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Review Article

Heterotic group theory: A thriving vitality in hybrid maize breeding

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Abstract

Heterotic group theory is one of the fundamental concepts which allow the maximum exploitation of the available genetic diversity in maize. The development of potential heterotic groups and identification of their subsequent heterotic patterns has been proved to be a crucial factor in hybrid maize breeding. Dissecting the genetic basis of a specific heterotic pattern is also useful in devising possible breeding strategies for the utilization of elite inbred lines in the breeding program. Genetic diversification of the available genetic pools is a key to develop genetically divergent heterotic groups which results into the development of superior heterotic patterns in maize. Estimation of combining ability (SCA and GCA) effects supplemented with pedigree information, phenotypic clustering and molecular marker characterization has been predominantly utilized to develop heterotic groups in maize. The present review will focus on (i) to explain the basic concept of heterotic group theory along with its evolution and advancements in accordance to hybrid maize breeding. (ii) Underpinning the genetic basis of heterosis in maize. (iii) Review different heterotic group classification strategies along with their relative efficiencies and their combined application through a proposed conceptual framework. (iv) A comprehensive review about different heterotic groups developed in maize up till now and (v) future directions for the improvement of heterotic group concept in maize.

Key words: Hybrid maize, heterotic group, heterotic pattern, genetic basis, genetic diversification and classification methods.

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Introduction

In plant breeding, utilization of heterosis is one of the most extensively studied phenomenon to increase the agricultural productivity (Zhang *et al*., 1998). In the beginning of the 20th century, Shull and East independently reported the development of maize hybrids by crossing superior genotypes with no inbreeding depression (East 1908; Shull 1908). After that in 1914, Shull proposed the term "heterosis" to address the phenomenon of hybrid vigor (Shull 1914). As a result of increased productivity of double cross hybrids, a dramatic shift was observed in utilizing double cross hybrids instead of open pollinated varieties (OPVs) in 1930s and 1940s (Mangelsdorf, 1975). Comprehensive and more focused research at that time led to the development of combining ability theory during 1940s. The theoretical basis of combining ability was further strengthened when Griffing proposed diallel mating design and statistical approach to select the superior genotypes on the basis of combining ability (Griffing, 1956a, b). Therefore, combining ability theory (Sprague *et al*., 1942) helped breeders in the development of superior inbred lines to produce high yielding single cross maize hybrids instead of double cross hybrids in 1960s (Troyer, 1999). Subsequently, it created the basis for maize heterotic group theory (Dubreuil *et al*., 1996; Hongtrakul *et al*., 1997; Segovia-Lerma *et al*., 2004).

Heterotic group is a set of genotypes that shows similar combining ability and heterotic response while crossing with genotypes from other genetically different groups. Whereas, heterotic pattern is one of the specific pair among the heterotic groups which expresses optimum level of heterosis and hybrid vigor upon crossing to each other (Melchinger and Gumber, 1998; Ertiro *et al*., 2017). The hybrid progeny derived from a heterotic pattern has been observed to be more productive as compared to the hybrids which produced upon crossing between the genotypes of the same heterotic group (Moll *et al*., 1965; Hallauer *et al.*, 1988; Melchinger, 1999).

Hybrid progenies produced from the crosses between Iowa stiff stock synthetic (BSSS) and Lancaster sure Crop (LSC) groups are probably the most exploited heterotic patterns (Hallauer and Miranda, 1988;

Holley and Goodman, 1988; Hallauer and Carena, 2014). Another heterotic group classified as "Iodent" is the third one to be exploited generally in maize seed industry (Nelson *et al*., 2008; Van Heerwaarden *et al*., 2012; Romay *et al*., 2013). The molecular marker-based study conducted by Romay *et al*. (2013) argued that tropical maize Germplasm has a different population structure therefore it should be considered as a distinct heterotic group for its efficient utilization in the maize breeding programs. Another successful heterotic pattern was developed between white maize African heterotic groups i.e., Salisbury White (N) and Southern Cross (SC) (Olver, 1988). In Zimbabwe (southern Rhodesia), hybrid named "SR52" was developed by crossing the inbreds of these two heterotic groups. Being the first commercialized hybrid in Zimbabwe, SR52 led the basis of maize breeding over there and in the eastern and southern African regions as well (Derera and Musimwa, 2015).

Increased heterozygosity is a key factor in determining the optimum heterotic response which clearly depicts the role of genetic diversity among the heterotic groups in increasing the level of heterozygous loci in hybrid progeny (Martin *et al*., 1995; Falconer and Mackay, 1996; Fu *et al*., 2014). Since Germplasm with high degree of genotypic variability is more prone to accumulate favorable dominant genes in the hybrid combinations by improving the selection in the target population. Therefore, classifying Germplasm into different heterotic groups is the basic criteria in order to utilize the available genetic diversity in a more productive way (Reif *et al*., 2003; 2007).

Several studies reported the significant contribution of heterotic group development in overall success of a particular breeding program (Hallauer and Miranda, 1988; Barata and Carena, 2006; Fan *et al*., 2014; Richard *et al*., 2016). As heterotic grouping involved in increasing the combining ability of specific parental combinations (Barata and Carena, 2006) therefore; selection for the best cross combination become much easier for a breeder (Reid *et al*., 2011). It also avoided the selection of undesirable crosses and decreases the number of duplicates during Germplasm storage (Richard *et al*., 2016). Concept of heterotic group development has been observed to be very successful in maize (*Zea mays*) (Reif *et al*., 2003; Menkir *et al*., 2004; Prasanna, 2012; Akinwale *et al*., 2014; Suwarno *et al*., 2014); pearl millet (*Pennisetum glaucum*) (Gupta *et al*., 2020); sorghum (Menz *et al*., 2004); rye (*Secale*) (Fischer *et al*., 2010); rice (Xie *et al*., 2013; Wang *et al*., 2015) and in sunflower (*Helianthus annuus*) (Reif *et al*., 2013).

Currently, molecular characterization of genetic variability and genotypic associations among the maize Germplasm is the most feasible and sustainable approach which help to select the promising inbred lines and further facilitate their classification into different heterotic groups (Wu *et al*., 2016). Among these molecular markers, single nucleotide polymorphism (SNPs) and simple sequence repeats (SSRs) or microsatellites are extensively utilized in assigning maize inbred lines into different heterotic groups (Barata and Carena, 2006; semagn *et al*., 2012 b; Wen *et al*., 2012; Mir *et al*., 2013; Wu *et al*., 2014; Wu *et al*., 2016; Richard *et al*., 2016; Devi *et al*., 2017). Whereas, SSRs are relatively more efficient than the random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLPs) and amplified fragment length polymorphism (AFLPs) markers (Pejic *et al*., 1998; Senior *et al*., 1998; Gethi *et al*., 2002). On the other hand, some authors characterized the maize Germplasm into different heterotic groups by using (RFLPs) (Ajmone-Marsan *et al*., 1998; Benchimol *et al*., 2000; Pinto *et al*., 2003; Warburton *et al*., 2005) and AFLPs (Oliveira *et al*., 2004; Legesse *et al*., 2007). The molecular approach to develop heterotic group in maize has several advantages over conventional methods e.g., pedigree analysis and quantitative genetic analysis. Through molecular characterization even few divergent lines are not neglected which results into the development of high degree of required variability among the heterotic groups. The present review will focus on to explain the significance of heterotic group theory along with the advancements taken place form the start of its utilization in hybrid maize breeding. Along with that, a comprehensive review about the heterotic group classification system has been done and a unified framework for the development of heterotic group in maize has been proposed.

Genetic basis of heterosis

Heterosis breeding has been observed to be a powerful tool in improving overall productivity of various food crops (Schnable and Springer, 2013). The strength of heterosis may vary from one species to another depending upon the mode of pollination and degree of variability between the selected inbred lines (Dwivedi *et al*., 1998; Melchinger, 1999). Therefore, the cross-pollinated species like maize show more productivity in hybrid cross combinations. Due to efficient utilization of heterosis, hybrid maize can increase the yield by 50-100% as compared to the (open pollinated varieties) OPVs (Kutka, 2011). Understanding the genetic basis of heterosis facilitate the breeders to identify various heterotic groups and patterns in maize.

Over the last century, the genetic basis of heterosis has been thoroughly discussed from all angles (Whaley, 1944; Sprague, 1946; Richey, 1946). The key factors responsible for the heterosis are based upon different hypotheses elucidating its genetic basis according to classical genetics model. Among these, dominance and over dominance hypothesis are the most extensively studied genetic mechanisms formulated and discussed by Bruce (1910) and Shull (1910; 1911) respectively. The dominance hypothesis explains the superior performance of the F_1 hybrid due to masking effect of dominant alleles of one parent over the deleterious recessive alleles of the other parent (Davenport, 1908; Bruce, 1910; Jones, 1917). The over dominance theory justifies heterosis as heterozygosity of the F_1 hybrid being superior in performance to either of the homozygous parental genotypes at specific loci (Hull, 1945; Crow, 1948). Whereas, another phenomenon called pseudo-overdominance persists in which favorable dominant alleles get closely linked with each other leading to an apparent over dominance of the chromosomal region (Jones, 1917; Crow *et al*., 1952). Finally, Brieger (1950) studied epistatic effects by indicating that the non-allelic interactions might be a consequence of producing heterosis.

For instance, two quantitative trait loci (QTLs) of two different inbreds of maize having genotypes "CCdd" and "ccDD" respectively (as illustrated in the figure 1: describing "C" and "D" as a dominant loci causing high maize grain yield whereas "d" and "c" as a recessive deleterious alleles which cause a reduced grain yield). Cross between these two inbreds will produce a hybrid having genotype "CcDd". According to the dominance complementation hypothesis, there would be no linkage between these two genes (C and D) and thus the additive effect of "CcDd" could be the possible reason to increase the grain yield as compared to "CCdd" and "ccDD". Guo *et al*. (2014) studied genetic mechanisms behind the increased grain yield in an elite maize hybrid "Yuyu22" by using an "immortalized F_2" population. High density linkage map comprising of 3,184 bins showed that the dominance effects are more important as compared to the other effects (over-dominance and epistatic) in improving all yield and yield contributing traits of the hybrid**.** Moreover, the pseudo-overdominance follow the same genetic mechanism like in case of dominance effects but the difference lies there is that two genes are closely linked with each other on the same chromosome. To avoid the effects of pseudo-overdominance, random mating of the F_2 population up to several generations may results into the breakdown of the undesirable linkages. However, sometimes it is very difficult to break the linkage among the dominant genes even after intercrossing up to several generations as reported by Romero-Severson and Bernardo (2003) in maize. Single locus over-dominance is the second hypothesis in which the heterozygote produced "Cc" exhibit superior performance as compared to both parental genotypes ("CC" and "cc") for a single yield attributing character. Single gene heterosis in maize has been identified by (Hollick and Chandler, 1998). The third hypothesis describing the genetic basis of heterosis in plants is epistasis effects. According to this genetic mechanism, increased productivity of the F_1 hybrid "CcDd" might be due to the mutual interaction of the two loci (C and D). Studies conducted by Kusterer *et al*. (2007) in Arabidopsis and by Wolf and Hallauer (1997) in maize are strong evidences of the role of epistasis in heterosis of polygenic characters.

Before the use of molecular approach, different experimental strategies were adopted to identify the genetic mechanisms responsible for heterosis in maize. Generation mean analysis proposed by Mather and Jinks (1982) provided the basis to estimate relative importance of different genetic effects (additive, dominance and epistasis) in accordance to their contribution in heterosis. Moreover, variance component analysis also helps to identify the genetic causes of heterosis by partitioning the genetic variance into different components due to additive, dominance and epistatic effects. The utilization of molecular markers to characterize the genetic mechanisms behind the heterosis has been proved to be a promising approach which also facilitates to identify major QTLs responsible for heterosis (Melchinger *et al*., 2007; Lariepe *et al*., 2012; Goff and Zhang, 2013).

Segregating F_2 population has been the most extensively utilized heterotic population to identify the heterotic loci and their genetic pattern (Yan *et al*., 2006; Yu *et al*., 1997). But due to high variability, lack of genetically similar plants for replications in an experiment is one of the major consequences while dealing with these primary populations. For underpinning molecular events responsible for heterosis, the F_2 heterotic population has been manipulated to generate more efficient populations i.e., "immortalized F_2" (IF_2) population, based on North Caroline Design III (Comstock and Robinson, 1952; Gardiner *et al*., 1993). While developing "immortalized F_2" population, recombinant inbred lines (RILs) were initially generated from the progeny of an F_1 hybrid cross. The developed RILs were then subjected to pair crossing in an experimental design to generate IF_2 population. In maize, IF_2 populations have been successfully

utilized to identify heterotic gene loci and genetic mechanisms responsible for heterosis (Tang *et al*., 2010; Guo *et al*., 2014a).

Although it should must be understood here that genetic cause of heterosis is not merely due to a single genetic mechanism but possibly due to a combination of all the discussed genetic models (dominance, over-dominance and epistasis) (Swanson-Wagner *et al*., 2006; Lippman and Zamir, 2007). Furthermore, it is very difficult to separately classify the genetic effects of dominance, over dominance and epistasis because the concept of heterosis in agriculture is due to non-linear combined effect of heterozygous genes. Therefore, with the use of novel genetic approaches, plant breeders should first identify the relative contribution of all genetic models in a hybrid and secondly the characterization of QTLs responsible for heterosis.

Heterotic group and pattern: a conceptual context

Hybrid vigor, may also be defined as "heterosis" is one of the key factors involved in the superior performance of the hybrid progeny in comparison with either of the parent inbred lines (Shull, 1952). Heterosis breeding has played significant role in maximizing heterotic effects to uplift the yield especially in maize crop (Duvick, 2005; Troyer, 2006; Schnable and Springer, 2013). Although all hybrid crosses do not necessarily lead to an increased productivity of the hybrid due to low genetic diversity between the parental inbred lines. Therefore, to avoid this setback, plant breeders classify the Germplasm into different heterotic groups on the basis of combining ability estimates and also through molecular characterization of the available Germplasm.

A heterotic group is a diverse population of different inbred lines with strong general combining ability (GCA), which when crossed with the inbred lines of another heterotic group produces superior hybrid progenies. Hybrids which are being generated by crossing the inbred lines from different heterotic groups are superior in performance as compared to the hybrids produced by crossing inbreds of the same heterotic group. On the other hand, heterotic pattern is a specific pair of inbred lines from two different heterotic groups which expresses significant heterotic response in a hybrid combination. Heterotic pattern plays an important role in efficient utilization of available Germplasm by maximizing the yield of hybrids derived from two genetically diverse populations' i.e., heterotic groups (Eberhart *et al*., 1995).

Reif *et al*. (2005) proposed basic criteria to develop a heterotic pattern i.e., the hybrid should exhibit high mean yield and heterosis and a decreased specific combining ability variance (σ^2 SCA) along with higher σ^2 GCA/ σ^2 SCA ratio. To maximize the affectivity of heterosis breeding based on the development of heterotic group and patterns, plant breeders should must focus on: 1) Germplasm diversification by the introduction of superior exotic lines to detect novel heterotic groups and patterns; 2) exploitation of different molecular breeding tools to develop an efficient heterotic group classification method; 3) to elucidate the genetic and molecular basis of heterotic patterns.

Heterotic group classification methods

Pedigree Analysis

Classification of diverse pool of inbred lines into different heterotic groups has been going on since the beginning of heterotic group theory. Various studies reported the utilization of pedigree analysis for assigning germplasm into various heterotic groups (Senior *et al*., 1998; Yu *et al*., 2007). The most extensively utilized heterotic groups i.e., Iowa stiff stock synthetic (BSSS) and Lancaster sure crop (LSC) group, were primarily developed through pedigree and geographical analysis (reviewed by Meena *et al*., 2017). Ajmone-Marsan *et al*. (1992) further investigated the intra-group genetic similarities among the individuals of these two heterotic groups by using RFLP molecular markers. In comparison with the previously available pedigree data, RFLP cluster analysis showed similar associations in BSSS group; while in case of LSC, co-ancestry data of pedigree analysis showed significant differences from the genetic similarity coefficients produced by RFLP analysis. Reif *et al*. (2003) classified sub-tropical maize Germplasm into two distinct heterotic groups i.e., flint and dent maize, by using SSR analysis and pedigree information and concluded that the results obtained through SSR analysis are in high concordance with the available pedigree data.

Combining ability analysis

Combining ability analysis has been proved to be a promising approach in classifying Germplasm into various heterotic groups. A large number of studies in maize have been focused on to assess the GCA and SCA effects of the inbred lines following a specific mating design (Table 1). Fan *et al*. (2002) comprehensively studied the inter-group relatedness among four temperate and five tropical/sub-tropical maize heterotic groups on the basis of combining ability estimates. They found that Tuxpeno and Antigua heterotic pools had close connections with BSSS (Reid group); POP32 (ETO) and Suwan1 were closely related with Ludahonggu and POP28 was

close to Sipingtou (SPT) heterotic group. Suwan1 (tropical maize Germplasm) was classified as a separate heterotic group due to significant differences from both BSSS (Reid) and Lancaster (non-Reid) heterotic groups (Fan *et al*., 2008a). After that, Suwan1 has been widely recognized as the primary source of Germplasm in South and East Asia.

The line × tester mating design has been generally utilized to compute the combining ability estimates (GCA and SCA effects) related to the grain yield and other agronomic characters in maize (Hallauer and Miranda, 1988; Menkir *et al*., 2004; Barata and Carena, 2006; Wu *et al*., 2007; Fan *et al*., 2008a). On the basis of combining ability estimates for grain yield, Menkir *et al*. (2004) classified 23 of the 38 evaluated inbred lines into two distinct heterotic groups by using line × tester mating design. Recently, Mosa *et al*. (2017) classified a core set of 25 Egyptian maize parental lines lines into two distinct heterotic groups i.e., group 1 (SK11) for grain yield and Group 2 (SK2) for disease resistance index, by estimating specific and general combining ability (HSGCA method) generated through line × tester analysis.

The diallel mating design proposed by Griffing (1956) led the basis to analyze the combining ability estimates thus it can help in the identification of heterotic groups and patterns among the parental lines. Bidhendi *et al*. (2012) used diallel and biplot analysis to identify the possible heterotic groups among the Iranian inbred lines and reported LSC × BSSS a potential heterotic pattern developed among the evaluated genotypes. The reciprocal crosses generated through diallel mating design significantly influence the grain yield and combining ability estimates (GCA and SCA effects). Fan *et al*. (2013) argued that the three heterotic group (TriHG) classification system (Suwan1, Reid and non-Reid) might be a perfect model for improving the efficiency of a maize breeding program. They studied the effects of reciprocal crosses on combining ability estimates through a diallel mating design which results into decreased GCA and residual variances but SCA variance along with grain yield was increased due to an increased no. of parents in a diallel cross. Therefore, reciprocal crosses play significant role in the development of heterotic groups due to their positive effects on grain yield and SCA effects. Recently, Yu *et al*. (2020) generated a large-scale multiple hybrid population (MHP) comprised of 724 hybrids derived from the crossing of parents with different ecological backgrounds (temperate and tropical ecotypes) by combine application of diallel and North Carolina design II. The hybrids generated by crossing parent lines of different ecotypes (temperate x tropic) showed higher heterotic response than the temperate and tropic hybrids. Moreover, they identified potential heterotic patterns i.e., BSSS (Reid) x Sipingtou (SPT), BSSS x LRC (Lyda Red Coda Chinese Landrace), SPT x PA (Group A Germplasm derived from modern US hybrids) and LSC (non-Reid) x LRC on the basis of SCA effects and superior heterosis related to agronomic characters.

Phenotypic clustering

Phenotypic clustering a conventional heterotic group classification method is of great importance especially when combined with pedigree data (Babic *et al*., 2008) and combining ability analysis (Mosa *et al*., 2017). Plant breeders apply multivariate analysis to classify a diverse genetic pool of Germplasm into various groups on the basis of different morphological and agronomic characters. However due to unknown genetic mechanism and environmental influences, several questions arise on the sole use of morphological markers while identifying possible heterotic groups and potential heterotic patterns. Therefore, along with phenotypic clustering, genotypic characterization through molecular markers of a diversified maize population has been proved to be an effective approach to assign Germplasm into various heterotic groups (Rebourg *et al*., 2001; Warburton *et al*., 2005; Wei *et al*., 2009). Babic *et al*. (2012) validated the morphological classification of 19 maize parental lines by molecular characterization using RAPD molecular markers. Cluster analysis of both (morphological and molecular) markers showed significant resemblance with the available pedigree data depicting the efficiency of morphological markers while estimating the genetic distances among the parental inbred lines.

Marker assisted heterotic group development

The availability of genetically diverse Germplasm is a key factor required by the plant breeder to develop high yielding hybrids. Therefore, it necessitates the assessment of genetic diversity among the available parental genotypes for their maximum exploitation in a hybrid breeding program. Classification of maize parental genotypes based on genetic diversity analysis has resulted into the development of "heterotic group" in most of the maize growing regions of the world (table 1). As discussed in the previous sections, several strategies have been adopted to develop heterotic groups in maize including pedigree analysis, combining ability analysis and phenotypic clustering. But limited pedigree information (by keeping information confidential) and environmental effects on plant morphology are some major limitations of these

approaches. In such conditions, genotypic clustering (based on genetic distance) by the application of molecular markers is the most feasible alternate strategy to assign the available Germplasm into different heterotic groups (Melchinger and Gumber, 1998; Reif *et al.*, 2003a, b). Marker assisted heterotic group development has several advantages over morphological classification due to whole genome coverage and environment neutral behavior. A large number of studies have indicated the effective use of molecular markers for the classification of maize germplasm into different heterotic groups (Reif *et al.*, 2003a; Aguiar *et al.*, 2008b; Reid *et al.*, 2011; Akinwale *et al.*, 2014; Suwarno *et al.*, 2014).

Initially, Restriction Fragment Length Polymorphism (RFLP) markers are generally utilized for the assessment of genetic diversity in maize. Several studies reported the use of RFLPs as an efficient way to classify maize Germplasm into various heterotic groups along with supplemented pedigree data (Boppenmaier *et al*., 1993; Dubreuil and Charcosset, 1999; Li *et al*., 2000; Livini *et al*., 1992; Melchinger *et al*., 1991) and SCA effects (Pinto *et al*., 2003). Contrary to this, use of RFLPs has been observed to be inefficient in evaluating the performance of F_1 hybrid (Boppenmaier *et al*., 1993; Melchinger *et al*., 1992). Moreover, Warburton *et al*. (2005) found RFLPs ineffective in identification of testers representing different heterotic groups and concluded that the use of RFLPs alone is not effective for the assessment of potential heterotic groups in maize.

Amplified Fragment Length polymorphism (AFLP) has also been reported to be effective in assigning maize Germplasm into different heterotic groups (Marsan *et al*., 1998; Chitto *et al*., 2000; Nagy *et al*., 2000, 2003; Li *et al*., 2004). Ajmone-Marsan *et al*. (1998) and Pejic *et al*. (1998) reported the relative efficiency of AFLP over RFLP markers while detecting polymorphism because of a greater number of genetic loci evaluated during a single PCR reaction. Barbasa *et al*. (2003) reported that the performance of hybrids (derived from genetically diverse intra-population hybridization) can be estimated by generating genetic distances (GDs) through AFLP marker application. Furthermore, Li *et al*. (2004) examined the usefulness of AFLP markers by classifying Italian and Chinese inbred lines into different heterotic groups. They found that most of the Italian and Chinese inbred lines have the background of Reid Yellow Dent (RYD) and Huangzaosi (HZS) heterotic groups respectively. In addition, they also identified a new heterotic group i.e., PN group derived from Pioneer maize hybrids and concluded that AFLP based markers are quite efficient for classification of maize Germplasm into various heterotic groups.

Simple sequence repeats (SSRs) or microsatellite are also a preferred choice to classify maize Germplasm into distinct heterotic groups along with the prediction of the hybrid performance. A number of studies have identified SSR markers as an effective tool (table 1) in predicting heterotic groups i.e., identification of Reid (BSSS) and non-Reid (LSC) parent genotypes (Choukan *et al*., 2006; Jambrovic *et al*., 2008); IITA and CYMMT lines (Dhliwayo *et al*., 2009) and tropical maize inbred lines (Aguiar *et al*., 2008). Recently, Devi *et al*. (2017) classified a core set of 24 waxy parental genotypes into three distinct heterotic groups by using 77 SSRs distributed throughout the genome. Along with the molecular profiling, all parental genotypes were also subjected to morphological characterization based on eight agronomic parameters. Therefore, SSR based molecular classification of these parental lines along with superior agronomic performance can be effectively utilized for identification of desirable heterotic patterns. Moreover, Wagery *et al*. (2018) applied SSR molecular markers to identify the genetic relatedness and heterotic structure among the core set of 35 elite inbred lines (32 Quality Protein Maize (QPM) and 3 non-QPM lines). The lines were screened with 40 SSR markers which results into clustering of genotypes into two to three distinct heterotic groups which will lead to effective utilization of QPM inbred lines in future breeding programs. On the other hand, results from some studies revealed the inefficiency of SSRs in classifying the maize Germplasm into heterotic groups, probably due to the mixed composition of inbred lines of diversified breeding populations (Adetimirin *et al*., 2008; Xia *et al*., 2004; Xia *et al*., 2005). Therefore, extensive field trials are still needed along with molecular profiling to identify heterotic groups and patterns in maize (Barata and Carena, 2006).

In recent years, due to advancements in molecular breeding, single nucleotide polymorphism (SNP) markers are widely utilized molecular markers for the classification of diversified maize Germplasm into different heterotic groups (Semagn *et al*., 2012b; Wen *et al*., 2012; Wu *et al*., 2014; Wu *et al*., 2016). Currently, SNPs are highly recommended for various molecular studies due to reduced chances of genotyping error, high genomic abundance and ability of high-throughput analysis (Wu *et al*., 2016). In maize due to abundance of gene-based SNP markers, the identification of heterotic loci and genotyping of a diverse gene pool is now practically suitable with high-cost effectiveness. Furthermore,

due to the advancement in next generation sequencing (NGS) and genotyping by sequencing (GBS), the applicability of SNP molecular markers has been increased up to a great extent (Elshire *et al*., 2011). The study conducted by Lu *et al*. (2009) is one of the best examples of utilizing SNPs for genetic diversity analysis accompanying global maize Germplasm. Diverse Germplasm of 770 inbred lines collected from maize breeding pools of CIMMYT, China and Brazil were screened with 1,034 SNPs. Genotypic clustering characterized the temperate and tropical/sub-tropical Germplasm into two distinct clusters with highest genetic divergence, followed by the divergence between white and yellow kernel genotypes; dent and flint lines exhibited the least genetic divergence. Recently, Richard *et al*. (2016) classified a core set of 45 diverse maize inbred lines into four heterotic groups by utilizing 129 SNPs. The characterized heterotic groups had close connections with N, SC, BSSS and LSC heterotic groups. Temperate genotypes were observed to be more genetically diverse as compared to the tropical/sub-tropical inbred genotypes. Recently, SNP based heterotic grouping has been shown to be the most efficient compared to SCA and GCA (HSGCA) heterotic grouping even over heterotic grouping based on GCA of multiple traits (HGCAMT) (Badu-Apraku *et al.*, 2015).

A three heterotic group (TriHG) classification system

The efficiency of a maize breeding program is solely depending upon optimum utilization of the available genetic resources through effective heterotic grouping and patterns (Richard *et al*., 2016). Two heterotic groups including Reid derived Iowa Stiff Stalk Synthetic and Lancaster sure crop group (non-Reid) have been utilized predominantly to develop high yielding maize hybrids (Hallauer and Carena, 2014). Molecular studies conducted by Romay *et al*. (2013) revealed that tropical maize Germplasm exhibit significant genetic distances as compared to the Reid and non-Reid heterotic groups and further suggested to classify the tropical maize into a separate heterotic group. Finally, Fan *et al*. (2014) classified the tropical maize Germplasm (Suwan1) into a distinct heterotic group and proposed the classification of maize Germplasm according to three heterotic group classification (TriHG) system i.e., Reid, non-Reid and Suwan1 heterotic groups. More recently, Fan *et al*. (2018) strengthened the applicability of TriHG over DiHG classification system as breeding efficiency was observed to be increased while utilizing TriHG system. Therefore, plant breeders can improve the efficiency of a maize breeding program by utilizing a three heterotic group (TriHG) system instead of a usual two heterotic group (DiHG) classification system which might effectively contribute in developing high yielding maize hybrids in the future.

Up till now, plant breeders have proposed various classification systems for heterotic group development some of which have been discussed in the previous sections along with their relative efficiencies. Among these methods, the phenotypic clustering and combining ability analysis are more likely to be affected by cultivation and environmental effects. Sometimes the data from pedigree analysis does not validate the results produced by molecular markers. Moreover, the use of molecular markers for the genotypic clustering of the available Germplasm is also limited by the kind and quantity of available primers. The results obtained from a single classification method are not much effective and reliable; therefore, an integrated application of different classification methods should be adopted for the development of potential heterotic groups and patterns in maize.

A conceptual framework for heterotic group development

On the basis of integrated application of various classification methods, we propose following conceptual framework for heterotic group development in maize (Fig. 2). (i) Collection of diversified maize Germplasm (with known pedigree) followed by phenotypic clustering and character association studies which ultimately lead to the identification of high yielding parental lines. (ii) To validate phenotypic clustering and the assessment of actual genetic diversity, molecular characterization of the Germplasm with the application of appropriate molecular markers. (iii) Combining ability analysis by using a suitable mating design to identify distinct maize heterotic pools on the basis of GCA effects. For effective utilization of combining ability estimates, GCA effects can also be predicted through the application of genome wide molecular markers. (iv) Reciprocal recurrent selection is carried out to improve the combining ability of the identified heterotic pools. Reciprocal recurrent selection also improves the genetic divergence among the identified heterotic pools; thus, lead to the development of potential heterotic patterns. (v) Continuous introduction and introgression of the new (exotic) Germplasm improves the intra-group genetic diversity; however, it must be noticed that the introduction of exotic Germplasm should not disturb the developed heterotic pattern and the genetic distances among the developed heterotic groups.

Thus, before the introduction, the exotic lines should must be molecularly characterized to uncover their genetic variability with respect to the identified heterotic groups. Along with that, genome-based prediction of GCA effects is carried out as the exotic lines are poorly adapted to the introduced conditions and field testing is not showing possible results. Based on genetic similarities with one of the developed heterotic groups and significant GCA effects with the opposite groups, the superior candidate lines are assigned into respective heterotic pools. But before the test crosses are being made, these introduced lines are crossed with the superior adapted parental lines from their respective heterotic group and evaluation was carried out for all agronomic characters as well as GCA effects. The selection of representative testers of a specific heterotic group is also of great importance to improve the efficiency of a maize breeding program. (vi) Exploitation of the potential heterotic pattern developed by the crossing of parental lines (having high GCA) from two genetically distinct and diverse heterotic groups.

Strengthening heterotic group theory: what to do?

The concept of heterotic groups has been effectively applied in plant breeding over the last 100 years. The development of first commercial maize hybrid i.e., SR52 in 1960s led the basis of utilizing potential heterotic patterns (N x SC) developed by classifying maize parental lines into different heterotic groups (table 1). So far, it has been well understood now that heterotic group classification cannot be accomplished effectively by utilizing a single breeding methodology based on either conventional or modern approaches; but rather a comprehensive and unified strategy with a combined application of different classification methods (Figure 2). The availability of an ultimate level of genetic diversity is of great interest in hybrid maize breeding as genetically divergent parental lines lead to the development of a high yielding F_1 hybrid. So, the inclusion of exotic Germplasm in the local breeding programs must be the top priority in order to develop genetically divergent heterotic pools (Reif *et al*., 2010, Fan *et al*., 2015).

Moreover, crossing inbred lines representing different ecotypes i.e., tropical x temperate, has been well documented to improve the performance of the hybrid produced. Maize breeding programs should focus on utilizing maize Germplasm representing different ecotypes to develop genetically divergent maize heterotic pools. In this context, a large-scale analysis of heterosis and combining ability based on developing multiple hybrid population (MHP) could help to estimate the genetic gains of a breeding program comprised of utilizing heterotic pools representing different ecotypes (Yu *et al*., 2020). In recent years, classification of tropical Germplasm into a distinct heterotic group i.e., Suwan1, has created new vistas of exploiting a three heterotic group (TriHG) classification system (Reid, non-Reid, Suwan1) over two heterotic group (DiHG) classification system (Ried and non-Reid). Thus, the concept of TriHG system should be applied by inclusion of testers representing Reid, non-Reid and Suwan1 heterotic groups in order to enhance the efficiency of a hybrid breeding program (Fan *et al*., 2018).

In countries like Pakistan where the maize cultivation significantly contribute in national economy, has been facing various setbacks in utilizing available genetic diversity to develop high yielding local maize hybrids. Therefore, by utilizing the concept of heterotic groups, maize breeders in countries like Pakistan, should have to classify the available maize genetic diversity into various heterotic pools which could help to produce high yielding maize hybrids developed by exploiting local genetic diversity. Furthermore, if the available local germplasm is not sufficient to produce genetically divergent heterotic pools, then introduction and introgression of exotic Germplasm could be the most feasible and appropriate solution for the genetic diversification of the available local Germplasm.

Conclusion:

Classification of maize Germplasm into various heterotic groups could significantly improve the exploitation of available genetic diversity. The development of heterotic group with superior heterotic pattern has a significant contribution in enhancing the overall breeding efficiency by minimizing the evaluation of undesirable hybrid combinations. Combined application of different heterotic group classification methods including pedigree data, combining ability analysis, phenotypic clustering and genotypic clustering through the application of molecular markers has been observed to be the most appropriate and comprehensive strategy to develop possible heterotic groups and to identify potential heterotic patterns. Moreover, Introduction and introgression of exotic Germplasm could lead to the development of genetically divergent heterotic pools which results into increased level of heterosis in the hybrid produced. Therefore, it has been concluded that identification of genetically divergent heterotic groups and their

subsequent heterotic patterns is a dire need for the maximum exploitation of the available genetic diversity in order to develop a long-term hybrid maize breeding program.

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Table 1: Different heterotic group and patterns developed in maize (*Zea mays* L.)

Heterotic Group	**Heterotic Pattern**	**Classification method**	**Country**	**References**
BSSS (Reid) and LSC (non-Reid)	BSSS (Reid) x LSC (non-Reid)	Pedigree and Geographical Analysis	USA	Hallauer and Carena, 2014
N and SC	N x SC		Zimbabwe	Derera and Musimwa, 2015
Suwan1, ETO and Tuxpeno	Suwan1 x Tuxpeno, ETO x Tuxpeno		Mexico	Bjamason, 1996
Flint and Dent maize	Flint x Dent	Combining ability analysis (Diallel mating design) Simple Sequence Repeat (SSR) markers application		Reif *et al*., 2003
B73 and Mo17	B73 x Mo17	Metabolomics	Germany	Römisch-Margl *et al*., 2010
Flint and Dent maize	Flint x Dent	Combining ability analysis (Diallel mating design) Simple Sequence Repeat (SSR) markers application	India	Rajendran *et al*., 2014
BSSS (Reid), Tem-tropic1, Zi330, SPT and LSC (non-Reid)	BSSS × Tem-tropic 1, Zi330 x Tem-tropic 1, BSSS x Zi330, BSSS x SPT, and LSC x SPT	Simple Sequence Repeat (SSR) markers application	China	Teng *et al*., 2004
BSSS (Reid), PA Group, PB Group LSC (non-Reid), LRC and SPT		Simple Sequence Repeat (SSR) markers application		Xie *et al*., 2008
Suwan1, BSSS (Reid) and LSC (non-Reid)	Suwan1 x BSSS (Reid), Suwan1 x LSC (non-Reid) and BSSS (Reid) x LSC (non-Reid)	Combining Ability Analysis (Line x Tester mating design)		Fan *et al*. 2018
Tem-tropic 1A and Tem-tropic 1B	Tem-tropic 1A x Tem-tropic 1B	Genotyping by Sequencing (GBS)		Leng *et al*., 2019
BSSS (Reid), PA Group, PB Group LSC (non-Reid), LRC and SPT	Reid x SPT, Reid x LRC, SPT x PA, and LSC x LRC	Combining Ability Analysis (Diallel and North Carolina II mating design)		Yu *et al*., 2020

BSSS = Iowa Stick Stock Synthetic Group; LSC = Lancaster Sure Crop Group; N = Salisbery White Group; SC = Southern Cross Group; ETO = POP32; SPT = Sipingtou Group; PA = Group A Germplasm derived from modern US hybrids; PB = Group A Germplasm derived from modern US hybrids; LRC = Lyda Red Coda Chinese Landrace; B73 = Representatives of Reid (BSSS) group; Mo17 = Representatives of non-Reid (LSC) group

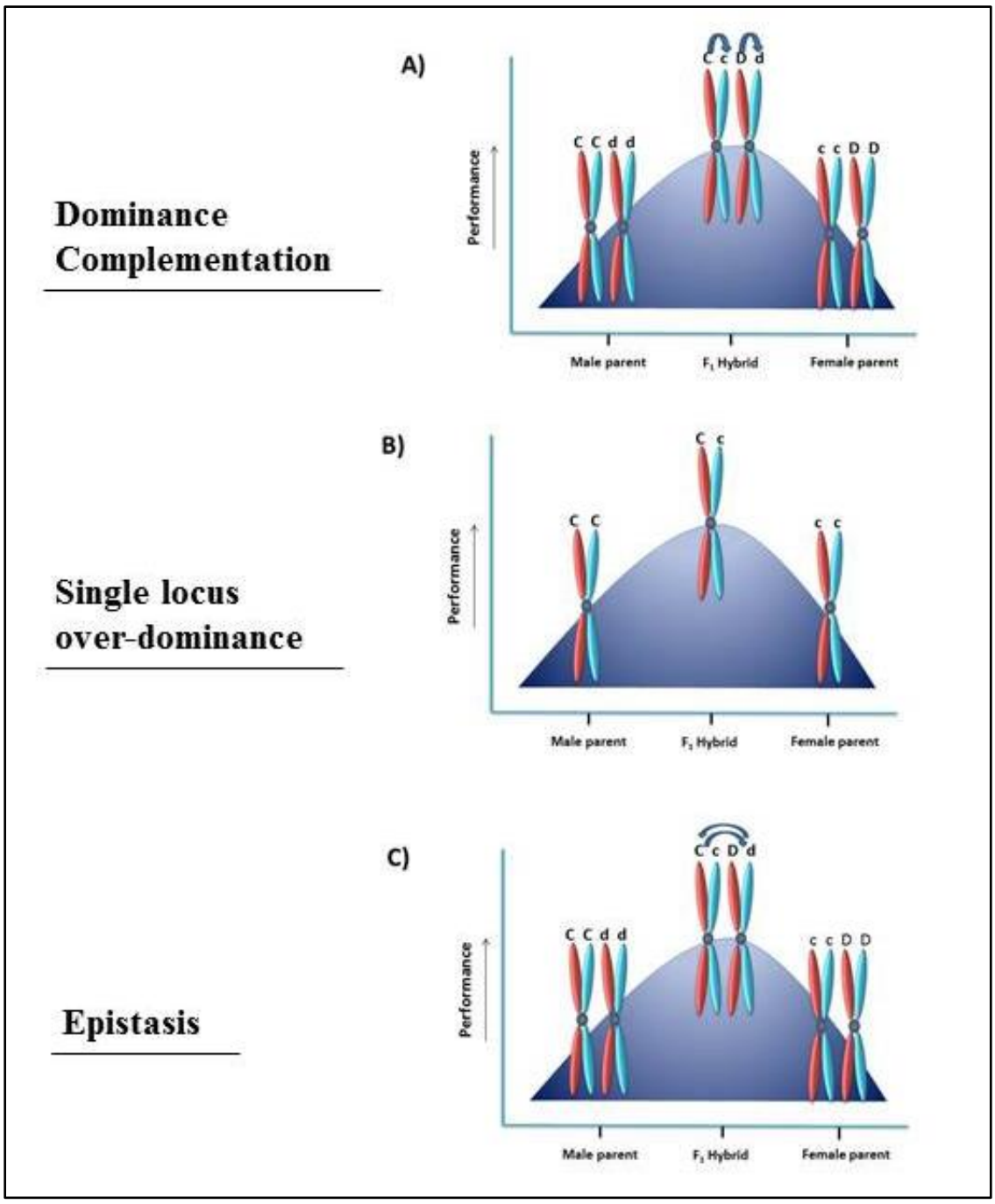

Fig. 1: Genetic basis of heterosis

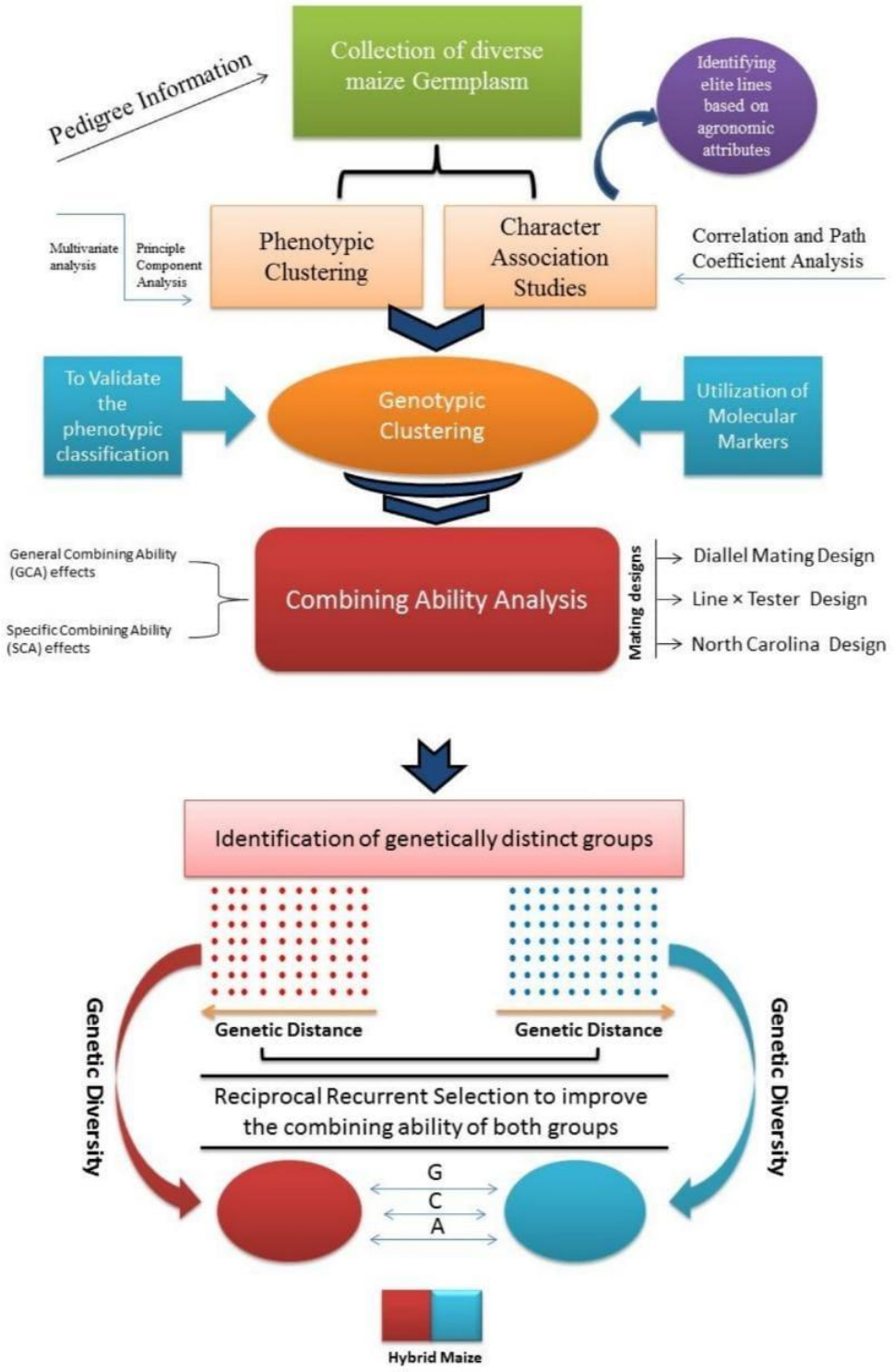

Fig. 2: Represents the schematic visualization of the conceptual framework for heterotic group development in maize