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Thirty First

Annual Meeting 1995

Barbados

Vol.XXXI

APPLICATIONS OF MOLECULAR BIOLOGY TECHNIQUES IN PLANT BREEDING

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INTRODUCTION

The techniques of molecular biology have become important tools in plant breeding. Associated with these new techniques are terms and acronyms that seem to form a separate language. This paper introduces some frequently used terminology in molecular biology while explaining applications in plant breeding.

GENE IDENTIFICATION AND ISOLATION

The first step in engineering a plant with a desirable agronomic trait is to identify and isolate the relevant gene for that trait. Examples of desirable agronomic traits include herbicide resistance, insect resistance, disease resistance, improved nutrient content, longer shelf-life, and unusual compounds like human hormones and biodegradable plastics. Generally, each trait is encoded by one gene. The isolation of the relevant gene can be difficult and time-consuming. All techniques for gene isolation are based on the following characteristics of the gene:

(i) a gene has specific nucleotide sequences
(ii) a gene occupies a particular location within the genome
(iii) a gene encodes RNA with a particular expression pattern
(iv) most genes have a function.

Isolating a gene is relatively simple if the corresponding gene from another plant is known. The gene can be isolated by heterologous hybridization (i.e. pairing of complementary DNA from different sources). If a gene exists in yeast or bacteria then the microbial gene could be used to probe the plant DNA to isolate the gene. Also, the relevant gene can be readily isolated if its function is known. This process involves protein isolation, purification, determination of the amino acid sequence followed by determination of the DNA sequence and location of this DNA sequence in the plant genome. However, for many genes their functions are not known or have not been established. To find out the function of a gene, one can create a transgenic plant that expresses antisense mRNA for that gene. This is done by cloning cDNA (DNA copied from mRNA) of the gene next to a highly expressed promoter in an antisense construct such that the mRNA is transcribed from the antisense strand of DNA when put into a plant. mRNA is normally transcribed from the sense strand. The antisense RNA lowers the level of sense RNA thereby decreasing the level of gene product. This technique has been used to identify two genes (the polygalacturonase gene and the ACC synthase gene) involved in fruit ripening in tomato.

USE OF MOLECULAR MARKERS

When nothing, or very little, is known of a plant genome then isolating genes for agronomically desirable traits becomes somewhat difficult. In such situations information about genes in the genome can be compiled in the form of a genetic map. A major advancement in plant breeding is the use of molecular markers to compile genetic maps (Landry, 1993). Molecular markers are also used in fingerprinting varieties, establishing phylogenies, tagging desirable genes and determining similarities in a population.

Isozymes were the first molecular markers to be used in plant breeding programmes but due to the small number of markers that could be generated by isozymes, they have been replaced by RFLP (Restriction Fragment Length Polymorphism) and RAPD-PCR (Random Amplified Polymorphic DNA using the Polymerase Chain Reaction) markers. Plant genetic maps generated by these molecular markers provide insights into genome organization, evolution, and function, and facilitate the management of genetic variation in natural and domesticated populations. Genetic maps also provide a means to identify the loci affecting quantitative traits involving adaptive or economically valuable attributes.

RFLPs are based on differences in fragment lengths obtained by digesting DNA samples with restriction endonucleases (enzymes that DNA at specific base sequences called restriction sites). Polymorphism is due to the presence or absence of restriction sites in the genomes being compared. RFLP markers are phenotypically neutral, codominantly inherited, nonspecific to growth stage and practically limitless in number. RFLP generated maps enable plant traits to be selected at the molecular level in the laboratory, thereby eliminating the need to grow large numbers of plants prior to selection. RFLP markers are presently being used to develop genetic maps for several agronomically important crops (e.g. Helentjaris, 1987). Also, RFLP markers are now being sequenced to identify suitable primers that would allow the amplification of specific polymorphism in DNA segments of known sequence. These primers are called STS (Sequence Tagged Sites).

The advent of PCR has made a new set of markers available for comparing organisms at the molecular level. The principle of PCR is outlined in Figure 1.

RAPD markers are obtained by PCR amplification of random genomic DNA segments using arbitrary primers of about 9–10 bases in length. These primers have a GC content of about 50–80% and do not contain palindromic sequences. RAPD primers are usually used singly and the number of fragments generated depends on the genomic DNA and the primer. The advantages of using RAPD markers are that a universal set of primers can be used for all species and that the process of generating a genetic map is much quicker than with RFLP markers.

Each amplification product by RAPD is derived from a region of the genome that contains two short DNA segments with some homology to the primer. These segments must be present on opposite DNA strands and be sufficiently close to each other to allow DNA amplification to occur. Using short primers and low annealing temperatures ensures

that several sites, randomly distributed in the genome, produce amplification products. The polymorphism between individuals results from sequence differences in one or both of the primer binding sites and are visible as the presence or absence of a particular RAPD band on an electrophoretic gel. Theoretically, the number of amplified fragments depends on the length of the primer, the size of the target genome, and is based on the probability that a given DNA sequence (complementary to that of the primer) will occur in the genome, on opposite DNA strands in opposite orientation within a distance that is readily amplifiable by PCR.

RAPD markers have some drawbacks. RAPDs are typically inherited as dominant traits, making it difficult to distinguish heterozygote and homozygote individuals with the dominant marker. As such RAPD markers are less informative and thus less efficient than codominant markers. In terms of genetic mapping, RAPDs are more useful for plant species that are inbred or backcrossed. However, dominance is a phenomenon associated with diploids and higher ploidy not haploids. Therefore, RAPD markers are very suitable for analyzing haploid plant genomes.

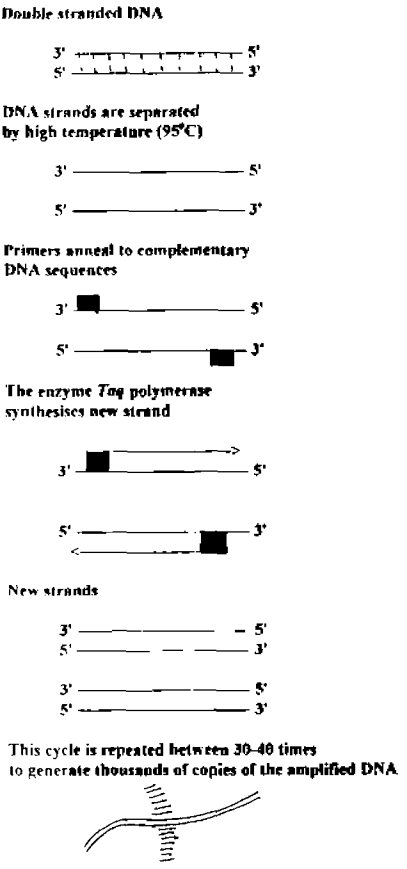

Figure 1 The principle of PCR (Polymerase Chain Reaction)

As additional molecular markers are located in the genome then the greater the chances are that markers will be found that are linked to the gene of interest. When the gene of interest has been chromosomally localized, the next step is to move towards it from a linked marker thereby creating a physical map and isolating new markers across the chromosome region. Molecular markers may enable the gene to be approached from either direction and define the region in which the gene lies. This minimizes the amount of DNA analyzes on the plant genomic DNA.

GENE TRANSFER

Once the agronomically important gene has been isolated, it is cloned into a transformation vector. This recombinant DNA vector could then be introduced into a plant by a plant transformation technique in an attempt to generate a transgenic plant that expresses the cloned gene. There are several transformation methods. A commonly used method is *Agrobacterium*-mediated transformation. The gene is cloned into the disarmed *Ti* plasmid of this bacterium and the plasmid is then used to infect the target plant. However, some plants (e.g. cereals) are recalcitrant to this method of transformation and a method based on the direct transfer of the gene by microprojectile bombardment of intact plant tissue is utilized. Transformation has also been achieved in plant protoplasts through facilitation of DNA uptake by calcium phosphate precipitation, polyethylene glycol treatment, electroporation and microinjection.

CONCLUSION

Presently the limiting factor in developing new plant varieties is the lack of knowledge of the genetic basis of most agronomic traits. The techniques of molecular biology, especially those that allow the detection and exploitation of naturally occurring DNA polymorphism, are likely to hasten the isolation of agronomically valuable plant traits. However, plant breeders need the expertise of plant physiologists, pathologists and geneticists to analyse these agronomic traits adequately.

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