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Evaluation of Root System Architecture of Some Rice Genotypes Under Artificial Saline Conditions

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Abstract

Root system architecture is critical for plant growth and crop yield, especially under abiotic stress conditions such as drought, salinity, and flooding. In this study, we evaluated the salt tolerance of thirteen rice genotypes by analyzing plant height, root length, root growth angle, and soil-surface rooting using the glass tube method with NaCl supplementation at concentrations of 0‰, 3‰, and 6‰. The results indicated that salinity negatively impacts the plant height, reducing it by 4.5% to 31.8%, and restricts root development, with root length reductions of 0.8 to 3.8 cm at 6‰ NaCl. Notably, rice genotypes 8C, Hom rau 6190, Cuom type 2, Lua ven, and Chanh trui exhibited strong salt tolerance at 3‰ NaCl concentration, as evidenced by a high soil-surface rooting ratio and a root growth angle below 30 degrees (salt tolerance scores of 1). These findings have provided valuable information for assessing salt tolerance in rice genotypes based on their root system architectures.

Keywords: *Oryza sativa* L., root growth angle, soil-surface rooting, salt tolerance

1. Introduction

Rice (*Oryza sativa* L.) plays a vital role as a staple food crop in Vietnam (Khanh et al., 2021). However, in recent years, the severe impacts of climate change have led to a serious problem: saltwater intrusion in coastal provinces. This phenomenon threatens both the livelihoods of farmers and the national food security. Arable land area and rice productivity have declined due to salinity. To address this urgent situation, it is crucial for worldwide scientists to actively research and develop rice varieties that are high-yielding, of good quality, highly salt-tolerant, and adaptable to climate change (Anh et al., 2016).

The 'Green Revolution' has led to the development of high-yielding rice varieties. While crop breeding has primarily focused on improving above-ground plant structures to maximize yield in water-rich environments (Khush, 2001), it is equally crucial to enhance below-ground plant structures. In unstable environments with limited water and nutrients, the root system plays a vital role in water and nutrient uptake and transport. Roots have a significant impact on yield, especially in adverse conditions like drought, flooding, and salinity. Excessive accumulation of Na+ in the soil can lead to oxygen deficiency in black soils, resulting in stunted rice root growth. Surface roots allow plants to access more oxygen from the higher-oxygen surface soil, reducing salt stress damage (Kitomi et al., 2020). Despite advancements in above-ground traits, root characteristics have been given less attention. Intuition and breeder experience play a significant role in selecting promising hybrid lines, but direct observation of roots remains challenging due to their hidden underground nature (Uga, 2021). Researchers are developing technologies for rapid field-based root trait evaluation. However, cost-effective methods for assessing root traits are limited, and comprehensive imaging of the root system is lacking (Atkinson et al., 2019). Recently, the soil-surface root (SOR) phenotype was discovered, which aids rice plants in coping with saline conditions. This suggests that SOR roots could benefit both saline rice fields and flooded paddies. Genes like *DRO1* and *qSOR1*, closely related to root system architecture, contribute to abiotic stress tolerance. DRO1 (on chromosome 9) and *qSOR1* (on chromosome 7) were the first quantitative trait loci (QTLs) identified for root angle development in crops (Uga et al., 2013; Kitomi et al., 2020). Our study evaluated root system architecture in selected rice genotypes under saline conditions to understand the relationship between root system architecture and salt tolerance

2. Materials and Methods

2.1. Material Collection

All rice genotypes were mainly provided by Agricultural Genetics Institute and the Plant Resource Center. This set comprised 12 genotypes characterized by their salt tolerance, with 'Khang Dan 18' referring to a non-tolerant control cultivar. The cultivars employed in this research are enumerated in Table 1.

Table 1. List of rice varieties and their information used in the study

No.	Genotype	Original collection

1	Khang dan 18	Thai Binh
2	8C	Agricultural Genetics Institute
3	Nep cai doc	Ha Giang
4	Khau tan pom	Ha Giang
5	Khau ray	Yen Bai
6	Hom rau 6190	Plant Resource Center
7	Khau nua cai	Ha Giang
8	Nep cam den	Ha Giang
9	Cuom type 2	Plant Resource Center
10	Ca bay	Ha Giang
11	Lua ven	Plant Resource Center
12	Te meo	Yen Bai
13	Chanh trui	Plant Resource Center

2.2. Methods

2.2.1. Experimental design

The study applied a modified version of the glass tube technique described by Hanzawa et al. (2013), specially tailored for saline environments. The methodology involved several steps: Firstly, the seeds were sterilized using 70% ethanol for 45 seconds, followed by rinsing twice in sterilized distilled water. Subsequent sterilization was then performed with a 20% hydrogen peroxide solution for 20 min, and then the seeds were rinsed three to four times with sterilized distilled water. Post-sterilization, seeds were allocated to individual Petri dishes and submerged in distilled water for a 24-hour period, followed by an incubation phase lasting 36-48 hours. Germination ensued, and the sprouted seeds were planted in glass tubes (1.8 x 18 cm) containing 10 ml of 0.7% (w/v) agar, which was fortified with varying salt concentrations. The tubes were hermetically sealed using aluminum foil. Plant cultivation occurred within a growth chamber, adhering to a 10-day experimental protocol. The chamber conditions were regulated to maintain a constant temperature of 24 ± 2°C and a photoperiod of 12 hours per day at a luminous intensity ranging from 2500 to 3000 lux. The experimental design was completely randomized, with each cultivar planted in triplicate, resulting in a total of 20 plants per trial.

2.2.2. The salt tolerance assessment of rice varieties

The salt tolerance assessment of rice varieties was conducted following the IRRI (1997) guidelines, with some modifications to align with the research context. The experiment was arranged in a completely randomized design, involving 13 rice varieties and three NaCl salt concentrations: 0‰, 3‰, and 6‰. The salt-sensitive variety Khang dan 18 was used as the control treatment. The salt tolerance levels were categorized as follows:

Level < 3: good salt tolerance.

Levels 3 – 5: moderate salt tolerance.

Levels 5 – 7: sensitivity to salt.

Levels 7 – 9: high sensitivity

2.2.3. Effect of salinity on growth and development of rice genotypes

In this study, we evaluated several parameters related to rice growth and development under varying saline conditions (NaCl 3‰ and NaCl 6‰) compared to their respective non-saline control groups over a 10-day period. The parameters included plant height, root length, root growth angle, and soil-surface root. For plant height, we measured from the base of the stem and roots to the tip of the tallest leaf (cm). Root length was done following the previously described report of Ramalingam et al. (2017) with some modifications. Specifically, we recorded from the root base, and to the tip of the longest root (cm) (Anh et al., 2016; Vu et al., 2016). For root growth angle, to assess the root growth angle, seedlings were removed from the growth chamber after 10 days of cultivation. The number of primary roots was counted in four angular zones: < 0°, 0–30°, 30–60°, and 60–90°. Gravity and horizontal direction corresponded to angles of 90° and 0°. For soil-surface roots, the number of soil-surface roots was counted, and the roots extending above the soil surface were considered as surface roots (Hanzawa et al., 2013).

2.2.4. Data analysis

One-way analysis of variance (ANOVA) was performed using SPSS software. Mean values in each treatment were compared using Duncan’s new multiple-range test.

3. Results and Discussion

3.1. Evaluation of salt tolerance in rice genotypes

Testing for salt tolerance among various rice varieties/landraces in agar-based environments under saline conditions revealed that after a period of 10 days, all tested varieties performed well in 3‰ saline environments, with a resistance score ≤ 3. In contrast, at a higher salinity level of 6‰, the Khang dan 18 and Ca bay cultivars displayed symptoms of leaf desiccation and mortality (resistance score 5-7). Only two cultivars, Cuom type 2 and Tranh chui, continued to grow and develop almost normally (resistance score ≤3). The remaining rice varieties experienced stunted growth, with initial signs of leaf yellowing and some basal leaves drying out and dying (tolerance score 3-5), as shown in Table 2. Yellowing and scorching of leaves, wilting, leaf decay and defoliation are all symptoms of salt stress and chloride toxicity, as previously described by Marschner (1995) and Alam et al. (2002). The main symptom observed in rice is leaf scorching, which can ultimately lead to the death of the plant due to differences in osmotic pressure. Elevated levels of Na^+ levels in the surrounding environment infiltrate the cells, causing water to exit and dehydrate and wilting the plant. Additionally, excessive uptake of Cl^- and Na^+ results in shortages of Ca_2^+ and K^+, which disrupts the nutritional balance in plants (Mensah et al., 2006). The findings of this study confirm the salt tolerance capabilities of different types of rice, corroborating the research published by Anh et al. (2016).

Table 2. Evaluating salinity tolerance of the rice genotypes in this study

No.	**Genotype**	**Salinity tolerance score (3‰)**	**Salinity tolerance score (6‰)**
1	Khang dan 18	3	7
2	8C	1	5
3	Nep cai doc	3	5
4	Khau tan pom	3	5
5	Khau ray	1	5
6	Hom rau 6190	1	5
7	Khau nua cai	3	5
8	Nep cam den	1	5
9	Cuom type 2	1	3
10	Ca bay	3	7
11	Lua ven	1	5
12	Te meo	3	5
13	Chanh trui	1	3

3.2.Effect of salinity on growth characteristics of rice types

3.2.1. Effect of salinity on plant height

Under identical conditions, the plant height of different rice varieties was primarily attributed to the genetic characteristics of the various genotypes (Hasamuzzaman et al., 2009). However, within the same variety under varying conditions, it was observed that salinity significantly reduced the plant height in most genotypes ($p < 0.05$). Two genotypes, Cuom type 2 and Chanh trui, exhibited a reduction in the plant height, although it was not statistically significant ($p > 0.05$), indicating that salinity did not have a significant effect on the plant height of these two genotypes. At 3‰ salinity, salt decreased the plant height of the varieties from 4.5% (Hom rau 6190) to 17.4% (Khang dan 18) (Figure 1). At 6‰ salinity, the plant height of Khang dan 18 was reduced the most (31.8%), followed by 8C (27.6%) and Khau nua cai (24.2%). Hom rau 6190 and Nep cam den experienced the least reduction in plant height, at 12.9% and 15.1%, respectively (Figure 1). According to Bo et al. (2016), the sensitivity of rice varieties to salinity correlates with the degree of reduction in plant height. The research findings on the impact of salinity on reducing the plant height of rice are consistent with the study by Nhuong et al. (2022), which assessed the salt tolerance of four rice varieties OM5451, IR29, OM18, and MTL316 at the germination and seedling stages.

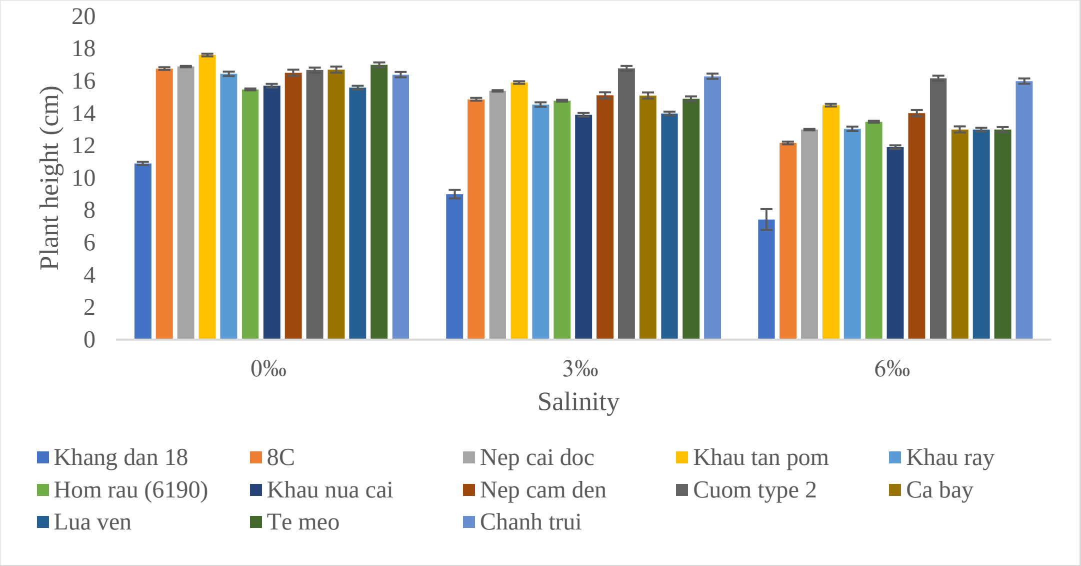

Figure 1. Effect of salinity on plant height across rice genotypes. Different letters above the bars indicate statistically significant differences ($p < 0.05$)

3.2. Effect of salinity on the root length

The analysis of root length in various rice genotypes at different salinity levels indicated that root growth of most genotypes was reduced in a medium with 3‰ and 6‰ salinity compared to a 0‰ salinity medium. Among the genotypes tested, the genotypes Khau nua cai, Nep cam den, Ca bay, Lua ven, and Te meo showed no statistically significant reduction in root length when subjected to the 3‰ salinity medium compared to the 0‰ salinity medium ($p < 0.05$). However, at the 6‰ salinity level, there was a remarkable reduction in root length for all rice genotypes ($p < 0.05$). The root length of the rice genotypes decreased from 0.8 cm (Ca bay) to 3.8 cm (8C) (Figure 2, Figure 3). These results clearly demonstrate that salinity negatively impacts on the root growth of rice. This phenomenon occurs due to the impact of salinity, where the root system reduces its development to adapt to saline conditions by creating barriers to minimize water loss within cells, thereby inhibiting root elongation (Saddiqe et al., 2016). Additionally, high Na^+ concentrations could replace $Ca2^+$ and reduce the bonds within lignin molecules, leading to a decrease in root cell elongation (Byrt et al., 2018). These research findings are in complete agreement with the studies conducted by Nhuong et al. (2022) and Tam et al. (2021).

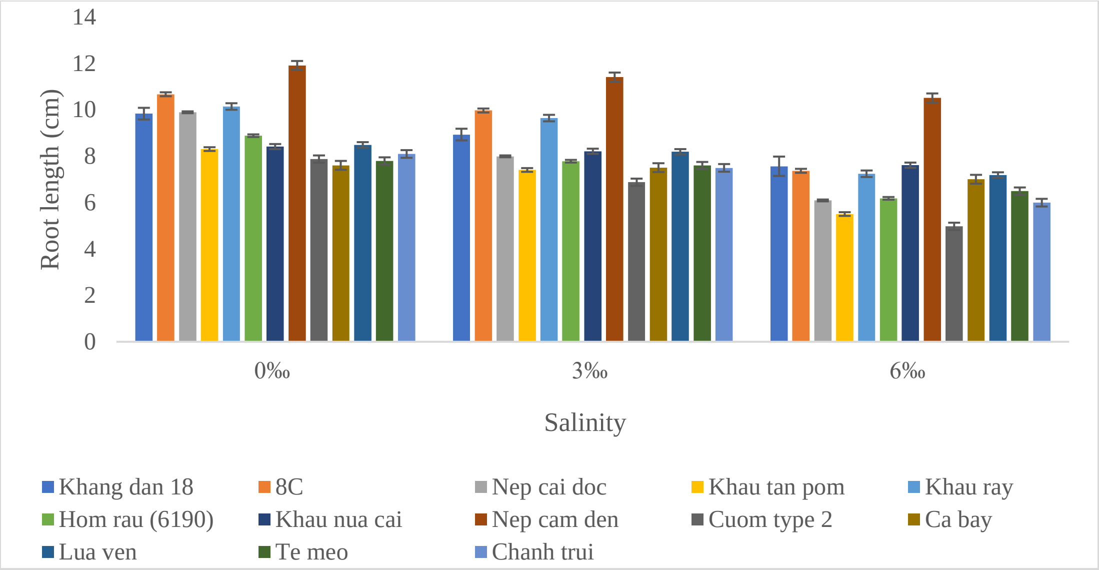

Figure 2. The effect of salinity on root length across rice genotypes. Different letters above the bars indicate statistically significant differences ($p < 0.05$)

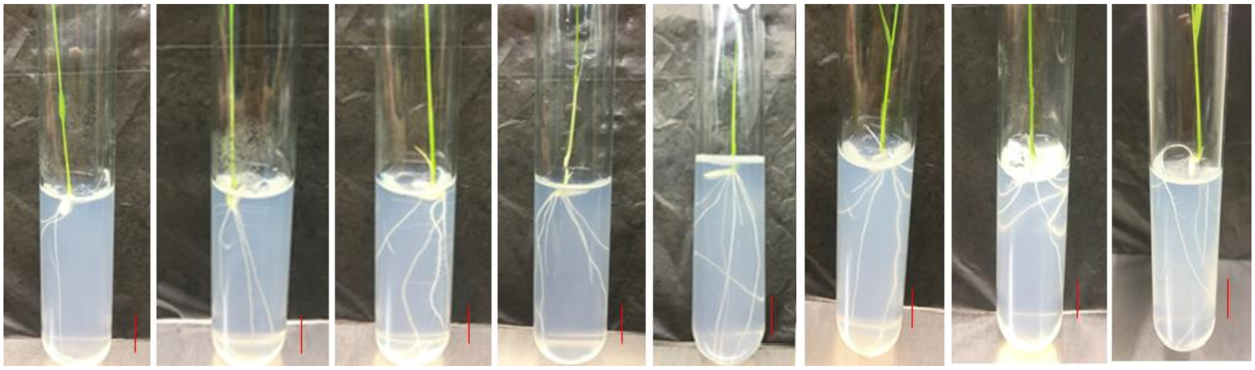

Figure 3. The root phenotype of rice genotypes (left to right: Khang dan 18, Nep cai doc, Khau tan pom, Khau ray, Khau nua cai, Nep cam den, Ca bay, Te meo). Scale bar: 5 cm

3.3. Effect of salinity on the root growth angle

To investigate the effect of salinity on root growth angle, we cultivated rice genotypes in agar supplemented with NaCl at different concentrations of 0‰, 3‰, and 6‰. Remarkably, over 60% of the roots in the rice genotypes were observed to fall within the root angle zone >60°, a characteristic outcome of the positive gravitropic response exhibited by root tips. Interestingly, when we compared the same genotypes across different salinity levels, the distribution of roots in angle zones below 30°, 30°-60°, or above 90° did not exhibit statistically significant differences.

Notably, certain rice genotypes, specifically, 8C, Hom rau 6190, Cuom type 2, Lua ven, and Chanh trui, displayed a higher ratio of roots in the angle zone below 30°. Intriguingly, these same genotypes also demonstrated greater salinity tolerance (score 3) compared to others at the

3‰ salt concentration (Figure 4). This correlation suggests that the development of shallow root angles may contribute to minimizing the adverse effects of salinity on crops.

In a related study, Kitomi et al. (2020) measured root angles (from the horizontal surface to the shallowest root part) in mutant rice lines and their original counterparts. The mutants exhibited smaller root angles than the originals. Surprisingly, despite having equivalent yields under normal conditions, the mutants achieved a 15% higher yield in 0.4% saline water fields over a four-year period (2015 - 2018). These findings highlighted the potential benefits of shallow root angles in mitigating the impacts of salinity.

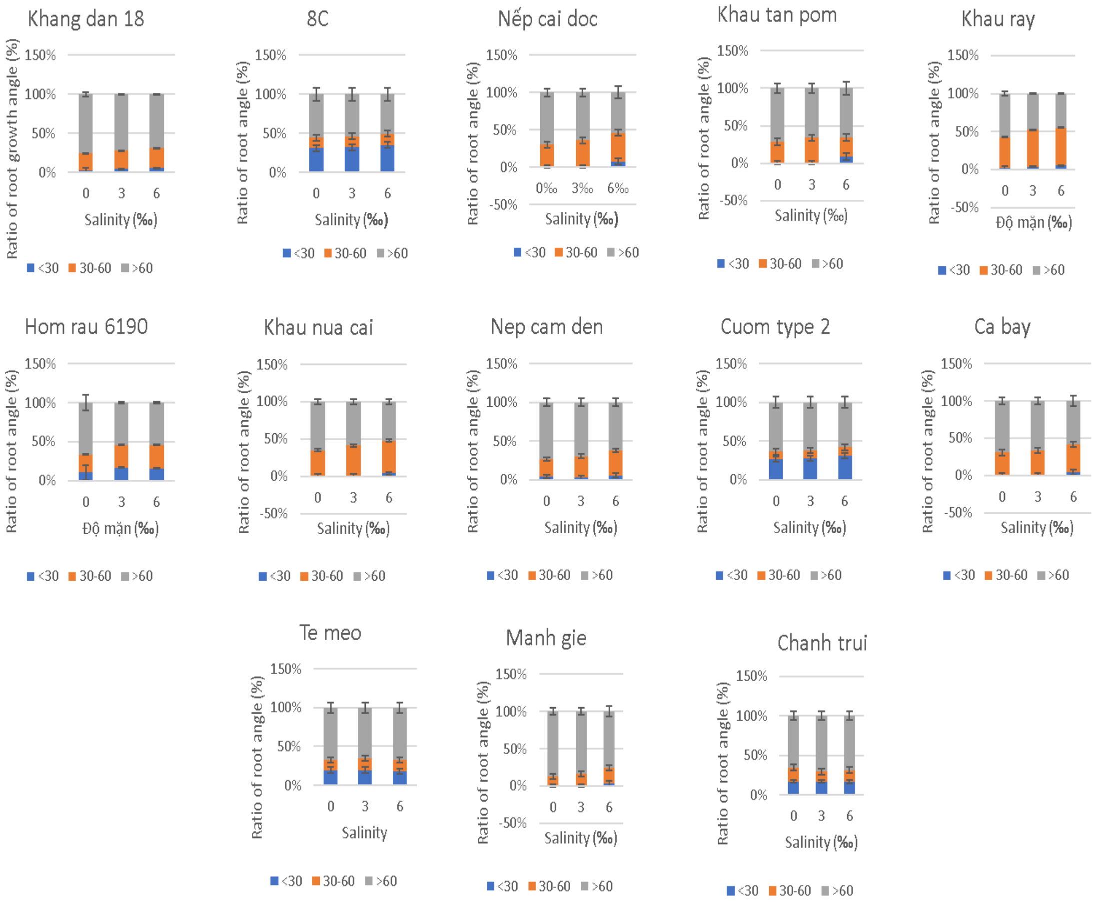

Figure 4. The effect of salinity on the root growth angle across rice genotypes

3.4. Effect of salinity on soil-surface root

Primary roots extending above the agar surface, referred to as soil-surface roots, were observed in five rice genotypes: 8C, Hom rau 6190, Cuom type 2, Lua ven, and Chanh trui. Among these, 8C and Cuom type 2 stood out with the highest percentages of soil-surface roots ratio, recorded at 35.0% and 31.3%**,** respectively. No significant difference in the soil-surface root ratio was observed across saline media with concentrations of 0‰, 3‰, and 6‰. Furthermore, even in the control medium containing 0‰ NaCl, genotypes that naturally lacked soil-surface roots did not develop them when exposed to 3‰ NaCl and 6‰ NaCl (as shown in Figure 5, Figure 6). These results implied that salinity does not directly induce the formation of soil-surface roots.

Kitomi et al. (2020) provided the evidence related to the formation of soil-surface roots, and found that physiological factors such as oxygen and light influence their emergence. On a molecular level, the presence of the qSOR1 gene was particularly linked to the formation of soil-surface roots. Experiments showed that lines lacking the *qSOR1* gene either do not have soil-surface roots or have fewer compared to those with the gene (Kitomi et al., 2020). Typically, roots extend into the agar in the direction of gravity. However, in this experiment, roots developed upwards, against the direction of gravity. The genotypes with soil-surface roots were those that exhibited high salinity tolerance at a concentration of 3‰ NaCl. Thus, the presence of soil-surface roots in rice plants is not a response to salt stress in plants but rather a formation that helps rice avoid the stress caused by salinity instead of directly resisting it. Our findings have provided further foundation for evaluating the salinity tolerance of rice varieties by analyzing the soil-surface root phenotype, in addition to assessing the salinity tolerance phenotype through the presence of the *Sal* gene (Le et al., 2012). However, further research is needed to understand the mechanisms and clarify the relationship between salinity tolerance and the structure of soil-surface roots in rice varieties.

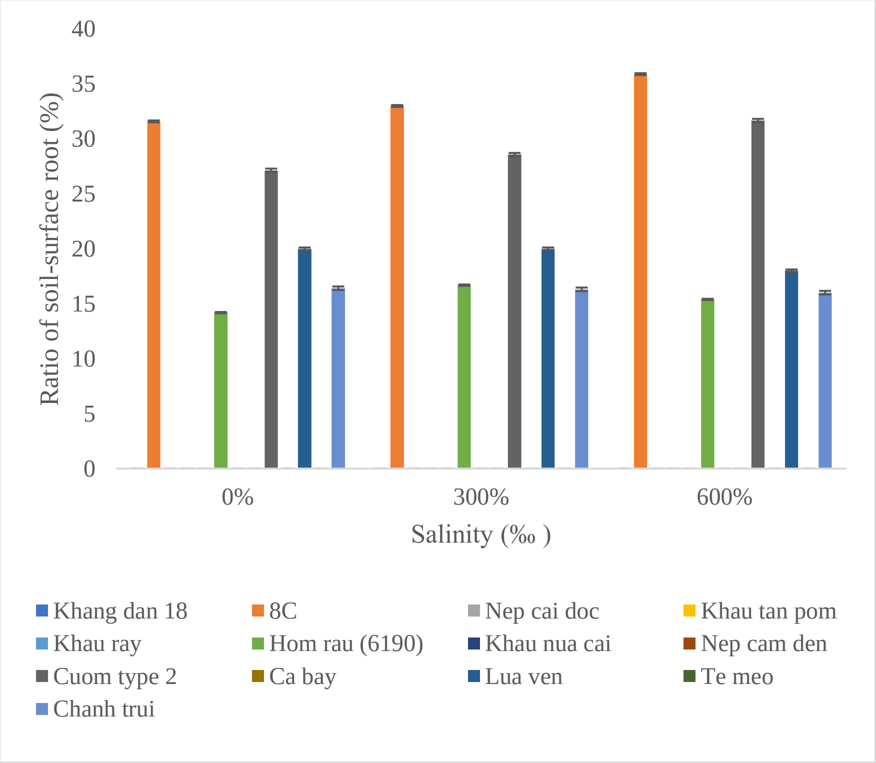

Figure 5. The effect of salinity on the ratio of soil-surface roots

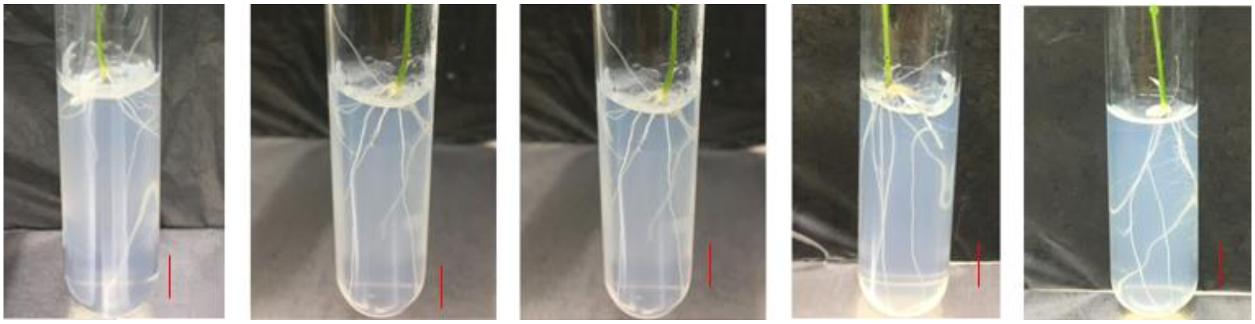

Figure 6. Soil-surface rooting phenotype of rice genotypes (left to right: 8C, Hom Rau 6190, Cuom Type 2, Lua Ven, and Chanh Trui). Scale bar: 5 cm

4. Conclusions

In agar media with different salinity levels, the study observed a decrease in plant height and root length in the rice genotypes as they adapted to saline conditions. The investigation focused on the root structure near the soil surface and found that genotypes with a significant presence of soil-surface roots and roots growing close to the ground showed better tolerance to salinity. Although the presence of soil-surface roots did not necessarily improve salinity tolerance, further research is needed to understand their role in reducing salinity stress in crops. These findings provide valuable insights for developing a method to evaluate salinity tolerance in rice varieties through the analysis of root system architecture.

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