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Effects of different concentrations of inorganic selenium on photosynthetic performance in Rosa rugosa 'Zizhi': a preliminary study

Abstract

This study examined the effects of inorganic selenium at two dilutions (2000-fold and 4000-fold) applied via foliar spray or root irrigation on photosynthetic performance in Rosa rugosa 'Zizhi' under field conditions. Compared with the control, the 2000-fold foliar treatment significantly reduced PSII maximum photochemical efficiency (Fv/Fm), performance index (PIABS), Sm, and Ψo, while increasing ABS/RC, DIo/RC, and TRo/RC, indicating lower PSII stability and higher sensitivity to light and temperature variations. Under root irrigation, these parameters also decreased but not significantly, suggesting more stable PSII structure and better adaptation. Therefore, root irrigation with the 4000-fold inorganic selenium dilution is considered the optimal practice.

Keywords: rubisco enzyme activity, photosynthesis, OJIP curve

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Introduction

Rosa rugosa Thunb. 'Zizhi' is a deciduous shrub belonging to the subfamily Rosoideae within the family Rosaceae, and represents a variant cultivar of rose.[1] It is characterized by a long flowering period, intense fragrance, large flower size, and the ability to bloom repeatedly. The species also exhibits strong drought tolerance, resistance to pests and diseases, cold hardiness, and broad environmental adaptability. The experimental material used in this study was obtained from the purple-branch rose plantation of Sichuan Shengxiang Rose Ecological Agriculture Development Co., Ltd., where the plants are primarily cultivated for essential oil extraction, tea scenting, medicinal purposes, and direct consumption. In autumn, both the leaves and stems of purple-branch rose turn purplish red, making it highly suitable for landscape greening. Consequently, this cultivar integrates ornamental, processing, and ecological functions, delivering ecological, economic, and social benefits simultaneously.[2] The test material specifically comprised double-petaled, purplish red flowers with purplish red canes, collected from the rose production base at Yongtongqiao Village, Lianchi Town, Xichong County, Nanchong City. In current agricultural practice, exogenous selenium supplementation commonly involves the use of inorganic selenium, amino acid-chelated selenium, organic chelated selenium, and humic acid complexed selenium.[3]

The inorganic selenium fertilizer (agricultural selenium fertilizer) contained no less than 45% elemental selenium.[4] Selenium is a nonmetallic chemical element that exists in nature in two principal forms: inorganic selenium and plant active selenium.[5] Inorganic selenium generally refers to selenite (Na_2SeO_3) and selenate (Na_2SeO_4), and includes products such as selenium enriched yeast and malt that retain substantial inorganic selenium residues derived as by products of metal ore refining.[6] As an essential trace element for humans, animals, and plants, selenium fulfills multiple vital physiological roles.[7] Notably, approximately 72% of China's cultivated land area is classified as selenium deficient, a condition that inevitably leads to insufficient selenium uptake by crops.[8]

Chlorophyll represents the most critical class of pigments involved in photosynthesis, participating directly in its core reactions. The fundamental principle of photosynthesis is the conversion of light energy into chemical energy through chlorophyll and associated compounds, ultimately leading to the synthesis of organic matter. Consequently, changes in chlorophyll fluorescence provide a direct reflection of photosynthetic activity.[9] In experimental settings, the time dependent variation in fluorescence intensity emitted by plants can reveal whether photosynthesis is being promoted or inhibited.[10] Following the transition from dark adaptation to actinic illumination, fluorescence intensity initially rises sharply and then gradually declines. This fluorescence intensity – time profile is known as the chlorophyll fluorescence induction kinetics curve.[11] During plant growth and development, chlorophyll plays a pivotal role in delaying leaf senescence and supporting reproductive development, while also directly modulating the photosynthetic rate.

Although selenium is beneficial at low concentrations, excessive or improper application can induce oxidative stress and cause damage to the photosynthetic apparatus in plants.[4,12] High levels of selenium have been reported to impair chlorophyll biosynthesis, disrupt chloroplast ultrastructure, and inhibit photosystem II (PSII) activity, leading to reduced photochemical efficiency and increased energy dissipation.[13,14] Moreover, foliar application of high concentration selenium may generate reactive oxygen species (ROS) that attack the D1 protein of PSII reaction centers, thereby accelerating photoinhibition.[15] Therefore, although selenium can promote Rubisco activation and carbon assimilation under certain conditions, its potential negative effects on the light dependent reactions, particularly on PSII performance, must be carefully evaluated. The present study aims not only to quantify the beneficial effects of inorganic selenium on Rubisco activity but also to identify any concomitant damage to the photosynthetic electron transport chain.

The purpose of this work was to quantify the carboxylation activity of Rubisco and to evaluate the effects of different concentrations of inorganic selenium on photosynthetic performance in leaves of Rosa rugosa Thunb. 'Zizhi'.

Materials and methods

Experimental site

The experiment was conducted at the rose production base in Yongtongqiao Village, Lianchi Town, Xichong County, Nanchong City, Sichuan Province (105°8′ E, 30°9′ N). This area is characterized by a subtropical humid monsoon climate. Perennial purple-branch rose (Rosa rugosa Thunb. 'Zizhi') plants were used, and 36 individuals with similar plant architecture were selected for the experiment. Inorganic selenium fertilizer (containing ≥45% Se) was diluted to 2000-fold and 4000-fold concentrations and then applied. Soil samples from plots without inorganic selenium application were also collected for analysis.

Application methods

The diluted inorganic selenium solutions (2000-fold and 4000-fold) were applied to the 36 selected plants (all with similar architecture) using two methods: foliar spray and root irrigation (soil drench). Each plant received 100 mL of the diluted selenium solution and was labeled accordingly. Foliar spraying was performed with a hand held sprayer directed at the abaxial (lower) leaf surfaces, spraying upward from beneath the canopy to minimize the risk of leaf scorch. For root irrigation, the solution was applied in a circular band around the root zone of each plant, taking care to avoid direct contact with the stem base.

The following precautions were observed during application:

1. Foliar spray was applied exclusively to the abaxial leaf surfaces, not to the adaxial surfaces or indiscriminately.

2. All selected plants were of comparable size and growth habit to minimize experimental error.

3. Each selenium treatment was assigned a specific code to avoid confusion. For example, "1-1-1" denoted inorganic fertilizer-foliar spray-2000-fold.

Fertilization schedule

The first fertilizer application was carried out on March 21, 2021, when the plants were at the budburst stage (first leaves just emerging), and subsequent applications were performed at 7-day intervals. Fertilization continued uninterrupted from March 28 to April 16, 2021. On April 16, foliar spraying was suspended, whereas root irrigation proceeded, and the first leaf sampling was conducted. Sampled leaves were immediately sealed in plastic bags and stored in an icebox for refrigerated preservation. A second leaf sampling event took place on April 27, 2021. Photosynthetic measurements were conducted from May 23 to June 6, 2021. In total, eleven fertilization events were performed, with the final application on June 6, 2021.

Leaf sampling protocol

The first leaf sampling was initiated in the fifth week post-treatment. For each sample group, the third and fourth fully expanded, intact functional leaves were excised. Leaf surfaces were gently wiped with tissue paper to remove dew and then immediately sealed in plastic bags and placed in a pre-chilled icebox. This procedure was intended to preserve cell viability and prevent sample degradation due to ambient environmental fluctuations. A minimum of six intact functional leaves, collected from multiple branches, was obtained per sample group.

Materials and reagents

Inorganic selenium (Hengshui Gemei Trace Elements Co., Ltd., 0.5 g and 0.25 g aliquots); TB buffer (Tris-HCl, pH 7.8); FB2 buffer (Tris-HCl, pH 8.0); FB1 buffer (Tris-HCl, pH 6.5); and 0.025 g ribulose-1,5-bisphosphate (RuBP).

Instruments and equipment

Electronic analytical balance (0.001 g precision); DHG-9140A electric forced-air drying oven (Shanghai Yiheng Scientific Instruments Co., Ltd.); 5424R benchtop refrigerated centrifuge; thermostatic water bath (Jintan Hengfeng Instrument Manufacturing Co., Ltd.); fully automatic microplate reader with 96-well plates; Handy PEA+ Plant Efficiency Analyser (Hansatech Instruments Ltd.).

Instrument operating conditions

Incubation time for initial Rubisco activity assay: 2 min; incubation for total activity assay: 20 min; reading interval: 15 s. Centrifugation conditions: 12 000 × g, 2 min, −4 °C. Fluorescence measurement duration: 20 min. The fluorescence analyzer was used on the third and fourth fully expanded, intact functional leaves.

Determination of rubisco carboxylation activity

Freshly collected purple-branch rose leaves (0.1 g) were excised, combining functional leaves from multiple branches of the same plant. Liquid nitrogen was added, and the tissue was immediately ground in a pre-chilled mortar to facilitate pulverization. Grinding proceeded until a homogeneous slurry free of visible coarse particles was obtained. One milliliter of TB buffer, pre-cooled to 4 °C, was added using a micropipette, and the mixture was stirred to form a homogenate. The homogenate was transferred to a PV tube and centrifuged at 12 000 × g for 2 min at −4 °C using the 5424R bench top refrigerated centrifuge. After filtration and volume adjustment, the supernatant was collected and stored immediately in a refrigerator for subsequent analysis. The crude enzyme extract was diluted 10-fold, and separate reaction mixtures were prepared for the determination of initial and total Rubisco activity. A CK control group (CK, without selenium application) was included in all assays.

Each sample was assayed in triplicate to minimize operational error, and all assays were performed in triplicate prior to data analysis. In the 96-well microplate, three wells were designated for distilled water as reagent blanks. The remaining wells were filled sequentially with the test solutions corresponding to each treatment, with three replicate wells allocated per treatment. The treatments included four combinations of selenium containing inorganic fertilizer (differentiated by application method and dilution factor) and two control treatments without selenium. Detailed information for each treatment is presented in Table 1.

Measurement of rapid chlorophyll fluorescence induction kinetics and JIP-test calculation

The Handy PEA+ Plant Efficiency Analyser (Hansatech Instruments) was employed for photosynthetic rate determinations. The instrument is characterized by high resolution, compact dimensions, portability, and robust durability. The main accessories consist of a carrying strap, main unit, charger, dark adaptation clips, and an optical probe. The probe integrates a high performance PIN photodiode detector coupled with a high precision RG9 long pass filter, ensuring maximal sensitivity to longer wavelength fluorescence signals and yielding an excellent signal to noise ratio.

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Table 1 Detailed description of each treatment group

Treatment code	Fertilizer type	Selenium-containing	Application method	Dilution factor
1 1 1	Inorganic fertilizer	Yes	Foliar spray	2000-fold
1 1 2	Inorganic fertilizer	Yes	Foliar spray	4000-old
1 2 1	Inorganic fertilizer	Yes	Root irrigation	2000-fold
1 2 2	Inorganic fertilizer	Yes	Root irrigation	4000-fold
CK-1	Inorganic fertilizer	No	Foliar spray	—
CK-2	Inorganic fertilizer	No	Root irrigation	—

Note: CK-1 and CK-2 are control treatments without selenium."—" indicates not applicable

On May 15, 2021, the Handy PEA+ analyzer was transported to the experimental field for in situ photosynthetic measurements. Ten indicators were recorded for each treatment group. The third and fourth fully expanded, intact functional leaves were clamped using dark adaptation clips and allowed to adapt in darkness for a minimum of 20 min. Subsequently, rapid chlorophyll fluorescence kinetics were acquired with the Handy PEA+ instrument.[16]

The leaf blade completely covered the measurement aperture, preventing any further preparation. The excitation source was 650 nm red light, with a maximum controllable intensity exceeding 3500 μmol m-2 s-1 (saturating irradiance). Data were exported using dedicated PEA Plus software and processed in a Windows environment. OJIP transients and radar plots were generated and graphically represented. Chlorophyll fluorescence transient parameters were analyzed according to the JIP-test methodology described by Saeedi et al.[17] The definitions and derived formulas for the JIP-test parameters are presented in Table 2.

Table 2 *Parameters of JIP-test rapid chlorophyll fluorescence induction kinetics curve (OJIP)*

Fluorescence parameter	Biological significance
F_o	Minimal recorded fluorescence intensity
F_m	Maximal recorded fluorescence intensity
F_v/F_m	Maximum photochemical efficiency of PSII
F_v/F_o	Potential photochemical activity of PSII
PI_{abs}	Photosynthetic performance index
RC	Reaction center
CS_m	Unit light area
ABS/RC	Light energy absorbed per reaction center
TR_o/RC	Energy captured per unit reaction center for QA reduction (t=0)
ET_o/RC	Energy captured per unit reaction center for electron transport (t=0)
DI_o/RC	Energy dissipated per unit reaction center at t = 0 (t=0)
ABS/CS_o	Absorption flux per CS_m
TR_o/CS_o	Trapped energy flux per CS_m at t = 0 (t=0)
ET_o/CS_0	Electron transport flux per CS_m at t=0 (t=0)
DI_o/CS_0	Dissipation energyflux per CS_m at t=0 (t=0)
RC/CS_0	Density of reaction centers per cross-section (t=0)
$V_j=(F_j-F_o)/(F_m-F_o)$	Relatively variable fluorescence at J
$V_I=(F_I-F_o)/(F_m-F_o)$	Relatively variable fluorescence at I
S_m	The normalized area between J~P phase and the line F=Fm
φE_o	PSII quantum ratio of electron transport
φP_o	Maximum photochemical efficiency of PSII
$\Psi_o=1-V_J$	Probability that a trapped exciton moves an electron transport chain beyond Q_A (t=0)
dV/dt_o	Net rate of cloure of reaction centers

Statistical analysis

Statistical analyses were performed using Microsoft Excel 2010 and SPSS 19.0 software. Graphical representations were prepared with WPS Office. One-way analysis of variance (ANOVA) and Duncan's multiple range test were applied to evaluate the significance of differences among treatments.[18]

Results

Effects of Different Concentrations of Inorganic Selenium on Rubisco Activity in Leaves of Purple-Branch Rose

Rubisco activase (RCA) is the key enzyme regulating Rubisco activity, and its activation level directly influences photosynthetic carbon assimilation. An increase in Rubisco activation state leads to enhancement of photosynthesis, and vice versa. As shown in Figure 1A, compared with the CK-1 control, Rubisco activation in purple-branch rose subjected to foliar application of 2000-fold inorganic selenium was markedly increased: at 16 s, the activation rate increased by 9.53%, and at 1 min, the increase reached 12.25%. Plants receiving foliar application of 4000-fold inorganic selenium also exhibited an increase in Rubisco activation, and the magnitude of increase was greater than that observed for the 2000-fold treatment: at 16 s, the

increase was 16.67%, and at 1 min, it amounted to 19.44%, both differences achieving statistical significance. These results indicate that both 2000-fold and 4000-fold foliar selenium applications enhanced Rubisco activity, with the 4000-fold treatment exerting a significantly more pronounced effect.

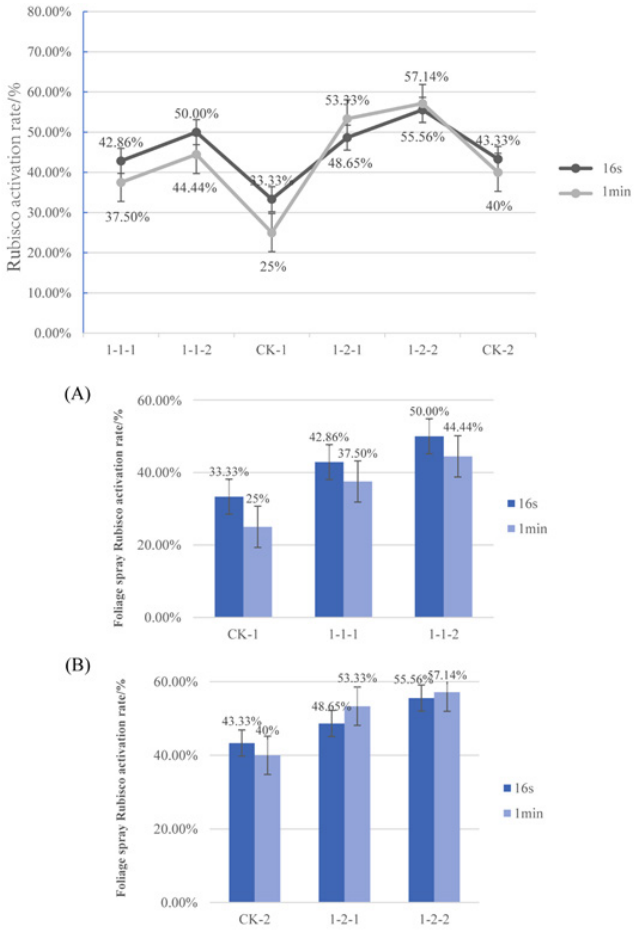

Figure 1 Effects of different concentrations of inorganic selenium on the activation rate of Rubisco of purple-branch rose (*Rosa rugosa* 'Zizhi').

Rubisco activity, thereby significantly enhancing photosynthesis.

As illustrated in Figure 1B, relative to the CK-2 control, Rubisco activation in plants receiving root irrigation with 2000-fold inorganic selenium was significantly elevated: at 16 s, the increase was 5.8%, and at 1 min, it was 8.65%. Root irrigation with 4000-fold selenium also increased Rubisco activation—by 17.34% at 16 s—while at 1 min, the value was 13.25% higher than the control, both differences achieving statistically significant levels. Thus, both 2000-fold and 4000-fold root-applied selenium enhanced Rubisco activity, with the 4000-fold treatment again displaying a more conspicuous effect.

In summary, changes in Rubisco activity paralleled trends in photosynthetic rate. Both foliar spray and root irrigation with 4000-fold inorganic selenium substantially promoted.

Effects of different concentrations of inorganic selenium on OJIP transients of leaves of purple-branch rose

The chlorophyll fluorescence induction kinetics curve, obtained when a dark adapted green plant is suddenly exposed to intense actinic light, typically exhibits four characteristic inflection points: O, J, I, and P. Analysis of this OJIP transient provides insight into the effects of different selenium concentrations and application methods on the photosynthetic performance of purple branch rose. Figures 2a and 2b present the rapid chlorophyll fluorescence induction transients (OJIP curves) between the O step (Fo) and the P step (Fm).

As shown in Figure 2a, relative to CK 1, foliar application of 2000-fold inorganic selenium caused a gradual elevation of the J, I, and P steps and the J–P amplitude, resulting in a more slowly rising OJIP curve. Under identical application method, compared with the 2000-fold foliar treatment, 4000-fold foliar application led to a moderate decline in the O , J , and I step fluorescence intensities and the O–I amplitude, whereas the I–P phase exhibited no marked change. Among the foliar treatments, the 2000-fold application produced the largest increase in fluorescence intensity in the O–I region and the highest J step value, whereas the 4000-fold treatment resulted in the greatest I–P amplitude and the highest I and P step values.

As illustrated in Figure 2b, compared with CK 2, root irrigation with 2000-fold inorganic selenium caused the J–I amplitude to decline slowly, then rise and finally fall to the I step, while the I , P steps and the I–P amplitude increased, thereby modifying the OJIP curve shape. When comparing within the root irrigation method, 4000-fold application induced a distinct, slow decline in the O and J steps and the O–J interval, with a gradual rise and subsequent fall in the J–I amplitude to the I step, accompanied by a reduction in the I step, P step, and I–P amplitude. Root irrigation with 2000-fold yielded the highest fluorescence increments in the O–J and I–P intervals, as well as the highest O , J , I , and P step values. In contrast, 4000-fold root irrigation notably elevated the O and K steps while producing the lowest I–P amplitude and the lowest I and P step values. Overall, root irrigation with 4000-fold inorganic selenium, relative to other treatments, increased the O step fluorescence to varying degrees.

Further analysis of basic fluorescence parameters revealed that different concentrations of inorganic selenium had a substantial impact on photosynthesis in purple branch rose leaves. As shown in Figure 2c, relative to CK 1, foliar application of 2000-fold inorganic selenium increased minimal fluorescence (Fo) but significantly decreased maximal fluorescence (Fm) and the maximum quantum yield of PSII (Fv/Fm), with reductions of 0.077 (relative units) and 328.8 (absolute units), respectively. Foliar application of 4000-fold resulted in a slight, non-significant decrease in Fv/Fm and an increase in Fm compared with CK 1. Among the three foliar treatment groups, the 4000-fold application produced the highest Fm value (an increase of 123.8), whereas the 2000-fold treatment gave the lowest Fv /Fm value (0.724).

As shown in Figure 2d, compared with CK 2, root irrigation with 2000-fold inorganic selenium increased both Fo and Fm, while Fv/Fm remained largely unchanged but exhibited a slight downward trend. Fm increased significantly by 218.3. In comparison with CK 2, root irrigation with 4000-fold resulted in reductions in Fo, Fm, and Fv/Fm to varying extents.

These findings demonstrate that foliar application of 2000-fold inorganic selenium causes more severe damage to the photosynthetic apparatus and greater impairment of PSII electron transport and photochemical efficiency than does foliar application of 4000-fold. Conversely, root irrigation with 4000-fold selenium exerts a more pronounced inhibition of the photosynthetic machinery and PSII electron transport than does root irrigation with 2000-fold.

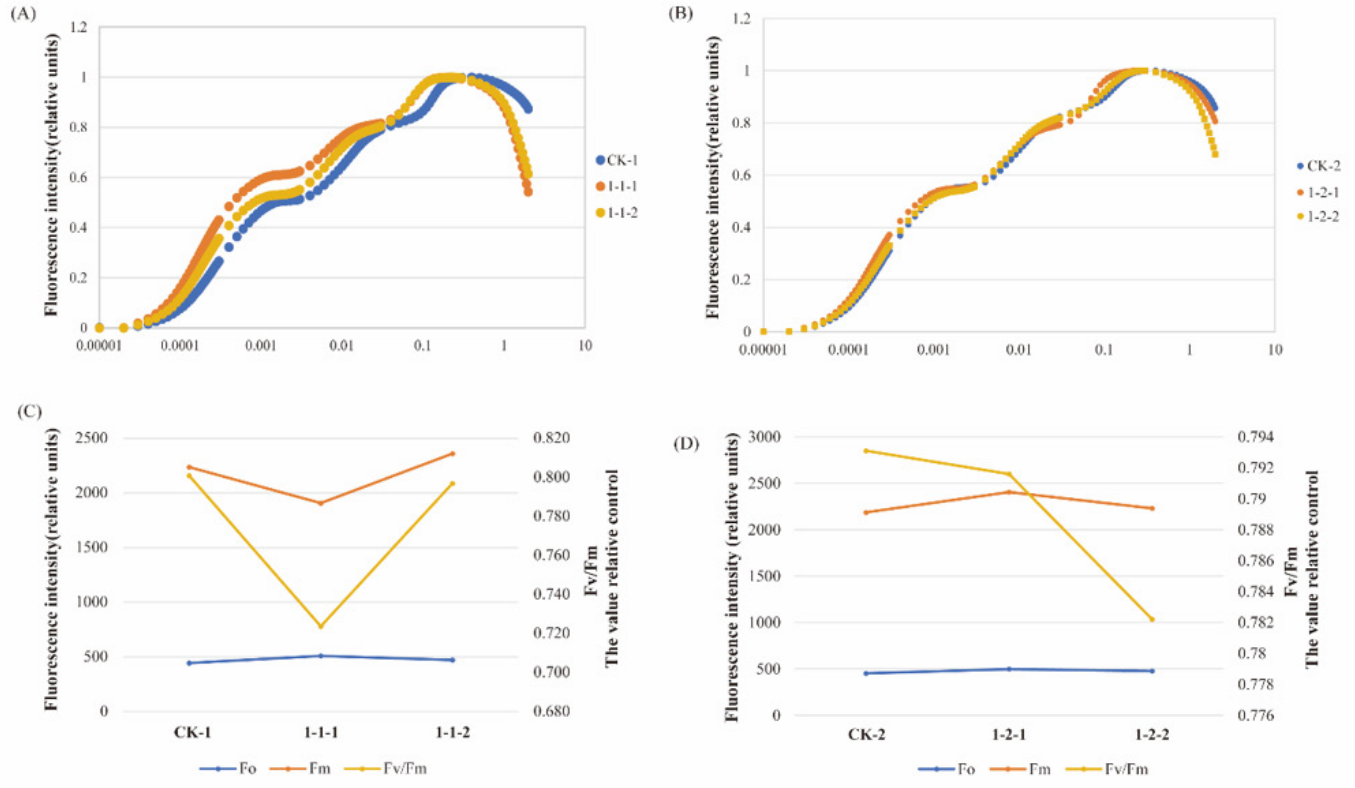

Figure 2 Effects of inorganic selenium on fluorescence intensity, F_o, F_m and F_v/F_m of purple-branch rose (*Rosa rugosa* 'Zizhi') leaves, at different concentrations.

Note: 0: Fluorescence at t=20μs; K: Fluorescence at t=300μs; J: Fluorescence at t=2ms; I: Fluorescence at t=30ms; P: Fluorescence at t=0.3s.

Effects of different inorganic selenium concentrations on leaf JIP-test parameters of purple-branch rose

PSII donor side and electron transport

Different dilutions of inorganic selenium induced changes on both the acceptor and donor sides of PSII in leaves of purple branch rose. As shown in Figures 3a and 3c, relative to CK 1, foliar application of 2000-fold inorganic selenium led to significant decreases in Sm, ψo, φPo, φEo, Fv/Fm, and PIABS. Notably, PIABS declined by approximately 3.33 fold, indicating that PSII reaction center efficiency and the overall state of the photosynthetic apparatus are highly sensitive to 2000-fold foliar selenium application. In parallel, the values of dV/dto, Vj, Vi, and Fo/Fm increased significantly. Treatment with 4000-fold foliar selenium also reduced Sm, ψo, φPo, φEo, Fv/Fm, and PIABS, while elevating dV/dto, Vj, Vi, and Fo/Fm. These results suggest that both 2000-fold and 4000-fold foliar selenium applications diminish the size of the plastoquinone (PQ) pool on the acceptor side of PSII and decrease the probability of electron transfer from Qa- to the downstream secondary quinone acceptor Qβ. Consequently, Qa- becomes over reduced, electron flow beyond Qa- is constrained, and quantum yields and photosynthetic performance decline—demonstrating that the acceptor side of PSII sustains severe damage.

As shown in Figures 3b and 3d, compared with CK 2, root irrigation with 2000-fold inorganic selenium exerted no significant effect on Sm, ψo, φPo, φEo, Fv/Fm, or Vj, but significantly lowered Vi and PIABS, while markedly increasing Fo/Fm and dV/dto. Thus, PSII reaction center efficiency and the photosynthetic apparatus were largely insensitive to root irrigation with 2000-fold selenium. In contrast, root irrigation with 4000-fold caused significant reductions in φPo, Fv/Fm, PIABS, and Vj, with no significant change in Vi, relative to CK 2. Hence, root irrigation with 4000-fold inorganic selenium produced a more pronounced inhibitory effect.

Specific activity per PSII reaction center and per excited cross section

The specific activity parameters of PSII reflect the functional status of the photosynthetic apparatus; quantum efficiencies expressed on a per active reaction center (RC) basis and per excited cross section (CS) basis allow comparison of photosynthetic structure across treatments. As shown in Figure 4a, relative to CK-1, foliar application of 2000-fold inorganic selenium increased the absorbed photon flux per reaction center (ABS/RC), dissipated energy flux per RC (DIo/RC), and trapped energy flux per RC (TRo/RC) by factors of 1.04, 0.48, and 0.57, respectively. The electron transport flux per RC (ETo/RC) did not differ significantly from CK-1 but was elevated slightly by 0.01. As shown in Figure 4c, DIo/CSo and ABS/CSo for the 2000-fold foliar treatment were 1.6 and 1.15, respectively, both significantly higher than the corresponding CK-1 values. In contrast, ETo/CSo for the 2000-fold foliar treatment was 0.8, significantly lower than that of CK-1.

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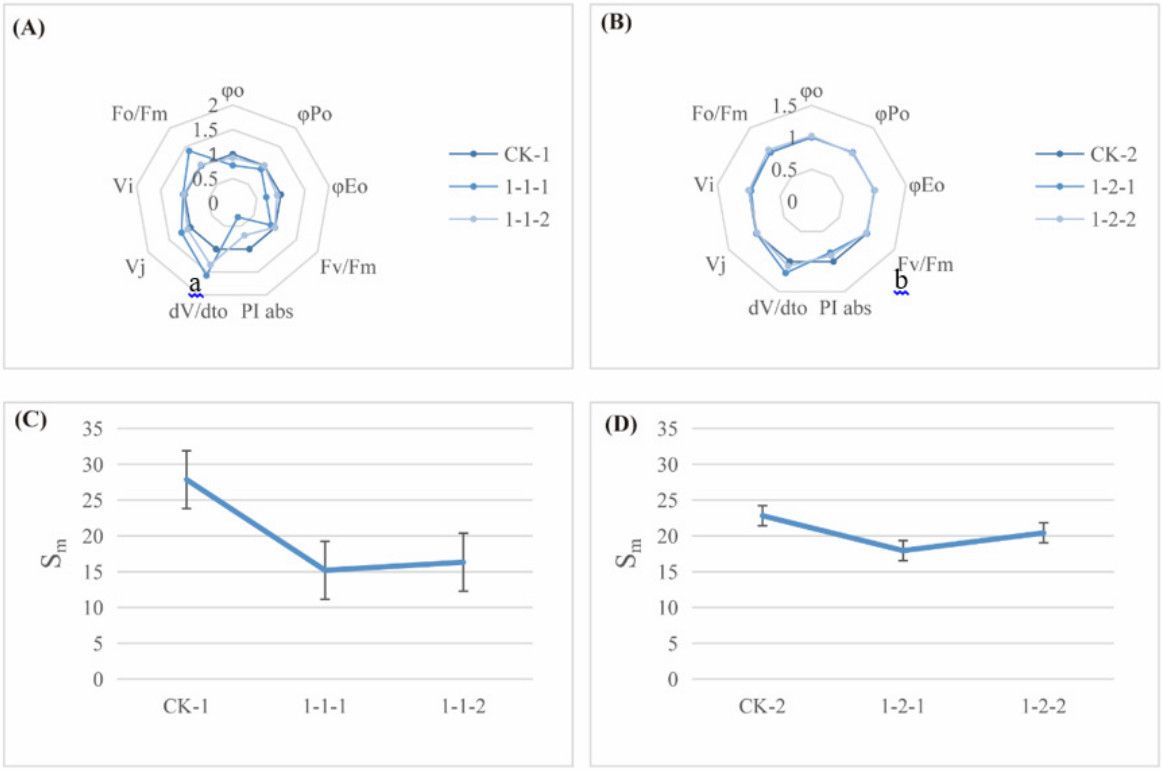

Figure 3 Effects of different concentrations of inorganic selenium on jiP-test parameters of leaves of *Rosa rugosa 'Zizhi'*.

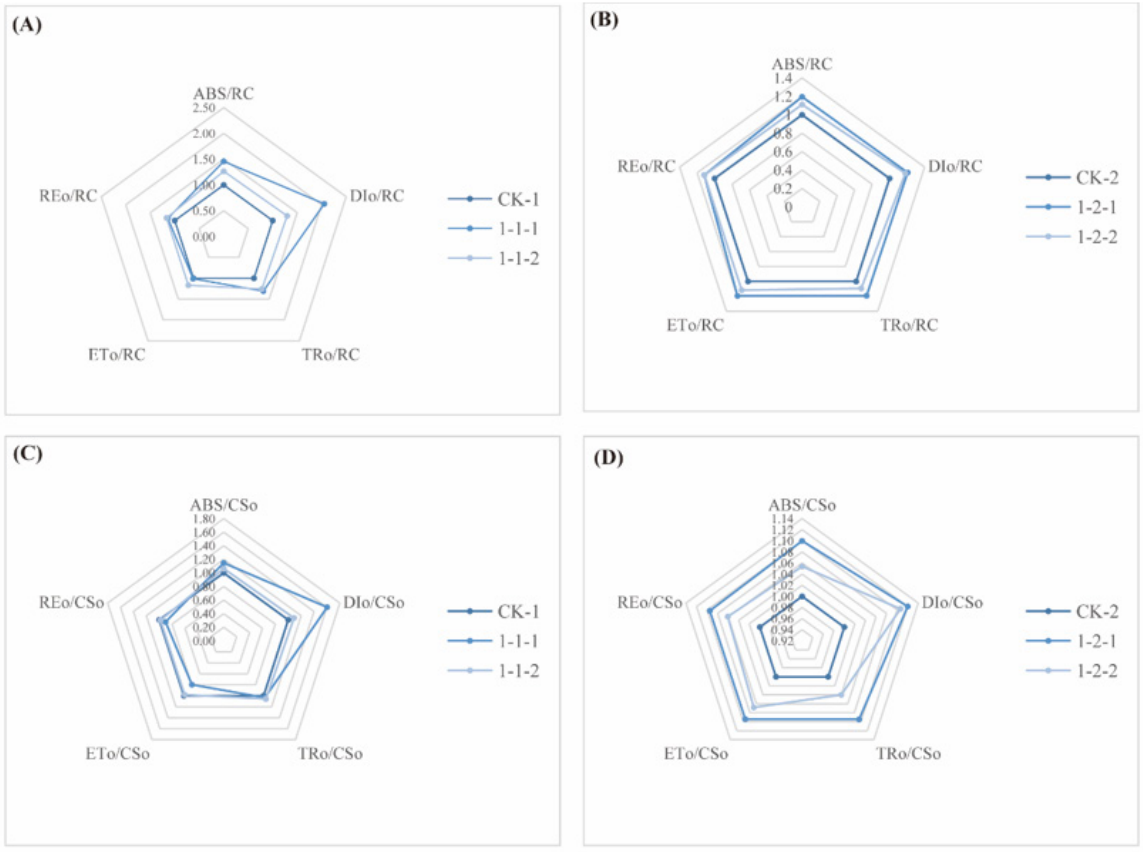

Figure 4 *Effects of different concentrations of inorganic selenium on PSII specific activity parameters in the leaves of purple branch rose (Rosa rugosa 'Zizhi').*

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For foliar application of 4000-fold inorganic selenium, the ratios of ABS/RC, DIo/RC, TRo/RC, and ETo/RC were 1.27, 1.29, 1.26, and 1.17, respectively, all significantly above CK-1. Similarly, DIo/CSo, TRo/CSo, and ABS/CSo were 1.09, 1.06, and 1.06, respectively, all exceeding CK-1, whereas ETo/CSo (0.98) was not notably different from the control. These observations indicate that both 2000-fold and 4000-fold foliar selenium treatments enhance reaction center activity and increase the proportion of energy allocated to electron transport, while simultaneously raising the fraction of energy dissipated as heat—a protective response that mitigates the photodamage induced by 2000-fold foliar application. Overall, the foliar selenium treatments alter light energy partitioning and strengthen electron transport capacity in purple branch rose leaves.

As shown in Figure 4b, compared with CK 2, root irrigation with 2000-fold inorganic selenium increased ABS/RC, DIo/RC, TRo/RC, and ETo/RC to 1.20, 1.21, 1.19, and 1.19 times the control value, respectively, all significant elevations. Correspondingly, as shown in Figure 4d, ABS/CSo, DIo/CSo, TRo/CSo, and ETo/CSo were 1.10, 1.12, 1.09, and 1.09, respectively, all significantly higher than CK 2. Root irrigation with 4000-fold inorganic selenium likewise increased ABS/RC, DIo/RC, TRo/RC, and ETo/RC to 1.11, 1.18, 1.09, and 1.12 times the CK 2 levels. The corresponding CS based parameters—ABS/CSo, DIo/CSo, TRo/CSo, and ETo/CSo—were 1.05, 1.11, 1.04, and 1.07 times the control, all significantly elevated.

Discussion

Various ecological factors that modulate photosynthesis ultimately exert their effects through Rubisco, which requires activation by Rubisco activase to become catalytically competent.[19] The present experiment examined the influence of 2000-fold and 4000-fold inorganic selenium applied via foliar spray and root irrigation on Rubisco activation in purple branch rose. The results demonstrated that, with respect to selenium concentration, the 4000 fold dilution conferred greater Rubisco activation than the 2000 fold dilution, regardless of the application method.[20,21] When compared across all treatments, both foliar and root applied 4000 fold selenium sustained higher Rubisco activation levels than their 2000 fold counterparts.[22] These findings indicate that selenium concentration profoundly affects Rubisco activation state and, consequently, photosynthetic rate.[7] Considering the comparable or superior activation effect of the 4000 fold concentration under both application methods, together with the greater stability of the photosynthetic apparatus afforded by root irrigation (as discussed later), root irrigation with the lower concentration (4000 fold) selenium fertilizer is considered the preferable practice.[23]

Dynamic changes in chlorophyll fluorescence directly reflect the impact of external environmental factors on plant photosynthetic activity and can indirectly or directly reveal photochemical alterations on both the donor and acceptor sides of PSII.[24] Such changes manifest as distinct differences in the typical O, J, I, and P phases of the rapid fluorescence induction kinetics curve, and the derived fluorescence parameters are indicative of the functional status of PSII.[25] The O step corresponds to the initial fluorescence Fo emitted when all PSII reaction centers are open, whereas the P step represents the maximal fluorescence Fm attained when all PSII centers are closed.[26] The J step is intimately linked to the primary quinone acceptor Qa; under stress conditions, the efficiency of electron transfer beyond Qa declines markedly. The magnitude of Fm is determined by the absorption, reflection, and re absorption of chlorophyll fluorescence within the leaf, and an increase in Vj signifies an accumulation of the reduced primary acceptor Qa-.[27]

In the present experiment, foliar application of 2000-fold inorganic selenium caused a significant decrease in Fm and Fv/Fm after the O step. Foliar application of 4000-fold also resulted in a marked reduction in Fv/Fm, despite a significant elevation in Fm. These observations attest that PSII reaction centers sustain damage or undergo reversible inactivation, culminating in photoinhibition of purple branch rose leaves.[28] Both foliar selenium treatments (2000-fold and 4000-fold) reduced the maximum quantum yield of PSII (Fv/Fm) and the performance index (PIABS) relative to CK 1. The 2000-fold foliar application produced the lowest Fv/Fm, indicating a stronger inhibition of photosynthesis than that induced by 4000-fold foliar application.[29] These results demonstrate that foliar selenium treatments suppress the photosynthetic apparatus, lowering the actual light trapping efficiency of PSII reaction centers and decreasing both maximum quantum yield and performance index.

In the case of root irrigation, 2000-fold treatment increased Fm significantly while reducing Fv/Fm, and 4000-fold treatment depressed both Fm and Fv/Fm significantly. Both root applied selenium concentrations therefore caused PSII damage or reversible inactivation, leading to photoinhibition. Both root irrigation treatments led to lower Fv/Fm and PIABS compared with CK 2, with the 2000-fold concentration yielding the lowest PIABS; hence, 2000-fold root irrigation exerted a stronger inhibitory effect on photosynthesis than did 4000-fold root irrigation.

In this study, both foliar selenium applications (2000-fold and 4000-fold) increased the dissipated energy flux per reaction center (DIo/RC) and per excited cross section (DI/CSo), suggesting a reduction in the number of active PSII reaction centers.[30] Similar increases in DIo/RC and DI/CSo were observed with root irrigation treatments.[31] Further analysis revealed that the 2000-fold concentration, whether applied foliarly or via root irrigation, more strongly enhanced ABS/RC, DIo/RC, TRo/RC, and ETo/RC than did the 4000-fold concentration, indicating that the 2000-fold concentration imposes a more severe photosynthetic inhibition.[32] In addition, foliar spray generally elevated energy dissipation per reaction center to a greater extent than did root irrigation, implying that foliar application is more detrimental to photosynthetic performance.

Conclusion

The results of this study reveal an apparent contradiction: both foliar and root applications of inorganic selenium (especially at the 4000 fold dilution) significantly enhanced Rubisco activation and carboxylation efficiency, indicating a positive effect on the carbon fixation (light independent) reactions of photosynthesis. However, the same selenium treatments simultaneously decreased PSII maximum photochemical efficiency (Fv/Fm), performance index (PIABS), and electron transport fluxes, while increasing energy dissipation (DIo/RC and DI/CSo). This indicates that PSII reaction centers were damaged or reversibly inactivated, leading to photoinhibition and reduced light use efficiency.

Several hypotheses may explain this discrepancy. First, selenium may directly promote Rubisco activase activity or stabilize the active conformation of Rubisco, thereby enhancing carboxylation independently of the light reactions. Second, the damage to PSII is likely caused by selenium induced oxidative stress: excessive selenite or selenate can trigger the overproduction of reactive oxygen species (ROS), which preferentially attack the D1 protein of PSII reaction centers and the oxygen evolving complex, impairing electron transport without immediately suppressing RuBP regeneration or Rubisco activation. Third, the positive effect on Rubisco might represent a

compensatory response to reduced photochemical energy supply, as plants attempt to maximize carbon capture under stressful conditions.

In practical terms, although the 4000 fold root irrigation treatment still caused some PSII damage, it was significantly less inhibitory than the 2000 fold foliar application. Moreover, root irrigated plants maintained more stable PSII structure and better adaptation to ambient light and temperature fluctuations. Therefore, root irrigation with 4000 fold diluted inorganic selenium is recommended as the optimal practice for balancing the positive effects on carbon assimilation with the negative impacts on the PSII reaction centers and light dependent reactions. Future studies should explore the molecular mechanisms linking selenium induced ROS production to the differential regulation of Rubisco activase and PSII core proteins.

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None

Conflict of Interest

All authors declare that there is no conflicts of interest.

References

1. Hurt RT, Vencill WK. Phytotoxicity and nutsedge control in woody and herbaceous landscape plants with manage (MON12037). *J Environ Hortic*. 1994;135–137.
2. Geneva MP, Stancheva IV, Boychinova MM, et al. Effects of foliar fertilization and arbuscular mycorrhizal colonization on *Salvia officinalis* L. growth, antioxidant capacity, and essential oil composition. *J Sci Food Agric*. 2010;90(4):696–702.
3. Skrypnik L, Feduraev P, Golubkina N, et al. Foliar spraying of selenium in inorganic and organic forms stimulates plant growth and secondary metabolism of sage (*Salvia officinalis* L.) through alterations in photosynthesis and primary metabolism. *Sci Hortic*. 2024;338:113633.
4. Gupta M, Gupta S. An overview of selenium uptake, metabolism, and toxicity in plants. *Front Plant Sci*. 2017;7:2074.
5. Dervisi I, Koletti A, Agalou A, et al. Selenium–binding protein 1 (SBP1): a new putative player of stress sensing in plants. *Int J Mol Sci*. 2024;25(17):9372.
6. Liu H, Xiao C, Qiu T, et al. Selenium regulates antioxidant, photosynthesis, and cell permeability in plants under various abiotic stresses: a review. *Plants (Basel)*. 2022;12(1):44.
7. Bandehagh A, Dehghanian Z, Gougerdchi V, et al. Selenium: a game changer in plant development, growth, and stress tolerance, via the modulation in gene expression and secondary metabolite biosynthesis. *Phyton*. 2023;92(8):2301–2324.
8. Zubek S, Stefanowicz AM, Błaszkowski J, et al. Arbuscular mycorrhizal fungi and soil microbial communities under contrasting fertilization of three medicinal plants. *Appl Soil Ecol*. 2012;59.
9. Reetnik I, Barievi D, Rusu DB, et al. Genetic diversity and demographic history of wild and cultivated/naturalised plant populations: evidence from Dalmatian sage (*Salvia officinalis* L., Lamiaceae). *PLoS One*. 2016;11(7):e0159545.
10. Da Silva DF, Cipriano PE, De Souza RR, et al. Anatomical and physiological characteristics of *Raphanus sativus* L. submitted to different selenium sources and forms application. *Sci Hortic*. 2020;260:108839.
11. El–Keltawi NE, Croteau R. Influence of herbicides and growth regulators on the growth and essential oil content of sage. *Phytochemistry*. 1987;26(3):675–679.
12. Hasanuzzaman M, Bhuyan MHM B, Raza A, et al. Selenium in plants: boon or bane? *Environ Exp Bot*. 2020;178:104170.
13. Hawrylak–Nowak B, Matraszek R, Pogorzelec M. The dual effects of two inorganic selenium forms on the growth, selected physiological parameters and macronutrients accumulation in cucumber plants. *Acta Physiol Plant*. 2015;37(2):41.
14. Kaur M, Sharma S. Influence of selenite and selenate on growth, leaf physiology and antioxidant defense system in wheat (*Triticum aestivum* L.). *J Sci Food Agric*. 2018;98(15):5700–5710.
15. Hossain A, Skalicky M, Brestic M, et al. Selenium biofortification: roles, mechanisms, responses and prospects. *Molecules*. 2021;26(4):881.
16. Harikrishnan R, Yang XB. Influence of herbicides on growth and sclerotia production in *Rhizoctonia solani*. *Weed Sci*. 2001;49(2):241–247.
17. Saeedi R, Seyedi A, Esmaeilizadeh M, et al. Improving the performance of the photosynthetic apparatus of *Citrus sinensis* with the use of chitosan–selenium nanocomposite (CS + Se NPs) under salinity stress. *BMC Plant Biol*. 2024;24(1):745.
18. Lahkim LT, Peretz Ion I. The influence of the inoculum source of *Rhizoctonia solani* on development of black scurf on potato. *J Phytopathol*. 2010;153(4):240–244.
19. Jiang C, Quick WP, Alred R, et al. Antisense RNA inhibition of Rubisco activase expression. *Plant J*. 1994;5(6):787–798.
20. Zhang W, He X, Chen X, et al. Exogenous selenium promotes the growth of salt–stressed tomato seedlings by regulating ionic homeostasis, activation energy allocation and CO2 assimilation. *Front Plant Sci*. 2023;14:1206246.
21. Wang Y, Zhu Q, Wang Z, et al. Effects of foliar application of amino acid–chelated selenite on photosynthetic characteristics of peanut (*Arachis hypogaea* L.) leaves at the podding stage. *Plant Soil Environ*. 2024;70(1):17–25.
22. Sekhurwane M, Moloi M. Variation in the selenium application methods influences the physiological and biochemical traits of drought–stressed edamame. *SSRN*. 2023.
23. Dennis A, Atkinson M, Development of *Rhizoctonia solani* on stems, stolons and tubers of potato II. Efficacy of chemical applications. *Am J Potato Res*. 2011.
24. Beyzi E, Güneş A, Karaman K, et al. Agronomic, biochemical and bioactive properties of sage (*Salvia officinalis* L.) affected by foliar selenium spraying. *J Plant Nutr*. 2024;47(12):1931–1942.
25. Haas C, Hengelhaupt KC, Kümmritz S, et al. *Salvia* suspension cultures as production systems for oleanolic and ursolic acid. *Acta Physiol Plant*. 2014;36(8):2137–2147.
26. Šatović Z. Legal protection, conservation and cultivation of medicinal and aromatic plants in Croatia. 2004.
27. Weathers KA. *The Role of Invasive Erodium Species in Restoration of Coastal Sage Scrub Communities and Techniques for Control*. Corporate Information Management; 2013.
28. Ning CJ, Ding N, Wu GL, et al. Proteomics research on the effects of applying selenium to apple leaves on photosynthesis. *Plant Physiol Biochem*. 2013;70:1–6.
29. Sun J, Sui X, Wang S, et al. The response of *rbcL*, *rbcS* and *rca* genes in cucumber (*Cucumis sativus* L.) to growth and induction light intensity. *Acta Physiol Plant*. 2014;36(10):2779–2791.
30. Habibi G. Selenium ameliorates salinity stress in *Petroselinum crispum* by modulation of photosynthesis and by reducing shoot Na accumulation. *Russ J Plant Physiol*. 2017;64(3):368–374.
31. Diao M, Ma L, Wang J, et al. Selenium promotes the growth and photosynthesis of tomato seedlings under salt stress by enhancing chloroplast antioxidant defense system. *J Plant Growth Regul*. 2014;33(3):671–682.
32. Moloi MJ, Khoza BM. The effect of selenium foliar application on the physiological responses of edamame under different water treatments. *Agronomy*. 2022;12(10):2400.