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DNA Finger Printing - A Tool for Identification of Cultivars

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An organism's DNA contains the blueprint of its characteristics - in the case of plants that would include features like yield, drought resistance, pest resistance, water logging tolerance etc. Making a DNA fingerprint of a particular crop enables the researchers to pinpoint specific fingerprint and accurately identify seed varieties. The ability to identify seed varieties will make the test important to guaranteeing the authenticity of a crop being purchased. Present article discusses shortly about DNA finger printing technique and its importance in plant varieties in respect to their true identity.

Introduction

DNA fingerprinting technique invented by Alec Jeffreys during 1985 helped in solving high-profile murder cases may now employed to solve the crop crimes. Several Research Organizations in India and across the globe have started offering DNA testing for plant varietal identification and DNA fingerprinting is used by plant breeders of both private and public sectors for identification for crop varieties. While it is very difficult to tell the differences in a crop variety just by looking at the seeds, DNA fingerprinting makes it possible for researchers to pinpoint specific fingerprint and accurately identify seed varieties. More importantly, Protection of Plant Variety and Farmers Right Authority (PPV & FRA, 2001), Govt. of India made a DNA fingerprint as an additional mandatory requirement for the release of new crop varieties released by the Central Varietal Release Committee.

Method of DNA Fingerprinting

A DNA fingerprint can be called a genetic photograph of an individual, whether that individual is a plant, animal or person. The technique of DNA fingerprinting has been developed by Alec Jeffreys using the science of genetics. An organism's DNA contains the blueprint of its characteristics- in the case of plants that would include features like yield, drought resistance, pest resistance, water logging tolerance etc. Making a DNA fingerprint of a particular crop involves several steps as described below:

- Selection of suitable molecular markers (e.g. SSR) covering the whole crop genome.
- Employing diversity analysis for all released varieties by selected molecular markers.
- Identification and selection of markers which will differentiate the maximum number of varieties and formation of set of consensus markers in order to differentiate all the released varieties.

- Make the amplification profile for released varieties with each of selected polymorphic marker from the core set.
- Amplify the new crop variety (going to be released) by core set of markers. Here keep few old varieties along with new variety during amplification to help in identification of unique profile of new variety.
- Compare the amplification profiles between new variety and available old varieties for the unique profile in the new variety.

Relevance of Fingerprinting

Conventional morphological characters such as pigmentation, disease resistance and presence of awns etc. have been successfully used to distinguish the uniqueness of the new varieties for the purpose of plant variety protection. However, with increasing number of cultivars and a finite number of conventional characters, it has become apparent that such traits will not be sufficient to establish the uniqueness of concerned crop variety. Enforcement of Trade Related aspect of Intellectual Property Rights (TRIPS), agreements under World Trade Organization resulted in worldwide shift from free exchange and unhindered exploitation to controlled accessions to plant genetic resources. After the Convention on Biological Diversity Act (CBD, 1993), India has enacted the PPV &FRA to provide legal frame work for the plant breeders' and farmers rights'. Novelty, distinctness, uniformity and stability are the essential requirements of the grant of protection to all the varieties. Enforcement of this act and increased private sector investment would lead to greater ownership related disputes in future. Therefore, a more precise system of identification for varieties, parents and hybrids is fundamental requirement to enforce this protection.

Utility of DNA Fingerprinting

Protecting plant breeders' rights (the breeders' patents on specific types of seed), is major utility of DNA fingerprinting technique. Disputes over the true identify of seed varieties can be easily resolved, because the test will be able to isolate the specific traits that distinguish one seed variety from another. The ability to identify seed varieties will make the test important to guaranteeing the authenticity of a crop being purchased. That is why it is referred as Intellectual property right (IPR) tool. Similarly, genetically modified crops are also tested by DNA fingerprinting technique to identify and keep track of transferred genes.

Another way that DNA fingerprints can be used is if a farmer grows a crop and its performance does not match the claims made for it. A fingerprint could be taken to show whether the seed of which the farmer planted was infact the variety that was purchased by him. Over two thousand varieties, parental lines and hybrids of 32 important crops have been fingerprinted using STMS, AFLP, ISSR and RAPD techniques at National Research Centre on DNA fingerprinting (Division of Genomic Resources) NBPGR, New Delhi (Bhat, NRC on DNA fingerprinting).

Practical Aspects of DNA Fingerprinting

Development of consensus set of markers to differentiate all released varieties in respective crops is a challenging task. Moreover, these markers developed must work across the labs and able to differentiate new candidate varieties from pre- existing varieties. So, validation of consensus core set of markers across the labs is most important activity in fingerprinting. The

non availability of the genetically pure and quality seeds of all released varieties is one of the major limitations for finger printing. The narrow amount of crop diversity represented in development of new varieties (i.e. narrow genetic base) is also a major factor in new cultivar identification. If the released varieties are developed from few germplasm material makes difficulty to fingerprint such cultivars with available minimum number of core set of markers. Therefore the number of markers increases in the core set list so that it is possible to differentiate the varieties under narrow genetic base condition, hence DNA fingerprinting is a continuous process. The different instruments employed for the analyzing the DNA fragments of cultivars is also one of major concern for accuracy of the fingerprinting. For instance, normal agarose, metaphore, vertical gel electrophoresis (PAGE), and recently, robotic (chips) are employed to detect the unique bands present among the cultivars. The size of the band may vary according to type of instrument used to detect the band sizes. Robotic chip can differentiate even 1bp difference in the DNA fragment size but others can't.

SNP markers are also better choice for DNA fingerprinting due to their abundance, time saving, and highly automated (speedy). More importantly SNPs found to posses higher efficiency in differentiating the cultivars. However, SNP markers demands complete sequence information of the crop to be fingerprint. According to the recent literature available only 22 crop genome sequences are available (Toad and Rob, 2012) and it may take several years to sequence all the major and minor crops. Moreover, high instrumentation cost as well as their bi-allelic nature may limit to exploit SNP markers in DNA fingerprinting.

Conclusion

The DNA fingerprinting is a robust tool in identification of crop varieties frequently developed by the plant breeders. Therefore a core set of consensus SSR markers can be developed in the respective crops to fingerprint the new crop varieties to be released near in future.

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