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Breeding of Herbicide-Resistant Thermosensitive Two-Line Sterile Rice Line "Z017S" and Its Potential Application in Weed Control in Direct-Seeded Rice Fields

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ABSTRACT. Rice, as one of the world's most important staple crops, plays a crucial role in ensuring food security. Currently, traditional rice production methods face immense pressure due to challenges such as labor shortages, aging agricultural workforces, and water scarcity caused by climate change. Against this backdrop, direct-seeded rice technology has garnered widespread attention for its advantages in labor savings and improved water-use efficiency. However, weed management in direct-seeded rice fields remains a significant challenge. While traditional chemical herbicides are effective, they pose environmental pollution risks and food safety concerns. To address this issue, this study successfully breeds a non-GMO two-line sterile line, "Z017S," through pedigree breeding using herbicide-resistant germplasm resources (such as Hua K01S) and superior two-line sterile lines as parents. This sterile line exhibits stable herbicide resistance and sterility traits, providing a new parental resource for breeding herbicide-resistant hybrid rice. It offers an eco-friendly, cost-effective, and easy-to-operate solution for weed control in direct-seeded rice fields compared to traditional herbicide methods. The results show that Z017S demonstrates excellent herbicide resistance and sterility across various growing environments, indicating a wide range of application prospects.

Key words: Direct-seeded rice; Herbicide resistance; Z017S; Weed management; Sustainable rice production.

INTRODUCTION

As one of the world's major food crops, rice plays a vital role in global food security (Rezvi et al., 2022; Chaudhary et al., 2023). It is estimated that about over half of the global per capita caloric intake comes from rice, underscoring its central place in global life (Muthayya et al., 2014). High and stable rice yields are not only crucial for ensuring global food security but also serve as an important foundation for maintaining economic stability and promoting social development. However, in recent years, rapid global economic development and accelerated urbanization have significantly altered the structure of the agricultural workforce (Ma et al., 2019). The large-scale shift of labor from the agricultural to industrial sectors has led to a shortage of able-bodied workers engaged in agricultural production and an increasingly aging agricultural workforce (Zhang et al., 2023). This shift poses new challenges to traditional agricultural production models, making it imperative to improve agricultural productivity and explore production models that can adapt to these new circumstances.

On the other hand, globally, increasingly severe water shortages have become one of the most urgent challenges facing agricultural production (Ingrao et al., 2023). The rapid decline in groundwater levels sharply contrasts with the high water demand associated with traditional rice cultivation methods (Jasechko et al., 2024). Climate change is increasing the variability of future rainfall patterns, elevating the risks of drought and water scarcity, and further exacerbating water shortages in rice cultivation. All these factors could have profound impacts on agricultural productivity (Hussain et al., 2022). Therefore, rice cultivation methods must continually adapt to changing conditions, sparking innovations in new models to meet the dual demands of resource conservation and food security. Against this backdrop, technologies such as machine transplanting and direct seeding have gained widespread attention and adoption due to their advantages in labor savings, increased efficiency, and reduced production costs (Cui et al., 2022).

Direct seeding technology for rice has significant advantages in agricultural production but also faces some challenges (Hafeez ur et al., 2019). The primary advantage of direct seeding lies in the elimination of the need for seedling raising and transplanting, which significantly reduces labor demand. This is particularly beneficial in regions with high labor costs, where it can significantly lower production expenses and improve farmers' economic returns (Chaudhary et al., 2023). Additionally, direct seeding is crucial in improving planting efficiency and conserving water resources (Hang et al., 2022; Jat et al., 2022). Direct seeding technology also faces challenges, such as lodging, low seed germination rates, and difficulties in managing weeds in paddy fields. To address issues like lodging and low germination rates, rice varieties with strong lodging resistance and high seed germination ability are usually chosen for direct seeding (Kaur, 2017). However, weed management in direct-seeded fields remains a persistent challenge and is currently a key focus of scientific research (Shekhawat et al., 2020).

Weeds in paddy fields not only compete with rice plants for sunlight, nutrients, and water but also serve as habitats for pests, severely affecting the growth and development of rice crops, which in turn leads to reduced yield and quality (Zhou et al., 2023). Statistics show that if weeds in paddy fields are not effectively managed, rice yields can drop by 10% to 30%, and in extreme cases, can even result in total crop failure (Xu et al., 2024). In China, there are over 200 common weed species found in paddy fields, of which about 40 cause significant damage (Luo et al., 2014). Among them, barnyard grass has the widest distribution and causes the most damage, significantly impacting rice yields. It also shares a high genetic affinity with rice in terms of evolutionary development. Currently, the primary methods for weed control in paddy fields include tillage management, manual weeding, chemical weeding, biological control, and mechanical weeding (Xu et al., 2024; Kumar et al., 2017; Paiman et al., 2020).

Recent advances in weed control research have produced some positive results. For example, to manage excessive growth in rice populations, controlling fertilizer application in the middle and late growth stages can reduce ineffective tillers and increase effective ones, thereby optimizing plant population structure. During the growth and development of rice, it is also crucial to consider the application of panicle fertilizers based on field fertility, crop growth, and climatic conditions to prevent excessive nutrition and delayed maturity of crops (Tian et al., 2017; Ma et al., 2020). Chemical herbicides remain the most common method for weed control in paddy fields. In rice cultivation under simplified planting models, the application of 2 to 3 selective herbicides is usually required to effectively manage weeds and ensure stable and high yields. However, the use of herbicides not only increases production costs but also causes environmental pollution and raises concerns about food safety (Parven et al., 2024; Kumar et al., 2017).

Genetic engineering offers another potential method for weed control. By breeding genetically modified (GM) rice varieties resistant to herbicides, broad-spectrum herbicides such as glyphosate and gluconate can be used during the rice growth period, broadening the scope of weed control and potentially reducing or eliminating the need for specific selective herbicides for grass-like malignant weeds. While the cultivation of GM crops has advantages such as reducing production costs, improving product quality, and decreasing ecological pollution, the planting of GM rice has long been a subject of social controversy (Dong et al., 2021; Kumam et al., 2023). As of now, China has not approved its large-scale promotion and application. Therefore, finding effective weed control solutions in directly sown rice fields without relying on genetic engineering has become a major challenge for agricultural development (Sen et al., 2021).

To address these challenges, this study successfully developed a non-genetically modified two-line male sterile line "Z017S" through pedigree selection, using herbicide-resistant germplasm resources (such as K01S) and excellent two-line sterile lines as parents. This sterile line exhibits stable herbicide resistance and sterility traits, providing a new solution to the weed control problem in directly sown rice fields. In this study, herbicide-resistant germplasm resources (Hua K01S) obtained through chemical mutagenesis were used as parents to hybridize with Ke 36S. Subsequently, Ke 36S was crossed with the F_1 generation, and after 4 years and 8 generations of systematic breeding, the two-line sterile line "Z017S" was identified in 2024 by the Anhui Province Sterile Line Identification. The herbicide-resistant sterile line bred in this study provides important parental and germplasm resources for breeding directly sown rice varieties, offering an efficient and convenient method for hybrid seed production and direct sowing of hybrid rice varieties, with broad practical application prospects and immense potential value.

MATERIALS AND METHODS

The breeding process of "Z017S", a new herbicide-resistant, temperature-sensitive sterile line in rice. The breeding process is described as follows (Figure 1):

A. Parental Lines Selection: Two germplasm resources were chosen as starting materials for the breeding process: HuaK 01S, a herbicide-resistant germplasm: Ke 36S, a two-line sterile line material.

B. Initial Cross: The breeding process began with crossing HuaK 01S (female parent) with Ke 36S (male parent).

C. Backcrossing: The resultant F_1 generation from the initial cross was then backcrossed with Kee 36S to introduce the desirable traits from Ke 36S into the progeny.

D. Systematic Breeding: This breeding process spanned 4 years and 8 generations of systematic selection and breeding to isolate the temperature-sensitive sterile lines.

E. Winter 2019 Cross: Another cross was performed using Huakai 01S with Kee 36S.

F. BC_1F_1 Generation: In the first quarter of 2020, the backcross between Kee 36S and the F_1 generation resulted in more than 2000 BC_1F_1 plants.

G. Planting and Selection: These BC_1F_1 plants were grown in Hainan in the winter of 2020. Over the next 3 years, the plants underwent further systematic breeding.

H. Final Selection: In the first quarter of 2023, a line named 23S017 was selected for its strong combination advantages, slightly shorter growth period, compact plant type, stable fertility, and longer grain size.

I. Seed Propagation: The original seed of the selected line 23S017 was propagated in Hainan during the winter of 2023.

J. Naming: The selected line was officially named "Z017S".

Herbicide spraying: The herbicide was imazamox, 0.375ml/m^2, and was observed 18 days after spraying.

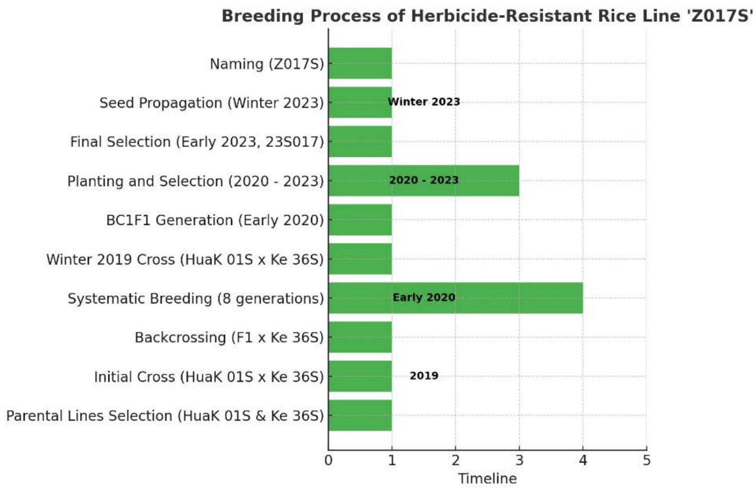

Figure 1. The breeding process of "Z017S".

RESULTS

The fertility performance of Z017S

In 2022, a staggered sowing trial was conducted in Hefei to observe the fertility dynamics of Z017S under natural conditions. The trial started on April 28 and ended on June 18, with sowing taking place every 10 days for a total of 6 sowing periods. From the heading stage, five panicles were collected every two days for microscopic examination, and the results are presented in Figure 2. Figure 2 shows that Z017S had a long sterility period with a significant fertility transition. From July 24 to September 3, the pollen sterility rate observed under the microscope ranged from 99.9% to 100.0%, predominantly displaying a pollen-free type of sterility. The bagged selfing trial was conducted from July 24 to September 3. In the bagged selfing conducted before September 3, all exhibited self-sterility, except for the selfing on September 3, which had a seed-setting rate of 0.1%.

In 2023, another staggered sowing trial was conducted in Hefei to observe the fertility dynamics of Z017S under natural conditions. The trial started on May 5 and ended on June 15, with sowing taking place every 10 days for a total of 5 sowing periods. From the heading stage, five panicles were collected every two days for microscopic examination, and the results are presented in Figure 3. Figure 3 shows that Z017S still had a long sterility period with a significant fertility transition. From July 26 to September 6, the pollen sterility rate observed under the microscope ranged from 99.8% to 100.0%, also predominantly showing a pollen-free type of sterility. The bagged selfing trial was conducted from July 26 to September 6. In the bagged selfing conducted before September 6, all exhibited self-sterility, except for the selfing on September 6, which had a seed-setting rate of 0.2%.

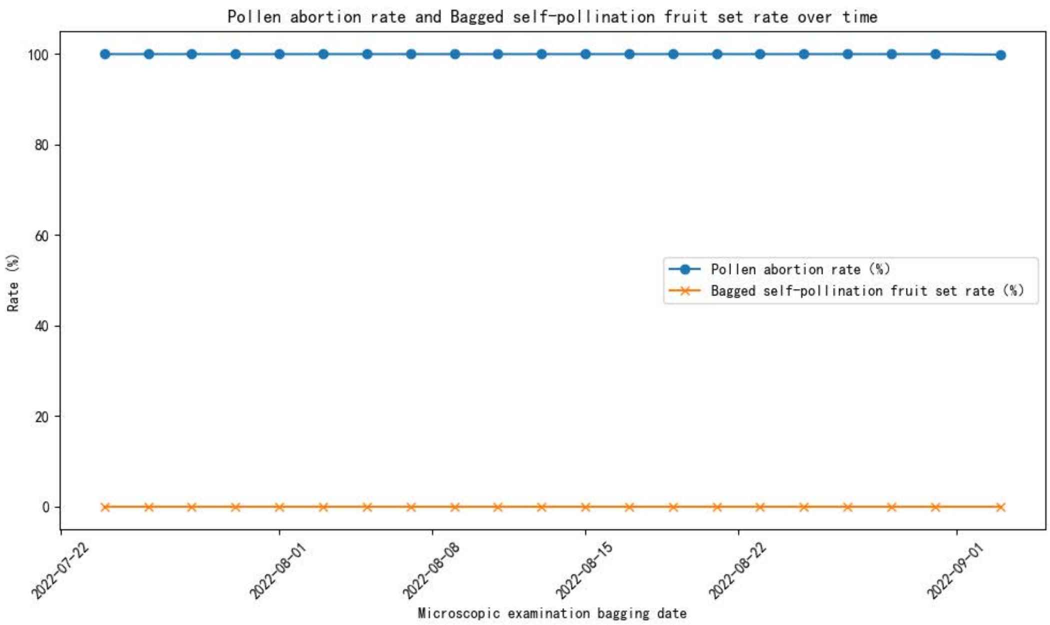

Figure 2. Fertility observations of Z017S under natural conditions in Hefei during 2022.

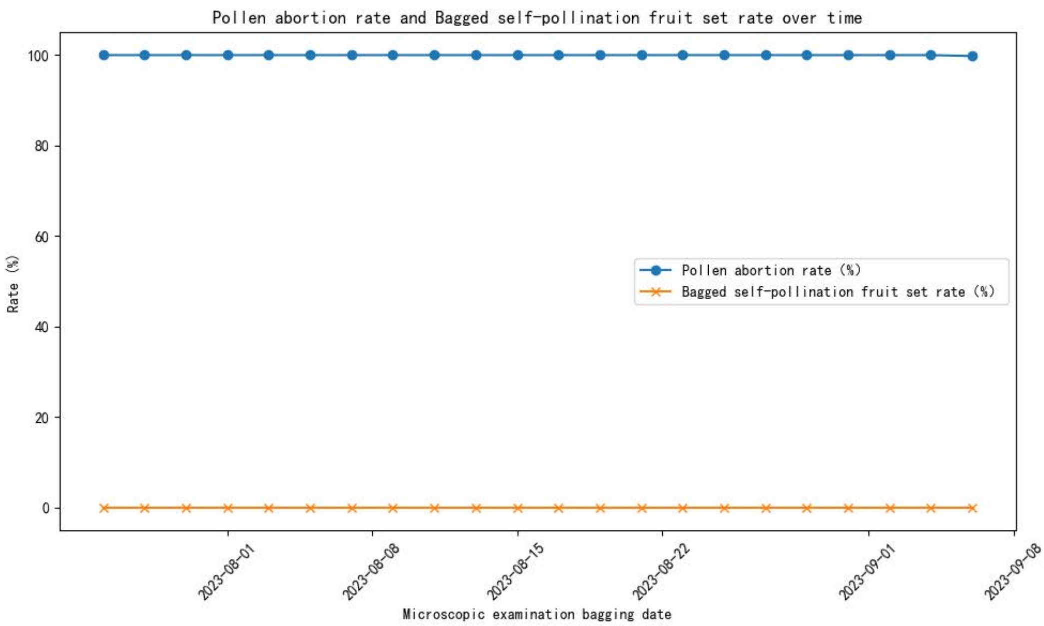

Figure 3. Fertility observation data for Z017S in 2023 under natural conditions in Hefei.

In the winter of 2022, a small-scale propagation of "Z017S" was conducted at the Nanbin Farm in Sanya, Hainan Province, at the South Breeding Base. The propagation covered an area of 0.2 mu (approximately 1/15 of an acre). The seeds were sown on November 15, and the panicles emerged on February 12. The effective panicle count per mu was 165,000, with a total of 143.0 grains per panicle and 78.0 filled grains per panicle. The thousand-grain weight was 25.5 grams, and the actual yield per mu was 320.5 kilograms. These results indicate that "Z017S" exhibits good fertility characteristics.

Analysis of the characteristic traits of Z017S

The average plant height of Z017S, when grown in Hefei, is 82.5 cm, with an average ear length of 22.4 cm. Each ear contains a total of 170.0 grains, of which 14 are necked, resulting in a necked grain rate of 8.24%. Individual plants produce 7 to 9 years, and the thousand-grain weight is approximately 25.5 grams. The grains are elongated, with a colorless stigma and a top awn. The plant has a compact structure, strong tillering ability, thick stems, upright flag leaves, and slightly inward-rolled leaves.

Under favorable weather conditions, flowering occurs between 9:30 and 11:30 AM. The stigma remains colorless, with an exposure rate of 54.5%. The cross-pollination rate for small-scale seed production can reach up to 48%.

In 2022, Z017S was sown in Hefei from May 10 to May 30, with a growth period ranging from 76 to 82 days, and the main stem displayed 14.1 to 14.8 leaves.

In 2023, the sowing of Z017S in Hefei took place from May 7 to May 26, resulting in a growth period of 77 to 84 days, and the number of leaves on the main stem varied from 14.2 to 14.9 leaves.

The combined advantages of Z017S

This sterile line exhibits strong combining ability and has yielded numerous promising hybrid combinations. Among them, the hybrid ‘Z017S/22HC1324’ demonstrated high yield, superior quality, and disease resistance during plot evaluations from 2022 to 2023. It achieved a yield increase of 7.50%-9.25% compared to Fengliangyou No. 4. The plant height reached 118 cm, with an average of 169,000 effective panicles per mu. Each panicle contained an average of 208.5 grains, with a fruit set rate of 93.6% and a 1000-grain weight of approximately 25.0 grams. The rice exhibited excellent grain quality. In field tests, it showed resistance to bacterial blight and was free from rice blast disease.

The primary distinctions between Z017S, Hua K01S, and Ke 36S

Through observation and comparative analysis, the key differences between Z017S and the control lines Hua K01S and Ke 36S are detailed in Tables 4 and 5. Table 4 reveals that Z017S has a less compact plant type compared to Ke36S, possesses a top awn that is absent in the control, extends the sowing period by over 10 days relative to Ke36S, exhibits a thousand-grain weight that is 2g heavier, and has a wider flag leaf than Ke36S.

Table 5 illustrates that Z017S is 9.1cm taller in plant height than HuaK 01S, features a longer top awn, and has larger dimensions in both length and width compared to HuaK 01S.

Eighteen days after the herbicide application at the seedling stage, the results showed that both Z017S and HuaK 01S demonstrated good herbicide resistance, with normal plant growth and no noticeable signs of herbicide damage (Figure 4 and Figure 5). In contrast, the control variety Ke 36S was sensitive to the herbicides used, with all plants dying within 15 days. This highlights the herbicide-resistant characteristics of Z017S.

Figure 4. Field phenotypes of sprayed and unsprayed imazamox for two materials Z017S and Hua K01S.

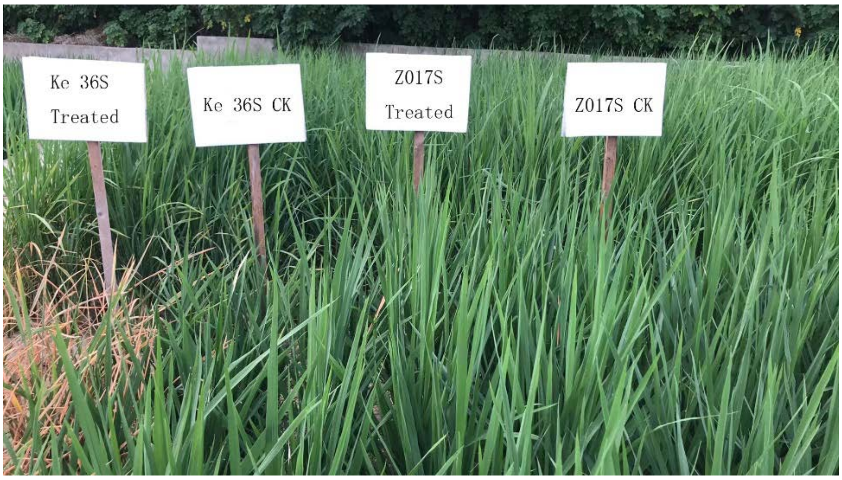

Figure 5. Field phenotypes of sprayed and unsprayed imazamox for two materials Z017S and Ke 36S.

DISCUSSION

This study utilized herbicide-resistant materials obtained through chemical mutagenesis as parents, combined with traditional pedigree breeding methods, to successfully breed a new herbicide-resistant, thermosensitive two-line sterile line, "Z017S." This sterile line shows significant application potential in weed control in direct-seeded rice fields and improving seed production efficiency of direct-seeded hybrid rice. Compared to traditional weed control methods, Z017S offers an effective non-GMO alternative that is environmentally friendly, low-cost, and easy to operate. This study provides a feasible solution to the global challenges of labor shortages and increased water pressure in rice production.

Direct-seeded rice planting technology has significant advantages over traditional transplanting methods, especially in terms of labor and water resource management (Shanmugam Vijayakumar et al., 2023). However, direct-seeded rice planting also faces some challenges. Since direct-seeded rice seedlings are directly exposed to natural conditions, they are more susceptible to weed invasion (Kaur, 2017; Li et al., 2023). Weeds compete with rice for nutrients, water, and sunlight, and serve as breeding grounds for pests and diseases, severely affecting rice yield and quality (Kumar et al., 2017; Shekhawat et al., 2020). Studies have shown that if weeds are not effectively controlled in direct-seeded fields, yield loss can reach 70-100%. Additionally, direct-seeded rice seedlings are less competitive than transplanted seedlings, further increasing the difficulty of weed control. Weed control in direct-seeded rice fields is particularly important, with current methods including manual weeding, mechanical weeding, chemical weeding, and biological weeding. Manual weeding is a primitive method that can prevent weed regrowth, and spread, and precisely remove specific types of weeds, such as barnyard grass, but it is labor-intensive and inefficient(Shekhawat et al., 2020). Chemical weeding is currently the most common and effective weed management method. Chemical herbicides are easier to control in the early growth stages of weeds and can significantly reduce the impact of weeds on rice. However, long-term reliance on chemical herbicides can lead to increased weed resistance, pesticide residues in rice, and soil compaction, among other negative effects (Kumar et al., 2017; Shekhawat et al., 2020). Developing modern tools such as intelligent robots and field mechanical weeding equipment can improve weeding efficiency and reduce the use of chemical agents, thereby reducing environmental pollution (Xiang et al., 2024); however, precision welding operations need further improvement. Bio-based herbicides and microbial herbicides are environmentally friendly herbicides and are one of the future directions of herbicide development (Parven et al., 2024; Ocan-Torres et al., 2024). However, issues such as susceptibility to environmental factors and poor stability hinder the commercialization of bioherbicides. Researchers have conducted extensive research on herbicide-resistant plants and their resistance mechanisms. Through artificial mutagenesis and mutant screening, herbicide-resistant gene-targeted mutagenesis, or the introduction of exogenous resistance genes into rice through transformation, a series of herbicide-resistant rice materials have been obtained (Dong et al., 2021). However, the promotion of genetically modified rice has not yet been approved in China. Based on chemically induced materials, this study developed a two-line sterile line Z017S with herbicide resistance. Compared with traditional weed control techniques in direct-seeded rice fields, Z017S, due to its herbicide resistance, can effectively solve weed problems in direct-seeded rice fields, reducing or eliminating the use of selective herbicides targeting noxious grass weeds. Traditional herbicide methods can easily cause environmental pollution and food safety issues, while this study provides a safer and more environmentally friendly solution by breeding herbicide-resistant sterile lines.

The research results indicate that Z017S exhibited stable herbicide resistance and sterility across various growth environments. In seedling-stage herbicide spraying trials, neither Z017S nor Hua K01S showed herbicide damage symptoms after 18 days, while all control varieties Ke 36S died under the same conditions, demonstrating the excellent herbicide resistance of Z017S. Moreover, Z017S maintained good growth performance and yield levels under different environmental conditions (such as Hefei and Hainan), further confirming its stability and adaptability for application.

The successful development of Z017S provides high-quality parental lines for hybrid seed production in direct-seeded rice significantly improving weeding efficiency in rice fields and reducing agricultural production costs. Farmers can significantly reduce labor input and herbicide usage through its application in direct-seeded hybrid rice, lowering both the economic and environmental costs of agricultural production. Additionally, this non-GMO herbicide-resistant rice variety avoids the controversies associated with genetically modified crops in societal applications, making it widely socially acceptable.

Although Z017S has demonstrated excellent herbicide resistance and sterility traits, further research is needed to study its long-term stability under different environmental and planting conditions. Moreover, for its promotion and application in actual agricultural production, comprehensive evaluations combining specific conditions of different regions are required to ensure its stability and efficiency in practical use. Future research can focus on optimizing the temperature threshold for fertility conversion, improving seed germination rates, and further enhancing plant stress resistance to enhance its application effects in practical production.

As the agricultural labor structure and global resource conditions change, agricultural production methods are gradually evolving toward mechanization and intensification. The introduction of Z017S provides new possibilities for agricultural mechanization; its excellent herbicide resistance can be combined with mechanical or chemical weeding to improve weeding efficiency and reduce production costs, thereby achieving the modernization transformation of rice production systems. This study not only enriches the germplasm resource pool of herbicide-resistant sterile lines but also provides new strategies and tools for future rice production.

CONCLUSION

In summary, this study successfully developed the herbicide-resistant, thermosensitive two-line sterile rice "Z017S," which can potentially address weed control challenges in direct-seeded rice fields and significantly improve production efficiency and economic benefits. In the future, as research on its application in different agricultural production systems deepens, Z017S is expected to become an important variety for improving rice production efficiency, reducing production costs, and promoting sustainable agricultural development.

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REFERENCES

Chaudhary A, Venkatramanan V, Kumar Mishra A, Sharma S. (2023). Agronomic and Environmental Determinants of Direct Seeded Rice in South Asia. *Circ Econ Sustain.* 3: 253-290.

Cui Y, Zhu M, Song J, Fan H, et al. (2022). Expression dynamics of phytochrome genes for the shade-avoidance response in densely direct-seeding rice. *Front Plant Sci.* 13: 1105882.

Dong H, Huang Y, Wang K. (2021). The Development of Herbicide Resistance Crop Plants Using CRISPR/Cas9-Mediated Gene Editing. *Genes (Basel).* 12: 912.

Hafeez ur R, Nawaz A, Awan MI, Ijaz M, et al. (2019). Direct Seeding in Rice: Problems and Prospects. *Agronomic Crops.* pp.199-222.

Hang X, Danso F, Luo J, Liao D, et al. (2022). Effects of Water-Saving Irrigation on Direct-Seeding Rice Yield and Greenhouse Gas Emissions in North China. *Agriculture.* 12: 937.

Hussain J, Hussain S, Tahir N, Rasool I, et al. (2022). Climate Change and Rice Production: Impacts and Adaptations. *Modern Techniques of Rice Crop Production*. pp. 585-603.

Ingrao C, Strippoli R, Lagioia G, Huisingh D. (2023). Water scarcity in agriculture: An overview of causes, impacts and approaches for reducing the risks. *Heliyon.* 9: e18507.

Jasechko S, Seybold H, Perrone D, Fan Y, et al. (2024). Rapid groundwater decline and some cases of recovery in aquifers globally. *Nature.* 625: 715-721.

Jat RK, Meena VS, Kumar M, Jakkula VS, et al. (2022). Direct Seeded Rice: Strategies to Improve Crop Resilience and Food Security under Adverse Climatic Conditions. *Land*. 11: 382.

Kaur J, Singh A. (2017). Direct Seeded Rice: Prospects, Problems/Constraints and Researchable Issues in India. *Current Agriculture Research Journal.* 5: 13-32.

Kumam Y, Trick HN, Vara Prasad PV, Jugulam M. (2023). Transformative Approaches for Sustainable Weed Management: The Power of Gene Drive and CRISPR-Cas9. *Genes (Basel).* 14: 2176.

Kumar V, Mahajan G, Chauhan BS. (2017). Rice Weeds and Their Management. *Rice Production Worldwide.* pp. 361-392.

Li X, Dong J, Zhu W, Zhao J, et al. (2023). Progress in the study of functional genes related to direct seeding of rice. *Mol Breed.* 43: 46.

Luo Y, Fu H, Traore S. (2014). Biodiversity Conservation in Rice Paddies in China: Toward Ecological Sustainability. *Sustainability.* 6: 6107-6124.

Ma L, Long H, Zhang Y, Tu S, et al. (2019). Agricultural labor changes and agricultural economic development in China and their implications for rural vitalization. *Journal of Geographical Sciences.* 29: 163-179.

Ma P, Lan Y, Lyu T, Zhang Y, et al. (2020). Improving Rice Yields and Nitrogen Use Efficiency by Optimizing Nitrogen Management and Applications to Rapeseed in Rapeseed-Rice Rotation System. *Agronomy.* 10: 1060.

Muthayya S, Sugimoto JD, Montgomery S, Maberly GF. (2014). An overview of global rice production, supply, trade, and consumption. *Ann N Y Acad Sci.* 1324: 7-14.

Ocan-Torres D, Martinez-Burgos WJ, Manzoki MC, Soccol VT, et al. (2024). Microbial Bioherbicides Based on Cell-Free Phytotoxic Metabolites: Analysis and Perspectives on Their Application in Weed Control as an Innovative Sustainable Solution. *Plants (Basel)* 13: 1996.

Paiman S, Ismail NF, Abdullah AS. (2020). Recent Developments of Weed Management in Rice Fields. *Reviews in Agricultural Science.* 8: 343-353.

Parven A, Meftaul IM, Venkateswarlu K, Megharaj M. (2024). Herbicides in modern sustainable agriculture: environmental fate, ecological implications, and human health concerns. International *Journal of Environmental Science and Technology.*

Rezvi HUA, Tahjib-Ul-Arif M, Azim MA, Tumpa TA, et al., (2022). Rice and food security: Climate change implications and the future prospects for nutritional security. *Food and Energy Security.* 12: e430.

Sen S, Kaur R, Das TK, Raj R, et al. (2021). Impacts of herbicides on weeds, water productivity, and nutrient-use efficiency in dry direct-seeded rice. *Paddy and Water Environment.* 19: 227-238.

Vijayakumar S, NN, AM, Saravanane P, et al. (2023). Revolutionizing Rice Farming Maximizing Yield with Minimal Water to Sustain the Hungry Planet. *IntechOpen.*

Shekhawat K, Rathore SS, Chauhan BS. (2020). Weed Management in Dry Direct-Seeded Rice: A Review on Challenges and Opportunities for Sustainable Rice Production. *Agronomy*. 10: 1264.

Tian G, Gao L, Kong Y, Hu X, et al. (2017). Improving rice population productivity by reducing nitrogen rate and increasing plant density. *PLoS One.* 12: e0182310.

Xiang M, Qu M, Wang G, Ma Z, et al. (2024). Crop detection technologies, mechanical weeding executive parts and working performance of intelligent mechanical weeding: a review. *Front Plant Sci.* 15: 1361002.

Xu S, Wang F, Ding Y, Liu W, et al. (2024). An Ecological Weed Control Strategy in Paddy Fields: Light Interception from Duckweed Mulching. *Agronomy.* 14: 670.

Zhang H, Li J, Quan T. (2023). Strengthening or Weakening: The Impact of an Aging Rural Workforce on Agricultural Economic Resilience in China. *Agriculture.* 13: 1436.

Zhou W, Song K, Sun X, Fu Q, et al. (2023). Design Optimization and Mechanism Analysis of Water Jet-Type Inter-Plant Weeding Device for Water Fields. *Agronomy.* 13: 1305