The Bioscan 9(4): 1707-1710, 2014 (Supplement on Genetics and Pland Breeding) www.thebioscan.in
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

# ISSR MARKER IN ACCESSING GENETIC DIVERSITY IN CHICKPEA GENOTYPES

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**KEYWORDS**
ISSR marker
Genetic diversity
Chickpea

**Received on :**
04.11.2014

**Accepted on :**
28.12.2014

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**ABSTRACT**
Genetic diversity is an essential component for Chickpea breeding. To access this genetic diversity in eighteen different genotypes, total of twenty ISSR markers were used, out of which seven ISSR primers, yielded a total of 37 amplified loci. Out of these 37 amplified loci, 32 loci were polymorphic whereas 5 loci were found monomorphic. The numbers of amplified loci varied from minimum of three to maximum of eight bands with an average of 5.28 loci per primer. It was lowest with Primer 8161-043 and Primer 8161-045 while highest with Primer 8161-049. One primers *viz*., Primer 8161-041 gave a total of one unique bands and this was unique to 612 bp for genotype PG105. Dendrogram based on UPGMA analysis of ISSR data grouped 18 genotypes in to four major clusters namely A, B, C and D. The cluster A had two genotypes. The major cluster B was sub divided into two groups $B_1$ and $B_2$ at the demarcation having 68.7 percent similarity. The group $B_1$ included ten genotypes while group $B_2$ comprised of four genotypes. The cluster C and D were found to be monogenotypic respectively. Based on estimated genetic similarity matrix, the highest genetic similarity value 0.930 was noticed between PG049 and PG081 while lowest similarity value 0.442 was noticed between PG105 and PG055. The study suggests that the genotypes PG105 and PG055are most genetically distant and beneficial for hybridization programmes to generate transgressive segregants. Current study also indicates that ISSR show mostly 100% polymorphism and acts useful tool in determining the genetic diversity and genotyping some of the chickpea accession regardless of environmental conditions.

## INTRODUCTION

Chickpea is the third most important pulse crop of the world after dry bean and dry pea, it is traditionally grown in different parts of the world covering Asia, Europe and north and South America. India is the largest producer of chickpea in the world.India contributes 67% of the global chickpea production. India ranks first in chickpea production and area with 8.25 million tons produced from 8.75 million hectare area with productivity hovers around 943kg/ha (Agriculture Statistics Division, 2010-11). But still chickpea is not at par with current requirement of Indian population. Therefore to develop high yielding genotypes from the available materials is major challenge for pulses breeding. Genetic diversity is one of the key prerequisite for increasing yields which is been most lacking in cultivated types of chickpea. 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. Thus information regarding genetic diversity and genetic relationships among different genotypes is very valuable in crop improvement. Morphological characteristics, is often difficult, since most of these characteristics are under the influence of environmental factors therefore molecular markers are useful for the genetic identification, estimating the genetic diversity among plant genotypes and allow more reliable differentiation of genotypes. Various molecular markers are available for analysis of genetic diversity, but Inter-simple sequence repeat (ISSR) is more consistent markers, as they generate a greater number of polymorphic loci per primer (Ratnaparkhe *et al.,* 1998; Aggarwal *et al.,* 2011). Unlike RAPDs, ISSR markers are detected using longer semiarbitrary SSR primers at highly stringent conditions. ISSR markers do not require a prior knowledge of the SSR targets sequences and are highly reproducible due to their primer length and to the high stringency achieved by the annealing temperature and were found to provide highly polymorphic fingerprints (Bornet and Branchard, 2001). ISSR markers have been employed to analyze genetic diversity and relationships in a number of crops (Ajibade *et al.,* 2000; Raina *et al.,* 2001; Bart *et al.,* 2002). In chickpea, Ratnaparkhe *et al.* (1998b) reported that the ISSR technique is helpful in finding markers closely linked to a disease-resistant gene. In addition, these markers have been successfully used to study diversity and phylogenetic relationships in chickpea for the last decade (Iruela *et al.,* 2002; Rajesh *et al.,* 2003; Rao *et al.,* 2007; Bhagyawant and Srivastava, 2008; Aggarwal *et al.,* 2011). Therefore present study was carried out to access the genetic diversity among the 18 genotypes using ISSR markers to obtain desirable parents for chickpea breeding programme.

## MATERIALS AND METHODS

The present investigation was carried out during *Rabi* 2012-

2013 at Norman E. Borlaug Crop Research Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India. The experimental material comprised of eighteen chickpea genotypesviz. PG063, PG049, PG119, PG120,PG081, PG101, PG105, IGB3, PG065, PG045, PG102,PG038, PG039, PG041, PG055, PG052, PG083, PG033. Fresh leaf samples from 10-15 days old seedlings were used for DNA extraction. CTAB procedure was used for isolation of DNA (Doyle *et al.*, 1987). The DNA amplification reactions were carried out in 0.2 mL PCR tubes using an eppendorf Thermalcycler with 17-18 mer screened ISSR primers. The amplification consisted a 5 min. initial denaturation step at 94°C followed by denaturation at 94ºC for 1.0 min. Annealing of the primer was done at 42ºC for 1.0 min, followed by an extension period for 2.0 min. at 72ºC. The reactions were subsequently subjected to 40 additional cycles after reaching the final annealing temperature. This was followed by a final extension at 72ºC for 10 min. Quantification of the DNA was done through electrophoresis on a 1% agarose gel. Twenty ISSR primers were tested for their ability to amplify scorable and reproducible DNA fragments. Primers resulting in faint or irreproducible bands were excluded from subsequent analysis. Finally seven primers were selected for these studies are given in Table 1.1. The data was analyzed using the SIMQUAL option to generate a similarity matrix among all possible pairs of genotypes and primers. The similarity matrix was run on SAHN clustering (Sokal R. R. and Michener C. D. 1958) and a dendrogram was created by using UPGMA and Jaccard's similarity coefficient (Jaccard P. 1908). PCA was done by calculating eigen values using EIGEN and PROJ modules. NTSys pc 2.0 program (Applied Biosystem Inc. USA) was used to perform the above mentioned functions (Rohlf R. J., 1990).

## RESULTS AND DISCUSSION

The ISSR banding pattern was observed among the eighteen genotypes using 20 primers. Out of the total number of 20 primers taken up for the study, only 7 primers gave amplification in all the 20 genotypes. Hence the analysis was carried out with the data generated from those seven primers (Table 1.1).They showed wide range of polymorphism ranging between 60-100%. A total of 37 loci were detected, out of which, 32 loci were polymorphic and5loci was monomorphic. The numbers of amplified loci varied from 3-8 with an average of 5.28 loci per primer. It was lowest with Primer 8161-043, Primer 8161-045while highest with Primer 8161-049.One primers *viz.*, Primer 8161-041 gave a total of one unique bands and this was unique to 612bp for genotype PG105. The banding profiles of four primers were depicted in Fig. 1.2. These ISSR primers gave unique bands in one genotypes which can be used for identification of the respective genotypes. But these primers failed to categorize the rest of genotypes. This may be due to less number of primers which failed to cover the whole genome. Further study using specific and high resolution molecular markers can be done, so that the differences at the DNA level can be achieved.

### Cluster analysis

ISSR marker based UPGMA analysis divided 18 genotypes into four major clusters A, B, C and D. The major cluster B was sub divided into two groups $B_1$ and $B_2$ at the demarcation having 68.7 percent similarity. The group $B_1$ included ten genotypes PG033, PG052, PG041, PG039, PG038, PG102, PG045, PG065 IGB-3and PG101. PG038 and PG102 together showed a similarity of 90.7 percent with PG045. While IGB-3 showed a similarity of 93.0 with PG065, PG045, PG038 and PG039. The group $B_2$ comprised of four genotypes PG049, PG119, PG120 and PG081. PG081 and PG049 which were 93.0 percent similar to each other.The cluster A had two genotypes are (PG063, PG083) and having 79.1 percent similarly. The cluster C contained one genotype PG055 having 53.4 percent similarly with PG063 which is member of cluster A and far from PG055. The cluster D had one genotype *i.e.* PG105 (Fig. 1.1).Based on estimated genetic similarity matrix, the highest genetic similarity value 0.930 was noticed between PG049 and PG081, PG038 and IGB3, IGB3 and PG065, IGB3 and PG045 followed by 0.907 between PG119 and PG120, PG120 and PG081, PG101 and IGB-3, PG081 and PG041, PG065 and PG045, PG065 and PG102, PG065 and PG038, PG045 and PG102, PG045 and PG038 (Table1.2). Based on these similarity coefficients it can be said that these genotypes were more similar genetically. The lowest similarity value 0.465 was noticed between PG105 and PG083 followed by 0.512 between PG055 and PG083, 0.535 between PG038 and PG083, PG065 and PG083, 0.406 between PG063 and PG055.Thus the above results suggest that crosses obtain from parents viz. PG105x PG055, PG083 x PG055, PG052 x PG105, PG063 x PG055, PG065 x PG083 are highly

**Table 1.1: Seven ISSR primers used for amplification of DNA**

| Primer code | Total no. of amplified loci | Total no of polymorphic loci | Total no. of monomorphic loci | Polymorphism (%) |
|---|---|---|---|---|
| 8161-041 | 8 | 8 | 0 | 100 |
| 8161-043 | 3 | 3 | 0 | 100 |
| 8161-045 | 3 | 3 | 0 | 100 |
| 8161-047 | 5 | 5 | 0 | 100 |
| 8161-049 | 8 | 5 | 3 | 62.5 |
| 8161-050 | 5 | 0 | 0 | 100 |
| 8161-054 | 5 | 3 | 2 | 60 |

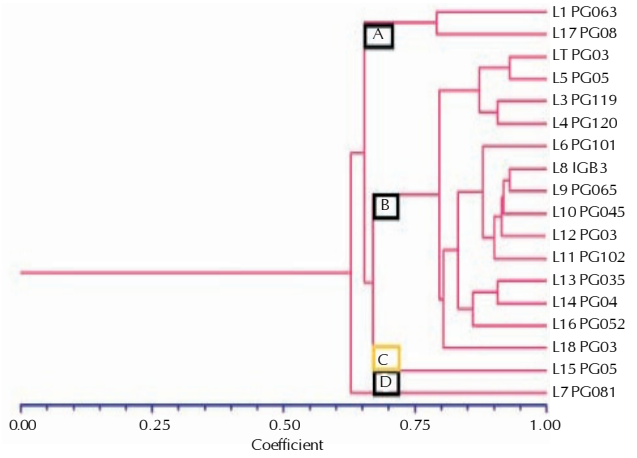


**Figure 1.1 - ISSR marker based UPGMA analysis**

productive and divergent enough to get fair amount of variability.

**Genetic Diversity**

The overall range of similarity was found to be 0.44 to 0.93 among the eighteen genotypes which is similar to that found by *Irula et al.,* where the similarity between 14 *Cicer* species ranged from 0.30 to 0.98. This indicated that large scope exists to use the different exotic germplasm to broaden the genetic base of Indian chickpea cultivars. Thus present study indicates that the parent PG105 and PG083 are divergent enough so that they can be crossed to obtain better sergeants. The conducted study also refers that ISSR maker either singly or in combination may be of great potential use in the establishing of identities of unknown genotypes and to monitor the designed crosses involving these genotypes. Similar types of findings had also been reported by Souframanien *et al.* (2002), Souframanien *et al.* (2004) in *Vigna*, Datta *et al.* (2007) in different pulses and Singh *et al.* (2008) in pigeon pea. Chattopadhyay *et al.* (2005) employed ISSR and RAPD to assess the genetic diversity among mungbean germplasm comprising varieties, landraces and wild accessions and reported efficiency of ISSR markers in giving higher frequency of polymorphic bands and polymorphic information content values. Similar studies in pulses were performed earlier by Sudupak *et al.* (2004), Gunjanjyoti *et al.* (2005), Choudhury *et al.* (2006) and Rao *et al.* (2007).

The results obtained in the present investigation show that the ISSR primers are informative markers which can be examined to correlate banding patterns and genetic relatedness. The unique band as produced by the primer in the PG-105 genotype may serve as unique identifier for this particular phenotype. Furthermore, we observe that using DNA marker technology like ISSR, identification of variability became relatively easier then morphological basis. It is more suitable for the selection of suited parental lines for transgressive breeding and combining favorable alleles. In current study we found these following suitable crosses PG105x PG055, PG083 x PG055, PG052 x PG105, PG063 x PG055 and PG065 x PG083 for crossing to get the heterotic $F_1$s as well as chances of getting transgresive segregents in $F_2$ generation.

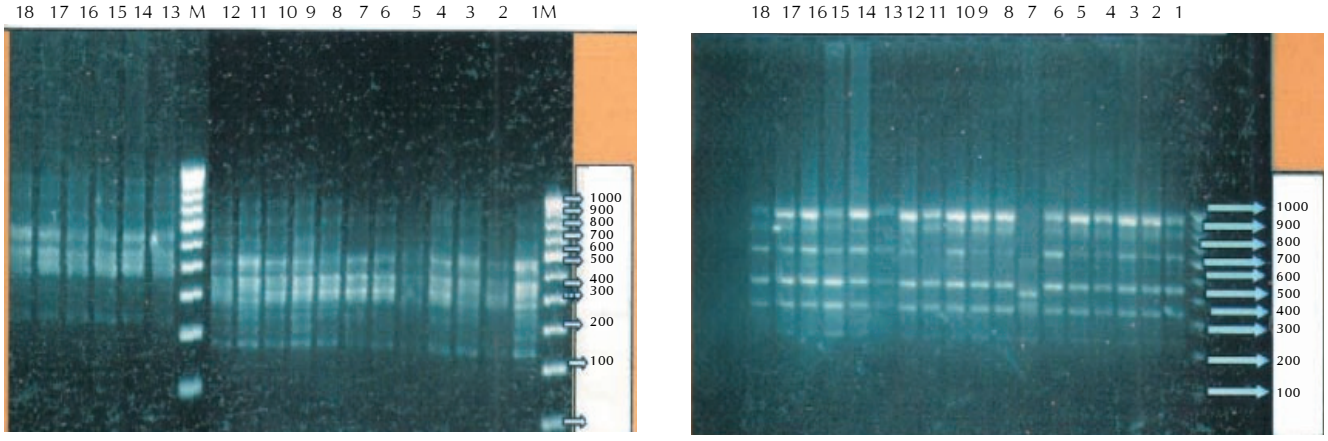


**Figure 1.2 - ISSR marker based Gel documentation of two primers**

**Table 1.2: Genetic relatedness through similarity index by ISSR marker**

| Genotype | | Relatedness Close relative | Percent similarity | Distant relative | Percent similarity |
|---|---|---|---|---|---|
| 1 | PG063 | PG083,PG102 | 79 | PG055 | 53.5 |
| 2 | PG049 | PG081 | 93 | PG083 | 58.1 |
| 3 | PG119 | PG120 | 90.7 | PG055 | 58.1 |
| 4 | PG120 | PG081 | 90.7 | PG055 | 58.1 |
| 5 | PG081 | PG041 | 90.7 | PG083 | 60.5 |
| 6 | PG101 | IGB3 | 90.7 | PG105 | 62.8 |
| 7 | PG105 | IGB3 | 72.1 | PG055 | 44.2 |
| 8 | IGB-3 | PG045, PG038, PG065 | 93 | PG083 | 60.5 |
| 9 | PG065 | IGB 3 | 93 | PG083 | 53.5 |
| 10 | PG045 | IGB 3 | 93 | PG083 | 62.8 |
| 11 | PG102 | PG065, PG045, PG038 | 90.7 | PG083 | 58.1 |
| 12 | PG038 | IGB 3 | 93 | PG083 | 53.5 |
| 13 | PG039 | IGB 3 | 90.7 | PG083 | 55.8 |
| 14 | PG041 | PG081, PG039,IGB3 | 90.7 | PG083 | 55.8 |
| 15 | PG055 | PG041 | 76.7 | PG105 | 44.2 |
| 16 | PG052 | PG039, PG041,PG101 | 86 | PG105 | 53.5 |
| 17 | PG083 | PG063 | 79.1 | PG055 | 51.2 |
| 18 | PG033 | IGB 3 | 86.0 | PG083 | 60.5 |

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