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THE APPLICATION OF MOLECULAR MARKERS TO PLANT BREEDING

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Abstract: Morphological characters are subject to change due to environment. However, DNA markers are less independent of environmental contiditions. They can be obtained from isozyme, RFLP and PCR tecniques. Data are used for cultivar identification, prediction of hybrid performance, measuring the genetic distance, finding heterotic patterns, selection for agronomic traits and mapping genes in chromosomes. The most widely used method for detection of DNA polymorphisms is RFLP and a recent technique, PCR. However, detection of polymorphisms by using PCR technology is faster and less laborious than by using RFLP technology.

Key Words: Polymorphisms, genetic variability, RFLP, and PCR

Moleküler Belirleyicilerin Bitki Islahına Uygulanması

Özet: Morfolojik karakterler çevreden dolayı değişim gösterme eğilimindedirler. Fakat DNA belirleyicileri çevreden daha az bağımsızdırlar. Bunlar isozim, RFLP ve PCR tekniklerinden elde edilebilir. Veriler çeşit belirlenmesinde, hibrid verimliliğinin tahmininde, genetik uzaklığın ölçülmesinde, heterotik grupların bulunmasında, agronomik özelliklerin seçiminde ve genlerin kromozom üzerinde haritalanmasında kullanılırlar. DNA polimorfizmin belirlenmesinde RFLP ve PCR teknikleri kullanılmaktadır. Fakat, polimorfizmin ortaya çıkarılmasında PCR teknolojisi RFLP'ye göre daha hızlı olup daha az işgücüne ihtiyaç duymaktadır.

Anahtar Kelimeler: Polymorfizm, genetik varyasyon, RFLP ve PCR.

INTRODUCTION

Plant breeding is a successful technology that changed the nature of agriculture. Plant breeders not only changed the performance of crop varieties,but also increased the value of germplasm. With increasing agricultural industrialization, plant breeding has evolved from primarily a public sector research concern to an increasingly private sector activity. A sample of genes only has practical use as germplasm when those genes are assembled into blocks that can function effectively in providing food resources. Crop improvement involves five basic steps: 1) Discover or create genetically stable variation for desired traits, yield, disease and pest resistance, stress tolerance,

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etc.) 2) select from these variations that individuals possessing the desired traits. 3) incorporate the desired trait into a suitable agronomic background 4) test the new variety over a wide range of habitats and 5) release the new variety. Traditional breeding and the new biotechnologies differ in the first two steps. Traditional breeding focuses on individuals and populations and relies primarily upon sexual reproduction to manipulate useful variability.

Hybrid breeders often face a continuing challenge to identify germplasm sources that will provide inbreds with superior heterosis in single cross combinations. Morphological diversity, including chromosomal and degree of unrelatedness has been found to be important in hybrid performance. A primary goal of most breeding programs is to optimize the effectiveness of selection for quantitatively inherited agronomic traits. Selection for these traits based on the phenotype or genetic population parameter estimates. Currently attempts are being made to integrate molecular biology technology and conventional breeding procedures. Molecular biology has provided methodologies that enable the list of useful markers to be used in selection, considerably. These methodologies are based on protein and DNA polymorphisms. Initially, research was done to determine the potential of isozyme-aided selection in maize. isozyme loci were used to study the relationship between isozyme dissimilarities in maize inbred lines and hybrid performance. The results showed that general combining ability for yield was correlated with the number of heterozygous isozyme loci. However, specific combining ability did not appeared to be related to isozyme patterns (1, 2). Pedigree diversity is often correlated with allozyme diversity. There was a general association of allozyme diversity with higher yield but reliability of the prediction depended on the pedigree background of the lines (3). In a study designed to determine the usefulness of different methods of accurately assess relatedness and relationships of corn inbreds, morphological, isozymic, and zein chromatographic distance measures were compared (4). The results indicated that these data may be a starting point in determining distinctiveness, but that heterosis measurements or analysis of RFLP data offer better resolution of relatedness of corn inbreds.

RFLP analysis:

With the exception of specific disease resistances and morphological and color pattern traits which are often determined by allelic differences at one or two loci, genetic variation in most traits of agricultural importance is attributed to allelic differences but generally unknown number of loci having relatively small individual effects. These loci are termed quantitative trait loci. Genetic analysis and improvement of such traits is generally based on biometrical approaches that treat the overall global effect of all genetic loci and environmental factors affecting the traits in question. These

biometrical approaches are, however, unable to identify, characterize or manipulate the specific loci involved on an individual basis.

Genotypic analysis using RFLP's has proven to be powerful tool for both plant genetists and breeders. Genetical variations are monitored as changes in the length of defined DNA fragments produced by digestion of the DNA sample with restriction endonucleases and been termed Restriction Fragment Length Polymorphisms (RFLP's) Seed companies around the world currently analyze an estimated five to ten million crop genotypes in the field every year, a significant fraction of these field-tested genotypes could instead be determined by RFLP analysis. RFLP's may also provide a convenient means of assessing heterogeneity within and between stocks retained as germplasm resources. The assumption would be that heterogeneity for RFLP's is indicative of heterogeneity for other characters as well. This kind of screening might be one way to ensure that germplasm resources do indeed represent a wide range of genetic variability. In maize approximately 95 % of all unique sequence clones either cDNA or genomic, revealed RFLP's when tested with only three restriction enzymes against a selection of Corn Belt Dent germplasm. Many important agronomic crop species such as wheat and soybean display low levels of variability. Although RFLP's are a reflection of DNA sequence variation, they are a rather crude and indirect measure of this variation, since the polymorphism relies on altered restriction enzyme recognition sites or on significant size differences of inserted or deleted DNA restriction sites. All of these problems have prompted a search for an alternative technology that would allow fast, low-cost analysis that could be applied to genotypic analysis of all species.

RFLP's offer several advantages over isozymes as molecular markers. Like isozymes RFLP's are unaffected by environment, and show codominant inhertitance. But, in contrast to isozymes, RFLP's are more polymorphic, more numerous, and are better distributed throughout the genome and are suggested as superior markers to assess diversity and relationships in corn breeding germplasm (5). Several researchers tried to determine if RFLP diversity was correlated with hybrid performance (6, 7, 8) Their results showed that there was no relationship between hybrid performance and RFLP distance, but stated that RFLP's could be used to assign inbreds to heterotic groups. However, both pedigree distance and RFLP distance were highly correlated with F1 grain yield and heterosis, indicating RFLP distance was better indicator of yield and heterosis than was pedigree distance (9). From the results of another study indicates that potentially successful utilization of RFLP alleles to assign maize inbreds to their respective heterotic groups and to investigate relationships among inbred lines (10). Allocation of maize inbreds, of unknown heterotic pattern, to heterotic groups before field testing may allow breeder the opportunity to reduce costs by avoiding

crosses within heterotic groups.

PCR analysis

PCR techniques which is known as Polymerase Chain Reaction has been developed that allows in vitro synthesis and amplification of specific segments of DNA. PCR can enzimatically amplify a specific region of DNA flanked by two oligonucleotide primers that share identity to the opposite DNA strands. Amplification is achieved by a repetetive series of cycles involving template denaturation primer annealing, and DNA synthesis by DNA polymerase. By using repetetive cycles, where the primer extension products of the previous cycle serve as new templates for the following cycle. Originally, temperature control was achieved by transferring samples among three water baths set at the appropriate temperatures: Denaturation (94 ^{0}C), primer annealing (37 to 65 ^{0}C) and DNA polymerase extension (37 to 40 ^{0}C) (11). However, the use of Taq polymerase greatly simplified the PCR technique and without the need to add new DNA polymerase after each cycle. It should be pointed out that one does not have to use an automated thermal cycling apparatus to perform PCR amplifications, because amplifications obtained by the manual transfer of samples among three water baths with temperature control is as precise as any of the automated thermal cycling apparatuses. A unique property of the PCR technique is that only small amounts of target DNA (usually between 100 ng to 1 μg) are needed. Reduction in the amount of DNA required for a reaction is particularly important in speeding up early screening (in maize e.g. screening could be done a few days after germination instead of 2-3 weeks). The DNA need not to be pure for amplification and amplified DNA product can generally be detected by gel electrophrosis followed by staining with ethidium bromide (thus, in many cases radioactive probes are not needed).

The utility of single short oligonucleotide primers of arbitrary sequence for the amplification of DNA segments distributed throughout the plant genome. PCR generates reproducible fingerprints from any organism, without the need for DNA sequence information. These fingerprints include DNA fragment for polymorphisms can be used for varietal identification and parentage determination, 2) followed in segregating populations by crosses, 3) used as markers for the construction of genetic maps and, used to generate dendograms of phylogenetic relationships, especially at the interspecific level (12).

Application of PCR on cultivar identification was studied for a number of plant species (13, 14, 15). Determination of the genetic purity of lines and of F1 hybrid cultivars is a quality-control requirement of critical importance in plant breeding and seed production. Knowledge of these dat is essential for the control and uniformity of yield, as well as for avoidance of unacceptable impurity levels in seed lots

prior to market release. DNA markers are independent of environmental conditions and are present in all plant cells.

CONCLUSION

Methods have been developed over the past two decades that allow the detection of polymorphisms in DNA. DNA polymorphisms can be used as molecular markers and long with isozymes are making a significant impact on applied plant breeding. The most widely used method for detection of DNA polymorphisms is restriction fragment length polymorphisms (RFLP's) which are the product of changes in the bases within restriction enzyme target site. Most recently, techniques based on the polymerase chain reaction (PCR) have been used to detect polymorphisms in various plant, animal and bacterial species Detection of polymorphisms by using PCR technology is faster and less laborious than by using RFLP technology as long as primers of approximately the same length and GC content are used in a given set of experiments. The main advantage of PCR over RFLP technology are increased speed of analysis and reduction in the amount required by analysis.

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