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Full Length Article

Use of RAPD Markers in Comparison with Agro-morphological Traits for Estimation of Diversity among Chickpea Genotypes

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Abstract

Genetic diversity was assessed among 38 chickpea (*Cicer arietinum* L.) genotypes on the basis of random amplified polymorphic DNA (RAPD) in comparison with agro-morphological traits. Evaluation of agro-morphological traits revealed highly significant differences among genotypes. Days to 50% flowering ranged from 92–118, plant height 54.16–87 cm, number of fruit bearing branches 4–17.25, number of pods per plant 7.6–27.4, and grain yield per plant 3.5–9.8 g. Ascochyta blight (caused by *Ascochyta rabiei*) score of these genotypes was recorded on 1–9 rating scale that varied from 3–9. Cluster analysis showing relationship based on morphological traits (scale: Euclidean distance) placed 35 genotypes into five distinct groups, while three genotypes namely Noor-91, Local Mankera and BR4 did not include in any cluster. RAPD analysis showed that 35 RAPD primers amplified a total of 212 fragments out of which 45 were polymorphic. Polymorphic bands were generated by 21 primers whereas 14 primers were monomorphic. Genetic similarity matrix based on Nei and Li's index revealed similarity coefficients ranging from 92–97% indicating lower level of genetic polymorphism revealed by RAPD primers. Dandrogram constructed on the basis of these coefficients grouped all the genotypes into 2 major and 3 small clusters at 92% similarity level. Two decamers, OPC5 and OPC14 distinguished between three Desi and two Kabuli genotypes. This study showed that the level of genetic variability observed in chickpea for agro-morphological traits was not reflected in DNA polymorphism obtained by RAPD analysis. © 2017 Friends Science Publishers

Keywords: Agro-morphological traits; Chickpea; Genetic variability; DNA polymorphism; RAPD

Introduction

Chickpea (*Cicer arietinum* L.), the only cultivated species of genus *Cicer,* is the 3rd most important food legume globally. It is cultivated on over 10 million hectares with average yield 0.78 t ha^{-1} and total production 12.2 Mt all over the world. India, Australia and Turkey contributed a total production of 7.7 Mt, 0.673 Mt and 0.518 Mt, respectively (FAOSTAT, 2012). Chickpea is an important grain legume crop in Pakistan where it is cultivated on an area of 0.284 Mt in arid and semi-arid regions, primarily under rain-fed conditions (FAOSTAT, 2012). Its production in Pakistan fluctuates across the years; therefore, yield stability is a major objective of chickpea breeding programs in the country. Instability in chickpea production may be attributed to moisture stress, diseases and insect pests. Therefore, an integrated approach with major emphasis on agronomic and genetic management of crop holds promise to the solution of this problem. Comprehensive knowledge of amount and pattern of genetic variation existing in the crop is an essential prerequisite for genetic improvement of chickpea (Collard *et al.,* 2003). Assessment of such variability on the basis of phenotypic expression of traits may not give the reliable picture of variation at genetic level as it also includes environmental components. The study of phenotypic variability in combination with DNA polymorphism could give more reliable information on genetic variability. For this purpose, various DNA-based markers have been used in different crops. Among these, RAPD markers are popular due to simplicity of application. They have been extensively used to study genetic variability in crop plants such as sorghum (Ayana *et al.,* 2000; Agrama and Tuinstra, 2004), potato (Alam *et al.,* 2012), rice (Dey *et al.,* 2012) and wheat (Kafeel, 2014). RAPD markers have also been used to find out phylogenetic relationship in the genus *Cicer* (Iruela *et al.,* 2002). Objective of the present investigation was to assess genetic variation in chickpea on the basis of RAPD markers in comparison with genetic variability for agro-morphological traits. This information will help to identify the parent genotypes to design crosses for pyramiding of

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genes regulating various traits with ultimate objective of genetic improvement for yield stability under water stress conditions.

Materials and Methods

Plant Material

This study was carried out using 38 genotypes of chickpea of Kabuli and Desi types obtained from various national and international institutes namely National Agricultural Research Centre (NARC) Islamabad, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, International Centre for Agricultural Research in Dry Areas (ICARDA), Syria and International Centre of Research in Semi-Arid Tropics (ICRISAT). These included Dasht, ILC72, ICCV97117, NCS9911, CMC 211s, ICCV2, Balkasar, C727, KK1, CM98, CM88, C44, Bittle98, Punjab91, CMC71s, CMC170T, PK 51830, ILC482, ILC201, ILC95, ILC200, NIFA88, K850, AUG424, PB2000, Local Mankera, PB1, AUG786, CM72, ILC263, CM72 x ILC3279, Parbat, NIFA88 x PK51814, Noor 91, 99CC-015, C235, BR4 and DC-1.

Evaluation of Agro-morphological Traits

The 38 chickpea genotypes were grown in 4 m long 2-rowed plots in an augmented design in the field area of National Institute of Biotechnology and Genetic Engineering (NIBGE), Faisalabad. Plant to plant and row to row distance was maintained at 10 and 30 cm, respectively. Data were recorded on days to 50% flowering, plant height, number of primary branches, number of secondary branches and pods per plant on 10 randomly selected plants, for each genotype. Data were statistically analysed to determine the level of significance of difference between genotypes.

Evaluation of Resistance against Ascochyta Blight

In order to evaluate their response to Ascochyta blight, the selected 38 chickpea genotypes were separately grown in single row plots of 4 m in length under controlled conditions. After every two rows of the check entries, one row of a susceptible line C-727 was cultivated for better spreading of the pathogen. At flowering initiation, field was sprayed with conidial suspension of *A. rabiei* at 5×10^5 conidia mL^{-1}. Spray was regularly done during evening hours till onset of the blight disease. To enhance humidity for better disease development spray of water was carried out using a knapsack sprayer. Data regarding blight disease severity was recorded at reproductive stage following Singh *et al.* (1981).

Total Genomic DNA Isolation

The 38 genotypes were grown in a glass house and their tender leaves were collected for DNA extraction. DNA extraction was carried out by CTAB method (Doyle and Doyle, 1990) and its concentration was determined by DyNA Quant $^{TM\ 200}$. For PCR analysis, dilution of total genomic DNA was done by adding double distilled water to achieve a concentration of 5 ng μL^{-1}.

PCR and Primers

PCR was carried out in 25 µL reaction volume consisting of 5.7 µL double distilled water, 2.5 µL PCR buffer (10×), 3 µL of 4 mM $MgCl_2$, 2.5 µL (10×) Gelatin, 4 µL of 200 µM DNTPs, 0.33 µL Taq DNA polymerase (1.5 units), 3 µL of 0.2 µM primer and 3 µL (15 ng μL^{-1}) of TDNA. A total of 35 RAPD primers of OP series from Operon Technology Inc. were used to amplify the DNA (Table 1). DNA amplification reactions were performed in appendolf gradient thermal cycling system adjusted to following cycling program. One cycle of 94°C for 5 min, 40 cycles of 94°C for 1 min, 36°C for 1 min, 72°C for 2 min, followed by one cycle of 72°C for 10 min. Separation of amplification products was carried out on 1.2% agarose gel in 0.5% TAE buffer and visualised under UV light after ethidium bromide staining. Similarity among the genotypes was worked out on number of shared amplification products basis following Nei and Li (1979).

Results

Means and standard deviation of agro-morphological traits recorded on 38 genotypes revealed wide variation among these genotypes (Table 2 and 3). Genotypic variation for days to 50% flowering ranged from 92 to 112. Number of pods per plant and number of fruit bearing branches, respectively ranged from 5±0.89 to 30.4±4.8 and 4.0±1.09 to 20.0±2.7, respectively. A range of 3.36±0.3–9.80±0.61 for grain yield per plant (g) and 3 to 9 for blight reaction on 1–9 rating scale, were recoded. Phylogenetic relationship between these genotypes on the basis of agro-morphological traits using Euclidean distance revealed five distinct groups of these 38 chickpea genotypes, whereas two Kabuli genotypes (Noor-91, Local Mankera) and one Desi genotype (BR4) were individually placed in the dandrogram. The same 38 genotypes were used for DNA fingerprinting with 35 RAPD primers. All the primers amplified a total of 162 DNA fragments out of which, 45 were polymorphic. On average 2.14 polymorphic bands were obtained from each polymorphic primer. The polymorphic bands were generated by 21 primers whereas 14 primers were monomorphic. Based on pair-wise comparison of amplification products, similarity between genotypes was estimated using similarity coefficients of Nei and Li (1979) that revealed 92% to 96% similarity among various genotypes. The dandrogram which was constructed from RAPD data using unweighted pair-group method with arithmetic averages (UPGMA) placed all the genotypes in a single group at 92% similarity level except BR4 and DC1 which were together separately (Fig. 1–4).

Table 1: List of RAPD primers used for DNA polymorphism studies

S.No.	Name of primer	S.No.	Name of primer
1	OPC1	19	OPG14
2	OPC2	20	OPG15
3	OPC3	21	OPG16
4	OPC4	22	OPG18
5	OPC5	23	OPG16
6	OPC6	24	OPG19
7	OPC7	25	OPG20
8	OPC9	26	OPM10
9	OPC10	27	OPM11
10	OPC11	28	OPM13
11	OPG3	29	OPM14
12	OPG4	30	OPM15
13	OPG5	31	OPM19
14	OPG6	32	OPP1
15	OPG8	33	OPP14
16	OPG10	34	OPP15
17	OPG12	35	OPP5
18	OPG13		

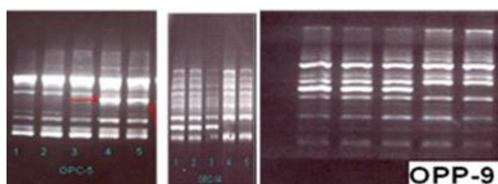

Fig. 1: Amplification profiles of blight resistant (Lane 1, 2, 3) and blight susceptible (Lane 4 & 5) genotypes with RAPD primers

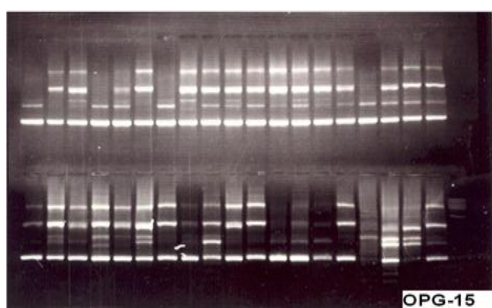

Fig. 2: RAPD fingerprinting of 38 cultivated chickpea genotypes with Primer OPG-15

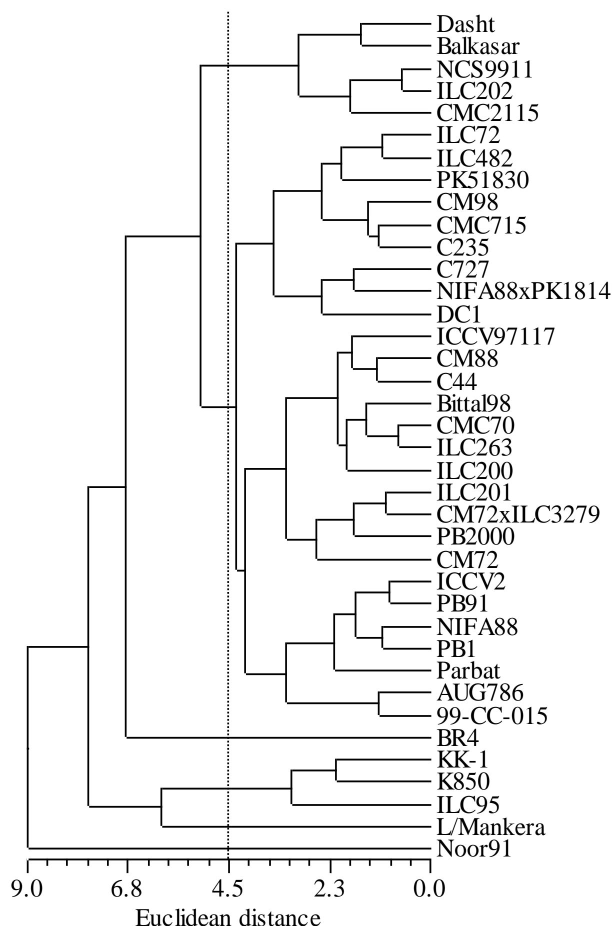

Fig. 3: Cluster analysis of 38 chickpea genotypes based on quantitative traits

Discussion

Evolution of crop varieties with broader genetic basis through hybridization requires involvement of divergent parents. Therefore, the information on variability between available germplasm lines is an important prerequisite for meaningful breeding of this legume for wide adaptability. Estimation of genetic variability based on agro-morphological traits may be misleading due to involvement of environmental component in the expression of such traits. The detection of variability at DNA level could be more reliable as it is not influenced by environmental changes. Various marker systems have been developed to study DNA polymorphism in various crop plants. Among these RAPD analysis is cheap and easy to apply, although its repeatability is not perfect with respect to amplification products profile. Careful optimization of the RAPD protocol has been shown to increase the reproducibility of the RAPD data (Micheli *et al.,* 1994). This marker system has been extensively used for variability study in various crops. In the present study, we assessed the ability of selected RAPD markers to reveal polymorphism at DNA level in comparison with variability observed at the level of various agro-morphological traits in 38 genotypes of chickpea. A wide variation was recorded among these genotypes on the basis of agro-morphological traits. Dendrogram constructed on the basis of these traits revealed maximum variability between Dasht (Desi) and Noor 91 (Kabuli). Grouping of genotypes in various clusters did not appear to be related to the origin or type (Desi or Kabuli) of genotypes. Previous studies have also revealed high level of variation, for morphological traits in chickpea (Bakhsh *et al.,* 1999; Shaukat *et al.,* 2002).

Table 2: Genetic variation for blight and other agronomic traits in 38 genotypes of chickpea

Genotype	Days to Flowering	Plant height (cm)	Primary branches per plant	Secondary branches per plant	Pods per plant	Blight score (1–9 scale)	Yield per plant (g)
Dasht	102	73.0±2.36	2.8±0.54	12.0±2.54	19.6±2.72	3.33±0.44	6.13±0.50
ILC72	95	56.8±1.72	2.2±0.4	6.6±2.4	16.6±1.72	6.33±0.94	4.10±0.3
ICCV97117	108	64.8±2.92	2.6±0.48	16.2±1.32	17.5±3.38	8.0±1.0	4.2±0.53
NCS9911	102	73±2.68	2.2±0.4	8.6±1.62	12.25±2.3	3.0±0.94	4.42±0.6
CMC 211s	102	75.6±3.61	1.4±0.48	4.0±1.09	9.75±2.95	5.33±1.0	4.44±1.0
ICCV2	93	62.8±3.31	3.0±0.63	14.75±2.95	18.25±4.5	7.66±0.74	6.60±0.4
Balkasar	111	80.8±1.16	3.0±1.2	8.0±2.23	9.4±1.74	3.0±0.0	3.50±0.6
C727	94	58.4±1.04	3.6±0.49	12.4±2.24	26.2±4.32	8.66±0.74	7.90±1.17
Kk1	104	58±1.9	6.0±0.4	8.0±2.04	25.0±3.6	7.33±0.88	6.50±1.4
CM98	92	68±5.25	3.0±0.70	10.5±2.06	23.5±2.69	9.0±0.0	8.20±1.9
CM88	102	67.25±6.3	2.2±0.4	13.5±3.61	21.5±4.76	5.33±0.74	8.3±1.89
C44	101	63±2	2.6±0.49	13.8±1.60	16.00±3.2	8.33±0.51	7.40±0.7
Bittle98	108	74.2±2.99	2.8±0.4	11.4±2.72	20.8±4.30	9.0±0.0	5.12±0.53
Punjab91	93	69.6±2.41	2.2±0.40	10.5±1.11	16.2±3.44	5.33±0.74	9.82±2.0
CMC71s	92	63.2±1.46	2.4±0.48	7.8±1.72	25.2±2.13	3.66±0.94	8.80±1.6
CMC170T	102	73.2±1.94	2.8±0.37	12.2±2.48	17.4±3.7	3.0±0.0	8.90±1.3
PK51830	95	54.8±2.13	2.2±0.74	9.30±3.31	27.4±2.05	4.66±0.74	8.50±0.4
ILC482	92	54.8±2.13	2.8±0.40	7.0±1.67	19.3±3.1	4.66±0.94	5.40±1.0
ILC201	100	63±4.11	3±1.03	10.2±3.76	13.0±1.80	7.66±1.49	7.53±0.69
ILC95	104	58.2±1.32	4.4±0.80	17.1±2.91	34.75±6.5	3±0.0	8.40±1.30
ILC200	103	79.6±3.26	2.8±0.40	15.0±2.28	13.25±1.0	4.33±0.94	4.70±0.55
NIFA88	92	69.6±1.01	3.0±0.0	12.4±2.72	15.4±2.61	5.33±0.94	5.10±0.26
K850	100	56.4±4.12	2.8±0.4	16.8±3.30	26.0±3.54	9.0±0.0	9.80±1.50
AUG424	110	54.16±2.11	2.5±0.5	20.0±2.7	30.4±4.8	8.0±1.0	9.30±2.27
PB2000	100	68.8±1.16	3.4±0.48	17.25±1.47	17.0±2.54	9.0±0.74	7.40±0.9
Local Mankera	110	72.8±1.72	3.2±0.4	12.6±3.44	19.6±2.72	6.33±1.54	6.50±1.3
PB1	90	63.8±1.16	2.6±0.48	10.8±1.46	16.6±2.3	9.0±0.0	4.35±0.1
AUG786	99	85.2±1.16	3.2±0.40	9.40±3.2	19.8±2.26	7.66±0.94	9.30±0.9
CM72	112	67.8±1.32	3.0±0.63	10.6±1.85	10.0±2.32	6.66±0.74	4.25±0.15
ILC263	101	75.2±2.48	2.80±0.4	12.2±2.13	16.25±3.4	8.0±0.74	5.96±0.38
CM72 x ILC3279	101	69±5.29	2.8±0.74	11.6±2.57	13.6±3.13	3.33±0.74	10.0±2.8
Parbat	110	72.8±1.72	3.0±0.0	12.6±3.44	23.6±4.06	8.0±1.0	8.50±1.5
NIFA88 x PK51814	98	72.2±2.99	3.6±0.49	12.6±2.44	26.2±2.71	4.20±0.74	8.20±1.25
Noor91	97	63±2.45	3.2±0.75	13.5±3.64	16.5±4.17	8.66±0.94	5.7±0.90
99CC-015	95	87±2.65	3.6±0.48	12.0±3.16	19.6±4.12	7.33±1.0	9.80±0.6
C235	96	66.4±2.05	2.6±0.8	7.20±2.03	20.5±4.01	9.0±0.0	8.40±0.9
BR4	104	66.4±1.50	3.0±0.00	9.0±1.67	7.60±2.38	6.5±0.86	3.7±0.50
DC-1	98	55±0	4.0±0.60	13.0±2.82	18.0±1.16	4.0±1.0	4.20±0.00

Table 3: Genetic variation for yield and yield related traits in 38 genotypes of chickpea

Traits	Range	
	Minimum	Maximum
Days to 50% flowering	92	112
Plant height (cm)	54.16±2.11	87.0±2.65
Primary branches per plant	01.4±0.48	06.0±0.4
Fruit bearing branches per plant	04.0±1.09	17.25±1.47
Number of pods per plant	7.60±1.38	27.4±2.05
Grain yield per plant (g)	03.50±0.6	09.8±0.61
Ascochyta blight rating on 1-9 scale	03.0±0.0	09.0±0.0

Level of DNA polymorphism obtained in the present study was quite low. This low level of polymorphism may be attributed to limited number of RAPD primers used, or to their inability to reveal polymorphism in cultivated chickpea. Previous studies have shown that RAPD fingerprinting of cultivated chickpea was unable to reveal DNA polymorphism. Low level of polymorphism with RAPD has been reported by Sonnante *et al.* (1997) and Sant *et al.* (1999). These workers studied the potential of DNA markers in detecting divergence in chickpea and reported the inability of RAPD markers to reveal a high level of DNA polymorphism. Chowdhary *et al.* (2002) compared chickpea and other pulses for the level of polymorphism with RAPD and reported low polymorphism in chickpea as compared to other pulses. Cultivated chickpea is characterized with limited genetic variability therefore, inter-specific crosses have been utilized for the construction of linkage maps (Simon and Muelbaur, 1997; Winter *et al.,* 1999). Despite low level of polymorphism obtained, in the present study, three primers, OPP9, OPC5 and OPC14 differentiated between Kabuli genotypes (ILC482, ICCV2, Pb1) and Desi genotypes (Dasht, Balkasar) of chickpea. It is suggested that STMS markers be used for variability studies in cultivated chickpea as RAPD markers are unable to reveal the DNA polymorphism that is responsible for genotypic variation with respect to agro-morphological traits. STMS markers have been extensively used for mapping in chickpea (Winter *et al.,* 1999). The grouping of genotypes in two dandrograms constructed on the basis of RAPD data and morphological data did not reveal any relationship with each other. The placement of various genotypes in two

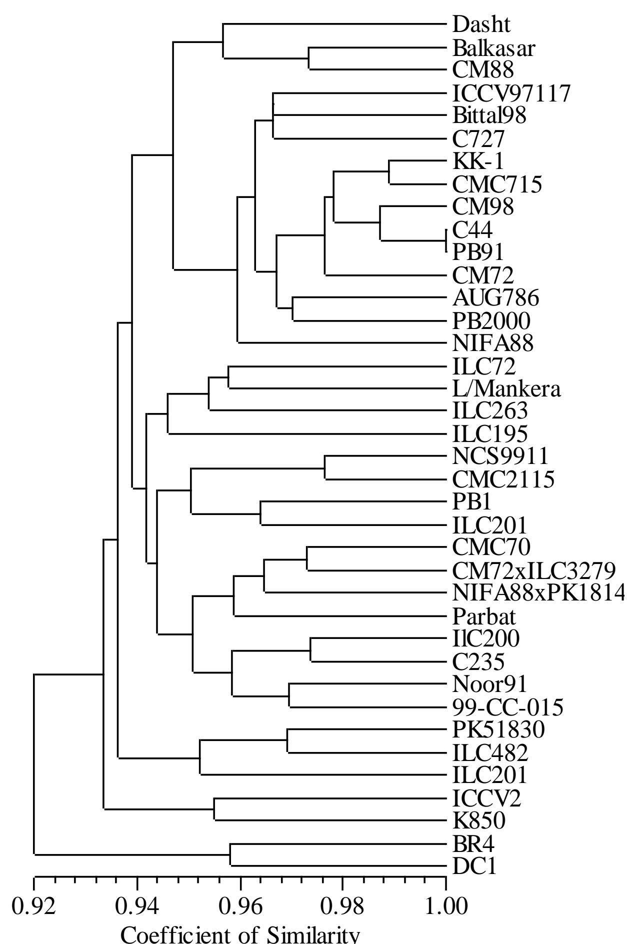

Fig. 4: Genetic relationship among 38 genotypes of chickpea based on similarity index values using RAPD markers

dandrograms was quite different e.g. DC1 and Dasht were the two most distinct genotypes on the basis of RAPD analysis whereas Dasht and Noor91 were the most divergent genotypes with respect to agro-morphological traits. However, Dasht and Balkasar appeared to be closely related in both the dandrograms.

Conclusion

RAPD markers were unable to reflect the level of genetic variability observed for quantitative traits. Therefore STMS markers in combination with agro-morphological traits could be a suitable approach to study genetic variability in cultivated chickpea.

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