pISSN 2302-1616, eISSN 2580-2909
Vol 9, No. 2, December 2021, pp. 126-136
Available online http://journal.uin-alauddin.ac.id/index.php/biogenesis
DOI https://doi.org/10.24252/bio.v9i2.20754

Growth and physiological response of rice 'Inpari 35' under salinity stress and application of silicate fertilizer

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ABSTRACT. Rice is an important staple food in Indonesia. Crop areas can be expanded to boost productivity by utilizing marginal lands, which are saline. This study aims to study the growth and physiological response of rice 'Inpari 35' to the application of silicate fertilizer under salinity stress conditions. This study used a completely randomized design (CRD) with two factors. The first factor is the difference in NaCl salt concentration consisting of N0: 0 mM; N1: 37.5 mM; N2: 50 mM, while the second factor is the difference in the concentration of silicate fertilizer ($CaSiO_3$) consisting of S0: 0 mM; S1: 1 mM and S2: 2 mM. Each treatment combination was repeated three times. Observed data were analyzed by analysis of variance (ANOVA). A significant difference between treatments is continued with Duncan's multiple distance test at a 95% confidence level. The results showed that NaCl treatment significantly ($p<0.05$) inhibited the growth of rice 'Inpari 35', which was indicated by a decrease in the plant height and number of leaves. The NaCl treatment caused a reduction in the levels of chlorophyll, carotenoids, proline, membrane stability index (MSI), and relative water content (RWC). The interaction between NaCl treatment and $CaSiO_3$ showed significant differences in physiological parameters by increasing the levels of chlorophyll, carotenoid, proline, membrane stability index, and relative water content.

Keywords: hydroponic system; membrane stability index; NaCl and $CaSiO_3$ treatment; relative water content; rice cultivar

Article History: Received 22 April 2021; Received in revised form 18 June 2021; Accepted 16 November 2021; Available online 30 December 2021
How to Cite This Article: Ramadhani AN, Rachmawati D. 2021. Growth and physiological response of rice 'Inpari 35' under salinity stress and application of silicate fertilizer. *Biogenesis: Jurnal Ilmiah Biologi*. vol 9(2): 126–136. doi: https://doi.org/10.24252/bio.v9i2.20754.

INTRODUCTION

Rice is a food crop commodity that produces rice, the staple food in Indonesia. However, the change in land use causes rice production to decline (Jiang *et al*., 2013; Suhartini & Harjosudarmo, 2017). One way to increase rice production is by expanding the planted area on marginal lands (Swinton *et al*., 2011; Hussain *et al*., 2019), one of which is saline land. In the future, the area of saline land will increase due to the decrease in water quality and rainfall. Salinity-tolerant rice cultivars are needed to take advantage of these marginal lands.

The major current challenge in Indonesia is the conversion of paddy fields to non-agriculture, especially for road infrastructure, airports, offices, housing, and industry (Mahbubi, 2013; Sunartomo, 2015; Mulyani *et al*., 2017; Septanti & Saptana, 2019). The lack of updated assessments of the extent, magnitude, and progress of soil salinity and sodicity at different scales is the primary knowledge gap to address the problem of soil salinity (Zhou *et al*., 2013; Machado & Serralheiro, 2017; Hassani *et al*., 2020). Salinity is one of the factors that constrain rice plant growth and production due to the high concentration of sodium chloride (NaCl) in the growing medium, which can interfere with plant physiological processes, affecting osmotic pressure and nutrient balance (Ghosh *et al*., 2011; Das *et al*., 2015; Reddy *et al*., 2017). Thus, other elements are needed that can absorb toxic ions due to salinity stress (Rad *et al*., 2012). Dry leaf tips, diminished tillers, root length, plant height, shoot dry weight, and root weight are all symptoms of salinity in plants. Salinity decreases the area and concentration of chlorophyll on leaves and reduces lipid peroxidase in cell wall membranes (Weisany *et al*., 2012; Shah *et al*., 2017). High salinity levels can induce chloroplast damage which causes a reduction in chlorophyll content and photosynthetic capacity (Rad *et al*., 2012; Gao *et al*., 2015).

Silicon (Si) is a well-known crucial element for rice plants, since it provides physical and mechanical protection and also modulates metabolic and physiological functions (Sahebi *et al*., 2015; Savvas & Ntatsi, 2015; Luyckx *et al*., 2017). Silicon contributes to increased plant tolerance to abiotic stress by altering water content levels, lowering water loss through transpiration, regulating nutritional sufficiency, and limiting the absorption of toxic ions such as Na^+ (Kafi & Rahimi, 2011; Rizwan *et al*., 2015; Suhartini & Harjosudarmo, 2017; Chen *et al*., 2018). The accumulation of silicic acid ($Si(OH)_4$) in the cell walls of leaves, stems, and roots supports plants by assisting in the reduction of ionic poisoning via increased antioxidant enzyme activity under stress situations (Wani *et al*., 2017; Ikhsanti *et al*., 2018; Bhat *et al*., 2019).

Rice plants that are subjected to salinity stress will produce proline and increased membrane permeability as an effort to adjust osmotic pressure (Teh *et al*., 2015; Chun *et al*., 2018). In line with Dolatabadian *et al.* (2011), Zhang *et al.* (2012), and Aini *et al.* (2014), salinity can affect the chlorophyll index of the leaf, shorten the root length and diameter, and anatomically disrupt cell division and the process of cell growth by decreasing the thickness of the apical meristem, cortex, and stele diameter. In addition, salinity also stimulates root exodermis and endodermis suberization (Fleck *et al*., 2011; Cheng *et al*., 2020).

By conducting this research, information about the role of silicate fertilizer on growth, yield, and tolerance levels of rice 'Inpari 35' to salinity stress can be obtained. Characters that indicate plant adaptive traits to salinity will be observed in growth and physiological changes. As a result, it is important to conduct this research to determine the tolerance of rice 'Inpari 35' under salinity stress conditions and the effect of silicate fertilizer application.

MATERIALS AND METHODS

This research was conducted at the Laboratory of Plant Physiology and the Laboratory of Plant Structure and Development, Faculty of Biology, Universitas Gadjah Mada. The research was conducted from December 2020 to March 2021. 'Inpari 35' rice seeds cultivar obtained from Indonesian Center for Rice Research (ICRC). The design used was a completely randomized design (CRD) with two factors. The first factor is the difference in NaCl concentration consisting of N0: 0 mM; N1: 37.5 mM; N2: 50 mM, while the second factor is the difference in $CaSiO_3$ concentration consisting of S0: 0 mM; S1: 1 mM; and S2: 2 mM.

Seed germination. Sterilized rice seeds are sown in a container filled with distilled water and grown at room temperature. Germination was carried out ± four days until the radicles appeared, then the seeds were transferred to the rockwool growing medium. Uniform germinating seeds were transferred to hydroponic styrofoam measuring 25 × 20 × 3 cm consisting of six holes of 2.5 cm in diameter with a distance of 2 cm and have been coated with plastic gauze on the bottom and installed in a plastic container containing 4 L of full concentration of Yoshida nutrient solution (Yoshida *et al*., 1976). Each hole was used to grow one seedling. Hydroponic Yoshida's solution in a plastic container was replaced once a week. Four days old seedlings were acclimatized hydroponically in Yoshida's solution full concentration until 14 DAP (days after planting). The salinity treatments used were 37.5 mM and 50 mM NaCl, while $CaSiO_3$ were 0, 1, and 2 mM. The salinity and calcium silicate treatment was carried out during the vegetative period from the age of 14 DAP to 35 DAP.

Plant growth. Plant height and number of leaves were measured every 3 days. Plant height was measured from the root base above the rockwool surface to the tip of the longest leaf. The number of leaves was determined by counting the number of whole leaves in each plant. The measurement of plant dry weight was carried out by drying the plants in an oven at 80°C until a constant weight was obtained. Plant dry weight measurements were conducted at the end of the research (21 days after treatment/DAT).

Plant photosynthesis pigments. Chlorophyll and carotenoid according to the

Harborne (1998) method with some modifications at 14 DAT A leaf sample of 0.3 g was ground with a mortar and homogenized with 2 ml of 80% cold acetone solution. Chlorophyll content was determined using a spectrophotometer (Genesys 10 UV Scanning, Thermo Scientific) at multiwavelength of 470, 645, and 664 nm. The chlorophyll and carotenoid level were calculated by the following formula:

$$\text{Chlorophyll a level } (\text{mg.L}^{-1}) = 12.21 \times A_{(663)} - 2.81 \times A_{(646)}$$

$$\text{Chlorophyll b level } (\text{mg.L}^{-1}) = 20.13 \times A_{(646)} - 5.03 \times A_{(663)}$$

Then, converted into mg.g^{-1}, by the following formula (Harborne, 1998):

$$\text{Chlorophyll level } (\text{mg.g}^{-1}) = \frac{1.100^{-1} \text{ x chlorophyll level}}{0.1 \text{ mg.g}^{-1}}$$

$$\text{Carotenoid level } (\text{mg.g}^{-1}) = \frac{(1000A6470) - 3.27(\text{ChA}) - 104(\text{Ch B})]}{227}$$

Membrane stability index. Measurement of the membrane stability index (Swapna & Shylaraj, 2017) was carried out on the 14th day after treatment. Leaf samples were taken from three plants from each replication as much as 0.1 g. The leaves were put into a clean test tube containing 10 ml of ddH_2O, then incubated for ± 24 h at room temperature under constant lighting. The initial conductivity value (EC1) is measured using an EC meter. After that, the tube was heated in boiling air (100°C) for 30 min and then cooled to 25°C for 15 min. The conductivity of the solution was measured with an EC meter as the final conductivity value (EC2). The membrane stability index (MSI) value is calculated by the following formula (Swapna & Shylaraj, 2017):

$$\text{MSI} = (1 - \frac{EC1}{EC2}) \times 100$$

Relative Water Content (RWC). Measurement of RWC was carried out after the 14th day of treatment (Polash *et al*., 2018). Leaf samples were taken from three plants from each replication as much as 0.1 g. Leaf samples were then incubated in 10 ml ddH_2O for ± 24 h and then the turgid weight was weighed. The leaf samples were then put in a brown paper bag and dried in an oven at a temperature of 70°C for ± 72 h, then the dry weight of the sample was weighed. The value of RWC is calculated using the following formula (González & González-Vilar, 2001):

$$\text{RWC} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}} \; x \; 100$$

Proline content measurement. Proline contents were measured according to the Bates *et al.* (1973) method at 19 DAT. A total of 0.25 g fresh leaves were frozen using liquid nitrogen and mashed with a mortar. The sample were extracted with sulfosalicylic acid 3% and mixed with ninhydrin reagents and heated in 95°C water bath for 60 min. About 2 ml of toluene were added after the sample has cooled to form two layers of solution. The absorbance of the extract was determined by spectrophotometry with toluene as blank. Proline levels were calculated as $\mu g.\text{ml}^{-1}$ using calibration with a proline standard curve. The level of proline µmol per gram of fresh sample weight was calculated using the following formula (Bates *et al*., 1973):

$$\text{Proline level} = \frac{[\mu g \; proline/.mlx \; ml \; toluen \; t \;] \; / [115{,}13 \; \mu g/\mu mol]}{0{,}25}$$

Data analysis. Following our previous studies (Rachmawati *et al*., 2021), the significance and interaction values of NaCl and $CaSiO_3$ treatment on plant growth and plant physiological parameters were tested with One-Way ANOVA and continued with Duncan Multiple Range Test (DMRT) conducted at 95% confidence level ($p \leq 0.05$) with IBM-SPSS ver. 26.0.

RESULTS AND DISCUSSION

The present study examined the growth and physiological responses of rice 'Inpari 35' when treated with $CaSiO_3$ as a source of silicon (Si) at varied concentrations and exposed to

salinity stress. Silicon is an inorganic fertilizer needed in rice cultivation since it contributes to the quality and quantity of agricultural products (Meena *et al*., 2014). Fig.1 showed the morphology of rice 'Inpari 35' with a combination of NaCl and $CaSiO_3$ treatments.

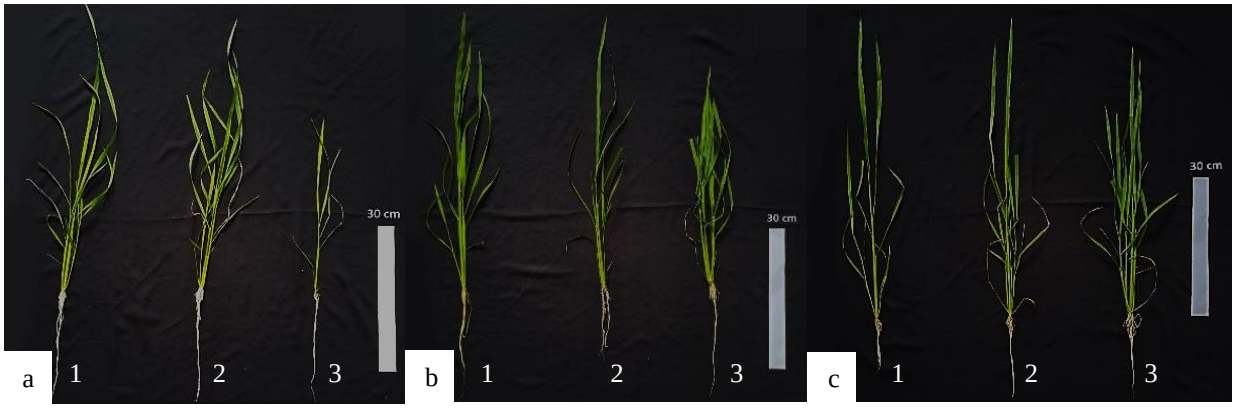

Fig 1. Morphology of rice 'Inpari 35' with NaCl and $CaSiO_3$ treatment: a. 0 mM CaSiO3; b. 1 mM $CaSiO_3$ (S1); c. $CaSiO_3$ 2 mM (S2); 1. 0 mM NaCl (N0); 2. 37.5 mM NaCl (N1); 3. 50 mM NaCl (N2); bars= 30 cm.

Plant height. Plant height parameters were measured every three days up to 21 days of treatment which is shown in Fig. 2-4. Based on the observations, treatment 1 mM and 2 mM $CaSiO_3$ increased plant height compared to control (Fig. 3).

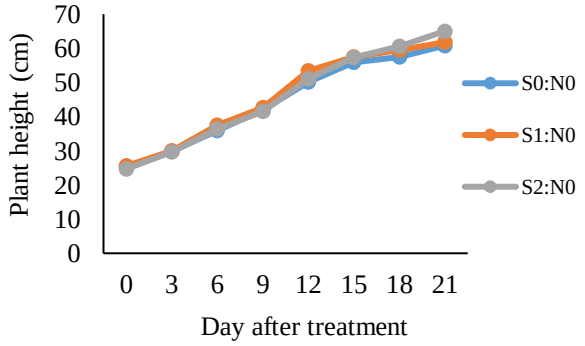

Fig 2. Plant height of rice 'Inpari 35' treated with NaCl and $CaSiO_3$ using 0 mM NaCl (N0) (S0: 0 mM $CaSiO_3$; S1: 1 mM $CaSiO_3$; S2: 2 mM $CaSiO_3$ for three weeks).

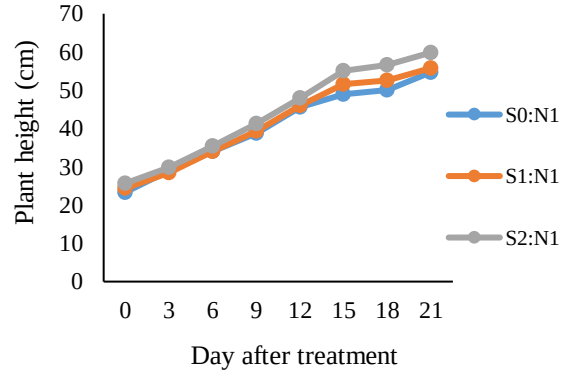

Fig 3. Plant height of rice 'Inpari 35' treated with NaCl and $CaSiO_3$ using 37.5 mM NaCl (N1) (S0: 0 mM $CaSiO_3$; S1: 1 mM $CaSiO_3$; S2: 2 mM $CaSiO_3$ for three weeks).

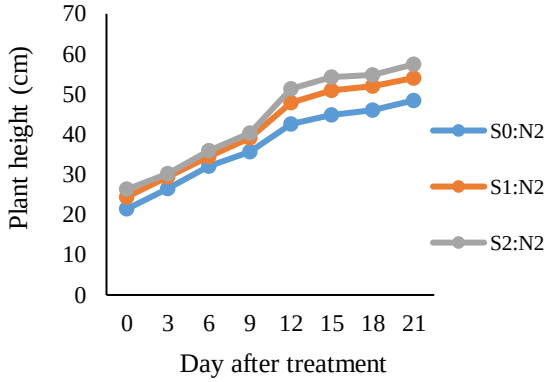

Fig 4. Plant height of rice 'Inpari 35' treated with NaCl and $CaSiO_3$ using 50 mM NaCl (N2) (S0: 0 mM $CaSiO_3$; S1: 1 mM $CaSiO_3$; S2: 2 mM $CaSiO_3$ for three weeks).

The treatment without NaCl by application of $CaSiO_3$ 1 mM and 2 mM increased plant height compared to control (Fig. 2). In Fig. 3, NaCl treatment at 37.5 mM with $CaSiO_3$ 2 mM showed a higher plant height than 37.5 mM NaCl treatment with $CaSiO_3$ 1 mM or without Si. Fig. 4 showed NaCl treatment at 50 mM with $CaSiO_3$ 2 mM showed a higher plant height increase than 50 mM NaCl treatment with $CaSiO_3$ or without $CaSiO_3$ 1 mM. The higher $CaSiO_3$ concentration given, the higher the plant height increase. High concentrations of Na^+ and Cl^- cause toxicity to plants and thus inhibit growth. Salinity reduces plant growth through osmotic stress, reduces the ability of plants to absorb water, and causes inhibition of plant growth (Rad *et al*., 2012).

Table 1. Plant height of rice 'Inpari 35' after treated with $CaSiO_3$ and NaCl for three weeks.

NaCl (mM)	$CaSiO_3$ (mM) 0	1	2	Average
0	60.82ef	61.78ef	65.03f	62.54r
37.5	54.79b	55.81bc	59.87de	56.82q
50	48.45a	54.02b	57.42bcd	53.29p
Average	54.69x	57.20y	60.77z	

Note: Numbers followed by the same letters in columns and rows show insignificant differences based on the DMRT test with a confidence level of 95%.

ANOVA analysis revealed that differences in NaCl and $CaSiO_3$ treatments had a significant influence on the plant height of rice 'Inpari35' (p0.05). According to Table 1, NaCl treatment without $CaSiO_3$ resulted in a reduction in rice plant height compared to control. This shows that NaCl treatment with a concentration of 37.5 mM and 50 mM inhibit the growth of rice plant height.

Number of leaves. The parameter number of leaves were measured every three days up to 21 days of treatment, which is shown in Fig. 5-7.

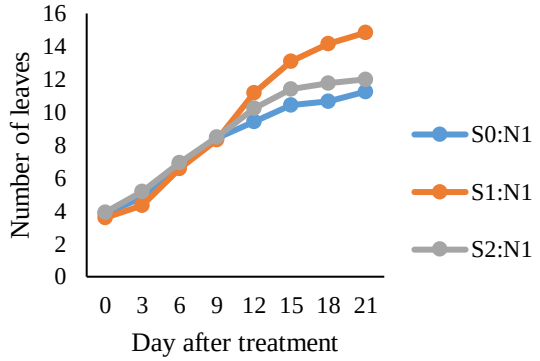

Fig 5. Number of leaves of rice 'Inpari 35' rice plant treated with NaCl and $CaSiO_3$ using 0 mM NaCl (N0) (S1: 1 mM $CaSiO_3$; S2: 2 mM $CaSiO_3$ for three weeks).

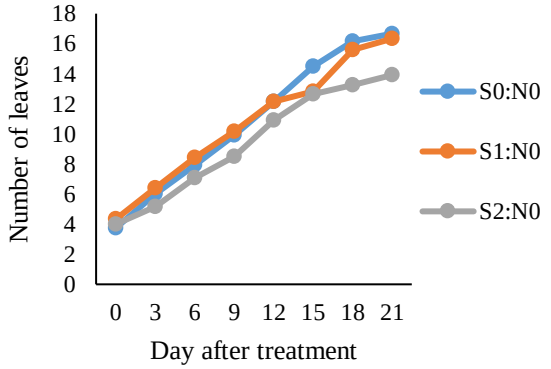

Fig 6. Number of leaves of rice 'Inpari 35' rice plant treated with NaCl and $CaSiO_3$ using 37.5 mM NaCl (N1) (S1: 1 mM $CaSiO_3$; S2: 2 mM $CaSiO_3$ for three weeks).

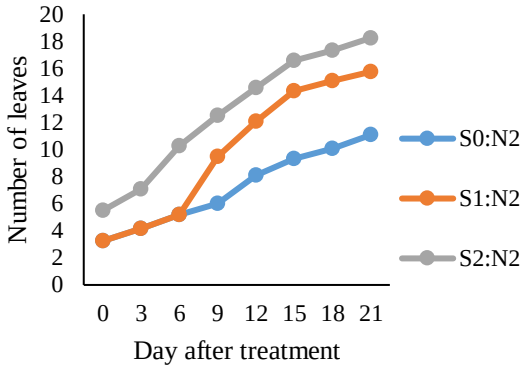

Fig 7. Number of leaves of rice 'Inpari 35' rice plant treated with NaCl and $CaSiO_3$ using 50 mM NaCl (N2) (S1: 1 mM $CaSiO_3$; S2: 2 mM $CaSiO_3$ for three weeks).

The control treatment without NaCl showed that the number of leaves was more increased in the treatment with 1 mM and 2 mM $CaSiO_3$ (Fig. 5). The treatment of 37.5 mM NaCl with 1 mM $CaSiO_3$ showed a higher number of leaves than that with 2 mM $CaSiO_3$ (Fig. 6). The treatment of 50 mM NaCl with 2 mM $CaSiO_3$ showed a higher number of leaves than without $CaSiO_3$ or with 1 mM $CaSiO_3$ (Fig. 7). $CaSiO_3$ concentration boosted silicon intake, resulting in an increase in the number of leaves on rice plants. Leaf damage due to NaCl treatment on rice seedling occurs as a result of the excessive transport of Na^+ and Cl^- ions which cause Na^+ and Cl^- to accumulate in the leaves tissues (Khare *et al*., 2015; Almeida *et al*., 2017). As a result of this excessive ion transport, leakage occurs through the apoplastic pathway to the xylem (Zhao *et al*., 2020).

Table 2. Number of leaves of rice 'Inpari 35' after treated with $CaSiO_3$ and NaCl for three weeks.

NaCl (mM)	$CaSiO_3$ (mM) 0	1	2	Average
0	16.67cd	16.33cd	13.92abc	15.64q
37.5	11.25a	14.83bc	12.00ab	12.69p
50	11.08a	15.75cd	18.25d	15.03q
Average	13.00x	14.72y	15.64y	

Note: Numbers followed by the same letters in columns and rows show insignificant differences based on the DMRT test with a confidence level of 95%.

According to Table 2, treatment with 37.5 mM and 50 mM NaCl without $CaSiO_3$ resulted in a reduction in the number of leaves compared to the control. The results of the ANOVA test, the number of leaves decreased significantly ($p<0.05$) with NaCl treatment without $CaSiO_3$

treatment. However, the 50 mM NaCl and 2 mM $CaSiO_3$ treatments showed a significantly different number of leaves compared to the 50 mM NaCl treatment without $CaSiO_3$. Application of 1 mM and 2 mM $CaSiO_3$ on rice 'Inpari 35' at a 50 mM NaCl treatment concentration showed that the number of leaves tended to increase compared to the NaCl treatment without $CaSiO_3$ application. Salinity treatment with NaCl inhibits the growth process of rice plants.

Dry weight. Plant dry weight represent the yield of growth. The increase in dry weight indicates that the plant is growing and developing more rapidly. Root and shoot dry weight of rice 'Inpari 35' at five weeks of age are presented in Tables 3.

Tabel 3. Root and shoot dry weight of rice 'Inpari 35' after treated with $CaSiO_3$ and NaCl for three weeks.

Parameter	NaCl (mM)	$CaSiO_3$ (mM)			Average
		0	1	2	
Root dry weight (gram)	0	0.142g	0.080d	0.040b	0.087p
	37.5	0.085d	0.081d	0.071c	0.079q
	50	0.028a	0.117e	0.129f	0.091p
	Average	0.085y	0.093z	0.080x	
Shoot dry weight (gram)	0	0.671d	0.615cd	0.406b	0.564q
	37.5	0.451b	0.553c	0.614cd	0.539q
	50	0.131a	0.636cd	0.660cd	0.476p
	Average	0.418x	0.599y	0.560y	

Note: Numbers followed by the same letters in columns and rows show insignificant differences based on the DMRT test with a confidence level of 95%.

Table 3 showed that the 0 mM and 37.5 mM NaCl treatment decreased root dry weight and increased $CaSiO_3$ application. However, at 50 mM NaCl treatment, the root dry weight increased along with the increasing $CaSiO_3$ application. The greater the dry weight indicated, the better the metabolism occurs in the plant cells, and vice versa (Jayantie *et al*., 2017). The measurement of rice shoot dry weight showed that 0 mM NaCl treatment and increasing $CaSiO_3$ application tended to decrease the shoot dry weight. However, at 37.5 mM and 50 mM NaCl treatment, increasing $CaSiO_3$ concentration increases the shoot dry weight (Table 3). The calcium can prevent the rate of leaves dropping to prevent plant weight loss (Gilliham *et al*., 2011). Plant under NaCl treatment with the application of $CaSiO_3$ has a larger leaf weight ratio than plants without $CaSiO_3$ application, indicated with loss of water transpiration. $CaSiO_3$ in nutrient solutions increased rice shoot dry weight (Sahebi *et al.*, 2015).

Plant photosynthesis pigments. Chlorophyll functions in the photosynthetic process of plants by collecting and converting sunlight into chemical energy. Chlorophyll parameter measurements offer information about the activity of photosystem II (PSII) and changes in the photosynthetic metabolism of plants under abiotic stress (Chen *et al*., 2011).

Table 4. Chlorophyll a and chlorophyll b levels of rice 'Inpari 35' rice plants after treated with $CaSiO_3$ and NaCl for three weeks.

Parameter	NaCl (mM)	$CaSiO_3$ (mM)			Average
		0	1	2	
Chlorophyll a $mg.g^{-1}$ Fresh weight	0	1.017a	0.871a	4.522b	2.137q
	37.5	0.698a	0.806a	1.224a	0.909p
	50	0.654a	1.108a	1.532a	1.098p
	Average	0.790x	0.928x	2.426y	
Chlorophyll b $mg.g^{-1}$ Fresh weight	0	1.522a	1.874a	3.347b	1.498p
	37.5	1.685a	1.825a	1.742a	1.209p
	50	1.472a	2.434ab	3.234b	1.587p
	Average	1.040x	1.363x	1.891y	

Note: Numbers followed by the same letters in columns and rows show insignificant differences based on the DMRT test with a confidence level of 95%.

Based on Table 4, NaCl treatment showed no significant difference ($p \geq 0.05$) to chlorophyll b levels. However, increasing the NaCl concentration can decrease chlorophyll a levels. Application of 2 mM $CaSiO_3$ to plant with 50 mM NaCl treatment increased chlorophyll a levels significantly ($p < 0.05$). However, the application of $CaSiO_3$ to plant with 37.5 mM NaCl treatment did not significantly increase chlorophyll a levels ($p \geq 0.05$). The highest chlorophyll a levels were found in plants treated without NaCl and given 2 mM of $CaSiO_3$. Saline conditions cause changes in chlorophyll levels resulting from the damage to the chloroplasts, which is indicated by the chlorophyll a level that is lower than the chlorophyll b level. Degradation of chlorophyll a in the seedling stage treated with NaCl was associated with photosystem II which caused a decrease in photosynthesis rate (Amirjani, 2011). Table 4 shows that chlorophyll levels decreased at 50 mM NaCl treatment. Application of 1 mM and 2 mM $CaSiO_3$ to plants treated with NaCl or without NaCl could increase chlorophyll b levels. About 2 mM $CaSiO_3$ increased chlorophyll b levels significantly ($p \geq 0.05$). The highest chlorophyll b was found in plants treated without NaCl with 2 mM $CaSiO_3$.

Our study showed that application of $CaSiO_3$ tends to increase the levels of chlorophyll a and chlorophyll b of rice plant under salinity stress (Table 4). Decrease in chlorophyll levels at high NaCI concentration (50 mM) can be caused by damage to chloroplasts or changes in the ratio of lipid protein complexes to pigment proteins, as well as an increase in chlorophyllase activity (Taïbi *et al*., 2016). In addition, NaCI treatment caused several structural changes in the leaves.

Table 5. Carotenoid levels of rice 'Inpari 35' after treated with $CaSiO_3$ and NaCl for three weeks.

NaCl (mM)	$CaSiO_3$ (mM) 0	1	2	Average
0	0.118a	0.110a	0.384b	0.204q
37.5	0.089a	0.101a	0.145a	0.112p
50	0.084a	0.140a	0.190a	0.138pq
Average	0.097x	0.117x	0.239y	

Note: Numbers followed by the same letters in columns and rows show insignificant differences based on the DMRT test with a confidence level of 95%.

The NaCl treatment did not show a significant difference ($p \geq 0.05$) to the carotenoid levels, but the NaCl treatment tended to reduce the carotenoid levels. Application of 2 mM $CaSiO_3$ without NaCl treatment increased carotenoid levels significantly (Table 5). Overall, the application of $CaSiO_3$ to plants treated with 37.5 mM NaCl, 50 mM NaCl, or without NaCl increased carotenoid levels. The application of $CaSiO_3$ to plants with NaCl treatment did not increase carotenoid levels significantly ($p \geq 0.05$). The highest carotenoid levels were found in plants without NaCl with the application of 2 mM $CaSiO_3$. Carotenoids consist of carotene and xanthophyll which represent the photosynthetic pigment group, play a role in harvesting photon energy for photosynthesis (Maoka, 2020), and as defense mechanisms against oxidative stress. The carotenoid pigments degenerate to maintain chlorophyll levels, which is one of the most important mechanisms in rice plants under salinity stress (Taïbi *et al*., 2016).

Membrane stability index (MSI). NaCl treatment increased the electrolyte leakage value and decreased the MSI value, which effects were more visible on plants as the NaCl concentration increased (Senguttuvel *et al*., 2013). The results of measuring the MSI parameters in the rice 'Inpari 35' with $CaSiO_3$ and NaCl treatment are presented in Table 6.

Table 6. Membrane stability index (MSI) of rice 'Inpari 35' after treated with $CaSiO_3$ and NaCl for three weeks.

NaCl (mM)	$CaSiO_3$ (mM) 0	1	2	Average
0	92.737b	92.493b	93.073b	92.768q
37.5	75.047a	92.450b	89.687b	85.728p
50	75.460a	92.14b	92.690b	86.763p
Average	81.081x	92.361y	91.817y	

Note: Numbers followed by the same letters in columns and rows show insignificant differences based on the DMRT test with a confidence level of 95%.

The MSI value of rice 'Inpari 35' treated with 50 mM NaCl increased with increasing $CaSiO_3$ concentration. The application of 1 mM and 2 mM of $CaSiO_3$ with 37.5 mM and 50 mM NaCl treatment showed a significant difference ($p < 0.05$) compared to the NaCl treatment without application of $CaSiO_3$. The control plant had the highest membrane stability index value compared to other treatments. Between

NaCl treatment and $CaSiO_3$ application, there was a significant difference ($p<0.05$). The MSI measurement results in Table 6 show that the MSI value tends to decrease with the increasing concentration of NaCl treatment. The decrease in the membrane stability index might be due to the accumulation of Na^+ and Cl^- ions. The reduction of Na^+ uptake and Na^+ accumulation by plant tissues is the most important plant resistance to salinity stress. It is well established that Si supply from $CaSiO_3$ reduces Na^+ absorption by plants under salt stress and increases the K^+/Na^+ ratio (Zargar *et al*., 2019).

Relative water content (RWC) is a salinity tolerance metric in plants that describes the relative water content of the leaves and indicates the plant's stress level. The results of measurement of RWC parameters in rice 'Inpari 35' with CaSiO3 and NaCl treatment are presented in Table 7.

Table 7. Relative water content (RWC) of rice 'Inpari 35' after treated with $CaSiO_3$ and NaCl for three weeks.

NaCl (mM)	$CaSiO_3$ (mM) 0	1	2	Average
0	92.440ab	92.230ab	95.99b	93.553p
37.5	82.563a	92.360ab	92.113ab	89.012p
50	82.357a	92.14ab	96.093b	90.197p
Average	85.787x	92.243y	94.732y	

Note: Numbers followed by the same letters in columns and rows show insignificant differences based on the DMRT test with a confidence level of 95%.

Table 7 shows that the NaCl treatment tends to decrease the RWC, but it increased by applying 1 mM or 2 mM $CaSiO_3$. Treatment of 37.5 mM NaCl by application of 1 mM $CaSiO_3$ increased the RWC compared without $CaSiO_3$. The 37.5 mM NaCl treatment slightly decreased in the RWC when given 2 mM $CaSiO_3$ but significantly different from 37.5 mM NaCl treatment without $CaSiO_3$. The RWC on 50 mM NaCl treatment tended to increase with increasing $CaSiO_3$ concentration. The highest values were found in plants treated with 50 mM NaCl and 2 mM $CaSiO_3$. The RWC increased by the application of $CaSiO_3$. Application of 2 mM $CaSiO_3$ to rice plants without NaCl treatment and 50 mM NaCl treatment showed significantly different changes in the RWC ($p<0.05$) compared to the control. Rice plants exposed to NaCl treatment showed lower RWC values. In line with Rahman *et al.* (2016), these results indicate an imbalance of water status in cells and the cell environment due to the excess Na^+ and Cl^- ion accumulation, resulting in osmotic stress occurring in plants.

Proline levels. Proline levels of rice 'Inpari 35', which were treated with 50 mM NaCl, decreased with 1 mM $CaSiO_3$ but increased by 2 mM $CaSiO_3$. The proline levels of rice 'Inpari 35' with 37.5 mM and 50 mM NaCl treatment increased along with the increase in the $CaSiO_3$ concentration given (Table 8). It is known that the application of 2 mM $CaSiO_3$ to 37.5 mM and 50 mM of NaCl showed a significant difference compared to the control. Control plants had the lowest proline levels compared to other treatment combinations. $CaSiO_3$ can increase proline levels of rice plants treated with NaCl or without NaCl.

Table 8 showed that NaCl treatment and $CaSiO_3$ application significantly ($p\leq0.05$) increased proline levels of rice 'Inpari 35' compared to the control. The proline accumulation in the rice 'Inpari 35' tended to increase in NaCl treatment with the application of $CaSiO_3$. Calcium silicate plays a role in increasing proline in plants treated with NaCl and without NaCl. The 2 mM $CaSiO_3$ application increased proline levels in the rice 'Inpari 35' compared to control and plants with NaCl treatment without $CaSiO_3$.

Table 8. Proline levels of rice 'Inpari 35' after treated with CaSiO3 and NaCl for 3 weeks.

NaCl (mM)	$CaSiO_3$ (mM) 0	1	2	Average
0	0.391a	0.722ab	0.619ab	0.576a
37.5	0.785b	0.843b	0.993b	0.874b
50	0.763b	0.664ab	0.782b	0.736ab
Average	0.646a	0.743a	0.798a	

Note: Numbers followed by the same letters in columns and rows show insignificant differences based on the DMRT test with a confidence level of 95%.

When plants are exposed to abiotic stress, such as NaCl, they store proline as a resistant strategy. Proline accumulation in cells has been shown to influence the osmotic equilibrium between the cell and cytosolic environments (Salsinha *et al*., 2020). Proline accumulation decreased significantly in the presence of Si in

all stress treatments. However, the drop was only significant for plants exposed to severe salinity stress (El Moukhtari *et al*., 2020). As Zargar *et al*. (2019) explain, although plants are grown hydroponically, other researchers have demonstrated that when silicates are supplied to plants under low salinity stress, yields do not alter, regardless of whether the plants are cultivated in pots using sand, soil, or substrate. This suggests that $CaSiO_3$ can be applied to plants in areas with high salinity.

CONCLUSION

NaCl treatment causes inhibit plant growth. The concentration of 50 mM NaCl reduced leaves number of rice 'Inpari 35'. Application of 2 mM $CaSiO_3$ increased plant height and leaves number under 50 mM NaCl treatment. Chlorophyll level, carotenoid level, proline level, relative water content, and membrane stability index increased due to NaCl and calcium silicate treatment combination.

ACKNOWLEDGEMENTS

The authors are thankful for the facility support provided by the Faculty of Biology, Universitas Gadjah Mada. This research was supported through Lecturer and Student Collaboration Research Grant Faculty of Biology, Universitas Gadjah Mada, No. 987/UN1/FBI/KSA/PT.01.03/2021.

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