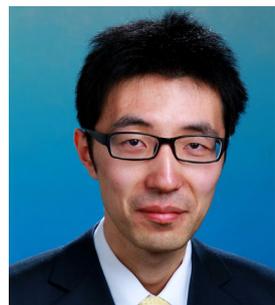


Exploring the molecular basis of heterosis for plant breeding

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Abstract Since approximate a century ago, many hybrid crops have been continually developed by crossing two inbred varieties. Owing to heterosis (hybrid vigor) in plants, these hybrids often have superior agricultural performances in yield or disease resistance succeeding their inbred parental lines. Several classical hypotheses have been proposed to explain the genetic causes of

heterosis. During recent years, many new genetics and genomics strategies have been developed and used for the identifications of heterotic genes in plants. Heterotic effects of the heterotic loci and molecular functions of the heterotic genes are being investigated in many plants such as rice, maize, sorghum, *Arabidopsis* and tomato. More and more data and knowledge coming from the molecular studies of heterotic loci and genes will serve as a valuable resource for hybrid breeding by molecular design in future. This review aims to address recent advances in our understanding of the genetic and molecular mechanisms of heterosis in plants. The remaining scientific questions on the molecular basis of heterosis and the potential applications in breeding are also proposed and discussed.

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INTRODUCTION

Heterosis, also known as hybrid vigor, refers to the phenomenon that the heterozygous hybrids show superior performance over their parents. This phenomenon is quite beneficial for plants on reproduction and adaptation to environmental changes. It is also of great importance on agriculture production, as hybrid breeding has been proved to be one of the most efficient ways to increase grain yield of various crops (Schnable and Springer 2013). For example, compared with the inbred lines, hybrid rice often has a 10%–20% rise in grain yield per unit area (Cheng et al. 2007a). The developments of the elite rice hybrids become important breakthroughs of rice modern breeding in China (Cheng et al. 2007b), e.g., the development of Shanyou63 of the three-line hybrid system since

1980s, LiangYouPeiJiu (LYP9) of two-line system since 2000s and YongYou12 of subspecies intercrossing system since 2010s. As an outcrossing plant, maize generally displayed stronger heterosis (e.g., The hybrid maize cultivars could get a 50%–100% yield rise over open-pollinated cultivars [Kutka 2011]). Consequently, hybrid breeding is very powerful in maize and nearly all commercial maize varieties are hybrid lines nowadays. Besides applications of heterosis in grain crops, hybrid breeding is also one of the great choices in many other plants including rapeseed, sunflower and tomato (Krieger et al. 2010; Girke et al. 2012; Seiler et al. 2017).

Since heterosis was found in plants more than a century ago, it has attracted many scientific researchers' attention, resulting in many classical experiments, hypotheses and discussions (Shull 1908; Bruce 1910; Jones 1917; East 1936). It needs to be clarified here that,

when crossing two inbred lines of the same species at random, the hybrid “often” shows greater vigor, but not true for all cases. The occurrence and degree of heterosis varies among different species, in which the cross-pollinated plants such as maize have more chances to show heterosis, and often show a higher degree of heterosis than the self-pollinated plants like rice. Even within the same plant species, the magnitude of heterosis is quite different as well. The hybrids from two divergent clades usually show stronger heterosis than the hybrids from two genetically close accessions (e.g., *indica-japonica* crosses > *indica-indica* crosses > *japonica-japonica* crosses) (Dwivedi et al. 1998; Melchinger 1999). However, divergent genetic backgrounds sometimes lead to reproductive isolation between two parental lines (Dopman et al. 2010; Ouyang and Zhang 2013).

With the rapid accumulation of knowledge on gene functions in plants and the advance of new genomics approaches, progress has been made on the studies of genetic and molecular basis of heterosis during recent years, although some aspects of its underlying mechanism remain enigmatic. We now provide a short review on the research strategies, recent scientific progresses and application perspectives for the molecular mechanisms of heterosis in plants.

GENETIC DISSECTION OF HETEROTIC LOCI AND GENES

To figure out the molecular underpinnings of heterosis magnitude in plants, it is crucial to characterize the gene loci responsible for heterosis through quantitative genetics methods. Different from conventional quantitative trait loci (QTL) identifications, the genetic populations for heterosis studies are usually generated

from elite hybrids, especially those widely-planted hybrid varieties with strong heterozygote advantages over their parental lines. Moreover, to facilitate the identifications of heterotic loci and the estimates of heterotic effects, the heterotic populations need to have a reasonable composition for different genotypes (that is, the heterozygous genotypes and both homozygous genotypes).

There are several types of heterotic population (Table 1). The segregation F_2 population from the elite hybrid crosses (F_1) is one of the best choices for heterosis analysis, because the F_2 population has the heterozygous genotypes (short for MF hereinafter, in which M refers to male parent allele and F refers to female parent allele) and both homozygous genotypes (short for MM and FF). The three genotypes are usually present with the proportion of 1:2:1 (MM:MF:FF) and uniform distributions of allelic combinations between different loci thus allowing for precise estimates of genetic effects. Benefiting from high-throughput sequencing technology, it now becomes feasible to genotype large F_2 populations at relatively low cost. For example, 17 populations containing totally 10,074 F_2 lines were used for searching the key heterosis-related gene loci in hybrid rice (Huang et al. 2016). The large sample size of the F_2 population and the high genotype density from whole-genome sequencing helped in a comprehensive identification of the heterotic contributors and discovery of about 16 key gene loci for heterosis in hybrid rice.

Since the F_2 population cannot be easily preserved, the “immortalized F_2 ” population was developed based on North Carolina Design III (Comstock and Robinson 1952; Gardiner et al. 1993). In the experimental designs for “immortalized F_2 ”, recombinant inbred lines (RILs) are firstly developed from one elite hybrid cross (F_1) and

Table 1. The comparison of different populations for heterotic studies

Populations for heterotic studies	Considering hybridization works, population sizes could be	Ideal genotypic ratio	Genotyped once, phenotyped repeatedly
F_2	Very high	Yes	No
Immortalized F_2	Modest	Yes	Yes
CSSL backcrossing	Modest	Yes, at target regions	Yes
RH- F_2	High	Yes, at target regions	Yes
Diverse F_1	Modest	No	Yes

subsequently well-designed pair-crosses of numerous RILs generate the “immortalized F_2 ”. Compared with commonly used F_2 populations, the “immortalized F_2 ” population can be genotyped only once but phenotyped many times in different locations through pair-crossing and growing repeatedly, thus becoming a very powerful population for heterosis studies. The designs of “immortalized F_2 ” populations have been implemented in many plants including rice (Hua et al. 2002; Hua et al. 2003; Zhou et al. 2012), maize (Tang et al. 2010; Guo et al. 2014a), oilseed rape (Chen et al. 2007), and cotton (Liu et al. 2011). In rice, the RILs from the cross between the maintainer line “Zhenshang97B” and the restorer line “Minghui63” (both parents of the elite hybrid “Shanyou63”, the classical hybrid of three-line system) was used to create the subsequent “immortalized F_2 ” population, which enabled the comprehensive evaluations of the genetic contributions of dominance, overdominance and epistatic effects across the whole rice genome (Hua et al. 2003). LiangYouPeiJiu (LYP9) from the cross between the sterile line “PA64s” and the restorer line “93-11” is a classical hybrid of two-line system in rice. Several studies using the RIL populations and the backcross population (RILBC₁, derived from crossing each RIL with PA64S) had been performed and identified several major QTLs underlying yield heterosis (Gao et al. 2013; Li et al. 2016). In maize, similar with the experimental design for the elite rice hybrid “Shanyou63”, the “immortalized F_2 ” population derived from an elite maize hybrid Yuyu22 was created (Tang et al. 2010). With a high-density bin map from a SNP genotyping chip, the genetic study in maize found that the yield heterosis in Yuyu22 relied on the cumulative effects of dominance, overdominance and epistasis, among which the dominance effect was the most important contributor to heterosis (Guo et al. 2014b).

How can we further precisely verify the genetic effects of the individual heterotic loci? One of the solutions is to construct test populations through backcrossing the chromosome segment substitution lines (CSSLs) of target gene loci with parental inbred lines. Recently, using a genetic population from crossing 184 CSSLs with two maize inbred lines (Zheng58 and Xun9058), 63 and 57 different heterotic loci were identified for four kernel traits in CSSL×Zheng58 and CSSL×Xun9058 population, respectively (Wang et al. 2018). Another way is to use the residual heterozygosity (RH) in heterosis analyses

(Yamanaka et al. 2001; Watanabe et al. 2011) (Zhuang et al. 2007). When screening the RIL populations (e.g., F_8 lines) with whole-genome high density genotypes, we can often find a few heterozygotes covering a small genomic interval. Selfing of the RILs of interest will produce the residual heterozygosity offspring (RH- F_2) segregating for the target QTL — exhibiting three genotypes only around the target loci but containing the same and homozygous genotypes in all the other genetic backgrounds. Similar to the CSSL test populations, the RH- F_2 lines can also be used in heterotic evaluations and testcrossing.

The population of diverse hybrid crosses (F_1 lines) can also be used in genetic dissection of heterotic loci. In *Arabidopsis thaliana*, totally 200 hybrids were used to characterize the genomic architecture of biomass heterosis through genome-wide association studies (GWAS), and several important heterosis candidate genes including *WUSCHEL* and *ARGOS* were found (Yang et al. 2017). In rice, many superior alleles underlying 38 agronomic traits were detected with a high-density genome map for 1,495 elite hybrid rice varieties generated (Huang et al. 2015). Moreover, using a genomic and metabolic approach for hundreds of hybrid maize lines, GWAS has been tested to dissect the underlying heterotic loci in maize and computation modeling has been applied to predict complex heterotic traits in hybrid maize (Riedelsheimer et al. 2012). Compared with F_2 , “immortalized F_2 ”, CSSL and RH- F_2 testcrossing populations, the population consisting of hybrid crosses from diverse varieties or ecotypes contains much more allelic variation and more comprehensive heterotic combinations. Moreover, GWAS can be used to investigate the heterotic loci underlying various yield traits. Hence, generally the mapping resolutions are very high in this kind of population. However, in the population of diverse F_1 lines, both the proportion of different genotypes and the distributions of allelic combinations are usually non-ideal for a precise evaluation of genetic effects of the heterotic loci. Low frequency alleles in the population could lead to a deviated genotype proportion. For example, for one heterotic gene with minor allele frequency of 10%, the proportion of three genotypes (MM, MF, FF) will not be 25%:50%:25% but 1%:18%:81%, in which it would be very difficult to precisely estimate the genetic effects of the MM genotype. Furthermore, the allelic combinations in F_1 lines are not reshuffled (no recombination between

inbred parental lines and heterotic groups). Thus, the effect estimates (e.g., dominance effects, overdominance effects) from F_1 populations may be affected by the population structures (Zhou and Huang 2019).

After the identifications of the heterotic loci, the causative genes need to be excavated. In plants including *Arabidopsis thaliana* and rice, there's already a lot of known functional information – the biological functions of many causative genes underlying complex yield traits are more and more clear, which certainly facilitates to find the candidate genes within the heterotic loci. In other plants, the characterization of causative genes for the heterotic loci is still a challenge. For the heterotic loci with dominant or recessive effects, characterizations of the causative genes are nearly the same with fine mapping and cloning of ordinary QTLs, in which CSSL or RH is used to generate advanced lines. The situation would be somewhat complicated for overdominant or pseudo-overdominant loci, because there may be only small phenotypic differences between two homozygous genotypes but large differences between the homozygous genotypes and the heterozygous genotype. However, we can expect that the functionally important causative genes, at most cases, control multiple traits simultaneously but displayed different heterotic effects. Hence, for the overdominant or pseudo-overdominant loci, the case could be simplified by choosing multiple suitable traits for mapping. Moreover, test crossing, screening and evaluation of candidate gene mutants should be very useful to detect the candidate genes (See the beautiful work in tomato (Krieger et al. 2010)).

GENETIC BASIS OF HETEROSIS

Since a century ago, the genetic basis of heterosis has been proposed and discussed (Shull 1908). There are now three major hypotheses explaining the genetic causes of heterosis based on the classical genetics model (Figure 1).

The first one is the dominance complementation hypothesis (Bruce 1910; Jones 1917). For example, two inbred lines contain the genotypes “ $A_1A_1B_1B_1$ ” and “ $A_2A_2B_2B_2$ ” at two major QTLs, respectively (Figure 1A, Note: “ A_1 ” and “ B_2 ” are the dominant alleles resulting high grain yield while “ A_2 ” and “ B_1 ” are the recessive alleles resulting low grain yield). The hybrid cross from the two parental lines will have the genotypic

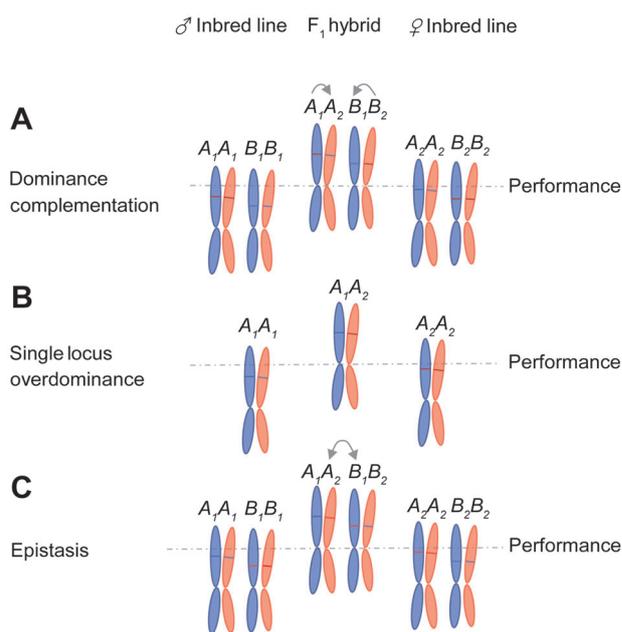


Figure 1. Genetic basis of heterosis

Three major hypotheses are supported to explain the genetic basis of heterosis based on the classical genetics model: **(A)** The dominance complementation hypothesis. Recessive deleterious alleles (A_2 and B_1 , shown as blue bars) from maternal and paternal inbred line will be complemented by the dominant superior alleles (A_1 and B_2 , shown as red bars) in F_1 hybrid (“ $A_1A_2B_1B_2$ ”), resulting in a better performance. **(B)** The single locus overdominance hypothesis. The combination of A_1 and A_2 alleles from paternal and maternal inbred line respectively leads to a superior trait in F_1 hybrid line (“ A_1A_2 ”) compared with both parents with homozygous genotypes (“ A_1A_1 ” and “ A_2A_2 ”). **(C)** Epistasis. The interaction between A and B loci (A_2 and B_1 , for example) makes the combined effect of “ $A_1A_2B_1B_2$ ” superior.

combination “ $A_1A_2B_1B_2$ ”. When supposing that the two loci (A and B) do not interact with each other and act additively, the overall effect of “ $A_1A_2B_1B_2$ ” should perform better than “ $A_1A_1B_1B_1$ ” and “ $A_2A_2B_2B_2$ ” in grain yield. There was a typical example in sorghum for this hypothesis, in which two major QTLs for plant height, *Dw3* and *qHT7.1*, have repulsion linkage between two parental lines. Further dissection showed that *Dw3* controls the height below the flag leaf while *qHT7.1* affects both the upper and lower parts (Li et al. 2015). In addition, the pseudo-overdominance hypothesis is actually the same with dominance complementation except that the two loci are tightly linked on one chromosome in pseudo-overdominance cases.

The second hypothesis is single locus overdominance (Shull 1908; East 1936), in which the heterozygous genotype “ A_1A_2 ” is superior to both homozygous genotypes (“ A_1A_1 ” and “ A_2A_2 ”) for the overall yield performance or one specific trait (e.g., one yield component) (Figure 1B). There are a few cases identified to result in single gene heterosis in tomato (Krieger et al. 2010), rice (Huang et al. 2016) and maize (Hollick and Chandler 1998). The *FT* gene is a typical example. *Flowering Locus T (FT)* in *Arabidopsis* encodes florigen (floral stimulus) and promotes the transition from vegetative growth to the flowering stage. When crossing the mutant line of *SFT* (its ortholog in tomato) with the wild-type line in tomato, strong yield overdominance could be observed (Krieger et al. 2010). Using the approach of large-scale F_2 populations in hybrid rice, the ortholog of *FT* in rice (*Hd3a*) was also found to play an important role in the heterosis of hybrids, majorly for the hybrids belonging to *O. sativa* ssp. *indica-indica* crosses of three-line system (Huang et al. 2016).

Epistasis (Minvielle 2009) (Schnell and Cockerham 1992) is the third hypothesis explaining the heterosis in plants. That is, two loci (A and B) may interact with each other, which makes the combined effect of “ $A_1A_2B_1B_2$ ” superior (Figure 1C). There is strong evidence shown that epistasis plays a significant role in heterosis for multiple traits in *Arabidopsis* (Kusterer et al. 2007), rice (Yu et al. 1997) and maize (Wolf and Hallauer 1997). In those cases, for a large number of loci, single locus based heterosis was not detected while the interactions of those loci were consistently detected.

It must be clarified here that the three models, dominance, overdominance and epistasis, are non-mutually exclusive and all involved in plant heterosis. Furthermore, it is difficult to divide heterosis into three separate proportions (that is, the contribution from dominance, overdominance, or epistasis), because the heterosis phenomenon in agriculture production is the nonlinear effect from multiple heterozygous genes combined. Hence, using genetic approaches we now need to (i) quantitatively evaluate the heterotic effects of heterozygous genotypes and (ii) make clear the regulation networks of the heterosis related genes.

In the classical model, complete dominance effect refers to a situation where the effect of “ A_1A_2 ” is equal to that of “ A_1A_1 ”, where “ A_1A_1 ” is the homozygous superior allele (superior allele – the allele which leads to more grain yield or better agricultural traits). Complete

recessive refers to “ A_1A_2 ” is equal to that of “ A_2A_2 ” (homozygous inferior allele) and complete additive refers to “ A_1A_2 ” is the average of “ A_1A_1 ” and “ A_2A_2 ”. Complete dominance means that a nonfunctional detrimental recessive allele was covered by a functional dominant allele. However, according to recent genetic studies in rice (Huang et al. 2015), most heterotic genes exhibit “partial dominance” or “partial recessive”, while complete dominance, complete recessive and complete additive effects are not common. Partial dominance or partial recessive illustrates that the alleles probably exhibit a dosage effect, all belonging to the reflections of complicated gene regulation networks (Birchler 2016). The dominance to additive variance ratio (d/a) could be used for quantitative evaluations at each single QTL through genetic analysis in the heterotic populations. Furthermore, the situation will become more complicated when meeting genetic interactions between two loci. There are nine genotypic combinations for two heterotic loci (with the proportion in F_2 populations shown 6.25%:12.5%:6.25%:12.5%:25%:12.5%:6.25%:12.5%:6.25%), the genetic effect for each genotypic combination and the interactions between them cannot be finely evaluated unless a large population is available. Currently, data and related knowledge on genetic interactions of heterotic genes are still not enough. The inclusion of more experiments on the genetic interactions in plants will extend our understanding of heterosis.

Owing to more and more high-profile omics data and functional genomics data (Sun et al. 2018; Zhao et al. 2018; Shao et al. 2019), the heterosis related allelic variation, especially the key causative variation, could be excavated, but more challenges may fall over the decoding of regulation networks of the heterosis genes. Near-isogenic lines for multiple important heterosis genes or the genome editing technology that mimics natural alleles need to be used to picture the developmental processes that underlie how the heterozygous states of genes play a role in gene regulation.

EPIGENETIC REGULATION IN HETEROSIS

Although classical genetic models have explained the genetic basis of heterosis, they are still not sufficient to predict heterosis. Epigenetic factors, including DNA methylation, small RNAs and histone modification, play

important roles in plant development and adaptation (He et al. 2011). Moreover, there is also growing evidence that epigenetics is one of the gap fillers in understanding of heterosis regulation (Groszmann et al. 2013; Greaves et al. 2015).

DNA methylation occurs in two different pathways (Law and Jacobsen 2010). *De novo* DNA methylation, known as RNA-directed DNA methylation (RdDM), is triggered by 24-nucleotide small interfering RNAs (siRNAs). The maintenance of DNA methylation, within symmetric CG and CHG context or asymmetric CHH context, is conducted by DNA METHYLTRANSFERASE 1 (MET1), CHROMOMETHYLASE 3 (CMT3) and DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2), respectively (Chan et al. 2005). Two studies reported a genome-wide DNA methylome alteration in *Arabidopsis* F₁ hybrids and indicated that DNA methylation was involved in the heterosis regulation since DNA demethylating compromised the heterosis (Greaves et al. 2012; Shen et al. 2012). Furthermore, DNA methylation defect mutants showed that DDM1 directed DNA methylation played an important role in heterosis regulation while MET1 mediated DNA methylation maintenance was not involved in this process (Kawanabe et al. 2016; Zhang et al. 2016). To overcome the confounding effect of genetic variations from both parents, the isogenic epigenetic recombinant inbred lines (epiRILs) were utilized to illustrate the potential role of DNA methylation on complex traits (Johannes et al. 2009; Cortijo et al. 2014; Lauss et al. 2018).

The accumulation of 24-nt small RNAs was reported to decrease in F₁ hybrids of *Arabidopsis*, rice or maize (He et al. 2010; Groszmann et al. 2011; Barber et al. 2012), which indicates the potential role of siRNAs in heterosis. However, RNA polymerase IV, a key enzyme for generating 24-nt siRNAs, was shown to be not involved in heterosis of *Arabidopsis* (Kawanabe et al. 2016). In addition, miRNA expression and histone modification also changes in F₁ hybrids (Ni et al. 2009; Chen et al. 2010; He et al. 2010). However, it is not easy to tell that those changes in epigenetic modifications are the causes or the results of heterosis.

MOLECULAR MECHANISM OF VARIOUS HETEROTIC EFFECTS

Although the gene loci controlling heterosis are becoming clearer and the classical hypotheses are

being confirmed by many cases of specific genes, there remain several unsolved basic questions for heterosis in plants from the view of modern molecular genetics. We know that the occurrences of dominance and recessive effects, either partial or complete ($-1 \leq d/a \leq 1$), are readily comprehensible based on the “gene dosage effects”. However, why do a few genes at the heterozygous states show overdominance effects? According to the overdominance case *IPA1*, the gene with overdominance effects can control several yield components (Huang et al. 2016). The superior alleles for specific components may differ from each other (e.g., the reference allele can produce more panicles while the other allele has better performances for grain number per panicle). For each yield component and each sub-factor, the gene display partial dominance effects. Because the composite trait “grain yield per plant” or “grain yield per unit area” is made up of multiple components and sub-factors, the overdominance effect appears. However, for those genes which display the overdominance effect for a specific component or sub-factor, a reasonable explanation could be that the heterotic effects are resulted from an optimal gene expression level although in-depth mechanisms on the gene regulation networks are still unclear. Further dissections to this question may rely on more molecular mechanisms revealed with physiological and developmental biology approaches.

Another question is, why the gene loci showing partial dominance effects (broadly speaking, $d/a > 0$) are much more than those of partial recessive effects (broadly speaking, $d/a < 0$) in number, especially for the QTLs controlling plant height and biomass. This trend could be found in many large-scale genetic studies (Hua et al. 2002; Tang et al. 2010; Zhou et al. 2012; Huang et al. 2015; Huang et al. 2016). Based on the “dominant complementation” hypothesis, the wild-type allele should be dominant while the mutant allele (often belonging to frameshift indels or some other large-effect mutations causing non-functional gene products) is recessive. Usually, at the heterozygous state, one copy of wild-type allele is nearly enough for the gene to take the biological functions. However, for some cases, the mutant allele may be the dominant one. For example, *Twisted dwarf1-1* mutation is semi-dominant for dwarfism and twisted growth in rice (Sunohara et al. 2009). Even assuming that one copy of wild-type allele is enough for taking functions, the partial dominance

effect also needs another assumed condition – the genes should be “positive regulators”. Although many QTL genes controlling plant height and grain yield are the “positive regulators” involved in gibberellin pathway or flowering time pathway (Davière and Achard 2013; Song et al. 2015), but there are also a number of exceptions in which the genes are “negative regulators” (for example, the wild-type functional *Gn1a* reduces grain number (Ashikari et al. 2005). Hence, either being positive/negative regulator for the gene or being dominant/recessive for the wild-type functional allele may not be the critical point. We guess that the critical point might be natural and human selections for hybrid plants. The hybrids with better performances in plant height and biomass have more chances to be selected for rising up seeds in natural conditions and selected for experimental studies of heterosis. In crop breeding, the selections must be more intensive – there are a large number of hybrid crosses to be generated for agriculture production testing, and only the hybrid crosses with strong heterosis performances will be reserved and become widely-planted hybrid varieties. As a result, the gene loci showing partial recessive effects (broadly speaking, $d/a < 0$) would tend to be homozygous genotypes in hybrids for genetic experiments on heterosis.

The third question is whether the heterosis related genes and pathways are conserved among various plants. In the other words, is it possible that a key gene controlling strong heterosis in one plant species can be applied in some other plants for hybrid breeding (e.g., by searching or creating the alleles of the heterotic gene ortholog and test crossing)? In tomatoes, it has been proved that overdominance of yield per plant from *SFT* heterozygosity is robust, which could appear in different genetic backgrounds and different environments (Krieger et al. 2010). *Hd3a*, the *SFT* homolog gene in rice, plays a very important role in hybrid rice yield as well (Huang et al. 2016). The heterozygous state of *Hd3a* gene showed an advantage in grain number per panicle, seed setting rate and grain yield per plant. In *indica-indica* crosses of three-line system (the most planted hybrid rice system), nearly all paternal lines harbored the wild type *Hd3a*, while most maternal lines carried the mutant allele (*hd3a*, a two-base-pair substitution leading to an amino acid substitution) (Huang et al. 2016). The underlying mechanism is still not very clear. The studies of molecular functions of this

gene had focused on its role as floral stimulus to promote plant flowering. A recent study demonstrated that *Hd3a* can also promote lateral branching (Tsuji et al. 2015), thus affecting panicle development and number of grains or fruits. We guess that, the heterosis related genes are likely to be the key regulation nodes for the pathways involved in flowering time, plant architecture and panicle developments, some of which should be conserved in plants (especially in the graminaceous crops). We may see more cases and evidence in the next few years. Moreover, it is still not clear that during the polyploidization events whether the conserved genes play a role in this process, although the underlying mechanism might be more complicated.

FUTURE PERSPECTIVES ON BREEDING

In plants, it is hoped that the knowledge derived from exploring the molecular basis of heterosis will be applied in agricultural breeding, including the genetic improvements of parental lines and the creation of superior hybrids efficiently by optimizing the designs of breeding (Figure 2). In conventional breeding, breeders usually have to screen a large number of hybrid crosses to obtain those with ideal yield performance. However, only a very small fraction of the tested hybrid crosses would be eligible as elite hybrid varieties. When the heterotic loci, the causative heterotic genes and even the causative variations are known clearly, it will be possible to analyze the genotypes of the key loci across the collection of parental lines and narrow down the potential hybrid combinations with strong heterosis performances ahead of time. Once the genotype-based predictions achieve relatively high levels of accuracy, the labor cost and the time cost of hybrid breeding will be greatly reduced. Certainly, the major difficulties in hybrid crop breeding by molecular design might be the complicated genetic interactions among the heterotic loci and the influence from many minor effect genes across the genome. In maize, predictions of complex heterotic traits using genome-wide and metabolome-wide data has been performed and applied into hybrid breeding (Riedelsheimer et al. 2012). A potential direction in future aiming to achieve stronger prediction power may be involved in the wide use of deep learning methods coupled with large-scale well-designed genetic data and in-depth biological insights of the heterotic genes.

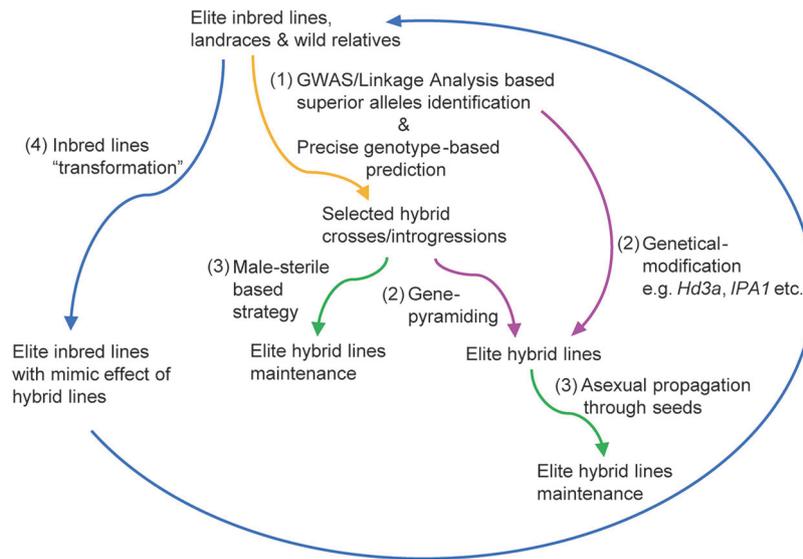


Figure 2. Future perspectives on breeding

(1) After superior alleles identified with GWAS or linkage analysis approaches and comprehensive understanding of the molecular mechanism of heterosis genes achieved, it will be possible to narrow down the potential hybrid combinations with strong heterosis performances by precise genotype-based prediction. (2) Elite hybrid lines could be generated by gene-pyramiding or genetical modification (genome-editing or transgenic technology) in selected hybrid crosses or introgressions events. (3) Those elite hybrid lines could be maintained by either traditional male-sterile based strategy or recently-developed approach – asexual propagation through seeds. (4) Furthermore, it would be possible to “transform” inbred lines into elite lines that perform equally with elite hybrid lines by mimicking heterosis effects of various loci in future.

Another opportunity in hybrid breeding is to implement the gene pyramiding for the superior alleles from multiple heterotic groups. In maize, there are many well-known heterotic groups including Reid, Lancaster, and Sipingtou (Wu et al. 2014). Situations in rice are quite similar to this. For example, there are at least three major cross systems – *indica-indica* rice crosses of three-line system, *indica-indica* rice crosses of two-line system, and *indica-japonica* rice crosses. According to the results from the genetic studies in all three types of hybrid rice (Huang et al. 2016), there exist several key heterotic genes conserved within the same combination types, but there is no single heterotic gene robust for all rice hybrid combination types. The superior alleles among different heterotic groups can be shared to further increase the degree of heterosis. Furthermore, we can even try to “transplant” the heterotic gene in one crop species into another crop. For example, *IPA1* and *hd3a* have been proved to be important heterotic genes in rice (Huang et al. 2016), so it is possible to apply them from Asian cultivated rice (*O. sativa*) to African cultivated rice (*O. glaberrima*), or even

to other crops, through genome editing or natural alleles screening.

In self-pollinated crops, the application of heterosis in agriculture greatly relies on the developments of male sterility technologies since artificial emasculation is labor-intensive. In China, three-line hybrid rice system depending on cytoplasmic male sterility and the two-line system depending on environmentally sensitive male sterility were successfully developed and applied (Li et al. 2007). Key genes and the molecular mechanisms for cytoplasmic male sterility and environmental-sensitive genic male sterility in rice were also well illuminated (Fujii and Toriyama 2009; Ding et al. 2012; Luo et al. 2013; Chen and Liu 2014; Zhou et al. 2014; Fan et al. 2016). Excitingly, an agricultural dream for maintaining heterosis through the one-line hybrid rice system has been proved to be feasible very recently (Khanday et al. 2019; Wang et al. 2019). In these two independent studies, MiMe approach (d’Erfurth et al. 2009) was utilized to substitute meiosis to mitosis which resulted in diploid gametes without recombination. Furthermore, ectopic expression of *BBM1* in egg

cell or knock out of *MTL* gene could induce formation of haploid seeds (parthenogenesis). Combination of MiMe and parthenogenesis make it possible to propagate diploid clonal seeds with heterozygosity of F_1 hybrid rice retained.

One step further, with more and more comprehensive molecular knowledge on heterosis, is it possible to create inbred lines in future whose yield performance are close to elite hybrid lines but without the need of hybrid crossing? We believe that it would be achievable even though it's hard to make it happen. Many QTLs show partial dominance effects, so the inbred lines with homozygous superior alleles would perform better than the hybrid lines with heterozygous alleles. For the overdominant genes, new alleles (e.g., with ideal expression levels or patterns) exceeding the performances of currently existing alleles could be created to mimic the effects of heterozygous genotypes. As for pseudo-overdominance or linkage drags, it is expected that suitable recombinants could be generated with samples large enough. The remaining challenge is the complexification of epistatic (or, generally speaking, the effects from genetic backgrounds) – the combined effects of multiple QTLs are not completely additive and linear, and a slight change in one heterotic gene may affect genetically interacted genes and then alter the performances as a whole. However, we believe a better understanding of heterosis and complex yield traits in plants will help to bring out new strategies for handling the complicated interactions and develop better breeding platforms and better plant varieties to meet global food security.

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