during this time, providing greater energy availability in the form of solar radiation and temperature (Camargo and Camargo, 2001). The number of groups formed by Ward's agglomerative method shows that there is wide variability among the evaluated genotypes. Guedes et al. (2013) verified genetic divergence among coffee trees of the Maragogipe germplasm in the Alto Paranaíba region of the State of Minas Gerais, using the Tocher method. This shows that the genetic divergence among coffee plants is mainly due to genetics, as recommended by Giles et al. (2019).

The cultivars Sacramento MG 1, Sarchimor MG8840, and Yellow Catucaí 2SL showed similar phenological traits and were included in the same group until 24 months, when Yellow Catucaí 2SL formed a new group.

Table 2. Phenological variables of coffee trees 12 months after the plantation was cultivated and irrigated in the Cerrado of Goiás.

Genotype	DST** (mm)	DCA (cm)	HEI (cm)	NOBN	ALOBI (cm)	TNPB	TLPB (cm)	ALPBI (cm)
Oeiras MG 6851	26.0^{c}*	102.3^{d}	99.6^{e}	17.9^{d}	5.6^{c}	32.9^{b}	103.9^{e}	3.1^{d}
Catiguá MG 1	26.6^{c}	104.8^{c}	100.4^{e}	18.3^{c}	5.5^{c}	33.3^{b}	114.3^{d}	3.5^{b}
Sacramento MG 1	30.0^{b}	139.8^{a}	116.3^{c}	20.8^{a}	5.6^{c}	41.0^{a}	150.4^{b}	3.7^{b}
Catiguá MG 2	26.7^{c}	118.3^{b}	98.0^{f}	18.9^{c}	5.2^{d}	38.2^{a}	127.6^{c}	3.3^{c}
Araponga MG 1	29.0^{b}	127.4^{b}	108.8^{d}	20.5^{b}	5.3^{d}	38.7^{a}	137.5^{c}	3.6^{b}
Paraíso MG 419-1	23.5^{d}	103.1^{d}	97.9^{f}	18.8^{c}	5.3^{d}	37.8^{a}	118.0^{d}	3.2^{d}
Pau Brasil MG 1	27.0^{c}	105.4^{c}	102.3^{e}	20.1^{b}	5.1^{e}	37.4^{a}	126.3^{c}	3.4^{c}
Catiguá MG 3	23.5^{d}	89.3^{e}	92.1^{f}	16.4^{e}	5.6^{c}	26.7^{c}	93.9^{e}	3.5^{b}
Topázio MG 1190	28.3^{b}	107.4^{c}	102.7^{e}	19.8^{b}	5.2^{d}	38.8^{a}	120.4^{d}	3.1^{d}
'23 II'	28.9^{b}	120.9^{b}	107.4^{d}	17.9^{d}	6.0^{b}	31.3^{b}	129.2^{c}	4.2^{a}
IPR 104	28.5^{b}	109.4^{c}	101.7^{e}	18.7^{c}	5.5^{c}	34.6^{b}	122.0^{d}	3.5^{b}
Sarchimor MG8840	29.0^{b}	132.3^{a}	109.3^{d}	17.6^{d}	6.2^{b}	37.6^{a}	148.3^{b}	3.9^{a}
Red Catucaí 20/1 pit 476	26.6^{c}	106.3^{c}	103.6^{e}	19.8^{b}	5.3^{d}	37.3^{a}	123.2^{d}	3.3^{c}
Tupi IAC 1669-33	26.2^{c}	122.2^{b}	98.2^{f}	20.5^{b}	4.8^{e}	35.6^{a}	127.9^{c}	3.6^{b}
Red Obatã IAC 1669-20	29.4^{b}	120.9^{b}	107.4^{d}	18.8^{c}	5.7^{c}	38.3^{a}	137.5^{c}	3.6^{b}
Yellow Obatã IAC 4932	26.1^{c}	99.5^{d}	100.3^{e}	18.3^{c}	5.5^{c}	38.8^{a}	130.1^{c}	3.4^{c}
Red Catuaí IAC 15	26.9^{c}	106.6^{c}	105.7^{d}	18.9^{c}	5.6^{c}	38.6^{a}	125.7^{c}	3.3^{c}
Yellow Catuaí IAC 062	28.2^{b}	108.4^{c}	110.3^{d}	20.1^{b}	5.5^{c}	40.2^{a}	131.9^{c}	3.3^{c}
IPR 98	29.4^{b}	112.6^{c}	100.0^{e}	18.9^{c}	5.3^{d}	40.9^{a}	136.5^{c}	3.4^{c}
IPR 99	28.8^{b}	108.6^{c}	103.2^{e}	18.7^{c}	5.5^{c}	38.0^{a}	130.4^{c}	3.5^{b}
IPR 100	27.9^{b}	110.0^{c}	106.9^{d}	20.5^{b}	5.2^{d}	42.6^{a}	139.6^{c}	3.3^{c}
IPR 103	27.8^{b}	108.1^{c}	112.9^{c}	19.5^{b}	5.8^{c}	38.4^{a}	135.3^{c}	3.5^{b}
Yellow Catucaí 2SL	32.8^{a}	136.3^{a}	145.8^{a}	19.2^{c}	7.7^{a}	38.7^{a}	164.3^{a}	4.3^{a}
Yellow Catucaí 24/137	26.4^{c}	106.8^{c}	106.9^{d}	19.1^{c}	5.6^{c}	33.3^{b}	113.9^{d}	3.4^{b}
Yellow Catucaí 20/15 pit 479	29.1^{b}	119.6^{b}	121.2^{b}	21.3^{a}	5.7^{c}	41.1^{a}	141.8^{b}	3.5^{b}
Red Catucaí 785/15	27.5^{b}	100.8^{d}	107.8^{d}	21.5^{a}	5.0^{e}	38.5^{a}	116.2^{d}	3.0^{d}
Acauã 2 and 8	27.3^{b}	102.6^{d}	96.3^{f}	18.2^{c}	5.3^{d}	31.9^{b}	106.3^{e}	3.4^{c}
Late Sabiá or Sabiá 398	28.0^{b}	117.1^{b}	103.6^{e}	19.8^{b}	5.2^{d}	40.0^{a}	143.6^{b}	3.6^{b}
Asa Branca	27.3^{b}	122.4^{b}	107.2^{d}	18.5^{c}	5.8^{c}	37.6^{a}	153.8^{b}	4.1^{a}
IBC - Palma 2	27.5^{b}	105.8^{c}	105.9^{d}	20.2^{b}	5.3^{d}	31.3^{b}	106.0^{e}	3.4^{b}
Acauã	28.4^{b}	108.1^{c}	104.1^{e}	20.1^{b}	5.2^{d}	34.5^{b}	118.8^{d}	3.5^{b}
New Acauã	28.8^{b}	114.1^{c}	107.6^{d}	21.6^{a}	5.0^{e}	39.0^{a}	126.2^{c}	3.3^{c}
'H-419-3-3-7-16-4-1'	27.4^{b}	120.3^{b}	104.6^{d}	18.6^{c}	5.7^{c}	39.7^{a}	133.6^{c}	3.3^{c}
'Paraíso H 419-10-6-2-12-1'	24.2^{d}	97.1^{d}	93.1^{f}	18.4^{c}	5.0^{e}	35.3^{b}	102.6^{e}	2.9^{d}
'Paraíso H 419-10-6-2-10-1'	25.4^{c}	108.3^{c}	97.8^{f}	17.6^{d}	5.6^{c}	37.8^{a}	126.2^{c}	3.3^{c}

*Averages followed by the same letter in the column do not differ by Scott-Knott test at 5% probability of error. **Stem diameter (DST), canopy diameter (DCA), plant height (HEI), number of orthotropic branch nodes (NOBN), average length (cm) of orthotropic branch internodes (ALOBI), total number of nodes at the 2nd plagiotropic branch pair (TNPB), total length (cm) at the 2nd plagiotropic branch pair (TLPB), and average length (cm) of plagiotropic branch internodes (ALPBI).

This result may be attributed to the fact that this genotype had the highest averages for phenology traits (DST = 56.2 mm, DCA = 210.4 cm, HEI = 226.2 cm, ALOBI = 6.9 cm, TNPB = 58, 6, and TLPB = 205.1 cm) compared to the other genotypes. High phenology averages for Yellow Catucaí 2SL may be due to that fact that this cultivar is a hybrid (Icatu × Catuaí) and is highly adaptable, which is a known characteristic of 'Catuaí' (Botelho et al*.,* 2010). However, densification between plants could have caused superior development of this cultivar. Pereira et al*.* (2011) found that the spacing between lines and between plants influenced the growth and architecture of *Coffea arabica* trees. However, this genotype-environment interaction is unique to this cultivar, since the other cultivars did not show the same pattern of development.

Three groups were identified at 6 months (Figure 1). Yellow Catucaí 2SL, Araponga MG 1, Sacramento MG 1,

Table 3. Phenological variables of coffee trees 18 months after the plantation was cultivated and irrigated in the Cerrado of Goiás.

Genotype	DST** (mm)	DCA (cm)	HEI (cm)	NOBN	ALOBI (cm)	TNPB	TLPB (cm)	ALPBI (cm)
Oeiras MG 6851	34.9[c]*	127.3[d]	120.9[e]	25[c]	6.8[e]	54.0[b]	185.8[d]	4.4[e]
Catiguá MG 1	36.0[c]	131.7[c]	123.4[d]	24[c]	6.0[d]	51.0[b]	167.3[c]	3.8[d]
Sacramento MG 1	41.3[b]	167.3[a]	144.1[b]	27[a]	6.0[d]	50.8[a]	164.7[a]	3.7[c]
Catiguá MG 2	35.9[c]	148.8[b]	123.7[d]	25[c]	5.9[e]	50.4[a]	164.2[b]	3.6[d]
Araponga MG 1	39.3[b]	138.3[c]	133.4[c]	28[a]	5.6[e]	49.6[a]	160.9[b]	3.5[c]
Paraíso MG 419-1	34.5[c]	135.7[c]	119.3[e]	26[b]	5.5[f]	49.3[a]	159.3[c]	3.5[f]
Pau Brasil MG 1	35.7[c]	127.6[d]	126.3[d]	26[b]	5.5[e]	48.9[a]	157.3[c]	3.4[d]
Catiguá MG 3	33.7[c]	115.6[d]	113.3[e]	22[d]	5.3[d]	48.7[c]	154.2[d]	3.4[d]
Topázio MG 1190	38.7[b]	136.8[c]	127.4[d]	27[a]	5.3[f]	48.6[a]	151.4[b]	3.4[f]
'23 II'	40.8[b]	152.6[b]	139.8[b]	23[d]	5.3[b]	48.0[b]	150.5[b]	3.3[a]
IPR 104	39.0[b]	142.8[c]	128.3[d]	27[b]	5.2[e]	47.6[a]	150.4[b]	3.3[e]
Sarchimor MG8840	41.0[b]	171.8[a]	138.9[b]	23[d]	5.1[b]	47.5[a]	150.1[a]	3.3[b]
Red Catucaí 20/1 pit 476	35.6[c]	134.0[c]	125.6[d]	27[a]	5.1[f]	47.4[a]	148.7[b]	3.2[e]
Tupi IAC 1669-33	35.0[c]	155.9[b]	129.8[d]	28[a]	5.1[f]	46.8[a]	147.1[b]	3.2[d]
Red Obatã IAC 1669-20	39.1[b]	157.0[b]	137.4[c]	25[c]	5.1[c]	46.8[a]	145.8[a]	3.2[d]
Yellow Obatã IAC 4932	37.3[c]	136.4[c]	127.8[d]	24[c]	5.0[d]	46.5[a]	144.8[b]	3.2[d]
Red Catuaí IAC 15	36.8[c]	138.6[c]	132.0[c]	26[b]	4.9[d]	45.4[a]	142.0[b]	3.2[e]
Yellow Catuaí IAC 062	38.2[b]	136.6[c]	134.1[c]	27[b]	4.9[d]	44.4[a]	141.3[b]	3.2[e]
IPR 98	38.8[b]	150.7[b]	127.8[d]	26[b]	4.9[e]	44.2[a]	136.9[b]	3.2[e]
IPR 99	39.8[b]	139.6[c]	135.7[c]	25[c]	4.9[c]	44.2[a]	135.9[b]	3.2[e]
IPR 100	38.2[b]	140.1[c]	130.9[c]	27[a]	4.9[e]	44.1[a]	135.1[a]	3.2[e]
IPR 103	38.2[b]	139.9[c]	139.2[b]	25[c]	4.9[c]	42.7[a]	135.0[a]	3.1[c]
Yellow Catucaí 2SL	46.4[a]	172.1[a]	179.1[a]	26[b]	4.8[a]	42.7[a]	134.0[a]	3.1[b]
Yellow Catucaí 24/137	35.4[c]	137.4[c]	136.9[c]	26[b]	4.8[d]	42.5[a]	133.6[c]	3.1[e]
Yellow Catucaí 20/15 pit 479	41.0[b]	144.6[c]	147.5[b]	28[a]	4.8[d]	42.3[a]	132.9[a]	3.1[d]
Red Catucaí 785/15	36.6[c]	127.8[d]	127.0[d]	29[a]	4.8[f]	41.3[a]	132.5[c]	3.1[f]
Acauã 2 and 8	37.0[c]	124.1[d]	123.6[d]	24[c]	4.8[d]	40.8[b]	130.9[c]	3.1[d]
Late Sabiá or Sabiá 398	37.6[c]	156.9[b]	131.4[c]	27[a]	4.8[e]	39.8[a]	125.4[a]	3.1[e]
Asa Branca	36.8[c]	155.6[b]	136.9[c]	23[d]	4.7[b]	39.1[a]	125.2[b]	3.0[b]
IBC - Palma 2	36.7[c]	117.9[d]	128.0[d]	27[a]	4.7[f]	39.1[b]	125.1[c]	3.0[c]
Acauã	38.7[b]	143.3[c]	130.6[c]	27[a]	4.6[e]	38.4[a]	121.6[b]	3.0[e]
New Acauã	38.4[b]	146.4[b]	134.1[c]	28[a]	4.6[e]	34.1[a]	116.8[b]	2.9[d]
'H-419-3-3-7-16-4-1'	37.4[c]	149.4[b]	124.9[d]	26[b]	4.6[e]	31.9[a]	109.6[b]	2.8[d]
'Paraíso H 419-10-6-2-12-1'	33.9[c]	123.9[d]	113.8[e]	25[c]	4.6[f]	31.6[a]	107.2[c]	2.8[f]
'Paraíso H 419-10-6-2-10-1'	34.4[c]	133.0[c]	119.3[e]	24[c]	4.4[e]	29.3[a]	98.6[b]	2.7[e]

*Averages followed by the same letter in the column do not differ by Scott-Knott test at 5% probability of error. **Stem diameter (DST), canopy diameter (DCA), plant height (HEI), number of orthotropic branch nodes (NOBN), average length (cm) of orthotropic branch internodes (ALOBI), total number of nodes at the 2nd plagiotropic branch pair (TNPB), total length (cm) at the 2nd plagiotropic branch pair (TLPB), and average length (cm) of plagiotropic branch internodes (ALPBI).

23 II, Yellow Catucaí 20/15 pit 479, Sarchimor MG8840, IBC-Palma 2, and New Acauã formed the first group, which had the highest averages for most analyzed variables (DST, DCA, NOBN, TNPB, and TLPB). The second group consisted of the genotypes 7, 28, 21, 18, 20, 14, 4, 15, 19, 33, 11, and 31, which had the highest averages of phenological development for DST, NOBN, and TNPB. The third group, whichconsisted of treatments 26, 9, 24, 22, 29, 2, 17, 8, 35, 6, 16, 1, 13, 27, and 34, had the smallest number of significant variables, with DCA and TLPB showing homogeneity. At this stage of growth, the phenological variables that showed significant differences for most genotypes were DST, NOBN, and TNPB (Table 1).

The dendrogram for the evaluation at 12 months shows three groups that were divided into subgroups (Figure 2). The first group comprised 8, 34, 1, 27, 26, 30, 2, 24, 6, 9, 11, 31, 16, 17, 7, 13, 20, and 35 and had the smallest

Table 4. Phenological variables of coffee trees 24 months after the plantation was cultivated and irrigated in the Cerrado of Goiás.

Genotype	DST** (mm)	DCA (cm)	HEI (cm)	NOBN	ALOBI (cm)	TNPB	TLPB (cm)	ALPBI (cm)
Oeiras MG 6851	41.2^{d}*	145.6^{e}	151.7^{e}	30.9^{b}	4.9^{d}	43.4^{c}	139.7^{e}	3.2^{d}
Catiguá MG 1	41.5^{d}	159.5^{d}	156.8^{e}	31.4^{b}	5.1^{c}	46.7^{c}	151.3^{d}	3.3^{d}
Sacramento MG 1	49.3^{b}	191.4^{b}	177.8^{c}	34.1^{a}	5.2^{c}	47.6^{c}	179.0^{b}	3.8^{b}
Catiguá MG 2	43.5^{d}	164.8^{d}	155.3^{e}	31.8^{b}	4.9^{d}	43.3^{c}	148.5^{d}	3.5^{c}
Araponga MG 1	45.7^{c}	173.5^{c}	171.8^{d}	35.8^{a}	4.8^{d}	49.6^{c}	167.2^{c}	3.5^{c}
Paraíso MG 419-1	41.4^{d}	156.5^{d}	150.0f	31.9^{b}	4.7^{d}	51.8^{b}	153.4^{d}	3.0^{e}
Pau Brasil MG 1	42.8^{d}	163.1^{d}	153.3^{e}	30.4^{c}	5.1^{c}	48.1^{c}	147.4^{d}	3.1^{d}
Catiguá MG 3	40.3^{d}	137.1^{e}	143.4f	28.5^{c}	5.1^{c}	35.1^{d}	129.2^{e}	3.7^{b}
Topázio MG 1190	45.8^{c}	173.3^{c}	165.3^{d}	32.1^{b}	5.2^{c}	58.5^{a}	169.7^{c}	2.9^{e}
'23 II'	46.6^{c}	177.9^{c}	166.9^{d}	27.9^{c}	6.1^{b}	40.9^{d}	161.3^{c}	4.1^{a}
IPR 104	44.3^{c}	178.8^{c}	163.1^{d}	32.1^{b}	5.1^{c}	58.4^{a}	167.3^{c}	2.9^{e}
Sarchimor MG8840	47.4^{c}	182.0^{c}	170.8^{d}	29.2^{c}	5.9^{b}	47.1^{c}	171.6^{c}	3.7^{c}
Red Catucaí 20/1 pit 476	42.2^{d}	170.9^{d}	162.4^{d}	32.9^{b}	4.9^{d}	52.7^{b}	157.8^{d}	3.0^{e}
Tupi IAC 1669-33	41.8^{d}	168.1^{d}	155.6^{e}	30.8^{b}	5.1^{c}	43.4^{c}	148.1^{d}	3.4^{c}
Red Obatã IAC 1669-20	47.2^{c}	193.1^{b}	162.9^{d}	29.3^{c}	5.6^{c}	54.1^{b}	175.4^{b}	3.3^{d}
Yellow Obatã IAC 4932	46.6^{c}	176.8^{c}	155.5^{e}	28.7^{c}	5.4^{c}	54.0^{b}	162.6^{c}	3.3^{d}
Red Catuaí IAC 15	43.8^{d}	176.9^{c}	165.1^{d}	31.3^{b}	5.4^{c}	53.6^{b}	163.6^{c}	3.1^{d}
Yellow Catuaí IAC 062	43.8^{d}	183.9^{c}	169.8^{d}	33.2^{b}	5.1^{c}	60.3^{a}	176.9^{b}	3.0^{e}
IPR 98	44.8^{c}	180.6^{c}	160.3^{d}	31.8^{b}	5.1^{c}	56.5^{a}	166.8^{c}	3.0^{e}
IPR 99	46.1^{c}	184.3^{c}	164.6^{d}	31.6^{b}	5.2^{c}	57.6^{a}	178.1^{b}	3.1^{d}
IPR 100	45.1^{c}	188.2^{b}	165.6^{d}	32.8^{b}	5.1^{c}	61.6^{a}	182.3^{b}	3.0^{e}
IPR 103	45.7^{c}	188.4^{b}	167.9^{d}	32.9^{b}	5.3^{c}	57.8^{a}	181.8^{b}	3.2^{d}
Yellow Catucaí 2SL	56.2^{a}	210.4^{a}	226.2^{a}	32.9^{b}	6.9^{a}	58.6^{a}	205.1^{a}	3.5^{c}
Yellow Catucaí 24/137	43.4^{d}	168.9^{d}	171.0^{d}	33.5^{b}	5.1^{c}	53.3^{b}	162.1^{c}	3.1^{d}
Yellow Catucaí 20/15 pit 479	49.5^{b}	194.1^{b}	188.4^{b}	36.4^{a}	5.2^{c}	59.3^{a}	181.5^{b}	3.1^{d}
Red Catucaí 785/15	45.3^{c}	157.2^{d}	163.5^{d}	31.3^{b}	5.3^{c}	55.2^{b}	150.3^{d}	2.8^{e}
Acauã 2 and 8	42.6^{d}	151.9^{e}	159.1^{e}	32.4^{b}	4.9^{d}	38.4^{d}	128.9^{e}	3.4^{c}
Late Sabiá or Sabiá 398	44.8^{c}	194.4^{b}	165.4^{d}	32.5^{b}	5.1^{c}	61.4^{a}	178.4^{b}	2.9^{e}
Asa Branca	45.3^{c}	197.4^{b}	163.9^{d}	26.8^{c}	6.2^{b}	52.9^{b}	189.3^{b}	3.6^{c}
IBC - Palma 2	42.4^{d}	144.1^{e}	160.2^{d}	32.1^{b}	5.0^{c}	47.0^{c}	144.6^{d}	3.3^{d}
Acauã	45.6^{c}	177.0^{c}	166.2^{d}	32.6^{b}	5.2^{c}	52.3^{b}	161.1^{c}	3.1^{d}
New Acauã	45.0^{c}	186.7^{b}	167.2^{d}	35.6^{a}	4.7^{d}	52.4^{b}	165.4^{c}	3.2^{d}
'H-419-3-3-7-16-4-1'	44.6^{c}	180.6^{c}	162.8^{d}	32.4^{b}	5.1^{c}	58.4^{a}	178.1^{b}	3.1^{d}
'Paraíso H 419-10-6-2-12-1'	41.9^{d}	157.8^{d}	145.3f	31.6^{b}	4.6^{d}	49.9^{c}	138.3^{e}	2.8^{e}
'Paraíso H 419-10-6-2-10-1'	41.8^{d}	167.4^{d}	148.4f	27.4^{c}	5.5^{c}	55.0^{b}	162.1^{c}	3.0^{e}

*Averages followed by the same letter in the column do not differ by Scott-Knott test at 5% probability of error. **Stem diameter (DST), canopy diameter (DCA), plant height (HEI), number of orthotropic branch nodes (NOBN), average length (cm) of orthotropic branch internodes (ALOBI), total number of nodes at the 2nd plagiotropic branch pair (TNPB), total length (cm) at the 2nd plagiotropic branch pair (TLPB), and average length (cm) of plagiotropic branch internodes (ALPBI).

averages for most variables, particularly HEI and TLPB. The second group had the highest average for TNPB and the lowest for HEI and consisted of treatments 4, 14, 10, 32, 18, 22, 28, 19, 21, 25, 5, 15, and 33. The third group was formed by Yellow Catucaí 2SL, Asa Branca, Sacramento MG1, and Sarchimor MG8840 cultivars. This group had the highest averages for most of the analyzed variables, especially DST, DCA, TNPB, TLPB, and ALPBI (Table 2).

The dendrogram of the 18-month evaluation had three groups (Figure 3). The first group had the lowest averages for ALOBI and ALPBI, but the TNPB variable had higher averages. This group consisted of genotypes 34, 35, 33, 31, 32, 19, 28, 29, 30, 26, 27, 25, 17, 18, 21, 24, 20, and 22. The second group consisted of genotypes 1, 8, 11, 16, 9, 13, 7, 2, 6, 4, and 5 and presented highest average for TNPB, whereas this group had the lowest averages for HEI, ALOBI, and ALPBI. The third group

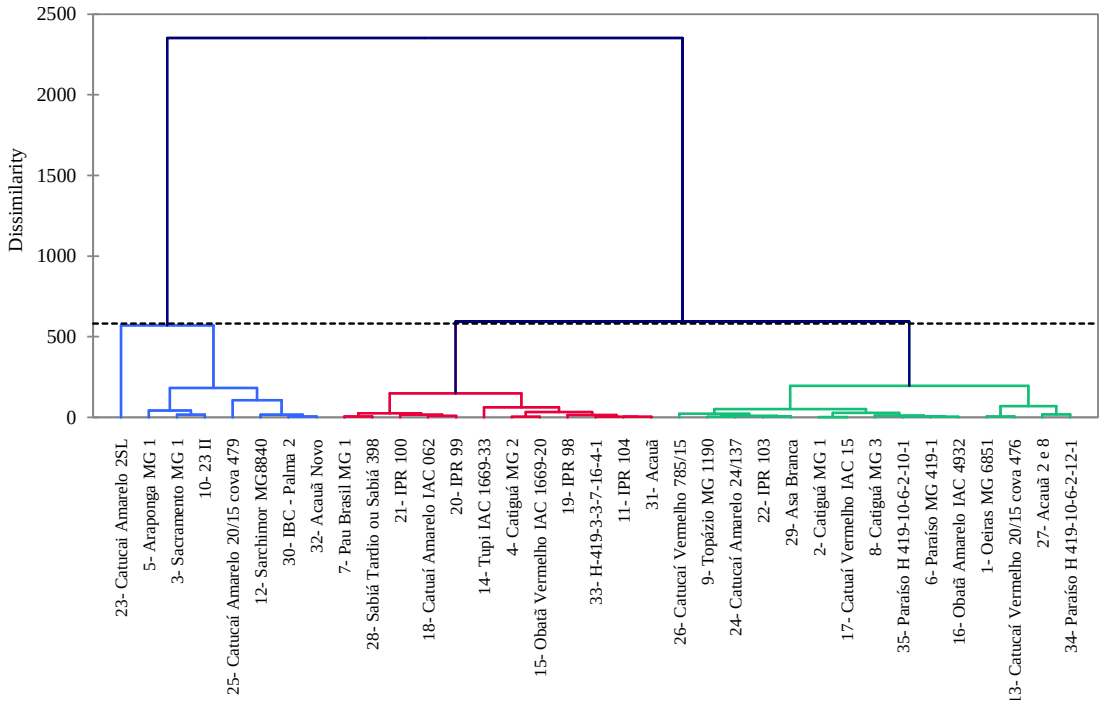

Figure 1. Dendrogram showing the grouping of 35 genotypes of *C. arabica* at 6 months. Hierarchical Agglomerative Cluster was used with mean Euclidian distance and Ward's agglomeration method to analyze 8 phenological characteristics.

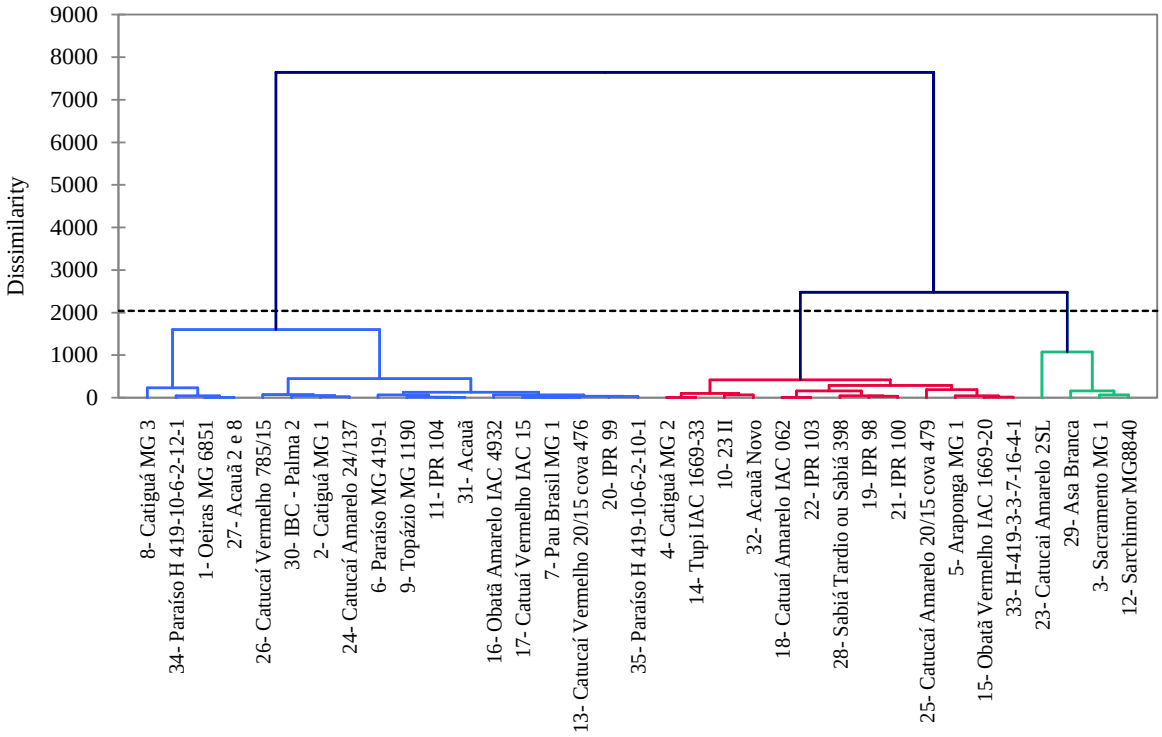

Figure 2. Dendrogram showing the grouping of 35 genotypes of *C. arabica* at 12 months. Hierarchical Agglomerative Cluster was used with mean Euclidian distance and Ward's agglomeration method to analyze 8 phenological characteristics.

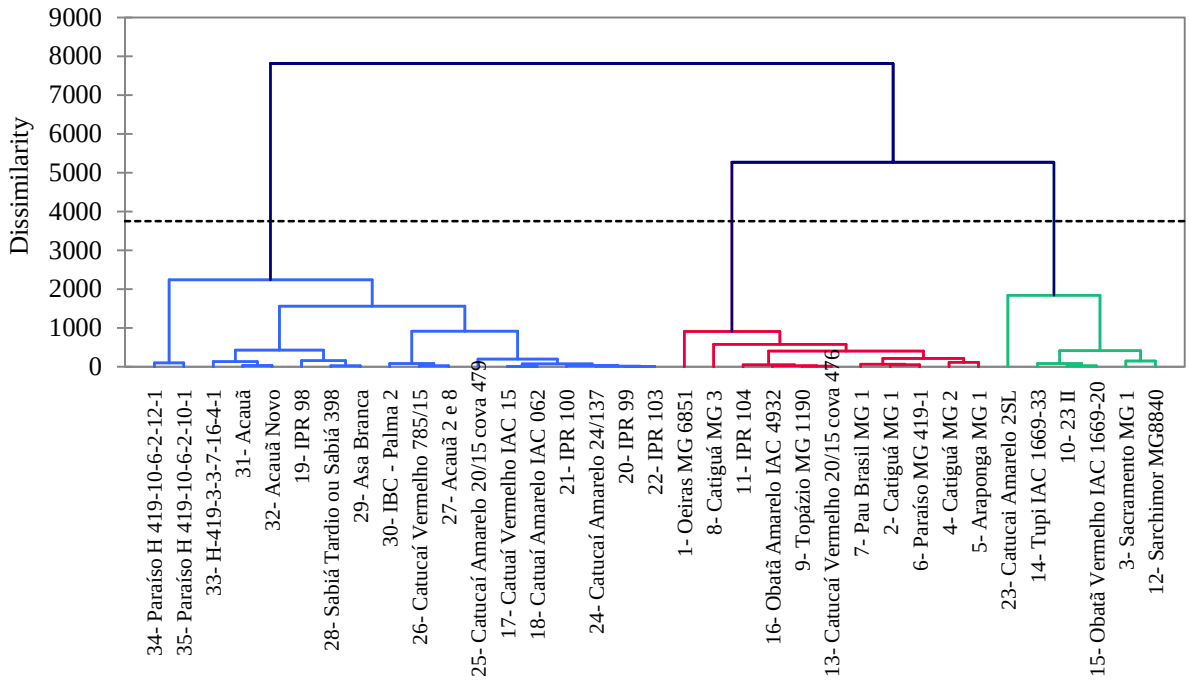

Figure 3. Dendrogram showing the grouping of 35 genotypes of *C. arabica* at 18 months. Hierarchical Agglomerative Cluster was used with mean Euclidian distance and Ward's agglomeration method to analyze 8 phenological characteristics.

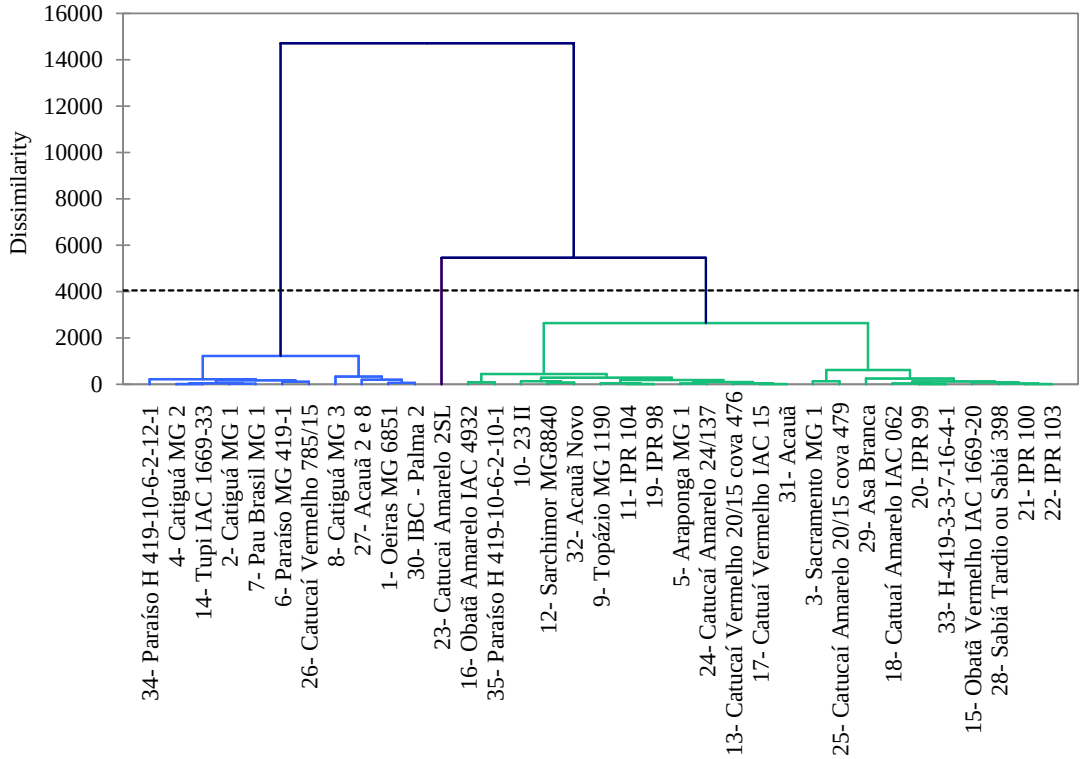

Figure 4. Dendrogram showing the grouping of 35 genotypes of *C. arabica* at 24 months. Hierarchical Agglomerative Cluster was used with mean Euclidian distance and Ward's agglomeration method to analyze 8 phenological characteristics.

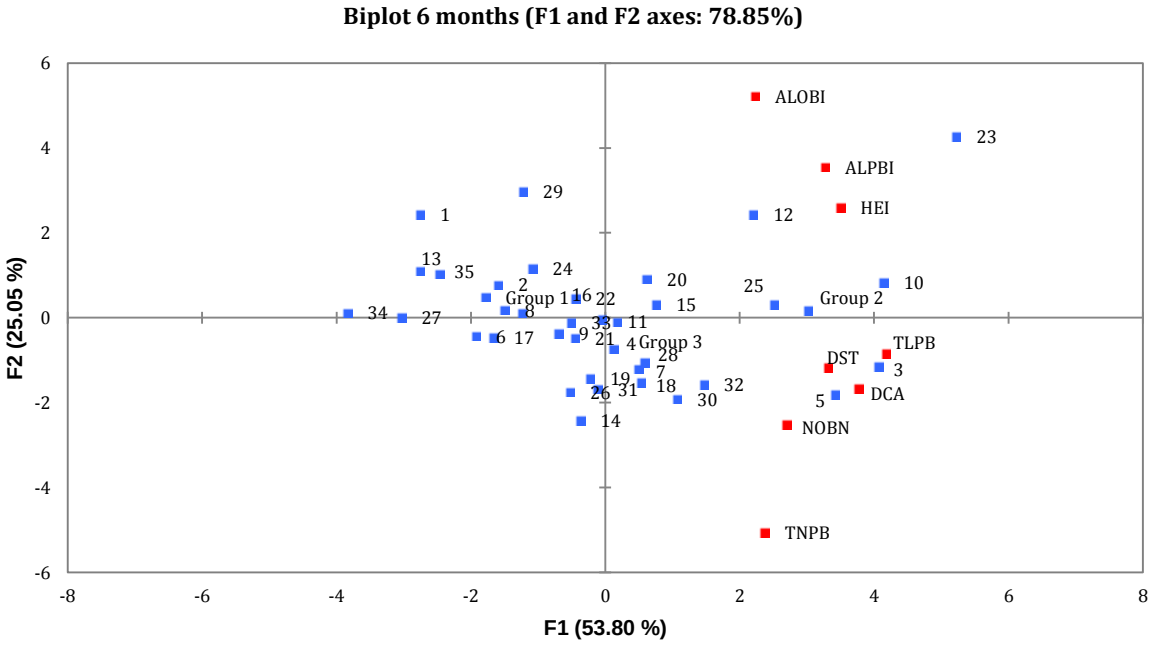

Figure 5. Biplot of the relative contribution of the variables to the genetic dissimilarity of each group in the phenological evaluation performed at 6 months, evidencing the frequencies.

consisted of Yellow Catucaí 2SL, Tupi IAC 1669-33, 23 II, Red Obatã IAC 1669-20, Sacramento MG 1, and Sarchimor MG8840 and had the highest average values for DCA, TNPB, and TLPB but the lowest averages for ALOBI. This group formed two subgroups when regrouped, one of which consisted of Yellow Catucaí 2SL, probably because it had high average values for DST, DCA, HEI, ALOBI, TNPB, and TLPB (Table 3).

The first group formed in the 24th month dendrogram consisted of the treatments 34, 4, 14, 2, 7, 6, 26, 8, 27, 1, and 30 (Figure 4). This group had the lowest averages, particularly for DST, DCA, HEI, and TLPB. The second group consisted entirely of the genotype Yellow Catucaí 2SL. The third group consisted of the treatments 16, 35, 10, 12, 32, 9, 11, 19, 5, 24, 13, 17, 31, 3, 25, 29, 18, 20, 33, 15, 28, 21, and 22 and had the lowest averages for HEI and ALPBI, with only TNPB having a greater amount of significant averages (Table 4).

PCA results showed that the relative contribution of phenological variables to genetic dissimilarity (frequency) was 78.85% at 6 months (Figure 5), with the F1 component contributing 53.80% and the F2 component contributing 25.05%. For the second evaluation period (12 months), a frequency of 81.38% was observed (Figure 6) with F1 contributing 57.99% and F2 contributing 23.39%. At 18 and 24 months, the relative contribution was 79.73% (F1 = 47.48% and F2 = 32.25%) and 81.97% (F1 = 47.48% and F2 = 32.25%), respectively (Figures 7 and 8). Thus, variability in the contribution of phenological variables was observed mainly at 18 and 24 months. This could be due to a decrease in photo-assimilated reserves, causing a decrease in the growth rate of the plants, as they were in the process of filling the grains, which is considered to be a substantial photo-assimilates drain (Arantes et al*.,* 2006).

The PCA shows that there was a large contribution of the phenological variables to genetic dissimilarity (>75%) in the four evaluation periods. Rodrigues et al. (2013) verified that evaluation methods of productivity, stability, and adaptability, the harmonic mean of the genetic values, the relative performance of the genetic values, and the harmonic mean of the relative performance of the predicted must be part of the selection criteria for recommendation of genotypes of coffee for commercial plantations.

However, phenological patterns can vary within the same plant species if evaluated in different ecosystems, and variation can occur between populations, individuals, and years (Mantovani et al*.,* 2003). Moreover, several factors can influence these phenological variations, such as exposure to light, leaf damage, water stress, or flower abortion. Thus, the influence of these factors on coffee phenology should be considered when examining a particular genotype in different regions and conditions. By analyzing the contribution rate of phenological variables over four evaluation periods (Figure 9), a contribution percentage equal to or greater than 25%was observed for: ALOBI, TNPB, and ALPBI at 6 months; NOBN, ALOBI, TNPB, and ALPBI at 12 months; DST, DCA, HEI,

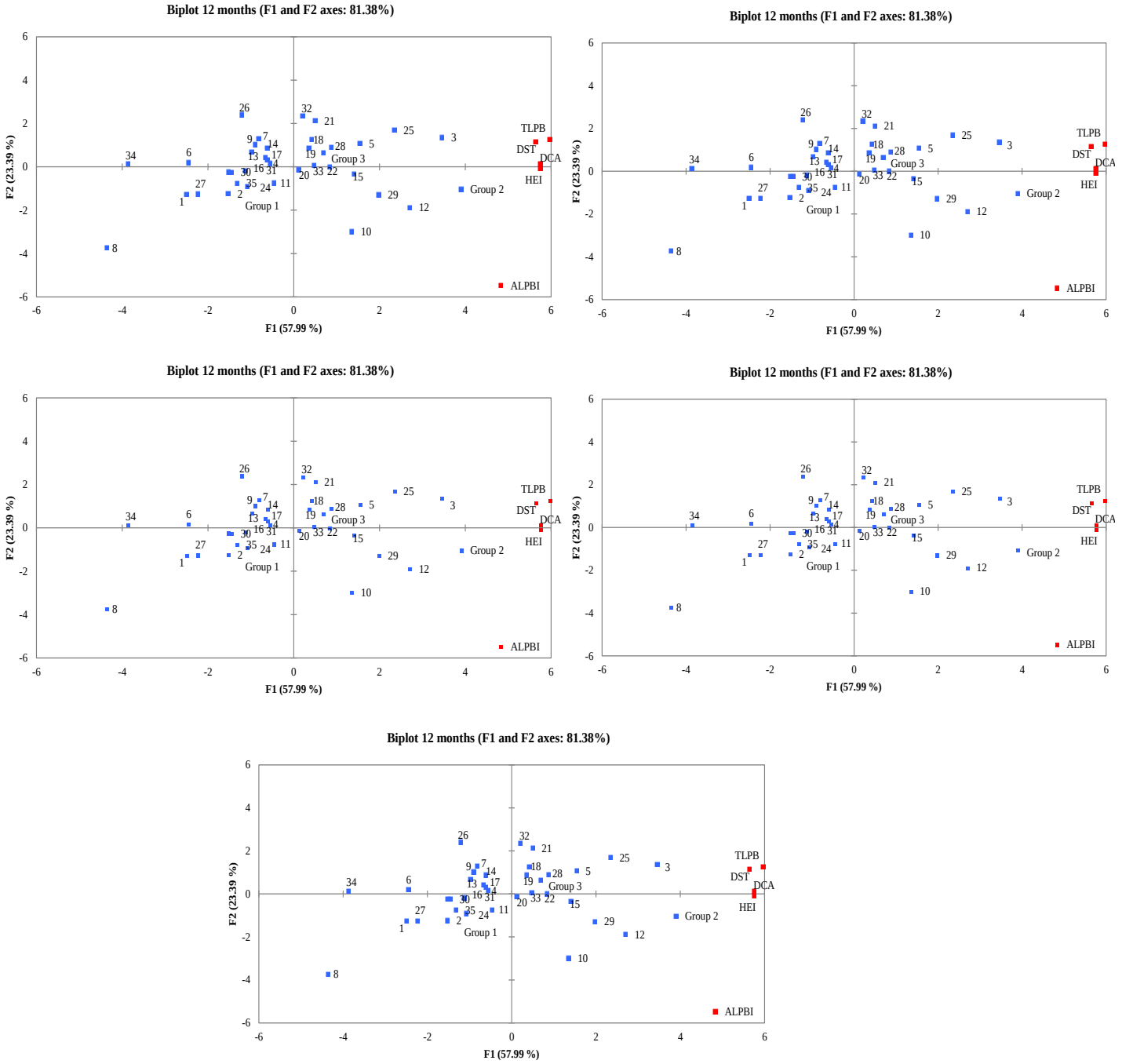

Figure 6. Biplot of the relative contribution of the variables to the genetic dissimilarity of each group in the phenological evaluation performed at 12 months, evidencing the frequencies.

TLPB, and ALPBI at 18 months; and ALOBI, TNPB, and ALPBI at 24 months. ALPBI contributed throughout the four evaluation periods. Moreover, the greatest number of variables contributing ≥25% was observed for the 18 month evaluation, showing that this may be the best stage of development to evaluate genotypes and examine genetic divergence under edaphoclimatic conditions.

Conclusion

Genetic dissimilarity was evidenced between the 35 genotypes of *C. arabica* in the Cerrado, under drip irrigation, using phenological data and multivariate statistics. At 24 months after planting, the genotype Yellow Catucaí 2SL shows great dissimilarity. There was a large percentage of the contribution of phenological

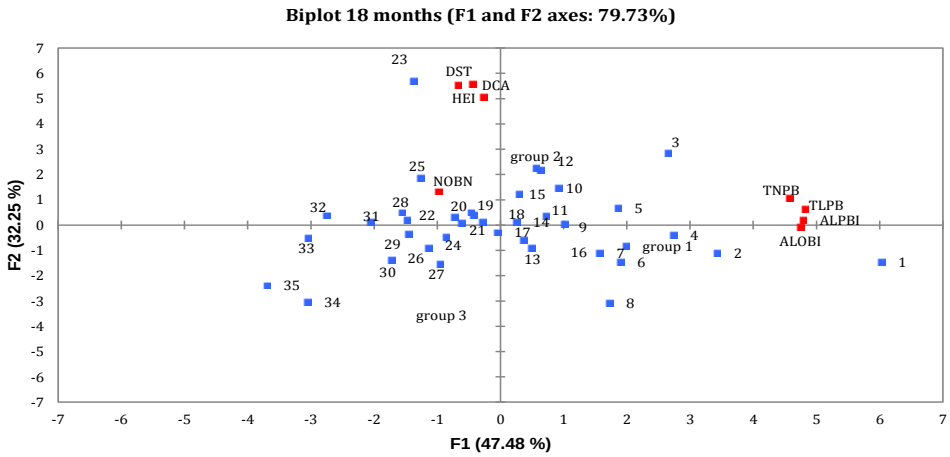

Figure 7. Biplot of the relative contribution of the variables to the genetic dissimilarity of each group in the phenological evaluation performed at 18 months, evidencing the frequencies.

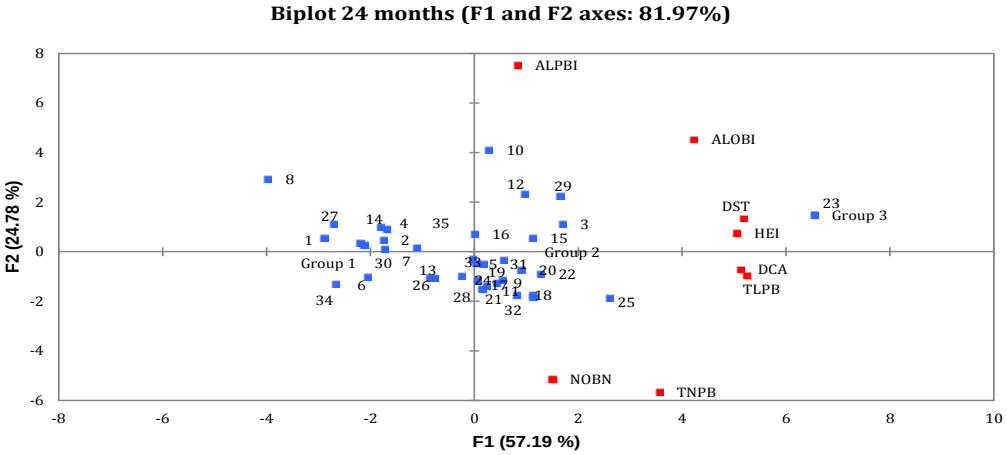

Figure 8. Biplot of the relative contribution of the variables to the genetic dissimilarity of each group in the phenological evaluation performed at 24 months, evidencing the frequencies.

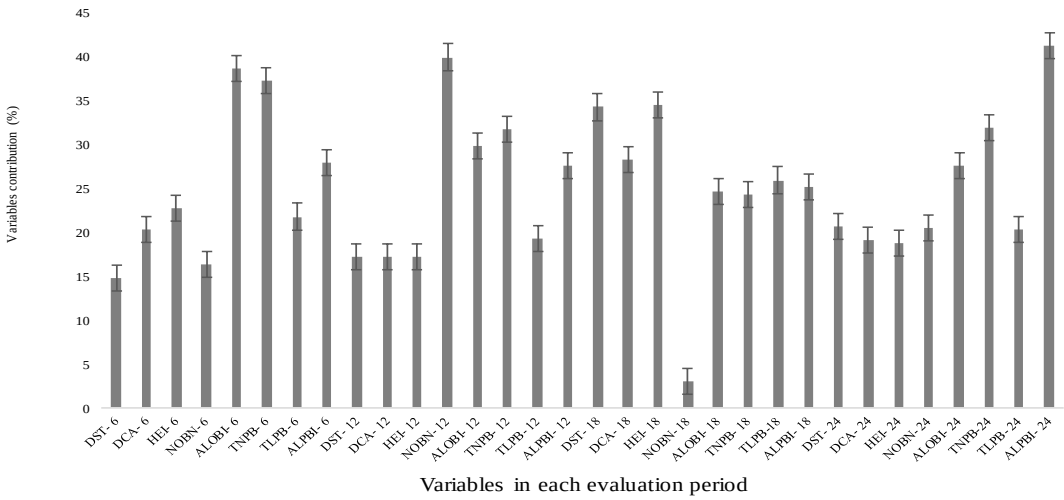

Figure 9. Contribution of the phenological variables to genetic dissimilarity in coffee over the four evaluation periods.

variables to genetic dissimilarity (> 75%), in the four evaluations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Arantes KR, Arantes SACM, Faria MA, Rezende FC (2006). Desenvolvimento do cafeeiro (*Coffea arabica* L.) podado sob irrigação. Revista de Ciências Agro-Ambientais 4:75-66.

Botelho CE, Rezende JC, Carvalho GR, Carvalho AM, Andrade VT, Barbosa CR (2010). Adaptabilidade e estabilidade fenotípica de cultivares de café arábica em Minas Gerais. Pesquisa Agropecuária Brasileira 45:1404-1411.

Camargo AP, Camargo MBP (2001) Definição e esquematização das fases fenológicas do cafeeiro arábica nas condições tropicais do Brasil. Bragantia 60:65-68.

CONAB- Companhia Nacional de Abastecimento. Acompanhamento da safra brasileira: café. v.5 - safra (2019) - n.2 - Segundo levantamento, maio 2019. Disponível em: http://www.conab.gov.br. Acesso em: 10 junho 2019.

Fernandes ALT, Partelli FL, Bonomo R, Golynski A (2012). A moderna cafeicultura dos cerrados brasileiros. Pesquisa Agropecuária Tropical 42:231-240.

Guedes JM, Vilela DJM, Rezende JC, Silva FL, Botelho CE, Carvalho SP (2013). Divergência genética entre cafeeiros do germoplasma Maragogipe. Bragantia 72:127-132.

Giles JAD, Ferreira AD, Partelli FL, Aoyama EM, Ramalho JC, Ferreira A, Falqueto AR (2019). Divergence and genetic parameters between *coffea* sp. genotypes based in foliar morpho-anatomical traits. Scientia Horticulturae 245:231-236.

Guerra AF, Rocha OC, Rodrigues GC, Sanzonowicz C, Sampaio JBR, Silva HC, Araújo MC (2005). Irrigação do Cafeeiro no Cerrado: estratégia de manejo de água para uniformização de florada. Embrapa Cerrados - Comunicado técnico n 122, 4p.

Guimarães PTG, Garcia AWR, Alvarez VVH, Prezotti LC, Viana AS, Miguel AE, Malavolta E, Corrêa JB, Lopes AS, Nogueira FD, Monteiro AVC Cafeeiro. In: Ribeiro AC, Guimarães, PTG, Alvarez VVH (1999). Recomendações para o uso de corretivos e fertilizantes em Minas Gerais, 5ª Aproximação. Comissão de Fertilidade do Solo do Estado de Minas Gerais. pp. 289-302.

Ivoglo MG, Fazuoli LC, Oliveira ACB, Gallo PB, Mistro JC, Silvarolla MB, Toma-Braghini M (2008). Divergência genética entre progênies de café robusta. Bragantia 67:823-831.

Mantovani M, Ruschel AR, Reis MS, Puchalski A, Nodari RO (2003). Fenologia reprodutiva de espécies arbóreas em uma formação secundária da floresta atlântica. Revista Árvore 27:451-458.

Meireles EJL, Camargo MBP, Pezzopane JRM, Thomaziello RA, Fahl JI, Bardin L, Santos JCF, Japiassú LB, Garcia AWR, Miguel AE, Ferreira RA (2009). Fenologia do Cafeeiro: condições agrometeorológicas e balanço hídrico do ano agrícola 2004–2005. Brasília, DF: Embrapa Informação Tecnológica.

Oliveira EL, Faria MA, Reis RP, Silva MLO (2010). Manejo e viabilidade econômica da irrigação por gotejamento na cultura do cafeeiro Acaiá considerando seis safras. Revista Engenharia Agrícola 30:887-896.

Paiva RN, Carvalho CHS, Mendes ANG, Almeida SR, Matiello JB, Ferreira RA (2010). Comportamento agronômico de progênies de cafeeiro (*Coffea arabica* L.) em Varginha-MG. Coffee Science 5:49-58.

Pereira SP, Bartholo GF, Baliza DP, Sobreira FM, Guimarães RJ (2011). Crescimento, produtividade e bienalidade do cafeeiro em função do espaçamento de cultivo. Pesquisa Agropecuária Brasileira 46:152-160.

Pinto MF, Carvalho GR, Botelho CE, Gonçalves FMA, Rezende JC, Ferreira AD (2012). Eficiência na seleção de progênies de cafeeiro avaliadas em Minas Gerais. Bragantia 71:1-7.

Rodrigues WP, Vieira HD, Barbosa DHSG, Souza FGR, Candido LS (2013). Adaptability and genotypic stability of *Coffea arabica* genotypes based on REML/BLUP analysis in Rio de Janeiro State, Brazil. Genetics and Molecular Research 12:2391-2399

Silva DFG, Coelho CJ, Romanek C, Gardingo JR, Silva AR, Graczyki BL, Oliveira EAT, Matiello RR (2016). Dissimilaridade genética e definição de grupos de recombinação em progênies de meios-irmãos de milho-verde. Bragantia 75:401-410.

Silva FL, Baffa DCF, Oliveira ACB, Pereira AA Bonomo VS (2013) Integração de dados quantitativos e multicategóricos na determinação da divergência genética entre acessos de cafeeiro. Bragantia 72:224-229.

Torres FE, David GV, Teodoro PE, Ribeiro LP, Correa CG, Luz Júnior RA (2015). Desempenho agronômico e dissimilaridade genética entre genótipos de soja. Revista de Ciências Agrárias 38:111-117.

XLSTAT 7.0 (2005). Addinsoft. Paris, France, 2005.

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Evaluation of coffee berry disease resistance (*Colletotrichum kahawae*) in F_2 populations derived from Arabica coffee varieties Rume Sudan and SL 28

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Coffee supports livelihoods of approximately 125 million families worldwide and over 700,000 households in Kenya. The epidemics of Coffee berry disease (CBD), caused by Colletotrichum kahawae, destroy up to 80% of the developing berries on susceptible varieties. The control of the disease using chemicals accounts for 30 to 40% of the production cost and contributes to environment pollution, hence the use of resistant varieties. Resistance to CBD is conferred by three genes; R, T that are dominant and k which is recessive, from coffee varieties Rume Sudan (RS), Hibrido de Timor (HDT) and K7 respectively. Although the T gene has been mapped, there is need for genetic mapping of the other genes to improve selection efficiency. The objective of this study was to evaluate F2 populations of RS x SL28 for their suitability to genetic mapping of the R gene in RS. Resistance to CBD was evaluated by hypocotyl inoculation on their F3 progenies. The data was subjected to Analysis of Variance (ANOVA) and Chi Square (χ^2) test. The ANOVA result showed significant differences ($P \leq 0.05$) between the genotypes to CBD resistance. The phenotypic ratio of resistance to susceptible plants fitted the 3:1 monohybrid inheritance ratio for a dominant gene using the χ^2 test ($\chi^2 = 1.0565$ and $P=0.30207$, $P \leq 0.05$), hence confirming the suitability of the F2 populations for the identification of the DNA marker for R gene in RS.

Key words: Rume Sudan, SL 28, Coffee Berry Disease, mapping population, hypocotyl, inoculation.

INTRODUCTION

Coffee (*Coffea* spp.) is among the most important commodities in the tropical countries of the world (Vieira et al., 2019). It is commercially grown in more than 10.5 million ha in 80 different countries worldwide (Van der Vossen et al., 2015). It supports livelihoods of approximately 125 million families in coffee producing countries (Zhou et al., 2016). Coffee is an important export crop and a major foreign exchange earner for

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Kenya, supporting over 700,000 smallholder farmers that are organized into about 435 farmers' co-operative societies. The cooperative societies account for 80% of the total coffee production while the remaining 20% is produced by 3000 coffee plantations (Kathurima, 2013; Gimase, 2014). Coffee ranks fifth in terms of economic importance after tea, tourism, horticultural sub-sector and diaspora remittance. It contributes about 1% to GDP and 8% of the total agricultural export revenue (Kenya National Bureau of Statistics, 2019). Of the 70% of Kenya's workforce engaged in agriculture, 30% are employed by the coffee industry (Minai et al., 2014). Kenya is known for production of some of the worlds' top grade and highly valued Arabica coffee beans that are usually used in small quantities by international coffee roasters to moderate and improve their blends (Kathurima, 2013).

Coffea arabica L., is a tetraploid (2n = 4x = 44) and 95% self-fertile (Bertrand et al., 2003). Coffee was introduced in Kenya at the beginning of 20th Century by missionaries (Omondi et al., 2016). It is mainly grown in three regions, the East of Rift Valley (areas around Mount Kenya, the Aberdare ranges and Machakos), West of Rift Valley (Kisii highlands, area around Mt Elgon and the North of the Rift valley) and Taita Hills in the coast (Kathurima, 2013).

Coffee berry disease caused by the fungal pathogen *Colletotrichum kahawae*, is a specialized hemibiotrophic pathogen of *C. arabica* L. (Vieira et al., 2019). Unlike other Arabica coffee diseases, CBD is still restricted to the African continent despite favourable climatic conditions in certain high-altitude Arabica coffee growing areas of Latin America and Asia (Agwanda et al., 1997; Van der Vossen et al., 2015). *Colletotrichum kahawae* infects green berries at the rapid expansion stage (4-16 weeks after flowering) and may also attack mature berries, 28 weeks after flowering (Gichimu et al., 2014, CRI, 2016). Epidemics of this disease can quickly destroy 50–80% of the developing berries on susceptible Arabica coffee cultivars during prolonged wet and cool weather conditions (Hindorf and Omondi, 2011). Preventive control by frequent fungicide sprays account for 30–40% of total production costs and leads to environmental pollution (Gichuru et al., 2008). Crop loss and cost of CBD control in Africa is estimated to be 300 – 500 million USD (Van der Vosen and Walyaro, 2009).

The first case of CBD in Kenya was reported in 1922, in newly established coffee plantations on the slope of Mt Elgon in Western Kenya (McDonald, 1926). The disease then spread to other parts of the country, East of Rift Valley by 1939 and to all other main coffee growing zones by 1951. It was from Kenya that CBD spread to Angola in 1930, Zaire in 1937, Cameroon between 1955 and 1957, Uganda in 1959, Tanzania in 1964, Ethiopia in 1971 and Malawi in 1985 (Hindorf and Omondi, 2011). Breeding for resistance to CBD in Kenya, started in 1971, following a serious outbreak of CBD in 1967-68 (Van Der Vossen and Walyaro, 1981). The CBD epidemics experienced at that time affected all the Kenyan commercial varieties and threatened to wipe out the coffee industry in the country (Van der Vosen and Walyaro, 1981; Hindorf and Omondi, 2011).

Marker-assisted selection is one of the best approaches to reduce the period taken to develop coffee varieties (Moncada et al. 2016). To implement this technique, one requires to develop a genetic map for markers that are associated with traits of interest, a process that also involves the development of a mapping population (Baison, 2014). The simplest population is the F_2 genotypes (Schneider, 2005). The mode of reproduction influences the choice of mapping populations and the relative ease of raising such populations. An ideal mapping population therefore, should be derived from parents with a wide variation in the trait to be analyzed (Gichuru, 2007). Self-fertile naturally inbreeding plants of Arabica coffee attain a high degree of homozygosity and well-varied pure line parents for generating mapping populations (Gichuru, 2007).

The discovery of hypocotyl infection on a six-week-old seedling using artificial inoculation with *Colletotrichum kahawae* spores contributes significantly to Arabica coffee breeding by shortening the time required to identify resistant progenies from crosses involving resistant and susceptible donors (Van Der Vossen et al., 1976; Agwanda et al., 1997; Gichuru et al., 2008). The method was found to be a reliable pre-selection test whose result was significantly correlated (r^2=0.73-80, P≤0.05) with mature plant resistance in the field (Van Der Vossen et al., 1976).

Inheritance studies on Arabica coffee genotypes have identified three genes that confer resistance to CBD in *C. arabica* as R-gene in the variety Rume Sudan, T-gene in Hibrido de Timor and k-gene in K7 (Van der Vossen and Walyaro, 1980). The T gene has an intermediate gene action, with the R gene dominant and the k gene recessive; therefore, the k gene only confers partial resistance to CBD (Van der Vossen and Walyaro, 2009).

Genetic resistance to CBD has been characterized by various studies in Kenya. The T gene was mapped by Agwanda et al. (1997) using random amplified polymorphic DNA (RAPD) markers while Gichuru et al. (2008) mapped the first locus for resistance to *Colletotrichum kahawae* using simple sequence repeats (SSR) markers and amplified fragment length polymorphisms (AFLP) markers and christened it as *Ck*-1. The locus was found to be linked to the highly repetitive and informative SSR primer locus (Sat 235) that has been widely adopted for marker assisted selection (MAS) in Arabica coffee (Gichimu et al. 2014; Mtenga, 2016; Alkimim et al. 2017). Although molecular markers for the T-gene (Ck-1) gene in HDT was detected, similar molecular research studies remain necessary for the detection and mapping of R genes in Rume Sudan. This will increase selection efficiency in the

rapid development of CBD resistant varieties to CBD that meet consumers preference for Arabica coffee growing countries in Africa and breeding programmes in Latin America, where CBD has not been reported but there remains a possible likelihood of its occurrence (Van der Vossen et al., 2015).

Evaluation of F_2 populations to determine their suitability for mapping of resistance to diseases in coffee has been carried out by various studies. Gichuru (2007) evaluated two F_2 populations derived from CBD resistance donor parent Catimor and susceptible cultivar SL 28; Brito et al. (2010) F_2 populations from HDT UFV 427-15 and the susceptible cultivar Catuai Amarelo UFV 2143-236 for their segregation on resistance to race II *Heimillea vastatrix,* the causal agent of coffee leaf rust. Similarly, Diola et al. (2011, 2013) studied an F_2 population obtained by crossing HDT UFV 427-15 (resistant) with Catuai Amarelo IAC 30 (susceptible) segregating for a dominant gene that confers resistance on coffee to race II of *Heimillea vastatrix* while Pestana et al. (2015) examined F_2 population of a cross between Catuai Amarelo IAC 64 (UFV 2148-57), a susceptible Arabica coffee variety and HDT UFV 443-03 as a donor variety of resistance to *Heimillea vastatrix.*

Breeding programs in various crops have led to release of genotypes with improved traits that often breaks up after a short period as different genes for resistance acts against different isolates, races or biotypes (Mekonnen et al., 2017). The process of accumulating various genes to a given genotype (pyramiding) broadens the number of races or isolates that one variety can resist or tolerate at the same time (Sundaram et al., 2009). Pyramiding of genes for resistance using conventional screening methods is limited by the dominance effects of genes governing disease resistance (Arunakumari et al., 2016); for DNA markers, it is possible to accurately identify genes of interest of the progenies at each generation, thus making pyramiding process faster and more efficient (Zhao et al., 2014). Despite the fact that resistance to CBD is controlled by three genes (Van der Vossen and Walyaro, 1980), only the T gene has been mapped (Gichuru et al., 2008) and adopted for MAS (Alkimim et al. 2017). It is therefore necessary to identify DNA markers for R and k genes to increase selection efficiency in the rapid development of CBD resistant varieties (Van der Vossen et al., 2015). The objective of this study was to evaluate F_2 populations derived from crosses between *C. arabica* varieties Rume Sudan and SL 28 for resistance to CBD and determine their suitability of genetic mapping of R gene that confer resistance to CBD in *C. arabica* L.

MATERIALS AND METHODS

This study was conducted at Coffee Research Institute (CRI) of the Kenya Agricultural and Livestock Research Organization (KALRO), Ruiru Centre. Ruiru is located within the upper midland (UM2) at 1° 06'S and 36° 45'E and at an altitude of 1620 m above sea level. The rainfall pattern is bimodal with 1063 mm per annum and the annual average temperature is 19°C with a range of 12.8 to 25.2°C (Jaetzold et al., 2006).

Study materials

This study utilized a total of 108 genotypes comprising 106 F_2 genotypes and their parents, Rume Sudan and SL28. F_2 segregating population of a cross between *C. arabica* varieties Rume Sudan (RS) and SL 28 was developed and established at CRI. In the development of RS x SL28 F_2 populations, the cultivar RS was used as female parent. Rume Sudan is a normal Arabica variety that is believed to possess the R gene in the R locus that confers resistance to CBD while the male parent SL 28 is *C. arabica* cultivar highly susceptible to CBD (Hindorf and Omondi, 2011).

Evaluation for resistance to CBD and classification of F_2 populations

The 108 genotypes were classified based on resistance to CBD of their F_3 progenies using hypocotyl inoculation method on a scale of 1 – 12, as described by Van der Vossen et al. (1976) (Table 1). The F_2 plants were selfed to generate F_3 progenies for two seasons (2017 and 2018). For each season, ripe and healthy F_3 berries were harvested from each of the F_2 Rume Sudan x SL 28 individual genotype. The harvested berries were manually pulped by hand-squeezing them between the fingers. The seeds were fermented for about 16 h, washed, dried to a moisture content of 15% and then the parchment was removed by hands. Three hundred seeds were planted in sterilized sand in plastic boxes and kept at room temperature under laboratory conditions. Twenty seedlings of the susceptible SL 28 were also sown alongside the test seedlings in each box. Coffee variety SL 28 was used as a susceptible control to verify success of infection. The experiment was set out in the laboratory in a completely randomized design (CRD) with three replicates each of 100 seeds. Watering was carried out twice per week using distilled water to ensure that the sand remained moist but not water logged. After 6 weeks, the germinated hypocotyl seedlings with unopened cotyledons were uprooted and immediately replanted in similar but clean boxes filled with sterilized sand at a spacing of 2.5 cm x 2.5 cm.

Isolates of *Colletotrichum kahawae* were obtained from freshly infected coffee berries in the field and multiplied on malt extract agar (MEA) for a period of about 15 days in the laboratory. To stimulate conidia production, isolates were cultured on coffee leaf extract agar medium for 7 days under a photoperiod of 12 h at 22°C and then sub-cultured on 90-mm polystyrene petri dishes containing malt extract agar (40 g L^{-1}, MEA; Oxoid) for 7 days under the same photoperiod (Vieira et al., 2019). Inoculum was obtained by dislodging and harvesting the conidia by flooding the plate with 5 mL of sterile distilled water and the suspensions passed through four layers of sterile muslin cloth to remove mycelia (Vieira et al., 2019). Concentrations of spore suspensions were determined using a haemocytometer (NEUBAUER Scientific International, Germany).

Six weeks after sowing the F_3 seeds in the sand boxes, inoculum suspension was adjusted and standardized to a concentration of 2 x 10^6 conidia per ml (Van der Vossen et al., 1976; Viera et al., 2019). The hypocotyls were inoculated using a hand sprayer, spraying them twice at 48-h interval with the inoculum. After every spray interval, the seedlings were incubated in the dark by covering them with black polythene sheet for 48 h at room temperature and then transferred to a temperature-controlled room at 18 to 20°C for 2 weeks. The seedlings were transferred back to room temperature for one week, after which disease symptom severity rating were

Table 1. Coffee Berry Disease (CBD) pathogenicity rating*.

Scale*	CBD symptom
1	No visible symptoms
2	A few scab lesions
3	Small scab or tiny brown lesions
4	Scab or brown lesions
5	Scab and brown lesions, and a few small black lesions
6	Brown and narrow black lesions
7	Narrow black lesions, some more than 1 centimeter long
8	Black lesions becoming wider and starting to coalesce
9	Large coalescing black lesions but not yet complete
10	Large coalescing black lesions, complete girdling of stem
11	Most of the stem affected, more than one third stem shriveled, seedling dead
12	Whole stem affected and shriveled and seedling dead

*Pathogenicity scale described by Van der Vossen et al. (1976).

carried out. The incubation period was determined by the full expression of disease on SL 28. Each seedling was assessed based on expression of disease symptoms on the hypocotyls. Average infection (AI) per replicate was calculated as follows:

$$AI = 1/\mathrm{N}\sum_{i=1}^{12} ini$$

where, ***i*** is the disease class, $\mathbf{n}_i$ is the number of seedlings in class ***i, N*** is the total number of seedlings scored (Van der Vossen et al., 1976) and mean grade data computed for each genotype.

Chi squared tests for goodness of fit on Mendelian monohybrid inheritance ratio for phenotypic segregation of 3:1 (resistant to susceptible) for a dominant gene was used to confirm genetic hypothesis on the mode of inheritance for CBD resistance of the genotypes (Fazel-Najafabadi et al., 2015; Kim and Reinke, 2019).

RESULTS

Phenotypic segregation of the F_2 Population for CBD resistance

The SL 28 seedlings were ranked highly susceptible to CBD with a rating between 11 and 12 and a mean of 11.9 (Table 2), an indication that the infection was highly successful. Rume Sudan recorded a disease rating of 2.97 and was considered highly resistant to CBD. The F_2 genotypes segregated showing various levels of resistance and susceptibility, however, no genotypes had a score of 12 (Table 2). The mean infection score for all the genotypes was 5.75. Based on the hypocotyl inoculation results, the genotypes were classified into two phenotypic classes by comparing the infection rates of the F_2 populations with SL 28. Seedlings with ratings of 7 and 12 were considered susceptible and those rated from 1 – 6, considered resistant (Van Der Vossen et al., 1976). The frequency curve of CBD resistance on the F_2 genotypes showed a continuous distribution from grade 1 to 12, but was slightly skewed towards more resistance genotypes (Figure 1).

The mean data on phenotypic segregation for CBD resistance of RS x SL28 F_2 populations was subjected to analysis of variance using SAS statistical software (Version 9) and means separated using least significant difference (LSD). The result showed significant variation (P≤0.05) among the genotypes for resistance to CBD (Table 2). Three genotypes namely 35, 5 and 14 were significantly more resistant (P≤0.05) to CBD than Rume Sudan whereas two genotypes, 71 and 33 were not significantly different (P≤0.05) from the susceptible check (SL 28).

The mean data was subjected to Chi-square test in order to check for goodness-of-fit for the various Mendelian monohybrid inheritance ratios for the segregation of a dominant gene (Table 3). The segregation ratios of resistant to susceptible genes (R:S) is used by breeders to determine conformity of populations to the expected genetic segregations (Baison, 2014). The phenotypic segregation of CBD infection distribution fitted the 3:1 monohybrid ratio (χ^2 = 1.0565 and P=0.30207, P≤0.05) for the F_2 mapping populations (Table 3).

DISCUSSION

The study revealed significant variations in the resistance to CBD within the F_2 populations derived from RS x SL28 crosses. Related results were reported by Gichuru (2007) who observed significant variation in resistance to CBD among F_2 population derived from CBD resistance donor parents Catimor and susceptible cultivar SL 28. Gichimu et al. (2014) observed variations in the resistance to CBD among coffee variety R11 parental genotypes and within different Ruiru 11 siblings. Mtenga (2016) also found

Table 2. Classification of F_2 genotypes based on their mean score for phenotypic expression of resistance to CBD**.**

Susceptible genotype		Resistant genotype	
Genotype	**Mean**	**Genotype**	**Mean**
SL 28	11.9366^{a}	38	6.7866^{l-p}
71	11.5000^{a}	64	6.7258^{l-q}
33	11.2849^{ab}	147	6.6575^{l-r}
85	10.6934^{bc}	42	6.6267^{l-r}
78	10.6771^{bc}	111	6.5886^{l-s}
49	9.8645^{cd}	12	6.5711^{m-t}
68	9.7422^{d}	45	6.5050^{n-u}
18	9.6250^{d}	105	6.3539^{o-v}
116	8.6889^{e}	101	6.1954^{p-w}
132	8.6132^{e-f}	83	6.1420^{p-w}
126	8.5235^{e-g}	117	6.1207^{p-w}
110	8.4638^{e-g}	48	6.0904^{p-w}
15	8.4415^{e-g}	41	6.0702^{p-w}
16	8.3521^{e-g}	87	5.9112^{q-x}
82	8.1966^{e-h}	95	5.8947^{q-x}
52	8.1816^{e-h}	55	5.8871^{q-x}
46	8.1595^{e-i}	153	5.8706^{r-x}
75	14830^{e-j}	106	5.7696^{s-y}
94	7.9334^{e-k}	47	5.7335^{t-z}
146	7.8265^{i-k}	60	5.7335^{u-z}
24	7.7059^{i-k}	96	5.6309^{v-A}
22	7.4325^{u-l}	143	5.6131^{v-A}
122	7.3795^{i-m}	51	5.4955^{x-B}
54	7.3200^{i-n}	10	5.4932^{x-B}
11	7.3101j^{-n}	112	5.4496^{x-B}
121	7.2980^{k-n}	108	5.4374^{x-B}
80	7.2806^{k-n}	86	5.3866^{x-B}
59	7.2800^{k-n}	50	5.3863^{x-B}
21	7.2011^{k-n}	27	5.2247^{x-C}
31	7.1543^{k-o}	99	5.2243^{x-C}
44	7.1518^{k-o}	92	5.2208^{x-C}
13	7.1489^{k-o}	144	5.2000^{x-C}
		65	5.1563^{x-D}
		1	5.0051^{y-E}
		67	4.9412^{y-F}
		72	4.9168^{z-G}
		25	4.8906^{z-H}
		76	4.8443^{A-H}
		37	4.7615^{B-I}
		89	4.4738^{C-J}
		66	4.4713^{C-J}
		93	4.3159^{E-J}
		9	4.1937^{E-L}
		8	4.1871^{E-L}
		63	4.1642^{E-L}
		104	4.1131^{F-L}
		3	4.1105^{F-L}
		120	4.0894^{I-L}
		77	4.0871^{I-L}

Table 2. Contd.

	61	4.0700^{I-L}
	29	3.974^{I-M}
	36	3.9612^{I-M}
	20	3.9386^{I-M}
	53	3.9193^{I-M}
	98	3.8871^{K-M}
	7	3.8788^{K-M}
	124	3.8788^{K-N}
	115	3.5385^{K-N}
	88	3.4938^{K-N}
	133	3.433^{L-N}
	123	3.3827^{L-N}
	102	3.1852^{M-O}
	113	3.1701^{M-P}
	91	3.1698^{M-P}
	30	3.1689^{M-P}
	28	3.0054^{N-Q}
	RS	2.9722^{N-R}
	97	2.9414^{N-R}
	79	2.4370^{O-S}
	118	2.4055^{O-S}
	23	2.3385^{P-S}
	103	2.3095^{Q-S}
	6	2.1341^{R-T}
	35	1.9092ST
	5	1.4286^{T}
	14	1.4274^{T}
Mean	5.74720	
CV	9.132596	

Mean values followed by same letter (s) in a column are not significantly different (P≤0.05); Mean = Mean score for CBD infection rating, RS = Rume Sudan.

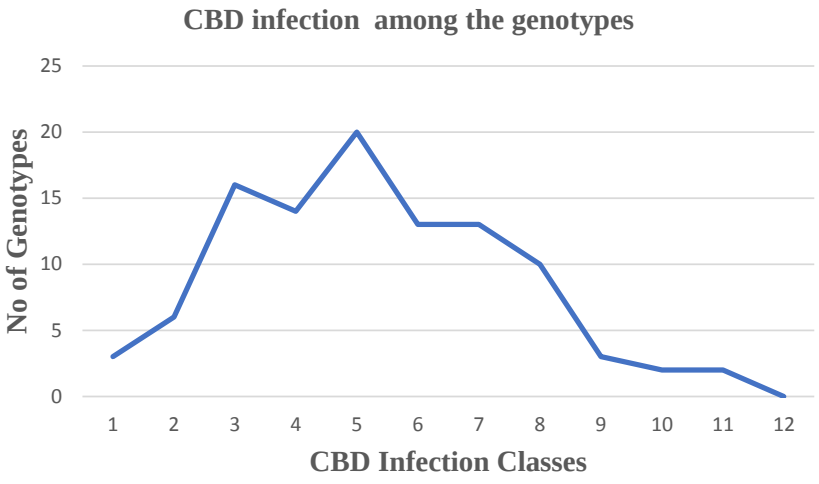

Figure 1. Distribution of CBD infection among the F_2 populations.

Table 3. Chi-square goodness of fit test for 3:1 Monohybrid inheritance ratio for phenotypic segregation of the F_2 genotypes based on hypocotyl inoculation results.

Phenotypic expression of the F_2 genotype						
Generation	**Genotype category**	**Observed**	**Expected**	**d.f**	**χ^2 (3:1)**	**P**
F2	Resistant (RR)	74	80	1	1.0565	0.30207
	Susceptible (rr)	32	26			
	Total	106	106			

χ^2 critical value (P≤0.05), d.f.=1) = 3.83; χ^2 =3:1.

significant differences for CBD resistance on progenies of a cross between Ethiopian accessions and *C. arabica* cultivar, KP423 for resistance to CBD.

The variety SL 28 had a high rating of 11 - 12 in the reaction to the inoculation of *Colletotrichum kahawae*, whereby at least most of the stem of the hypocotyl was affected, with more than one third of the stem found to have shriveled, leading to the death of the seedling and confirming its high susceptibility to the disease. A related result was reported by Gichimu et al. (2014) recorded a disease rate of 11.59 – 11.72 for SL 28; Gichuru (2007), who recorded 11.8 for the susceptible cultivar Catura; Omondi et al. (2001), 10.5 – 12 for SL 28 and Van Der Vossen et al. (1976), 10 - 12 for SL 28. A relatively recent study by Mtenga (2016), using cultivar KP423, which is a susceptible *C. arabica* commercial variety in Tanzania recorded the highest CBD scores among the F_1 progenies. Rume Sudan was rated as highly resistant with a disease rating of 2.97. Similar results were reported by Van Der Vossen et al. (1976) with disease rating of 4.1 and Gichimu et al. (2014) who reported 4.6. Rume Sudan and SL 28 scores ranged from highly resistant class to most susceptible class rating, respectively. An ideal mapping population should be derived from parents with a large variation in the trait to be analyzed (Gichuru, 2007; Baison, 2014; Moncada et al. 2016). Therefore, in this study, Rume Sudan x SL 28 could be an ideal parental combination for a mapping population following their wide rating range for CBD resistance.

The phenotypic ratios of resistant to susceptible genotypes were 74:32 which fitted a 3:1 monohybrid inheritance ratio for a major/dominant gene (χ^2 = 1.0565 and P=0.30207, P≤0.05) for the F_2 populations (Table 3). A study by Gichuru (2007) on a dominant T gene that confer resistance to CBD in HDT using two F_2 population derived from susceptible cultivar SL 28 and Catimor as a donor of the resistance gene, revealed that the ratio of resistant to susceptible for two populations were 96:35 and 103:44 which fitted the 3:1 Mendelian ratio for a major gene action (χ^2 = 0.206; P=0.650 and χ^2 = 1.907; P=0.167) for the two respective populations. Brito et al. (2010) evaluated 160 F_2 genotypes derived from a cross between the resistant genotype Hibrido de Timor UFV 427-15 and the susceptible cultivar Catuai Amarelo UFV 2143-236 for their segregation on resistance to race II *Heimillea vastatrix*, the causal agent for coffee leaf rust and reported 124:36 for R:S that fitted in the 3:1 Mendelian ratio for a dominant gene (χ^2= 0.5336, P = 0.4652). Diola et al. (2011) reported phenotypic ratio of 166:58 that also fitted on the 3:1 segregation pattern expected for a single, dominant gene (χ^2=0.09524) on F_2 population from a crossing of Hibrido de Timor UFV 427-15 (resistant) with Catuaí Amarelo IAC 30 (susceptible), for a dominant gene that confers resistance on coffee to race II of *Heimillea vastatrix*. Diola et al. (2013) reported a Mendelian ratio of resistant to susceptible genotypes of 3:1, indicating that one gene is involved in the resistance of the HDT to pathotype of race II of *Heimillea vastatrix* using 224 F2 plants derived from resistant parent HDT UFV 427-15 and the susceptible parent Catuaí Amarelo UFV 2143-236 (IAC 30). In a related study, Pestana et al. (2015) also reported 3:1 (P≤0.05) ratio for dominant gene in an F_2 population of a cross between Catuai Amarelo IAC 64 (UFV 2148-57), a susceptible Arabica coffee variety and Hibrido de Timor UFV 443-03, a donor variety for resistance *Heimillea vastatrix*. Kim and Reinke et al. (2019) reported expected phenotypic ratio of 3:1 (χ^2 = 4.15 and P = 0.12, P≤0.05) on F_2 rice genotypes evaluated for resistance to bacterial blight.

The distribution of the infection rating among the F_2 genotypes was close to normal with a skew toward the lower level of CBD infection. The skewness is a necessary for determination of Mendelian inheritance ratios. A study by Kim and Reinke et al. (2019) revealed a skewed result towards resistant genotypes on F_2 rice genotypes evaluated for resistance to bacterial blight.

Conclusion

This study revealed significant variation in the symptoms of *Colletotrichum kahawae* among the F_2 populations derived from Arabica coffee varieties Rume Sudan and SL 28. The CBD disease rating was skewed towards the resistant genotypes, demonstrating an ideal segregation of the populations for resistance. There was a significant contrast in terms of resistance to CBD between RS and SL 28, indicating that the choice of the parents was justified. This study also showed that the phenotypic

segregation within the populations for resistant to susceptible genotypes fitted 3:1 Mendelian ratio expected from a dominant gene. The F_2 populations derived from RS and SL 28 were suitable for genetic mapping of a dominant gene. Therefore, RS x SL 28 F_2 populations are recommended for mapping of R gene in the variety RS that confers resistance to CBD to *C. arabica* L.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Agwanda CO, Lashermes P, Trouslot P, Combes MC, Charrier A (1997). Identification of RAPD markers for resistance to coffee berry disease, *Colletotrichum kahawae*, in arabica coffee. Euphytica 97:241-248.

Alkimim ER, Caixeta ET, Sousa TV, Pereira AV, Oliveira ACB, Zambolim L, Sakiyama NS (2017). Marker-assisted selection provides Arabica coffee with genes from other Coffea species targeting on multiple resistance to rust and coffee berry disease. MoleculaR Breeding 37(6):1-10.

Arunakumari K, Durgarani CV, Satturu V, Sarikonda KR, Chittoor PDR, Vutukuri B, Laha GS, Nelli APK, Gattu S, Jamal M, Prasadbabu A, Hajira S, Sundaram RM (2016). Marker-Assisted Pyramiding of Genes Conferring Resistance against Bacterial Blight and Blast Diseases into Indian Rice Variety MTU1010. ScienceDirect Rice Science 23(6):306-316.

Baison J (2014). Mapping and identification of disease resistance candidate genes in three Malus populations using SSRs, DArT and Infinium SNP markers and Illumina sequencing technology (PhD thesis), University of the Western Cape, South Africa. p. 343.

Bertrand B, Guyot B, Anthony F, Lashermes P (2003). Impact of Coffea canephora gene introgression on beverage quality of C. arabica. Theoretical and Applied Genetics 107(3):387-394.

Brito GG, Caixeta ET, Gallina AP, Zambolim EM, Zambolim L, Diola V, Loureiro ME (2010). Inheritance of coffee leaf rust resistance and identification of AFLP markers linked to the resistance gene. Euphytica 173:255-264.

Coffee Research Institute (2016). Coffee Production Recommendation, 4th Ed. p 107.

Diola V, Brito GG, Caixeta ET, Zambolim EM, Sakiyama NS, Loureiro ME (2011). High-density genetic mapping for coffee leaf rust resistance. Tree Genetics and Genomes 7(6): 1199-1208.

Diola V, Brito GG, Caixeta ET, Pereira LFP, Loureiro ME (2013). A new set of differentially expressed signaling genes is early expressed in coffee leaf rust race II incompatible interaction. Functional and Integrative Genomics 13(3):379-389.

Fazel-Najafabadi M, Peng J, Peairs FB, Simkova H, Kilian A, Lapitan NLV (2015). Genetic mapping of resistance to Diuraphis noxia (Kurdjumov) biotype 2 in wheat (Triticum aestivum L.) accession CI240. Euphytica 203:607-614.

Gichimu BM, Gichuru EK, Mamati GE, Nyende AB (2014). Occurrence of *Ck*-1 gene conferring resistance to Coffee Berry Disease in *Coffea arabica* cv. Ruiru 11 and its parental genotypes. Journal of Agricultural and Crop Research 2(3): 51-61.

Gichuru EK. (2007). Characterization of genetic resistance of Coffee berry disease (*Colletotrichum kahawae*, Waller and Bridge) in Arabica coffee (*Coffea arabica* L.) that is introgressed from *Coffea canephora* (PhD thesis), University of Nairobi, Kenya. P. 224.

Gichuru EK, Agwanda CO, Combes MC, Mutitu EW, Ngugi ECK, Bertrand B, Lasherme, P (2008). Identification of molecular markers linked to a gene conferring resistance to Coffee berry disease (*Colletotrichum kahawae*) in C*offea arabica.* Plant Pathology 57:1117-1124.

Gimase JM (2014). Genetic Diversity of Arabusta coffee and their parental genotypes (MSc thesis), Kenyatta University, Kenya. p 171.

Hindorf H, Omondi CO, (2011) A review of three major fungal diseases of *Coffea arabica* L. in the rainforests of Ethiopia and progress in breeding for resistance in Kenya. Journal of Advance Research 2(2):109-120.

Jaetzold R, Schimidt H, Hornez H, Shisanya C (2006). Farm Management Handbook of Kenya, Vol. II/C: Natural Conditions and Farm Management Information, Central Kenya. Ministry of Agriculture, Nairobi; Kenya. p.573.

Kathurima CW (2013). Characterization of the Diversity of Coffee Genotypes in Kenya by Genetic, Biochemical and Beverage Quality Profile, (PhD thesis) Jomo Kenyatta University of Agriculture and Technology, Kenya. P. 240.

Kenya National Bureau of Statistics (2019). Economic survey Report. www.knbs.or.ke

Kim SM, Reinke RF (2019). A novel resistance gene for bacterial blight in rice, *Xa43(t)* identified by GWAS, confirmed by QTL mapping using a bi-parental population. PLoS ONE 14(2):e0211775.

McDonald J (1926). A preliminary account of a disease of green berries in Kenya colony. Transactions of the British mycological Society 11(1-2):145-154.

Mekonnen T, Haileselassie T, Tesfaye K (2017). Identification, Mapping and Pyramiding of Genes/Quantitative Trait Loci (QTLs) for Durable Resistance of Crops to Biotic Stresses. Journal of Plant Pathology and Microbiology 8:412.

Minai JM, Nyairo N, Mbataru P (2014). Analysis of socio-economic factors affecting the coffee yields of smallholder farmers in Kirinyaga County, Kenya. Journal of Agricultural and Crop Research 2(12):228-235.

Moncada MP, Tovar E, Montoya JC, González A, Spindel J, McCouch S (2016). A genetic linkage map of coffee (*Coffea arabica* L.) and QTL for yield, plant height, and bean size. Tree Genetics and Genomes 12:5.

Mtenga JD (2016). Diversity, combining ability and Coffee Berry Disease (*colletotrichum kahawae*) resistance among Ethiopian and Tanzanian Arabica coffee genotypes, (PhD thesis), Sokoine University of Agriculture, Tanzania. p 117

Omondi CO, Ayiecho PO, Mwang'ombe AW, Hindorf H (2001). Resistance of Coffea arabica cv. Ruiru 11 tested with different isolates of Colletotrichum kahawae, the causal agent of coffee berry disease. Euphytica 121:19-24.

Omondi CO, Gichimu BM, Cheserek J, Gimase J (2016). Leveraging germplasm acquisition for Arabica Coffee improvement in Kenya. Journal of Agriculture and Crop Research 4(1):9-16.

Pestana KN, Capucho AS, Caixeta ET, de Almeida DP, Zambolim EM, Cruz CD, Zambolim L, Pereira AA, Oliveira ACB, Sakiyama NS (2015). Inheritance study and linkage mapping of resistance loci to *Hemileia vastatrix* in Híbrido de Timor UFV 443-03. Tree Genetics and Genomes 11:72.

Schneider K (2005). Genetic and Physical Mapping; The Handbook of Plant Genome Mapping; WILEY-VCH Verlag GmbH and Co., KGaA, Weinheim, p 22.

Sundaram RM, Vishnupriya MR, Laha GS, Rani NS, Srinivasa RP, Balachandran SM, Reddy GA, Sharma NP, Sonti RV (2009). Introduction of bacterial blight resistance into Triguna, a high yielding, mid-early duration rice variety by molecular marker assisted breeding. Biotechnology Journal 4(3):400-407.

Van der Vossen HAM, Cook RTA, Murakaru GNW (1976). Breeding for resistance to Coffee Berry Disease caused by *Colletotrichum*

coffeanum Noack *sensu* Hindorf in *Coffea arabica* L. I. Methods of pre-selection for resistance. Euphytica 25:733-756.
Van der Vossen, HAM, Walyaro DJA (1980). Breeding for resistance to coffee berry disease in coffea arabica L. II. Inheritance of the resistance. Euphytica 29:777-791.
Van der Vossen, HAM, Walyaro DJA (1981). Coffee breeding programme in Kenya. A review of progress made since 1971 and a plan of action for the coming years. Kenya Coffee 46:113-130
Van Der Vossen H, Walyaro D (2009). Additional evidence for oligogenic inheritance of durable host resistance to coffee berry disease (*Colletotrichum kahawae*) in Arabica coffee (Coffea arabica L.). Euphytica 165(1): 105–111.
Van der Vossen H, Bertrand B, Charrier A (2015). Next generation variety development for sustainable production of arabica coffee (*Coffea arabica* L.): a review. Euphytica 204:243.
Vieira A, Diniz I, Loureiro A, Pereira AP, Silva MC, Varzea V, Batista D (2019). Aggressiveness profiling of the coffee pathogen *Colletotrichum kahawae.* Plant Pathology 68:358-368.
Zhao GZ, Jiang CM, Liu JX, Chen YM, Yu TQ, Cheng ZQ (2014). Identification and analysis on the rice blast resistance gene *Pi-ta* in wild rice from Yunnan, China. China Journal of Rice Science 28(6):675-680.
Zhou L, Vega FE, Tan H, Lluch RA., Meinhard LW, Fang W, Mischke S, Irish B, Zhang D (2016). Developing Single Nucleotide Polymorphism (SNP) Markers for the Identification of Coffee Germplasm. Tropical Plant Biology 9:82-95.

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

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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.

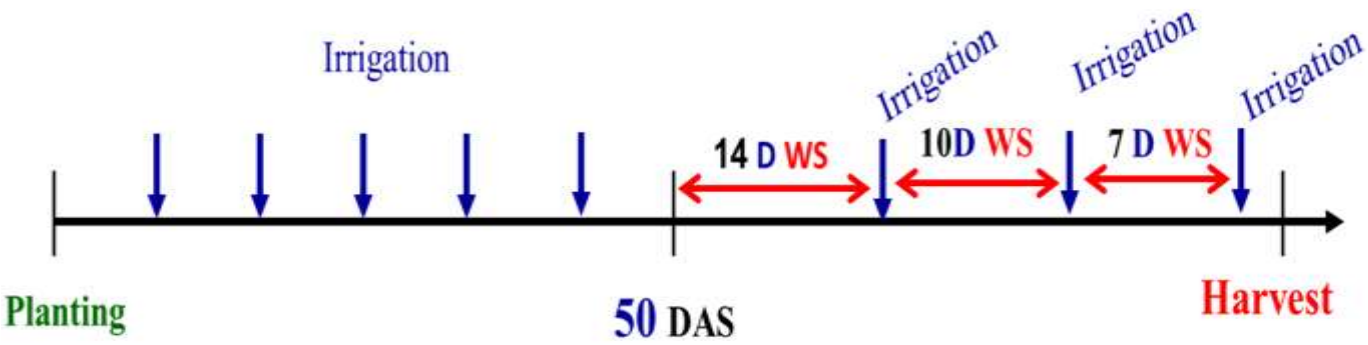

Figure 1. Drought stress imposition and irrigation frequencies (Adapted from; Mamadou, Coulibaly Adama, PhD. Thesis, 2013; http://ugspace.ug.edu.gh). *D: Days, *WS: Water-Stressed, *DAS: Days after Sowing, 14 D: 14 days, 10 D: 10 days, 7 D: 7 days.

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

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

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

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

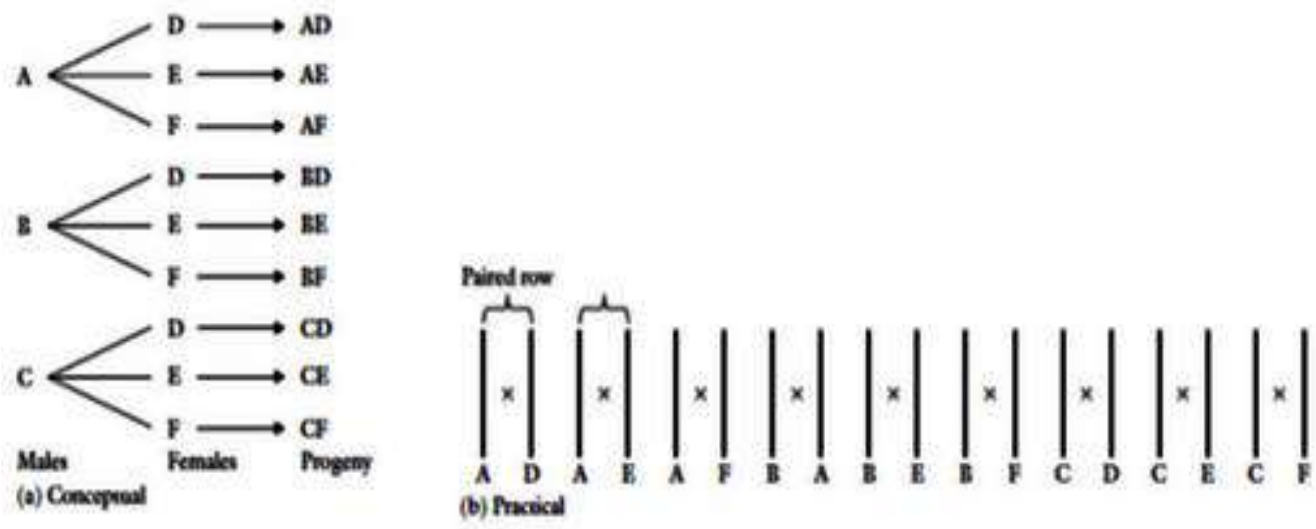

Figure 2. NC II Design (factorial design with paired rows).
Source: Kearsey and Pooni, 1996; Acquaah, 2012.

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 × 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 ≤ 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 ≤ 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 ≥ 0.05) association among many of the measured traits (Table 9).

Pod yield recorded a significant (F ≤ 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 ≤ 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 ≤ 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 ≤ 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 ≤ 0.05) in SCMR80DAP and fresh biomass but non-significantly (P ≥ 0.05) in SCMR60DAP, pod yield, dry biomass and harvest index (Table 11). The parents (male and female) showed significant (P ≤ 0.05) difference in SCMR80DAP, pod yield, fresh biomass and dry biomass but no significant (P ≥ 0.05) difference was observed for SCMR60DAP and harvest index (Table 11). Based on the combined ANOVA, no significant (P ≥ 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.

REFERENCES

Acquaah G (2012). Principles of Plant Genetics and Breeding. 2nd ed. Wiley-Blackwell, Oxford. P 740.

Ali N, Wynne JC (1994). Heritability Estimates and Correlations Studies of Early Maturity and other Agronomic Traits in Two Crosses of Peanut (*Arachis hypogaea* L.). Pakistan Journal of Botany 26:75-82.

Arunyanark A, Jogloy S, Akkasaeng C, Kesmala T, Nageswara RRC, Wright G, Patanothai A (2008). Chlorophyll Stability is an Indicator of Drought Tolerance in Peanut. Journal of Agronomy and Crop Science 194(2):113-125.

Ashraf M (2010). Inducing Drought Tolerance in Plants: Recent Advances. Biotechnology Advances 28:169-183.

Athanase N, Pangirayi T, Slyvestre H (2013). Mating Designs: Helpful Tool for Analysis. Journal of Plant Breeding and Genetics 1(3):117-129.

Bindu MH, Sheshshayee MS, Shankar AG, Prasad T G, Udayakumar M (2003). Use of SPAD Chlorophyll Meter to Assess Transpiration Efficiency of Peanut. In: Cruickshank AW, Rachaputi NC, Wright GC, Nigam SN (eds) Breeding of Drought Resistant Peanuts. ACIAR Proceedings No. 112. Australian Centre for International Agricultural Research, Canberra pp. 3-9.

Dwivedi SL, Gurtu S, Chandra S, Yuejin W, Nigam SN (2001). Assessment of Genetic Diversity among Selected Groundnut Germplasm. I: RAPD Analysis. Plant Breeding 120:345-349.

Falconer DS, Mackey TFC (Eds.) (1996). Introduction to quantitative genetics. Longman Group Ltd., London, UK. 386 p.

Fernandez GC, Miller JC (1985). Estimation of Heritability by Parent-Offspring Regression. Theoretical and Applied Genetic 70:650-654.

Girdthai T, Jogloy S, Vorasoot N, Akkasaeng C, Wongkaew S, Holbrook CC, Patanothai A (2010). Associations between Physiological Traits for Drought Tolerance and *Aflatoxin* Contamination in Peanut Genotypes under Terminal Drought. Plant Breeding 129:693-699.

Girdthai T, Jogloy S, Vorasoot N, Akkasaeng C, Wongkaew S, Patanothai A, Holbrook CC (2012). Inheritance of the Physiological Traits for Drought Resistance under Terminal Drought Conditions and Genotypic Correlations with Agronomic Traits in Peanut. Journal of Breeding and Genetic 44:240-262.

Griffing B (1956). Concept of General and Specific Combining Ability in Relation to Diallel Crossing Systems, Division of Plant Industry, C.S.I.R.O., Australian Journal of Biological Sciences 9:463-493.

He G, Prakash CS (2001). Evaluation of Genetic Relationships among Botanical Varieties of Cultivated Peanut (*Arachis hypogaea* L.) using AFLP Markers. Genetic Resources and Crop Evolution 48(4):347-352.

Hill J, Becker HC, Tigerstedt PMA (1998). Quantitative and Ecological Aspects of Plant Breeding. Chapman and Hall, UK, London 124 p.

Jogloy C, Jaisil P, Akkasaeng C, Kesmala T, Jogloy S (2011). Heritability and Correlation for Maturity and Pod Yield in Peanut. Journal of Applied Sciences Research 7:134-140.

Kearsey MJ, Pooni HS (1996). The Genetical Analysis of Quantitative Traits. 1st ed. Chapman and Hall, London, UK. http://dx.doi.org/10.1007/978-1-4899-4441-2, 249 p.

Lal C, Hariprasanna K, Rathnakumar AL, Samdur MY (2006). High Yielding, Water-Use Efficient Spanish Groundnut (Arachis hypogaea) Genotypes for Rainfed Production System. Indian Journal of Agricultural Science 76:148-150.

Mace ES, Phong DT, Upadhaya HD, Chandra S, Crouch JH (2006). SSR Analysis of Cultivated Groundnut (*Arachis hypogaea* L.) Germplasm Resistant to Rust and Late leaf Spot Diseases. Euphytica 152(3):317-330.

Ndunguru BJ, Ntare BR, Williams JH, Greenberg DC (1995). Assessment of Groundnut Cultivars for End-of-Season Drought Tolerance in a Sahelian Environment. Journal of Agricultural Science 125:79-85.

Nageswara RRC, Sadar SRC, Sivakumar MVK, Srivastava KL, Williams JH (1985). Effect of Water Deficit at Different Growth Phases of Peanut: I. Yield Responses. Journal of Agronomy 77:782-786.

Nageswara RRC, Talwar HS, Wright GC (2001). Rapid Assessment of Specific Leaf Area and Leaf N in Peanut (*Arachis hypogaea* L.) using Chlorophyll Meter. Journal of Agronomy and Crop Science 189:175-182.

Narasimhulu R, Kenchanagoudar PV, Gowda MVC (2012). Study of Genetic Variability and Correlations in Selected Groundnut Genotypes. International Journal of Applied Biology and Pharmaceutical Technology 3(1):355-358.

Nigam SN, Upadhyaya HD, Chandra S, Nageswara RRC, Wright GC, Reddy AGS (2001). Gene Effects for Specific Leaf Area and Harvest Index in Three Crosses of Groundnut (*Arachis hypogaea* L.). Annals of Applied Biology 139:301-306.

Nigam SN, Aruna R (2008). Stability of Soil Plant Analytical Development (SPAD) Chlorophyll Meter Reading (SCMR) and Specific Leaf Area (SLA) and their Association across Varying Soil Moisture Stress Conditions in Groundnut (*Arachis hypogaea* L.). Euphytica 160:111-117.

Oppong-Sekyere D, Laary JK, Asumboya G (2018a). Assessing Farmers' Knowledge, Perceptions, Varietal Preferences and Constraints to Groundnut Production; the PRA Approach. International Journal of Research 05(21):310-338. https://pen2print.org/index.php/ijr/

Oppong-Sekyere D, Akromah R, Kena AW, Larweh V, Ozias-Akins P (2018b). Screening and Selection of Drought-Tolerant Groundnut Varieties Based on Yield Performance. International Journal of Plant Breeding and Crop Science 5(3):463-473.

Painawadee M, Jogloy S, Kesmala T, Akkasaeng C, Patanothai A (2009). Identification of Traits Related to Drought Resistance in Peanut (*Arachis hypogaea* L.). Asian Journal of Plant Sciences 8:120-128.

Panhwar SA, Baloch MJ, Jatoi WA, Veesar NF, Majeedano MS (2008). Combining Ability Estimates from Line x Tester Mating Design in Upland Cotton. Proceedings of the Pakistan Academy of Sciences 45:69-74.

Ravi KD, Sekhar MR, Reddy KR, Ismail S (2012). Character Association and Path Analysis in Groundnut (Arachis hypogaea L.). International Journal of Applied Biology and Pharmaceutical Technology 3:385-389.

Ravi K, Vadez V, Isobe S, Mirr SS, Guo Y, Nigam SN, Gowda MVC, Radhakrishnan T, Bertioli DJ, Knapp SJ, Varshney RK (2010). Identification of Several Small Main-Effect QTLs and a Large Number of Epistatic QTLs for Drought Tolerance Related Traits in Groundnut (*Arachis hypogaea* L.). Theoretical and Applied Genetics 122(6):1119-1132.

Rawlings JO, Thompson DL (1962). Performance Level as Criterion for the Choice of Maize Testers. Crop Science 5:217-220.

Reddy BPK, Begum H, Sunil N, Reddy MT (2013). Variance Component Analysis of Quantitative Traits in Musk melon (*Cucumis melo* L.). Trakia Journal of Science 2:118 124.

Reddy TY, Reddy VR, Anbumozhi V (2003a). Physiological Responses of Groundnut (*Arachis hypogea* L.) to Drought Stress and its Amelioration: A Critical Review. Plant Growth Regulation 41:75-88.

Reddy TY, Reddy VR, Anbumozhi V (2003b). Physiological Responses of Groundnut (*Arachis hypogaea* L.) to Drought Stress and its Amelioration: A Review. Acta Agronomica Hungarica 51:205-227.

Richardson AD, Duigan SP, Berlyn GP (2002). An Evaluation of Non-Invasive Methods to Estimate Foliar Chlorophyll Content. New Phytologist 153:185-194.

Roychowdhury R, Tah J (2011). Genetic Variability Study for Yield and Associated Quantitative Characters in Mutant Genotypes of *Dianthus caryophyllus*. International Journal of Biosciences 1:38-44.

Sarfaraz M, Saif-ul-Malook, Wasi-Ul-Din, Sajjad M, Ali A, Iram A, Shahzadi SMK, Ahmed S, Samiullah TR, Junaid JA, Azam S, Qasrani SA, Ali Q (2014). Role of Nitrogen and Sowing Date on Sunflower (*Helianthus annuus* L.) to Improve Yield and Oil Content – An Overview. International Journal of Advanced Life Sciences 7(4):691-698.

Selvaraj MG, Narayana M, Schubert AM, Ayers JL, Baring MR, Burow MD (2009). Identification of QTLs for Pod and Kernel Traits in Cultivated Peanut by Bulked Segregant Analysis. ElectronicJournal Biotechnology 12(2):13.

Sheshshayee M, Bindumadhava H, Rachaputi N, Prasad T, Udayakumar M, Wright G, Nigam S (2006). Leaf Chlorophyll Concentration Relates to Transpiration Efficiency in Peanut. Annals of Applied Biology 148:7-15.

Shoba D, Manivannan N, Vindhiyavarman P (2010). Genetic Diversity Analysis of Groundnut Genotypes using SSR Markers. Electronic Journal of Plant Breeding 1(6):1420-1425.

Smith JD, Kinman ML (1965). The Use of Parent-Offspring Regression as an Estimator of Heritability. Crop Science 5:595-596.

Songsri P, Jogloy S, Kesmala T, Vorasoot N, Akkasaeng C, Patanothai A, Holbrook CC (2008). Heritability of Drought Resistance Traits and Correlation of Drought Resistance and Agronomic Traits in Peanut. Crop Science 48:2245-2253.

Songsri P, Jogloy S, Holbrook CC, Kesmala T, Vorasoot N, Akkasaeng C, Patanothai A (2009). Association of Root, Specific Leaf Area and SPAD Chlorophyll Meter Reading to Water Use Efficiency of Peanut Under Different Available Soil Water. Agricultural Water Management 96:790-798.

Sprague GF, Tatum LA (1942). General versus Specific Combining Ability in Single Crosses of Corn. Journal of American Society of Agronomy 34:923- 932.

Sumathi P, Madineni S, Veerabadhiran P (2010). Genetic Variability for Different Biometrical Traits in Pearl Millet Genotypes (*Pennisetum glaucum* L. R. BR.). Electronic Journal of Plant Breeding 1:437-440.

Surihan B, Patanothai A, Jogloy S (2005) Gene Effect for Specific Leaf Area and Harvest Index in Peanut (*Arachis hypogaea* L.). Asian Journal of Plant Sciences 4:667-672.

Tang R, Gao G, He L, Han Z, Shan S, Zhong R, Zhou C, Jiang J, Li Y, Zhuang W (2007). Genetic Diversity in Cultivated Groundnut Based on SSR Markers. Journal of Genetics and Genomics 34(5):449-459.

Tsaur WL, Yang KH, Lu HS (1989). Heritability and Correlation Based on the F2 Population from Two Crosses Between Large Seeded Virginia Bunch Type and Small-Seeded Spanish Type Peanuts. Journal Agricultural Research of China 38:191-200.

Upadhyaya HD, Mallikarjuna BP, Swamy PV, Goudar K, Kullaiswamy BY, Singh S (2005). Identification of Diverse Groundnut Germplasm through Multi-Environment Evaluation of a Core Collection for Asia. Field Crops Research 93:293-299.

Warunyuwat A, Tongsri S (1990). Correlations of Yield and Yield Component Traits in Advanced Generations of Peanut Crosses. Thai Journal of Agriculture Science 23:19-22.

Wright GC, Nageswara RRC, Farquar GD (1994). Water-Use Efficiency and Carbon Isotope Discrimination in Peanut under Water Deficit Conditions. Crop Science 34:92-97.

Wuma H, Jogloy S, Toomsan B, Sanitchon J (2009). Response to Early Drought for Traits Related to Nitrogen Fixation and their Correlation to Yield and Drought Tolerance in Peanut (*Arachis hypogaea* L.). Asian Journal of Plant Sciences 8:138-145.

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

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Full Length Research Paper

Efficacy of insecticides and crop critical stage for the management of chickpea pod borer (*Helicoverpa armigera*) in Central Zone of Tigray, Ethiopia

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Chickpea pod borer (*Helicoverpa armigera*) is a major insect pest constraining chickpea production in Tigray, northern Ethiopia, as there is no recommended management option in the area. Therefore the present study was conducted to assess the efficacy of insecticides and to determine the critical growth stage of the crop for effective spray at Axum Agricultural Research Center. Results indicated that in laboratory profit 72% EC (profenofos), abema 3% EC (abamectin 20 g/L + emamectin benzoit 10 g/L), perfecto (imedachloprid + lambda-cyhalothrin) and hamectin (abamectin) reduced the number of larvae by 75, 55, 44 and 34%; 86, 82, 65, 56% and 83, 83, 66 and 83% at 24, 48 and 72 h after spray, respectively. Similarly abema 3%EC and profit (Profenofos) 72% EC were the most effective insecticides to give high mortality of pod borer on chickpea under field conditions. These insecticides reduced the number of larva per plant by 51 to 56.7% five days after spray. The number of damaged pods per plant was very low in both insecticides (0.91 and 1.05) but on the untreated check 3.05. The highest yield was also obtained from chickpea treated with abema 3%EC at podding stage (23.92 qt/ha). Comparatively the most effective insecticides against pod borer were abema and profit and the best application time were at podding stage of the crop. Thus chickpea growers in the area should prefer these insecticides for better pod borer management.

Key words: Chickpea, *Helicoverpa armigera*, insecticides, growth stage.

INTRODUCTION

Chickpea (Cicer arietinum L.) is a legume crop of the Fabaceae family originated in present day South eastern Turkey and adjoining Syria (Sexena and Singh, 1987). It is the second most important food legume in the world after common bean. The major chickpea-producing countries are India (67.41%), Australia (6.21%), Pakistan (5.73%), Turkey (3.86%), and Myanmar (3.74%) (FAOSTAT 2015). Ethiopia is considered as secondary center of genetic diversity for chickpea and the wild relative of cultivated chickpea (C. arietinum L.), is found in Tigray region (Yadeta and Geletu, 2002; Dagne et al., 2018). In Ethiopia the area coverage and the volume of production of chickpea in 2017/2018 are 242703.73 ha and 4994255.5 quintal with average productivity of 2.05

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ton/ha. It contributes 15.18% of Ethiopia's total pulse production and is second after fababeans (CSA, 2017/2018). It has the ability to grow on residual moisture which gives farmers the opportunity to engage in double cropping, since chickpea is sown at the end of rainy season.

Nutritionally chickpea contains 24% protein, 59.6% carbohydrates, and 3.2% minerals (Bakr et al., 2004). Its fiber reduces cholesterol and regulates blood sugar. Hence, it is an important crop as source of food and income commonly used as a green vegetable (Yasin, 2014). It is very important component of cropping systems which can fix up to 140 kg N per ha from air and meet most of its nitrogen requirement. It increases substantial amount of residual nitrogen for subsequent crops and adds some amount of organic matter to maintain and improve soil health and fertility. It saves the fertilizer input cost not only for chickpea but also for subsequent crops. Chickpea production is important for crop rotation with cereals such as wheat and tef which are widely grown in relatively well-drained black soils (Menale et al., 2009)

However, the production of chickpea is challenging because of different insect pests and diseases such as pod borers, cut worms, aphids, jassids, thrips, whitefly and the storage pests (bruchids) which are the most devastating pests of chickpea in Asia, Africa, and Australia. Among these gram pod borers *H. armigera* (Hubner) (Lepidoptera: Noctuidae) is a serious obstacle and a global concern for the production of chickpea.This pest is a cosmopolitan, multi-voltine and highly polyphagous, which attacks a number of crops which have agricultural importance throughout the world (Dabhi and Patel, 2007). Fitt (1989) recorded the crops of maize, sorghum, cotton, common bean, peas, chickpeas, tomatoes, capsicum, vicia and to a lesser extent, okras, cabbages, lettuces, strawberries, tobacco, sunflowers, and many of the other legumes as host plants of the pest. Pod borer is a key pest of chickpea causing 90-95% total damage (Sachan and Lal, 1994). It can cause damage up to 100% in unprotected chickpea fields (Tsedeke et al., 1982; Sarwar et al., 2009). A single *H. armigera* larva can damage up to 40 pods throughout its larval stage (Khan et al., 2009). The chickpea economic threshold is one pod borer larva per one meter row length (Zahid et al., 2008).

Different management options have been practiced against pod borer in different areas and years. Cultural practices such as inter cropping, deep ploughing, trap crops and sowing date have been reported to reduce the survival and damage of *H. armigera* (Romeis et al., 2004). Extracts from different parts of neem tree (neem leaf, neem oil and neem seed kernel 5%) influenced negatively both the survival and feeding of the larva of *H. armigera* (Mesfin et al., 2012). Insecticides monocrotophos 36 WC, endosulfan 35 EC, carbaryl WP, cypermethrin 25 EC, indoxacarb 14.5 SC, Profenofos 50 EC and coragen 20 SP showed the highest mortality of *H. armigera* larvae on chickpea (Iqbal et al., 2014). Mesfin et al. (2012) reported synthetic insecticides have resulted in fast and effective pest control and the present study was initiated to select the best insecticides as well as to determine the growth stage of the crop for effective foliar spray against chickpea pod borer.

MATERIALS and METHODS

Description of the study area

The experiment was conducted at Axum Agricultural Research Center (AxARC) in Laelay-mychew district which is 3 km east of Axum town. The study area is located at 13°15'40.2'' N latitude and 38°34'45.8''E longitude with an altitude of 2148 masl. It is located in northern part of the country, central zone of Tigray region in the semiarid tropical belt of Ethiopia with "*weinadega*" agro climatic zone. It is characterized by low and erratic rainfall with mean minimum and maximum range of 500 to 782.8 mm. The rainy season is mono modal concentrated in one season from July to September. The daily average minimum and maximum temperatures are 12.6 and 25.51°C, respectively. The soil type is classified as vertisol with a characteristic feature of clay soil type with pH 7.19.

Treatments and experimental design

The experiment was conducted both in field and laboratory in the same season. It was designed in a factorial randomized complete block design (RCBD) with three replications at field and CRD in the laboratory. Chickpea seed (Dalota variety) was used as planting material.The field was ploughed using oxen and harrowed manually to bring the soil to fine tilth. Fertilizer NPSZnB at the rate of 100 kg/ha was used during sowing date. The plot size was 3 × 3 m^2. To manage the chemical drift among plots, spacing between reps and plots were 2 and 1.5m; spacing between rows and plants 30 and 10 cm, respectively. One liter capacity hand sprayer was used for each insecticide to manage the chemical mixtures. Each insecticide was sprayed twice at different growth stages of the crop. Spraying was done at wind free time of the day early in the morning up to 2 o'clock. The insecticides were applied at manufacturer rates. Cultivation, weeding and all recommended agronomic practices were performed accordingly (Table 1).

Data collection

Number of pod borer larva, damaged pods and total pods per plant were collected from five randomly selected and tagged plants in each treatment. The yields were taken from the harvested net plot area excluding the borders. The infestation percentage was captured using the formula,

$$\text{Infestation percentage} = \frac{Totalnumberofdamagedpods \quad per\ plant}{Totalnumberofpodsoft \quad heplant} \text{x100}$$

$$\text{Pod borer larva reduction percentage} = \frac{\text{Mean of untreated} \quad \text{Mean of treated}}{\text{Mean of untreated}} \text{x100}$$

All collected data were analyzed using SAS version 9.1 software and the insect data were transformed using square root transformation before analysis.

Table 1. Treatment combinations.

Trade name	Common name	Chemical group	Dose Lha^{-1}	Application time
Profit 72% EC	Profenofos	organophosphate	0.75	A,B,C
Agrothoate40% EC	Dimethoat	organophosphate	1	A,B,C
Con-fidence	Imedachloprid	neonicotinoids	0.4	A,B,C
Perfecto	imedachloprid+lambda-cyhalothrin	-	0.4	A,B,C
Hamectin3.6% EC	Abamectin	avermectins	1	A,B,C
Abema3% EC	Abamectin 20 g/L+emamectin benzoit 10 g/L	avermectins	1	A,B,C
Untreated	--	-	-	-

Where A,B,C were each insecticide applied twice; (A) Before flowering, (B) at 50% flowering stage and (C) at podding stage.

Table 2. Effect of different insecticides on 3rd-4th instars larva of chickpea pod borer after spray in laboratory.

Treatment	No. of larva before spray	24h after spray		48h after spray		72h after spray	
		No. of alive larva	Reduction %	No. of alive larva	Reduction %	No. of alive larva	Reduction %
Profit	30	7^{d}	75.86	3^{d}	86.95	3^{c}	83.33
Agrothoate	30	21^{b}	27.58	15^{b}	34.78	10^{b}	44.44
Confidence	30	21^{b}	27.58	12^{bc}	47.83	10^{b}	44.44
Perfecto	30	16^{bc}	44.83	8^{cd}	65.22	6^{c}	66.67
Hamectin	30	19^{bc}	34.48	10^{bc}	56.52	3^{c}	83.33
Abema	30	13^{c}	55.17	4^{d}	82.61	3^{c}	83.33
Un treated	30	29^{a}	-	23^{a}	-	18^{a}	-
Lsd(0.05)		5		5		3.8	
Cv(%)		17		27		28	

RESULTS AND DISCUSSION

The data collected on the comparative efficacy of different insecticides against chickpea pod borer larva tested in laboratory and at field are presented in Tables 2 to 4.

Efficacy of treatments on *H. armigera* larvae population in laboratory and field

The result showed that all treatments were significantly different ($P<0.05$) from the untreated control after treatment application in the laboratory. Profenofos and abema were effective in killing the larvae 24 h after spray. Moreover, effectiveness of these insecticides varied with time intervals; maximum effect was found after 72 h interval. Out of thirty 3rd-4th instar larvae only three alive larvae were observed on treatments with profenofos, abamectin 20 g/L+ emamectin benzoit 10 g/L and hamectin after 72 h of spray. However, the immediate killing action within 24 h was observed on profenofos and then abamectin 20g/L+emamectin benzoit 10 g/L which reduced the larva by 75 and 55% respectively. The highest reduction percentage up to 83% was observed 72 h after spraying with profenofos and abamectin 20 g/l+emamectin benzoit 10 g/L treated plots (Table 2).

In the field experiment insecticide treated plots were significantly different from the untreated control even though there was difference in effectiveness between insecticides. The number of larvae increased with the crop phenological growth. The highest larvae population was recorded at podding stage before treatment application. There was statistical difference in larvae population among treatments before insecticide application; before flowering, at 50% flowering and podding. The lowest number of larvae per plant was observed on the treated plots and the highest on the untreated plots. Three days after treatment application before flowering all insecticides were effective to reduce the larvae population; but after time intervals the insecticides lost their effectiveness and consequently the infestation increased again to damage the pods. However, these insecticides were also applied at 50% flowering and podding stages of the crop. Table 3 indicated that the lowest number of larva per plant (0.91, 0.95 and 1.2) was observed on abema (abamectin 20 g/L + emamectin benzoit 10 g/L) 3%EC, profenofos 72%EC and perfecto treated plots respectively at five days intervals applied before flowering. Similarly, at podding

Table 3. Field efficacy of different insecticides on chickpea pod borer larva after spray.

Treatment	No. of larva before spray	No of larva 3days after spray	Reduction %	No of larva 5 days after spray	Reduction %
Profit x A	1.27^{f}	1.05ij	49.76	0.95^{jk}	54.76
Profit x B	1.75^{abc}	1.31^{ghf}	37.32	1.05^{hijk}	50.00
Profit x C	1.68^{cb}	1.27^{gh}	39.23	1.02^{ijk}	51.43
Agrothoate xA	1.47^{de}	1.37^{efgh}	34.45	1.29^{cdef}	38.57
Agrothoate xB	1.69^{bc}	1.57^{bcde}	24.88	1.43^{c}	31.90
Agrothoate xC	1.86^{ab}	1.78^{b}	14.83	1.70^{b}	19.05
Confidence xA	1.43^{ef}	1.32^{ghf}	36.84	1.25^{efg}	40.48
Confidence x B	1.78^{abc}	1.49^{def}	28.71	1.36^{cde}	35.24
Confidence x C	1.85^{ab}	1.71^{b}	18.18	1.69^{b}	19.52
Perfecto x A	1.32^{ef}	1.24^{hi}	40.67	1.1^{ghij}	47.62
Perfecto x B	1.79^{abc}	1.55^{cde}	25.84	1.33^{cdef}	36.67
Perfecto x C	1.81^{abc}	1.51^{cdef}	27.75	1.22^{efgh}	41.90
Hamectin x A	1.35^{ef}	1.24^{hi}	40.67	1.16^{fghi}	44.76
Hamectin x B	1.63^{cd}	1.48^{defg}	29.19	1.29^{cdef}	38.57
Hamectin x C	1.92^{a}	1.61^{cbd}	22.97	1.41^{c}	32.86
Abema x A	1.29^{ef}	1.02^{j}	51.20	0.91^{k}	56.67
Abema x B	1.72^{bc}	1.25^{hi}	40.19	1.01^{ijk}	51.90
Abema x C	1.79^{abc}	1.27^{h}	39.23	1.02^{ijk}	51.43
Control (untrt	1.94^{a}	2.09^{a}	0.00	2.10^{a}	0.00
Lsd(0.05)	0.19	0.21		0.17	
Cv%	7.16	8.17		8.17	

Where A,B,C were each insecticide applied twice; A = Before flowering, B = at 50% flowering stage and C = at podding stage.

stage the number of larva per plant was 1.02 on abamectin 20 g/L+emamectin benzoit 10 g/L and profenofos treated plots. These insecticides reduced the larval population by 83% after five days of spray intervals at podding stage.

The result showed that all treatments were significantly different from the untreated plot in number of damaged pods and infestation percentage. The lowest damage was recorded in treatments sprayed with abema (abamectin 20 g/L + emamectin benzoit 10 g/L) and profenofos (0.91 and 1.05) at podding. Comparatively the best insecticides effective against pod borer were Abema (abamectin 20 g/L + emamectin benzoit 10 g/L) and profenofos. The best application time was at podding stage of the crop. Yield was significantly higher on treatments sprayed with abamectin 20 g/L + emamectin benzoit 10 g/L at podding stage and abamectin at 50% flowering stage (Table 4).

DISCUSSION

The current study was carried out to examine the effect of different insecticides against *H. armigera* on chickpea in laboratory and under field conditions. The result in the laboratory showed that insecticide treatments were significantly effective on killing the *H. armigera* larvae. Profenofos, abema, perfecto and hamectin reduced the number of larvae by 75, 55, 44 and 34% after 24 h of spray; (86, 82, 65 an 56%) after 48 h and (83, 83, 66 and 83%) 72 h after spray, respectively. This result is in agreement with Iqbal et al. (2014) who studied the efficacy of emamectin 1.9 EC. (emamectin benzoate), lannate 40 SP. (methomyl), coragen 20 SP. (rynaxypyr), match 50 EC. (lufenuron), profenofos 50 EC. Profenofos tested against *H.armigera* on chickpea had the highest mortality of larvae in plots treated with profenofos (85%, 90% and 94%) and rynaxypyr (85, 90 and 92%) at 3, 5 and 7 days after treatment, respectively. The field efficacy of different treatments against *H. armigera* larvae was determined on the basis of number of larvae per plant. The data revealed that all the treatments were significantly superior to control. The lowest number of larvae per plant (0.91, 1.01, 1.02) and (0.95, 1.05, 1.02) was recorded on chickpea treated with abema (abamectin 20 g/L + emamectin benzoit 10 g/L) 3%EC and profenofos 72%EC before flowering, at 50% flowering and podding stage of the crop five days after spray reduced the number of larva by (56.7, 51.9 and 51%) and (54.8, 50 and 51%), respectively; whereas the highest number of H. *armigera* larva per plant (2.10) was recorded on untreated control. The present results

Table 4. Field efficacy of insecticides on chickpea yield and yield components.

Treatments	No. of Damaged pods/P	No. of un damaged pods/P	Total no of pods/p	Infestation percentage	Yield qt/ha
Profit x A	1.09^{de}	1.97^{a-d}	1.97^{abc}	1.14^{e}	18.93 $^{a-d}$
Profit x B	1.19^{de}	1.91^{a-e}	1.92^{abc}	1.28^{de}	19.04 $^{a-d}$
Profit x C	1.05^{de}	1.98^{abc}	1.98^{abc}	1.05^{e}	19.41 $^{a-d}$
Agrothoate x A	2.64^{ab}	1.94^{a-e}	1.96^{abc}	2.72^{ab}	18.33 $^{a-d}$
Agrothoate x B	1.26^{de}	1.95^{a-d}	1.96^{abc}	1.29^{de}	16.82^{cbd}
Agrothoate x C	1.64^{cd}	1.78^{f}	1.79^{d}	2.01^{bcd}	17.33 $^{a-d}$
Confidence x A	2.89^{ab}	1.87^{def}	1.92^{abc}	3.17^{a}	19.33 $^{a-d}$
Confidence x B	2.23^{bc}	1.94^{a-e}	1.96^{abc}	2.32^{bc}	17.33 $^{a-d}$
Confidence x C	2.49^{ab}	1.91^{bcde}	1.94^{abc}	2.70^{ab}	20.44 $^{a-d}$
Perfecto x A	1.49^{de}	1.99^{ab}	2^{a}	1.51^{de}	22.59^{a}
Perfecto x B	1.29^{de}	1.89^{cde}	1.90^{bc}	1.41^{de}	18.44 $^{a-d}$
Perfecto x C	1.35^{de}	1.88^{cde}	1.89^{cd}	1.49^{de}	15.96^{cbd}
Hamectin x A	1.63^{cd}	1.98^{abc}	1.99^{ab}	1.67^{cde}	20.85^{abc}
Hamectin x B	1.29^{de}	1.94^{a-e}	1.94^{abc}	1.35^{de}	23.96^{a}
Hamectin x C	1.27^{de}	1.89^{b-e}	1.90^{abc}	1.39^{de}	18.67 $^{a-d}$
Abema x A	1.26^{de}	2.01^{a}	1.99^{ab}	1.29^{de}	14.85^{cd}
Abema x B	1.15^{de}	1.91^{a-e}	1.90^{bc}	1.23^{e}	20.70 $^{a-d}$
Abema x C	0.91^{e}	1.94^{a-e}	1.93^{abc}	0.93^{e}	23.92^{a}
Control (untrt)	3.05^{a}	1.84^{ef}	1.89^{cd}	3.46^{a}	13.78^{d}
Lsd (0.05)	0.70	0.1	0.09	0.77	6.97
Cv (%)	25.9	3.2	3.1	26.5	22.2

Where A,B,C were each insecticide applied twice; A = Before flowering, B = at 50% flowering stage and C = at podding stage.

revealed with findings by Digne et al. (2018) who reported that the highest pod borer larval reduction (90.63%) was found in Diazenon sprayed plot followed by Karate 5% EC (71.87%) sprayed plot. Similarly, Khan et al*.* (2009) conducted a trial against gram pod borer and to assess comparative efficacy of insecticides (thiodan 40EC, lorsban 40EC, ripcord 10EC, nurell-D (chlorpyrifos + cypermethrin 50 + 500 g/L EC) and methomyl 45 WP). Methomyl was found most effective against the tested pest under field conditions.

The current study showed that all insecticides were effective to reduce the number of damaged pods per plant applied before flowering, at 50% flowering and podding stages of the crop, compared to the untreated check. But before flowering application insecticides lost their effectiveness and increased the pod damage. The lower damaged pods and infestation percentage were recorded on insecticides applied at podding stage of the crop. Abema (abamectin 20 g/L + emamectin benzoit 10 g/L) applied at podding stage gives the minimum damaged pods per plant (0.91) and lower infestation percentage (0.93%) with the highest yield (23.9 qt/ha). Savita and Pandurang (2014) reported that the lowest number of surviving population of larvae 0.70 larvae/plant, highest yield recorded 15.00 q/ha, lower pod damage 8.10% were recorded on chickpea treated with rynaxypyr 20 SC at 40 g/ha.

Conclusion

The experiment was conducted to assess the efficacy of insecticides against *H. armigera* on chickpea and to determine the critical growth stage of the crop for spray. From the present research study, it was concluded that approaches for chemical management of *H. armigera* were found effective. Spraying insecticides at podding stage of the crop were important. The result revealed that abema 3% EC (abamectin 20 g/L + emamectin benzoit 10 g/L) and profit (Profenofos) 72% EC were the most effective insecticides to give high mortality of pod borer on chickpea under field conditions. These insecticides were highly effective in reducing the number of larva, damaged pods and infestation percentage per plant. The highest yield was also obtained from chickpea treated with abema (abamectin 20 g/L + emamectin benzoit 10 g/L) 3%EC at podding stage.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Bakr MA, Afzal MA, Hamid A, Haque MM, Aktar MS (2004). Blackgram

in Bangladesh. Lentil, Blackgram and Mungbean Development Pilot Project, Pulses Research Centre, BARI, Gazipur.
Central Statistics Authority (CSA) (2007/2018). Agricultural Sample Survey. Report Area and production of major crops (Private Peasant Holdings, Meher Season). Statistical Bulletin 1(586). CSA, Addis Ababa, Ethiopia.
Dabhi MV, Patel CC (2007). Life expectancy of *Helicoverpa armigera* on chickpea. Journal of SAT Agricultural Research 5(1):1-2.
Dagne K, Ermias T, Amare B (2018). On Farm Demonstration and Evaluation of Synthetic Insecticides for the Control of Pod Borer (*Helicoverpa armigera* Hubner) on Chickpea in Bale Zone. American Journal of Plant Biology 3(3):29-32.
Food and Agricultural Organization of the United States (FAOSTAT) (2015). www://faostat.fao. org/site/339/default. aspx.. Accessed in June 12, 2015.
Fitt GP (1989). The ecology of *Heliothis* species in relation to agroecosystems. Annual Review of Entomology 34:17-52.
Iqbal J, Farooq SU, Jamil M, Khan HAA, Younis M (2014). Relative efficacy of selective insecticides against gram pod borer, (*Helicoverpa armigera*) of chickpea. Mycopath 12(2):119-122.
Khan SM, Muhammad YI, Muhammad AK (2009). Varietal screening of chickpea and the efficacy of different insecticides against chickpea pod borer *Helicoverpa armigera*. Gomal University Journal of Research 25(1):20-24.
Menale K, Bekele S, Solomon A, Tsedeke A, Geoffrey M, Setotaw F, Million E, Kebebew A (2009). Current situation and future outlooks of the chickpea subsector in Ethiopia. International Crops Research Institute for the SemiArid Tropics, Nairobi, Kenya.
Mesfin W, Emana G, Geremew T (2012). Neem *Azadirachta indica* (A. Juss) extracts negatively influenced growth and development of African bollworm, *H.armigera* (Hubner) (Lepidoptera: Noctuidae). Academic Journal of Entomology 5(1):22-27.
Romeis J, Sharma HC, Das KKS, Sharma BK (2004). The potential of transgenic chickpeas for pest control and possible effects on non-target arthropods. Crop Protection 23(10):923-938.
Sachan JN, Lal SS (1994). Role of Botanical Insecticides in *Helicoverpa armigera* management in pulses. Botanical Pesticides in Integrated Pest Management, ISTS, Rajahmundry, India pp. 261-269.
Sarwar M, Ahmad N, Toufiq M (2009). Host plant resistance relationships in chickpea (*cicerarietinum* L.) against gram pod borer, *Helicoverpa armigera* (Hubner). Pakistan. Journal Botany 41(6):3047-3052.
Savita PA, Pandurang BM (2014). Efficacy of newer molecules of insecticides against gram pod borer, Helicoverpa armigera (Hub) on chickpea. International Journal of Science and Research 4(12):1283-1285.
Sexena M, Singh K (1987). The chickpea. pp. 163-189. In: Saxena, MC., Singh, KB. (ed.): CAB international, UK.
Tsedeke A, Tadesse G, Kemal A (1982). Arthropod pests of grain legumes in Ethiopia: Their importance and distribution. Institute of Agricultural Research (IAR), Addis Abeba, Ethiopia. pp. 22-28.
Yadeta A, Geletu B (2002). Evaluation of Ethiopian chickpea landraces for tolerance to drought. Genetic Resources and Crop Evolution 49:557-564.
Yasin G (2014). Evaluation of chickPea (*Cicer arietinum* L.) Varieties for yield Performance and adaptability to Southern Ethiopia. Journal of Biology, Agriculture and HealthCare 4(17):34-38.
Zahid MA, Islam MM, Reza MH, Prodhan MHZ, Rumana BM (2008) Determination of economic injury levels of *Helicoverpa armigera* (Hubner) in chickpea. Bangladesh Journal of Agricultural Reserch 33(4):555-563.

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Evaluation of hot pepper (*Capsicum annuum* L.) varieties for green pod yield and yield components in Western Tigray, Northern Ethiopia

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A field experiment was conducted at Humera Agricultural Research Center experimental site for two consecutive years (2011/2012 and 2012/2013 cropping season) under irrigation condition to evaluate performance of hot pepper varieties for green pod yield and yield components in western Tigray, Northern Ethiopia. A total of six hot pepper varieties were used as test genotypes. Least Significant Difference (LSD) and Pearson correlation were used to compare treatment means and association of characters. Combined analysis of variance explained that all the traits except days to 50% flowering and days to 50% fruiting showed highly significant difference ($p<0.01$) among the varieties. Among the six varieties the highest marketable green pod yield was found from Jeju (19.47 t ha $^{-1}$) which is statistically at par with marecofana (19.35 t ha $^{-1}$). Marecofana scored the largest green pod weight (7.3 gram) followed by Jeju (6.2 g). Correlation analysis showed that marketable green pod yield per hectare had highly significant positive association with fruit yield per plant (r=0.705), single fruit weight (r=0.668) and fruit diameter (r=0.675) indicating that selection based on these trait improves marketable green pod yield of hot pepper in the specific agroecology.

Key words: Humera, Jeju Marecofana, Pearson correlation.

INTRODUCTION

Capsicum is a high value crop used as vegetables and spice in Ethiopia. Different pepper types such as bell (*sweet*) pepper which is non -pungent, chili (*mitimita*) and hot pepper (*berbere*) which is pungent are produced in which hot pepper is dominantly produced. The pungency is due to high capsaicin ($C_{18}H_{27}O_3N$) content in the fruit. It is important in local dishes, karia, berbre and processing industries (coloring agent); it is exported in the form of oleoresin (red pigment) and ground powder in different forms (Girma et al., 2001). Capsicum is grown in most part of the county. The central (Eastern and Southern Shoa), Western, North Western (Wellega, Gojjam) and the Northern part of the country are the potential capsicum producing areas in the country (Girma et al., 2001). Peppers are a warm-season crop and require similar growing conditions as tomato and eggplant. The crop grows at wide range of altitudes with rainfall between 600-1250 mm per annum.

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Seeds germinate best at 25-30°C. Optimal temperatures for productivity range between 18-30°C. Peppers are tolerant to a wide range of soil conditions. However, fertile medium loams and well-drained soils with a pH of 5.5-6.8 are generally considered most suitable (Brandenberger et al., 2012).

Pepper is one of the most important vegetable crops in the world. In 2018 the total cultivated area under pepper in Ethiopia was 8001 hectare with a production quantity of 4889 tons (FAOSTAT, 2018). Tigray region is one of the potential areas for cultivation of the crop. In 2017/2018 the total area covered by green pepper was 689.28 hectare with a production quantity of 40,571.18 quintal (CSA, 2018)

The productivity of the crop in Ethiopia is 6.11 t ha^{-1} (FAOSTAT, 2018) and in Tigray 4.06 ton ha^{-1}(CSA, 2018) which is very low as compared to the national average. The major reasons associated with yield reduction are shortage of improved varieties, infestation of disease and pests, poor agronomic practices, poor post-harvest handling. To reduce the production challenges of hot pepper the Ethiopian Agricultural Research Institute has so far released a number of varieties that include 3 for fresh and dry market and 2 for fresh market (green fruit) and 2 for processing. Moreover, many authors viz; Tegene, 2009; Melakui et al. (2015), Tibebu and Bizuayehu (2014); Gebremeskel et al. (2015); Kirk and Gu (2011); Keneth (2017); Sameer et al. (2017) and Sibhatu et al. (2016) have studied evaluation trial of hot pepper varieties for specific agro ecology However, in the study areas farmers used to grow unknown sources of seed. This revealed no effort was made to recommend agro ecologically adaptable, better quality and high yielding pepper variety for the specific area. Thus, the objective of this study is to evaluate performance of nationally released hot pepper varieties and recommend adaptable and high yielding variety/ies for Western Tigray.

MATERIALS AND METHODS

Experimental location

The experiment was conducted in Humera Agricultural Research Center experimental site, Northern Ethioipia for two years 2011/2012 and 2012/2013 cropping calendar under irrigation. The experimental site is located at 14° 06' N latitudes and 38° 31' E longitudes with an altitude of 604 meter above sea level. It has chromic vertisol black color. Agro-ecologically it is described as hot to warm semiarid plain sub agro-ecology (SA1-1). The mean maximum temperature varies from 42°C in April to 33°C in August, while the mean minimum temperature is from 22.2°C in May to17.5°C in July (EARO, 2002).

Experimental material

The experimental materials comprise five hot pepper varieties obtained from Melkassa Agricultural Research Center (Marecofana, Melkaawaze, Bakolocal and Odaharo) and one cultivar widely used by farmers (Jeju).

Experimental design and management

The trial was laid out in randomized complete block design (RCBD) with three replications. Each variety was planted in the main field in a gross plot size of 14 m^2 (5 rows * 4 m row length* 0.7 m spacing between rows). Spacing between row and plants was maintained at 70*30 cm, respectively. The middle three rows were used for data collection leaving the two rows as borders. All agronomic practices (irrigation, cultivation, weeding and fertilization) were applied uniformly for all plots according to the recommendation of the crop.

Data collection

Ten plants were randomly sampled from middle three rows. Data on number of green pods per plant, pod yield per plant (g), single green pod weight (g), pod length (mm), pod width (mm) were recorded per plant and fruit basis. While measurements such as days to flowering, days to maturity, marketable green pod yield $hectare^{-1}$ (tons) were taken on plot basis.

Data analysis

Statistical analysis was done using statistical analysis software (SAS version 9.2) package (SAS Institute, 2008) and treatment means were compared using least significant difference (LSD) at 5% probability level. Pearson correlation was used to measure association of characters among themselves and green pod yield per hectare. Correlation analysis was done using Proc Corr procedures of SAS.

RESULTS AND DISCUSSION

Two years combined analysis of variance on evaluation of hot pepper varieties demonstrated that there were significant differences ($p<0.01$) among the varieties for number of fruits per plant, green pod yield per plant, pod weight, pod length, pod diameter and marketable green pod yield. While days to 50% flowering and 50% fruiting showed nonsignificant differences among the varieties (Table 1). This might be because divergent genotypes are included in the evaluation trial. In line with this, Delelegn et al. (2014) reported highly significant different for days to 50% flowering, days to first harvest, number of fruits per plant, fruit length, fruit width and marketable yield for 9 varieties evaluated in Jimma and Seka chekorsa areas of Ethiopia. Similarly, Mangoel et al. (2012) found significant difference among seven varieties for days to first flowering, number of fruits per plant and fresh fruit yield per hectare. Moreover, Gebremeskel et al. (2015) found significant differences among three varieties evaluated for two years in Raya valley, Northern Ethiopia for plant height, fruit diameter, fruit length and marketable fruit yield. The combined mean value of the six varieties evaluated in western lowland of Tigray showed a wide range of variation for most of the traits.

Table 1. Combined mean square results of pod yield and yield components of hot pepper varieties obtained from ANOVA.

Source of variation	Df	Mean square							
		Days to 50% flowering	Days to 50% fruiting	No. of green pods per plant	Green pod yield per plant (g)	Single green pod weight (g)	Pod length (mm)	Pod diameter (cm)	Marketable green pod yield (t ha^{-1})
Block	2	0.151	92.13	103.2	10865	0.2677	171.91	4.133	8.962
Varieties	4	8.670ns	70.99ns	1033.2**	62344**	5.9601**	114.58*	41.113**	46.171**
Year	1	383.83**	15.28ns	87.7ns	1249920**	3.0959**	867.70**	75.632**	52.396**
Variety*year	4	28.314	99.13*	385.5*	109752**	2.6327**	14.81ns	4.855ns	4.286ns
Residual	18	4.724	33.55	106.7	72679	0.344	36.57	2.539	2.439
Total	29								

Df= degree of freedom, **=highly significant at (p< 0.01), *= significant at (p< 0.05) and ns=non-significant at (p< 0.05) probability level respectively, cm= centimeter, mm= millimeter, g= gram.

This might be the difference of the genotypes in expressing their genetic potential to the specific agro ecology.

Combined mean response showed that Odaharo was the earliest (67days) in days to 50% fruit setting and jeju was the late one (76 days). The highest number of green pods per plant (143) was scored from bako local, but statistically at par with Jeju (134) and Marecofana (135) varieties; while Melka awaze scored the least (109) number of green pods per plant. The highest green pod yield per plant was obtained from Marecofana (608.7 g), which is statistically not significant with jeju (576.5 g) and the least yield was observed from Odaharo (357.3 gram). In addition, Marecofana had the largest fruit size (7.3 g) while Melkashote had the least pod weight (4.5 g). The finding is in agreement with the result of Awole et al. (2011) who reported wide range of pod size difference (6.6-17.0 gram) for five hot pepper varieties evaluated in Diredawa, Ethiopia.

The highest marketable green pod yield was recorded from Jeju (19.47 t ha $^{-1}$) which is statistically at par with Marecofana (19.35 t ha $^{-1}$) whereas, the least yield was obtained from Odaharo (13.36 t ha $^{-1}$) (Table 2). Similar result was also reported by Delelegn et al. (2014) who found a wide range of variation on marketable fruit yield (5.11 -19.00 qt ha^{-1}) for nine varieties of hot pepper varieties. Tesfaw et al. (2013) also obtained significant fresh fruit yield difference (6.42 and 10.92 t ha^{-1} for Melkazala and Marecofana varieties) of hot pepper varieties evaluated in Bure, Northwestern Ethiopia. Moreover, Awole et al. (2011) found a wide range of mean marketable yield variation (6.6-20.0 t ha^{-1}) for five hot pepper varieties.

Pearson correlation (r) of marketable green pod yield with other traits revealed that marketable yield (t ha $^{-1}$) had a very highly significant positive correlation with fruit yield per plant (r=0.705), fruit diameter (r=0.675) and single fruit weight (r=0.668). On the contrary, it had highly significant negative correlation with days to 50 % flowering (r=-0.485) (Table 3). This indicated that fruit yield per plant, fruit diameter and single fruit weight the most important yield component traits in the specific agroecology showed that any improvement in these traits increases marketable green pod yield per hectare. This is in agreement with the finding of Yadeta et al. (2011) who report a highly significant positive association of fruit yield ha^{-1} with fruit weight, fruit diameter and fruit length. Similarly, Zhani et al. (2015) obtained a highly significant positive interrelation of single fruit weight with fruit diameter. Association among other characters indicated that days to 50% flowering had highly significant negative correlation with fruit length (r=-0.573), fruit diameter (r=-0.527) and fruit yield per plant (r=-0.716). While single green pod weight had highly significant positive interrelation with fruit diameter (r=0.784) and fruit yield per plant (r=0.576) (Table 3).

Conclusion

Of the six hot pepper varieties evaluated in Humera Jeju scored highest marketable green pod yield (19.47 t ha^{-1}), which was statistically at par with yield of Marecofana (19.35 t ha^{-1}). Traits

Table 2. Combined mean response of hot pepper varieties for growth, marketable green pod yield and green pod characteristics.

Variety	Days to 50% flowering	days to 50% fruiting	No. of green pods per plant	Pod yield per plant (g)	Single pod weight (g)	Pod length (mm)	Pod diameter (mm)	Marketable green pod yield (t ha^{-1})
Jeju	40	76^{a}	133.6^{a}	576.5^{a}	6.2^{b}	74.6^{a}	13.5^{b}	19.47^{a}
Markofana	40	75^{a}	135.4^{a}	608.7^{a}	7.3^{a}	75.4^{a}	15.6^{a}	19.35^{a}
melka awaze	42	75^{a}	109.2^{b}	504.4^{b}	6.1^{b}	77.5^{a}	11.8^{b}	14.66^{b}
Melkashote	42	75^{a}	118.1^{b}	384.6^{cd}	4.5^{d}	78.8^{a}	8.3^{c}	14.44^{b}
Bako local	42	72^{ab}	142.9^{a}	443.5^{bc}	5.0^{cd}	74.4^{a}	9.9^{c}	13.97^{b}
Oda haro	43	67^{b}	116.9^{b}	357.3^{d}	5.5^{bc}	66.3^{b}	12.7^{b}	13.36^{b}
SEM(+)	0.9	2.4	4.2	23.46	0.24	2.47	0.65	0.638
CV (%)	5.2	7.9	8.2	12	10.2	8.1	13.3	9.8
Level of sig.	ns	*	**	**	**	*	*	**

SEM= standard error of the mean, CV= coefficient of variation ns= non-significant, *=significant, **=highly significant, g=gram, mm= millimeter, t ha^{-1}= ton per hectare. Means in the same columns connected by the same letter are not significantly different at p≤0.05.

Table 3. Pearson correlation (r) of marketable green pod yield and yield components of hot pepper varieties evaluated in Humera.

	DFl	DFr	NGPP	SPW	FL	FD	FYPP	YLD
DFl		0.001	-0.064	-0.302	-0.573**	-0.527**	-0.716**	-0.485**
DFr			0.198	-0.221	0.057	-0.217	-0.038	-0.006
NGPP				0.089	-0.058	0.012	0.109	0.311
SPW					0.21	0.784**	0.576**	0.668**
FL						0.231	0.572**	0.399*
FD							0.664**	0.675**
PYPP								0.705**
YLD								

DFl= days to 50% flowering, DFr= days to 50% fruit setting, NPPP= number of green pods per plant, SPW= single green pod weight, FL= fruit length, FD= fruit diameter, PYPP= green pod yield per plant and YLD= marketable green pod yield per hectare.

such as fruit yield per plant, fruit diameter and single fruit weight were among the most important yield components which had highly significant positive association with marketable green pod. Thus, selection based on these traits improves fruit yield per hectare. Generally, two years evaluation indicated that Jeju and Marecofana varieties outsmart the other varieties hence, the two varieties should further be shown to farmers in Western Tigray for them to select the best one.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Awole S, Woldetsadik K and Workneh TS (2011). Yield and storability of green fruits from hot pepper cultivars (*Capsicum* spp.). African Journal of Biotechnology 10(56):

12662-12670.
Brandenberger L, Brian AK, Eric R, John D (2012). Pepper production. Oklahoma Cooperative Extension Service HLA-6030. Available at http://pods.dasnr. okstate.edu/docushare/dsweb/Get/Document-1041/F-6030web.pdf. Accessed on March 2016
Central Statistical Agency (CSA) (2018). Report on area and production of major crops. Agricultural Sample Survey 2017/2018. Addis Ababa, Ethiopia. Statistical Bulletin 586(1):19-20.
Delelegn S, Belew D, Mohammed A, Getachew Y (2014). Evaluation of elite hot pepper varieties (*Capsicum* Sp.) for growth, dry pod yield and quality under Jimma Condition, South West Ethiopia. International Journal of Agricultural Research 9(7):364-374.
Ethiopian Agricultural Research Organization (EARO) (2002). An assessment of the agricultural production base, technological packages and innovation and intervention strategies for commercial farmers in Kafta- Humera woreda of Tigray Regional State, Addis Ababa.
FAOSTAT (2018). Statistical Database of the Food and Agriculture Organization of the United Nations.FAO, Rome, Italy. Available at http://faostat.fao.org.Accessed on August 2018.
Gebremeskel H, Abebe H, Biratu W, Jelato K (2015). Performance evaluation of hot pepper (*Capsicum annum* L.) varieties for productivity under irrigation at Raya Valley, Northern, Ethiopia. Basic Research Journal of Agricultural Science and Review 4(7):211-216.
Girma T, Lidet S, Damtrew M, Daniel B (2001) Berebere production in Ethiopia. Ethiopian Agricultural Research Organization. Extension Material 5:03-04.
Keneth VAR (2017). Evaluation of three pepper varieties (*Capsicum annuum* L.) varieties. Gladstone Road Agricultural Center. Crop Research Report No. 26. Available athttps://www.bahamas.gov.bs/wps/wcm/connect/b91f0ffd-efd9-4591-b0f2-a1ae 25f18 e9a/ Evaluation+of+Three+Pepper+Varieties+2016 +%2826%29.pdf?MOD= AJPERES. Accessed on December 2019
Kirk S, Gu S (2011). Preliminary evaluation of 48 chili pepper cultivars in central Missouri. Midwest Vegetable Trial Report. Lincoln University Cooperative Extension.
Mangoel W, Ndam ON, Badi SH, Daar JW, Mamzing D (2012). Evaluation of hot pepper (*Capsicum* Spp) varieties for some growth and yield parameters at Garkawa, Northern Guinea Savanna Zone of Nigeria. Crop Science Society of Nigeria: Second National Annual Conference Proceedings. Available at https://africaneditors. org/journal /NJCS/ abstract/D637016121. Accessed on December 2019.
Melaku FT, Alemayehu TG, Lidet BT (2015). Adaptation trail of different improved hot pepper (*Capsicum* species) varieties under Gedeo Zone, Dilla, Ethiopia. International Journal of Life Sciences 4(4):216-220.
Sameer HMD, Laximinarayan H, Sharatababu AG, Hadge NK, Shantapa T, Gura murthy SB, Manju MI, Shirakumar KM (2017). Evaluation of local black pepper (*Piper Nigrum* L.) geneotypes for yield and quality under arecanut based cropping system. International Journal of Pure Applied Bioscience 5(5):1396-1400.
SAS Institute Inc. (2008). Statistical analysis Software version 9.2, Cary, NC: SAS Institute Inc. USA.
Sibhatu B, Tekle G, Harife M (2016). Hot pepper varietal evaluation for growth, dry pod yield and yield related parameters at Kola Tembien District, Northeren Ethiopia. Research Journal of Agriculture and Environmental Management 5(4):126-131.
Tegene G (2009). Pepper Production and management. Melkassa Agricultural Research Center, Ethiopia. FRG Extension Material Series No.14
Tesfaw A, Dechassa N, W/TSadik K (2013). Performance of hot pepper (*Cupsicum annuum*) varieties as influenced by nitrogen and phosphorus fertilizers at Bure, Upper Watershed of the Blue Nile in Northwestern Ethiopia. International Journal of Agricultural Sciences 3(8):599-608.
Tibebu S, Bizuayehu T (2014). Growth and productivity of hot pepper (*Capsicum annuum* l.) as affected by variety, nitrogen and phosphorous at Jinka, Southern Ethiopia. Research Journal of Agriculture and Environmental Management 3(9):427-433.
Yadeta B, Belew D, Gebreselassie W, Marame F (2011). Genetic association among some attributes of hot pepper (*Capsicum annum* L.) genotypes in West Shoa, Ethiopia. Middle-East Journal of Scientific Research 7(4):567-573.
Zhani K, Hamdi W, Sedraoui S, Fendri R, Lajimi O, Hannachi C (2015). Agronomic Evaluation of Tunisian Accessions of Chili Pepper (*Capsicum Frutescens* L.). International Research Journal of Engineering and Technology 2(4):29-30.

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Evidences that polyploidization and hybridization affected resveratrol content in *Arachis* interspecific hybrids

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To broaden the genetic base of the allotetraploid peanut (*Arachis hypogaea* L.), pre-breeding programs have produced interspecific synthetic allotetraploids resulting from the chromosome duplication of hybrids between peanut related diploid species. These allotetraploids were highly cross-fertile with peanut making it possible to access the extensive genetic variability harbored by the wild species. This study aims to evaluate the impact of polyploidization and hybridization in resveratrol content in *Arachis* hybrids. Resveratrol is a potent antioxidant that has been shown to be useful in the treatment of many human diseases. For that, resveratrol was characterized in five synthetic allotetraploids of wild *Arachis*, six diploid wild species, three cultivars of *A. hypogaea* and three backcross (BC) hybrids between synthetic allotetraploids and *A. hypogaea.* Leaves from these genotypes were ultraviolet (UV) light irradiated for 2 h 30 min and their resveratrol contents were determined by high performance liquid chromatograph (HPLC). Resveratrol was found in all genotypes, but at variable concentrations. Synthetic allotetraploids and peanut did not differ and diploid species had the lowest resveratrol content. The highest concentrations were observed in hybrids between allotetraploids and cultivars of *A. hypogaea* that were probably the most heterozygous among the genotypes analyzed since their chromosome sets came from different species. This study data suggest a positive effect of polyploidy and hybridization in resveratrol content.

Key words: Peanut, wild relatives, polyploidy, pre-breeding.

INTRODUCTION

The cultivated peanut *Arachis hypogaea* L. is an allotetraploid (AABB) that originated from a single crossing event between the diploid wild species *Arachis duranensis* and *Arachis ipaënsis* (Kochert et al., 1996;

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Bertioli et al., 2016). These two species belong to section *Arachis*, which also comprises the cultivated and its most close relatives (Krapovickas and Gregory, 1994). All the other species of section *Arachis* are diploids with the single exception of *Arachis monticola*, which is also a tetraploid (Smartt et al*.,* 1978; Fernández and Krapovickas, 1994; Peñaloza and Valls, 2005; Stalker, 2017).

Section *Arachis* species constitute the secondary gene pool of the cultivated peanut (Stalker and Moss, 1987) and because of that, many accessions of those species have been characterized and evaluated for several agronomic traits, including resistance to biotic (Stalker, 1984; Pande and Rao, 2001; Michelotto et al., 2015) and abiotic stresses (Nautiyal et al., 2008; Leal-Bertioli et al., 2012).

The reproductive barrier between cultivated and wild *Arachis* due to ploidy level difference has been overcomed using interspecific synthetic allotetraploids. Sterile diploid hybrids obtained by crossing A and B genome *Arachis* species have been turned fertile after their tetraplodization with colchicine, which allowed their crossing with peanut and the introgression of alleles from the wild species into the cultivated (Simpson , 1991).

Peanuts are among the few plant species that produce resveratrol (Lanz et al., 1990; Sobolev and Cole, 1999; Arora and Japlan, 2018). This phenolic compound is a potent antioxidant (Frankel et al., 1993) whose healing and preventive potential for many human diseases were described in some recent reviews (Colica et al*.,* 2018; Galiniak et al*.,* 2019). Resveratrol is also a phytoalexin that has been associated with resistance to major peanut diseases (Sobolev et al*.,* 2007). Moreover, ten species of section *Arachis* also synthesizes resveratrol and three of them had levels higher than those found in cultivar Caiapó of *A. hypogaea* (Lopes et al*.,* 2013).

The effect of polyploidization and hybrization on different traits in *Arachis* interspecific synthetic allotetraploids have been studied (Burow et al., 2001; Fávero et al., 2009, 2015; Leal-Bertioli et al*.,* 2012, 2017; Michelotto et al*.,* 2015, 2016, 2017). The characterization of resveratrol content in *Arachis* allotetraploids could add new value to these genotypes, which have been developed to be used in peanut pre-breeding programs (Bertioli et al*,* 2011). In this context, the objective of the present study was to evaluate the impact of polyploidization and hybridization in the resveratrol content analyzing synthetic allotetraploids, their respective diploid wild parentals and hybrids between two synthetic allotetraploids and three *A. hypogaea* cultivars.

METHODOLOGY

Plant material

Seventeen *Arachis* genotypes were analyzed for resveratrol content being six wild diploid species, three tetraploid peanut (*A. hypogaea*) cultivars and eight tetraploid hybrids that comprised five interspecific synthetic allotetraploids, and three hybrids resulting from crosses between peanut and synthetic allotetraploids (Table 1). The *Arachis* wild species analyzed harbor different types of genome: A (*villosa, stenosperma, A. duranensis*), B (*A. ipaënsis and Arachis gregoryi*) and K (*batizocoi*). The cultivars of peanut analyzed were 'IAC Caiapó', 'Runner IAC 886' and 'IAC 505'. All synthetic allotetraploids and hybrids analyzed were developed by Santos (2013). The plants were grown in greenhouses at Embrapa Genetic Resources and Biotechnology, Brasília, Brazil.

Induction of resveratrol synthesis using UV

The experiments were performed using detached leaves collected from six-month-old *Arachis* plants in greenhouse conditions, as previously described (Lopes et al., 2013). In short, detached leaves were exposed to an ultraviolet light for 2 h 30 min and maintained in the dark for additional 15 h at room temperature. UV-treated and non-treated control leaves of each genotype were divided into three aliquots of 1 g and stored at -80°C.

Resveratrol extraction and sample preparation

The resveratrol extraction protocol was based on Potrebko and Resurreccion (2009). Prior to high performance liquid chromatograph (HPLC) injection, the dried residue was reconstituted in 6.8 ml of 15% (v/v) ethanol. The samples were vortexed for 1 min and left in ultrasonic bath for four minutes. The procedure was repeated twice to ensure the complete recovery of the extract. The samples were then transferred to 2.0 ml tubes and centrifuged for 15 min at 25°C at 13,400 rpm. The supernatant of the centrifuged material was conditioned in a 2-ml tube and then used for injection in a HPLC (CLAE, Varian®) with ternary pump, automatic dial and coupled photodiode array detector (PDA Varian® PS- 240 / PS-410 / PS-335 / Galaxie Software 1.9).

HPLC analysis

The column used in HPLC was Zorbax XDB Agilent (250 x 4.6 mm, 5 µm), without guard column. A gradient of acetonitrile and a 0.02% aqueous phosphoric acid (J. T. Baker) were used as mobile phase. The conditions were: acetonitrile for 0 min at 13%; 6 to 9 min at 15%; 17 min at 17%; 28 to 33 min at 28%; 40 min at 50%; 45 min at 60%; 46 to 48 min at 80%; 49 to 54 min at 13%; flow rate of 1.0 ml/min. The UV absorption was monitored at 308 nm, 280 nm and also at the maximum absorption wavelength of each eluent (PDA). The injection volume of each sample was 10 µl.

The peak of resveratrol was identified by comparison with the retention time of the commercial standard solutions of resveratrol (> 99%, 230-240 µg/ml, Sigma-Aldrich) and phenolphthalein (> 98%, 2927-2835 µg/ml, Sigma-Aldrich) that were injected daily for area verification. Additional procedures for resveratrol identification were analysis of the spectrum provided by the diode array detector, and the quantification by co-elution with the resveratrol pattern and further comparison of the chromatograms of the induced and control samples. The final concentration of resveratrol per gram of leaf was calculated according to Potrebko and Resurreccion (2009).

Data analysis

The means of the resveratrol production were compared using the Scott and Knott test at 5% probability, considering the groups of plants over time (3 blocks) as covariate, aiming at filtering the variability observed due to these repetitions. The analysis was

Table 1. *Arachis* species and hybrids analyzed, their accession number, genome type, and concentration of resveratrol estimated after UV exposure.

Species / Hybrid	Accession*	Genome	Resveratrol content ± SD* (µg/g)
A. batizocoi	K9484	KK	162.1 ± 147.0^{f}
A. duranensis	V14167	AA	415.8 ± 118.0^{d}
A. ipaënsis	K30076	BB	84.5 ± 46.4^{f}
A. gregoryi	V6389	BB	390.3 ± 30.9^{d}
A. hypogaea	cv. IAC 505	AABB	212.7 ± 88.0^{e}
A. hypogaea	cv. Runner IAC 886	AABB	275.2 ± 88.0^{e}
A. hypogaea	cv. IAC Caiapó	AABB	579.5 ± 85.8^{b}
A. stenosperma	V10309	AA	42.5 ± 12.5^{f}
A. villosa	V12812	AA	61.7 ± 26.8^{f}
A. batizocoi x *A. stenosperma*	K9484 x V10309	AAKK	290.0 ± 85.4^{e}
A. batizocoi x *A. duranensis*	K9484 x Se2848	AAKK	513.3 ± 145.0^{c}
A. batizocoi x *A. duranensis*	K9484 x V14167	AAKK	627.0 ± 213^{b}
A. ipaënsis x *A. villosa*	K30076 x V12812	AABB	88.9 ± 44.9^{f}
A. gregoryi x *A. stenosperma*	V6389 x V10309	AABB	261.6 ± 70.0^{e}
cv.886x[cv 886 x (*A. batizocoi* x *A. stenosperma*)]**		AABK	351.7 ± 151.6^{e}
cv. 505 x [(*A. gregoryi* x *A. stenosperma*)]		AABB	526.8 ± 91.5^{c}
Caiapóx[Caiapóx (*A. batizocoi* x *A. stenosperma*)]**		AABK	743.0 ± 103.9^{a}

* Collectors: K=A. Krapovickas; Se=G.J. Seijo; V=J.F.M. Valls. ** Backcrossings (BC_1). Means followed by the same letter do not differ (α<0.05) according to Scott-Knott test.

developed in the statistical language program R, free for download at the site http://www.r-project.org/.

RESULTS AND DISCUSSION

All genotypes analyzed were able to produce resveratrol in response to UV induction (Table 1). Traces of resveratrol (below 0.1 µg) were detected in the samples not exposed to UV (data not shown).

The resveratrol content varied greatly among the six wild diploid species analyzed going from 42.53±12.5 µg/g in *A. stenosperma* to 415.8±118.0 µg/g in *A. duranensis* (Table 1). Lopes et al. (2013) detected 370.0 µg/g of resveratrol in UV-treated plants of *A. gregoryi* (accession V6389) which was very similar to the value found in the present study (390.3±30.9 µg/g). Conversely, for *A. batizocoi* (accession K9484) and *A. ipaënsis* (accession K30076), Lopes et al. (2013) detected higher contents (524.5 and 314.0 µg/g, respectively) than those found here (162.1±147.0 µg/g and 84.5±46.4 µg/g, respectively). Also, Carvalho et al. (2017) detected resveratrol in UV-treated leaves of *A. duranensis* (accession V14167) in concentration (371.97 µg/g) similar to that observed here (415.8±118.0 µg/g), whereas in *A. stenosperma,* (accession V10309) resveratrol concentration was at least 13-times higher (512.6 µg/g) than in our study (42.49±12.5 µg/g). The differences in the resveratrol content of a same accession observed in these studies may be due to different factors, such as the intrinsic nature of resveratrol as a secondary metabolite, whose production is prone to changes according to the environment temperature (Wang and Zheng, 2001), plant age (Chung et al., 2001), water availability in the soil (Esteban et al., 2001), and cultivation season (Chen et al., 2002). Genetic, ontogenic, morphogenetic, and environmental factors that could cause variation on plant secondary metabolite content in different species were reviewed by Yang et al. (2018).

Concerning the three peanut cultivars evaluated, 'IAC Caiapó' presented the highest resveratrol concentration (579.5 ± 85.8^{b} µg/g), followed by 'Runner IAC 886' (275.2 ± 88.0^{e} µg/g) and 'IAC 505' (212.7 ± 88.0^{e} µg/g) that showed similar concentrations to each other. Over the years, many studies have shown a variable resveratrol content among *A. hypogaea* varieties/cultivars, with differences due to the plant organ studied, crop location, annual season and pathogens infestation levels. Sanders et al. (2000) found differences among resveratrol content (from 0.03 to 0.147 µg/g) in seeds without coat of three peanut market types (Virginia, Runner, and Spanish) produced in different areas and without any specific induction of resveratrol, as UV used in this study. Significant variations in resveratrol content (from 0.125 to 1.626 µg/g) was also found when seeds of 20 germplasm accessions of *A. hypogaea* harvested from the same field were analyzed using HPLC (Wang and Pittman, 2009). Variation on resveratrol content was also found in roots of three peanut cultivars grown in 2000 fall and 2001 spring

being the content of fall crops much higher than those of spring (Chen et al., 2002). Peanut cultivar 'IAC Caiapó' higher resveratrol content (ranging from 300 to 600 µg/kg) when compared to the cultivar 'IAC 886' (Zorzete et al., 2011). This last cultivar is less susceptible to thrips *Enneothrips flavens* infection (Moraes et al., 2005). Considering that resveratrol is a phytoalexin, peanut cultivars with higher concentrations of this metabolite are likely more resistant against pathogen attack. Resveratrol content in peanut seeds was negatively correlated with aflatoxin production and *in vitro* trials demonstrated that resveratrol could inhibit aflatoxin production (HouMiao et al., 2012). An association between total phytoalexin production and genotype resistance to major peanut diseases was observed being trans-resveratrol was one of the main compounds found in stress-resistant genotypes (Sobolev et al*.,* 2007).

The grouping of the genotypes according to their ploidy level (Table 2), helped to observe that the tetraploids genotypes (peanut cultivars and hybrids) showed significantly higher resveratrol contents than the wild diploid species. The effect of polyploidization has been studied in *Arachis* comparing synthetic allotetraploids, their corresponding diploid parental species and peanut cultivars. The comparison among *A. duranensis* (V14167), *A. ipaënsis* (KG30076), a synthetic allotetraploid (*A. duranensis* V14167 × *A. ipaënsis* K 30076)^{4x} and *A. hypogaea* subsp. *hypogaea* var. *hypogaea* 'Runner IAC 886' showed some diploid traits such as chlorophyll meter readings are maintained through hybridization and polyploidization and most characters are substantially modified (Leal-Bertioli et al., 2012). An increase in resistance to the foliar diseases rust (*Puccinia arachidis*) and late leaf spot (*Cercosporidium personatum*) was observed in the synthetic allotetraploids compared to their diploid parental species (Kumari et al., 2014). More recently, it was also demonstrated that *Arachis* allotetraploids have some general phenotypic trends that are common, regardless of the combination of their wild parental diploid suggesting that nucleotypic effect is more important than new allelic combination (Leal-Bertioli et al., 2017). The effect of polyploidization on the increase of bioactive compounds has also been studied in some other species. The concentrations of some phytoconstituents, such as emodin, physcion, piceatannol, resveratrol and rutin were determined by LC–MS in three species of *Rumex* and a positive correlation could be detected with the increasing ploidy status in different chromosomal races (Jeelania et al., 2017).

Our data suggested that the polyploidization could be one of the causes of the increase in the resveratrol content observed in the polyploidy compared to diploid samples analyzed.

The resveratrol content among the synthetic allotetraploids (Table 1) ranged from 88.9 ± 44.9^{f} µg/g for *A. ipaënsis* x *A. villosa* to 627.0 ± 213.2^{b} µg/g for *A. batizocoi* x *A. duranensis* V14167. Interestingly, the parental diploids of *A. ipaënsis* X *A. villosa* that had the lowest resveratrol content (88.9 ± 44.9^{f} µg/g for) among the synthetic allotetraploids displayed the second and third lowest concentrations of resveratrol among the wild diploids (61.7 ± 26.8^{f} and 84.5 ± 46.4^{f} µg/g for *A. villosa* and *A. ipaënsis*, respectively). Likewise, the synthetic allotetraploid with the highest resveratrol content (513.3 ± 145.0^{c} µg/g for *A. batizocoi* X *A. duranensis* V14167) had at least one parental diploids with high content (162.1 ± 147.0^{f} and 415.8 ± 118.0^{d} µg/g for *A. batizocoi* and *A. duranensis,* respectively). Overall, we observed that the hybrids that produced high quantities of resveratrol resulted from crosses between parents with the highest levels of resveratrol. Increase on ginsenoside content was also obtained using a interspecific *Panax* F_1 hybrids (Kim et al., 2016). The use of hybridization to increase flavonoids using wild relatives in many cultivated species was recently reviewed (D'Amelia et al., 2018). Thus, our results suggest that, besides the polyploidization, the allelic composition of the allotetraploids might also be positively related to the production of resveratrol in *Arachis.*

The three hybrids resulting from the crosses between peanut cultivars and synthetic allotetraploids presented significant differences compared to the other genotypes analyzed, showing the highest resveratrol content averages (Table 2). Those hybrids were the most heterozygous among genotypes analyzed in this study since each of their four chromosomes sets came from peanut and two of the wild species used in the synthetic allotetraploids synthesis. This suggested that increase in heterozygosity might also have contributed to increase of resveratrol content. Besides, those hybrid chromosomes were the only ones among the material evaluated that had their chromosomes resultin from the recombination between A and B genomes from the cultivated with A and B or K genomes from the wild species. The other genotypes (diploid and synthetic polyploidy) were most probably homozygous since wild species are most autogamous and because of that recombination would not result in any new allelic combination as it happened in BC1 hybrids.

On average, the three hybrids between synthetic allotetraploids and peanut cultivars had the highest resveratrol content. Previous study showed that hybrids of peanut with interspecific synthetic allotetraploids showed an increased concentration of flavonoids than their parental that resulted in an increased larval mortality of *Spodoptera litura* (Mallikarjuna et al., 2004).

Variation on resveratrol content was found among the three BCs hybrids analyzed (Table 1). The hybrid ['cv 886' X ['cv 886' X (*A. batizocoi* X *A. stenosperma*)] that had 'cv 886' (275.2 ± 88.0^{e} µg/g) as a parental showed lower resveratrol content concentration (351.7 ± 151.6^{e} µg/g) than the one that had Caiapó' (579.5 ± 85.8^{b} µg/g) as parental ['Caiapó' X ['Caiapó' X (*A. batizocoi* X *A stenosperma*)] that had 743.0 ± 103.9^{a} µg/g). This result

Table 2. Average of resveratrol content in the different genotypes evaluated by level of significance between groups.

Genotype	Average resveratrol content (µg/g)	SK (%)
Synthetic allotetraploids x peanut cv	540.5±199.0	a
Synthetic allotetraploids	356.2±225.6	b
Peanut cultivars	355.82±183.5	b
Wild diploid species	192.80±173.5	c

The analysis was done with Scott-Knott's (SK) 5% Test.

suggested that peanut cultivar used as the parental in these crosses highly influences the resveratrol content in the resulting hybrids.

Conclusion

This study data suggest a positive effect of polyploidy and hybridization in resveratrol content in *Arachis* hybrids. Resveratrol can be synthesized by a few species and the major dietary natural sources include grapes, wine, peanuts, and soybeans (Burns et al., 2002). Our data opens the possibility to create and provide new sources of natural resveratrol by the used of interspecific synthetic *Arachis* hybrids analyzed, mainly the BCs genotypes, which displayed higher resveratrol contents than wild and the cultivated species.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Arora D, Jaglan S (2018). Therapeutic applications of resveratrol nanoformulations. Environmental Chemistry Letters 16:35-41.

Bertioli D, Seijo G, Freitas F, Valls J, Leal-Bertioli S, Moretzsohn M (2011). An overview of peanut and its wild relatives. Plant Genetic Resources 9:134-149.

Bertioli DJ, Cannon SB, Froenicke L, Huang G, Farmer AD, Cannon EKS, Liu X, Gao D, Clevenger J, Dash S, Ren L, Moretzsohn MC, Shirasawa K, Huang W, Vidigal B, Abernathy B, Chu Y, Niederhuth CE, Umale P, Araújo ACG, Kozik A, Kim KD, Burow MD, Varshney RK, Wang X, Zhang X, Barkley N, Guimarães PM, Isobe S, Guo B, Liao B, Stalker HT, Schmitz RJ, Scheffler BE, Leal-Bertioli SCM, Xun X, Jackson SA, Michelmore R, Ozias-Akins P (2016). The genome sequences of *Arachis duranensis* and *Arachis ipaënsis*, the diploid ancestors of cultivated peanut. Nature Genetics 48:438-446.

Burns J, Yokota T, Ashihara H, Lean ME, Crozier A (2002). Plant foods and herbal sources of resveratrol. Journal of Agricultural and Food Chemistry 50:3337-3340.

Burow MD, Simpson CE, Starr JL, Paterson AH (2001). Transmission genetics of chromatin from a synthetic amphidiploid to cultivated peanut (*Arachis hypogaea* L.): Broadening the gene pool of a monophyletic polyploid species. Genetics 159: 823-837.

Carvalho PASV, Brasileiro AC, Leal-Bertioli S, Bertioli DJ, Silva JP, Agostini-Costa TS, Gimenes MA (2017). Coupled transcript and metabolite identification: insights on induction and synthesis of resveratrol in peanut, wild relatives and synthetic allotetraploid. Genetics and Molecular Research 16:3.

Chen RS, Wu PL, Chiou RYY (2002). Peanut roots as a source of resveratrol. Journal of Agricultural and Food Chemistry 50:1665-1667.

Chung I-M, Park MR, Rehman S, Yun SJ (2001). Tissue specific and inducible expression of resveratrol synthase gene in peanut plants. Molecules and Cells 12:353-359.

Colica C, Milanovićb M, Milićb N, Aielloc V, De Lorenzo A, Abenavolie AA (2018). Systematic review on natural antioxidant properties of resveratrol. Natural Product Communications 13:1195-1203.

D'Amelia V, Aversano R, Chiaiese P, Carputo D (2018). The antioxidant properties of plant flavonoids: their exploitation by molecular plant breeding. Phytochemistry Reviews 17: 611.

Esteban MA, Villanueva MJ, Lissarrague JR (2001). Effect of irrigation on changes in the anthocyanin composition of the skin of cv Tempranillo (*Vitis vinifera* L) grape berries during ripening. Journal of the Science of Food and Agriculture 81:409-420.

Fávero AP, Moraes SAD, Garcia AAF, Valls JFM, Vello NA (2009). Characterization of rust, early and late leaf spot resistance in wild and cultivated peanut germplasm. Scientia Agricola 66: 110-117.

Fávero AP, Pádua JG, Costa TS, Gimenes MA, Godoy IJ, Moretzsohn MC, Michelotto MD (2015). New hybrids from peanut (*Arachis hypogaea* L.) and synthetic amphidiploid crosses show promise in increasing pest and disease tolerance. Genetics and Molecular Research 14:16694-16703.

Fernández A, Krapovickas A (1994). Cromosomas y evolucion en *Arachis* (*Leguminosae*). Bonplandia 8:187-220.

Frankel EN, German JB, Kinsella JE, Parks E, Kanner J (1993). Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. The Lancet 341:454-457.

Galiniak S, Aebisher D, Bartusik-Aebishe D (2019). Health benefits of resveratrol administration. Acta Biochimica Polonica 66:13-21.

HouMiao W, Huang J, Lei Y, Yan L, Wang S, Jiang H, Ren X, Lou Q, Liao B (2012). Relationship of resveratrol content and resistance to aflatoxin accumulation caused by *Aspergillus flavus* in peanut seeds. Acta Agronomica Sinica 38:1875-1883.

Jeelania SM, Farooqb U, Guptac AP, Lattooa SK (2017). Phytochemical evaluation of major bioactive compounds in different cytotypes of five species of *Rumex L*. Industrial Crops and Products 109: 897-904.

Kochert G, Stalker H, Gimenes M, Galgaro L, Lopes C, Moore K (1996). RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut *Arachis hypogaea* (*Leguminosae*). American Journal of Botany 83:1282-1291.

Krapovickas A, Gregory WC (1994). Taxonomia del genero *Arachis*

(*Leguminosae*). Bonplandia 8:1-186.
Kim YJ, Silva J, Zhang D, Shi J, Joo SC, Jang M-G , Kwon W-S, Yan D-C (2016). Development of interspecies hybrids to increase ginseng biomass and ginsenoside yield. Plant Cell Report 35:779-790.
Kumari V, Gowda MVC, Tasiwal V, Pandey MK, Bhat RS, Mallikarjuna N, Upadhyaya HD, Varshney RK (2014). Diversification of primary gene pool through introgression of resistance to foliar diseases from synthetic amphidiploids to cultivated groundnut (*Arachis hypogaea* L.). The Crop Journal 2:110-119.
Lanz T, Schröder G, Schröder J (1990). Differential regulation of genes for resveratrol synthase in cell cultures of *Arachis hypogaea* L. Planta 181:169-175.
Leal-Bertioli SC, Bertioli DJ, Guimarães PM, Pereira TD, Galhardo I, Silva JP, Brasileiro ACM, Oliveira RS, Silva PIT, Vadez V, Araujo ACG (2012). The effect of tetraploidization of wild *Arachis* on leaf morphology and other drought-related traits. Environmental and Experimental Botany 84:17-24.
Leal-Bertioli SC, Moretzsohn MC, Santos SP, Brasileiro AC, Guimarães PM, Bertioli DJ, Araujo ACG (2017). Phenotypic effects of allotetraploidization of wild *Arachis* and their implications for peanut domestication. American Journal of Botany 104:379-388.
Lopes RM, Silveira D, Gimenes MA, Vasconcelos PAS, Rosa de Belem NA, Silva JP, da Silveira Agostini-Costa T (2013). Characterization of resveratrol content in ten wild species of section *Arachis*, genus *Arachis*. Genetic Resources and Crop Evolution 60:2219-2226.
Mallikarjuna N, Kranthi KR, Jadhav DR, Kranthi S, Chandra S (2004). Influence of foliar chemical compounds on the development of *Spodoptera litura* (Fab.) in interspecific derivatives of groundnut. Journal of Applied Entomology 128: 321-328.
Michelotto MD, Barioni Jr W, de Resende MDV, de Godoy IJ, Leonardecz E, Fávero AP (2015). Identification of fungus resistant wild accessions and interspecific hybrids of the genus *Arachis*. PloS One 10:0128811.
Michelotto MD, de Godoy IJ, dos Santos JF, Martins ALM, Leonardecz E, Fávero AP (2016). Identifying amphidiploids resistant to foliar fungal diseases. Crop Science 56:1792-1798.
Michelotto MD, de Godoy IJ, Pirotta MZ, dos Santos JF, Finoto EL, Fávero AP (2017). Resistance to thrips (*Enneothrips flavens*) in wild and amphidiploid *Arachis* species. PloS One 12: 0176811.
Moraes ARAD, Lourenção AL, Godoy IJD, Teixeira GDC (2005). Infestation by *Enneothrips flavens* Moulton and yield of peanut cultivars. Scientia Agricola 62:69-472.
Nautiyal PC, Rajgopal K, Zala PV, Pujari DS, Basu M, Dhadhal BA, Nandre BM (2008). Evaluation of wild *Arachis* species for abiotic stress tolerance: I. Thermal stress and leaf water relations. Euphytica 159:43-57.
Pande S, Rao JN (2001). Resistance of wild *Arachis* species to late leaf spot and rust in greenhouse trials. Plant Disease 85:851-855.
Peñaloza APS, Valls JFM (2005). Chromosome number and satellite chromosome morphology of eleven species of *Arachis* (Leguminosae). Bonplandia 14:65-72.
Potrebko I, Resurreccion AVA (2009). Effect of ultraviolet doses in combined ultraviolet−ultrasound treatments on trans-resveratrol and trans-piceid contents in sliced peanut kernels. Journal of Agricultural and Food Chemistry 57:7750-7756.
Sanders TH, McMichael RW, Hendrix KW (2000). Occurrence of resveratrol in edible peanuts. Journal of Agricultural and Food Chemistry 48:1243-1246.
Santos SPD (2013). Produção e caracterização de alotetraploides sintéticos entre espécies silvestres do gênero *Arachis*. Dissertação de mestrado Universidade de Brasília 89. http://repositorio.unb.br/handle/10482/12978
Simpson CE (1991). Pathways for introgression of pest resistance into *Arachis hypogaea* L. Peanut Science 18:22-26.
Smartt J, Gregory WC, Gregory MP (1978). The genomes of *Arachis hypogaea*. Cytogenetic studies of putative genome donors. Euphytica 27:665-675.
Sobolev VS, Cole RJ (1999). Trans-Resveratrol content in commercial peanuts and peanut products. Journal of Agricultural and Food Chemistry 47:1435-1439.
Sobolev VS, Guo BZ, Holbrook CC, Lynch RE (2007). Interrelationship of phytoalexin production and disease resistance in selected peanut genotypes. Journal of Agricultural and Food Chemistry 55:2195-2200.
Stalker HT, Moss JP (1987). Speciation, citogenetics and utilization of *Arachis* species. Advances in Agronomy 41:1-40.
Stalker HT (1984). Utilizing *Arachis cardenasii* as a source of *Cercospora* leafspot resistance for peanut improvement. Euphytica 33:529-538.
Stalker HT (2017). Utilizing wild species for peanut improvement. Crop Science 57:1102-1120.
Wang SY, Zheng W (2001). Effect of plant growth temperature on antioxidant capacity in strawberry. Journal of Agricultural and Food Chemistry 49:4977-4982.
Wang ML, Pittman RN (2009). Resveratrol content in seeds of peanut germplasm quantified by HPLC. Plant Genetic Resources 7:80-83.
Zorzete P, Reis TA, Felicio JD, Baquião AC, Makimoto P, Corrêa B (2011). Fungi, mycotoxins and phytoalexin in peanut varieties, during plant growth in the field. Food Chemistry 129:957-964.
Yang L, Wen K-S, Ruan X, Zhao Y-X, Wei F, Wang Q (2018). Response of Plant Secondary Metabolites to Environmental Factors. Molecules 23:762.

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