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Full Length Research Paper

Genetic fingerprinting of chickpea (*Cicer arietinum* L.) germplasm using ISSR markers and their relationships

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Genetic diversity analysis of chickpea germplasm can provide practical Information for selection of parental material and thus, assist in forecasting breeding strategies. Released cultivars and breeding lines of total twelve chickpea genotypes were subjected to ISSR analysis for assessment of genetic diversity. A total of 10 ISSR primers were used in the present investigation. Amplification of genomic DNA of the 12 genotypes using ISSR analysis yielded 492 fragments that could be scored. The total number of bands amplified by 3' anchored primers varied from 36 to 96. The primers based on poly (ATG) and (GAA) motifs produced least number of fragments (36) whereas, primers (AC) T and (AC) TT, produced maximum number of fragments (96). The unique band as produced by the GGAGA primer in the BCP-15 genotype may be attributed to temperature tolerance phenotype.

Key words: Chickpea, Genetic diversity, ISSR.

INTRODUCTION

The utility of DNA markers for marker-assisted selection (MAS) is the current trend in 'Modern Agriculture'. These DNA markers allow the construction of complete genome map and can be integrated with conventional linkage maps, which play an important role in plant breeding strategies (Simon and Muehlbauer, 1997). Improvement of crop through utilization of available genetic diversity among the germplasm is the key to successful breeding programmes (Renganayaki et al., 2001). Variance of relatively highly heritable quantitative genetic markers provides estimates of genetic diversity and thus, genetic variation among diverse genotypes could be practical to select parents to be crossed.

Inter-simple sequence repeat (ISSR) markers have been proposed as a new source of genetic markers that are inherited in Mendelian fashion and are scored as dominant markers (Ratnaparkhe et al., 1998). ISSR analyses offer breeders and geneticists with competent means to link phenotypic and genotypic variations and it is rapidly being used by the research community in various fields of plant improvement (Godwin et al., 1997). Inter simple sequence repeat analysis (ISSR) analysis involves the polymerase chain reaction reaction (PCR) amplification of the regions between adjacent, inversely oriented microsatellites, using a single simple sequence repeat (SSR) motifs (di,- tri,- tetra,- pentanucleotides) containing primers anchored at 3' or 5' end by two or four arbitrary, often degenerate nucleotides (Zietkiewicz et al., 1994). ISSR markers linked to the traits of agronomic importance have been sequenced and used as STS markers in marker aided selection (Reddy et al., 2002). ISSR fingerprinting has been successfully applied to determine genetic diversity and relationships in a number of crop species (Joshi et al., 2000; Fernandez et al., 2002; Souframanien and Gopalakrishna, 2004).

Chickpea is the third most important pulse crop of the world and ranks first in the Indian subcontinent (FAO, 2004). Information about the relationships among elite breeding populations and the genetic diversity in available germplasm is requisite for design and establishing new chickpea breeding programs. Therefore, improvement of chickpea can be undertaken vis-a-vis genetic diversity statistics. The present investigation focuses molecular polymorphism as assessed by ISSR-PCR fingerprinting between desi and kabuli type chickpea genotypes differing in yield, grain quality and other

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Table 1. Details of chickpea (*Cicer arietinum* L.) germplasm used for diversity analysis.

S/No	Genotype	Type	Characteristics
1	BDN 9-3	Desi	Early maturity, drought and wilt tolerant, high yield
2	BDNG 57-3	Desi	Spreading plant type, wilt resistant
3	BCP-15	Desi	Bold seeded, temperature and wilt tolerant
4	BCP-17	Desi	Wilt resistant, extra bold seeded
5	BCP-28	Kabuli	White flowered, medium grain size and wilt resistant
6	BCP-48	Desi	Erect plant type, wilt resistant
7	JG-62	Desi	Most susceptible to wilt, twin podded
8	BCP-54	Desi	Medium seeded, wilt tolerant, low yield
9	BCP-73	Desi	White flowered, wilt tolerant
10	BCP-201	Kabuli	Wilt tolerant and high yield
11	Vishal	Desi	Wilt resistant and high yield
12	ICCV-2	Kabuli	Early maturity, rain fed as well as irrigated, wilt resistant

agronomic traits.

MATERIALS AND METHODS

Plant materials and DNA extraction

Table 1 lists the chickpea germplasm collected from 'Agricultural Research Station' Badnapur. Dist. Jalna (M.S.) India, used in the present study with their Agronomic characteristics. DNA was isolated using soaked seeds according to the procedure of Krishna and Jawali (Krishna and Jawali., 1997).

ISSR-PCR amplifications

PCR amplifications were carried out using a Perkin Elmer Cetus thermocycler. Ten primers (UBC primers) were chosen and allowed us to distinguish the germplasms (Table 2). Amplifications were performed for 40 cycles with denaturation at 94°C for 1 min, annealing at 50°C for 45 s and extension at 72°C for 2 min. Initial denaturation was done at 94°C for 5 min and a final extension step of 5 min at 72°C was also included. The reaction mixture (20 µl) contained 20 ng of DNA template, 10 mM of Tris-Hcl (pH 9.0), 2 mM $MgCl_2$, 50 mM KCl, 200 mM dNTP mix, 0.25 µM primer, 5 mM of spermidine and 0.8 units of *Taq* DNA polymerase. Mastermixes of each reaction were overlaid with 10 µl of mineral oil to prevent evaporation
. ISSR amplified PCR products were resolved on 2% agarose gel in 1X TBE buffer (89 mM Tris-Hcl, pH 8.3, 89 mM boric acid, 5 mM EDTA) at a constant voltage of 50 V for 4 h. The bands were scored quantitatively for presence (1) or absence (0) in each genotype.

RESULTS

Chickpea genotypes used in the present study include desi and kabuli type cultivars. The desi genotype possesses important agronomic characters like higher yield potential with early maturity and wilt resistance. Use of PCR-based markers may enhance the efficiency of conventional breeding methods for the introgression of these traits among the different chickpea cultivar types. As a pre-requisite, development of molecular marker polymorphism between the chickpea genotypes has been worked out.

The genetic fingerprinting of chickpea (*Cicer arietinum* L.) genotypes was performed using ISSR primers. Twelve *Cicer* genotypes were screened using 10 ISSR primers for PCR-studies. The primers tested were six dinucleotide [$(TC)_8C$, $(AC)_7T$, $(AC)_8TT$, $(AC)_8GG$, $(TG)_8GA$, $(TG)_8AA$] and three trinucleotide [$(ATG)_6$, $(CTC)_6$ and $(GAA)_6$] 3' anchored repeat primers. One pentanucleotide $(GGAGA)_3$ repeat primer was also included in the ISSR-PCR studies.

Among the dinucleotide repeats, $(TC)_8C$, $(AC)_7T$ and $(AC)_8TT$ produced better amplification patterns. A total of 492 bands were amplified across 12 cultivars with 7 primers, revealing an average of 70.28 bands per primer and 5.85 bands per primer per genotype. The total number of bands amplified by 3 anchored primers varied from 36 to 96. The primers sequences $(ATG)_6$ and $(GAA)_6$ amplified least number of bands (36). Whereas, primers $(AC)_7T$ and $(AC)_8TT$, amplified maximum number of bands (96) each. Figure 1 is the representative of the polymorphism observed among the chickpea genotypes as revealed by UBC-880.

DISCUSSION

The importance and need of chickpea cultivars at global level requires evaluation of germplasm to assist the future breeding programs. Hence, it is essential to characterize chickpea germplasm using markers like PCR-based marker such as RFLPs, RAPDs and microsatellites.

In the present study, out of the 10 primers used for PCR-Studies, 3 primers did not amplify DNA of any chickpea genotypes. The primers, which did not amplify DNA of all the genotypes, may not have found complementary sequences on the genomic DNA. Such non-amplifying primers are also reported in other crop plants (Tao et al., 1993). Of the 10 primers, two primers UBC-825 and UBC-855 revealed dinucleotide repeats $(AC)_7T$ and $(AC)_8TT$. These primers have amplified maximum

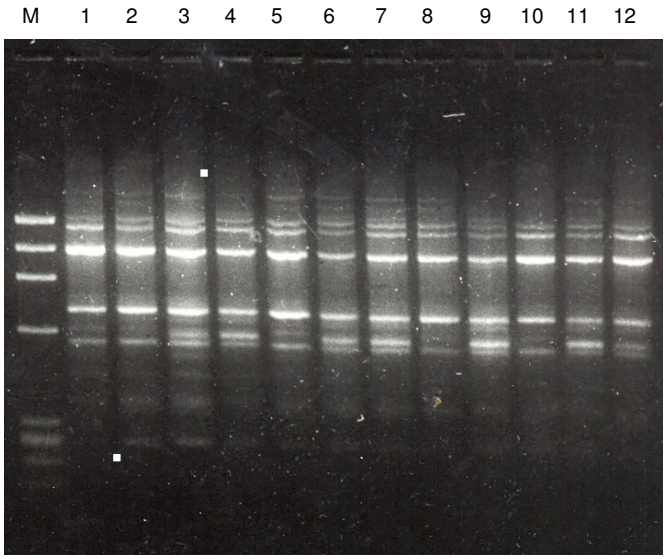

Figure 1. Agarose gel patterns of PCR products amplified with the primer UBC-880. *M* represents the phi X 174 *Hae III* molecular weight marker. Lane identification refers to the genotypes as listed in Table 1.

number of bands, while primer repeats $(ATG)_6$ and $(GAA)_6$ have amplified least number of bands. The results obtained from present ISSR-PCR studies indicate that $(AC)_8$ repeats are more frequent in *Cicer* genome than the TG repeats.

Of the ten primers used, the pentanucleotide $(GGAGA)_3$ repeat primer UBC-880 gave the polymorphy in comparison with the other primers for dinucleotide and trinucleotide repeats. The pattern generated by pentanucleotide primer UBC-880 was irrespective of the 3' anchor. However, in the present case $(GGAGA)_3$ repeat generated unique bands in the BCP-15, BCP-17, BCP-48, JG-62, BCP-54 and Vishal cultivars. Amongst these, BCP-15 is a temperature tolerant variety. The polymorphy visualized may be attributed to temperature tolerance phenotype.

The trinucleotide repeats $(ATG)_6$ and $(GAA)_6$ gave least amplification in the present studies. This may be due to the reason that chickpea genomic sequences may not be in the range of amplification by *Taq* DNA polymerase. Joshi et al. (2000) studied the genetic diversity and phylogenetic relationships as revealed by ISSR polymorphism in the genus *Oryza*, where they could find that the poly (TG) and poly (CA) generated least number of bands by PCR-amplifications.

Applications of ISSR technique in gene tagging and marker assisted selections are becoming more popular. The advantage in the use of ISSR markers lies in their linkage to SSR loci and likely to mark gene rich regions (Kojima et al., 1998). Ratnaparkhe et al. (1998) studied the inheritance of Inter-simple sequence repeat polymorphisms and linkage analysis with *Fusarium* resistance gene in chickpea. They demonstrated that a simple sequence repeat $(AC)_8YT$ (marker UBC-885_{500}) was linked to the gene for resistance to *Fusarium* wilt race 4.

Table 2. List of ISSR primers.

S/ No	Primer	Anchor sequence
1.0	UBC-823	5' TCTCTCTCTCTCTCTCC 3'
2.0	UBC-825	5' ACACACACACACACT 3'
3.0	UBC-855	5' ACACACACACACACACTT 3'
4.0	UBC-857	5' ACACACACACACACAC GG 3'
5.0	UBC-860	5' TGTGTGTGTGTGTGTGGA 3'
6.0	UBC-860	5' TGTGTGTGTGTGTGTGAA 3'
7.0	UBC-864	5' ATGATGATGATGATGATG 3'
8.0	UBC-866	5' CTCCTCCTCCTCCTCCTC 3'
9.0	UBC-868	5' GAAGAAGAAGAAGAAGAA 3'
10	UBC-880	5' GGAGAGGAGAGGAGA 3'

The marker concerned, UBC-885_{500} was found to be linked in repulsion with the *Fusarium* wilt resistance gene at a distance of 5.2 cM. ISSR markers are as well practical to study hybrid seed genotyping and varietal identification. Ranade et al. (2000) studied the hybrid seed genotyping and plant varietal identification using ISSR markers in cotton for GOT (Grow Out Test) for genetic purity. Rao et al. (2007) reported the ISSR fingerprinting in cultivated chickpea and its wild progenitor to correlate the relationship measures based on pedigree data and morphological traits for the selection of good parental material in chickpea breeding programmes. However, utility of such ISSR marker actually lies in converting it into sequence tagged microsatellite sites (STMS) in marker aided selection.

The results obtained in the present investigation show that the ISSR primers are informative markers which can be examined to correlate banding patterns and agronomic characteristics. However, this necessitates effective collaboration between plant breeders and molecular biologists to tag the gene of interest. The unique band as produced by the (GGAGA) primer in the BCP-15 genotype may serve as unique identifier phenotype for temperature tolerant characteristics. However, this needs to be further investigated using more number of primers. The measured and tailored fingerprints could be generated to find cultivar specific marker which can be exploited in planning the chickpea crosses and consequently it may enhance chickpea germplasm management and conservation.

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REFERENCES

FAO (Food and Agricultural Organization) (2004). Available at: http://faostat.fao.org.

Fernandez ME, Fygueiras AM, Benito C (2002). The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. Theor. Appl. Genet. 104: 845-851.

Godwin ID, Aitken EAB, Smith LW (1997). Application of inter-simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis 18: 1524-1528.

Joshi SP, Gupta VS, Aggarawal PK, Ranjekar PK, Brar DS (2000). Genetic diversity and phylogenetic relationship as revealed by inter-simple sequence repeat (ISSR) polymorphism in the genus *Oryza.* Theor. Appl. Genet. 100: 1311-1320.

Kojima T, Nagaoka T, Noda K, Ogihara (1998). Genetic linkage map of ISSR and RAPD markers in Einkorn wheat in relation to that of RFLP markers. Theol. Appl. Genet. 96: 37-45.

Krishna TG, Jawali N (1997). DNA isolation from single or half seeds suitable for Random Amplified Polymorphic DNA analyses Anal. Biochem. 250: 125-127.

Ranade R, Vaidya UJ, Kotwal SA, Bhagwat A, Gopalakrishna T (2000). Hybrid seed genotyping and plant varietal identification using DNA markers. In: DAE-BRNS symposium on the use of nuclear and molecular techniques in crop improvement, 6-8 December 2000, Mumbai, pp. 338-345.

Rao LS, Usha Rani P, Deshmukh PS, Kumar PA, Panguluri SK (2007). RAPD and ISSR fingerprinting in cultivated chickpea (*Cicer arietinum* L.) and its wild progenitor *Cicer reticulatum Ladizinsky* Genet. Res. Crop Evol. 54: 1235-1244.

Ratnaparkhe MB, Santra DK, Tullu A, Muehlbauer FJ (1998). Inheritance of inter simple sequence repeat polymorphism and linkage with fusarium wilt resistance gene in chickpea. Theor. Appl. Genet. 96: 348-353.

Reddy MP, Sarla N, Siddiq EA (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica 128: 9-17.

Renganayaki K, Read JC, Fritz AK (2001). Genetic diversity among texas bluegrass (Poa arachnifera Torr.) revealed by AFLP and RAPD markers Theor. Appl. Genet. 102: 1037-1045.

Simon CJ, Muehlbauer FJ (1997). Construction of chickpea linkage map and its comparison with the maps of pea and lentil. J Herid. 88: 115-119.

Souframanien J, Gopalakrishna T (2004). A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. Theor. Appl. Genet. 109: 1687-1693.

Tao Y, Mannes JM, Ludlow MM, Henzell RG (1993). DNA polymorphisms in grain sorghum (*Sorghum bicolor (L.) Moench*) Theor. Appl. Genet. 86: 679-688.

Zietkiewicz E, Rafalaski A, Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)- anchored polymerase chain reaction amplification. Genomics 20: 176-183.