The effect of silicate fertilizer on the root development of rice and its tolerance to salinity stress

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Abstract. The problem of salinity stress in agricultural land is increasing rapidly, mainly due to climate change. Salinity in the soil has a detrimental effect on the root growth of rice and reduces the ability of the plant to absorb water and other nutrients from the soil resulting in stunted growth. The application of silicate fertilizers is an effort to reduce the negative effects of salinity stress. Absorption of beneficial element silicon (Si) by rice plants can reduce salinity stress. The objective of this research is to analyze the effect of calcium silicate on rice root growth and its tolerance to salinity stress. This research was conducted with the addition of silicate fertilizer consisting of three levels of CaSiO₃ (0 mM, 2 mM, 4 mM) in red rice (Oryza sativa L. ‘Sembada Merah’) under salinity stress treatment at the level of 0 dS m⁻¹ (control), 3 dS m⁻¹ (low), 7 dS m⁻¹ (moderate) and 10 dS m⁻¹ (high). The treatment with a salinity level of 10 dS m⁻¹ reduced root length, fresh and dry weight of the root. The addition of calcium silicate in salinity stress conditions was able to improve the root anatomical characteristics of rice ‘Sembada Merah’ by increasing the epidermis thickness, cortex thickness, stele diameter and root diameter. Silicate fertilizer is indicated to play a role in increasing suberin and lignin to form apoplast defenses in order to prevent the entry of Na⁺ ions into the stele.

1. Introduction
Changes in climate patterns have an impact on increasing soil salinity in some agricultural areas. Increased salinity in the soil causes a decrease in rice growth and yield due to osmotic stress and inhibition of water and nutrient absorption from the soil [1]. High salinity conditions affect plant photosynthesis due to stomatal closure, chlorophyll breakdown, decreased enzymatic activity and damage to photosynthetic membranes and chloroplasts ultrastructure [2]. Accordingly, a strategy for rice cultivation must be developed to minimize the negative effects of salinity and to maintain rice productivity. Silicate fertilizer, which is a source of silicon, is known to increase the growth of rice plants under stress conditions [3]. The application of silicate fertilizer is expected to improve the resistance and productivity of rice plants under salinity stress conditions.

Root is a primary plant organ responsible for water and nutrient absorption. With regard to salinity tolerance, the root organ is one of the supporting parameters of the plant tolerance to salinity stress [4][5]. When the roots experience a water deficit due to salinity stress, growth and differentiation of root cells are inhibited which reduces root length and root diameter [4][6]. Changes in root development and anatomical characteristics are a response of plant to adapt to the salinity stress. Anatomically, salinity...
affords cell division and elongation thereby reducing the size of the apical meristem, cortex and diameter of the stele. In addition, salinity stimulates suberization in the exodermis and endodermis walls [6][7]. Krishnamurthy [8] reported that plants under salinity stress showed a thickening of the casparian strip as a barrier to the transport of Na⁺ ions into the stele. Therefore, the analysis of the anatomical characteristics of the root organ provides a new perspective on the effect of salinity stress on root structure.

Silicon is not an essential nutrient but is beneficial for plants as it plays a role in increasing plant growth and development, particularly when under stress conditions. Silicon also has a role in increasing nutrient availability, reducing nutrient toxicity, and helping plants survive in abiotic stress conditions such as salinity, radiation, high temperatures, etc [9][10]. Previous studies reported that the application of silicon can affect cell density and help increase cell strength [10]. Silicon makes the stems stronger and resistant to drop as it increases the lignification and silicification processes of sclerenchyma cells as well as increases the content of cellulose. In addition, silicon is effective in increasing the thickness of the cell wall and the size of the vascular bundle which makes the stem stronger [9]. Fleck et al. [11] also reported that Si treatment could increase suberization and lignification of sclerenchymes in root endodermis and exodermis tissues and contribute to the reduction of radial oxygen loss and oxidation power in mature roots.

The potential of silicate fertilizers in relation to tolerance to salinity stress can be determined by observing and analyzing changes in the development and anatomical root characteristics after the application of silicate fertilizers under salinity stress conditions. In this study, we investigated the effect of silicate fertilizer in root development based on the following parameters, root length, fresh and dry weight of the root, and root anatomical characteristics including epidermis thickness, cortex thickness, stele diameter and root diameter. Through the study of root development and anatomical characteristics, the important role of beneficial silicon element under salinity stress can be defined.

2. Materials and methods
This experiment used a completely randomized design with two factors, calcium silicate and salinity stress. Calcium silicate consisted three level of CaSiO₃ (0 mM, 2 mM, 4 mM) and salinity stress consisted of 0 dS m⁻¹ (control), 3 dS m⁻¹ (low), 7 dS m⁻¹ (moderate) and 10 dS m⁻¹ (high) with 5 replications for each treatment combination. Si application is given after 14 DAP, while salinity stress is given at the age of 28 DAP. The measurement of root growth and morphology, including root length, root fresh and dry weight, as well as anatomical characteristics of the root, were conducted at the end of the vegetative phase. The root length is measured at the longest part of the root from the base to the tip of the root. Root fresh weight was measured by weighing the roots after harvesting, while dry weight was measured by weighing after the roots had been dried and reached a constant weight.

For observation of root anatomical characteristics, root samples approximately 1.5 cm from the tip were collected at 70 DAP and prepared for anatomical slides using the paraffin embedding method with a simple staining method with safranin [12]. The root anatomical characteristics including epidermis thickness, cortex thickness, stele diameter and root diameter were observed using a binocular light microscope (Boeco BM-180/SP, Germany). The analysis of the root anatomical characteristics was performed using the Optilab and Image Raster 3.0 (Miconos). Analysis data were conducted using ANOVA and followed by Duncan’s test at 95% confidence level.

3. Results and discussion
Salinity stress has a negative effect on root growth such as root length and root weight, since roots are plant organs which are in direct contact with salt ions in the soil [3][4]. High salt content in the soil, particularly in root areas, can cause a decrease in groundwater potential and water availability. This disorder contributes to cellular dehydration and osmotic stress [13]. Salinity also causes the reduction of water absorption by the root cells, which results in inhibition of the division process and expansion of the root cells causing a decrease in root length and root weight.
Figure 1. Root morphology of rice ‘Sembada merah’ after calcium silicate and salinity treatment. Bar = 10 cm. S: CaSiO\textsubscript{3} application 0 mM (S0), 2 mM (S1), and 4 mM (S2). N: salinity stress 0 (N0), 3 dS m\textsuperscript{-1} (N1), 7 dS m\textsuperscript{-1} (N2) and 10 dS m\textsuperscript{-1} (N3).

Salinity stress treatment causes inhibition of the growth of the root system. Based on morphological observations, the salinity level of 10 dS m\textsuperscript{-1} without calcium silicate (S0N3) showed that the root growth of rice ‘Sembada Merah’ appeared to be thinner, smaller and shorter. The application of calcium silicate to 10 dS m\textsuperscript{-1} salinity-treated plant showed a thicker root growth and a higher fresh weight compared to calcium silicate-free treatment (Figure 1). The addition of calcium silicate is a source of silicon, a beneficial element for plants. It indicates that silicon functions in minimizing the impact of salinity by increasing root length, root fresh and dry weight (Table 1).

The root fresh weight of rice treated with calcium silicate tended to be higher than that of control (Table 1). This might be due to accumulation or compartmentation of water and salt into the rice root vacuole, as shown in Figure 1, the condition of the rice roots in the 10 dS m\textsuperscript{-1} salinity treatment appeared dark brown and wetter than the other treatments. Munns [14] reported that the increase in fresh weight of the root is probably due to the ability of the plants to increase the size of the vacuole to store water. Meanwhile, if confirmed from the results of the root dry weight, it was shown that the salinity treatment without calcium silicate showed that the root dry weight was lower than the control (Table 1). Thus it can be stated that the photosynthate in the roots was lower in the salinity stress treatment compared to the control and calcium silicate treatment. This result is in line with the research of Nabati et al. [15] salinity stress reduces the amount of photosynthetic pigments, inhibits the photosynthesis process and reduces the dry weight of rice roots.

Table 1. Root length, root fresh and dry weight of red rice (Oryza sativa L. ‘Sembada Merah’) subjected to application of calcium silicate and salinity stress

| Parameter                  | Salinity level (dS m\textsuperscript{-1}) | Level of CaSiO\textsubscript{3} (mM) | 0     | 2     | 4     |
|----------------------------|------------------------------------------|------------------------------------|-------|-------|-------|
| Root length (cm)           |                                          |                                    |       |       |       |
| 0                          |                                          | 30.12\textsuperscript{ab}          | 31.37\textsuperscript{b}          | 39.62\textsuperscript{b}          |
| 3                          |                                          | 32.25\textsuperscript{ab}          | 39.87\textsuperscript{b}          | 35.87\textsuperscript{b}          |
| 7                          |                                          | 30.50\textsuperscript{ab}          | 36.12\textsuperscript{b}          | 33.50\textsuperscript{ab}         |
| 10                         |                                          | 25.62\textsuperscript{a}           | 34.00\textsuperscript{b}          | 37.00\textsuperscript{b}          |
| Root fresh weight of root (g) |                                           | 6.20\textsuperscript{a}           | 9.63\textsuperscript{ab}          | 9.18\textsuperscript{ab}          |
| 3                          |                                           | 9.55\textsuperscript{ab}           | 11.55\textsuperscript{bcd}        | 6.98\textsuperscript{ab}          |
| 7                          |                                           | 7.23\textsuperscript{ab}           | 13.02\textsuperscript{bcd}        | 10.72\textsuperscript{abcd}       |
| 10                         |                                           | 9.45\textsuperscript{a}           | 16.30\textsuperscript{cd}         | 16.95\textsuperscript{d}          |
| Root dry weight (g)        |                                           | 3.00\textsuperscript{abcd}         | 5.10\textsuperscript{cd}          | 5.13\textsuperscript{d}          |
| 3                          |                                           | 4.63\textsuperscript{bcd}          | 4.28\textsuperscript{bcd}         | 3.28\textsuperscript{abcd}        |
| 7                          |                                           | 2.95\textsuperscript{ab}           | 3.93\textsuperscript{abcd}        | 4.20\textsuperscript{bcd}         |
| 10                         |                                           | 2.08\textsuperscript{a}           | 4.50\textsuperscript{bcd}         | 4.60\textsuperscript{bcd}         |

Note. The number followed by the same letters in the same parameter does not show significant difference at 95% confidence level based on the Duncan’s test.
Changes in plant root anatomical characteristics are a response to the presence of salinity stress. Table 2 shows that an increase in salinity level decreases the thickness of epidermal root cells. In the treatment without calcium silicate, some epidermal cells were damaged so that the thickness of the epidermis was smaller than that of the control (Figure 2). Under normal conditions, the epidermis has a circular shape, closed and organized around the cortex. Meanwhile, when the plant is in a condition of salinity stress, the epidermis is compressed so that the shape is flattened with an irregular arrangement. In addition, the thickness of the cortex will also change due to salinity stress. The thickness of the cortex decreases as cells shrink in size [16]. The decrease in cortex thickness is directly proportional to the increase in NaCl concentration [17]. The stele diameter also decreased due to the salinity stress as the parenchyma cells of the stele had decreased in size [16]. This was also mentioned by Krishnamurthy et al. [8] that plants under salinity stress conditions showed a thickening of the casparian strips as a barrier to the transport of Na+ ions to the stele. The combination of Si and salinity stress has significant effect on epidermis thickness, cortical thickness, stele diameter and root diameter (Table 2).

Table 2. Epidermis thickness, cortex thickness, stele diameter, and root diameter of the roots of rice ‘Sembada Merah’ subjected to application of calcium silicate and salinity stress

| Parameter               | Salinity level (dS m⁻¹) | 0          | 2          | 4          |
|-------------------------|-------------------------|------------|------------|------------|
| Epidermis thickness (μm)| 0                       | 17.00 cde  | 19.50 df   | 16.25 cde  |
|                         | 3                       | 12.25 b    | 14.00 bc   | 22.75 f    |
|                         | 7                       | 17.25 cde  | 15.75 bnde | 18.25 de   |
|                         | 10                      | 7.00 a     | 14.50 bde  | 17.75 cde  |
| Cortex thickness (μm)   | 0                       | 301.55 b   | 353.00 bcd | 303.92 b   |
|                         | 3                       | 209.06 a   | 408.57 de  | 432.44 e   |
|                         | 7                       | 333.46 bc  | 406.42 de  | 372.14 bde |
|                         | 10                      | 364.30 bde | 377.03 cde | 434.33 e   |
| Stele diameter (μm)     | 0                       | 206.90 cde | 194.62 abc | 180.24 a   |
|                         | 3                       | 183.90 a   | 203.12 bcd | 221.14 e   |
|                         | 7                       | 251.74 f   | 219.99 de  | 203.09 bcd |
|                         | 10                      | 189.10 ab  | 210.14 cde | 246.12 f   |
| Root diameter (μm)      | 0                       | 833.93 b   | 880.57 bc  | 843.34 bc  |
|                         | 3                       | 629.87 a   | 1018.00 cde| 1059.40 def|
|                         | 7                       | 1002.40 bde| 1130.60 ef | 932.20 bcd |
|                         | 10                      | 914.03 bde | 932.44 bcd | 1219.40 f  |

Note. The number followed by the same letters in the same parameter does not show significant difference at 95% confidence level based on the Duncan’s test.

The salinity treatment significantly affected (p = 0.05) epidermis thickness, cortex thickness, stele diameter, and root diameter (Table 3). Cortex thickness and root diameter tend to increase with increasing salinity, probably because rice plants have the ability to localize Na+ ions into the cortex. The compartmentation of Na+ ions into the cortex will limit the number of Na+ ions that enter the xylem [18]. Increasing the cortex thickness under salinity stress is a plant tolerance mechanism by improving the storage capacity of Na+ ions [19]. The addition of calcium silicate significantly (p = 0.05) increased epidermis thickness, cortex thickness and root diameter, but does not affect the diameter of the stele (Table 3).
Table 3. Growth and anatomical root characteristics of rice (*Oryza sativa* L. ‘Sembada Merah’) treated with calcium silicate under salinity stress

| Treatment | Root length (cm) | Root fresh weight (g) | Root Dry weight (g) | Epidermis thickness (μm) | Cortex thickness (μm) | Stele diameter (μm) | Root diameter (μm) |
|-----------|------------------|-----------------------|---------------------|--------------------------|-----------------------|---------------------|---------------------|
| CaSiO₃ level |                  |                       |                     |                          |                       |                     |                     |
| 0         | 29.62⁺          | 8.106⁺                | 29.62⁺              | 13.38⁺                   | 302.09⁺               | 207.92⁺             | 845.06⁺             |
| 2 mM      | 35.34⁽⁄⁾        | 12.62⁽⁄⁾             | 35.34⁽⁄⁾            | 15.94⁽⁄⁾                 | 386.25⁽⁄⁾            | 206.96⁽⁄⁾           | 990.15⁽⁄⁾           |
| 4 mM      | 36.50⁽⁄⁾        | 10.95⁽⁄⁾             | 36.50⁽⁄⁾            | 18.75⁽⁄⁾                 | 385.71⁽⁄⁾            | 212.65⁽⁄⁾           | 1013.6⁽⁄⁾           |
| Salinity level |                  |                       |                     |                          |                       |                     |                     |
| 0         | 33.70⁽⁄⁾        | 8.333⁽⁄⁾             | 4.408⁽⁄⁾            | 17.58⁽⁄⁾                 | 319.49⁽⁄⁾            | 193.93⁽⁄⁾           | 852.62⁽⁄⁾           |
| 3 dS m⁻¹  | 36.00⁽⁄⁾        | 9.258⁽⁄⁾             | 4.058⁽⁄⁾            | 16.33⁽⁄⁾                 | 350.02⁽⁄⁾            | 202.72⁽⁄⁾           | 902.41⁽⁄⁾           |
| 7 dS m⁻¹  | 33.37⁽⁄⁾        | 10.32⁽⁄⁾             | 3.691⁽⁄⁾            | 17.08⁽⁄⁾                 | 370.67⁽⁄⁾            | 224.94⁽⁄⁾           | 1021.7⁽⁄⁾           |
| 10 dS m⁻¹ | 32.20⁽⁄⁾        | 14.23⁽⁄⁾             | 3.725⁽⁄⁾            | 13.08⁽⁄⁾                 | 391.89⁽⁄⁾            | 215.12⁽⁄⁾           | 1021.6⁽⁄⁾           |

Note: Means within the column with different letters showed significant difference at p<0.05 using the Duncan’s test.

Figure 2. Cross section of periphery root of rice (*Oryza sativa* L. ‘Sembada Merah’) treated with calcium silicate under salinity stress. CaSiO₃ application 0 mM (S0), 2 mM (S1), and 4 mM (S2). Salinity stress 0 (A), 3 dS m⁻¹ (B), 7 dS m⁻¹ (C) and 10 dS m⁻¹ (D). Bar: 30 μm; Ep = epidermis; Ex = eksodermis; C = cortex.
Figure 3. Cross section of the root stele of rice (Oryza sativa L.) ‘Sembada Merah’ treated with calcium silicate under salinity stress. CaSiO$_3$ application 0 mM (S0), 2 mM (S1), and 4 mM (S2). Salinity stress 0 (A), 3 dS m$^{-1}$ (B), 7 dS m$^{-1}$ (C) and 10 dS m$^{-1}$ (D). Bar = 20 µm. en=endodermis; f = phloem; x = xylem p = pericycle; e = empulur; (*) = the wall thickening.

The application of silicon can increase the thickening of endodermis cell wall in rice plants under salinity stress (Figure 3). Salinity stress affects the diameter of the stele. Increased salinity level without calcium silicate resulted in a decrease in the stele diameter. The stele structure of ‘Sembada Merah’ rice in all treatments was not damaged, only there was a difference in the diameter of the stele. Kozlowski [20] reported that salinity stimulates suberization in hypodermis and root endodermis. For most plants, suberin is deposited as a secondary wall of the casparian strip in endodermal cells. Casparian strips are a special feature of roots that have an important function, namely as barriers or preventing the entry of
ions through the apoplastic pathway from the cortex to the stele [21]. Suberin forms hydrophobic covering around the cells (except plasmodesmata) which prevents the absorption of ions from the apoplasts into the endodermal cells.

Silicon plays a role in increasing the cell wall suberization of the casparian strip in the endodermis and exodermis as well as lignification in the sclerenchyma cells [11]. Suberin and lignin formation was clearly visible in the anatomy of Si-treated rice roots (Figure 3). Si helps in the development of secondary and tertiary cells from the endodermis which induces thickening of the endodermic wall to form U type. Therefore, it allows increased tolerance and strengthens root growth [22].

4. Conclusion
The application of silicate fertilizer under salinity stress increased root length, fresh and dry weight of roots. It also improve the root anatomical characteristics of rice including epidermis thickness, cortex thickness, stele diameter and root diameter. Through the application of silicate fertilizer, the impact of salinity stress can be minimized. Silicate fertilizer is suggested as potential fertilizer to increase salinity tolerance in rice plant.

Acknowledgment
This research was supported by the Research Directory of Universitas Gadjah Mada through Final Project Recognition Programs (RTA). Letter Task No. 2488/UN1.P.III/DIT-LIT/PT/2020

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