The potential of silicate fertilizer for salinity stress alleviation on red rice (*Oryza sativa* L. ‘Sembada Merah’)

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**Abstract.** Climate change causes an increase in soil salinity, hence reduce rice growth and productivity. The application of silicate fertilizer as a source of silicon (Si) can be encouraged to enhance the rice tolerance against biotic and abiotic stresses. This study aimed to analyze the potential of silicate fertilizer to alleviate the salinity stress on red rice. A pot experiment was conducted to observe the physiological and biochemical aspects. Rice seedlings were treated with three doses of rice husk ash/RHA (0, 4, and 8 tons ha⁻¹), and three levels of salinity stress, namely control (0 dS m⁻¹), low (3 dS m⁻¹), moderate (7 dS m⁻¹), and high (10 dS m⁻¹). The observed parameters were plant height, number of tillers, plant biomass, activity of superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT), levels of chlorophyll, carotenoid and proline. The results showed the growth of red rice 'Sembada Merah' was inhibited by moderate and high salinity stress as indicated by a decrease in plant height, number of tillers and plant biomass. Photosynthetic pigment chlorophyll and carotenoid levels decreased with the increasing salinity stress. RHA application increased SOD activity as a response to enhance salinity stress tolerance. At doses of 4 t ha⁻¹, RHA could maintain rice growth up to moderate salinity stress.

1. **Introduction**

Salinity stress can be resulted by the global climate change, and may has an impact rice growth and productivity. Rice is sensitive to high salt concentrations in the soil. Salinity stress may lead to disruption on growth and production of susceptible rice cultivar. The effects of high concentration of soil NaCl on plant including three aspects, namely osmotic pressure, nutrient balance, and ion toxicity [1]. Salinity stress promoting the drought effect on plants which causes the accumulation of reactive oxygen species (ROS) to increase and disrupt the normal metabolism, as well as damage to the plasmalemma and endomembrane system [2]. An increase in ROS levels is a signal to initiate the activation of the intracellular oxidative defense system.

The response of plants in minimizing the negative impact of ROS is through the enzymatic and non-enzymatic oxidative defense systems. Non-enzymatic oxidative defenses consist of a group of hydrophilic and lipophilic antioxidant compounds including ascorbic acid, glutathione, carotenoids and α-tocopherol. Enzymatic antioxidant included superoxide dismutase, ascorbate peroxidase, catalase, and glutathione reductase as regulatory scavenging ROS [3].
Silicon (Si) is known as an effective beneficial element for Gramineae to enhance plant tolerance under biotic and abiotic stress [4]. In this study, the rice husk ash (RHA) used as silica fertilizer, which is a potential source of silicon. RHA solubility is relatively high, as it can reaches 40%-60% to provide Si that can be absorbed by plants [5]. Application of RHA from brick burning waste is reported to increase rice growth and productivity in optimal environmental conditions [6]. However, the potential of RHA to increase the tolerance of local red rice cultivars on salinity stress has not been reported. Therefore, this study presented the investigation of enzymatic defense related to salinity stress, as well as the physiological aspect of rice plants treated with rice husk ash. Furthermore, the result is expected to be an additional reference for the application of rice husk ash, as a promising Si source for alleviating salinity stress on rice.

2. Materials and methods
Materials used for this research including local red rice seeds (Oryza sativa L. ‘Sembada Merah’) obtained from Yogyakarta Assessment Institute for Agricultural Technology. RHA is prepared by burning rice husk from local rice milling at 500°C temperature to ashes. The content of SiO₂ in rice husk ash was 55.02%. Loamy sand soils containing 0.42% of SiO₂ were used for seed germination and growing medium.

This research was conducted at the Sawit Sari Research Station and Central Research Laboratory Faculty, Universitas Gadjah Mada, Yogyakarta, Indonesia. This experiment used completely randomized design with two factors including RHA doses (0, 4, and 8 tons ha⁻¹) and salinity stress (0 dS m⁻¹: control, 3 dS m⁻¹: low, 7 dS m⁻¹: moderate, 10 dS m⁻¹ high), hence resulted in 12 treatment combinations with 3 replications for each treatment. Twenty days-old seedlings were transplanted into pot containing media with rice husk ash was applied into growing medium at one week before the rice seedlings were transferred. Treatment of salinity stress was conducted one week after rice transplanting by adding the NaCl solutions. Soil salinity level was determined using a 1 water : 5 water suspension (EC1:5). The value appeared on the EC meter was converted to ECe by multiplying the value of EC1:5 according to the soil texture. The ECe of Sandy loam soil was 14 [7].

The observed parameters were plant height, number of tillers, plant biomass, activities of superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT), levels of chlorophyll, carotenoid, and proline. The plant growth including plant height and number of tiller were also observed every week during experiment. The plants were harvested 6 weeks after treatments. The shoots and roots were dried at 70°C until reached a constant weight. SOD activity in leaves was measured by improved pyrogallol autoxidation method. Absorbance of the reaction mixtures was read at λ325 nm by a spectrophotometer (Genesis UV- Scanning Thermo-Scientific). One unit of SOD activity was shown by SOD in the extract that caused 50% inhibition of pyrogallol autoxidation [8]. Catalase was measured using the method of Bergmeyer [9]. Measurement of APX used the method described by Nakano and Asada [10]. Proline levels were determined spectrophotometrically according to methods of Bates [11]. The total chlorophyll and carotenoid content were measured by a method of Yoshida [12]. Chlorophyll and carotenoid content was expressed by mg. g⁻¹ of fresh weight (FW).

The data were analyzed with ANOVA using the IBM SPPS Statistics 22. The significant difference between the means was analyzed by Duncan's Multiple Range Test with p values < 0.05.

3. Results and discussion
Rice husk ash is a promising source of Si, and a beneficial element to reduce the effects of both biotic and abiotic stress in plants [4]. The growth inhibitory effects caused by the salinity stress were observed through plant height, number of tillers and plant biomass as shown in Table 1. High salinity level (10 dS m⁻¹) generally inhibited the plant height, number of tillers, shoot and root dry weight. Salinity treatment has a significant effect on plant height. The plant height in salinity treatment was different than control, especially at moderate and high salinity levels. The increased salinity significantly decreased the plant height.
The high salinity level as environmental stress reduced the rice growth by decreasing the root capacity to absorb water because of osmotic effect [13]. Osmotic stress disrupts water balance in plants, causing plant cells to lose water and gradually reduce cell lengthening, cell division, and leaf stomata closure. Closing the stomata reduces the rate of photosynthesis due to CO₂ deficiency, thus photosynthates translocation from source to sink and carbohydrate metabolism in leaves resulting in plants growth decrease [3]. High salinity level significantly reduced shoots and roots dry weight (p<0.05).

**Table 1.** Plant height, number of tillers and biomass of red rice "Sembada Merah" at 6 weeks after rice husk ash and salinity application

| Parameter                     | Doses of RHA (tons ha⁻¹) | Salinity Level (dS m⁻¹) |
|-------------------------------|--------------------------|-------------------------|
|                               | 0 (control) | 3 (low) | 7 (moderate) | 10 (high) |
| Plant height (cm)              |             |         |              |           |
|                               | 0           | 89.50 c | 82.00 bc     | 71.67 ab  |
|                               | 4           | 84.97 bc| 81.00 bc     | 83.40 bc  |
|                               | 8           | 89.17 c | 74.50 ab     | 71.33 ab  |
| Number of tillers              |             |         |              |           |
|                               | 0           | 8.00 abc| 8.00 abc     | 7.00 ab   |
|                               | 4           | 9.00 abc| 6.00 a       | 11.00 c   |
|                               | 8           | 7.00 ab | 7.00 ab      | 10.00 bc  |
| Shoot Dry weight (g)           |             |         |              |           |
|                               | 0           | 11.37 b | 8.49 abc     | 8.52 ab   |
|                               | 4           | 11.70 b | 9.03 ab      | 10.36 b   |
|                               | 8           | 9.63 ab | 8.93 ab      | 13.77 b   |
| Root Dry weight (g)            |             |         |              |           |
|                               | 0           | 1.24 bc | 0.81 abc     | 0.65 ab   |
|                               | 4           | 1.08 abc| 1.31 bc      | 1.38 c    |
|                               | 8           | 0.78 abc| 0.79 abc     | 1.10 abc  |

The means followed by the same letters in the same parameter show no significant different based Duncan test at α = 5%

Table 1 indicates the application of RHA 4 tons ha⁻¹ showed no differences in growth parameters (plant height, shoot and root dry weight) at various levels of salinity. In general, application of RHA can increase the growth of red rice plants in salinity stress as indicated in shoot and root dry weight. The application of RHA significantly affected shoot and root dry weight. At doses 4 tons ha⁻¹ significantly increased shoot and root dry weight (Table 3).

Salinity affect the level of chlorophyll and carotenoid. Increased salinity levels tended to reduce chlorophyll and carotenoid levels of leaves (Table 2). The application of RHA increased the level of chlorophyll with a significant difference under salinity conditions (Table 3). Chlorophyll level in RHA treated plants had a higher value compared to control. Si has a role in reducing salt absorption by plants. Si element is able to increase the concentration of K⁺ in leaves by increasing the affinity of K⁺ transport [14]. Furthermore K⁺ helps to maintain high stromal pH levels that can reduce chloroplast damage due to photooxidation and protect chlorophyll degradation during stress [15]. Chlorophyll content is indirectly related to the character of plant productivity due to the role of chlorophyll in photosynthesis.

**Table 2.** Chlorophyll and carotenoid levels of leaves of red rice (*Oryza sativa* L. ‘Sembada Merah’) at 6 weeks after rice husk ash and salinity application

| Parameter   | Doses of RHA (tons ha⁻¹) | Salinity Level (dS m⁻¹) |
|-------------|--------------------------|-------------------------|
|             | 0 (control) | 3 | 7 | 10 |
| Chlorophyll |             | | | |
| mg.g⁻¹ FW   | 0           | 1.77 a | 1.35 a | 1.35 a | 1.26 a |
|             | 4           | 2.62 a | 2.21 a | 1.97 a | 1.74 a |
|             | 8           | 1.56 a | 1.99 a | 1.97 a | 1.82 a |
| Carotenoid  |             | | | |
| mg.g⁻¹ FW   | 0           | 0.35 ab| 0.19 ab| 0.12 a | 0.10 a |
|             | 4           | 0.32 ab| 0.23 ab| 0.25 ab| 0.16 a |
|             | 8           | 0.53 b | 0.27 ab| 0.33 ab| 0.26 ab |

The means followed by the same letters in the same parameter show no significantly different based Duncan test at α = 5%

Carotenoid is an accessory light harvesting pigments in photosynthesis. The treatment of salinity levels reduce carotenoid levels. A significant decrease in carotenoid levels observed in line with the
increased level of salinity stress, while the RHA application does not affect carotenoid levels (Table 3). At different level of salinity, application of RHA showed an increase of carotenoid levels. The highest levels of carotenoid is shown in plant treated with 8 tons ha\(^{-1}\) of RHA.

**Table 3.** Growth parameters of red rice "Sembada Merah" at different doses of RHA and salinity stress

| Treatment          | Plant height (cm) | Number of tiller | Shoot Dry weight (g) | Root Dry weight (g) | chlorophyll | carotenoid |
|--------------------|------------------|------------------|----------------------|---------------------|-------------|------------|
| **Doses of RHA**   |                  |                  |                      |                     |             |            |
| 0                  | 79.23            | 7.16             | 8.33                 | 0.78                | 1.43        | 0.19       |
| 4 tons ha\(^{-1}\) | 83.25            | 8.5              | 12.2                 | 0.86                | 1.83        | 0.24       |
| 8 tons ha\(^{-1}\) | 75.45            | 7.25             | 10.5                 | 1.21                | 2.14        | 0.35       |
| **Salinity level** |                  |                  |                      |                     |             |            |
| 0                  | 87.87            | 7.55             | 10.9                 | 1.07                | 1.98        | 0.40       |
| 3 dS m\(^{-1}\)   | 79.16            | 6.88             | 8.82                 | 0.85                | 1.85        | 0.23       |
| 7 dS m\(^{-1}\)   | 75.46            | 9.44             | 10.9                 | 1.16                | 1.76        | 0.23       |
| 10 dS m\(^{-1}\)  | 74.74            | 6.67             | 8.36                 | 0.74                | 1.61        | 0.17       |

Means within the column followed by different letters differ significantly at p < 0.05 using Duncan test

4. Biochemical Response

Plants respond to oxidative stress due to salinity stress through activate enzymatic and non-enzymatic defense systems [3]. Oxidative enzymes to reduce ROS include SOD, CAT and APX. Superoxide dismutase has main function to scavenge reactive oxygen species as impact of salinity stress. Plants treated with salinity stress increased SOD activity (Table 4). Application of RHA in salinity-exposed plants also increased SOD activity. The highest SOD activity was observed in plants treated with RHA (8 tons ha\(^{-1}\)) under high salinity levels (10 dS m\(^{-1}\)).

**Table 4.** Oxidative enzyme activities of red rice (*Oryza sativa* L. ‘Sembada Merah’) at 6 weeks after rice husk ash and salinity application

| Parameter | Doses of RHA (tons ha\(^{-1}\)) | Salinity Level (dS m\(^{-1}\)) | 0 (control) | 3 (low) | 7 (moderate) | 10 (high) |
|-----------|--------------------------------|------------------------------|-------------|---------|---------------|-----------|
| SOD \(^{1}\) | (U L\(^{-1}\)) | 0 | 20.868 | 20.426 | 15.848 | 14.491 |
|           |                  | 4 | 22.163 | 31.760 | absd | 41.010 | 54.775 |
|           |                  | 8 | 28.950 | 33.402 | absd | 28.761 | 46.251 |
| CAT \(^{1}\) | (U L\(^{-1}\)) | 0 | 0.238 | 0.300 | 0.204 | 0.779 |
|           |                  | 4 | 0.171 | 0.254 | 0.204 | 0.358 |
|           |                  | 8 | 0.275 | 0.183 | 0.242 | 0.079 |
| APX \(^{1}\) | (U L\(^{-1}\)) | 0 | 2.529 | 4.411 | 5.347 | 1.409 |
|           |                  | 4 | 2.714 | 2.032 | 3.603 | 2.922 |
|           |                  | 8 | 5.520 | 1.848 | 2.610 | 3.684 |
| Proline \(^{2}\) | (umol/g FW) | 0 | 0.584 | 0.589 | 0.599 | 0.602 |
|             |                  | 4 | 0.588 | 0.586 | 0.567 | 0.589 |
|             |                  | 8 | 0.589 | 0.687 | 0.589 | 0.590 |

The numbers followed by the same letters in the same parameter show no significantly different based Duncan test at α = 5%

Increased activity of antioxidant enzymes is a defense mechanism against stress. As shown in Table 4, RHA applications tend to increase CAT and APX activities under salinity stress. CAT activity increases in RHA doses of 4 tons ha\(^{-1}\) under salinity stress treatment, although it is not significantly different (p≥0.05) among salinity treatments. This shows that the Si application can increase the oxidative defense response to salinity stress. An increase in RHA dose can reduce oxidative damage as indicated by the increased activity of superoxide dismutase, catalase, and ascorbate peroxidase. These antioxidant enzymes are important components in the mechanism of neutralizing ROS to increase tolerance to stress [3, 16].

Some studies reported that Si had a role in reducing salinity effect. Sahebi et al [17] reported that application of Si concentrations in tomato leaves reduce oxidative damage as indicated by increased
activity of the enzyme catalase, superoxide dismutase, and increased protein content in tomato leaves. Daoud et al. [18] also reported the role of Si in wheat in saline conditions significantly increase the activity of superoxide dismutase and catalase in plant leaves. Increased SOD activity is shown by the decrease in hydrogen peroxide.

The results also showed the application of rice husk ash and its interaction with salinity increased SOD activity, but did not affect proline levels (Table 4). Plant synthesized proline to reduce the osmotic potential of cytoplasm, scavenging ROS, and maintain the cell turgor [18]. Proline accumulation plays a role to stabilize osmotic pressure by maintaining water balance in plants and stabilizing complex protein-enzymes. In addition, proline functions as a protective enzyme, stabilizes cell membranes and cellular structure of plants during stress conditions, and detoxifies free radicals. Proline known as an osmoprotectant to protect the biological structure of macromolecules during salinity conditions [19].

5. Conclusion
RHA is a potential source of Si, which alleviated the negative effects of salinity on red rice. Si improved plant growth indicated by the chlorophyll level, plant biomass and oxidative enzyme activity such as SOD increase. Therefore, the application of rice husk ash is a potential strategy to reduce salinity in rice.

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