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The Application of Molecular Marker on Crop Heterosis Development: A Review Paper

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ABSTRACT: *Heterosis has historically been exploited in plants; however, its underlying genetic mechanisms and molecular basis remain elusive. The use of molecular markers to identify quantitative trait loci (QTL) affecting agriculturally important traits has become a key approach in plant genetics-both for understanding the genetic basis of these traits and to help design novel plant improvement programs. Hybrids are commercially successful in many crops, including sorghum. Development of hybrids through the exploitation of heterosis involves evaluation of hundreds of test crosses in the field, making it input and resource intensive. Therefore, plant breeders are interested in methods that can forecast the potential parental combinations so that only limited test crosses can be evaluated for heterosis. The availability of genomic tools such as DNA markers and gene expression platforms has encouraged research groups globally to work toward the prediction of heterosis. This review is intended to be a summary of recent developments in molecular markers and their applications in plant breeding and is devoted to early researchers with a little or no knowledge of molecular markers. Overall, this review reveals about the role of various recently developed molecular markers in the improvement of crop. Molecular markers act as a breakthrough for the researchers who aim to enhance crop breeding and its role in heterosis.*

KEYWORDS: DNA, Heterosis, MAS, Molecular marker

INTRODUCTION

Heterosis occurs in variety of species and has been observed and recorded in china since ancient times. The exploitation of heterosis through hybrid breeding is one of the landmark achievements in plant breeding (Duvick, 2001). The genetic basis of the phenomenon of heterosis has been studied intensively during the past century. Vast data sets were generated, analyzed, and the gathered information was discussed at three International conferences, at Iowa State College in Ames (Gowen, 1952), at CIMMYTin Mexico City (Coors and Pandey, 1999), and at the University of Hohenheim in Stuttgart (Melchinger, 2010).

Genetic hypotheses are amongst the oldest but still most prevailing explanations for heterosis (Lamkey and Edwards, 1999). The dominance hypothesis explains heterosis by the cumulative effect of favorable alleles exhibiting either partial or complete dominance. The over dominance

hypothesis assumes over dominant gene action at many loci and the epistasis hypothesis attributes heterosis to epistatic interactions between non-allelic genes. The relevance of the three hypotheses has been investigated intensively using phenotypic data (Reif *et al.*, 2005) and also through molecular marker-aided QTL mapping. Molecular marker aided genetic analyses provided a new and powerful tool to study the genetic basis of heterosis in more detail.

Reliable prediction of single-cross performance is very important in hybrid breeding, because it is difficult to evaluate inbred lines in numerous cross combinations. Several prediction approaches have been suggested using phenotypic data with coancestry coefficients calculated from pedigree records or marker data (Schrag *et al.*, and 2009). Moreover, genomic selection based on dense molecular marker profiles has the potential to assist breeders in the selection of the most promising hybrids for field evaluation (Piepho, 2009). For less complex traits, mid-parent performance serves as a good predictor for hybrid performance. In contrast, for complex traits, predicting the performance of single crosses based on the line *per se* performance is expected to be severely hampered by masking non-additive effects (Smith, 1986). This was also confirmed in sunflower with several experimental studies (Ortis *et al.*, 2005).

An effort has been made to critically assess the level of success and future strategies in the development and adoption of hybrid technology in selected field crops under three categories: ***(i)*** Extension of hybrid technology to new niches and situations (maize, sorghum and pearl millet); ***(ii)*** Sustenance of hybrid technology in new crops of proven potential (rice, cotton and sunflower), and ***(iii)*** Extending hybrid technology to new prospective crops (*Brassica*, wheat, pigeon pea and castor). Maize closely followed by sorghum and pearl millet have been the pioneer crops in India since mid-fifties. However, lack of single cross short duration hybrids in maize; lack of higher yielding dual purpose hybrids suitable for *rabi* season in sorghum and narrow cytoplasmic base of CMS system in pearl millet are certain limitations which we need to overcome. Failure to produce and supply targeted quantity of hybrid seed, especially of public bred hybrids warrants urgent attention. High yielding cotton hybrids combining early maturity and resistance to leaf curl virus and boll worm complex for irrigated north and north-west regions having limited growing season are yet to be developed. Dissemination of transgenic *Bt.* cotton hybrids/varieties all over to effectively manage bollworm complex and thereby stabilize yield and reduce cost on plant protection is required. One of the major concerns in sunflower hybrids is slow pace of development of heterotic gene pools, exclusive dependence on a single CMS source (*PET-1*) and narrow genetic diversity for seed yield and oil content. Broadening of genetic base through enrichment of source nursery with germplasm for yield vigour is important keeping in view the fact that available genetic variability in Indian mustard is too inadequate to realize the desired level of yield heterosis; at the same time, it is worth searching for cytoplasmic-genetic male sterility source. First wheat hybrid "Pratham" is being commercialized. However, viable seed production technology either through CMS/chemical based system is yet to be perfected.

Mapping and tagging of agriculturally important genes have been greatly facilitated by an array of molecular markers in crop plants. Marker-assisted selection (MAS) is gaining considerable importance as it would improve the efficiency of plant breeding through precise transfer of

genomic regions of interest (foreground selection) and accelerating the recovery of the recurrent parent genome (background selection).

MAS has been more widely employed for simply inherited traits than for polygenic traits, although there are a few success stories in improving quantitative traits through MAS. The success of MAS depends upon several critical factors, including the number of target genes to be transferred, the distance between the flanking markers and the target gene, the number of genotypes selected in each breeding generation, the nature of germplasm and the technical options available at the marker level. With the advent of third generation marker technologies, such as the single nucleotide polymorphisms, the power and efficiency of genotyping are expected to improve in the coming decades.

The various methods are in use to predict heterosis and can be grouped into (i) per se performance, (ii) combining ability and (iii) genetic diversity as determined through geographic origin, morphological and agronomic traits as well as molecular markers. The experimental data indicate that heterosis is a function of heterozygosity in a higher number of loci and that the increase of the heterozygous loci number by crosses to genetically distant lines or populations increases the level of heterosis in the crosses. Based on this hypothesis Hallauer *et al*., (1988) assumed that the magnitude of heterosis could be predicted on the basis of inbred lines differences obtained after use of molecular markers. Different classes of molecular markers have been used to analyze the genetic relationships among maize inbred lines and to examine the relationship between marker-based GD and heterosis in maize (Lee, 1995; Smith *et al*., 2004; Boppenmar *et al*.., 1992; Melchinger *et al*., 1991). Correlation level varies depending on the analyzed material and various types of gene effects, pointing to the complexity of the genetic background of heterosis.

Justification and/or rationale for the paper

Its know that Hybrid crop varieties vastly outperform their inbred progenitors in economically important species ranging from maize (Zea mays) to oil palm (Duvick, 2005; Fu *et al.,* 2014; Cros *et al.,* 2015). Infact, hybrid breeding requires more time and resources than inbred breeding (Troyer and Wellin, 2009; Longin *et al.,* 2014; Cros *et al.,* 2018). The effectiveness of hybrid breeding can be improved by genomic selection, in which marker information partially replaces phenotypes in estimation of breeding values (Heffner *et al.,* 2009). Genomic selection can shorten the breeding cycle, reduce the costs of phenotyping, and improve selection accuracies (Lorenz *et al.,* 2011; Heslot *et al.,* 2015; Zhao *et al.,* 2015b; Schulthess *et al.,* 2017; Kadam and Lorenz, 2018). Genomic selection also opens new opportunities to establish hybrid breeding programs in crops which are widely cultivated as inbreds, such as wheat (Triticum aestivum; Zhao *et al.,* 2015b). Understanding heterosis from the perspective of molecular genetic mechanisms alone may be elusive, because heterosis is likely an emergent property of populations. Hybrid breeding is a process of recurrent population improvement to maximize hybrid performance. Genetic differences between hybrid parents can be expressed by GD, and the degree of heterosis is strongly correlated with the GD of both parents. In a broad sense, the degree of heterosis increases with the increase of GD (Usatov *et al., 2014;* Boeven *et al.,* 2020), and the farther the GD between the parent inbred lines is, the more scattered the gene expression in the hybrids will be (He *et al.,* 2010; and Paschold *et al.,*

2012). Studies on a variety of plants have shown that GD has a considerable correlation with heterosis. The present researches on plant heterosis were summarized from different aspects including parental genetic distance (GD), quantitative trait loci (QTL), genomics and modern molecular markers can provide theoretical reference for improving plant yield in breeding in the future.

Structural genomics: random, targeted and whole genome approaches

Structural genomics is an approach in molecular genetics that enables researchers to detect segments of DNA with allelic variations, correlate those polymorphisms with phenotypic data and determine causative mutations underlying important traits. The scope of "structural genomics" discussed here needs to be distinguished from that coined by protein community where similarly-named approach has been used to investigate the comprehensive repertoire of protein folds to infer molecular functions of the proteins (Burley *et al*., 1999). Although the main goal of structural genomics is similar in both cases, i.e. from structure to function, researchers use different paths to achieve the final goal.

Molecular markers: development and applications

Structural allelic alterations, or polymorphisms, of a genome can be grouped into three major categories that include differences in the number of tandem repeats at a particular locus [microsatellites, or simple sequence repeats (SSRs)] (Weber and May, 1989), segmental insertions/deletions (InDels) (Ophir and Graur, 1997) and single nucleotide substitutions [single nucleotide polymorphisms (SNPs)] (Wang *et al*., 1998). In order to detect and track allelic variations in progeny, the scientific community has been developing genetic tools, called molecular markers, since the late 1980s (Botstein *et al*., 1980). Although SSRs, InDels and SNPs are the three major allelic variations discovered so far, a plethora of molecular markers have been developed to detect the above-mentioned polymorphisms (Bernardo, 2008; Gupta *et al*., 1999). The main drivers for the evolution of molecular markers have been throughput, level of reproducibility and cost reduction (Bernardo, 2008). Depending on the detection method and throughput, all molecular markers can be divided into three major groups: (1) low-throughput, hybridization-based markers such as restriction fragment length polymorphism [RFLP (Botstein *et al*., 1980)], (2) medium-throughput PCR-based markers, that include random amplification of polymorphic DNA (RAPD) (Welsh and McClelland, 1990; Williams *et al*., 1990), amplified fragment length polymorphism (AFLP) (Vos *et al*., 1995) and SSRs (Wang *et al*., 1998), and (3) HTP sequence-based markers: SNPs (Wang *et al*., 1998). In late eighties, RFLPs were the most popular molecular markers and were widely used in plant molecular genetics, because they were reproducible and codominant.

However, the detection of RFLPs was very expensive, labor- and time-consuming process, which made these markers eventually obsolete. Additionally, RFLP markers werenot amenable for automation. Invention of PCR technology and application of this method for the rapid detection of polymorphisms overthrew low-throughput RFLP markers, and new generation of PCR-based markers emerged in the beginning of 1990s. RAPD, AFLP and SSR markers are the major PCR-

based markers that research community has been using in various plant systems. RAPDs were able to simultaneously detect polymorphic loci in various regions of a genome.

The fundamental basis of plant breeding is the selection of specific plants with desirable traits. Selection typically involves evaluating a breeding population for one or more traits in field or glasshouse trials (e.g. agronomic traits, disease resistance or stress tolerance), or with chemical tests (e.g. grain quality). The goal of plant breeding is to assemble more desirable combinations of genes in new varieties.

There are five main considerations for the use of DNA markers in MAS: reliability; quantity and quality of DNA required; technical procedure for marker assay; level of polymorphism; and cost (Mackill & Ni 2000; Mohler & Singrun 2004).

1. **Reliability**. Markers should be tightly linked to target loci, preferably less than 5 cM genetic distance. The use of flanking markers or intragenic markers will greatly increase the reliability of the markers to predict phenotype.
2. **DNA quantity and quality**. Some marker techniques require large amounts and high quality of DNA, which may sometimes be difficult to obtain in practice, and this adds to the cost of the procedures.
3. **Technical procedure**. The level of simplicity and the time required for the technique are critical considerations. High-throughput simple and quick methods are highly desirable.
4. **Level of polymorphism**. Ideally, the marker should be highly polymorphic in breeding material (i.e. it should discriminate between different genotypes), especially in core breeding material.
5. **Cost**. The marker assay must be cost-effective in order for MAS to be feasible.

The most widely used markers in major cereals are called simple sequence repeats (SSRs) or microsatellites (Gupta *et al.*, 1999; Gupta & Varshney 2000). They are highly reliable (i.e. reproducible), co-dominant in inheritance, relatively simple and cheap to use and generally highly polymorphic. The only disadvantages of SSRs are that they typically require polyacrylamide gel electrophoresis and generally give information only about a single locus per assay, although multiplexing of several markers is possible. These problems have been overcome in many cases by selecting SSR markers that have large enough size differences for detection in agarose gels, as well as multiplexing several markers in a single reaction. SSR markers also require a substantial investment of time and money to develop, and adequate numbers for high-density mapping are not available in some orphan crop species. Sequence tagged site (STS), sequence characterized amplified region (SCAR) or single nucleotide polymorphism (SNP) markers that are derived from specific DNA sequences of markers (e.g. restriction fragment length polymorphisms: RFLPs) that are linked to a gene or quantitative trait locus (QTL) are also extremely useful for MAS (Shan *et al*., 1999; Sanchez *et al.*, 2000; Sharp *et al*., 2001).

Marker-assisted evaluation of breeding material prior to crossing (hybridization) and line development, there are several applications in which DNA marker data may be useful for breeding, such as cultivar identity, assessment of genetic diversity and parent selection, and confirmation of

hybrids. Traditionally, these tasks have been done based on visual selection and analyzing data based on morphological characteristics.

(i) Cultivar identity/assessment of 'purity'

In practice, seed of different strains is often mixed due to the difficulties of handling large numbers of seed samples used within and between crop breeding programmes. Markers can be used to confirm the true identity of individual plants. The maintenance of high levels of genetic purity is essential in cereal hybrid production in order to exploit heterosis. In hybrid rice, SSR and STS markers were used to confirm purity, which was considerably simpler than the standard 'grow-out tests' that involve growing the plant to maturity and assessing morphological and floral characteristics (Yashitola *et al.*, 2002).

(ii) Assessment of genetic diversity and parental selection

Breeding programmes depend on a high level of genetic diversity for achieving progress from selection. Broadening the genetic base of core breeding material requires the identification of diverse strains for hybridization with elite cultivars (Xu *et al*., 2004; Reif *et al.*, 2005). Numerous studies investigating the assessment of genetic diversity within breeding material for practically all crops have been reported. DNA markers have been an indispensable tool for characterizing genetic resources and providing breeders with more detailed information to assist in selecting parents. In some cases, information regarding a specific locus (e.g. a specific resistance gene or QTL) within breeding material is highly desirable. For example, the comparison of marker haplotypes has enabled different sources of resistance to Fusarium head blight, which is a major disease of wheat worldwide, to be predicted (Liu & Anderson 2003; McCartney *et al*., 2004).

(iii) Study of heterosis

For hybrid crop production, especially in maize and sorghum, DNA markers have been used to define heterotic groups that can be used to exploit heterosis (hybrid vigour). The development of inbred lines for use in producing superior hybrids is a very time-consuming and expensive procedure. Unfortunately, it is not yet possible to predict the exact level of heterosis based on DNA marker data although there have been reports of assigning parental lines to the proper heterotic groups (Lee *et al.*, 1989; Reif *et al.*, 2003). The potential of using smaller subsets of DNA marker data in combination with phenotypic data to select heterotic hybrids has also been proposed (Jordan *et al.*, 2003).

(iv) Identification of genomic regions under selection

The identification of shifts in allele frequencies within the genome can be important information for breeders since it alerts them to monitor specific alleles or haplotypes and can be used to design appropriate breeding strategies (Steele *et al*., 2004). Other applications of the identification of genomic regions under selection are for QTL mapping: the regions under selection can be targeted for QTL analysis or used to validate previously detected marker–trait associations (Jordan *et al*., 2004). Ultimately, data on genomic regions under selection can be used for the development of new varieties with specific allele combinations using MAS schemes such as marker-assisted backcrossing or early generation selection (Ribaut *et al*., 2001; Steele *et al.*, 2004).

SUMMARY AND CONCLUSION

It's known that genetic diversity is important to identify groups of inbred lines for further utilization for heterosis. It is also useful for studying the diversity of different germplasms as possible sources of genes that can improve the performance of cultivars. Genetic diversity markers help to determine the uniqueness and distinctness of phenotypic and genetic characters. DNA-based molecular marker technologies are useful tools for genetic similarity studies. Different molecular markers have their own advantages and disadvantages. For instance, AFLP markers have the capacity to give several bands in a particular amplification. Depending on the purpose of the study and the availability of resources, SSR and SNP are still recognized as markers of choice to study the application of molecular markers in heterosis development.

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