

Stability analysis and molecular profiling of Desi chickpea (*Cicer arietinum* L.)

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Abstract

A study was conducted during the 2021 rabi season to investigate and identify stable genotypes of desi Chickpea (*Cicer arietinum* L.). The investigation involved employing 15 SSR primers for screening and assessing thirty genotypes under three distinct sowing dates to evaluate their grain yield performance. The findings highlighted significant variance components associated with genotype, environment, and G x E interactions, signifying variability in both genotypes and environments and variations in performance among different genotypes. Linear components were also notable, suggesting the potential to anticipate variations in genotype performance. Particularly, genotype ICCV-18-1664 exhibited stability for grain yield per plant in favourable environments, while genotypes DC-17-1111 and DC-17-1115 showed stability in unfavourable environments. The dissimilarity index, derived from SSR data clustering, categorized the 30 genotypes into five primary clusters. Genetic structure analysis, based on 15 SSR primer loci, unveiled that the 30 desi chickpea genotypes comprise a blend of four major ancestral components in diverse combinations.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) holds significant importance as a key food legume, globally ranking third after pea and common bean in terms of cultivation. Particularly in India, it stands as one of the most vital pulse crops, occupying a significant position by being cultivated across 9.69 million hectares, with a remarkable production of 11.07 million tons and an impressive productivity of 1142 kg ha⁻¹. This crop plays a crucial role in fulfilling the protein needs of the vegetarian population and contributes to the restoration of soil fertility. In Bihar, it covers an area of 0.58 million hectares, yielding 0.06 million tons, and exhibiting a productivity of 1154 kg ha⁻¹. The performance of crop plants varies based on the region, indicating a lack of stability in the mean performance of crop varieties across different environments (Dasgupta et al. 1987). Consequently, the breeding of crop varieties tailored to the specific climates of regions and focusing on genotypes exhibiting stability across diverse environments becomes an essential endeavour (Yilmaz and Tugay. 1999). Analysing and identifying stable genotypes with consistent high yields across diverse environments necessitates a thorough examination with current environmental conditions influence genotype performance (Tuteja, 2006). The Eberhart and Russell model for genotype-environment (G x E) interactions and yield-stability analysis remains a crucial tool for assessing varietal stability and their suitability for cultivation across various seasons and ecological zones. Advancements in statistical techniques have provided methods to analyse G x E interactions, allowing for the categorization of genotypes based on their responses in different environmental settings (Singh *et al.* 1987).

Molecular markers have emerged as pivotal tools in crop improvement initiatives, facilitating efficient marker-assisted selection of crucial agronomic traits. Understanding the genetic foundation of chickpea varieties holds immense significance, providing breeders valuable insights for future crossing programs and guiding focused efforts to broaden the genetic diversity of chickpea varieties. However, cultivated chickpea exhibits a limited level of genetic polymorphism. The abundant availability of microsatellite markers presents a significant opportunity to assess diversity and leverage diverse lines for constructing genetic maps. Characterizing genetic diversity in plant species is vital, given that they constitute a foundational resource for genetic information that is yet to be fully uncovered. Adapting varieties to environmental fluctuations is a key factor in stabilizing crop production, both regionally and annually.

Consequently, it is crucial to enhance the genetic potential for increased yield while also ensuring production stability. High productivity and stability stand as the utmost desirable traits for any crop variety (Costa et al. 2004). Hence, the primary goal of this study was to assess the impact of G x E interaction on yield and its attributing traits under various sowing date conditions. Additionally, the study aimed to analyse the molecular diversity of all chickpea genotypes, with the intention of leveraging these genotypes in future breeding programs.

MATERIAL AND METHODS

The field experiment took place at the TCA Dholi research farm, RPCAU, Pusa, during the rabi season of 2021, encompassing three distinct sowing dates (E1–15th November, E2–30th November, and E3–15th December). The experimental materials comprised 30 genotypes of desi chickpea. Sowing was carried out using a randomized block design with three replications. The plots were sized at 4m x 1m, and row-to-row and plant-to-plant spacing were set at 30cm and 10cm, respectively. Mean values were utilized for the analysis of variance, and the effects of genotype, environment, and G x E were assessed using the method outlined by Eberhart and Russel (1966).

For the molecular analysis, fresh young leaf samples were collected during the winter season. DNA isolation was conducted using a modified CTAB method. A set of fifteen SSR primers (Table 6) were utilized. The amplifications were carried out in 10 µl volumes using a Thermal Cycler. Each volume contained 1 µl of plant genomic DNA, 1 µl of 5X PCR buffer, 1 µl of (10 mM MgCl₂), 1 µl of 200mM dNTP mix, 1.2 µl of primer, and 0.1 µl of 1U/µl Taq polymerase. The PCR analysis initiated with an initial denaturation temperature of 94°C for 5 minutes, followed by denaturation at 94°C for 30s, annealing at 55°C for 30 s, elongation at 72°C for 1 minute, and final elongation at 72°C for 8 minutes.

Band patterns corresponding to each SSR marker were documented for every genotype. Quantitative scoring of PCR products from SSR analyses was performed to determine the presence (scored as '1') or absence (scored as '0') of amplicons. These scores were then analysed to estimate polymorphism information content and assess genetic diversity among the 30 genotypes based on the data from 15 SSR markers. Nei's Genetic diversity index (h) and Shannon index (I) values were computed using POPGENE Version 1.31 software.

The software DARWIN Version 6 (<http://darwin.cirad.fr>) was employed to conduct clustering based on the dissimilarity index derived from the SSR data matrix. The data was then subjected to factorial and dendrogram analysis. To calculate the genetic structure of the chickpea genotypes, STRUCTURE VERSION 2.3.4 software and the online tool "STRUCTURE HARVESTER"(<http://taylor0.bioogy.ucla.edu/structureHarvester/>) were utilized.

RESULT AND DISCUSSION

The genotypes displayed significant variation across all the analysed traits as observed in the pooled ANOVA for the various studied traits (Table 1). This clearly indicated the presence of genotypic variation in the desi chickpea genotypes under study. Environmental variation was also notable for all the traits, evident from the significant mean squares attributed to the environment, signifying fluctuating environmental conditions. This aligns with similar findings reported by Gazal *et al.* (2013). The significant G x E interaction, including environmental (linear) effects for all the traits, revealed that the average performance of genotypes in terms of yield and other characteristics varied significantly. This variation due to G x E interaction and linear components was also observed by Baker (1969) and Byth *et al.* (1976), demonstrating similar observations regarding the proportion of interaction between G and E through linear regression (bi) in environment indices. The highly significant G x E interaction and linear components for most characters indicated that these characters were unstable and displayed varied expression with changes in the environment. This finding was in line with observations from Langer *et al.* (1979) and Ramagosa and Fox (1993). Additionally, a significant pooled deviation (non-linear) was present for all characters, indicating considerable genetic diversity in the material. The significance of genotype and G-E (linear) components emphasized that genotypes differing slightly from the regression line of unit slope could be identified. Accordingly, these linear responses, namely $bi = 1$, $bi > 1$, and $bi < 1$, were generally observed.

The estimation of environmental indices for fourteen traits among present study showed that E_3 (15th December) revealed positive and the maximum environmental indices for nearly all the important yield contributing traits excepting plant height and number of primary branches per plant. E_1 (15th November) was the favourable environment for the characters *viz.*, plant height, number of primary branches per plant, number of secondary branches per plant, number of pods per plant and nodules dry weight. E_2 (30th November) favourable for the traits plant height, number of nodules per plant, nodule fresh weight, and grain yield per plant.

The stability parameters assessed for diverse traits (Tables 2, 3 & 4) facilitate to recognize genotypes with overall and specific adaption. Studying the stability of yield components is essential in this regard.

Among the 30 genotypes studied, a total of three genotypes exhibited yield stability in favourable and unfavourable environments. In a favourable environment, genotype ICCV-18-1664 displayed below-average stability, while in an unfavourable environment, genotypes DC-17-1111 and DC-17-1115 exhibited stability with above-average ratings. Regarding plant height, genotypes BG-372 and JG315 demonstrated below-average stability, whereas genotypes DC-18-1104, DC-21-1203, DC-21-1107, and ICCV-2012-11 displayed above-average stability. For days to 50% flowering, genotypes DC-18-1104, DC-17-1111, GNG-1558, DMHC-21-1104, and Pant-G-114 showcased below-average stability, while genotypes DC-18-1107, RVG-203, DC-21-1203, DMHC-18-1664, and JG-315 exhibited above-average stability. In terms of days to maturity, genotypes DC-19-1, DC-2011-1211, ICCV-95311, ICCV-201203, and KPG-59 demonstrated below-average stability, while DC-21-1203, DC-17-1111, ICCV-18-1664, BG-3048, JG-315, ICCV-10102, and BG-256 exhibited above-average stability. For the number of primary branches per plant, genotypes DC-2011-1211 and DMHC-21-1108 showed below-average stability. Concerning the number of secondary branches per plant, genotypes DC-21-1107 and ICCV-2012-11 demonstrated below-average stability, whereas genotypes DMHC-21-1108, C-235, and BG-256 exhibited above-average stability. These similar findings reported by Khan *et al.*, 2002.

For number of pods per plant genotypes ICCV-201108 and ICCV-201308 showed below average stability and ICCV-17-1115, GNG-1558, JG-315 and BG-256 shown above average stability. For No of nodules per plant genotype KPG-59 revealed above average stability. For nodules fresh weight. genotype DMHC-21-1108 shown above average stability. For nodule dry weight genotypes DC-2021-1211, DMHC-21-1108, GNG-1558, ICCV-95311 and GNG-2264 showed below average stability and DC-18-1107, ICCV-2012-11, ICCV-95311 and DC-2021-1664 shown above average stability. For root length genotypes RVG-203, ICCV-17-1115, BG-3048 and DC-2021-1664 showed below average stability and genotypes DC-2011-1211, ICCV-95311 and KPG-59 shown above average stability. For root volume genotype ICCV-10102 showed below average stability and genotypes DC-19-1, BG-372, RVG-203, DC-21-1203, ICCV-201308 and ICCV-201203 shown above average stability. For the character 100 seed weight genotype DC-2021-1211 shown above average stability. For harvest index genotype DMHC-21-1104 showed below average stability and genotypes DC-21-1203, ICCV-2012-11, DC-2021-1664 and GNG-2264 shown above average stability (Table 5). None of the genotypes have shown average and absolute stability and none of genotypes found stable for all the traits under study.

Genotyping of 30 desi chickpea genotypes were done based on amplification with 15 SSR marker *viz.*, GA2, GA9, G20, GA21, GA24, GA108, GA117, TA176, GA4, GAA41, GAA50, GAA51, GAA54, GAA60 and TA3 (Table 6). The PIC across markers ranged from 0.14 to 0.85 with an average of 0.50. Maximum PIC was observed with primer GA117 (0.85) followed by GA4 (84), GAA54 (068), GA2 (0.60). PIC value of each of the amplified loci is calculated for each marker and average PIC of all the loci of a marker is considered as a PIC of the marker. For the codominant markers, the PIC value ranges from 0 to 1. Primer GA117 has highest PIC value of 0.86 and primer GA24 has the lowest PIC value of 0.14 (Fig. 1 & 2).

Nei's Genetic Diversity (h) and Shannon Index (I) was calculated to check the genetic diversity among the 30 genotypes based on 15 SSR marker data. The h and I value were calculated using the software POPGENE Version 1.31. The mean h and I among 30 genotypes of desi chickpea were observed as 0.318 and 0.446 respectively. The value of 'h' ranges from 0–1 with no diversity as 0 to completely diverse as 1. The h value of 0.318 which was observed between 30 genotypes revealed that the genotypes under study are moderately diverse. Dissimilarity computation provides the generic function dissimilarity to compute and returns distances for binary data in a matrix which can be used for grouping and clustering. Dissimilarity index from SSR data matrix was calculated and subjected for factorial and dendrogram analysis (Fig. 3). Through factorial analysis

also, the 30 genotypes were found to be majorly clustered in five clusters (Table 7). The genetic structure results obtained for 10 different k values were subjected to structure harvester for determining the best K value. The resultant graph obtained from structure harvester suggested that the genetic structure of 30 desi chickpea genotypes was best explained by the cluster value (k value) of 4 (*i.e.*, $k = 4$). The genetic structure explained by 15 SSR primers loci showed that the genetic structure of 30 desi chickpea genotypes is composed of four major ancestral components (Figs. 4 & 5). Genotypes are the admixture of different combinations of four ancestral components at the loci explained by 15 SSR primers.

CONCLUSION

This study demonstrated substantial genotype-environment interaction and variability in grain yield performance of desi chickpea. Noteworthy stable genotypes were identified for both favourable and unfavourable environments. The genetic analysis using SSR markers revealed moderate diversity and allowed categorization of genotypes into distinct clusters.

Declarations

Ethical Approval:

Not approval.

Competing interests:

The authors declare no competing interests.

Declaration of competing interest:

This manuscript has not been published in whole or in part and is not being considered for publication elsewhere. This manuscript does not violate or infringe upon any existing copyright/s license/s from any third party. All authors contributed significantly to work and agree with the manuscript's content. There are no conflicts of interest. All authors reviewed the manuscript.

Author Contribution

Author demonstrated substantial genotype-environment interaction and variability in grain yield performance of desi chickpea. Noteworthy stable genotypes were identified for both favourable and unfavourable environments. The genetic analysis using SSR markers revealed moderate diversity and allowed categorization of genotypes into distinct clusters.

Availability of Data and Materials:

Not applicable.

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Tables

Table 1

Poole ANNOVA (Mean sum squares) for different characters over the environment. *, ** significance at p = 0.05 and P = 0.01 respectively.

Source of variation	d.f.	Plant height (cm)	Days to 50% flowering	Days to maturity	No of primary branches per plant	No of secondary branches per plant	No of pods per plant	No of nodules per plant
Genotype	29	217.7227**	23.2389*	6.4784**	0.0972*	11.7731*	41.4478**	45.6801**
Environment	2	318.1500**	46.5010**	1.2458*	0.2355*	20.9865*	58.9711**	47.2057**
G x E	58	14.7175*	4.9226*	0.2394	0.1693*	0.7011*	6.5154*	1.2693*
Evt. + (G X E)	60	24.8319*	6.3085	0.2729	0.1715	1.3773*	8.2639	2.8005*
Evt. (Linear)	1	636.3000**	93.0021	2.4917*	0.4711	41.9731**	117.9422**	94.4115**
G x E (linear)	29	23.6081*	3.1158*	0.1865	0.1455	0.6955	5.3758	1.4641
Pooled deviation	30	5.6339*	6.5044*	0.2794	0.1867*	0.6831*	7.4000*	1.0387*
Pooled Error	174	3.6533	5.0441	0.4734	0.0578	0.4080	2.7897	0.0521
Table 1 continued.								
Source of variation	d.f.	Nodule fresh weight (g)	Nodule dry weight(g)	Root length (cm)	Root volume (ml)	100 seed weight (g)	Harvest index (%)	Grain yield per plant (g)
Genotype	29	1.3275*	0.0832*	4.5482*	0.4562*	43.5130**	64.2722**	36.7154**
Environment	2	0.1038*	0.1543*	18.5075*	0.5142*	9.2867*	24.4497**	37.2436**
G x E	58	0.0153*	0.0134*	0.9309*	0.0720*	0.9895*	3.4936*	8.3998*
Evt. + (G X E)	60	0.0182	0.0181	1.5168*	0.0867	1.2660	4.1921*	9.3613
Evt. (Linear)	1	0.2076*	0.3085*	37.0150**	1.0284*	18.5734*	48.8995**	74.4872*
G x E (linear)	29	0.0090	0.0130	1.1542	0.0420	0.8257	3.9201	8.1386
Pooled deviation	30	0.0208*	0.0133*	0.6841*	0.0985*	1.1147*	2.9649*	8.3723*
Pooled Error	174	0.0007	0.0240	0.6796	0.0437	0.7714	0.1024	0.3227

Table 2
Mean and stability parameters of different genotypes in three environments. *, ** significance at 5% and 1% respectively.

Genotypes	Plant height (cm)			Days to 50% flowering			Days to maturity			No of primary branches/ plant			No of secondary branches/ plant		
	– x i	bi	S ² di	– x i	bi	S ² di	– x i	bi	S ² di	– x i	bi	S ² di	– x i	bi	S ² di
DC-19-1	43.64	-0.27	5.25**	76.78	2.27	2.73	121.67	1.34	-0.14	2.78	1.79	0.40*	5.58	0.64	2.74*
DC-18-1104	52.30	-0.40	0.85	73.60	1.41	-0.63	121.27	1.91	0.62*	2.85	-3.21	0.06*	8.09	1.11	0.02
DC-18-1107	56.25	1.04*	-1.67	71.42	0.22	0.94	122.09	1.11	0.38	2.95	5.37	0.58*	6.14	1.20	0.08
DC-2021-1211	54.27	2.58	6.74**	76.03	1.17	-1.15	124.59	0.54	-0.04	3.09	-1.32	0.17*	5.87	0.76	1.45*
BG-372	48.78	1.30	-0.53	75.72	1.03	0.02	122.94	1.56	0.05	2.87	1.91	-0.01	8.44	2.71	1.09*
RVG-203	56.39	-0.12	15.29**	73.51	0.75	-1.06	122.51	0.18	-0.15	3.09	0.35	0.41*	7.88	1.71*	-0.12
ICCV-201108	67.51	0.92	2.68	77.47	0.21	12.14**	123.47	-0.20	-0.15	2.86	1.00	0.14*	13.17	0.01	0.43*
ICCV-17-1115	75.41	1.33	3.87	78.52	2.34	0.77	123.19	0.62	0.15	2.96	1.98	0.16*	9.08	1.09	0.45*
DC-21-1203	58.87	0.56	0.57	70.85	0.49	-1.20	120.85	-1.65	0.39	2.97	-0.98	0.17*	7.55	0.83	1.82*
DC-17-1111	68.83	-0.41	5.12**	72.91	2.04	-0.90	120.58	-0.05	-0.14	3.00	-7.75	0.06*	6.70	1.33	-0.11
ICCV-201308	54.69	2.04*	-1.31	70.53	0.08	13.45**	121.87	3.22	0.01	3.01	0.36	0.43*	5.73	1.03*	-0.13
DC-21-1107	67.79	-0.13	-0.95	78.33	-0.09	3.39	123.00	2.71	-0.05	2.53	1.24	-0.01	8.54	1.78**	-0.13
ICCV-2012-11	66.32	0.15	-0.45	75.80	0.44	12.06**	122.80	1.70	-0.10	3.17	2.05	0.07*	7.48	0.58	0.23
ICCV-18-1664	58.91	0.95	7.64*	75.41	-0.46	13.67**	120.08	0.34	0.35	2.81	0.72	0.48*	6.24	1.70	0.54*
DMHC-18-1664	67.55	1.99	10.11**	71.46	0.77	-1.14	122.13	1.60	0.50*	2.80	5.27	0.09*	6.21	1.26	-0.07
DC-2011-1211	61.87	1.30	2.17	71.40	3.57	89.46**	121.73	1.98	-0.11	3.19	1.50	-0.01	9.38	0.39	0.68*
DMHC-21-1108	64.90	2.38*	-1.61	79.11	2.66	1.42	124.11	5.25	0.41	3.09	3.58	0.00	9.53	0.96	-0.11
BG-3048	44.24	0.45	9.25*	76.69	-1.05	-0.14	120.36	0.05	-0.14	2.71	-2.83*	-0.01	10.66	0.53	1.90*
GNG-1558	46.99	-0.46	-1.38	73.79	1.27*	-1.21	123.12	-2.19	0.03	2.87	2.10	0.26*	8.47	2.24*	-0.11
DMHC-21-1104	62.04	2.42	1.42	73.24	1.62*	-1.20	122.91	-0.90	0.34	2.62	3.12	0.01	7.18	0.83	-0.05
JG-315	55.51	2.45	-0.46	70.90	0.04	2.89	118.23	-2.04	-0.01	2.58	0.10	-0.01	6.32	-0.25	0.56*
ICCV-10102	72.21	-0.19	1.19	77.56	1.52	-0.55	121.56	0.12	-0.15	2.95	2.16	0.26*	7.13	1.15*	-0.12
ICCV-95311	60.73	3.31	12.02**	76.92	1.24	-1.02	120.92	2.01	-0.09	2.92	-4.12	-0.00	11.06	1.51	-0.08
DC-2021-1664	44.32	1.76	30.54**	79.51	0.88	0.72	123.18	1.04	-0.15	3.33	5.73	0.24*	6.51	1.04	1.42*
ICCV-201203	46.63	0.17	2.65	76.42	1.00	3.41	120.42	1.01	-0.15	2.99	-1.23	0.17*	12.64	-0.66	0.23
Pant-G-114	53.40	1.84	5.25**	73.60	1.27	-0.91	118.93	2.86	2.30*	3.00	4.96	0.48*	7.92	1.11	0.14
GNG-2264	57.72	0.60	1.68	74.97	0.07	6.87*	122.63	-0.16	-0.14	3.05	-0.10	0.18*	7.21	1.06	0.10
C-235	55.25	0.61	-1.40	77.03	1.72*	-1.20	123.36	-0.13	-0.15	2.90	-1.34	0.14*	6.95	1.34	3.46*
BG-256	55.60	0.29	2.90	79.54	1.52	-0.77	121.54	0.40	-0.15	2.95	4.81*	-0.01	8.54	-0.14	-0.11
KPG-59	52.65	1.47	1.06	77.54	-0.14	7.71*	120.88	2.13	0.17	2.72	2.71	0.06*	5.32	1.00	0.19
SE (bi)	1.71			1.83			0.38			0.31			0.59		
Mean	57.72			75.21			121.89			2.92			7.92		

Table 3
Mean and stability parameters of different genotypes in three environments. *, ** significance at 5% and 1% respectively.

Genotypes	No of pods per plant			Number of nodules/ plant			Nodule fresh weight (g)			Nodule dry weight (g)			Root length (cm)		
	– x i	bi	S ² di	– x i	bi	S ² di	– x i	bi	S ² di	– x i	bi	S ² di	– x i	bi	S ² di
DC-19-1	25.13	1.91	24.96**	22.47	0.80	0.59*	1.97	1.52	0.02*				9.85	-0.73	0.03
DC-18-1104	15.98	1.12	28.33**	22.47	0.70	0.60*	1.21	2.72	0.00	0.615	2.2915*	-0.0078	12.58	0.01	0.97*
DC-18-1107	21.23	3.30	5.46*	19.88	0.78	2.10*	2.20	0.29	0.01*	0.433	0.4419	-0.0034	12.40	0.47	1.24*
DC-2021-1211	14.49	0.26	16.11**	18.49	1.52	1.77*	2.49	-0.08	0.00*	0.779	0.0772	-0.0061	12.66	1.05	0.01
BG-372	21.18	-0.63	2.84*	20.62	-0.78	0.40*	1.52	1.57	0.02*	0.549	1.1169	-0.0015	9.25	1.04	0.09
RVG-203	16.50	3.38	-0.56	24.08	1.92	1.12*	2.26	-0.87	0.01*	0.394	-0.5014	-0.0078	11.14	1.68	0.05
ICCV-201108	25.42	1.83	-0.35	31.79	1.72	4.23*	0.75	2.38	0.14*	0.440	2.4249	0.0007	10.43	1.65	0.21
ICCV-17-1115	23.02	0.02	-0.85	17.46	0.77	0.90*	0.95	1.49**	0.00	0.462	0.0997	0.0080	11.83	2.13	0.41
DC-21-1203	16.17	0.88	1.05	21.80	1.58	0.60*	1.63	-0.73	0.02*	0.371	0.3145	0.0892*	9.60	1.14	-0.03
DC-17-1111	18.96	1.11	16.07**	31.17	1.96	1.61*	1.24	1.10	0.01*	0.426	2.7222	-0.0043	10.13	1.32	0.72*
ICCV-201308	20.45	1.22**	-0.92	22.85	1.56	0.68*	1.45	1.26*	-0.00	0.2921	-0.8298	-0.0056	11.17	1.59	1.26*
DC-21-1107	13.99	0.14	3.24*	28.10	1.17	0.37*	1.28	0.26	0.02*	0.473	0.7060	-0.0042	9.62	0.04	0.04
ICCV-2012-11	19.60	2.41	3.57*	19.81	1.60	0.38*	2.56	0.75	0.01*	0.209	1.2936	-0.0067	9.13	1.28	1.30*
ICCV-18-1664	18.46	0.83	4.27*	25.33	1.17	0.36*	1.55	-0.79	0.01*	0.494	0.5565	-0.0014	10.62	0.74	0.76*
DMHC-18-1664	19.20	1.19	21.44**	18.83	1.10	1.75*	1.63	1.01	0.04*	0.205	1.9820	-0.0050	10.16	1.78	1.19*
DC-2011-1211	17.77	1.26	13.18**	18.67	0.33	0.65*	0.95	2.66	0.01*	0.328	1.8459	-0.0063	12.06	0.45	0.22
DMHC-21-1108	17.30	2.11	0.90	23.01	1.88	2.57*	2.12	0.91	0.00	0.342	-0.5588	-0.0057	12.23	-1.60	0.95*
BG-3048	20.80	0.27	5.39*	22.72	1.43	1.66*	2.55	1.23	0.01*	0.604	1.7621	-0.0061	10.51	2.46	-0.04
GNG-1558	23.58	0.44	0.02	24.41	0.63	0.55*	1.45	1.90*	-0.01	0.911	1.4329	0.0001	10.86	1.48	1.85*
DMHC-21-1104	18.93	0.52	0.73	18.83	0.19	0.63*	1.12	1.00	0.01	0.658	0.5672	0.0775*	10.12	1.57	-0.16
JG-315	22.87	-1.94	-0.50	23.97	1.79	0.43*	0.85	1.74	0.01	0.436	0.4983	0.0649*	9.60	0.54	0.96*
ICCV-10102	27.48	1.58	6.54*	16.76	0.72	1.93*	0.92	2.35	0.00*	0.261	0.1325	-0.0074	8.17	0.91	0.32
ICCV-95311	23.16	3.03	22.54**	27.47	0.73	0.43*	2.85	-0.90	0.02*	0.362	-0.1150	-0.0078	11.81	0.31	-0.18
DC-2021-1664	14.85	-0.15	2.05	23.18	1.05	0.10*	2.81	0.14	0.03*	0.672	2.9111	0.0100	11.97	1.62	-0.10
ICCV-201203	15.93	0.63	4.06	27.14	0.50	1.49*	1.82	2.87	0.00*	0.718	0.9656	0.0221	10.34	0.54	1.35*
Pant-G-114	16.81	1.20	-0.75	17.81	0.28	0.33*	1.62	0.78	0.03*	0.569	0.5844	-0.0019	9.98	2.98	-0.11
GNG-2264	18.20	-0.66	13.90**	20.59	0.69	0.50*	1.92	0.02	0.00*	0.486	2.4453	-0.0070	8.75	1.51	0.41
C-235	14.94	1.22	-0.83	18.04	1.05	0.46*	1.08	2.58	0.04*	0.641	2.2675	0.0002	9.10	1.77*	-0.22
BG-256	22.79	0.71	0.96	22.37	1.54	1.25*	3.19	-0.59	0.08*	0.426	-0.8379*	-0.0080	9.15	-0.60	-0.15

Genotypes	No of pods per plant			Number of nodules/ plant			Nodule fresh weight (g)			Nodule dry weight (g)			Root length (cm)		
KPG-59	13.44	0.68	1.15	24.89	-0.49	0.04	1.25	1.34*	-0.01	0.421	2.8082	-0.0043	10.77	0.76	0.29
SE (bi)	1.96			0.73			0.10			0.08			0.59		
Mean	19.29			22.50			1.71			0.479			10.53		

Table 4

Mean and stability parameters of different genotypes in three environments. *, ** significance at 5% and 1% respectively.

Genotypes	Root volume (ml)			100 seed weight (g)			Harvest index (%)			Grain yield per plant (g)		
	–	bi	S ² di	–	bi	S ² di	–	bi	S ² di	–	bi	S ² di
	x i			x i			x i			x i		
DC-19-1	3.55	0.72	-0.01	28.83	-0.54	0.28**	23.24	-0.63	-0.02	18.00	-0.34	2.68*
DC-18-1104	2.39	1.04	0.05*	25.09	2.62	0.35**	29.39	-0.26	2.59*	22.60	1.96	10.79**
DC-18-1107	3.19	0.65	0.22*	23.15	0.48	0.38*	37.30	2.06	1.58*	20.43	-0.69	8.35*
DC-2021-1211	3.00	4.46	0.43*	31.11	0.12	-0.02	30.08	0.76	3.69*	18.03	-0.40	4.25*
BG-372	3.49	0.85	-0.01	27.45	1.48	0.37*	32.34	-0.23	0.54*	24.18	-0.52	-0.18
RVG-203	3.18	0.80	0.57	24.63	0.15	3.17*	28.07	1.28	1.95*	14.03	0.63	55.57**
ICCV-201108	3.08	0.54	0.24*	24.19	1.76*	-0.02	36.09	1.13	1.66*	17.42	2.22	7.09*
ICCV-17-1115	2.68	1.81	-0.01*	29.67	4.74	0.69*	27.00	2.39	0.44*	22.66	-0.91	13.93*
DC-21-1203	3.28	-0.17	-0.01	22.23	0.62	0.17*	39.26	0.49	0.17	12.95	-0.66	0.38
DC-17-1111	2.87	-1.15	0.17*	31.72	1.56*	-0.03	27.12	4.20	0.95*	14.51	-1.08	9.58**
ICCV-201308	3.57	0.26	-0.00	33.85	1.17	2.06*	26.96	1.49	0.26	14.39	0.35	-0.16
DC-21-1107	2.52	0.46	-0.01	26.61	1.32	0.40*	32.26	-0.63	1.54*	19.03	3.30	5.08*
ICCV-2012-11	2.86	2.94*	-0.01	23.18	2.21*	-0.02	39.10	-0.59	-0.06	16.74	-2.34	3.04*
ICCV-18-1664	2.32	1.93	0.03	29.12	-0.06	0.56*	25.13	3.93	0.05	19.59	1.91	-0.10
DMHC-18-1664	2.42	0.97	0.03	30.16	-1.07	7.08*	27.05	1.34	5.08*	10.90	-0.69	0.45
DC-2011-1211	2.59	-0.20	0.01	31.25	2.01	1.87*	27.39	2.59	1.13*	13.38	-0.27	6.53*
DMHC-21-1108	2.58	0.69	-0.01	32.50	1.80	0.73*	29.22	1.95	0.23	15.69	2.37	25.19**
BG-3048	2.49	-0.37	-0.01	29.84	-0.08	2.83*	26.25	1.98	6.46*	14.66	2.86	2.43*
GNG-1558	2.57	0.97	-0.01	22.54	0.00	0.01	35.47	1.74	12.56**	16.40	3.59*	-0.24
DMHC-21-1104	3.04	1.02	0.15*	25.18	0.41	0.52*	36.46	3.04	0.16	11.43	-1.24	0.70
JG-315	3.39	0.79	-0.01*	28.13	0.70	0.52*	30.66	-0.42	5.48*	17.90	2.09	1.67*
ICCV-10102	2.89	2.40*	-0.01	26.57	0.06	1.09*	31.76	-0.52	0.25	21.32	4.11	6.79*
ICCV-95311	2.47	0.76	-0.00	23.92	1.09	-0.02	32.63	-0.52	6.27*	14.68	2.11	31.28**
DC-2021-1664	2.62	0.25	-0.00	19.56	1.35	0.36*	36.20	-1.67	0.21	14.70	2.74	1.10*
ICCV-201203	3.23	0.20	-0.00	19.88	2.30	0.23*	32.11	-0.87	5.06*	17.32	4.36	5.02*
Pant-G-114	2.59	0.46**	-0.01	21.87	-0.00	0.22*	37.66	-0.48	6.01*	18.69	0.80	0.16
GNG-2264	2.24	2.60	0.18*	23.26	0.89	0.39*	35.72	0.47	-0.06	13.41	-0.59	5.17*
C-235	3.23	1.58	0.25*	23.38	1.90	1.90*	35.95	1.95	2.29*	22.78	0.09	3.28*
BG-256	3.17	1.97	0.29*	25.79	0.69	2.26*	38.37	0.56	4.82*	14.28	1.43	25.20**
KPG-59	2.72	0.65	-0.01	26.43	0.19	3.98*	34.95	3.43	14.30**	18.04	2.80	8.30**
SE (bi)	0.23			0.76			1.24			2.08		
Mean	2.87			26.37			32.04			17.00		

Table 5
Stable genotypes (in favourable, unfavourable and all environments) for morphological traits in desi chickpea

Sl No	Characters	Stable genotypes		
		Average stability (suitable for all environments)	Below average stability (bi > 1) (suitable for favourable environment)	Above average stability (bi < 1) Suitable for unfavourable environment
1	Plant height		BG-372, JG315.	DC-18-1104, DC-21-1203, DC-21-1107, ICCV-2012-11.
2	Days to 50% flowering		DC-18-1104, DC-17-1111, GNG-1558, DMHC-21-1104, Pant-G-114.	DC-18-1107, RVG-203, DC-21-1203, DMHC-18-1664, JG-315.
3	Days to maturity		DC-18-1107, ICCV-2012-11, DMHC-21-1108, DC-2021-1664, BG-372.	DC-2021-1211, RVG-203, ICCV-201108, GNG-1558, DMHC-21-1104, GNG-2264, C-235, Pant-G114.
4	Number of primary branches Per plant		DC-2011-1211, DMHC-21-1108	
5	Number of secondary branches Per plant		DC-21-1107, ICCV-2012-11.	DMHC-21-1108, C-235, BG-256.
6	Number of pods per plant		ICCV-201108, CCV-201308.	DC-17-1115, GNG-1558, JG-315, BG-256.
7	Number of nodules per plant			KPG-59.
8	Nodule fresh weight			DMHC-21-1108.
9	Nodule dry weight		DC-2021-1211, DMHC-21-1108, GNG-1558, ICCV-95311, GNG-2264.	DC-18-1107, ICCV-2012-11, ICCV-95311, DC-2021-1664
10	Root length		RVG-203, DC-17-1115, BG-3048, DC-2021-1664.	DC-2011-1211, ICCV-95311, KPG-59.
11	Root volume		ICCV-10102.	DC-19-1, BG-372, RVG-203, DC-21-1203, ICCV-201308, ICCV-201203.
12	100 seed weight			DC-2021-1211.
13	Harvest index		DMHC-21-1104.	DC-21-1203, ICCV-2012-11, DC-2021-1664, GNG-2264.
14	Grain yield per plant		ICCV-18-1664	DC-17-1111, DC-17-1115

Table 6
Analysis of 15 SSR primers for Nei's Gene diversity (h*), Shannon's information index (I*) and polymorphism information content (PIC):

S. No.	Primers	Total no of observed alleles	No of effective alleles (ne*)	Nei's Gene diversity (h*)	Shannon's information index (I*)	PIC
1	GA2	4	1.44	0.29	0.45	0.60
2	GA9	4	1.4	0.24	0.35	0.38
3	GA20	5	1.28	0.42	0.37	0.47
4	GA21	5	1.48	0.49	0.46	0.47
5	GA24	3	1.67	0.40	0.59	0.14
6	GA108	3	1.67	0.39	0.56	0.52
7	GA117	3	1.58	0.33	0.49	0.85
8	TA176	7	1.15	0.12	0.24	0.33
9	GA4	5	1.47	0.29	0.43	0.84
10	GAA41	4	1.48	0.28	0.42	0.47
11	GAA50	4	1.85	0.45	0.65	0.77
12	GAA51	7	1.58	0.29	0.45	0.28
13	GAA54	3	1.38	0.26	0.43	0.68
14	GAA60	9	1.35	0.22	0.34	0.25
15	TA3	3	1.53	0.30	0.46	0.58

Table 7: Clustering pattern obtained by SSR analysis in desi chickpea genotypes

Cluster	Number of genotypes	Composition of genotypes different clusters
1	11	ICCV-201308, BG-372, RVG-203, ICCV-201108, DC-17-1115, DC-21-1203, DC-17-1111, DC-21-1107, ICCV-2012-11, ICCV-18-1664, DMHC-18-1664
2	9	ICCV-10102, ICCV-95311, DC-2021-1664, ICCV-201203, Pant-G-114, GNG-2264, C-235, BG-256, KPG-59
3	7	DC-19-1, DC-18-1104, DC-18-1107, DC-2021-1211, DC-2011-1211, DMHC-21-1108, BG-3048
4	3	GNG-1558, DMHC-21-1104, JG-315

Figures

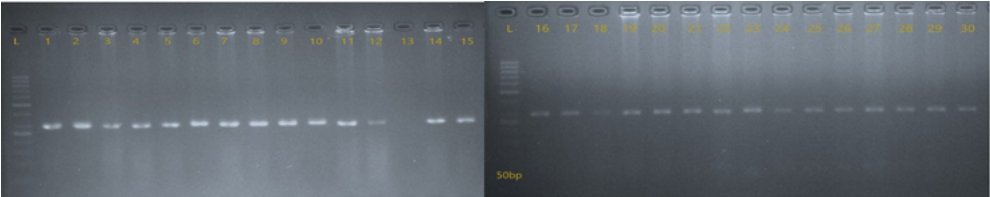


Figure 1

GA117 Primer pair directed amplification of genomic DNA region extracted from leaves of desi chickpea genotypes

1-DC-9-1	7-ICCV-201108	13-ICCV2012-11	19-GNG-1558	25-ICCV-201203
2-DC18-1104	8-ICCV-17-1115	14-ICCV-18-1664	20-DMHC-21-1104	26-Pant-G-114
3-DC-18-1107	9-DC-21-1203	15-DMHC-18-1664	21-JG-315	27-GNG-2264
4-DC-2021-1211	10-DC-17-1111	16-DC-2011-1211	22-ICCV-10102	28-C-235
5-BG-372	11-ICCV-201308	17-DMHC-21-1108	23-ICCV-95311	29-BG-256
6-RVG-372	12-DC-21-1107	18-BG-3048	24-DC-2021-1664	30-KPG-59

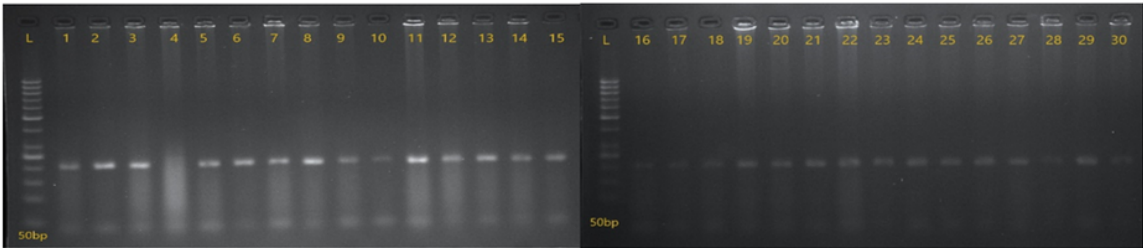


Figure 2

GA24 Primer pair directed amplification of genomic DNA region extracted from leaves of desi chickpea genotypes

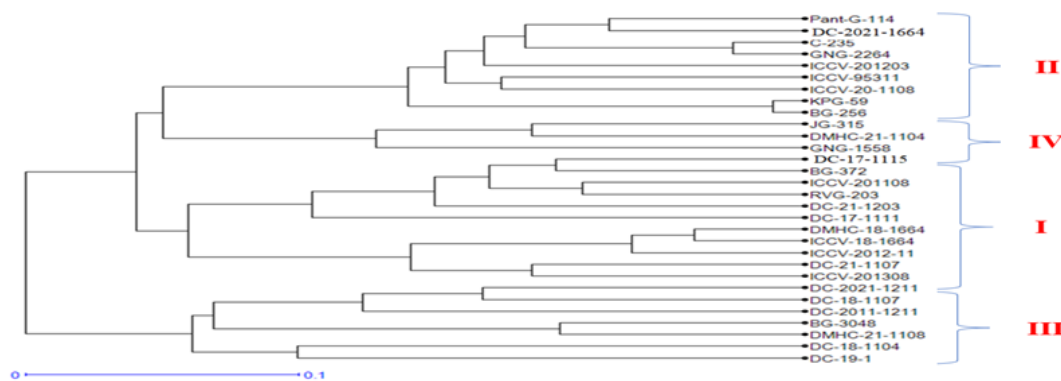


Figure 3

Dendrogram of 30 desi chickpea genotypes constructed based on Dissimilarity Index from 15 SSR data by DARWIN Software

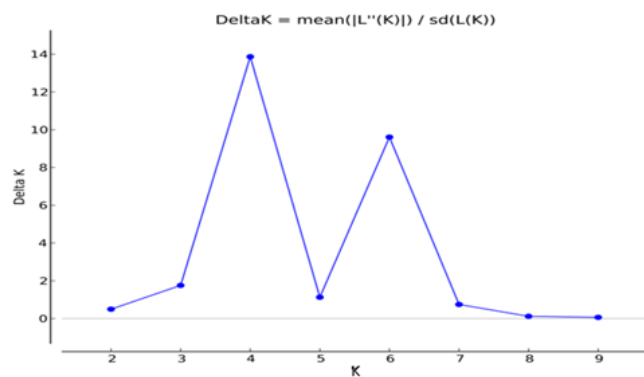


Figure 4

STRUCTURE HARVESTER results to determine the most likely K value compares the ΔK between sequential K values showing the best K value as result K=4.

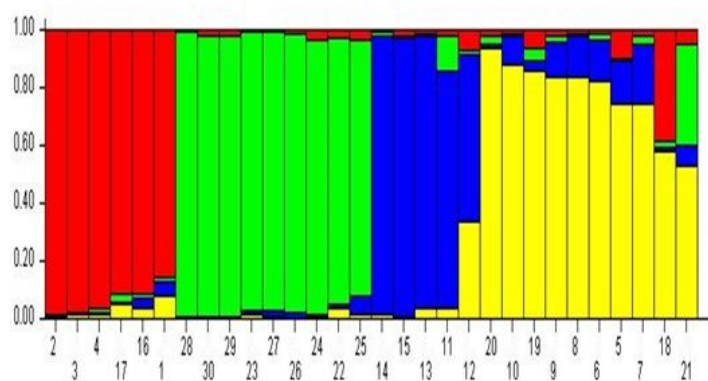


Figure 5

A bar graph produced by the Structure Software (at K = 4) using the SSR molecular data show the membership coefficient of the 30 genotypes of desi chickpea in its respective group.