The Usefulness of Molecular Markers Approach for Developing Heterotic Groups in Maize

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Abstract

The phenomenon of heterosis provides a great opportunity for plant breeders to gain greater performance and yield in the hybrids compared to their inbred line parents. In most cross-pollinated crops like maize, heterosis plays an important role as the performance of the hybrids is of a great value. Heterosis gain much interest until recently and current studies still attempt to elucidate one of these is utilizing molecular markers to estimate genetic distances among inbred lines followed by developing putative groups. In a well-defined heterotic group, between-groups mating are expected to produce better hybrids than within-groups mating. Some studies of marker-aided heterotic group development resulted in functional heterotic groups; while some other reported that the groups failed to provide evidence of heterosis in the hybrids. Considering the prediction of hybrids' performance will depend on genetic background of inbred lines, and markers technology are continuously improved, there is still a good opportunity to obtain useful heterotic groups for a particular breeding population.

Keywords: maize breeding, genetic distance, heterosis, molecular markers.

Hybrid Maize Breeding

Excellent maize hybrid varieties are obtained through development and use of inbred lines with superior combining ability for grain yield and other agronomic traits. Hybrid maize breeding includes development of stable, vigorous, high-yielding inbred lines with the extensive evaluation of combining ability during the process of developing the lines, followed by use of selected inbred lines in development of improved hybrids (Singh, 1987). Characterization of maize lines for their combining ability is routinely conducted for numerous traits, including adaptation to drought and low nitrogen stress conditions (Betran et al., 2003; Medici et al., 2004), soil acidity (Welcker et al., 2005), aflatoxin accumulation (Williams et al., 2008), resistance to pathogens causing lodging (Moreno-Gonzalez et al., 2004), mite (Bynum et al., 2004), Striga hemonthica and Striga asiatica (Gethi and Smith, 2004), maize weevil (Dhliwayo et al., 2005), and many more. Combining ability of maize lines is also important for value adding traits, including nutritional characteristics, such as grain's iron and zinc density (Long et al., 2004), pro vitamins A, protein, oil and starch content, and of course, grain yield.

The increase in size, vigor, or productivity of a hybrid plant over its parents is known as hybrid vigor or heterosis (Allard, 1960; Poehlman, 1995). Genetic diversity is important in maize breeding because crosses among two similar inbred lines are expected to result in small levels of heterosis. Inbred lines are therefore typically developed from two or more genetically different populations to obtain high levels of heterosis in their hybrids (Singh, 1987). Instead of naturally exists in the germplasm, strong evidences revealed that heterotic pattern were developed by the plant breeders (Tracy and Chandler, 2006).

The use of molecular plant breeding tools has become routine in many variety development programs. These methods can enhance breeding efficiency by replacing phenotypic with genotypic selection during some stages of the breeding process, thereby reducing overall phenotyping costs, biases (e.g. caused by environment factors and genotype by environment interactions), and measurement errors (Moose and Mum, 2008). In hybrid maize breeding programs, development of heterotic groups and assignment of inbred lines into the established heterotic groups are very important. Development of heterotic groups and assignment of experimental inbred lines to heterotic groups before making hybrid crosses is time and cost efficient, because the number of crosses to be made and evaluated will decrease substantially. The use of molecular marker data and genetic distances (GDs) to predict heterosis, however, has given inconsistent results (Collard and Mackill, 2008).

Molecular Marker Approaches for Heterotic Groups Development

Molecular marker methods have been used extensively to estimate genetic distance between maize populations and lines and to assign maize inbreds to heterotic groups. The molecular marker technologies have been...
evolving rapidly, thus the preferred molecular marker types for these and other applications have been changing every few years. Beginning around 1991, researchers commonly used Restriction Fragment Length Polymorphism (RFLP) markers for genetic relationship studies in maize. The use of RFLPs was followed by a preference for use of Amplified Fragment Length Polymorphism (AFLP) markers, and after that Simple Sequence Repeat (SSR) or microsatellites have been the most commonly used markers for diversity work in maize. Most recently, Single-Nucleotide Polymorphism (SNP) markers are becoming the markers of choice for many applications in maize breeding.

RFLP markers were proven as a convenient method to distinguish maize populations based on geographic origins (Gauthier et al., 2002; Rebourg et al., 1999). To some extent, researchers also found that RFLPs were also suitable for predicting heterotic groups and measuring genetic diversity between lines. Several studies found that RFLP is useful for assigning inbreds into heterotic groups, indicated by consistency of the GDs grouping with pedigree information (Boppenmaier et al., 1993; Dubreuil and Charcosset, 1999; Li et al., 2000; Livini et al., 1992; Melchinger et al., 1991) and SCAs estimation (Pinto et al., 2003). However, different studies reported that RFLP is only effective for predicting F, performance for crosses between lines from the same heterotic group (Boppenmaier et al., 1993; Melchinger et al., 1992). On the other hand, Warburton et al. (2005) had difficulty to figure out potential heterotic groups using RFLPs only as they could not differentiate the testers from opposite heterotic groups.

Genetic distance based on RFLP markers could have positive correlation with f values, hybrid performance, SCA, and heterosis (Betran et al., 2003). However, correlation between GDs and heterosis were low for pairs from different heterotic groups (Benchimol et al., 2000). Correlation between GD and performance-based relationship was high (Rebourg et al., 2001; Williams and Hallauer, 2000), especially when the parents were distantly related (Dudley et al., 1991). Genetic similarity (GS) and F values were highly correlated for related lines (Ajmonemarsan et al., 1992; Messmer et al., 1993), and GS mean for line combinations from different population was smaller than unrelated line combinations from the same population (Livini et al., 1992; Marsan et al., 1998; Melchinger et al., 1991).

Studies that demonstrated successful heterotic grouping using AFLP marker were reported by Chitto, et al. (2000), Li et al. (2004) and Marsan et al. (1998). On the contrary, a study by We and Dai (2000) reported that AFLP’s based GDs have low reliability to predict hybrid yield and heterosis because there is either no correlation between them for unrelated lines (Wu and Dai, 2000) or the correlation is too small (Marsan et al., 1998). In a study with 51 European maize lines representing flint and dent heterotic groups, GS estimates from RFLPs and AFLPs were correlated for both related and unrelated pairs (Lubberstedt et al., 2000). Low correlation between GD and grain yield could be caused by small range of GD due to previous selection for combing ability (Barbosa et al., 2003). Kumar (1999) suggests that in order to obtain significant relationship between GDs and hybrid performance several conditions must be met, i.e. adequate phenotypic evaluation and choice of markers used for analysis, and sufficient number of parental inbred lines (Stuber et al.in Kumar, 1999). GD revealed by SSRs tends to show greater degree of polymorphism compared to AFLPs (Heckenberger et al., 2006). Finding an appropriate set of SSR loci is also an important consideration because it could provide similar accuracy with the use of a greater number of loci (Enoki et al., 2005).

More recent studies on genetic distances and heterotic grouping have been using SSR markers. Several studies found that SSR markers perform high polymorphism and could be used for predicting heterotic groups, i.e. for CIMMYT and IITA lines (Dhliwayo et al., 2009), tropical maize lines (Aguiar et al., 2008), and Reid Yellow Dent and Lancaster Sure Crop lines (Choukan et al., 2006; Jambrovic et al., 2008). In contrast, some studies revealed that SSR markers could not predict heterotic groups precisely (Adetimirin et al., 2008; Barata and Carena, 2006; Xia et al., 2004; Xia et al., 2005), where one of the possible reasons is because the lines studied have incorporated mixed composition from quite diverse populations (Xia et al., 2004; Xia et al., 2005).

SSR-based genetic distances were correlated well with specific combining ability in temperate inbreds (Zheng et al., 2008) and tropical hybrids (Phumichai et al., 2008). However, evidence of correlation in inter- and intrapopulational crosses were vary. Amorim et al. (2006) reported that interpopulational crosses tend to have more significant correlation between GDs and grain yield, whereas Barbosa et al. (2006) reported low correlation at interpopulation level. Meanwhile, SSR-marker GDs are not significantly correlated with heterosis in the study of CIMMYT and IITA inbred lines (Dhliwayo et al., 2009) and Chinese inbred lines (Xu et al., 2004).

Comparison studies between markers revealed that AFLPs and SSRs found that there were significant correlation between marker-based and morphological-based GDs for both marker platforms (Beyene et al., 2005), as well as the correlation between those two...
platforms’ GDs (Beyene et al., 2005; Heckenberger et al., 2003). Both marker platforms can successfully differentiate early- from late-maturing *Striga*-resistant inbred lines (Menkir et al., 2005). Another successful result was obtained from a study with 18 tropical maize inbred lines, which reported that AFLPs, SSRs, and RFLPs’ GD were correlated with hybrid performance and heterosis in intra population crosses (Garcia et al., 2004). However, in some cases, AFLP and SSR GDs were poorly correlated (Laborda et al., 2005) and could not perform satisfying heterotic groups for both tropical maize (Laborda et al., 2005; Menkir et al., 2004). Bernardo et al. (2000) suggested that SSRs are more reliable than RFLPs to study the genetic relationships in maize.

Low correlation between GD and grain yield could be caused by small range of GD due to previous selection for combing ability (Barbosa et al., 2003). Kumar (1999) suggests that in order to obtain significant relationship between GDs and hybrid performance several conditions must be met, i.e. adequate phenotypic evaluation and choice of markers used for analysis, and sufficient number of parental inbred lines (Stuber et al. in Kumar, 1999). GD revealed by SSRs tends to show greater degree of polymorphism compared to AFLPs (Heckenberger et al., 2006). Finding an appropriate set of SSR loci is also an important consideration because it could provide similar accuracy with the use of a greater number of loci (Enoki et al., 2005).

Recently, SNPs are known as new marker technology provides automation and more precise results. Clustering of genotypes based on SSR markers has successfully differentiated the tropical from temperate maize germplasm, and provided consistency with pedigree and information and combining ability (Lu et al, 2009). Another study reported that SNP loci should be available in large number to replace highly polymorphic SSRs (Hamblin et al., 2007). Moreover, large parental genetic diversity makes precise measurement of the association among lines more difficult (Hamblin et al., 2007).

**Prospects on Developing Heterotic Groups for a Novel Breeding Population**

Heterotic groups for tropical maize are not yet as establish as those for temperate maize, and molecular markers can be a good advance to help in setting up such groups. An applicable heterotic pattern can benefit a maize breeding program for development of good-performing hybrids. Well-developed heterotic groups will allow plant breeders to acquire maximum benefit from heterosis, yet the question is whether the use of molecular markers approach for building such groups is efficient. As mentioned above, there are several studies confirming the usefulness of molecular markers approach for developing heterotic groups, despite of some unsuccessful ones. It is important to note that the heterotic pattern can vary from population to population, since such pattern is developed by breeders rather than naturally exists (Tracy and Chandler, 2006). The predictability of F, performance using a marker-aided heterotic pattern depends on the genetic background of inbred lines that one working on (Boppenmaier et al, 1993).

Other important consideration is selection of marker platform to be used. In some of the previous studies, RFLP, AFLP, and SSR markers had been reported helpful for developing heterotic groups. As markers technology advances rapidly, exploitation of the most current marker technology such as SNP for various genetic studies is interesting to many researchers. A recent methods, Genotyping by Sequencing (GBS) (Elshire, 2011), allows utilization of thousands of SNP markers for heterotic pattern studies, which may increase the probability to develop a useful one. One interesting exercise would be to estimate how many markers are needed to perform such analysis (K. Pixley, CIMMYT, pers. comm.), followed by defining a core set of SNPs that suitable for estimation of genetic distances among lines having certain adaptation (e.g. lowland tropical, temperate, or highland). With the availability of recent bioinformatics tools with capability to handle large data sets, genetic distance between inbred lines can be calculated in a timely manner.

A putative heterotic group developed using marker-based GDs can be further refined using estimates of specific combining ability (SCA) and discriminant analysis of principal components (DAPC). A hierarchical clustering using the complete linkage method can be used as DAPC’s prior information. As expected, results from the analysis based on SCA (actual) estimates were more satisfactory than that based on GD (predictive) data in terms of F, yield comparison among between- versus within groups mating (Suwarno., et al, 2014).

Previous studies suggested that marker-based GDs are correlated with heterosis and F, yield only up to a certain distance point, and after that there are no associations between those (Melchinger, 1999; Suwarno, 2014). This implies that heterosis is not expected to arise from crosses among two genetically similar lines, yet it is challenging to predict heterosis and F, yield for crosses among two genetically dissimilar lines using molecular marker information. Considering the usefulness of heterosis in hybrid maize production further investigations using more recent approaches should be conducted to better understand this phenomenon.

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References


