Research article

Morphophysiological and anatomical responses of rice to water deficit stress after silicon seed priming

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Article Info

Abstract

Importance of the work: Silicon acts as a priming agent that enhances plant tolerance to water deficit stress.

Objective: To analyze the interactive effect of sodium metasilicate concentrations and field capacity levels on the morphophysiological and anatomical responses of rice (*Oryza sativa* L. 'Inpari 24 Gabusan') under drought stress.

Materials and Methods: A completely randomized design with three replications was applied using four silicon priming concentrations (0, 20, 40, 60 mM) and three field capacities (100%, 75%, 50%). Growth, pigment content, stress biomarkers and stomatal characters were analyzed at 42 d after transplanting.

Results: Significant interaction effects were observed between silicon concentration and field capacity for all measured parameters. The combination of 60 Mm Na_2SiO_3 and 100% or 75% field capacity enhanced plant height, root length, chlorophyll content and membrane stability, while reducing malondialdehyde and proline accumulation. In contrast, under 50% field capacity, the benefits of silicon were reduced but remained superior to the untreated control. Stomatal density and aperture size peaked with 60 Mm Na_2SiO_3 at 100% field capacity, contributing to optimized water use and gas exchange.

Main finding: Silicon priming at 60 Mm Na_2SiO_3 improved drought resistance in rice, primarily through a synergistic effect with higher soil moisture levels. The results from this study should provide valuable insights for developing rice varieties with improved drought resistance.

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Introduction

Rice productivity in Indonesia faces increasing challenges due to climate change, particularly prolonged drought and extreme temperatures, which significantly impact the growth of rice (*Oryza sativa* L.) (Ramadhan, 2024). As a water deficit sensitive crop, rice experiences reduced vigor and yield under water stress, threatening national food security (Naylor et al., 2007).

'Inpari 24 Gabusan', a local rice cultivar from Yogyakarta, is valued for its high nutritional content and short cultivation cycle, making it a promising candidate for climate-resilient agriculture (Romdon et al., 2014). Drought tolerance has been explored using seed priming, particularly with silicon, as a strategy to improve plant resilience and growth (Mauad et al., 2016; Koentjoro et al., 2022).

Silicon, an abundant element in soil, plays a critical role in various physiological processes in plants. For example, silicon enhances resilience to drought by strengthening cell walls, improving nutrient uptake and enhancing the antioxidant defenses to oxidative stress (Ali et al., 2021). In addition, it minimizes cell membrane damage and improves water use efficiency, which is particularly beneficial under limited water availability (Pereira et al., 2021; Hernandez-Apaolaza, 2022). To further explore the potential of 'Inpari 24 Gabusan' rice in managing water deficit stress, this study aimed to evaluate the effects of priming with sodium metasilicate on morphophysiological and anatomical responses. Silicon is a potential element to enhance plant resistance to drought stress. The focus was to assess the role of silicon priming in improving drought tolerance under varying levels of water availability.

Materials and Methods

Preparation of priming solutions and field capacity

The rice seeds (*O. sativa* L. 'Inpari 24 Gabusan') were obtained from the Ngudi Makmur II Farmer Group in Yogyakarta, Indonesia. Sodium metasilicate (Na_2SiO_3, from Sigma-Aldrich, Germany) was dissolved in distilled water to prepare a 60 mM stock solution, which was further diluted to obtain 40 mM and 20 mM concentrations. Seeds were primed by soaking in each solution for 8 hr, followed by air drying and pre-germination. Soil field capacity was determined following the gravimetric method described by FAO (1990), using a loamy soil medium. To calculate field capacity, oven dried soil samples were first saturated and then allowed to drain until no more water dripped from the bottom (field capacity moisture content, FCMC). The air dry moisture content (ADMC) was determined by measuring the weight of naturally dried soil. The difference between FCMC and ADMC was used to calculate the soil water holding capacity. Based on this, three water regimes were established to represent 100%, 75% and 50% field capacity. These corresponded to 800 mL, 600 mL and 400 mL of water per 3 kg of soil in each polybag, respectively. Soil moisture was maintained by weighing each pot daily and replenishing water to the designated capacity.

Planting of samples

The experiment was conducted in a greenhouse at Sawitsari Station I Yogyakarta, Indonesia under natural light conditions. During the experimental period, the average temperature was 28°C. Uniform seedlings were selected and transplanted into 20 cm × 25 cm polybags filled with 3 kg of loamy soil. A completely randomized design was used with two treatment factors: sodium metasilicate (Na_2SiO_3) concentrations (0, 20, 40 or 60 mM) and field capacity levels (100%, 75% or 50%). The experiment consisted of 12 treatment combinations, each with three replications. Water was applied daily to maintain soil moisture at designated field capacity levels (800 ml, 600 ml or 400 ml per 3 kg soil).

Observation of morphophysiological responses

Growth parameters were measured at 42 d after transplanting (DAT). Plant height and root length were measured using a ruler and recorded in centimeters. Biomass was determined by separating roots and shoots, blotting to remove surface moisture and weighing them separately using an analytical balance. The results were recorded as fresh weight (grams FW), then after oven-drying at 70°C for 72 hr, as dry weight (grams DW). Physiological characters (chlorophyll and carotenoid content) were analyzed using a spectrophotometer (Genesys 10S UV-Visible; Thermo Scientific; Madison Winconsin; USA) at 470, 645 and 664 nm (Holden, 1965) and expressed in milligrams per gram FW. The proline content (expressed in $\mu mol.g^{-1}$ FW) was determined following the method of Bates et al. (1973) based on the absorbance measured at 520 nm using a spectrophotometer (Genesys 10S UV-Visible; Thermo Scientific; Madison Winconsin; USA) and a proline standard curve (Equation 1):

Proline content = (Proline × Toluene / 115.13) / FW   (1)

where the proline is measured in $\mu mol.g_{-1}$ FW, the toluene in milliliters and 115.13 represents the molecular weight of proline.

The membrane stability index (MSI) as a percentage was measured following Swapna and Shylaraj (2017) and calculated using Equation 2:

MSI = (1 – ICV × FCV) × 100   (2)

where ICV is the initial conductivity value after incubation at 40°C and FCV is the final conductivity value after boiling at 100°C, both measured using a digital electrical conductivity meter (EC Meter CM-21P; TOA corp; Japan). The malondialdehyde (MDA) levels were analyzed using a thiobarbituric acid (TBA) reaction at 532 nm, based on the method of Hodges et al. (1999).

Stomatal density and size of stomatal aperture

The stomatal density and the stomatal aperture size were evaluated on the third leaf of the three plants for each treatment. Nail polish as liquid replica was applied to the abaxial side of the leaves and allowed to dry completely. Subsequently, the replica was gently peeled off using forceps and placed on the slide to ensure imprints from the surface were on the upper side. One drop of water was added for proper spreading of the replica and then covered with cover slip. The number of stomata and the aperture size were observed under a microscope (BM-180/SP; Boeckel + Co.; Hamburg, Germany) using the Miconos software (version 1.0; Optimos LLC; Pheonix, AZ, USA) at magnifications of 60× and 100×, respectively. The number of stomata present in the microscopic view field was recorded for calculating the stomatal density expressed in terms of stomata per square millimeter (Sakoda et al., 2020).

Data Analysis

Data were analyzed using analysis of variance (ANOVA) followed by Duncan's multiple range test to assess significant differences between treatments means. The analysis was performed using SPSS software (version 26.0; IBM Corp.; Armonk, NY, USA). Correlation analysis between parameters was conducted based on Pearson's correlation coefficient (r) in (GraphPad; Boston, MA, USA) and visualized using a correlation matrix withof -1 (perfect negative correlation),

0 (no correlation) and +1 (perfect positive correlation). According to Evans (1996) the degree of correlation between parameters : very strong (> 0.80), strong (0.60–0.79), moderate (0.40–0.59), weak (0.20–0.39) and very week (< 0.20). All results were considered significant at $p < 0.05$

Results

Growth attributes

Silicon priming with Na_2SiO_3 mitigated the deleterious effects of drought, particularly in plant height and root length (Fig. 1 and Fig. 9, respectively). The two way ANOVA indicated significant interaction effects between silicon concentration and field capacity on key growth parameters (plant height, root length, shoot and root biomass). Therefore, the results were presented and interpreted based on treatment combinations rather than single-factor comparisons. Higher silicon concentrations resulted in greater growth across all drought levels, with 60 mM producing the greatest increases in plant height under optimal, moderate and severe drought by 12.5% of S0, 13.52% of S0 and 9.37% of S0, respectively, whereas the increases in root length were 117.85% of S0, 65.53% of S0 and 53.52% of S0, under optimal, moderate and severe drought, respectively. Fig. 2 shows the enhanced root-to-shoot fresh and dry weight ratios, particularly under severe drought.
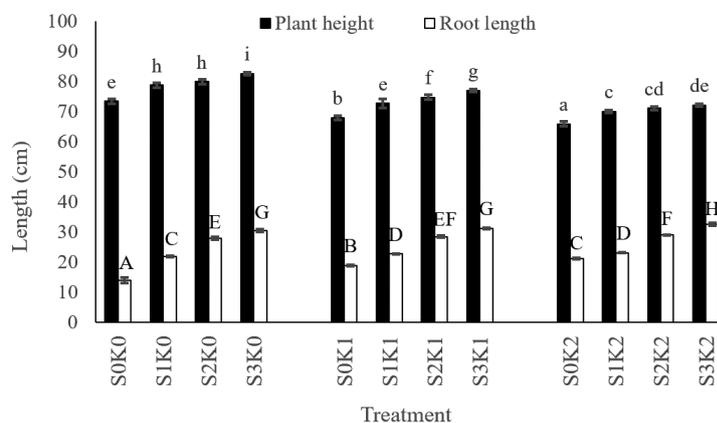

Fig. 1 Plant height and root length values of rice 'Inpari 24 Gabusan' at 42 d after transplanting with different levels of silicon priming concentration and field capacity.S0 = sodium metasilicate at 0 mM, S1 = at 20 mM, S2 = at 40 mM and S3 = at 60 Mm, K0 = field capacity at 100%, K1 = at 75% and K2 = at 50%. Bars represent means (n = 3) and error bars represent ±SD. Different lowercase (plant height) or uppercase (root length) letters above bars indicate significant ($p < 0.05$) differences among meansbased on Duncan's multiple range test
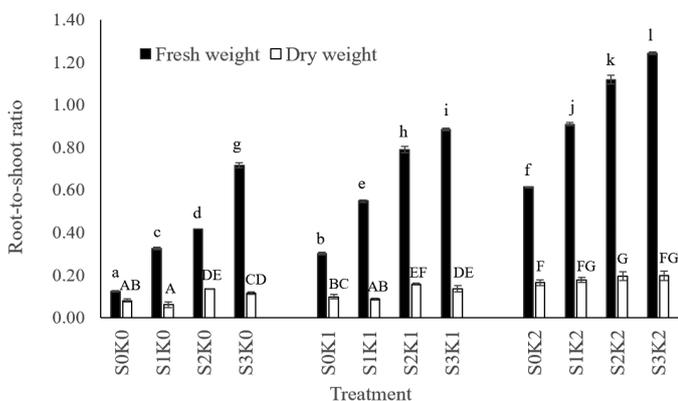
Fig. 2 Root-to-shoot ratios for fresh and dry weight of rice 'Inpari 24 Gabusan' at 42 d after transplanting with different levels of silicon priming concentration and field capacity. S0 = sodium metasilicate at 0 mM, S1 = at 20 mM, S2 = at 40 mM and S3 = at 60 Mm, K0 = field capacity at 100%, K1 = at 75% and K2 = at 50%. Bars represent means and error bars represent ± SD (n = 3). Different lowercase (fresh weight) or uppercase (dry weight) letters above bars indicate significant ($p < 0.05$) differences among means based on Duncan's multiple range test.

The increased root-to-shoot FW ratio suggested that silicon priming improved the root structure by enhancing water and nutrient absorption capacity. Increasing concentrations of silicon led to greater root-to-shoot FW ratios across all field capacity levels, with 60 mM Na_2SiO_3 producing the greatest improvements. At 100% field capacity, the ratio increased from 0.13 (0 mM) to 0.72 (60 mM), at 75% from 0.30 to 0.89 and at 50% from 0.61 to 1.25. These findings indicated a substantial shift in biomass allocation toward the roots under silicon priming, particularly under moderate and severe drought. Enhanced root development relative to shoot biomass suggests improved adaptive capacity for water and nutrient uptake under stress conditions. Based on these results, silicon not only alleviated drought-induced biomass reduction but also enhanced biomass production beyond the control levels in all water regimes.

Physiological responses

Drought stress reduced the total chlorophyll and carotenoid contents, particularly at 50% field capacity; however, silicon priming maintained higher pigment levels, improving photosynthetic efficiency (Table 1). At 100% field capacity, 60 Mm resulted in 3.36 mg/g FW, up from 1.95 mg/g FW in 0 Mm (+72.3%). At 75%, chlorophyll increased from 1.74 mg/g FW to 2.40 mg/g FW (+37.93%) and at 50%, from 1.68 mg/g FW to 2.38 mg/g FW (+41.67%). The carotenoid content followed a similar pattern. Under 100% field capacity, levels rose from 0.51 mg/g FW (0 Mm) to 0.61 mg/g FW (60 Mm; +19.6%). At 75% field capacity, the carotenoid contents increased by +46.81% and at 50% field capacity, by +22.2%. Proline accumulation, an indicator of osmotic stress, decreased with increasing silicon, dropping from 0.12 mg/g FW (0 mM) to 0.03 mg/g FW (60 mM) at 100% field capacity. However, under 50% field capacity (severe drought), proline increased significantly (0.36 µmol/g FW). At 75% field capacity, proline decreased by 61.9% and at 50% field capacity, by 52.78% (Table 1).

In terms of lipid peroxidation, silicon priming reduced the MDA content across all field capacities (Fig. 3). At 100% field capacity, MDA decreased from 12.24 nmol/g FW to 10.67 nmol/g FW (–12.83%). At 75%, it declined from 15.01 nmol/g FW to 12.75 nmol/g FW (–15.07%) and at 50%, from 19.72 nmol/g FW to 13.76 nmol/g FW (–30.2%). Based on these results, there was a consistent mitigation of oxidative stress through silicon application, with the greatest effect under severe drought. Concurrently, the MSI increased significantly with silicon (Fig. 4). At 100% field capacity, the MSI improved from 46.83% (0 mM) to 54.52% (60 mM) (+16.4%). At 75%, the MSI rose from 43.16% to 51.43% (+19.15%) and at 50%, from 39.01% to 42.27% (+8.35%). Though the effect was less pronounced under extreme drought, silicon still improved membrane integrity compared to the untreated controls.

Table 1 Total chlorophyll, carotenoid and proline levels in leaves of rice 'Inpari 24 Gabusan' at 42 d after treatment with varying silicon priming concentrations and field capacities

Parameter	Field capacity	Sodium metasilicate priming concentration (mM)			
		0	20	40	60
Total chlorophyll content	100%	1.95±0.01[f]	2.36±0.03[c]	2.92±0.05[b]	3.36±0.00[a]
(mg/g fresh weight)	75%	1.74±0.02[g]	2.22±0.03[d]	2.39±0.03[d]	2.40±0.04[c]
	50%	1.68±0.03[h]	2.05±0.04[e]	2.24±0.04[c]	2.38±0.03[c]
Carotenoid content	100%	0.51±0.00[e]	0.61±0.02[c]	0.75±0.04[a]	0.61±0.01[c]
(mg/g fresh weight)	75%	0.47±0.02[f]	0.56±0.0[d]	0.63±0.02[c]	0.69±0.02[b]
	50%	0.45±0.00[f]	0.45±0.02[f]	0.57±0.00[d]	0.55±0.04[d]
Proline content	100%	0.12±0.02[e]	0.12±0.01[e]	0.07±0.03[fg]	0.03±0.02[g]
(µmol/g fresh weight)	75%	0.21±0.03[bc]	0.13±0.03[de]	0.13±0.02[e]	0.08±0.02[f]
	50%	0.36±0.03[a]	0.22±0.02[b]	0.20±0.03[bc]	0.17±0.00[cd]

Values (mean ± SD, n = 3) within each column with different lowercase superscripts (response of field capacity for each sodium metasilicate priming concentration) or each row (response of sodium metasilicate priming concentration for each field capacity) in each parameter indicate significant ($p < 0.05$) differences
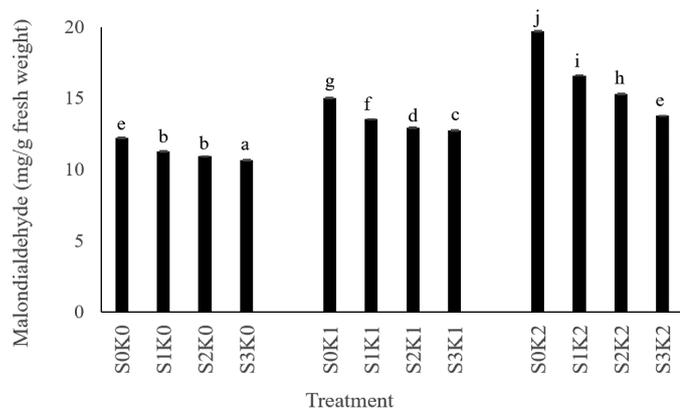
Fig. 3 Malondialdehyde levels in rice 'Inpari 24 Gabusan' at 42 d after transplanting with different levels of silicon priming concentration and field capacity, where S0 = sodium metasilicate at 0 mM, S1 = at 20 mM, S2 = at 40 mM and S3 = at 60 Mm, K0 = field capacity at 100%, K1 = at 75% and K2 = at 50%. Bars representmeans and error bars represent ±SD (n = 3). Different lowercase letters above bars indicate significant ($p < 0.05$) differences among means based on Duncan's multiple range test

Fig. 4 Membrane stability index of rice 'Inpari 24 Gabusan' at 42 d after transplanting with different levels of silicon priming concentration and field capacity, where S0 = sodium metasilicate at 0 mM, S1 = at 20 mM, S2 = at 40 mM and S3 = at 60 Mm, K0 = field capacity at 100%, K1 = at 75% and K2 = at 50%. Bars represent mean and error bars represent ± SD (n = 3). Different lowercase letters above bars indicate significant ($p < 0.05$) differences among means based on Duncan's multiple range test
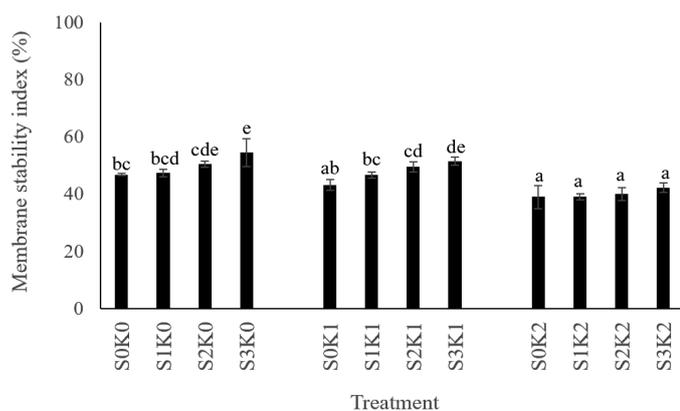
Leaf anatomical attributes

Silicon priming with Na_2SiO_3 influenced stomatal characteristics (Fig. 5 and Fig. 6), which play a critical role in water regulation under drought stress (Ahmed et al., 2013). Increasing the silicon priming concentration resulted in increased stomatal density across all field capacity levels. At 60 mM Na_2SiO_3, the stomatal density increased under optimal,

moderate and severe drought by 38.67%, 24.89% and 23.70% of S0, respectively. In addition, the stomatal aperture size was greatly enhanced with higher silicon concentrations. Under the 60 mM treatment, the aperture size increased by 70.00%, 128.09% and 140.79% of S0 under optimal, moderate and severe drought, respectively, indicating enhanced stomatal functioning and potential transpiration regulation in drought conditions.
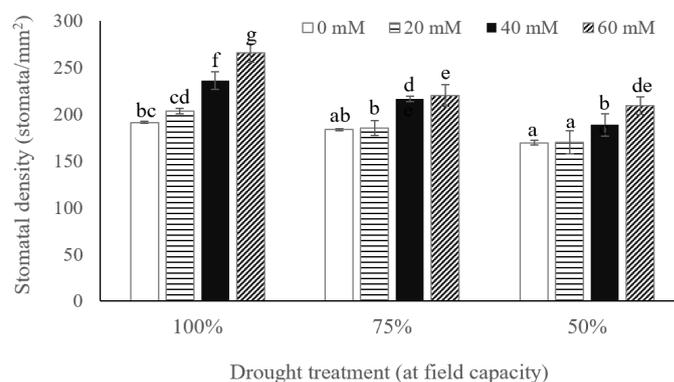

Fig. 5 Stomatal density of 'Inpari 24 Gabusan' rice leaves at 42 d after transplanting with different levels of sodium metasilicate priming treatment (0 mM, 20 mM, 40 mM, 60 Mm) at different field capacities (100%, 75%, 50%). Bars represent means and error bars represent ±SD (n = 3). Different lowercase letters above bars indicate significant ($p < 0.05$) differences among based on Duncan's multiple range test

Fig. 6 Stomatal aperture size of 'Inpari 24 Gabusan' rice leaves at 42 d after transplanting with different levels of sodium metasilicate priming treatment (0 mM, 20 mM, 40 mM, 60 Mm) at different field capacities (100%, 75%, 50%). Bars represent mean and error bars represent ±SD (n = 3). Different lowercase letters above bars indicate significant ($p < 0.05$) differences among means based on Duncan's multiple range test
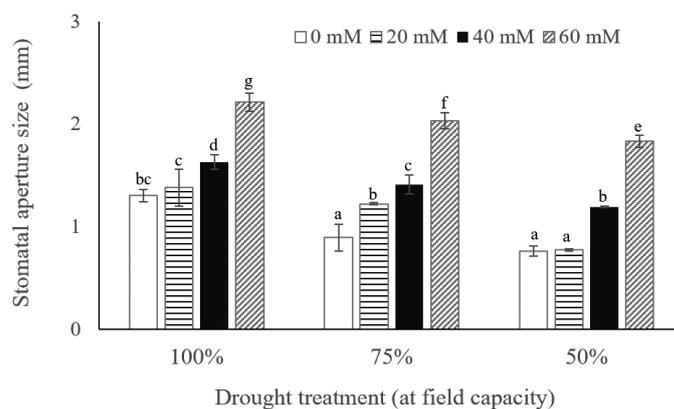
Correlation analysis

Based on the results of the correlation analysis (Fig. 7), the MSI was strongly positively correlated with the chlorophyll and carotenoid contents but negatively correlated with the proline and MDA levels. Silicon priming has been reported to reduce oxidative damage, improve stomatal density and aperture size, enhance CO_2 uptake and boost drought tolerance (Ahmed et al., 2013). However, in the current study under severe drought (50% field capacity), silicon's effectiveness declined, indicating its limits in mitigating extreme stress. There was a very strong correlation between total chlorophyll content and plant height, as well as a strong correlation between total chlorophyll content and root length, MSI, carotenoid content. Silicon priming enhanced
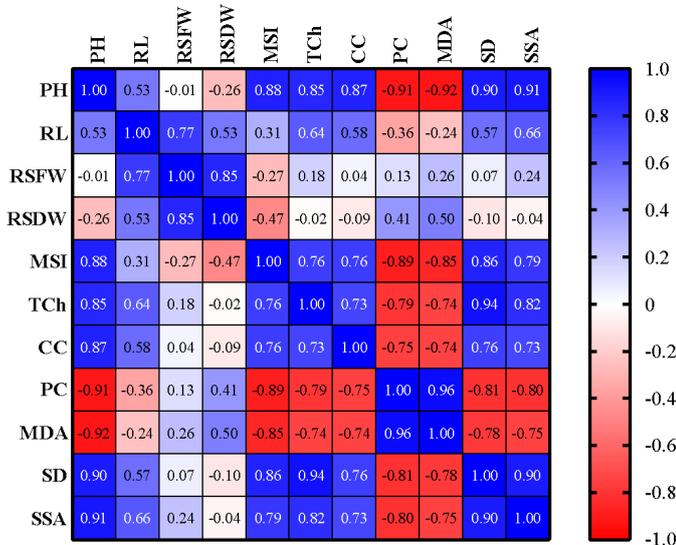
the stomatal density and stomatal aperture size, which helped to increase CO_2 uptake for photosynthesis. Under drought conditions, the plants allocated photosynthate to the roots, as indicated by an increase in the root-to-shoot FW ratio.

Discussion

The interaction between silicon priming concentration and field capacity significantly influenced growth, physiological and anatomical parameters, indicating the effectiveness of silicon in improving drought tolerance in rice varies with moisture conditions, depending on the specific combination of silicon level and water availability.

Vegetative growth characters (plant height and root length; Figs. 1, 8–9) exhibited strong interaction effects.

	PH	RL	RSFW	RSDW	MSI	TCh	CC	PC	MDA	SD	SSA
PH	1.00	0.53	-0.01	-0.26	0.88	0.85	0.87	-0.91	-0.92	0.90	0.91
RL	0.53	1.00	0.77	0.53	0.31	0.64	0.58	-0.36	-0.24	0.57	0.66
RSFW	-0.01	0.77	1.00	0.85	-0.27	0.18	0.04	0.13	0.26	0.07	0.24
RSDW	-0.26	0.53	0.85	1.00	-0.47	-0.02	-0.09	0.41	0.50	-0.10	-0.04
MSI	0.88	0.31	-0.27	-0.47	1.00	0.76	0.76	-0.89	-0.85	0.86	0.79
TCh	0.85	0.64	0.18	-0.02	0.76	1.00	0.73	-0.79	-0.74	0.94	0.82
CC	0.87	0.58	0.04	-0.09	0.76	0.73	1.00	-0.75	-0.74	0.76	0.73
PC	-0.91	-0.36	0.13	0.41	-0.89	-0.79	-0.75	1.00	0.96	-0.81	-0.80
MDA	-0.92	-0.24	0.26	0.50	-0.85	-0.74	-0.74	0.96	1.00	-0.78	-0.75
SD	0.90	0.57	0.07	-0.10	0.86	0.94	0.76	-0.81	-0.78	1.00	0.90
SSA	0.91	0.66	0.24	-0.04	0.79	0.82	0.73	-0.80	-0.75	0.90	1.00

Fig. 7 Correlation matrix between plant growth and physiological and anatomical characteristics, where blue and red colors indicate positive and negative correlations, respectively, color intensity is proportional to correlation coefficient (magnitude of relation), as presented in legend, PH = plant height, RL = root length, RSFW = root-to-shoot shoot fresh weight, RSDW = root-to-shoot dry weight, MSI= membrane stability index, TCh = total chlorophyll content, CC = carotenoid content, PC = proline content, MDA = malondialdehyde, SD= stomatal density and SSA = size stomatal aperture
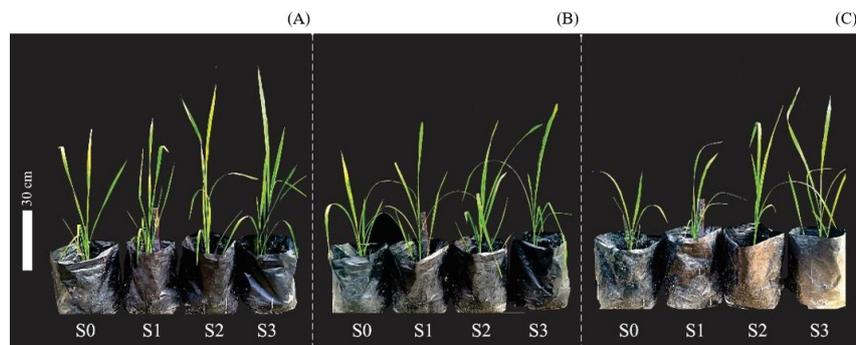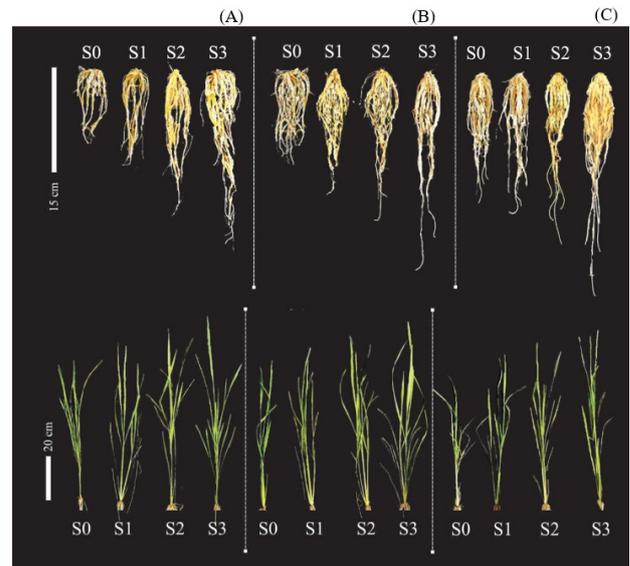

Fig. 9 Root morphology of 'Inpari 24 Gabusan' rice plants at 42 d after transplanting with different levels of sodium metasilicate priming treatment (S0 = sodium metasilicate at 0 mM, S1 = at 20 mM, S2 = at 40 mM, S3 = at 60 Mm) at different field capacities of: (A) 100%; (B) 75%; (C) 50%

Fig. 8 Morphology of 'Inpari 24 Gabusan' rice plants at 42 d after transplanting with different levels of sodium metasilicate priming treatment (S0 = sodium metasilicate at 0 mM, S1 = at 20 mM, S2 = at 40 mM, S3 = at 60 Mm) at different field capacities of: (A) 100%; (B) 75%; (C) 50%

At 100% field capacity, increased plant height was most pronounced at 60 mM silicon, indicating enhanced cell expansion under optimal moisture conditions (Dien et al., 2019). However, at 75% field capacity, the growth-promoting effect of silicon was even more evident in root development as limited carbohydrate synthesis under drought reallocates resources to survival rather than development (Agurla et al., 2018; Kuromori et al., 2022). At 50% field capacity, root elongation remained high at 40–60 mM silicon, as Si upregulates aquaporin genes (*PIP*) and reduces reactive oxygen species (ROS)-induced inhibition of water channels, increasing the osmotic driving force through K^+ accumulation in xylem sap (Chen et al., 2018; Saja-Garbarz et al., 2024). This Si × FC interaction shows that silicon not only promotes overall growth but fine tunes biomass allocation based on environmental cues, aligning with findings by Siangliw et al. (2022) and Karim et al. (2024).

Stomatal characteristics (Figs. 5–6) also demonstrated significant interaction patterns. Under 100% field capacity, higher silicon levels increased both stomatal density and aperture size, promoting optimal gas exchange. Under 75% field capacity, 40–60 mM silicon maintained a balance between density and aperture to support photosynthesis with reduced water loss. However, under 50% field capacity, although stomatal density remained stable, the aperture size was significantly reduced, particularly at higher silicon levels, suggesting that the silicon modulated stomatal behavior in response to water availability, supporting photosynthetic performance under mild stress while minimizing transpiration under severe drought. This confirmed the interactive role of the silicon priming concentration and field capacity in coordinating anatomical and physiological stomatal characters for drought adaptation, as described in Sakoda et al. (2020).

In addition, there was a clear interaction with the photosynthetic pigment content. The retention of total chlorophyll and carotenoids (Table 1) was highest at 60 mM silicon under 100% and 75% field capacity, indicating protection of the photosynthetic apparatus under non severe stress. However, under 50% field capacity, the pigment content declined across all treatments but was still significantly higher in the silicon-primed plants than in the controls. In tomato plants (*Lycopersicon esculentum* L.), silicon application (0.25–1.75 μmol) under water deficit increased total chlorophyll levels, with a strong positive correlation (r = 0.71) between leaf water potential and chlorophyll content (Silva et al., 2012). Under severe water deficits, chlorophyll degradation genes (such as *SGR*) are upregulated;

however, silicon application in pepper plants suppressed these genes and reduced ROS, delaying chlorophyll breakdown (Mushtaq et al., 2024). Silicon improves membrane stability and reduces electrolyte leakage, indirectly protecting chlorophyll under moderate stress. However, under extreme drought, the benefits of silicon are limited by irreversible cellular damage, such as excessive ROS accumulation and nutrient uptake inhibition (Rehman, 2021; Verma et al., 2021). While silicon mitigates chlorophyll degradation across drought intensities, its stabilization effects are most pronounced under mild-to-moderate stress, where physiological processes remain partially functional. Under extreme deficits, systemic damage overwhelms silicon's protective mechanisms, reducing its efficacy. Such trends highlight the conditional efficacy of silicon that is influenced by water status, in line with reports by Pérez-Gálvez et al. (2020) and Jarin et al. (2024).

Similarly, oxidative stress indicators, particularly MDA and the MSI, showed significant interaction effects (Figs. 4–5). Under well-watered conditions, the MDA levels were relatively low across treatments but decreased further with silicon. Under moderate drought stress (75% field capacity), MDA reduction and MSI improvement were most significant at 60 mM silicon, indicating enhanced antioxidant defense induced by the interaction of silicon and moderate water stress. However, at 50% field capacity, MDA remained elevated, even with silicon, suggesting that the protective capacity of silicon is constrained under extreme drought. These findings aligned with Ali et al. (2021) and Irfan et al. (2023), who emphasized that antioxidant activation by silicon was most effective when the plant is under moderate but not lethal stress.

The interaction of silicon and field capacity had a considerable effect on proline accumulation, indicating osmotic adjustment. At 50% field capacity, the proline content increased substantially in the silicon-treated plants, particularly at 40 and 60 mM. At 100% and 75% field capacity, Mauad et al. (2016) evaluated the effects of supplemental silicon on proline accumulation in upland rice (*Oryza sativa*) under drought stress. Based on their results, under water deficit conditions, silicon application reduced proline levels during both the vegetative and reproductive phases compared to the non-silicon controls. This reduction in proline content could be interpreted as evidence that silicon alleviated drought-induced stress, thereby diminishing the plant's need to accumulate proline as an osmotic adjustment mechanism. The effect of silicon on osmotic regulation is context-dependent—it mitigates stress under optimal conditions but promotes compatible solute accumulation when stress is present.

These patterns were consistent with the interactive model proposed by Dien et al. (2019) and Li et al. (2024), where osmolyte responses are triggered only beyond certain stress thresholds and enhanced by silicon priming.

In the current study, silicon increased the stomatal density and aperture size under optimal water conditions, which enhances photosynthesis by improving gas exchange efficiency (Verma et al., 2021). This effect helps to maintain higher photosynthetic rates when water is sufficient. Silicon is deposited on stomatal guard cells and the cuticle, which reduces transpirational water loss by decreasing stomatal conductance and increasing stomatal sensitivity to closure signals such as abscisic acid. This mechanism involves enhanced K^+ efflux from guard cells, leading to reduced stomatal aperture size under stress, thus conserving water (Vandegeer et al., 2021), while reducing both under low field capacity to conserve water. This modulation enhances drought stress tolerance and optimizes water use efficiency (Sakiroh and Aunillah, 2020). Silicon can help to maintain the stomatal aperture at an optimal size even under stress, allowing plants to continue photosynthesis even with a more limited opening. Research by Daszkowska-Golec and Szarejko (2013) showed that silicon increased the turgor pressure in guard cells, widening the stomatal aperture when water is available. Under drought, stomata closed to minimize water loss; however, prolonged closure limits CO_2 uptake, ultimately reducing photosynthesis. The findings of the current study suggested that silicon priming optimized stomatal behavior by increasing the stomatal density and adjusting the aperture size, allowing a better balance between water retention and gas exchange (Figs. 5–6). This supports other research indicating that silicon strengthened guard cell structure and enhanced water transport efficiency in plants (Wang et al., 2021).

The effects of silicon on drought tolerance in rice are strongly modulated by water availability and that silicon's physiological functions are only completely realized when combined with certain field capacity conditions. The interaction affects multiple biological levels from anatomical adaptations (stomata, roots), to biochemical responses (MDA, proline), to pigment stability (chlorophyll, carotenoids). Therefore, future research and recommendations for silicon use in agriculture must be based on an understanding of these interactive dynamics rather than individual component responses. The current study has provided compelling evidence that silicon priming with sodium metasilicate enhanced the drought tolerance of rice through numerous physiological, biochemical and anatomical mechanisms. The most effective concentration identified was 60 mM, which resulted in improved growth, a stronger root system,

greater pigment stability, enhanced antioxidant activity and better osmotic regulation under water-deficit conditions. These findings are consistent with and expand upon recent international research, placing the work in the broader context of sustainable agricultural adaptation to climate change. As water scarcity becomes an increasingly critical issue for global food security, silicon-based seed priming offers a scientifically validated and practically applicable strategy to enhance crop resilience and productivity in drought prone environments.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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