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Effects of Low Temperature and Potassium Chloride on Physiological and Biochemical Indices of Rice (*Oryza sativa* L.) at the Seedling Stage

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ABSTRACT

Ambient temperature directly affects growth and development of rice (*Oryza sativa* L.), especially at the seedling stage. This study aimed to determine the effects of temperature and potassium Chloride (KCl) on certain physiological and biochemical indicators at the seedling stage of two improved rice cultivars (CR203 and DT10). Under the influence of low temperature (10°C), degradation of carbohydrates in seeds was inhibited and the total organic acid content and α-amylase activity decreased, resulting in higher starch content and lower reducing sugar content in both rice cultivars, compared to treatment at the optimal temperature (28°C). For seeds treated with KCl, the α-amylase activity and total organic acid content of the two rice cultivars increased; therefore, the reducing sugar content increased while the starch content decreased. The peroxidase and catalase activities of CR203 decreased under a low-temperature treatment, while those of DT10 increased under the same conditions. Meanwhile, treating seeds with KCl increased the peroxidase and catalase activities of both rice cultivars; however, the peroxidase activity of DT10 increased slightly. The results also suggest that low temperature inhibits the physiological activities of CR203 and DT10 at the seedling stage, while treatment of seeds with KCl increases the physiological activities of both rice cultivars. These results suggest that treating seeds with KCl contributed to improve the tolerance to low temperature stress of the CR203 and DT10 rice cultivars at the seedling stage. In general, treatment of rice seeds with KCl could be recommended to increase cold tolerance at the seedling stage.

Keywords: Catalase, cv. CR203, cv. DT10, Seed treatment.

INTRODUCTION

Rice (*Oryza sativa* L.) is an ancient and popular food crop worldwide (Bouman, 2007). It is the main food crop of many countries, such as India, China, Japan, and Vietnam. Rice is an export commodity that brings significant economic value to many countries worldwide, including Vietnam (Bandumula, 2018). However, Vietnam's rice production industry currently faces many difficulties. For example, its productivity and output are not high due to extreme changes in weather conditions, including the effects of low temperatures. The winter-spring crop in northern Vietnam often experiences severe and damaging cold spells since the ambient temperature can drop below 10°C, with many places experiencing temperatures of 5–6°C. Such low temperatures make rice grow and develop poorly, thereby reducing its yield and quality (Vien, 2011).

Many studies have shown that rice is susceptible to poor environmental conditions, especially at the seedling stage (Vergara,

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1991; Wang and Cai, 2011; Huang *et al*., 2013; Shi *et al*., 2016). The minimum critical temperature for rice is 10–12°C. When the ambient temperature drops below 10°C, chloroplasts will be damaged and chlorophyll decomposition will increase rapidly, resulting in reduced photosynthesis that affects plant growth (Kabaki, 1983). Low temperature also disturbs the nitrogen exchange process, increases protein breakdown, and reduces protein nitrogen content, thereby increasing soluble nitrogen forms (e.g., NH_3) and other toxic substances to the protoplasm (Allen and Ort, 2001; Ahmed *et al*., 2008; Theocharis *et al*., 2012; Hussain *et al*., 2019). In practice, several methods are often used to increase the cold tolerance of rice at the seedling stage, such as fertilizing with ash and covering plants with nylon. Among these methods, potassium fertilization is also a reasonably popular method (Wang *et al*., 2013). Potassium exists in the form of ions so that it can penetrate between organelles, promote nutrient transport, and aid in enhancing rice respiration (Cakmak, 2005; Pettigrew, 2008; Oosterhuis *et al*., 2014). Potassium also helps the root system increase water absorption, increase drought and cold tolerance, reduce the risk of damage caused by pests, and contribute to increasing the productivity and quality of rice (Pettigrew, 2008; Zain and Ismail, 2016). Balanced fertilization of nitrogen, phosphorus, and potassium makes rice absorb more nutrients and creates good conditions for plant health and high yields, while simultaneously creating favorable conditions for microorganisms to develop and provide essential nutrients for rice. Many studies have been conducted on the role of potassium fertilizers in rice production in Vietnam and abroad (Ren *et al.*, 1992; Sarwar, 2012; Islam *et al.*, 2015). However, studies on the effects of low temperature and potassium fertilizers on physiological and biochemical metabolism at the seedling stage of rice have received little interest. Therefore, the present experiment was conducted to determine the effects of low temperature and potassium Chloride (KCl) on certain physiological and biochemical indicators at the seedling stage for two rice varieties (CR203 and DT10).

MATERIALS AND METHODS

Research Materials

The experiment was performed on two improved rice cultivars, CR203 and DT10, which were provided by the Agricultural Genetics Institute (Vietnam) in 2017. These were two direct seeded Indica cultivars. The CR203 cultivar was released in 1985. Its growing season is 130–140 days, the average yield is 4,500–5,000 kg ha^{-1}, and it has poor cold tolerance (Cuong *et al.*, 2015). DT10 cultivar was released in 1990. Its growing season is 180–190 days, the average yield is 5,000–5,500 kg ha^{-1}, and it has good cold tolerance (Cuong *et al.*, 2015). Moreover, KCl was purchased from Thanh Hoa Chemical Company (Vietnam), while the Knop solution was provided by Hung Nguyen Equipment Joint Stock Company (ingredients: $Ca(NO_3)_2$, KH_2PO_4, $MgSO_4.7H_2O$, KCl, and $FeCl_3$).

Experimental Design

The experiment was conducted at a greenhouse of Hong Duc University (Vietnam). The experiment was arranged in a Completely Randomized Design (CRD) for three factors with four replicates. Factor 1 was the chemical (KCl and distilled water (control)), while Factor 2 was the temperature (28 and 10°C), and Factor 3 was rice cultivars (CR203 and DT10). The total number of small experimental plots in each lot was 16. Each plot had an area of 5 m^2, and the total area of each large plot was 80 m^2.

Chemical Treatments

Grains that were plump and unscathed were selected. The selected number of seeds of

each variety was divided into two equal parts. The first part was immersed in distilled water (control), while the rest was immersed in KCl solution (concentration 1.3 g L^{-1}). This is the optimal concentration for the germination and growth of seedling plants selected through the probe experiment. Seeds were soaked for 2 days and then picked out, washed with distilled water, and placed in Petri boxes lined with distilled water-soaked paper at the bottom. Seeds were incubated to germinate at 28°C in a Binder incubator. After the seeds germinated for 2 days, the even seeds were chosen and placed into Petri boxes lined with absorbent paper (each box contained 100 seeds). Then, 15 mL of Knop solution was added to each box and the seeds were further treated at low temperature (10°C) and optimal temperature (28°C).

Temperature Treatments

A low-temperature treatment experiment (10°C) was conducted in a Hitachi refrigerator, while an optimal temperature treatment experiment (28°C) was conducted in a Binder incubator. The duration of the temperature effect was 2 days. After 2 days of temperature treatment, seedlings were planted in plastic boxes (50 plants box^{-1}) containing 500 g of sand (washed and dried). Each day, Knop solution was added evenly to the boxes (20 mL box^{-1}). Seedlings were grown for 4 days (seedlings with two real leaves) in a Knop nutrient environment. Then, samples were collected as previously described by Trong *et al.* (2020) to analyze their physiological and biochemical criteria.

Determination of Plant Height and Root Length

Plant height and root length were measured using rulers to an accuracy of millimeters (Trong *et al.*, 2020).

Determination of Dry Matter

The plants of each variety were placed into plastic bags according to the treatment formula and transported to the laboratory. The plants were placed into an oven at 105°C for 24 hours until the weight was constant, and then weighed. The dry weight of each plant was determined using an electronic balance with an accuracy of 10^{-4}. Dry matter was calculated in mg $plant^{-1}$ according to Chen *et al.* (2013)

Determination of Chlorophyll Content

Concentrations of chlorophyll a, chlorophyll b, and total chlorophyll in leaves were determined via the spectroscopic method described by Trong *et al.* (2020). First, the fresh leaf samples were cooled with liquid nitrogen, then, crushed into a fine powder. Thereafter, 5 mg of fine powder was placed into a tube, 100 µL of distilled water was added, and the ingredients were absorbed for 10 minutes. Then, 8 mL of 80% acetone was added to separate the chlorophyll. The filtrate (10 mL) was then centrifuged and the optical density was measured using a spectrophotometer at 663 and 644 nm, respectively. The chlorophyll content was then calculated as Fresh Weight (FW).

Determination of Reducing Sugar Content

To determine the reducing sugar content, 2 g of fresh leaves were placed into a ceramic mortar and crushed. Then, 50 mL of distilled water was added to a 250 mL triangle. This was boiled in a water bath at 80°C for 15 minutes, with occasional shaking during boiling to extract the sugar. The triangle was then removed and cooled to room temperature. Impurities were then reduced and a filtrate was obtained. Then, 10 mL of the test solution was added to a 1,000-mL conical flask, followed by the addition of 10

mL of Fehling solution. The mixture was then boiled for 3 minutes. To settle the precipitate, it was filtered into a Buchner vacuum filter. The flask and filter funnel were then washed with hot distilled water 3–4 times. The Cu_2O precipitate was then dissolved in the Buchner flask by adding a small quantity (5 mL) of $Fe_2(SO4)_3$ solution in H_2SO_4 and stirring it carefully with a glass rod to dissolve the Cu_2O precipitate entirely in the funnel. The flask and filter funnel were then rinsed and placed into a conical flask. The obtained solution was then titrated with $KMnO_4$ 1/30N until a persistent pale pink color appeared (20–30 seconds). The amount of $KMnO_4$ 1/30N was then calculated using titration and the table was checked to determine the amount of reducing sugar in the experimental sample. The reducing sugar content was calculated using the formula previously described by Trong *et al.* (2020).

Determination of Starch Content

To determine the starch content, 2 g of crushed and dried leaves were placed into a 100-mL triangle with 50 mL of distilled water, shaken, and left for 30–45 minutes. This was then filtered through filter paper and the residue was washed with distilled water 2–3 times. The filter paper was then punctured and the powder was transferred to a triangle containing 25 mL HCl 5%, which was boiled in a water bath for 3 h. After the starch had completely hydrolyzed, the solution was cooled and then neutralized with 0.5% NaOH solution to pH 5.6–6.0. The mixture was then transferred to a 100-mL volumetric flask and decontaminated with $Pb(CH_3COO)_2$ 30%. Excess lead salt was removed with 20 mL of saturated Na_2SO_4 solution. The solution was then made up to volume with distilled water, shaken, and filtered. The amount of glucose in the solution was quantified *via* the Bertrand method, from which starch content was calculated according to the formula of Trong *et al.* (2020).

Determination of Total Organic Acid Content

The total organic acid content was determined as described by Trong *et al.* (2020). First, 2 g of fresh leaves were crushed in a ceramic mortar, ground thoroughly, placed into a 50-mL volumetric flask, and made up to volume with distilled water. This was then stirred and 10 mL of filtrate was added to a 100-mL conical flask. Then, a few drops of phenolphthalein reagent were added to the solution and titrated with NaOH 0.1N solution until a stable pink color appeared. The total organic acid content was determined by the titration value.

Determination of Peroxidase Activity

To determine peroxidase activity, 2 g of fresh leaves were crushed in a ceramic mortar and veronal buffer was slowly added to a volume of 10 mL. Cold centrifugation was then conducted (20,000 rounds for 20 minutes), and the clear fluid was taken to determine the enzyme activity. A test tube was then filled with 0.1 mL of pyrogallol 0.2M solution, 1 mL of veronal buffer, 1.2 mL of water, 0.5 mL of H_2O_2 solution, and 0.2 mL of enzyme extract. The reaction mixture was then incubated at 30°C for 10 minutes and 1 mL of 5% H_2SO_4 was added to stop the reaction. The optical density was determined by comparing it to the amount of purpurogallin from the standard curve at a wavelength of 430 nm. The peroxidase activity was calculated using the formula of Trong *et al.* (2020).

Determination of Catalase Activity

To determine the catalase activity, 2 g of fresh leaves were crushed in a ceramic mortar with glass powder and 0.3 g of $CaCO_3$. Then, 20 mL of distilled water was added and carefully ground to form a homogeneous solution that was placed into a

50-mL volumetric flask. The distilled water was drained to the volume. The mixture was shaken well and then centrifuged after 30 minutes. Thereafter, two 100 mL conical flasks were taken, and 10 mL of the filtrate was added to each flash. The control flask was boiled for 3 minutes (to deactivate the enzyme), and the flask was then cooled. Thereafter, 20 mL of distilled water and 3 mL of 1% H_2O_2 were added to each flask and set at 25°C for 30 minutes. Then, 5 mL of 10% H_2SO_4 solution was added and titrated with $KMnO_4$ 0.1N solution until a stable light pink color appeared for 1 minute. The catalase activity was then determined from the titration value.

Determination of α-Amylase Activity

To determine the α-amylase activity, 2 g of the crushed study sample was placed into a 250-mL conical flask, to which 10 mL of buffer solution and 90 mL of distilled water were added. The mixture was then maintained at 30±2°C for 1 hour. The enzyme extracts were then filtered and recovered. A 1% starch solution was then prepared by dissolving 1 g of starch with 50 mL of distilled water in a 100-mL volumetric flask and shaking it well. The mixture was then placed in a boiling water bath and shaken continuously until the starch was completely dissolved. The flask was then cooled and 10 mL of buffer solution was added, with distilled water added to make it up to volume. The mixture was then placed into two 50-mL conical flasks and 10 mL of 1% starch solution was added to each flask and incubated at the appropriate temperature for 10 minutes. Then, 5 mL of enzyme extract was added to flask 2 and stirred for 10 minutes. From each flask, 0.5 mL of the mixture was taken and placed into two other flasks containing 50 mL of analytical iodine solution and shaken well. The absorbance was measured at a wavelength of 656 nm using a spectrophotometer to determine the α-amylase activity.

Statistical Analysis

The analysis of physiological and biochemical indicators in this study was independently conducted three times. The collected data were processed using IRRISTAT 5.0 statistical software to Analyze Variance (ANOVA).

RESULTS AND DISCUSSION

Effects of Temperature and KCl on the Content of Starch, Reducing Sugar, and Total Organic Acid

Starch is the most common plant reserve polysaccharide and is mainly accumulated in nuts as well as rosemary seeds and tubers (Isherwood, 1973; Shannon *et al*., 1984). During germination, starch is broken down into sugar. Through respiration, starch provides energy and raw materials for the biosynthesis processes of new organics required for the germination and growth of seedlings (Murata *et al.*, 1968). The results presented in Table 1 show that low temperature (10°C) inhibits the degradation of starch stored in seeds during germination. Thus, the starch content remaining in the two studied rice varieties is greater than that of seeds treated at 28°C. However, when the seeds are treated with KCl, the starch metabolism is better (Table 1). This result is consistent with the study of Farooq *et al.* (2008) since the treatment of seeds with the appropriate concentration of KCl effectively improved starch metabolism under normal and cold stress conditions.

Along with the starch content, the reducing sugar content was also analyzed, as shown in Table 1. Low temperature (10°C) caused a decrease in both rice varieties' reducing sugar content. However, KCl limited the decrease in reducing sugar content of the two rice varieties, but to different degrees. The level of reducing sugar accumulation in rice treated with KCl was highest in CR203. Under the influence

Table 1. Effects of temperature and KCl on the content of starch, reducing sugar, and total organic acid for the CR203 and DT10 rice varieties.[a]

Variety	Treatment		Starch (%)	Reducing sugar (%)	Total organic acid (mg 100 g^{-1})
	Chemical	Temperature (°C)			
CR203	Distilled water	10	19.95 ± 0.63^{c}	3.01 ± 0.25^{d}	30.22 ± 0.49^{e}
		28	18.76 ± 0.58^{d}	4.23 ± 0.28^{c}	40.21 ± 1.08^{d}
	KCl	10	19.13 ± 0.72^{d}	4.75 ± 0.18^{b}	32.55 ± 0.45^{e}
		28	17.65 ± 0.68^{e}	5.37 ± 0.20^{ab}	47.05 ± 1.37^{b}
DT10	Distilled water	10	22.63 ± 0.60^{a}	4.87 ± 0.13^{b}	44.22 ± 0.88^{c}
		28	21.08 ± 0.58^{bc}	5.02 ± 0.29^{ab}	50.57 ± 1.41^{a}
	KCl	10	21.78 ± 0.84^{b}	5.20 ± 0.32^{ab}	44.53 ± 1.03^{c}
		28	21.54 ± 0.57^{b}	5.61 ± 0.17^{a}	50.96 ± 1.55^{a}

[a] The values are expressed as mean±SD (n= 3), and different superscript letters represent significant difference ($P< 0.05$).

of KCl, the reducing sugar content in CR203 was close to that in DT10, while the difference in DT10 was not significant. Notably, the increase in the reducing sugar content due to treatment of seeds with KCl is consistent with a previous report (Trong and Thinh, 2020). The accumulation of sugar in these tissues is one means used by plants to help resist cold since the sugar inhibits protein inactivation at low temperatures (Dong and Beckles, 2019).

The results presented in Table 1 also show that low temperature (10°C) reduces the total organic acid content in rice at the seedling stage. While seed treatment with KCl increased the total organic acid content of these two rice varieties, this increase was not apparent under low temperatures. When the seeds were treated with KCl and planted at 28°C, the most substantial increase in total organic acid content was observed in CR203 (Table 1).

Effects of Temperature and KCl on the Activity of α-Amylase, Peroxidase, and Catalase

The breakdown of starch into sugar is due to the action of the enzyme α-amylase (Murata *et al*., 1968). The results of the present study suggest that activity of α-amylase highlights differences in starch resolution for the different rice varieties. Low temperature (10°C) reduces the α-amylase activity of these two rice varieties (Table 2). Specifically, when treating seeds at 10°C, the α-amylase activity of CR203 reaches 12.03 IU g^{-1} h^{-1}, which is lower than that at 28°C (13.24 IU g^{-1} h^{-1}). When treating seeds at 10°C, the α-amylase activity of DT10 reaches 12.42 IU g^{-1} h^{-1}, which is lower than that at 28°C (13.17 IU g^{-1} h^{-1}). KCl increases the α-amylase activity for all treatments of the two rice varieties to varying degrees (Table 2). Notably, α-amylase activity in CR203 reaches 12.87 IU g^{-1} h^{-1} at 10°C and 15.43 IU g^{-1} h^{-1} at 28°C. In DT10, the corresponding indices reach 13.32 IU g^{-1} h^{-1} at 10°C and 14.86 IU g^{-1} h^{-1} at 28°C. When seeds germinate at low temperatures, the effects of potassium on starch degradation are due to potassium being the cofactor of more than 40 enzymes, including phosphate-based reactive activators that are necessary for a germinated metabolism (Cakmak, 2005). Additionally, potassium maintains a neutral state of electricity in the cell and increases the ability of the cell to retain water, thereby helping to stabilize the cell's structure under adverse temperature conditions (Pettigrew, 2008).

Under stressful conditions, including cold stress, accumulation of Reactive Oxygen Species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radical has been reported (Mittler, 2002; Suzuki and Mittler, 2006). Previous reports have shown that elevated ROS levels can damage cell

Table 2. Effects of temperature and KCl on the α-amylase, peroxidase, and catalase activities of the CR203 and DT10 rice varieties.[a]

Variety	Treatment		Alpha-amylase (IU g^{-1} h^{-1})	Peroxidase (IU g^{-1} s^{-1})	Catalase (μM H_2O_2 g^{-1} min^{-1})
	Chemical	Temperature (°C)			
CR203	Distilled water	10	12.03 ± 0.06^{d}	2.01 ± 0.07^{e}	0.97 ± 0.05^{d}
		28	13.24 ± 0.08^{b}	2.53 ± 0.09^{d}	1.27 ± 0.02^{b}
	KCl	10	12.87 ± 0.16^{c}	3.22 ± 0.04^{c}	1.12 ± 0.02^{c}
		28	15.43 ± 0.12^{a}	3.40 ± 0.07^{c}	1.35 ± 0.03^{a}
DT10	Distilled water	10	12.42 ± 0.05^{cd}	4.52 ± 0.22^{ab}	0.89 ± 0.01^{e}
		28	13.17 ± 0.04^{b}	4.03 ± 0.17^{b}	0.80 ± 0.01^{f}
	KCl	10	13.32 ± 0.15^{b}	4.75 ± 0.20^{a}	1.06 ± 0.04^{c}
		28	14.86 ± 0.06^{a}	4.32 ± 0.25^{ab}	0.95 ± 0.01^{d}

[a] The values are expressed as mean±SD (n= 3), and different superscript letters represent significant difference ($P< 0.05$).

structure and macromolecules, thereby leading to cell death (Apel and Hirt, 2004; Krasensky and Jonak, 2012). Peroxidase and catalase are two enzymes that are very important in protecting cells from damage caused by increased ROS levels (Anjum *et al*., 2016). The peroxidase and catalase activities of rice varieties CR203 and DT10 under the influence of temperature and KCl are presented in Table 2. The study results show that the effect of low temperature (10°C) on the peroxidase and catalase activities of the two rice varieties is not the same. Notably, low temperature (10°C) reduces the peroxidase and catalase activities of CR203. In contrast, the activity of these two enzymes for DT10 increases under the influence of low temperature (10°C). This difference may be due to DT10 being a good cold-tolerant variety, while CR203 is a non-cold-tolerant variety. Studies have also demonstrated that enzymatic activity only increases at a specific limit temperature, beyond which enzymatic activity will decrease (Liu *et al*., 2013). The treatment of seeds with KCl increases peroxidase and catalase activities in both rice varieties (Table 2). However, the increase in peroxidase activity of KCl-treated DT10 seed is unclear. Potassium's role in reducing ROS production and maintaining photosynthetic electron transport has been demonstrated in previous studies (Cakmak, 2005). The increased activity of peroxidase and catalase under the action of KCl contributes to improving the resistance to cold stress of the two studied rice varieties.

Effect of Temperature and KCl on Chlorophyll Content

Chlorophyll biosynthesis is influenced by many factors, including temperature (Sharma *et al*., 2005; Zhao *et al*., 2020). Previous studies have reported that temperature is one of the main environmental factors that can reduce the actual rate of photosynthesis, stomatal conductivity, intracellular CO_2 concentration, and plant water efficiency (Han *et al*., 2017; Zhao *et al*., 2020). In this study, low temperatures (10°C) reduced the content of chlorophyll a, chlorophyll b, and total chlorophyll in both rice varieties (Table 3). This result is consistent with other studies suggesting that low temperature reduces chlorophyll content (Liu *et al*., 2013; Shi *et al*., 2016; Zhao *et al*., 2020). The decrease in chlorophyll content was greatest in CR203 (chlorophyll a reached 0.457 mg g^{-1}, chlorophyll b reached 0.603 mg g^{-1}, and total chlorophyll reached 1.060 mg g^{-1}). These contents in the DT10 variety decreased by less than in the CR203 variety (chlorophyll a reached 0.741 mg g^{-1}, chlorophyll b reached 0.956 mg g^{-1}, and total

Table 3. Effects of temperature and KCl on the chlorophyll content of the CR203 and DT10 rice varieties.[a]

Variety	Treatment		Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})
	Chemical	Temperature (°C)			
CR203	Distilled water	10	0.457 ± 0.012^{e}	0.603 ± 0.014^{d}	1.060 ± 0.026^{e}
		28	0.676 ± 0.016^{d}	0.844 ± 0.025^{c}	1.520 ± 0.041^{d}
	KCl	10	0.633 ± 0.015^{d}	0.929 ± 0.024^{b}	1.562 ± 0.039^{d}
		28	0.721 ± 0.026^{c}	1.106 ± 0.017^{a}	1.827 ± 0.043^{b}
DT10	Distilled water	10	0.741 ± 0.029^{c}	0.956 ± 0.040^{b}	1.697 ± 0.068^{c}
		28	0.824 ± 0.020^{ab}	1.066 ± 0.029^{ab}	1.890 ± 0.049^{ab}
	KCl	10	0.797 ± 0.032^{b}	1.042 ± 0.051^{ab}	1.839 ± 0.083^{b}
		28	0.849 ± 0.036^{a}	1.094 ± 0.054^{a}	1.943 ± 0.090^{a}

[a] The values are expressed as mean±SD (n= 3), and different superscript letters represent significant difference ($P< 0.05$).

chlorophyll reached 1.697 mg g^{-1}). This is because when non-cold-tolerant varieties are exposed to prolonged cold, their chloroplasts often break down their structure faster, which damages the photosynthetic apparatus and reduces the chlorophyll content in leaves (Liu *et al*., 2017).

Treatment of rice seeds with KCl increases the content of chlorophyll a, chlorophyll b, and total chlorophyll (Table 3). The highest increase in chlorophyll content was found in CR203. Previous reports also confirmed that chlorophyll content could be increased with the optimum concentration of KCl (Chen *et al*., 2013). In DT10, KCl increased chlorophyll (but not significantly) at 28°C. Specifically, the total chlorophyll content increased slightly from 1.890 to 1.943 mg g^{-1} at 28°C. When treating seeds with KCl at a low temperature (10°C), the total chlorophyll content of DT10 increased markedly from 1.697 to 1.839 mg g^{-1}. This shows that treating rice kernels with KCl increases photosynthesis under cold stress, thereby contributing to the plant's tolerance to adverse environmental conditions.

Effects of Temperature and KCl on Plant Height, Root Length, and Dry Matter Weight

The effects of temperature and KCl on the plant height and root length of the CR203 and DT10 rice varieties are shown in Table 4 and Figure 1. In both varieties, plant height decreased when seeds were treated at low temperatures (10°C). The plant height of CR203 decreased from 63.05 to 61.20 mm, while that of DT10 decreased from 86.21 to 84.32 mm. Meanwhile, treating seeds with KCl at low temperatures increased plant height in the two rice varieties. Table 4 also shows that low temperature and KCl also influence the root length of the two rice varieties by different degrees. Notably, low temperature (10°C) reduces the root length of the two studied rice varieties. Research by Li-qun *et al.* (2018) also confirmed that low temperature reduces the root length of rice. In contrast, KCl increased the root length in all treatments, especially in the low-temperature treatment. When treating seeds with KCl, the root length of CR203 reached 50.01 mm at 10°C and 56.87 mm at 28°C. The root length also increased for DT10, reaching 61.74 mm at 10°C and 63.28 mm at 28°C. The effects of KCl on plant height and root length were most obvious in CR203. Thus, low temperature inhibits the metabolic and energy processes in plants, thereby reducing the plant growth rate (Hussain *et al*., 2019). In contrast, KCl promotes metabolic and energy processes, thereby promoting plant growth and development (Pettigrew, 2008).

When the seeds were treated at a low temperature (10°C), the dry matter weight of

Table 4. Effects of temperature and KCl on the plant height, root length, and dry matter weight of the CR203 and DT10 rice varieties.[a]

Variety	Treatment		Plant height (mm)	Root length (mm)	Dry matter weight (mg)
	Chemical	Temperature (°C)			
CR203	Distilled water	10	61.20 ± 1.12^{f}	38.41 ± 0.93^{f}	14.17 ± 0.50^{f}
		28	63.05 ± 1.30^{e}	49.32 ± 1.03^{e}	15.20 ± 0.55^{e}
	KCl	10	63.38 ± 1.20^{e}	50.01 ± 1.98^{e}	16.02 ± 0.53^{de}
		28	65.73 ± 1.13^{d}	56.87 ± 2.01^{d}	16.75 ± 0.48^{d}
DT10	Distilled water	10	84.32 ± 1.25^{c}	58.56 ± 0.98^{c}	18.52 ± 0.75^{c}
		28	86.21 ± 1.23^{ab}	62.82 ± 2.05^{ab}	19.31 ± 0.81^{b}
	KCl	10	85.17 ± 1.15^{b}	61.74 ± 0.90^{b}	20.00 ± 0.92^{ab}
		28	86.77 ± 1.18^{a}	63.28 ± 0.93^{a}	20.67 ± 0.84^{a}

[a] The values are expressed as mean±SD (n= 3), and different superscript letters represent significant difference (P< 0.05).

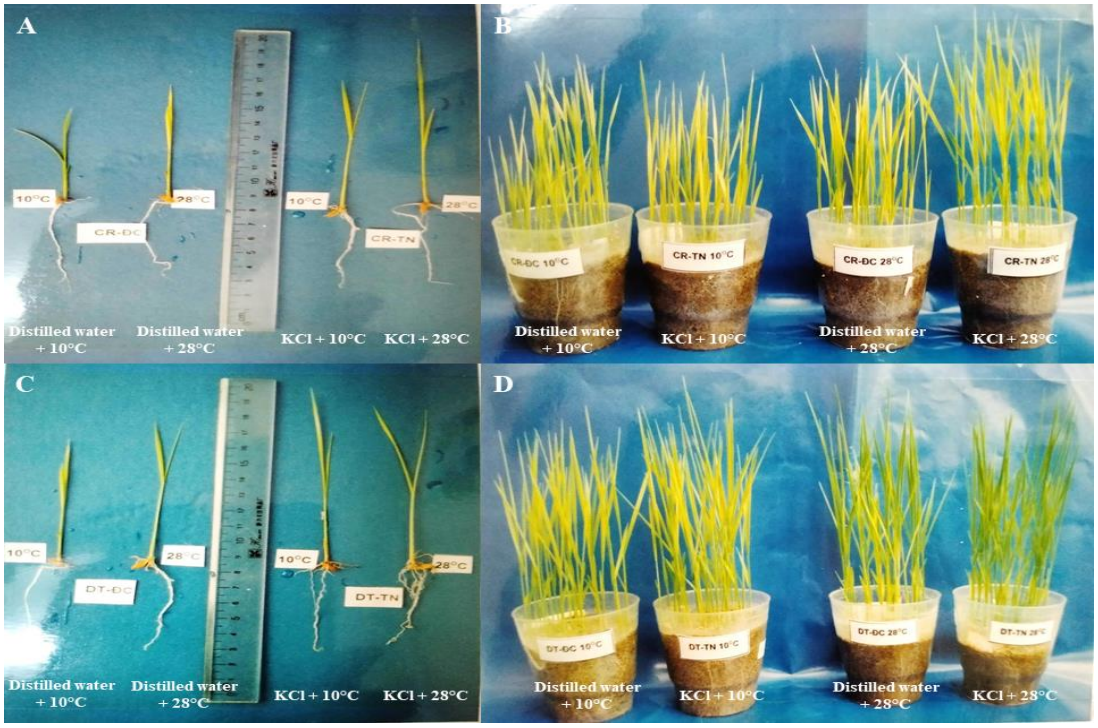

Figure 1. Effects of temperature and KCl on the growth of the CR203 (A and B) and DT10 (C and D) rice varieties.

both rice varieties tended to decrease (Table 4). Research by Mollo *et al.* (2011) and Zhao *et al.* (2020) also showed that low temperature reduces the dry matter weight of plants. Specifically, at 10°C, the dry matter weight of CR203 reached 14.17 mg, which was lower than that at 28°C (15.20 mg). For DT10, dry matter weight reached 18.52 mg at 10°C, which was lower than that at 28°C (19.31 mg). In contrast, treatment with KCl resulted in an increase in dry matter weight across all treatments (Table 4). This result is consistent with the study of Chen *et al.* (2013), which showed that the addition of KCl at the appropriate concentration increased the dry matter weight of plants. The treatment of seeds with KCl resulted in the dry matter weight of CR203 reaching

16.02 mg at 10°C and 16.75 mg at 28°C. For DT10, treating seeds with KCl resulted in dry matter weights of 20.00 and 20.67 mg at 10 and 28°C, respectively.

CONCLUSIONS

Under the influence of low temperature, degradation of carbohydrates in seeds was inhibited and the total organic acid content and α-amylase activity were reduced, which resulted in higher content of the remaining starch and lower content of reducing sugar when compared to the control. Low temperature caused the peroxidase and catalase activities of CR203 to decrease enormously, while those of DT10 increased under the same conditions. Additionally, low temperature also affected some of the physiological activities of rice, such as reducing chlorophyll content, plant height, root length, and dry matter weight. For seeds treated with KCl, the physiological and biochemical activities during the seedling stage of rice were higher than those of the control at both 10 and 28°C. KCl promoted the breakdown of carbohydrates and increased the total organic acid content, level of enzymatic activity, chlorophyll content, plant height, root length, and dry matter weight.

Thus, low temperature slowed the physiological and biochemical processes of the CR203 and DT10 rice varieties at the seedling stage, while KCl treatment increased the physiological and biochemical activities of seedlings, which allowed them to grow better than those in the control group.

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اثرهای دمای پایین و کلرید پتاسیم بر نمایه‌های فیزیولوژیکی و بیوشیمیایی برنج (*Oryza sativa* L.) در مرحله گیاهچه

ا. و. ترانگ، ب. ب. تین، ه. و. چینه، و ت. و. جیانگ

چکیده

دمای محیط مستقیما بر رشد و نمو برنج (*Oryza sativa* L.)، به ویژه در مرحله گیاهچه، تأثیر می گذارد. هدف این پژوهش تعیین اثر دما و کلرید پتاسیم (KCl) بر برخی نمایه های فیزیولوژیکی و بیوشیمیایی دو کولتیوار اصلاح‌شده برنج (CR203 و DT10) در مرحله گیاهچه‌ای بود. بر اساس نتایج، در دمای پایین (10 درجه سانتیگراد)، از تخریب کربوهیدرات‌ها در بذر جلوگیری شد و محتوای کل اسید آلی و فعالیت آلفاآمیلاز کاهش یافت. در نتیجه، در این تیمار، در هر دو رقم برنج، افزایش محتوای نشاسته و کاهش قند نسبت به تیمار دمای بهینه (28 درجه سانتیگراد) مشاهده شد. برای بذر های تیمار شده با KCl، فعالیت آلفاآمیلاز و محتوای اسید آلی کل هر دو کولتیوار برنج افزایش یافت. بنابراین، محتوای قند کاهنده زیاد شد و محتوای نشاسته کاهش یافت. فعالیت های پراکسیداز و کاتالاز در تیمار دمای پایین در کولتیوار CR203 کم شد، در حالی که در شرایط مشابه این فعالیت ها در

کولتیوار DT10 افزایش یافت. همچنین، تیمار بذر با KCl باعث افزایش فعالیت پراکسیداز و کاتالاز درهر دو کولتیوار برنج شد، هرچند که افزایش فعالیت پراکسیداز در DT10 کم بود. نیز، نتایج نشان می دهد که دمای پایین، از فعالیت های فیزیولوژیکی CR203 و DT10 در مرحله گیاهچه جلوگیری می کند، در حالی که تیمار بذر با KCl فعالیت فیزیولوژیکی هر دو کولتیوار برنج را افزایش می دهد. نتایج حاکی از آن است که تیمار بذر با KCl به بهبود تحمل تنش دمای پایین کولتیوارهای برنج CR203 و DT10 در مرحله گیاهچه ای کمک کرد. به طور کلی، برای افزایش تحمل سرما در مرحله گیاهچه برنج، تیمار بذر با KCl را می توان توصیه کرد.