

Shoot Growth and Yield of Rice (*Oryza sativa* var. *Indica*) in the Combined Submergence and Salinity

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Abstract: The field experiment was conducted from June to September 2015 at the Field Research Facility of Faculty of Agriculture, Gadjah Mada University, Yogyakarta. The study aims to investigate the response of the shoot growth and yield of rice on a combination of salinity and submergence. The study consisted of three submerged treatments and two salinity treatments. Overall there are six combination treatments with three replications, arranged in a split plot design. There was an interaction between duration of submergence and salinity on growth parameters (leaf area, the density of stomata, and the number of tillers) and yield parameters (number of productive tillers, harvesting age, the percentage of filled grain per clump, panicle length, and grain weight per clump). Combination of salinity and submergence increased leaf area, leaf sodium content, and time of harvest, but decreased stomatal density, number of tillers, percentage filled grain per clump, and grain weight per clump, compared to non-saline condition. Two weeks submergence in salinity decreased leaf area and percentage of filled grain per clump, compared to those at one week submergence. In non-saline water, submergence decreased the total number of tillers including productive tillers.

Keywords: rice, *Oryza sativa*, submergence, salinity, shoot, yield

1. Introduction

Rice is a staple food crops that provide energy for more than three billion people in the world [35] and become a source of income for the 200 million households in the world [34]. By 2050 the world population is expected to increase 34% compared to 2014, reaching about 9.1 trillion [6]. Demand for rice will increase following population growth.

Meeting the needs of food, including rice facing increasing challenges due to climate change that threatens the sustainability and productivity of agricultural production systems [6]. Climate change causes the rice exposed to various abiotic stress under field conditions, including immersion [5], [21], [24], [33] and high salinity stress [10], [12], [13], [15], [26]–[29]. Submerged, high salinity, and other abiotic stresses cause decreasing rice yields by more than 50%.

Submergence causes a complex abiotic stresses on plants [3], [25]. The crops level of tolerance depends on the stage of plant development, submergence depth, duration of submergence, water and air temperature, and the penetration of light through water [20]. Level of submergence tolerance is likely to decrease when other adverse factors exist [19].

According to the USDA Salinity Laboratory, saline soils can be defined as soil that has electrical conductivity of saturated paste extract (EC_e) over 4.9 dS m⁻¹ measured at a temperature of 25 °C [15], [30]. Salinity increases when there are a lot of mineral salt dissolved in water. The types of mineral salts include NaCl, Na₂SO₄, NaNO₃, MgSO₄, MgCl₂, K₂SO₄, and CaCO₃ [7]. Although in germination and early growth stages rice tolerated up to 16 dS m⁻¹ [18], however, in older growth stages, rice as a glycophyte crop have salinity tolerance limit only up to 4.9 dS m⁻¹ [15].

It is estimated that the area affected by submergence and high salinity will double in the next 50-100 years [4]. Globally

estimated at about 800-953 million ha, or 8% of the total land surface, including 20-30 million hectares of irrigated agricultural land severely damaged by an increased concentration of salt above the level of tolerance of crops [2]. Every year there is an increase of between 0.25 to 0.50 million hectares of irrigated land in the world can not produce optimally due to high salinity [25]. Salinity on agricultural land causing economic losses worth US\$ 12 billion per year [6].

Submergence and salinity stress can take place simultaneously due to sea water intrusion and overflow of water bodies that are directly related to the sea. Therefore, the problem of high salinity and submergence stress can be regarded as a unity problem. However, not much is known about the interaction of the two types of stress in influencing the shoot growth and yield of rice. Through this research, we want to know the influence of submergence and high salinity as a single factor or a combination to the shoot growth and yield of rice.

2. Materials and Methods

2.1 Experimental site

Research was conducted in Field Research Facility of Faculty of Agriculture, Gadjah Mada University, located in District Banguntapan, Bantul Regency, Yogyakarta Province, located at 113 meters above sea level. Soil used for research was regosol. Soil was slightly acidic with pH H₂O value 6.24. The soil have low available nitrogen content, organic materials, CEC value, C-organic and salinity level (Table 1). Soil nutrient deficiency was ameliorated using appropriate fertilization recommendation from Institute for Agricultural Technology (BPTP) Yogyakarta i.e. 15 t ha⁻¹ organic fertilizer, 300 kg ha⁻¹ NPK, and 150 kg ha⁻¹ Urea.

Table 1: The soil physical and chemical properties measured before the experiment was carried out

| Parameter | Value | Description |
|--|-------|-----------------|
| <i>Physical Properties</i> | | |
| - Texture | | |
| Sand (%) | 81.61 | Loamy sand |
| Dust (%) | 12.97 | |
| Clay (%) | 5.42 | |
| - Particle density (g cm ⁻³) | 2.84 | |
| - Bulk density (g cm ⁻³) | 1.6 | |
| - Porosity | 43.76 | |
| - Moisture level (%) | | |
| Original | 12.62 | |
| 0.5 mm diameter | 0.56 | |
| 2 mm diameter | 0.72 | |
| <i>Chemical Properties</i> | | |
| CEC (me 100 g ⁻¹) | 6.04 | low |
| Organic matter (%) | 1.62 | low |
| C-Organic (%) | 0.94 | low |
| pH H ₂ O | 6.24 | Slightly acidic |
| N-available (ppm) | 70.95 | Very low |
| EC soil solution (dS m ⁻¹) | 0.30 | low |

Humidity in the study site ranged from 42%–98%. Temperatures during the research ranged from 25 °C–35 °C. The rain causes higher humidity and air temperature, while lower humidity and air temperatures occur in bright daylight.

2.1. Treatment details

The design applied in this research was split plot design. The duration of submergence (R), which consists of three levels (without submergence (R0); a week submergence (R1); and a two-week submergence (R2)) as the main plot. Salinity (S) consisting of two levels (low salinity (S1) and high salinity (S2)) as the sub plot. High salinity using NaCl until the value reaches 4.9 dS m⁻¹ according to the standard test of salinity endurance by IRRI and USDA, and guarded until the end of the study, while low salinity treatment maintain salinity level at 0.3 dS m⁻¹. The main plot is arranged by using the basic design of RCBD (randomized complete block design). The experiment is repeated three times.

The experimental plot consists of 9 main plots, divided into 3 blocks as replicates. Each plot has 2 x 1 m wide, the distance between blocks is 0.5 m length and the distance between plots within a block is 0, 3 m. The distance between pot is 0.3 m and each pot consists of three plants. Dendang varieties that have resistance to high salinity selected as planting material.

2.2. Parameters record

Measure conducted twice, namely when plant enter the maximum vegetative phase (49 DAT) and the time of harvest, each represented by a single pot of each sub-plot. Measuring leaf area, stomatal density, leaf sodium content, leaves sugar content, plant dry weight and number of tillers conducted at maximum vegetative phase. Measuring number of productive tillers, harvesting age, the percentage of filled grain per clump, panicle length, and grain weight per clump conducted during harvest.

2.3. Statistical analysis

The obtained data will be tabulated and analyzed by F test to determine the effect of treatment attempted in the observed variables. If there is a significant difference between the treatments, there will be a further Duncan's Multiple Range Test (DMRT) at 5% level. Correlation analyzes were conducted to determine the relationship between variables.

3. Result and discussion

3.1 The growth

There is an interaction between the duration of submergence and salinity to the leaf area, stomatal density, and the number of seedlings at 49 DAT (Table 2). Two weeks low salinity submergence caused the death of the plant. High salinity induces expansion of the leaves on the entire duration of the submergence. This is an adaptation mechanisms call dilution, when sodium ions distributed to plants tissue and is under a toxic concentration [1].

Table 2: The combined effect of submergence and salinity on leaf area, stomatal density, and the number of tillers at 49 DAT (day after transplanting)

| Treatment | Parameter | | |
|-------------|------------------------------|--------------------------------------|-------------------|
| | Leaf area (cm ²) | Stomatal density (cm ⁻²) | Number of tillers |
| R0 S1 | 9330 b | 9822 a | 24.0 a |
| R0 S2 | 11400 a | 7422 d | 14.3 c |
| R1 S1 | 4244 d | 9822 a | 19.7 b |
| R1 S2 | 5609 c | 8489 c | 16.7 bc |
| R2 S1 | 0 e | 0 e | 0 d |
| R2 S2 | 4089 d | 9111 b | 15.7 bc |
| Interaction | + | + | + |
| CV (%) | 11.51 | 5.77 | 10.02 |

Description: (+) there is interaction; Figures followed by the same letters in the same column are not significantly different at Duncan's Multiple Range test level of 5%.

The decline in leaf area on the submergence conditions thought to be caused by damage old leaves and the low rate formation of young leaves. According to [16] submergence cause mechanical damage to the membrane due to softening of the leaf tissue. Discontinuation access plant to obtain CO₂ as photosynthesis substrate and O₂ as respiration substrates are major factors hindering the expansion of the leaves.

Submergence with low salinity inhibits leaf widening. These results are in accordance with the opinion of [31] which states that the widening of leaves in submergence conditions is due to reduced turgor pressure. Submergence cause softening plant tissue and further causes cell fluid out and lower turgor pressure. This condition is reinforced by visual inspection showed me that looked wilted leaves and roll up.

A week submergence does not cause changes in stomatal density, while high salinity lowering stomatal density on the duration of a week's submergence and without submergence. High saline submergence give the number of stomata higher than non-saline submergence.

Stomatal density is closely related to transpiration. High salinity causes plants experience drought apparent, triggering a decline in the number of stomata. In contrast, crops in low salinity submergence suffer excess water thus increasing the density of stomata. High salinity submergence is between these two conditions. Submergence can reduce the effect of high salinity that causes plants experience apparent drought due to high dissolved salt ions. Pseudo-drought occurs when the plant is not able to absorb the water from the rooting zone despite the fact that there is enough water [13], [32].

In normal conditions the solute concentration in plant cells is higher than in the soil solution. In high salinity conditions, osmotic balance between cells and the soil solution becomes impaired [36]. The osmotic pressure in soil solution is lower than the osmotic pressure in plant cells because of their high content of salt ions. It would reduce the ability of plants to take up water and mineral salts such as K^+ and Ca^{2+} [11].

Stomata density depends on the availability of water in the planting medium. As water availability decreases, the density of stomata will fall [23]. The density of stomata which can be an indicator of plant water status is closely linked to the percentage of filled grain per clump (0.84), harvesting (0.95), days to flowering (0.94), and number of tillers (0.93).

Low salinity submergence decrease the number of tillers, while high salinity just reduce the number of tillers on treatment without submergence. According to [13] although the number of tillers remained decreased at high salinity but tillering phase is the phase that is less sensitive to high salinity stress. At the end of the vegetative phase, plant face a week of submergence is able to recover and produce a number of tillers that are not significantly different from the non-submergence. High salinity decrease the number of tillers on treatment without submergence and one week submergence, while on a two-week submergence, high salinity able to produce more tillers than low salinity submergence. Two weeks low salinity submergence cause death of all tillers. These results are consistent with reports [33] that the submergence causing death in tillers due to mechanical damage. High salinity can reduce mechanical damage by increasing solute that can lowering water pressure to plant [9], [14].

There is no interaction between the submergence duration and salinity on the content of sodium and total leaves sugar content (Table 3). Different submergence duration do not give a significant difference to the sodium content of the leaf, while the saline submergence increase the leaf sodium content.

These results indicate that sodium ions can get into the plants through transpiration stream [17]. Salt ions will accumulate in the shoot and in time will reach toxic concentrations [8]. Ion toxicity can decrease cell membrane function. Damage to the cell membrane can then become the new entrance for the sodium ions.

Table 3: The combined effect of submergence and salinity on the leaf sodium and sugars content

| Treatment | Parameter | |
|--------------------|--|---|
| | Leaf sodium content (mmol g ⁻¹ DW) | Leaf sugar content (mg g ⁻¹ FW) |
| Submergence | | |
| R0 | 14.6 a | 4.8 a |
| R1 | 18.2 a | 3.6 ab |
| R2 | 14.3 a | 2.5 b |
| Salinity | | |
| S1 | 13.3 q | 3.1 p |
| S2 | 18.2 p | 4.1 p |
| Interaction | - | - |
| CV (%) | 10.61 | 2.67 |

Description: (-) no interaction; Figures followed by the same letters in the same column are not significantly different at Duncan's Multiple Range test level of 5%; DW (dry weight); FW (fresh weight)

Total leaves sugar content can indicate the plant photosynthesis rate, because the sugar is primary products of photosynthesis. The declining of photosynthesis primary products caused by interruption of the carbon dioxide supply as a substrate of photosynthesis in waterlogged conditions. In this study, there is no interaction between the duration of submergence and salinity to the sugar content. Submergence with longer duration significantly lowering the total sugar content of the leaves. High salinity proved not to cause a decrease in total sugar content of leaves than low salinity.

3.2 Yield

There is an interaction between submergence and salinity to the number of productive tillers, harvesting age, the percentage of filled grain per clump, panicle length, and grain weight per clump (Table 4). One week low and high salinity submergence did not cause a significant difference to the yield parameters compared with no submergence, except percentages of filled grain per clump. The number of tillers that can become productive tillers is determined primarily by the adequacy of growth factors, such as nutrients, water, CO₂ and O₂ gas, as well as light. Abiotic stresses such as submergence or high salinity cause the growth factor become unavailable or un-adequate.

Table 4: The combined effect of submergence and salinity on rice yield parameters

| Treatment | Parameters | | | |
|-------------|--|-----------------------|--|----------------------------|
| | The number of productive tillers (stems) | Harvesting time (DAT) | Percentage of filled grain per clump (%) | Grain weight per clump (g) |
| R0 S1 | 19.0 ab | 112 c | 94.7 a | 26.87 a |
| R0 S2 | 12.0 bc | 119 b | 71.9 d | 12.23 b |
| R1 S1 | 23.0 a | 111 c | 86.8 b | 29.59 a |
| R1 S2 | 19.3 ab | 119 b | 78.8 c | 15.13 b |
| R2 S1 | 0.0 d | 0 d | 0 f | 0.00 c |
| R2 S2 | 9.3 c | 125 a | 37.3 e | 5.81 bc |
| Interaction | + | + | + | + |
| CV (%) | 2.87 | 1.39 | 5.4 | 3.24 |

Description: (+) there is interaction; Figures followed by the same letters in the same column are not significantly different at Duncan's Multiple Range test level of 5%; DAT (day after transplanting)

One week high salinity submergence delay ripening compare to low salinity. Two weeks submergence resulting longest harvesting time. These results are consistent with the findings [14] which states that the submergence stress cause changes in the duration of the phase of rice flowering and ripening. Elongation of harvesting time is caused by delaying crop enters the generative phase, while the duration of the generative phase is not change. The analysis showed that the submergence and high salinity stress will prolong the vegetative phase. This is thought to be the implication of the slow pace of plants net assimilation rate. Plants require great energy to switch from vegetative to the generative phase.

High salinity submergence for a week increased the percentage of grain filled per clump above of the high salinity stress without submergence. These results indicate that the submergence is able to reduce the intensity of stress by high salinity. Longer duration of submergence at the same salinity level reduced the percentage of filled grains per clump higher. High salinity also lowers grain filled per clump higher than the low salinity. High salinity reduce rice yields by reducing the total number of grains per panicle and filled grain. Percentage of filled grain per clump correlated fairly closely with a sugar content of leaves (0.84). These results demonstrate the source and sink close relationship.

Submergence and high salinity stress caused an increase in empty grain through several mechanisms. Empty grain can be caused by the failure of fertilization and low translocation assimilate towards grain. In the constantly saline condition, grain yield lost is a combination of a decline in the habitus performance, increase non-productive tillers, reduction in the panicle number and length, failure of panicle initiation, number of grains per panicle, spikelet fertility, and harvest index [13], [22]. Among all these components, spikelet fertility is the biggest cause of the decline in total grain yield. High salinity is thought to cause a decrease in pollen viability because it is not able to germinate so fail to fertilize the stigma.

High salinity without submergence lowering panicles length

per hill is greater than the one week submergence. Total panicle length difference between combination treatment cause by the number of productive tiller ($R = 0.99$) and not panicle length as individual. Total panicles length per clump is also influenced by the percentage of filled grain per clump (0.89).

Grain weight per clump as the ultimate yield of rice cultivation has decreased significantly decreased in high salinity conditions. One week submergence does not cause significant changes in grain weight per clump, but the two weeks submergence causing death of the plant. High salinity stress can lower the intensity of two weeks submergence so the plants still able to enter a generative phase. The amount of grain weight per panicle is affected by the number of productive tillers ($R = 0.84$), the percentage of grain filled per clump ($R = 0.86$), and total panicle length per clump ($R = 0.86$).

4. Conclusion

According to the findings above, it can be concluded that longer duration of submergence and salinity reducing growth and yield. Adding salinity during submergence increased leaf area, leaf sodium content, and time of harvest, but decreased stomatal density, number of tillers, percentage filled grain per clump, and grain weight per clump compared to non-saline condition. Longer submergence at the same level in saline water decreased leaf area and percentage of filled grain per clump. Longer submergence in non-saline water decreased the total number of tillers including productive tillers.

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