

EFFECTS OF FOLIAR SPRAYING OF BIOLOGICAL NANO SELENIUM ON THE BLOOMING TRAITS IN HYBRID FRAGRANT RICE SEED PRODUCTION

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(Received 2nd Jan 2025; accepted 11th Jun 2025)

Abstract. Selenium is an important trace element for plant growth and development, and plays a key role in improving crop stress resistance, yield and quality. As a new type of high-efficiency selenium fertilizer, the regulatory mechanism of biological nano-selenium (nano-Se) in the reproductive development stage of rice still needs to be further studied. This study was conducted to investigate the effects of foliar spraying of biological nano-Se on flowering traits of hybrid aromatic rice seed production. In this study, a field experiment was carried out, with two seed production combinations (Qing A×19Xiang and Sheng A×19Xiang) and four biological nano-Se treatments: a control group (CK, no spray), a group sprayed 3 days before young panicle differentiation (T1), a group sprayed at the ear break stage (T2), and a group sprayed at both stages (T3). The results showed that compared with CK, the pollen grain number per stamen and percentage of pollen germination in vitro of T1 and T3 treatments were significantly increased by 8.40%, 7.56% and 31.06%, 31.46%, respectively. Meanwhile, the biological nano-Se treatments significantly improved the superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activity of rice leaves and reduced the content of malondialdehyde (MDA) in the leaves, indicating that it may promote the development of floral organs by reducing oxidative damage. In contrast, due to the short intervention time of biological nano-Se, the regulation effect of T2 treatment on rice flowering traits was significantly lower than that of T1 and T3 treatments, and there was no significant difference in the regulation effect of T1 and T3 treatments on rice flowering traits. Considering the cost of biological nano-Se, T1 treatment was identified as the best choice.

Keywords: *biological nano-selenium, hybrid seed production, flowering trait, antioxidant, pollen*

Introduction

Fragrant rice is highly valued for its unique aroma, with volatile aromatic compounds distributed in all plant organs except the roots (Zhao and Tang, 2011). Previous studies have shown that natural environmental factors (such as temperature, light, and moisture), agricultural practices (such as planting density, fertilization methods, and irrigation management), and the application of external nutrients (such as trace elements and growth regulators) affect the physiological characteristics related to rice blooming traits (Qin et al., 2022; Zhu et al., 2022). Among them, selenium has attracted much attention due to

its dual effects of “low promotion and high inhibition” (Poonlaphdecha et al., 2016). Its application effect depends not only on the total concentration of exogenous selenium, but also on its chemical form (Wu, 2018).

When exploring the transformation process of Se into biological macromolecules (such as DNA and protein), researchers have frequently observed the formation of red substances (Zhang et al., 2005), which have been identified as biological nano-selenium (nano-Se) ranging from 5–200 nm in diameter. The emergence of biological nano-Se not only solves the problems of high cost of traditional selenium fertilizer, low utilization rate of selenium by crops, and narrow range of safe concentration, which leads to “selenium poisoning” of crops, but also attracts wide attention of researchers because of its high safety, easy absorption and high biocompatibility (Malyugina et al., 2021; Zambonino et al., 2023). The results of Yu (2023) research showed that spraying biological nano-Se could simultaneously increase the seed setting rate and 1000-grain weight of rice to achieve yield increase, while spraying sodium selenite interfered with the grain filling process of rice and resulted in a decrease in seed setting rate. Li (2023) reported that biological nano-Se treatment increased the activities of peroxidase, polyphenol oxidase and catalase in cowpea pods while reducing the content of malondialdehyde. Liao (2023) review revealed that the application of biological nano-Se in agriculture is to improve plant growth (Wu et al., 2021; Liang et al., 2022), enhance plant stress resistance (Li et al., 2020) and inhibits the accumulation of heavy metals in plants (Qi et al., 2021).

In this study, biological nano-Se was applied in hybrid aromatic rice seed production system for the first time, focusing on the key seed production link of hybrid rice flowering characteristics, which filled the research gap of biological nano-Se on reproductive development regulation. It is also revealed that biological nano-Se can regulate the development process and antioxidant activity of floral organs, which provides a new perspective for improving the efficiency of hybrid seed production, and promotes biological nano-Se from basic research to hybrid breeding practice, which provides theoretical and technical support for the upgrading of aromatic rice industry in South China.

Materials and methods

Experimental background

This study carried out field trials for hybrid rice seed production in the late season of 2023 at the Agronomic Experiment Station (23°09'N, 113°22'E) of South China Agricultural University, Tianhe District, Guangzhou city, Guangdong Province. The female sterile lines used in this experiment were Sheng A and Qing A, and the male parent of the restorer line was 19Xiang, both of which were provided by the Guangdong Agricultural Technology Extension Center. The biological Nano-Se phytonutrient (Datian Special No.1) was used as the test material. The selenium content of this stock solution was 2 g·L⁻¹, and the average particle size was 13.767 nm, which was provided by LvHuinong Biotechnology (Shenzhen) Co., Ltd. The company showed that the best spraying specification was to spray 750 mL of stock solution per hectare, and 300 times of water should be added before spraying, that is, the spraying concentration was 6.67 mg·L⁻¹. The physical and chemical properties of the experimental soil were as follows: the organic matter content was 23.19 g·kg⁻¹, the total nitrogen content was 1.84 g·kg⁻¹, the total phosphorus content was 1.42 g·kg⁻¹, the total potassium content was

20.05 g·kg⁻¹, and the pH was 6.54. The meteorological data collected during the test are detailed in *Table 1*, weather station: 592870 (23 39' N, 113 30' E, altitude of 15.24 m).

Table 1. Monthly meteorological data during the experiment

Month	Daily average temperature (°C)	Daily maximum temperature (°C)	Daily minimum temperature (°C)	Precipitation amount (mm)	Rainfall days (d)
8	29.50	36.70	24.40	434.60	19
9	28.30	35.20	24.20	757.30	16
10	24.90	35.70	16.10	129.00	13

Experimental design

To examine the effects of foliar spraying of biological nano-Se on blooming traits in hybrid fragrant rice, a field experiment was conducted using two seed production combinations (Qing A×19Xiang and Sheng A×19Xiang) and four treatments: a control group (CK, no spray), a group sprayed 3 days before young panicle differentiation (T1), a group sprayed at the ear break stage (T2), and a group sprayed at both stages (T3). Four replications were performed, and each plot had an area of 16 m². The sowing period was divided into two periods. The female parent Qing A and the first-stage male parent were sown on July 8 for the first time. The second sowing of the female parent Sheng A and the second-stage male parent occurred on July 18. The seeds were sown by a rice pot seedling machine (2BD-300), while the male parent seeds were sown by hand. Mechanical transplanting was performed on July 31, with the spacing between the rows and the plants being 30 cm×14 cm, and the row ratio of the parents for seed production was 2:6. Xinxiang rice fertilizer (N:P₂O₅:K₂O = 15:4:6) was used, the base fertilizer application rate was 450 kg·hm⁻², the tillering fertilizer application period was 10 days after transplanting, and the application rate was 300 kg·hm⁻². The male parent fertilizer was applied 2 times (the first time was 5 days after tillering fertilizer; the second time was 10 days after tillering fertilizer. The amount of fertilizer was 225 kg·hm⁻² each time). Before rice heading, a 2-meter-high transparent film was installed around each plot to prevent cross-pollination between different treatments. During the flowering period, manual pollination was conducted using bamboo poles. In addition, biological nano-Se (750 mL of stock solution per hectareml, 300 times diluted with water) were sprayed on the foliar surface as an additional field farming operation, and the remaining water and fertilizer management and blooming period regulation were implemented according to the “three-line” hybrid seed production technology (DB44/T 434-2007).

Determination indexes and methods

Determination of the female parent's non-flowering panicle rate, panicle emergence rate, and unemerged panicle rate

The proposed method was performed according to Shu (2016) and Yan (2016). At the pre-anthesis stage of female plants, primary panicles with homogeneous growth vigor were selected as observation subjects. The following parameters were recorded: (i) panicle emergence dates, and (ii) total grain number per panicle. Additionally, we quantified: (a) the count of emergence panicles, and (b) the number of non-flowering

panicles (defined as panicles that emerged during the entire flowering period but failed to complete floral opening, with all anthers persistently retained within the spikelet structures).

Non-flowering panicle rate (%) = number of unflowered flowers outside the leaf sheath / total number of spikelets outside the leaf sheath $\times$ 100%

Panicle emergence rate (%) = total number of spikelets outside the leaf sheath / total number of spikelets $\times$ 100%

Unemerged panicle rate (%) = 1 - panicle emergence rate

Determination of the male parent's Pollen grain number per stamen and Percentage of pollen germination in vitro

The Pollen grain number per stamen and Percentage of pollen germination in vitro were determined according to the methods of Wen (2010), Sun (2015) and Zhao (2023). Rice panicles were randomly collected and immersed in containers with room-temperature clear water between 7:00 and 8:00 in the morning. then, the samples were quickly returned to the laboratory for observation. During sample processing, the flowering spikelets that had already bloomed were removed, and the spikelets that were not completely opened in the upper part (the anthers had extended to the top) were selected as the measurement objects. The anthers were separated and made into microscope slides to count the number of pollen grains. An appropriate amount of pollen (more than 200 pollen grains) was placed in culture medium (ingredients: 40 mg $\cdot$ LH₃BO₃⁻¹, 3 mmol $\cdot$ L⁻¹Ca(NO₃)₂, 20% sucrose, 10% PEG4000, 10 mg $\cdot$ L⁻¹ V-B1), the slides were placed in a Petri dish cushioned with wet filter paper, the Petri dish lid was covered with wet gauze, and after being incubated in a 33°C incubator for 2 h, the samples were observed under a microscope, and the germination threshold was that the pollen tube elongation exceeded 50% of the maximum diameter of the original pollen grains.

Determination of antioxidant indicators at the blooming stage

Spikelets and flag leaves that had essentially the same growth process were collected during the flowering stage, quickly frozen in liquid nitrogen, and then stored in a -80°C freezer in the laboratory for subsequent detection of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activity, and malondialdehyde (MDA) content (Gu et al., 2024).

Statistical analysis of data

The trial data were entered and collated via WPS Office software. IBM SPSS Statistics 21 software was used for analysis of variance (ANOVA) and least significant difference (LSD) multiple comparisons. The data analysis results were converted into charts via Microsoft Excel 2021 software. In the charts, different lowercase letters indicate that the difference is significant according to the LSD test ($P < 0.05$); in ANOVA, ** indicates that the difference is very significant ($P < 0.01$), and * indicates that the difference is significant ($0.01 < P < 0.05$). ns indicates that the difference was not significant ($P > 0.05$).

Results and analysis

Effects of the sterile lines's non-flowering panicle rate, panicle emergence rate, and unemerged panicle rate

The results of the ANOVA (Table 2) revealed that variety (C), treatment (T), and the interaction (C×T) of variety and treatment had significant effects on the agronomic traits associated with the female parent blooming stage. For Sheng A, compared with CK treatment, different biological nano-Se treatments reduced the rate of non-flowering panicle rate and unemerged panicle rate. Among them, the non-flowering panicle rate significantly decreased by 71.81% under the T1 treatment; Compared with CK, the Panicle emergence rate increased under different biological nano-Se treatments, but the difference was not significant. For Qing A, Compared with CK, the non-flowering panicle rate under the T1 and T3 treatments significantly decreased, by 46.86% and 43.96%, respectively, whereas the T2 treatment had no significant effect on the non-flowering panicle rate; Compared with CK, the panicle emergence rate in the T1, T2, and T3 treatments significantly increased by 2.79%, 2.96% and 5.93%. In summary, the biological nano-Se treatments had a significant regulatory effect on the flowering agronomic traits of the two sterile rice varieties, and the T1 treatment showed the most prominent effect.

Table 2. Effects of different biological nano-Se treatments on the non-flowering panicle rate, panicle emergence rate, and unemerged panicle rate of rice sterile lines.

Female parent	Treatment	Non-flowering panicle rate (%)	Panicle emergence rate (%)	Unemerged panicle rate (%)
Sheng A	CK	1.49 ± 0.26a	99.18 ± 1.04a	0.82 ± 1.04a
	T1	0.42 ± 0.05b	99.89 ± 0.22a	0.11 ± 0.22a
	T2	1.29 ± 0.13a	99.31 ± 0.71a	0.69 ± 0.71a
	T3	1.18 ± 0.33a	99.50 ± 0.58a	0.50 ± 0.58a
Qing A	CK	2.07 ± 0.39a	89.45 ± 1.05c	10.56 ± 1.05a
	T1	1.10 ± 0.15b	91.95 ± 1.42b	8.06 ± 1.42b
	T2	2.14 ± 0.29a	92.10 ± 1.82b	7.90 ± 1.82b
	T3	1.16 ± 0.23b	94.75 ± 0.56a	5.25 ± 0.56c
ANOVA	C	**	**	**
	T	**	**	**
	C×T	*	**	**

Different lowercase letters after the data in the same column indicate that the difference among different treatments of the same variety reaches the 0.05 significance level. In the ANOVA, ** indicates that the difference is very significant ($P < 0.01$), and * indicates that the difference is significant ($0.01 < P < 0.05$); ns indicates that the difference was not significant ($P > 0.05$)

Effects of the restorer lines's pollen grain number per stamen and percentage of pollen germination in vitro

As shown in (Fig. 1), biological nano-Se significantly affected the pollen grain number per stamen and percentage of pollen germination in vitro of male parents. Compared to CK, T1 and T3 treatments significantly increased pollen grain number per stamen by 8.40% and 7.56%, and improved percentage of pollen germination in vitro by 31.06% and 31.46%, whereas T2 treatment exhibited no significant effect.

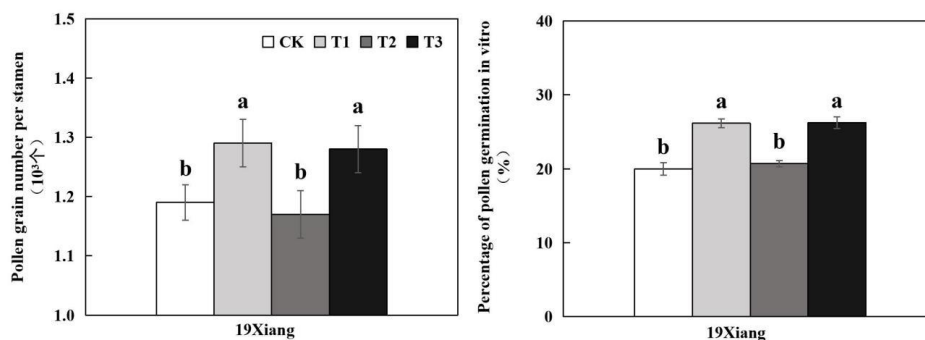


Figure 1. Effects of different biological nano-Se treatments on Pollen grain number per stamen and Percentage of pollen germination in vitro of the rice recovery lines. Bars that share a common letter have no significant difference ($p \leq 0.05$) according to the LSD test. The same is true afterward

Effects of leaf antioxidant system during the blooming period

Foliar application of biological nano-Se significantly impacted the antioxidant system in terms of the Superoxide dismutase (SOD), peroxidase (POD), Catalase (CAT) and Malondialdehyde (MDA) of rice leaves during the flowering period of hybrid seed production (Figs. 2–4).

For 19Xiang, compared to CK, T1 and T3 treatments significantly increased the CAT activity by 29.42% and 31.27%, respectively; T1 and T3 treatments significantly increased the SOD activity by 6.35% and 4.2%, respectively; T2 and T3 treatments significantly increased the POD activity by 21.36% and 20%, respectively; T1, T2 and T3 treatments significantly decreased the MDA content by 27.74%, 11.56% and 22.3%, respectively.

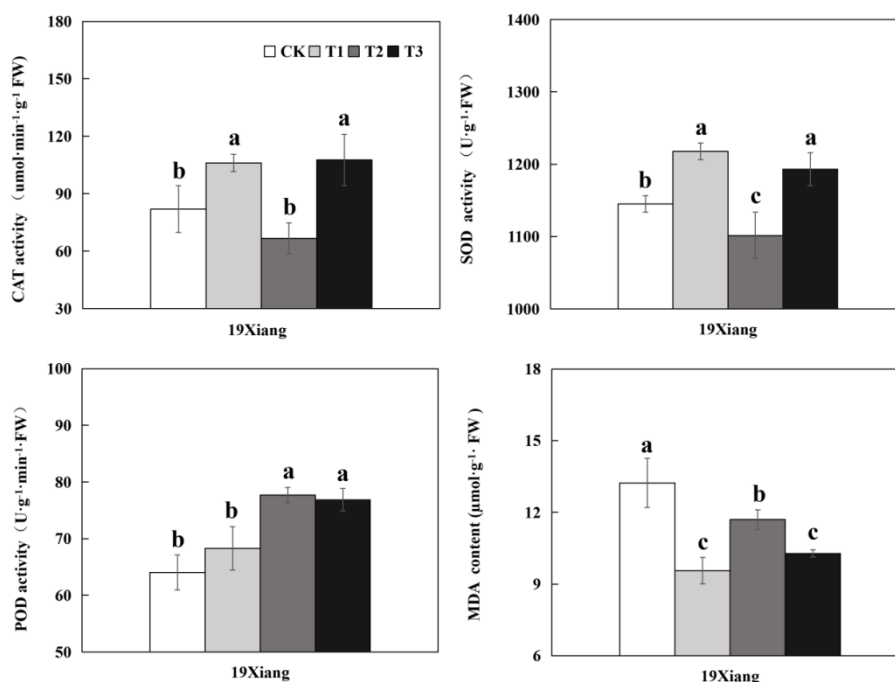


Figure 2. Effects of different biological nano-Se treatments on the leaf antioxidative activity of the rice recovery lines 19 Xiang

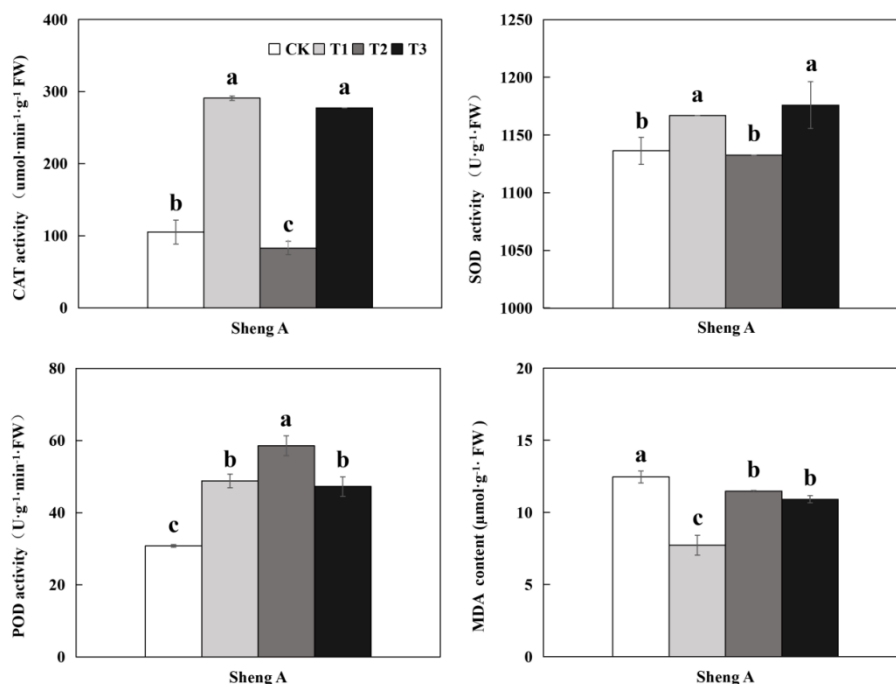


Figure 3. Effects of different biological nano-Se treatments on the leaf antioxidative activity of the rice sterile lines Sheng A

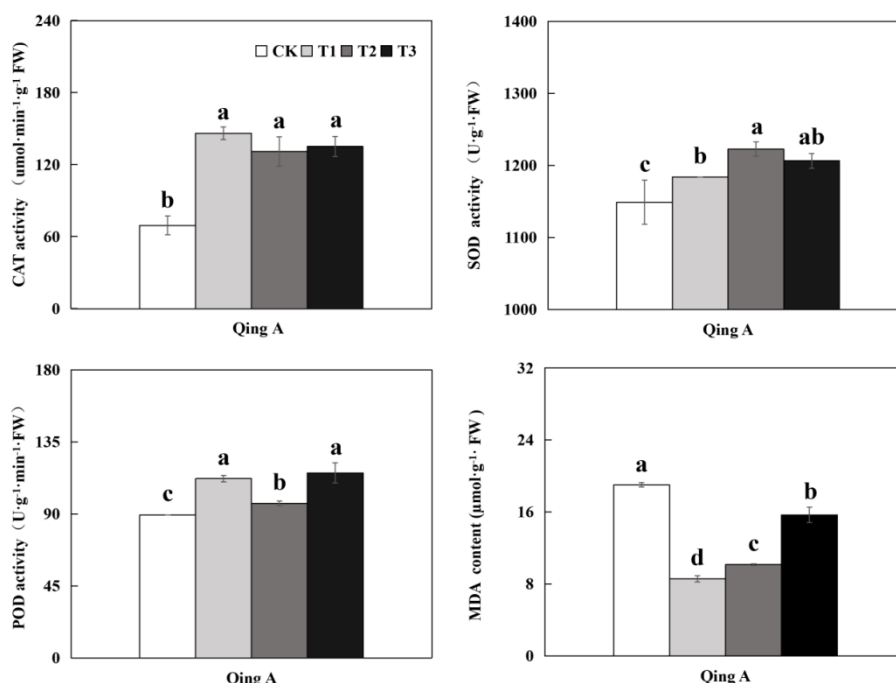


Figure 4. Effects of different biological nano-Se treatments on the leaf antioxidative activity of the rice sterile lines Qing A

For Sheng A, compared to CK, T1 and T3 treatments significantly increased the CAT activity by 176.38% and 163.54%, respectively; T1 and T3 treatments significantly increased the SOD activity by 2.69% and 3.49%, respectively; T1, T2, and T3 treatments

significantly increased the POD activity by 58.76%, 90.34%, and 53.69%, respectively; T1, T2 and T3 treatments significantly decreased the MDA content by 37.96%, 7.87%, and 12.44%, respectively.

For Qing A, compared to CK, T1, T2 and T3 treatments significantly increased the CAT activity by 110.70%, 88.82% and 94.82%, respectively; T1, T2 and T3 treatments significantly increased the SOD activity by 3.04%, 6.41% and 5.01%, respectively; T1, T2 and T3 treatments significantly increased the POD activity by 25.36%, 8.05% and 29.39%, respectively; T1, T2 and T3 treatments significantly decreased the MDA content by 54.92%, 46.66% and 17.57%, respectively.

Discussion

Effects of biological nano-Se on agronomic traits at the blooming stage of hybrid seed production

Yi (2009) noted that the pollen amount of the male parent is a key factor affecting the yield of seed production. In addition, Zhou (1998) noted that the pollen involved in insemination must be sufficiently vigorous. In hybrid rice seed production, sterile lines may appear closed during the blooming period; that is, some flowers remain closed throughout the blooming period and are unable to participate in pollination (Chen et al., 2018). In addition, in the process of internode differentiation and elongation of rice sterile lines, the cell division and elongation of the vertical layer may be affected by the dwarfing gene, resulting in some grains in the panicle cannot be completely exposed from the leaf sheath. (Yang et al., 2016). Therefore, reducing the non-flowering panicle rate and unemerged panicle rate of sterile lines and increasing the pollen amount and pollen vitality of restored lines are essential for increasing seed production.

This study revealed that, compared with the CK treatment, spraying biological nano-selenium on the leaves 3 days before young panicle differentiation significantly reduced the non-flowering panicle rate and unemerged panicle rate of rice sterile lines, and significantly increased the Pollen grain number per stamen and Percentage of pollen germination in vitro of rice restorer lines. These results are highly consistent with those of Dai (2019) and Barman (2023), indicating that biological nano-Se can significantly improve the traits of rice flowering. However, the regulation effect of T2 treatment on rice flowering traits was significantly lower than that of T1 and T3 treatments, and there was no significant difference with CK. This is because the time interval between T2 treatment and rice flowering is very short, so it is difficult for rice to make full use of the biological nano-Se absorbed by leaves. The results showed that spraying biological nano-Se at different growth stages of rice could significantly regulate the distribution and utilization of photosynthetic products in rice (Huang et al., 2017; Hou et al., 2021). Specifically, foliar spraying of biological nano-selenium during the vegetative growth period can promote the transport of photosynthetic products to roots, stems and leaves, thereby improving plant growth; in the reproductive growth period, it mainly promotes the transport of photosynthetic products to leaves and grains, thereby significantly increasing the selenium content in rice.

Effects of biological nano-Se on the leaf antioxidative activity during the blooming period

Reactive oxygen species (ROS) play an important role in rice growth. Under normal physiological conditions, ROS acts as a signaling molecule to regulate the

growth and development of rice. However, when rice is subjected to adversity, ROS is metabolically unbalanced to destroy the biological macromolecules such as DNA, lipids and proteins of rice cells, which in turn triggers apoptosis or necrosis of rice cells (Kumar and Dasgupta, 2024). Therefore, in order to maintain the balance of ROS in rice, rice eliminates the negative effects of excessive ROS accumulation by regulating the expression level and activity of antioxidant enzymes. The enzymes involved in the ROS scavenging mechanism include superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) (Yang et al., 2016). In addition, excessive accumulation of ROS may lead to peroxidation or degreasing of the inner membrane of rice cells, and the content of malondialdehyde (MDA) produced by lipid peroxidation or degreasing of the inner membrane will also increase accordingly (Chi et al., 2023).

The results of this study showed that compared with CK, spraying biological nano-Se on the leaves 3 days before young panicle differentiation could significantly increase the activities of SOD, POD and CAT in rice leaves at flowering stage, and significantly decrease the content of MDA. This result is consistent with the research results of Wen (2022) in citrus, which indicates that selenium treatment can generally enhance the antioxidant capacity of citrus and maintain cell membrane integrity by scavenging membrane lipid peroxidation product MDA. It is worth noting that T2 treatment had no significant effect on the antioxidant activity of rice leaves at flowering stage. It is speculated that it may take some time for the absorption and transformation of biological nano-Se in leaves to fully play its role, which is consistent with the research conclusions of Luo (2021) and Khan (2023). In addition, biological nano-Se may also regulate floral organ development by improving the antioxidant activity of flowering leaves (Liu and Wu, 2023), which provides new insights into the mechanism of biological nano-Se affecting rice reproductive growth.

Conclusion

The results of this study based on the double cropping rice area in South China and specific rice seed production combinations (Qing A×19Xiang and Sheng A×19Xiang) showed that foliar spraying of biological nano-Se requires a certain absorption time before it can be effectively used and transferred to other rice tissues to play a role. Therefore, foliar spraying 3 days before young panicle differentiation (T1 and T3 treatments) could increase the pollen number and viability of male parents and reduce the non-flowering panicle rate and unemerged panicle rate of female parents. At the same time, this treatment can also enhance the antioxidant capacity of rice. By enhancing the floral organ performance and antioxidant capacity of rice, biological nano-selenium may indirectly increase the yield of rice, especially under stress conditions, its antioxidant effect may be further highlighted. Considering the cost of biological nano-selenium, the T1 treatment (a group sprayed 3 days before young panicle differentiation) was determined to be the best choice.

Funding. This study was supported by the National Natural Science Foundation of China (31971843), the Special Rural Revitalization Funds of Guangdong Province (2021KJ382), Guangzhou Science and Technology Project (202103000075).

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