Plant Archives Vol. 19 No. 1, 2019 pp. 1974-1977 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

CURRENT STATUS OF GENETIC DIVERSITY ASSESSMENT IN CHICKPEA USING MOLECULAR MARKERS- A REVIEW

Ashwani Kumar[1] and R.S. Yadav[2*]

[1]U.P. Council of Agricultural Research, Lucknow (UP), India
[2]Department of Botany, K.K. P.G. College, Etawah (UP), India

Abstract

In this review, we have discussed most significant molecular markers that can be routinely employed in various aspects of plant genome analysis and their use in chickpea enhancement. Molecular markers have diverse applications in chickpea enhancement in the areas of genetic diversity. The knowledge of genetic diversity has a significant impact on the chickpea enhancement and this information has been successfully used for efficient germplasm management, fingerprinting and genotype selection. Genetic diversity can be made by diverse methods. It is generally believed that the use of molecular markers is more reliable and repeatable as compared to other methods. Molecular markers give extremely discriminatory information and hence, are regularly used for genetic studies.

Key words: Chickpea, Molecular marker, RAPD, SSR and Genetic diversity.

Introduction

Chickpea is a cool season grain legume, serves as a chief economical source of protein and energy in India and plays a major role in the improvement of soil fertility. Two types of chickpea are grown: desi and kabuli. It is a valuable and essential agricultural crop but yield potential is restricted by biotic and abiotic stresses with *Ascochyta* blight, *Fusarium* wilt, drought, cold and salinity. Assessment of genetic variability within chickpea is necessary for chickpea improvement and the conservation of genetic resources. Information of genetic diversity is a supportive tool in gene-bank management and breeding experiments such as germplasm tagging, identification or eradication of duplicates in the gene store and establishment of core collections.

Molecular markers can explore the genetic diversity in plants the utility of DNA-based markers for equitable assessment of molecular diversity and establishing exact phylogenetic associations along with species as compared to morphological, cytological and biochemical markers has been well understood. Genetic diversity can be estimated by phenotypic identification or molecular markers. On the other hand, morphological traits have a number of limitations as well as low polymorphism, low heritability and late appearance and controlled by epistatic and pleiotropic gene effects (Eivazi *et al.,* 2008). Molecular markers are cooperative and complement to morphological characterization of accessions because they are abundant, independent of environmental effects and permit cultivar recognition very early in crop improvement (Manifesto *et al.,* 2001).

***Author for correspondence :** E-mail: ramarsy@rediffmail.com

Genetic diversity assessment using molecular markers

Various types of molecular markers are utilized to assess DNA polymorphism and are generally classified as hybridization-based markers and polymerase chain reaction (PCR)-based markers. Different types of molecular markers have been developed in different crops and have been used for the study of genetic diversity, population structural analysis and gene discovery (Gupta *et al*., 2008, 2013). In chickpea, diverse molecular markers including Amplified fragment length polymorphism (AFLPs) (Nguyen *et al*., 2004), Sequence tagged microsatellite site (STMS) (Sethy *et al*., 2006b)), Random amplified polymorphic DNA (RAPDs) (Talebi *et al*., 2008a), Inter simple sequence repeat (ISSRs) (Jida *et al*., 2018) and SSRs (Sefera *et al*., 2011; Keneni *et al*., 2012; Ghaffari *et al*., 2014; Aggarwal *et al*., 2018) have been used for genetic diversity analysis. Simple sequence

repeats (SSRs) are common and informative molecular markers used for genetic diversity studies because of their simplicity, high levels of polymorphism, high reproducibility and co-dominant inheritance patterns (Powell *et al.,* 1996; Castro *et al*., 2011; Thudi *et al*., 2014).

DNA markers have been used widely for fingerprinting of plant genomes, genetic diversity analysis and to understand the evolutionary associations among crop species. Ahmad (1999) and Sudupak *et al*. (2002) used RAPD markers to examine genetic relations among the annual *Cicer* species. AFLP markers have also been used to study the genetic relations in the nine annual species (Sudupak *et al*., 2004) and the assemblage was similar as found with the RAPD markers (Sudupak *et al*., 2002; Shan *et al*., 2005). Genetic diversity in the chickpea has been find out by a class of molecular markers such as SSR (Saeed *et al*., 2011; Ghaffari *et al*., 2014), RAPD (Talebi *et al*., 2008b) and AFLP (Talebi *et al*., 2008a). Modern studies have demonstrated that highly polymorphic and Co-dominant SSR markers can be used for gene mapping and genetic diversity in chickpea (Winter *et al*., 2000; Saeed *et al*., 2011; Jannatabadi *et al*., 2014; Ghaffari *et al*., 2014). Hence, SSR markers can be extensively used for constructing chickpea related genetic linkage maps (Winter *et al*., 2000; Lichtenzveig *et al*., 2005; Jamalabadi *et al*., 2013).

Most of the RAPD polymorphisms appeared as single major band polymorphisms. RAPD also represents a source of genetic variation that is easy to access, very fast, capable in the production of DNA polymorphism. Comparable to the morphological and biochemical (isozyme) markers, RFLP and RAPD markers also demonstrate extremely low level of genetic variation between chickpea cultivars (Van Rheenen, 1992; Singh *et al*., 2003). The low polymorphism may have been caused by narrow pedigree and self-pollinated crop (Van Rheenen, 1992). Sant *et al*. (1999) engaged RAPD and oligo-nucleotide probes to assess genetic diversity between 29 elite Indian chickpea cultivars. In another study, out of 78 RAPD primers tested, only 20 primers revealed polymorphisms between the chickpea cultivars (Singh *et al*., 2003).

RAPD markers are cost effective and easy to operate, lack of reproducibility and dominant inheritance limit their use in plant breeding. However, the problem can be overcome by converting RAPD markers to more robust SCAR markers (Paran and Michelmore, 1993). Due to availability of huge numbers of expressed sequence tags (ESTs) in several plant species, SSRs have been developed from ESTs. Such SSRs are referred as EST-SSRs and development of such SSR markers involves small cost and less time as compared to conventional process of SSR development (Varshney *et al*., 2005). In recent years, Microsatellites or STMS markers have been shown to be more useful and highly polymorphic in comparison with other molecular markers (Akkaya *et al*., 1992; Morgante and Olivieri, 1993). In chickpea, several hundreds of SSR markers have been developed and mapped on intra- and inter-specific mapping populations (Winter *et al*., 1999; Huttel *et al*., 1999; Lichitenzveiz *et al*., 2005; Choudhary *et al*., 2005; Sethy *et al*., 2006a, 2006b). Large numbers of SSR markers have been identified, characterized and utilized extensively to identify genetically diverse germplasm with traits of interest for use in chickpea improvement (Keneni *et al*., 2012; Choudhary *et al*., 2012).

Conclusion

Molecular markers are widely used in chickpea improvement due to its simplicity, reproducibility and precise location. It is not affected by environmental effect. In recent times many molecular markers have been developed and are powerful tools for successful chickpea breeding. There are different genetic markers for evaluating genetic variation: morphological, biochemical and DNA markers. Molecular markers have several advantages over morphological markers. Molecular markers such as SSR and SNP are useful for construction of high density genetic maps of chickpea.

References

Aggarwal, H., S.P. Choudhary, M.K.Rana and R. Choudhary (2018). Assessment or Evaluation of Genetic Diversity among 66 Cultivars of Chickpea (*Cicer arietinum* L.) of Indian Origin Using SSR Markers. *Int. J. Curr. Microbiol. Appl. Sci.*, **7(2):** 523-533.

Ahmad. F. (1999). Random amplified polymorphic DNA (RAPD) analysis reveals genetic relationships among the annual *Cicer* species. *Theoretical and Applied Genetics*, **98:** 657-63.

Akkaya, M.S., Bhagwat A.A. and Cregan P.B. (1992). Length polymorphisms of simple sequence repeat DNA in soybean. *Genetics*, **132(4):** 1131-1139.

Castro, P., Millan T., Gil J., Merida J., Garcia M.L., Rubio J. and Fernadez-Romero MD (2011). Identification of chickpea cultivars by microsatellite markers. *J. Agric. Sci.,* **149(4):** 451-460.

Choudhary, P., Khanna S.M. and Jain P.K. (2012). Genetic structure and diversity analysis of the primary gene pool of chickpea using SSR markers. *Genet. Mol. Res.*, **11(2):** 891-905.

Choudhary, S., Sethy N.K., Shokeen B. and Bhatia S. (2005). Development of sequence tagged microsatellite site markers for chickpea (*Cicer arietinum* L.). *Molecular Ecology Notes*, **6:** 93-95.

Eivazi, A.R., Naghavi M.R., Hajheidari M., Pirseyedi S.M., Ghaffari M.R., Mohammadi S.A., Majidi I., Salekdeh G.H. and Mardi M. (2008). Assessing wheat (*Triticum aestivum* L.) genetic diversity using quality traits, amplified fragment length polymorphisms, simple sequence repeats and proteome analysis. *Annals of Applied Biology,* **152:** 81-91.

Ghaffari, P., Talebi R. and Keshavarz F. (2014). Genetic diversity and geographical differentiation of Iranian landrace, cultivars and exotic chickpea lines as revealed by morphological and microsatellite markers. *Physiol Mol Biol Plant*, **20(2):** 225-233.

Gupta, P.K., Rustgi S. and Mir R.R. (2008). Array-based high-throughput DNA markers for crop improvement. *Heredity*, **101:** 5-18.

Gupta, P.K., Rustgi S. and Mir R.R. (2013). Array-based high-throughput DNA markers and genotyping platforms for cereal genetics and genomics. In: P.K Gupta and R.K Varshney (Eds.), Cereal Genomics II. Springer: The Netherlands.

Huttel, B., Winter P., Weising K., Choumane W., Weigand F. and Kahl G. (1999). Sequence-tagged microsatellite markers for chickpea (*Cicer arietinum* L.). *Genome*, **42:** 210-217.

Jamalabadi, J.G., Saidi A., Karami E., Kharkesh M. and Talebi R. (2013). Molecular mapping and characterization of genes governing time to flowering, seed weight and plant height in an intraspecific genetic linkage map of chickpea (*Cicer arietinum* L.). *Biochem. Genet.*, **51:** 387-397.

Jannatabadi, A.A., Talebi R., Armin M., Jamalabadi J. and Baghebani N. (2014). Genetic diversity of Iranian landrace chickpea (*Cicer arietinum* L.) accessions from different geographical origins as revealed by morphological and sequence tagged microsatellite markers. *J Plant Biochem Biotechnol.*, **23(2):** 225-229.

Jida, Z., Alemu A. and Mullualem D. (2018). Genetic diversity among elite chickpea (*Cicer arietinum* L.) varieties of Ethiopia based on inter simple sequence repeat markers. *Afr. J. Biotechnol.*, **17(34):** 1067-1075.

Keneni, G., Bekele E., Imtiaz M., Dagne K., Getu E. and Assefa F. (2012). Genetic diversity and population structure of Ethiopian chickpea (*Cicer arietinum* L.) germplasm accessions from different geographical origins as revealed by microsatellite markers. *Plant Mol. Biol. Rep*, **30(3):** 654-665.

Lichitenzveiz, J., Scheuring C., Dodge J., Abbo S. and Zhang H.B. (2005). Construction of BAC and BIBAC libraries and their application for generation of SSR markers for genome analysis of chickpea (*Cicer arietinum* L.). *Theoretical and Applied Genetics*, **110:** 492-510.

Manifesto, M.M., Schlatter A.R., Hopp H.E., Suarez E.Y. and Dubcovsky J. (2001). Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. *Crop Sci.,* **41:** 682-690.

Morgante, M. and Olivieri A.M. (1993). PCR-amplified microsatellites as markers in plant genetics. *The Plant Journal*, **3(1):** 175-182.

Nguyen, T.T., Taylor P.W.J., Redden R.J. and Ford R. (2004). Genetic diversity estimates in *Cicer* using AFLP analysis. *Plant Breed*, **123(2):** 173-179.

Paran, I. and Michelmore R.W. (1993). Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. *Theoretical and Applied Genetics*, **85:** 985-993.

Powell, W., Morgante M., Andre C., Hanafey M., Vogel J., Tingey S. and Rafalski A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding,* **2:** 225-238.

Saeed, A., Hovsepyan H., Darvishzadeh R., Imtiaz M., Panguluri S.K. and Nazaryan R. (2011). Genetic diversity of iranian accessions, improved lines of chickpea (*Cicer arietinum* L.) and their wild relatives by using simple sequence repeats. *Plant Mol Biol Report*, **29:** 848-858.

Sant, V.J., Patankar A.G., Sarode N.D., Mhase L.B., Sainani M.N., *et al.* (1999). Potential DNA markers in detecting divergence and in analyzing heterosis in Indian elite chickpea cultivars. *Theo App Gene.*, **98(8):** 1217- 1225.

Sefera, T., Abebie B., Gaur P.M., Assefa K. and Varshney R.K. (2011). Characterisation and genetic diversity analysis of selected chickpea cultivars of nine countries using simple sequence repeat (SSR) markers. *Crop Pasture Sci*., **62(2):** 177-187.

Sethy, N.K., Choudhary S., Shokeen B. and Bhatia S. (2006a). Identification of microsatellite markers from *Cicer reticulatum*; molecular variation and phylogenetic analysis. *Theoretical and Applied Genetics*, **112:** 347-357.

Sethy, N.K., Shokeen B., Edwards K.J. and Bhatia S. (2006b). Development of microsatellite markers and analysis of intraspecific genetic variability in chickpea (*Cicer arietinum* L.). *Theoretical and Applied Genetics*, **112:** 1416-1428.

Shan, F., Clarke H.C., Plummer J.A., Yan G. and Siddique K.H.M. (2005). Geographical patterns of genetic variation in the world collections of wild annual *Cicer* characterized by amplified fragment length polymorphisms. *Theoretical and Applied Genetics*, **110:** 381-391.

Singh, R., Durga Prasa C., Singhal V. and Randhawa G.J. (2003). Assessment of genetic diversity in chickpea cultivars using RAPD, AFLP and STMS markers. *Journal of Genetics and Breeding*, **57:** 165-174.

Sudupak, M.A., Akkaya M.S. And Kence A. (2002). Analysis of genetic relationships among perennial and annual *Cicer*

species growing in Turkey using RAPD markers. *Theoretical and Applied Genetics*, **105:** 1220-1228.

Sudupak, M.A., Akkaya M.S. and Kence A. (2004). Genetic relationships among perennial and annual *Cicer* species growing in Turkey assessed by AFLP fingerprinting. *Theoretical and Applied Genetics*, **108:** 937-944.

Talebi, R., Fayaz R., Mardi M., Pirsyedi S.M. and Naji A.M. (2008a). Genetic relationships among chickpea (*Cicer arietinum*) elite lines based on RAPD and agronomic markers. *Int J Agric Biol.*, **8:** 301-305.

Talebi, R., Naji A.M. and Fayaz F. (2008b). Geographical patterns of genetic diversity in cultivated chickpea (*Cicer arietinum* L.) characterized by amplified fragment length polymorphism. *Plant Soil Environ*, **54:** 447-452.

Thudi, M., Upadhyaya H.D., Rathore A., Gaur P.M. *et al.* (2014). Genetic dissection of drought and heat tolerance in chickpea through genome-wide and candidate gene-based association mapping approaches. *PloS one*, **9(5):** e96758.

Van Rheenen, H.A. (1992). Biotechnology and chickpea breeding. *International Chickpea Newsletter*, **26:** 14-17.

Varshney, R.K., Graner A and Sorrells ME (2005). Genomic-assisted breeding for crop improvement. *Trends in Plant Science*, **10:** 621-630.

Winter, P., Benko-Iseppon A.M., Hüttel B., Ratnaparkhe M., Tullu A., Sonnante G., Pfaf T., Tekeoglu M., Santra D., Sant V.J., Rajesh P.N., Kahl G. and Muehlbauer F.J. (2000). A linkage map of chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. arietinum* × *C. reticulatum* cross: localization of resistance genes for *Fusarium wilt* races 4 and 5. *Theor. Appl. Genet.*, **101:** 1155-1163.

Winter, P., Pfaff T., Udupa S.M., Huttel B., Sharma P.C., Sahi S., Arreguin-Espinoza R., Weigand F., Muehlbauer F.J. and Kahl G. (1999). Characterization and mapping of sequence tagged microsatellite sites in the chickpea genome. *Molecular and General Genetics*, **262:** 90-91.