HETEROSIS PREDICTION THROUGH MOLECULAR MARKERS

Ms. Reginah Pheirim,

Assistant Professor – School of Agriculture, Rai University, Saroda-Dholka Road, Ahmadabad

Dr. Rehana Niyaria,

Assistant Professor – Junagadh Agricultural University, Junagadh

Mr. Pradeep Kumar Singh

Assistant Professor – School of Life Science, Rai University, Saroda-Dholka Road, Ahmedabad

ABSTRACT

Heterosis has been an important method of increasing yield and improving quality in crops. Development of hybrids through the exploitation of heterosis is considered as one of the greatest breakthrough in plant breeding, as is evident from the fact that area under hybrids for several crops is rapidly increasing. However, the use of heterosis requires the selection of a large number of cross-combinations which is labor and time consuming and the results are prone to be erratic in different environments. Parameters like *per se* performance, combining ability, genetic diversity by pedigree analysis and biochemical markers were used to predict heterosis, but the prediction accuracy was low. Therefore, to increase the efficiency of hybrid breeding, various approaches for heterosis prediction without large scale field evaluation becomes more necessary.The use of molecular marker led heterosis prediction into a new phase since it appeared in 1980s, but the conclusions were not consistent. Several efforts were made to improve the power of heterosis prediction. The success of which may lead to the selection of the limited number of parental combinations for synthesizing experimental hybrids, and for the identification of highly heterotic hybrids, thus increasing the efficiency of hybrid development.

KEY WORDS: *Genetic distance, heterosis, heterosis prediction, molecular markers.*

INTRODUCTION

Heterosis is ubiquitous and is of great practical value in plant and animal breeding. The commercial exploitation of heterosis or hybrid vigor through the development and cultivation of hybrid cultivars is one of the landmark achievements in plant breeding. Ever since the two pioneering publications by George H. Shull more than century years ago, in which he scientifically described heterosis and laid the foundation of modern hybrid breeding in maize, the exploitation of heterosis in crop and tree species has greatly expanded and the acreage under hybrid cultivars has steadily increased. Thus, hybrid breeding has made commendable contributions in meeting the food, feed, and fiber needs of the burgeoning population of the world, and benefitted farmers and consumers. Several hybrids in cereals and various crops were developed by hybridization and exploitation of heterosis. The overwhelming importance of heterosis attracted many scientists to study the underlying genetic causes of this unique phenomenon.

In recent years, there have been new developments in agriculture. On one hand, intensive agriculture is adversely affecting the natural resources and the arable land available for food production is decreasing with the increasing demand of plants for industrial purposes and renewable resources of energy. On the other hand, our needs for food and feed production are increasing, because of an ever growing population and improving living standards. Further, future agriculture is expected to face serious challenges due to the impending climate changes. Consequently, serious concerns are being expressed about the food security of our planet. A viable option to meet the increasing demands is the enhancement of productivity per unit of resources on a sustainable basis. The answer for these challenges could be heterotic hybrids because heterosis is credited for large increase in production per unit area and crops from heterotic hybrids are found 10-30% better than the ordinary varieties. In this scenario, optimal exploitation of heterosis can play a key role for improvement in yield, quality, adaptability and other traits of economic importance.

Hybrid breeders have always been interested in the selection of potential lines among the available parental lines which are expected to give heterotic hybrids bypassing the field evaluation of a large number of crosses involving these parental lines. Over the years, several methods were employed to predict heterosis, such as, *per se* performance of parental lines, combining ability, genetic diversity, biochemical markers and molecular markers.

PHENOTYPIC DATA- BASED PREDICTION OF HETEROSIS

Line *per se* performance of parents:

 Several studies in different crops reported contrasting conclusions regarding the effectiveness of *per se* performance in the prediction of heterosis and was concluded that there was no association between *per se* performance of parental lines and heterosis in F1 hybrids. Several studies in different crops reported contrasting conclusions regarding the effectiveness of *per se* performance in the prediction of heterosis. Predicting the performance of hybrids from the *per se* performance of their parental inbred lines has not been effective due to masking dominance effects.

Combining ability

Combining ability is the capacity or ability of a genotype to produce superior progeny when crossed with the other genotypes. Identification of superior parental lines was generally done by employing combining ability tests such as top cross test, poly-cross test, single cross test, and diallel mating and line × tester analysis (Virmani *et al*. 1994). In general, high general combining ability (GCA) effects resulted in the development of heterotic hybrids in any crop and shows moderate to strong correlation between combining ability and *per se* performance as well as mid-parent heterosis, but in varying degrees (Rajendra kumar *et al.* 2015).

Genetic diversity:

Genetic diversity is of paramount importance for heterosis and was assessed, up till 1970, mostly by Pedigree analysis, morphological markers, and biochemical markers cytological/Physiological markers. Biometric analysis of variance in crosses, conventionally, parental lines with superior cross performance are identified by genetic divergence (D2) analysis, metroglyph analysis etc.

MOLECULAR-MARKER BASED PREDICTION OF HETEROSIS

The genetic divergence can be more efficiently and effectively studied or derived with the help of molecular markers. Molecular markers are identifiable DNA sequences, found at specific locations on the genome, and transmitted by the standard laws of inheritance from one generation to the next. These sequences may be associated with a certain trait of an organism and help to trace out gene for a particular trait.

Further use of molecular marker has helped research groups to dissect heterosis into genetic components and identification of markers and genomic regions. Currently, several molecular marker techniques are available serving various purposes in several crops. The use of molecular markers led heterosis prediction into a new phase since it appeared in 1980s (Rajendra kumar *et al.* 2015) However, the result were found inconsistent with each other. Genetic distance (GD) based on SSR data in maize (Xu *et al*. 2014), morphological and AFLP marker based in sunflower Usatov *et al. (*2014) , SSR-Based molecular marker in Cotton F1 Inter Specific Hybrids Performance for Seed Cotton Yield and Fiber Properties (Alkuddsi *et al*. 2013), was found significantly correlated with hybrid yield/yield heterosis. On the other hand, low predictive value or weak correlation were found with AFLP measured genetic distance in chilli (Krishnamurthy *et al*. 2010), RAPD Markers based estimation in pop corn (Silvia *et al*. 2012), RFLP based in Sorghum (Jordan *et al.*2003). Several other studies were made to predict the heterosis in various crops using molecular markers.

Possible Reasons of Inconsistency in the Prediction:

The possible reasons of inconsistency in the prediction of heterosis in various crops may be due to the following points listed below:

- Prediction of heterosis was based on genetic diversity of anonymous molecular markers, which may not be associated with functional regions of the genome.
- The hybrid heterosis performance is prone to be influenced by environment whereas the molecular markers are not influenced by environmental effects.
- Different experimental materials (composition of the material or crosses) used in the experiment, experimental designs, and molecular markers.
- There must be linkage between genes controlling the trait and most markers used to estimate genetic distance for high correlations to occur and it was not known if the markers used were linked to genes controlling traits.
- There is a relationship between gene expression and hybrid heterosis therefore the gene loci screened out at DNA level may not expressed at RNA level.

The above shortcomings could be overcome by the improvement of DNA marker based approaches led to a prediction accuracy that is comparable to phenotype based estimates. To further increase prediction abilities for hybrid breeding, several efforts were made to improve the power of heterosis prediction and other approaches were also made. These include:

1. Use of varied types of Molecular Marker approach

A study based on computer simulations concluded that at least 30-50% of the molecular markers should be linked to QTL while not more than 20-30% of the molecular markers should be randomly and also use of molecular markers associated with heterosis will help in the reliable prediction of heterosis (Bernerdo *et al.* 1992). Heterosis is presumed to be due to heterozygosity and hence the

selection of the appropriate type of marker is very important for assessing the genetic diversity among the parents. Co-dominant and single locus markers such as SSRs and SNPs are advantageous, since they could estimate the heterozygosity based on the parental alleles. This will also help in the identification of specific alleles associated with heterosis of various traits that can be employed in the improvement of inbred parents. Moreover, with the sequencing of crop genomes, these markers can be easily located in the physical map. In contrast, the dominant markers are not informative, since they possess information on the allele of only one parent at a particular locus. Such lack of complete allelic information will be a great setback for the parental line improvement aimed at enriching of superior alleles.

(A) Emerging Concepts In Marker Based Prediction Of Heterosis

A series of studies in rice for the past two decades resulted in the development of the concept of heterosis prediction. The concept has evolved from anonymous or random markers to specific or informative markers. Specific heterozygosity of positive markers (markers with significant effects on the target traits) showed a high correlation with heterosis for grain yield in rice. The importance of significant correlations of specific heterozygosity based on the small number of positive markers (markers associated with heterotic yield response) with hybrid performance than those with general heterozygosity in the prediction of heterosis. Favourable and unfavourable SSR alleles significantly affecting yield heterosis were identified with a possible use in hybrid rice breeding. Another study revealed that the presence of loci with positive or negative effects, and their counteraction during the prediction of heterosis. Markers associated with hybrid vigour and hybrid weakness were identified in rice and they proposed a new concept of 'key DNA markers' for the prediction of heterosis (xu *et al.* 2004) In line with this, the concept of 'effect-increasing' loci was proposed recently for the prediction of hybrid performances in *indica* rice (Renning *et al.* 2008). Comparative analysis of genomic and EST-SSR markers in rice revealed that the latter exhibited a significant positive correlation with heterosis. A set of informative markers was identified for the prediction of heterosis by a careful and critical analysis of EST-SSR markers (Jaikishan *et al.* 2012). The concept of heterosis (Rajendra kumar *et al.* 2015) is summarized in **Fig 1.**
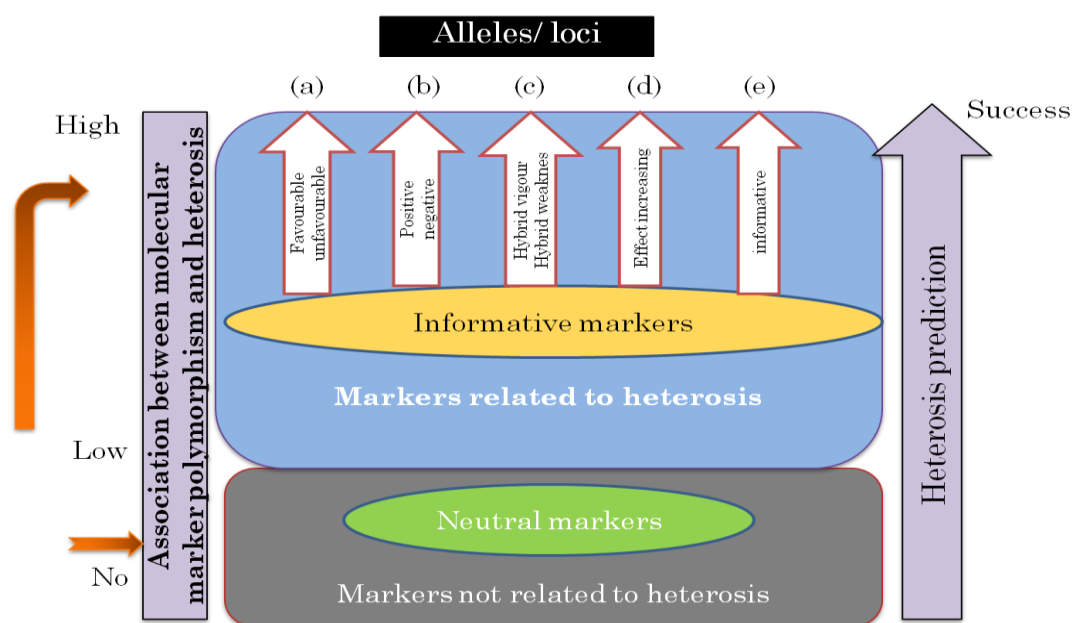
Fig 1. Emerging concepts in marker based prediction of heterosis

(B) Transcriptome and Metabolome data based Prediction Methods

Heterotic diagnostic and predictive potential may be increased by use of various molecular compounds of the plant, like DNA methylation states, transcripts, proteins and metabolites and new markers with modified levels of gene expression in hybrids.

This can be attributed to the fact that heterosis is a genome-wide phenomenon involving a network of genes and proteins leading to changes in the plant metabolism. Rapid advances in this area provide new avenues for the expression-based prediction of heterosis. Several projects investigating the phenomenon of heterosis in different crop species have contributed to the identification and characterization of differentially expressed genes (DEGs) related to heterosis. Among the various techniques applied to identify genes possibly associated with heterosis, differential display analysis, cDNA-AFLP and microarrays have been more popularly used. Analysis of the differential expression of genes at different developmental stages of hybrids and their parental lines have proven to be a useful methodology to identify the genes associated with heterosis. Recent studies on the transcriptome based prediction of heterosis showed promising results (Frisch *et al.* 2010). At present, transcriptome analysis is expensive, but a decrease in lab costs is expected and with such a cost decrease, the suggested pre-selection can increase the cost efficiency of breeding programs.

2.   TM-BLUP approach

TM Best linear unbiased prediction (BLUP) takes into account the molecular marker data together with the trait data for the prediction of hybrid performance. It predicts performance of untested single crosses using phenotypic information of related single crosses. BLUP is useful for routinely identifying superior single crosses prior to making actual crosses and evaluating them yield trials. This approach slightly improved the quality of the prediction (Bernardo 1998, 1999).

CONCLUSIONS

Molecular marker assayed genetic variation of the parental lines may be useful for predicting heterosis and this concept has been refined since it appeared in 1980s. The concept has evolved from using random markers to specific or informative markers which increased the reliability and accuracy of prediction but, there is need to improve strategies for selection of DNA molecular markers. A careful selection of molecular markers may be one that has a judicious mix of anonymous, EST/ gene-based markers and QTL-associated markers. Heterosis prediction precisely at DNA level has difficulties. Therefore besides molecular marker data, the transcriptomic and metabolic data also have the potential for the prediction of heterosis. Recent studies on transcriptome- based prediction of heterosis showed promising results. However, gene expression based approaches are expensive and require expertise therefore there is a need for development of easy, cheap, rapid assays for routine screening of hybrids and consequent heterosis prediction. The future prediction strategies should include the valuable information data from transcriptome analysis along with the molecular marker information. Further development of functional genomics and establishment of saturated genetic maps and physical maps is required. The methodology of heterosis prediction also needs improvement e.g. Exploitation of methods like TM- BLUP.

An accurate and reliable prediction of heterosis may become a reality soon with the advances in transcriptomics, proteomics and metabolomics as analysis of the differential expression of genes at different developmental stages of hybrids and their parental lines have proven to be a useful methodology to identify the genes associated with heterosis and in the case of genes with minor effect, more integrated markers such as the levels of plant metabolites, contribute a decisive part of information, since they represent the combined effect of many genes.The identification of the

complete network of genes responsible for different pathways and identification of genes associated with heterosis from the approach involving whole transcriptome analysis may also led to a better prediction of heterosis. Moreover, development of faster processors, better compilers, and more efficient computing algorithms which would decrease computing time will lead to an easier and more rapid method to predict heterosis in different crops.

Sustained research resulting in improved tools and techniques in the various fields may one day make this approach a reliable and accurate one, which would immensely improve the hybrid breeding and thus help the breeders meet the demands of future.

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