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RESEARCH ARTICLE

Genetic Diversity in chickpea using various Molecular Markers: First step towards Molecular Breeding

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Summary:

Chickpea (*Cicer arietinum* L.) is an ancient self-pollinated legume crop possibly originated in south-eastern Turkey and the adjoining part of Syria. The major objectives of chickpea breeding are to increase yield either by upgrading the genetic potential or by eliminating the effects of disease, pests and stress. Numerous selection techniques for pest resistance and agronomic traits have been developed and Molecular markers is one of them. Progress in breeding necessitates a better understanding of the genetics underlying these traits. Marker assisted selection (MAS) would allow a superior targeting of required genes. Genetic mapping in chickpea is hampered by little polymorphism for a long time but today, it is facilitated by highly polymorphic and co-dominant microsatellite based markers. This paper reviews the analysis of genetic diversity in chickpea by using different approaches of molecular marker system.

Chickpea: An important grain legume

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop worldwide. It is the only cultivated species belonging to the *Cicer* genus, which is a member of the Leguminosae family *Cicereae* Alef tribe (van der Maesen, 1987). Chickpea is an annual, winter-grown legume that stands between 20 cm and 1 m tall (Muehlbauer and Tullu, 1997). Among grain legumes, chickpea, the major crop of our country, occupies 82% of total area under pulses and contribute 79% of the total pulse production in the world. In spite of various measures taken for the improvement of yield in chickpea, the annual growth rate of chickpea production has been very slow at 1.9% and yield have risen at the rate of only 0.6% annually. World production is 8.6 million metric tons annually from 11.2 million hectares (FAOSTAT data, 2005). It is one of the most important legume crops in the Indian sub-continent. India produces 5.97 million tons of chickpea during 2008, which was 75% of the world's production (FAO, 2008). The major growing areas of the crop are the Indian sub-continent and the Mediterranean basin because large number of cultivars and landraces subsists there. Regardless of its agronomic importance, the seed productivity of chickpea is quite low i.e. nearly 700 kg/ha (Singh et al., 1994). Currently, productivity of chickpea is very low (World average apprx. 0.8 t/ha, FAOSTAT, 2005). The main reason for decreasing yield is the susceptibility of the plant to many pests like pod borer, most importantly to fungal disease such as ascochyta blight caused by *Ascochyta rabiei* and fusarium wilt caused *Fusarium oxysporum* (Singh and Saxena, 1992). Both these diseases are very damaging and account for major (10 to 90%) crop losses every year.

The crop is a self pollinated diploid (2n = 2x =16) with a quite small genome (740 Mb, Arumuganathan and Earle, 1991). The major producers India, Pakistan and Turkey contribute 65%, 9.5% and 6.7% respectively; to the world harvest (FAOSTAT, 2005). Chickpea seeds contain 20-30% protein, about 40% carbohydrates and only 3-6% oil (Gil et al., 1996) and furthermore, they are a good source of calcium, magnesium, potassium,

phosphorus, iron, zinc and manganese (Ibrikci et al., 2003). Compared to other grain legumes, anti-nutritive components are almost absent (Williams and Singh, 1987). In some countries, chickpea is used as a feed for livestock (Oplinger et al., 1997) and as a traditional medicine (Muehlbauer and Tullu, 1997). Thus, chickpea is considered as a functional food or nutraceutical (Agharkar, 1991; McIntosh and Topping, 2000; Charles et al., 2002). Still, there is a need of more research to illuminate and enlarge the food and nutraceutical benefit of this crop through breeding. The cultivated species has many common names including chickpea(English), Bengal gram (Indian), garbanzo (Latin America), homes, hamaz (Arab world), nohud, lablabi (Tukey) and shimbra (Ethiopia) (Muehlbauer and Tullu, 1997).

Programs for breeding resistance gene into high yielding cultivars are underway in some parts of world, but these are time consuming and it is to achieve pyramidal resistance by conventional means. In the past decade, biotechnology have led to the development of a number of novel tools that offer the promise of making plant breeding more precise and faster. Among the most promising are molecular markers, which are segments of plant DNA that breeders use to detect the presence or absence in experimental plants of specific alleles of interest and thus use them as selection tools. Such a selection of desirable plants based on linked markers is termed as Marker-Assisted Selection (MAS). By using molecular markers, breeders can by-pass traditional phenotype-based selection methods, which involve growing plants to maturity and closely observing their physical characteristics in order to infer underlying genetic makeup (Varshney et al., 2004).

In recent years there has seen remarkable improvement in the development of novel genetic tools such as DNA molecular markers and genetic maps profiling techniques. Although they can be fully applied only to a few model species but these new technologies are spectacularly improving our understanding of basic ideology of plant metabolism. Now, it is up to researchers and breeders to exploit these new opportunities to improve chickpea yield for the benefit of farmers and consumers.

Diversity analysis in chickpea: a major prerequisite for molecular breeding

Knowledge of the inheritance of agronomic traits is a basic requirement to identify and integrate genes in linkage maps for marker–assisted selection (MAS). In recent years, molecular markers have been extensively used to assess genetic diversity within plant species and tagging of important genes for agronomic traits. In order to improve the productivity of chickpea, the use of DNA based molecular markers has been proposed for marker assisted selection (MAS), mapping of QTL (Quantitative Trait Loci) and positional cloning of genes in chickpea (Winter and Kahl, 1995). Molecular markers, both biochemical (Ahmad et al., 1992; Labdi et al., 1996) and DNA based like RFLP (Udapa et al., 1993), RAPD (Sant et al., 1999; Iruela et al., 2002; Sudupak et al., 2002; Chowdhary et al., 2002) were unable to address the genetic variation within chickpea. The low polymorphic ability of isozymes, RAPD and RFLP markers may be due to lesser polymorphism in structural genes in chickpea genome (Rheenen et al., 1993). It was also reported that amplified fragment length polymorphisms (AFLPs) were also rare within *Cicer arietinum* (Huttel et al., 1999).

In recent years, the microsatellite and STMS (Sequence tagged microsatellite site) markers has been shown to be more useful and to be highly polymorphic. Microsatellites 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; Morgante and Olivieri, 1993; Wang et al., 1994). Another technique based on microsatellites employs sequence regions to design locus – specific PCR primer pairs. This so called "sequence tagged microsatellite site" (STMS) approach produces almost optimal molecular markers, as they are co-dominant, highly abundant, and often polymorphic within populations. This increases the probability of detecting polymorphism many folds. Therefore, a study on genetic diversity is essentially the first step for any genetic improvement programme.

Genetic Diversity reported using various markers:

Quantitative trait loci (QTL) mapping is a highly effective approach to identify and tag, disease resistance genes in plants (Young, 1996). QTL mapping may be appropriate for identifying the number and position of genes conferring disease resistance, because previous reports suggest that resistance is controlled by multiple genes (Tekeoglu et al., 2000). Knowledge of the extent of genetic diversity within a species is a critical factor when selecting parents for QTL mapping, because sufficient DNA polymorphism must exist between parents for segregation analysis and genetic mapping (Collard et al., 2003). But little or no polymorphisms have been detected among chickpea genotypes using traditional morphological and biochemical markers.

Ahmed et al., (1992) studied isozyme polymorphism in the genus *Cicer* L. They observed

alloenzyme variation among 50 accessions representing the cultivated chickpea and 8 wild annual *Cicer* species. Sixteen enzymes revealed 22 putative and scorable loci of which 21 showed polymorphism. They further reported that no isozyme polymorphism was detected in 25 accession of cultivated chickpea accession originating from 6 different geographical regions. This is surprising in view of the diverse in Asia, the Middle East and several Mediterranean countries, some of which have grown chickpea for at last 7000 years (Van der Maesen, 1987) and the presence of abundant genetic variation for other qualitative and quantitative traits (Muehbauer and Singh, 1987). Previously, Oram et al., (1987) studied isozyme variability for 27 isozyme loci in 20 cultivated chickpea accession and concluded that as a species, chickpea was relatively poor in genetic variation at isozyme loci. Tuwafe et al., (1988) surveyed isozyme variability in 1392 accession of cultivated chickpea from 25 countries and found polymorphism for only 4 isozyme loci. Gaur and Slinkard (1990 a,b) did not find any genetic variation for isozyme loci in *Cicer arietinum* and consequently utilized interspecific hybrids of *Cicer arietinum* with *Cicer reticulatum* and *Cicer echinospurmum* to study their genetics and linkage. This again indicates the limited variability present at isozyme loci in cultivated chickpea.

The abundance and polymorphism of 38 different SSR motifs was studied in 4 accessions of cultivated chickpea by in gel hybridization of synthetic oligonucleotide to genomic DNA, digested with 14 different restriction enzymes. Among 38 probes tested, 35 yielded detectable hybridization signals. The abundance and level of polymorphism of the target sequences varied considerably. The high level of intraspecific polymorphism detected by oligonucleotide fingerprinting suggests the suitability of SSR probes as molecular markers for gene mapping (Sharma et al., 1995).

Ratnaparkhe et al., (1998) studied the inheritance of an inter simple sequence repeat (ISSR) polymorphism in a cross of cultivated chickpea *(Cicer arietinum* L.) and closely related wild species *(Cicer reticulatum* L.) using primer that anneal to a simple repeat of the various lengths. The ISSR loci showed virtually complete agreement with expected Mendelian ratios. 22 primers were tested for analysis and yield a total of 31 segregating loci. They reported an ISSR marker linked to the gene for resistance to fusarium wilt race 4. Sudupak, (2004) studied intra-species ISSR variations in 43 chickpea genotypes and showed a gene diversity of 0.24.

Singh et al., (2002) employed PCR based RAPD markers to assess genetic diversity in 23 chickpea genotypes. Forty out of 100 random primers screened revealed polymorphism among the genotypes. Most of the primers revealed single polymorphic bands and only 14.2 % of the products are polymorphic. Estimate of genetic similarity based on jaccard's coefficient ranged from 0.92 to 0.99 indicating narrow genetic variability among genotypes based on RAPD markers. Rao et al., (2007) investigated genetic relationship between 19 chickpea cultivars and five accessions of its wild progenitor *Cicer reticulatum* L. using RAPD and ISSR markers. On an average, six bands per primer were observed in RAPD analysis and 11 bands per primer in ISSR analysis. Taran et al., (2007) conducted research to map genetic factors for resistance to ascochyta blight using a linkage map constructed with 144 sample sequence repeat markers and 1 morphological marker (*fc*, flower colour). A total of 556 cutting-derived plants were evaluated for their reaction to ascochyta blight under controlled conditions. Composite interval mapping identified 3 genomic regions that were associated with the reaction to ascochyta blight. One quantitative trait locus (QTL) on each of LG3, LG4, and LG6 accounted for 13%, 29% and 12%, respectively, of the total estimated phenotypic variation for the reaction to ascochyta blight. Together, these loci controlled 56% of the total estimated phenotypic variation. The QTL on LG4 and LG6 were in common with the previously reported QTL for ascochyta blight resistance, whereas the QTL on LG3 was unique to the current population.

Huttel et al., (1999) used 22 STMS primer pairs proved to be informative at an intraspecific level in *Cicer arietinum*. Galvez et al., (2003) showed that out of 164 primers/primer pairs screened between the parents, only 65 (39.6%) revealed DNA polymorphism, generating a total of 82 reproducible and segregating markers for linkage analysis. Bhatia et al., (2006) used 74 functional STMS primer pairs. This analysis revealed 66% intraspecific polymorphism within chickpea.

Shan et al., (2004) reported 98% polymorphism using 146 *Cicer* accessions. Analysis of the 146 *Cicer* accessions with six selective amplification primer pairs identified a total of 455 AFLP markers, of which 447 are polymorphic in the whole collection. In another study, the six primer combinations detected substantial polymorphism in all species of the primary and secondary gene pools. The total number of bands observed ranged from 180 to 231 within a species, and the number of polymorphic loci detected ranged from 158 to 213 accounting for 77.5 to 92.2% of total AFLP bands generated (Shan et al., 2007).

Conclusion:

The present review demonstrates the potential of different molecular markers in detecting polymorphism among chickpea cultivars. The STMS and AFLP analysis clearly indicated that even with few polymorphic primers reliable estimation of genetic diversity could be obtained and these markers successfully identified genetic variation in chickpea. The variation identified was greater than that revealed by the other biological markers like isozymes, RFLPs, RAPD etc used in studies of genetic relationships among *Cicer* species. Therefore, AFLP and STMS markers comes very close to the ideal molecular marker system because they are highly polymorphic, highly reproducible, occur abundantly in eukaryotic genomes and inherited in a co-dominant fashion.

This information could be very valuable in the management of genetic resources in this species. Further, large amount of genetic variation which exists between chickpea cultivars and its wild accessions can be used efficiently for gene tagging and genome mapping of wild and cultivar crosses to intrigues the disease and insect resistance into the cultivated genotypes. Increasing and stabilising seed yield is the major aim of chickpea breeding. The development of molecular markers and more efficient tools in plant breeding will continue to be a very dynamic process in future.

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