

Seminar report

Heterotic grouping

By

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(2018-11-053)

Presented on 21/11/2019

Submitted in partial fulfilment of the requirement for course

GP 591: Master's Seminar (0+1)



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DECLARATION

I, Minnu Thomas (2018-11-053) hereby declare that the seminar entitled '**Heterotic grouping**' has been prepared by me, after going through various references cited at the end and has not copied from seminar reports of any seniors, juniors or fellow students.

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25/01/2020

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CERTIFICATE

This is to certify that the seminar report entitled '**Heterotic grouping**' has been solely prepared by Minnu Thomas (2018-11-053) under my guidance and has not been copied from fellow students.

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CONTENTS

Sl. No.	Title	Page No.
1	Introduction	9
2	Heterosis	9
2.1	The phenomena of heterosis	10
2.2	Exploitation of heterosis	10
3	Heterotic group	11
3.1	Heterotic grouping	11
3.2	Heterotic pattern	11
3.3	Importance of maintaining Heterotic Groups	12
3.4	Objectives of heterotic groups and heterotic patterns development	12
4	Experimental arguments supporting the concept of heterotic grouping	12-15
5	Methods to develop heterotic groups	16
5.1	Geographical isolation inference	16-17
5.2	Pedigree analysis	17
5.3	Quantitative genetic analysis	18
5.4	Use of molecular markers	18-19
6	Strategies for the establishment of Heterotic groups	19-21
7	Criteria for the identification of new heterotic patterns	21
7.1	Criteria by Melchinger and Gumber (1998)	21
7.2	Criteria by Cress (1967)	21-22

7.3	Case 1: Small Number of Populations	22
7.4	Case 2 : Large Number of Germplasm Accessions without heterotic patterns	22-23
8	Steps involved in the development of heterotic groups	23
9	Achievements	24
10	HyBFrame: A new approach	24
10.1	Proposed framework	25
10.1.2	Steps	25-28
11	Case study	28
11.1	Materials and methods	28-29
11.2	Results	30
12	Limitations of heterotic grouping	31
13	Future prospects	31
14	Coclusion	31-32
15	Discussion	32-33
16	References	34-37
17	Abstract	38-39

List of tables

Table No.	Title	Page No.
1	Yield advantage of inter group crosses over intra group crosses	15
2	Pedigree data of Chinese rice accessions	18
3	Heterotic groups of different crops	24

List of figures

Fig No.	Title	Page No.
1	Heterosis in cob size	10
2	Graphical representation	10
3	U.S maize	13
4	European maize	14
5	Comparison of inter and intra population crosses	15
6	Heterotic groups of maize in China	17
7	Available germplasm	19
8	Cress strategy	20
9	Melchinger and Gumber strategy	20

10	Schematic visualization of the proposed unified framework for hybrid wheat breeding and the establishment of heterotic groups.	25
11	Clustering of 147 hybrid parents into 8 groups using SSRs, and selection of representative 17 parents from different marker 3based groups	29
12	Heterotic pools in hybrid parents of pearl millet	30

1. Introduction

Heterosis breeding promises to boost yield and stability. Hybrid breeding is superior to line breeding with respect to exploitation of heterosis resulting in higher grain yield and an enhanced yield stability (Xu *et al.*, 2014). Production of hybrids works exploiting the phenomenon of heterosis. Both cost and time wise, the most expensive phase in hybrid breeding programme would be the identification of parental lines that in turn results in superior hybrids when they are crossed. According to Reif *et al.*, 2007, for an efficient hybrid breeding programme, it is desirable to organize the germplasm into heterotic groups.

The genetic basis of heterosis given as $HF1 = dy^2$ highlights the importance of enhancing genetic diversity for maximizing the heterosis. Mere increase in genetic diversity doesn't increase the heterosis, the combining ability of the lines has to be routinely checked. So development of hybrid oriented heterotic populations (genetically diverse populations) and application of schemes for improving combining ability is an integral part of hybrid breeding. Good heterotic group classification method can be defined as one which allow inter-heterotic group crosses to produce more superior hybrids than the within- group crosses.

2. Heterosis

Heterosis is a natural phenomenon whereby hybrid offspring from genetically diverse individuals show increased vigor relative to their parents. Heterosis has been increasingly applied in crop production for nearly a century, with the aim of developing more vigorous, higher yielding and better performing cultivars.

Shull (1952) defined heterosis as the increased vigour, speed of development, resistance to disease and insect pests or to climatic rigours of any kind, manifested by crossbred organisms as compared with corresponding inbreds as the specific result of unlikeness in the constitutions of the uniting parental gametes.

2.1 The phenomena of heterosis

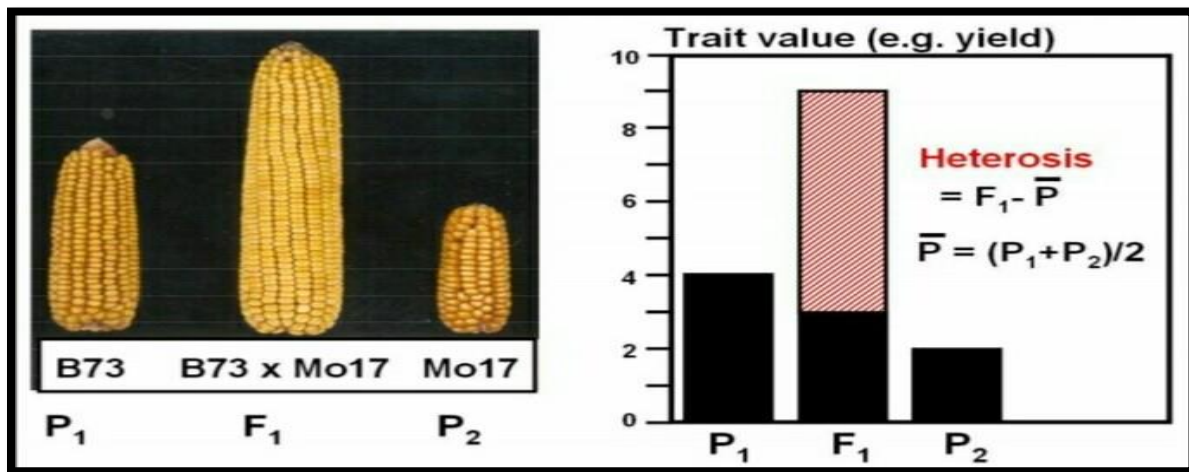


Fig 1: Heterosis in cob size

Fig 2 : Graphical representation

The figure 1 shows a clear cut improvement of hybrid in terms of cob size when crossed between lines B73 and Mo17. From the figure 2, we could explain heterosis as the deviation of F1 hybrid from the mean of two homozygous parental lines.

2.2 Exploitation of heterosis

Schnell (1992) pointed out that heterosis is a major yield factor in all breeding categories except line breeding; however, only in hybrid and clone breeding it is possible to have maximum exploitation of heterosis.

High heterosis can be expected from a hybrid if the source populations have:

- (i) A high frequency of partially or completely dominant genes
- (ii) Maximum difference in gene frequencies for overdominant loci.

Consequently, for optimum exploitation of heterosis in hybrid breeding, the seed and pollen parents should be derived from genetically unrelated germplasm pools, commonly referred to as heterotic groups.

3. Heterotic group

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 (Melchinger and Gumber,1998).

The term heterotic group can be easily understood by taking an example; consider three lines A,B and C.

- $A \times B \rightarrow$ Heterosis
- $A \times C \rightarrow$ Heterosis
- $B \times C \rightarrow$ No Heterosis

Now, we could say that A belongs to one heterotic group and the related B and C belongs to another heterotic group

3.1 Heterotic grouping

Heterotic grouping is defined as Identifying germplasm groups that are genetically distinct from each other and that produce superior hybrids when crossed

3.2 Heterotic pattern

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. The concept of heterotic patterns includes the subdivision of the germplasm available in a hybrid breeding program in at least two divergent populations, which are improved with inter-population selection methods. Heterotic patterns have a strong impact in crop improvement because they predetermine to a large extent the type of germplasm used in a hybrid breeding program over a long period of time.

Heterotic pattern is a key factor for utilizing germplasm to maximize performance of the population crosses and derived hybrids. The development of successful maize (*Zea mays* L.) hybrids requires establishment of heterotic patterns, defined as the cross between known genotypes that expresses a high level of heterosis.

3.3. Importance of maintaining Heterotic Groups

- Developing parental lines that are unrelated by descent
- Increased allelic diversity among heterotic groups
- Increased degree of heterozygosity in the resulting hybrids
- Sustainable hybrid breeding

Heterotic grouping leads to a reduced specific combining ability (SCA) variance and a lower ratio of SCA variance to general combining ability (GCA) variance. Thus early testing becomes more effective and superior hybrids can be identified and selected mainly based on their prediction from GCA effects. Assigning lines to heterotic groups would avoid the development and evaluation of crosses that should be discarded, allowing maximum heterosis to be exploited by crossing inbred lines belonging to different heterotic groups.

Heterotic grouping help in effective management of available germplasm (Melchinger and Gumber, 1998). Reciprocal recurrent selection helps to improve the combining ability of the inbred line and simultaneously increases the genetic diversity between the inbreds of the opposite populations .

3.4. Objectives of heterotic groups and heterotic patterns development

- To get higher mean heterosis and hybrid performance
- To reduce the specific combining ability (SCA) variance and SCA to GCA variance
- To avoid the development and evaluation of crosses that should be discarded
- To save the time of hybrid development

4. Experimental arguments supporting the concept of heterotic grouping

The superiority of inter-group over intra-group crosses in terms of mean performance and heterosis or heterotic deviation for grain yield and other heterotic traits has been already studied. In U.S maize (Fig 3), with genetically balanced sets of crosses, inter-group hybrids out yielded the respective intragroup hybrids by 21 % in Reid Yellow Dent (RYD) x Lancaster Sure Crop (LSC) crosses (Dudley *et al.*, 1991) and by 16% in Flint x Dent crosses of European maize (Fig 4) (Dhillon *et al.*, 1993). In both studies, the percentage of increase in heterosis for yield of inter-group over intra-group crosses was about twice as large as for hybrid yield itself.

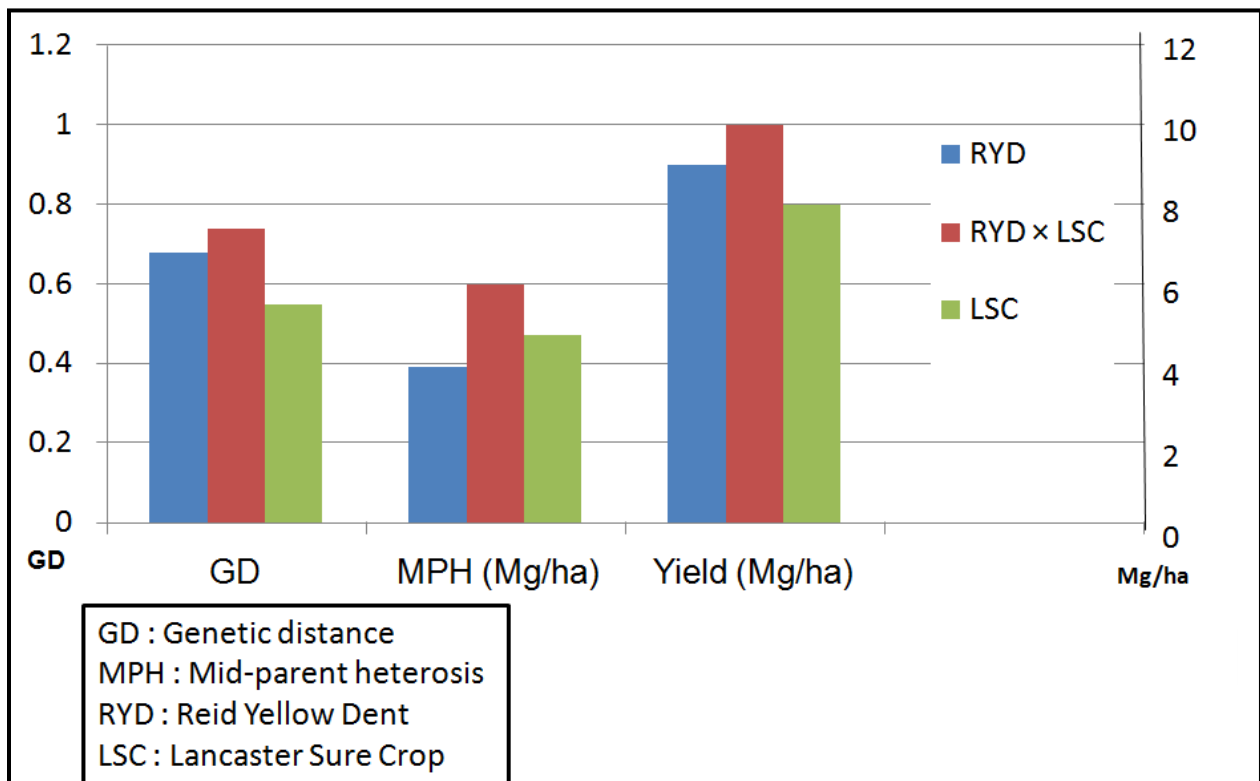


Fig 3: U.S maize

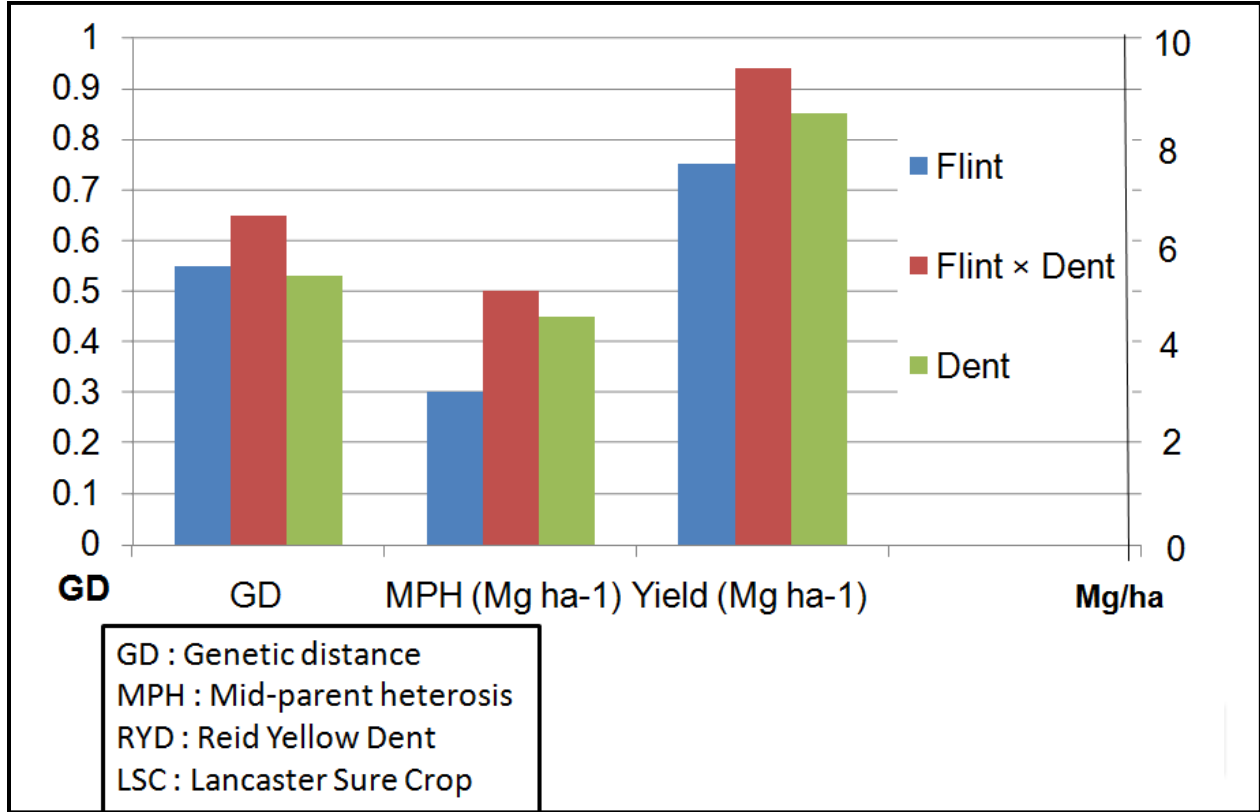


Fig 4: European maize

In two studies with maize germplasm from the U.S. Com Belt, inter-population crosses out yielded intra-population crosses by 16 to 24% with an average positive heterotic deviation of about 20% (Mungoma & Pollak, 1988).

In rye, crosses among populations from different germplasm pools such as Petkus and Carsten had on average 11 % higher yield than intra-pool population crosses (Grant & Beversdorf, 1985). In spring oilseed rape, two inter-population crosses between open-pollinated cultivars of Canadian and European origin outyielded their parent populations by 50%, the average heterotic deviation across all inter-population crosses amounted to 16% (Grant & Beversdorf, 1985).

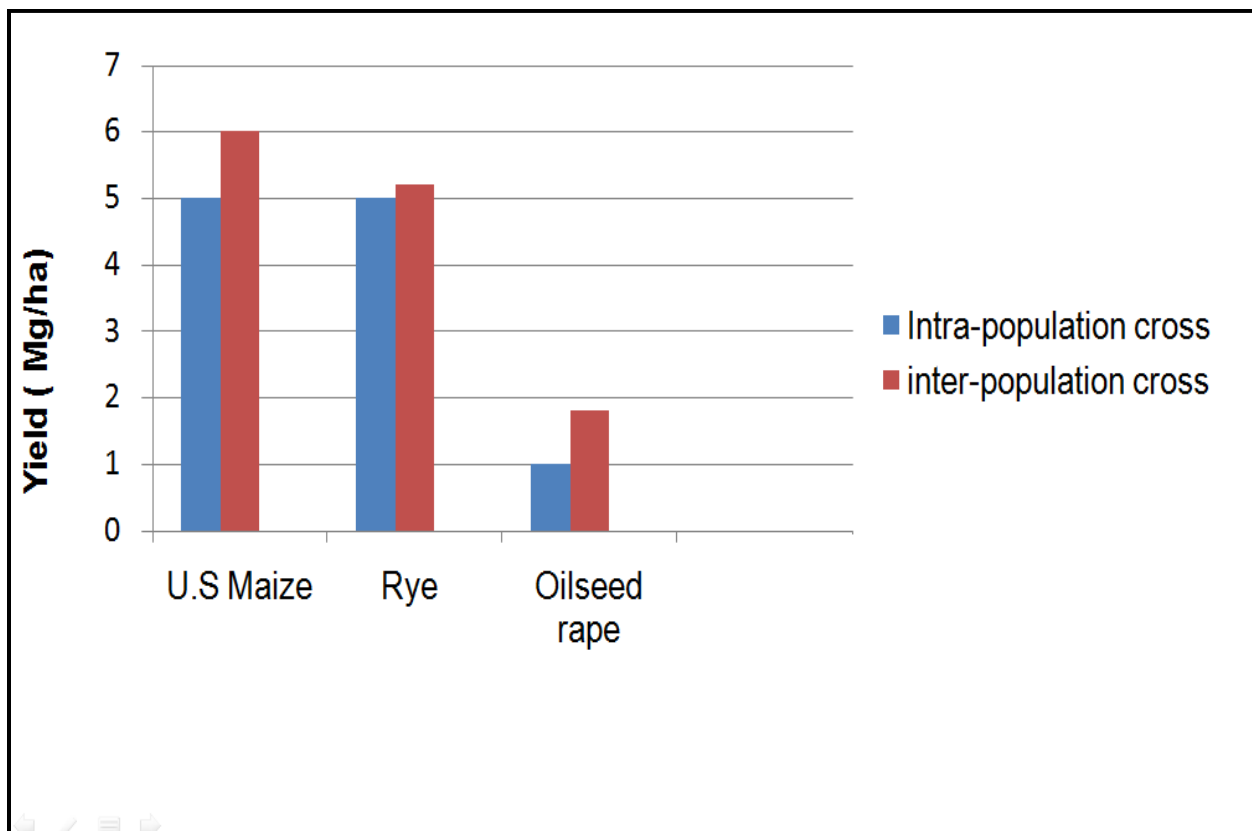


Fig 5: Comparison of inter and intra population crosses

Crop	Inter group crosses	Mean yield advantage over intra group crosses	Reference
Rye	Petkus × Carsten	11 %	Grant and Beversdorf, 1985
Oilseed	Canadian × European	16.3 %	Grant and Beversdorf, 1985
Maize	1. Reid Yellow Dent × Lancaster Sure Crop	21 %	Dudley <i>et al.</i> , 1991
	2. Flint × Dent	16 %	Dhilon <i>et al.</i> , 1993

Table 1: Yield advantage of inter group crosses over intra group crosses

5. Methods to develop heterotic groups

A number of procedures have been used by breeders to establish heterotic groups and patterns. These include pedigree analysis, geographic isolation inference, quantitative genetic analysis and use of molecular markers. Some have used diallel analysis to obtain preliminary information on heterotic patterns. This procedure is used for 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.

The most exploited heterotic pattern is the cross between Iowa Stiff Stalk Synthetic (BSSS) and Lancaster Sure Crop heterotic groups. Crosses among inbred lines that derive from unrelated heterotic groups are known to have better grain yield performance than those crosses among lines belonging to the same group.

5.1 Geographical isolation inference

Geographical isolation results in the formation of genetically diverse genotypes. Selection of lines from geographically distant origins results in harnessing higher heterosis, The geographical origin of the two populations contributed to the high grain yield (Reif et al., 2007).

Heterotic rice hybrids are generally derived from distant parents by geographic origin or different ecotypes (Wang *et al.*, 2018). 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 (Meena *et al.*, 2017).



Fig 6: Heterotic groups of maize in China (Yifeng *et al.*, 2019)

5.2 Pedigree analysis

Pedigree analysis shows the occurrence and appearance of phenotypes of a particular gene or organism and its ancestors from one generation to the next. So the pedigree data helps to avoid the repetition of parents.

The heterotic pattern increases the efficiency of hybrid development, inbred recycling and population improvement. The Reid and Lancaster groups were identified based upon pedigree and geography analysis of inbred lines used in the Corn Belt. Wu (1983) attempted to classify inbred lines into 4 or 5 groups based on pedigree analysis and to predict heterotic patterns used in China.

Wang *et al* (2018) classified rice accessions into four heterotic groups (G1, G2, G3 and G4) based on pedigree data.

Rice Accession	Pedigree	Group
Tianfeng B	Bo B / Zhe 9428	G1
Taifeng B	Bo B/ G9248	G1
IR 79632-21-2-2-1	IR79532-21-2-2--1	G2
IR 68058-64-1-2	IR68058-64-1-2	G2
Minghui 70	IR54/ Minghui 63	G3
Shuhui 881	R 6323 / japonica	G4
Shuhui 527	1318 / 88-R3360	G4

Table 2: Pedigree data of Chinese rice accessions

5.3 Quantitative genetic analysis

Melchinger (1999) reviewed different approaches to classify and identify heterotic groups. Diallels or factorial designs have been used when the number of populations or groups was small in tropical (Vasal *et al.*, 1999) and temperate corn (Ordas, 1991). Development of hybrid oriented heterotic populations and application of schemes for improving combining ability is an integral part of hybrid breeding in maize and other cross pollinated crops.

Basis of grouping the germplasms into different heterotic groups was specific combining ability (SCA) effects for grain yield (Gurung *et al.*, 2009). Cluster analysis based on SCA can be used to classify inbred lines into heterotic groups. Fourteen maize inbred lines, used in maize breeding programs in Iran, were crossed in a diallel mating design for investigation of combining ability of genotypes for grain yield and to determine heterotic patterns among germplasm sources, using both, the Griffing's method and the biplot approach for diallel analysis (Bidhendi *et al.*, 2012).

5.4 Use of molecular markers

Diverse datasets have been used to analyze genetic diversity in crop plants. Molecular marker data provide a more reliable differentiation of genotypes (Mohammadi and Prasanna 2003), since these data are less affected by environmental effects. Molecular marker data classified a set of germplasm based on genetic similarities, however Melchinger and Gumber (1998) emphasized that it has been challenging to predict heterotic relationships based on these data. Additionally, researchers agreed that field experiments are still needed to validate groupings of germplasm based on molecular marker data (Carena, 2006).

Genotyping and cluster analysis of extracted genotypic DNA from the mutants and respective parents from their young leaves (1 to 2 weeks after seed germination), using the Cetyltrimethyl ammonium bromide (CTAB) method. These genotypes were further genotyped using twenty one Simple Sequence Repeats (SSR) markers on GenBank data base (Yu *et al.*, 2000). Genetic diversity studies determine the variation among individuals or groups of individuals using a specific method or combination of methods to analyze multivariate datasets (Mohammadi and Prasanna, 2003).

We concluded that the relationships between the populations obtained by SSR analyses are in excellent agreement with pedigree information. SSR markers are a valuable complementation to field trials for identifying heterotic groups and can be used to introgress exotic germplasm systematically (Aguiar *et al.*, 2008).

6. Strategies for the establishment of Heterotic groups

Two well-known strategy were available for the establishment of heterotic groups. The first one by Cress in 1967 (Cress strategy) and another one by Melchingner and Gumber in 1998 (Melchingner and Gumber Strategy).

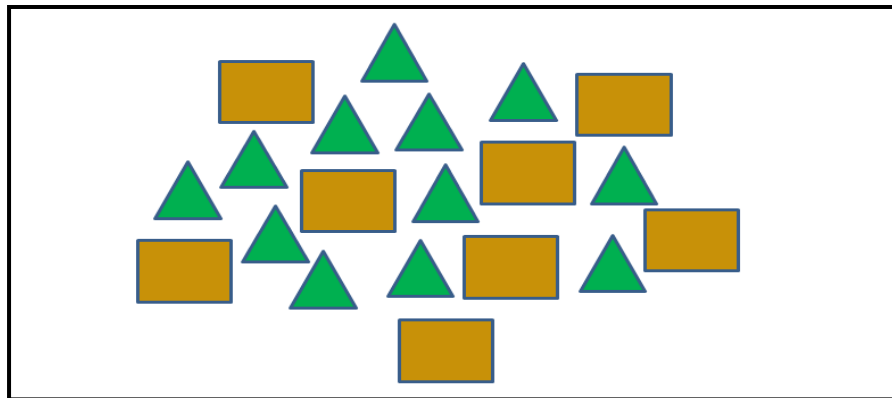


Fig 7: Available germplasm

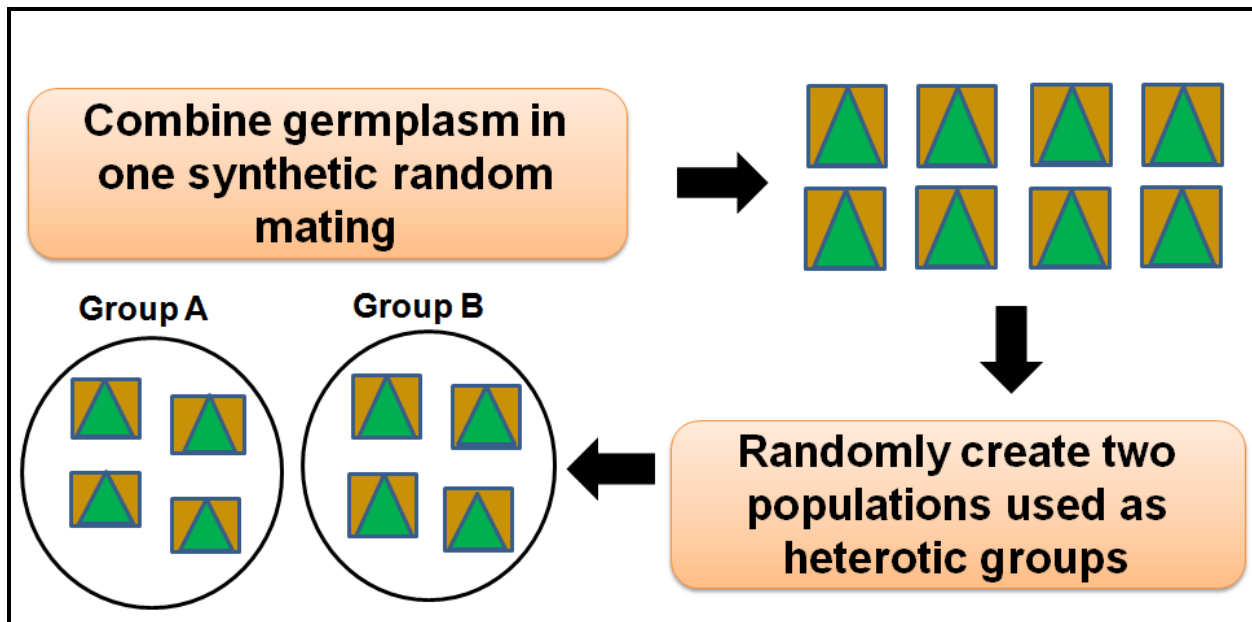


Fig 8: Cress strategy

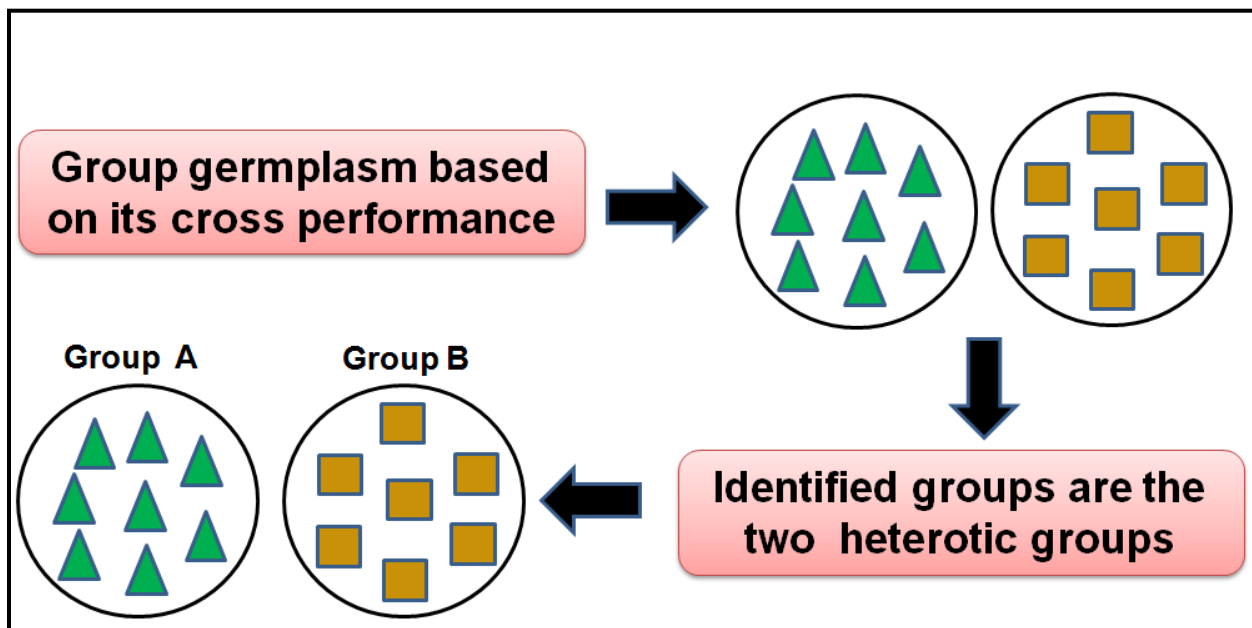


Fig 9: Melchinger and Gumber strategy

The decision which of both strategies is superior depends on several factors such as:

- (i) The genetic basis of heterosis
- (ii) The applied selection intensities for QTL
- (iii) The importance of favorable linkages.

Further research is required incorporating recent advances on the genetic architecture of quantitative traits and on the genetic basis of heterosis to develop optimal procedures for establishing and maintaining heterotic patterns.

7. Criteria for the identification of new heterotic patterns

The fundamental principle underlying the criteria for the identification of heterotic patterns is to select, from a large number of germplasm sources, parent populations of crosses with highest hybrid performance. Diallel and testcrosses are generally used for this purpose. The various steps involved in the identification of heterotic groups and patterns depend on the source materials and the availability of established heterotic patterns..

7.1 Criteria by Melchinger and Gumber (1998):

- (i) High mean performance and large genetic variance in the hybrid population
- (ii) High per se performance and good adaptation of the parent populations to the target environment
- (iii) Low inbreeding depression, if hybrids are produced from inbreds.

In practice, the choice of heterotic patterns is mainly based on the performance of the corresponding hybrid population.

7.2 Criteria by Cress (1967):

Based on results of a simulation study, that all genetic material entered into a long-term program of inter-population selection should be combined into one synthetic population. Any

subsequent populations required would be obtained by sampling this synthetic. However, the results reported by Cress (1967) were based on a rather simple genetic model assuming

- (i) A low number of quantitative trait loci (QTL)
- (ii) Absence of linkage between the QTL
- (iii) Two alleles per QTL
- (iv) No epistasis.

Cress strategy involves random mating and mixing of genotypes, so it is not followed for developing heterotic groups and patterns. In principle, these approaches could also be used for enlarging the genetic base of existing heterotic groups:

7.3 Case 1: Small Number of Populations

In this case, produce a complete diallel with a small number of populations. The diallel crosses together with their parent populations are evaluated in replicated field trials for hybrid performance and heterotic deviation. Using the identification criteria given above, parent populations of cross combinations with high performance are selected as potential heterotic groups and patterns.

If well established heterotic patterns are available, their performance is compared with the new heterotic patterns. New heterotic patterns in the U.S. Corn Belt such as Midland x Leaming and Midland x LSC or BSSS(R) CIO x Mexican Dent were identified by this approach. Similarly, in rye and in oilseed rape identified promising heterotic patterns using diallel crosses of open-pollinated populations.

7.4 Case 2 : Large Number of Germplasm Accessions without heterotic patterns

With large number of inbred lines or populations available, it is not feasible in most crops to make diallel crosses and produce sufficient F₁ seed for multi-environment field testing. Therefore, we suggest a multi-stage procedure to identify heterotic groups, which consists of the following steps:

1. Grouping the germplasm based on genetic similarity

2. Selection of representatives genotypes (e.g., two to four lines or one population) from each subgroup for producing diallel crosses
3. Evaluation of diallel crosses among the subgroups together with the parents in replicated field trials
4. Selection of the most promising cross combinations as potential heterotic patterns using the identification criteria.

A preliminary classification of germplasm into subgroups of genetically similar lines could be based on the geographic origin, morphological data, pedigree information and breeding history of the crop. An extremely Powerful tool for grouping of germplasm are molecular markers such as RFLPs, AFLPs, and RAPDs (Melchinger, 1999). Using genetic similarities determined from molecular data, relationships between genotypes can be represented graphically by cluster analysis or principal coordinate analysis.

8. Steps involved in the development of heterotic groups

- Phenotypic characterization and diversity studies
- Studies on plant types and physiological process
- Identifying ideal plant types for high productivity
- Studies on hybrid performance
- Development of heterotic groups
- Exploitation and validation of heterotic groups
- Broad based population of each heterotic group

9. Achievements

Crop	Heterotic groups	Reference
Maize	Lancaster, Reid, SPT and P (introduced from Pioneer hybrids)	Wang <i>et al.</i> , 2008
Rapeseed	Asian and European winter-type Canadian and European spring type	Qian <i>et al.</i> , 2009
Rice	<i>Indica</i> varieties of southeast china and southeast Asia	Yifeng <i>et al.</i> , 2019
Cotton	Compact, robust, RGR and stay green	Patil <i>et al.</i> , 2011

Table 3: Heterotic groups of different crops

10. HyBFrame: A new approach

Hybrid wheat breeding has great potential to increase the global wheat grain yield level particularly in view of the increasing abiotic and biotic stress challenges as well as variable climatic conditions. For the long-term success of hybrid wheat breeding and the maximum exploitation of heterosis, high-yielding heterotic patterns must be established. Philip *et al* (2016) proposed a unified framework for hybrid breeding and the establishment of heterotic groups in autogamous crops and exemplified it for hybrid wheat breeding in Germany.

10.1 Proposed framework

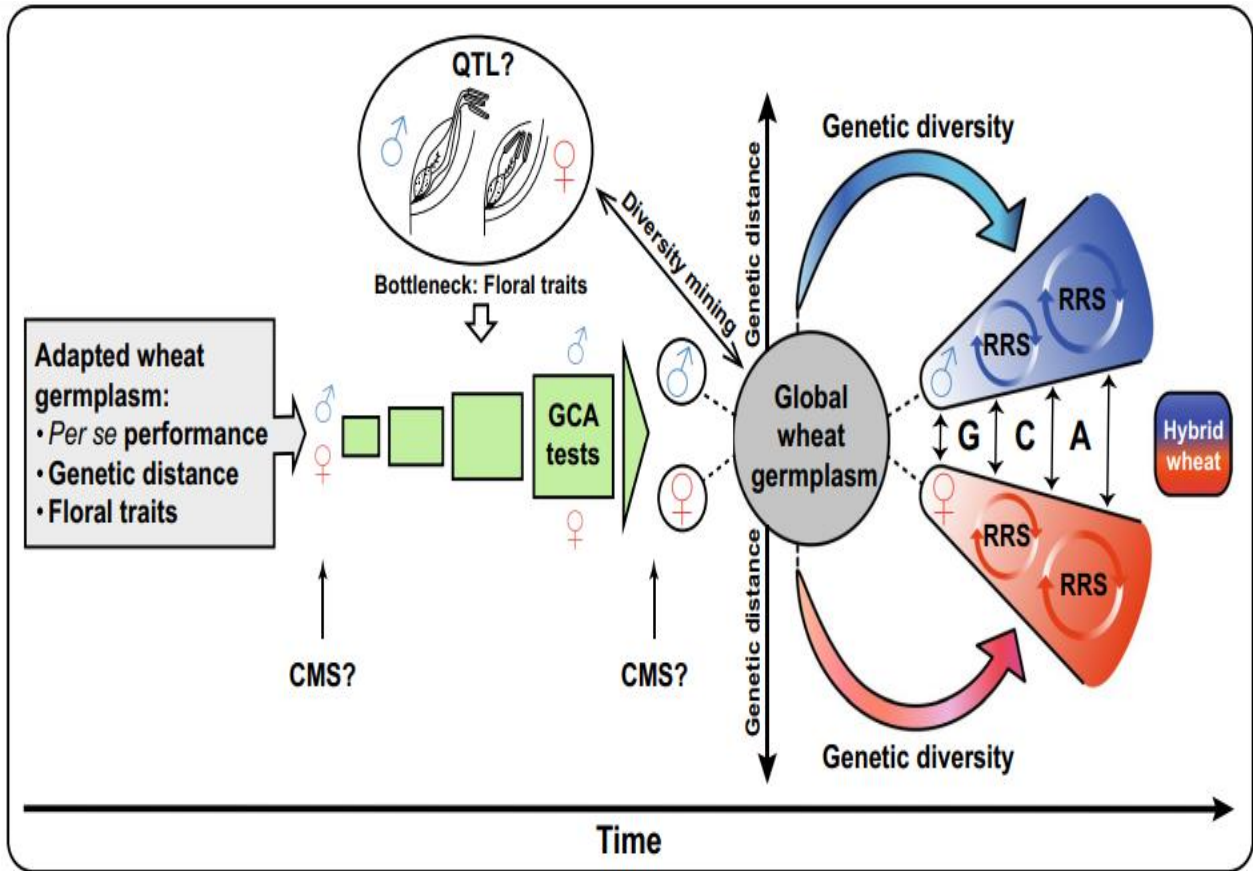


Fig 10: Schematic visualization of the proposed unified framework for hybrid wheat breeding and the establishment of heterotic groups.

CMS (cytoplasmic male sterility), GCA (general combining ability), QTL (quantitative trait loci), RRS (reciprocal recurrent selection)

10.1.2 Steps

(1) Adapted germplasm serves as starting material and due to the moderate to high correlations between line per se performance and general combining ability (GCA) observed in wheat (Longin *et al.*, 2013), high-yielding elite lines are selected. These lines are molecularly characterized to ensure a high level of genetic diversity in the initially selected lines.

(2) The floral characteristics display a bottleneck for hybrid breeding in autogamous crops and hence, a prerequisite for efficient hybrid seed production is the redesign of floral characteristics to enforce outcrossing. Consequently, male and female lines allowing a good cross-pollination must be identified (Whitford *et al.*, 2013).

(3) These lines are next tested for their GCA in factorial crosses.

(4) Lines displaying a high GCA can then serve as starting material for the development of the male and female pools.

(5) In wheat gametocides can be employed for hybrid seed production but in case a cytoplasmic male sterility (CMS) system is used, restorer loci must be introgressed into the male pool and females introgressed into the respective cytoplasm (Kempe and Gils, 2011).

(6) Next, the combining ability of the lines in both pools is improved by reciprocal recurrent selection, which over time will also result in a divergence of the two groups.

(7) To support this process but also to improve intra-group genetic diversity, the pools are not closed but rather new germplasm should be continuously added. It is crucial, however, that this process of introgressing new diversity does not disrupt the established heterotic pattern and the genetic distance between both groups. Therefore, before new material is allowed to enter the male or female pool, the lines should be molecularly characterized to assess their genetic distance to the two groups and to allow the genomics-based prediction of their GCA or the hybrid performance (Seifert *et al.*, 2016).

Lines with genetic similarity to either of the two groups and a predicted high GCA with the respective opposite group are identified as promising candidates. These candidate lines can, however, in many cases not be directly evaluated for their GCA in field trials, as they lack in adaptation as well as traits required for hybrid production. Thus, these candidate lines are first crossed with adapted elite lines from their pool, the progeny selected in the field for all relevant traits and subsequently for predicted GCA, before testcrosses are made. The selection of testers which optimally represent the respective groups is thereby of utmost importance (Acquaah 2012).

(8) Genotypes with a high observed GCA can then enter their group and be crossed with additional lines, if required with a further selection of the progeny for floral characteristics, for CMS-related

traits, and adaptation traits. Theoretically, the analysis of genetic distance followed by GCA tests can also result in the identification of additional heterotic patterns, i.e. pre-existing groups of lines showing high hybrid performance with one or even both established groups. Thus, different genetic patterns might be established for different target regions.

The proposed HyBFrame approach illustrates the importance of genetic diversity analyses for hybrid breeding in general and for the establishment, maintenance and improvement of heterotic groups in wheat. For wheat, the initial steps of the framework outlined above have already been made and are currently ongoing. The redesign of the wheat flower appears possible owing to the observed variation and the next step will be the identification of QTL underlying these traits towards their markerassisted improvement. Following the initial GCA tests and the establishment of a male and female pool, reciprocal recurrent selection is employed to improve the pools, particularly with regard to their combining ability.

Zhao *et al.*, (2016) recently suggested that a group size of only 16 individuals guarantees a long-term selection gain for grain yield in wheat. Given this seemingly low number of individuals is purposefully selected, there is indeed an almost infinite number of possible allele combinations that will take very long to exhaust. However, apart from targeted introgressions of major resistance genes, the introgression of new genetic diversity appears required in hybrid wheat breeding.

Zhao *et al.* (2016) focused mainly on grain yield, but in wheat many traits have to be combined in the final hybrid. In addition to quality characteristics, adaptation traits and abiotic stress will likely gain in importance illustrating the need to continuously introgress new variability into the pools. This process is of additional importance, as it can expedite the genetic divergence between the initially established male and female pools. Thus, new germplasm should be identified that can be fed into the two pools, however under the strict condition of not disrupting the established heterotic pattern and coevolved gene complexes.

As not all genotypes can be tested for their GCA with both groups, a preselection must be done. One approach to preselect genotypes is to predict the hybrid performance based on genome-wide molecular markers. This approach has recently been shown to yield high prediction accuracies for rice (Xu *et al.*, 2014) and wheat (Zhao *et al.*, 2016). For genomic approaches, however, knowledge on hybrid performance of at least a subset of lines must be already available. In contrast, no information on hybrid performance is required for the second approach, where the

preselection of lines is based on genetic diversity. Thus, a consequent next step for the establishment of hybrid wheat is the analysis of the genetic diversity in a large germplasm set.

11. Case study

Formation of heterotic pools and understanding relationship between molecular divergence and heterosis in pearl millet [*Pennisetum glaucum* (L.) R. Br.]

-(Singh and Gupta, 2019)

Pearl millet breeding program at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) India and other programs in the public and private sector in India are continuously engaged in enhancing genetic diversity of hybrid parents utilizing a significant amount of breeding material of African and Asian origin.

To continue the momentum of genetic gains in this crop, information on the heterotic pool patterns in existing germplasm pool of hybrid parents and on the heterosis prediction are needed. Several concepts have been proposed to increase heterosis further; one of them is to increase heterozygosity by crossing genetically distinct parental materials, i.e. materials belonging to the distinct heterotic pools. To achieve this, available germplasm needs to be organized into heterotic pools to increase the efficiency of any hybrid breeding program.

11.1 Materials and methods

- 147 pearl millet hybrid parents
- 56 SSR markers for genotyping
- Genetic distance (GD) was estimated based on the simple matching allele frequency
- Cluster diagram was developed for all the 147 hybrid parents using DARwin-5.0 software
- A trial comprising of 136 hybrids, 17 parents and four standard hybrid checks (HHB 67 Improved, HHB 226, Pioneer 86M88 and Pro Agro 9444) was evaluated during rainy season (July to October) of 2015 at two locations, CCS HAU-Hisar and ICRISAT-Patancheru in alpha lattice design with two replications
- Statistical and molecular analysis were done

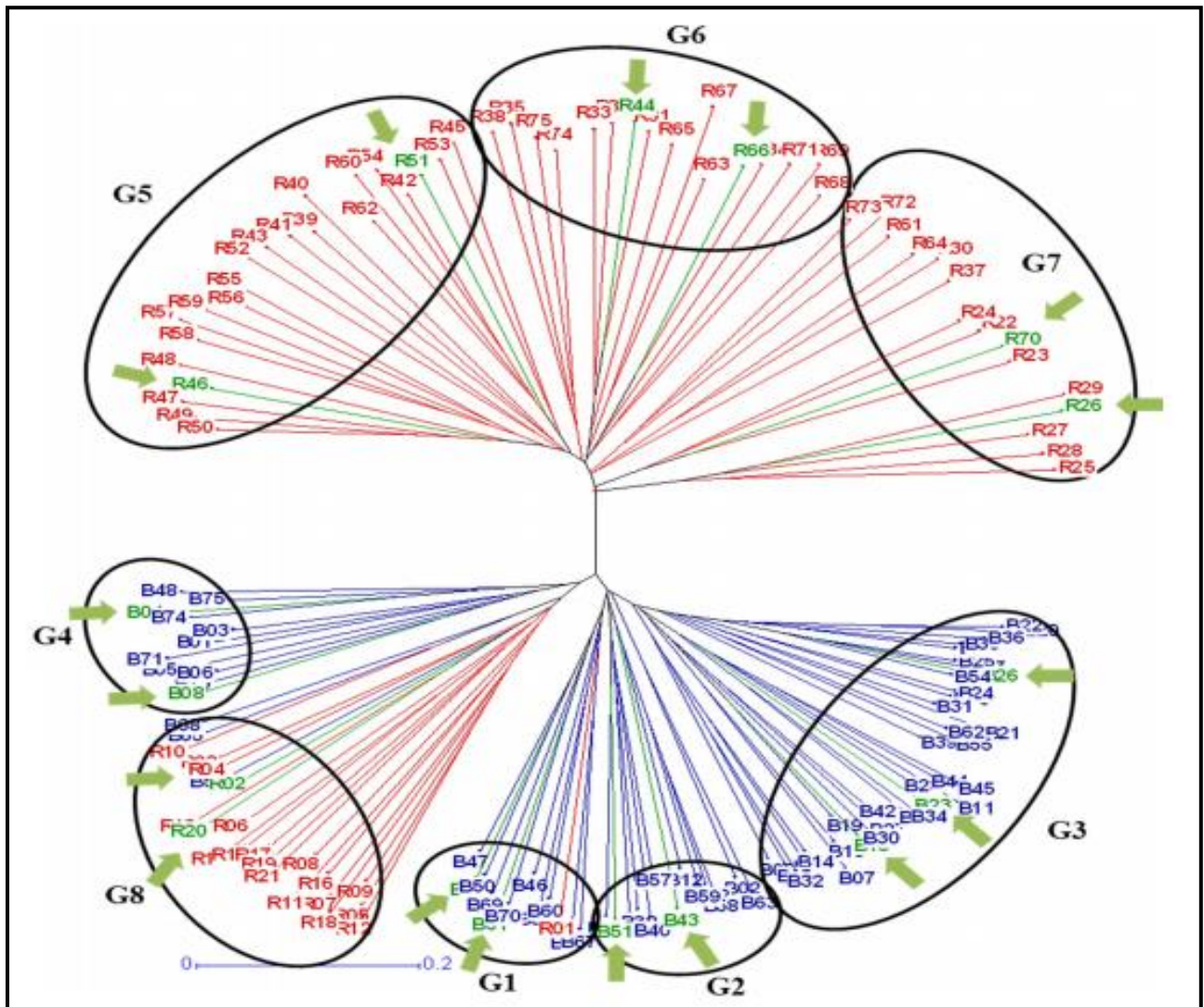


Fig 11: Clustering of 147 hybrid parents into 8 groups using SSRs, and selection of representative 17 parents from different marker based groups (as shown by arrow mark).

11.2 Results

- Inter group hybrids showed high performance than intra group
- Clustering pattern shows B and R lines in two separate groups
- Based on B × R hybrid studies, 4 heterotic pools were formed

- 4 heterotic groups were formed:
 1. HPB1 (combining G1 and G2)
 2. HPB2 (G3)
 3. HPR3 (G6 and G7)
 4. HPR4 (G8)

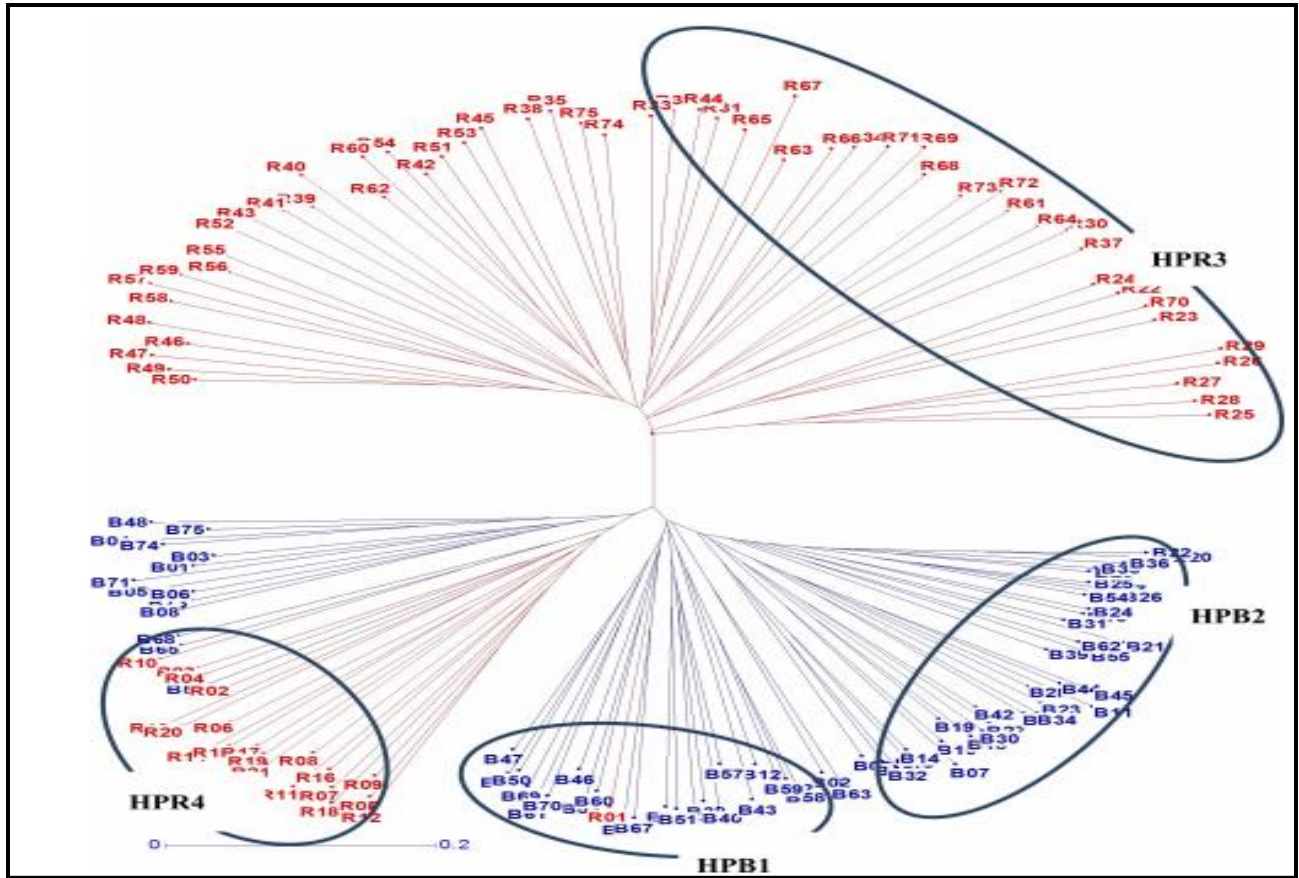


Fig 12: Heterotic pools in the hybrid parents of pearl millet

12. Limitations of heterotic grouping

- Time consuming
 - As it is a pre-breeding tool, it requires large number of years to formulate heterotic groups

- Difficult in autogamous crops due to their typical floral characters
- Large number of initial crosses is needed

13. Future prospects

- To enrich germplasm pool
- To develop simple, more efficient method of identifying heterotic groups and pattern
- To define the genetic interactions and molecular mechanisms involved in heterotic patterns

14. Conclusion

Heterotic groups are the backbone of successful hybrid breeding. The decision regarding the choice of heterotic groups is of fundamental importance and must be made at the beginning of a crop improvement program. Once the heterotic groups are established and improved over a number of selection cycles, it is extremely difficult to develop new competitive ones, because at an advanced stage, the gap in performance between improved breeding materials and unimproved source materials is usually too large. Only if the breeding goals change, is there a good chance to change or develop new heterotic groups with reasonable effectiveness. Moreover, if hybrid breeding is based on CMS, the selection of a heterotic group representing the seed parents (male steriles) and pollen parents (restorers) must be made at an early stage, because considerable efforts are needed for the transfer of eMS and restorer genes.

It is evident from the review of various studies that genetic diversity caused by isolation in space and/or time is essential for the establishment of promising heterotic patterns. Heterotic groups should not be considered as closed populations, but should be broadened continuously by introgressing unique germplasm to warrant medium and long-term gains from selection. While characterization of germplasm is possible with molecular markers, heterosis and hybrid performance among unrelated germplasm is generally not predictable and requires field evaluation of crosses among them (Melchinger, 1999).

15. Discussion

1. Which was the most exploited heterotic pattern?

Heterotic groups were initially formulated in maize. The most exploited heterotic pattern is the cross between Iowa Stiff Stalk Synthetic (BSSS) and Lancaster Sure Crop heterotic groups.

2. Which is the most efficient method for developing heterotic groups?

The most efficient method for developing heterotic groups is the use of molecular markers. Molecular markers have shown to be useful classifying unrelated inbred lines into heterotic groups and it is the most systematic approach.

3. Why Cress strategy is not followed for the development of heterotic groups?

The Cress strategy involves random mating which leads to the mixing of genotypes. This method itself is a destructive step and cannot be used for developing heterotic group. Cress strategy is just a proposed method.

4. In HyBframe approach, what is RRS and why it is used?

RRS is reciprocal recurrent selection and it is a population improvement method. Through RRS, we could check both GCA variance and SCA variance.

5. In HyBframe approach, what do you mean by redesign of floral characters?

In autogamous crops, floral characters are not favourable for outcrossing. Redesign of floral characters means enriching the genotype of a self-pollinated crop with characters suitable for outcrossing. This is done through backcrossing. Suitable

male characters includes high pollen load, increased plant height over female line *etc.* Favourable female characters include feathery stigma, well exposed panicles *etc.*

6. Developing a heterotic group or heterotic pattern, which is more important?

Heterotic pattern could be developed only if well established heterotic groups are available. So development of heterotic groups is the most important step

7. On what basis heterotic groups are developed? Is it only based on yield parameters?

It depends upon the types of crops. In cotton, the main parameter is cotton ball yield of fibre fineness. So heterotic group may be formed based on that. If a heterotic group is formed on the basis of yield, then other characters will be incorporated into each group through backcrossing.

8. Is there any heterotic groups available in horticultural crops?

So far no any reports were available. Due to their high heterozygous nature, development of inbreds is difficult in plantation and trees.

16. References

- Acquaah, G .2012. Principles of plant genetics and breeding. Wiley-Blackwell, Hoboken, 5(2): 38-50
- Aguiar, C. G., Schuster, Amaral-Júnior, A. T., Scapim, C. A., and Vieira E. S. N., 2008. Heterotic groups in tropical maize germplasm by test crosses and simple sequence repeat markers. *Genet. Mol. Res.* 7 (4): 1233-1244.
- Bidhendi, M. Z., Choukan, R., Darvish, F., Mostafavi, K., and Majidi, E., 2012. Classifying of Maize Inbred Lines into Heterotic Groups using Diallel Analysis. *World. Aca. Sci, Engi and Tech.* 6: 07- 25.
- Carena, M. J. and Hallauer, A. R. 2001. Response to inbred progeny selection in Leaming and Midland Yellow Dent maize populations. *Maydica* 46:1–10
- Dhillon, B. S., Boppenmaier, J., Pollmer W. G., Herrmann R. G., and Melchinger A. E. 1993. Relationship of restriction fragment length polymorphisms among European maize inbreds with ear dry matter yield of their hybrids. *Maydica* 38: 245-248.
- Dudley, J. W., Saghai-Maroo, M. A., and Rufener, G. K, 1991. Molecular markers and grouping of parents in a maize breeding program. *Crop Sci.* 31: 718-723.
- Grant, I. and Beversdorf , W. D. 1985. Heterosis and combining ability estimates in spring oilseed rape (*Brassica napus* L.). *Can. J. Genet. Cytol.* 27: 472-478.
- Gurung, D. B., George, L.C. And Delacruz, Q. D. 2009. Determination of Heterotic Groups in Nepalese Yellow Maiz Populations. *Nepal. J. of Sci and Tech.* 10:1-8.
- Kempe K, Gils M,. 2011.Pollination control technologies for hybrid breeding. *Mol Breed* 27: 417–437
- Longin C. F. H., Gowda, M., Mühleisen, J., Ebmeyer, E., Kazman, E., Schachschneider, R., Schacht, J., Kirchhoff, M., Zhao, Y., and Reif, J. C. 2013. Hybrid wheat: quantitative genetic parameters and consequences for the design of breeding programs. *Theor. Appl. Genet.* 126: 2791–2801

- Melchinger, A. E. and Gumber, R. K. 1998. Overview of heterosis and heterotic groups in agronomic crops. In: Lamkey, K. R. and Staub, J. E. (eds.), Concepts and Breeding of Heterosis in Crop Plants. CSSA, Madison, WI. pp. 29-44.
- Melchinger, A. E. 1999. Genetic diversity and heterosis. In: Coors, J. G., Pandey, S. (eds), The genetics and exploitation of heterosis in crops. ASA, CSSA & SSSA, Madison, WI.
- Meena, A. K., Gurjar, D., Patil, S. S., and Khumar, B. L. 2017. Cocept of heterotic group and its exploitation in hybrid breeding. *Int. J. Curr. Microbiol. App. Sci.*6(6): 61-73. Available: <https://doi.org/10.20546/ijcmas.2017.606.007> [Accessed 25 Oct.2019].
- Mohammadi, S. A. and Prasanna, B. M. 2003. Analysis of genetic diversity in crop plants—salient statistical tools and considerations. *Crop Sci* 43:1235–1248.
- Mungoma, C. and L. M. Pollak. 1988. Heterotic patterns among ten Com Belt and exotic maize populations. *Crop Sci.* 28: 500-504.
- Ordas, A. 1991. Heterosis in crosses between American and Spanish populations of maize. *Crop Sci.* 31:931-935.
- Patil, S. S., Ramakrishna, V., Manjula, S. M., Swati, P., Ranganatha, H. M., Kencharaddi, H. G., and Deepakbabu, H. 2011. Deploying reciprocal selection for combining ability for improving performance of hybrids in cotton (*G. hirsutum*). *Indian J. Genet.*, 71(2): 180- 184
- Philipp, H. G., Boeven. C., Friedrich, H. L., and Tobias, W. 2016. A unified framework for for hybrid breeding and the establishment of heterotic groups in wheat. *Theor. Appl. Genet.* Available: <https://doi.org/10.1007/s00122-016-2699-x> [Accessed 25 Oct.2019].
- Qian, W., Sass, O., Meng, J., Li, M., Frauen, M., and Jung, C. 2007. Heterotic patterns in rapeseed (*Brassica napus* L.): I. Crosses between spring and Chinese semi-winter lines. *Theor. Appl. Genet.*, 115: 27–34.

- Reif, J. C., Gumpert, F. M., Fischer, S., and Melchinger, A. E. 2007. Impact of interpopulation divergence on additive and dominance variance in hybrid populations. *Genetics* 176:1931–1934.
- Schnell F. W., Cockerham, C. C. 1992. Multiplicative vs. arbitrary gene action in heterosis. *Genetics* 131: 461-469.
- Seifert F, Thiemann A., Grant-Downtown, R., Edelmann, S., Schrag, T., Gutierrez-Marcos, J. F., Frisch M, Dickinson H.G., Melchinger A. E., and Scholten, S. 2016. Pericentromeric 22-nt small RNAs are negatively associated with yield heterosis of maize.
- Shull, G. H. 1952. Beginnings of the heterosis concept. In: J. W. Gowen (Ed.), *Heterosis*. Iowa State College Press, Ames, IA, pp. 14- 48.
- Singh, S. and Gupta, S. K. 2019. Formation of heterotic pools and understanding relationship between molecular divergence and heterosis in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Available: PLoSONE14(5):e0207463.<https://doi.org/10.1371/journal.pone.0207463> [Accessed 15 Oct.2019]
- Vasal S. K., Cordova, H. S. Pandey, S., Srinivasan, G. 1999. Tropical maize and heterosis. In: J.G. Coors, S. Pandey (Eds.), *the Genetics and Exploitation of Heterosis in Crops*. ASA, CSSA, SSSA, Madison, WI. pp. 363-373.
- Wang, Y., Cai, Q., Xie, H., Wu, F., Lian, L., He, W., Chen, L., Xie, H. A., and Zhang, J. 2018. Determination of heterotic groups and heterosis analysis of yield performance in *indica* rice. *Rice Sci.* 25(5): 261-269. Available: www.sciencedirect.com [Accessed 15 Oct.2019].
- Whitford R, Fleury D, Reif J. C, Garcia M, Okada T, Korzun V, Langridge P.2013. Hybrid breeding in wheat: technologies to improve hybrid wheat seed production. *J. Exp. Bot.*64: 5411–5428
- Xu, S., Zhu, D., and Zhang, Q. 2014. Predicting hybrid performance in rice using genomic best linear unbiased prediction. *Proc. Natl. Sci.* 111:56-61.

- Yifeng, L., Cheng, L., Lujiang, L., Yong, X., Chao, X., Rujun, W., Tingzhao, R and Hai, L.2019. Heterotic grouping based on genetic variation and population structure of maize inbred lines from current breeding program in Sichuan province, Southeast China using genotyping by sequencing(GBS). *Mol. breeding*. 39:38. Available: <https://doi.org/10.1007/s11032-019-0946-y> [Accessed 25 Oct.2019].
- Yu, K., Park, J., Poysa, V. and Gepts, V. 2000. Integration of Simple Sequence Repeat (SSR) markers into a molecular linkage map of common bean. *The J. Heredity* 9: 429 - 433.
- Zhao, Y., Li, Z., Liu, G., Jiang, Y., Maurer, H. P., Würschum, T., Mock, H. P, Matros, A., Ebmeyer, E., Schachschneider, R., Kazman, E., Schacht, J., Gowda, M., Longin, C. F. H., and Reif, J. C.2016. Genomebased establishment of a high-yielding heterotic pattern for hybrid wheat breeding. *Proc Natl Acad Sci USA*. doi:10.1073/ pnas.151454711.

17. Abstract

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Department of Plant Breeding and Genetics**

GP 591: Master's Seminar

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Admission No : 2018-11-053
Major Advisor : Dr.Jiji Joseph

Venue : Seminar Hall
Date : 21-11-2019
Time : 10.00 am

Heterotic grouping

Abstract

Heterosis breeding promises to boost yield and stability in agricultural crops. Hybrid breeding is superior to line breeding with respect to exploitation of heterosis resulting in higher grain yield and an enhanced yield stability (Xu *et al.*, 2014). Heterosis is a natural phenomenon whereby hybrid offspring from genetically diverse individuals show increased vigour relative to their parents. Heterosis has been increasingly exploited in crop production for nearly a century, with the aim of developing more vigorous, higher yielding and better performing cultivars.

Narrow genetic base limits the phenomenon of heterosis and acts as a bottleneck in breeding programme. Hence it is always desirable to maintain genetic diversity between the parents, which can be made possible by assigning germplasm into different heterotic groups. A heterotic group is 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 (Melchinger and Gumber, 1998). 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.

A number of procedures have been used by breeders to establish heterotic groups. These include pedigree analysis, quantitative genetic analysis, geographical isolation inference and use of molecular markers. In pearl millet, SSR marker based classification resulted in the formation of four heterotic groups (Singh and Gupta, 2019). The newly identified heterotic patterns of rice out-yielded the currently used hybrid populations in China (Beukert *et al.*, 2017). Development of hybrids is a tedious process in self-pollinated crops. Recently, HyBFrame was established as a

unified framework for hybrid breeding and the establishment of heterotic groups in autogamous crops (Boevan *et al.*, 2016).

The lack of systematic study in the development of heterotic groups could be one of the main reason for observed low yield heterosis in tropical hybrid rice (Ambati and Singh, 2017). Heterotic groups could be enhanced when new parents with new traits or germplasm are integrated and adapted to the targeted cropping region. Once heterotic groups and patterns are identified, large number of hybrid combinations can be achieved in a short period of time. Heterotic groups are the backbone for successful hybrid breeding, and hence a decision about them should be made at the beginning of crop improvement programme.

References

- Ambati, S. and Singh, T. V. J. 2017. Heterotic grouping and its importance in tropical hybrid rice breeding. *Int. J. Multidiscip. Adv. Re. Trends*. [e-journal]. Available: <https://www.researchgate.net>.ISSN: 2349-7408 [Accessed 7 Oct. 2019].
- Beukert, U., Zuo, L., Guozheng, L., Yusheng, Z., Ramachandra, N., Mirdita, V., Pita, F., Pillen, K., and Reif, J. C. 2017. Genome based identification of heterotic patterns in rice. *Springer Nature* [e-journal]. Available: DOI 10.1186/s12284-017-0163-4 [Accessed 7 Oct. 2019].
- Boeven, P. H. G., Longin, C. F. H., and Wurschum, T. 2016. A unified framework for hybrid breeding and the establishment of heterotic groups in wheat. *Theor. Appl. Genet.* [e-journal]. Available: DOI 10.1007/s00122-016-2699-x [Accessed 15 Oct. 2019].
- Melchinger, A. E. and Gumber, R. K. 1998. *Overview of heterosis and heterotic groups in agronomic crops*. In: Lamkey, K. R. and Staub, J. E. (eds), *Concepts and Breeding of Heterosis in Crop Plants*. Crop Science Society of America, USA, pp. 29-44.
- Singh, S. and Gupta, S. K. 2019. Formation of heterotic pools and understanding relationship between molecular divergence and heterosis in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Available: PLoSONE14(5): e0207463.<https://doi.org/10.1371/journal.pone.0207463> [Accessed 15 Oct. 2019].
- Xu, S., Zhu, D., and Zhang, Q. 2014. Predicting hybrid performance in rice using genomic best linear unbiased prediction. *Proc. Natl. Acad. Sci.* 111:56–61.