Journal of Food Legumes 25(2): 147-149, 2012

Short Communication

Genetic and molecular diversity analysis of chickpea (*Cicer arietinum* L.) genotypes grown under rice fallow condition

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(Received: November 30, 2011; Accepted : June 05, 2012)

ABSTRACT

Fifteen agro-morphological traits and seventeen SSR primers were used in sixteen chickpea cultivars to study genetic diversity among the accessions and their subsequent classification. Sixteen genotypes were grouped into 5 clusters, out of which cluster I was the largest comprising 11 genotypes. Out of 17 primers screened, 15 showed polymorphism with an average 0.16 hetrozygocity and 0.763 similarity coefficients. Cluster analysis revealed two and five groups based on morphological and molecular markers, respectively. Genetic diversity assessment with different methods and their comparison could provide complementary information for improvement of chickpea.

Key words: Chickpea, Cluster analysis, Genetic diversity, Molecular markers

Chickpea is one of the most important pulse crop of India grown in 7.89 million ha area with 7.06 million tonnes production and 895kg per ha productivity (DAC 2011). A substantial part of *Kharif* rice area remains fallow (11.65 m ha) during the *Rabi* season in India. Of this 82% of the rice fallows are located in the states viz., Bihar, Madhya Pradesh, Chhattisgarh, Orissa and Assam (Subbarao *et al*. 2001). Chickpea is most suitable crop that can be grown profitably on residual soil moisture in rice fallow with minimum irrigation. There is ample scope for expansion of high yielding, disease resistant and short duration chickpea varieties in rice fallow lands.

Knowledge of genetic variation is important to understand the genetic variability available and its potential use in breeding program. Morphological traits, despite the problems associated with this method, continue to play a major role in studying and characterizing germplasm since it requires no complicated laboratory facilities and procedures. Molecular analyses in conjunction with morphological and agronomic evaluation of germplasm are recommended to increase the resolving power of genetic diversity analyses and provide complementary information (Singh *et al*. 1991). In recent years, more sensitive DNA-based techniques like SSRs are developed which are most suitable because of easy in handling, reproducibility, multiallelic nature, co-dominant inheritance, relative abundance and genomic wide coverage (Powell *et al.*, 1996). Therefore, present investigation was carried out to assess the genetic variability and interrelationship of the traits using morphological and molecular markers among chickpea varieties/genotypes grown under rice fallow condition.

Sixteen genotypes including 12 cultivars of chickpea recommended for central zone and 4 promising lines from ICRISAT were evaluated in rice fallow fields in Randomized Block Design (RBD) with three replications during *Rabi* 2010-11 at Seed Breeding Farm, JNKVV, Jabalpur (MP). Each genotype was comprised of 12 rows plot of 4m length with spacing of 30x10cm. The assessment of divergence for a set of characters using multivariate analysis (D^2) was done (Table 1). The intra cluster distance was higher in cluster I (11.40) which is having the higher number of genotypes followed by cluster II (9.00). The inter cluster distance is highest between the cluster III and cluster IV (32.70) followed by between cluster II and cluster III (28.57) and cluster III and cluster IV (27.83). The lowest inter cluster distance were found in cluster I and cluster III (15.83). Clustering pattern of chickpea genotypes confirmed the quantum of diversity present in material. The cluster I was the largest among all the clusters, consisting 11 genotypes. It was closest to cluster III followed by cluster II. Cluster II comprised of 2 genotypes viz., 'ICCV 05106', 'ICCV 06107' and was nearest to cluster IV ('AIG21') followed by cluster V. Cluster III consisting of one genotype 'DCP 92-3' was closest to cluster V which contained 'Subhra' (Table 2). These results indicate existence of some homology between closely situated clusters. Therefore, crossing genotypes from different clusters would produce more genetic variability.

Maximum contribution towards genetic divergence was by 100 seed weight followed by harvest index, total pods per plant, days to pod initiation, days to maturity and primary branches per plant. However, seeds per pod did not show any contribution towards genetic divergence (Table 3). The crosses among genotypes of the cluster IV and V are likely to produce desirable recombinants for phenological traits, while crossing between cluster III ('DCP 92-3') and cluster IV ('AIG 21') might provide more pods/plant and better seed traits. These results are in conformity with the findings of Dwivedi and Gaibriyal (2009). Different clusters have higher mean values for different traits indicating that none of the cluster contained genotypes with all the desirable characters, therefore recombination breeding between genotypes of different clusters is suggested.

For molecular characterization, DNA extraction was done from young leaves collected from randomly sampled individual plants of each cultivar. Total genomic DNA was isolated using a modified cetyl trimethyl ammonium bromide (CTAB) extraction technique (Borsch *et al*., 2003). Determination of the quantity and quality of DNA was done by comparing DNA samples with Lambda *Hind* III ladder. A total of 17 SSR primers were used (Table 4). The optimum reaction components were 6.5 ìl dH_2O, 100 ìM of each dNTP 1 ìl of 10X *Taq* buffer, 0.5 U *Taq* polymerase, 5 pmol each forward and reverse primer and 20 ng template DNA. The final reaction volume per sample was 10 ìl. PCR amplification conditions were set as: Initial denaturing at 94°C for 4 min followed by 40 cycles of 94°C for 30s, 45-60°C for 30s, and 72°C for 45s and ended with extension phase of 72°C for 5 min. Amplification products of all the 17 SSR markers were denatured and resolved on 4% denatured polyacrylamide gel as described by Chen *et al*. (1997). Electrophoresis (PAGE) was run at 2000 V for about 2:00 hours in 0.5X TBE buffer. The resultant gel was visualized after staining with silver nitrate. The PCR products were scored qualitatively by comparing with the 20bp ladder. Power Marker version 3.25 (Liu & Muse, 2005) was used to calculate the average number of alleles, gene diversity, and polymorphic information content (PIC) values. Genetic similarities between the genotypes were measured by Similarity Coefficient based on the proportion of shared alleles using 'simqual' sub-program of NTSYS-PC version 1.8 (Exeter Software, Setauket, NY, U.S.A.) software package (Rohlf, 1993). The resultant similarity matrix data was used to construct dendrograms by using the un-weighted pair-group method with an arithmetic average (UPGMA) subprogram of NTSYS-PC. Based on standardized morphological traits value, Euclidian distances between chickpea genotypes were calculated. The Mantel test of significance (Mantel, 1967) was also used to compare the molecular and morphological traits matrices produced above.

Table 1. Intra and inter distance of chickpea genotypes

Cluster	I	II	III	IV	V
I	**11.40**	18.89	15.83	21.28	20.32
II		**9.00**	28.57	18.86	18.88
III			**0.00**	32.70	27.83
IV				**0.00**	15.49
V					**0.00**

Table 2. Distribution of chickpea genotypes in different clusters based on morphological characters

Cluster no	No. of genotypes	Genotype
I	11	Rajas, JG 14, JG 11, PUSA 547, Vaibhav, GCP 105, ICCV 07111, PG 186, PUSA 372, JAKI 9218, JG 16
II	2	ICCV 05106, ICCV 06107
III	1	DCP 92-3
IV	1	AIG 21
V	1	Subhra

Table 3. Contribution of different characters towards clustering of chickpea

S. No.	Character	Time ranked	Percentage contribution towards divergence (%)
1.	DFF	0	0.00
2.	DFI	0	0.00
3.	PI	0	0.00
4.	DM	0	0.00
5.	PH (cm)	1	0.83
6.	PB	0	0.00
7.	SB	1	0.83
8.	TP	17	14.17
9.	EP	1	0.83
10.	100SW (g)	59	49.17
11.	S/P	0	0.00
12.	SY/plant (g)	2	1.67
13.	BY(g)	1	0.83
14.	HI (%)	31	25.83
15.	Seed yield (kg/ha)	7	5.83

DFI= Days to flower initiation, DFF= Days to 50% flowering, PI= Pod Initiation

DM= Days to maturity, PH= Plant Height, PB= Primary Branches, SB= Secondary Branches, TP= Total Pods/plant EP= Effective Pods/ plant, 100 SW= Hundred Seed Weight, S/P= Seeds/Pod, SY= Seed Yield/ plant, BY (g)= Biological Yield, HI (%)= Harvest Index

Table 4. Details of SSR markers, number of bands, major allele frequency, gene diversity, hetrozygocity, and PIC values obtained using SSR markers

Marker	Total allele	Polymor-phic allele	Unique allele	Range	Gene diversity	Heteroz ygocity	PIC
STMS 11	1	0	0	261-281	0	0	0
GAA 47	1	0	0	160186	0	0	0
TA 72	8	8	3	259-292	0.843	0	0.825
TA 2	9	9	5	140-176	0.851	0	0.835
TA 146	8	8	1	143-195	0.835	0	0.814
TR 20	6	6	1	156-169	0.767	0.312	0.73
TS 72	6	6	2	288-315	0.734	0	0.702
TS 54	7	7	4	267-320	0.773	0	0.741
ICCM 0293	6	6	1	278-320	0.83	0.062	0.809
ICCM 0127	6	6	1	320-375	0.818	0.062	0.792
TA 103	7	7	1	196-225	0.82	0	0.796
TA 194	8	8	4	120-169	0.82	0	0.799
TA 200	6	6	4	389-421	0.742	0	0.713
TR 58	7	7	2	248-280	0.835	0	0.814
TA 96	7	7	1	264-300	0.843	0	0.823
GA 16	7	7	2	238-267	0.828	0	0.805
GA 20	7	7	4	167-187	0.812	0	0.789
Mean	6.294	6.176	2.117	160	0.714	0.025	0.693

Out of 17 SSR markers screened, fifteen markers were found polymorphic and two monomorphic. The maximum numbers of alleles (nine) were found in primer TA2 followed by eight alleles by primers TA194 and TA72. The size of amplified markers ranged from 120bp (TA194) to 421bp (TA200) (Table 4). The average numbers of allele were 6.52 which is similar to findings of Sethy *et al.* (2006). Besides, a very low hetrozygocity was detected with an average of 0.025 which is similar to findings of Chaudhary *et al.* (2009). The range of polymorphic information content of SSR markers ranged between 0.000 to 0.835 with an average 0.693 where the range of PIC was 0.04 to 0.92 and the markers detected 71% gene diversity in the investigated materials.

Some SSR markers were found to have higher discriminating power for differentiation of genotypes, 36

Table 5. SSR markers amplified specific alleles in chickpea

Primers	Genotypes	Alleles	Size (bp)
TA 103	PUSA 372	E	218
	JG 11	C	139
TA 194	JAKI 9218	D	149
	JG 14	E	152
	Rajas	F	154
	Rajas	A	389
TA 200	JG 11	B	393
	Vaibhav	E	407
	AIG 21	F	421
TR 58	DCP 92-3	D	262
	AIG 21	G	280
TR 96	Rajas	D	276
GA 16	JG 11	A	238
	PUSA 547	C	248
	DCP 92-3	B	169
GA 20	PUSA 547	C	173
	Subhra	D	178
	GCP 105	E	180
TA 72	DCP 92-3	D	271
	GCP 105	E	275
	AIG 21	H	292
	PUSA 547	A	140
	AIG 21	B	142
TA 2	PUSA 372	E	160
	JG 14	F	162
	GCP 105	H	172
TA 146	JAKI 9218	F	180
TR 20	Subhra	A	156
TS 72	AIG 21	A	288
	GCP 105	F	315
	AIG 21	A	267
TS 54	ICCV 07111	B	280
	Vaibhav	F	312
	GCP 105	G	320
ICCM 0293	Rajas	B	285
ICCM 0127	Rajas	A	320

Table 6. SSR markers amplified multiple alleles in chickpea

Primers	Genotypes	Size (bp)
TR 20	JG 16	160/163
	PG 186	163/166
	DCP 92-3	163/166
	PUSA 372	163/166
	AIG 21	166/169
ICCM 0293	ICCV 06107	278/301
ICCM 0127	JG 16	356/375

specific alleles (Table 5) were amplified by fifteen primers. These primers separated specific chickpea genotype from remainings which is similar to findings of Joshi *et al.,* (2010). Multiple alleles were amplified by three primers *viz,* TR20, ICCM0127 and ICCM0293 (Table 6). Vurul and Akcin (2010) also found multiple alleles in five primers. Based on banding pattern of SSR markers, all the genotypes of chickpea were grouped into two clusters. The first cluster consisted of four genotypes namely Rajas, JG 14, JG 11 and GCP105. Second cluster divided into four subgroups. Subgroup A contained PUSA 372, DCP92-3, PG 186 and PUSA 547, whereas subgroup B contained Vaibhav, ICCV06107, ICCV05106 and ICCV 07111 genotypes. Subgroup C consisted Subhra, JG 16 and JAKI 9218, whereas subgroup D contained a single genotype namely AIG21. UPGMA cluster analysis showed all ICCV genotypes in same subcluster that indicated higher genetic similarity among them, similarly both PUSA genotypes were in same cluster and JG11 and JG14 were also together in another cluster.

The correlation between morphological similarity matrix and molecular similarity matrix (GS) were not significant (Mantel test, r = -0.057; P = 0.2728). There was also no correlation between dendrograms generated by morphological and molecular data except that both the dendrogram placed the two genotypes ICCV 05106 and ICCV 06107 in the same group. The reason may be that the very few markers were used in the present study. There was close relationship between some of the genotypes, presumably they might have been collected from similar locations. The present study suggested that genotypes which were found to be diverse based on both morphological and molecular diversity analysis can be used for making crosses for getting better recombinants to develop chickpea varieties suitable for rice fallow situation of Madhya Pradesh, India.

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