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IMPROVEMENT OF CHICKPEA USING MOLECULAR MARKERS

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Abstract:
Food crops exhibited different traits of agronomic importance. Chickpea is one such food crops which preferred in Asia; being major source of protein after milk. Chickpea being self-pollinated crop hence is supposed to display little genetic variation. A number of both protein and DNA based markers are available for the effective quantification of genetic variations in plant genomes. The protein based markers having sizable limitations hence, DNA based markers are more suitable as a choice. Thus genetic marker assisted selection is becoming a more reliable current trend in the modern day agriculture. Such DNA markers are fingerprints of a species in revealing polymorphism at molecular level. Molecular techniques using DNA polymorphism has increasingly been used to characterize and identify a novel germplasm for use in the crop enhancement programs.
Key Words: *RAPD, ISSR, DNA polymorphism, Chickpea.*

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INTRODUCTION

Legume grains occupy an important place in human nutrition because of being low in fat and rich in proteins, complex hydrocarbons, and minerals [1] exhibiting lower glycaemic index compared to other starchy foods. In addition, they contain a rich variety of phytochemicals, including phytosterols, natural antioxidants and bioactive compounds [2]. Epidemiological and intervention studies indicate that legume consumption is inversely associated with the risk of coronary heart disease [3] type II diabetes mellitus [4] and obesity [5]. Consumption of legumes also results in lower LDL cholesterol and higher HDL cholesterol [6].

Chickpea, pigeonpea and mungabean are the major cash pulse crops that are used as the sources of dietary protein. Chickpea seed, in particular, is the most preferred protein legume for its palatability and pliability [7]. Chickpea is a good source of carbohydrates and protein. Further, it has certain medicinal properties and is also used as an aphrodisiac, in treating bronchitis, catarrh, cutamenia, cholera, constipation, diarrhea, dyspepsia, flatulence, snakebite, sunstroke and warts [8]. The chickpea grains are rich in minerals and vitamins [9]. It also constitutes a good source of livestock feed. Apart from dietary benefits to both human and liverstock, it is very useful in the management of soil fertility due to its nitrogen fixation ability [10]. Ever increasing population pressures, an urgent and increased need to catalogue the available genotype of the majority of food crops; this includes chickpea. The genotype manipulation will go a long way in maintaining the food security of added mouth to feed [11].

Molecular markers have proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. A number of DNA based markers are now available for the effective quantification of genetic variation in plant species. A DNA marker may be defined as an assay for the detection of polymorphism in DNA sequence between samples. A number of different DNA marker systems have been described [12] most of which now rely on the sensitivity and specificity of the polymerase chain reaction [12]. DNA markers offer the advantages of being applicable to any developmental stage and any tissue of the plant, and of producing results that are independent of environment. Various types of molecular markers are utilized to evaluate DNA polymorphism and are generally classified as hybridization-based markers and polymerase chain reaction (PCR)-based markers. In the former, DNA profiles are visualized by hybridizing the restriction enzyme-digested DNA to a labeled probe, which is a DNA fragment of known origin or sequence. PCR-based markers involve in vitro amplification of particular DNA sequences or loci, with the help of specifically or arbitrarily chosen oligonucleotide sequences (primers) and a thermo-stable DNA polymerase enzyme. The amplified fragments are separated electrophoretically and banding patterns are detected by using methods such as staining and autoradiography.

TYPES OF DNA MARKERS

Randomly-Amplified Polymorphic DNA Markers (RAPD)

Welsh and McClelland [13] developed a new PCR-based genetic assay namely randomly amplified polymorphic DNA (RAPD). This procedure detects nucleotide sequence polymorphisms in DNA using a single primer of arbitrary nucleotide sequence. In this reaction, a single species of primer anneals the genomic DNA at two different sites on complementary strands of DNA template. If these priming sites are within an amplifiable range of each other, a discrete DNA product is formed through thermo cyclic amplification. On an average, each primer directs amplification of several discrete loci in the genome, making the assay useful for efficient screening of nucleotide sequence polymorphism between individuals [14]. However, due to the stochastic nature of DNA amplification with random sequence primers, it is important to optimize and maintain consistent reaction conditions for reproducible DNA amplification. These are dominant markers and hence have limitations in their use as markers for mapping, which can be overcome to some extent by selecting those markers that are linked in coupling [15]. RAPD assay has been used by several groups as efficient tool for identification of markers linked to agronomically important traits, which are introgressed during the development of near isogenic lines. The application of RAPDs and their related modified markers in variability analysis and individual specific genotyping has largely been carried out, but is less popular due to problems such as poor reproducibility faint or fuzzy products, and difficulty in scoring bands, leading to inappropriate inferences.

Sequence Characterized Amplified Regions for Amplification of Specific Band (SCAR)

Michelmore [16] and Martin [17] introduced this technique wherein the RAPD marker termini are sequenced and longer primers are designed (22–24 nucleotide bases long) for specific amplification of a particular locus. These are similar to STS markers [18] in construction and application. The presence or absence of the band indicates variation in sequence. These are better reproducible than RAPDs. SCARs

are usually dominant markers, however, some of them can be converted into co-dominant markers by digesting them with tetra cutting restriction enzymes and polymorphism can be deduced by either denaturing gel electrophoresis. Compared to arbitrary primers, SCARs exhibit several advantages in mapping studies (co-dominant SCARs are informative for genetic mapping than dominant RAPDs), map-based cloning as they can be used to screen pooled genomic libraries by PCR, physical mapping, locus specificity, etc. SCARs also allow comparative mapping or homology studies among related species, thus making it an extremely adaptable concept in the near future.

Cleaved Amplified Polymorphic Sequences (CAPs)

These polymorphic patterns are generated by restriction enzyme digestion of PCR products. Such digests are compared for their differential migration during electrophoresis [19]. PCR primer for this process can be synthesized based on the sequence information available in databank of genomic or cDNA sequences or cloned RAPD bands. These markers are co-dominant in nature.

Randomly Amplified Microsatellite Polymorphisms (RAMPO)

In this PCR-based strategy, genomic DNA is first amplified using arbitrary (RAPD) primers. The amplified products are then electrophoretically separated and the dried gel is hybridized with microsatellite oligonucleotide probes. Several advantages of oligonucleotide fingerprinting [20], RAPD [21] and microsatellite-primed PCR [22] are thus combined, viz., the speed of the assay, the high sensitivity, high level of variability detected and the non-requirement of prior DNA sequence information [23]. This technique has been successfully employed in the genetic fingerprinting of tomato, kiwi fruit and closely-related genotypes of *D. bulbifera [23]*.

Amplified Fragment Length Polymorphism (AFLP)

A recent approach adopted by Zabeau [24] known as AFLP. It is a technique based on the detection of genomic restriction fragments by PCR amplification and can be used for DNAs of any origin or complexity. The fingerprints are produced, without any prior knowledge of sequence, using a limited set of generic primers. The number of fragments detected in a single reaction can be 'tuned' by selection of specific primer sets. AFLP technique is reliable since stringent reaction conditions are used for primer annealing. This technique therefore ingenious combines RFLP and PCR techniques [25] and is extremely useful in detection of polymorphism between closely related genotypes. AFLP analysis depicts unique fingerprints regardless of the origin and complexity of the genome. Most AFLP fragments correspond to unique positions on the genome and hence can be exploited as landmarks in genetic and physical mapping [26]. AFLPs are extremely useful as tools for DNA fingerprinting [27] and also for cloning and mapping of variety specific genomic DNA sequences [28]. Similar to RAPDs, the bands of interest obtained by AFLP can be converted into SCARs. Therefore, AFLP provides a newly developed, important tool and finds a variety of applications.

Expressed Sequence Tag Markers (EST)

This term was introduced by [29]. Such markers are obtained by partial sequencing of random cDNA clones. Once generated, they are useful in cloning specific genes of interest and synteny mapping of functional genes in various related organisms. ESTs are popularly used in full genome sequencing and mapping programmes underway for a number of organisms and for identifying active genes thus helping in identification of diagnostic markers. Moreover, an EST that appears to be unique helps to isolate new genes. EST markers are identified to a large extent for rice, Arabidopsis, etc, wherein thousands of functional cDNA clones are being converted in to EST markers [30].

Single Strand Conformation Polymorphism (SSCP)

This is a powerful and rapid technique for gene analysis particularly for detection of point mutations and typing of DNA polymorphism [31]. SSCP can identify heterozygosity of DNA fragments of the same molecular weight and can even detect changes of a few nucleotide bases as the mobility of the single stranded DNA changes with change in its GC content due to its conformational change. To overcome problems of reannealing and complex banding patterns, an improved technique called asymmetric-PCR SSCP was developed [32], wherein the denaturation step was eliminated and a large-sized sample could be loaded for gel electrophoresis, making it a potential tool for high throughput DNA polymorphism. It was found useful in the detection of heritable human diseases. In plants, however, it is not well developed although its application in discriminating progenies can be exploited, once suitable primers are designed for agronomically important traits [33].

Application of Molecular Markers in Plant Genome Analysis and Breeding

Molecular markers have been looked upon as tools for a large number of applications ranging from localization of a gene to improvement of plant varieties by marker assisted selection. They have also become extremely popular markers for phylogenetic analysis adding new dimensions to the evolutionary theories. Genome analysis based on molecular markers has generated a vast amount of information and a number of databases are being generated.

Mapping and Tagging Of Genes

Plant improvement, either by natural selection or through the efforts of breeders, has always relied upon creating, evaluating and selecting the right combination of alleles. Molecular markers are routinely used to trace valuable alleles in a segregating population and mapping. These markers once mapped enable dissection of the complex traits into component genetic units more precisely [34] thus providing breeders with new tools to manage these complex units more efficiently in a breeding program. The very first genome map in plants was reported in maize [35], followed by rice [36] and Arabidopsis [37] etc. using RFLP markers. Maps have since then been constructed for several other crops like potato, barley, banana, members of *Brassicaceae,* etc [38]. Once mapped, these markers are efficiently employed in tagging several individual traits that are extremely important for a breeding programme like yield, disease resistance, stress tolerance, seed quality, etc.

CONCLUSION

The crop improvement using biotechnological PCR based tools assist in overcoming the challenges of increasing the crop yields [39]. Novel genetic technologies employ several molecular marker techniques, which permit establishment of linkage maps in crop plants [40]. These linkage maps also assist plant/crop breeders in the identification and characterization of genetic variability [41]. Molecular markers have large number of applications ranging from localization of gene to improvement of these plant varieties through marker assisted selection (MAS). Such studies therefore, are extremely useful for phylogenetic analysis. Hence add new dimensions to the evolutionary understanding. The objectives of a breeder are to develop cultivars of high with yield an enhance protein content through application of recent tools available under biotechnology.

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REFERENCES

1 Geil PB, Anderson JW. Nutrition and health implications of dry beans: A review. J. of the Ame. College of Nutri., 1994;13: 549–558.
2 Rochfort S, Panozzo J. Phytochemicals for health, the role of pulses. J. of Agri. and Food Chem., 2007; 55: 7981–7994.
3 Bazzano LA, He J, Ogden LG, Loria C, Vupputuri S, Myers L. Legume consumption and risk of coronary heart disease in US men and women. NHANES I epidemiologic follow-up study. Archives of Internal Medicine., 2001; 161: 2573–2578.
4 Villegas R, Gao YT, Yang G, Li HL, Elasy TA, Zheng W. Legume and soy food intake and the incidence of type 2 diabetes in the Shanghai Women’s Health Study. Ame. J. of Clinical Nut., 2008; 87: 162–167.
5 Rizkalla SW, Bellisle F, Slama G. Health benefits of low glycaemic index foods, such as pulses, in diabetic patients and healthy individuals. Br. J. of Nutri., 2002; 88: S255–S262.
6 Bazzano LA, He J, Ogden LG, Loria C, Vupputuri S, Myers L. Legume consumption and risk of coronary heart disease in US men and women. NHANES I epidemiologic follow-up study. Archives of Int. Med., 2008; 161: 2573–2578.
7 Mahesh K, Vinod C, Anil K, Sarla Vikas B, Himanshu A. A Comparative Study of Genetic Diversity in Chickpea Based Upon Touchdown and Non-touchdown PCR Using ISSR Markers. Chiang Mai J. Sci., 2015; 42(1) : 117-125
8 Duke, JA. Handbook of legumes of world economic importance. Plenum Press, New York., 1981; 52-57.
9 Miao M, Zhang T, Jiang B. Characterizations of kabuli and desi chickpea starches cultivated in China. Food Chem., 2009; 113: 1025–1032.
10 Ali Q, Ahsan M, Farooq J. Genetic variability and trait association in chickpea (*Cicer arietinum* L.) genotypes at seedling stage. Elect. J. Plant Breed., 2010; 1: 334-341.
11 Pramod KS, Himanshu S, Nidhi S, Sameer SB. Analysis of Genetic Diversity among Wild and Cultivated Chickpea Genotypes Employing ISSR and RAPD Markers American Journal of Plant Sciences., 2014; 5: 676-682.
12 Himanshu A, Alka R , Anil K, Jasbir S, Jogender SR, Pradeep KN, Vinod C.

Assessment of genetic diversity among 125 cultivars of chickpea (*Cicer arietinum* L.) of Indian origin using ISSR markers. Turk J Bot., 2015; 39: 218-226.

13 Welsh J, McClelland M. DNA fingerprinting by arbitrarily primed PCR. Nucleic Acids Res., 1991., 19: 861–866.
14 Tingey SV, del Tufo JP. Genetic analaysis with random amplified polymorphic DNA markers. Plant Physiol., 1993; 101: 394-352.
15 Williams JGK, Hanafey MK, Rafalski JA, Tingey SV.Genetic analysis using random amplified polymorphic DNA markers. Meth. Enzymol., 1993; 218:705– 740.
16 Michelmore RW, Paran I, Kesseli RV. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proc. Natl. Acad. Sci. USA., 1991; 88: 9828–9832.
17 Martin GB, Williams JGK, Tanksley SD. Rapid identification of markers linked to a Pseudomonas resistance gene in tomato by using random primers and near-isogenic lines. Proc. Natl. Acad. Sci. USA., 1991; 88: 2336–2340.
18 Olson M, Hood L, Cantor C, Botstein D. Monomorphism in humans and sequence differences among higher primates for a sequence tagged site (STS) in homeo box cluster 2 as assayed by denaturing gradient electrophoresis. Science., 1989; 245: 1434–1435.
19 Jarvis P, Lister C, Szabo V, Dean C. Integration of CAPS markers into the RFLP map generated using recombinant inbred lines of *Arabidopsis thaliana*. Plant Mol. Biol., 1994; 24: 685-687.
20 Epplen, JT. Advances in Electrophoresis. Eds. Inchrambach, A., Dunn, N. J. and Radola, B. J., VCH, Cambridge., 1992; 5: 59-112.
21 Williams JGK, Kubelik AR, Livak KJ, Rafalski KJ, Tingey SV. DNA polymorphism amplified by arbitrary primers is useful as genetic markers. Nucleic Acids Res., 1990; 18: 6531-6535.
22 Weising K, Nybom H, Wolff K, Kahl G. DNA Fingerprinting in Plants. Principles, Methods and Applications. 2nd Edition. Taylor and Francis Group., 2005; ISBN 0-8493-1488-7.
23 Richardson T, Cato S, Ramser J, Kahl G, Weising K. Hybridization of microsatellites to RAPD: a new source of polymorphic markers. Nucleic Acids Res., 1995; 23: 3798-3799.
24 Zabeau M, Vos P. Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application number: 92402629.7., 1993; Publ. No. EP0534858.
25 Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT. Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science., 1988; 239: .487-491.
26 Vos P, Hogers R, Bleeker M, Rijans M, Van de Lee T, Hornes M, Frijters A, Pots J, Peleman J, Kuiper M, Zabeau M. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res., 1995; 23: 4407-4414.
27 Hongtrakul V, Huestis GM, Knapp, SJ. Amplified fragment length polymorphisms as a tool for DNA fingerprinting sunflower germplasm: genetic diversity among oilseed inbred lines. Theor. Appl. Genet., 1997; 95: 400- 407.
28 Paglia GP, Olivieri AM, Morgante M. Towards second-generation STS linkage maps in conifers: a genetic map of Norway spruce (*Picea abies* K.). Mol. Gen. Genet., 1998; 258: 466-478.
29 Adams MD, Kelley JM, Gocayne JD, Dubnick M, Polymeropoulos MH, Xiao H, Merril CR, Wu A, Olde B, Moreno RF, Kerlavage AR, McCombie WR, Venter JC. Complementary DNA sequencing: expressed sequence tags and human genome project. Science., 1991; 252: 1651 – 1656.
30 Sasaki T. Rice Genome. Newsletter for Rice Genome analysis., 1994., 3/1, Tsukuba.
31 Orita M, Suzuki Y, Sekiya T, Hayashi K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics., 1989; 5(4): 874.
32 Ainsworth PJ, Surh LC, Coulter-Mackie MB. Diagnostic single strand conformational polymorphism, (SSCP): a simplified non-radioisotopic method as applied to a Tay-Sachs B1 variant. Nucleic Acids Res., 1991; 19:405-406.
33 Fukuoka S, Inoue T, Miyao A, Monna L, Zhong MS, Sasaki T, Minobe Y. Mapping of sequence-tagged sites in rice by single strand conformation polymorphism. DNA Res., 1994; 1: 271-277.
34 Hayes PM, Liu BH, Knapp SJ, Chen F, Jones B, Blake T, Franckowiak J,

Rasmusson D, Sorrells M, Ullrich SE, Wesenberg D, Kleinhofs A. Quantitative trait locus effects and environmental interaction in a sample of North American barley germplasm. Theor. Appl. Genet., 1993; 87: 392-401.

35 Helentjaris T, Slocum M, Wright S, Schaefer A, Nienhuis J. Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphism. Theor. Appl. Genet., 1986; 72: 761-769.

36 McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD. Molecular mapping of rice chromosomes. Theor. and Appl. Genet., 1988; 76: 815-824.

37 Nam HG, Giraudat J, Den Boer B, Moonan F, Loos WDB, Hauge BM, Goodman HM. Restriction fragment length polymorphism linkage map of *Arabidopsis thaliana* Plant Cell., 1989; 1: 699-705.

38 Winter P, Kahl G. Molecular marker technologies for plant improvement. World J. of Microbiol. and Biotech., 1995; 11: 438-448.

39 Lalitha SK. DNA markers in plant improvement: An overview. Biotech Adv., 1999; 17: 143 -182.

40 Cobos, MJ, Rubio J, Strange RN, Moreno MT, Gil J, Millan, T. A new QTL for *ascochyta blight* resistance in a RIL population derived from a interspecific cross in chickpea. Euphytica., 2006; 149: 105–111.

41 Cobos MJ, Fernandez MJ, Rubio J, Kharrat M, Moreno MT, Gil J, Millan T. A linkage map of chickpea (*Cicer arietinum* L.) based on populations from Kabuli x Desi cross; location of genes for resistance to *fusarium wilt* race. Theor Appl Genet., 2005; 110: 1347-1353.