

APPLICATIONS OF RAPD AND ISSR MARKERS IN PLANT GENOME ANALYSIS

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ABSTRACT

Plant genome analysis requires a good genetic marker for tagging the gene of interest. PCR-based markers are the preferred techniques available due to their obvious advantages over conventional phenotypic markers. Random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) are ideal genetic markers employed for various studies, mostly in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology. These markers are simple and no sequence knowledge is required. This review reports the usefulness of RAPD and ISSR markers for the assessment of genetic diversity and its application in plant genetics in a wide range of crop plants.

Key words: RAPD, Polymorphic DNA.

INTRODUCTION

There is an ample genetic diversity of plants with traits of agronomics importance. Fast and efficient methods are needed to tap genetic variation as a base for selection as well as for plant improvements. The genetic markers that can be employed in plant systems include morphological, biochemical and DNA variants for direct/indirect selection. Morphological markers can be scored conveniently without any sophisticated laboratory facility. However, their expression at specific growth stages and analyzing only a limited number of markers in a single population limits their use in plant breeding programmes. Many protein isozymes, are also one of the useful genetic markers. These markers can be resolved by electrophoresis. The alleles of most isozyme markers segregate in a codominant manner and rarely show epistatic interactions. This allows accumulation of many polymorphic isozyme loci in a single F₂ population. Once their map locations are known, they can be efficient as biochemical markers for mapping genes for morphological, physiological and phyto-pathological traits [1].

Molecular markers i.e DNA makers are the best choices existing for plant genome analysis. DNA markers have an advantage of not being influenced by environment and therefore exhibit epistatic interactions. High stability of DNA markers therefore, permits construction of linkage maps with hundreds or even thousands of markers from single mapping populations. DNA markers are being widely used to: a) identify and discriminate between closely related cultivars, b) assess taxonomic and phylogenetic relationships of a crop species and its cultivars c) construction of high density genetic linkage maps, d) study genome organization and e) tagging of loci affecting qualitative or quantitative traits [2].

The major DNA markers employed in plant genome characterization includes restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites or simple sequence repeats (SSRs), diversity array technology (DART) and single nucleotide polymorphisms (SNPs) etc. [3]. These molecular markers represent a class of molecular tools that are sensitive to technical advancements and therefore, subject to continuous evolution. Most molecular marker techniques are employed in the evaluation of genetic diversity and construction of genetic and physical maps [4].

RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD)

The basis of RAPD method is the differential PCR amplification of genomic DNA. It infers DNA polymorphisms generated through rearrangements or deletions at or between oligonucleotide primer binding sites in the genome via short random oligonucleotide sequences mostly of ten bases long. RAPD markers can provide simple and reproducible fingerprint of germplasm by using single

arbitrary chosen primers [5]. These markers detect nucleotide sequence polymorphisms in DNA by using a single primer of arbitrary nucleotide sequence. In this reaction, a single species of primer anneals to 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 [6]. They 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 [7]. RAPD assay has been used by several groups as efficient tools for identification of markers linked to agronomical important traits. These markers can detect genetic polymorphism and when linked to major genes it can be potentially used to identify morphological traits. It is also used in variability analysis and individual-specific genotyping but is less popular due to poor reproducibility, faint products, and difficulty in scoring bands, that leads to inappropriate inferences.

PCR-based RAPD markers require small amounts of DNA have been developed in recent past [8, 9]. SSR markers have proved to be polymorphic but require nucleotide information for primer design [10]. However, RAPD methodology overcomes this limitation; considerable polymorphic markers can be obtained with relative ease from minute amounts of genomic DNA without prior knowledge of sequence information.

The RAPD protocol usually uses a 10 bp arbitrary primer at constant low annealing temperature (generally 34 - 37 °C). RAPD primers can be purchased as a sets or individually from different sources, such as the University of British Columbia (http://www.michaelsmith.ubc.ca/services/NAPS/Primer_Sets) and the Operon Biotechnologies (<http://www.operon.com>). Although the sequences of RAPD primers are arbitrarily chosen, two basic criteria indicated by Williams et al. (1990) must be met: a minimum of 40% GC content (50 - 80% GC content is generally used) and the absence of palindromic sequence (a base sequence that reads exactly the same from right to left as from left to right). A flow chart depicting RAPD and ISSR DNA fingerprinting is shown in the figure 1.

RAPDs were the earliest PCR-based molecular markers employed in genetic variation analyses. RAPD markers are generated through the random amplification of genomic DNA using short decamer primers. As this approach requires no prior knowledge of the genome analyzed, it can be employed across species using universal primers. Lineage preferring primers, in RAPD analysis for certain species, are still absent. This hinders the potential of RAPD analysis as a high-throughput molecular research tool [11]. RAPD markers been demonstrated to be useful for the studies of taxonomic identities,

systematic relationship, population genetic structure, species hybridization and parentage identification [12, 13].

RAPD technique are highly polymorphic and useful in studies on chickpea for genetic diversity [14], phylogeny [15], gene tagging [16] and evolutionary biology [17]. Abundance and polymorphism of 38 different microsatellite have been studied in four chickpea accessions [18]. Random amplified polymorphic DNA (RAPD) analysis has been applied to study genetic relationship among nine annual *Cicer* species [15, 19, 20]. It has been shown that RAPD markers can be a useful tool for studies of phylogenetic relationships within *Cicer* cultivars with other types of analyses used to determine relationships between species. For example, expectations based on karyotype analysis [21, 22, 23] agree with the results of crossability studies [24,25].

INTER SIMPLE SEQUENCE REPEATS (ISSR)

In this technique, primers based on microsatellites are utilized to amplify an inter-SSR DNA sequence which involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. This technique uses microsatellites, usually 16–25 bp long, as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly the inter- SSR sequences of different sizes. These are mostly dominant markers occasionally few of them exhibit co-dominance. An unlimited number of primers can be synthesized for various combinations of di-, tri-, tetra- and penta-nucleotides [26, 27, 28, 29, 30] or more usually anchored at 3' or 5' end with 1 to 4 degenerate bases extended into the flanking sequences.

Anchored ISSRs have high reproducibility possibly due to the use of longer primers (16–25 mers) or anchored technique. As compared to RAPD primers (10-mers), which permits the subsequent use of high annealing temperature (45–60°C) leading to higher stringency. The studies on reproducibility show that it is only the faintest bands that are not reproducible. Flow chart depicts the approach of ISSR-

PCR analysis and its subsequent scoring (figure 1). About 92–95% of the scored fragments could be repeated across DNA samples of the same cultivar and across separate PCR runs when detected using polyacrylamide gels [31, 32]. It is reported that the 10 ng template DNA yielded the same amplification products as did 25 or 50 ng per 20µl PCR reaction. The annealing temperature depends on the GC content of the primer used and usually ranges from 45 to 65°C [33]. ISSRs segregate mostly as dominant markers following simple Mendelian inheritance [34, 35]. However, they have also been shown to segregate as co-dominant markers in some cases thus enabling distinction between homozygotes and heterozygotes [35, 36].

ISSR-PCR technique overcomes most of the limitations of other molecular markers [37]. In this method SSRs are used as primers to amplify mainly the inter-SSR regions. Now a day, it is rapidly being used by the research community in various fields of plant improvement [38, 39]. This technique is equally useful in assessing genetic diversity, phylogenetic studies, gene tagging, genome mapping and evolutionary biology in a wide range of crop species.

Several research groups used inter-simple sequence repeat (ISSR) as a better alternative DNA fingerprinting technique to appraise the genetic framework and diversity of wild and cultivated medicinal plants. The evolutionary rate of change within microsatellites is considerably higher than most other types of DNA, so the likelihood of polymorphism in these sequences is greater. ISSR markers have been applied to determine genetic diversity and relationships in a number of crop species [40, 41]. In *Cicer*, Taran, *et al.* (2007) [42] have reported that ISSR approach is useful in finding markers closely linked to a disease resistance gene in chickpea whereas Rao *et al.* (2007) [43] has recently used ISSR markers to study relationships in a chickpea accession collection. However, till date, there is no report of using ISSR markers in determination of genetic relationships among and within wild species in the genus *Cicer*. Table 1 compare the technical features of RAPD and ISSR markers.

Table 1: Comparative features of RAPD and ISSR markers employed in plant genome analysis

Features	RAPD	ISSR
Plant material required	Little	Little
Information generated	High and nonspecific	High and specific
Time required for detection	Short	Short
Loci (single/multiple)	Multiple	multiple/Single
Principle	DNA amplification	DNA amplification
PCR based Markers	Yes	Yes
Reproducibility	Moderate	Moderate
Part of genome	Whole genome	Whole genome
Use and development	Moderate	Moderate
Reliability	Low	High
Use of Radioactivity	Yes/No	No
Development time	Short	Long

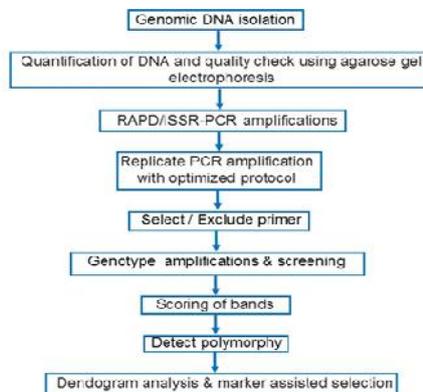


Fig.1: Flow chart depicting RAPD/ISSR genotyping

Employing ISSR and RAPD markers, the inter genetic relatedness of crop plants can be assessed. This polymorphic marker data can hence be converted into discrete character data and analyzed subsequently by using various bioinformatics softwares for assessing inter-genetic relationships. It is therefore, aimed that the genome analysis using such PCR-based markers may help to produce a detailed linkage map of crop plants. The study and the work therefore, can be planned to characterize the germplasm of interest.

A comprehensive study and research is a demand for all round use of genomics for authentic crop plants and planning future breeding strategies. With the advent of new DNA sequencing platforms that achieve an ever increasing degree of speed, elite genotypes of agronomic importance can be developed in short period of time employing RAPD and ISSR marker assisted selection. Therefore, RAPD and ISSR are the low cost preferred PCR-based markers that could be used for a wide range of applications in plants.

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