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Comparative study of root characteristics revealed distinctive responses between Moroberekan and MR297 rice varieties subjected to drought stress

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Abstract. *Karim MF, Tajudin NHN, Salmin SA, Rosely NFSM, Saimi NN, Amri CNAC. 2024. Comparative study of root characteristics revealed distinctive responses between Moroberekan and MR297 rice varieties subjected to drought stress. Nusantara Bioscience 16: 29-36.* In light of the growing concern over climate change, it is crucial to comprehend how the rice plant, *Oryza sativa* L., responds to various environmental stress, particularly prolonged drought. This study investigated the morphological and anatomical characteristics of the roots of rice plants following a continuous drought on two selected varieties: MR297, known to produce a high yield but is highly sensitive to low water potential, and Moroberekan, known to be drought tolerant. The drought treatment was initiated on day 24 after sowing and continued for seven days or until any plant exhibited curled leaves. There was no significant difference in root length between the two varieties, but MR297 had substantially lower fresh and root dry weights (32.5% and 40%, respectively) than Moroberekan under drought stress. Drought also significantly affected root electrolyte leakage and MDA content, especially in MR297 compared to Moroberekan. Meanwhile, root anatomy studies have revealed differences between the control and drought treatments. While the root diameter of the control plants was greater, their aerenchyma cells were less developed than those of the drought-induced plants, which had a higher ratio of aerenchyma cells per sectioned area. The number of metaxylem was reduced by drought, but the effect was more pronounced in MR297 than in Moroberekan. This study provides evidence of the impact of drought on both Moroberekan and MR297, as observed through their root morpho-physiology and anatomical structure.

Keywords: Aerenchyma cells, MDA, metaxylem cells, *Oryza sativa*, root anatomy

INTRODUCTION

Rice, scientifically known as *Oryza sativa* L., is an essential staple crop that serves as a primary food source for more than half of the world's population. The majority of the production, accounting for almost 90%, is concentrated in the Asian region (Ali et al. 2017; Landi et al. 2017). Rice is known to be the most susceptible to drought among cereal crops (Panda et al. 2021). Water availability has been one of the most devastating abiotic stresses that have shaped the evolution of plants in general and rice in particular. According to Sandhu et al. (2012), 15% of Asia's 75 million acres of irrigated rice crop could face water shortages by 2025.

Drought has been one of the most significant stresses on rice growth over the past two decades, reducing global rice production by 25.4% (Zhang et al. 2018). According to Nahar et al. (2018), drought reduces crop yield and inhibits plant growth and development, resulting in more severe conditions and plant death. Numerous studies have examined plants' morphological, physiological, and biochemical responses in a drought environment to identify rice varieties with enhanced drought tolerance (Singh et al. 2017, 2018; Swapna and Shylaraj 2017). Several adverse effects can be caused by prolonged exposure to drought stress, including a shorter plant height, plant wilting, leaf rolling, leaf senescence, stomatal closure, reduced leaf elongation, and decreased dry matter production (Singh et al. 2017; Swapna and Shylaraj 2017; Karim et al. 2021). However, it is still challenging to determine whether they are drought-resistant or vulnerable based merely on their agronomic properties, even though the effects of drought can vary depending on the rice variety (Singh et al. 2017; Swapna and Shylaraj 2017).

The architecture of the root system, which is appropriately referred to as the "hidden half", has a significant impact on crop production, as the roots are principally responsible for adaptation and responses to varied stress circumstances through complex gene interactions. Knowledge about plant roots' growth and structure presents opportunities for leveraging and controlling root traits to enhance crop productivity and maximize agricultural land use efficiency (Den Herder et al. 2010). The root system architecture is depicted by a range of root traits such as root type, elongation rate, root thickness, growth duration, root density, root surface area, root volume, root gravitropism, and longevity (Schneider and Lynch 2020; Shamsuddin et al. 2021; Tajima 2021). These root behaviors provide insights into optimizing water acquisition and plant adaptation to various environmental conditions (Kadam et al. 2015). Plants utilize their root plasticity to survive while producing stable yields even under adverse external factors (Suseela et al. 2020). Phenotypic selection based on root plasticity could be a potential target for extracting genetic variation for breeding stress-tolerant programs (Schneider and Lynch 2020).

Nevertheless, the interaction between root plasticity and a dynamic environment is intricate and varies depending on the genotype and the type and severity of environmental stress. Concerning water scarcity, recent evidence of Southeast Asian rice has shown that the highest grain yield was attained by higher root numbers and smaller stele diameters (Siangliw et al. 2022). Similarly, the drought-stressed root of maize alters the morphological traits by multiplying the fine roots to optimize water absorption (Yan et al. 2022). This phenotypic plasticity differs in response to adverse external factors. Fluctuation of Na^+ accumulation in soil resulted in the anatomical plasticity of apoplastic barriers (Shelden and Munns 2023). The rapid formation of suberin lamellae and Casparian bands as apoplastic barriers in barley and rice plants alleviate salt accumulation, thus promoting salt tolerance (Mehmet 2016; Chen et al. 2018; Ho et al. 2020).

Meanwhile, root architecture patterns during heat stress displayed compact, consistent, and longer roots to enhance the deeper root in the soil horizon (Yadav et al. 2022). Such deeper roots enable soil moisture uptake, resulting in transpirational cooling and mitigating the impact of heat stress. Thus, the dynamic of root plasticity provides advantageous insights for developing stress-tolerant cultivars. Therefore, from an anatomical perspective, it is crucial to support a comprehensive investigation into how the root systems contribute to mechanisms that make rice plants more resistant to drought stress.

This study has employed a morphological and anatomical method to investigate the adaptive strategies of rice roots under drought-stress conditions. The main objective of this study is to assess the phenotypic and morphological changes in the roots of rice plants as they are exposed to prolonged periods of drought.

MATERIALS AND METHODS

Experimental design

The experiment was conducted at the Glasshouse & Nursery Complex (GNC) of the International Islamic University of Malaysia in Kuantan, Pahang, Malaysia. Two different rice genotypes, Moroberekan (an upland rice variety) and MR297 (domestically cultivated and drought-susceptible) were subjected to two irrigation conditions: well-watered and drought stress. Seeds were pretreated by soaking in distilled water overnight before germinating on wet tissues. After a week, seedlings were transferred into 6 cm × 9 cm polybags with 100% topsoil. The standing water of about 3±1 cm was maintained above the soil surface in each tray during the planting period. The fertilizers were applied once every two weeks.

Drought stress treatment

Drought stress treatment was exposed by removing the standing water from the soil surface when rice seedlings reached the end of the pre-tillering stage. The pre-tillering stage can be defined as the period from the development of the first leave to the fourth leave stage (Hardke 2013). Rice plants typically enter the tillering stage between 15 and 25 days after seedling emergence. In this study, drought stress treatments were applied after 21 Days After Sowing (DAS). The standing water was maintained approximately 3±1 cm above the soil surface for control treatment throughout the study period.

Root morphology and physiology

The root was harvested on day 29 DAS after a few plants showed signs of rolled leaves. Root systems were cleaned thoroughly to remove excessive soil and dirt to determine their fresh weight. The length of the roots was measured from the plant's base to the top of the longest root branch. Then, root samples were oven-dried at 72°C for 3 days to get the constant weight.

Root leakage was assessed through the Root Electrolyte Leakage (REL) method (Radoglou et al. 2007). The root system was cut and washed in cold tap water to remove soil. Then, the root was rinsed in distilled water to remove adsorbed ions. The root sample was made certain to have as little soil contamination in the root as possible. Fresh samples were selected from a portion of the root system between 100 to 500 mg weights. The samples were then submerged in 28 mL universal glass bottles of distilled water. The bottles were capped, shaken, and left at room temperature for 24 hours. Next, the bathing solution's first conductivity (C1) was measured using a conductivity probe with a built-in temperature compensation system. The samples were then autoclaved at 110°C for 10 minutes. The samples were cooled to room temperature, and a second conductivity (C_2) was measured. REL was expressed as:

$$REL = (C_1/C_2) \times 100$$

Meanwhile, malondialdehyde (MDA) content was measured following the method by Hodges et al. (1999). 0.25-0.50g of root samples were homogenized in 3 mL of 0.1% (w/v) trichloroacetic acid (TCA) before being centrifuged at 10,000 g for 10 minutes. Then a 750 µl aliquot was pipetted and mixed with the same volume of either (a) a +thiobarbituric acid (TBA) solution containing 20% (w/v) TCA and 0.5% (w/v) TBA or (b) -TBA containing only 20% (w/v) TCA in a 2-ml capped microcentrifuge tube. All samples were then heated in a water bath for 25 minutes at 95°C before being brought to room temperature. After a centrifugation for 10 minutes at 10,000 g, the absorbances were recorded at 440, 532, and 600 nm.

Root anatomy

Root samples were cut 5cm from the root tip for root anatomy. Anatomical specimens were prepared with a sliding microtome, and the thickness was adjusted to 100 µm. As rice roots were very small and fragile, the clearing process was unnecessary. The root sections were stained immediately in Methylene Blue for 30 seconds after sectioning. All the sections were observed under a light microscope LEICA ICC50 HD and captured by Leica LAS EZ Software.

RESULTS AND DISCUSSION

Effect of drought on root morphology and physiology

Shoot-related morpho-physiological assessments have been established in numerous research conducted on rice. Nevertheless, root architecture has traditionally been largely ignored by plant breeders in terms of potential yield increases and was not a major selection criterion as part of the crop development programs because of the absence of simple and effective techniques for investigating root systems in soil (Den Herder et al. 2010). This bias is extremely unfortunate, as the dearth of discoveries in root phenes could limit our understanding and ability to predict how crops and their surrounding environments respond to climate change-induced abiotic stress, particularly frequent drought. The present study subjected rice seedlings to normal water levels before drought treatments. No discernible difference in root growth was observed between the Moroberekan and MR297 varieties under control conditions (Figure 1.A).

Nevertheless, the root system in both varieties experienced significant impairment when the seedlings were exposed to drought-induced stress. The occurrence of drought stress resulted in a notable decrease in root length, with Moroberekan and MR297 exhibiting reductions of 33.3% and 36.2%, respectively, in comparison to MR297 plants that were subjected to regular watering (Figure 1.B). A longer root length was associated with drought resistance in plants. Soil exploration during drought necessitates a longer root system for water and nutrient search, resulting in a greater allocation of carbohydrates to root growth (Djanaguiraman et al. 2019). Although early studies have shown that drought-treated plants have longer roots than those with normal irrigation, the severity of the drought exposure and growth stage also affect the outcome (Karim et al. 2021). In this study, rice plants were exposed to drought stress at an early stage of tillering, which could be crucial for growth and development, resulting in the opposite result.

The root system is a crucial plant organ responsible for the absorption of water and nutrients, significantly influencing plant growth and yield productivity (Azmi et al. 2020; Cochavi et al. 2020). Drought impairs root development, particularly root branching, essential for increasing the root system's surface area. Furthermore, the reduced root length under drought stress also limits the enhanced access to subsoil water, resulting in lower grain yield when water is limited (Bodner and Robles 2017). In the control, no significant difference was seen in root fresh weight across all plants (Figure 2.A). On the other hand, drought stress resulted in a notable decrease in the fresh weight of plant roots compared to plants cultivated under controlled water conditions. Moroberekan and MR297 exhibited reductions of 47.5% and 67.3%, respectively, when exposed to drought, compared to the control.

Similarly, while root dry weight under normal watering showed no difference, drought substantially impacted the root biomass of all plants, with a more notable effect detected in MR297. The MR297 exhibited a significant decrease of 40% compared to the Moroberekan under drought stress and nearly 81% compared to its performance on the control. This observation implies that MR297 demonstrated a higher vulnerability to drought stress (Figure 2.B). The continuation of root growth in dry weight was important in yield determination and positively correlated with grain filling in rice (Chen et al. 2021).

Under drought, the roots can grow with sufficient sugars from the leaves. Miller et al. (2017) stated that this necessitates a dynamic acclimation within the leaf proteome, specifically photosynthetic-related proteins, to overcome the challenges. The decrease in plant dry weight, including root biomass, observed in response to drought stress can be attributed to a decline in photosynthetic activity. The impact of drought on the physiological metabolism of photosynthesis has been reported, leading to disruptions in the electron transport chain and reduced assimilation of carbon dioxide (Wang et al. 2018).

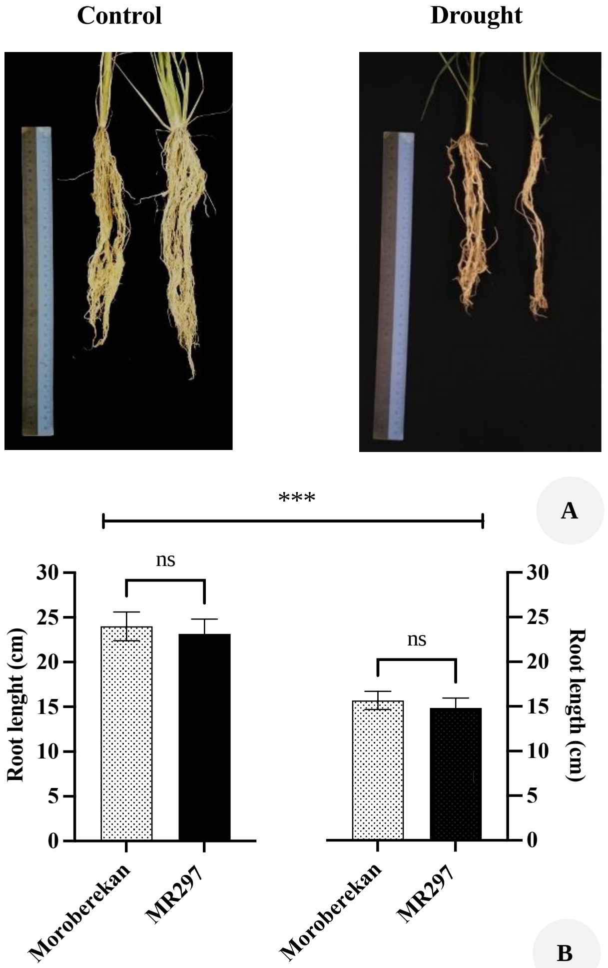

Figure 1. A. Root system and B. length of Moroberekan and MR297 rice seedlings subjected to normal irrigation and drought stress. Data was analyzed using a t-test to assess the significance level between means (p ≤0.05). Values represent the mean ± SE of n = 3. *** indicates the significant level of drought treatment based on a two-way ANOVA

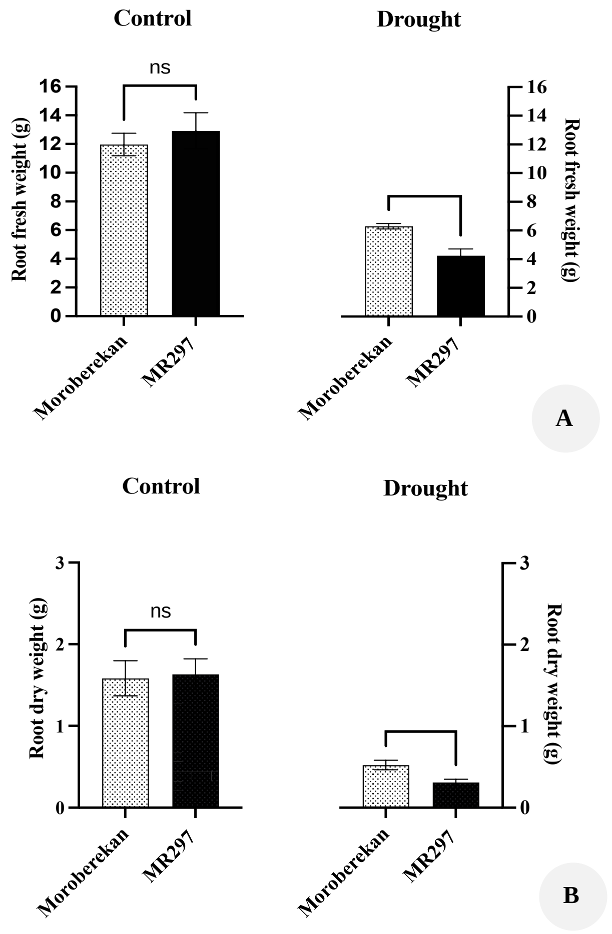

Figure 2. A. Root fresh weight and B. dry weight of Moroberekan and MR297 rice seedlings subjected to normal irrigation and drought stress. Data was analyzed using a t-test to assess the significance level between means (p ≤0.05). Values represent the mean ± SE of n = 3

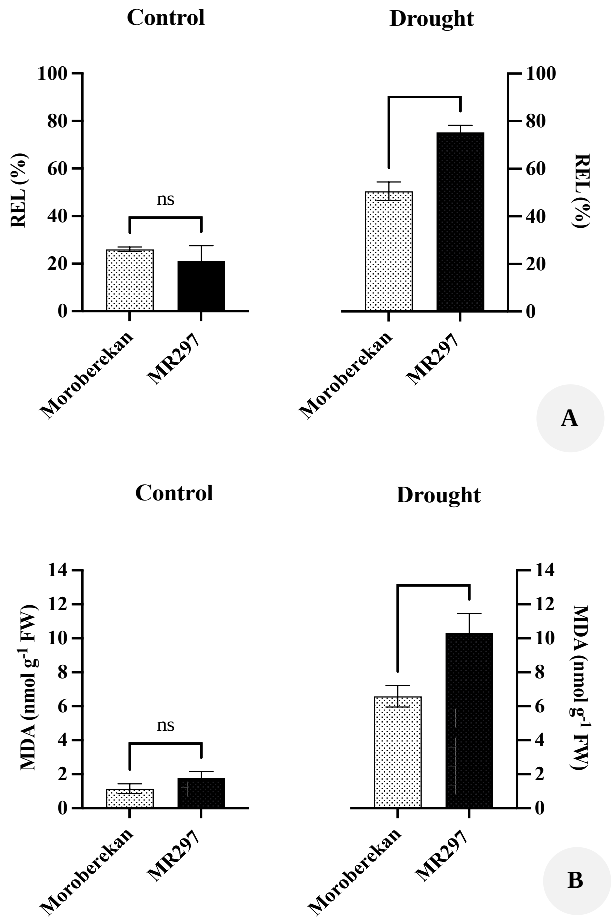

Figure 3. A. REL and B. MDA content of Moroberekan and MR297 rice seedlings subjected to normal irrigation and drought stress. Data was analyzed using a t-test to assess the significance level between means (p ≤0.05). Values represent the mean ± SE of n = 3

The interruption of the electron transport chain can impede the synthesis of ATP by ATP synthase, potentially impacting the Calvin cycle's metabolic processes (Simkin et al. 2019). All of these processes depend on the acclimation capacity of plant species and cultivars in response to drought, which may explain why Moroberekan still recorded a significantly higher value than MR297 despite a lower root biomass under drought.

Evaluating root electrolyte leakage can provide insights into the resistance of root systems to drought-induced stress. Cells normally lose their membrane integrity; thus, electrolytes, such as K^+ ions, leak out of the cell, indicating the extent of cell death in the tissue, especially under abiotic stresses (Demidchik et al. 2014). Moroberekan and MR297 show no difference in the control, with an average REL value of 21-25%. However, a significant increase was observed in those plants exposed to drought ($p \leq 0.05$), with the effect being greater in MR297 compared to Moroberekan ($p \leq 0.01$) (Figure 3.A). According to Assaha et al. (2016), drought stress induces the overproduction of reactive oxygen species, which damage cellular membranes and can increase electrolyte leakage. The REL results correspond to the MDA level in roots where drought significantly increased lipid peroxidation compared to control (Figure 3.B). The oxidative stress was known to cause a higher accumulation of MDA (Karim and Johnson 2021). The production of MDA was more pronounced in MR297, with a significant 1.6-fold higher than that of Moroberekan. Hence, considering the root physiology, it can be inferred that Moroberekan exhibits higher drought resistance than MR297, indicating that the response to drought may differ depending on the specific variety and ecotype.

Effect of drought on root anatomy

The present study examines root anatomy to determine whether there were any alterations in root structure between two rice varieties, an upland rice variety (Moroberekan) and a domestically grown rice variety (MR297). In the absence of stress exposure, it was shown that Moroberekan naturally exhibited a larger root diameter than MR297, with an average difference of 16% (Figure 4). However, the exposure to drought resulted in a substantial decrease in the root diameter of the MR297 and Moroberekan varieties. Specifically, the root diameter of MR297 was reduced by 50.1%, while Moroberekan had a loss of 33.4% when compared to their respective control. Analysis of rice roots under drought stress revealed that Moroberekan had more root hairs surrounding its epidermis cells than MR297. According to Hernández et al. (2010), certain plant species have been reported to decrease their root diameter but with longer root systems for subsoil water exploration.

Nevertheless, the present study did not observe such an outcome, as both diameter and root length were impacted by drought, which aligns with the findings reported by Boguszewska-Mańkowska et al. (2020). A separate study observed that drought environments resulted in a reduction in root length and an increase in root diameter (Zhou et al. 2018). In contrast, another study found no significant alterations in fine root morphology (Mrak et al. 2019).

Rice has special tissue that allows it to live in a root submergence environment called aerenchyma. Aerenchyma cells in rice roots are formed by cell lysis and cell deflation to provide air channels that allow gas diffusion from above-part to below-part organs to maintain aerobic respiration in submerged conditions. This study observed aerenchyma formation in rice roots regardless of treatments. While the control plants exhibited greater root diameter, the aerenchyma cells in these plants were not fully developed compared to the drought-induced plants, which displayed a higher ratio of the number of aerenchyma cells present per sectioned area. This indicates that the formation of aerenchyma cells continues despite the prolonged exposure of the root system to drought. According to Schneider et al. (2023), the development of cortical aerenchyma formation is predominantly regulated by a root cortex-expressed gene-encoding transcription factor bHLH121.

Meanwhile, in a study conducted by Ni et al. (2019), it was observed that the presence of ethylene and reactive oxygen species facilitated the process. The transformation of live cortical cell tissue into a porous structure can decrease respiration activity and, hence, lower the metabolic expenditure associated with soil exploration during drought (Schneider et al. 2023). This is because root exploration in the soil is metabolically costing, sometimes surpassing 50% of the daily photosynthetic activity (Lynch et al. 2021).

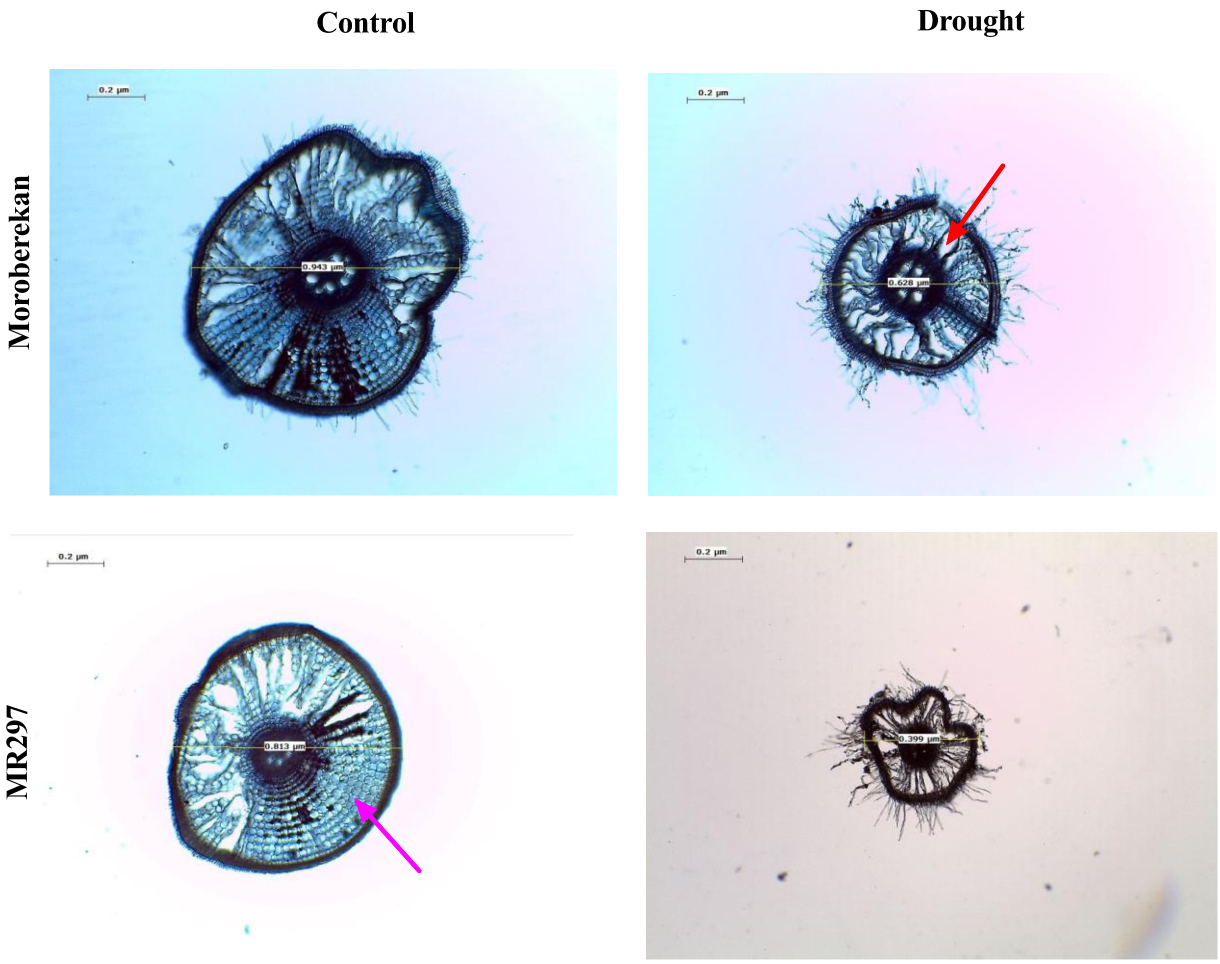

Figure 4. Cross-sectioned roots of Moroberekan and MR297 were subjected to control and drought conditions. The samples were obtained approximately 5 cm from the root tip and observed under a compound microscope, 4x magnificent with scale bar = 0.2 µm. The red and magenta arrows indicate specific anatomical structures: Aerenchyma and undeveloped aerenchyma cells

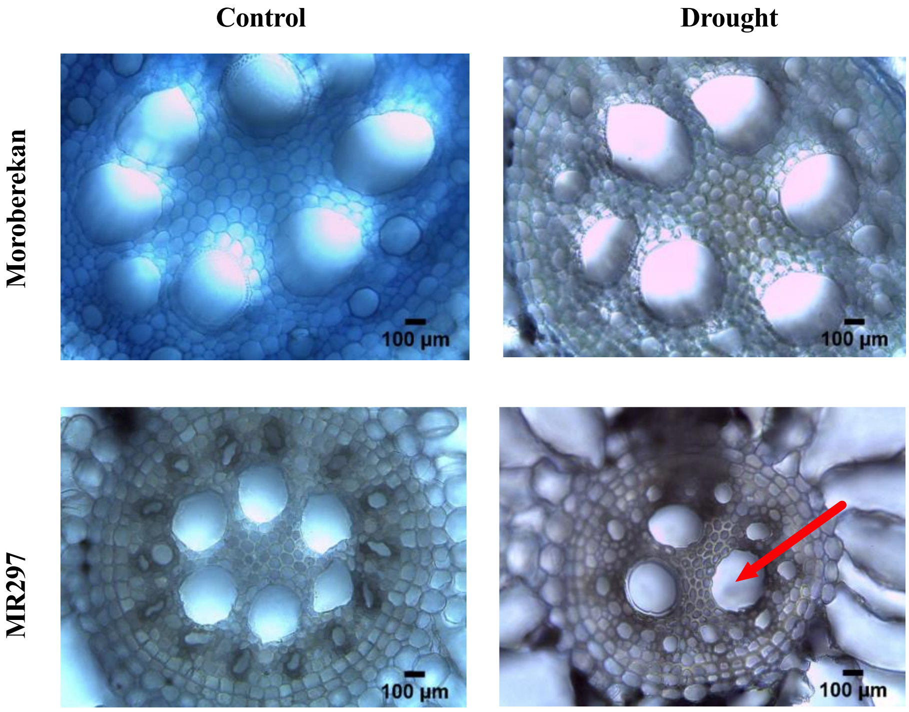

Figure 5. Anatomical analysis of metaxylem cells in Moroberekan and MR297 under control and drought treatment, respectively. The sections were obtained 5 cm from the root tip and observed under a compound microscope, 4x magnificent with scale bar = 100 µm. The red arrow indicates a specific anatomical structure: metaxylem cells

In addition, the drought resistance features in rice have also been assessed on the root xylem. Metaxylem cells originate from primary xylem tissue, formed during the primary growth phase. The current study examined metaxylem cells and the number in rice roots, revealing different responses under normal and stress conditions (Figure 5). The results of the study showed that there were variations in the number of metaxylem cells in both varieties. MR297 showed a decrease in metaxylem numbers, in contrast to Moroberekan, which showed only a slight decrease in response to drought conditions relative to control. The average number of metaxylem cells in MR297 under drought conditions ranged from 3 to 4, which was lower than its performance in the control, where the average number was 6 to 7.

In contrast, Moroberekan developed 5 to 6 metaxylem cells, a count nearly equivalent to the amount observed under normal water level. The studies conducted by Prince et al. (2017) and Cornelis and Hazak (2022) have demonstrated a positive correlation between increased xylem number and diameter and enhanced drought tolerance. Root metaxylem traits were found to have different outcomes in previous research. While there are reports that the smaller metaxylem vessel and number benefit the plants reduced hydraulic conductance per root and lower risk of cavitation and collapse (Klein et al. 2020; Reeger et al. 2021), the greater diameter and number of metaxylem vessels were typically found in upland rice, where it could be the phenotype of tolerance in upland rice varieties (Gowda et al. 2011). This observation may account for the greater drought tolerance exhibited by Moroberekan in comparison to MR297. It suggests that further research on drought tolerance traits based on root phenes could be expanded to include upland rice varieties commonly cultivated in areas with limited water resources.

In conclusion, the availability of water is crucial for facilitating the normal growth and development of plants. Even a minor decrease in soil moisture levels can significantly impact the physiological processes of less resilient plant species. Hence, identifying appropriate candidates with superior root traits that can withstand adverse conditions is crucial in breeding programs, particularly in challenging climate uncertainty. The present study revealed that the root morpho-physiology of the MR297 and Moroberekan was impacted by drought stress. This includes the significantly lower root fresh and dry weight, maximal root length, and % of REL and MDA accumulation compared to the plants maintained in the control condition. However, the impact was more pronounced in MR297 in all the mentioned parameters except for the maximal root length. Similarly, the root anatomical study results indicate that drought stress had reduced root diameter and metaxylem number compared to the control, but not the aerenchyma formation. Furthermore, it was demonstrated that Moroberekan exhibited better root structure than MR297 when subjected to drought stress.

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