# Asian Journal of Plant Sciences

ISSN 1682-3974

OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2023.250.259

## Research Article

# Salinity Tolerance Evaluation of Rice (*Oryza sativa* L.) 'Tubtim Chumphae' Seedling and Early Vegetative Stage

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## Abstract

**Background and Objective:** Salinity is abiotic stress that affects rice growth and results in lower yields. This research studied the effects of salinity on Tubtim Chumphae rice growth. **Materials and Methods:** Seedlings were cultivated for 35 days in the soil before being stressed by salinity (0, 25, 50, 75 and 100 mM NaCl) for 4 weeks. Growth performance and some physiological traits were recorded weekly for 4 weeks. **Results:** Under salinity stress, fresh and dry weight of shoot and root, relative dry weight, plant length and root length all decreased as salt solution concentration increased. Electrolyte leakage percentage and malondialdehyde content increased when rice was exposed to the salt solution for 4 weeks, indicating that high salinity damaged the lipid membrane. The PCA results revealed positive correlations among growth parameters that negatively correlated with MDA content and electrolyte leakage. The HCA results confirmed the PCA biplot showing decreasing expression of growth parameters with rising salt stress levels, while MDA content and electrolyte leakage continuously increased. Results suggested MDA content and electrolyte leakage as indicators for salt stress in Tubtim Chumphae rice at seedling and early vegetative stage. **Conclusion:** Findings in this study indicated that Tubtim Chumphae rice plants can grow in slight to moderate salinity soil. Results will deliver advantageous data that can be integrated with additional agronomical characters in rice growth or breeding plans for abiotic stress-tolerant cultivars improvement.

Key words: Hierarchical cluster analysis, principal component analysis, rice growth, salinity stress, sodium chloride

Citation: Taratima, W., S. Chaisuwan, K. Plaikhuntod, P. Maneerattanarungroj, N. Kunpratum and A. Trunjaruen, 2023. Salinity tolerance evaluation of rice (*Oryza sativa* L.) 'Tubtim Chumphae' seedling and early vegetative stage. Asian J. Plant Sci., 22: 250-259.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Rice is the most widely consumed global food crop, ahead of maize and wheat, providing roughly 3.5 billion individuals with a significant source of calories[1]. Nevertheless, rice is categorized as a crop that is sensitive to salinity in both the seedling and reproductive phases[2]. Soil saltiness is a serious agricultural issue that affects plant growth, impacting over 20% of irrigated land[3] and resulting in global crop output reductions. Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES)[4] estimated 1 billion hectares of salinity-affected soil in approximately 100 countries. High salt concentration in plant cells results in ion toxicity, osmotic stress, oxidative stress and loss of antioxidant system balance, with the inability to eliminate reactive oxygen species (ROS)[5]. An increase in abiotic stress damages DNA, lipids and proteins in plant cells, directly affecting growth and productivity and possibly death due to lipid peroxidation[6]. Osmotic stress and nutritional imbalance caused by excessive salt concentration in plant cells inhibit water absorption of soluble salts in the root zone, especially for Potassium ($K^+$) and Calcium ($Ca^+$)[7]. Many plants modify their metabolic processes in response to stress by increasing proline, ethylene, glycine betaine and ROS contents that impact lipid peroxidation and Malondialdehyde (MDA) content[8].

Rice producers are increasingly concerned about how climate change is affecting rainfall patterns. Rice production is impacted by a variety of environmental conditions, making breeding extremely complex. Basic knowledge is essential to better understand plant responses to salinity stress under unpredictable temperature adaption processes. Plants undergo cellular, molecular and morphological adaptations, as well as physiological and biochemical alterations, to overcome stress. These variations are influenced by factors such as stress severity, developmental stage and genotype[9].

Tubtim Chumphae (SRN06008-18-1-5-7-CPA-20), the Thai hybrid rice cultivar (KDML105 (Mother)×Sangyod Phatthalung (Father)) has high vitamin E, phenolic, antioxidant and flavonoid contents and also contains compounds such as kaempferol, myricetin, gallic acid, luteolin, cyanidin-3-glucoside and apigenin[10]. This cultivar has low amylose content[11], making it ideal for diabetics and people who wish to limit their sugar intake. Tubtim Chumphae rice nutrition values are well known but knowledge about stress physiology and plant response to salinity stress is limited. This study investigated the effects of salinity stress on Tubtim Chumphae rice growth and investigated the physiological and biochemical adaptations of Tubtim Chumphae rice at seedling and early vegetative stage under salinity stress and unpredictable temperature. Findings will serve as a model for future rice breeding researchers and farmers interested in growing this cultivar and provide basic strategies to deal with salinity stress, especially the seedling preparation step for transplanting.

## MATERIALS AND METHODS

**Study area:** This research project was performed from July, 2021 to March, 2022.

**Plant materials and NaCl treatment:** Healthy Tubtim Chumphae rice seeds provided by Chumphae Rice Research Center, Thailand were soaked in water for 1 day and then germinated in moist filter paper in Petri dishes under room temperature and dark conditions for 2 days. The germinated seeds were cultivated in seedling trays containing peat moss for 7 days before transfer to culture pots (10 inches across). Each container included two kilograms of a 2:1 volume mixture of peat moss and semi-loamy clay soil. After being watered every day for 35 days, one seedling per pot was treated once every other day for 4 weeks with 100 mL of different NaCl solution concentrations (0, 25, 50, 75 and 100 mM) rather than water.

**Growth performance:** All treated and control plants were cultivated in a greenhouse at the Department of Biology, Faculty of Science, Khon Kaen University, Thailand from December, 2021 to February, 2022. Growth performance in terms of root length, shoot length, fresh and dry weight were recorded weekly for 4 weeks. Relative dry weight percentage was calculated based on Islam and Karim[12] as follows:

$$\text{ReDW (\%)} = \frac{\text{Total dry weight at salt level}}{\text{Total dry weight at control level}} \times 100 \qquad (1)$$

**Chlorophyll content:** After 4 weeks of treatment, total chlorophyll, chlorophyll a and chlorophyll b contents were studied by pulverizing 0.1 g of mature and healthy leaves in a mortar before dissolving in 5 mL of 80% acetone and filtrating the solution over filter paper. Another 20 mL of 80% acetone was filled once all of the green matter had completely dissolved. A spectrophotometer (Spectronic 20) was used to determine the supernatant using 80% acetone for a blank at 645 and 663 nm absorbance. The following formulae were used to calculate chlorophyll content based on Arnon[13]:

$$\text{Total chlorophyll (mg g}^{-1}\text{ tissue)} = \frac{20.2\ (\text{A645})+8.02\ (\text{A663})\times \text{V}}{1000\times \text{W}} \quad (2)$$

$$\text{Chlorophyll a (mg g}^{-1}\text{ tissue)} = \frac{12.7\ (\text{A663})-2.69\ (\text{A645})\times \text{V}}{1000\times \text{W}} \quad (3)$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ tissue)} = \frac{22.9\ (\text{A645})-4.68\ (\text{A663})\times \text{V}}{1000\times \text{W}} \quad (4)$$

Where:
V = Total solution volume (mL)
W = Leaves weight (g)

Chlorophyll fluorescence of treated and control plants was evaluated and mature leaves were measured as dark-adapted leaves (30 min dark) (Fv'/Fm' units) and light condition (Fv/Fm units) using a Chlorophyll Fluorimeter Handy PEA[14]. All measurements were conducted in triplicate.

**Electrical conductivity ($EC_e$):** Soil electrical conductivity during NaCl treatment was investigated based on the method of Rayment and Higginson[15]. Every other day, 3 g of soil samples were taken and transferred to 15 mL of deionized water and left to settle for 24 hrs. A PL-700 Series Bench Top Meter (Gondo: PL-700PC(S)) was used to measure electrical conductivity.

**Electrolytic leakage (EL) measurement:** Electrolytic leakage value was established based on the method of Dionisio-Sese and Tobita[16]. After 2, 3 and 4 weeks of treatment, the plant samples were chopped into small pieces (approximately 1 cm). Six pieces were placed in a test tube with 10 mL of deionized distilled water and kept in the dark for 24 hrs before the EC1 measurement was determined. The test tubes were then autoclaved for 20 min at 121°C before being measured for EC2. Percentages of electrolytic leakage were calculated as follows:

$$\text{Electrolytic leakage} = \frac{\text{EC1}}{\text{EC2}}\times 100 \quad (5)$$

**Malonaldehyde (MDA) estimation:** Malonaldehyde (MDA) content was evaluated following the protocol of Sunohara and Matsumoto[17]. After 1 to 4 weeks of treatment, plant samples were cut and frozen at -80°C. Aliquots of 0.1 g of plant samples were ground with 2 mL of 0.1% (w/v) TCA (trichloroacetic acid) and centrifuged at 14000 rpm for 5 min. After that, 0.5 mL of the supernatant was transferred to a new test tube containing 1.5 mL of 0.5% (w/v) TBA (thiobarbituric acid) and heated for 25 min at 95°C. The absorbance of the solutions was examined at 532 nm (A532) and 600 nm (A600) using 20% TCA as the blank. The MDA content was calculated as follows:

$$\text{MDA } (\mu\text{mole g FW}^{-1}) = \frac{\left[8\times\left(\frac{A_{532}-A_{600}}{155}\right)\right]}{\text{Fresh weight}} \quad (6)$$

Where:
MDA = Malonaldehyde
FW = Fresh weight

**Data analysis:** Each treatment was conducted in triplicate using a Completely Randomized Design (CRD). Data from each week were submitted to One-way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test ($p<0.05$) was used to differentiate the differences between means using IBM SPSS Statistics version 28. Principal Component Analysis (PCA) and hierarchical cluster analysis (HCA) were conducted using Origin 2022 software to investigate the growth and physiological response of Tubtim Chumphae rice plants.

## RESULTS

**Growth performance:** Shoot length, root length, fresh and dry weight of shoots and roots and relative dry weight were recorded for 4 weeks old plants to estimate the effects of salinity stress on Tubtim Chumphae rice growth. Shoot lengths of treated plants were not significantly different from the control after 4 weeks of culture, while shoot fresh and dry weight, root fresh and dry weight of treated plants decreased as NaCl concentration increased (Table 1). Root lengths of all treated plants were not significantly lower than the control during the first week of the experiment but decreased in the 75 mM and 100 mM NaCl treatments after the 4th week of culture. Root fresh and dry weight of the treated plants were lower than the control at every week of culture. The 75 and 100 mM NaCl treatments had significantly lower root weights than other treatments and the control. Relative dry weights of the whole plant of treated plants significantly reduced related to the control. The growth performance of treated plants differed from the control after 4 weeks of treatment (Fig. 1). Results demonstrated that higher NaCl concentrations significantly retarded the shoot and root growth of Tubtim Chumphae rice plants. During the experimental duration between December, 2021 and February, 2022, temperature and humidity percentages were recorded (Fig. 2a-b).


Fig. 1: Rice seedlings after various NaCl concentration treatments for 4 weeks

Table 1: Performance during growth and physiological traits of control and treated plants after 4 weeks of NaCl treatment

| | NaCl (mM) | | | | |
|---|---|---|---|---|---|
| Characteristics | 0 | 25 | 50 | 75 | 100 |
| Survival (%) | 100 | 100 | 100 | 100 | 100 |
| SL (cm) | 80.06±8.05[a] | 74.60±3.08[a] | 74.26±3.05[a] | 74.23±0.75[a] | 73.36±3.67[a] |
| SFW (g) | 43.02±1.40[a] | 35.92±6.10[ab] | 32.11±1.39[bc] | 28.14±2.65[cd] | 21.76±5.36[d] |
| SDW (g) | 11.99±0.42[a] | 9.82±2.24[ab] | 8.74±0.76[bc] | 7.08±0.62[cd] | 5.10±1.34[d] |
| RL (cm) | 37.50±0.10[a] | 34.06±3.64[ab] | 33.70±2.42[ab] | 32.40±2.59[b] | 27.80±1.65[c] |
| RFW (g) | 26.10±6.44[a] | 17.87±3.43[b] | 17.81±3.47[b] | 14.02±2.04[bc] | 10.16±2.69[c] |
| RDW (g) | 3.68±0.47[a] | 2.39±0.22[b] | 2.09±0.07[bc] | 1.71±0.10[c] | 1.16±0.34[d] |
| ReDW (%) | 100.00±0.00[a] | 77.92±3.11[b] | 69.08±4.37[bc] | 56.06±4.57[c] | 39.94±0.47[d] |
| Cha (mg $g^{-1}$ tissue) | 0.17±0.05[a] | 0.14±0.02[a] | 0.18±0.05[a] | 0.24±0.07[a] | 0.16±0.05[a] |
| Chb (mg $g^{-1}$ tissue) | 0.05±0.02[a] | 0.04±0.00[a] | 0.06±0.01[a] | 0.07±0.02[a] | 0.06±0.01[a] |
| TC (mg $g^{-1}$ tissue) | 0.23±0.07[a] | 0.18±0.03[a] | 0.24±0.06[a] | 0.32±0.10[a] | 0.22±0.07[a] |
| Fv/Fm | 0.79±0.01[a] | 0.79±0.02[a] | 0.75±0.04[a] | 0.77±0.00[a] | 0.75±0.04[a] |
| Fv'/Fm' | 0.71±0.01[a] | 0.70±0.03[a] | 0.67±0.05[a] | 0.65±0.03[a] | 0.65±0.02[a] |
| EL (%) | 27.38±1.62[c] | 32.94±2.16[b] | 35.91±5.80[b] | 42.40±2.42[a] | 44.67±2.35[a] |
| MDA (µmole g $FW^{-1}$) | 0.02±0.02[a] | 0.03±0.02[a] | 0.04±0.05[a] | 0.04±0.02[a] | 0.06±0.05[a] |

Means±SE with different letters of the same row is significantly different according to ANOVA and Duncan's Multiple Range Test ($p < 0.05$), SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, ReDW: Relative dry weight, SL: Shoot length, RL: Root length, Cha: Chlorophyll a content, Chb: chlorophyll b content, TC: Total chlorophyll content, Fv/Fm: Chlorophyll fluorescence measurement in light condition and Fv'/Fm': Chlorophyll fluorescence measurement in dark condition

**Physiological analysis:** Physiological characteristics including chlorophyll contents, chlorophyll fluorescence, electrolyte leakage (EL) and Malondialdehyde (MDA) content were determined to study the responses of Tubtim Chumphae rice plants to salinity stress. Results demonstrated that chlorophyll a, chlorophyll b and total chlorophyll contents of treated plants were not substantially different from the control after 1-4 weeks of treatment. In comparison to the other treatments and the control, the 75 mM NaCl treatment had the highest chlorophyll concentration.

After 4 weeks of culture, chlorophyll fluorescence values slightly decreased compared to the control but the difference was not significant. Higher EL values were observed in all treatments after 4 weeks of culture than in the control group, with significant differences. After 4 weeks of culture, MDA contents of all treatments were higher than the control but with no significant differences (Table 1). These results indicated that salinity stress negatively influenced the EL of Tubtim Chumphae rice plants, whereas other variables were not affected by increased NaCl concentrations.

**Principal Component Analysis (PCA) and hierarchical cluster analysis (HCA):** Data of growth and physiological alterations of Tubtim Chumphae rice plants due to salinity stress were subjected to Principal Component Analysis (PCA) to investigate the relationships between parameters and rice seedling response to stress. The correlation coefficients were used to analyze PCA and the relationships were represented as a biplot. Fourteen components were used to explain the data variances. The biplot created from the first two

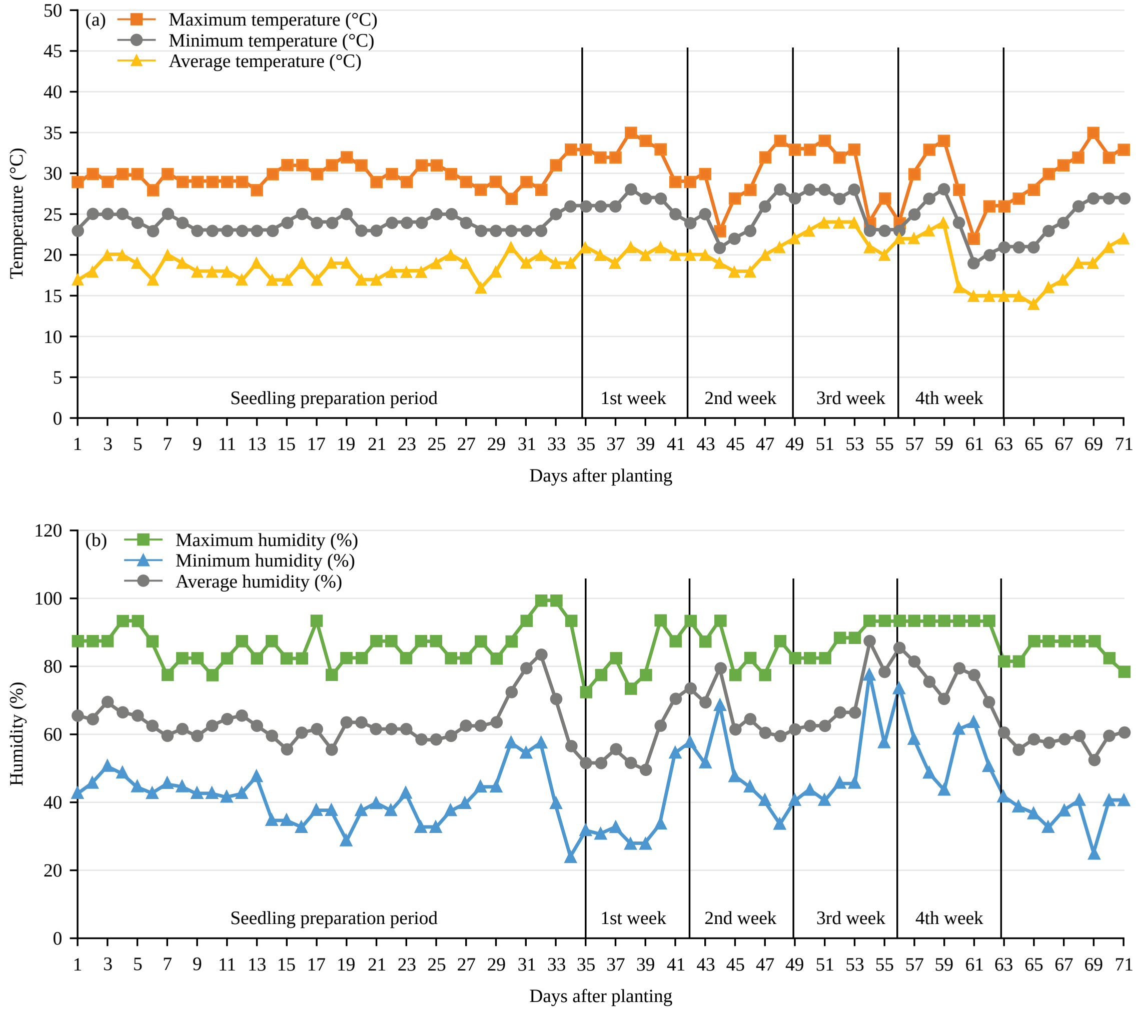


Fig. 2(a-b): Maximum, minimum and average (a) Temperature and (b) Humidity percentage of the study area during the first 35 days as seedling preparation period before treatment with NaCl for 1-4 weeks

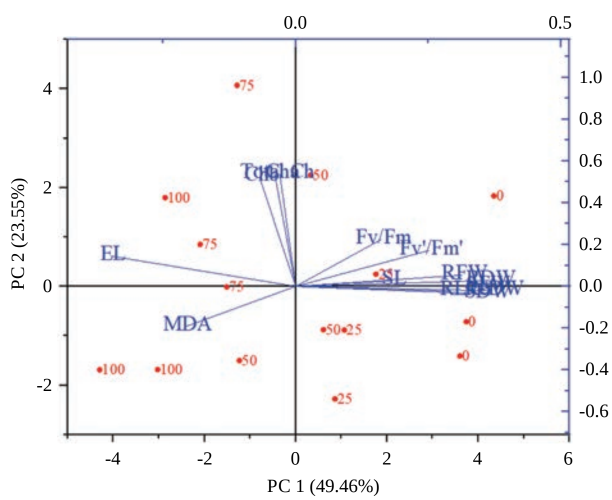


Fig. 3: Principal Component Analysis (PCA) biplot exhibiting correlations among growth and physiological parameters of Tubtim Chumphae rice seedlings treated with different NaCl concentrations

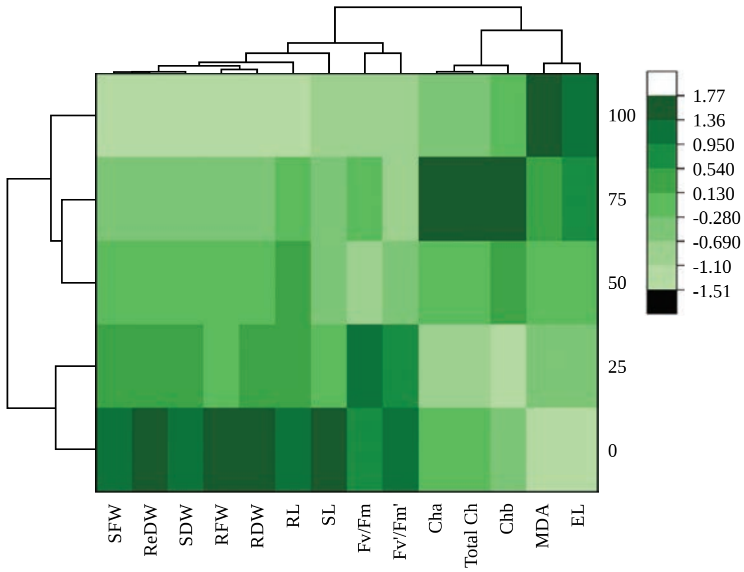


Fig. 4: Hierarchical cluster analysis (HCA) heatmap demonstrating response patterns of growth and physiological parameters of Tubtim Chumphae rice seedlings treated with different NaCl concentrations

components, PC1 and PC2, explained 73.01% of the variance. The biplot also demonstrated the relationships among the parameters. All growth parameters, Fv/Fm and Fv'/Fm' were positively correlated and located on the positive side of PC1. However, they negatively correlated with EL and MDA, which were located on the left side of PC1. Chlorophyll content had no significant relationships with the other parameters with vertical plots against all of them (Fig. 3).

Growth performance and physiological parameter results analyzed with HCA were visualized using a heatmap to demonstrate the response pattern of the rice plants to salinity stress. Results showed that all parameters could be classified into three clusters. Cluster I contained all growth parameters, Fv/Fm and Fv'/Fm' while MDA content and electrolyte leakage were grouped in cluster II. Cluster III consisted of chlorophyll a, b and total chlorophyll content. Salinity stress treatments were also displayed in three groups. Treatments with 0 and 25 mM NaCl showed healthy plants reflected by high growth performance (cluster I) and low levels of MDA and electrolyte leakage (cluster II). The second group contained treatments of 50 and 75 mM NaCl, while the last group received 100 mM NaCl (Fig. 4).

## DISCUSSION

Treated plants did not experience considerable salinity stress in the first 1-2 weeks (data not shown) but cumulative salinity rose after 3-4 weeks, affecting plant growth. Accumulation of salt in the soil profile poses a threat to long-term agricultural production[18]. Furthermore, an abrupt drop in temperature during the 2nd and 3rd weeks impacted rice growth, particularly when the plants were challenged by both salinity stress and cold shock. During the experimental duration, temperature and humidity fluctuate. The lowest temperature was recorded during the 4th week of treatment, while lowest humidity percentage was noted during the 1st week of treatment. Rice growth was impacted by multiple abiotic stressors. Bhar[19] reported that abiotic stressors such as salt, heat stress, drought, UV damage, cold shock and mineral harmfulness cause considerable yield loss in rice plants.

Generally, rice growth decreased under high NaCl concentration and length of culture. In all rice cultivars, salt stress reduced germination energy and speed and germination rate, resulting in shorter shoots, roots and dry weight[20]. Current results concurred with Quí[21], who found that increasing saline levels reduced the lengths of numerous Vietnamese rice cultivars rice shoots and roots. Liu *et al.*[22] conducted a physiological and biochemical investigation and found that salinity stress hindered rice seedling growth. When plants are exposed to salinity, ionic toxicity is produced by the accumulation of Sodium ions ($Na^+$) and Chloride ions ($Cl^-$), thus reducing plant growth[23]. Moreover, salt stress also causes osmotic stress imbalance, thereby limiting cell wall extensibility and decreasing plant growth[24].

Salinity screening for the stress level of rice involves the germination stage, seedlings or early vegetative stage and the productive stage[25]. This study focused on the effect of salinity stress during the seedling and early vegetative stage. Many farmers in Thailand prefer to cultivate rice following the transplanting rice cultivation system. These findings indicated that Tubtim Chumphae rice seedlings can grow in slight to moderate salinity soil. When compared to the control group, several salinity values were not statistically significant. Farmers can prepare Tubtim Chumphae rice seedlings for transplanting rice production systems in slight to moderate salinity conditions. However, other factors such as soil fertility, amount of available water or other environmental parameters must also be considered. Seedlings of the 100 mM NaCl treatment thrived but their growth was not better than the control group and the 25 and 50 mM NaCl treatments. Other factors may influence soil salinity levels, while some soil microorganisms play a vital role in soil nutrient balance[26].

After 4 weeks of treatment, the chlorophyll content and green intensity of some treated plants increased. This consequence concurred with Pamuta *et al.*[27] who discovered that after 3 weeks of treatment, salt stress resulted in an increase in leaf green intensity relative to the control in KDML105 rice. Salinity caused a loss in photosynthetic pigments such as carotenoids, chlorophyll a and chlorophyll b but chlorophyll content increased in plants under salinity stress for a short period or at low salt concentrations[14]. A decrease in chlorophyll content is caused by salt stress, resulting in decreased plant water absorption efficiency, the stomata close to reduce water loss from plant transpiration. As a result, the amount of carbon dioxide available for photosynthesis is reduced[28].

The chlorophyll fluorescent value can be used as an indicator of Photosystem II (PSII) efficiency. The PSII is the most vulnerable factor of the photosynthetic device that conveys the effect of abiotic stress. Generally, salinity stress impacts photosynthetic efficiency by reducing stomatal conductance, photosynthetic pigments, transpiration rate and PS II performance[29]. Non-significant differences in chlorophyll fluorescence on Tubtim Chumphae rice seedlings and early vegetative stage in this study concurred with previous reports that this occurrence indicated that PSII was unaffected by salinity stress[30]. However, previous studies reported that salinity stress decreased chlorophyll fluorescence in many plant species such as rice[31] and wheat[32].

Generally, MDA is formed after plant cell and tissue damage as the lipid membrane is peroxidized. Increasing MDA content and EL percentage enhanced NADPH oxidase activity, resulting in ROS production[33]. To effectively lower ROS and preserve plant cells during times of high salinity, the plants need to establish antioxidant defense systems involving enzyme activity such as ascorbate peroxidases (APX), superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (POD)[34]. Further studies on enzyme activities are required.

Unstable temperature and humidity percentage during the experimental period may cause multiple stressors such as chilling or drought stress with salinity stress. During the 4th week of treatment, there was a sudden drop in temperature (less than 15°C) 4 days before the end of the study. Chilling is a natural environmental stress that can directly alter chloroplast physiological activities by changing enzyme activities and lipid membrane states, resulting in decreased photosynthetic rate and ROS production[22]. Low temperature during the experimental period in this study caused chilling stress that impacted Tubtim Chumphae rice seedling growth but did not affect physiological and biochemical traits.

The PCA biplot confirmed the negative effect of salt stress on seedling growth and also revealed the relationships between growth and physiological parameters in response to salt stress. The biplot showed a strong positive correlation among growth parameters and a negative correlation with MDA content and electrolyte leakage (Fig. 3). Although each treatment was not distinctly grouped together, a pattern between the treatment scores plotted on the biplot was observed. Scores distributed on the biplot depended on increasing NaCl concentrations along the PC1 axis. The treatment without salinity stress was plotted on the right side and the highest level of stress was plotted on the left side (Fig. 3). Results implied that Tubtim Chumphae rice seedlings that did not encounter salinity stress gave the highest growth performance while higher NaCl concentration treatments showed lower growth performance. Adverse impacts of salinity stress on rice growth parameters were also reported in LYP9 and NPBA rice. Roots were fully grown in the treatment without salt stress but growth decreased when NaCl concentration increased[35]. Similar results were also recorded in shoots and roots of Cuban and Thai rice landraces[36,37]. The HCA heatmap also revealed similar results, with decreasing growth parameters at higher salt stress levels (Fig. 4).

Salt stress results from high contents of soluble salts in the soil (saline soil) or high exchangeable sodium (sodic soil). The major adverse effects of salinity on rice are ion imbalance and toxicity due to excessive $Na^+$ uptake[38]. Excessive $Na^+$ accumulation and $Na^+/K^+$ ratio in roots, stems and grain were observed in the reproductive stage of Japonica and Indica rice[39]. High $Na^+$ content accumulated in rice shoots and grain resulted in defective growth and low grain yield[40,41]. Osmotic potential and root water uptake are disrupted by salinity stress. Rice roots in direct contact with soil can be easily harmed by high salt concentration[38]. Therefore, the decrease in growth parameters of Tubtim Chumphae rice seedlings was directly related with an increase in salinity levels, as shown by the PCA and HCA results.

Interesting trends of chlorophyll fluorescence response were discovered by PCA and HCA. The PCA biplot demonstrated that Fv/Fm and Fv'/Fm' showed positive correlation with growth parameters, consistent with HCA, showing that the parameters decreased when NaCl concentration increased. High $Na^+$ concentration in plant cells directly impacted water uptake and interrupted water potential and stomatal conductance, leading to a decrease in photosynthesis efficiency[42]. In rice, net photosynthesis rate decreased when rice seedlings were treated with NaCl[43]. The adverse effects of increasing NaCl concentration on photosynthesis efficiency were also noticed in sorghum[44]. Excessive $Na^+$ accumulation also damages chloroplasts and disrupts the electron transport chain in Photosystem II (PSII), resulting in reduced photosynthesis[38]. However, chlorophyll contents, which are directly involved with photosynthesis, did not show response patterns among the treatments. Chlorophyll fluorescence parameters showed an interesting trend but one-way ANOVA results indicated no significant decrease in chlorophyll content and photosynthesis parameters (Table 1). This implied that salinity stress did not cause significant negative effects on photosynthesis and chlorophyll contents of Tubtim Chumphae rice seedlings.

In addition to growth and photosynthesis, membrane integrity, indicated by MDA content and electrolyte leakage, was directly disrupted by salinity stress, as indicated by the PCA and HCA results. In the biplot, MDA content and electrolyte leakage showed strong negative correlation with growth parameters, while normal seedlings without salinity stress provided low MDA content and electrolyte leakage. By contrast, elevated MDA levels and electrolyte leakage accompanied by interrupted growth and photosynthesis were observed in seedlings treated with NaCl (Fig. 4). Overaccumulation of $Na^+$ damages both growth and development and induces displacement of $Ca^{2+}$, which plays a role in maintaining membrane integrity[39]. Ion imbalance also leads to the overproduction of reactive oxygen species (ROS), inducing membrane damage[44]. The ROS are molecules with high potential to accept electrons from others. This causes defective biomolecules, especially phospholipid bilayers. Malondialdehyde (MDA) is a byproduct of the reaction between free radicals and phospholipids in lipid membrane peroxidation. Membrane instability due to ion toxicity and lipid peroxidation causes leakage of ions from plant cells[45]. Higher MDA content and electrolyte leakage were observed in KDML105 rice lines treated with NaCl than in the control group[46]. Similar responses to salt stress in MDA content and electrolyte leakage were also found in other plant species including wheat and ryegrass[47,48]. Therefore, higher MDA content and electrolyte leakage can be considered indicators in plants that suffer from salt stress, while growth and physiological response of Tubtim Chumphae rice seedlings under salt stress can be clarified by PCA and HCA results.

## CONCLUSION

These investigations revealed that, Tubtim Chumphae rice plants showed salinity stress, especially during fluctuations in temperature and humidity. Growth and physiological traits decreased, except for electrolytic leakage rate and malondialdehyde content. The PCA and HCA results also confirmed seedling response to salt stress and proved that MDA content and electrolyte leakage are potential indicators for salinity stress. This is the first physiological and biochemical study of Tubtim Chumphae rice salinity stress under temperature and humidity fluctuations. Current findings indicated that Tubtim Chumphae rice seedlings can grow in slight to moderate salinity soil. Knowledge gained from this study can be used to improve rice breeding plans and ameliorate multiple stress situations.

## SIGNIFICANCE STATEMENT

This research exposed an optimal salinity tolerance level for seedling preparation of Tubtim Chumphae rice in transplanting rice production systems that many researchers have yet to investigate. As a result, a new theory on Tubtim Chumphae rice seedling preparation in transplanting rice production systems under slight to moderate salinity conditions could be developed.

## ACKNOWLEDGMENTS

This research was funded by the National Science, Research and Innovation Fund (NSRF), Thailand (Fiscal year: KKU-2022). We are also very grateful to Department of Biology, Faculty of Science, Khon Kaen University and the National Research Council of Thailand (NRCT) through the Senior Research Scholar Project of Prof. Dr. Piyada Theerakulpisut for facility support.

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