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Barbara Hawrylak-Nowak; University of Life Sciences in Lublin, Poland; https://orcid.org/0000-0002-2935-1491

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ORIGINAL RESEARCH PAPER in PHYSIOLOGY

Comparison of the Yield of Different Rice Varieties Treated with L-Ascorbic Acid on Site-Specific Saline Soil

Wan Arfiani Barus [1*], Abdul Rauf [2], Rosmayati [2], Chairani Hanum [2]

[1] Universitas Muhammadiyah Sumatera Utara, Indonesia
[2] Universitas Sumatera Utara, Indonesia

[*] To whom correspondence should be addressed. Email: wanarfianibarus@umsu.ac.id

Abstract

We conducted a comparative study of the effect of vitamin C (L-ascorbic acid) treatment on the yield of selected rice varieties grown in site-specific saline soil; the study area was Paluh Merbau, which is situated on the east coast of North Sumatra and has a salinity of 5.9 dS/m. Salinity causes osmotic, salt-specific, and oxidative stress, which negatively affects crop yields. The aim of this study was to compare the effects of L-ascorbic acid treatment on the yield parameters of eight varieties of rice grown on saline soil. This study was based on a randomized block design with two factors. The first factor was the rice variety (Ciherang, IR 64, Lambur, Batanghari, Banyuasin, IR 42, Inpara 10, and Margasari), and the second factor was the concentration of L-ascorbic acid (0, 500, 1,000, or 1,500 mg/L). The number of empty grains per panicles, grain weight per clump, and number of filled grains per panicles increased as the concentration of L-ascorbic acid increased. L-Ascorbic acid at a concentration of 1,500 mg/L exhibited the best results, and the grain weight of Banyuasin and Batanghari varieties was 33.22 and 30.18 g, respectively; however, there was no significant difference in grain weight between the varieties. Batanghari and Ciherang were the most salt-tolerant genotypes, with a high ability to produce high number of filled grains compared to other varieties. L-Ascorbic acid treatment can promote the yields of rice varieties grown under salinity stress.

Keywords

antioxidants; *Oryza sativa*; salinity stress; vitamin C

1. Introduction

Rice is the leading staple food crop in Indonesia. Rice plantations in Percut Sei Tuan District in 2015 covered 792 hectares; farmers planted Ciherang varieties twice a year. However, the rice area has decreased each year owing to land conversion, and it affected the number of grains produced. The average grain yield is 5.6 tons/ha, whereas the potential yield is 8.5 tons/ha. The harvest is lower than the potential yield even under optimal soil conditions.

Based on the definition by the U.S. Salinity Laboratory, soil is classified as saline if the saturated extract from saline soil has an electrical conductivity (EC) $\geq$ 4 dS/m (Corwin et al., 1989). Soil conditions in the Percut Sei Tuan District are salinized owing to seawater intrusion. Soil with clay texture, pH level of 8.2, and electrical conductivity of 5.9 dS/m is classified as saline. Saline soil causes poor growth and affects rice production, and yield under this soil condition is still below its actual potential (Sulistiani, 2010). The nutrient contents are as follows: nitrogen, 0.45%; phosphorus, 76 mg/L; potassium, 1.36%; organic carbon, 2.35%; and C/N 5.20. Provision of adequate nutrients is essential to avoid reduction in rice yield (Mahmoodi et al., 2020).

The main problem with rice cultivation in saline soil is its low production. Therefore, novel methods are required to overcome the effect of salinity stress so that production can increase. Moreover, salinity stress affects yield components, such as the number of grains per panicle, number of panicles, number of active tillers, and number of filled grains per panicle (Aref & Rad, 2012; Kalita & Tanti, 2020). Salinity stress can reduce yield (Khan et al., 2019) by as much as 30% (Ashraf & Harris, 2004). Although intensification is one of the ways to increase or decrease rice production, fertile areas are no longer available. Therefore, this study aimed to optimize the use of suboptimal land, even though saline soils are widely spread over the country. Soil and water salinity are crucial in agriculture because they affect crop yields, both with respect to quantity and quality (Zamani et al., 2019).

Plants undergo physiological and biochemical modifications such as ABA accumulation and stomata closure in response to salinity stress (Ben Abdallah et al., 2016). Under salinity stress, excessive accumulation of reactive oxygen species (ROS) damages membranes, proteins, and nucleic acids (Billah et al., 2017). To prevent plants from oxidative damage, plant cells are fitted with ROS scavenging systems and enzymatic and nonenzymatic ROS scavenging systems.

Vitamin C, which is chemically referred to as L-ascorbic acid, is a vitamin found in a variety of foods and is used as a supplement. Vitamin C prevents and treats canker sores (American Society of Health-System Pharmacists, 2020), and it is involved in tissue repair and production of certain neurotransmitter enzymes to induce the functioning of several enzymes and the immune system (American Society of Health-System Pharmacists, 2020; Institute of Medicine, 2000). Plants commonly synthesize vitamin C as a part of their antioxidant defense mechanisms. Vitamin C functions through reduction mechanism to control ROS accumulation (Akram et al., 2017). It is a water-soluble small molecule that is used as a primary substrate in the detoxification of hydrogen peroxide in the ascorbate-glutathione pathway (also called the Asada–Halliwell pathway) (El-Afry et al., 2018).

In this study, vitamin C was used to prevent salt-induced oxidative stress in different rice varieties. Almost all rice varieties are unable to survive under high salt concentrations in saline areas, owing to nutritional deficiencies (ionic pressure) and inhibition of oxidative stress-induced photosynthesis. The use of salinity-resistant rice varieties and suitable methods for increasing salt tolerance may result in satisfactory grain yield under salinity conditions. The physiological and biochemical responses of plants are influenced by salinity stress, and crop yields are significantly reduced (Alami-Milani & Aghaei-Gharachorlou, 2015).

Several rice varieties in saline soils have shown differences in growth. Moreover, ascorbic acid (L-ascorbic acid) application has resulted in an interaction between L-ascorbic acid and varieties that affects the growth and yield of rice plants under salt stress. Therefore, we investigated the best varieties and favorable L-ascorbic acid concentrations to apply in paddy fields with saline soils.

2. Material and Methods

The experiment was conducted in Dusun Paluh Merbau, Sumatera Utara, Indonesia in Deli Serdang District, from July to September 2017. The location of the study area is 3°45′18″ N, 98°43′39″ E, and its altitude is 1.5 m above sea level and 1.5 to 2 km from the shore (Figure 1).

Rice (*Oryza sativa* L.) was used as the test plant. The experiment was conducted using a two-factor randomized block design (RBD) with factorial arrangement. The factorial arrangement included eight rice varieties and four L-ascorbic acid levels, and each treatment had three replicates. The first experimental factor was the rice variety, which consisted of V_1, Ciherang; V_2, IR 64; V_3, Lambur; V_4, Batanghari; V_5, Banyuasin; V_6, IR 42; V_7, Inpara 10; and V_8, Margasari. The second factor was vitamin C concentration at four levels: 0 (A_0), 500 (A_1), 1,000 (A_2), and 1,500 (A_3) mg/L. The investigated yield parameters were as follows: the number of filled grains per panicle, number of empty grains per panicle, and grain weight/clump.

Implementation of the study consisted of several phases:

Figure 1 Map of the location and appearance of the experimental plots.

- **Plot Research:** We made 96 plots (size of 1.5 m × 1.5 m and spacing of 25 cm × 25 cm) in the paddy fields, with 16 plants per plot. Of the total 16 plants per plot, eight plants were taken to be observed as samples. The sample plants were selected diagonally from each plot, ensuring that the selected sample was more objective, i.e., provided an equal opportunity for each element of the population to be selected as a sample.
- **Seedbed:** Eight rice varieties were sown in each plot. Untreated seeds were soaked for 24 hr in distilled water, then drained, and each variety was inserted into a small sack for 2 days for germination. Beds measuring 2 m × 2 m were prepared for the seeds of all eight varieties, and the seedling beds were fenced with plastic to prevent the entry of mice. Fertilizer containing urea was applied a day before the seedlings were transferred to the plots. Furthermore, the germinated seeds were sown directly in the field to help the plants adapt better during germination and early vegetative growth. The daytime temperature was 31.4 °C with 2,148 mm/year of rainfall, air humidity of 84.5%, and sunlight intensity of 166 W/m^2. Generally, farmers cultivate conventional rice without the application of L-ascorbic acid.

  After 3 weeks, the seedlings were transplanted into the research plot, which had the following conditions: EC of 5.9 dS/m and pH of 8.2. For the measurement of EC and pH values, 50 mg of soil was dissolved in 50 mL of water and stirred. The solution was poured into a glass beaker, and a pH and EC measuring instrument (the Milwaukee Instruments T76 High Range Total Dissolved Solids Pen; TDS) was dipped into the beaker. The EC and pH readings were automatically visible on the screen.
- **Treatments with L-ascorbic acid:** The experimental stages comprised plotting, seeding, application of L-ascorbic acid, and determination of yield parameters. L-Ascorbic acid in crystal form, produced by General Nutrition Corp (USA), was applied to the leaves with a 15-L Solo Mist Sprayer. The acid was applied at concentrations of 0, 500, 1,000, or 1,500 mg/L (spray volume: 15 L per treatment) at 15, 35, 55, and 75 days after the seedlings were transferred to the study area.

Data were subjected to one-way analysis of variance (ANOVA) after testing the normality of the distribution and homogeneity of variance using IBM SPSS ver. 24 software. Thereafter, Duncan's test was performed at a probability level of 0.05.

3. Results

3.1. Empty Grains per Panicle of L-Ascorbic Acid-Treated Rice Varieties

L-Ascorbic acid treatment significantly reduced the number of empty grains. Treatment of the plants with the antioxidant, especially at the highest concentration in this study, reduced the amount of empty grains in all the varieties, under saline conditions. Compared to that in the other varieties and treatments, the lowest value was obtained in Ciherang with 14.84 empty grains after the application of 1,500 mg/L vitamin C. In addition to Ciherang, other varieties that also had low number of empty grains were Inpara 10 and Banyuasin, but their values were significantly higher than that of Ciherang. Meanwhile, the amount of empty grains was generally higher in the eight varieties grown in saline soil without vitamin C treatment than those of the varieties that were treated with vitamin C (500, 1,000, or 1,500 mg/L). The different varieties with respect to the number of empty grains in decreasing order were as follows: Margasari, IR 64, Batanghari, Lambur, Banyuasin, Inpara 10, and Ciherang due to increased concentrations of vitamin C sprayed onto the leaves (Figure 2).

Treatment with 500 mg/L L-ascorbic acid resulted in a significant decrease in the number of empty grains. The number of empty Margasari grains decreased significantly compared to that of the plants that were not sprayed. The pattern of the number of empty grains of different varieties after treatment with 500 mg/L L-ascorbic acid was the same as that in the plants that were not treated; the varieties in decreasing order were as follows: Margasari, IR 64, Batanghari, IR 42, Lambur, Ciherang, Banyuasin, and Inpara 10.

Treatment with 1,000 mg/L L-ascorbic acid significantly reduced the amount of empty Margasari grains compared to that of other varieties. The decreasing order of different varieties on the basis of the number of empty grains, considering the increasing concentrations of L-ascorbic acid, was as follows: Margasari, IR 64, Batanghari, Lambur, Banyuasin, Inpara 10, and Ciherang.

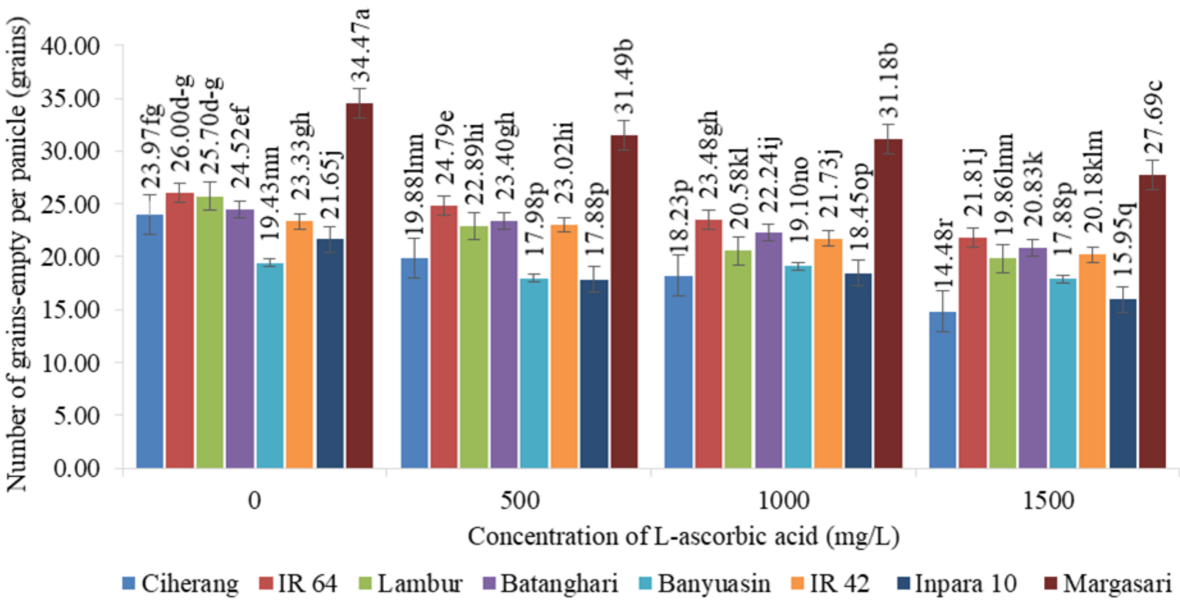

Figure 2 Effect of L-ascorbic acid treatment on the number of empty grains per panicle of eight rice cultivars grown under salinity stress. The mean values followed by different letters indicate significant difference between treatments according to Duncan's test.

In addition, 1,500 mg/L L-ascorbic acid treatment significantly reduced the number of empty grains compared to those in other concentrations, starting from Margasari and followed by IR 64, Batanghari, IR 42, Lambur, Banyuasin, Inpara 10, and Ciherang. Compared to that of the other varieties and treatments, the lowest value was observed in Ciherang, with 14.84 empty grains after the application of 1,500 mg/L L-ascorbic acid. In addition to Ciherang, other varieties that also had a low number of empty grains were Inpara 10 and Banyuasin, but their values were significantly higher than that of Ciherang.

3.2. Weight of Grain per Clump of L-Ascorbic Acid-Treated Rice Varieties

The weight of grain per clump of L-ascorbic acid-treated rice varieties is presented in Figure 3, and it shows that, in general, the Banyuasin variety had higher grain weight than other varieties, but no significant difference was observed between the plants treated with or without vitamin C.

The lowest grain weight was observed in the IR 64 variety, but the value was not significantly different from that of other varieties grown under saline soil, with or without vitamin C treatment. The order of the rice varieties on the basis of grain weight per clump from the highest to the lowest was as follows: Banyuasin, Batanghari, IR 42, Inpara 10, Lambur, Margasari, Ciherang, and IR 64.

3.3. Number of Filled Grains per Panicle of L-Ascorbic Acid-Treated Rice Varieties

Treatment with vitamin C solution caused a significant difference in the number of filled grains among the eight rice varieties. The highest number of filled grains was observed in the plants treated with 1,500 mg/L vitamin C, namely Ciherang and Batanghari, compared to that of the other six varieties. Both treatment with a lower concentration of vitamin C and no treatment resulted in a significantly lower amount of filled grains (Figure 4).

Generally, the number of filled grain per panicle was higher in the following four varieties grown in saline soil: Batanghari, followed by Ciherang, IR 64, and Lambur. Meanwhile, the number of filled grains was the lowest in Banyuasin, followed by IR 42, Inpara 10, and Margasari (Figure 4).

Treatment with 500 mg/L L-ascorbic acid caused the number of filled grains to be significantly different between the varieties tested. Batanghari had the highest number of filled grains; however, it was not significantly different from that of

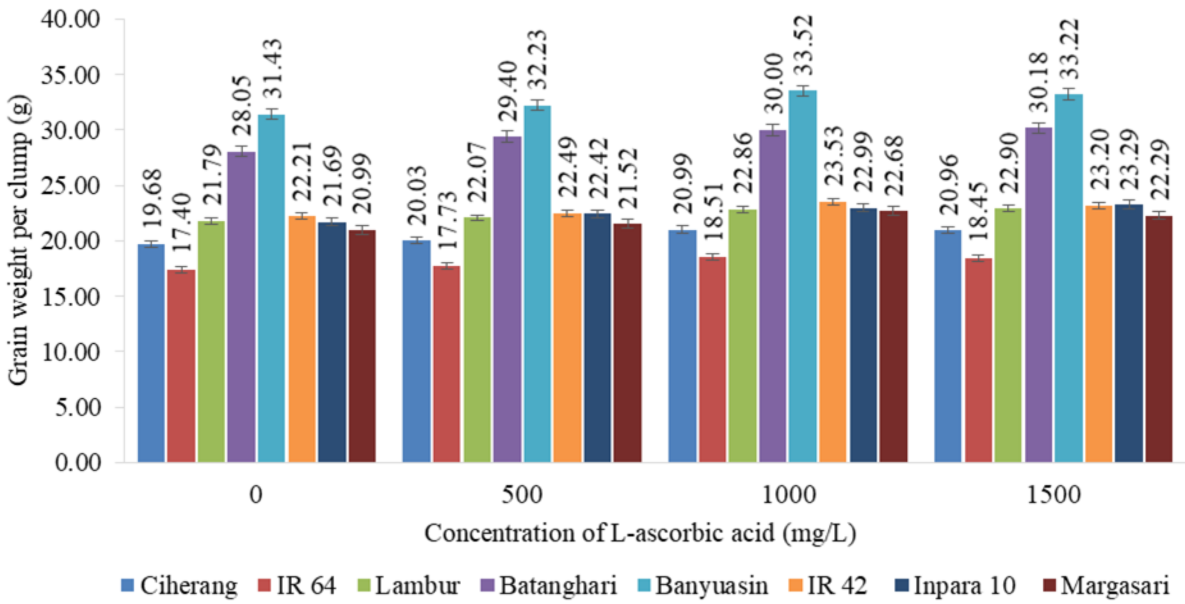

Figure 3 Effect of L-ascorbic acid treatment on the grain weight per clump of eight rice varieties grown under salinity stress. The mean values followed by different letters indicate significant difference between treatments according to Duncan's test.

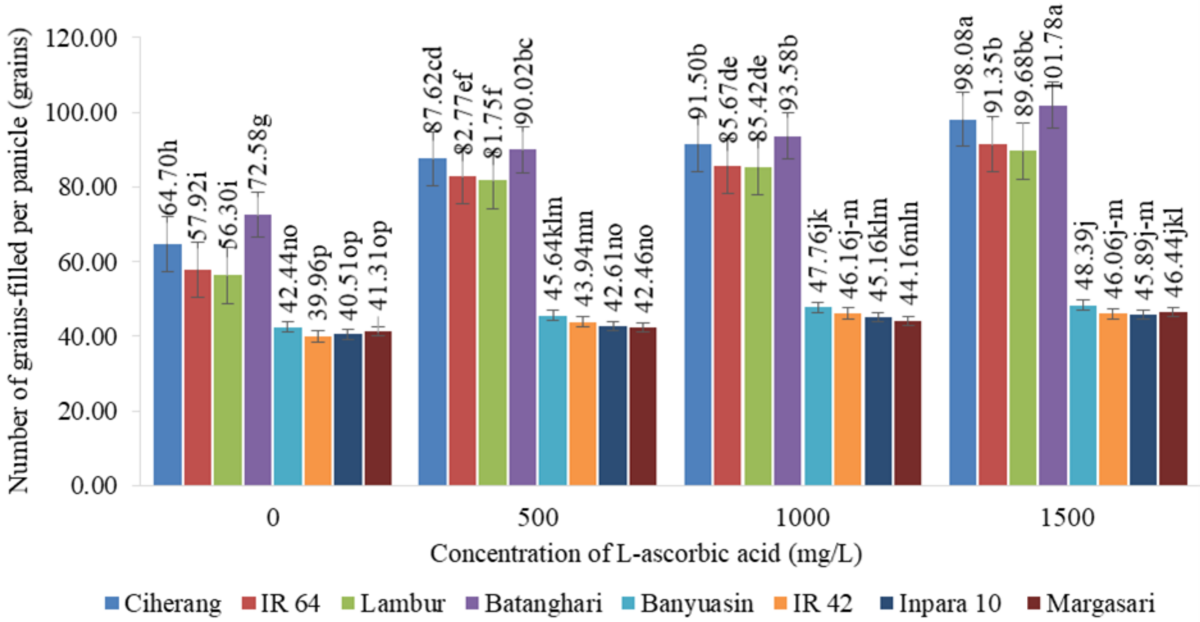

Figure 4 Effect of L-ascorbic acid treatment on the number of filled grains per panicle of eight rice varieties grown under salinity stress. The mean values followed by different letters indicate significant difference between treatments according to Duncan's test.

Ciherang, but it was significantly different from that of IR 64, Lambur, Banyuasin, IR 42, Inpara 10, and Margasari. In addition, treatment with 1,000 mg/L L-ascorbic acid exhibited the same number of filled grains for each variety as that for the 500 mg/L treatment. The application of 1,500 mg/L L-ascorbic acid caused a difference in the number of filled grains.

Compared to that of the other six varieties, we obtained the highest number of filled grains in the Batanghari and Ciherang plants treated with 1,500 mg/L L-ascorbic acid. Both treatment with a lower concentration of L-ascorbic acid and no treatment resulted in a significantly lower number of filled grains (Figure 4). Generally, the number of filled grains per panicle was higher in the four varieties grown in saline soil in the following order: Batanghari, followed by Ciherang, IR 64, and Lambur. However, the number of filled grains was lower in Banyuasin than in IR 42, Inpara 10, and Margasari.

4. Discussion

The area of cultivable rice land in the Percut Sei Tuan Subdistrict was 792 hectares in 2015, and it has decreased every year due to conversion of land into ponds and residential areas. Moreover, rice production has also decreased owing to salinity and land conversion. High levels of salinity disrupt the photosynthetic process in leaves, which is involved in the formation of assimilated and other plant products. According to Ismail et al. (2018), soybean varieties showed the highest yield reduction at 3.51 dS/M salinity. This shows that the soil salinity in Paluh Merbau village (5.9 dS/M) can reduce rice production by approximately 34%. In Percut Sei Tuan District, rice production is 6.5 tons/ha is lower than the expected harvest of 8.5 tons/ha.

Biotic and abiotic stresses inhibit the growth and development of rice plants. The increasing effects of abiotic stresses are mainly because of climate change, and rice production continues to decline, especially in areas grown under rainfed conditions (Phansak et al., 2021) and specific locations under salinity stress.

The increased concentration of foliar-application of L-ascorbic acid was associated with an increase in the number of filled grains. These findings indicate that foliar spraying with L-ascorbic acid helps to maintain rice production in areas subjected to salinity. We found that growth, cell division, and cell wall expansion of different genotypes vary when subjected to salt stress (Fageria et al., 2012). Another role of vitamin C in plant metabolism is its involvement in the ascorbate–glutathione pathway and scavenging of ROS in the chloroplast and cytosol. Foyer and Noctor (2011) reported the ascorbate–glutathione or Foyer–Halliwell–Asada pathway. The resulting scheme has been recognized as a critical player in H_2O_2 metabolism in animals and plants.

The eight rice varieties investigated in this study exhibited varying numbers of empty grains. The decreasing or increasing pattern in the number of empty grains depended on the concentration of the applied L-ascorbic acid. When the concentration of L-ascorbic acid was increased to 1,500 mg/L, the number of empty grains decreased, and when the plants were not treated with L-ascorbic acid, the number of empty grains generally increased for all varieties. This shows that genetic factors influence plant reactions (Radanielson et al., 2018).

The highest grain weight was found in Banyuasin, followed by Batanghari, IR 42, Inpara 10, Lambur, Margasari, Ciherang, and IR 64. This shows the different abilities of plants to produce different grain weight. Banyuasin had a higher grain weight than other varieties, but there was no significant difference between the plants treated with or without L-ascorbic acid.

This study shows that L-ascorbic acid can stimulate plant growth and increase the rate of photosynthesis. Ascorbic acid is an important metabolite in plants and functions as an antioxidant, enzyme cofactor, and modulator of cell signaling in various critical physiological processes (Wolucka et al., 2005), such as photosynthesis.

Grain weight is closely related to photosynthetic activity. Antioxidant molecules, such as L-ascorbic acid, are crucial for photosynthesis. L-Ascorbic acid protects organelles and cells from ROS, which accumulate excessively due to oxidative damage induced by stress (Barus & Rosmayati, 2012; Mukhtar et al., 2016; Naz et al., 2016). Disruption of photosynthesis inhibits the grain weight, and thus the plant

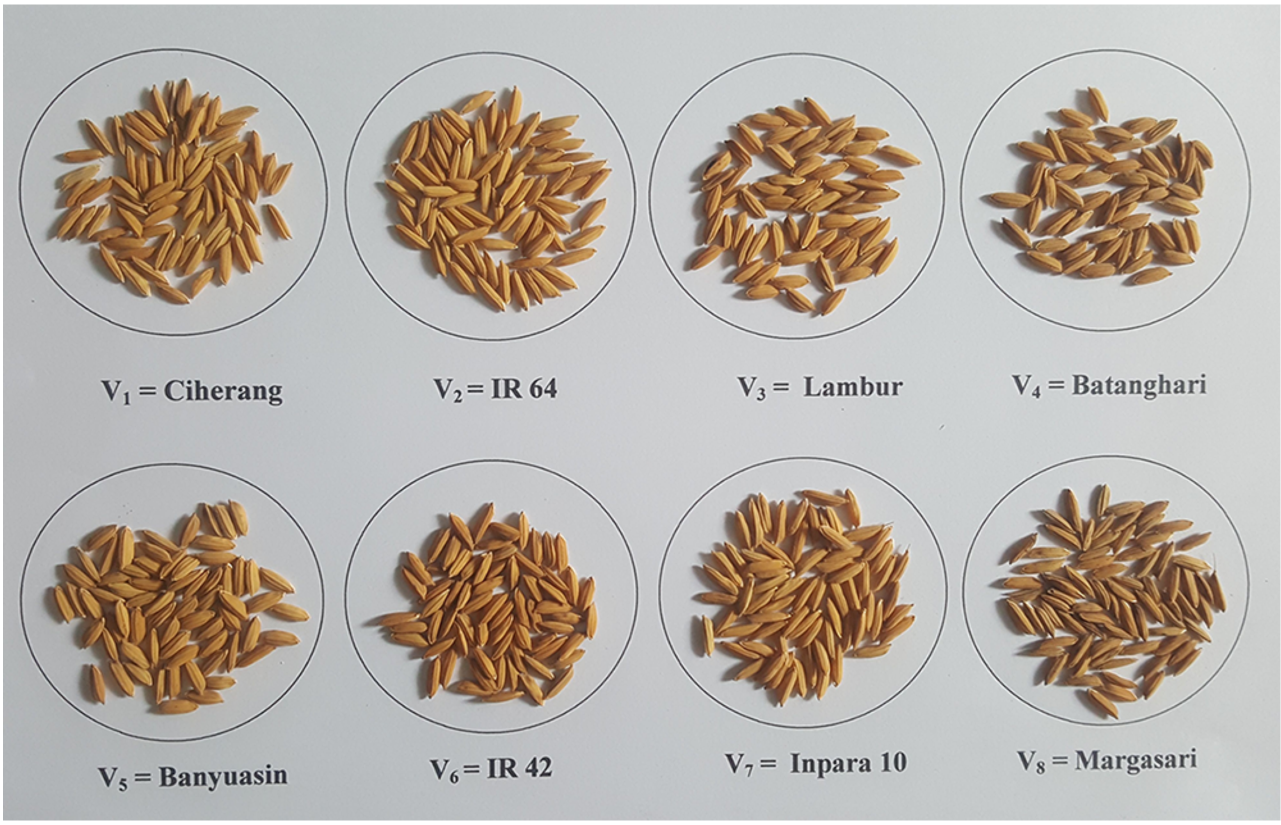

Figure 5 Morphology of grains of eight rice varieties used for experiments.

production. Abiotic stress can increase the production of secondary metabolites, such as phenolics (Sulistiani et al., 2020), consequently decreasing grain yields.

The treatment of rice with L-ascorbic acid resulted in a significant difference in the number of filled grains among the eight varieties. We obtained the highest number of filled grains in Ciherang and Batanghari under 1,500 mg/L L-ascorbic acid treatment compared to that of other six varieties. This shows that the responses of the eight rice varieties grown in saline land were different under L-ascorbic acid treatment. In general, the administration of high doses of L-ascorbic acid helps plants survive under salt stress conditions. However, the ability of plants to produce filled grains was different because grain filling is determined by genetic characteristics. Salinity affects the components of rice harvest (Aref & Rad, 2012; Khan et al., 2019) and decreases yields by up to 30% (Ashraf & Harris, 2004), especially grain production. Climate change has caused rice yields to decline to 57% lower than the baseline level (Monkham et al., 2015).

The resistance and tolerance of rice varieties to salt stress can be attributed to their ability to produce filled grains. According to Alami-Milani and Aghaei-Gharachorlou (2015), the physiological and biochemical responses of plants to salt stress can be seen from the significant reduction in crop yields. The results of this study showed that Batanghari had the highest number of filled grains, followed by Ciherang, IR 64, Lambur Banyuasin, IR 42, Inpara 10, and Margasari. The sizes of the grains of the eight rice varieties treated with L-ascorbic acid in saline soil were different (Figure 5). Genetic factors affect the response of rice to salinity stress.

5. Conclusions

Treatment with 1,500 mg/L L-ascorbic acid was the most effective in mitigating salinity-induced stress and exhibited the best results in terms of the number of grains filled per panicle. Batanghari and Ciherang genotypes were tolerant to saline soil judging based on their ability to produce the highest number of filled grains compared to that of other varieties. Banyuasin and Batanghari varieties exhibited the highest weight of grains per clump; however, there was no significant differences in the weight of grains per clump between the plants treated with or without L-ascorbic acid.

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