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Research Article

GENETIC DIVERSITY ASSESSMENT IN CHICKPEA USING ISSR MARKERS

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ABSTRACT

Chickpea (*Cicer airetinum* L.) is an important and most preferred food legume in many parts of the world especially in the Indian sub-continent. Utilization of diverse germplasm is required to enhance the genetic variability of cultivars. Genetic diversity analysis of chickpea germplasm can provide practical information for selection of parental material and thus, assist in forecasting breeding strategies. Fourteen chickpea desi and kabuli genotypes were subjected to ISSR analysis for assessment of genetic diversity using seven ISSR markers. Total number of loci amplified using 7 ISSR primers were found to be 22with an average of 3.14 amplified loci per primer. The PIC value, gene diversity, size of amplified products and percentage polymorphism were found to be in the range of 0–0.364, 0–0.479, 350–2000 bp and 0–100% respectively.

Keywords: Chickpea, Genetic diversity, Germplasm, Breeding strategy.

INTRODUCTION

Chickpea (*Cicer arietinum* L., 2n=2x=16) derived from the Fabaceae family, subfamily Faboideae is the third most important cool season food legume worldwide after dry bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.) and is consumed as a high quality protein food.. It is an annual plant having high nutritive value and serves as an important cheap source of protein in developing countries in addition to improving land fertility (Saeed *et al*., 2011). Chickpea being self-pollinated crop hence is supposed to display little genetic variation. Thus seeds exhibiting genetic variation among diverse genotypes of Cicer could be a practical way to

select parents to be crossed (Ahmad *et al*., 2010).

ISSR or simple sequence repeats (SSRs) are short tandem repetitive DNA sequences with a repeat length of few (1-6) base pairs (Litt and Luty, 1989). These sequences are abundant, dispersed throughout the genome and are highly polymorphic in comparison with other molecular markers (Akkaya *et al*., 1992; Wang *et al*., 1994). Due to their short repeat length and limited interaction at individual loci, ISSR markers were used in the present study to investigatethe genetic polymorphism among chickpea cultivar.

MATERIALS AND METHODS

Plant material and DNA extraction

Seeds of 14 chickpea varieties viz., 'Pant G 186', 'PG 043', 'PG 071', 'Pant G 114', 'PG 99', 'PG 120', 'PG 81', 'PG 100 ', 'ILWC 115', 'ILWC 141', 'ILWC 292', 'ILWC 115R', 'PG 65', 'PG 126' developed by G.B Pant University of Agriculture and Technology, Pantnagar were grown in a greenhouse located at the N. E. Borlaug Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar during *Rabi* season of2013-14. Fresh leaves from were used for DNA extraction. Total genomic DNA was isolated from young plants at the 8 to 10 leaf stage using the CTAB method. DNA quality and quantity was analyzed using Nanodrop spectrophotometer.

PCR procedure

Ten ISSR markers were selected to assess genetic diversity of the chickpea genotypes on the basis of their high polymorphism information content and the quality scores reported in earlier studies.The molecular marker analysis was carried out at Molecular Cytogenetics Laboratory, Department of Molecular Biology & Genetic Engineering, College of Basic Sciences & Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar. The

PCR was carried out in a 15-µL volume of a master mixture containing 20-25 ng genomic DNA, 200 µM deoxyribonucleotide triphosphates, 2 mM $MgCl_2$, 1 U Taq DNA polymerase, 1X Taq buffer, and 0.6 mM reverse and forward primers. DNA amplification was carried out in a thermal cycler. The PCR program included an initial denaturation step at 94°C for 3 min; this was followed by 40 cycles of denaturing at 94°C for 30 s, annealing at optimum annealing temperature for 30 s, and extension at 72°C for 1 min. After the last cycle, samples were kept at 72°C for 5 min for final extension. The samples of PCR amplification were stored at 4°C before loading. The amplified products were electrophoretically separated at 70 V in a 1.2% agarose gels for 2 h along with the low range DNA ruler, stained with ethidium bromide, visualized by Bio-Rad Gel Documentation System and documented thereafter.

Statistical analyses

ISSR markers were scored for the presence (1) and absence (0) of the corresponding band among the genotypes in the form of a binary matrix, and the data matrix was subjected to further analysis using NTSYS-pc version 2.11W (Rohlf, 1992). The SIMQUAL program was used to calculate Jaccard's similarity coefficients. The resulting similarity matrix was used for unweighted pair-group method with arithmetic mean (UPGMA)-based dendrogram construction. Polymorphism information content (PIC) for

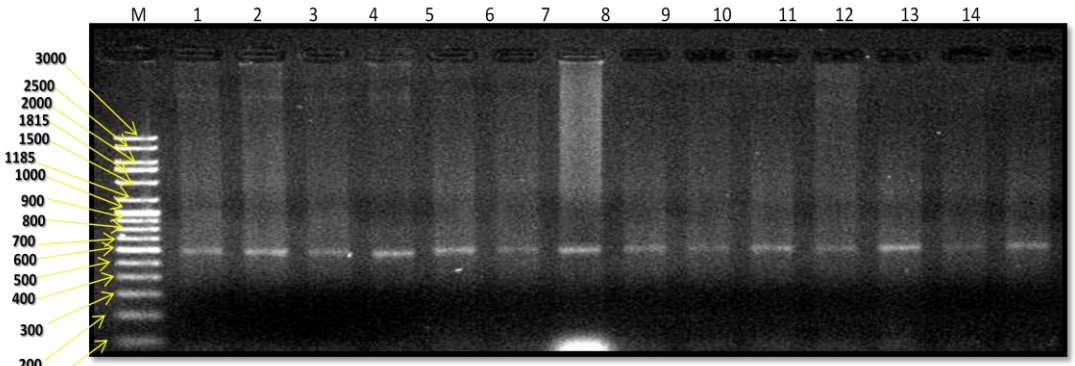
SSR markers was calculated using the following formula: PIC*i*= 1 - ΣP*ij*2 where *PICi* is the PIC of marker *i*; *Pij* is the frequency of the *j*th pattern for marker *i*, and the summation extends over *n* patterns.

RESULTS AND DISCUSSION

The PCR amplification of DNA extracted from 14 genotypes of chickpea was performed with 7 primers. The PCR products run on agarose gel were scored manually. The amplification profile generated by each primer was compared and the relative molecular size of each band was examined by comparing with low range DNA ruler.Total number of loci amplified using 7 ISSR primers were found to be 22 with an average of 3.14 amplified loci per primer. The PIC value, gene diversity, size of amplified products and percentage polymorphism were found to be in the range of 0–0.364, 0–0.479, 350–2000 bp and0–100%, respectively (Table 1). Figure 1 reveals the amplification profile of the ISSR primers used under study. No monomorphic loci were amplified by the primers UBC848, UBC823 and UBC807 therefore the percentage polymorphism shown by these primers were found to be 100%. One unique band was generated from the primer UBC827.

Cluster analysis

Association among 14 genotypes, revealed by UPGMA cluster analysis is presented in Figure 2. The dendrogram separated genotypes at the demarcation of 0.6 similarity coefficient value into two major groups, Group A and Group B.



AMLIFICATION PROFILE OF PRIMER UBC 848

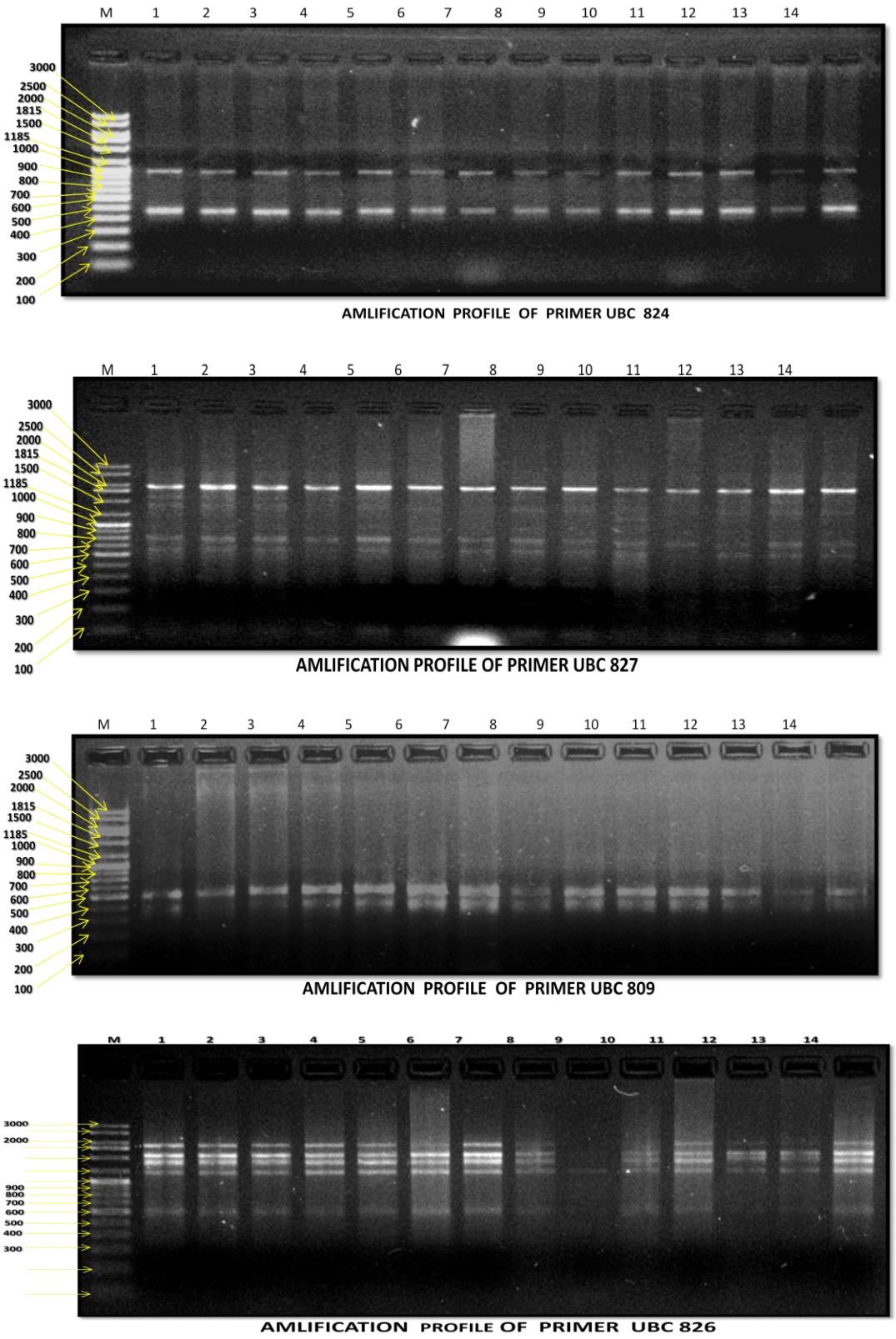


Figure 1. Amplification profile of Chickpea ISSR primers

Lanes denote: **M**, Marker; **1**, PG 186; **2**, PG 043; **3**, PG 071; **4, PG 114** ; **5**, PG 99; **6**, PG 120; **7**, PG 81; **8,** PG 100; **9**, ILWC 115; **10**, ILWC 141; **11**, ILWC 292 ; **12**, ILWC 115R; **13**, PG 65; **14**, PG 126.

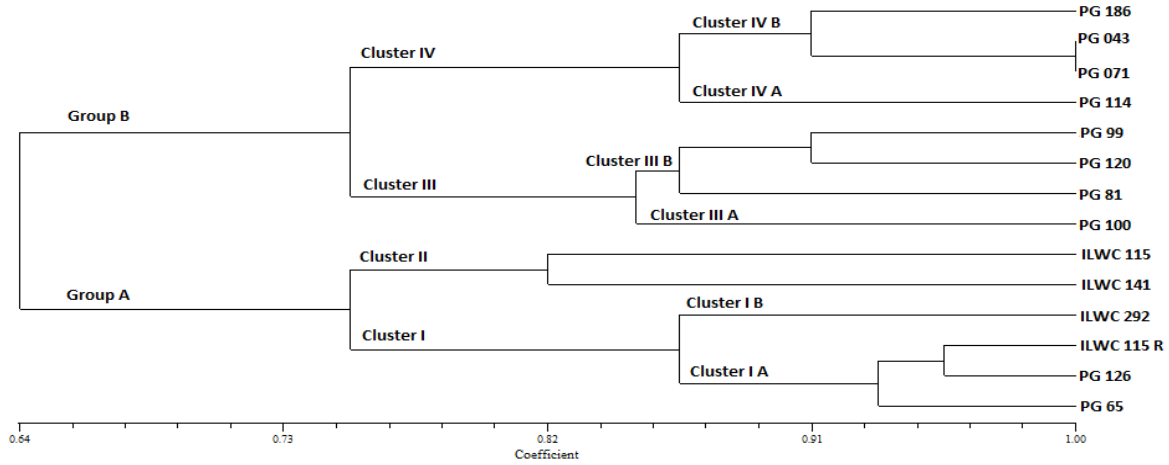


Figure 2.Dendrogram depicting the classification of 14 genotypes constructed through UPGMA method and based on ISSR primers. The scale at the bottom is Jaccard's coefficient of genetic similarity.

Table 1. ISSR Primers used for the amplification of fourteen genotypes of Chickpea

S. No.	Primer Code	Number of locia mplified	Number of monomorphicloci	Number of polymorphicloci	Polymorphism (%)	Polymorphic Information Content (PIC)	Gene diversity	Number of unique band	Range of amplifiedloci (bp)
1	**UBC 848**	2	0	2	100	0.324	0.408	0	600-625
2	**UBC 824**	2	2	0	0	0	0	0	450-1000
3	**UBC 827**	6	1	5	83.33	0.218	0.267	1	600-2000
4	**UBC 823**	2	0	2	100	0.353	0.459	0	1185-1500
5	**UBC 807**	3	0	3	100	0.364	0.479	0	350-800
6	**UBC 809**	2	1	1	50	0.107	0.122	0	400-550
7	**UBC 826**	5	1	4	80	0.130	0.146	0	600-1900

Table 2. Similarity matrix of 14 genotypes of chickpea using Jaccard's coefficient calculated from ISSR banding pattern using 7 markers.

Genotypes	PG 186	PG 043	PG 071	PG 114	PG 99	PG 120	PG 81	PG 100	ILWC 115	ILWC 141	ILWC 292	ILWC 115R	PG 65	PG 126
PG 186	1.000													
PG 043	0.909	1.000												
PG 071	0.909	1.000	1.000											
PG 114	0.863	0.863	0.863	1.000										
PG 99	0.863	0.863	0.863	0.818	1.000									
PG 120	0.772	0.772	0.772	0.727	0.909	1.000								
PG 81	0. 681	0. 681	0. 681	0.727	0.818	0.909	1.000							
PG 100	0.772	0. 681	0. 681	0. 636	0.818	0.909	0.818	1.000						
ILWC115	0.590	0.500	0.500	0. 454	0. 636	0.727	0. 636	0.818	1.000					
ILWC141	0.590	0.500	0.500	0.545	0. 636	0.727	0.727	0.818	0.818	1.000				
ILWC292	0.590	0.500	0.500	0. 636	0. 636	0.727	0.727	0.727	0. 636	0.727	1.000			
ILWC115 R	0. 681	0.590	0.590	0.545	0.727	0.818	0.727	0.818	0.727	0.727	0.909	1.000		
PG 65	0.590	0.500	0.500	0.454	0. 636	0.727	0. 636	0.818	0.818	0.818	0.818	0.909	1.000	
PG 126	0. 636	0.545	0.545	0.500	0. 681	0.772	0. 681	0.863	0.772	0.772	0.863	0.954	0.954	1.000

The lowest similarity value of 0.454 was noticed between genotypes ILWC 115 and PG 114 and between PG 65 and PG 114. The similarity value of 0.500 was recorded between ILWC 115 and PG 043, ILWC 115 and PG 071, ILWC 141 and PG 043, ILWC 141 and PG 071, ILWC 292 and PG 043, ILWC 292 and PG 071, PG 65 and PG 043, PG 65 and PG 071 and between PG 126 and PG 114, 0.545 between PG 126 and PG 043, PG 126 and PG 071, ILWC 141 and PG 114 and between ILWC 115R and PG 114, 0.590 between ILWC 115 and PG 186, PG 141 and PG 186, ILWC 292 and PG 186, PG 65 and PG 186 and ILWC 115R and PG 043 and between ILWC 115R and PG 071 (Rao *et al*., 2007) reported the similarity coefficient values between 0.76 and 1.00 and between 0.58 and 0.76 for cultivated chickpea lines and wild accessions, respectively.

CONCLUSION

Molecular markers are useful in germplasm management as well as in genetic diversity studies as a tool for rapid variety identification. The knowledge of genetic relationships is useful in plant breeding

programs since it allows making informed decisions, especially regarding the choice of genotypes to cross for the development of new populations or to facilitate the identification of parents to cross in hybrid combinations in order to maximize the expression of heterosis. In addition, molecular markers may be an important tool in plant variety protection (Reis *et al.*, 2012).

In this study, molecular diversity analysis of 14 genotypes of chick pea varieties released from Pantnagar University with 7 ISSR primers was carried out. No monomorphic loci were amplified by the primers UBC848, UBC823 and UBC807 therefore the percentage polymorphism shown by these primers were found to be 100% Primer UBC823being the most informative one.

On the basis of Jaccard's similarity coefficient genotypes The lowest similarity value of 0.454 was noticed between genotypes ILWC 115 and PG 114 and between PG 65 and PG 114 were found genetically more distant suggesting that these varieties can be used in future hybridization programs to generate desirable segregates. Rao *et al*., (2007) reported the similarity coefficient values between 0.76 and 1.00 and between 0.58 and 0.76 for cultivated chickpea lines and wild accessions, respectively. Primers UBC 827 gave one unique band (500 bp) for different genotypes which clearly indicates that these primers can be used to identify this genotype.

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