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GENETIC VARIABILITY ASSESSMENT AMONG ELITE CHICKPEA (*CICER ARIENTINUM*) GENOTYPES UNDER WATER DEFICIT CONDITIONS

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Abstract
Gene drift, recurrent selections, unintended ways during domestication and natural mutations had led to narrow genetic base of available chickpea cultivars. Exploration and employment of naturally occurring genetic variability is most basic requirement for a genetic improvement program because it provides raw material to chickpea breeders. Limited progress for exploration of chickpea genetic variability has been made so far. A research study involving 50 chickpea genotypes (46 elite breeding lines and 4 standard varieties) was carried out at Gram Breeding Research Station, Kallur kot, Pakistan under water stress conditions during 2021-22 crop season. Data were subjected to D2 statistics, principle component analysis (PCA) and cluster analysis. Through D2 statistics, wide dispersion of data for range, standard deviation and coefficient of variation was found indicating that significant amount of variability exists among genotypes in performance of different traits. PCA distinguished the genotypes into nine principal components on the basis of studied traits. PCA confirmed that first three components (PC1, PC2 and PC3) extracted >1 Eigen values indicating that the genotypes possess sufficient genetic variation. Cluster Analysis distributed the genotypes into four different groups. Dendrogram of agglomerative clustering on the basis of Euclidean distance illustrated that cluster-III and IV were more diverse. Mean performance of different traits in cluster analysis was evident that member of cluster-III (TGP-005, TGP-022, D-86030, D-88023, D-89033, D-04025, D-02055, D-02060, D-13031) possess higher yield potential coupled with more diversity. Therefore, these genotypes may be favored while making selections for genetic variation and to accelerate the chickpea genetic advancement program.
Keywords: *Chickpea, Cluster analysis, Genetic variation, PCA*

INTRODUCTION

Chickpea (*Cicer arientinum* L.), is one of widely cultivated pulse legume crop of tropical and subtropical climates across the globe. Its origin and domestication by humans dates back to old world (10,000 years ago) however its formal cultivation in Anatolia, Turkey, dates back to 6000 years ago (1, 2). Chickpea grains are cheapest source of dietary protein and often termed as "poor man's meat" (3, 4). Chickpea is soil restorative crop because of symbiotic nitrogen fixation and is best suited in cropping system of Pakistan. Chickpea yields have been hampered seriously due to vulnerability of available commercial cultivars to global climatic changes and several biotic and abiotic stresses (5, 6). Lack of high yielding cultivars and narrow genetic base of available chickpea varieties are the basic reasons for declined chickpea productivity across the world (7, 8).

Genetic variability is key drive for evolution of new improved genotypes and it assures the survival of species in nature (9, 10). Variability provides adequate breeding options for researchers to exploit specific desired genetic improvement i.e. yield potential, resilience to climate, tolerance to biotic and abiotic stresses (11, 12, 13). Characterization, breeding and development of most fittest types of crop plants guarantees the food security, zero hunger and availability of high level per-capita food to ever increasing world population (14). Detection of genetic variability occurring naturally in genetic stock of a species is most valuable

resource for crop breeders (4). Variability among germplasm of a species provides more opportunities for breeders to bring desirable genetic changes. Assessment of patterns and amount of such variability provides basis while making selection for genetic improvement in chickpea (7, 15).

Genetic diversity occurring naturally in crop plants is an important source of alleles. Genetic advancement programs require assessment of genetic diversity of available genetic stock for selection of appropriate parental types (16-18). Several former researcher employed series statistical methods i.e. Mahalanobis D2 statistics, principal component and cluster analysis for estimation of genetic variation (13, 16, 19).

In Pakistan very limited scientific efforts have been done so far to characterize and screen out the chickpea germplasm. Genetic variability provides sufficient raw material for selection and development of improved chickpea cultivars (20-22). The present study was planned to sort out the novel genetic resources possessing more variability and high yield potential for future chickpea genetic improvement program.

MATERIALS AND METHODS

The study involving 46 elite breeding lines of Chickpea along with four commercial varieties was carried out at Gram Breeding Research Station, Kallur kot (71.165 0E and 32.920 0N) during rabi season of 2021-22. Experimental material was sown by dibbler under tri-replicate RCBD design. Research experiment was done under water deficit conditions, only initial moisture was created necessary for germination. No supplementary watering was done to evaluate the entries under rain fed conditions. Total precipitation during crop season was recorded 148 mm in five spells. Each entry was sown in 30 cm apart 4 rows measuring 4 meter in length and plant to plant distance was maintained 10 cm thinning of plants was done to ensure one plant in each hole. Trial was protected from pod borer by repeated chemical spray of Chlorantraniliprole @ 200 ml per hectare. All recommended agronomic practices were performed as per recommendation for the crop.

Data for nine morpho-yield traits were recorded for plant population, days taken to 50 % flowering, primary branches, secondary branches, plant height, pod numbers, days required to maturity, 100 seed weight and yield kg ha-1. Mahalanobis (23, 24) D2 statistics was employed to study the standard deviation and to compare the variance among different chickpea genotypes. Principal Component analysis was performed for genetic variability following the outlines of Pearson K. (25) and cluster analysis following Wards J.H. (26) by applying STAR (version 2.0.1).

RESULTS AND DISCUSSION

Results regarding D^2 Statistics confirmed higher values for coefficient of variation and standard deviation (σ) as presented in Table I. Wide range, higher values for coefficient of variation and standard deviation were evident that the studied genotypes possess extended variability. Wide dispersion of data for range, coefficient of variation and standard deviation were also reported by Malik *et al.* (15) and Johnson *et al*. (19).

Table I. D^2 analysis of different chickpea traits

Traits	Range	Mean (μ)	Standard Deviation (σ)	CV values
Plant Population	23-38	28.67	6.31	9.33
Days to 50 % flowering	102-115	108.4	9.24	6.65
Primary Branches	3-9	5.4	2.25	4.62
Secondary branches	6-18	10.78	7.5	16.7
Plant height (cm)	43-78	58.9	8.5	18.5
Number of pods	25-60	38.74	8.65	21.4
Maturity days	149-158	152.2	5.87	17.2
100 Grains weight (g)	16.2-30.4	21.2	4.86	8.84
Yield kg ha^{-1}	1086-3570	2401	267.69	27.2

Principle component analysis (PCA) was distributed the genotypes into nine components. Data also showed that first three components (PC1, PC2, PC3) exhibited >1 Eigen values explaining that the maximum variation was contributed by these components. Eigen values explained by a principal component measure the amount of variation (27). Principal components extracting higher Eigen values (>1) have major share in creation of variability (28). From the data it was obvious that 70 percent cumulative percentage of variance was shared by first three PCs (Table II).

Table II. Eigen values extracted by different principal components

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigen values	1.8009	1.3372	1.1480	0.9563	0.7652	0.6248	0.5672	0.5263	0.4021
Percentage of variance	36.04	19.87	14.64	10.16	6.51	4.34	3.57	3.08	1.80
Cumulative percentage	36.04	55.90	70.55	80.71	87.21	91.55	95.13	98.2	100

For graphical illustration of data, Scree plot was constructed denoting that maximum Eigen values was exhibited by PC1 (1.8) followed by PC2 (1.34), PC3 (1.15) while least was contributed by PC9 (0.40) (Fig. 1). Data also revealed that in PC1 higher positive loadings were presented by plant height (0.4814), yield kg ha^{-1} 0.4648), 100 seed weight (0.3688) and number of pods (0.3663) (Table III). While, days taken for 50 % flowering and maturity days extracted negative loading. Likewise, in second component, maximum positive

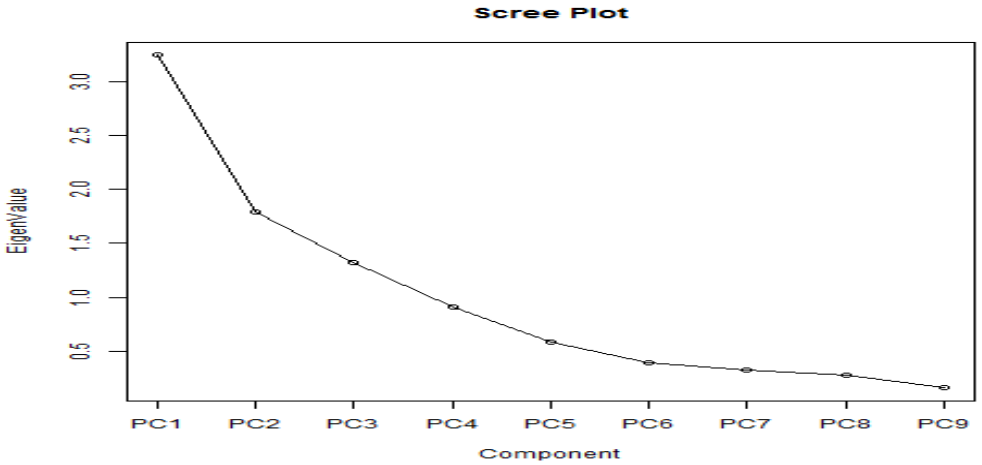

Fig. 1. Scree Plot showing Eigen values of principal components

Table III. Performance of different variables in PCA

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
pp	0.3336	0.0066	0.4985	-0.0255	0.6085	-0.3739	0.3059	-0.1698	0.0702
DF	-0.2837	-0.03713	0.4633	-0.1955	0.1383	-0.0201	-0.7102	0.0397	-0.0568
PH	0.4814	0.4599	0.0164	-0.1273	-0.1438	0.2749	-0.3239	-0.7395	-0.0150
PB	0.1098	0.1034	-0.5218	-0.1361	0.2949	-0.1513	0.0213	-0.1167	-0.5564
SB	0.1894	0.1946	-0.1330	0.1051	0.0070	0.1540	0.0964	0.0854	0.6856
NPP	0.3663	0.2172	0.2636	-0.0324	-0.6721	-0.4647	0.0702	0.1499	-0.1975
MD	-0.2057	-0.1284	0.0735	0.9433	0.0468	-0.1291	-0.0378	-0.0175	-0.1596
GW	0.3688	0.3037	-0.3636	-0.1080	-0.1747	-0.4110	-0.5277	0.3150	0.2253
YLD	0.4648	0.2447	0.1988	0.1136	0.1359	0.5797	-0.0179	0.5289	-0.3111

PP =Plant population, DF=Days to flowering, SB=Secondary branches, PH= Plant height, NPP= Number of pods plant^{-1}, MD= Maturity days, GW=100seed weight, YLD= Yield kg ha^{-1}

loading were exhibited by plant height (0.4599), 100 seed weight (0.3037) yield kg ha^{-1} (0.2447) and number of pods (0.2147) while negative loadings were exhibited and number of pods plant^{-1}. In PC3 comparatively higher positive values were extracted by plant population while minimum were recorded in days to

maturity. From above results it was found that major variability share was contributed by plant height, yield kg ha^{-1}, 100 grain weight and number of pods plant^{-1}. Findings of this study agree to the former reports of Bisht *et al*., 2005; Agrawal *et al*., 2018; Arora *et al*., 2018.

Biplots were constructed to represent the data graphically by showing the direction and patterns of data sets (Fig. 2). Biplot view illustrated that the vectors for plant height, yield kg ha^{-1}, 100 seed weight and number of pods plant^{-1} were superimposed on plots showing that they have significant role in creation of total genetic variation. Therefore selection criteria for may be based on these traits. In agreement to this study plant height, yield kg ha^{-1}, 100 seed weight were narrated highest contributors towards genetic diversity by Sharifi *et al*., Arora *et al*. and Singh *et al*. (5, 29, 30).

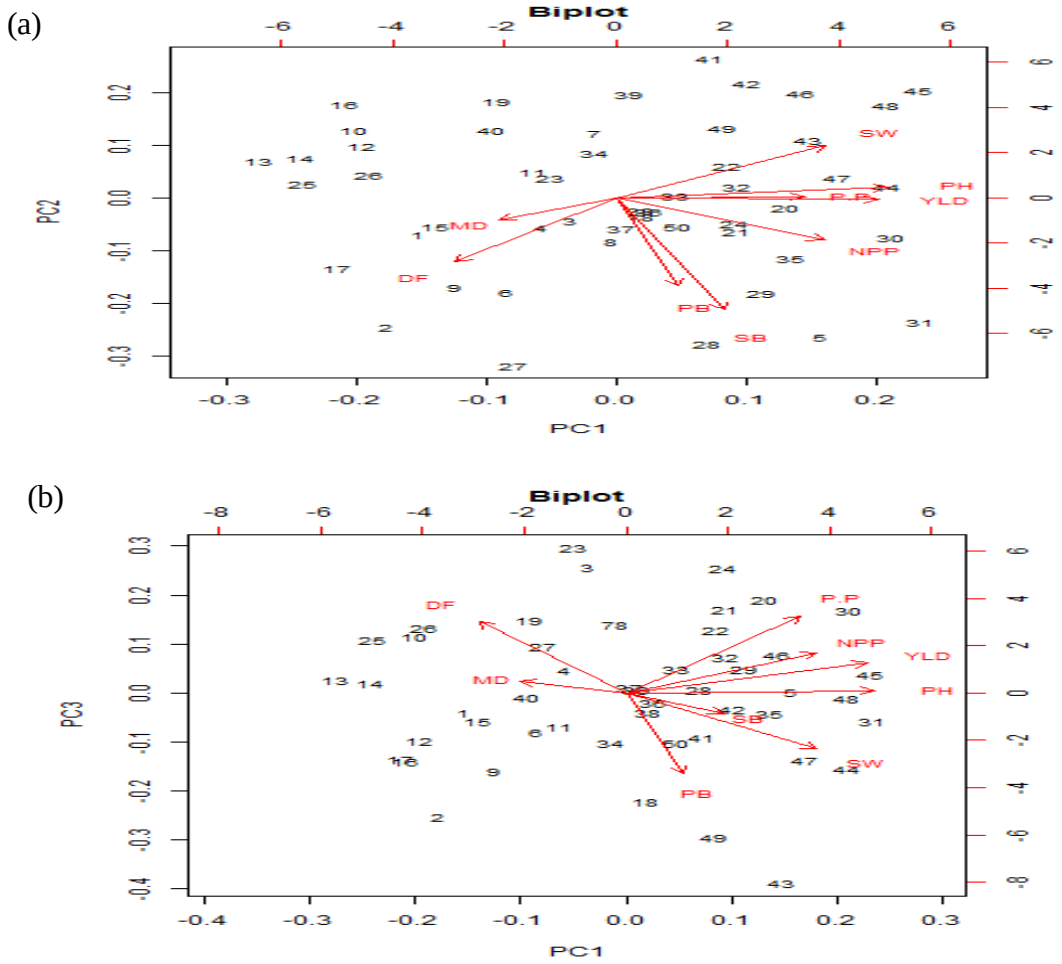

Fig. 2. (a). Biplot among PC1 and PC2, **(b).** Biplot among PC1 and PC3

Through cluster analysis the genotypes were distributed into four different clusters. Data in table (4) denotes that the genotypes having higher mean values yield kg ha^{-1} (3045 kg), plant height (63.9 cm), 100 grain weight (28.3 g) and number of pods (47) were grouped in cluster-III followed by cluster-IV in which yield kg ha^{-1} was found (2563 kg), plant height (63.5 cm), 100 grain weight (27.7 g) and number of pods (38.5). Our results agree to Gautam *et al*., Agrawal *et al*. and Pavan *et al*. (10, 29, 32).

On the basis of Euclidean distance Ward's Dendrogram of agglomerative clustering was constructed. Dendrogram of genotypes illustrated that the members of cluster-III and IV were genetically most diverse. Cluster analysis also showed that 14 genotypes (TGP-001, TGP-002, TGP-006, TGP-009, TGP-010, TGP-012, TGP-015, TGP-016, TGP-017, TGP-018, TGP-019, TGP-020, D-89020, D-89027) were gathered in cluster-I. Similarly in cluster-II, 13 genotypes (TGP-003, TGP-004, TGP-007, TGP-008, D-86060, D-86205, D-89031, D-04043, D-01032, D-05002, D-03019, Bittle 2016, Noor-19) were grouped, in cluster –III contained 9 genotypes (TGP-005, TGP-022, D-86030, D-88023, D-89033, D-04025, D-02055, D-02060, D-13031). Likewise in cluster-IV, 14 genotypes (TGP-011, TGP-021, D-05007, BAKHAR 2011, PB -2008, PCK-18006, PCK-18021, PCK-18005, PCK-18023, PCK-18007, PCK-20010, PCK-20016, PCK-20024, PCK-18022) were grouped as shown in table (5). From cluster analysis it was found that the members of cluster-III (TGP-005, TGP-022, D-

86030, D-88023, D-89033, D-04025, D-02055, D-02060 and D-13031) possess high yield potential apart from diversity. Therefore selection of genotypes from this cluster may be more useful for further utilization in genetic advancement program.

Table IV. Performance of various traits in different clusters

Traits	Cluster-1		Cluster-2		Cluster-3		Cluster-4	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
PP	23-29	26.4	24-30	27.5	25-38	34.62	28-36	33.4
DF	103-113	109.6	107-115	109.4	108-110	109.1	102-109	105.8
PH	43.6-58.2	49.56	52-67.5	60.55	56.5-78.4	63.9	52.7-77.3	63.5
PB	3-6	4.1	3-7	5.0	5-8	6.94	3-8	6.33
SB	6-15	9.93	9-17	11.5	11-18	14.2	6-13	8.79
NPP	25-40	33.6	32-52	39.1	42-60	47.11	32-52	38.51
MD	151-158	153.2	152-155	153.3	151-153	151.9	149-154	151.5
GW	16.2-24.5	19.9	21.8-25.8	23.5	23.4-30.4	28.3	25.4-29.9	27.7
YLD	1086-2522	1635	2029-3456	2607	2497-3570	3045	2046-3017	2563

*PP =Plant population, DF=Days to flowering, SB=Secondary branches, PH= Plant height, NPP= Number of pods plant^{-1}, MD= Maturity days, GW=100seed weight, YLD= Yield kg ha^{-1}

Table V. Membership of different genotypes in clusters

Cluster Names	Number of members	Membership
Cluster-1	14	TGP-001, TGP-002, TGP-006, TGP-009, TGP-010, TGP-012, TGP-015, TGP-016, TGP-017, TGP-018, TGP-019, TGP-020, D-89020, D-89027.
Cluster-2	13	TGP-003, TGP-004, TGP-007, TGP-008, D-86060, D-86205, D-89031, D-04043, D-01032, D-05002, D-03019, Bittle 2016, Noor-19
Cluster-3	9	TGP-005, TGP-022, D-86030, D-88023, D-89033, D-04025, D-02055, D-02060, D-13031
Cluster-4	14	TGP-011, TGP-021, D-05007, BAKHAR 2011, PB -2008, PCK-18006, PCK-18021, PCK-18005, PCK-18023, PCK-18007, PCK-20010, PCK-20016, PCK-20024, PCK-18022.

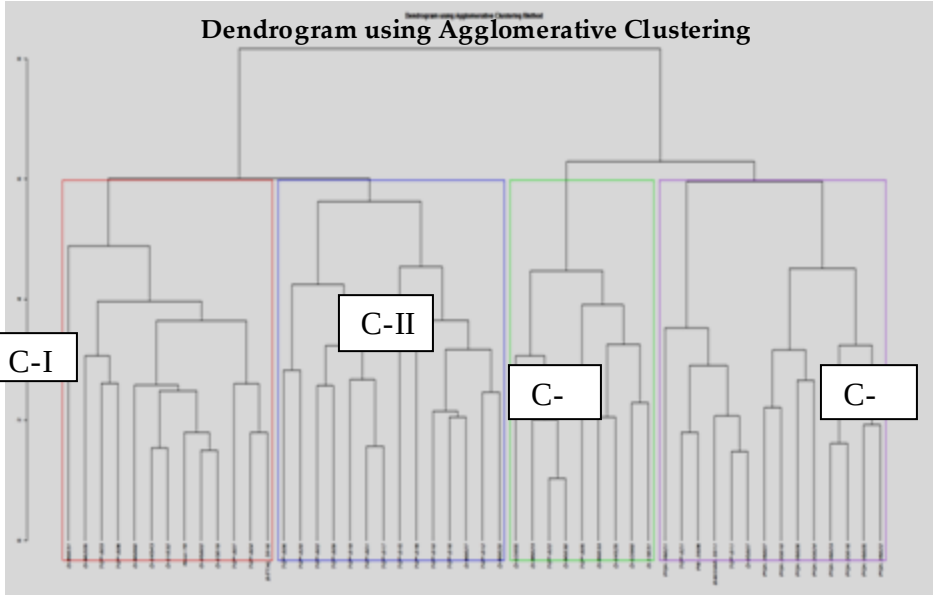

Fig. 4. Ward's dendrogram of agglomerative clustering

CONCLUSION

Wide dispersion of data and higher values of coefficient of variation were evident that the genotypes varied significantly in performance of different traits. Principal component analysis of studied genotypes also confirmed that first three components (PC1, PC2 and PC3) extracted >1 Eigen values showing that the included genotypes exhibited sufficient genetic variability. PCA also denoted that the

vectors for plant height, yield kg ha-1, 100 grain weight and number of pods plant-1 were superimposed on plots indicating that they have significant role in creation of genetic variation. Cluster Analysis of genotypes illustrated that the member of cluster-III and IV were more diverse. Cluster analysis also revealed that member of cluster-III (TGP-005, TGP-022, D-86030, D-88023, D-89033, D-04025, D-02055, D-02060 and D-13031) possess high yield potential coupled with more genetic variability. Therefore, these genotypes may be further utilized in chickpea genetic advancement program.

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Conflict of interest:

Authors have no conflict of interest.

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