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Multivariate Analysis for Identification of Heat Tolerant Superior Chickpea (*Cicer arietinum* L.) Genotypes

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ABSTRACT

Background: Chickpea being a winter season crop, often experiences high temperature during its reproductive phase resulting in yield losses due to the direct effect of it, on different physiological processes. Therefore, identification and development of heat stress tolerant genotypes is an important aspect in chickpea breeding especially in view of the changing climate scenario.

Methods: An experiment was conducted during *Rabi* season 2017-18 at pulses research field, CCS HAU, Hisar, Haryana comprising 60 genotypes in randomized block design with three replications under two environmental conditions namely, normal and late sown to find out heat stress tolerant genotypes. A total of eleven morpho-physiological yield attributes noted down under both normal and late sown conditions and various multivariate statistical methods were used to find out tolerant chickpea genotypes.

Result: The present study has led the understanding of many inter-related traits involved in the genetic variation of chickpea seed yield under normal as well as late sown conditions. This would certainly provide guidelines for selection of parents as well as effective selection of promising chickpea genotypes and also have paramount importance in formulating plant model for selection of segregating generations in chickpea breeding programmes for development of high yielding varieties.

Key words: Chickpea, Correlation, Cluster analysis, Multivariate, PCA, Seed yield.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop globally after common bean and field pea. This annual legume is a significant contributor to agricultural sustainability through N_2-fixation and as a rotation crop allowing the diversification of agricultural production system. Chickpea being a winter season crop, often experiences abnormally high temperature ≥35°C during its reproductive phase (flowering and pod development) resulting in severe yield losses due to the direct effect of high temperatures on different physiological processes (Stoddard *et al*., 2006). Due to increase in 1°C of seasonal temperature there is decrease in 53 kg ha^{-1} of chickpea yield occurred (Kalra *et al.,* 2008).

Identification and development of high temperature tolerant genotypes is an important aspect of chickpea breeding. It can be done by studying the genetic variability present in the germplasm lines of chickpea. PCV, GCV, heritability and genetic advance are important selection parameters should be used in predicting the ultimate effect for selecting high temperature tolerant varieties. Correlation and path analysis determine the association between yield and its components and also bring out the relative importance of their direct and indirect effects, thus proving an understanding of their association with yield. Principal component score strategy has been employed for the identification of a set of accessions which captures grain maximum genetic diversity of the whole collection (Gireesh *et al*., 2017) and has been successfully used in the germplasm evaluation of crops for understanding the relationship and correlation among the variables studied (Zafar *et al*., 2008).

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Considering the above scenario, the present investigation is carried out with the objective of studying the genetic variability, diversity and trait relationship associations in chickpea genotypes for yield improvement along with identification of genotypes showing tolerance mechanism and exhibit minimum reduction in yield in heat stress condition.

MATERIALS AND METHODS

A sixty chickpea genotypes included advance breeding lines, released varieties and genetic stocks taken from pulses section CCS HAU, Hisar, Haryana. The details of experimental materials mentioned in Table 1. The experiment was conducted in randomized block design (RBD) with three replications under two environmental conditions (Normal sown: 13th November, 2017 and late sown: 9th December, 2017) at Research Area of Pulses Section, during *Rabi* 2017-

18 in a plot of 4 m length with spacing of 30 cm × 10 cm for each of the genotype.

The traits *viz*., days to 50% flowering (DF), days to physiological maturity (DM), plant height (PH), number of secondary branches per plant (SB), number of pods per plant (PP), number of seeds per pod (SP), 100 seed weight (SW) and seed yield (SY) were recorded under field conditions. Along with that three physiological parameters namely, Canopy temperature measurement (CTD), relative stress injury (RSI) and total chlorophyll content (CHL) were recorded. The multivariate parameters were estimated using various packages of Rstudio software (R Core Team, 2021). The analysis of variance, correlation, path analysis and regression estimated using 'agricolae' package (deMendiburu and Yaseen, 2020). Principle component analysis carried out using 'factoextra' package (Kassambara, 2017). The corrplot and box plot were constructed using 'corrplot' (Wei, 2010) and 'ggplot2' (Wickham, 2016) packages.

RESULTS AND DISCUSSION

Anova

Analysis of variance indicated that the significant difference existed among the genotypes for all the eleven studied traits evaluated under both the environmental conditions (Table 2).

Table 1: ANOVA among different yield contributing traits in both normal and late sowing condition.

Source of variation		Replication		Genotypes		Error	
Degree of freedom		2		59		118	
Sowing condition		NS	LS	NS	LS	NS	LS
Mean sum of square	DF	2.517	23.172	64.036**	30.046**	2.59	1.974
	DM	2.867	0.517	61.65	18.675**	1.353	0.703
	PH	59.062	58.394	84.163**	124.114**	20.189	33.724
	SB	0.783	3.533	3.631**	0.88**	0.524	0.206
	PP	230.444	422.93	1399.699**	455.17**	104.151	93.905
	SP	0.001	0.042	0.504**	0.299**	0.03	0.022
	SW	0.506	3.717	76.116**	50.405**	1.432	1.044
	CTD	0.056	0.023	0.432**	0.286**	0.013	0.006
	RSI	1.848	0.73	17.764**	19.673**	0.53	0.378
	CHL	0.032	0.025	1.637**	1.088**	0.062	0.022
	SY	30.441	41.872	74.781**	24.16**	8.025	5.471

Table 2: Genetic variability parameters among different yield contributing traits in both normal and late sowing condition.

Traits	Sowing condition	Mean	Range		GCV	PCV	Heritability B.S. (%)	Genetic advance (% of mean)
			Min.	Max.				
DF	NS	91.53	71.67	97.00	4.94	5.25	88.77	9.60
	LS	80.43	68.00	87.00	3.80	4.19	82.58	7.12
DM	NS	131.60	124.00	141.33	3.41	3.52	93.70	6.79
	LS	120.03	110.00	127.00	2.04	2.16	89.50	3.97
PH	NS	84.82	59.80	94.40	5.44	7.60	51.37	8.04
	LS	70.84	52.20	84.33	7.75	11.28	47.19	10.96
SB	NS	6.63	4.33	9.20	15.34	18.83	66.40	25.75
	LS	4.64	3.67	6.07	10.22	14.15	52.21	15.22
PP	NS	73.91	31.73	129.33	28.12	31.33	80.57	51.99
	LS	57.15	16.93	86.00	19.20	25.62	56.19	29.65
SP	NS	1.61	1.11	2.60	24.68	26.92	84.02	46.60
	LS	1.48	1.07	2.15	20.58	22.87	81.03	38.17
SW	NS	18.03	10.67	37.33	27.68	28.46	94.56	55.44
	LS	17.45	12.00	33.67	23.25	23.97	94.03	46.43
CTD	NS	2.25	1.30	2.80	16.66	17.39	91.76	32.87
	LS	1.51	0.90	2.20	20.22	20.89	93.70	40.31
RSI	NS	12.32	7.43	17.27	19.46	20.34	91.55	38.36
	LS	15.09	10.67	22.23	16.80	17.29	94.44	33.64
CHL	NS	3.05	1.80	5.27	23.75	25.12	89.40	46.27
	LS	2.48	1.47	4.30	24.01	24.76	94.07	47.98
SY	NS	15.87	7.00	30.88	29.73	34.68	73.49	52.51
	LS	11.61	3.67	18.00	21.50	29.46	53.24	32.31

This indicated the existence of substantial amount of genetic variability for all studied traits among 60 chickpea genotypes. These ample variations in eleven quantitative traits could be utilized for selection of superior genotypes, improvement in SY through selection, heterosis and combination breeding in chickpea germplasm. Similar findings are reported by Peerzada *et al.* (2014) and Bala *et al.* (2015).

Mean performance analysis

The mean performance of all the studied traits were depicted using boxplot (Fig 1). There is a high difference in flowering traits, DF and DM in both sowing condition. Flowering duration and maturity days more in normal case compared to late sown condition. It can be concluded that in late sown condition plants get less vegetative period as compared to normal sowing condition and this is clearly depicted in seed yield. SY was less in late sown condition. Flowering and podding in chickpea is known to be very sensitive to changes in external environment and drastic reduction in seed yields were observed when plants were exposed to high temperature (Bahuguna *et al*., 2012; Devasirvatham *et al.,* 2012). Moving to physiological traits, CHL and CTD values more in normal sowing condition while RSI lower in normal sown condition. The same behaviour of physiological traits were also observed by Kumar *et al*. (2012). In case of remaining traits, performance of genotypes in normal sown higher than late sown condition.

GCV and PCV

Table 3 showed a small difference between GCV and PCV in all studied traits indicating negligible influence of environment in expression of these traits. The traits *viz,* SY, PP, SW and CHL content all exhibited greater variability under both the environmental conditions. The highest variability was exhibited by SY under both condition normal as well as late sown condition followed by PP and CHL. The highest value of GCV was exhibited by SY followed by PP in case of normal sown condition but under late sown condition highest value was exhibited by CHL content followed by SW. The highest value of GCV indicated that exploitable genetic variability present for the character that can facilitate the selection.

Heritability and genetic advance

The proportion of genetic variability which is transmitted from parents to offspring is known as heritability. The characters *viz* CTD, RSI, DM and CHL exhibited high heritability under both the environmental conditions. But high heritability coupled with high genetic advance was found for the character *i.e*. PP and SW content under normal sown condition whereas under late sown condition it was sown by PP and RSI. So special attention should be given to these characters for selection as they were controlled by additive gene action. The above report is akin with the findings of Dev *et al*. (2017); Paul *et al*. (2018) and Yucel (2020).

Correlation

Correlation is the mutual association between several variables. Under both the environmental condition SY has positive correlation with CTD, CHL, PP, SB, SW, SP and PH (Fig 2). The information on traits association with SY would be additional assistance to plant breeders in deciding the selection criteria and developing effective breeding strategy for evolving high yielding varieties.

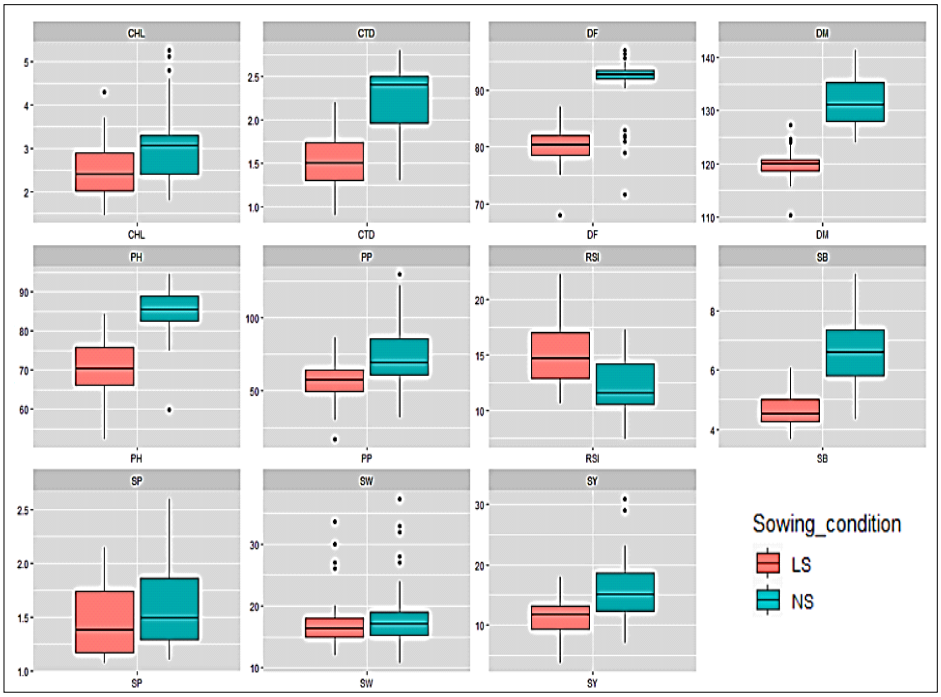

Fig 1: Box-plots showing the variation of the data from the eleven quantitative traits of two seasons evaluated in 60 accessions of chickpea during *rabi* season 2017-18.

Under late sown condition DF exhibited negative correlation this means that this trait by increasing the number in DF this will lead to decline in grain yield. Thus the selection of this trait for improving ST may not be rewarding. RSI exhibited negative association with SY under both the environmental condition this means that lesser the RSI higher will be the seed yield. These all results were in agreement with Jha *et al*. (2012) and Kumar *et al*. (2012).

Regression analysis

Regression analysis of SY showed that SY has positive association with DF, DM, SB, PP, number of seeds per pod, SW, canopy temperature depression, CHL under both the environmental conditions and negative with relative stress injury. Coefficient of determination gives information on how much variation of dependent variable was due to the independent variable, which gave a value of R^2 = 0.88 and

Table 3: Genetic relationships and principal component analysis in elite chickpea (*Cicer arietinum* L.) genotypes for seed yield.

		PC1	PC2	PC3	PC4	PC5
Eigenvalue	NS	5.06	1.74	1.11	0.79	0.75
	LS	4.66	1.58	1.13	0.85	0.63
Proportion	NS	45.97	15.79	10.08	7.18	6.78
	LS	46.65	15.79	11.30	8.46	6.30
Cumulative	NS	45.97	61.77	71.84	79.02	85.80
proportion	LS	46.65	62.44	73.74	82.20	88.49
DF	NS	0.10	-0.76	-0.25	0.43	-0.20
	LS	1.09	0.22	0.74	1.41	0.78
DM	NS	-0.07	-0.86	-0.06	-0.17	-0.22
	LS	-0.06	0.78	-0.29	0.32	-0.34
PH	NS	0.44	0.24	-0.63	0.43	0.31
	LS	0.35	-0.66	0.32	-0.01	-0.53
SB	NS	0.82	0.09	0.11	0.27	-0.14
	LS	0.68	0.12	0.16	0.56	-0.16
PP	NS	0.82	0.29	0.10	0.13	-0.33
	LS	0.75	-0.35	0.07	0.31	0.14
SP	NS	0.46	-0.33	0.55	0.26	0.41
	LS	0.36	-0.23	-0.77	-0.28	-0.29
SW	NS	0.59	-0.30	-0.45	-0.40	0.35
	LS	0.31	0.54	0.54	-0.45	-0.23
CTD	NS	0.74	0.20	-0.24	-0.20	-0.27
	LS	0.91	0.13	-0.13	-0.04	0.10
RSI	NS	-0.93	0.02	-0.04	0.12	0.13
	LS	-0.93	-0.03	0.04	0.13	-0.07
CHL	NS	0.81	-0.08	0.23	-0.16	0.21
	LS	0.89	0.11	0.03	-0.10	0.18
SY	NS	0.94	-0.14	0.10	-0.06	0.02
	LS	0.89	0.15	-0.06	-0.12	0.03

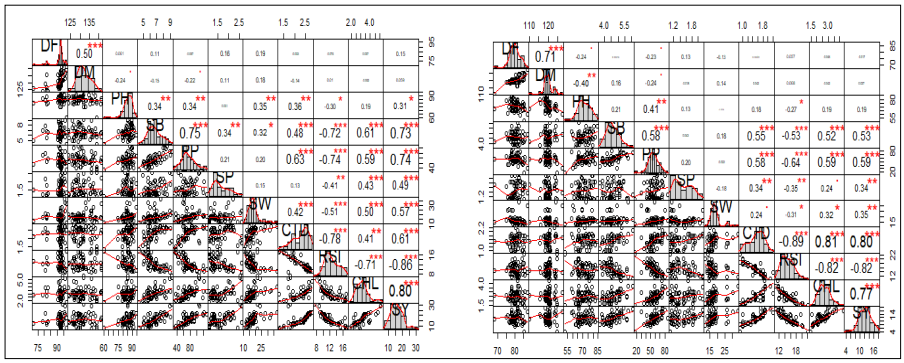

Fig 2: Correlation coefficient analysis of yield and yield attributing traits in *Cicer arietinum* L. accessions under normal and late sown conditions.

0.75, indicating 88 and 75% variation of the yield parameter was due to the independent traits under normal as well as late sown conditions, respectively. The regression equation is as follow:

SY at normal sown condition =

-15.83 + 0.02(DF) + 0.11(DM) + 0.01(PH) + 0.29 (SB) + 0.07(PP) + 1.98 (SP) + 0.18 (SW) + 0.23(CTD) - 0.43(RSI) + 1.73(CHL).

SY at late sown condition =

-0.37 + 0.04(DF) + 0.01(DM) - 0.02(PH) + 0.23(SB) + 0.04(PP) + 1.16(SP) + 0.13(SW) + 2.49(CTD) - 0.24(RSI) + 0.74(CHL).

Path coefficient analysis

Under both the environmental conditions, the maximum positive directs effects on SY were exhibited by traits *viz*., total chlorophyll content, canopy temperature depression, number of seed per pod, SW, number of pod per plant and SB (Fig 3). Therefore, selection for these will likely to bring about improvement in grain yield. Maximum indirect effects on SY were exhibited by SB (0.2522) through CHL under normal sown condition, whereas in case of late sown conditions by number of secondary branches (0.2479) through RSI under both the environmental conditions.

A residual effect on dependent variable measures the error and unexplained variance by other possible independent variables. Residual effects were observed 0.162 and 0.226 in normal and late sown conditions for genotypic path coefficient analysis which is too low.

PCA analysis

In present investigation, PCA was performed for yield and its contributing traits in chickpea in which principal components greater than one Eigen value were selected for interpretation (Kaiser, 1958 and Jeffers,1967). Here, PCA showed the 85% total variability was explained by first five PC in both the sowing condition (Table 3). In case of normal sown, PC1 contributed for 45.97% of the total variation with SY having the highest positive and RSI with the highest negative loadings. PC2 accounted 15.79% of the total variation with PP having the highest positive and DM with the highest negative coefficients. Furthermore, 10.07% variation was explained by PC3 with SP and PH, having highest and lowest loading, respectively. The remaining two PC, *i.e.,* PC4 and PC5 were captured 7.17% and 6.77% variability. While, in case of late sown, PC1 account for 46.64% variation to the total variation with SY having the highest positive and RSI with the highest negative loadings. Moreover, 15.79% of the total variation accounted by PC2 with DM having the highest positive and PH with the highest negative coefficients. The remaining three PC, *i.e.,* PC3, PC4 and PC5 were captured 11.29%, 8.45% and 6.29% variability. The PC3 having the highest positive loadings for SW while, the highest negatives loadings for SP. The comparison of PC1 from both the sowing condition showed that, the highest and lowest loading present in traits, SY and RSI. Thus, it can be concluded that, these two traits tend to be very important. The results were in support with the Jha *et al*. (2015).

In both the sown condition, higher vector length was observed in most of traits indicating the presence of large variability, while SP, PH and SW in case of normal sown and SP in case of late sown had smaller vector length indicating low variability (Fig 4). The vector of SY and RSI were diverging and form a large angle (close to 180°), which indicating they were negative correlated in both the sowing condition. The vector of SY meet vectors of DF, DM and PH almost at 90° indicating non-significant or low negative association with these traits. Conversely, vectors of remaining traits forming a small angle means they were positively correlated. Same situation was observed in both sowing condition. These relationships of traits present in PCA biplot were in accordance with the pearson correlation values. Based on PCA results it found that genotype *viz*., HC-5, H04-87, GNG 2267 under normal sown conditions and HC-1302, CSG 8962 and HC 5 under late sown condition have highest value of seed yield. Genotype *viz*., H 08-71, H07-120 and H 07-75 under normal sown conditions and H 08-18 and H 08 71 under late sown conditions have higher value of number of pod per plant.

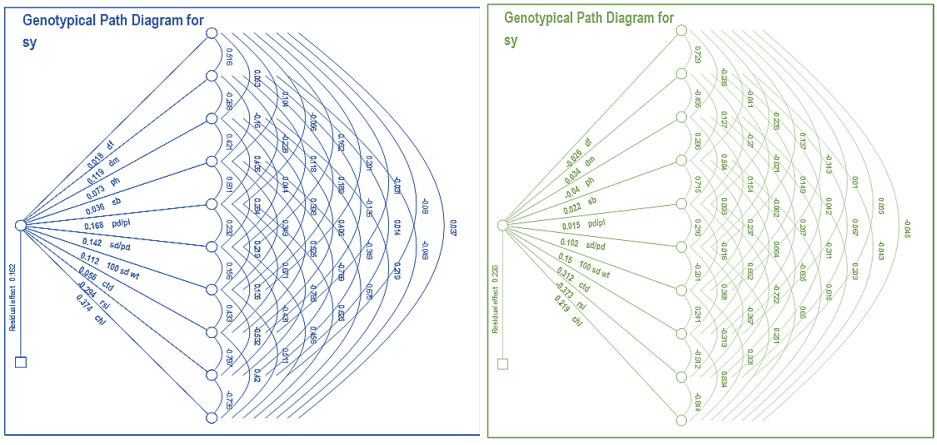

Fig 3: Path diagram for SY in *Cicer arietinum* L. accessions.

Cluster analysis

D^2 analysis grouped the germplasm lines into eight clusters under normal sown conditions and into seven clusters under late sown conditions (Fig 5). Under normal sown conditions, cluster I contain maximum number of 19 genotypes, whereas cluster II comprises 14, cluster III-5, cluster IV-6, cluster V-9, cluster VI-2, cluster VII-1 and cluster VIII have 4 genotypes but under late sown condition, cluster I comprises of 39 genotypes, cluster II-9 genotypes and cluster VI-2 genotypes whereas cluster III, cluster V and cluster VII comprises only 1 genotype this indicate huge variability also exist among the genotypes under both environmental conditions. The maximum intra cluster distance was found between the cluster V under normal sown condition whereas it was shown by cluster IV. The maximum inter cluster distance was observed between cluster IV and cluster VII. But under late sown condition it was observed between cluster VI and cluster VII. This indicated that genotypes present in this cluster group were highly divergent from each other implying large amount of diversity within and between groups, which could be exploited in breeding programmes. Genotypes GL 12003 and GNG 2300 would be consider as very rewarding on the basis of cluster mean analysis under normal sown condition because these genotypes present in cluster VI showed highest value of cluster mean of different traits. In case of late sown condition, genotype GNG 1958, GL 120 21 and HC 3 would be considering very best as these

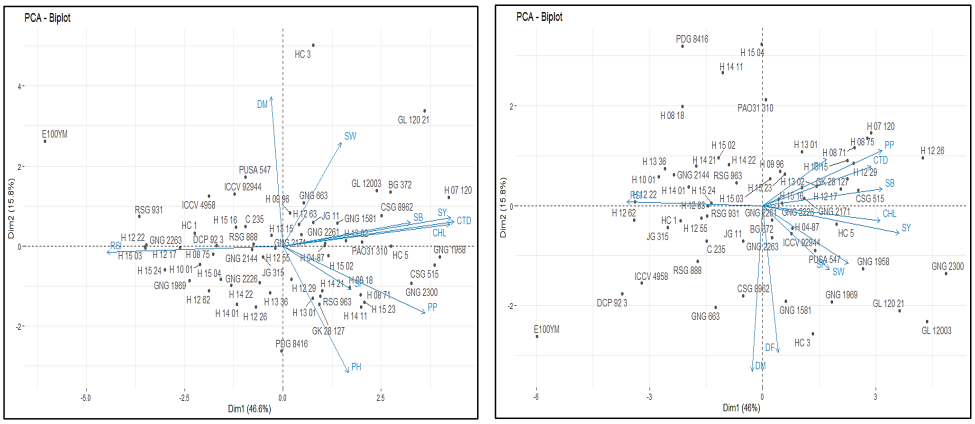

Fig 4: PCA biplot constructed based on eleven morpho-physiological traits left under normal sown condition (right) late sown condition.

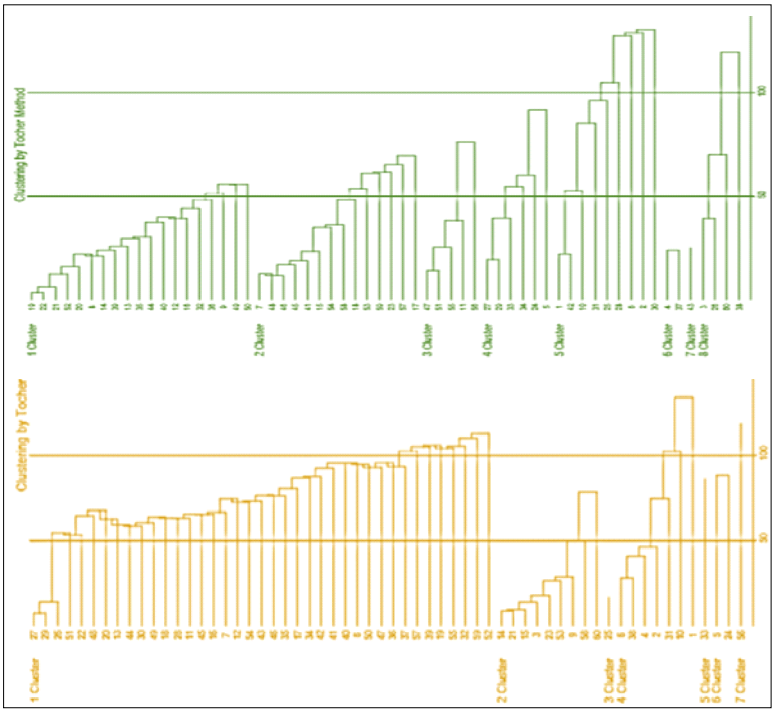

Fig 5: Grouping of 60 genotypes of *Cicer arietinum* into different clusters based on yield and yield contributing traits by D^2 analysis (upper side) under normal sown conditions (lower side) late sown conditions.

genotypes were present in cluster V and cluster VI having highest value of cluster mean for different traits. D^2 statistics has been used in several crops for identifying diverse parents for hybridization programmes. These results were in agreement with Verma and Walia, (2013).

CONCLUSION

The present study revealed high level of genetic variability exist for traits *viz*., SY, DF, DM, PH, PP, SP, SW, under normal sown conditions as compare to late sown conditions. Based upon the mean value it was found that genotypes *viz* H 12-29, H 12-55, H 12-17, H 08-18, H 07-120, HC 1, HC 5 and HC 3 showed less reduction in yield hence can be consider as more tolerant. SY exhibit positive and highly significant association with CTD, CHL, SB, SW, PP, SP under both the environmental conditions. Therefore, selection would be effective for increasing the yield potential of traits. Highest direct positive effects on yield were exerted by CHL, PP, CTD and SP but negative direct effects were exerted by RSI, hence the direct selection under these traits would be effective for increasing the yield of plants. PCA identified SY, PP, CHL, DF, DM, DF, in different principal components similar to D^2 statistics playing a prominent role in classifying the variation existing in the germplasm genotypes. Thus, the results of the present study revealed high level of genetic variation existing in the population along with the traits contributing to this diversity, which can find immense applications in chickpea improvement programme.

Conflict of interest

All authors declared that there is no conflict of interest.

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