

Effects of Fulvic Acid-Selenium Treatment on Growth of Different Rice Varieties

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Abstract
Selenium is an essential trace element for humans, with rice being a major dietary source. Different rice varieties respond differently to selenium, affecting selenium enrichment. This study investigated the effects of humic acid selenium on seed germination, seedling growth, and physiological characteristics of three rice varieties: Qiantaiyou 851, Qianliangyou 1606, we used the thiobarbituric acid colorimetric method to determine malondialdehyde content and the guaiacol method to measure peroxidase activity, aiming to clarify their interactions and guide selenium-enriched rice cultivation.Results showed varietal differences in responses. Humic acid selenium improved germination rates, especially in Qianyouxiang 88, while Qianliangyou 1606 maintained the highest rate. It promoted seedling growth in Qianyouxiang 88 (height and stem diameter) and Qiantaiyou 851 (root length) but inhibited growth in Qianliangyou 1606. Peroxidase (POD) activity increased, and malondialdehyde (MDA) content decreased in all varieties, with the most significant changes in Qiantaiyou 851. Qianyouxiang 88 showed strong antioxidant capacity after treatment. These results support variety selection and technical improvement for selenium-enriched rice cultivation.

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Keywords: Fulvic acid selenium, Rice varieties, Seed germination, Growth morphology, Physiological and biochemical, Selenium-enriched cultiva

1. Introduction

Selenium is an essential trace element for the human body and plays important roles in the body's antioxidant defense system, immune system, and other aspects, while rice is the main food source for more than half of the world's population and also a major pathway through which people obtain selenium [1]. Selenium-enriched rice has a high selenium content and is more easily absorbed and utilized by the human body. A 12-year follow-up study by the Chinese Academy of Agricultural Sciences showed that regularly eating selenium-enriched rice can maintain people's blood selenium levels at a relatively stable high level, greatly reducing the incidence of hypothyroidism, cardiovascular and cerebrovascular diseases, and certain tumors, and delaying the aging process [2]. Studies have shown that the uptake and accumulation of selenium by rice exhibit obvious differences in genetic background, and different rice genotypes show different patterns in selenium uptake, transport, and translocation to grains [3]. In addition, root spraying can also effectively increase the selenium content in rice, but the effects differ among rice varieties [4].
Fulvic acid is a naturally occurring macromolecular organic substance. Among humic substances, it is a component with relatively small molecular weight, abundant functional groups, and high biological activity. It has many advantages in agricultural production: it can significantly increase crop yields and enhance plant stress resistance while also improving soil structure and increasing soil fertility. It is a biostimulant widely valued in green agriculture [5]. Fulvic acid selenium combines the functions of fulvic acid with those of selenium and is a new type of selenium-enriched fertilizer with great application prospects in agricultural production. Due to differences in genetic background and physiological characteristics, different rice varieties also differ in selenium uptake, transport, and accumulation in the body.

At present, domestic and international studies on the effects of exogenous selenium on rice growth mostly focus on inorganic selenium or single organic selenium types. For fulvic acid selenium composite selenium fertilizer, its effects on seed germination, plant growth and development, and physiological and biochemical characteristics of different rice varieties have not yet been systematically and deeply investigated.

Regarding the use of selenium fertilizer abroad, many studies have confirmed the relationships among reasonable application rate, application method, and variety. Relevant literature [6] reports that an appropriate amount of selenium fertilizer can promote crop growth and development, increase yield and income, and give agricultural products higher nutritional value, but excessive application inhibits crop growth. Regarding selenium application methods [7], relevant foreign scholars analyzed the effects of foliar spraying, root irrigation, and their combination. The results showed that combined application is conducive to selenium uptake and accumulation and also helps alter the rhizosphere microbial flora and maintain the stability of the rhizosphere microecological environment. From the perspective of interactions among elements, spraying selenium together with other trace elements such as zinc and iron onto leaves can achieve two goals at once, biofortifying agricultural products with multiple nutrients without affecting crop yield [8]. In addition, soil texture, fertilizer type, and pH all have major impacts on selenium bioavailability in the soil–crop system and thereby affect crop selenium uptake and distribution [9]. A large number of experiments have also shown that different crops differ to some extent in selenium enrichment capacity and tolerance, as reflected in crops such as maize, soybean, and grape [10]. Moreover, exogenous selenium can interact with phosphate fertilizer or mycorrhizal fungi to alter selenium distribution and antioxidant metabolism in crops. Under harsh environments such as heavy metals or high salinity, it can alleviate plant damage by regulating rhizosphere microbial communities and enhance crop stress resistance [11]. Overall, biofortifying crops with selenium through agricultural measures is considered an effective way to address human diseases caused by selenium deficiency and improve the quality of agricultural products, and it is a hot topic in plant nutrition and modern agriculture [12]. In China, research has mainly focused on the study and extension of cultivation techniques for selenium-enriched rice. Feng Xiaolong et al. [13] found that spraying selenium-enriched organic water-soluble fertilizer significantly increased rice yield and selenium content in brown rice. Ling Li et al. [14] pointed out that applying exogenous selenium can significantly improve selenium uptake and transport efficiency in rice and reported selenium enrichment in various parts of rice such as roots, stems, leaves, and grains.

From the overall application of selenium fertilizer, existing studies show that an appropriate application of selenium fertilizer can increase crop yield and selenium content, but excessive application inhibits crops, as reported in crops such as daylily, rice, and forage mulberry. In addition, different types of selenium fertilizer have their own advantages and disadvantages. For example, foliar spraying of nano-selenium or organic selenium fertilizer [15] is beneficial for optimizing rice plant architecture, increasing yield, and increasing selenium content in grains, and has a better yield-increasing effect on indica rice; soil application of selenium [16] has a long-lasting effect and is more suitable for perennial plants such as forage mulberry that are harvested multiple times. In rice studies, some scholars have found that exogenous selenium can affect antioxidant enzyme activity, thereby improving plant stress resistance, and have analyzed the selenium content in various parts of rice; in daylily studies, the application of organic selenium fertilizer [17] can also significantly increase the selenium content in flower buds and is superior to traditional amino acid water-soluble fertilizer. Regarding varietal differences, Yang Bin et al. [18] compared several different selenium fertilizer treatments and found large differences in their effects on rice growth and development and selenium enrichment. They also proposed that suitable rice varieties could be selected to obtain rice varieties with higher selenium content [19].

Therefore, some research has been conducted on the mechanisms of exogenous selenium action on rice, selenium-enriched cultivation techniques, and the application of fulvic acid. However, there is currently limited research on the effects of fulvic acid selenium, a composite selenium fertilizer, on rice, especially different types of rice. This is a problem faced in the precision and variability of fulvic acid selenium application in selenium-enriched rice cultivation and is also the research content of this paper.

Using fulvic acid selenium as the treatment agent, experiments were conducted on Quantaiyou 851, Quanliangyou 1606, and Quanyouxiang 88 to explore the effects of fulvic acid selenium on the growth of these three varieties and their different responses, select the most suitable variety, and lay a foundation for the rational use of fulvic acid selenium in selenium-enriched rice production.

2. Materials and Methods

2.1. Rice Varieties Tested and Assay Kits

The rice varieties tested were Quantaiyou 851, Quanliangyou 1606, and Quanyouxiang 88, which are commonly planted in Hubei. The seeds tested were all healthy seeds with plump grains, uniform size, no mechanical damage, no pests or diseases, and no mildew or deterioration. Shriveled grains, empty shells, and damaged grains were removed so that the seeds had good viability and germination vigor.

Experimental reagents: Fulvic acid selenium, with a selenium content of 910 mg·kg^{-1} and a fulvic acid proportion of 48.9%, was provided by Humusi (Heilongjiang) Biotechnology Co., Ltd. The POD kit (plant) and malondialdehyde (MDA) assay kit were provided by Nanjing Jiancheng Bioengineering Institute.

2.2. Methods

2.2.1 Seed Germination and Seedling Raising/ transplanting Treatments

Experimental design: Three rice varieties, Quanliangyou 1606, Quanyouxiang 88, and Quantaiyou 851, were selected in this experiment. For each rice variety, three experimental groups and three control groups were set up. The experimental groups were treated with fulvic acid selenium solution, and the control groups were sprayed with an equal amount of clean water. Reagent concentration setting: the treatment concentration of fulvic acid selenium in the experimental groups was 4 mg/L. Preparation method: 2 mL of fulvic acid selenium stock solution was added to 1000 mL of clean water, stirred evenly, and set aside for use. The control groups used clean water without any addition.

For each variety, 100 plump and healthy seeds were taken and divided into 2 portions, which were soaked in the

corresponding liquids. The seeds were cultured at a constant temperature of 28°C, a light intensity of 3000 lx, and a light/dark cycle of 12 h/12 h for 48 h. The same amount of treatment solution was added daily, and the germination status was observed. When the seeds germinated to a bud length of 0.5–1.0 cm and a root length of 1.0–2.0 cm, well-growing seedlings were selected and transplanted into seedling substrate for further cultivation for subsequent determination of various indicators.

2.2.2 Determination of Morphological Indicators of Seedling Plant Architecture

Rice seedlings with basically consistent growth status were selected and transplanted into seedling trays and managed normally in a greenhouse, without artificial intervention to regulate temperature and humidity. After seedling recovery, they were divided into different lines according to different seed soaking times and subjected to uniform water and fertilizer management: the experimental groups were treated with 4 mg/L fulvic acid selenium solution, and the control groups were treated with an equal amount of clean water, with the same irrigation frequency and irrigation volume. During growth, weeds and diseased leaves were removed in a timely manner. When the seedlings reached the three-leaf stage, samples were collected in batches according to line and treatment, with three biological replicates set for each treatment. Before sampling, the seedlings were removed, washed, and air-dried. A ruler was used to measure plant height (from the ground to the tip of the topmost leaf) and root length (from the base of the main root to the root tip), and a vernier caliper was used to measure the diameter of the lowest internode, taking care to avoid compression affecting the results. All data were original data, and their means were taken as the effects of fulvic acid selenium treatment on rice seedling morphology.

2.2.3 Determination of Leaf Physiological and Biochemical Indicators

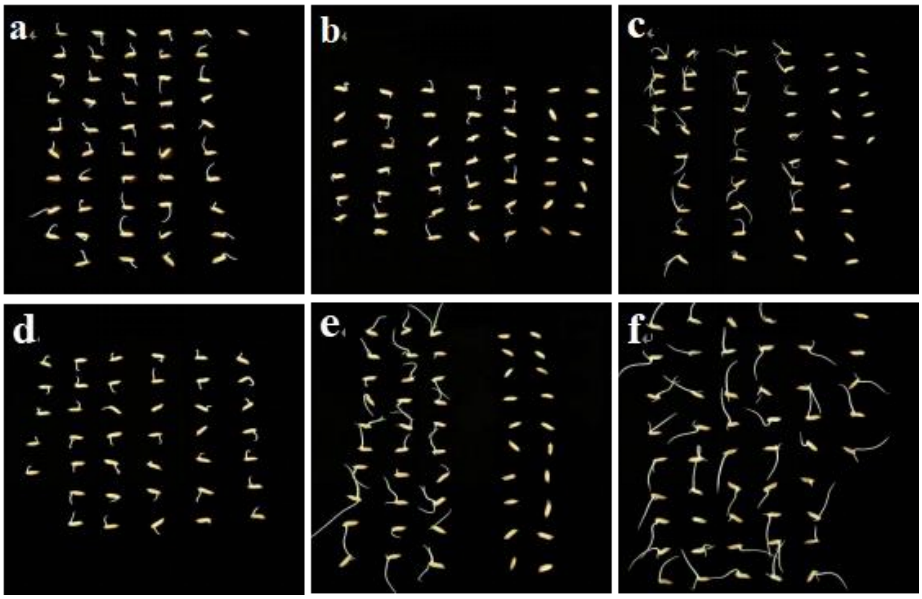
Peroxidase (POD) activity was determined by the guaiacol method [20] using a POD kit (plant) provided by Nanjing Jiancheng Bioengineering Institute. Fresh rice leaves (1.0 g) were added at a 1:9 ratio to pre-cooled 0.01 mol/L phosphate buffer (pH 7.0–7.4), ground into a homogenate in an ice bath, and centrifuged at 4°C and 3500 r/min for 10 min. The supernatant was collected as the crude enzyme extract. The reaction system was prepared according to the kit instructions. Corresponding reagents were added to the blank tube and test tube, respectively, followed by incubation in a constant-temperature water bath at 37°C for 30 min, after which Reagent IV was added to terminate the reaction. The absorbance was read at 420 nm after zeroing with the blank tube. Three technical replicates were performed for each sample.

Malondialdehyde (MDA) content was determined by the thiobarbituric acid (TBA) method [21] using an MDA assay kit provided by Nanjing Jiancheng Bioengineering Institute. Fresh rice leaves (1.0 g) were added at a 1:9 ratio to pre-cooled 0.01 mol/L phosphate buffer (pH 7.0–7.4), ground into a homogenate in an ice bath, and centrifuged at 4°C and 3500–4000 r/min for 10 min. The supernatant was collected as the sample solution for determination. The reaction system was prepared according to the kit instructions. Absolute ethanol, 10 nmol/mL MDA standard, and the sample solution were added to the blank tube, standard tube, and test tube, respectively, after which the corresponding reagents were added. After mixing, the tubes were incubated in a boiling water bath at 95°C for 40 min, cooled in an ice bath, and centrifuged at 3500 r/min for 10 min. The supernatant was collected, and the absorbance was measured at 532 nm. Three technical replicates were set for each sample.

3. Results

3.1. Effects of Fulvic Acid Selenium Treatment on the Germination of Different Rice Varieties

In this experiment, three rice varieties-Quanliangyou 1606, Quanyouxiang 88, and Quantaiyou 851 were selected to investigate the effects of seed soaking with fulvic acid selenium on seed germination. The results showed that different varieties responded differently to the fulvic acid selenium treatment (Figure 1).



Note: a, Quanliangyou 1606 + Se; b, Quanyouxiang 88 + Se; c, Quantaiyou 851 − Se; d, Quantaiyou 851 + Se; e, Quanyouxiang 88 − Se; f, Quanliangyou 1606 − Se.

Fig 1: Effects of fulvic acid selenium treatment on the germination of different rice varieties

Table 1: Germination rates of different rice varieties under fulvic acid selenium treatment

Rice varieties	Se(x̄±s,%)	CK(x̄±s,%)
Quanliangyou1606	98.00±0.00	97.33±1.15
Quanyouxiang88	72.00±2.00	60.00±2.00
Quantaiyou851	80.00±2.00	74.000±2.00

Note: The P value for Quanliangyou 1606 is 0.42; the P value for Quanyouxiang 88 is 0.01; the P value for Quantaoyou 851 is 0.02; P<0.05 indicates a significant difference between the two; P>0.05 indicates no significant difference between the two.

As shown in Table 1, seed soaking with fulvic acid selenium had different effects on the germination rates of different rice varieties. The germination rates of Quanliangyou 1606 under selenium-added and non-selenium conditions were 98.00% ± 0.00% and 97.33% ± 1.15%, respectively, with no significant difference between the two (P = 0.42 > 0.05), indicating that this variety has strong inherent germination ability and is insensitive to exogenous selenium. For Quanyouxiang 88, the germination rate increased highly significantly from 60.00% ± 2.00% to 72.00% ± 2.00% after selenium treatment (P = 0.01 < 0.05), an increase of 12 percentage points, indicating that seed soaking with fulvic acid selenium can effectively promote seed germination in this variety. For Quantaiyou 851, the germination rate increased significantly from 74.00% ± 2.00% to 80.00% ± 2.00% after selenium addition (P = 0.02 < 0.05), an increase of 6 percentage points.

Overall, fulvic acid selenium treatment had a strong promoting effect on Quanyouxiang 88, which had a low germination rate, but had little effect on Quanliangyou 1606, which had a high germination rate. Different varieties responded differently to selenium treatment, which may be determined by the genetic basis of the varieties themselves and the extent to which selenium is absorbed and utilized.

3.2. Effects of Fulvic Acid Selenium Treatment on the Growth and Morphological Indicators of Different Rice Varieties

All morphological indicators were measured at the three-leaf stage. In terms of root length, fulvic acid selenium had the greatest effect on different varieties: Quantaiyou 851 had well-developed roots and long root length, while Quanliangyou 1606 and Quanyouxiang 88 had poorly developed roots and short root length. This indicates that the effect of fulvic acid selenium on roots is mainly related to the variety itself. In terms of plant height, different varieties performed differently: Quanyouxiang 88 grew well above ground with a moderate plant height; Quanliangyou 1606 showed little change in plant height; while Quantaiyou 851, which originally had the tallest plants, showed a substantial decrease in plant height after treatment, and its above-ground growth was inhibited. This indicates that the effect of fulvic acid selenium on above-ground growth is also selective. In terms of stem diameter, the treatment also had opposite effects: Quanyouxiang 88, which originally had the thinnest stems, showed an increase in stem diameter and became more robust; while Quanliangyou 1606 and Quantaiyou 851, which originally had thicker stems, showed a decrease in stem diameter, with a tendency for stems to become thinner.

Overall, the effects of fulvic acid selenium on the morphology of rice seedlings exhibited a clear "variety-specific response," namely: for Quanliangyou 1606, above-ground parts (plant height and stem diameter) were promoted but below-ground parts (root length and root area) were inhibited; for Quantaiyou 851, below-ground parts were promoted but above-ground parts were inhibited; and for Quanyouxiang 88, overall growth was inhibited.

Table 2: Effects of fulvic acid selenium treatment on morphological indicators of different rice varieties

Rice varieties/Treatment	Root length（cm）	Plant height（cm）	Stem diameter（cm）
Quanliangyou1606-Se	13.67±2.43	32.83±0.44	0.57±0.03
Quanliangyou1606+Se	11.13±2.74	32.27±0.84	0.40±0.30
Quanyouxiang88-Se	19.57±0.30	30.57±0.81	0.37±0.03
Quanyouxiang88+Se	13.83±2.77	34.97±0.52	0.47±0.03
Quantaiyou851-Se	12.87±1.81	40.00±0.58	0.57±0.03
Quantaiyou851+Se	16.13±2.98	32.40±1.23	0.47±0.03

Note: -Se represents the water control, +Se represents the selenium treatment; data are the arithmetic means of six biological replicates, with the unit in cm.

3.3. Effects of Fulvic Acid Selenium on the Physiological and Biochemical Indicators of Seedlings of Different Rice Varieties

It can be seen from Table 3 that under the clear water control condition, differences in POD activity exist among different rice varieties. Quantaoyou 851 had the highest POD activity, while Quanyouxiang 88 had the lowest POD activity, indicating that differences exist in the antioxidant levels of different varieties.

After selenium treatment, the POD activity of all three materials increased to some extent, and the ability of the plants to scavenge reactive oxygen species was enhanced, which can to a certain extent alleviate the damage caused by oxidative stress. Moreover, the three varieties differed in their degree of response to selenium. After selenium treatment, the order of POD activity among the three varieties was the same as before selenium application, with Quantaoyou 851 still being the highest and Quanyouxiang 88 the lowest, and the differences among the three varieties became more pronounced, indicating that this effect has obvious variety specificity.

Under the clear water control, the MDA content differed among the three rice varieties, with Quantaoyou 851 having the highest MDA content and Quanyouxiang 88 having the lowest MDA content, indicating that the degree of cell membrane lipid peroxidation differs among different varieties.

After the application of fulvic acid selenium, the MDA content of all varieties decreased. Fulvic acid selenium can effectively inhibit lipid peroxidation of the cell membrane in rice seedlings, reduce MDA content, and thereby alleviate the damage to the cell membrane caused by oxidative stress. At the same time, different varieties also differed in their degree of response to fulvic acid selenium treatment. Among them, the greatest reduction in MDA was found in Quanyouxiang 88, and the smallest reduction was found in Quantaoyou 851, indicating that this effect shows considerable inter-variety differences. In this experiment, three rice varieties were

selected to study the effects of fulvic acid selenium on rice seed germination, seedling growth, and physiological and biochemical aspects. The results showed that the effects of fulvic acid selenium on morphological indicators such as rice seed germination rate, seedling plant height, and root length, as well as physiological indicators such as POD and MDA, exhibited significant differences among different varieties, that is, variety specificity. In this paper, three rice varieties were selected to study the effects of fulvic acid selenium treatment on rice seed germination, seedling morphological growth, and physiological and biochemical characteristics. It was found that the effects of fulvic acid selenium on morphological indicators such as rice seed germination rate, seedling plant height, and root length, as well as physiological indicators such as POD and MDA, differed considerably among different varieties, showing variety specificity.

Table 3: Determination of POD activity and MDA content

Rice varieties	Treatment	POD activity（U/g）	MDAcontent（μmol/g）
Quanliangyou1606	-Se	264.44±2.22	8.20±0.32
Quanliangyou1606	+Se	338.22±2.78	4.50±0.22
Quangyouxiang88	-Se	248.89±1.78	7.90±0.30
Quangyouxiang88	+Se	314.22±2.33	4.20±0.20
Quantaiyou851	-Se	271.11±2.67	8.40±0.34
Quantaiyou851	+Se	346.33±3.22	4.70±0.24

Note: -Se represents the clear water control, +Se represents selenium treatment; the data are the arithmetic means of three biological replicates.

4. Discussion

In this study, three rice varieties were selected to investigate the effects of fulvic acid selenium treatment on seed germination, seedling morphological growth, and physiological and biochemical characteristics. The results showed that the effects of fulvic acid selenium on rice seed germination rate, morphological indicators such as seedling plant height and root length, and physiological indicators such as POD and MDA differed significantly among varieties, exhibiting a pattern of variety-specific responses.

Fulvic acid selenium treatment had certain effects on rice seed germination and was beneficial to seed germination, with considerable differences in effectiveness among varieties. Selenium can participate in the decomposition processes within seeds and in cell membrane repair, reducing membrane lipid peroxidation damage; when combined with fulvic acid, it can jointly promote the decomposition of storage substances in seeds and promote the development of radicles and plumules, thereby improving germination uniformity and increasing germination rate. Li Chanqiong et al. [22] also found that exogenous selenium application can regulate selenium content in plants and has a positive effect on seed germination, which is consistent with the results obtained in this study.

In terms of seedling morphogenesis, fulvic acid selenium affected rice plant height and root length, showing either promoting effects or no significant changes. Due to differences among rice varieties in selenium uptake, transport, and tolerance, their responses to exogenous selenium and fulvic acid composite treatment also differed, resulting in different plant height and root development. Wang Jinyan et al. [23] reached the same conclusion in a foliar selenium spraying experiment, namely that selenium treatment has variety-specific effects on crop agronomic traits, which is consistent with the variety-specific responses of morphological indicators found in this study.

Regarding physiological and biochemical indicators, fulvic acid selenium can regulate the antioxidant metabolism of rice seedlings, affecting POD activity and MDA content. POD is an important antioxidant enzyme that can scavenge reactive oxygen species in plants and alleviate damage caused by oxidative stress, while MDA content is a measure of the degree of cell membrane damage. After appropriate selenium application, rice seedlings can maintain higher POD activity and lower MDA accumulation, enhancing their ability to resist adverse environments. Wu Xiaozhuo et al. [24] found that different selenium sources greatly affect plant antioxidant enzymes and physiological and biochemical indicators; Jiang Xiaogang et al. [25] also showed that exogenous selenium can improve plant growth and enhance their physiological antioxidant capacity, which is consistent with the results of this study.

Exogenous selenium can also benefit the growth and development of rice seedlings by altering the rhizosphere soil microenvironment and enzyme activities, as indicated by Lu Pengwei's [26] study on the effects of exogenous selenium application on soil enzyme activities.

Fulvic acid selenium has certain effects on rice seed germination, seedling growth, and physiological characteristics, but the degree of influence is related to the genotype of the rice variety. In production, corresponding measures should be taken according to the differential effects on different rice varieties, applying fulvic acid selenium during seed dressing or at the seedling stage. This study only conducted indoor seedling-stage experiments; field experiments can be carried out in the future to further explore the effects of fulvic acid selenium on the mid-to-late stage development, yield, and selenium enrichment of rice.

5. Conclusion

During seed soaking, fulvic acid selenium had only a minor effect on rice seed germination but improved seed vigor to some extent, raising germination rates of different varieties to varying degrees, with some varieties showing marked increases; plumule length, germination potential, and germination index also increased, though considerable differences existed among varieties. During the seedling stage, fulvic acid selenium regulated plant height, stem diameter, and root length across varieties; in some varieties, root length and stem diameter increased significantly, favoring water and nutrient uptake and the transport of photosynthetic products. Moreover, fulvic acid selenium significantly enhanced POD activity and effectively inhibited MDA production in seedlings, thereby alleviating cell membrane lipid peroxidation and improving stress resistance. Considering germination rate, seedling growth, and antioxidant capacity comprehensively, Quanyouxiang 88 performed best under fulvic acid selenium treatment, showing good promotion of seed germination, increased plant height and stem diameter at the seedling stage, and better physiological adaptation to environmental changes; it is therefore recommended as the target variety for fulvic acid selenium application.

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