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Autotetraploid Rice Hybrids: Overcoming Sterility Barriers for Enhanced Heterosis

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Abstract Autotetraploid rice, developed through whole genome duplication of diploid rice, offers potential advantages such as larger grains, higher nutrient content, and increased resistance. However, its low fertility has been a significant barrier to its commercial viability. Recent advancements have focused on understanding and overcoming the sterility issues in autotetraploid rice hybrids to harness their heterosis potential. The development of high-fertility tetraploid rice lines and the application of molecular breeding techniques have shown promise in improving hybrid performance. This study synthesizes the genetic mechanisms underlying the low fertility of autotetraploid rice and its F_1 hybrids from both cytological and molecular biological perspectives. It introduces the main types of high-fertility tetraploid rice and the latest research progress. Lastly, the study proposes ideas for future research on exploiting the heterosis of high-fertility autotetraploid rice, aiming to provide insights for polyploid rice breeding.

Keywords Autotetraploid rice; Hybrid sterility; Heterosis; Fertility restoration; Molecular breeding

1 Introduction

Autotetraploid rice, characterized by having four sets of chromosomes ($2n = 4x = 48$, AA to AAAA), has emerged as a promising avenue in rice breeding due to its potential to overcome sterility barriers and enhance heterosis. The development of autotetraploid rice hybrids has been suggested as a novel method to exploit heterosis more effectively, as these hybrids can exhibit significant improvements in yield components such as productive panicles per plant, total kernels per panicle, and seed set (Tu et al., 2007). Moreover, autotetraploid rice hybrids have shown better chromosome pairing and genetic stability, which are crucial for maintaining high fertility and yield (Luan et al., 2007).

Heterosis, or hybrid vigor, refers to the phenomenon where hybrid offspring exhibit superior biological and agricultural traits compared to their parents. In rice, heterosis is a critical factor for achieving high yields and improving crop performance. Traditional diploid rice hybrids, especially those resulting from intersubspecific crosses between *indica* and *japonica* varieties, often face challenges related to poor fertility, which significantly limits the practical utilization of heterosis due to hybrid sterility (Tu et al., 2007; Guo et al., 2017; Bei et al., 2019; Liu et al., 2023). Recent studies have highlighted the potential of autotetraploid rice hybrids to overcome these sterility barriers, thereby unlocking higher levels of heterosis. This suggests that polyploidy can be a viable strategy for enhancing rice breeding programs (Cai et al., 2007; Guo et al., 2017; Chen et al., 2020).

The objective of this study is to provide a comprehensive analysis of the current state of research on autotetraploid rice hybrids, focusing on their ability to overcome sterility barriers and enhance heterosis. This study aims to summarize the key findings from recent studies on the production and performance of autotetraploid rice hybrids; explore the genetic and molecular mechanisms underlying the fertility and heterosis observed in these hybrids and discuss the potential applications and future directions for autotetraploid rice breeding. By synthesizing the available literature, this study expects to offer valuable insights into the advantages and challenges associated with autotetraploid rice hybrids, ultimately contributing to the development of more robust and high-yielding rice varieties.

2 Autotetraploidy in Rice

2.1 Historical of autotetraploids rice

The phenomenon of polyploidy, characterized by the multiplication of chromosome sets (often from diploid to tetraploid), is a widespread feature of higher plants. Polyploids are traditionally recognized as autopolyploids (originating from a single parent species) and allopolyploids (arising from two hybridizing species) (Clark and Donoghue, 2018). Autotetraploids are plants that have four sets of chromosomes derived from the doubling of a diploid genome. The concept of autotetraploidy in rice dates back to the 1930s when researchers first reported spontaneously autotetraploid rice and then attempted to create polyploid rice to exploit its potential yield advantages (Nakamori, 1933; Ichijima, 1934).

In rice, autotetraploids are created by colchicine treatment, induceing chromosome doubling in diploid rice lines, resulting in plants with larger cell sizes, increased biomass, and often showing enhanced heterosis compared to their diploid counterparts (Luan et al., 2007; Tu et al., 2007; Chen et al., 2022). However, despite facing challenges such as high pollen sterility and low seed fertility, which have historically limited their commercial use (Oka, 1955; Wu et al., 2015; Guo et al., 2017; Chen et al., 2020), recent advancements have led to the development of fertile autotetraploid lines to overcome early setbacks.

2.2 Development of fertile autotetraploids rice

To address the bottleneck issue of low fertility in autotetraploid rice, Chinese rice researchers have made significant efforts over many years. Through the hybridization of different types of autotetraploid rice, they have bred multiple varieties of highly fertile tetraploid rice, exhibiting high fertility and strong hetrosis, thus rekindling interest in autotetraploid rice breeding. Three representative categories are noteworthy:

(1) PMeS (Polyploid Meiosis Stability) Polyploid Rice: Developed by Cai et al. (2007) at Hubei University, produced through *indica-japonica* hybridization, followed by backcrossing and selection, and subsequent chromosome doubling and breeding. Two *japonica*-type tetraploid cultivars, designated PMeS-1 and PMeS-2, both display an average seed setting rate above 70%.

(2) High-Fertility Tetraploid Rice: From Chengdu Institute of Biology Chinese Academy of Sciences, Tu et al. (2003) reported high-fertility tetraploid rice sterile and restorer lines, achieving a three-line system for autotetraploid rice. They have created over 100 hybrid combinations.

(3) Neo-Tetraploid Rice: Derived from the crossing of autotetraploid rice Jackson-4x with tetraploid rice line 96025, followed by multiple generations of selfing and selection. Given that the genome of autotetraploid rice can be represented as AAAA, the genome of the neo-tetraploid rice should theoretically be $A^1A^1A^2A^2$, indicating a combination of two distinct autotetraploid genomes. Unlike allotetraploid rice, where chromosome pairing is exclusively bivalent, in autotetraploid rice, chromosome pairing is primarily a mix of bivalents and quadrivalents.

The meiotic prophase I chromosome pairing in all three high-fertility tetraploid rice varieties is primarily characterized by bivalents and quadrivalents, with a low frequency of univalents, trivalents, and multivalents (pentavalents and above), as well as reduced abnormal chromosome behavior (Table 1) (Liu et al., 2023). This is likely one of the key reasons for their high fertility.

2.3 Potential advantages of autotetraploid rice

Autotetraploid rice offers several potential advantages over diploid rice. The most significant benefit is the enhanced heterosis, or hybrid vigor, observed in autotetraploid hybrids. Studies have shown that autotetraploid hybrids can exhibit significantly higher yields, improved biomass, and better stress tolerance compared to diploid hybrids (Figure 1) (Tu et al., 2007; Chen et al., 2020; Ghaleb et al., 2020). Additionally, autotetraploid rice lines have been found to possess greater genetic diversity, which can be harnessed to develop new and improved rice varieties (Wu et al., 2013). The development of fertile autotetraploid lines, such as the neo-tetraploid rice, has further demonstrated the feasibility of using autotetraploids in commercial rice breeding programs (Guo et al., 2017; Wu et al., 2019). By overcoming the sterility barriers and leveraging the advantages of autotetraploidy, researchers aim to develop high-yielding, resilient rice varieties that can contribute to global food security.

Table 1 Pollen fertility and abnormal frequency of chromosome behavior of pollen mother cells in autotetraploid and high fertility tetraploid rice (Adopted from Liu et al., 2023)

Line type	Pollen fertility (%)	Seed set rate (%)	Chromosome configuration	
Diploid	96.32±2.67	85.06±8.06	Diakinesis	(0.20~0.83) I+(11.17~11.90) II
			Metaphase I	(0.16~1.18) I+(11.41~11.92) II
Autotetraploid	69.10±17.61	33.83±16.05	Diakinesis	(0.09~1.03) I+(5.67~13.21) II+(0.04~1.23) III+(4.83~8.71) IV+ (0.00~0.19) V + (0.00~0.36) VI①
			Metaphase I	(0.25~4.13) I+(5.36~14.54) II+(0.13~1.26) III+(4.49~8.29) IV+ (0.00~0.28) V + (0.00~0.37) VI②
Diploid hybrid F_1 (*indica x japonica*)	30.51±1.63	30.34±19.70	Diakinesis	(0.74±0.38) I+(11.26±0.19) II
			Metaphase I	(0.23±0.04) I+(11.77±0.11) II
Autotetraploid hybrid F_1 (*indica x japonica*)	52.96±38.49	47.91±12.09	Diakinesis	(0.23~0.72) I+(10.26~13.00) II+(0.08~0.64) III+(5.23~6.65) IV
			Metaphase I	(0.57~1.33) I+(10.89~15.79) II+(0.05~0.53) III+(3.74~6.27) IV
PMeS polyploid	--	77.14±3.71	Diakinesis	(0.03~0.04) I+(15.83~15.86) II+(0.03~0.04) III+(4.15~4.38) IV+(0.02~0.03) VI
			Metaphase I	--
High fertility tetraploid restore line	81.27±0.72	72.35±1.91	Diakinesis	--
			Metaphase I	(0.05~0.11) I+(19.17~19.96) II+(0.01~0.09) III+(2.20~2.26) IV+(0.00~0.01) VI
High fertility tetraploid hybrid F_1	86.69±1.01	80.5±2.12	Diakinesis	--
			Metaphase I	(0.06~0.02) I+(14.36~17.67) II+(0.01~0.06) III+(3.10~4.80) IV +(0.00~0.01) VI+(0.00~0.01) VIII
Neo-tetraploid	90.79±5.13	75.21±5.81	Diakinesis	(0.06~0.53) I+(4.89~10.17) II+(0.02~0.37) III+(6.85~9.45) IV+(0~0.02) VI
			Metaphase I	--
Neo-tetraploid hybrid F_1	80.34±4.73	76.42±5.64	Diakinesis	(1.62±0.22) I+(8.71±0.40) II+(0.33±0.06) III+(6.84±0.73) IV+(0.01±0.01) V+(0.07±0.03) VI
			Metaphase I	--

Figure 1 Morphological characteristics of the parents and two hybrids (Adopted from Ghaleb et al., 2020)

Image caption: a, c: Plant appearance of parents and two F_1 hybrids (H1 × H8 and T485 × H8); b, d: Performance of the two hybrids in the field (maturity stage); e: Panicles of H1, H8 and T485. T485, H1and H8 indicate Huanghuazhan-4x, Huaduo 1 and Huaduo 8, respectively (Adopted from Ghaleb et al., 2020)

3 Genetic Basis of Autotetraploid Rice

3.1 Chromosomal doubling and genome stability

Chromosomal doubling is a fundamental process in the development of autotetraploid rice, which involves the duplication of the entire set of chromosomes, resulting in a tetraploid organism with four sets of chromosomes. This process is typically induced using colchicine, a chemical that disrupts microtubule formation during cell division, thereby preventing chromosome segregation and leading to genome doubling (Tu et al., 2007).

Understanding meiotic stability and chromosome pairing is essential for overcoming sterility barriers in autotetraploid rice. Abnormal chromosome behavior in meiocytes of autotetraploid rice during meiosis compared to diploid hybrids is one of the key reasons for reduced fertility (Wu et al., 2015).

Extensive cytological studies have demonstrated that during meiosis in autotetraploid rice, pollen mother cells (PMCs) exhibit various abnormalities in chromosome pairing and segregation. These include the presence of univalents and multivalents during prophase I, chromosome dragging in metaphase I, lagging chromosomes in anaphase I and II, and the formation of micronuclei in telophase II (Luan et al., 2007). These abnormalities result in the formation of non-haploid gametes, leading to decreased pollen fertility, affecting fertilization, and ultimately reducing seed setting rates. Therefore, the stability of the genome is crucial for the successful development and fertility of autotetraploid rice hybrids (Luan et al., 2007).

3.2 Genetic and epigenetic changes

The transition to autotetraploidy involves not only chromosomal doubling but also significant genetic and epigenetic changes. Transcriptome analyses have revealed that autotetraploid rice hybrids exhibit differential gene expression profiles compared to their diploid counterparts. For instance, a study identified 807, 663, and 866 differentially expressed genes (DEGs) in the anther, ovary, and leaf tissues of F_1 hybrid developed by crossing neo-tetraploid with autotetraploid rice, respectively (Guo et al., 2017). These DEGs are associated with various biological processes, including photosynthesis, metabolic processes, and transport, which are crucial for the enhanced heterosis observed in autotetraploid hybrids (Guo et al., 2017). Additionally, epigenetic modifications, such as DNA methylation and histone modifications, play a significant role in regulating gene expression in autotetraploid rice (Guo et al., 2017; Li et al., 2018). Specific genes related to meiosis and epigenetic regulation, such as *RAD51* and *SMC2*, have been identified as key players in maintaining fertility and heterosis in autotetraploid rice (Guo et al., 2017).

3.3 Polyploidy and gene expression

Polyploidy significantly impacts gene expression in autotetraploid rice, leading to both additive and non-additive gene expression patterns. Comparative studies between diploid and autotetraploid rice hybrids have shown that polyploidy enhances the interaction of pollen sterility loci, resulting in increased meiosis abnormalities and pollen sterility (Wu et al., 2015; Chen et al., 2020). Moreover, polyploidy induces non-additive gene expression, where certain genes exhibit expression levels that are not simply the sum of their expression in the diploid parents. This non-additive expression is often associated with heterosis and improved agronomic traits in autotetraploid rice hybrids (Guo et al., 2017; Chen et al., 2020). Gene ontology and pathway analyses have identified key genes involved in amino acid metabolism, photosynthesis, and meiosis, which contribute to the enhanced yield and fertility observed in autotetraploid rice hybrids (Bei et al., 2019; Chen et al., 2020).

In summary, the genetic basis of autotetraploid rice involves complex interactions between chromosomal doubling, genetic and epigenetic changes, and polyploidy-induced gene expression. These factors collectively contribute to the stability, fertility, and enhanced heterosis of autotetraploid rice hybrids, making them a promising resource for future rice breeding programs.

4 Challenges of Sterility in Autotetraploid Rice

Autotetraploid rice hybrids, while promising for their potential to enhance heterosis and increase yield, face significant challenges related to sterility. These challenges are primarily due to complex genetic and cytological factors that affect pollen and embryo sac development, leading to reduced fertility and seed set.

4.1 Mechanisms of sterility in autotetraploids

Many studies have found that during meiosis and subsequent pollen development in autotetraploid rice, a large number of genes are abnormally differentially expressed, leading to reduced pollen fertility. Studies indicated that the interaction between pollen sterility loci (*Sa*, *Sb*, and *Sc*) exerts an "epistasis-like" effect in F_1 hybrids of autotetraploid rice, influencing pollen fertility through meiotic abnormalities that can result in partial abortion of male gametes and reduced seed set (He et al., 2011; Wu et al., 2015). Gene expression profiling identified meiosis-related genes, such as *OsPUB73*, and genes involved in photosystem I, whose downregulated expression severely impacted the coordination of the meiosis-related gene network, leading to reduced pollen fertility in the F_1 hybrids (Wu et al., 2015).

In the pollen mother cells of the autotetraploid rice Taichung 65-4x during meiosis, 786 DEGs (compared to its diploid progenitor) were detected (Wu et al., 2014), with 125 of these being downregulated, including genes involved in chromosome pairing and recombination, such as *PAIR2* and *OsDMC1B*.

Additionally, 75 meiosis-related DEGs were identified in the autotetraploid rice T449, including the important genes *OsMTOPVIB* for meiotic DNA double-strand breaks and *OsMOF*, which is involved in homologous chromosome pairing and synapsis, both of which were significantly downregulated (Chen et al., 2018).

Differentially expressed miRNAs (DEM) and small RNAs have been identified as key players in the development of pollen and embryo sacs. Specific miRNAs, such as *osa-miR1436_L+3_1ss5CT* and *osa-miR167h-3p*, are associated with female meiosis, while *osa-miR159a.1* and *osa-MIR159a-p5* are related to male meiosis, indicating their role in sterility (Li et al., 2017).

In the autotetraploid rice 02428-4x, important fertility-regulating genes such as *OsMYB80*, *OsABCG15*, *PTC1*, and *CYP703A3* were found to be significantly downregulated (Li et al., 2018). The role of *OsMND1* in PMeS autotetraploid rice lines was investigated. It was found that overexpression of *OsMND1* improved pollen fertility and viability, early normal embryo development, and seed set rates in Balilla-4x. However, knocking down *OsMND1* significantly hindered pollen and embryo development (Xiong et al., 2019).

Furthermore, 941 differentially expressed proteins (DEPs) were identified in the meiotic anthers of newly synthesized autotetraploid rice, including 489 upregulated and 452 downregulated proteins, some of which may be related to the early degradation of the tapetum, ultimately affecting pollen fertility (Ku et al., 2022).

4.2 Environmental factors affecting fertility

Environmental factors also influence the fertility of autotetraploid rice hybrids. It is well-known that factors such as temperature, humidity, and soil conditions can impact the expression of genes related to fertility and sterility (Yan et al., 2010).

It is reported that seasons have a significant impact on both pollen and embryo sac fertility in both diploid and autotetraploid rice. This seasonal variation affects the overall seed setting rate, underscoring the importance of considering environmental conditions when evaluating fertility in autotetraploid rice varieties (Shahid et al., 2010).

4.3 Overcoming sterility barriers

4.3.1 Breeding strategies to enhance fertility

Selection and screening of fertile lines are crucial steps in overcoming sterility barriers in autotetraploid rice hybrids. Hybridization techniques play a pivotal role in enhancing fertility in autotetraploid rice. Studies have shown that the development of three types of autotetraploid rice lines mentioned above (2.2), which exhibit high fertility and heterosis, can be achieved through rigorous selection processes.

Cytogenetic studies have demonstrated that two hybrids of autotetraploid rice show extremely high pollen fertility, seed set and heterosis, further highlighting the importance of hybridization techniques in breeding strategies (Luan et al., 2007; He et al., 2011).

The development of Huaduo1 to Huaduo8 (H1-H8) involved crossing generations of autotetraploid rice, resulting in high pollen fertility (>92.29%) and stable chromosome configurations during pollen development (Guo et al., 2017; Bei et al., 2019; Chen et al., 2019; Ghaleb et al., 2020). Additionally, the identification of double neutral genes, such as Sa^n and Sb^n, has been instrumental in overcoming pollen sterility barriers and achieving higher pollen fertility in autotetraploid rice hybrids (Chen et al., 2020).

4.3.2 Biotechnological approaches

Genetic engineering, such as CRISPR technologies offer innovative solutions to address fertility issues in autotetraploid rice by targeting specific genes involved in meiosis and pollen development. The use of CRISPR/Cas9 technology to knock out specific genes, such as *TMS9-1* and *TMS5*, has been shown to significantly impact fertility and pollen development. Mutants of these genes displayed low fertility and abnormal pollen development, indicating their crucial role in fertility regulation (Wu et al., 2020).

Additionally, transcriptome analysis has identified DEGs associated with fertility, providing targets for genetic engineering to enhance fertility in autotetraploid rice (Guo et al., 2017). For example, the up-regulation of meiosis-related genes, such as *RAD51D*, and tapetal-related genes, such as *MIL2*, *OsAP25*, and *OsAP37*, has been associated with high fertility in newly developed tetraploid rice lines (Wu et al., 2020). Furthermore, the identification of meiosis-specific and meiosis-related genes through transcriptome analysis provides valuable insights into the molecular mechanisms underlying fertility restoration in autotetraploid rice (Wu et al., 2015; Guo et al., 2017).

Moreover, transcriptome analysis has identified DEGs and specific molecular markers associated with fertility, such as meiosis-related genes and genes involved in photosynthesis and amino acid metabolis (Guo et al., 2017; Chen et al., 2020). These molecular markers can be used to screen and select fertile lines, thereby improving the efficiency of breeding programs.

By integrating these breeding strategies, biotechnological approaches, and a deeper understanding of meiotic behavior, researchers can effectively overcome sterility barriers and enhance heterosis in autotetraploid rice hybrids.

5 Case Studies and Field Trials

5.1 Experimental design and methodology

In the pursuit of overcoming sterility barriers and enhancing heterosis in autotetraploid rice hybrids, several experimental designs and methodologies have been employed. One notable study focused on the cytogenetics and transcriptome comparison between diploid and autotetraploid rice hybrids harboring double neutral genes, Sa^n and Sb^n. This study involved detailed cytological and transcriptome analyses during meiosis and single microspore stages to understand the molecular mechanisms underlying pollen fertility in autotetraploid rice hybrids (Chen et al., 2020).

Another significant approach utilized CRISPR/Cas9 technology to edit the *TMS5* gene in neo-tetraploid rice, developing thermo-sensitive genic male sterile (TGMS) lines. These lines were then crossed with other tetraploid rice lines to generate F_1 hybrids, which were evaluated under both controlled and natural growing conditions (Chen et al., 2022; Samonte et al., 2023). Additionally, previous studies have demonstrated that the sterility of F_1 hybrids in autotetraploid rice is primarily due to embryo sac abortion, which is also influenced by the genotype of the S_5 gene (Fimanekeni et al., 2023).

5.2 Examples of successful autotetraploid hybrids

Field trials have demonstrated promising results in overcoming sterility barriers and enhancing heterosis in autotetraploid rice hybrids. Several studies have demonstrated significant yield improvements in autotetraploid rice hybrids. For example, hybrids such as T461A/T4002 and T461A/T4193 have shown yield increases of 46.3% and 38.3%, respectively, compared to the commercial diploid hybrid Shanyou 63 (Tu et al., 2007).

Two photoperiod- and thermo-sensitive genic male sterile lines of PMeS lines, PS006 and PS012, were bred and found to have desirable agronomic traits, high outcrossing rates, and good combining abilities. These lines show strong heterosis in their hybrids and have great potential for increasing rice productivity and quality, providing valuable resources for further research into polyploidy and hybrid vigor in rice (Zhang et al., 2017).

Autotetraploid rice hybrids also exhibit enhanced stress tolerance and disease resistance. The development of neo-tetraploid lines with high fertility and heterosis has been a significant breakthrough. These lines display strong heterosis when crossed with other autotetraploid rice lines, resulting in hybrids with improved stress tolerance and disease resistance (Bei et al., 2019; Chen et al., 2022). For instance, the use of CRISPR/Cas9 technology to develop thermo-sensitive genic male sterile lines in neo-tetraploid rice has led to hybrids with high levels of hybrid vigor and resilience to environmental stresses (Chen et al., 2022).

These autotetraploid rice hybrids hold great promise for future rice breeding programs, as they collectively underscore the potential to achieve higher yields, improved fertility, and better stress tolerance, thereby paving the way for their broader application in rice breeding programs.

6 Future Prospects and Challenges

6.1 Potential applications in rice breeding

China's research on utilizing the heterosis of rice hybrids is at the forefront globally, making significant contributions to food security, particularly in China and worldwide. However, with the continuous global population growth and reduction in arable land, increasing rice yields has become increasingly important (Zhang et al., 2017). Furthermore, global climate warming, extreme weather events, droughts, floods, soil degradation, and frequent pest and disease outbreaks pose severe threats to rice growth and harvest (Chen et al., 2021). Therefore, there is an urgent need to develop new high-yielding rice varieties that can enhance yield and improve resilience to climate change and disasters. To address these challenges, scientists have proposed two approaches: First, the polyploidization of cultivated rice, focusing on the development of tetraploid rice, to leverage its high resistance and strong heterosis; Second, the acceleration of the domestication of allotetraploid wild rice using molecular biology techniques to create new rice species (Figure 2) (Yu et al., 2021).

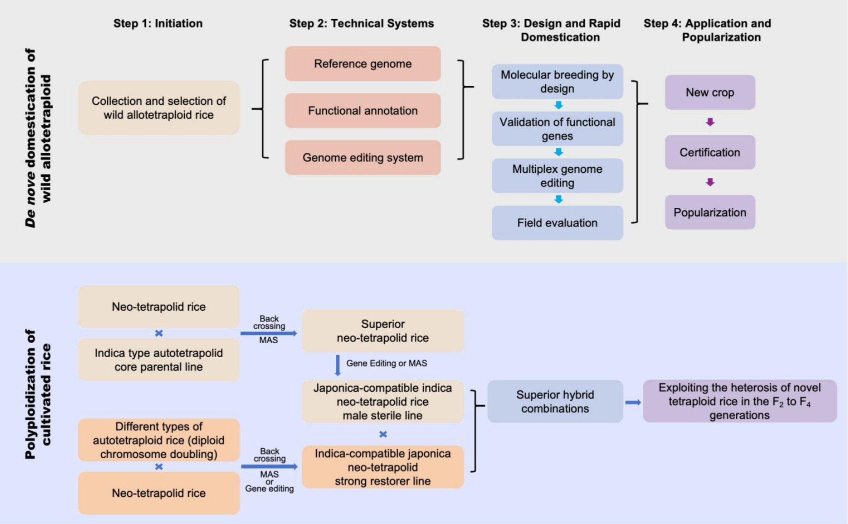

Figure 2 The novel approaches for creating superior new germplasms in rice, MAS: Molecular marker assisted selection (Adapted from Yu et al., 2021; Liu et al., 2023)

The high-fertility tetraploid rice hybrids exhibit distinct advantages that can be maintained across multiple generations, offering significant potential for breakthroughs in polyploid rice breeding. To harness the heterosis of high-fertility tetraploid rice in agricultural production, three bottlenecks need to be addressed:

(1) Development of efficient autotetraploid rice sterile lines. In addition to traditional hybridization methods, CRISPR/Cas9 gene editing technology can be used to knockout the *TMS5* gene in novel tetraploid rice to efficiently breed thermo-sensitive sterile lines (Song et al., 2021; Chen et al., 2022).

(2) Cultivation of strong restorer lines. Utilize existing high-quality restorer lines from diploid rice, directly doubling their chromosomes, and then crossing them with high-fertility tetraploid rice to obtain strong restorer lines through backcrossing and self-breeding.

(3) Utilization of multi-generational heterosis. Studies have shown that the heterosis of hybrid combinations involving neo-tetraploid rice can be maintained at least until the F_4 generation. Thus, F_1 hybrids can be used for seed propagation, and the F_2 to F_4 generations can be utilized for production, addressing the issue of only being able to use the F_1 generation's advantage in traditional diploid rice and significantly reducing seed production costs (Chen et al., 2022).

It should be noted that, to date, the precise molecular mechanisms underlying the high fertility of both PMeS tetraploid rice and neo-tetraploid rice remain unclear. Long-term research is needed to further understand their fertility. Interestingly, recent studies have observed a small number of meiocytes showing 24 bivalent pairing in neo-tetraploid rice, suggesting the possibility of developing new diploid rice germplasm containing 48 chromosomes (Liu et al., 2023).

6.2 Addressing ecological and environmental concerns

While the potential benefits of autotetraploid rice hybrids are significant, it is essential to consider the ecological and environmental impacts of their widespread adoption. The introduction of genetically modified organisms (GMOs) into the environment can have unforeseen consequences on biodiversity and ecosystem balance. For instance, the use of TGMS lines developed through gene editing may require careful monitoring to prevent unintended gene flow to wild rice species or other crops (Chen et al., 2022). Additionally, the increased vigor and yield of autotetraploid rice hybrids could lead to changes in agricultural practices, such as increased use of fertilizers and water resources, which may have environmental repercussions. Therefore, it is crucial to conduct comprehensive ecological risk assessments and develop sustainable agricultural practices to mitigate potential negative impacts.

6.3 Policy and regulatory considerations

The successful implementation of autotetraploid rice hybrids in agriculture will require supportive policy and regulatory frameworks. Given the use of gene editing technologies, such as CRISPR/Cas9, in developing these hybrids, it is essential to address regulatory challenges related to GMOs. Policymakers need to establish clear guidelines for the approval, cultivation, and commercialization of genetically modified autotetraploid rice hybrids to ensure their safe and responsible use (Chen et al., 2022). Additionally, there should be policies in place to protect the intellectual property rights of researchers and breeders who develop these innovative hybrids. Public awareness and acceptance of genetically modified crops will also play a critical role in the successful adoption of autotetraploid rice hybrids. Therefore, transparent communication and education efforts are necessary to inform stakeholders about the benefits and risks associated with these advanced breeding technologies.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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