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## Morphophysiological Characters of Black Rice on Application of Several Sources of Antioxidants and NaCl

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### Abstract

This study aims to determine the characteristics of the morphophysiology of black rice with the use of several antioxidant sources at several levels of salinity. This study uses a randomized factorial group design, which is an antioxidant source and soil salinity level. Sources of antioxidants, consisting of; without antioxidants (A0), garlic extract (A0), Kepok banana peel extract (A0) and ascorbic acid (A0). NaCl concentration consists of 0 mM (N0), 20 mM (N1), 40 mM (N2) and 60 mM (N3). The results showed that the source of antioxidants affected the morphophysiological characteristics of black rice with different NaCl concentrations. The physiological activity of black rice decreases with an increased concentration of NaCl salt. The NaCl concentration of 60 mM causes the highest decrease in morphophysiological character and the best antioxidant source to improve the morphological and physiological character of black rice to NaCl salt stress is garlic extract.

Keywords: Black Rice, Morphophysiological Characters, NaCl, Source of Antioxidant.

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### Introduction

Coloured rice has become a strong source of antioxidants and encouragement as a decent source of antioxidants for functional food (Yawadio et al, 2007). Like black rice which is a local variety that contains pigment. Although the local rice cultivar is tested, only the callus of mountain lemongrass can develop into a planlet in the regeneration media (Muktimanur et al., 2022; Nurhayati et al., 2022). The nutritional value of black rice means has the potential to be used in healthy drinks and food production, thus providing health benefits for consumers (Ito & Lacerda, 2019; Purba et al., 2020). Of the varieties of pigmented rice, black rice receives

increased attention due to its sensory characteristics, high nutritional value, protein content, vitamins, high minerals and properties that are beneficial for health (Ito et al., 2019; Kushwaha, 2016; Suzuki et al, 2004). The main content extracted from black rice will also provide the effect of inhibiting cell invasion in various cancer cells (Chen et al., 2006). Black rice has a delicious aroma and taste with a unique and unique appearance. The color of rice is regulated genetically, due to differences in genes that regulate the colour of aleuron, endospermia, and starch composition in endospermia. Given the importance of black rice both for the sustainability of germplasm diversity and for health, its existence must be preserved immediately.

At present black rice, cultivation is still classified as rare because conservation efforts and potential results from farmers are considered still lacking (Shelley et al., 2016; Syamsafitri et al., 2023). Farmers are less interested in planting black rice because of their relatively long age, easily exposed to bacterial blight, and low results, so it is also needed the use of cultivars as one method of overcoming it (Inayah et al, 2022; Kwanwah et al., 2020; Hanifah et al., 2023). In addition, the condition of rice plants with extreme weather can cause a decrease in rice production to be very weak, even up to 0.92% (Dulbari et al., 2021). This causes the existence of local rice genetic resources to be increasingly rare. Therefore, there needs to be good conservation and utilization. Efforts to increase black rice production can be done with extensification. Therefore, marginal land is an alternative such as saline land. Barus (2016) reports that some rice varieties can grow well in saline soils in production after being applied to antioxidant acid ascorbic acid. Some antioxidants are not only in the form of ascorbic acid but in the form of other compound groups that are categorized as antioxidants. The use of ascorbic acid as an antioxidant is not cheap. On this basis, the alternative is the field of using natural antioxidants. Sources of antioxidants can be obtained synthetically or naturally, but the problem is high production costs. Another solution to this problem can also be carried out simultaneous selection phenotypics to introduce several resistance genes in rice (Wangsawan et al., 2018).

Efforts to increase rice production on saline land require a study of several aspects of land quality. It will affect the height of the plant, the condition of photosynthesis, harvest index, flowering schedule, biological results and mature age are very related to rice results (Dhakal et al., 2021; Mareza et al., 2016). Even some studies have shown that the stress of salinity can reduce enzyme activity, physiological processes and crop yields (Shereen et al., 2005; Munns et al., 2006; Ashraf, 2009; Azooz, 2009). While the application of ascorbic acid can increase the activity of antioxidant enzymes (Sakr & Arafa, 2009). In addition, it is also important to conduct other research on the technology and treatment of black rice that has been adopted to improve rice quality (Meng et al., 2018; Laokuldilok & Kanha, 2015; Li et al., 2017) and its application in food functions (Mau et al., 2018). al., 2017; SUI et al., 2016). This process certainly requires research that leads to the concept of land adjustment or soil quality to the conditions found in black rice.

## Materials and Methods

The research was carried out at the LLDIKTI-1 Greenhouse Growth Center, Ministry of Research and Technology of Higher Education of the Republic of Indonesia. This study used a factorial randomized block design (RAK) with 2 treatment factors and 3 replications. The first factor is the treatment of salinity levels which consists of 3 levels, namely: 0 (N0), 20 (N1), 40 (N2) and 60 mM NaCl (N3). The second factor is the source of antioxidants (A), namely: Control = Without Antioxidants (A0), Garlic Extract (A1), Kepok Banana Peel Extract (A2) and Ascorbic Acid (A3).

The ingredients used were Kepok banana peel, garlic, ascorbic acid, ammonium fertilizer, SP-36, KCl, acetone, fungicide, insecticide and black rice of Compo Ireng variety. While the tools used are digital scales, oven, hoe, name tag, stationery, plastic, meter, DHL meter, plastic bucket, large envelope, plastic bag and hand sprayer.

Based on the two treatments, it is obtained as follows:

Number of Treatment Combinations	= 16 combination
Number of pots in each treatment combination	= 4 pots
Number of sample plants for each treatment combination	= 2 pots
Total number of pots	= 3 x 64 = 192 pots
Number of plants per pot	= 1 plant

**The analysis used** to see the general effect of treatment is the F test at a 5% level and followed by Duncan's level test at 5%.

**Observed parameters include:**

1. Morphological characteristics, consisting of: Plant height, number of tillers, leaf area and panicle length.

2. Physiological Characteristics, consisting of: The content of chlorophyll a and b (fruit/mm<sup>2</sup>), ascorbic acid content and proline content.

3. The characteristics of the results consist of:

The number of productive tillers (saplings), the number of empty grains per panicle, the number of filled grains per panicle, the weight of dry grain per panicle, the weight of dry grain per clump (g) and the weight of 1000 grains.

**Research Implementación**

**Seed Preparation**

Dry the black rice seeds for ± 1 hour, then soak for ± 12 hours and discard the floating seeds. Drain the seeds, put the seeds in a sack that has been lined with banana leaves and close tightly, store them in a damp place. The seeds are ripened for 2-3 days until the seeds begin to germinate. Germinated seeds are ready to be stocked in plastic tray seedlings.

**Making planting media in pots according to the salt concentration NaCl treatment**

The planting media was made by slurring the top soil along with compost, manure and NaCl salt according to the treatment. Black Rice cannot use chemical fertilizers. The ratio between humus: compost: cow manure is 2:1:1.

Move the seedlings into polybags (pots).



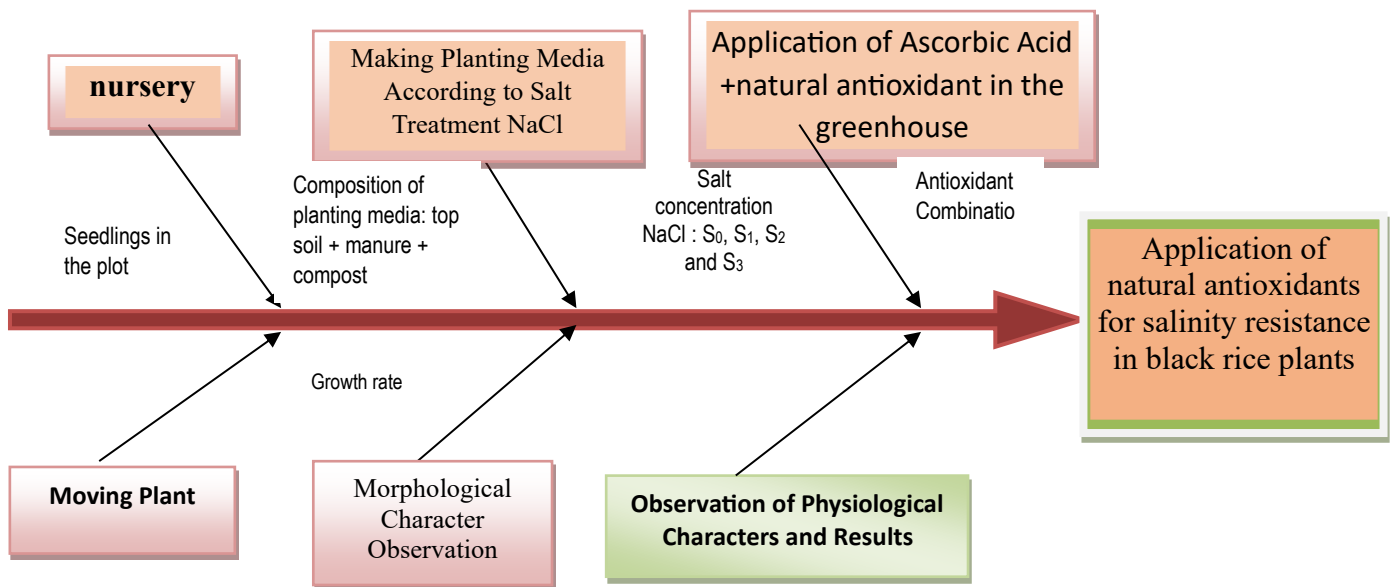
Figure 1. Illustration of Black Rice Planting in a Polybag

- **Antioxidant Application**

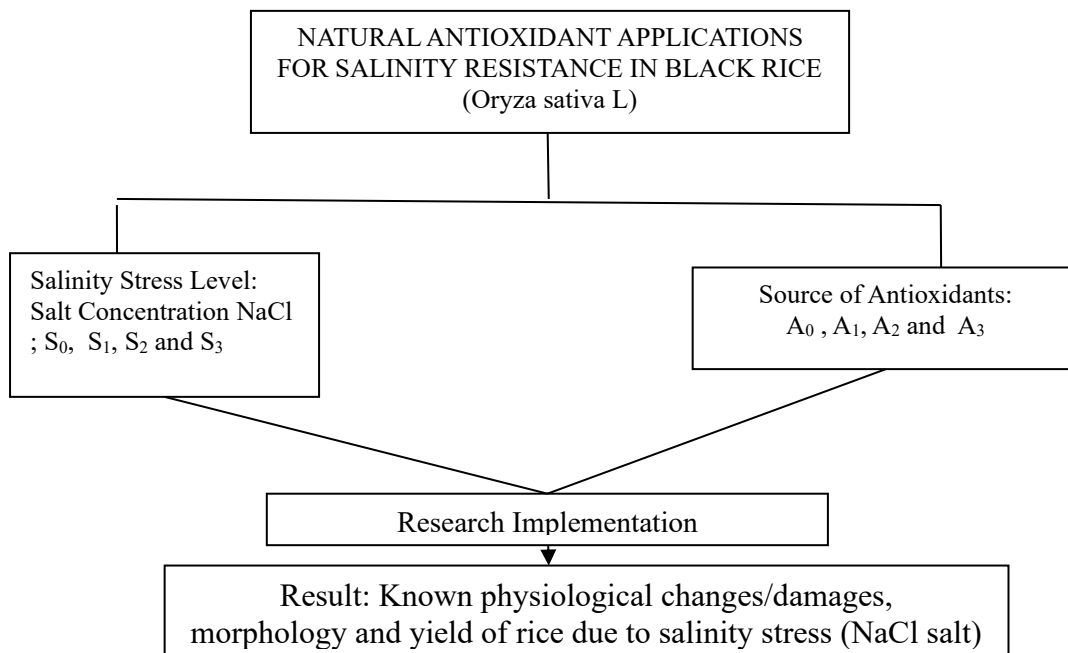
Application of ascorbic acid, kepok banana peel extract and garlic extract each with a concentration of 1000 ppm, at the age of 14, 28, 42 and 56 days after transplanting.

- **Plant maintenance and harvest.**

The system can be seen as shown in diagram 1 and chart 1 which are listed below:



**Diagram 1.** Fishbone Diagram (*Fishbone*)



**Chart 1.** Research Flow

**Results and Discussion**

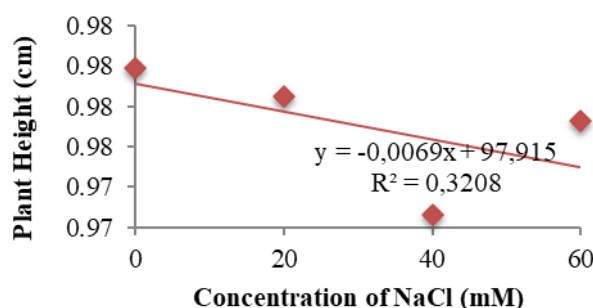
Several observational variables observed in this study were morphological characters consisting of plant height and leaf area. Another study showed that the combination of Nominee together with adjuvants can produce higher biological yields to more grain with better grain yields (Saeed et al., 2021). Components of this physiological character consist of the amount of chlorophyll, K uptake, Na uptake and proline content. Morphological characters of black rice at several levels of NaCl concentration with several sources of antioxidants can be seen in table 1.

**Table 1.** Morphological Characteristics of Black Rice at Several Levels of NaCl Concentration with Some.

Treatment	Plant Height (cm)				Leaf Area (mm <sup>2</sup> )
	2 WAP	4 WAP	6 WAP	8 WAP	
Source of Antioxidants (A)					
A <sub>0</sub> : Without Antioxidant	43.75	60.36	95.47	109.15	78.68 b
A <sub>1</sub> : Banana peel extract	42.79	60.88	97.37	110.53	85.03 a
A <sub>2</sub> : Ascorbic Acid	45.09	60.22	98.38	109.05	70.08 c
A <sub>3</sub> : Garlic extrac	50.83	64.96	99.60	105.66	88.94 a
Concentration of NaCl (N)					
N <sub>0</sub> : 0 mM	43.58 b	63.00 a	97.99	109.70	84.39 a
N <sub>1</sub> : 20 mM	54.07 a	67.73 a	97.85	105.65	83.91 ab
N <sub>2</sub> : 40 mM	41.81 b	55.01 b	97.20	108.01	77.86 bc
N <sub>3</sub> : 60 mM	43.00 b	61.68 ab	97.73	111.02	76.58 c
Interaction AxN					
A <sub>0</sub> N <sub>0</sub>	42.18	62.58	99.16	109.52	87.82
A <sub>0</sub> N <sub>1</sub>	43.89	57.13	95.46	109.97	79.23
A <sub>0</sub> N <sub>2</sub>	43.74	55.92	95.41	108.41	74.09
A <sub>0</sub> N <sub>3</sub>	45.19	65.79	91.87	108.72	73.60
A <sub>1</sub> N <sub>0</sub>	38.11	58.11	96.79	112.87	85.36
A <sub>1</sub> N <sub>1</sub>	50.42	67.22	100.40	110.06	89.97
A <sub>1</sub> N <sub>2</sub>	39.39	55.38	93.90	108.16	83.44
A <sub>1</sub> N <sub>3</sub>	43.24	62.80	98.40	111.06	81.34
A <sub>2</sub> N <sub>0</sub>	40.37	56.19	94.74	111.19	70.09
A <sub>2</sub> N <sub>1</sub>	61.64	75.53	98.99	107.24	76.07
A <sub>2</sub> N <sub>2</sub>	37.41	50.37	97.57	107.19	64.92
A <sub>2</sub> N <sub>3</sub>	40.92	60.78	102.34	110.57	69.24
A <sub>3</sub> N <sub>0</sub>	53.66	75.13	101.28	105.24	94.29
A <sub>3</sub> N <sub>1</sub>	60.31	69.03	96.67	95.36	90.37
A <sub>3</sub> N <sub>2</sub>	46.71	58.36	102.17	108.29	88.99
A <sub>3</sub> N <sub>3</sub>	42.63	57.33	98.29	113.74	82.13

Description: Number that followed by different letters in the same column indicate significantly different according to Duncan's Multiple Range Test Test on the level of  $\alpha = 5\%$

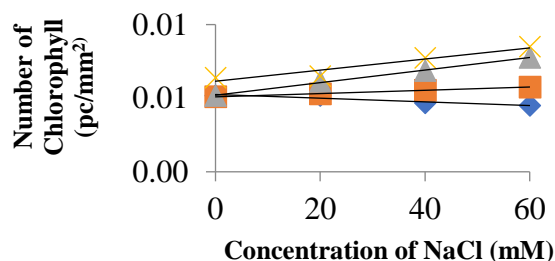
Based on Table 1, it can be seen that the application of NaCl salt had a significant effect on the morphological character of black rice plants. There is a tendency that by focusing on NaCl, there will be a decrease in morphological characters (height and leaf area) of black rice plants. Furthermore, the relationship between NaCl salt concentration and plant height (6 WAP) can be seen in graph 1.



Graph 1. Relationship between the Height of Black Rice Plants Age 6 Weeks After Moving Planting Inside Some Levels of NaCl Concentration

Based on Figure 1, it can be seen that the application of NaCl salt with a concentration of 60 mM gave the lowest growth in plants. NaCl concentration of 60 mM caused black rice plants to experience salinity stress. Based on the results of several previous studies, it was found that salinity stress resulted in inhibition of the impact on plant growth decline. In line with this, Jaleel et al. (2008) found that plants subjected to NaCl salt stress decreased plant heights of *Catharanthus roseus* by 7% and 34%, respectively. Plant growth occurs mainly due to the spatial expansion mediated by the increase in vacuole volume and the blocking of osmotic-adjusting Na+

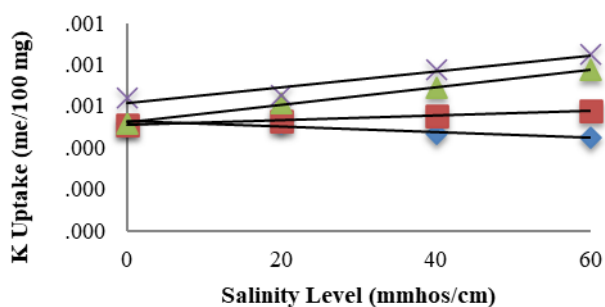
and Cl ions important for cell development (Shuji et al., 2002). Choi et al (2003) observed that plant height decreased significantly in salinity (affected). Reports that plant height will decrease along with salinity stress through osmotic effects, thereby reducing the ability of plants to absorb air (Munns, 2008; Motamed et al, 2008). Furthermore, NaCl salt stress with several sources of antioxidants also affects the physiological characteristics of black rice plants, namely the amount of chlorophyll, K uptake, Na uptake and proline content. Physiological characteristics of black rice plants at various concentrations of NaCl salt with several sources of antioxidants can be seen in Graphs 2, 3, 4 and 5.



◆ Without Antioxidant ■: Banana Peel Extract ▲ : Ascorbic Acid × : Garlic Extract

Graphs 2. Chlorophyll Number of Black Rice in several NaCl Concentration With Some Sources of Antioxidants

The salinity relationship affects two main processes, namely water and ionic relationships. At the beginning of salinity stress, plants experience water stress, which in turn reduces leaf expansion. In the long term due to salinity stress, plants experience ion stress which can accelerate the leaf ageing process (Amirjani, 2011). Salinity has three potential effects on plants: Reduced water potential, direct toxicity of any absorbed Na and Cl and associated inhibition of absorption of essential nutrients. Based on Graph 2, it can be seen that the amount of chlorophyll decreases with the increasing concentration of NaCl salt. The content or amount of chlorophyll in plants is directly correlated with plant health (Zhang et al., 2005). The resistance of the photosynthetic system to salinity is related to the plant's capacity to effectively limit the cytoplasm, vacuoles and chloroplasts (Croser et. al., 2001). Low chlorophyll content under stress conditions is caused by membrane damage (Djanaguiraman et al., 2006). The absorption of K at several concentrations of NaCl salt with several sources of antioxidants can be seen in Graph 3.

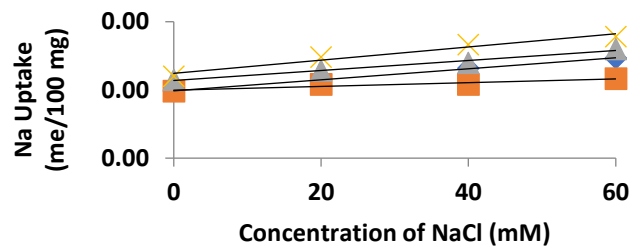


◆ Without Antioxidant ■: Banana Peel Extract ▲ : Ascorbic Acid × : Garlic Extract

Graph 3. K Uptake of Black Rice in several NaCl Concentration with Some Sources of Antioxidants

Graph 3 can be seen that the absorption of K tends to decrease with the concentration of NaCl. Priyansyah (2012) reported that plant physiological processes, especially genetic, environmental factors and genetic and environmental interactions. The air factor in plant physiology is a very important main factor because without plants there will be no life. Air is part of the protoplasm (85-90%) of the total weight of the green part (growing tissue) is air. Furthermore, it is said that water is an important reagent in the process of photosynthesis and the hydraulic process. The very important role of water has the consequence that water shortages directly or indirectly in plants will affect the entire metabolic process so that it can reduce plant growth (Sinaga, 2008).

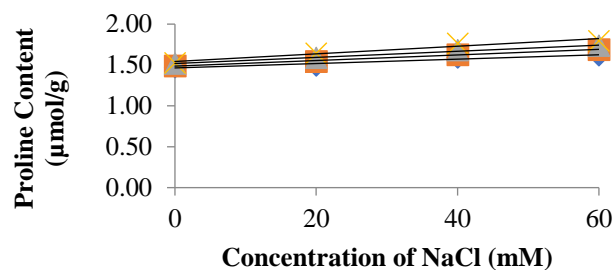
Based on the research also obtained the dynamics of the Na uptake value of black rice at several levels of NaCl concentration with the application of several sources of antioxidants can be seen in Graph 4.



◆ : Without Antioxidant   ■ : Banana Peel Extract   ▲ : Ascorbic Acid   × : Garlic Extract

Graph 4. Na Uptake of Black Rice in several NaCl Concentration With Some Sources of Antioxidants

The results of other studies showed an increase in the content of proline with a source of antioxidants from garlic extract. The relationship between the proline content of black rice leaves at various concentrations of NaCl salt with several sources of antioxidants can be seen in Graph 5 below :



◆ : Without Antioxidant   ■ : Banana Peel Extract   ▲ : Ascorbic Acid   × : Garlic Extract

Graph 5. Proline Content of Black Rice in several NaCl Concentration With Some Sources of Antioxidants

Based on Graph 5, it can be seen that the highest proline content of black rice leaves is found in antioxidant sources derived from garlic extract. Proline content is related to plant tolerance to salt stress. Organosulfur and phenolic compounds as antioxidants contained in garlic play a very important role in preventing damage to cells and organs from the oxidation process. Phenolic compounds from garlic have one or more groups, namely hydrogen proton donors and neutralize free radicals. Organosulfur and phenolic compounds as antioxidants contained in garlic play a very important role in preventing damage to cells and organs from the oxidation process. Phenolic compounds from garlic have one or more groups, namely hydrogen proton donors and neutralize free radicals.

## Conclusion

The treatment of several levels of NaCl concentration and the application of several sources of antioxidants affected the morphophysiological character of black rice plants. Morphophysiology of black rice tends to decrease with increasing NaCl concentration. The best sources of antioxidants to increase the resistance of black rice on saline soils were garlic extract, then ascorbic acid and banana peel extract. The results showed that the source of antioxidants affected the morphophysiological characteristics of black rice with different concentrations of NaCl. Physiological activity of black rice decreased with increasing concentration of NaCl salt. At a concentration of 60 mM NaCl caused the highest decrease in morphophysiological characteristics and the best source of antioxidants to improve the morphological and physiological characters of black rice against NaCl salt stress was garlic extract.

## Acknowledgement

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