



HETEROTIC GROUPING AND ITS IMPORTANCE IN TROPICAL HYBRID RICE BREEDING

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ABSTRACT

Heterosis and heterotic patterns are important factors exploited to the most in corn breeding. Rice might be the only crop where hybrids used extensively but studies done on heterotic groupings were very limited. High yielding hybrids have been produced from crosses between *O. indica* and *O. japonica*; however, hybrids between *indica* and *japonica* rices show a variable degree of sterility. Moreover, fertility restorer frequency is lower in *japonica* rice than *indica* rice. Presently, hybrids involving *indica* x *indica* crosses are under cultivation and express as much as 70% heterosis. The three subspecies of *O. sativa* (i.e., *indica*, *japonica* and *javanica*) have different morphological and physiological characteristics and ecogeographical distribution and, therefore, may serve as distinct heterotic groups. In the earlier stage of hybrid rice development in China, two heterotic groups, that is, early season *indica* from southern China and mid or late-season *indica* from Southeast Asia were identified for three-line hybrid rice based on wild abortive (WA) male sterile cytoplasm. More heterotic groups were studied and identified for three-line hybrids derived from other male sterile cytoplasm and for two-line hybrid rice based on thermo-sensitive genic male sterility. For other types of rice hybrids, however, such as tropical *indica*, and temperate and tropical *japonica*, no clear information is available for a definition of heterotic groups. Parents of tropical *indica* hybrid rice are still categorized by fertility reaction (restorer or maintainer of male sterility). The lack of a systematic study aimed at heterotic groups could be one of main reasons for observed low yield heterosis in tropical hybrid rice resulting from the unpredictable combination of parents. Therefore there is an enormous need to work in this direction.

Keywords: Heterotic group, heterotic pattern, heterosis and hybrid rice.

Introduction:

In any breeding program, genetic diversity of the germplasm affects the potential genetic gain achievable through selection. Both cost and time wise, the most expensive phase in a hybrid breeding program would be the identification of parental lines that in turn result in superior hybrids when they are crossed. Production of hybrids works exploiting the phenomenon of heterosis. Genetic distance between parents plays an important role in determining the extent of heterosis. In general, heterosis is considered as an expression of the genetic divergence among cultivars. When heterosis or some of its components are significant for all traits, it may be concluded that there is genetic divergence among the parental cultivars. Information on the genetic diversity and genetic distance among the breeding lines, and the correlation between genetic distance and hybrid performance, are important for



determining breeding strategies, classifying the parental lines, defining heterotic groups, and predicting the future hybrids performance.

For potential exploitation of heterosis, it is important to identify the heterotic groups and patterns. The heterotic grouping importance was been advocated by many scientists since earlier times. *“The single most important element of a breeding program is the recognition and utilization of heterotic pattern. This recognition both simplifies and increases the efficiency of all subsequent operations”* (SPRAGUE, 1984).

What is heterotic group and heterotic pattern?

Lee (1995) defines a heterotic group as “a collection of germplasm which, when crossed to germplasm external to its group (usually another heterotic group), tends to exhibit a higher degree of heterosis (on average) than when crossed to a member of its own group.” Two reciprocal heterotic groups define a heterotic pattern”

Melchinger and Gumber (1998) defined a heterotic group as a group of related or unrelated genotypes from the same or different populations, which display similar combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups. By comparison, the term heterotic pattern refers to a specific pair of two heterotic groups, which express high heterosis and consequently high hybrid performance in their cross. Knowledge of the heterotic groups and patterns is help the breeders to utilize their germplasm in a more efficient and consistent manner through exploitation of complementary lines for maximizing the outcomes of a hybrid breeding program. Breeders may use heterotic group information for cataloging diversity and directing the introgression of traits and creation of new heterotic groups.

Heterotic groups initially became relevant in the United States because related inbreds were used as single-cross parents for double-cross hybrids (Hallauer, 1990).The existence of heterotic groups has been attributed to the possibility that populations of divergent backgrounds might have unique allelic diversity that could have originated from founder effects, genetic drift, or accumulation of unique diversity by mutation or selection. Interallelic interaction (over dominance) or repulsion phase linkage among loci showing dominance (pseudo-over dominance) could explain the observance of significantly greater heterosis following a cross between genetically divergent populations. Experimental evidence supports the concept of heterotic patterns. Such research has demonstrated that intergroup hybrids significantly out-yielded intragroup hybrids. In maize, one study showed that intergroup hybrids between Reid Yellow Dent x Lancaster Sure Crop out-yielded intragroup hybrids by 21%.

D. Melchinger and R.R. Gumber advocated that heterotic groups are the backbone for successful hybrid breeding, and hence a decision about them should be made at the beginning of a hybrid crop improvement program. They further



commented that once established and improved over a number of selection cycles, it is extremely difficult to develop new and competitive heterotic groups. This is because, at an advanced stage, the gap in performance between improved breeding materials and unimproved source materials is often too large. However, the chance to develop new heterotic groups could be enhanced with a change in breeding objectives. Once developed, heterotic groups should be broadened continuously by introgressing unique germplasm in order to sustain medium and long term gains through selection.

Methods for developing heterotic groups

A number of procedures have been used by breeders to establish heterotic groups and patterns. These include pedigree analysis, geographic isolation inference, measurement of heterosis, and combining ability analysis. Some have used diallel analysis to obtain preliminary information on heterotic patterns. The procedure is recommended for use with small populations. The technology of molecular markers may be used to refine existing groups and patterns or for expediting the establishment of new ones, through the determination of genetic distances.

To establish a heterotic group and pattern, breeders make crosses between or within populations. Intergroup hybrids have been shown to be superior over intragroup hybrids in establishing heterotic relationships. In practice, most of the primary heterotic groups were not developed systematically but rather by relating the observed heterosis and hybrid performance with the origin of parents included in the crosses. One of the earliest contributions to knowledge in the areas of developing heterotic patterns was made in 1922. Comparing heterosis for yield in a large number of intervarietal crosses of maize, it was discovered that hybrids between varieties of different endosperm types produced a higher performance than among varieties with the same endosperm type. This discovery, by F.D. Richey, suggested that crosses between geographically or genetically distant parents expressed higher performance and, hence, increased heterosis. This information led to the development of the most widely used heterotic pattern in the US Corn Belt the Reid Yellow Dent x Lancaster Sure Crop.

Heterotic groups and patterns in various crops

Heterotic patterns have been studied in various species. For certain crops, breeders have defined standard patterns that act as a guide in the production of hybrids. As previously indicated in maize, for example, a widely used scheme for hybrid development in temperate maize is the Reid x Lancaster heterotic pattern. These heterotic populations were discovered from pedigree and geographic analysis of inbred lines used in the Corn Belt of the United States. In Europe, a common pattern for maize is the European flint x Corn Belt Dent, identified based on endosperm types. In France, F₂ x F₆ heterotic pattern derived from the same open pollinated cultivars was reported. Other patterns include ETO-composite x Tuxpeno and Suwan 1 x Tuxpeno in tropical regions. Alternate heterotic patterns continue to be sought as per peoples requirements. For example to enhance CIMMYT's



provitamin A maize breeding efforts, a study done by Suvarno *et al.* 2014 which evaluated whether separation of experimental maize lines into groups based on maximizing their molecular-marker based genetic distances (GD) resulted in heterosis for among-group crosses, assessed genetic effects (general and specific combining ability, GCA and SCA) for grain yield and provitamin A concentrations in hybrids among 21 inbred lines representing the three proposed groups, and assessed the association between grain yield and provitamin A concentrations. They found Grain yield was not significantly correlated with provitamin A concentration, indicating that both traits could be improved simultaneously. It was observed that a small but significant yield advantage of crosses among versus within putative heterotic groups formed by maximizing GDs confirmed that molecular-marker-determined GD, while not a panacea, can provide an effective starting point for further breeding work to develop useful heterotic groups. They concluded a breeding program developing and exploiting three heterotic groups would support the objective of CIMMYT– Harvest Plus’s provitamin A biofortification breeding program to develop excellent three-way cross hybrids with high yield and provitamin A concentration.

In rice, some research suggests two heterotic groups within *O. indica*, one including strains from S.E. China and another containing strains from S.E. China and another containing strains from S.E. Asia. In rye, the two most widely used germplasm groups are the Petkus and Carsten, while in faba bean three major germplasm pools are available, namely, Minor, Major, and Mediterranean.

Even though various approaches are used for the identification of heterotic patterns, they generally follow certain principles. The first step is to assemble a large number of germplasm sources and then make parent populations of crosses from among which, the highest performing hybrids are selected as potential heterotic groups and patterns. If established heterotic patterns already exist, the performance of the putative patterns with the established ones is compared. Where the germplasm accession is too large to permit the practical use of a diallel cross, the germplasm may first be grouped based on genetic similarity i.e., based on geographical origin, morphological data, pedigree information and breeding history or molecular marker based grouping, Cluster analysis or PCA. For these groups, representatives are selected for evaluation in a diallel cross and evaluated in replicated yield trials among the subgroups along with parents as such select the most promising cross combinations.

Criteria for the identification of new heterotic groups and patterns

The choice of a heterotic group or pattern in a breeding program should be based on the following criteria:

1. High mean performance and large genetic variance in the hybrid population to ascertain future selection response by adopting the usefulness concept of Schnell (1978).



2. High per se performance and good adaptation of both or at least one of the parental heterotic groups.
3. Low inbreeding depression in the source materials for the development of inbreds and
4. A stable CMS system without deleterious side effects, as well as effective restorers and maintainers, if hybrid breeding is based on cytoplasmic male sterility.

Recently heterotic groups were been established successfully that includes Brazilian popcorn (Miranda et al. 2008), Rye (Fischer et al. 2010), Wheat (Fischer et al. 2010; Zhang et al. 2011), Pigeon Pea (Hari D. Upadyaya et al., 2014) - K, B, S, KB, KS and KBS groups and Cotton H x B (Yanal Alkuddsi et al. 2013 and Ranganatha et al. 2013).

Heterotic grouping in rice

Rice might be the only crop where hybrids used extensively but studies done on heterotic groupings were very limited. High yielding hybrids have been produced from crosses between *O. indica* and *O. japonica* (Xiao et al. 1995); however, hybrids between indica and japonica rices show a variable degree of sterility. Moreover, fertility restorer frequency is lower in japonica rice than indica rice. Presently, hybrids involving indica x indica crosses are under cultivation and express as much as 70% heterosis.

The three subspecies of *O. sativa* (i.e., indica, japonica, and javanica) have different morphological and physiological characteristics and ecogeographical distribution and, therefore, may serve as distinct heterotic groups. Genotypes belonging to indica and japonica are adapted to tropical and temperate climates, respectively, whereas javanica rice has intermediate characteristics. The average genetic distance measured by RFLPs between indica lines was three to four times higher than that between japonica lines (Zhang et al., 1992). Yuan (1992) pointed out that heterosis for grain yield in crosses among the three subspecies has the following trend: indica x japonica > indica x javanica > javanica x japonica > indica x indica > japonica x japonica. This is in harmony with the increased genetic distance between parents of indica x japonica crosses compared with indica x indica and japonica x japonica crosses found in a recent study with RAPD markers (Xiao et al., 1996). Zhang et al. (1995) studied molecular divergence and hybrid performance in diallel crosses among eight *O. indica* lines representing the parents of the best performing commercial rice hybrids grown in China. Their results suggest the existence of two heterotic groups within *O. indica*, one comprising rice strains from southeast China and the other comprising strains from Southeast Asia. In the earlier stage of hybrid rice development in China, two heterotic groups, that is, early season *indica* from southern China and mid or late-season *indica* from Southeast Asia were identified for three-line hybrid rice based on wild abortive (WA) male sterile cytoplasm (Yuan 1977). More heterotic groups were studied and identified for three-line hybrids



derived from other male sterile cytoplasm and for two-line hybrid rice based on thermo-sensitive genic male sterility (Wang and Lu 2007; Lu and Xu 2010).

NECESSITY OF HETEROTIC GROUPING IN TROPICAL RICE

For other types of rice hybrids, however, such as tropical *indica*, and *temperate and tropical japonica*, no clear information is available for a definition of heterotic groups. Parents of tropical *indica* hybrid rice are still categorized by fertility reaction (restorer or maintainer of male sterility). The lack of a systematic study aimed at heterotic groups could be one of main reasons for observed low yield heterosis in tropical hybrid rice resulting from the unpredictable combination of parents. Information on heterotic groups and patterns is a fundamental prerequisite for hybrid crop breeding; however, no such clear information is available for tropical hybrid rice breeding after more than 30 years of hybrid rice commercialization. Based on a study conducted for assessing genetic diversity using molecular markers, 18 parents representing hybrid rice populations historically developed at the International Rice Research Institute (IRRI) were selected to form diallel crosses of hybrids and were evaluated in tropical environments (Xie *et al.* 2014)

Results revealed that intergroup hybrids yielded higher, with higher yield heterosis than intra-group hybrids. Four heterotic patterns within two heterotic groups based on current IRRI B- and R-line germplasm were identified. Parents in two marker-based groups were identified with limited breeding value among current IRRI hybrid rice germplasm because of their lowest contribution to heterotic hybrids. Heterotic hybrids are significantly correlated with high-yielding parents. The efficiency of breeding heterotic hybrids could be enhanced using selected parents within identified marker-based heterotic groups. This information is useful for exploiting those widely distributed IRRI hybrid rice parents.

Association of marker-based GD and hybrid performance that the correlation of the two was too small to be used for predicting hybrid performance (Dudley *et al.* 1991; Xie 1993, Zhang *et al.* 1995; Saghai Maroof *et al.* 1997; Zhao *et al.* 2010). However, recent results showed that the association and prediction could be enhanced when parental groups are formed first by molecular markers, which may not predict the best hybrid combination, but it reveals a practical value of assigning existing and new hybrid rice germplasm into heterotic groups and increasing opportunities to develop desirable hybrids from the best heterotic groups, which is consistent with a previous study in maize (Lanza *et al.* 1997).

The following general conclusions were being drawn from the previous studies related to heterotic groups in hybrid rice (Yuan 1977; Liu *et al.* 2002; Wang and Lu 2006a, b; Wang and Lu 2007; Xu *et al.* 2002)



- (1) Two major heterotic groups in *indica* WA three-line hybrid rice, i.e., early season *indica varieties* from central and southern China and *indica varieties* from Southeast Asia, mostly from IRRI;
- (2) Two major groups as represented by Xie Qinzhao and Zhenshan97 in the female pool, and two major groups as represented by IR24 and IR26, and some other breeding lines with IRRI variety ancestors, such as Ming Hui 63 (an IR30 offspring) in the male pool; and
- (3) R-lines are more divergent than B-lines (in China). However, a previous study showed that all WA R-lines used in China could be considered as one single group because they shared many ancestors and clustered closely together (Xie et al. 2012) with similar heterotic response to WA-based female parents. The study conducted by Xie *et al.* 2014 also shows that IRRI-bred B-lines are more divergent genetically than the R-lines.

The following observations were being made for the tropical hybrid rice parents generated from IRRI, that two heterotic groups could be classified based on current B and R-line germplasm categories, as represented by parents in G5 and G6 as females and parents in G2 and G3 as males. The parents with low or no possibility of producing heterotic hybrids, such as those in the G1 and G4 groups, have limited value for developing heterotic hybrids and they have to be further improved to combine with germplasm from other possible heterotic pools or to be changed to diverge from current R-line pools. G5 and G6 B-line groups combined with G2 and G3 R-line groups are their preferred choices of hybrids for current IRRI-bred hybrid rice germplasm. On average, the hybrids derived from these four crossing patterns produced 27.4, 11.9, and 14.9 % of mid parent, better parent and standard heterosis respectively. They also tracked the pedigrees of all 11 IRRI bred hybrids released in the Philippines for commercial production, and found that 6 parents were from G2 and G3 (36 %), 10 parents were from G5 and G6 (59 %), and Only 1 parent was from G1, but none from G4.

Conclusion

It should be noted that the core set of parents from the study conducted by Xie *et al.* 2014 as advocated may generally be fit to tropical Asian environments based on current IRRI hybrid rice parents. Further heterotic groups could be changed or enhanced when new parents with new traits/germplasm are integrated and adapted to the targeted cropping region as shown in case of provitamin A maize breeding by Suvarno *et al.* 2014. We may see such kind of trait related heterotic grouping may be for Fe and Zn, may be for quality or so also in rice. Heterotic grouping can fasten the breeding and hybrid production based on the preferences. So, there is a great need for more work in this area of rice in general and tropical hybrid rice in particular to exploit the full potential of heterosis.



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