

Original Research Article

Screening of chickpea ~~germplasm genotypes~~ for growth, ~~and~~ yield related traits ~~and yield~~ under agro-climatic conditions of Thal

Abstract

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A field experiment comprising of fifty chickpea ~~germplasm genotype-genotypes entries~~ along with two commercial varieties (Punjab-2008 and Bittle-2016) was carried out ~~with the objective to for characterization of chickpea germplasm genotype and screening of superior screen~~ chickpea genotypes under agro-climatic conditions of Kallurkot (~~71.153°E and 32.923°N~~) (~~Thal, Punjab, Pakistan~~) at research area of Gram Breeding Research Station, ~~Kallurkot India(71.153°E and 32.923°N)~~. D2 Statistics, principal component and cluster analysis were employed for screening of chickpea genotypes. Results showed wide range and higher values of variance for the included traits. PCA results demonstrated that the first four principal components extracted more than 1 Eigen values with a cumulative share of 74% of total variation. Cluster analysis distributed the genotypes into five distinguished clusters. Dendrogram constructed on the basis of Euclidean distance showed that the members of cluster IV (GP-17215, GP-16548) and V (GP-16929, GP-01937, GP-01974) possess higher genetic variation. Results confirmed that these genotypes possess the best combination of morpho-agronomic and may be utilized further for chickpea yield improvement program.

Key words: PCA, Cluster analysis, Variability, D² statistics, Dendrogram, Eigen value, Euclidean distance

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Introduction

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Chickpea (*Cicer arietinum* L.) is one of the major grain legume ~~crop~~ crops contributing for food requirements of ~~ever increasing~~ ever-increasing global population. It is a winter season crop mostly cultivated in arid and semi arid tropical regions throughout the world (Varshney *et al.*, 2019). Globally, it ranks 3rd most important pulse crop after peas and beans (Varshney *et al.*, 2013). Among top producers, Pakistan ranks 3rd in term of production and 2nd for overall area under chickpea cultivation (Anon., 2012). The production of chickpea in Pakistan is 444 kg ha⁻¹ which is far lower than the average global production of 969 kg ha⁻¹ (Nadeem *et al.*, 2019). Several biotic and abiotic factors drastically influence the overall productivity of the crop (Vrignon-Brenas *et al.*, 2016; Roorkiwal *et al.*, 2017). In Pakistan, drought stress is the prime constraining factor for production due to its farming on sand dunes and dry land of Thal

where it faces extreme moisture stress conditions (Mahmood *et al.*, 2018).

Drought stress is primary limiting factor for growth and economic production of the crop (Garg *et al.*, 2004; Talebi *et al.*, 2013). Insufficient and irregular distribution of rainfall together with intensifying rate of temperature influence and extended dry spells result in unstable crop production (Irshad *et al.*, 2013). It is direly needed to develop more advance varieties with improved performance and higher yield potential under stress environmental conditions (Lobell *et al.*, 2009; Varshney *et al.*, 2017; Rubiales *et al.*, 2018). This can be achieved by utilizing screening techniques for assessing [germplasmgenotype](#) so as to recognize the genetic basis of drought tolerance and identify genotypes that have the potential to withstand with drought stress and raised the level of chickpea yield production (Dixit *et al.*, 2019).

In Pakistan, crop is mainly cultivated in arid and semi arid region thus, development and release of chickpea varieties with minimum moisture requirements are generally successful. Genetic variation among crop plants in performance of different growth and yield related traits serve as significant source for development of new varieties (Sharifi *et al.*, 2018 and Rybinski *et al.*, 2019). Genetic variability among parental genotype gives fundamental basis that helps the researchers to distinguished genetics resources and identify the suitable [germplasmgenotype](#) (Varshney *et al.*, 2019). Superior chickpea genotypes were also identified that are adaptable to drought prone areas of the country (Rafiq *et al.*, 2020).

Statistical analysis including principal component analysis and cluster analysis have been found most suitable sequence method to find out genetic variability of a large scale data of genotypes by grouping and identifying the pattern and range of diversity (Sharifi *et al.*, 2018). Principal component analysis and cluster analysis have already been used for assessment of genetic variability of agronomic traits by many researchers (Farshadfar and Farshadfar, 2008; Johnson *et al.*, 2015 and Chen *et al.*, 2017).

The main focus of current study was to explore genetic variability and performance of morpho-agronomic traits for reorganization of more appropriate parental genotypes having higher yield potential under drought stress so that, the most divergent types with best genetic constitution can be utilized further in breeding program.

Materials and Method

The experimental material comprising of 50 ~~superior~~ chickpea genotypes collected from diversified locations of Thal along with two commercial varieties (Punjab-2008 and Bittle-2016) was laid down in ~~randomized complete block design~~ with three replications at Gram Breeding Research Station, Kallurkot, Punjab, Pakistan located at 71.153°E and 32.923°N during Rabi (~~include here the English equivalent of Rabi~~) season of the year 2019-20. Each entry was sown in ~~experimental~~ plot of 4 meter in length with 4 rows having 30 cm row to row spacing. Sowing was done by dibbler by maintaining 10 cm plant to plant spacing. Initially 2 seeds were sown in each hole and after germination thinning was done to ensure single plant in each hole. Only one irrigation was applied to provide initial moisture for germination of seed and no supplementary irrigations was applied throughout the ~~crop~~ ~~growing~~ ~~period~~ ~~of~~ ~~crop~~. Total rainfall recorded during the crop period was 46 mm in three spells. At pod formation stage insecticide Emamectin @ 600 ml ha⁻¹ was sprayed twice to prevent pod borer attack. Manual hoeing was done twice to keep the crop weed free. Data ~~for~~ ~~on~~ ~~plant~~ ~~population~~, ~~days~~ ~~population~~, ~~days~~ to 50% flowering, days to 90% maturity, plant height (cm), primary branches, secondary branches, number of pods plant⁻¹, 100 seed weight (g), ~~biological yield~~, harvest index and yield kg ha⁻¹ were recorded for each ~~experimental~~ genotype. Data recorded for all traits ~~was~~ ~~were~~ subjected to analysis of variance following Steel et al., 1997, while principal component analysis and cluster ~~was done~~ ~~analysis~~ by STAR (Statistical Tool for Agricultural Research version 2.0.1).

Results and discussion

Data related ~~to~~ range, mean, standard deviation and coefficient of variation were ~~measured~~ ~~calculated~~ using D² Statistics (Table1). From the data, it is evident that traits presented broad dispersion for range, standard deviation and coefficient of variation. All the studied traits showed wide range values in different studied traits. Similar findings were also noticed by Malik *et al.*, (2010) and Khan *et al.*, (2011) representing the significance of these traits in yield enhancement. ~~Higher values of standard deviation and coefficient of variation demonstrated the existence of sufficient amount of variation among the genotypes for included different traits.~~ These findings are in line with the past results of Syed *et al.*, (2012) and Malik *et al.*, (2014).

Table 1: ~~Mean~~ ~~performance~~ ~~of~~ ~~different~~ ~~traits~~ ~~of~~ ~~chickpea~~ ~~germplasm~~ ~~genotype~~ Performance of chickpea genotypes for different parameters.

Comment [HZ2]: What is the justification to use RCBD (randomized complete block design when you have 50 genotypes). You need to justify strongly why RCBD is used. Are there no other design that are more efficient than RCBD when the treatment numbers are greater than 20? **THIS IS CRITICAL!** Thus, **justify stringly** since you might have had your justification.

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Comment [HZ3]: How easy was it to record this data since biological yield includes practically all parts of the plant. It will be good if the approach used is explained in this case to avoid confusion between Above ground Biomass yield and Biological yield. Since this is scientific writing we should be worried about the terms and phrases we are using.

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Comment [HZ4]: Revisit this statement again since the values of S.D and C.V (%) are not entirely of genetic. Let us not forget the role of the growing condition on the expression of traits, particularly quantitative traits like yours.

Comment [HZ5]: Is this the way we discuss our work in light of their findings? Please make your discussion more sensible. Thus revisit and discuss properly! Yes you may find such way of writing by some, but that doesn't guarantee us to write as they did. We scientists can be more scientific in writing than others. I guess you are a young scientist who wants to grow. Thus follow what is more scientific.

<u>Variables/Traits</u>	<u>Parameter</u>			
	<u>Range</u>	<u>Mean (μ)</u>	<u>S.D (σ)</u>	<u>C.V (%)</u>
Plant Population	30-84	64.6	10.24	21.17
Days to 50% Flowering	92-106	97.94	4.06	20
Primary Branches plant ⁻¹	2-5	2.6	0.63	12.69
Secondary Branches plant ⁻¹	2-13	7.77	3.1	12.35
Plant Height (cm)	47-75	63.37	5.38	15.98
Number of pods plant ⁻¹	19-106	69.13	17.44	25
Days to Maturity	114-174	167.52	11.1	23.44
100-Seed Weight (g)	18-30	24.19	2.69	13.25
Harvest Index	15-48	29.33	6.58	24.5
YLDha ⁻¹	202-789	510.79	146.08	28.23

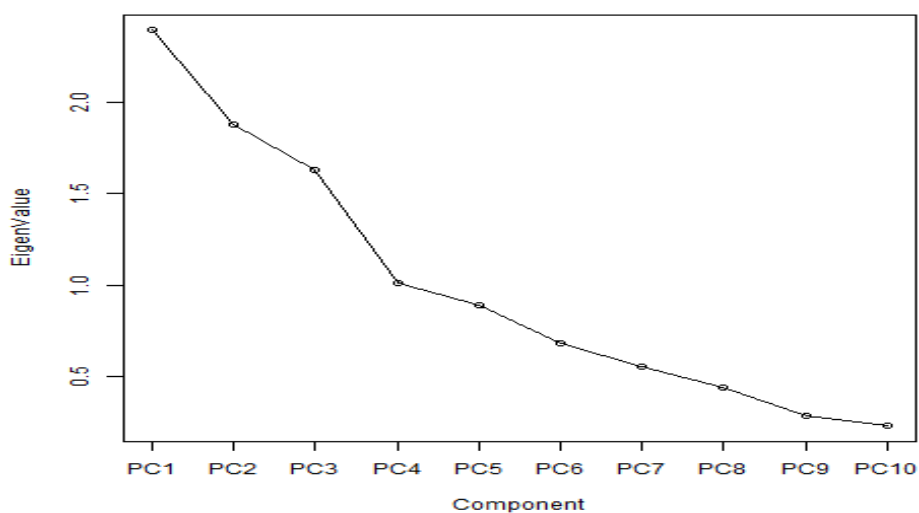
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Principal component analysis extracted ten PCs, among which first four PCs showed Eigen values more than 1 (Table2). A Scree plot (Fig.1) between Eigen values and principal component was constructed to illustrate results and also summarized the involvement of PCs. Maximum variation was present in PC1 with maximum Eigen value of 2.393 followed by PC2 (1.879), PC3 (1.633), and PC4 (1.014). Data showed that PC1, PC2, PC3 and PC4 contributed 24%, 21%, 17% and 12% respectively and 74% cumulative variation. Similar results were reported earlier (Talebi and Rokhzadi, 2013; Malik *et al.*, 2014 and Agrawal *et al.*, 2018)

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Scree Plot



contribution of more than two PCs in variability.

Fig. 1. Scree plot showing contributions of PCs in variability

Results (Table.2) also showed that in PC1 significant positive values were exhibited by plant population (0.079), primary branches (0.137), secondary branches (0.475), plant height (0.503), number of pods plant⁻¹ (0.386), days to maturity (0.5030 and yield kg ha⁻¹(0.045) while days to flowering, 100 seeds weight and harvest index contributed negative loadings. 2nd component was associated positively to days to 50% flowering (0.327), secondary branches (0.0163) and days to maturity (0.043) while all other traits expressed negative loadings. In 3rd component positive contribution of plant population (0.213), plant height (0.300), days to maturity (0.090) and 100 seeds weight (0.429) was noted while negative share was observed by remaining traits.

Table (2) Principal component analysis of various traits of chickpea germplasm genotype

Variables/Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
PP	0.079	-0.554	0.231	-0.181	0.328	-0.037	-0.073	0.595	0.148	-0.331
DFD	-0.072	0.327	-0.079	0.467	0.726	0.340	-0.052	0.114	0.058	0.049
PB	0.137	-0.092	-0.538	-0.105	-0.312	0.682	-0.226	0.213	0.109	-0.035
SB	0.475	0.0163	-0.194	-0.299	0.288	0.064	0.437	-0.427	0.207	-0.379
PH	0.503	-0.033	0.300	0.088	0.018	0.179	-0.364	-0.154	-0.659	-0.157
NPP	0.386	-0.015	-0.386	0.329	-0.088	-0.306	0.437	0.430	-0.301	0.157
DM	0.503	0.043	0.090	0.347	-0.143	-0.247	-0.396	-0.044	0.607	0.087
100-SW	-0.032	-0.274	0.429	0.474	-0.289	0.415	0.479	-0.097	0.127	-0.064
HI	-0.293	-0.317	-0.395	0.433	-0.025	-0.232	-0.193	-0.274	-0.093	-0.543
YLD (kg/ha)	0.045	-0.632	-0.159	-0.004	0.269	0.028	-0.047	-0.331	-0.036	0.623
Eigen Value	2.393	1.879	1.633	1.014	0.890	0.680	0.552	0.439	0.289	0.232
Percent of variance	24	21	17	12	7	8	4	3	2	2
Cumulative % of variance	24	45	62	74	81	89	93	96	98	100

PP(plant population), DFD(days to 50% flowering), PB(primary branches), SB(secondary branches), PH(plant Height), NPP(number of pods plant⁻¹), DM(days to maturity), 100 SW(100 seed weight), HI(harvest index), YLD(yield in kgha⁻¹).

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Biplot between PC1 and PC2 depicted that vectors for days to maturity and secondary branches revealed that these [characteristics/traits](#) have considerable involvement in determination of variability (Fig 2).

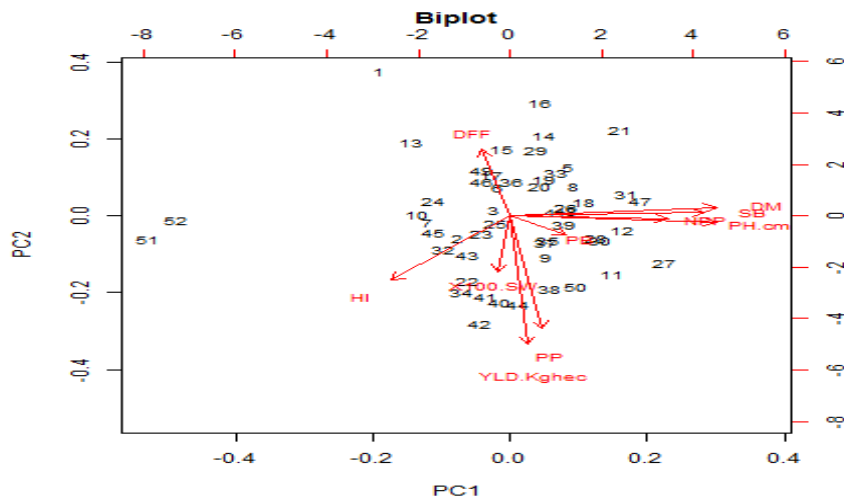


Fig.2. Biplot of PC1 and PC2 showing contribution of various traits in variability

Biplot (Fig.3) among PC1 and PC3 depicted that plant population, plant height (cm) and days to maturity expressed most significant contributions to genetic variation in examined chickpea genotypes.

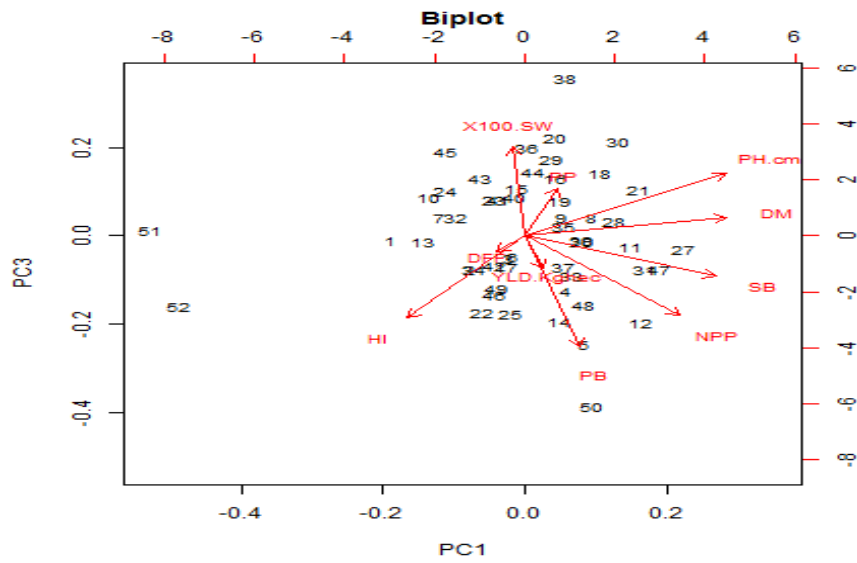


Fig.3. Biplot of PC1 and PC3 showing contribution of various traits in variability

Another biplot (Fig.4) for PC2 and PC3 depicted days to 50% flowering and days to maturity expressed more significant contributions to genetic diversity in studied chickpea genotypes. Malik *et al.*, (2014) also recorded assortment of genotype from first three PCs will be most important for the success of a breeding program for chickpea improvement and reported similar results in agreement to current study.

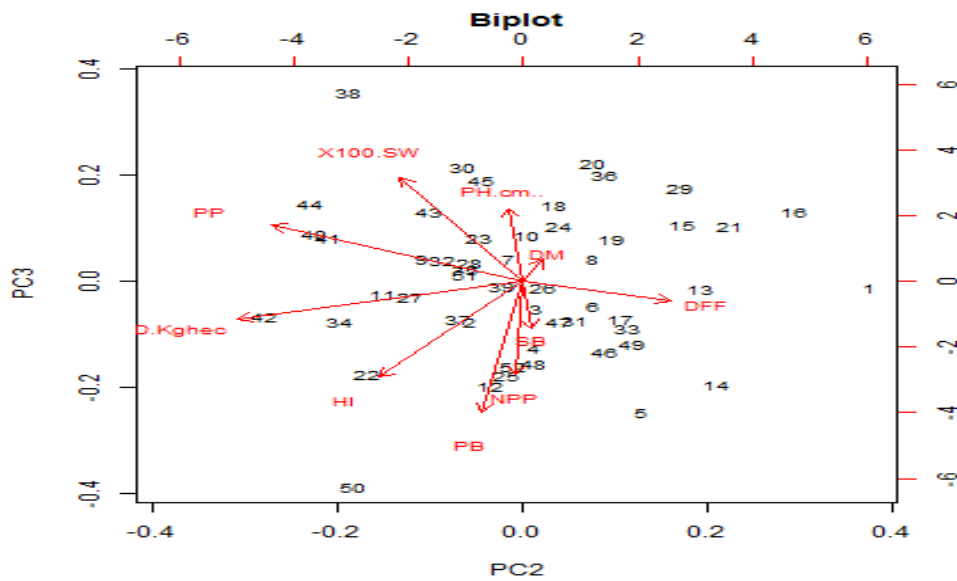


Fig.4 Biplot of PC2 and PC3 showing contribution of various traits in variability

Cluster analysis grouped the genotypes into five clusters on the basis of similarity in characters. Cluster I comprised of ten genotypes viz; GP-16553, GP-01982, GP-02561, GP-16832, GP-16848, GP-16802, GP-16847, GP-01873, GP-01879, GP-01888. Cluster II consisted of fifteen genotypes viz; GP-1891, GP-01930, GP-0 2133, GP-16550, GP-16587, GP-16615, GP-16712, GP-16726, GP-16815, GP-17007, GP-17210, GP-16827, GP-17026, GP-17076, GP-02722 while cluster III included twenty two genotypes viz; GP-01883, GP-01928, GP-01967, GP-16664, GP-16674, GP-16719, GP-17077, GP-02563, GP-02576, GP-01975, GP-01976, GP-01896, GP-01991, GP-01903, GP-02054, GP-00207, GP-03003, GP-03011, GP-16539, GP-16689, GP-16711, GP-16744. Cluster IV comprised of two genotypes viz; GP-17215 and GP-16548 and the cluster V consisted of three genotypes viz; GP-16929, GP-01937 and GP-01974. Similar results of 40 genotypes also recorded three clusters by Talebi and Rokhzadi (2013).

Table.3. Cluster Membership of Chickpea Genotypes

Clusters	Members Genotypes
Cluster I	GP-16553, GP-01982, GP-02561, GP-16832, GP-16848, GP-16802, GP-16847, GP-01873, GP-01879, GP-01888
Cluster II	GP-1891, GP-01930, GP-0 2133, GP-16550, GP-16587, GP-16615, GP-16712, GP-16726, GP-16815, GP-17007, GP-17210, GP-16827, GP-17026, GP-17076, GP-02722

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- | Cluster III GP-01883 , GP-01928, GP-01967, GP-16664, GP-16674, GP-16719, GP-17077, GP-02563, GP-02576, GP-01975, GP-01976, GP-01896, GP-01991, GP-01903 , GP-02054, GP-00207 ,GP-03003, GP-03011 ,GP-16539, GP-16689 ,GP-16711, GP-16744
- | Cluster IV GP-17215, GP-16548
- | Cluster V GP-16929, GP-01937, GP-01974

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It is obvious that G-genotypes in cluster V showed overall maximum high values for secondary branches (10), number of pods plant⁻¹ (88), days to maturity (171), harvest index (36) and yield Kgha⁻¹ (576) indicating that these genotypes have significant role in diversity (Table.4). Genotypes in cluster IV expressed highest values for days to 50% flowering and number of pods plant⁻¹ indicating that these are early maturing genotypes. Cluster III included genotypes with highest plant population (65).The remaining three clusters provided lower mean values indicating that these genotypes had little or no contribution in diversity. Similar findings were also narrated by Ghafoor *et al.*, (2003) and Malik *et al.*, (2014).

Comment [HZ6]: Here what is your justification to say "overall maximum". I don't see significant difference between the values in cluster IV (9. 170, 33) versus V (10, 161, 36) for the traits you indicated. Making such statements may be dangerous. Why after to use words such maximum and minimum where don't hve the justification for the genotypes attaining their highest genetic potential for the traits.

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Table.4 Cluster mean values Analysis of for various traits (mean values) of chickpea genotypes.

Variabletraits	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Plant Population	45	64.4	65	67	65
Days to 50% Flowering	95	98	95	100	99
Primary Branches	3	3	3	2	3
Secondary Branches	4	6	4	9	10
Plant Height	58	48	65	65	63
No. of pods plant ⁻¹	59	33	67	69	88
Days to maturity	169	114	169	170	171
100 Seed Weight	21	24	23	26	24
Harvest Index	29	24	28	33	36
yield kg ha ⁻¹	291	444	528	535	576

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Genotypes of cluster IV and V were more diverse in performance of different characteristics traits therefore addition utilization of these genotypes will be more helpful-useful for chickpea enhancement-breeding program (Fig.5). Similar results were already detailed reported by Pavan *et al.*, 2017 and Sharifi *et al.*, 2018-in agreement to this consider.

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