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Genetic Diversity of Soybean in India: A Review

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ABSTRACT

Success of a crop breeding programme depends on the extent of variability present in the germplasm, choice of the parents and the selection procedure. Even though soybean has rich source of germplasm, narrow spectrum of variability exists among the cultivars. Much of the work in soybean improvement has been restricted due to the non-existence of broad genetic base among cultivars. This problem is further aggravated due to high degree of self-pollination in this crop. The rapid advancement in molecular marker technology has provided new classes of genetic markers at the DNA level. The advent of DNA based markers have opened up new avenues and uncommon opportunities for biological science in the area of evolutionary studies.

1. Introduction

Soybean offers multiple utility as pulse, oil seed and vegetable and has vital importance of its own as a cash crop of large number of people in the different states of India. Soybean seeds contain an average of 20 per cent oil and 36 per cent protein. It yields more usable protein than any other cultivated crop. Besides oil and high-quality protein, it fixes atmospheric nitrogen in the soil at the rate of 65-100 kg/ha with the help of *Rhizobium japonicum* bacteria. Due to its multifaceted advantages, soybean has progressed by leaps and bounds as an oil seed crops and contributes a major part of the total supply of the world vegetable oil. Success of a crop breeding programme depends on the extent of variability present in the germplasm, choice of the parents and the selection procedure. Even though soybean has rich source of germplasm, narrow spectrum of variability exists among the cultivars. Much of the work in soybean improvement has been restricted due to the nonexistence of broad genetic base among cultivars. This problem is further aggravated due to high degree of self-pollination in this crop. An improvement in yield and quality of self-pollinated crops like soybean is effected mainly through selection of genotypes with desirable characters from the germplasm or by creating the variation through recombination and / or induced mutagenesis followed by selection. The basic requirement for adopting a suitable breeding method is sound knowledge of the genetic make-up of the characters and their expression in different genetic backgrounds. Genetic diversity is normally assessed by common morphological traits. However, such traits are affected by effects of environment, development stage of the plant, also the type of plant material and require several replications to establish the genotypic contributions. Hence, there is a need to go in for a highly reliable and precise method for assessment of genetic variability with no environmental effects. Assessment of genetic diversity with molecular markers overcomes this problem. They are devoid of environmental effects and provide a true representation of the entire genome.

2. Genetic diversity by molecular markers

Molecular genetic markers have brought phenomenal changes in the area of plant biotechnology by their ability to produce unique DNA profiles in various crops. Simple sequence repeat markers (SSR) are being extensively used in genome studies, marker-assisted selection, and cultivar identification and are well-known for their versatility in providing a quick assay and for their highly informative data (Song *et al.,* 1999; Cregan *et al.,* 1999a; Cregan *et al.,* 1999b). The use of molecular markers allows the direct assessment of genotypic variation at the DNA level.

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As genes involved in traits of agronomic importance are mapped and tagged, markers assist in breeding programs and even to isolate the gene. Marker analysis helps to understand the genetic make-up of the accessions and also make it possible to analyze the global organization of genetic diversity within a species. Several statistical techniques are available for the analysis of genetic diversity using DNA fingerprinting data. Ultimately the phenotypic variations observed can be correlated to the molecular marker profile.

Borthakur (1989) described the genetic diversity of the soybean genotypes of North East region of India. Kisha *et al*. (1998) reported that soybean diversity pattern may serve as a valuable guide for finding and incorporating new lines into elite soybean genotypes. Access to and use of genetic diversity is the basis of genetic improvement through plant breeding and can be estimated by different methods. The precision of genetic variation estimates depends on the method used. The polymorphism can be detected at morphological, biochemical and at molecular levels. The rapid advancement in molecular marker technology has provided new classes of genetic markers at the DNA level. The advent of DNA based markers have opened up new avenues and uncommon opportunities for biological science in the area of evolutionary studies (Faivre-Rampant *et al*., 1992; Gupta *et al*., 1992; Kapila *et al*., 1996 b), plant systematics and more recently in the tagging of genes coding for traits of agronomic importance (Michelmore *et al*., 1991; Mohan *et al*., 1994; Reamon-Buttner *et al*., 1998). It plays a vital role in enhancing global food production by improving the efficiency of conventional plant breeding programs (Kasha, 1999). These developments have stimulated new interest in exploring the applications of genetic markers in plant breeding and allow breeders to dissect complex traits without having to measure the phenotype, thus reducing the need for extensive field-testing over time and space.

The markers are typically small regions of DNA, often showing sequence polymorphism in different individuals within a species and transmitted by the simple Mendelian laws of inheritance from one generation to the next. These include Restriction Fragment Length Polymorphism (RFLP) (Botstein *et al*., 1980), Oligonucleotide Polymorphism (OP) (Beckmann, 1988), Single Strand Confirmation Polymorphism (SSCP) (Orita *et al.,* 1989), Mini satellite Simple Sequence Length Polymorphism (SSLP) (Jarman *et al.,* 1989), Random Amplified Polymorphic DNA (RAPD) (Williams *et al.,* 1990), Allele Specific PCR (AS PCR) (Sarkar *et al.,* 1990), DNA Amplification Fingerprinting (DAF) (Caetano *et al.,* 1991), Sequence Characterized Amplified Region (SCAR) (Williams *et al.,* 1990), Sequence Specific Amplicon Polymorphism (SSAP) (Williams *et al.,* 1990), Single Sequence Repeats (Hearne *et al.,*1992), Arbitrarily Primed PCR (AP-PCR) (Welsh and Mc Clelland, 1992), Cleaved Amplified Polymorphic Sequences (CAPS) (Lyamicher *et al.,* 1993), Single Nucleotide Polymorphisms (SNP) (Jordan and Humphries, 1994), Microsatellite Simple Sequence Length Polymophism (SSLP) (Saghai- Maroof *et al.,* 1994), Sequence Tagged Sites (STS) (Fukuoka *et al.,* 1994), Amplified Fragment Length Polymorphism (AFLP) (Vos *et al.,* 1995), Amplicon Length Polymorphism (ALP) (Ghareyazie *et al.,* 1995), Retro Transposon Based Insertion Polymorphism (RBIP) (Flavell *et al.,* 1998) and Three Endonuclease (TE)- AFLP) (Wurff *et al.,* 2000).

Molecular markers have several advantages over the morphological markers. This however requires identification of molecular markers tightly linked to the trait of interest. Tanksley *et al.* (1989) have discussed the relative advantages of molecular markers over morphological markers for most genetic and breeding applications. The first type of molecular marker applied to soybean was restriction fragment length polymorphism (RFLP). Unfortunately, owing to the lack of polymorphism, this approach was not as successful in soybean as in other crop species (Tanksley *et al.*, 1989). The use of polymerase chain reaction (PCR) and PCR-based markers, *i.e.* random amplified polymorphic DNA (RAPD) and microsatellite sites study (SSR), could be alternative methods in the search for polymorphism in soybean. Microsatellites have been widely applied in mammalian (Weisenbach *et al.,* 1992) and plant genomes (Thomas *et al.,* 1993; Wang *et al.,* 1994). They are reported to show a high level of polymorphism and have been successfully used in soybean, (Morgante *et al.,* 1994; Akkaya *et al.,* 1995). Baranek *et al.* (2002) used random amplified polymorphic DNA (RAPD) technique to evaluate both genetic diversity among 19 soybean accessions included in the Czech National Collection of Soybean Genotypes and their potential as a new source of genetic variation for soybean breeding programs. Only 22 of all the 40 random primers used
in RAPD reactions showed polymorphism acceptable for an effective characterisation of these accessions. Altogether 122 highly reproducible RAPD fragments were generated, 55 of them were polymorphic (46%).

The genetic diversity and relationships were assessed among 35 North American soybean ancestors (NASA), 66 high yielding NASC, 59 modern Chinese cultivars, and 30 modern Japanese cultivars. Five AFLP primer-pairs produced 90 polymorphic (27%) and 242 monomorphic AFLP fragments. Polymorphic information content (PIC) scores ranged from zero to 0.50. Only 53 of the 332 AFLP fragments provided

PIC scores 0.30. Genetic distance (GD) between pairs of genotypes was calculated on the basis of the similarity indices determined by the 332 AFLP fragments. Within each of the cultivar groups, the average GD between pairs of genotypes was 6.3% among the Japanese cultivars, 7.1% among the NASC, 7.3% among the NASA, and 7.5% among the Chinese cultivars. The average GD between the NASC and the Chinese cultivars was 8.5% and between the NASC and the Japanese cultivars was 8.9% (George *et al.,* 2003). Several studies were conducted in the past for assessing the genetic diversity in germplasm collections of crops using various types of molecular markers such as Restriction Fragment Length Polymorphism (RFLP, Cui *et al.,* 1995; Dubreuil and Charcosset, 1998), Random Amplified Polymorphic DNA (RAPD) (De Oliveira *et al.,* 1996; Fofana *et al.,* 1997), Amplified Fragment Length Polymorphism (AFLP, Tohme *et al.,* 1996; Zhu *et al.,* 1998) and Microsatellites (Garland *et al.,* 1999; Dje *et al.,* 2000).

3. Simple Sequence Repeats (SSR)

Simple Sequence Repeats (SSR), also known as Microsatellites; Short Tandem Repeats (STRs) or Simple Sequence Length Polymorphism (SSLPs), are tandem repeats of mono-, di-, tri-, tetra- or penta nucleotide units dispersed throughout the genome of eukaryotic organisms. It has originated because of slippage during DNA replication (Schlotterer and Tautz, 1992). Slippage is thought to be depend on mispairing of tandem repeats during replication (Levinson and Gutman, 1987), and it may not occur when there are a few tandem repeats. These repeated motifs are flanked by unique or single copy sequences, which provide a foothold for specific amplification via PCR. Primer pairs (forward and reverse) are designed based on conserved flanking regions. The primers are usually 15-18 bp in length, and polymorphisms are usually based on variation in the number of specific repeat units at a locus (Morgante and Olivieri, 1993; Saghai Maroof *et al.,* 1994; Wang *et al.,* 1994; Rongwen *et al.,* 1995; Yang *et al.,* 1995; Brown *et al.,* 1996). Simple Sequence Repeats (SSR), are currently considered the molecular markers of choice, due to high level of polymorphism and information content, high reproducibility, codominance, abundant, rapid and simple genotyping assays (Kong *et al.,* 2000) and are rapidly being adapted by plant researchers. These markers are chromosome specific, often amplifying a single locus with multiple alleles and are used by researchers to tag useful genes. A total of 391 simple sequence repeat (SSR) markers designed from genomic DNA libraries, 24 derived from existing Gen Bank genes or ESTs, and five derived from bacterial artificial chromosome (BAC) end sequences were developed. The 420 newly developed SSR loci were added to the 606 SSR loci published by Cregan *et al.* (1999a) and SRs were mapped in one or more of five soybean mapping populations: 'Minsoy' X 'Noir 1', 'Minsoy' X 'Archer', 'Archer' X 'Noir 1', 'Clark' X 'Harosoy', and A81- 356022 X PI468916 (Song *et al.,* 2004). Well-saturated microsatellite maps have been developed for rice (Temnykh *et al.,* 2001), wheat (Roder *et al.,* 1995), helianthus (Brunel, 1994), soybean (Cregan *et al.,* 1999), tomato (Brown and Tanksley, 1996) and sorghum (Taramino *et al.,* 1997; Brown *et al.,* 1996; Kong *et al.,* 2000; Schloss *et al.,* 2002).

Sneller *et al.* (1997) detected genetic diversity in soybean from the analysis of 31 southern (MG V–VI) PIs, 15 southern (MG IV–VI) elite genotypes, and five northern (MG I–III) cultivars at 60 RFLP loci. They found that the greatest amount of genetic relative diversity was between the southern elite genotypes and the southern PIs. The lowest average SMC within a germplasm group was 0.72 and the average SMC between the southern elite lines and southern PIs was 0.65. but more genetic diversity based on SMC was detected by Narvel *et al.* (2000) with SSRs. The use of molecular markers to facilitate the introgression of plant introduction (PI) germplasm into elite soybean [Glycine max (L.) Merr.] cultivars will depend on the amount of polymorphism that exists between elite genotypes and PIs. Narvel *et al.* (2000) assessed the simple sequence repeat (SSR) diversity of 39 elite soybean genotypes (Elites) and 40 PIs that were selected for high yield potential. Average marker diversity among the PIs was 0.56 and ranged from 0.0 to 0.84. Average marker diversity among the Elites was 0.50 and ranged from 0.0 to 0.79. Genetic similarity estimates based on simple matching coefficients revealed more genetic diversity among the PIs than among the Elites. The greatest genetic diversity was between the PIs and Elites.

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