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MOLECULAR MARKERS IN WHEAT

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ABSTRACT: The progress made in DNA markers technology has been tremendous and exciting. DNA markers have provided valuable tools in various analysis ranging from polygenetic analysis to the positional cloning of genes. The development of high density molecular maps which has been facilitated by the PCR-based markers have made the mapping of almost any trait possible. The size and structure of the wheat genome makes it one of the most complex crop species for genetic analysis. The development of molecular technique for genetic analysis, in particular the use of molecular markers to monitor DNA sequence variation between varieties, and races, and wild relatives of wheat and related grass species, has led to a dramatic expansion in our understanding the structure and behavior of wheat genome.

Key words: wheat, DNA, Molecular, Markers

INTRODUCTION: There are two types of markers systems in crop plants. They are phenotypic/ morphological and molecular markers. Phenotypic/ morphological markers are the expression dependent. Morphological markers are also used for genetic mapping. These phenotypical/ morphological markers are not an ideal markers due to their several drawbacks. The morphological markers are environment dependent. Their expression can be modified by the environmental factors secondly; other genes (epistasis and pleiotropism) can modify the expression of the phenotypic markers. Molecular markers are useful because they are generally stable, numerous and informative. The molecular markers for genome mapping should have additional properties such as, highly abundant and evenly distributed within entire genome, highly polymorphic information content (PIC), high multiplex ratio, co-dominant and neutral.

A large number of molecular markers are now available to detect polymorphism, each of which has advantages and disadvantages. They are RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), SSR (simple sequence repeat), SNP (single nucleotide polymorphism), STS (sequence tagged sites).

RFLP (restriction fragment length polymor - phism)

RFLP is the basic molecular markers (Botstein et al. 1980) detect polymorphism on the basis of differences in restriction fragment length. These

differences may be due to mutations or insertion-deletion, which can create or delete restriction endonuclease recog- nition sites. These RFLP assays are performed by the radio active labelled DNA probes to a southern blot. These markers (RFLP) are specific to single clone/ restriction enzyme combinations and most of them are co-dominant in nature, highly locus specific and often multiallelic. These markers can be used across a wide range of related species. In wheat mapping, probes from wheat, rye (Secale cereale), barley, and oats (Avena sativa) are widely used. Further, wheat sequences successfully hybridize to genomic DNA from rice, maize, sorghum, and several grass species. The use of RFLP probes has shown the relationship between chromosomes of a number of gene order (synteny) across the grosses (Moore et al. 1995). However, RFLPs have disadvantages such as tedious and inefficient due to low multiplex ratio, low genotyping throughput, high labour intensity, and the requirement for the large amounts of high quality template DNA.

RAPD (random amplified polymorphic DNA)

RAPD is a PCR-based technique. RAPD markers were generated by using random primers in the range of 10-20 nucleotides to detect complementary sites across relatively short distances within the genome (Williams et al. 1990). Polymorphisms result from either base changes that alter the primer binding sites, rearrangements or insertion-deletions at or between oligonucleotide primer binding sites in the genome. This type of marker system is dominant, as a band is either produced or not produced and the inability to distinguish heterozygotes from a homozygous class is a major disadvantage of the system.

AFLP (amplified fragment length polymor - phism):

AFLP assays are based on a combination of restriction digestion and PCR amplification. AFLPs caused by mutation or insertion-deletions in a restriction site that create or abolish restriction endonuclease recognition sites. One of the principal applications of AFLP (Zabeau and Vos 1993; Vos et al. 1995) is in genetic mapping, either in map construction or identifying linkages using bulk segregant analysis. The high multiplex ratio of AFLP technique is now being exploited in wheat. This marker system has the potential to improve the efficiency of genetic map construction and to generate high density map around loci that control commercially important traits. The reported levels of AFLP polymorphism observed in cereal mapping populations differ widely among cereal species from 26.6% in rice (cho et al. 1998), up to 48% in maize (Vuylsteke et al. 1999) 11.3% in barely (Becker et al. 1995), 23.8% in durum wheat (Lotti et al. 2000) and 12.8% in bread wheat (Chalmers et al. 2001). AFLP loci appear to be evenly distributed across the A and B genomes but there is a significant reduction in the level of polymorphisms detected in the D genome of hexaploid wheat (Marino et al. 1996; Roder et al. 1998). AFLP is a robust and reliable technique that is finding increased use in wheat genetics and is rapidly becoming the preferred molecular technique has now been widely demonstrated across most of the cereals.

SSRs (simple sequence repeats)

SSRs, or microsatellite, are short tandem repeats of mono-di, tri and tetra-nucleotides although more complex repeats have been detected. They are

abundant in mammals (Weber and may 1989) and have been found by searching sequence databases (Lagercrantz et al. 1993). They can be found by the sequencing through random clones of genomic DNA. The polymorphism among individuals is due to the variation in number of repetitive units. The main advantage of SSRs over RAPDs and AFLPs is that they are, like RFLPs, co-dominant markers and can therefore reliably identify heterozygotes as being multiallelic. SSRs are highly abundant and randomly dispersed throughout most genomes. SSRs provide an excellent framework for markers with locus identity. The main advantage of SSRs is their high development cost, requiring extensive DNA cloning and sequencing for their identification. The production of libraries enriched for specific SSR motifs has been found to greatly increase the rate of SSR discovery (Ramsay et al. 2000). A number of groups have invested resources in developing SSRs and 230 are available for wheat (Roder et al. 1998) and more than 560 for barely (Ramsay et al. 2000). Due to their high PIC values, SSRs are proving to be excellent anchor loci for comparing various studies and are the current marker system of choice within species.

SNPs (single nucleotide polymorphisms)

Single nucleotide polymorphism (SNP) markers are becoming widely used in human genetics to investigate linkages of genes to specific disease conditions (Weiss, 1998). The technique involves the identification of single base pair changes and designing PCR-based amplification protocols revealing polymorphism. It is estimated that human genome includes one SNP every 1058 base pairs (Durrett and Limic, 2001). This marker class has not been widely exploited in plants, but represents a rich source of polymorphism's application.

STS (sequence tagged sites):

RFLP clones isolated from related cereal species quite often hybridize with homologous sequences in other species. Conservation of RFLP markers to a sequence tagged sites (STS) markers have an edge over the conventional RFLP analysis, including relative ease, greater throughput, sharing of primer sequence and it also needs less genomic DNA (Erpelding et al. 1996). This type of PCR is carried out using primers designed to flank at a known DNA sequence. These markers have particular importance when designed to identify the genes for genomic characters. In contrast to SSRs, STSs derived from conversion of RFLP markers are widely transferable between wheat and barely (Erpelding et al. 1996).

Marker assisted selections:

Molecular markers are specifically important for genomic traits that are difficult to tag such as resistance to pathogen, insects, tolerances to abiotic stresses, quality parameters and quantitative traits. Molecular marker studies using near isogenic lines and bulk segregant analysis or recombinant inbred lines have accelerated the mapping of many genes in different plant species. Screening the germplasm for agronomically important genes is difficult. Availability of tightly linked gene markers will play a pivotal role in selecting progenies in a segregating population. A plant breeder can save time, efforts and public funds by only growing and multiplying the required segregants and rejecting the undesired populations even in F_2 generation.

CONCLUSION:

The development of DNA marker technology has enabled the breeders to

use Mendelian genetic approach to complement breeding. Tremendous progress has been achieved in comparative mapping of cross-incompatible species. Conservation of certain regions on homologous chromosomes that has been observed among different members of a family certainly points to fact that such regions are important to the plant and have therefore been conserved through evolution. However, such regions, genes controlling agronomical important characters have been localized in related species. It will be interesting to find out what other conserved regions, where no genes as such have been mapped code for, by employing approaches of fine mapping and positional cloning. The gross family "Gramineae" is one of the best studied families from this aspect and each of its members has certain unique characteristics which makes it possible to manipulate genes across the family. For example, aneuploid stocks are available in wheat, which can be used to assign DNA markers to specific chromosomal arms. Genome synteny relationship studies were conducted to analyse the genome structure of gramineae family (Moore et al. 1995; Plaschke et al. 1995). These approaches will add to the number of genes that could be available for manipulation by genetic engineering. In the light of this progress, markers assisted selections (MAS) and transgenes will be increasingly used for wheat improvement. Although biotechnology has immense importance in agriculture, the fact that over 50% of agriculture production been achieved through conventional plant breeding should not be ignored. However, DNA marker technology cannot replace the plant breeding, certainly it will help the plant breeders by providing new tools to ease the many problems faced by plant breeders.

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