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Research Article
Effects of NaCl on Growth, Chlorophyll and Sugar Contents in Rice (*Oryza sativa* L.) Malidum and Malidang Cultivars

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Abstract
Background and Objective: Thailand encounters saline soil problems scattered in many areas which affect the agricultural area and causes a decrease in productivity especially growing rice, which is the main economic crop. Rice is the staple food of the Thai people. The rice that was used in this research is a native rice cultivar, which has a special feature: The Malidum cultivar with black grain and the Malidang rice grain with red color. It has high nutritional value in color grains, especially with antioxidants, which today's consumers are more attentive and consuming this rice. To study the impact of salinity stress in rice (*Oryza sativa* L.) using two cultivars (Malidum and Malidang). **Materials and Methods:** Seeds germinated for 7 days. After that, the seedlings were transplanted into grown in Hoagland's solution (modified) with NaCl added at concentrations of 0 (control), 50, 100 and 200 mM. Until the plants were 35 days old, the results were recorded and analyzed. The experiment consisted of four replicates in each treatment. **Results:** The NaCl had an effect on rice growth. The height, fresh weight, dry weight and chlorophyll A and B contents decreased when increasing the NaCl concentration significantly at the 95% confidence level and found that when plants were exposed to increased salinity concentrations, Malidum had a percentage reduction (compared to control) of height and chlorophyll B was higher than Malidang, while in Malidang there was a higher percentage reduction of fresh weight, dry weight and chlorophyll A than Malidum. In Malidang the total sugars increased to the highest concentration at 50 mM NaCl. **Conclusion:** A comparison of salt tolerance between two cultivars of rice found that the Malidang cultivar had a greater tolerance than the Malidum cultivar.

Key words: Rice (*Oryza sativa* L.), salinity, chlorophyll content, sugars content, fresh weight, dry weight

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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 a cereal grain belonging to the Poaceae family with the scientific name *Oryza sativa* L. Since rice is an important economic crop in Thailand but the plantations for rice production often suffer from saline soil problems, therefore, a comparative study of suitable and salt-tolerant rice varieties is necessary because it will affect the yield of rice varieties. In the future, especially in rice varieties that are color-producing plants that tend to be more popular. Rice is the main food source that provides carbohydrates that are essential to the sustenance of the world's population[1]. Rice is the staple food of many countries around the world. More than 100 countries grow rice for agriculture and 90% of the world's rice production comes from Asia[2]. There are many countries where rice is the main agricultural crop[3]. In addition, rice is an important staple food in Indonesia, India and Japan, etc. The rice used in this research is a local rice cultivar, which has the distinctive feature of being colored rice in the grain. Colored rice has a high content of free radicals and is popular for current and future consumption.

In Thailand, it is found that agricultural areas suffer from widespread saline soils and this affects plant growth and agricultural productivity, especially in the Northeast of Thailand. It is an important source of rice cultivation but found that it is an area that suffers from saline soil problems. Soil salinity is an important limiting factor that can cause crop damage and reduce the viability of crops. Most of the salt found in saline soils is in the form of Sodium chloride (NaCl), which greatly reduces the productivity of most crops[4]. Excessive concentrations of dissolved salts in soil negatively affect farmland and crops. More than 100 countries are facing soil salinity along with groundwater salinity[5]. Soil salinity is one of the most important ecological stressors. Soil salinity reduces agricultural production[6]. Saline soils affect the growth and productivity of many crops such as rice, tomatoes, peppers, etc. Plant responses under salt stress are similar to plant responses under drought stress because when plants are in saline conditions, the plants cannot absorb water from the soil into the roots. Salt stressed conditions, plants will have Na^+ and Cl^- ions accumulated in the cells, which will affect the ions exchange in the cells, causing plants to lose balance and may be toxic to plants. A high salinity level results in symptoms causing toxicity due to osmotic pressure, such as limited root and shoot growth, leaf curl, cell membrane damage and leaf tip burn[7]. In addition, plants in saline soils had a decrease in the rate of photosynthesis. The amount of chlorophyll is reduced as well when plants are under stress in some plants, there may be an accumulation of certain substances such as proline or sugar in plants to adjust the osmotic balance. Global population growth is expected to increase. This puts more pressure on agricultural production in saline soil areas, especially as rice is the most salt-sensitive grain[8]. Therefore, this research aims to compare the growth of the two rice cultivars as a guideline for considering salt tolerant plants.

MATERIALS AND METHODS

Study area: The research was during November, 2020 to March, 2021. The greenhouse of the Biology Department, Faculty of Science, Mahasarakham University.

Materials: Seeds of two rice (*Oryza sativa* L.) cultivars (Malidum and Malidang) were taken from Maha Sarakham Province, Thailand. Seeds were prepared for four treatments, each of which had four replicates.

Methodology: Seeds were germinated for 7 days, exposing the plants to 16 hrs of artificial light per day (200 μmol m^{-1} sec^{-1}). Seven-day-old seedlings were transplanted into Hoagland solution (modified) containing sodium chloride at concentrations of 0, 50, 100 and 200 mMol. During the experiment, solutions were changed every 5 days under greenhouse conditions. Four replicates of plants were planted for each salinity treatment until the plants were 35 days old to determine the height, fresh weight, dry weight, chlorophyll A and B content and total sugars in the 35 days old plants.

Chlorophyll A and chlorophyll B content: Plants were analyzed 35 days after exposure to 0, 50, 100 and 200 mM of NaCl solution. Sample tissue (0.1g) was ground on ice with a mortar and pestle with 6 mL of 80% buffered acetone. Then, the mixture was incubated at 4°C for 3 hrs. The mixture was centrifuged 5 min at 2500 rpm and the supernatant was transferred to a 100 mL volumetric flask and the volume was made to 100 mL with the addition of 80% acetone and the solution was used for the estimation of chlorophyll. The extracted chlorophyll solution was then measured for absorbance at wavelengths of 645 and 663 nm with a spectrophotometer (Shimadzu UV-Vis 1201).

Sugar content: Sample plants (50 mg) were extracted with 5 mL of 80% ethanol. Samples were boiled in a glass tube in a water bath at 95°C for 10 min each and centrifuged at 2500 rpm for 5 min extracted three times, then the supernatant of the three extractions was combined for sugar determination. After that, the test tube was stood for 10 min, then it was vortexed for 30 sec and placed for 20 min at room temperature by placing it in a water bath to observe the color development. The color absorbance of the solution was estimated by a spectrophotometer at the 490 nm wavelength.

Statistical analysis: Statistical data were analyzed using the SPSS program and the mean was compared using Duncan's New Multiple Range Method[9]. Then, the growth results, chlorophyll content and total sugar in the rice leaf results were summarized for both cultivars of rice.

RESULTS

From the experiments, it was found that in the Malidum and Malidang cultivars, the height decreased inversely with the concentration of NaCl. The height was statistically different at the 95% confidence level. The average height of the two cultivars was the highest without NaCl treatment. When considering the percentage decrease in height with increasing NaCl, it was found that Malidang had a small percentage reduction compared to control than Malidum for each NaCl concentration (Table 1).

The fresh weight content of the Malidum and Malidang cultivars was the highest without NaCl treatment. The fresh weight of the rice two cultivars decreased inversely with the concentration of NaCl. The fresh weight of the Malidum cultivar was statistically different at the 95% confidence level compared to the control. In addition, it was found that the Malidang cultivar at 50 mM it had a 2.36 times lower fresh weight percentage reduction compared to the control than the Malidum cultivar. More than that, it was found that the Malidang cultivar at the NaCl concentration of 50 mM was not statistically different from the control. Both cultivars at concentrations of 100 and 200 mM showed a greater than 70% reduction for the fresh weight compared to the control (Table 2).

The dry weight content of the Malidum and Malidang cultivars was the highest without NaCl treatment. The experimental results were similar to fresh weight but the Malidum cultivar found that dry weight content at the NaCl concentrations of 50 mM and 100 mM were the same. The dry weight of the two cultivars decreased inversely with the concentration of NaCl. The dry weight of Malidum cultivar was statistically different at the 95% confidence level compared to the control but the Malidang cultivar found that the NaCl concentration of 50 mM was not statistically different compared to the control. The Malidang cultivar at a concentration of 50 mM decreased by 29.41% while, the Malidum cultivar decreased by 72.73%, equivalent to 2.47 times that of the Malidang cultivar (Table 3).

The chlorophyll A content of the Malidum and Malidang cultivars was the highest without NaCl treatment. The chlorophyll A content of the two cultivars decreased inversely with the concentration of NaCl. The chlorophyll A content of the Malidum was statistically different at the 95% confidence level compared to the control. The Malidang cultivar found that the NaCl concentration of 100 and 200 mM were statistically different but the NaCl concentration of 50 mM was

Table 1: Average heights (cm) of two rice cultivars under salinity conditions with different concentrations of NaCl

Cultivar/concentration (NaCl)	0 mM	50 mM	100 mM	200 mM
Malidum	47.04 ± 4.50^{a}	22.27 ± 2.493^{b}	19.20 ± 2.09^{b}	17.82 ± 1.89^{b}
Percentage reduction compared to control	-	52.66	59.19	62.12
Malidang	51.67 ± 4.37^{a}	37.77 ± 1.76^{a}	23.52 ± 2.50^{b}	21.40 ± 2.32^{b}
Percentage reduction compared to control	-	26.9	54.48	58.58

Different characters in a row show different values by statistical significance at the 95% confidence level from the DMRT mean comparisons

Table 2: Fresh weight content (g) in two rice cultivars under salinity conditions with different concentrations of NaCl

Cultivar/concentration (NaCl)	0 mM	50 mM	100 mM	200 mM
Malidum	0.39 ± 0.06^{a}	0.14 ± 0.03^{b}	0.10 ± 0.01^{b}	0.09 ± 0.01^{b}
Percentage reduction compared to control	-	64.1	74.36	76.92
Malidang	0.59 ± 0.12^{a}	0.43 ± 0.06^{a}	0.13 ± 0.02^{b}	0.11 ± 0.01^{b}
Percentage reduction compared to control	-	27.12	77.97	81.36

Different characters in a row show different values by statistical significance at the 95% confidence level from the DMRT mean comparisons

Table 3: Dry weight content (g) in two rice cultivars under salinity conditions with different concentrations of NaCl

Cultivar/concentration (NaCl)	0 mM	50 mM	100 mM	200 mM
Malidum	0.11 ± 0.02^{a}	0.03 ± 0.01^{b}	0.03 ± 0.01^{b}	0.06 ± 0.04^{ab}
Percentage reduction compared to control	-	72.73	72.73	63.64
Malidang	0.17 ± 0.04^{a}	0.12 ± 0.03^{a}	0.04 ± 0.01^{b}	0.03 ± 0.00^{b}
Percentage reduction compared to control	-	29.41	76.47	82.35

Different characters in a row show different values by statistical significance at the 95% confidence level from the DMRT mean comparisons

Table 4: Chlorophyll A content (mg g^{-1}) in two rice cultivars under salinity conditions with different concentrations of NaCl

Cultivar/concentration (NaCl)	0 mM	50 mM	100 mM	200 mM
Malidum	0.14 ± 0.03^{a}	0.07 ± 0.02^{b}	0.05 ± 0.02^{b}	0.03 ± 0.01^{b}
Percentage reduction compared to control	-	50	68.75	78.57
Malidang	0.16 ± 0.04^{a}	0.11 ± 0.03^{ab}	0.07 ± 0.02^{b}	0.05 ± 0.01^{b}
Percentage reduction compared to control	-	31.25	56.25	68.75

Different characters in a row show different values by statistical significance at the 95% confidence level from the DMRT mean comparisons

Table 5: Chlorophyll B content (mg g^{-1}) in two rice cultivars under salinity conditions with different concentrations of NaCl

Cultivar/concentration (NaCl)	0 mM	50 mM	100 mM	200 mM
Malidum	0.19 ± 0.04^{a}	0.10 ± 0.02^{b}	0.07 ± 0.02^{b}	0.04 ± 0.01^{b}
Percentage reduction compared to control	-	47.37	63.16	78.95
Malidang	0.21 ± 0.05^{a}	0.15 ± 0.03^{ab}	0.10 ± 0.02^{b}	0.07 ± 0.02^{b}
Percentage reduction compared to control	-	28.57	52.38	66.67

Different characters in a row show different values by statistical significance at the 95% confidence level from the DMRT mean comparisons

Table 6: Total sugar content (mmol g^{-1} fresh weight) in two rice cultivars under salinity conditions with different concentrations of NaCl

Cultivar/concentration (NaCl)	0 mM	50 mM	100 mM	200 mM
Malidum	0.61 ± 0.13^{a}	0.48 ± 0.12^{a}	0.50 ± 0.11^{a}	0.47 ± 0.10^{a}
Percentage reduction compared to control	-	21.31	18.03	22.95
Malidang	0.59 ± 0.12^{a}	0.69 ± 0.15^{a}	0.50 ± 0.11^{a}	0.55 ± 0.12^{a}
Percentage reduction (-) on increase (+) compared to control	-	+14.49	-14.25	-6.78

Different characters in a row show different values by statistical significance at the 95% confidence level from the DMRT mean comparisons

not statistically different compared to the control. The Malidang cultivar had a reduction percentage compared to control less than the Malidum cultivar at all concentrations of NaCl (Table 4).

In the same way, the chlorophyll B content of the Malidum and Malidang cultivars were the highest without NaCl treatment. The chlorophyll B content of two cultivars decreased inversely with the concentration of NaCl. The chlorophyll B content of the Malidum was statistically different at the 95% confidence level compared to the control. The Malidang cultivar found that the NaCl concentration of 100 and 200 mM were statistically different but the NaCl concentration of 50 mM was not statistically different compared to the control. The Malidang cultivar had a reduction percentage compared to the control less than the Malidum cultivar at all concentrations of NaCl (Table 5).

The total sugar content of the Malidum cultivar was higher than that of the Malidang cultivar without NaCl and the highest total sugar content for Malidang was at the 50 mM concentration. For Malidum when NaCl was added, the total sugar content decreased with increasing concentration of NaCl compared to that without NaCl. For both cultivars, the total sugar content was not statistically different at the 95% confidence level compared to the other treatments. For the Malidang cultivar at the concentration of 50 mM, the total sugar content was 14.49% higher than the control but it was not statistically different (Table 6).

DISCUSSION

In this work, salinity affected plant growth. When salinity increased, plant growth decreased. Salinity affects plant metabolism in many ways, for example, it reduces the rate of photosynthesis in plants. Salt increases the osmotic stress, making rice water less usable. Salt also affects various metabolic processes, causing the plant to lack some essential nutrients[10]. Saline soils reduce the productivity of rice cultivation[11,12]. If rice is not mature, the leaves are dry and crispy and the plants cannot absorb nutrients which also causes the plants to lack water, currently, the cultivation of salt-tolerant rice does not have a clear solution. It also increases the intensity of the growth and yield of rice[13]. Salinity in rice plants causes metabolic changes, so an intensive and comprehensive study of plant parts, such as stems, leaves and roots, affected by salt stress should be undertaken[14]. Salinity-induced ionic and osmotic stresses reduce the rate of photosynthesis and consequently cause oxidative stress, which is also responsible for growth reduction[15,16]. Salinity exposes plants to primary osmotic stress and secondary ionic stress. Excess salt in the soil can have a negative effect on plant growth. Either through the inhibition of root uptake or the effects of specific ions. The effects of specific ions can lead to toxicity, in one way or another. Water solubility or ion adsorption can affect the nutritional balance of plants[17,18].

When the NaCl concentration increased, the chlorophyll A and chlorophyll B content in the leaves decreased to their lowest levels at 200 mM NaCl in both cultivars. Increasing the concentration of salt resulted in a decrease in water content in the plant leaves and chlorophyll content. When plants lack water, the cells wither and the stomata close, reducing the potential for absorbing carbon dioxide, which affects the photosynthetic process of the plant. The amounts of chlorophyll A and chlorophyll B, the light energy receptor in photosystem II, were reduced. The efficiency of photosynthesis in the plant is reduced. Salinity decreased the chlorophyll content[19-22]. Salt reduces the resistance of the mesophyll[23]. It affects the thickness of the leaves and decreases the intercellular space, resulting in reduced CO_2 fixation.

As the NaCl concentration increased, the total sugar content in the leaves decreased to the lowest at 200 mM NaCl in both cultivars. The Malidum cultivar had the highest total sugar content at 0 mM NaCl. While, the Malidang cultivar had the highest sugar content at 50 mM. When the concentration of NaCl was increased, the total sugar content decreased. Salinity may influence the sugar content in plants. In plants exposed to salinity stress, the vacuolar content of glucose, fructose, sucrose and fructan is greater in salinity-tolerant varieties than in sensitive ones[24] and a low concentration of salt stress may increase the rice quality, all of which are meant for agricultural production[25].

The experimental results, when the rice was salted, it resulted in a decrease in growth. Similar to the study in the rice genotype (IR29), it showed decreased growth when grown under saline conditions[26]. In bean and alfalfa, growth also decreased when plants were exposed to salt[27]. Plant biomass decreases when exposed to salt stress. Salinity impairs plant growth and development caused by water stress. Cytotoxicity due to excessive adsorption of Sodium (Na^+) and Chloride (Cl^-) ions and reactive oxygen species (ROS)[28]. This was due to the reduction in carbohydrate production caused by decreased carbon uptake but also severely affected the photosynthetic activity in the leaves[29]. It was also found that salted radish resulted in a decrease in the amount of chlorophyll[30]. In addition, in saline soil conditions, it will inhibit the microbial inoculants, resulting decrease inefficient nitrogen that may affect plant growth and yield under salt stress[31]. Introducing rice cultivars between Malidum and Malidang to farmers for use in cultivation in saline soil areas should introduce Malidang cultivar because it was found to have a better growth rate in saline soil conditions, especially in low salt concentrations. Malidang cultivar has fewer effects than Malidum cultivar. There is also an adaptation by accumulating sugar to adjust the osmotic value. As a result, plants can better transport water. Compatible solutes such as polyethylene glycol, mannitol, salicylic acid, sorbitol and sucrose can be indicators of salt tolerant plants[32]. Research on rice showed that the sugar content increased when the plant was salted[33,34]. A similar study on tomatoes showed an increase in sugar content when grown in salty conditions[35]. The plant physiological characteristics expressed after salt exposure can allow evaluation of plant tolerance[36]. It was similar to the research that selected salt-tolerant plants by biomass and chlorophyll content as indicators[37].

After the seeds germinate, strong seedlings should be selected before transplanting. Plants will grow completely for all treatments. The place for planting should have proper ventilation for good plant growth. Plant samples should be selected with mature leaves, (leave position 4-6) because if the young leaves are taken to measure the amount of substances, it may not be suitable.

CONCLUSION

The NaCl affected rice growth, the height, fresh weight, dry weight and chlorophyll A and B contents decreased when increasing the NaCl concentration. The research shows that the Malidang cultivar was more salt tolerant than the Malidum cultivar due to its better growth. It has a higher chlorophyll content, especially when the NaCl concentration was low and the Malidum cultivar has a higher amount of sugar accumulation than the Malidang cultivar.

SIGNIFICANCE STATEMENT

Rice is a widely cultivated crop in the northeastern part of Thailand but the area has been found to have saline soils, which is a problem for rice cultivation. The objective of this research was to study the effect of salinity on rice growth and physiology. Including the comparison of 2 local rice cultivars grown under salt-tolerant conditions. From the research, it was found that salinity had an effect on growth and decreased chlorophyll content. Malidang rice was more salt tolerant than Malidum. In addition, it was found that at low NaCl concentrations, Malidang rice was less affected, possibly due to better adaptation such as accumulation of sugar in plants to improve water transport.

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