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SIGNIFICANCE OF RAPD AND ISSR PRIMERS

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RAPD (Random amplified polymorphic DNA) primers; RAPD is a type of polymerase chain reaction. Amplification of genomic DNA is done by PCR using short arbitrary primers (generally 10 bp) of random sequence. These oligonucleotides serve as both forward and reverse primer, and are usually able to amplify fragments from 1-10 genomic sites simultaneously. Amplified fragments, usually within the 0.5-5 kb size range, are separated by agarose gel electrophoresis, presence or absence of bands is detected based on polymorphism by using ethidium bromide staining. These polymorphisms are considered to be primarily due to variation in the primer annealing sites, but they can also be generated by length differences in the amplified sequence between primer annealing sites. Differentiation of the organisms is based on the presence or absence of bands.

ISSR (Inter sequence simple repeat) primers; ISSR is also a type of polymerase chain reaction. Amplification of genomic DNA is done by PCR using short tandem repetitive DNA motifs of 16-18 bp. Inter simple sequence repeat technique, which involves the use of microsatellite sequences as primers in a polymerase chain reaction to generate multilocus markers. The ISSR marker system detects polymorphisms in inter-microsatellite DNA regions without any prior sequence knowledge. About 10-60 fragments from multiple loci are generated simultaneously, separated by gel electrophoresis and scored as the presence or absence of fragments of particular sites.

Comparison Between RAPD and ISSR Markers

- RAPD and ISSR both are polymerase based and dominant molecular markers used for genetic variation among the species.

- Banding pattern is same for both the markers, presence of band is indicated as '1' and absence of band is indicated as '0'. Bands with the same migration distance are considered as homologous.
- Genetic similarity matrix is calculated using the Jaccard's similarity coefficient and a dendrogram is constructed using an unweighted pair-group method with arithmetic mean (UPGMA).

Difference Between RAPD and ISSR Markers

RAPD relies on the amplification of genomic DNA using short primers (10 nucleotides) with a random sequence, whereas ISSR is based on the amplification of regions flanked by repeating sequences (microsatellites or SSR), so the primers used contain those 2-6 nucleotides repeats with usually 2 varying nucleotides to the 3' end. RAPD markers are considered to be uniformly distributed along the genome, whereas ISSR are found only between microsatellite loci.

Pros and Cons of RAPD Marker

Pros

- No prior knowledge of DNA sequences is required
- Random distribution throughout the genome
- Very small amount of DNA is required
- Random decamer primers are commercially available for all type of species
- RAPD bands can often be cloned and sequenced to make SCAR (sequence-characterized amplified region) markers
- Cost effectiveness as compared to other markers.

Cons

- Dominant marker (Cannot separate heterozygous individuals from dominant homozygous individuals
- Sensitivity to changes in reaction conditions, which affects the reproducibility of banding patterns
- Co-migrating bands can represent non-homologous loci
- The results are not easily reproducible between laboratories.

Pros and Cons of ISSR Marker

Pros

- ISSR markers are more reproducible than RAPD
- These are easy to use, cheaper and have high throughput
- They yield multiple polymorphic loci
- A prior knowledge of the template DNA sequence is not required
- Generally, ISSR markers are dominant, but the use of a larger 50 anchored primer can yield co dominant ISSR marker.

Cons

- They aren't highly reproducible
- Some primers generate poorly reproducible band patterns
- Dominant markers

Advantages of ISSR Markers over RAPD

1. Number of polymorphic bands is more generating more percentage of polymorphism among the species.
2. ISSR markers are more reproducible than RAPD.
3. The multiplex ratio (MR) and the effective multiplex ratio (EMR) is more in ISSR marker as compared to RAPD marker. The multiplex ratio (MR) is estimated by dividing the total number of bands amplified by the total number of assays. The effective multiplex ratio (EMR) is the number of polymorphic fragments detected per assay.
4. Polymorphic information content (PIC) is calculated PIC=2fi (1-fi) where fi is the frequency of amplified allele. When average PIC values are calculated, ISSR marker exhibited higher level of polymorphism as compared to RAPD.
5. ISSR marker is a convenient tool for identifying genetic diversity among the genotypes.

Conclusion

Both the primers are used in molecular variability studies. Precise and more accurate information regarding molecular variability will be obtained by using ISSR primers.

Commercial availability of ISSR markers is more as compared to RAPD markers for all the species of disease causing organisms.

References

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