iScience

CellPress
OPEN ACCESS

Review

Toward understanding and utilizing crop heterosis in the age of biotechnology

Wenwen Liu,[1,2] Guangming He,[1,*] and Xing Wang Deng[1,2,*]

SUMMARY

Heterosis, a universal phenomenon in nature, mainly reflected in the superior productivity, quality, and fitness of F_1 hybrids compared with their inbred parents, has been exploited in agriculture and greatly benefited human society in terms of food security. However, the flexible and efficient utilization of heterosis has remained a challenge in hybrid breeding systems because of the limitations of "three-line" and "two-line" methods. In the past two decades, rapidly developed biotechnologies have provided unprecedented conveniences for both understanding and utilizing heterosis. Notably, "third-generation" (3G) hybrid breeding technology together with high-throughput sequencing and gene editing greatly promoted the efficiency of hybrid breeding. Here, we review emerging ideas about the genetic or molecular mechanisms of heterosis and the development of 3G hybrid breeding system in the age of biotechnology. In addition, we summarized opportunities and challenges for optimal heterosis utilization in the future.

INTRODUCTION

Heterosis is a prevalent genetic phenomenon observed in diverse species. It refers to the better performance of F_1 hybrids compared with their inbred parents in terms of growth rate, yield, quality, and dealing with unsuitable growth conditions.[1] Although the utilization of heterosis in major food crops, including maize and rice, has greatly improved grain yield in the past,[2,3] the exact mechanism of heterosis has been puzzling, which has hindered the efficient exploitation of heterosis. Dominance, overdominance, and epistasis have been proposed as potential genetic reasons for the heterotic traits of F_1 hybrids. However, only limited heterosis loci functioning in these modes have been uncovered through traditional genetic approaches. Currently, with the development of convenient molecular detection and quantification technologies, these genetic hypotheses have been supported at the molecular level for many traits among diverse species.[4–8] In particular, population genetic studies on heterosis performance variation provided new insights into the genetic basis of heterosis and the principles for parental selection in hybrid breeding.[9,10] Recent studies integrating new tools such as genome-wide and transcriptome-wide association studies,[10–14] long-read sequencing,[15,16] and three-dimensional (3D) genome scanning[17] have uncovered many genetic loci for genomics-based hybrid breeding. Strategies like gene regulatory network analysis[8] and single-cell transcriptome profiling[18,19] have dug out the key gene expression changes behind heterosis. Moreover, the new information on the molecular mechanisms underlying male sterility and new biotechnological strategies also greatly promoted the efficiency of heterosis utilization. Notably, by combining molecular biology tools and traditional breeding theories, the third-generation (3G) hybrid breeding system was developed to overcome the disadvantages of prior "three-line" and "two-line" hybrid breeding systems and has become the most efficient method for hybrid production.[20–23] The 3G system introduced a smart maintainer line, self-crossing of which can produce nontransgenic recessive nuclear male sterile seeds that are easily distinguished by seed features. Together with gene editing tools, genetic transformation, and integrative genomics-based molecular design, the 3G system can be applied flexibly in various species, and the future for hybrid breeding appears to be promising, where excellent hybrids with good traits can be generated conveniently and securely to meet the food needs of humans with lower costs of labor and agricultural resources. In this review, we summarize recent progresses toward understanding and utilizing heterosis catalyzed by new biotechnologies and emphasize the development of 3G hybrid breeding as well as its challenges and opportunities in the future.

Deeper understanding of genetic basis underlying heterosis

The genetic and molecular bases of heterosis have been widely characterized in the past two decades with the rapid development of high-throughput sequencing techniques and bioinformatics tools. Many innovative strategies have been found to be effective for dissecting the mechanism of heterosis. How heterosis is generated has been explored at multiple levels of biology, including the community, population, organism, organ, tissue, and single cell (Figure 1A). Multiomics profiles such as those of the genome,[10,12,13,24–27] epigenome,[28–37] transcriptome,[8,28,29,31,33,38–41] proteome,[42,43] and metabolome[37,42] of hybrid combinations in different kinds of species have revealed

[1]School of Advanced Agricultural Sciences and School of Life Sciences, State Key Laboratory of Protein and Plant Gene Research, Peking-Tsinghua Center for Life Sciences, Peking University, Beijing 100871, China
[2]National Key Laboratory of Wheat Improvement, Peking University Institute of Advanced Agricultural Sciences, Shandong Laboratory of Advanced Agricultural Sciences in Weifang, Weifang, Shandong 261325, China
*Correspondence: heguangming@pku.edu.cn (G.H.), deng@pku.edu.cn (X.W.D.)
https://doi.org/10.1016/j.isci.2024.108901

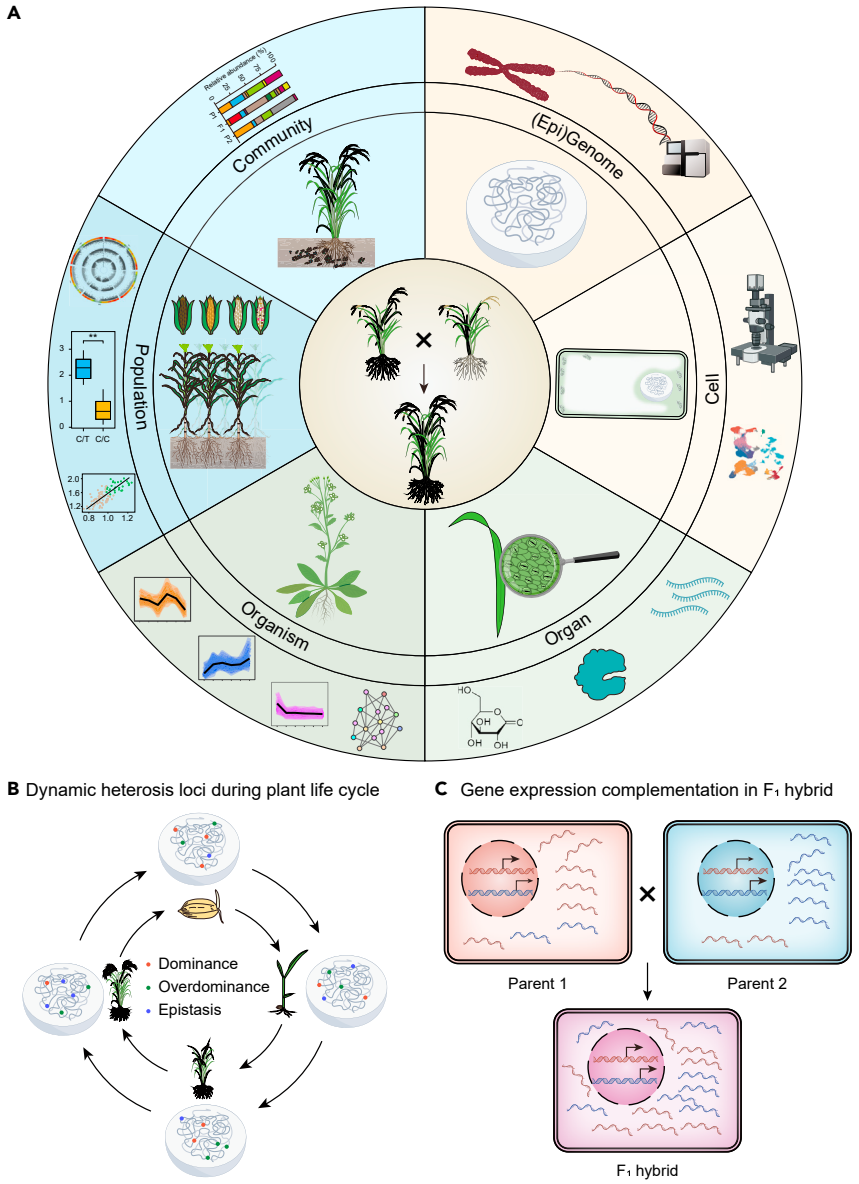

Figure 1. Mechanisms underlying heterosis revealed by novel tools and strategies

(A) Combined new biotechnologies and innovative strategies provide insights into the underpinnings of heterosis from multiple levels of life, including single cells, organs, organisms, populations, and communities, and accelerate the mechanism-guided utilization of heterosis.

(B) Dynamically altered functional loci for heterotic traits during plant development identified by genomic and genetic tools.

(C) Gene expression complementation of different biological pathways in F_1 hybrid as an important reason for heterotic phenotypes revealed by extensive gene expression profiles.

some important biological pathways or key genes associated with heterosis. According to these results, the underpinnings of heterosis appear to vary for different combinations and traits. However, some of these studies provided insight into the molecular regulation of gene actions related to heterosis in hybrids.

One of the most fundamental and important tools for understanding heterosis is long-read genome sequencing. It has facilitated the completion of high-quality genome sequences and collection of annotation information for inbred lines in several crops and vegetables.[15] Accurate genetic variations, such as SNPs, insertions or deletions, structural variants (SVs), presence/absence variations, inversions, and translocations, identified between the two parental genomes in hybrids provide detailed information on the genetic basis of heterosis.[15,16] For example, pangenome construction of maize founder inbred lines revealed a predominant role of SVs in heterosis, where strong positive correlations were observed between the yield better-parent heterosis of diallel-cross F_1 hybrids and the SV number between the parental lines, emphasizing the contribution of genetic complementation to heterosis.[15] The highly accurate maize genome also enables the identification

of novel genes responsible for yield heterosis via overdominance.[15] In addition to the comparative genomics strategy, genomic scanning of the diallel-cross population was also proven to be helpful in understanding heterosis and determining heterosis loci. For instance, why various heterosis levels are observed among hybrids from intraspecific strain crosses has remained elusive. Studies using a unique design of half-sibling F_1 hybrid populations uncovered the underlying molecular regulatory basis and identified key genetic components.[10,11] In *Arabidopsis*, new information regarding heterosis regulation was obtained by association studies on the transcriptomic landscape of a half-sibling hybrid population using transcriptome-wide association studies and expression quantitative trait loci (eQTL).[11] How substitution of half the parental genome in the hybrid generates different degrees of leaf size heterosis was found to be attributable to a distinctly increased number of photosynthetic cells due to the expression upregulation degree of cell cycle genes, where the expression change in the F_1 hybrid with high-level heterosis was found to be optimized by superior heterozygous eQTL accumulation.[11] In maize, through a design of multiple linked F_1 populations containing 42,840 F_1 hybrids and genome-wide association study, an instance of epistasis was revealed where one recessive, deleterious maternal allele, *Brachytic2,* repressed the favorable *Ubiquitin3* locus in the maternal lines, while the paternal allele alleviated this repression and thus recovered plant height and ear weight in hybrids.[10] The genomic architectures of heterosis in rice, maize, wheat, and other crops were also characterized using the population-based method,[6,7,12,13,26,27,44] which uncovered many novel loci functioning in heterosis via dominance, overdominance, and epistasis. These loci were dynamically predominant for different heterotic traits during plant life cycle and could serve as important references for genotype-based parent selection in hybrid breeding (Figure 1B). One excellent practice of identifying and utilizing heterotic loci in crop breeding is RiceNavi, which identified heterosis-related superior alleles in rice and could be used to improve parental lines to achieve stronger heterosis in hybrid by well-designed crossing.[45] Moreover, comparison of dynamic 3D chromatin architecture between hybrid and parents using 3D genomics approaches like high-throughput chromosome conformation capture was also proven to be effective in heterosis mechanism dissection. For example, in *Brassica napus*, it was found that F_1 hybrids with superior heterosis tended to contain more transcriptionally active A compartments than hybrids with inferior heterosis, which correlated with the genetic variance among parents to influence plant growth-related gene expression and contributed to leaf size heterosis.[17]

In addition to genomic analysis, novel sequencing technologies and bioinformatic analysis approaches were also used to uncover gene expression regulation in hybrids and its roles in heterotic traits. Differential analysis of transcripts and proteins in various kinds of organs in hybrids compared to parents revealed relationships between gene expression regulation and heterotic phenotypes.[8,31,36,37,42] Epigenetic comparison between hybrids and parents also indicated chromosome modification changes correlated with gene expression changes.[28–31,33,36] Notably, molecular-level complementation was found to be a common reason for hybrid gene expression performance[38,39,46–48] and a key driver of the heterotic phenotype (Figure 1C).[8] Gene regulatory network analysis in *Arabidopsis* showed that the regulatory network changes in hybrid compared to parents and the network hub gene expression complementation between different biological pathways occurred in hybrid during different developmental periods and in different organs contribute to biomass heterosis.[8] This study provided an example where high-resolution spatial-temporal omics profiling of hybrids and their parents for a specific heterotic trait was necessary for understanding the complex and dynamic regulation of hybrid vigor.

A deeper understanding of the molecular mechanisms underlying heterosis requires improving the accuracy and specificity of phenotypic and gene expression regulation analyses in hybrids at the organ, tissue, and even single-cell levels. Recently, tissue-level comparisons between hybrids and parents were performed using a new design with experimental methods, such as *in situ* staining and microscopy techniques. For instance, GUS staining of leaves of *Arabidopsis* mitotic cell division reporter lines realized *in situ* visualization for the area difference of mitotic cell division regions between hybrid and parents, demonstrating the roles of cell division and cell number increase in leaf area heterosis formation.[11] Moreover, high-throughput microscopic examination also uncovered the cellular mechanisms for biomass heterosis,[11] in particular the exact roles of cell size and cell number, where cell number increase was shown to make a prevalent contribution to leaf size heterosis. In addition, the cellular mechanism for plant heterosis was also investigated using single-cell sequencing technologies.[18,19] In one study, single-cell RNA sequencing (RNA-seq) was used to investigate the allelic expression patterns in mesophyll cells of two rice subspecies and their reciprocal F_1 hybrids, and pervasive monoallelic gene expression was observed in individual mesophyll cells.[19] In another study, single-nucleus RNA-seq of caryopses from a rice hybrid combination revealed allele-specific expression in the endosperm and transcriptional divergence in each endosperm cell type between F_1 and its parents.[18] These finer transcriptional differences at the single-cell level offered novel insights into heterosis from the perspective of the basic unit of an organism.

In addition to regulation via the growth and development processes of the plant itself, plant heterosis for root biomass or biotic stress defense was also found to be affected by its ecological interaction with the belowground microbial environment.[49,50] A study using amplicon sequencing of bacterial 16S rDNA and fungal internal transcribed spacer quantified the rhizosphere microbiome of a maize combination and found differences in the composition of bacteria and fungi between the hybrid and parents.[51] Furthermore, root biomass heterosis in maize was found to be influenced by the belowground microbial environment,[49] where heterosis can be observed under inoculation with a synthetic community of seven bacterial strains but not with growth under sterile conditions, indicating a contribution of microbiome composition to heterosis formation. In addition, the rice hybrid variety LYP9 was found to exhibit heterosis in the diversity and composition of the root microbiota, where the root-derived bacterial community in the hybrid but not the parents could protect rice seedlings against pathogens.[50] Understanding the genetic mechanisms behind these ecological phenomena would provide additional novel guidance for yield and resistance improvement in hybrid breeding.

Taken together, these findings supporting a progressive understanding of heterosis have led to the development of fundamental theory for better heterosis utilization. Although no consensus has been reached for heterosis mechanisms, all these findings consistently proved that not all heterozygous loci are involved in heterosis and that ingenious experimental designs and analysis strategies are helpful for identifying

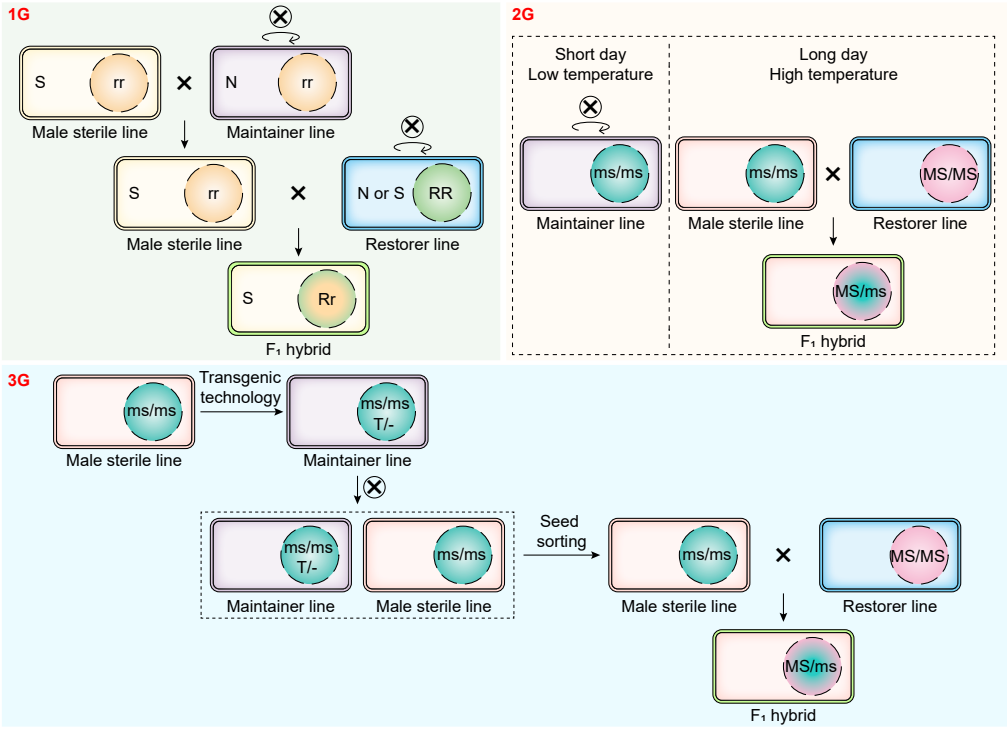

Figure 2. Development of the 3G hybrid seed production method in crops with new biotechnologies

Schematic representation of the procedure of hybrid breeding methods (1G, 2G, and 3G). R or MS, functional (dominant) male fertility restorer gene; r or ms, nonfunctional (recessive) male fertility restorer gene; S, sterile cytoplasm; N, normal cytoplasm; T, transgenic cassette.

the genetic loci that are dynamic for different heterotic traits. Notably, the integrated data and conclusions in these studies provide useful resources and guidelines for parent selection in practical hybrid breeding for the achievement of ideal crops.

Biotechnological approaches for utilizing heterosis in crop breeding

Since the successful utilization of hybrid breeding in maize production in the 1930s,[2,52] heterosis has been widely exploited in diverse crops, ensuring worldwide food yield and security. Because heterosis is exhibited only in the F_1 hybrid generation and not in later offspring, the means of F_1 hybrid seed production is crossing between parental inbred lines. Crossing is relatively easy for diclinous plant species with stamens and pistils in separate flowers, such as maize. However, for monoclinous plants that have stamens and pistils in the same flower, such as rice and wheat, artificial emasculation is the core-limiting factor for the mass production of F_1 seeds by machines. The best solution to this problem is using a male-sterile line as the maternal parent for cross-pollination. Nevertheless, the production of male-sterile line seeds is also a large difficulty in hybrid breeding practices. For resolving this issue, "three-line" and "two-line" hybrid breeding methods have been proposed and implemented in main crops and other species.[53–55] The "three-line" method refers to the cytoplasmic male sterility (CMS) system and is also called the 1G (1st generation) hybrid breeding system. The CMS line has a mutation in the mitochondrial genome, so its male sterility is inherited as a dominant, maternally transmitted trait. Thus, a CMS line (♀) and CMS maintainer line (♂) are used for the production of CMS line seeds, while a CMS line (♀) and CMS restorer line (♂) are used for F_1 hybrid seed production (Figure 2). This method first solved the problems in the production of male-sterile lines and in the recovery of sterility in F_1 hybrids and therefore has been most widely used in the production of hybrid varieties in crops such as rice. However, this system relies heavily on the availability of CMS maintainer and restorer lines, so there is a real lack of flexibility in applying it in any parental variety to breed hybrids with desirable traits. Meanwhile, the CMS mutation might be associated with a yield penalty and affect the performance of hybrid. This complex breeding procedure of the "three-line" method was simplified by the "two-line" method, which refers to the photoperiod/thermosensitive genic male sterility system, which was also called the 2G (2nd generation) hybrid breeding system. This system introduced a photoperiod/thermosensitive recessive GMS line that acts as either a sterility line or a maintainer line depending on the photoperiod/thermal conditions of the planting environment during seed propagation. The GMS line is generated by self-pollination under short-day and low-temperature conditions when fertility occurs, while the GMS line is sterile under long-day and high-temperature conditions and thus can be used as a maternal parent (♀) to breed F_1 hybrids by crossing with other varieties with normal fertility (♂) (Figure 2). However, in hybrid breeding practices, the photoperiod/thermal conditions of the natural environment are not always controllable and predictable. Meanwhile, the threshold of temperature for sterile/maintainer line switch is sometimes changeable. Those risks may lead to unstable yields of sterile line seeds, low purities of hybrid seeds, or even substantial negative impacts on breeding security in severe cases.

Fortunately, the intrinsic limitations of 1G and 2G hybrid breeding systems have been addressed recently because of the development of the 3G hybrid breeding system (Figure 2). The 3G system allowed the propagation of a stable recessive nuclear male sterility (NMS) line by a molecular design-based transgenic approach that can produce a nontransgenic male sterility line and thus a nontransgenic F_1 hybrid (Figure 2). Briefly, a smart maintainer line can be generated by transforming a homozygous recessive NMS plant with a cassette containing three functional elements: the corresponding fertility restorer gene, a pollen killer gene that specifically "kills" the transgenic pollen, and a color marker gene for seed sorting. Then, self-crossing of the transgenic maintainer plant can yield 50% transgenic fertile seeds and 50% nontransgenic sterile seeds that are distinct in color.[20,22,23] Finally, using a color-based seed sorting machine, these fertile and sterile seeds can be easily distinguished (Figure 2). Fertile seeds can be used for propagating the maintainer line itself, while sterile seeds can be used for breeding nontransgenic hybrids. The core genetic design using the pollen lethality gene in the 3G concept was first proposed by PLANT GENETIC SYSTEM in 1993,[56] and then the color-based sorting design essential for 3G system establishment was proposed in 2002, which provided a valuable theoretical basis for the 3G practice.[57] In 2006, this molecular design-mediated breeding technology was first successfully utilized in maize and called Seed Production Technology by PIONEER-HI-BRED INTERNATIONAL.[58] The 3G system was first proven effective in rice in 2010,[20] which offered great possibilities for the effective employment of distinct NMS mutations and numerous excellent rice cultivars to breed ideal hybrid rice varieties with combinations of good traits, including high yield, good quality, multiple resistance, and fitness in various environments. Currently, because of the development of CRISPR-Cas9 technology and the identification of different recessive NMS genes, the 3G system and its derived NMS propagation methods have been widely improved in the three main crops maize, rice, and wheat,[21,59–61] and have also been developed or are under exploration in other plant species such as soybean,[62] tomato,[63] and alfalfa.[64]

The core technology of the 3G system includes identifying stable male sterility mutations, determining the corresponding fertility restorer gene, and designing a construct with an applicable transgenic cassette. Any breakthroughs in these aspects are important for the application of the 3G system in crop breeding progress. With the improvement of genetic engineering and gene editing technologies in plants, many stable recessive nuclear male sterility genes and their fertility restorer genes have recently been characterized in many crops. The mutation of these genes impacts male gametophyte development, so the stamens of mutants cannot produce functional pollen, which provides valuable resources for hybrid seed production in crops using the 3G system. Another example of key progress in 3G system application is the biotechnology needed to propagate the stable recessive nuclear male sterility lines, which mainly relies on the identification of effective pollen killer genes and color marker genes for seed sorting. Progress in this aspect was recently accelerated by gene function studies in different plant species. The pollen killer gene is an extremely important module that specifically "kills" transgenic pollen to generate nontransgenic NMS seeds and prevents the transmission of transgenic components to the environment. The selection of an appropriate gene and promoter is essential for the successful function of the pollen killer gene and therefore the security of breeding and the purity of NMS seeds. Studies on pollen development-related genes and their promoters[65–67] provide an important reference for pollen killer gene expression cassette design. In particular, a multicontrol sterility system was proven effective in securing male sterility lines and hybrid seed production in maize.[68] The multicontrol system transforms the NMS plant with a construct containing five functional modules, namely, the fertility restorer gene, two pollen-killer genes, the red fluorescence protein-encoding gene, and an herbicide resistance gene.[68] The function of dual pollen-killer genes is to efficiently devitalize transgenic pollen and thus greatly reduce the transgene transmission rate and the transgene flow risk, ensuring high purity of NMS seeds and hybrid seeds. This strategy serves as a good example of 3G system improvement in other crops. In terms of transgenic/nontransgenic seed sorting, the red fluorescence protein (*RFP*) gene was first utilized to mark transgenic seeds.[58] However, in some cases, the *RFP* signals are too weak to be detected in practical breeding, leading to impure NMS seeds mixed with maintainer seeds. Recently, some emerging innovations have proven efficient in solving this problem.[69] In rice, a weight-based seed sorting system was constructed by inhibiting the expression of *OsAGPL2* and *OsAGPS2* encoding ADP-glucose pyrophosphorylase, which is essential for endosperm starch biosynthesis, via endosperm-specific expression of artificial miRNA. Then, the NMS seeds (with normal endosperm and heavy weight) and the transgenic maintainer seeds (with shrunken endosperm and light weight) can be distinguished efficiently and accurately by weight-sorting machines. This method has obvious advantages in increasing the purity of NMS seeds and transgenic security of 3G hybrid rice technology. In addition, in wheat, seed sorting was improved by a method using a gene from blue-grained wheat,[21,70] where the transgenic maintainer line seeds appear blue, obviously different from the nontransgenic NMS seeds. In addition, the use of the herbicide resistance *Bar* gene and anthocyanin synthesis gene was also proven effective.[63,71] These innovations circumvented the weakness of the *RFP* gene and offered an important reference for seed sorting strategies in other crops.

In short, the 3G method overcomes the problems of both the 1G and 2G methods and displays obvious advantages in terms of male-sterile seed production and breeding efficiency. Its high utilization efficiency of abundant crop germplasm resources and high security of the hybrid breeding process significantly influence hybrid seed production worldwide. With advances in biotechnologies, 3G is fulfilling its potential to breed excellent hybrid crops with high yield and multiple resistance. Moreover, the simple procedure of the 3G method offers valuable references for hybrid breeding of self-pollinated crops in which the "three-line" and "two-line" methods cannot be applied. Crop domestication and breeding has generated considerable varieties readily available for heterosis utilization in hybrid breeding. The 3G method indeed provides a simple and high-efficient procedure method for commercial hybrid seed production with flexible selection of parental lines, but the selection of the most suitable parents for applying the 3G method to breed excellent new hybrid varieties with ideal traits by experimental tests is still time-consuming. Thus, an efficient tool to aid in selecting parents for crossing is important for hybrid breeding. Recently, increased knowledge of heterosis mechanisms and developed bioinformatics tools have provided possibilities to resolve this problem by accurate prediction of heterosis performance, in which large-scale multiomics data or hybrid population data play important roles.[10,11,45,72–74] Recently, a deep neural network-based tool for genomic prediction using multiomics data in plant was constructed,[75] improving the prediction ability in

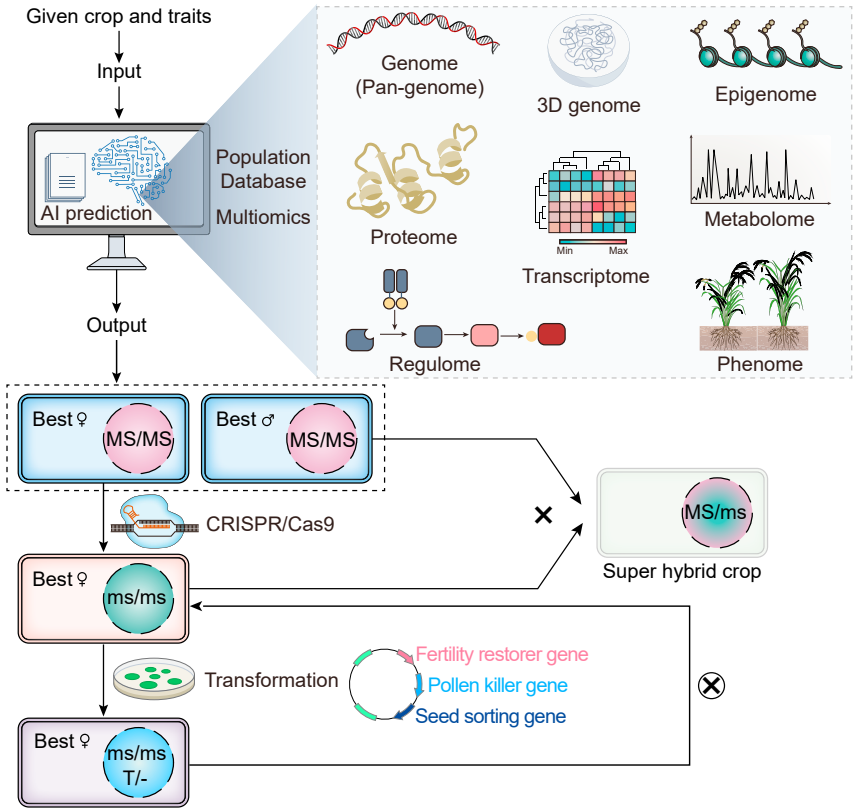

Figure 3. Heterosis utilization: a new age is coming

An efficient hybrid breeding procedure proposed based on the opportunities provided by the new age of biotechnology. For a given crop and desirable traits, large-scale information such as multiomics profiles or population breeding data in databases could enable selection of the best parents for crossing by accurate prediction. Then, an NMS plant could be generated from the best maternal parent through CRISPR-Cas9 technology, and transformation of the core 3G transgenic cassette into the NMS mutant could yield an effective maintainer line. Finally, an ideal, excellent hybrid could be mass produced by crossing the best NMS line (♀) and the best restorer line (♂). MS, functional (dominant) male fertility restorer gene; ms, nonfunctional (recessive) male fertility restorer gene; T, transgenic cassette.

genomic selection for molecular design breeding. The optimized deep learning method of this tool also offers an important reference for the analysis of complex relationships between heterotic genotypes and heterosis phenotypes and for the prediction of heterosis performance to realize smart parent selection in hybrid breeding.

In addition, some dominant NMS genes were recently identified,[76–80] which provides novel opportunities to develop new hybridization breeding systems to improve the yield of hybrid crops. Meanwhile, genetic manipulation technologies, including CRISPR-Cas9-mediated gene editing and ectopic gene expression, allowed exploration for fixing heterosis and cloning F_1 hybrid seeds in crops by "one-line" method through apomixis without meiosis and fertilization,[81–83] which is an important breakthrough in the field of heterosis utilization and hybrid seed production. High-efficiency apomixis is essential for stable yield of clonal hybrid seeds. In "one-line" method, genetic manipulation technologies could either be used to achieve the key step to convert meiosis to mitosis through induction of the triple *MiMe* (mitosis instead of meiosis) mutation or be used to explore novel strategies to improve the clonal propagation efficiency.

Heterosis utilization in the future: Challenges and opportunities

The progress in both understanding and utilizing heterosis in the past two decades has significantly promoted hybrid breeding practices to a new age. In particular, the establishment and innovation of the 3G hybrid breeding system have enabled efficient propagation of stable recessive male sterility in various plant species. With numerous studies on crop genomes and rapidly updated new biotechnologies, such as CRISPR-Cas9 technology, great opportunities have emerged for the improvement of the 3G system and the generation of NMS mutants in diverse germplasms of different crops,[84–86] which could greatly accelerate breeding progression soon. Meanwhile, based on the yield heterosis-related alleles identified by previous studies, gene editing could also help to modify those alleles for improving cross-compatibility and hybrid yield traits. With these booming biotechnologies, we conceived a fast and efficient hybrid breeding procedure based on integrating the great majority of multiomics data, abundant crop germplasm resources, CRISPR-Cas9-mediated gene editing, and 3G hybrid breeding technology to achieve more efficient utilization of heterosis (Figure 3). For a given crop and multiple desirable traits, a pair of best parents to

generate high levels of heterosis could be recommended by an artificial intelligence (AI) prediction tool based on a database integrating large-scale multiomics profiles and heterosis loci identified through population-level studies. Through CRISPR-Cas9-mediated gene editing, an NMS mutant could be obtained. Then, it could be transformed with the linked transgenic cassette according to the 3G system to generate the corresponding maintainer line. Mass production of nontransgenic hybrid seeds could be achieved using the nontransgenic NMS lines, propagated by self-crossing of the maintainer line, as the maternal parent and the AI-predicted best restorer line as the paternal parent for crossing. However, there are still some challenges for scientists and breeders to meet and overcome in this technology, such as the accuracy of AI prediction ability for parent selection, the effectiveness of the CRISPR-Cas9 system for fertility gene editing, efficient NMS gene characterization, and the functional perfection of the transgenic cassette in the 3G method to ensure transgenic security in hybrid breeding. Moreover, synthetic apomixis also accelerated the beginning of a new era for hybrid seed production to preserve hybrid vigor.[87] Though some challenges in widely applying "one-line" technology to the field still exist, like the establishment in limited species and low frequency of clonal seed, the advantages of this system in hybrid seed propagation would be manifested in more crops such as wheat, barley, and soybean with increased investigation on the mechanism of apomixis and improved efficiency of engineering synthetic apomixis. All these advances with the help of various updating biotechnologies will together propel the utilization of heterosis to the 4G period.

Concluding remarks

Heterosis is one of the most successfully utilized genetic phenomena in agriculture, the application of which guaranteed crop food demand, quality, and security worldwide in the past several decades. However, the intrinsic disadvantages of traditional hybrid breeding methods restrict the efficiency of heterosis utilization. In this century, the development of substantial biotechnologies has enabled significant improvement in the understanding and utilization of heterosis in numerous crop species. Specifically, the progress in plant genomics and 3G hybrid breeding technology driven by rapid innovation in high-throughput sequencing technologies and molecular biology tools has played fundamentally important roles. In this new age of biotechnology, desirable super hybrid crops with multiple excellent traits can be generated efficiently. Moreover, the widely established and continuously improved 3G hybrid breeding system in a variety of crops will greatly simplify commercial super hybrid seed production in agriculture. Altogether, these processes will enable extremely efficient heterosis utilization in the future.

ACKNOWLEDGMENTS

We thank the researchers in the heterosis field for their great efforts in understanding heterosis mechanisms and great contributions to the development of heterosis utilization methods. This work was supported by the Key Program of National Natural Science Foundation of China (32230006), the Provincial Technology Innovation Program of Shangdong, the funding from the Peking University Institute of Advanced Agricultural Sciences, and the Boya Postdoctoral Fellowship Program of Peking University.

AUTHOR CONTRIBUTIONS

X.W.D., G.H., and W.L. conceived the idea. W.L., G.H., and X.W.D. collected the literature and drafted the paper. All authors commented on and approved the final manuscript.

DECLARATION OF INTERESTS

The authors have no conflicting interests to declare.

REFERENCES

1. Birchler, J.A., Yao, H., Chudalayandi, S., Vaiman, D., and Veitia, R.A. (2010). Heterosis. Plant Cell *22*, 2105–2112.
2. Duvick, D.N. (2001). Biotechnology in the 1930s: the development of hybrid maize. Nat. Rev. Genet. *2*, 69–74.
3. Virmani, S.S., Aquino, R.C., and Khush, G.S. (1982). Heterosis Breeding in Rice (Oryza-Sativa-L). Theor. Appl. Genet. *63*, 373–380.
4. McKeown, P.C., Fort, A., Duszynska, D., Sulpice, R., and Spillane, C. (2013). Emerging molecular mechanisms for biotechnological harnessing of heterosis in crops. Trends Biotechnol. *31*, 549–551.
5. Liu, H., Wang, Q., Chen, M., Ding, Y., Yang, X., Liu, J., Li, X., Zhou, C., Tian, Q., Lu, Y., et al. (2020). Genome-wide identification and analysis of heterotic loci in three maize hybrids. Plant Biotechnol. J. *18*, 185–194.
6. Jiang, Y., Schmidt, R.H., Zhao, Y., and Reif, J.C. (2017). A quantitative genetic framework highlights the role of epistatic effects for grain-yield heterosis in bread wheat. Nat. Genet. *49*, 1741–1746.
7. Boeven, P.H.G., Zhao, Y., Thorwarth, P., Liu, F., Maurer, H.P., Gils, M., Schachschneider, R., Schacht, J., Ebmeyer, E., Kazman, E., et al. (2020). Negative dominance and dominance-by-dominance epistatic effects reduce grain-yield heterosis in wide crosses in wheat. Sci. Adv. *6*, eaay4897.
8. Liu, W., He, G., and Deng, X.W. (2021). Biological pathway expression complementation contributes to biomass heterosis in Arabidopsis. Proc. Natl. Acad. Sci. USA *118*, e2023278118.
9. Labroo, M.R., Studer, A.J., and Rutkoski, J.E. (2021). Heterosis and Hybrid Crop Breeding: A Multidisciplinary Review. Front. Genet. *12*, 643761.
10. Xiao, Y., Jiang, S., Cheng, Q., Wang, X., Yan, J., Zhang, R., Qiao, F., Ma, C., Luo, J., Li, W., et al. (2021). The genetic mechanism of heterosis utilization in maize improvement. Genome Biol. *22*, 148.
11. Liu, W., Ren, D., Yang, W., Xu, M., Zhang, Y., Wang, X., He, G., and Deng, X.W. (2023). Genetic and molecular regulation of increased photosynthetic cell number contributes to leaf size heterosis in Arabidopsis. iScience *26*, 107366.
12. Huang, X., Yang, S., Gong, J., Zhao, Y., Feng, Q., Gong, H., Li, W., Zhan, Q., Cheng, B., Xia, J., et al. (2015). Genomic analysis of hybrid rice varieties reveals numerous superior alleles that contribute to heterosis. Nat. Commun. *6*, 6258.
13. Huang, X., Yang, S., Gong, J., Zhao, Q., Feng, Q., Zhan, Q., Zhao, Y., Li, W., Cheng, B., Xia, J., et al. (2016). Genomic architecture of heterosis for yield traits in rice. Nature *537*, 629–633.
14. Xie, J., Wang, W., Yang, T., Zhang, Q., Zhang, Z., Zhu, X., Li, N., Zhi, L., Ma, X., Zhang, S., et al. (2022). Large-scale genomic and

transcriptomic profiles of rice hybrids reveal a core mechanism underlying heterosis. Genome Biol. *23*, 264.
15. Wang, B., Hou, M., Shi, J., Ku, L., Song, W., Li, C., Ning, Q., Li, X., Li, C., Zhao, B., et al. (2023). De novo genome assembly and analyses of 12 founder inbred lines provide insights into maize heterosis. Nat. Genet. *55*, 312–323.
16. Zhang, Y., Fu, J., Wang, K., Han, X., Yan, T., Su, Y., Li, Y., Lin, Z., Qin, P., Fu, C., et al. (2022). The telomere-to-telomere gap-free genome of four rice parents reveals SV and PAV patterns in hybrid rice breeding. Plant Biotechnol. J. *20*, 1642–1644.
17. Hu, Y., Xiong, J., Shalby, N., Zhuo, C., Jia, Y., Yang, Q.Y., and Tu, J. (2022). Comparison of dynamic 3D chromatin architecture uncovers heterosis for leaf size in Brassica napus. J. Adv. Res. *42*, 289–301.
18. Zhou, H., Deng, X.W., and He, H. (2023). Gene expression variations and allele-specific expression of two rice and their hybrid in caryopses at single-nucleus resolution. Front. Plant Sci. *14*, 1171474.
19. Han, Y., Chu, X., Yu, H., Ma, Y.K., Wang, X.J., Qian, W., and Jiao, Y. (2017). Single-cell transcriptome analysis reveals widespread monoallelic gene expression in individual rice mesophyll cells. Sci. Bull. *62*, 1304–1314.
20. Chen, H., Zhou, J., Wang, H., Xu, Z., Tang, X., Chen, L., Deng, X., and He, G. (2013). Hybrid Rice Breeding Welcomes a New Era of Molecular Crop Design. Sci. Sin. Vitae. *43*, 864–868.
21. Li, J., Zhou, K., Wang, Z., Zhou, J., and Deng, X.W. (2022). Research progress, problems, and prospects in hybrid wheat seed production technology based on recessive nuclear genetic male sterile lines. Chin. Sci. Bull. *67*, 3140–3151.
22. Liao, C., Yan, W., Chen, Z., Xie, G., Deng, X.W., and Tang, X. (2021). Innovation and development of the third-generation hybrid rice technology. Crop J. *9*, 693–701.
23. Wang, H., and Deng, X.W. (2018). Development of the "Third-Generation" Hybrid Rice in China. Dev. Reprod. Biol. *16*, 393–396.
24. Lai, J., Li, R., Xu, X., Jin, W., Xu, M., Zhao, H., Xiang, Z., Song, W., Ying, K., Zhang, M., et al. (2010). Genome-wide patterns of genetic variation among elite maize inbred lines. Nat. Genet. *42*, 1027–1030.
25. Sun, S., Zhou, Y., Chen, J., Shi, J., Zhao, H., Zhao, H., Song, W., Zhang, M., Cui, Y., Dong, X., et al. (2018). Extensive intraspecific gene order and gene structural variations between Mo17 and other maize genomes. Nat. Genet. *50*, 1289–1295.
26. Lv, Q., Li, W., Sun, Z., Ouyang, N., Jing, X., He, Q., Wu, J., Zheng, J., Zheng, J., Tang, S., et al. (2020). Resequencing of 1,143 indica rice accessions reveals important genetic variations and different heterosis patterns. Nat. Commun. *11*, 4778.
27. Li, C., Guan, H., Jing, X., Li, Y., Wang, B., Li, Y., Liu, X., Zhang, D., Liu, C., Xie, X., et al. (2022). Genomic insights into historical improvement of heterotic groups during modern hybrid maize breeding. Nat. Plants *8*, 750–763.
28. He, G., Zhu, X., Elling, A.A., Chen, L., Wang, X., Guo, L., Liang, M., He, H., Zhang, H., Chen, F., et al. (2010). Global Epigenetic and Transcriptional Trends among Two Rice Subspecies and Their Reciprocal Hybrids. Plant Cell *22*, 17–33.
29. He, G., Chen, B., Wang, X., Li, X., Li, J., He, H., Yang, M., Lu, L., Qi, Y., Wang, X., and Deng, X.W. (2013). Conservation and divergence of transcriptomic and epigenomic variation in maize hybrids. Genome Biol. *14*, R57.
30. Shen, Y., Sun, S., Hua, S., Shen, E., Ye, C.Y., Cai, D., Timko, M.P., Zhu, Q.H., and Fan, L. (2017). Analysis of transcriptional and epigenetic changes in hybrid vigor of allopolyploid Brassica napus uncovers key roles for small RNAs. Plant J. *91*, 874–893.
31. Sinha, P., Singh, V.K., Saxena, R.K., Kale, S.M., Li, Y., Garg, V., Meifang, T., Khan, A.W., Kim, K.D., Chitikineni, A., et al. (2020). Genome-wide analysis of epigenetic and transcriptional changes associated with heterosis in pigeonpea. Plant Biotechnol. J. *18*, 1697–1710.
32. Li, P., Su, T., Zhang, D., Wang, W., Xin, X., Yu, Y., Zhao, X., Yu, S., and Zhang, F. (2021). Genome-wide analysis of changes in miRNA and target gene expression reveals key roles in heterosis for Chinese cabbage biomass. Hortic. Res. *8*, 39.
33. Ma, X., Xing, F., Jia, Q., Zhang, Q., Hu, T., Wu, B., Shao, L., Zhao, Y., Zhang, Q., and Zhou, D.X. (2021). Parental variation in CHG methylation is associated with allelic-specific expression in elite hybrid rice. Plant Physiol. *186*, 1025–1041.
34. He, G., He, H., and Deng, X.W. (2013). Epigenetic Variations in Plant Hybrids and Their Potential Roles in Heterosis. J. Genet. Genom. *40*, 205–210.
35. Luo, J.H., Wang, M., Jia, G.F., and He, Y. (2021). Transcriptome-wide analysis of epitranscriptome and translational efficiency associated with heterosis in maize. J. Exp. Bot. *72*, 2933–2946.
36. Jaiswal, V., Rawoof, A., Gahlaut, V., Ahmad, I., Chhapekar, S.S., Dubey, M., and Ramchiary, N. (2022). Integrated analysis of DNA methylation, transcriptome, and global metabolites in interspecific heterotic Capsicum F1 hybrid. iScience *25*, 105318.
37. Li, D., Lu, X., Zhu, Y., Pan, J., Zhou, S., Zhang, X., Zhu, G., Shang, Y., Huang, S., and Zhang, C. (2022). The multi-omics basis of potato heterosis. J. Integr. Plant Biol. *64*, 671–687.
38. Paschold, A., Larson, N.B., Marcon, C., Schnable, J.C., Yeh, C.T., Lanz, C., Nettleton, D., Piepho, H.P., Schnable, P.S., and Hochholdinger, F. (2014). Nonsyntenic Genes Drive Highly Dynamic Complementation of Gene Expression in Maize Hybrids. Plant Cell *26*, 3939–3948.
39. Paschold, A., Jia, Y., Marcon, C., Lund, S., Larson, N.B., Yeh, C.T., Ossowski, S., Lanz, C., Nettleton, D., Schnable, P.S., and Hochholdinger, F. (2012). Complementation contributes to transcriptome complexity in maize (Zea mays L.) hybrids relative to their inbred parents. Genome Res. *22*, 2445–2454.
40. Li, D., Huang, Z., Song, S., Xin, Y., Mao, D., Lv, Q., Zhou, M., Tian, D., Tang, M., Wu, Q., et al. (2016). Integrated analysis of phenome, genome, and transcriptome of hybrid rice uncovered multiple heterosis-related loci for yield increase. Proc. Natl. Acad. Sci. USA *113*, E6026–E6035.
41. Shao, L., Xing, F., Xu, C., Zhang, Q., Che, J., Wang, X., Song, J., Li, X., Xiao, J., Chen, L.L., et al. (2019). Patterns of genome-wide allele-specific expression in hybrid rice and the implications on the genetic basis of heterosis. Proc. Natl. Acad. Sci. USA *116*, 5653–5658.
42. Li, Z., Zhu, A., Song, Q., Chen, H.Y., Harmon, F.G., and Chen, Z.J. (2020). Temporal Regulation of the Metabolome and Proteome in Photosynthetic and Photorespiratory Pathways Contributes to Maize Heterosis. Plant Cell *32*, 3706–3722.
43. Li, H., Jiang, S., Li, C., Liu, L., Lin, Z., He, H., Deng, X.W., Zhang, Z., and Wang, X. (2020). The hybrid protein interactome contributes to rice heterosis as epistatic effects. Plant J. *102*, 116–128.
44. Ye, J., Liang, H., Zhao, X., Li, N., Song, D., Zhan, J., Liu, J., Wang, X., Tu, J., Varshney, R.K., et al. (2023). A systematic dissection in oilseed rape provides insights into the genetic architecture and molecular mechanism of yield heterosis. Plant Biotechnol. J. *21*, 1479–1495.
45. Wei, X., Qiu, J., Yong, K., Fan, J., Zhang, Q., Hua, H., Liu, J., Wang, Q., Olsen, K.M., Han, B., and Huang, X. (2021). A quantitative genomics map of rice provides genetic insights and guides breeding. Nat. Genet. *53*, 243–253.
46. Marcon, C., Paschold, A., Malik, W.A., Lithio, A., Baldauf, J.A., Altrogge, L., Opitz, N., Lanz, C., Schoof, H., Nettleton, D., et al. (2017). Stability of Single-Parent Gene Expression Complementation in Maize Hybrids upon Water Deficit Stress. Plant Physiol. *173*, 1247–1257.
47. Li, Z., Zhou, P., Della Coletta, R., Zhang, T., Brohammer, A.B., O'Connor, C., Vaillancourt, B., Lipzen, A., Daum, C., Barry, K., et al. (2021). Single-parent expression drives dynamic gene expression complementation in maize hybrids. Plant J. *105*, 93–107.
48. Baldauf, J.A., Marcon, C., Lithio, A., Vedder, L., Altrogge, L., Piepho, H.P., Schoof, H., Nettleton, D., and Hochholdinger, F. (2018). Single-Parent Expression Is a General Mechanism Driving Extensive Complementation of Non-syntenic Genes in Maize Hybrids. Curr. Biol. *28*, 431–437.e4.
49. Wagner, M.R., Tang, C., Salvato, F., Clouse, K.M., Bartlett, A., Vintila, S., Phillips, L., Sermons, S., Hoffmann, M., Balint-Kurti, P.J., and Kleiner, M. (2021). Microbe-dependent heterosis in maize. Proc. Natl. Acad. Sci. USA *118*, e2021965118.
50. Zhang, M., Wang, Y., Hu, Y., Wang, H., Liu, Y., Zhao, B., Zhang, J., Fang, R., and Yan, Y. (2023). Heterosis in root microbiota inhibits growth of soil-borne fungal pathogens in hybrid rice. J. Integr. Plant Biol. *65*, 1059–1076.
51. Wagner, M.R., Roberts, J.H., Balint-Kurti, P., and Holland, J.B. (2020). Heterosis of leaf and rhizosphere microbiomes in field-grown maize. New Phytol. *228*, 1055–1069.
52. Sarton, G. (1936). The Beginnings of Plant Hybridization. Conway Zirkle. Isis *25*, 507–508.
53. Xu, Y., Yu, D., Chen, J., and Duan, M. (2023). A review of rice male sterility types and their sterility mechanisms. Heliyon *9*, e18204.
54. Tang, H., Xie, Y., Liu, Y.G., and Chen, L. (2017). Advances in understanding the molecular mechanisms of cytoplasmic male sterility and restoration in rice. Plant Reprod. *30*, 179–184.
55. Bohra, A., Jha, U.C., Adhimoolam, P., Bisht, D., and Singh, N.P. (2016). Cytoplasmic male sterility (CMS) in hybrid breeding in field crops. Plant Cell Rep. *35*, 967–993.
56. Mark, W., and Jan, L. (1993). Maintenance of male-sterile plants. Patent Application EP19930912959, 1993/06/11/ Application Date.
57. Perez-Prat, E., and van Lookeren Campagne, M.M. (2002). Hybrid seed production and the challenge of propagating male-sterile plants. Trends Plant Sci. *7*, 199–203.
58. Albertsen Marc, C., Fox Timothy, W., Hershey Howard, P., Huffman Gary, A., Trimnell, M., and Wu, Y. (2006). Nucleotide sequences mediating plant male fertility and method of

using same. patent Application WO2006US24273, 2006/06/22/ Application Date.
59. Wu, Y., Fox, T.W., Trimnell, M.R., Wang, L., Xu, R.J., Cigan, A.M., Huffman, G.A., Garnaat, C.W., Hershey, H., and Albertsen, M.C. (2016). Development of a novel recessive genetic male sterility system for hybrid seed production in maize and other cross-pollinating crops. Plant Biotechnol. J. *14*, 1046–1054.
60. Song, S., Wang, T., Li, Y., Hu, J., Kan, R., Qiu, M., Deng, Y., Liu, P., Zhang, L., Dong, H., et al. (2021). A novel strategy for creating a new system of third-generation hybrid rice technology using a cytoplasmic sterility gene and a genic male-sterile gene. Plant Biotechnol. J. *19*, 251–260.
61. Qi, X., Zhang, C., Zhu, J., Liu, C., Huang, C., Li, X., and Xie, C. (2020). Genome Editing Enables Next-Generation Hybrid Seed Production Technology. Mol. Plant *13*, 1262–1269.
62. Chen, X., Yang, S., Zhang, Y., Zhu, X., Yang, X., Zhang, C., Li, H., and Feng, X. (2021). Generation of male-sterile soybean lines with the CRISPR/Cas9 system. Crop J. *9*, 1270–1277.
63. Du, M., Zhou, K., Liu, Y., Deng, L., Zhang, X., Lin, L., Zhou, M., Zhao, W., Wen, C., Xing, J., et al. (2020). A biotechnology-based male-sterility system for hybrid seed production in tomato. Plant J. *102*, 1090–1100.
64. Ye, Q., Meng, X., Chen, H., Wu, J., Zheng, L., Shen, C., Guo, D., Zhao, Y., Liu, J., Xue, Q., et al. (2022). Construction of genic male sterility system by CRISPR/Cas9 editing from model legume to alfalfa. Plant Biotechnol. J. *20*, 613–615.
65. Wang, M., Yan, W., Peng, X., Chen, Z., Xu, C., Wu, J., Deng, X.W., and Tang, X. (2020). Identification of late-stage pollen-specific promoters for construction of pollen-inactivation system in rice. J. Integr. Plant Biol. *62*, 1246–1263.
66. Wilson, Z.A., and Zhang, D.B. (2009). From Arabidopsis to rice: pathways in pollen development. J. Exp. Bot. *60*, 1479–1492.
67. Shi, J., Cui, M., Yang, L., Kim, Y.J., and Zhang, D. (2015). Genetic and Biochemical Mechanisms of Pollen Wall Development. Trends Plant Sci. *20*, 741–753.
68. Zhang, D., Wu, S., An, X., Xie, K., Dong, Z., Zhou, Y., Xu, L., Fang, W., Liu, S.S., Liu, S.S., and Liu, S. (2018). Construction of a multicontrol sterility system for a maize male-sterile line and hybrid seed production based on the ZmMs7 gene encoding a PHD-finger transcription factor. Plant Biotechnol. J. *16*, 459–471.
69. Wu, J., Qiu, S., Wang, M., Xu, C., Deng, X.W., and Tang, X. (2021). Construction of a weight-based seed sorting system for the third-generation hybrid rice. Rice *14*, 66.
70. Ligeng, M.A., Wang, Z., Chen, Z., Yanfang, H.E., and Xingwang, D.E. (2021). Blue-grained Genes in Wheat and Application Thereof. United States patent application 20210180075.
71. An, X., Dong, Z., Tian, Y., Xie, K., Wu, S., Zhu, T., Zhang, D., Zhou, Y., Niu, C., Ma, B., et al. (2019). ZmMs30 Encoding a Novel GDSL Lipase Is Essential for Male Fertility and Valuable for Hybrid Breeding in Maize. Mol. Plant *12*, 343–359.
72. Riedelsheimer, C., Czedik-Eysenberg, A., Grieder, C., Lisec, J., Technow, F., Sulpice, R., Altmann, T., Stitt, M., Willmitzer, L., and Melchinger, A.E. (2012). Genomic and metabolic prediction of complex heterotic traits in hybrid maize. Nat. Genet. *44*, 217–220.
73. Dan, Z., Chen, Y., Li, H., Zeng, Y., Xu, W., Zhao, W., He, R., and Huang, W. (2021). The metabolomic landscape of rice heterosis highlights pathway biomarkers for predicting complex phenotypes. Plant Physiol. *187*, 1011–1025.
74. Fu, C., Ma, C., Zhu, M., Liu, W., Ma, X., Li, J., Liao, Y., Liu, D., Gu, X., Wang, H., and Wang, F. (2023). Transcriptomic and methylomic analyses provide insights into the molecular mechanism and prediction of heterosis in rice. Plant J. *115*, 139–154.
75. Wang, K., Abid, M.A., Rasheed, A., Crossa, J., Hearne, S., and Li, H. (2023). DNNGP, a deep neural network-based method for genomic prediction using multi-omics data in plants. Mol. Plant *16*, 279–293.
76. Xu, C., Xu, Y., Wang, Z., Zhang, X., Wu, Y., Lu, X., Sun, H., Wang, L., Zhang, Q., Zhang, Q., et al. (2023). Spontaneous movement of a retrotransposon generated genic dominant male sterility providing a useful tool for rice breeding. Natl. Sci. Rev. *10*, nwad210.
77. Lei, D., Jian, A., Huang, X., Liu, X., Chen, L., Bai, W., Cheng, S., He, X., Xiong, Y., Yu, X., et al. (2023). Anther-specific expression of OsRIP1 causes dominant male sterility in rice. Plant Biotechnol. J. *21*, 1932–1934.
78. Fox, T., DeBruin, J., Haug Collet, K., Trimnell, M., Clapp, J., Leonard, A., Li, B., Scolaro, E., Collinson, S., Glassman, K., et al. (2017). A single point mutation in Ms44 results in dominant male sterility and improves nitrogen use efficiency in maize. Plant Biotechnol. J. *15*, 942–952.
79. An, X., Ma, B., Duan, M., Dong, Z., Liu, R., Yuan, D., Hou, Q., Wu, S., Zhang, D., Liu, D., et al. (2020). Molecular regulation of ZmMs7 required for maize male fertility and development of a dominant male-sterility system in multiple species. Proc. Natl. Acad. Sci. USA *117*, 23499–23509.
80. Wan, X., Wu, S., and Li, X. (2021). Breeding with dominant genic male-sterility genes to boost crop grain yield in the post-heterosis utilization era. Mol. Plant *14*, 531–534.
81. Wang, C., Liu, Q., Shen, Y., Hua, Y., Wang, J., Lin, J., Wu, M., Sun, T., Cheng, Z., Mercier, R., and Wang, K. (2019). Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes. Nat. Biotechnol. *37*, 283–286.
82. Khanday, I., Skinner, D., Yang, B., Mercier, R., and Sundaresan, V. (2019). A male-expressed rice embryogenic trigger redirected for asexual propagation through seeds. Nature *565*, 91–95.
83. Vernet, A., Meynard, D., Lian, Q., Mieulet, D., Gibert, O., Bissah, M., Rivallan, R., Autran, D., Leblanc, O., Meunier, A.C., et al. (2022). High-frequency synthetic apomixis in hybrid rice. Nat. Commun. *13*, 7963.
84. Okada, A., Arndell, T., Borisjuk, N., Sharma, N., Watson-Haigh, N.S., Tucker, E.J., Baumann, U., Langridge, P., and Whitford, R. (2019). CRISPR/Cas9-mediated knockout of Ms1 enables the rapid generation of male-sterile hexaploid wheat lines for use in hybrid seed production. Plant Biotechnol. J. *17*, 1905–1913.
85. Sheng, X., Ai, Z., Tan, Y., Hu, Y., Guo, X., Liu, X., Sun, Z., Yu, D., Chen, J., Tang, N., et al. (2023). Novel Salinity-Tolerant Third-Generation Hybrid Rice Developed via CRISPR/Cas9-Mediated Gene Editing. Int. J. Mol. Sci. *24*, 8025.
86. Chen, G., Zhou, Y., Kishchenko, O., Stepanenko, A., Jatayev, S., Zhang, D., and Borisjuk, N. (2021). Gene editing to facilitate hybrid crop production. Biotechnol. Adv. *46*, 107676.
87. Xiong, J., Hu, F., Ren, J., Huang, Y., Liu, C., and Wang, K. (2023). Synthetic apomixis: the beginning of a new era. Curr. Opin. Biotechnol. *79*, 102877.