

MOLECULAR MARKERS AND THEIR APPLICATIONS IN CEREALS BREEDING

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Summary

The development of molecular techniques for genetic analysis has led to a great increase in our knowledge of cereal genetics and our understanding of the structure and behaviour of cereal genomes. These molecular techniques, in particular the use of molecular markers, have been used to monitor DNA sequence variation in and among the species and create new sources of genetic variation by introducing new and favourable traits from landraces and related grass species. Improvements in marker detection systems and in the techniques used to identify markers linked to useful traits, has enabled great advances to be made in recent years. While RFLP markers have been the basis for most work in crop plants, valuable markers have been generated from RAPDs and AFLPs. Simple sequence repeats (SSR) or microsatellite markers have been developed more recently for major crop plants and this marker system is predicted to lead to even more rapid advances in both marker development and implementation in breeding programs. Identification of markers linked to useful traits has been based on complete linkage maps and bulked segregant analysis. However, alternative methods, such as the construction of partial maps and combination of pedigree and marker information, have also proved useful in identifying marker/trait associations. A revision of current breeding methods by utilising molecular markers in breeding programmes is, therefore, crucial in this phase. This review provides an overview of current stage of these developments, examines some of special issue that have used in applying molecular techniques to molecular breeding of cereals species.

Key Words

Cereals, molecular markers, fingerprinting, marker assisted breeding

Introduction

Conventional cereal breeding is time consuming and very depended on environmental conditions. Breeding a new variety takes between eight and twelve years and even then the release of an improved variety cannot be guaranteed. Hence, breeders are extremely interested in new technologies that could make this procedure more efficient. Molecular marker technology offers such a possibility by adopting a wide range of novel approaches to improving the selection strategies in cereal breeding.

Molecular markers

In recent years, different marker systems such as Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Sequence Tagged Sites (STS), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) or microsatellites, Single Nucleotide Polymorphisms (SNPs) and others have been developed and applied to a range crop species including cereals. The relative advantages and disadvantages of these techniques are summarised in Table 1.

Table 1.
Comparison of the most common used marker systems in cereals.

| Feature | RFLPs | RAPDs | AFLPs | SSRs | SNPs |
|-----------------------------------|----------|------------|----------|----------|------|
| DNA required (μg) | 10 | 0.02 | 0.5-1.0 | 0.05 | 0.05 |
| DNA quality | high | high | moderate | moderate | high |
| PCR-based | no | yes | yes | yes | yes |
| Number of polymorph loci analysed | 1.0-3.0 | 1.5-50 | 20-100 | 1.0-3.0 | 1.0 |
| Ease of use | not easy | easy | easy | easy | easy |
| Amenable to automation | low | moderate | moderate | high | high |
| Reproducibility | high | unreliable | high | high | high |
| Development cost | low | low | moderate | high | high |
| Cost per analysis | high | low | moderate | low | low |

This information suggests that RFLP, SSR and AFLP markers are most effective in detecting polymorphism. However, given the large amount of DNA required for RFLP detection and the difficulties in automating RFLP analysis, AFLPs and SSRs are currently the most popular markers in cereals. An increasing amount of sequence information and the determination of the gene function in cereals will lead in the near future to the preferred use of new marker types, such as SNPs. Application of these markers for genetic studies of cereals have been so much diverse. Main uses include:

- assessment of genetic variability and characterisation of germplasm;
- identification and fingerprinting of genotypes;
- estimation of genetic distances between population, inbreds and breeding material;
- detection of monogenic and qualitative trait loci (QTL);
- marker-assisted selection;
- identification of sequences of useful candidate genes, ect.

Genotype identification and genetic diversity

It is vital for plant breeding programmes to have sufficient diversity available to allow for the production of new varieties that are aimed towards the improvement of crop productivity and able to withstand damage from biotic and abiotic factors. In this respect, efforts have also been made to predict the prospects of developing superior genotypes from a cross by the measurement of genetic similarity (GS) or genetic distance (CD) between the parents, since the later can be used as an estimation of expected genetic variances in different sets of segregating progenies derived, from different crosses.

DNA fingerprinting of cereals species and cultivated varieties has a long scientific history. When DNA profiling technology first came into use, restriction fragment length polymorphism (RFLP) were considered state-of-art. RFLP technology was followed random amplification of polymorphic DNA (RAPD), followed by amplified length polymorphism (AFLP) and most recently use microsatellite markers or single sequence repeat (SSR).

Advantage of SSR markers are:

- the method is relatively simple and can be automated;
- most of the markers are monolocus and show Mendelian inheritance;
- SSR markers are high informative;
- a high number of public SSR primer pairs are available;
- effective cost per genotype and primer (similar to that for RAPD).

Example 1.

Application of molecular markers for genotype identification and genetic

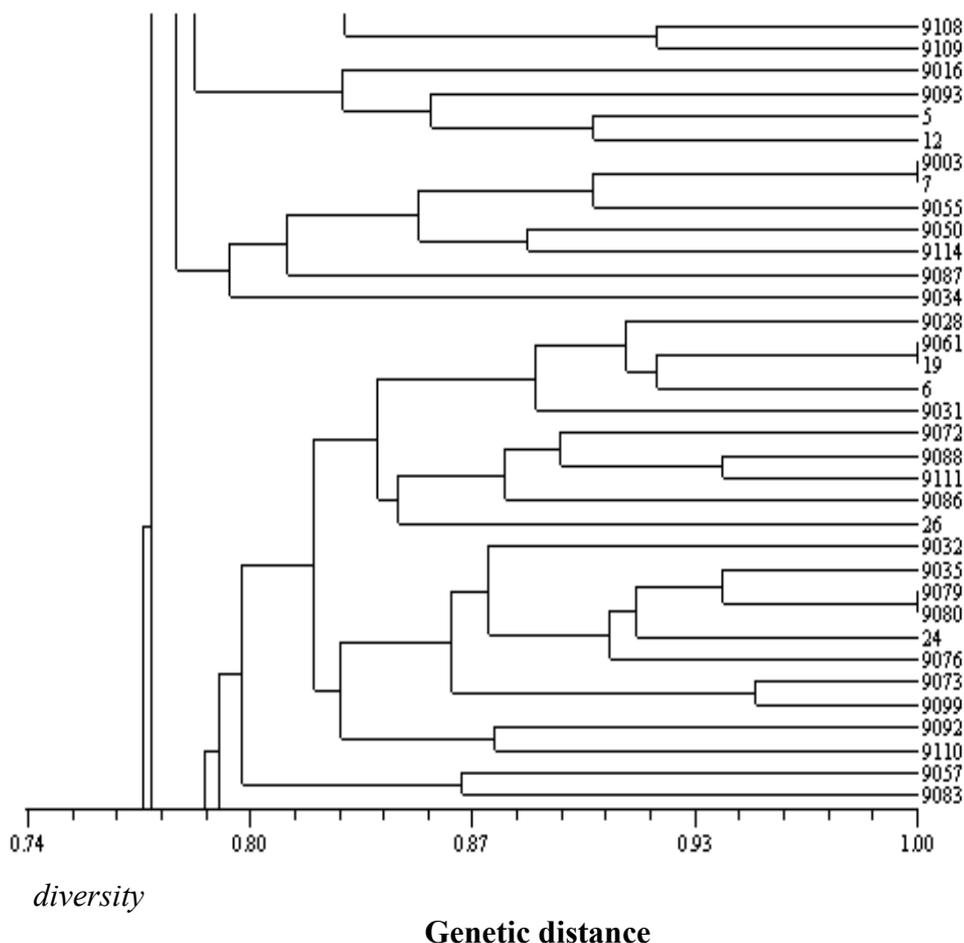


Fig.1
Dendrogram of wheat genotypes based on data generated using 22 microsatellites primers pair. Genotypes 9003 and 7, 9061 and 19 are duplicates; 9079 and 9080 are not distinguish

Trait tagging and marker-assisted selection

A large number of cereal studies have used markers as a tool to identify major genes, QTLs, or to introduce new characters in elite germplasm. In wheat, for example, molecular markers have been identified that are associated with around 40 traits of economic importance (Gurta et al. 1999). Knowing the location of these genes/traits and specific alleles offers the possibility to apply marker-assisted selection (MAS) in cereals, because one of the main objectives of plant breeding is the introgression of one or more favourable genes from a donor parent into the background of an elite variety. Marker-assisted selection allows plant selection at the juvenile stage from an early generation. For simply inherited traits, conventional PCR, which requires a small amount of DNA, is becoming very useful for screening large populations of segregating progenies. Unfavourable alleles can be eliminated or greatly reduced during the early stages of plant development through MAS, focusing the selection in the field on reduced numbers of mature plants.

Example 2. *Application of molecular markers in breeding for resistance to Barley yellow mosaic virus.*

Barley yellow mosaic virus disease – caused by *Barley mild mosaic virus* (BaMMV) and *Barley yellow mosaic virus* (BaYMV) – has to be considered as one of the most important

diseases of winter barley in Europe and East Asia. Because of the transmission by the soil-borne fungus *Polymyxa graminis*, chemical treatments to control the disease are neither efficient nor economic. Therefore, breeding for resistance to this disease is of special importance. However, field selection for resistance genotypes is often difficult to perform because of unpredictable environmental conditions. Consequently, the application of closely linked PCR-based markers for the transmission of resistance gene(s) against barley yellow mosaic virus is now successful and efficient (Table 2.).

Table 2

Application of PCR-based marker MG3H001 for detecting resistant plants allele. 169 bp corresponds to the susceptible plants, alleles 146 bp and 150 bp to the ym4 or ym5 resistant plants, respectively.

| Nrs | Plants | Allele | MG3H1_146 | MG3H1_150 | MG3H1_169 | Resistance |
|-----|------------|-------------|-----------|-----------|-----------|----------------|
| 1 | Ge0043_001 | MG3H001_146 | 1 | | | resistance ym4 |
| 2 | Ge0043_002 | MG3H001_169 | | | 1 | susceptible |
| 3 | Ge0043_003 | MG3H001_146 | 1 | | | resistance ym4 |
| 4 | Ge0043_004 | MG3H001_169 | | | 1 | susceptible |
| 5 | Ge0043_005 | MG3H001_146 | 1 | | | resistance ym4 |
| 6 | Ge0043_006 | MG3H001_146 | 1 | | | resistance ym4 |
| 7 | Ge0043_007 | MG3H001_169 | | | 1 | susceptible |
| 8 | Ge0043_008 | MG3H001_169 | | | 1 | susceptible |
| 9 | Ge0043_009 | MG3H001_150 | | 1 | | resistance ym5 |
| 10 | Ge0043_010 | MG3H001_150 | | 1 | | resistance ym5 |
| 11 | Ge0043_011 | MG3H001_150 | | 1 | | resistance ym5 |
| 12 | Ge0043_012 | MG3H001_169 | | | 1 | susceptible |

Example 3. Application of molecular markers for male fertility restoration in Pampa CMS in breeding of rye.

Hybrid rye breeding and seed production require a cytoplasmic male sterility (CMS) system as a hybridisation mechanism. On the other hand, for the complete restoration of pollen fertility, effective, nuclear encoded restorer genes for CMS-inducing cytoplasm are indispensable.

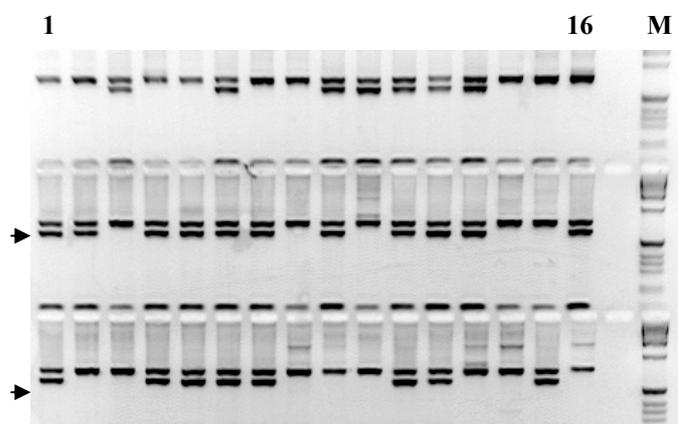


Fig. 2. Application of PCR-based marker SR4R04 for selecting fertile plants. M – 1 kb ladder; lanes 1-48 rye plants, fertile plants detected by two fragments.

Partial restoration of male fertility causes a reduction in the amount of viable pollen, thus encourages infection by the ergot fungus (*Claviceps purpurea*). Ergot infection contaminates rye grains with sclerotia containing toxic alkaloids. To reduce or avoid this risk, rye hybrids need effective restorer genes.

Recently, a new restorer source was found in IRAN IX, an Iranian primitive rye population. This exotic material displays a significantly higher level of restoration than the currently used European lines. However, despite the excellent restoration ability, the material contains many undesirable agronomic characters. In this case the breeding process can only be hastened by applying molecular markers for developing new material, which should combine an excellent pollen restoration with high agronomic performance. Recently, tightly linked markers to the restorer gene have been found and specific PCR-based assays have been developed (Figure 2).

Example 4. *Application of molecular markers in breeding for resistance to Fusarium head blight in wheat.*

Fusarium head blight is a serious disease of wheat (*Triticum aestivum* L.) in humid and semi humid areas of the world. In Central Europe, severe natural epidemics of *Fusarium* head blight (FHB) occur once or twice in a decade and can sharply reduce yield and quality of susceptible genotypes. Deoxynivalenol (DON) are harmful to humans, because they are highly heat stable and cannot be eliminated totally once they entered the food chain.

Evaluation of *Fusarium* head blight resistance is time consuming, laborious and costly because the inheritance of resistance is complex and phenotypic expression is significantly affected by environmental factors. Molecular markers closely linked to the major QTL involved in FHB resistance have recently been found (Buerstmayer et al. 2002) and raise the possibility of using MAS for introducing resistance alleles into elite wheat varieties as have been confirmed also by us. However, due to the multifactorial nature of FHB resistance, the combination of MAS on the major QTL during seedling stage with phenotypic selection on the particular plants after flowering stage could be at the moment more sufficient and safety strategy in breeding of a new varieties combining a high level of yield performance and high level of resistance to *Fusarium* head blight.

Conclusion

In recent times many DNA markers have been developed and are powerful tools for successful cereal breeding. The promise of marker-assisted selection in crop breeding still remains but achieving practical benefits is taking longer than expected. The main reasons for this delay are the insufficient quality of markers (regarding their predictive and/or diagnostic value), inadequate experimental design, high costs and complexity of quantitative traits.

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