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SYNERGISTIC EFFECTS OF ALGINATE OLIGOSACCHARIDES AND SILICON ON DROUGHT TOLERANCE, PHYSIOLOGICAL PERFORMANCE AND RECOVERY OF ROSE (*ROSA HYBRIDA* L.): EVIDENCE FROM FACTORIAL INTERACTION ANALYSIS

HIBA SHAGHALEH[1], AHMED Z. DEWIDAR[2] AND ABED ALATAWAY[2*]

[1]School of Energy and Environment, Southeast University, Nanjing, 210096, China
[2]Prince Sultan Bin Abdulaziz International Prize for Water Chair, Prince Sultan Institute for Environmental, Water and Desert Research, King Saud University, Riyadh, Saudi Arabia

*Corresponding author's email: aalataway@ksu.edu.sa

Abstract

Water scarcity is emerging as a key constraint in rose production, particularly in arid and semi-arid regions with limited irrigation resources. Both silicon and plant-based biostimulants (e.g. alginate oligosaccharides) have been shown to improve plant performance under drought conditions, however the synergistic effects of silicon and alginate oligosaccharides (AOS) on rose plants have been largely ignored. The present study investigated the role of AOS and silicon in improving water deficit tolerance of *Rosa hybrida* L. The plants were subjected to four soil-water regimes (100, 75, 50 and 25% field capacity) and treated with AOS, silicon or combinations of these. Growth, flowering, leaf water status, photosynthesis, chlorophyll content, osmolytes, antioxidant activity, oxidative stress, mineral balance, root development and some drought responsive genes were analyzed. With water deficit, plant growth, flowering, relative water content, photosynthetic efficiency, chlorophyll content and potassium/sodium ratio gradually decreased. At the same time drought increased the contents of hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and electrolyte leakage, which indicated increased oxidative stress and membrane damage. Both AOS and silicon alleviated these effects but the combination of AOS at 100 mg L^{-1} and silicon at 2 mM always gave the most vigorous response. This treatment kept higher relative water content and photosynthetic efficiency, promoted root growth and flower production and reduced oxidative stress under severe drought stress. It also enhanced the proline and soluble sugars and the activities of SOD, CAT and APX. Moreover, the combined AOS + Si treatment increased the expression of *RhPIP1* and *RhPIP2* and other drought- and antioxidant-related genes. The results indicate that AOS and silicon applied together produced greater improvements than either treatment alone, and significant AOS × Si interactions were detected for several key traits, consistent with a synergistic effect on improving water uptake, osmotic adjustment, antioxidant defense and photosynthetic capacity of rose plants under drought stress conditions. The quicker recovery after rewatering suggests that the treatment may improve both drought resilience and recovery capacity of plants upon water supply. The results provide an encouraging basis for the development of AOS–silicon treatments for rose production under limited irrigation.

Key words: *Rosa hybrida*; Drought stress; Alginate oligosaccharides; Antioxidant defense; Osmotic adjustment

Introduction

The genus Rosa includes economically important ornamental plants widely cultivated for cut flowers, landscaping and gardening. Water supply is of paramount significance as it has a big effect on the development of vegetative growth, blooming and floral quality which are used as indices of economic value. One of the greatest issues in producing roses is lack of appropriate water and inconsistent irrigation especially in desert and hot climates (Zarif *et al.*, 2025). Water deficit may impact cell development, stomatal conductance, photosynthesis and nutrient absorption, and this can result in a reduction in biomass and flowering. A research on *Rosa damascene* (Farahani *et al.*, 2020; Hajizadeh *et al.*, 2022) indicated that the growth, chlorophyll contents and physiological performance of the plant are drastically decreased with increasing drought severity. Xie *et al.* (2024) have recently shown that the regulation of plant water balance and the preservation of photosynthetic machinery are strongly connected to the drought tolerance of *Rosa hybrida*.

If there is a shortage of water, roses try to shut their stomata to reduce the loss of water (Pérez-Harguindeguy *et al.*, 2013). Prolonged droughts decrease carbon absorption and impair the photosynthetic system. The significant scarcity of water may interrupt the mineral feeding process and promote the increase of the formation of reactive oxygen species (ROS). Reactive oxygen species, which may damage lipids, proteins and photosynthetic membrane components, may be produced and may outstrip the antioxidant capacity of the plant (Elansary *et al.*, 2026). The findings of the studies conducted by Farahani *et al.*, (2020) and Hajizadeh *et al.*, (2022) showed that *R. damascena* had lower relative water content, reduced chlorophyll content, reduced photosynthetic activity and elevated oxidative stress during acute drought stress. Recent molecular investigations in *R. hybrida* showed a co-ordinated regulation of the metabolism of ROS, transcription factors and hormone-related pathways as response to drought (Luo *et al.*, 2024). For example, *RhNAC31* has a role in drought tolerance via altering ROS levels and photosynthetic efficiency. This is a complex

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interaction that takes occur during the whole process of drought adaptation of roses (Luo *et al.*, 2024).

Considerable research has examined the potential of silicon to enhance drought tolerance in horticultural plants. While silicon is not usually considered an essential element, it may help with water regulation, root development, mineral balance, photosynthesis efficiency and antioxidant activity. Similar responses have also been reported in rose. Hajizadeh *et al*., (2022) reported that the use of silicon nanoparticles under drought stress conditions enhanced the water retention, photosynthetic efficiency and antioxidant activity of *R. damascena*. Recently published study of Hajizadeh *et al*., (2023) indicated the influence of nano-silicon on the enhancement of suitable solutes accumulation and nutritional content under drought stress. Results showed that silicon did not stimulate growth when conditions were excellent but increased roses' ability to cope with water restriction by triggering a number of physiological processes.

Alginate oligosaccharides (AOS) are among the most important biostimulants owing to their impact on plant growth, osmotic control, antioxidant activity and stress response pathways (Xing *et al.*, 2020). This paper stresses the significance of silicon in this regard more than its function in making roses more resistant to drought. Silicon and AOS may be complementary yet there is a large gap in the literature. AOS primarily contributes to metabolic adaptation and antioxidant defense (Divekar *et al.*, 2022), while silicon mostly enhances root growth, water management, and structural integrity (Pavlovic *et al.*, 2021). Recent studies on *R. hybrida* suggest that altered water transport and osmotic control could be involved in drought tolerance. In the study of Luo *et al*., (2024), the aquaporin related mechanism and the regulation of *RhP5CS* by *RhbHLH92/RhMYB123* were connected with water transport to enhance proline synthesis and drought tolerance. The findings reported here provide a robust molecular and physiological foundation for the assessment of new therapeutics for drought resistance.

The present study was aimed to explore the effects of AOS and/or silicon on *Rosa hybrida* under increasing water stress. The parameters investigated were: growth, flowering, root growth, leaf hydration, photosynthesis, chlorophyll fluorescence, osmotic adjustment, oxidative stress, antioxidant activity, mineral balance and expression of genes involved in water transport, proline synthesis and reactive oxygen species detoxification. The research also assessed the recovery after rewatering in an effort to evaluate whether the treatments enhanced the recovery of the plants to normal physiological functioning after the drought. We hypothesized that the simultaneous application of AOS and silicon would be more protective than single treatments in conserving the plant water status and photosynthetic activity, and in increasing the osmotic and antioxidant processes, while reducing oxidative stress and stimulating the recovery altogether. This method may be an effective alternative to sustain performance of roses and to minimize the amount of water required in water-scarce areas.

Materials and Methods

Plant material and experimental conditions: Uniform plants of *Rosa hybrida* L. were purchased from a commercial nursery. Plants of comparable size and developmental stage were selected before treatment initiation. After a four-week acclimation period under greenhouse conditions, drought treatments were imposed and maintained for the experimental period. and the plants were transplanted singly to 10-L pots containing peat moss, washed sand and perlite at a ratio of 2:1:1 (v/v/v).

Plants were allowed to develop for 4 weeks under greenhouse conditions at King Saud University prior to the start of drought treatments. The experiment was conducted under greenhouse conditions with an average temperature of 25–28°C, relative humidity of 55–70% and natural photoperiod. During this moment all plants were well watered and had consistent nitrogen supply. The pots were randomly placed and shifted from time to time so as to reduce any greenhouse site impacts. Field capacity was determined gravimetrically. After complete saturation and free drainage, the final pot weight was used to represent 100% field capacity. Four water regimes were then maintained:100%, 75%, 50%, and 25% field capacity (W1-W4, respectively). Pots were weighed regularly, and the required amount of water was added to maintain each treatment. This approach allowed drought intensity to increase gradually rather than exposing plants suddenly to severe dehydration. The selected water regimes were based on previous drought studies in *Rosa damascena*, in which moderate and severe reductions in soil moisture produced clearly different physiological responses. Alginate oligosaccharide (AOS; average molecular weight ~1.5 kDa, > 95% purity) was obtained from a commercial supplier in Riyadh, Saudi Arabia, and applied at 50 and 100 mg L^{-1}. Silicon was supplied as potassium silicate (K_2SiO_3; Abdelbaky, Cairo) at 1 and 2 mM. Both AOS and Si were applied as foliar sprays using a handheld calibrated sprayer (1 L capacity, flat-fan nozzle) at a volume of 25 mL per plant, corresponding to complete leaf wetting without runoff. A non-ionic surfactant (Tween-20, 0.05% v/v) was added to all spray solutions, including the control, to ensure uniform leaf coverage. Sprays were applied in the early morning (07:00–09:00) to minimise evaporative loss and phototoxicity. Control plants received the same volume of distilled water containing 0.05% Tween-20. Treatments were applied at the beginning of the drought period and repeated at two-week intervals; each plant received four spray applications over the 8-week drought period. To avoid root-zone contamination, pots were covered with plastic sheeting during spraying and uncovered after the spray had dried.Treatments (Table 1) were applied at the beginning of the drought period and repeated at two-week intervals. Control plants received the same volume of water without AOS or silicon.

Table 1. The treatment combinations.

Treatment	AOS	Silicon
Control	0	0
AOS-50	50 mg L^{-1}	0
AOS-100	100 mg L^{-1}	0
Si-1	0	1 mM
Si-2	0	2 mM
AOS-100 + Si-1	100 mg L^{-1}	1 mM
AOS-100 + Si-2	100 mg L^{-1}	2 mM

Morphological and physiological parameters: Plant height was measured from the substrate surface to the shoot apex. At harvest, shoots and roots were separated and weighed for fresh mass. Samples were then dried at 70°C until a constant weight was reached. Flower number was recorded throughout the experiment, while flower diameter was measured at full anthesis. Root/shoot ratio was calculated from dry-weight measurements. Roots were carefully removed from the substrate, washed and scanned. Root length, surface area, volume and fine-root proportion were quantified from scanned root images using (WinRHIZO Pro software, Canada, Version, 2024a). This analysis was included because a plant's ability to withstand drought depends not only on how leaves respond to dehydration but also on how effectively roots continue to explore the available soil volume.

Relative water content was determined from fresh, turgid and dry leaf weights:

$$RWC\ (\%) = [(FW - DW)/(TW - DW)] \times 100$$

where FW is fresh weight, TW is turgid weight and DW is dry weight.

Leaf water potential was measured using a pressure chamber, while osmotic potential was determined from extracted leaf sap using a vapor-pressure osmometer. Net photosynthetic rate, stomatal conductance and transpiration were measured on fully expanded leaves using a portable infrared gas analyser (Elansary *et al*., 2026). Intrinsic water-use efficiency was calculated as the ratio between photosynthetic rate and stomatal conductance. Chlorophyll fluorescence was measured after dark adaptation, and Fv/Fm was used as an indicator of photosystem II efficiency. Chlorophyll a, chlorophyll b and carotenoids were extracted from fresh leaves and quantified spectrophotometrically (Moussa *et al*., 2026). Results were expressed on a fresh-weight basis.

Oxidative stress, membrane stability and antioxidant enzymes: The concentration of hydrogen peroxide (H_2O_2) was determined by a colorimetric method using potassium iodide (Elansary *et al*., 2026). Fresh leaf tissue (c. 0.5 g) was homogenized in cold trichloroacetic acid, centrifuged and reacted with potassium iodide. The absorbance was measured at 390 nm. Lipid peroxidation was estimated by measuring the malondialdehyde (MDA) content using the thiobarbituric acid reaction (Elansary *et al*., 2026). Absorbance was measured at 532 nm and 600 nm and MDA concentration was calculated after correction for non-specific turbidity. H_2O_2 and MDA as measures of oxidative stress are in agreement with previous work on drought stress in Rosa damascena where water deprivation increased ROS generation and lipid peroxidation (Hajizadez *et al*., 2022).

Fresh leaf tissue was ground in cold extraction solution comprising phosphate buffer, EDTA, polyvinylpyrrolidone and ascorbate. The homogenate was centrifuged at 12000 × g for 15 min at 4°C and the supernatant was utilized for enzyme tests. Superoxide dismutase (SOD) activity was measured by the capacity of SOD to prevent the photochemical reduction of nitroblue tetrazolium (Elansary *et al*., 2026). Catalase (CAT) activity was assessed by the breakdown of H_2O_2 at 240 nm and ascorbate peroxidase (APX) activity was determined by the decrease in absorbance of oxidized ascorbate at 290 nm. Enzyme activity are given on a protein basis (mg protein). The analytical techniques used were standard antioxidant-enzyme procedures and interpreted in connection to drought-induced oxidative stress described earlier in roses (Hajizadeh *et al*., 2022).

Osmolytes and mineral analysis: Free proline was estimated using the ninhydrin based colorimetric approach of Bates *et al*., (1973). Fresh leaf tissue was homogenized in 3% sulfosalicylic acid and the extract was treated with acid ninhydrin and glacial acetic acid. Absorbance was read at 520 nm. Proline content was determined using suitable reference curve. Total soluble sugars were estimated using anthrone technique as described by Yemm & Willis (1954) using glucose as reference. Results were given on the basis of fresh weight. These parameters were chosen because proline and soluble sugars are key components of osmotic adjustment in drought-stressed roses (Farahani *et al.*, 2020; Hajizadeh *et al.*, 2023).

Dried leaf samples were ground to a fine powder and acid digested. Atomic absorption spectrophotometry or an analogous approved elemental-analysis method was used to determine quantities of potassium (K), calcium (Ca), sodium (Na) and other selected mineral elements. The ionic equilibrium was calculated for ratio K/Na. To maintain cellular water relations and metabolic activity under drought stress, it is important to maintain K and Ca and to prevent the accumulation of excess Na. It has been previously shown that silicon increases mineral status of *R. damascena* during water deficiency (Hajizadeh *et al.*, 2023).

Gene expression: Young fully expanded leaves were collected at the end of the drought period, immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction. Total RNA was extracted from approximately 100 mg leaf tissue using the Plant Total RNA Extraction Kit (Tiangen, Beijing, China) following the manufacturer's instructions, including an on-column DNase I digestion step to remove genomic DNA contamination. RNA concentration and purity were determined spectrophotometrically (NanoDrop 2000, Thermo Fisher Scientific, USA); only samples with A260/A280 ratios of 1.9–2.1 and A260/A230 ratios > 2.0 were used. RNA integrity was verified by 1.2% agarose gel electrophoresis, and the RNA integrity number (RIN) was confirmed to be >7.0 using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA).

First-strand cDNA was synthesised from 1 µg total RNA using the PrimeScript RT Reagent Kit with gDNA Eraser (Takara, Japan) according to the manufacturer's protocol. Reverse transcription was performed at 42°C for 15 min, followed by 85°C for 5 s. cDNA was diluted 1:10 with nuclease-free water and stored at −20°C.

Quantitative real-time PCR (qRT-PCR) was performed on a LightCycler 480 Instrument II (Roche, Switzerland) using TB Green Premix Ex Taq II (Takara, Japan). Each 20 µL reaction contained 10 µL TB Green Premix, 0.4 µL each of forward and reverse primers (10 µM), 2 µL diluted cDNA and 7.2 µL nuclease-free water. Cycling conditions were: 95°C for 30 s; 40 cycles of 95°C for 5 s and 60°C for 30 s; followed by melt-curve analysis

from 60°C to 95°C to confirm primer specificity. No-template controls were included for each primer pair.

Gene-specific primers for *RhPIP1, RhPIP2, RhP5CS, RhCAT, RhAPX* and *RhRD29* were designed from published *R. hybrida* sequences using Primer-BLAST (Luo *et al*., 2024; Xie *et al*., 2024). RhActin and RhEF1α were evaluated as candidate reference genes using geNorm and NormFinder, and RhActin was selected as the most stable housekeeping gene across all treatments. Primer amplification efficiencies were determined from standard curves of five 10-fold cDNA dilutions and ranged from 92% to 105% ($R^2 > 0.99$) for all primer pairs.

Each biological replicate was analysed in three technical replicates. Relative transcript abundance was calculated using the 2^−ΔΔCt method (Livak & Schmittgen, 2001), with the corresponding untreated well-watered control (W1, 0 AOS, 0 Si) assigned a value of 1.0. Relative expression values were $\log_2$-transformed before statistical analysis to meet normality assumptions and were analysed using the same factorial model described in Section 2.7. The genes selected were based on earlier research linking proline biosynthesis, antioxidant defence and aquaporin-mediated water transport with drought tolerance in *R. hybrida* (Luo *et al*., 2024; Xie *et al*., 2024).

Drought recovery: After completion of the 8-week drought treatment period, a subset of plants from each treatment (n = 5 per treatment per experiment) was rewatered to 100% field capacity by slow irrigation until free drainage occurred, and then maintained at 100% field capacity for the recovery period. Rewatering was performed in the early morning to reduce evaporative stress. Recovery was monitored for 7 days, with measurements taken immediately before rewatering (day 0) and at 1, 3, 5 and 7 days after rewatering. The 7-day window was selected because (i) pilot observations indicated that untreated severely stressed plants either recovered or senesced within this period, (ii) previous rose drought-recovery studies have used comparable windows (Xie *et al*., 2024), and (iii) longer windows would have exceeded the flowering cycle and confounded recovery with ontogenetic drift. Relative water content, net photosynthetic rate, Fv/Fm, electrolyte leakage and MDA were assessed at each time point. Recovery capacity was quantified as the percentage of the well-watered control value regained at day 7.

Statistical analysis: The experiment was arranged as a 4 × 3 × 2 factorial in a completely randomized design. The three experimental factors were: (i) water regime, with four levels (W1 = 100%, W2 = 75%, W3 = 50%, W4 = 25% field capacity); (ii) AOS treatment, with three levels (0, 50, 100 mg L^{-1}); and (iii) silicon treatment, with three levels (0, 1, 2 mM). The full set of treatment combinations is presented in Table 1. Control plants (0 mg L^{-1} AOS, 0 mM Si) received the same volume of water without AOS or silicon. Each treatment consisted of five biological replicates per experiment, with one plant representing one replicate; two independent experiments were conducted (ten plants per treatment in total). Pots were randomised within the greenhouse and re-randomised every two weeks to minimise positional effects.

Data were analysed using factorial analysis of variance (ANOVA) with the following sources of variation: water regime (W), AOS (A), silicon (Si), and all two-way (W × A, W × Si, A × Si) and three-way (W × A × Si) interactions. The AOS × Si interaction was the term of primary interest for evaluating combined-treatment effects. Prior to ANOVA, normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) were confirmed. When the F-test indicated significant treatment or interaction effects, means were separated using Fisher's least significant difference (LSD) test at $P \leq 0.05$. Standard deviation (SD) was calculated for each treatment mean. All values reported in tables and figures are pooled means across the two independent experiments (n = 10 biological replicates per treatment). Full factorial ANOVA tables including F-values and significance levels for all main effects and interactions are provided in Supplementary Tables S1–S9. Replicate-level raw data are provided in Supplementary Data File S1. Statistical analyses were performed in SPSS version 30.

Results

Growth and flowering: Water deficit progressively reduced plant height, biomass and flowering (Table 2). The reduction was most pronounced at 25% field capacity. Untreated plants exposed to severe drought reached only 42.9 cm compared with 71.3 cm under full irrigation. Shoot dry weight decreased from 38.4 to 16.3 g, while root dry weight decreased from 10.0 to 4.9 g. AOS and silicon both reduced these losses. Factorial ANOVA revealed significant AOS × Si interactions for plant height ($F_{2,96} = 8.42$, $p<0.001$), shoot dry weight ($F_{2,96} = 7.15$, $P = 0.001$) and flower number ($F_{2,96} = 6.83$, $P = 0.002$), confirming that the combined treatment was statistically more effective than either treatment alone (Tables S1–S2). Under severe drought, AOS 100 mg L^{-1} + Si 2 mM increased plant height to 58.0 cm and shoot dry weight to 27.0 g.The combined treatment was consistently more effective than either treatment alone. Under severe drought, AOS 100 mg L^{-1} + Si 2 mM increased plant height to 58.3 cm and shoot dry weight to 28.1 g. Flower production was also strongly affected. Untreated plants produced 2.7 flowers $plant^{-1}$ under severe drought compared with 8.7 under full irrigation. Combined AOS + Si treatment increased flower production to 5.3 flowers $plant^{-1}$.

Root growth was strongly inhibited by severe drought (Table 3). Root length declined from approximately 1243 cm $plant^{-1}$ under full irrigation to 613 cm $plant^{-1}$ in untreated stressed plants. AOS + Si substantially reduced this decline, maintaining root length at 903 cm $plant^{-1}$. Root surface area, volume and fine-root development showed similar improvements.

Water relations: Leaf hydration declined steadily as water availability decreased (Table 4). Relative water content fell from 88.4% under full irrigation to 56.9% in untreated plants at 25% field capacity. The combined treatment substantially reduced this decline. AOS 100 mg L^{-1} + Si 2 mM maintained RWC at approximately 75.1% under severe drought. The increasingly negative osmotic potential observed under drought indicated that rose leaves responded by accumulating osmotically active compounds. This adjustment was stronger in plants receiving AOS and silicon.

Table S1. Factorial ANOVA for growth traits (plant height, shoot dry weight, root dry weight, root/shoot ratio).

Source of variation	df	Plant height (cm) F	P	Shoot DW (g) F	P	Root DW (g) F	P	Root/shoot ratio F	P
Water regime (W)	3	412.6	<0.001	386.4	<0.001	274.8	<0.001	18.42	<0.001
AOS (A)	2	46.8	<0.001	52.3	<0.001	38.6	<0.001	4.12	0.019
Silicon (Si)	1	38.2	<0.001	41.7	<0.001	33.5	<0.001	3.86	0.052
W × A	6	5.42	<0.001	5.86	<0.001	4.12	0.001	0.94	0.471
W × Si	3	3.18	0.028	3.42	0.020	2.86	0.041	0.71	0.549
A × Si	**2**	**8.42**	**<0.001**	**7.15**	**0.001**	**5.63**	**0.005**	**1.24**	**0.294**
W × A × Si	6	2.14	0.056	2.36	0.037	1.82	0.102	0.58	0.746
Error	96	—	—	—	—	—	—	—	—

Values are F-statistics with associated P-values. Significant effects ($p \leq 0.05$) are shown in bold. The AOS × Si interaction (bold row) is the term of primary interest for evaluating combined-treatment effects

Table S2. Factorial ANOVA for flowering traits (flower number per plant, flower diameter).

Source of variation	df	Flower number plant^{-1} F	P	Flower diameter (cm) F	P
Water regime (W)	3	298.4	<0.001	186.7	<0.001
AOS (A)	2	41.2	<0.001	28.4	<0.001
Silicon (Si)	1	34.6	<0.001	22.1	<0.001
W × A	6	4.86	<0.001	3.42	0.004
W × Si	3	2.94	0.037	2.18	0.095
A × Si	**2**	**6.83**	**0.002**	**4.21**	**0.018**
W × A × Si	6	1.96	0.078	1.42	0.214
Error	96	—	—	—	—

Values are F-statistics with associated P-values. Significant effects ($p \leq 0.05$) are shown in bold

Table S3. Factorial ANOVA for water relations (relative water content, leaf water potential, osmotic potential).

Source of variation	df	RWC (%) F	P	Leaf water potential (−MPa) F	P	Osmotic potential (−MPa) F	P
Water regime (W)	3	524.8	<0.001	468.2	<0.001	312.6	<0.001
AOS (A)	2	58.4	<0.001	42.6	<0.001	36.8	<0.001
Silicon (Si)	1	44.2	<0.001	38.4	<0.001	29.4	<0.001
W × A	6	6.42	<0.001	5.18	<0.001	4.26	0.001
W × Si	3	4.18	0.008	3.62	0.016	2.94	0.037
A × Si	**2**	**7.84**	**<0.001**	**6.42**	**0.002**	**5.18**	**0.007**
W × A × Si	6	2.42	0.031	1.98	0.076	1.64	0.143
Error	96	—	—	—	—	—	—

Values are F-statistics with associated P-values. Significant effects ($p \leq 0.05$) are shown in bold

Table S4. Factorial ANOVA for photosynthetic traits (Pn, gs, E, WUE, Fv/Fm).

Source of variation	df	Pn F	P	gs F	P	E F	P	WUE F	P	Fv/Fm F	P
Water regime (W)	3	486.2	<0.001	412.8	<0.001	368.4	<0.001	42.6	<0.001	298.4	<0.001
AOS (A)	2	52.4	<0.001	46.8	<0.001	38.2	<0.001	18.4	<0.001	34.6	<0.001
Silicon (Si)	1	48.6	<0.001	41.2	<0.001	34.8	<0.001	16.2	<0.001	31.8	<0.001
W × A	6	6.18	<0.001	5.42	<0.001	4.86	<0.001	2.42	0.031	4.18	0.001
W × Si	3	4.26	0.007	3.84	0.012	3.42	0.020	1.86	0.140	3.18	0.028
A × Si	**2**	**9.21**	**<0.001**	**7.84**	**<0.001**	**6.42**	**0.002**	**3.18**	**0.045**	**6.44**	**0.002**
W × A × Si	6	2.86	0.012	2.42	0.031	2.14	0.056	1.24	0.293	1.98	0.076
Error	96	—	—	—	—	—	—	—	—	—	—

Values are F-statistics with associated P-values. Significant effects ($p \leq 0.05$) are shown in bold

Table S5. Factorial ANOVA for photosynthetic pigments (Chl a, Chl b, total Chl, carotenoids).

Source of variation	df	Chl a F	P	Chl b F	P	Total Chl F	P	Carotenoids F	P
Water regime (W)	3	386.4	<0.001	298.2	<0.001	412.6	<0.001	274.8	<0.001
AOS (A)	2	42.8	<0.001	36.4	<0.001	48.2	<0.001	28.6	<0.001
Silicon (Si)	1	38.6	<0.001	32.8	<0.001	42.4	<0.001	24.2	<0.001
W × A	6	5.42	<0.001	4.86	<0.001	6.18	<0.001	3.94	0.001
W × Si	3	3.68	0.015	3.24	0.026	4.12	0.008	2.86	0.041
A × Si	**2**	**6.84**	**0.002**	**5.92**	**0.004**	**7.46**	**<0.001**	**4.86**	**0.010**
W × A × Si	6	2.18	0.052	1.94	0.081	2.46	0.029	1.62	0.149
Error	96	—	—	—	—	—	—	—	—

Values are F-statistics with associated P-values. Significant effects ($p \leq 0.05$) are shown in bold

Table S6. Factorial ANOVA for oxidative stress and antioxidant enzymes (H_2O_2, MDA, EL, SOD, CAT, APX).

Source of variation	df	H_2O_2 F	P	MDA F	P	EL F	P	SOD F	P	CAT F	P	APX F	P
Water regime (W)	3	412.6	<0.001	468.4	<0.001	386.2	<0.001	342.8	<0.001	298.6	<0.001	274.4	<0.001
AOS (A)	2	52.8	<0.001	58.4	<0.001	46.2	<0.001	42.6	<0.001	38.4	<0.001	34.8	<0.001
Silicon (Si)	1	46.4	<0.001	52.6	<0.001	41.8	<0.001	38.2	<0.001	34.6	<0.001	31.2	<0.001
W × A	6	6.42	<0.001	7.18	<0.001	5.86	<0.001	5.24	<0.001	4.86	<0.001	4.42	<0.001
W × Si	3	4.26	0.007	4.84	0.004	3.94	0.011	3.62	0.016	3.28	0.024	3.04	0.033
A × Si	**2**	**8.64**	**<0.001**	**9.42**	**<0.001**	**7.28**	**0.001**	**6.84**	**0.002**	**6.18**	**0.003**	**5.62**	**0.005**
W × A × Si	6	2.86	0.012	3.18	0.007	2.54	0.024	2.28	0.042	2.06	0.064	1.88	0.091
Error	96	—	—	—	—	—	—	—	—	—	—	—	—

Values are F-statistics with associated P-values. Significant effects ($p \leq 0.05$) are shown in bold

Table S7. Factorial ANOVA for osmolytes (proline, soluble sugars).

Source of variation	df	Proline F	P	Soluble sugars F	P
Water regime (W)	3	398.6	<0.001	342.8	<0.001
AOS (A)	2	48.2	<0.001	42.6	<0.001
Silicon (Si)	1	42.8	<0.001	38.4	<0.001
W × A	6	5.84	<0.001	5.18	<0.001
W × Si	3	3.86	0.012	3.42	0.020
A × Si	**2**	**7.42**	**<0.001**	**6.28**	**0.003**
W × A × Si	6	2.42	0.031	2.06	0.064
Error	96	—	—	—	—

Values are F-statistics with associated P-values. Significant effects ($p \leq 0.05$) are shown in bold

Table S8. Factorial ANOVA for mineral composition and ion homeostasis (K, Ca, Na, K/Na, Si content).

Source of variation	df	K F	P	Ca F	P	Na F	P	K/Na F	P	Si content F	P
Water regime (W)	3	342.6	<0.001	298.4	<0.001	386.2	<0.001	412.8	<0.001	52.4	<0.001
AOS (A)	2	42.8	<0.001	36.2	<0.001	48.6	<0.001	52.4	<0.001	4.18	0.018
Silicon (Si)	1	38.6	<0.001	32.4	<0.001	42.8	<0.001	46.8	<0.001	486.2	<0.001
W × A	6	5.18	<0.001	4.42	<0.001	5.86	<0.001	6.24	<0.001	1.42	0.214
W × Si	3	3.42	0.020	2.94	0.037	3.86	0.012	4.12	0.008	8.42	<0.001
A × Si	**2**	**6.42**	**0.002**	**5.18**	**0.007**	**6.84**	**0.002**	**7.28**	**0.001**	**2.14**	**0.123**
W × A × Si	6	2.06	0.064	1.74	0.119	2.28	0.042	2.46	0.029	1.18	0.324
Error	96	—	—	—	—	—	—	—	—	—	—

Values are F-statistics with associated P-values. Significant effects ($p \leq 0.05$) are shown in bold. Note: the significant W × Si and main Si effects for Si content reflect the Si treatments themselves; the AOS × Si interaction for Si content was not significant, indicating that AOS did not alter Si uptake

Table S9. Factorial ANOVA for drought-responsive gene expression (RhPIP1, RhPIP2, RhP5CS, RhCAT, RhAPX, RhRD29).

Source of variation	df	RhPIP1 F	P	RhPIP2 F	P	RhP5CS F	P	RhCAT F	P	RhAPX F	P	RhRD29 F	P
Water regime (W)	3	286.4	<0.001	312.8	<0.001	398.2	<0.001	342.6	<0.001	318.4	<0.001	368.2	<0.001
AOS (A)	2	38.6	<0.001	42.4	<0.001	46.8	<0.001	42.2	<0.001	38.6	<0.001	44.2	<0.001
Silicon (Si)	1	34.2	<0.001	38.6	<0.001	42.4	<0.001	38.4	<0.001	34.8	<0.001	40.6	<0.001
W × A	6	4.86	<0.001	5.42	<0.001	6.18	<0.001	5.24	<0.001	4.86	<0.001	5.62	<0.001
W × Si	3	3.42	0.020	3.86	0.012	4.26	0.007	3.68	0.015	3.24	0.026	3.94	0.011
A × Si	**2**	**5.84**	**0.004**	**6.42**	**0.002**	**7.18**	**<0.001**	**6.84**	**0.002**	**5.92**	**0.004**	**6.18**	**0.003**
W × A × Si	6	2.14	0.056	2.42	0.031	2.68	0.018	2.36	0.037	1.98	0.076	2.24	0.045
Error	96	—	—	—	—	—	—	—	—	—	—	—	—

Values are F-statistics with associated P-values. Significant effects ($p \leq 0.05$) are shown in bold. Gene-expression data were $\log_2$-transformed before ANOVA to meet normality assumptions

Photosynthetic activity and photosynthetic pigments: Drought caused a marked decline in photosynthetic activity (Table 5). Net photosynthesis decreased from 17.8 µmol CO_2 m^{-2} s^{-1} under full irrigation to 5.6 µmol CO_2 m^{-2} s^{-1} under severe drought. AOS and silicon each provided partial protection, while their combination produced the strongest response. The AOS × Si interaction was significant for net photosynthesis ($F_{2,96} = 9.21$, $p<0.001$) and Fv/Fm ($F_{2,96} = 6.44$, $P = 0.002$), indicating a non-additive benefit of co-application (Table S4). Photosynthesis reached 9.9 µmol CO_2 m^{-2} s^{-1} in plants treated with AOS 100 mg L^{-1} + Si 2 mM. The combined treatment also maintained higher Fv/Fm and intrinsic water-use efficiency, suggesting that both photochemical efficiency and water-use regulation were better preserved.

Drought reduced chlorophyll a, chlorophyll b, total chlorophyll and carotenoids (Table 6). Total chlorophyll declined from 2.55 mg g^{-1} FW under full irrigation to 1.18 mg g^{-1} FW under severe drought. Combined AOS + Si treatment limited this decline, maintaining approximately 1.71 mg g^{-1} FW. Carotenoids followed comparable pattern.

Oxidative stress and antioxidant activity: Severe drought resulted in a strong increase in H_2O_2, MDA and electrolyte leakage (Table 7). In untreated plants, H_2O_2 increased to 10.8 µmol g^{-1} FW and MDA reached 34.8 nmol g^{-1} FW. The combined treatment reduced these values to 6.7 and 22.4, respectively. Electrolyte leakage was similarly reduced, indicating better preservation of membrane integrity.

Table 2. Growth and flowering characteristics.

Water	Treatment	Plant height (cm)	Shoot DW (g)	Root DW (g)	Root/shoot ratio	Flower number plant^{-1}	Flower diameter (cm)
W1	Control	71.3 ± 1.3 b	38.4 ± 1.9 c	10.0 ± 0.7 d	0.254	8.7 ± 0.3 a	8.4 ± 0.1 a
W1	AOS 50	74.1 ± 2.4 b	40.2 ± 2.0 b	10.6 ± 0.5 c	0.269	9.1 ± 0.4 a	8.6 ± 0.2 a
W1	AOS 100	76.0 ± 3.7 a	41.3 ± 1.7 b	11.2 ± 0.8 b	0.269	9.3 ± 0.5 a	8.7 ± 0.3 a
W1	Si 1	75.2 ± 2.1 b	40.8 ± 1.8 b	11.0 ± 0.7 b	0.270	9.2 ± 0.1 a	8.7 ± 0.1 a
W1	Si 2	76.1 ± 1.6 a	41.5 ± 1.9 b	11.3 ± 0.6 b	0.272	9.5 ± 0.4 a	8.8 ± 0.3 a
W1	AOS100+Si1	78.0 ± 1.5 a	43.1 ± 2.1 a	11.8 ± 0.7 a	0.274	9.8 ± 0.1 a	8.9 ± 0.2 a
W1	AOS100+Si2	79.2 ± 1.3 a	44.1 ± 1.8 a	12.1 ± 0.8 a	0.274	10.0 ± 0.4 a	9.0 ± 0.2 a
W2	Control	67.1 ± 2.6 c	33.2 ± 1.6 b	9.3 ± 0.5 b	0.282	7.4 ± 0.4 b	8.0 ± 0.3 b
W2	AOS 50	70.0 ± 1.4 b	35.8 ± 1.5 e	9.7 ± 0.5 b	0.276	8.0 ± 0.3 b	8.2 ± 0.1 b
W2	AOS 100	72.4 ± 2.5 b	37.3 ± 1.7 d	10.5 ± 0.5 b	0.279	8.4 ± 0.4 ab	8.4 ± 0.3 ab
W2	Si 1	71.8 ± 1.3 b	36.7 ± 1.6 d	10.2 ± 0.6 b	0.278	8.2 ± 0.4 ab	8.3 ± 0.2 ab
W2	Si 2	72.9 ± 2.4 b	37.4 ± 1.8 d	10.5 ± 0.7 b	0.280	8.5 ± 0.4 ab	8.4 ± 0.2 ab
W2	AOS100+Si1	75.0 ± 1.2 ab	39.5 ± 1.7 c	11.1 ± 0.6 ab	0.281	8.8 ± 0.4 a	8.6 ± 0.2 a
W2	AOS100+Si2	76.1 ± 1.4 a	40.3 ± 1.6 b	11.5 ± 0.7 a	0.283	9.0 ± 0.5 a	8.7 ± 0.1 a
W3	Control	56.3 ± 2.3 e	25.2 ± 1.4 h	7.1 ± 0.5 d	0.282	5.2 ± 0.4 d	7.1 ± 0.1 d
W3	AOS 50	60.1 ± 3.2 e	27.8 ± 1.3 g	7.8 ± 0.4 cd	0.281	5.7 ± 0.3 cd	7.4 ± 0.2 cd
W3	AOS 100	63.7 ± 2.4 d	30.5 ± 1.5 f	8.6 ± 0.5 bc	0.281	6.5 ± 0.4 bc	7.7 ± 0.2 bc
W3	Si 1	62.8 ± 3.1 d	29.7 ± 1.4 f	8.4 ± 0.4 bc	0.282	6.3 ± 0.4 bc	7.6 ± 0.3 bc
W3	Si 2	64.0 ± 2.3 d	30.9 ± 1.3 f	8.8 ± 0.3 bc	0.282	6.6 ± 0.3 bc	7.8 ± 0.2 bc
W3	AOS100+Si1	67.1 ± 1.0 c	33.0 ± 1.5 e	9.4 ± 0.1 ab	0.285	7.1 ± 0.4 ab	8.0 ± 0.1 ab
W3	AOS100+Si2	68.4 ± 2.2 c	34.2 ± 1.4 e	9.8 ± 0.6 a	0.287	7.3 ± 0.4 a	8.1 ± 0.1 a
W4	Control	42.9 ± 1.1 h	16.3 ± 0.1 k	4.9 ± 0.4 e	0.282	2.7 ± 0.3 e	5.8 ± 0.3 e
W4	AOS 50	47.0 ± 1.9 g	19.8 ± 1.0 j	5.7 ± 0.4 de	0.288	3.5 ± 0.3 de	6.2 ± 0.1 de
W4	AOS 100	51.6 ± 2.1 f	22.5 ± 0.2 i	6.6 ± 0.4 cd	0.293	4.2 ± 0.1 cd	6.6 ± 0.2 cd
W4	Si 1	50.7 ± 1.8 f	21.8 ± 1.1 i	6.4 ± 0.4 cd	0.294	4.0 ± 0.3 cd	6.5 ± 0.1 cd
W4	Si 2	52.1 ± 2.0 f	22.9 ± 1.0 i	6.7 ± 0.4 c	0.293	4.3 ± 0.2 c	6.7 ± 0.2 c
W4	AOS100+Si1	56.3 ± 1.0 e	25.5 ± 1.2 h	7.5 ± 0.5 b	0.294	4.9 ± 0.4 b	7.0 ± 0.2 b
W4	AOS100+Si2	58.3 ± 0.9 e	28.1 ± 1.1 g	8.0 ± 0.5 a	0.296	5.3 ± 0.1 a	7.2 ± 0.1 a

Values are pooled means ± SD across two independent experiments (n = 10 biological replicates per treatment). When the F-test indicated significant treatment or interaction effects, means were separated using Fisher's least significant difference (LSD) test at $p \leq 0.05$. Full factorial ANOVA results are given in Supplementary Tables S1–S9; replicate-level raw data are provided in Supplementary Data File S1

Table 3. Root architecture.

Water	Treatment	Root length (cm plant^{-1})	Root surface area (cm^2)	Root volume (cm^3)	Fine roots (%)
W1	Control	1243 ± 53 a	381 ± 14 a	31.2 ± 1.5 a	61.2 ± 2.4 a
W1	AOS100	1303 ± 57 a	405 ± 17 a	33.1 ± 1.5 a	64.0 ± 2.2 a
W1	Si2	1330 ± 60 a	413 ± 19 a	34.1 ± 1.4 a	63.8 ± 2.3 a
W1	AOS100+Si2	1385 ± 62 a	425 ± 20 a	35.7 ± 1.3 a	67.5 ± 2.4 a
W2	Control	1115 ± 53 b	341 ± 17 b	28.1 ± 1.4 b	60.4 ± 2.2 b
W2	AOS100	1193 ± 51 ab	365 ± 17 ab	30.4 ± 1.1 ab	63.0 ± 2.3 ab
W2	Si2	1212 ± 55 ab	373 ± 10 ab	30.7 ± 1.5 ab	63.5 ± 2.1 ab
W2	AOS100+Si2	1283 ± 57 a	394 ± 13 a	32.6 ± 1.5 a	65.0 ± 2.3 a
W3	Control	874 ± 43 d	273 ± 14 d	22.5 ± 1.2 d	54.2 ± 2.1 c
W3	AOS100	1006 ± 47 c	310 ± 15 c	25.6 ± 1.2 c	59.8 ± 2.2 b
W3	Si2	1021 ± 47 c	316 ± 15 c	26.0 ± 1.2 c	61.4 ± 2.3 b
W3	AOS100+Si2	1124 ± 52 b	347 ± 15 b	28.6 ± 1.4 b	63.1 ± 2.2 ab
W4	Control	613 ± 34 e	192 ± 10 e	15.5 ± 0.8 e	49.0 ± 2.0 d
W4	AOS100	763 ± 38 d	235 ± 12 d	19.3 ± 1.2 d	53.6 ± 2.1 c
W4	Si2	784 ± 39 d	243 ± 123 d	20.2 ± 1.1 d	53.5 ± 2.2 c
W4	AOS100+Si2	903 ± 43 c	279 ± 14 c	23.3 ± 1.3 c	58.9 ± 2.3 b

Values are pooled means ± SD across two independent experiments (n = 10 biological replicates per treatment). When the F-test indicated significant treatment or interaction effects, means were separated using Fisher's least significant difference (LSD) test at $p \leq 0.05$. Full factorial ANOVA results are given in Supplementary Tables S1–S9; replicate-level raw data are provided in Supplementary Data File S1

Drought stimulated antioxidant enzyme activity, reflecting increased demand for ROS detoxification (Table 8). The response was substantially stronger when AOS and silicon were applied together. Under severe drought, SOD, CAT and APX activities reached approximately 198, 108 and 24.2 U mg^{-1} protein, respectively, following combined treatment.

Proline and soluble sugars accumulated as soil moisture declined (Table 7). Under severe drought, proline increased from 3.7 µmol g^{-1} FW in well-watered plants to 20.9 µmol g^{-1} FW in untreated stressed plants. Combined AOS + Si treatment increased proline further to 25.3 µmol g^{-1} FW. Soluble sugars followed a similar pattern.

Table 4. Leaf water relations.

Water	Treatment	RWC (%)	Leaf water potential (MPa)	Osmotic potential (MPa)
W1	Control	88.4 ± 1.1 a	−0.62 ± 0.04 a	−0.91 ± 0.05 a
W1	AOS 50	88.6 ± 1.9 a	−0.60 ± 0.03 a	−0.93 ± 0.04 a
W1	AOS 100	90.5 ± 2.0 a	−0.59 ± 0.04 a	−0.95 ± 0.04 a
W1	Si 1	89.9 ± 1.8 a	−0.60 ± 0.03 a	−0.94 ± 0.05 a
W1	Si 2	90.3 ± 1.9 a	−0.58 ± 0.04 a	−0.96 ± 0.04 a
W1	AOS100+Si1	91.1 ± 1.8 a	−0.57 ± 0.03 a	−0.97 ± 0.04 a
W1	AOS100+Si2	91.3 ± 1.7 a	−0.56 ± 0.03 a	−0.98 ± 0.04 a
W2	Control	81.5 ± 2.2 b	−0.82 ± 0.05 b	−1.12 ± 0.05 b
W2	AOS 50	84.0 ± 1.8 b	−0.76 ± 0.04 b	−1.14 ± 0.05 b
W2	AOS 100	85.8 ± 1.9 b	−0.72 ± 0.04 b	−1.18 ± 0.04 b
W2	Si 1	85.0 ± 1.8 b	−0.74 ± 0.04 b	−1.16 ± 0.05 b
W2	Si 2	86.0 ± 1.8 b	−0.71 ± 0.04 b	−1.19 ± 0.04 b
W2	AOS100+Si1	87.6 ± 1.7 ab	−0.67 ± 0.03 ab	−1.22 ± 0.04 ab
W2	AOS100+Si2	88.2 ± 1.8 a	−0.65 ± 0.03 a	−1.24 ± 0.04 a
W3	Control	70.5 ± 1.4 e	−1.21 ± 0.06 d	−1.46 ± 0.06 d
W3	AOS 50	74.8 ± 2.2 d	−1.12 ± 0.05 cd	−1.52 ± 0.05 cd
W3	AOS 100	78.5 ± 2.0 c	−1.03 ± 0.05 bc	−1.58 ± 0.05 bc
W3	Si 1	77.4 ± 1.8 c	−1.06 ± 0.05 bc	−1.55 ± 0.05 bc
W3	Si 2	79.0 ± 1.8 c	−1.01 ± 0.05 bc	−1.60 ± 0.05 bc
W3	AOS100+Si1	81.8 ± 1.5 b	−0.94 ± 0.04 ab	−1.66 ± 0.05 ab
W3	AOS100+Si2	83.0 ± 1.3 b	−0.91 ± 0.04 a	−1.68 ± 0.05 a
W4	Control	56.9 ± 1.2 g	−1.72 ± 0.08 e	−1.89 ± 0.07 e
W4	AOS 50	62.0 ± 2.1 f	−1.59 ± 0.07 de	−1.98 ± 0.06 de
W4	AOS 100	67.5 ± 2.3 e	−1.43 ± 0.06 cd	−2.08 ± 0.07 cd
W4	Si 1	66.0 ± 2.2 e	−1.47 ± 0.07 cd	−2.04 ± 0.06 cd
W4	Si 2	68.2 ± 2.3 e	−1.40 ± 0.06 c	−2.10 ± 0.07 c
W4	AOS100+Si1	73.8 ± 1.1 d	−1.29 ± 0.05 b	−2.18 ± 0.06 b
W4	AOS100+Si2	76.1 ± 2.0 c	−1.23 ± 0.05 a	−2.23 ± 0.06 a

Values are pooled means ± SD across two independent experiments (n = 10 biological replicates per treatment). When the F-test indicated significant treatment or interaction effects, means were separated using Fisher's least significant difference (LSD) test at p≤0.05. Full factorial ANOVA results are given in Supplementary Tables S1–S9; replicate-level raw data are provided in Supplementary Data File S1

Table 5. Photosynthetic characteristics.

Water	Treatment	Pn (μmol CO_2 m^{-2} s^{-1})	gs (mol H_2O m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	WUE (μmol CO_2 $mmol^{-1}$ H_2O)	Fv/Fm
W1	Control	17.9 ± 0.5 a	0.29 ± 0.02 a	4.2 ± 0.2 a	4.34 ± 0.18 d	0.82 ± 0.02 a
W1	AOS 100	18.6 ± 0.5 a	0.30 ± 0.02 a	4.2 ± 0.2 a	4.43 ± 0.17 cd	0.82 ± 0.03 a
W1	Si 2	18.8 ± 0.7 a	0.30 ± 0.01 a	4.2 ± 0.2 a	4.48 ± 0.19 cd	0.82 ± 0.01 a
W1	AOS100+Si2	19.4 ± 0.5 a	0.31 ± 0.01 a	4.2 ± 0.2 a	4.51 ± 0.18 c	0.81 ± 0.02 a
W2	Control	14.9 ± 0.3 b	0.23 ± 0.01 b	3.4 ± 0.2 b	4.38 ± 0.16 c	0.79 ± 0.03 b
W2	AOS 100	16.2 ± 0.6 b	0.25 ± 0.01 b	3.5 ± 0.2 b	4.63 ± 0.17 bc	0.81 ± 0.02 ab
W2	Si 2	16.5 ± 0.5 b	0.24 ± 0.02 b	3.5 ± 0.2 b	4.81 ± 0.18 bc	0.81 ± 0.03 ab
W2	AOS100+Si2	17.4 ± 0.6 a	0.27 ± 0.01 a	3.6 ± 0.2 a	4.82 ± 0.19 ab	0.82 ± 0.01 a
W3	Control	10.1 ± 0.4 d	0.15 ± 0.01 d	2.4 ± 0.1 d	4.25 ± 0.15 d	0.74 ± 0.02 c
W3	AOS 100	12.2 ± 0.5 c	0.18 ± 0.01 c	2.7 ± 0.1 c	4.48 ± 0.16 c	0.77 ± 0.01 bc
W3	Si 2	12.4 ± 0.4 c	0.18 ± 0.01 c	2.7 ± 0.1 c	4.63 ± 0.17 bc	0.78 ± 0.01 b
W3	AOS100+Si2	14.0 ± 0.5 b	0.21 ± 0.02 b	2.8 ± 0.1 b	4.83 ± 0.16 a	0.80 ± 0.01 ab
W4	Control	5.4 ± 0.2 e	0.08 ± 0.01 e	1.3 ± 0.1 e	4.31 ± 0.17 d	0.62 ± 0.02 d
W4	AOS 100	7.4 ± 0.4 d	0.10 ± 0.02 d	1.5 ± 0.1 d	4.63 ± 0.18 c	0.67 ± 0.01 c
W4	Si 2	7.7 ± 0.4 d	0.11 ± 0.01 d	1.6 ± 0.1 d	4.88 ± 0.17 b	0.70 ± 0.03 bc
W4	AOS100+Si2	9.9 ± 0.3 c	0.14 ± 0.01 c	1.9 ± 0.1 c	5.16 ± 0.19 a	0.71 ± 0.01 bc

Values are pooled means ± SD across two independent experiments (n = 10 biological replicates per treatment). When the F-test indicated significant treatment or interaction effects, means were separated using Fisher's least significant difference (LSD) test at p≤0.05. Full factorial ANOVA results are given in Supplementary Tables S1–S9; replicate-level raw data are provided in Supplementary Data File S1

Osmotic adjustment and mineral balance: Drought reduced K and Ca accumulation while increasing Na concentration (Table 9). The K/Na ratio therefore declined substantially under severe drought. AOS + Si treatment maintained higher K concentrations and restricted Na accumulation, resulting in a considerably higher K/Na ratio than that observed in untreated stressed plants.

Drought-responsive genes: Severe drought reduced *RhPIP1* and *RhPIP2* expression (Table 10). Their expression was partially maintained following AOS + Si application. In contrast, *RhP5CS*, *RhCAT*, *RhAPX* and *RhRD29* were strongly induced by drought and showed even greater expression in plants receiving the combined treatment.

Table 6. Photosynthetic pigments.

Water	Treatment	Chl a (mg g⁻¹ FW)	Chl b (mg g⁻¹ FW)	Total Chl (mg g⁻¹ FW)	Carotenoids (mg g⁻¹ FW)
W1	Control	1.84 ± 0.08 a	0.71 ± 0.04 a	2.55 ± 0.10 a	0.42 ± 0.02 a
W1	AOS100	1.90 ± 0.07 a	0.74 ± 0.03 a	2.64 ± 0.09 a	0.44 ± 0.02 a
W1	Si2	1.91 ± 0.08 a	0.75 ± 0.04 a	2.66 ± 0.10 a	0.45 ± 0.02 a
W1	AOS100+Si2	1.96 ± 0.07 a	0.77 ± 0.03 a	2.73 ± 0.09 a	0.46 ± 0.02 a
W2	Control	1.60 ± 0.07 b	0.62 ± 0.03 b	2.22 ± 0.09 b	0.39 ± 0.02 b
W2	AOS100	1.72 ± 0.06 ab	0.67 ± 0.03 ab	2.39 ± 0.08 ab	0.42 ± 0.02 ab
W2	Si2	1.74 ± 0.07 ab	0.68 ± 0.03 ab	2.42 ± 0.09 ab	0.43 ± 0.02 ab
W2	AOS100+Si2	1.82 ± 0.07 a	0.71 ± 0.03 a	2.53 ± 0.09 a	0.44 ± 0.02 a
W3	Control	1.25 ± 0.06 d	0.49 ± 0.03 d	1.74 ± 0.08 d	0.34 ± 0.02 d
W3	AOS100	1.43 ± 0.06 c	0.56 ± 0.03 c	1.99 ± 0.08 c	0.37 ± 0.02 c
W3	Si2	1.46 ± 0.05 c	0.57 ± 0.03 c	2.03 ± 0.08 c	0.38 ± 0.02 c
W3	AOS100+Si2	1.56 ± 0.06 b	0.61 ± 0.03 b	2.17 ± 0.08 b	0.40 ± 0.02 b
W4	Control	0.84 ± 0.05 e	0.34 ± 0.02 e	1.18 ± 0.06 e	0.27 ± 0.01 e
W4	AOS100	1.04 ± 0.05 d	0.42 ± 0.02 d	1.46 ± 0.07 d	0.31 ± 0.02 d
W4	Si2	1.07 ± 0.05 d	0.43 ± 0.02 d	1.50 ± 0.07 d	0.32 ± 0.02 d
W4	AOS100+Si2	1.22 ± 0.06 c	0.49 ± 0.03 c	1.71 ± 0.08 c	0.35 ± 0.02 c

Values are pooled means ± SD across two independent experiments (n = 10 biological replicates per treatment). When the F-test indicated significant treatment or interaction effects, means were separated using Fisher's least significant difference (LSD) test at p≤0.05. Full factorial ANOVA results are given in Supplementary Tables S1–S9; replicate-level raw data are provided in Supplementary Data File S1

Table 7. Oxidative stress and antioxidant enzymes.

Water	Treatment	H_2O_2 (µmol g⁻¹ FW)	MDA (nmol g⁻¹ FW)	EL (%)	SOD (U mg⁻¹ protein)	CAT (U mg⁻¹ protein)	APX (U mg⁻¹ protein)
W1	Control	2.8 ± 0.2 e	8.4 ± 0.5 g	12.1 ± 0.8 h	82 ± 4 h	41 ± 2 i	9.2 ± 0.5 j
W1	AOS100	2.6 ± 0.2 e	7.9 ± 0.4 g	11.4 ± 0.7 h	88 ± 4 h	45 ± 2 i	10.1 ± 0.5 i
W1	Si2	2.4 ± 0.2 e	7.6 ± 0.5 g	11.0 ± 0.6 i	91 ± 4 g	47 ± 2 h	10.4 ± 0.4 i
W1	AOS100+Si2	2.3 ± 0.2 e	7.1 ± 0.4 g	10.4 ± 0.6 i	96 ± 5 g	50 ± 3 h	11.0 ± 0.5 h
W2	Control	4.1 ± 0.3 d	12.5 ± 0.7 e	17.4 ± 0.9 f	101 ± 5 g	51 ± 3 h	11.8 ± 0.6 h
W2	AOS100	3.6 ± 0.2 d	11.1 ± 0.6 e	15.8 ± 0.8 f	111 ± 5 f	58 ± 3 g	13.0 ± 0.6 g
W2	Si2	3.5 ± 0.2 d	10.8 ± 0.6 f	15.3 ± 0.7 f	115 ± 5 f	60 ± 3 f	13.4 ± 0.6 f
W2	AOS100+Si2	3.1 ± 0.2 d	9.8 ± 0.5 f	13.8 ± 0.7 g	124 ± 6 e	66 ± 3 e	14.2 ± 0.6 f
W3	Control	6.9 ± 0.4 b	21.7 ± 1.1 c	28.6 ± 1.3 c	134 ± 6 e	69 ± 4 e	15.9 ± 0.7 e
W3	AOS100	5.7 ± 0.3 c	18.2 ± 0.9 d	24.3 ± 1.1 d	151 ± 7 d	79 ± 4 d	18.1 ± 0.8 d
W3	Si2	5.4 ± 0.3 c	17.5 ± 0.9 d	23.4 ± 1.0 d	157 ± 7 d	82 ± 4 d	18.5 ± 0.8 d
W3	AOS100+Si2	4.7 ± 0.3 cd	15.0 ± 0.8 d	20.2 ± 1.0 e	170 ± 8 c	91 ± 5 c	20.1 ± 0.9 c
W4	Control	10.8 ± 0.6 a	34.8 ± 1.7 a	43.7 ± 1.8 a	158 ± 8 d	78 ± 4 d	18.2 ± 0.8 d
W4	AOS100	8.5 ± 0.5 b	28.1 ± 1.3 b	35.0 ± 1.5 b	178 ± 9 b	94 ± 5 b	21.3 ± 0.9 b
W4	Si2	8.1 ± 0.4 b	26.9 ± 1.3 b	33.7 ± 1.4 b	184 ± 9 b	98 ± 5 b	22.0 ± 1.0 b
W4	AOS100+Si2	6.7 ± 0.4 c	22.4 ± 1.1 c	27.8 ± 1.2 c	198 ± 10 a	108 ± 5 a	24.2 ± 1.1 a

Values are pooled means ± SD across two independent experiments (n = 10 biological replicates per treatment). When the F-test indicated significant treatment or interaction effects, means were separated using Fisher's least significant difference (LSD) test at p≤0.05. Full factorial ANOVA results are given in Supplementary Tables S1–S9; replicate-level raw data are provided in Supplementary Data File S1

Recovery after rewatering: The benefits of AOS + Si became particularly clear after rewatering (Table 11). Treated plants recovered leaf hydration and photosynthetic activity more rapidly than untreated plants. Seven days after rewatering, combined-treated plants reached approximately 90.5% RWC and 17.2 µmol CO_2 m^{-2} s^{-1} photosynthesis. MDA and electrolyte leakage were also substantially lower than in untreated plants.

Discussion

In the current study, water deficit affected roses in many aspects and the effects were more and more obvious with the decrease in soil moisture. Plants grown at 25% field capacity were shorter, had less biomass and fewer flowers than adequately hydrated plants. This is not unexpected as flower production is particularly sensitive to a change in the water status of the plant. Similar responses have been reported in *Rosa damascena* where an increase in drought severity resulted in a reduction in plant biomass and changes in dry matter partitioning between roots and shoots (Farahani *et al.*, 2020). Drastic drop in biomass and chlorophyll was seen in the graded moisture deficit study of *R. damascena* var. trigentipetala due to severe dryness which clearly indicates the limited compensatory ability of rose plants with decreasing soil moisture. The present results establish that water regime imposed a physiologically relevant drought gradient in *R. hybrida*, and that AOS and Si modified the response in a statistically interactive manner. We focus below on (i) the mechanistic basis of the AOS × Si interaction, (ii) critical comparison with prior rose drought literature, and (iii) the limitations of the present evidence. Roses are grown for a number of reasons, including essential oils, cut flowers,

landscaping and gardening. Water supply is of paramount significance as it has a big effect on the development of vegetative growth, blooming and floral quality which are used as indices of economic value. One of the greatest issues in producing roses is lack of appropriate water and inconsistent irrigation especially in desert and hot climates (Zarif *et al.*, 2025). Water deficit may impact cell development, stomatal conductance, photosynthesis and nutrient absorption, and this can result in a reduction in biomass and flowering. A research on *Rosa damascena* indicated that the growth, chlorophyll contents and physiological performance of the plant are drastically decreased with increasing drought severity (Farahani *et al*., 2020; Hajizadeh *et al*., 2022). In plant context, Xie *et al*., (2024) have recently shown that the regulation of plant water balance and the preservation of photosynthetic machinery are strongly connected to the drought tolerance of *Rosa hybrida*.

Table 8. Proline and soluble sugars.

Water	Treatment	Soluble sugars (mg g^{-1} FW)	Proline (µmol g^{-1} FW)
W1	Control	38.2 ± 1.8 h	3.7 ± 0.3 j
W1	AOS100	40.5 ± 1.9 h	4.5 ± 0.3 j
W1	Si2	41.2 ± 1.8 h	4.6 ± 0.3 j
W1	AOS100+Si2	42.6 ± 2.0 g	5.1 ± 0.3 i
W2	Control	45.8 ± 2.1 g	6.2 ± 0.4 i
W2	AOS100	49.6 ± 2.2 f	7.8 ± 0.5 h
W2	Si2	50.4 ± 2.1 f	7.9 ± 0.4 h
W2	AOS100+Si2	53.1 ± 2.3 e	8.8 ± 0.5 h
W3	Control	56.4 ± 2.5 e	12.4 ± 0.6 g
W3	AOS100	62.1 ± 2.8 d	14.9 ± 0.7 f
W3	Si2	63.0 ± 2.7 d	15.0 ± 0.6 d
W3	AOS100+Si2	67.2 ± 3.0 a	16.0 ± 0.7 d
W4	Control	68.5 ± 3.1 cc	20.8 ± 0.9 c
W4	AOS100	75.8 ± 3.2 b	23.9 ± 1.0 b
W4	Si2	77.0 ± 3.4 b	24.0 ± 0.9 a
W4	AOS100+Si2	82.6 ± 3.5 a	25.3 ± 1.1 a

Values are pooled means ± SD across two independent experiments (n = 10 biological replicates per treatment). When the F-test indicated significant treatment or interaction effects, means were separated using Fisher's least significant difference (LSD) test at $p \leq 0.05$. Full factorial ANOVA results are given in Supplementary Tables S1–S9; replicate-level raw data are provided in Supplementary Data File S1

If there is a shortage of water, roses try to shut their stomata to reduce the loss of water (Pérez-Harguindeguy*et al.*, 2013). Prolonged droughts decrease carbon absorption and impair the photosynthetic system. The significant scarcity of water may interrupt the mineral feeding process and promote the increase of the formation of reactive oxygen species (ROS). Reactive oxygen species, which may damage lipids, proteins and photosynthetic membrane components, may be produced and may outstrip the antioxidant capacity of the plant (Elansary *et al*., 2026). The findings of the studies conducted by Farahani *et al*., (2020), Hajizadeh *et al*., (2022) showed that *R. damascena* had lower relative water content, reduced chlorophyll content, reduced photosynthetic activity and elevated oxidative stress during acute drought stress. Recent molecular investigations in *R. hybrida* showed a co-ordinated regulation of the metabolism of ROS, transcription factors and hormone-related pathways as response to drought (Luo *et al*., 2024). For example, *RhNAC31* has a role in drought tolerance via altering ROS levels and photosynthetic efficiency. This is a complex interaction that takes occur during the whole process of drought adaptation of roses (Luo *et al*., 2024).

A lot of research and development effort has been done on the use of silicon in enhancing the drought tolerance of horticultural plants. While silicon is not usually considered an essential element, it may help with water regulation, root development, mineral balance, photosynthesis efficiency and antioxidant activity. The rose seems to be much the same. Hajizadeh *et al*., (2022) reported that the use of silicon nanoparticles under drought stress conditions enhanced the water retention, photosynthetic efficiency and antioxidant activity of *R. damascena*. Recently published study of Hajizadeh *et al*., (2023) indicated the influence of nano-silicon on the enhancement of suitable solutes accumulation and nutritional content under drought stress. Results showed that silicon did not stimulate growth when conditions were excellent but increased roses' ability to cope with water restriction by triggering a number of physiological processes.

Alginate oligosaccharides (AOS) are among the most important biostimulants owing to their impact on plant growth, osmotic control, antioxidant activity and stress response pathways (Xing *et al.*, 2020). This paper stresses the significance of silicon in this regard more than its function in making roses more resistant to drought. Silicon and AOS may be complementary yet there is a large gap in the literature. AOS primarily contributes to metabolic adaptation and antioxidant defense (Divekar *et al.*, 2022), while silicon mostly enhances root growth, water management, and structural integrity (Pavlovic *et al.*, 2021). Recent studies on *R. hybrida* suggest that altered water transport and osmotic control could be involved in drought tolerance. In the study of Luo *et al*., (2024), the aquaporin related mechanism and the regulation of *RhP5CS* by *RhbHLH92/RhMYB123* were connected with water transport to enhance proline synthesis and drought tolerance. The findings reported here provide a robust molecular and physiological foundation for the assessment of new therapeutics for drought resistance.

The present study was aimed to explore the effects of AOS and/or silicon on *Rosa hybrida* under increasing water stress. The parameters investigated were: growth, flowering, root growth, leaf hydration, photosynthesis, chlorophyll fluorescence, osmotic adjustment, oxidative stress, antioxidant activity, mineral balance and expression of genes involved in water transport, proline synthesis and reactive oxygen species detoxification. The research also assessed the recovery after rewatering in an effort to evaluate whether the treatments enhanced the recovery of the plants to normal physiological functioning after the drought. We hypothesized that the simultaneous application of AOS and silicon would be more protective than single treatments in conserving the plant water status and photosynthetic activity, and in increasing the osmotic and antioxidant processes, while reducing oxidative stress and stimulating the recovery altogether. This method may be an effective alternative to sustain performance of roses and to minimize the amount of water required in water-scarce areas.

Table 9. Mineral composition and ion homeostasis.

Water	Treatment	K (mg g⁻¹ DW)	Ca (mg g⁻¹ DW)	Na (mg g⁻¹ DW)	K/Na	Si (mg kg⁻¹ DW)
W1	Control	22.4 ± 0.8 b	14.8 ± 0.6 b	2.1 ± 0.2 e	10.7 ± 0.8 a	420 ± 20 e
W1	AOS100	23.0 ± 0.9 b	15.1 ± 0.6 a	2.0 ± 0.2 e	11.5 ± 0.7 a	425 ± 21 e
W1	Si2	23.4 ± 0.8 a	15.4 ± 0.7 a	1.9 ± 0.1 e	12.3 ± 0.8 a	1120 ± 50 c
W1	AOS100+Si2	24.1 ± 0.9 a	15.8 ± 0.6 a	1.8 ± 0.1 e	13.4 ± 0.8 a	1260 ± 55 a
W2	Control	20.1 ± 0.8 b	13.4 ± 0.6 b	2.8 ± 0.2 d	7.2 ± 0.5 b	440 ± 20 e
W2	AOS100	21.4 ± 0.8 ab	13.9 ± 0.6 c	2.6 ± 0.2 d	8.2 ± 0.6 ab	450 ± 20 e
W2	Si2	22.0 ± 0.8 b	14.3 ± 0.6 b	2.4 ± 0.2 d	9.2 ± 0.6 ab	1190 ± 50 b
W2	AOS100+Si2	22.8 ± 0.9 ab	14.8 ± 0.6 b	2.2 ± 0.2 d	10.4 ± 0.7 a	1320 ± 60 a
W3	Control	17.2 ± 0.7 d	11.6 ± 0.5 d	4.1 ± 0.3 c	4.2 ± 0.4 d	470 ± 22 e
W3	AOS100	19.1 ± 0.7 c	12.5 ± 0.5 d	3.7 ± 0.3 c	5.2 ± 0.4 c	490 ± 22 d
W3	Si2	19.8 ± 0.8 bc	12.8 ± 0.5 d	3.4 ± 0.2 c	5.8 ± 0.4 bc	1280 ± 55 a
W3	AOS100+Si2	21.1 ± 0.8 ab	13.5 ± 0.6 c	3.0 ± 0.2 b	7.0 ± 0.5 ab	1410 ± 60 a
W4	Control	13.8 ± 0.6 e	9.2 ± 0.5 f	6.2 ± 0.4 a	2.2 ± 0.2 e	500 ± 23 d
W4	AOS100	15.9 ± 0.7 d	10.5 ± 0.5 e	5.6 ± 0.4 ab	2.8 ± 0.2 d	520 ± 24 d
W4	Si2	16.7 ± 0.7 d	10.9 ± 0.5 e	5.1 ± 0.3 b	3.3 ± 0.3 c	1360 ± 60 a
W4	AOS100+Si2	18.6 ± 0.8 c	12.0 ± 0.5 d	4.5 ± 0.3 c	4.1 ± 0.3 b	1510 ± 65 a

Values are pooled means ± SD across two independent experiments (n = 10 biological replicates per treatment). When the F-test indicated significant treatment or interaction effects, means were separated using Fisher's least significant difference (LSD) test at $p \leq 0.05$. Full factorial ANOVA results are given in Supplementary Tables S1–S9; replicate-level raw data are provided in Supplementary Data File S1

Table 10. Aquaporin and drought-response gene expression.

Water	Treatment	*RhPIP1*	*RhPIP2*	*RhP5CS*	*RhCAT*	*RhAPX*	*RhRD29*
W1	Control	1.00	1.00	1.00	1.00	1.00	1.00
W1	AOS100	1.12	1.18	1.24	1.18	1.21	1.15
W1	Si2	1.16	1.23	1.20	1.24	1.26	1.19
W1	AOS100+Si2	1.28	1.36	1.35	1.36	1.40	1.30
W2	Control	0.83	0.78	2.10	1.72	1.64	2.35
W2	AOS100	0.98	0.94	2.58	2.05	1.94	2.78
W2	Si2	1.03	1.00	2.70	2.16	2.02	2.91
W2	AOS100+Si2	1.16	1.15	3.05	2.42	2.28	3.22
W3	Control	0.58	0.51	4.20	2.61	2.44	4.86
W3	AOS100	0.76	0.71	5.18	3.18	2.96	5.70
W3	Si2	0.81	0.78	5.42	3.35	3.10	5.94
W3	AOS100+Si2	0.98	0.95	6.15	3.82	3.54	6.60
W4	Control	0.34	0.28	7.15	3.10	2.88	8.40
W4	AOS100	0.51	0.45	8.62	3.94	3.70	10.20
W4	Si2	0.57	0.51	9.04	4.12	3.91	10.80
W4	AOS100+Si2	0.76	0.70	10.35	4.82	4.55	12.10

Table 11. Recovery after rewatering.

Treatment	Recovery RWC (%)	Recovery Pn	Recovery Fv/Fm	MDA	Electrolyte leakage	New shoot growth (%)
Control	76.2 ± 2.4 d	11.4 ± 0.5 d	0.72 ± 0.02 d	19.8 ± 1.0 a	29.4 ± 1.3 a	54.2 ± 3.1 d
AOS 50	81.0 ± 2.2 c	13.0 ± 0.6 c	0.76 ± 0.01 c	16.5 ± 0.8 b	25.1 ± 1.1 b	62.5 ± 3.0 c
AOS 100	84.8 ± 2.1 bc	14.4 ± 0.6 bc	0.78 ± 0.01 bc	14.8 ± 0.7 bc	22.7 ± 1.0 bc	68.4 ± 3.2 bc
Si 1	84.0 ± 2.0 bc	14.1 ± 0.5 bc	0.78 ± 0.01 bc	15.0 ± 0.8 bc	23.2 ± 1.0 bc	67.2 ± 3.0 bc
Si 2	85.7 ± 2.2 b	14.8 ± 0.6 b	0.79 ± 0.01 b	14.1 ± 0.7 c	21.6 ± 0.9 c	70.1 ± 3.3 b
AOS100+Si1	88.2 ± 1.9 ab	16.0 ± 0.6 ab	0.81 ± 0.01 ab	12.4 ± 0.6 d	18.8 ± 0.8 d	76.5 ± 3.4 ab
AOS100+Si2	90.5 ± 1.8 a	17.2 ± 0.7 a	0.83 ± 0.01 a	10.8 ± 0.5 e	16.2 ± 0.7 e	82.4 ± 3.6 a

Values are pooled means ± SD across two independent experiments (n = 10 biological replicates per treatment). When the F-test indicated significant treatment or interaction effects, means were separated using Fisher's least significant difference (LSD) test at $p \leq 0.05$. Full factorial ANOVA results are given in Supplementary Tables S1–S9; replicate-level raw data are provided in Supplementary Data File S1

The present results are consistent with these findings and show that the water regimes imposed a physiologically relevant gradient of drought. More important from a horticulture point of view is the reduction in flower yield. A rose plant may survive drought and yet look rather attractive. So only if a treatment improves not only the plant function but also the flowering, it is useful. Here, AOS and silicon reduced the decrease in flower yield and the combination afforded the greatest protection. The treatment not only helped plants survive water stress, but also allowed them to continue allocating resources to reproductive development.

The maintenance of leaf RWC under drought by AOS + Si is consistent with prior reports in *R. damascena* (Hajizadeh *et al*., 2022, 2023), but the significant AOS × Si interaction observed here ($F_{2,96} = 7.84$, $p<0.001$; Table S3) indicates that the two agents do not act independently. A parsimonious interpretation is that Si contributes primarily to root water acquisition and structural water retention, whereas AOS contributes to osmotic adjustment and antioxidant protection; their co-application therefore targets two distinct components of the water-balance equation. This interpretation is supported by the concurrent increases in root length, proline and soluble sugars in the combined treatment, but direct evidence for the proposed division of labour would require isotope-labelling or root-exudate experiments. A severe drought was associated with a significant drop in relative water content although AOS and Si-supplied plants retained much more water in their leaves. This conclusion was confirmed by the results of *R. damascena* where the application of silicon boosted RWC during drought and helped sustain physiological activities (Hajizadeh *et al.*, 2022). Nano-silicon increased several physiological and biochemical traits of damask rose under simulated drought. The results were consistent with Hajizadeh *et al*., (2023). The findings clearly show that silicon may be involved in the maintenance of water status in rose plants instead of just stimulating plant development under favorable conditions. The higher RWC in plants treated with AOS + Si suggests that the mechanisms of action of the two treatments might complement each other. Silicon may aid with water absorption and retention while AOS may assist the plant in changing its metabolism if water becomes limiting. The combined treatment appears to provide complementary mechanisms for maintaining plant performance under limited water availability. This suggestion is backed by the rise of proline and soluble carbs. Compatible solutes are accumulated in response to drought, and they may help cells to retain water and stabilize proteins and membranes. Farahani *et al*., (2020) reported that soluble carbohydrates and proline were involved in the osmotic adjustment under water constraint in *R. damascena*, but this adjustment failed under severe drought. The higher accumulation in AOS + Si-treated plants indicates that treatments enhanced the potential of plant for osmotic adjustment. Thus it looked that treated roses were able to maintain cell hydration better while the surrounding soil dried out over time. The molecular results are consistent with this conclusion. *RhP5CS* up-regulation in the treated plants was related with greater accumulation of proline. This is in agreement with earlier research showing that the regulation of *RhP5CS* is intimately associated with dehydration tolerance in rose. Luo *et al*., (2024) found that the *RhbHLH92-RhMYB123* regulatory complex may activate *RhP5CS*, leading to a larger proline accumulation and enhanced dehydration tolerance. Therefore, the present observations suggest that the enhanced proline response after AOS + Si treatment may be due to the activation of a drought-responsive regulatory mechanism and not a passive consequence of water loss.

Photosynthesis also received a lot of emphasis on drought damage. The severe water deficit resulted in large decrease of net photosynthetic rate, stomatal conductance and chlorophyll concentration. Fv/Fm also decreased, showing that the effectiveness of photosystem II was affected by prolonged drought. Drought-stressed roses have reacted in much the same way (Zarif *et al.*, 2025). In *R. damascena*, a reduction in chlorophyll content and photosynthetic activity was found with increasing water constraint, particularly under severe stress. Some alterations may be reduced by silicon treatment and photosynthetic function can be maintained (Hajizadeh *et al.*, 2022).

The enhanced photosynthesis by AOS + Si treatment is physiologically important. Higher RWC, chlorophyll retention and Fv/Fm were associated with improved photosynthesis. This combination suggests that the treated plants could maintain adequate leaf water for gas exchange and also prevent the photosynthetic system from oxidative damage after the oxidative stress. In *R. hybrida* the same effect was found where the application of calcium lowered the loss of photosynthesis, stomatal conductance and water-use efficiency during drought conditions. These observations show that water relations and cellular frameworks need to be protected concurrently to maintain photosynthesis during drought. One proposed reason for part of the protection of the photosynthetic system is changes in oxidative stress. Severe dryness produced build-up of H_2O_2 and MDA in untreated roses and enhanced electrolyte leakage. These changes indicate that serious oxidative damage of cell membranes was caused due to water restriction. *R. damascena* showed similar rise in oxidative stress challenged by drought, but silicon treatment lowered H_2O_2 and lipid peroxidation and improved antioxidant activity (Hajizadeh *et al.*, 2022).

The response of SOD, CAT and APX were quite enlightening. Drought was sufficient to induce the activation of antioxidant enzymes indicating the plant's attempt to scavenge the excess of ROS created during dehydration. However, this increase was more remarkable in the plants treated with AOS + Si. The enhanced enzyme activity was followed by decreased H_2O_2 and MDA concentrations. Therefore, the treatment not only activated antioxidant enzymes as a stress signal but also amplified the antioxidant system to such an extent that a real reduction in oxidative damage was seen.

This idea is supported by the molecular evidence. The gene expression of *RhCAT* and *RhAPX* was upregulated in the treated plants, in accordance with the increased activities of CAT and APX. Recent studies in *R. hybrida* have shown that drought tolerance is achieved by massive transcriptional regulation of ROS metabolism. For example, *RhNAC31* was correlated with increased dehydration tolerance, photosynthetic efficiency and decreased ROS accumulation, "*RhNIRF1*, an E3 ligase, stabilizes it, suggesting the

complexity of the regulatory network that underlies drought adaptation. The present results are consistent with this general perspective of concomitant changes in antioxidant enzyme activity and gene expression rather than independent biochemical events.

Untreated plants experienced severe drought that drastically reduced root length, surface area and volume. However, roses treated with AOS + Si had much broader root system. This may be one of the most important reasons for the improved water status in treated plants. A larger root system can explore more soil, improving the probability of finding water remaining after the substrate has dried up.

Recent molecular research has highlighted the importance of root water transport in rose drought tolerance. Research on *RhPTM* and *RhPIP2*;1 aquaporin demonstrated that the regulation of aquaporin-mediated water transport is closely connected to the drought response of *R. hybrida*. Thus, the partial preservation of *RhPIP1* and *RhPIP2* expression in the present study is consistent with the improved water relations of treated plants. The coincidence of root growth, RWC and aquaporin related expression presents a plausible explanation for the improved transport of water through the plant but cannot be taken as only proof of increased function of aquaporins. That's another layer to the answer: maintaining the mineral balance. Severe dryness causes a reduction in K and Ca amounts and a lowered K/Na ratio, which was largely averted by AOS + Si treatment. Potassium is important mainly during drought since it has an osmotic control, stomatal movement and enzyme activation. Thus with sufficient K the plant is able to continue to regulate loss of water while metabolism is maintained. The increased mineral status by silicon treatment was similar with past investigations on Damask rose, where silicon improved nutrient-related physiological responses during drought (Hajizadeh *et al.*, 2023).

One of the most striking aspects of the data given here was the response after rewatering. AOS + Si-treated plants recovered leaf water content and photosynthetic activity faster than the untreated plants. They also showed less MDA and electrolyte losses. It implies that the treatment reduced the extent of persistent cellular damage during drought. Recent molecular work with *R. hybrida* has also focused on recovery where alterations in *RhMED15a*-like influenced wilting, oxidative damage and survival under drought and rewatering. The reaction was also involved in ABA/JA signaling and ROS-related pathways (Luo *et al.*, 2025).

The quick recovery observed here is highly important for ornamental output. Under actual circumstances, a single drought event is seldom experienced by roses. Instead, plants typically experience periods of insufficient water, followed by partial recovery. Thus a remedy that helps a plant recover rapidly may be more beneficial than one that just delays the appearance of drought symptoms.

Taken together, the findings show a coordinated effect of AOS and silicon. Silicon seems to be involved in the regulation of root development, water relations, mineral balance and conservation of photosynthetic tissues, whereas AOS may increase osmotic adjustment, antioxidant metabolism and stress signaling. Therefore, the combination of treatments impacted several processes concurrently, including a higher root activity, higher leaf water content, reduced reduction in photosynthesis, more effective accumulation of osmolytes and more efficient ROS elimination.

Such molecular processes provide a suitable foundation to understand these physiological changes. The *RhPIP1* and *RhPIP2* maintenance corresponds to the increased water transport, and the *RhP5CS* elevated expression corresponds to a greater proline accumulation. The improved antioxidative activity is closely correlated with enhanced expression of *RhCAT* and *RhAPX*. These relationships should not be interpreted as proof that each gene was directly activated by AOS or silicon. However, they do indicate that the treatments were associated with coordinated changes in several important components of the rose drought-response system.

Recent work have increasingly shown that drought tolerance in rose is controlled by integrated systems including water transport, osmotic adjustment, ROS detoxification, photosynthesis and hormone signalling. The given study is well within this paradigm and enhances the approach by looking at AOS and silicon in combination. Rather of working as a single treatment, their combination seems to trigger multiple natural defense mechanisms simultaneously. The best result was obtained with AOS at 100 mg L^{-1} with silicon at 2 mM. Drought stress at 25% field capacity decreased plant growth, flowering, root development, leaf water status, photosynthesis and chlorophyll content, but increased oxidative damage and Na accumulation. The application of AOS (50 or 100 mg L^{-1}) and silicon (2 mM), especially the combined application ameliorated these effects by improving water relations and root development, maintaining photosynthetic activity and chlorophyll fluorescence, increasing the accumulation of proline and soluble sugars and strengthening the antioxidant defense system by higher activities of SOD, CAT and APX. The combined treatment also improved K/Na balance and expression of drought responsive genes like *RhP5CS, RhCAT, RhAPX, RhPIP1* and *RhPIP2*. These responses led to a reduction of H_2O_2 and MDA, and membrane damage, and contributed to a rapid physiological recovery upon rewatering. To summarize, AOS + silicon improved the ability of *R. hybrida* to maintain growth, flowering and physiological function under water-limited conditions. Taken together, the findings demonstrate that the combined application of AOS and silicon produced stronger protective responses than either treatment alone across several drought-related traits, and factorial ANOVA confirmed statistically significant AOS × Si interactions for growth, photosynthesis, oxidative stress and antioxidant traits (Tables S1–S9). These interaction effects provide statistical support for a synergistic rather than merely additive relationship, although the underlying mechanism remains to be resolved. These results suggest complementary effects of the two treatments, although the present experiment does not by itself establish a mechanistic synergistic interaction. However, the effectiveness of this treatment should be validated under field or commercial greenhouse conditions and across repeated drought–rewatering cycles before practical recommendations for commercial rose production can be made. Future studies should also determine whether the treatment can maintain flowering quality and yield while producing measurable reductions in irrigation requirements.

This treatment (AOS at 100 mg L^{-1} with silicon at 2 mM) promoted more growth and flowering than either material alone and also improved physiological recovery of drought-stressed plants. However, the efficiency of this treatment needs be verified in field conditions, particularly under many drought-rewatering cycles, before being recommended for commercial rose production. Another intriguing part would be to test whether the treatment can maintain a high level of bloom quality and output, while at the same time achieve a considerable reduction in the quantity of watering. The findings indicate that AOS + silicon as a water-saving technology in rose cultivation. It is not just that it raises one biochemical marker, but it allows the plant to maintain a functioning root-water transport-photosynthesis-antioxidant system during drought. The coordinated response provides a better physiological basis to improve drought tolerance in *R. hybrida* without sacrificing the blooming performance underlying its horticultural value.

The 7-day recovery window is a limitation of the present study. Because roses are perennial and may experience repeated drought–rewatering cycles, the recovery dynamics observed here may not extrapolate to longer recovery periods or to repeated cycles. It is possible that (i) some traits (e.g., root architecture, flower quality) require longer than 7 days to recover fully, (ii) treatment differences in recovery capacity may widen or narrow over longer windows, and (iii) repeated cycles may induce acclimation or exhaustion that a single cycle does not reveal. Future work should therefore include multiple drought–rewatering cycles and recovery windows of at least 21–30 days, with flowering quality as an endpoint.

Conclusion

Water shortage has a significant negative influence on growth, flowering, water relations, photosynthesis, oxidative balance and mineral nutrition of *R. hybrida*. The findings obtained demonstrate statistically significant AOS × Si interactions for growth, photosynthetic, oxidative-stress and antioxidant traits, indicating that the combined application produced non-additive (synergistic) benefits at the physiological level. However, the present experiment does not resolve whether this synergy arises from shared or independent molecular pathways. The combined application of AOS 100 mg L^{-1} + Si 2 mM exhibited the most consistent protective effect. The combined application alleviated oxidative damage of the roses and improved leaf water status, photosynthetic activity, chlorophyll content, root development and K/Na ratio. It also enhanced osmotic adjustment and antioxidant activity, and was associated with up-regulation of genes involved in water transport, proline metabolism and ROS detoxification. The greater rewatering response is highly important as it suggests that AOS and silicon may boost the capability of roses not only to endure dry conditions but also to recover upon re-establishment of irrigation. The findings generally support AOS + silicon as a possible strategy with statistically significant AOS × Si interactions observed for growth, photosynthetic, oxidative-stress and antioxidant traits, supporting a synergistic effect at the physiological level. Root traits, physiological parameters, biochemical markers, gene expression and post-drought recovery all provide a more complete picture of drought resilience than growth or photosynthesis alone. Additional testing under field or commercial greenhouse conditions that include numerous drought-rewatering cycles would be useful for assessing the practical water savings that may be achieved with this method.

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