Early Seedling Vigor and Morphology of Five Malaysian Indica Rice Cultivars under Water Deficit

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Abstract: This study evaluated the early seedling vigor and growth morphology of five Malaysian indica rice cultivars (MR263, MR219, MR220CL2, MR269, MR284) under simulated water deficit using polyethylene glycol (PEG 6000) treatments. Seeds were germinated on Petri dishes with four PEG solutions (0, –0.3, –0.9, –1.5 MPa) at 25°C. After 14 days, germination percentage, seedling vigor index, root length, and shoot (seedling) length were measured. Increasing osmotic stress significantly reduced germination speed and seedling growth for all cultivars, with the most severe stress (–1.50 MPa) causing drastic declines. Seedling vigor index decreased sharply as water potential decreased. Among cultivars, MR263 and MR219 maintained significantly higher vigor and longer roots/shoots under stress than the others. At –1.50 MPa, MR263 exhibited the highest vigor index (~3.6), root length (~5.8 cm) and shoot length (~3.8 cm), whereas MR284 had the lowest (~0.5, ~2.7 cm, ~0.3 cm, respectively). These results indicate genetic variation in drought tolerance at early stages. Our findings suggest MR263 and MR219 may be better suited for environments prone to water deficit, and highlight the importance of seed vigor and root development as selection criteria for drought resilience.

Keywords: Rice, Drought stress, Seedling vigor, Root length, Seedling growth.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most important staple crops, feeding nearly half of the global population. Approximately 90% of rice is consumed in Asia, where it provides a major portion of calories and nutrition (IRRI, 2013). In Malaysia, rice is a key food security crop which is can be considered as the country's "rice bowl" and source of food security (Rusli *et al*., 2023), yet rice production is vulnerable to abiotic stresses, especially water deficiency. Drought can severely limit rice yields; under severe stress, crop production losses may reach up to 70%. Drought stress during early growth stages is particularly problematic because it restricts water uptake and disrupts physiological processes crucial for germination and seedling growth (Akram *et al*., 2013; Toosi *et al*., 2014). Reduced soil moisture leads to lower imbibition, slow enzyme activity, and limited nutrient mobilization, all of which inhibit seed germination and subsequent seedling development (Farooq *et al*., 2009; Fahad *et al*., 2017).

The earliest phase of a crop's life – seed germination and seedling establishment – is highly sensitive to water stress. Under osmotic stress, germination rates, seedling height, and biomass accumulation often decline markedly (Toosi *et al*., 2014). Fahad *et al.* (2017) noted that reduced water availability lowers tissue water potential and enzymatic activity, interrupting nutrient flow and cell division, thereby diminishing germination and seedling elongation. Moreover, rice crop in all stages from seed germination to flowering and grain filling, leading to yield losses, which might sometimes be over 50% under severe water deficit (Yang *et al.,* 2019, 2024). Reduced seedling vigor under drought is a well-documented phenomenon in many crops. Thus, measuring indices such as the germination index and seedling vigor index (which combines germination percentage with seedling length) provides a useful assessment of seed quality and stress response (Rahman *et al*., 2014; Abiri *et al*., 2016).

Root traits also play a critical role in drought tolerance. Rice plants often adapt to limited moisture by altering root architecture. In particular, stress can stimulate the formation of additional lateral and finer roots, increasing the soil surface area available for water uptake (Kim *et al*., 2020). Kim *et al.* (2020) reviewed rice root responses and observed that drought-tolerant genotypes tend to develop more lateral roots and narrower root diameters under stress to conserve resources and enhance water absorption. Deep and extensive root systems help maintain growth under water deficit by reaching deeper soil moisture. Conversely, sensitive cultivars often show curtailed root elongation when osmotic potential declines.

Drought stress is a major threat to global environmental sustainability, directly influencing water resources, climate adaptation, and food security. Prolonged water deficits reduce streamflow, groundwater recharge, and irrigation capacity, making efficient water resource management essential for resilience (FAO, (2022); Organisation for Economic Co-operation and Development [OECD], 2025). Under

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climate change, drought becoming more frequent and severe, intensifying evapotranspiration and reducing soil moisture, which undermines agricultural productivity and ecosystem stability (Shayanmehr *et al*., 2022). Drought stress causes water resources for agriculture, ecosystems, and communities becomes constrained. As the results the socio-economic and environmental costs of drought will continue to rise globally, with cascading and potentially irreversible consequences for societies, economies, and ecosystems in all continents. Biswas *et al*. (2024) reported that water scarcity poses a major barrier to sustainable development, as the lack of adequate water resources undermines the ability to consistently support agriculture, maintain healthy ecosystems, and meet human needs.

Many studies reported that drought stress significantly limits crop by affecting physiological, growth, development and yield, making it a major threat to global food security and sustainability. Drought significantly reduces crop yields due to lack of sufficient water which leads to lower photosynthesis, reduced growth of plants, less biomass, reduce nutrient assimilation, reproductive development (flowering, grain filling), and lower quality of crop produce. Staple crops such as wheat, maize and rice, yield losses due to drought could exceed 21 %, 40 % and 50 % respectively under rising dry conditions (Orek, 2023).

Therefore, given the importance of early vigor and root growth in drought adaptation, screening rice cultivars for seedling vigor under water deficit can help identify tolerant genotypes. Malaysian indica rice varieties exhibit genetic diversity in their response to stress (Lum *et al.*, 2014). This study therefore assessed five locally important indica cultivars (MR263, MR219, MR220CL2, MR269, and MR284) for germination performance, seedling vigor, and morphology under four PEG-induced water potentials. To investigate plant responses to water deficit under controlled conditions, osmotic agents such as polyethylene glycol (PEG) are frequently employed to simulate drought stress *in vitro*. Polyethylene glycol 6000 (PEG6000), a high-molecular-weight, water-soluble, non-ionic polymer, is particularly suitable for this purpose because it cannot easily penetrate plant cell walls. When dissolved in the growth medium, PEG6000 decreases the osmotic potential (water potential) of the solution, thereby reducing water availability to plant roots or cells. This creates a controlled osmotic environment that effectively mimics the physiological effects of drought stress, enabling the study of plant adaptive and tolerance mechanisms under water-limited conditions (Ahmad *et al*., 2022). The objective was to quantify how water deficit affects seed vigor index, root length, and shoot length at the seedling stage, and to identify cultivars with superior early drought tolerance.

2. MATERIALS AND METHODS

2.1. Plant Material and Treatments

Five Malaysian indica rice cultivars (MR263, MR219, MR220CL2, MR269, MR284), obtained from the Malaysian Agricultural Research and Development Institute (MARDI), were used. Seeds of uniform size and weight were selected and surface-sterilized by soaking in 10% sodium hypochlorite for 5 minutes, followed by three rinses with sterile distilled water. Seed moisture was allowed to equilibrate.

Polyethylene glycol (PEG 6000) was used to impose controlled osmotic stress by lowering the water potential of the growth medium, thereby restricting water uptake by plant tissues and mimicking drought (water-deficit) without causing direct toxicity to the seedlings (Elmaghrabi *et al*., 2017). PEG solutions at four osmotic potentials (0, –0.30, –0.90, –1.50 MPa) were prepared according to the method of Michel and Kaufmann (1973). Briefly, PEG 6000 was dissolved in distilled water at concentrations of 0 g (control), 5 g (–0.30 MPa), 15 g (–0.90 MPa), or 25 g (–1.50 MPa) per 100 mL water. The resulting solutions were measured to confirm the desired water potentials.

2.2. Experimental Design

Germination tests were conducted in a completely randomized design with two replicates per treatment. For each replicate, 10 seeds were placed on moistened Whatman No. 1 filter paper in a 9-cm Petri dish. Each dish received 8 mL of one of the PEG solutions (0, –0.3, –0.9, –1.5 MPa) or distilled water (control). The dishes were sealed with Parafilm to prevent evaporation and maintained at 25±1°C in a germination chamber for 14 days. The experiment was repeated three times for robustness.

2.3. Measurements

Seeds were scored as germinated when the radicle protruded at least 2 mm from the seed coat. Germination counts were recorded daily for 14 days. At day 14, germination percentage (GP) was calculated as the proportion of seeds germinated. The Germination Index (GI) was computed to account for speed and uniformity of germination (AOSA, 1983). The seedling vigor index (SVI) was calculated as: SVI = GP × (mean root length + mean shoot length) (Abdul-baki & Anderson (1970).

Five normal seedlings were randomly selected from each Petri dish to measure morphological parameters. Root length (distance from seed base to root tip) and shoot length (seedling height from base to tip of the oldest leaf) were measured with a ruler (in cm). Root and shoot lengths were averaged per dish. The data from two replicates were averaged for each cultivar and treatment.

2.4. Data Analysis

Data were subjected to Two-way Analysis of Variance (ANOVA) at confidence level $p < 0.05$ using SPSS Statistic window version 25. Mean comparisons among cultivars and treatments were performed by Tukey's HSD test at $p < 0.05$ to identify significant differences. The effects of water potential and cultivar, as well as their interaction, were evaluated. Graphs of seed vigor index, root length, and seedling length versus water potential were prepared for visualization of trends.

3. RESULTS

3.1. Seed Germination and Vigor

Figure **1** shows the all five rice cultivars exhibited high germination rates under non-stress conditions, but germination declined as PEG-induced water potential decreased. Under control (0 MPa), germination percentages ranged from ~76% (MR284) to ~92% (MR263). Mild stress (–0.30 MPa) slightly lowered germination in sensitive cultivars (*e.g.*, MR284 from 76% to 69%) but had little effect on tolerant ones (MR263 remained ~91%). Severe stress (–1.50 MPa) caused a pronounced drop: MR263 fell to ~80%, MR219 to 78%, and MR284 to ~44%.

Seed vigor index, which integrates germination and seedling growth, showed a clear downward trend with increasing stress (Figure **1**). At 0 MPa, MR263 had the highest vigor index (~15.6), followed by MR219 (13.4) and MR220CL2 (11.6), whereas MR284 had the lowest (8.4). As water potential decreased, vigor index declined sharply for all cultivars. Under moderate stress (–0.90 MPa), MR263 and MR219 maintained higher vigor (7.6–7.7) than the others (MR269: 3.5; MR284: 2.5). At the most severe level (–1.50 MPa), MR263 (3.6) and MR219 (2.6) still had measurable vigor, while MR220CL2 dropped to 1.7 and MR269 to 1.3. MR284 was nearly non-vigorous (0.5) under –1.50 MPa. Statistical analysis confirmed that both water potential and cultivar had significant effects on seed vigor index ($p<0.05$). In summary, drought stress uniformly reduced seed vigor, but MR263 and MR219 showed comparatively greater maintenance of vigor under stress (Figure **1**).

The graph of seed vigor index for the five cultivars across water potentials is shown in Figure **1**. Under zero stress (0 MPa), all cultivars began with relatively high vigor, but as osmotic stress intensified, seed vigor declined steeply. The more drought-tolerant MR263 and MR219 retained higher vigor values at each stress level than MR269 and MR284. Notably, MR284's vigor fell almost to zero at the most negative potential. These trends reflect each cultivar's ability to support seedling growth under limited water.

3.2. Root Length

Figure **2** shows the root length across PEG-induced water potentials. Root elongation was less sensitive to mild stress: at –0.30 MPa, MR263's mean root length remained ~7.53 cm (vs 7.72 cm at control) and MR219 ~6.28 cm (down from 7.22 cm). In contrast, MR269 and

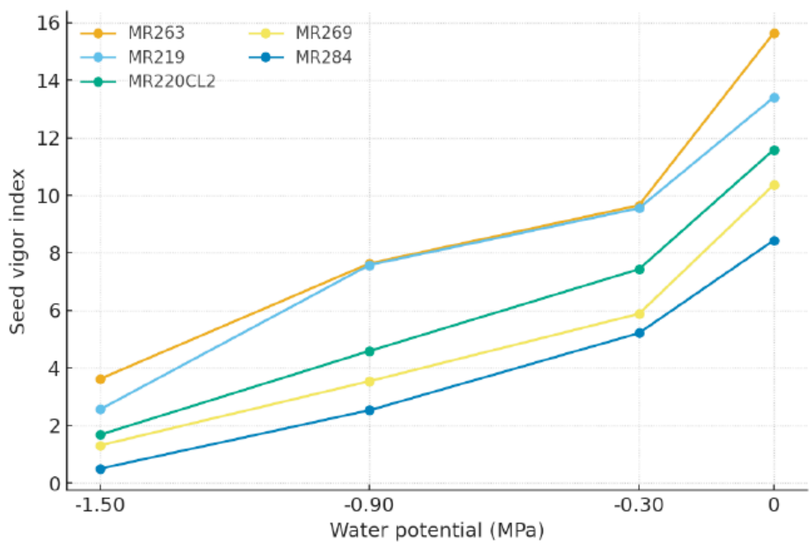

Figure 1: Seed vigor index across PEG-induced water potentials.

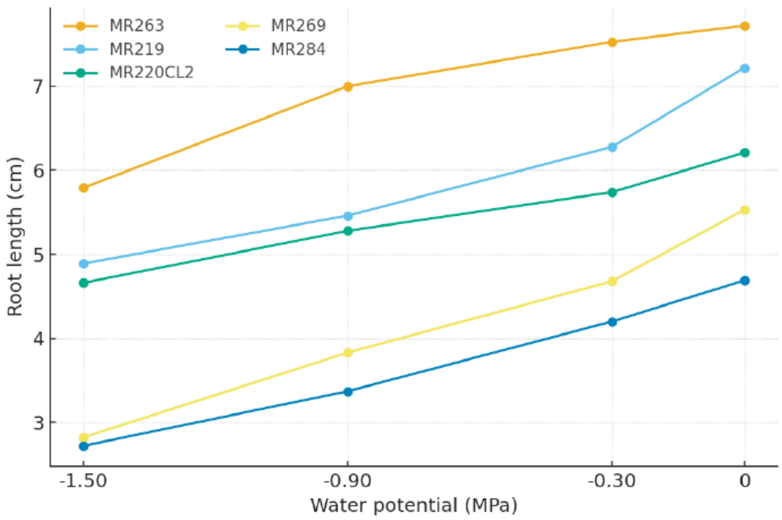

Figure 2: Root length across PEG-induced water potentials.

MR284 roots shortened more at –0.30 MPa (MR269: 5.53→4.68 cm; MR284: 4.69→4.20 cm). Under moderate stress (–0.90 MPa), roots of all cultivars were significantly reduced ($p<0.05$). For example, MR263 root length declined to 7.00 cm and MR219 to 5.46 cm. The severe stress (–1.50 MPa) had the most drastic impact: MR263 roots were 5.79 cm long, MR219 4.89 cm, MR220CL2 4.66 cm, MR269 2.82 cm, and MR284 2.72 cm. Throughout, MR263 maintained the longest roots at each stress level, whereas MR284 always had the shortest. In summary, root length decreased progressively with increasing water deficit, but MR263's roots were relatively least inhibited (Figure **2**). The cultivar × stress interaction was significant, indicating differential sensitivity.

3.3. Seedling (shoot) Length

Figure **3** shows the seedling (shoot) length across PEG-induced water potentials. Shoot growth was strongly inhibited by water stress. Under control conditions, shoot lengths ranged from 3.35 cm (MR284) to 6.21 cm (MR263). Even mild stress (–0.30 MPa) reduced shoot length in sensitive cultivars: MR269 fell from 4.27 to 3.36 cm, MR219 from 5.22 to 4.65 cm. Moderate stress (–0.90 MPa) caused MR219 to drop further to 3.71 cm and MR269 to 2.42 cm. At the extreme –1.50 MPa, shoot elongation was nearly arrested for MR269 and MR284: MR269 shoots averaged only 0.69 cm and MR284 0.34 cm, indicating severe stunting. In contrast, MR263 and MR220CL2, while reduced, still formed appreciable shoots at –1.50 MPa (3.84 cm and 3.23 cm, respectively). Among cultivars, MR263 consistently had the tallest seedlings under all conditions, whereas MR284 was shortest. The decline in shoot length with decreasing water potential was significant for all cultivars. Figure **3** illustrates these patterns: each line slopes downward as stress increases, with MR263 and MR220CL2 lines staying above the others.

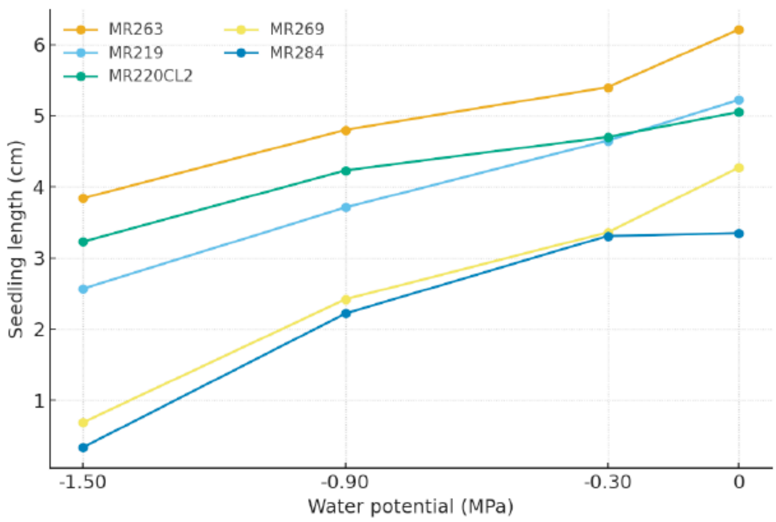

Figure 3: Seedling (shoot) length across PEG-induced water potentials.

In all measured traits, increased PEG-induced osmotic stress led to diminished performance. Seedling vigor index, root length, and shoot length all declined as water potential decreased. However, genotypic variation was evident: MR263 and MR219 outperformed the other three cultivars under stress, maintaining higher vigor indices and longer roots and shoots (Figures **1**–**3**). MR284 was most susceptible, with the steepest reductions. These results suggest inherent differences in drought tolerance at the early growth stage among the five cultivars.

4. DISCUSSION

4.1. Effects of Water Deficit on Germination and Seed Vigor

Water stress markedly impairs seed germination and vigor, consistent with previous findings. As osmotic potential declined, all cultivars showed reduced germination rates and slowed seedling emergence. This is in agreement with Toosi *et al.* (2014), who reported that drought (simulated by PEG) decreases germination percentage and delays radicle growth in *Brassica* and other specie (Toosi *et al.* (2014). The reduction in germination under stress arises from limited water imbibition and metabolic inhibition (Farooq *et al.*, 2009; Murillo-Amador *et al.*, 2002). Fahad *et al.* (2017) noted that under osmotic stress, seed water absorption and hydrolytic enzyme activity are suppressed, cutting off nutrient supply to the embryo and thus reducing germination speed and uniformity. Our observation of lower germination and vigor index at –1.50 MPa aligns with this mechanism.

Seedling vigor index (SVI) combines germination and growth attributes and is sensitive to stress. In this study, SVI declined sharply as stress intensified (Figure **1**), reflecting both fewer germinated seeds and smaller seedlings. Zheng *et al.* (2016) likewise found that rice seedling length and biomass were significantly reduced under drought, yielding lower vigor indices (Evamoni *et al*., 2023).

The present results support that concept: drought "markedly altered" early seedling growth, leading to a significant reduction in shoot and root length (thus in SVI) in all cultivars (Evamoni *et al*., 2023). Notably, MR263 and MR219 maintained higher SVI across treatments, which suggests these genotypes preserved better metabolic function and growth under water limitation. Conversely, MR284's SVI nearly collapsed at severe stress, indicating a low tolerance of the germination-phase to drought. This cultivar-dependent variation echoes findings in other rice studies (*e.g.*, Islam *et al.*, 2018) where genotypes differed widely in germination performance under water deficit. The strong cultivar × treatment interaction in our ANOVA underscores that conclusion.

4.2. Root Growth Responses

The root system showed adaptive but variable responses. At mild stress (–0.30 MPa), MR263's roots remained almost as long as control, implying this cultivar resisted moderate drought-induced growth retardation. For most cultivars, only severe stress caused substantial root shortening. This supports the idea that young rice roots can initially withstand moderate water stress, but severe stress quickly limits cell elongation (Lum *et al.*, 2014). The more tolerant MR263 maintained significantly greater root length at all stress levels (Figure **2**). Long roots under drought can enhance water uptake (Kadam *et al.*, 2013), so MR263's root growth under stress likely contributed to its superior vigor index.

Drought can also induce qualitative changes in root architecture. Kim *et al.* (2020) reviewed rice root responses, noting that drought-tolerant genotypes often develop more lateral roots and narrower nodal roots to conserve resources while maximizing uptake (Kim *et al.*, 2020). Although we measured only total root length, the consistently greater length of MR263 roots under stress suggests a better adaptive root response. In contrast, MR284's root growth was heavily inhibited by –1.50 MPa, implying this cultivar may lack effective stress-induced root adaptation. Our findings thus align with the general principle that enhanced root development contributes to drought resilience (Kim *et al.*, 2020). The reduction in root length under high PEG is also reported in other cereals: water deficit disrupts cell expansion, leading to shorter roots (Lum *et al.*, 2014).

4.3. Shoot (Seedling) Growth Responses

Shoot length declined even more steeply than root length under stress. By –1.50 MPa, two cultivars (MR269, MR284) produced barely measurable shoots, indicating that photosynthetic and growth processes were severely compromised. Drought typically inhibits shoot elongation by lowering turgor and limiting cell division (Fahad *et al.*, 2017). Zheng *et al.* (2016) found a similar pattern in rice, with drought causing "significant reduction in shoot … length" (as well as root length) (Evamoni *et al.*, 2023). Our data confirm that shoot growth is a highly drought-sensitive trait. Again, genotype matters: MR263's shoots, though reduced, remained longest at each stress level (*e.g*. 3.84 cm at –1.50 MPa), whereas MR284's shoots were almost nil. This suggests MR263 retained greater leaf expansion under stress, perhaps via better water status or osmotic adjustment.

Interestingly, MR220CL2 showed moderate shoot inhibition but not as drastic as MR269 or MR284. The chemistry of Clearfield varieties (imidazolinone residues) might affect growth, but under water stress, its shoot growth tracked closer to MR263's curve than to the more sensitive cultivars. This indicates that MR220CL2 possesses some drought resilience. Overall, our results confirm that shoot elongation under drought varies by genotype, matching reports in other rice and crop species (*e.g.*, Toosi *et al.*, 2014; Gola, 2018). Reduced shoot growth under stress is a common response, as plants close stomata and divert resources to root maintenance which is consistent with the steep declines observed here (Toosi *et al.*, 2014).

4.4. Genotypic Variation and Implications

The contrasting responses of the five cultivars highlight genetic differences in early drought tolerance. MR263 and MR219 clearly outperformed the others under water deficit, retaining higher germination, vigor, and growth (Figures **1**–**3**). These cultivars may possess favorable alleles or physiological traits (*e.g.* osmotic adjustment, efficient resource use) that sustain early growth when water is limiting. Lum *et al.* (2014) reported that some Malaysian upland rice varieties likewise showed superior root and shoot growth under PEG stress, pointing to inherent diversity in stress response. By contrast, MR284 was consistently weakest; its rapid decline in all traits suggests low inherent drought tolerance at the seedling stage. This pattern is reminiscent of other studies where certain indica rice varieties were identified as drought-sensitive (Islam *et al.*, 2018).

The ability of MR263 and MR219 to maintain root length under stress likely aided their relative success. Increased root length improves water acquisition, which in turn helps sustain shoots and improve vigor (Kim *et al.*, 2020). Cultivar-specific drought response has been noted in many rice screening studies. For example, Zheng *et al.* (2016) found wide variation among genotypes for germination and seedling vigor under PEG, and a principal component analysis indicated that seedling vigor indices were key discriminators of tolerant lines (Evamoni *et al.*, 2023). Our results suggest that simple measures like seedling vigor index, root length, and shoot length under PEG screening can effectively distinguish tolerant vs. sensitive cultivars.

From a breeding and agronomy perspective, our data imply that MR263 and MR219 may be better suited for direct-seeding or early establishment in fields prone to transient drought. Focusing on seed vigor and root development as selection criteria could accelerate the identification of drought-resilient lines. The clear differences observed also suggest that combining these traits into composite indices (or selecting QTLs related to seedling vigor and rooting traits) may help improve drought tolerance in Malaysian rice.

In summary, water deficit at germination slows or arrests early growth by limiting water uptake and cell division. These fundamental effects are well documented (Fahad *et al.*, 2017; Murillo-Amador *et al.*, 2002) and explain the general decline in all measured parameters. Our contribution is to quantify these effects for locally important cultivars and to identify the relatively tolerant genotypes. Future work could assess whether MR263 and MR219 also perform well under field drought conditions and investigate the physiological or genetic mechanisms underlying their tolerance.

5. CONCLUSION

The different responses of the five Malaysian indica rice cultivars to polyethylene glycol–induced drought stress highlight the importance of selecting varieties with high early growth and seedling vigor under limited water conditions. The superior performance of MR263 and MR219 suggests their potential in breeding programs aimed at improving drought resilience. Promoting such tolerant cultivars can enhance water-use efficiency, stabilize yields under drought condition, and support more sustainable rice production systems. Integrating these findings into breeding and cultivation strategies contributes directly to long-term agricultural sustainability and water resource conservation in rice-growing regions.

CONFLICTS OF INTEREST

We declare no conflicts of interest or competing interests.

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