

Investigation on root anatomy of rice plantation on coastal land

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ABSTRACT. Rice fields in coastal areas mainly threaten saline soil due to seawater intrusion. Tegaldlimo is a sub-district in Banyuwangi with agricultural land located in coastal areas. Rice plants on saline land will adjust as a form of survival. The research aims to investigate the salinity level of coastal land and rice plants' root anatomical structure profile (IR64, Ciherang, Pioneer) in coastal and typical areas. DHL (electrical conductivity (EC)) test to determine the salinity level and anatomical preparation observation by paraffin double staining method. EC score on the coastal land had the highest value of 1.234 dS/m, higher than the usual land, which was 0.190 dS/m. Changes in the anatomical structure of the roots were observed, i.e., the reduction of epidermal tissue shown by all varieties of rice. The expansion of the cortex functions to deviate more ions to maintain osmotic balance identified in IR 64 and Pioneer varieties. Furthermore, IR64 and Ciherang varieties performed an exodermis lignification. Wide variation among varieties in anatomical changes probably results from plant adjustment regarding higher salt content than typical land.

Keywords: Anatomy; rice varieties; root modification; salinity level; Tegaldlimo

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INTRODUCTION

Tegaldlimo, a sub-district in Banyuwangi with a large coastal area, is indicated to have relatively high soil salinity (Endarwati *et al.*, 2021; Hidayah *et al.*, 2021). Land use in Tegaldlimo is primarily agricultural (BPS Kabupaten Banyuwangi, 2021). Rice fields in coastal areas mainly threaten saline soil (Fahad *et al.*, 2018; Yichie *et al.*, 2018; Hua Qin, 2020). Some factors of high soil salinity include seawater intrusion, saline irrigation water, and high evaporation with low rainfall (Kusmiyati *et al.*, 2015; Zeeshan *et al.*, 2020). Rice on saline land will experience stress in vegetative and generative phases, with yield losses reaching 4-5 tons/ha (Reddy *et al.*, 2017; Fahad *et al.*, 2018; Yichie *et al.*, 2018). Optimizing rice production on saline land is essential due to the continuous reduction of agricultural land and increased food demand per year.

Rice plants in saline conditions will generally experience stress, namely osmotic stress and ion toxicity, causing the absorption decrease of soil minerals (Reddy *et al.*, 2017; Zeeshan *et al.*, 2020). The higher the salt concentration in the soil, the more water will move out of the plant cells. This causes the protoplasmic wall to shrink and the cell to plasmolyze. The ideal limit of saline tolerance for rice plants is an Electrical Conductivity (EC) score of fewer than four ds/m (Rachman *et al.*, 2018; Sembiring *et al.*, 2020). Electrical conductivity reflects the dissolved salt content. So that the electrical conductivity value increases as the salt content increases.

Plants in saline stress conditions will alter physiology, anatomy, and morphology to improve plant water status. The physiological response of saline-stressed plants is indicated by low photosynthetic ability, reduced chlorophyll content, and active antioxidant enzyme activity (Yichie *et al.*, 2018; Nassar *et al.*, 2020; Zeeshan *et al.*, 2020; Cárdenas-Pérez *et al.*, 2022). Changes in morphological and anatomical characteristics, i.e., a decreased thickness of epidermal tissue, both cortex and vascular cell increment, as well as thickening of the endodermis and sclerenchyma tissue in both roots and stems (Kusmiyati *et al.*, 2015; Kuster *et al.*, 2018; Wasim & Naz, 2020). In leaves, there is a reduction in stomata and bears saline-specific tissues such as bulliform cells and trichomes. The modified form generally aims to balance cell osmosis pressure through larger cell storage space and suppressed transpiration. This research investigates changes in the root anatomy of three rice varieties of IR 64, Ciherang, and Pioneer in the coastal lands of the Tegaldlimo sub-district.

MATERIALS AND METHODS

Location and rice varieties. The study duration was three months and was located in the Tegaldlimo sub-district Banyuwangi East Java (-8°36'47.3"S 114°16'17.8"E) which targeted the coastal area (< 4 km from the coastline) and typical land. The rice varieties observed were 2-month-old of IR 64, Ciherang, and Pioneer.

Soil analysis. Soil analysis was observed using soil characteristics, including pH, structure and texture, and salinity. Soil pH was measured with Takemura DM-15 Soil Tester. The soil pH clustering uses Indriana *et al.* (2020) as a reference with a value range of 3-8. Soil texture and structure were determined following the Rayes method (2017), Rinaldi and Irawan (2020), and Neswati *et al.* (2020). Soil texture is the relative ratio between sand, dust, and clay fractions whose effective diameter is ≤ 2 mm. Soil structure is used to describe the arrangement of soil particles. Soil salinity score was analyzed by DHL (Electrical Conductivity (EC)) test using Crison EC-Meter Basic with pre-treatment of the extract method by Muliawan *et al.* (2016). The soil samples were taken at a depth of 20 cm, and then the soil was dissolved in distilled water in a ratio of 1:1 for 30 minutes. The value read on the Crison EC-Meter Basic device in units of $\mu\text{S}/\text{cm}$ (micro-Siemens per centimeter) or dS/m (decisiemens per meter) indicates the number of electrolytes dissolved in the soil. The values become a basis for soil salinity categorizing, according to Abrol *et al.* (1988).

Root analysis. Root anatomy observations took 10 cm long root samples from the root tip fixed with FAA solution. Root tissue was prepared with the paraffin method with safranin and fast green staining. Root imagination (epidermal, exodermis, and cortex) was observed by an Olympus Bx53 microscope calibrated with the Image Raster 3.0 (Miconos, 2021).

RESULTS AND DISCUSSION

Soil characteristics. Based on the pH measurement, the entire land used in this research was neutral. The average pH score on the coastal land is 6,6, while the soil pH on the typical land averages 6,3. The pH on the coastal land is generally more alkaline than the typical land. The soil texture is non-coarse loam with soil structure in the form of crumbs (size <1 mm). Soil characteristics with a comparison of the root anatomy of rice were presented in Table 1.

Table 1. Soil and root characteristics of the rice farmland and the root anatomy of three varieties

Rice variety	Land type	pH	Soil texture/size	DHL test (dS/m)	Epidermis thickness	Cortex area	Exodermis lignification
Ciherang	Coastal	6,8	Clay (< 1 mm)	1,234	5,96 μm	436,00 μm^2	+
	Typical	6,5	Sandy clay (< 1 mm)	0,190	6,97 μm	498,38 μm^2	-
IR64	Coastal	6,4	Clay (< 1 mm)	1,056	6,09 μm	247,85 μm^2	+
	Typical	6,2	Clay (< 1 mm)	0,133	6,72 μm	169,21 μm^2	-
Pioneer	Coastal	6,8	Clay (< 1 mm)	0,879	5,70 μm	364,41 μm^2	-
	Typical	6,3	Clay (< 1 mm)	0,131	6,78 μm	319,01 μm^2	-

Notes: + = Present; - = Absent

Soil characteristics in pH showed that both control and coastal land have a neutral pH range 6 6,8. Coastal land has a higher pH value of 6.6, positively correlated with more salt content in the soil. The possible reason is the proximity of coastal land to the sea coastline (Fahad *et al.*, 2018; Rachman *et al.*, 2018). Increased salinity in rice farmland occurs near the beach or directly connected to waterways (Rachman *et al.*, 2018). Salinity levels in Indramayu rice fields become higher with a closer distance of 1-7 km (Erfandi, D., 2009). In general, the pH factor affects the availability of minerals in the soil in the growth process (Neina, 2019; Ghazali *et al.*, 2020). Soil texture and structure where crumbly clay also contributes to maintaining the availability of water and nutrients in the soil. Clay, in which particles are smaller than 2 μm in diameter, has much greater surface areas

and smaller channels between particles. Both make water does not freely drain from them; it is held more tightly (Taiz *et al.*, 2015).

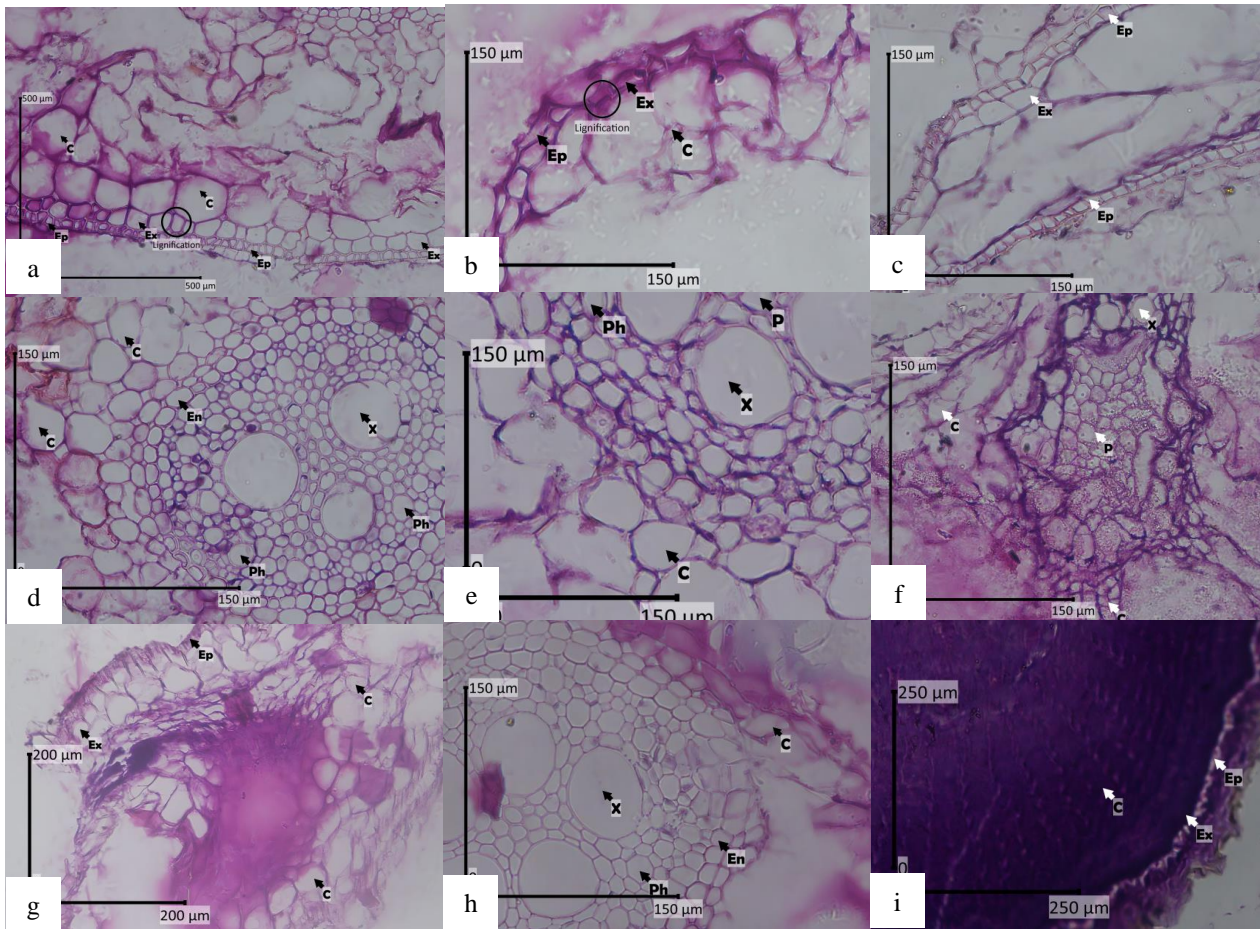


Fig. 1. Root anatomy of three rice varieties found in coastal land in epidermal part (a-c), dermal part (d-f), and typical land (g-i) (Rounded area = lignification; Ep = Epidermis, Ex = Exodermis, En = Endodermis, C = Cortex, X = Xylem, Ph = Phloem, P = Pith)

The level of soil salinity based on the EC test shows that the coastal area has the highest EC value, reaching 1.234, and the lowest is 0.879, where the score is categorized as moderate ($EC > 0.7$) according to Ayers R.S & Westcot D.W., (1985). Furthermore, according to Abrol *et al.* (1988), a salinity score < 4 dS/m is grouped as non-saline soil with negligible influence of diversity. Rice is a glycophytic plant that is not tolerant of high soil salinity. However, based on the calculation of the salinity score in Tegaldlimo District, the land can be used for rice cultivation. The three rice varieties used are not a type of variety that is tolerant of increased salinity.

Root anatomy description. The anatomical structures of rice roots are presented in Figure 1. The epidermal tissue of rice roots in coastal areas is minor compared to typical areas with a thickness of $6.83 \mu\text{m}$. In the exodermis tissue, lignification was found in IR64 and Ciherang rice varieties grown on coastal land. In comparison, all rice varieties on typical land did not the exodermis tissue lignification. The size of the cortex varies between varieties. IR64 and Pioneer varieties are larger in coastal areas than in the control. In the Ciherang variety, the cortex area is smaller than normal.

Symptoms seen in salinity-stressed rice are leaf curling, yellowing of leaf tips, leaf drying, decreased root growth, and stunted shoot growth that causes a decrease in total growth and seedling death (Singh dan Chaturvedi, (2014); Minh *et al.*, (2016)). Decreased plant growth can occur due to a decrease in turgor potential due to the water deficit produced by high concentrations of salt in the soil (Hussain *et al.*, 2010; Banerjee & Roychoudhury, 2017)). The impact of increasing soil salinity

was studied through anatomical observations of the roots. Roots are sensitive to changes in soil salinity compared to other organs (Dawood *et al.*, 2014; Acosta-Motos *et al.*, 2017; Hua Qin, 2020). Anatomical changes can also interfere with the plant's ability to deliver water and nutrients in high salinity (Farhana *et al.*, 2014). The epidermal tissue of the three types of rice varieties on coastal land shows a lower thickness than on typical land. Similar observations were also observed in the species *Cenchrus ciliaris*, a saline grass plant showing a reduction in root thickness (Wang *et al.*, 2018; Kuster *et al.*, 2018; Wasim & Naz, 2020). The highest reduction was in the Pioneer variety compared to the control, and the lowest was in the IR64 variety. In contrast, the observation of cortex tissue showed a larger tissue area than in the typical land, especially for two rice varieties, IR64 and Pioneer. However, the Ciherang is not. Modifying the cortex tissue can increase the storage space of water and solutes to maintain balance in osmosis stress conditions (Dolatabadian *et al.*, 2011; Naz *et al.*, 2015; Reddy *et al.*, 2017; Wasim & Naz, 2020). However, the cortex of Ciherang variety is less thick than that of typical land. This is consistent with the findings of Singh and Chaturvedi (2014); Rachmawati *et al.*, (2021), which showed that plants experiencing salinity stress had reduced cortex cells due to water loss in the vascular network causing shrinkage. Another form of adaptation observed and thought to be the impact of increased salinity is the lignification of exodermis tissue in IR64 and Ciherang varieties (rounded area in Figure 1). Meanwhile, no lignification in the exodermis tissue on rice roots in normal soil existed. Lignification is an essential modification in saline-stressed roots because it functions as a mechanical strength provider and protects against root damage (Hameed *et al.*, 2010). Although changes were observed in the three rice varieties, including epidermal reduction, larger cortex, and lignification, these parameters varied widely. It is possible by the presence of salinity that is still tolerable with a minor effect of change.

CONCLUSION

Agricultural land in the coastal areas of the Tegaldlimo sub-district has higher salinity levels than typical land, with a highest of 1,234 dS/m. However, the EC score shows Tegaldlimo land as non-saline soil capable of supporting optimal rice growth reinforced by neutral PH and clay soil structure. The root anatomy observations showed an epidermal reduction, a cortex expansion, and an exodermis lignification as a form of environmental adaptation. Yet those adaptations are in great variety among varieties. Further research is needed to determine whether the adapted IR 64, Ciherang, and Pioneer can support higher salinity fields.

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