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Original Research Article

Agromorphological and Biochemical Markers' Selection in Groundnut (*Arachis hypogaea* L.) Cultivars

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Abstract

The existence of genetic diversity should have to be supplemented with the associated traits as efficient modern breeding method. The crop was sown during 2015 wet season across four locations in Ethiopia. The Euclidean distances matrix (D) was worked out for 16 groundnut genotypes evaluated for 16 agromorphological and oil traits to study the association of traits with respective genotypes based on principal component analysis and clustering methods. The Eigen vectors based on euclidean distance matrix (D) worked out for agromorphological and oil traits has shown that the first principal component had high positive component loading from both agromorphological and high oil quality traits/parameters including number of branches per plant, above ground biomass per plant, pod weight per plant, 100seed weight, grain yield, oil yield, oleic acid, O/L ratio, and oil content. These traits found to associate with Behajidu, Bulki, Lote, Manipeter, NC-343, Oldhale, Roba, Shulamith, Tole-1 and Werer-962 genotypes with high positive PCA1 scores indicating that breeding for high oil quality traits can be conducted via improvement of grain yield, oil yield, 100 seed weight, seed weight per plant and/or pod weight per plant. On the other hand, the first component has got high negative load from agromorphological traits like number of seeds per pod and number of seed per plant, and low oil quality traits/parameters including linoleic acid, TPUS and TPUS/TS. The corresponding genotype loads were Fetene, Sedi, Werer-961 and Werer-963 indicating that genotypes with low oil quality can be identified through selection for number of seeds per pod and number of seeds per plant. The PCA and cluster analysis has shown that breeding for oil content, oil yield, grain yield and oil quality traits can be conducted through selection for seed weight per plant, pod weight per plant, number of branches per plant, above ground biomass per plant and number of mature pods per plant. Thus, this study has substantially shown that breeding for oil traits can be conducted through selection for agromorphological traits.

Keywords: Oil content, Oil yield, Oil quality, PCA, O/L ratio, grain yield, Oil yield, Genotypes.

INTRODUCTION

Groundnut is used as oilseed, food and animal feed, as a legume it improves soil health through nitrogen fixation as well as a source of fuel for rural population (Cummins and Jackson, 1982). Thus, groundnut cultivation contributes to the sustainability to mixed crop-livestock production systems, the most predominant system of the semi-arid areas (Upadhyaya *et al*., 2006). Information on the nature and degree of genetic diversity helps plant breeders in choosing the diverse parents for hybridization (Singh, 2015). Selection of genetically diverse parents in any breeding program is of immense importance for successful recombination breeding (Arunachalam, 1981). Within the available methods for genetic diversity studies, the average Euclidean distance is used as a measure of dissimilarity, which underlies multivariate analytical methods like hierarchical clustering methods such as UPGMA (unweighted pair group method with arithmetic mean), nearest neighbor, and the Tocher optimization method (Silva *et al*., 2011; Azevedo *et al.,* 2013).

The principal component analysis (PCA), one of multivariate analysis methods, shows which of the traits were decisive in genotype differentiation (Kovacic, 1994). PCA enables easier understanding of impacts and connections among different traits by finding and explaining them. Ferreira Júnior *et al.* (2015) pointed out that the reference information regarding genetic divergence is typically not sufficient for selecting parents for hybridization. Instead, the reference information should be accompanied by information about the genotype performance in relation to the desirable traits. The loading of both genotypes and the associated traits into PCA helps to associate and select genotypes with their corresponding traits (Singh *et al*., 2013; Cantelli *et al.,* 2016). If meticulous efforts have undertaken multivariate methods can be used as an alternative to modern molecular markers since the cost for molecular laboratory analysis is ever increasing and difficult to handle with local funds especially in developing countries.

In Ethiopia the area under groundnut cultivation was estimated about 79,947.03 ha and annual production of 112,088.724 tons with a productivity of 1.402 tons per ha (CSA, 2014). There is significant yield gap between the farmers' fields and the research centers, which are due to either the improved groundnut varieties are not reaching the farmers or lack of knowledge of agronomic management practices and because of various biotic and abiotic stresses like drought, insect pests, diseases etc (Chala *et al*., 2012). Moreover, groundnut production is far lower than production of other countries though there are huge potential in Ethiopia. Thus, further breeding effort is required to improve groundnut yield in Ethiopia so as to satisfy the growing demand of groundnut for domestic consumption, oil and confectionary industries. In light of such justifications the present study was aimed to study association of traits with respective genotypes and identifying those genotypes that can be used for the improvement of important traits in oil crops including grain yield, oil yield and quality trait.

MATERIALS AND METHODS

The experiment was carried out across four locations viz Fedis, Mechara (Eastern Ethiopia), Pawe and Guba (locations in Western Ethiopia) in 2015 growing season under rain fed condition. The experimental materials consisted of sixteen groundnut genotypes including local variety and varieties which were released by Ethiopian Institute of Agricultural Research (EIAR) from 1976 to 2012. Before starting laboratory experiment moisture content of seeds was reduced to 5%. Oil content and fatty acid profile determination was carried out based on the following techniques:

Total lipid from the seed sample was quantitatively extracted, according to the method of Folch *et al*. (1957). Iodine value was determined with the Hanus method (AOAC nr. 920.158, 1990). Fatty acids were transesterified to form methyl esters using 0.5 N NaOH in methanol and 14 % boron trifluoride in methanol (Slover and Lanza, 1979; Diaz *et al.,* 2005; Hur *et al*., 2004.). FAMEs from fat were quantified using a Varian 430 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 μm film thicknesses).

Fatty acid methyl ester samples were identified by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). Fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids present in the sample. The following fatty acid combinations were calculated: total saturated fatty acids (SFA), total monounsaturated fatty acids (TMUS), polyunsaturated fatty acids (TPUS), total unsaturated fatty acids (TUS) and TPUS/TS ratio. Data were recorded for oil content and major fatty acid composition and other oil quality parameters. Data for quality trait were analyzed by SAS proc means (SAS, 2011). Mean performance were tested among genotypes and across locations.

Genetic divergence was measured using Euclidean distance (D). Agglomerative Hierarchical cluster analysis was used to determine differences and similarities among the traits, and the distance measure used was Euclidean distance as the parameter that best reflects the differences existing among the genotypes (Kendall, 1980). Factor analysis used the covariance matrix of traits (Harman, 1967; Ariyo, 1992) to generate factor loadings and communalities using the method of principal component extraction. All statistical analysis was carried out based on twelve agro-morphological and 17 oil traits using SAS 9.2 software (SAS Institute, 2011) and Genes software VS 2016.6.0 (Cruz, 2013).

RESULT AND DISCUSSION

The PCA analysis for 16 groundnut varieties evaluated for 16 oil traits and grain yield was shown in Table 1. The first factor retained the information contained in 12.59 of the original variables while the second component retained 3.18 of the original variable. Two principal components, PC1 and PC2, which were extracted from

Table 1. Eigen values and Eigen vectors for correlation matrix, based on Euclidean distance(D)(left) and genetic correlation of 16 oil and agromorphological traits together with grain yield (kg/ha) (right), evaluated for 16 groundnut varieties

Genotypes	PC1	PC2	Traits	PC1	PC1
Eigen value	12.59	3.18	Eigen value	11.83	2.05
Difference	9.40	3.07	Difference	9.78	0.68
Proportion	0.79	0.20	Proportion	0.74	0.13
Cumulative	0.79	0.99	Cumulative	0.74	0.87
Bahagudo	0.06	-0.55	NBP	0.27	0.18
Bahajidu	0.27	0.15	NMP	0.12	0.54
Bulki	0.26	0.22	AGBP	0.24	0.27
Fetene	-0.27	0.11	PWP	0.25	0.07
Lote	0.27	0.14	SWP	0.27	-0.09
Manipeter	0.27	0.12	100SW	0.26	-0.26
NC-343	0.27	0.17	NSP	-0.14	0.56
Oldhale	0.28	0.05	NSPOD	-0.27	-0.19
Roba	0.26	0.22	Oleic	0.26	0.13
Sedi	-0.26	0.23	TPUS	-0.29	-0.06
Shulamith	0.28	0.005	O/L	0.26	0.08
Tole-1	0.24	-0.30	Oil	0.22	-0.03
Tole-2	0.12	-0.49	TPUS/TS	-0.27	0.19
Werer-961	-0.28	0.01	Linoleic	-0.29	-0.06
Werer-962	0.27	0.13	GY	0.27	-0.23
Werer-963	-0.23	0.33	OY	0.27	-0.22

NMP: mature pods per plant; NSP: seeds per plant; NBP: primary branches per plant; AGBP: above ground biomass per plant; PWP: pod weight per plant; SWP: seed weight per plant; 100SW: 100 seed weight; NSPOD: number of seeds per pod; O/L: oleic to linoleic acids ratio; TPUS: total polyunsaturated fatty acids; TPUS/TS: total poly to monounsaturated fatty acids; OY: oil yield; GY: grain yield.

the original data and having Eigen values greater than one, account for 99% based on Euclidean distance (D) suggesting these principal component scores might be used to summarize the original 16 variables in any further analysis of the data. The PCA in this study, was shown that the first component has got high positive loading from Behajidu, Bulki, Lote, Manipeter, NC-343, Oldhale, Roba, Shulamith, Tole-1 and Werer-962 cultivars, but high negative loading from Fetene, Sedi, Werer-961 and Werer-963 genotypes. The second component has high positive loading from Werer-963 and Sedi genotypes while high negative load were from Behagudo, Tole-2 and Tole-1.

The Eigen values and Eigen vectors for the first two principal components based on genotypic correlation among 16 oil traits and grain yield (kg/ha) were indicated in Table 1. The first principal component had high positive component loading from both agromorphological and high oil quality traits/parameters including NBP, AGBP, PWP, 100SW, GY, OY, oleic acid, O/L ratio, and oil content. These traits found to associate with Behajidu, Bulki, Lote, Manipeter, NC-343, Oldhale, Roba, Shulamith, Tole-1 and Werer-962 genotypes with high positive PCA1 scores based on euclidean distance indicating that breeding for high oil quality traits can be conducted via improvement of GY, OY, and 100SW,SWP and PWP.

On the other hand, the first component has got high negative load from NSPOD, NSP and low oil quality traits/parameters including linoleic acid, TPUS and TPUS/TS. The corresponding genotype loads were Fetene, Sedi, Werer-961 and Werer-963 indicating that genotypes with low oil quality can be identified through selection for NSPOD and NSP. Similarly, PCA2 had high positive component loading from agromorphological traits like NMP and NSP. The corresponding genotypes Werer-963, Sedi, Roba and Bulki. On the other hand, negative component loads for PC2 were from 100SW, GY and OY. The corresponding genotypes were Behagudo, Tole-2 and Tole-1 indicating these genotypes were associated with high grain yield and oil yield traits. These findings were in accordance with previous report by Ajay *et al.*

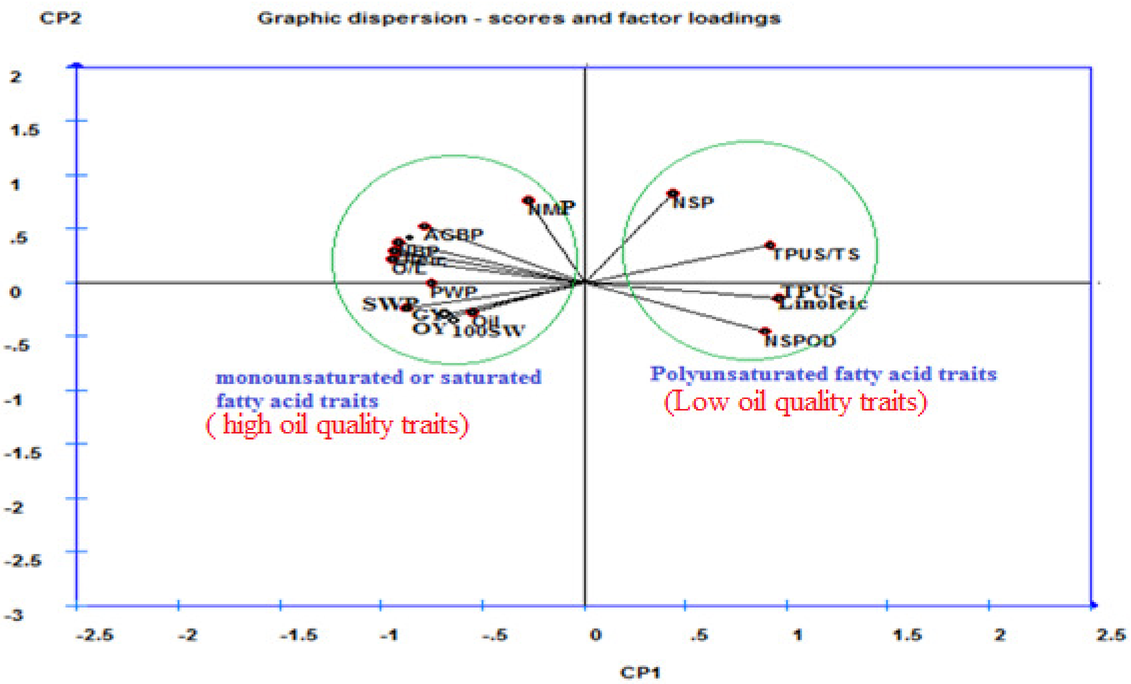

Figure 1. Principal component analysis of morphological and oil traits. NMP: mature pods per plant; NSP: seeds per plant; NBP: primary branches per plant; AGBP: above ground biomass per plant; PWP: pod weight per plant; SWP: seed weight per plant; 100SW: 100 seed weight; NSPOD: number of seeds per pod; O/L: oleic to linoleic acids ratio; TPUS: total polyunsaturated fatty acids; TPUS/TS: total poly to monounsaturated fatty acids; OY: oil yield; GY: grain yield.

(2012) who found divergent genotypes for confectionary quality traits in groundnut.

The graphical plot of PCA for oil and agromorphological traits (Figure 1.) was shown that GY, oil content, oil quality traits: oleic acid and O/L ratio were more closely related since the angle between them relative to principal axis was less than 90°. Grain yield, OY and oil content has shown acute angle with oil quality traits showing that it is possible to make selection of a genotype having all the important traits in oil crops including high grain yield, high oil yield, high oil content and oil quality. Since frequent evaluation of genotypes for oil traits is costly, breeding for oil traits can be conducted through selection for agromophological traits. Thus, breeding for oil content, OY, GY and oil quality traits can be conducted through selection for SWP, PWP, NBP, AGBP and NMP. The result obtained from PCA is as supplement to that of genetic correlation and path coefficient analysis. However, PCA and cluster analysis have further advantage of association genotypes to their respective traits.

Figure 2. has shown the clustering dendrogrm based on UPGMA for genetic correlation of agromorphological and oil traits. The cutting point for grouping dendrogam into clusters were based on the mean euclidean distance of the traits plus standard deviation which was found to be 4.39 in this case, that resulted in the grouping of 16 agromorphological and oil traits into 3 clusters. In the first cluster low oil quality traits like linoleic acid, TPUS and TPUS/TS were grouped with NSPOD and NSP agromorphological traits indicating that low oil quality genotypes can easily be identified through selection for such agromorphological traits. Even though high oil quality determinant traits: O/L ratio oleic acid, were grouped with GY, OY and oil content traits.

The high oil quality traits were found to be more closely related with agromorphological traits like NBP and AGBP indicating that breeding for high oil quality traits can be achieve via selection for NBP and AGBP. Such use of agromorphological traits to breed for oil traits helps breeders easily handle oil traits in the field and make the cost effective. Furthermore in the second cluster oil content, OY and oil content were found to closely associated with 100SW, PWP and SWP indicating that breeding for grain yield, oil yield and oil content can be conducted through selection for such highly heritable agromorphological traits including PWP, SWP and 100SW. The third cluster consisted of only one trait namely NMP, such trait considered divergent and it is not important for breeding of GY and oil traits. Similar findings were reported by Ashish (2013), Sarvamangala *et al*. (2011) and Sharma and Gupta (2011) who found association of various agromorphological and

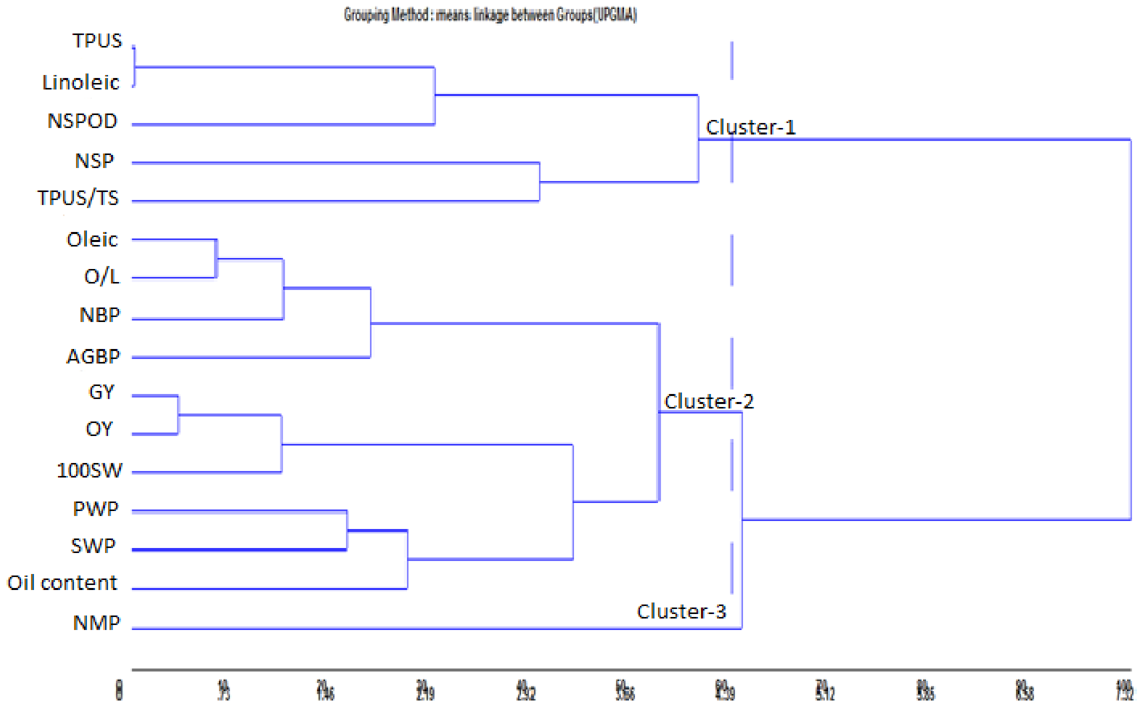

Figure 2. Dendrogram for agromorphological and oil traits. NMP: mature pods per plant; NSP: seeds per plant; NBP: primary branches per plant; AGBP: above ground biomass per plant; PWP: pod weight per plant; SWP: seed weight per plant; 100SW: 100 seed weight; NSPOD: number of seeds per pod; O/L: oleic to linoleic acids ratio; TPUS

biochemical characters with kernel yield, oil yield and oil quality traits.

CONCLUSION AND RECOMMENDATION

The component analysis has identified major yield, oil quality and related traits and their association with genotypes. High negative loading traits especially in PC1 shows inverse relationship and/or divergence to the rest variables therefore such traits are not mainly used for breeding since they have usually low heritability. This study has well identified interrelationship among oil traits and grain yield with respective genotypes and will have significant contribution for future groundnut breeding. The present result obtained through morphological and biochemical markers should be confirmed by molecular markers.

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