Rice growth in a combined submergence and salinity stresses

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Abstract. The occurrence of submergence in agricultural land is increased by the climate change. The level of tolerance in crops depends on many factors including plant stages, duration of submergence. Level of crop tolerance to submergence is likely to decrease when other adverse factors such as salinity exist. Two experiments have been conducted to study the effect of a combined submergence and salinity on rice growth. First experiment studied the salt tolerance at early seedling stage while submerged. Split plot design was used with oxygen concentrations (with and without aeration) as the main plot and salinity levels (0.2, 50, 100 dan 150 mM NaCl) as the sub plot. Second experiment evaluated the length of submergence (one and two weeks) and salinity levels (0.2 and 50 mM NaCl) at older stages rice plants. The results showed that at early stages of seedlings, almost all growth variables have been decreased by salinity. The oxygen deficit reduced salt tolerance in rice seedlings. At older stages of rice, one-week submergence did not reduce rice growth. However, for two weeks submergence, rice submerged in saline water showed better growth compared to those submerged in non-saline water.

1. Introduction

Rice is a staple food that provides energy sources to more than three billion people in the world [1] and hold the central place in Indonesian culture. Rice cultivation covered a total of around 10 million hectares throughout Indonesia, primarily in rice fields. The greatest amount of productivity is found in this irrigated rice, which is the most intensified production system. Southeast Asia including Indonesia are expected to be seriously affected by the adverse effect of climate change. In Indonesia, the annual average temperature is projected to rise by 4.8°C by 2100 and the global mean sea level will rise by 70 cm during the time [2].

Climate change causes agricultural land to be exposed to various abiotic stresses, including flooding [3, 4] and saline condition [5-7]. Rice production system of Indonesia has become increasingly threatened by the impact of climate change [8, 9], since majority of the rice growing areas are in vulnerable regions [10, 11]. The increasing temperature and aggravating climate change might result in a decrease in rice productivity [12] and ultimately a decrease in the national rice production [13].

Rice is considered to have moderate salinity resistance [14]. Salt injury in rice is indicated by some specific symptoms, such as stunted growth, leaves rolling, white tips, and grain sterility [15]. Soil salinity restricted the rice plants growth and development and resulting in yield losses of more than 50% [16]. Some reports indicated that rice yields are reduced by 12 percent for every unit of salinity (dS m⁻¹) [17].
Salinity and waterlogging can take place simultaneously. This can occur when seawater overflows containing salt hit crops on the coastal agricultural land. However, it is not yet known about the interaction of these two limiting factors in affecting rice growth and yield.

2. Methods

2.1. First experiment
The first experiment was conducted in the glasshouse of Agricultural Faculty, Universitas Gadjah Mada, whereas the second experiment was carried out at Banguntapan experimental field at Bantul, Yogyakarta. Plant analysis was carried out at Ecology Laboratory, Faculty of Agriculture, Universitas Gadjah Mada. Dendang varieties that have resistance to high salinity were selected as planting material.

The first experiment was arranged in a split plot design, with aeration as main plot and salinity levels as sub plot. Main plot was consisted of two oxygen levels, i.e. fully aerated, and without aeration. Sub plot was consisted of three four NaCl levels, i.e. 0.2, 50, 100 dan 150 mM NaCl. The combined treatments were repeated three times. Rice seeds (var. Dendang) were germinated on plastic growth containers, which were supported with metal screens inside, to locate the seeds. NaCl treatments were imposed since starting germination. Nutrition solution consisted of total N 21.6%; Ca 17.3%; K 34.7%; Mg 6.5%; S 11.4%; P 8.6%; Fe 3.35%; Mn 1.7%; Cu 1.7%; Bo 0.87%; Zn 0.6%; dan Mo 0.023%. After 14 days in hydroponic nutrient solution, seedlings were transplanted to pots filled with mixed of non-saline soil and organic fertilizer. Seedlings were grown in the glasshouse for one month.

2.2. Second experiment
The second experiment was also arranged in split plot design, with duration of submergence as main plot and salinity levels as sub plot. Main plot consisted of three levels, i.e. without submergence, a week submergence, and two-weeks submergence. In sub plot, two levels of salinity included low salinity and high salinity. Fresh water (Electrical Conductivity of 0.3 dS m\(^{-1}\)) was used as low salinity. Whereas for the high salinity saline water was maintained at Electrical Conductivity of 4.9 dS m\(^{-1}\) throughout the experiment. Three replications were applied in all treatments. Humidity in the study site ranged from 42%–98%. Temperatures during the research ranged from 25 °C–35 °C. Variables observed included chlorophyll concentrations, leaf dry weight, crop Relative Growth Rate which were recorded at 49 days after planting, as well as Harvest Index, calculated at harvest.

2.3. Data analysis
The observation data was analyzed with variant analysis in a split-plot block design at a 95% confidence level to determine the effect of treatment on the observed variables. If significant differences were found among the treatments, data was further tested using Duncans Multiple Range Test (DMRT).

3. Results and Discussion

3.1 Early growth of seedlings in salinity and different oxygen supply
During the experiment, environmental factors were recorded. Temperature was in a range from 26°C up to 35°C. Relative humidity was from 70% - 81%. Electrical Conductivity (EC) was recorded regularly to keep the level stable along the experiment. The recorded EC measurements were 0.2, 7, 12, dan 17 dS/m for 0, 50, 100 and 150 mM NaCl, respectively. The oxygen concentration in aerated solution from time to time was between 7.5 and 9 mg/L, whereas without aeration, oxygen concentration was from 5.3 to 7.5 ppm.

The effect of oxygen supply on some growth variables was shown in Table 1. Oxygen supply did not affect germination rate or vigor index. However, seedling height, number of leaves and root length were inhibited by the low oxygen supply. The largest salinity suppression was on germination rate. The highest salinity level (150 mM NaCl) only reduced germination rate. by less than 1%, meanwhile Vigor
Index, seedling height, number of leaves and root length were inhibited by 10%, 45%, 37%, and 59% for 0.2, 50, 100 and 150 mM NaCl, respectively.

Stunted seedling growth in low oxygen conditions indicated a disturbance in the supply of energy for growth. Low oxygen could reduce the rate of respiration required to provide energy for cell maintenance, as well as for seedling growth. This was indicated by the lower seedling height, less number of leaves and shorter roots in plants under hypoxia than those in aerated condition.

### Table 1. Germination Rate (%), Vigor Index, seedling height (cm), number of leaves and root length (cm) at 14 days after sowing

|                | Germination Rate (%) | Vigor Index | Seedling height (cm) | No of leaves | Root length (cm) |
|----------------|----------------------|-------------|----------------------|--------------|------------------|
| Aerated        | 99.68p               | 67.06p      | 19.50p               | 2.37p        | 8.60p            |
| No aeration    | 99.76p               | 60.15p      | 11.34q               | 1.02q        | 4.37q            |
| 0 mM NaCl      | 99.84a               | 68.03a      | 18.33a               | 2.06a        | 10.35a           |
| 50 mM NaCl     | 100.00a              | 64.69ab     | 17.98a               | 1.78ab       | 6.90b            |
| 100 mM NaCl    | 100.00a              | 60.31b      | 15.25b               | 1.61ab       | 4.47c            |
| 150 mM NaCl    | 99.04b               | 61.37b      | 10.13c               | 1.33b        | 4.25c            |
| Average        | 99.72                | 63.60       | 15.42                | 1.69         | 6.49             |
| CV (%)         | 0.42                 | 7.57        | 12.85                | 26.14        | 26.37            |

*Interaction ( )

*Different letters indicate significant differences at P=0.05 (DMRT)

**Symbol in parentheses indicates interaction between treatments

Water and oxygen are essentially required by germinating rice to produce energy during seed respiration. Energy produced will be allocated to initiate metabolism processes in relation to plumule and radicle protrusion through the micropylar endosperm. Deficiency of oxygen may reduce the energy production from seed respiration. Having energy limitation, growth activity of the new cells is suspected to be prioritized in forming organs which play important roles in cell survival under hypoxia. Since plumule is likely to have more important role to reach water surface as soon as possible, thus the growth of rice seedlings at water deficit is merely dominated by plumule rather than radicle.

### 3.2. Plant growth in salinity and different duration of submergence

In the second experiment, a14-days rice seedlings were planted in the field. In all submergence treatments, rice leaves were kept submerged using metal screen clamped under water surface. When rice plants reached maximum vegetative phase (~ 49 days after planting), an interaction between submergence duration and salinity levels were recorded. The longer the submerged time was applied, the lower the chlorophyll was formed. The combined salinity and two weeks submergence stresses resulted in a lower chlorophyll concentration compared to those in non-submerged salinity (Figure 1). Low chlorophyll content in plants under two weeks submergence caused disruption in the photosynthesis process which results in a decrease in plant biomass accumulation, as seen in Table 2. In plants, leaves are the main source that produces assimilate products to be distributed to all parts. The greater the volume of the green leaves, the greater the proportion of assimilates can be allocated by other parts of the plant. Adding salinity to two weeks of inundation conditions could increase plant resistance to waterlogging as shown by a high leaf dry weight in salinity than those in non-saline condition (Table 2). Salinity provides many beneficial ions that can be used as additional nutrients for plants. In addition, these ions might act as cheap osmolytes which can help plants to manage the osmoregulation process to survive from immersion stress.
**Figure 1.** Chlorophyll concentrations at a combined submergence and salinity stresses on 49 days after planting. *Different letters indicate significant differences at P=0.05 (DMRT)

**Table 2.** Leaf dry weight at a combined submergence and salinity stresses on 49 days after planting

| Submergence           | Leaf dry weight (g) |       |       |
|-----------------------|---------------------|-------|-------|
|                       | Salinity (dS m⁻¹)   | 0.3   | 4.9   |
| No submergence        | 3.85                | 3.52  | 3.69 a|
| One-week submergence  | 2.74                | 2.36  | 2.55 b|
| Two weeks submergence | 0.00                | 0.64  | 0.32 c|
| Rerata                | 2.20 p              | 2.17 p| (-)   |

**Table 3.** Relative Growth Rate (RGR) at a combined submergence and salinity stresses on 49 days after planting

| Submergence           | RGR ((mg g⁻¹ DW week⁻¹)) |       |       |
|-----------------------|--------------------------|-------|-------|
|                       | Salinity (dS m⁻¹)        | 0.3   | 4.9   |
| No submergence        | 2960 a                   | 2807 a| 2884  |
| One-week submergence  | 2780 a                   | 2653 a| 2717  |
| Two weeks submergence | 0 c                      | 1647 b| 824   |
| Rerata                | 1913                     | 2369  | (+)   |

Greater leaf dry weight implied the ability of the plant to form greater biomass. This could be seen from the relative growth rate of plants which was also greater in plants that were submerged for a week and plant that were not submerged, compared to those in plants that were submerged for two weeks. At 49 days after planting, an interaction between submergence time and salinity was identified on crop relative growth rate. Rice grown in two weeks submergence in saline water had higher RGR compared to that of rice submerged in non-saline water (Table 3).
Plants that were submerged for a week or aerial plants that were not submerged had higher relative growth rate which indicated a faster accumulation of dry matter. The higher RGR would benefit plants in entering their generative phase. On the other hand, plants that were inundated for two weeks had a delay biomass accumulation due to disruption in the photosynthesis process as well as solute translocation in plant tissue. This slower biomass formation caused a delay in plant flowering (Table 4).

| Submergence               | Flowering age (days after planting) |
|---------------------------|-------------------------------------|
|                           | Salinity (dS m⁻¹) | Average |
| No submergence            | 78.0 c               | 85.7 b   | 81.8 |
| One-week submergence      | 78.3 c               | 85.3 b   | 81.8 |
| Two weeks submergence     | 0.0 d                | 93.7 a   | 46.8 |
| Rerata                    | 52.1                 | 88.2     | (+)  |
| CV (%)                    | 1.81                 |          |      |

*Different letters indicate significant differences at P=0.05 (DMRT)
**Symbol in parentheses indicates interaction between treatments.

As shown in Table 5, there was interaction between duration of submergence and salinity on harvest index. Harvest index did not decrease in one-week submergence, whereas in two weeks submergence none of the plants in non-saline condition were survive. Adding salinity cause a reduction in harvest index, both in aerial and in one-week submergence. However, when the submergence was prolonged up to two weeks in salinity, harvest index was similar to that in non-saline condition.

| Submergence               | Harvest Index |
|---------------------------|---------------|
|                           | Salinity (dS m⁻¹) | Average |
| No submergence            | 0.48 a        | 0.34 b   | 0.41  |
| One-week submergence      | 0.57 a        | 0.30 b   | 0.44  |
| Two weeks submergence     | 0.00 c        | 0.49 a   | 0.24  |
| Mean                      | 0.35          | 0.37     | (+)   |
| CV (%)                    | 19.49         |          |      |

*Different letters indicate significant differences at P=0.05 (DMRT)
**Symbol in parentheses indicates interaction between treatments.

Salinity is one of the limiting factors which inhibit rice seedlings growth. The higher NaCl concentration is added, the more energy is required by plant cells to exclude Na⁺ ions from cytoplasm to avoid ion toxicity [18]. The existence of other adverse factor such as oxygen deficiency could possibly cause reduction in the respiration processes, hence limit the energy production [19]. This was indicated by the decrease of growth variables under hypoxia. Previous studies also reported that Na⁺ ions can get into the plants through transpiration stream [20]. Salt ions will accumulate in the shoot and in time will reach toxic concentrations [21]. Adding submergence as an adverse factor to salinity will further suppress plant growth to some extent [22].

On the other hand, when the combination of these two stresses was applied at later stage of rice growth, our results were different from what had been reported previously. Treatment of salinity which was combined by submergence was initially thought to exacerbate salinity stress. This research showed...
that to some extent, the presence of inundation in saline conditions could help plant increase its resistance to survive from the two coinciding stresses.

4. Conclusion
Almost all growth variables of rice seedlings were decreased by salinity. Submergence as an adverse factor further reduce the resistance of young rice seedlings to salinity. At older vegetative stage, duration of submergence become an important factor which determine the rice survival ability. Rice growth and yield was not affected by one-week submergence. However, in two weeks submergence, rice submerged in saline water showed better growth compared to those submerged in non-saline water.

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References
[1] Wassmann R, Jagadish S V K, Heuer S, Ismail A, Redona E, Serraj R, Singh R K, Howell G, Pathak H and Sumfleth K 2011 Climate change affecting rice production: the physiological and agronomic basis for possible adaptation strategies. In: Donald L. Sparks (eds.). Advances in Agronomy. Academic Press. Burlington 111 59-122.
[2] Asean Development Bank 2009 Climate change and migration in Asia and the Pasific. Executive Summary .
[3] Miro B and Abdelbagi M I 2013 Frontiers in Plant Science 4 1-18.
[4] Talpur M A, Changying J, Junejo S A, Tagar A A and Ram B K 2013 African Journal of Agricultural Research 8 4654-4659.
[5] Kurniasih B, Greenway H and Colmer T D 2013 Physiologia Plantarum 149 222–233.
[6] Rad H E, Aref F, Rezaei M, Amiri E and Khaledian M R 2011 Ecology, Environment and Conservation Paper 17 455-462.
[7] Rao P S, Mishra B and Gupta S R 2013 Rice Science 20 284–291.
[8] Masutomi T, Takahashi K, Harasawa H, and Matsuoka Y 2009 Agriculture, Ecosystem, and Environment 131 281–291.
[9] Fahad S, Hussain S, Matloob A, Khan F A, Khaliq A, Saud S, Hassan S, Shan D, Khan F, Ullah U, Faiq M, Khan M R, Tareen A K, Khan A, Ullah A, Ullah N and Huang J 2014 Plant Growth Regulation 1-14.
[10] Suryanto P, Kurniasih B, Faridah E, Nurjanto H H, Rogomulyo R, Handayani S, Kastono D, Muttaqien A S and Alam T 2020 Biodiversitas 21 780-791.
[11] Boy R, Indradewa D, Putra E T S and Kurniasih B 2020 Biodiversitas 21 2555-2565.
[12] Budiastuti K, Chalida N, Mohamad N E A P and Tohari 2020 IOP Conference Series: Earth and Environmental Science 458 012006 .
[13] Li T, Wassmann R 2011 Modeling approaches for assessing adaptation strategies in rice germplasm development to cope with climate change (available at:http://www.fao.org/fileadmin/templates/agphome/documents/IRRI_website/irri_workshop/LP_16.pdf).
[14] Tarigan I B 2016 Ketahanan bibit padi (Oryza sativa, L.) terhadap salinitas pada ketersediaan oksigen yang berbeda Thesis (Yogyakarta, Indonesia: Universitas Gadjah Mada).
[15] Firmansyah E 2016 Tanggapan padi (Oryza sativa L. var. indica) terhadap cekaman rendaman dan salinitas Thesis (Yogyakarta, Indonesia: Universitas Gadjah Mada).
[16] Zeng L and Shannon M C 2000 Crop Science 40 996-1003 .
[17] Maas E V and Grattan S R 1999 Crop yields as affected by salinity In R. W. Skaggs and J. van Schilfgaarde (eds) Agricultural Drainage. Agron. Monograph 38. ASA, CSSA, SSA, Madison, WI pp. 55–108.
[18] Firmansyah E, Kurniasih B and Indradewa D 2016 International Journal of Science and Research 5 1880-1884.
[19] Kurniasih B, Greenway H and Colmer T D 2017 *Annals of Botany* **119** 129–142.
[20] Yeo A R and Flowers T J 1982 *Physiologia Plantarum* **56** 343-348.
[21] Kronzucker H J and Britto D T 2010 *New Phytology* **189** pp. 54–81.
[22] García de Blas B, Sen M E, Bañuelos M A and Rodríguez-Navarro A 2003 *Plant Journal* **34** 788–801.