Indian J. Genet. Plant Breed., (2023); 83(4): 526-534
https://doi.org/10.31742/ISGPB.83.4.9
ISSN: 0975-6906
www.isgpb.org

RESEARCH ARTICLE

Unclenching the potentials of global core germplasm for root nodulation traits for increased biological nitrogen fixation and productivity in chickpea (*Cicer arietinum* L.)

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Abstract

Chickpea being an important food legume crop is able to fix the atmospheric nitrogen and form root nodules that support biological nitrogen fixation- a sustainable alternative for nitrogen supply to agriculture worldwide. In order to support findings on nodule formation in chickpeas, a diverse core set of 300 chickpea genotypes, including four checks, was evaluated for morphological and nodulation traits in four different environments. The maximum genotypic and phenotypic coefficient of variation was observed for a number of nodules, nodule fresh weight, shoot fresh weight, and number of seeds per plant. The heritability for most of the characters ranged from 26.56 for days to 50% flowering to 99.61 for a number of pods per plant. The genotypic and phenotypic correlation coefficient analysis revealed that the number of nodules was positively correlated with seed yield. Further, seed yield was partitioned into direct and indirect effects at genotypic and phenotypic levels through path coefficient analysis. Significant genotypic correlations and direct positive effects were exhibited by a number of nodules and number of pods per plant on grain yield. Based on the D^2 statistics, the number of seeds per plant, number of pods per plant, shoot fresh weight and number of nodules have been identified as top-ranking yield contributing traits. The genotypes ICC1013 and ICC16569 for a number of nodules and ICC1049 for the trait number of seeds per plant were identified as potential stable donors for the respective traits with high mean yield, heritability and genetic advance across the locations and can be used as donors in the chickpea breeding programs for increasing biological nitrogen fixation and enhancement of the crop productivity.

Keywords: Chickpea, correlation, nodulation traits, nitrogen fixation, path analysis

Introduction

India being the largest producer of chickpeas produces 70% of the total world production having around 9.21 mha area under cultivation and 8.88 mt production (FOASTAT 2021). Chickpeas are valued for their high (18.89-28.75%) dietary protein, 40% carbohydrates, fibers and 3 to 6% fats (Pushpavalli et al. 2015). Given its high nutritional content, market value, adaptability and nitrogen fixation ability, chickpea is being increasingly recognized as a staple "food crop of the future". The predicted genome size of chickpeas is approximately 738 Mb with 28, 269 genes (Varshney et al. 2013). Root nodule symbiosis (RNS) is a metabolism-dependent most successful symbiosis on the earth. Leguminous plants absorb nitrogen directly from RNS at the expense of photosynthate (Werner et al. 2015). Initiation of root nodulation occurs when modulation factor (NF) signals are secreted by the rhizobia and are perceived by root hairs curling followed by initiation of cell division and nodule primordium formation which finally develops into the new organ called 'nodule'. Nodulation factors NFR1 and NFR5 have been identified, cloned and phylogenetically

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How to cite this article: Chandana B.S., Mahto R.K., Singh R.K. Singh K.K., Kushwah S., Lavanya G.R., Tripathi S., Hegde V.S. and Kumar R. 2023. Unclenching the potentials of global core germplasm for root nodulation traits for increased biological nitrogen fixation and productivity in chickpea (*Cicer arietinum* L.). Indian J. Genet. Plant Breed., **83**(4): 526-534.

Source of support: DST-SERB, Govt. of India, project No. CRG/2019/006273

Conflict of interest: None.

Received: June 2023 **Revised:** Oct. 2023 **Accepted:** Nov. 2023

characterized showing significant homology with chickpea NFR receptors (Palaka et al. 2021) and chickpeas are nodulated by chickpea-compatible *Meso-rhizobium* strains (Wanjofu et al. 2022). Some of the genes involved at different stages in root nodule formation have been characterized in *L. japonicus*, *Medicago truncatula* and *Glycine max*. *However,* the only disadvantage is that except *Glycine max* most of these model legumes are not crop species (Endre et al. 2002; Madsen et al. 2003; Radutoiu et al. 2003).). In spite of being the most important grain legume in tropical and subtropical countries (Jukanti et al. 2012) information on the chickpea nodule development is quite limited (Mandal and Sinharoy 2019) and most of the studies were on rhizobium instead of molecular and genomic regions controlling nodulation based on a limited number of chickpea genotypes. However, some of the significant studies based on root nodule transcriptome resulted in development of many genic SSR markers and candidate genes for nodulation in chickpea (Kant et al.2016). Chickpea lines PM233 and PM405 have been undertaken to find out the genes for the nodulation namely RN1 and RN4 respectively through mutation (Frailey et al. 2022).

Further, seed-to-seed generation time in chickpeas is short (85 to 100 days), which makes it even more suitable for future studies (Upadhyaya et al. 2007). There are some studies on very limited studies that identified potential genotypes for nodulation in chickpea (Plett et al. 2021). There is an urgent need for the development of high nodule chickpea genotypes that can produce higher yield per unit area (Singh et al. 2016). However, till date, the Indian and global core germplasm collections of chickpeas have been negligibly used for the identification of nodulation-specific genomic regions. Thus, execution of a systematic and fully designed phenotyping approach is required for the evaluation of chickpea germplasm for identifying new donors for high nodulation traits and in this study, a set of 300 chickpea germplasm lines including checks was evaluated to identify new sources for high nodulation.

Materials and methods

Planting material and experimentation

A set of 300 diverse chickpea germplasm (Supplementary) consisting of exotic lines, landraces, and global germplasm lines including checks was used for developing an association panel. The Experimental trials of the Association panel were conducted during 2020-21 rabi season at four environmental locations namely; IARI, New Delhi (*28°38'24.0252" N latitude, 77°10'26.328"* E longitude and 228.6 m AMSL) having sandy clay loam soils. SHUATS, Allahabad (25°24'41.27" N latitude, 81°51'3.42"E longitude and 98 m AMSL) with a soil type of clay loam to sandy loamy. RPCAU, Samastipur (25°86'29.679" N latitude, 85°78.10' 263" E longitude and 52 m AMSL) with sandy loamy soil and IARI Regional Station, Pusa, Bihar (25°54'56.16" N latitude, 85°40'24.956" E longitude and 52 m AMSL) with alluvial soils. Each genotype was grown with a row-to-row distance of 60 cm following augmented randomized complete block design with repeated checks namely; BG372, BG3022, BG547 and BG1053. Plant materials were harvested after the pods reached physiological maturity and were completely dried in the field. The list of the recorded traits for each randomly selected five plants for each genotype includes Days to 50% flowering (DFF), plant height (PH) in cm, number of primary branches (PB) and secondary branches (SB), number of pods per plant (NOP), number of seeds per plant (NOS) and yield per plant (yield), number of nodules per plant (NON), nodules per plant (NON), nodule fresh weight (NFW in gram) and root fresh weight (RFW in gram) root dry weight (RDW in gram), Stem fresh weight (SFW in gram), stem dry weight (SDW in gram). The phenotyping for nodulation traits was done by uprooting whole plants without disturbing root systems at 60 days after sowing, which is the optimum stage for nodule phenotyping in legumes (Fig. 1). The root nodules were counted on five uniform plants by washing the roots followed by immediate transfer to white butter paper for measuring nodule fresh weight for genotype wise each plant. Further, these uprooted plants were stored in an oven at 50°C for 15 days for further drying to measure nodule dry weight. The genotype-wise yield and related data were taken on five uniform plants similar to uprooted ones from the same row and pods were weighed.

Phenotypic data analysis

The data was analyzed through R software (version 4.2.0**).** The Genotypic and phenotypic coefficient of variations were calculated using the method suggested (Burton 1953) and were categorized as low, moderate and high (< 10% = Low, 10 to 20% = Moderate, and > 20% = High) by following. The heritability in a broad sense by Allard (1960) and was categorized as (0-30% = Low, 31 to 61% = Medium, 61 to 100% = High) as suggested (Robinson et al. 1949). The expected genetic advance as a percentage of mean were calculated and categorized as low (< 10%), moderate (10–20%) and high (> 20%) as suggested (Johnson et al. 1955). Analysis of variance was done as per (Panse and Sukhatme1978). The genotypic (r_g) and phenotypic (r_p) correlation coefficients were calculated by adopting the procedure (Miller et al. 1958). The path analysis was done as suggested (Wright 1921); Dewey and Lu 1959). Diversity analysis was done as per (Mahalanobis 1936) and the genotypes were grouped into different clusters according to Tocher's method as described (Rao 1952).

Results and discussion

Analysis of variance

The analysis of variance revealed that mean sum of squares due to genotypes was significant for all the fourteen traits

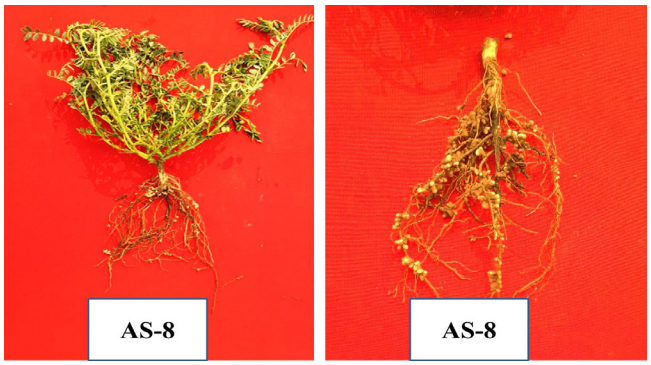

Fig. 1. Phenotyping for number of nodules

under study for the locations. Similar results were found in chickpeas for days to flowering (Nunavat et al. 2022), shoot /root fresh weight (Jha et al.2023), nodule fresh/dry weight and yield (Istanbuli et al. 2022). The GCV, PCV, heritability in broad sense and percent genetic advance over mean for all the traits under study mean, range, standard error, CV are presented in . The mean values ranged for days to flowering 79.31 to 90.79, days to maturity 129.20 to 150.89, number of nodules 4.89 to 51.26, plant height 25.02 to 50.19 cm, number of primary branches 1 to 3, number of secondary branches 4.2 to 10.4 with a mean of 6.7, shoot fresh weight 16 to 80.33 g, shoot dry weight 7.55 to 18.11 g, root fresh weight 3.23 to 11.60 g, root dry weight 1.34 to 5.38 g, number of pods per plant 7.55 to 96.33, number of seeds 14.31 to 104.31and yield per plant 12.38 to 23.56 g.

The high genotypic and phenotypic coefficient of variations were observed for primary branches, nodule fresh weight, root dry weight, number of seeds per plant and number of nodules indicating less amenability of these traits to environmental fluctuations. Hence, greater emphasis should be given to these characters, while selecting genotypes from the present material to be utilized in future crop improvement programs. High GCV for the number of seeds per plant and number of nodules fresh weight was also reported earlier (Priyadarshini et al. 2017). The heritability for most of the characters ranged from 26.56 to 99.67 for days to flowering and root fresh weight respectively. The heritability estimates were recorded high for all the traits under study except for days to flowering and days to maturity suggesting that except these two characters other characters are highly heritable from one generation to another and least influenced by environmental factors. Similar results were also reported earlier (Younis et al. 2008). The expected genetic advances were high for the number of pods per plant, number of seeds per plant, number of nodules, nodule fresh weight and moderate for shoot fresh weight. High heritability coupled with high genetic advance over mean was observed for nodulation traits (Girma et al. 2023). High heritability coupled with high genetic advance over means were observed for most of the traits under study which may be due to additive gene actions and can be considered as favorable attributes for crop improvement through selection without progeny testing. The estimates of the phenotypic coefficient of variation (PCV) in general were higher than the estimates of the genotypic coefficient of variation (GCV) for all the characters which suggested that the apparent variation is not only due to the genotypes but also due to the influence of environment.

Correlation and path analysis

The correlation coefficient analysis helps to understand the nature and magnitude of the interaction between various quantitative traits to determine the component traits on which selection can be based for genetic improvement in yield. The corresponding phenotypic and genotypic correlation coefficients of nodulation and seed yield have been presented in Table 2. The results of correlation coefficients revealed that nodule fresh weight and nodule dry weight were positively and significantly correlated with yield plant^{-1} at genotypic and phenotypic levels. These results are in accordance with Elias et al. (2009).

This indicated that the development of effective and promising nodules of chickpeas was due to uptake of atmospheric nitrogen through the process of biological nitrogen fixation which ultimately enhanced the final yield of chickpeas. Moreover, the positive and significant association between seed yield plant^{-1} and different

Table 1. Estimates of genetic parameters for quantitative traits in chickpea

Traits	DFF	DTM	PH	PB	SB	NON	NFW	SFW	SDW	RFW	RDW	NOP	NOS	Yield
Mean	84.37	137.99	37.70	2	13.5	13.52	326.34	31.02	12.29	5.19	2.41	49.44	55.70	17.43
Range	79.31-90.79	129.20-150.89	25.02-50.19	2-4	4-21	4.89-51.26	56.6-10 96.6	16.00-82.33	7.55-18.01	3.24-11.60	1.34-5.38	7.98-96.33	14.31-104.31	12.38-23.54
CV	4.53	6.71	20.04	18	15	64.73	4.56	47.15	41.31	56.07	65.42	62.53	55.65	29.17
GCV	35.33	6.43	26.92	86.43	38.54	55.36	77.82	50.63	45.84	61.57	64.58	60.11	61.04	32.22
PCV	11.45	9.07	27.82	86.60	38.85	55.71	78.68	50.77	46.52	61.67	64.862	61.57	61.19	36.66
h^2 (Broad Sense)	26.56	50.2	93.64	99.61	98.41	98.74	97.83	99.46	97.1	99.67	99.15	95.26	99.53	97.62
GA	3.35	13.37	22.28	6.94	10.65	32.48	50.44	32.39	7.51	2.51	0.608	87.01	84.61	12.58
GAM	6.27	9.40	53.67	177.70	78.75	113.33	158.57	104.02	93.05	126.63	132.482	120.83	125.46	73.72

Table 2. Estimation of genotypic and phenotypic correlation coefficients in chickpea

Traits	DFF	DM	PH	PB	SB	NON	NFW	SFW	SDW	RFW	RDW	NOP	NOS	YPP
DFF	1.000	0.076	-0.001	-0.165*	-0.074	-0.023	-0.141*	0.152*	-0.187*	0.035	-0.111	-0.104	-0.119	-0.014
DM	0.1049	1.00	-0.176*	-0.194*	-0.015	0.044	-0.132	-0.023	-0.215*	-0.088	-0.159*	0.055	0.042	0.167*
PH	-0.003	-0.403**	1.000	0.059	0.092	-0.035	0.021	0.180*	0.209*	0.057	0.163*	-0.045	-0.022	-0.036
PB	-0.197*	-0.427**	0.057	1.000	0.626**	0.053	0.093	0.066	0.221*	0.109	0.230*	0.127	0.128	-0.042
SB	-0.081	-0.065	0.097	0.636**	1.000	0.069	0.044	0.028	0.105	-0.061	0.117	0.020	-0.018	0.041
NON	-0.022	0.092	-0.035	0.052	0.069	1.000	-0.082	0.054	0.027	0.361**	0.365**	0.550**	0.477**	0.719**
NFW	-0.162*	-0.292**	0.020	0.093	0.045	-0.082	1.000	0.079	0.586**	0.135	0.197*	0.169*	0.217*	-0.072
SFW	0.170*	-0.028	0.182*	0.067	0.030	0.0542	0.079	1.000	0.015	0.048	0.069	0.217*	0.245**	0.008
SDW	-0.209*	-0.475**	0.210*	0.221*	0.107	0.027	0.586**	0.015	1.000	0.218*	0.537**	0.095	0.136	-0.033
RFW	0.039	-0.184*	0.058	0.110	-0.062	0.363**	0.135	0.048	0.218*	1.000	0.639**	0.416**	0.401**	0.521**
RDW	-0.123	-0.365**	0.165*	0.231*	0.119	0.365**	0.198*	0.0692	0.538**	0.641**	1.00	0.358**	0.333**	0.452**
NOP	-0.115	0.112	-0.045	0.128	0.021	0.553**	0.169*	0.217*	0.095	0.417**	0.359**	1.000	0.941**	0.619**
NOS	-0.142*	0.084	-0.021	0.128	-0.0164	0.480**	0.219*	0.247**	0.136	0.403**	0.336**	0.947**	1.000	0.523**
YPP	-0.012	0.356**	-0.038	-0.042	0.0409	0.724**	-0.0737	0.009	-0.034	0.523**	0.454**	0.622**	0.529**	1.000

Note 1: Upper diagonal values are phenotypic and lower diagonal values are genotypic correlation coefficients
2. Asterisks (*) indicate as *= Significance at 5%, **=Significance at 1%, ***=significance at 0.01%

Table 3. Direct and indirect genotypic effects of component characters on grain yield

Traits	DFF	DM	PH	PB	SB	NON	NFW	SFW	SDW	RFW	RDW	NOP	NOS	rgYPP
DFF	0.008	0.001	0.001	-0.002	-0.001	0.000	-0.001	0.001	-0.002	0.000	-0.001	-0.001	-0.001	-0.012
DM	0.054	0.523	-0.210	-0.223	-0.034	0.048	-0.152	-0.015	-0.248	-0.096	-0.190	0.058	0.044	0.356**
PH	-0.006	-0.070	0.173	0.010	0.017	-0.006	0.003	0.031	0.036	0.010	0.028	-0.008	-0.003	-0.038
PB	-0.019	-0.042	0.005	0.099	0.063	0.005	0.009	0.006	0.022	0.011	0.022	0.001	0.012	-0.042
SB	0.005	0.004	-0.006	-0.040	-0.063	-0.004	-0.002	-0.001	-0.006	0.003	-0.007	-0.001	0.001	0.040
NON	-0.009	0.037	-0.014	0.021	0.028	0.408	-0.033	0.022	0.011	0.148	0.149	0.225	0.196	0.724**
NFW	-0.012	-0.021	0.001	0.007	0.003	-0.006	0.074	0.005	0.043	0.010	0.014	0.012	0.016	-0.073
SFW	-0.015	0.002	-0.016	-0.006	-0.002	-0.004	-0.007	-0.090	-0.001	-0.004	-0.006	-0.019	-0.022	0.009
SDW	0.021	0.049	-0.021	-0.023	-0.011	-0.002	-0.060	-0.001	-0.103	-0.022	-0.055	-0.009	-0.014	-0.034
RFW	0.007	-0.036	0.011	0.022	-0.012	0.072	0.027	0.009	0.043	0.198	0.127	0.082	0.080	0.523**
RDW	-0.038	-0.114	0.051	0.072	0.037	0.115	0.062	0.021	0.169	0.201	0.314	0.113	0.105	0.454**
NOP	-0.052	0.051	-0.020	0.058	0.009	0.252	0.077	0.099	0.043	0.190	0.163	0.456	0.431	0.622**
NOS	0.045	-0.026	0.006	-0.040	0.005	-0.153	-0.069	-0.078	-0.043	-0.128	-0.107	-0.301	-0.318	0.529**
Partial R^2	0.000	0.186	-0.006	-0.004	-0.002	0.296	-0.005	-0.008	0.003	0.104	0.143	0.283	-0.168	-

Note: Asterisks (*) indicate as*= Significance at 5%, **= Significance at 1%, ***=significance at 0.01%

parameters of nodule suggested that seed yield can be enhanced through direct selection of these traits. Similar results were obtained for a number of nodules by (Hazra et al. 2021). In the current investigation, the genotypic correlations for most of the traits were slightly higher than their corresponding phenotypic correlations which would be beneficial in a selection of traits because they exclude the environmental influences. It also revealed significant and positive correlation values for seed yield with the number of pods per plant, seeds per plant, shoot fresh weight, days to flowering and days to maturity (Roy et al.2019). However, negative correlation values for seed yield with shoot dry weight and nodule fresh weight were observed. Significant and positive correlations were observed for the trait SY with NPB, NSB, NPP, indicating that the seed yield directly depends on these traits. Earlier studies have also reported that the yield per plant was closely related with the number of pods per plants (Barmukh et al. 2011). The positive significant correlation in these traits implicate that in addition to SY, the other traits can also serve as good criteria to select the genotypes for high yield.

The genotypic and phenotypic correlation coefficients (Table 3) of the fourteen quantitative characters with grain yield were partitioned into direct and indirect effects at phenotypic and genotypic levels through path coefficient analysis. Among all component traits the RDW (0.49), NON (0.40), RDW (0.207), RFW (0.20), SB (0.11), DTM (0.11), PH (0.11) showed directpositive effect on yield, while NOS (-0.22), PB(-0.18), SDW(-0.15), SFW(-0.07), DFF(-0.01) reflected direct negative effect at phenotypic level. However, at genotypic level among all the component traits the DTM (0.52) has exhibited the highest direct and positive effect on grain yield followed by NFW (0.45), NON (0.40), RDW (0.31), RFW (0.19), PH (0.17) and PB (0.09). The estimate of residual effect being very low magnitude (0.21) explains most of the variability for the trait grain yield. The traits such as SB and SDW had positive correlation with yield plant^{-1}, although they had direct negative effect on yield plant^{-1} with the values (-0.063), (–0.10). Plant height showed a low negative direct effect on grains yield plant-1 but the correlation co-efficient was significantly positive. Similar results were reported by several others (Singh et at al. 2022) and it indicated that the number of characters chosen for the study were very much appropriate for yield determination in the present study. Thus, path analysis indicated that the number of characters chosen for the study were very much appropriate for yield determination. Hence, the selection of genotypes based on these characters as selection criterion would be helpful in

Table 4. Clusters for a set of association panel comprising of 100 genotypes in chickpea

Cluster group	No. of genotypes	List of genotypes
Cluster 1	80	ICC 111,ICC 440, ICC 619, ICC7200, ICC7235, ICC7269, ICC3684, BG 547, ICC13185, ICC 1083, ICC 1172, ICC3093, ICC6579, ICC6995, ICC7167, ICC7167, ICC13185, ICC14002, ICC16069, ICC 2, ICC42, ICC 440, ICC 442, BG 1053, ICC 1009, ICC 1013, ICC 1026, ICC 1043, ICC 1049, ICC 1052, ICC 1059, ICC 1069, ICC 1093, ICC 1118, ICC 1122, ICC 1124, ICC 1127, ICC 1128, ICC 1164, BG 372, ICC7200, ICC7235, ICC7269, ICC7295, ICC7308, ICC7315, BG3022, ICC7744, ICC7818, ICC8265, ICC1852, ICC1891, ICC1896, ICC2083, BG3022, ICC6661, ICC9085, ICC9137, ICC9032, ICC9175, ICC9242, BGM 547, ICC14566, ICC14787, ICC14881, ICC15014, ICC15061, ICC15103, ICC15186, ICC15452, ICC15657, BG 372, ICC15823, ICC15825, ICC15851, ICC15717, ICC16569, ICC16853, ICC96288, ICC14569.
Cluster 2	10	ICC3631,BG 372,ICC 5,ICC 437,ICC 1145,ICC7185,ICC4638,ICC9002,ICC9362,ICC11378.
Cluster 3	3	ICC 1070, ICC 1098, ICC7737.
Cluster 4	2	ICC 506, BG1053.
Cluster 5	2	BG 372, ICC7764.
Cluster 6	2	ICC 448, ICC 1092.
Cluster7	1	ICC3571.

Table 5. Average intra and inter cluster distances among clusters for a set of 100 genotypes in chickpea

Clusters	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
Cluster 1	1451.84	2882.73	6180.92	2909.57	3056.04	4819.66	4926.16
Cluster 2	2882.73	0.00	12923.30	6778.37	7451.80	1915.10	4714.36
Cluster 3	6180.92	12923.30	688.61	4292.57	4740.28	15549.69	12417.68
Cluster 4	2909.57	6778.37	4292.57	0.00	1674.72	8509.57	3173.10
Cluster 5	3056.04	7451.80	4740.28	1674.72	0.00	11888.56	4054.95
Cluster 6	4819.66	1915.10	15549.69	8509.57	11888.56	0.00	8570.74
Cluster 7	4926.16	4714.36	12417.68	3173.10	4054.95	8570.74	0.00

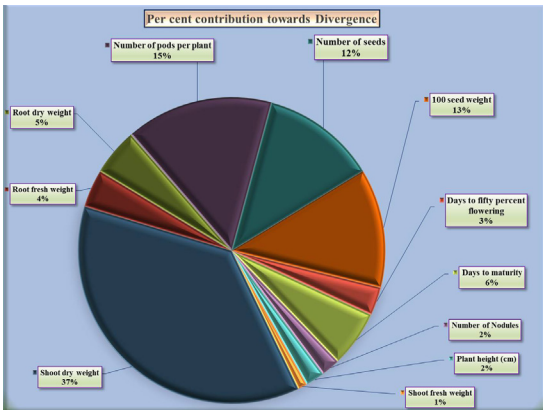

Fig. 2. Per cent contribution of individual traits of a set of association panel towards Divergence in chickpea

improving the seed yield potential of chickpea.

Genetic diversity analysis

Mahalanobis's D^2 statistics is a powerful tool in quantifying the degree of variability at the genotype level. Out of the 300, selected 100 genotypes were used for assessing diversity analysis and enough variability was observed as discussed in the following sections.

Cluster analysis

Based on the D^2 values the 100 genotypes were grouped into seven clusters (Table 4) revealing significant variations for all the characters and germplasm. Cluster I consisted of maximum 80 genotypes, followed by cluster II with 10 genotypes, cluster III with 3 genotypes remaining each cluster with two genotypes and cluster VI with one genotype. Cluster I contained maximum genotypes indicating narrow genetic divergence among themselves. The near uniformity observed in the cluster I might be either due to similarity in the base population from which they were evolved or unidirectional selection for one particular trait or a group of linked traits may produce similar phenotypes which can be aggregated into one cluster irrespective of their geographic origin (Parashi et al. 2013).

Intra and Inter cluster values of 7 clusters

The intra cluster distance ranged from 0.00 to 1451.84. The maximum intra-cluster distance was found in cluster I (1451.84) followed by cluster III (688.61) as indicated in Table 5. In the present study the highest inter-cluster distance (15549.69) was found between clusters III and VI followed by12923.30 between clusters II and III,12417.68between clusters II and VII, and 11888.56between the clusters V and VI**.** These clusters are quite divergent from each other and the genotypes belonging to them can be used for hybridization program as crosses between genotypes belonging to the clusters with maximum inter cluster distances may give high heterotic response resulting in better recombinants. Thus, hybridization programs involving genetically diverse parents belonging to different distant clusters would provide an opportunity for bringing together gene constellations of diverse nature and promising hybrid derivatives may be obtained probably due to complementary interaction of divergent genes in parents (Anand and Murthy1968**).**

Cluster means performances

Based on the cluster means performances for 11 characters for 100 chickpea genotypes the cluster I, II and VI varied considerably from the other clusters which are being revealed by the differing cluster means. The genotypes belonging to such clusters possess different genetic architectures as compared to other genotypes of the clusters. The characters like, plant height, number of nodules, shoot fresh weigh, shoot dry weight, number of seeds per plant possess high variability among the different clusters. The 100 genotypes were grouped into 7 clusters on the basis of D^2 values. Cluster I consisted of maximum genotypes (80). The near uniformity observed in the cluster I might be either due to similarity in the base population from which they were evolved or unidirectional selection for one particular trait or a group of linked traits may produce similar phenotypes which can be aggregated into one cluster irrespective of their geographic origin (Parashi et al. 2013). The clusters which are having high inter cluster distance indicate that these clusters are quite divergent from each other and the genotypes belonging to them can be used for hybridization programs as crosses between the genotypes belonging to the divergent clusters with maximum inter cluster distances may give high heterotic response yielding better recombinants. Similar findings have also been reported by many others in case of chickpea (Lal et al. 2001; Dwivedi and Lal 2001).

Per cent contribution of each character for diversity

Maximum expression of genotypes towards diversity was observed for shoot dry weight (36.8), number of pods per plant (15.5), 100 seed weight (12.6), number of seeds (12.1), days to maturity (6%), followed by root dry weight (5%) as presented in the (Fig. 2). Similarly, several others have also reported maximum contribution of number of pods per plant and number of sees per plant towards total divergence.

Stability analysis

In this research we have evaluated an association panel of chickpea in four different environments and recorded observation for various quantitative traits in order to identify the stable genotypes across the locations and stability analysis was carried out by Eberhart and Russel model (1966). The joint regression analysis (Table 6) revealed that variations due to varieties were found to be significant only for the traits number of nodules, plant height and shoot fresh weight. However, the variations due to environments were remarkable for all the traits under

Table 6. Joint regression analysis for nodulation and component traits in chickpea

Sources of Variation / raits	DF	DFF	DM	NON	PH	SFW	SDW	RFW	RDW	NOP	NOS	Yield
Rep.withinEnv.	4	52.21 *	899.43 **	154.68	193.00 *	124.14	211.857**	33.91 **	9.00**	982.39	1727.14	22.58
Varieties	99	21.58	58.26	134.26 **	82.10 *	251.28	25.47 **	6.838	2.048	1039.28	1148.06	24.95
Env.+(Var×Env.)	300	509.56 **	312.22 **	125.80 **	105.75**	927.18 **	236.76 **	11.089**	5.284 **	1728.23 **	1671.692**	37.81
Environments	3	49404.79 **	21336.37 **	4108.22 **	5127.40 **	68324.98 **	20978.28 **	360.77 **	305.972**	63173.47 **	57105.77 **	802.72 **
Var×Env.	297	15.66	99.85	85.57	55.03	246.35	27.256**	7.55	2.24	1107.57	1111.75	30.08
Environments (Lin.)	1	148214.40**	64009.11 **	12324.66**	15382.20**	204975.00 **	62934.83 **	1082.316**	917.915**	189520.400**	171317.30 **	2408.18**
Var×Env.(Lin.)	99	12.55	38.69	82.75	40.51	165.41	61.168**	7.566	2.65	1305.70	975.21	28.52
Pooled Deviation	200	17.05 **	129.13 **	86.12 **	61.66 **	284.016**	10.19 **	7.47 **	2.020 **	998.42 **	1168.22 **	30.56 **
Pooled Error	396	1.00	0.18	2.83	8.69	2.402	2.126	1.75	0.48	5.56	6.93	0.04
Total	399	388.48	249.20	127.90	99.88	759.47	184.34	10.03	4.481	1557.29	1541.76	34.61

Note: Asterisks (*) indicate as *= Significance at 5%, **= Significance at 1%, ***=significance at 0.01%

study. Considering the stability performance of genotypes for different characters across the environments, it was observed that the variance due to non-linear component of environments (pooled deviations) was significant for all the traits under study except for days to 50% flowering. Thus, the results showed that genotypic and environmental main effects were significant indicating that there is more variability present in the germplasm for different traits and also the response of these genotypes will vary across the environments as the environment also had a significant impact on the performance of the genotypes A meticulous perusal of regression coefficients (bi) and their deviations (S2di) indicated that the genotypes ICC1013 and ICC16569for the number of nodules and genotype ICC1049 for a number of seeds per plant showed bi =1 and S2di = 0.00, qualifying the criterion of stability and thus these genotypes were stable across the environments for a number of nodules and a number of seeds per plant respectively. On the other hand, the genotypes namely, ICC1092, ICC718 and BG1053 for the trait number of nodules, ICC6579, ICC7185and ICC14569 for number of pods per plant also had bi =1 but did not match the requirements in terms of S2di = 0.00, although they had good population means very near to average population mean values. Thus, these genotypes can also be considered as potential donors for the respective traits. The results obtained are in accordance with the earlier reports of Babar Manzoor Atta and Tariq Mahmud Shah (2009). In stability analysis, it was found that there is significant genotype and environmental interactions for all the traits under study. Earlier studies also support the high genotypic variability in the landraces showed that it is possible to identify high nodulating genotypes and the differential performance of the genotype under the influence of the environment as reported by others (Arifet al.2021). Even though most of the varieties had b=1 but majority of the genotypes could not satisfy both the parameters such as b = 1 and $Sd^2 = 0$. However, as high mean of yield is also considered to be one of the main selection parameters for the genotypes which are having high number of nodules with b = 1 value.

As discussed, above nodulation traits and the genotypes that are mentioned for better nodulation efficiency are very relevant in present context as global demand for nitrogen fertilizer is predicted to increase 1.4% annually. The loss of billions of dollars in farm profit has drawn attention to the need for alternative sources of N. Chickpeas like other legumes can fix atmospheric nitrogen through root nodule symbiosis on an average of 60 kg/ha under suitable crop growth conditions (Unkovich and Pate 2000) and 19–24 kg/ha under drought stress conditions (Carranca et al. 1999). It also been reported that in case of the pulses-cereals crop rotation, cereals yield 1.5 tonnes more yield per hectare than those not preceded by pulses, Biological nitrogen fixation is also having the importance in soil conservation as one

third of the world's soils are now deemed degraded due to a range of causes including acidification, salinization, erosion, urbanization, and pulses ability to fix the atmospheric nitrogen through root nodule symbiosis helps in restoring the soil health. Chickpea can grow with fewer nutrients than many others, while providing nitrogen, soluble phosphates and other needed compounds to the soils. In the efforts to advocate for and raise awareness about sustainable soil management, it's important to understand the process of biological nitrogen fixation in chickpeas and other pulses whose deep root systems boost their resilience to drought are intrinsically "climate-smart" as they simultaneously adapted to climate change and contribute towards mitigating its effects by boosting soil carbon sequestration capacities. Thus, present findings facilitate sustainable agriculture through identifying potential genotypes viz., ICC- 111, ICC506, ICC7200, ICC363 ICC6995, ICC7167, ICC7305ICC7764, ICC7744, ICC 932, ICC1369 and ICC13696, these genotypes were having for high number of nodules, high number of seeds and higher yield identified in our study. The genotypes ICC1013 and ICC16569 for a number of nodules and ICC 1049 for the trait number of seeds per plant are identified as stable genotypes and can be further used as parents in breeding programs for increasing biological nitrogen fixation and enhancement of crop productivity.

Supplementary material

Supplementary Table S1 with the names of genotypes with their origin is provided, www:isgpb.org

Authors' contribution

Conceptualization of research (RK); Designing of the experiments (RK); Contribution of experimental materials (RK, VSH); Execution of field/lab experiments and data collection (CBS, RKM, RKS, KKS, SK, GRI); Analysis of data and interpretation (CBS, RKM, RKS ST); Preparation of the manuscript (GOS, RKM, RKS, RK).

Acknowledgments

The Corresponding author sincerely acknowledges the DST-SERB, for financial support for the financial support by granting the project CRG/2019/006273 entitled utilization of germplasm for identification of genes/QTLs for nodulation through association mapping in chickpea.

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