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Commentary

Molecular Characterization of Aloevera through Biochemical Markers

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DESCRIPTION

Most of the limitations of morphological and biochemical markers can be overcome by using molecular markers. An important application of DNA based markers is the development of unique genetic fingerprints, which help in characterization and identification. Currently, several molecular marker based techniques are used for precise and speedy characterization of crop varieties. The theory exploits the fact that the marker locus identifies a chromosomal segment and enables that segment to be monitored in subsequent generations of selfing or crossing.

Basically molecular marker technology is based on two types of system;

- PCR (Polymerase Chain Reaction) based,
- Non PCR based.

PCR-based markers

The various PCR-based techniques are of two types depending on the primers used for amplification;

- Arbitrary / semi-arbitrary Primed PCR techniques that developed without prior sequence information (eg: AP-PCR, DAF (DNA Amplification Fingerprinting), RAPD, AFLP, ISSR)
- Site targeted PCR techniques that developed from known DNA sequence (e.g. EST, CAPS, SSR, SCAR, STS)

Non-PCR based markers

The two main non-PCR-based techniques are Restriction Fragment Length Polymorphism (RFLP) and Variable Number of Tandem Repeats (VNTRs). Among the various markers developed, RFLP was the first to be used for plant genome analysis. Base substitutions in a restriction endonuclease site or insertions or deletions between sites can give size to detectable differences in the length of DNA when digested with restriction enzymes. These differences constitute a RFLP. RFLP are co-dominant markers and are more informative than dominant markers. However, this technique requires relatively large amounts of purified and high molecular weight DNA, is time consuming and laborious, uses probe that is difficult to handle and dispose etc. Orndorff developed PCR technique for the detection and identification of Aloe species. Considerable attempts have been made to evaluate genetic diversity among Aloe species using RAPD and AFLP based marker. AFLP (Amplified Fragment Length Polymorphism) techniques assess the diversity in 12 elite accessions of Aloevera collected from different locations. Among the twelve genotypes the AFLP primer combinations generated a total of 410 fragments per primer combination. AFLP analysis was used to assess the genetic similarity among 34 accessions of *Chlorophytum borivilianum*. Nine primer sets of AFLP amplified 612 fragments, of which 246 fragments were found to be polymorphic. Cluster analysis based on AFLP data revealed limited genetic variation within the thirty four accessions collected from various parts of Central Indian forests. The discovery of Polymerase Chain Reaction (PCR) has led to the development of RAPD (Random Amplified Polymorphic DNA) marker system. RAPD involves PCR amplification of total genomic DNA using a single random primer of about 10 bases and separating amplified products by Agarose gel electrophoresis.

RAPD is a quick and easily adaptable technique and can be carried out without any prior knowledge of molecular sequence of the genome. Large amount of banding information is obtained very quickly. Therefore, RAPD is considered good starting point for learning other molecular techniques such as SSR and AFLPs. The following are the advantages of RAPDs over conventional RFLP technique.

- Requirement for a small amount of genomic DNA (25-100 mg per reaction).
- An Ethidium bromide detection system.
- Many primers can be screened on a single PCR run.
- RAPDs may provide markers in regions of the genome inaccessible to RFLP analyses due to presence of repetitive DNA sequence.

However, unlike RFLPs, RAPDs are dominant in nature. RAPD markers to be 4-6 times more efficient on per assay basis than screening for the same polymorphisms using RFLP technology and over 10 fold more efficient in time and labour. RAPD overcomes many of the technical limitations of RFLP analysis. It neither requires previous knowledge of any genomic sequence such as general PCR nor tedious procedure. The technique has been found successful to resolve various levels of inter- and intraspecific polymorphism, which facilitates assessment of genetic relationships, definition of regional grouping and identification of individual accession.

In RAPD analysis, genetic differences are reflected as the presence or absence of RAPD fragments and a huge number of

bands can be obtained in a limited time.

The RAPD has been widely used:

- For determining the genetic relationships between different related species.
- For the identification of cultivars and genotypes; and
- For estimating the genetic relationships and diversity among crop germplasm.

The technical ease of RAPD markers and the facility of their application to new species have led their employment in many organisms including forest trees, crop as well as medicinal plants and lower plants for genetic linkage mapping, phylogeny and systematics and population genetics use RAPD marker to distinguish botanical species of Aloe used in commercial food products. Genomic DNA was extracted using a modification of the method used. RAPD-PCR technique has revealed that *Lomatophyllum* species and Aloevera share some genetic similarities. Some biologically active compounds within the *Lomatophyllum* were also identified and their possible similarities with Aloevera were unveiled. In nature, there are two types of Aloe; bitter and non-bitter. Aloes of medicinal importance are bitter in taste. RAPD analysis is used to distinguish non-bitter and bitter types of Aloevera. Based on aloin content and RAPD analysis, it could be concluded that non-bitter type of Aloevera is a different ecotype that can be used as a vegetable. Eleven Aloe germplasm accessions; Aloevera, *A. perryi*, *A. lotus*, *A. zeylanicum* and seven strains of Aloevera were subjected to RAPD analysis in relation to morphometric parameters for estimating the extent of diversity within and between species. The RAPD analysis revealed comparable inter and intra species variation. A total of 192 bands were amplified with 7 primers. Out of 192 bands amplified, 89% was polymorphic and 10.9% was unique to a particular accession which made it distinct from all other accessions.