

Effects of Salicylic Acid on Some Physiological Characteristics in Rice (*Oryza sativa* L.) under Drought and Re-watering

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ABSTRACT

This research aimed to study the effects of salicylic acid (SA) on physiological characteristics in rice (*Oryza sativa* L.) cv. KDML 105 under drought stress and re-watering. In drought stress, the results showed that SA increased plant growth as shown by the significant increases in shoot fresh weight. SA also significantly increased relative water content and significantly decreased electrolyte leakage. Moreover, total chlorophyll, fructose and sucrose contents were slightly increased. As re-watering, the results showed the recoveries of plants as shown by the significant increases in root fresh weight, shoot fresh weight, shoot dry weight, relative water content and sucrose content, and the significant decrease in electrolyte leakage. The recoveries of root length, relative water content, total chlorophyll accumulation and membrane permeability were enhanced after re-watering by SA. The results suggested that SA can improve some physiological characteristics of rice under drought and re-watered conditions.

Keywords: Salicylic acid, Drought stress, Re-watering

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Introduction

Rice (*Oryza sativa* L.) represents the food of 3 billion people worldwide. Ninety percent of the global annual production of rough rice is produced and consumed in Asia. Main cropping countries are China, India, Indonesia, Bangladesh, Viet Nam, Myanmar, Thailand and Philippines (Food and Agriculture Organization of the United Nations [FAO], 2012). Rice is an important economics crop in Thailand. Rice cv. KDML 105 or Thai jasmine rice is one of main export products of Central and North Eastern of Thailand, which approximately half of total rice production areas are in the North Eastern (Agricultural Futures Trading Commission, 2007). However, the rice yield per rai of the North Eastern is much lower than other regions of Thailand (Thai Rice Exporters Association, 2015) because of problems of irrigation. Therefore, drought stress is a major constraint for rice production in the north eastern region.

Drought is a major environmental stress factor that affects the growth and development of plant. Plant growth and productivity are adversely affected by water stress, especially photosynthetic capacity. If the stress is prolonged, plant growth, and productivity are severely diminished. Plants have evolved complex physiological and biochemical adaptations to adjust and adapt to a variety of environmental stresses (Osakabe Y et al., 2014). Plant drought tolerance involves changes at morphological and physiological levels. 1) Morphological mechanisms include drought escape and drought avoidance. Drought escape is attained through a shortened life cycle or growing season, allowing plants to reproduce before the environment becomes dry. Flowering time is an important trait related to drought adaptation, where a short life cycle can lead to drought escape. Drought avoidance consists of mechanisms that reduce water loss from plants, due to stomatal control of transpiration, and also maintain water uptake through an extensive and prolific root system. 2) Physiological mechanism is involved in water uptake and maintaining water status of plants, namely drought resistance, plants close their stomata by abscisic acid (ABA) signaling mechanism and decrease osmotic potential by osmotic adjustment and, as a consequence, increase the gradient for water influx and maintenance of plant cell turgor. Osmotic adjustment is a mechanism to maintain water balance under osmotic stress. It involves the accumulation of osmotically active molecules/ions including soluble sugars, sugar alcohols, proline, glycinebetaine, organic acids, calcium, potassium, chloride ions, etc. Scavenging of reactive oxygen species (ROS) by enzymatic and non-enzymatic systems, cell membrane stability, expression of aquaporins and stress proteins are also vital mechanisms of drought tolerance (Farooq et al., 2009b).

As re-watering, plants can recover their growth and photosynthesis immediately through growing new plant parts, re-opening the stomata, and the decreasing of peroxidation. However, recovery extents and magnitude following re-watering depend on pre-drought severity, drought duration and various species (Xu et al., 2010). The recovery of plant upon re-watering after drought also has been reported. As re-watering after drought, Xu, Zhou (2007) showed that the long-term drought imposed negative effects on PSII and photosynthetic ability in *Leymus chinensis* by decreasing chlorophyll content, chlorophyll a/b ratio, carbonic anhydrase activity, net photosynthetic rate, and leaf area compared to the control. After re-watering, the ratio of chlorophyll a/b, carbonic anhydrase activity and net photosynthetic rate were increased, but leaf area and electrolyte leakage from the cut leaf pieces were decreased. Moreover, the long-term drought reduced the maximal efficiency of PSII (F_v/F_m), actual quantum yield,



and photochemical quenching, but these characters were increased by following re-watering, though nonphotochemical quenching was also decreased when compared to the control. However, plant growth substances, including plant hormones, polyamines and phenolic compounds have been reported to play an important role in drought tolerance (Farooq et al., 2009a).

Phenolic compounds play an important role in scavenging free radicals and protect plants against the damaging effects of increased reactive oxygen species (ROS) levels due to drought stress (Petridis et al., 2012). Salicylic acid (SA) is one of a diverse group of phenolic compounds, consisting of an aromatic ring bearing a hydroxyl group or its functional derivative, which is synthesized by plants (Dempsey et al., 1999). SA plays an important role in the regulation of growth, development and defense responses. SA can also improve drought tolerance in many plants, such as increasing antioxidative protection in barley (Fayez, Bazaid, 2014), increasing of plant growth characters, photosynthetic pigments, RWC and proline and decreasing electrolyte leakage (EL) in sweet basil (Kordi et al., 2013), increasing root and shoot length, fresh weight and dry weight in okra seedling (Baghizadeh, Hajmohammadrezaei, 2011) and increasing plant growth characters, RWC, chlorophyll contents and decreasing sugar and starch accumulations in waxy corn (Wisedsri, 2012). In addition, SA can improve the performance of rice cv. Basmati 2000 under both normal and drought conditions by increasing of compatible solute accumulations and antioxidative responses (Farooq et al., 2009a). Although the effects of SA in many plants under drought stress were studied, there were only a few reports on the effects of SA in rice under drought and re-watered condition. This research aims to study the effects of SA on some physiological characteristics in rice cv. KDML 105 under drought stress and re-watering, which will be the benefits of improving the production process of rice under drought stress in the north eastern of Thailand.

Objective of the study

To study effects of salicylic acid on physiological characteristics in rice cv. KDML 105 under drought and re-watering.

Materials and Methods

Plant materials and experimental conditions

Seeds of rice cv. KDML 105 were grown in plastic pots, containing 3 kg of soil in greenhouse. All pots were irrigated daily to keep them well watered. Fourteen-day-olds seedlings were separated into 5 groups, including control, SA0, SA50, SA100 and SA150, which were foliar sprayed with 0, 0, 50, 100 and 150 μ M of SA, respectively. The seedlings were then subjected to drought stress by withholding irrigation for 7 days while the control group was irrigated daily, and then re-watered thereafter. Physiological characteristics of plants under drought and re-watered conditions were then measured. The experiment was laid out in a completely randomized design with five replicates of each treatment.



Growth parameters

The seedlings were collected from each group (control, drought, SA50, SA100 and SA150) and used for various growth parameters, including root length, root fresh weight, root dry weight, shoot length, shoot fresh weight and shoot dry weight. Root and shoot were separated and length and fresh weight were then measured. For the determinations of dry weight, root and shoot were dried in an oven at 80°C for 3 days and then weighted.

Leaf relative water content (RWC)

Relative water content of fully expanded leaves was determined using 10-15 pieces of leaves (1 cm. long). Leaf tissues were weighed and recorded as fresh weight (FW) and then immediately floated on deionized water for 12 hours, turgid weight (TW) was determined. The leaf tissues were then dried in an oven at 80°C for 3 days to determine dry weight (DW). RWC was calculated using the following formular (Turner, 1981);

 $RWC = [(FW - DW) / (TW - DW)] \times 100$

Electrolyte leakage (EL)

EL was determined according to the method described by Baninasab and Ghobadi (2011). Six pieces of leaf tissue (1 cm.) were incubated with deionized water for 24 hours in dark and then electrical conductivity was measured as EC1. The samples were then heated at 100°C for 20 minutes and the electrical conductivity was again recorded as EC2. EL was calculated using the formula given below;

Electrolyte leakage (%) = $(EC1 / EC2) \times 100$

Chlorophyll contents

Total chlorophyll content was measured; leaf samples 0.03 g were extracted with 5 ml of acetone (80% v/v) for 48 hours under dark condition. The absorbance of supernatants was recorded at 645 (A645) and 663 nm (A663). And then chlorophyll content was calculated according to Arnon (1949).

Soluble sugar accumulations

Soluble sugar contents were determined; 50 mg of fresh leaf tissue was incubated with 3 ml ethanol (80% v/v) and was then boiled in water bath at 65°C for 1 h, this tissue was extracted three times repeatedly. The extracts were removed to the same container and stored at 4°C. The extracts were used for the determination of fructose and sucrose contents using resorcinol-HCL method.

Data analysis and summarize

Data were subjected to One-Way ANOVA analysis and Duncan's multiple range tests (p < 0.05) to compare the mean value of different treatments of each conditions (drought and re-watering) by SPSS (version 21.0). The difference between drought and re-watering of each treatment were analyzed using paired sample t-test (p < 0.05). Each data point would be expressed as the average \pm SE of five independent replicates.

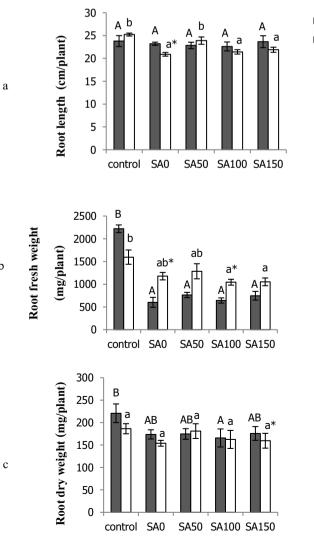


Results

Plant growth

b

Drought stress dramatically reduced rice seedling growth when compared to well-watered condition (control group). As shown in fig. 1, root fresh weight, shoot length, shoot fresh weight and shoot dry weight were decreased (p<0.05) (fig. 1b, 1d, 1e and 1f) and root dry weight was slightly decreased (fig. c) as compared to the control. Foliar spraying with various concentrations of SA affected plant growth under drought stress as shown by the increase in shoot fresh weight when foliar sprayed with 50 μ M of SA (SA50) (p<0.05) (fig. 1e) and the slight increases in shoot length (SA50), shoot fresh weight (SA100 and SA150) and shoot dry weight (SA150) (fig. 1d, 1e and 1f) as compared to the drought without SA application treatment (SA0). Nevertheless, foliar spraying with 100 μ M of SA slightly decreased root dry weight and shoot length as compared to the drought without SA application treatment (SA0). Nevertheless, foliar spraying with 100 μ M of SA slightly decreased root dry weight and shoot length as compared to the drought without SA application group. As re-watering, root fresh weight, shoot fresh weight and shoot dry weight were significantly increased (fig. 1b, 1e and 1f) but root length was decreased in re-watered SA0 (p<0.05) (fig. 1a) as compared to drought SA0. However, SA affected rice seedling growth under re-watering as shown by the significant increase in root length in SA50 (p<0.05) (fig. 1a), and slight increase in shoot fresh weight and decrease in root fresh weight in SA100 and SA150 (fig. 1b and 1e) as compared to the re-watered SA0.







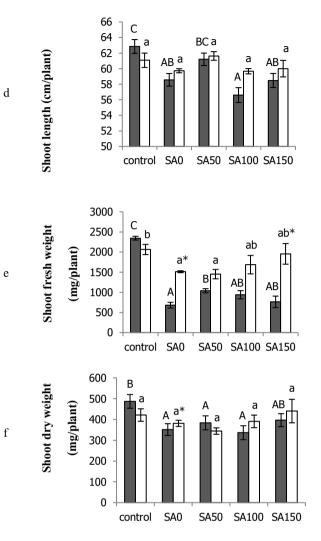


Figure 1 Effects of SA application on plant growth, (a) root length, (b) root fresh weight, (c) root dry weight (d) shoot length, (e) shoot fresh weight and (f) shoot dry weight in rice under re-watered (white bars) and drought conditions (gray bars). Different letters indicate significant differences between treatments within drought (capital letter) and re-watering (small letter) (p<0.05). Star (*) means significant difference of re-watering versus drought within each treatment (p<0.05). SA0, SA50, SA100, and SA150 = foliar applications of 0, 50, 100 and 150 μ M of SA, respectively.

Relative water content

The result showed that drought stress significantly decreased RWC as compared to the control (p<0.05). However, 50 µM of SA application significantly increased RWC as compared to SA0 (p<0.05). Following rewatering increased the RWC in all treatments and it was significant in the re-watered SA0 and SA100 as compared to its drought condition (p<0.05). Moreover, all various treatments of SA increased the RWC under re-watering as compared to the SA0 (p<0.05) (fig. 2a).



Electrolyte leakage

Drought stress affected membrane permeability by the increasing of EL. However, foliar application of SA significantly reduced EL in the SA50 and SA100 under drought stress (p<0.05). As re-watering, EL of SA0 and SA150 were significantly decreased as compared to its drought condition while EL was significantly decreased in re-watered SA50 (p<0.05) and slightly decreased in re-watered SA100 and SA150 as compared to the re-watered SA0 (fig. 2b).

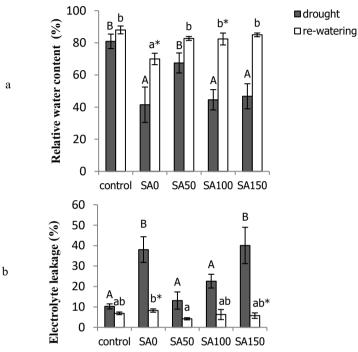


Figure 2 Effects of SA application on (a) RWC and (b) EL in rice under re-watered (white bars) and drought conditions (gray bars). Different letters indicate significant differences between treatments within drought (capital letter) and re-watering (small letter) (p<0.05). Star (*) means significant difference of re-watering versus drought within each treatment (p<0.05). SA0, SA50, SA100, and SA150 = foliar applications of 0, 50, 100 and 150 μ M of SA, respectively.

Total chlorophyll content

In water deficit stress, the concentration of photosynthetic pigments (chlorophyll a and b) of rice leaves was slightly decreased as compared to the control. In presence of SA, total chlorophyll content was slightly increased (SA50) and decreased (SA100 and SA150) as compared to the drought without SA application group. However, the re-watering after drought significantly increased total chlorophyll content in all SA treatments as compared to re-watered SA0 (p<0.05) (fig. 3).

Sugar accumulations

Soluble sugar contents (fructose and sucrose) under drought condition were decreased as compared to the control (p < 0.05). Applications of SA affected sugar accumulations by the slight increasing of fructose and sucrose contents in SA50. However fructose content was slightly decreased in the SA100 and SA150, and sucrose content



was slightly increased in the SA100 as compared to the SA0 (fig. 4a and 4b). Nevertheless, re-watering significantly increased soluble sugar by the slight increasing of fructose content and the significant increasing of sucrose content (p<0.05). Following re-watering, the application of 150 µM of SA significantly increased fructose and sucrose contents as compared to the drought SA150 (p<0.05). However, re-watered SA100 slightly decreased fructose content as compared to the re-watered SA0 (Fig. 4b).

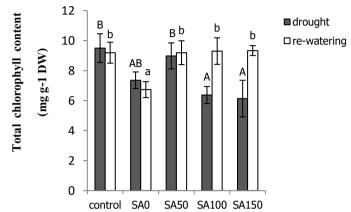


Figure 3 Effects of SA application on photosynthetic pigments, total chlorophyll content in rice under re-watered (white bars) and drought conditions (gray bars). Different letters indicate significant differences between treatments within drought (capital letter) and re-watering (small letter) (p<0.05). Star (*) means significant difference of re-watering versus drought within each treatment (p<0.05). SA0, SA50, SA100, and SA150 = foliar applications of 0, 50, 100 and 150 μ M of SA, respectively.

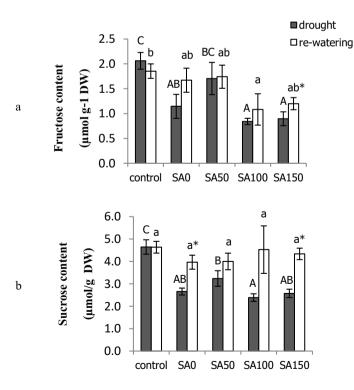


Figure 4 Effects of SA application on soluble sugar accumulations, (a) fructose content and (b) sucrose content in rice under re-watered (white bars) and drought conditions (gray bars). Different letters indicate significant



differences between treatments within drought (capital letter) and re-watering (small letter) (p<0.05). Star (*) means significant difference of re-watering versus drought within each treatment (p<0.05). SA0, SA50, SA100, and SA150 = foliar applications of 0, 50, 100 and 150 μ M of SA, respectively.

Discussion

Drought stress is one of main serious abiotic stress which leads to growth reduction of rice seedling as shown by the decreases in root fresh weight, shoot length, shoot fresh weight and shoot dry weight. Under drought stress condition, reduced water uptake results in a decrease in tissue water content and plant cell turgor, cell elongation in plant is inhibited by reduced turgor pressure of plant cells. Moreover, a decrease in stomatal opening, which limits CO₂ uptake, also reduces the photoassimilation and metabolites required for plant growth (Farooq et al., 2009b; Osakabe et al., 2014). Foliar spraying with SA affected plant growth under drought stress as shown by the significant increase in the shoot fresh weight and slight increases in shoot length and shoot dry weight. Previous studies also found that exogenous application of SA improves various plant growth under drought condition in okra, cucumber, cowpea, sweet basil and barley (Baghizadeh, Hajmohammadrezaei, 2011; Mardani et al., 2012; Afshari et al., 2013; Kordi et al., 2013; Fayez, Bazaid, 2014). However, we found that high concentrations of SA slightly decreased root dry weight and shoot length under drought stress. A reduction of plant growth in high concentrations of SA is affected by the diminished photosynthetic activity caused by disrubtion on the thylakoid membranes and light-induced reactions (Rivas-San Vicente, Plasencia, 2011)

The water status of plants is an important physiological indicator of plant response to water deficit stress so RWC was usually determined to indicate water status in plants. The result showed that RWC was decreased by drought stress. The findings of Saneoka et al. (2004) and Kordy et al. (2013) also suggested that drought stress reduced RWC of plant leaves. Low concentrations of SA application led to the increase in RWC but high concentrations of SA application were not effect on RWC. Agarwal et al. (2005) also demonstrated that SA increased RWC in wheat under drought stress, lower concentrations of SA were more effective than higher concentrations.

Environmental stresses lead to the generation of reactive oxygen species, including superoxide anion radicals (O_2^{\square}), hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), alkoxy radicals (RO) and singlet oxygen (O_2^{\square}). ROS may react with proteins, lipids and DNA, causing oxidative damage and impairing the normal functions of cell membrane (Farooq et al., 2009b; Golldack et al., 2014). EL was determined to assess membrane permeability under drought condition. The result showed that drought dramatically increased EL due to oxidative damage and low concentrations of SA improved drought response by increase in EL but high concentrations of SA had no effect on EL under drought stress. Nazarli et al. (2014) also suggested that SA decreased EL in chamomile plants. In contrast, SA increased EL under water deficit stress in maize (Nemeth et al., 2002). According to Miura, Tada (2014) concluded that low concentrations or the transient applications of SA promote plant tolerance to abiotic stress but high concentrations or the continual applications of SA induce inhibitory effects on plant growth and plants tolerance.

In the present studies, drought condition led to remarkable decreases in chlorophyll a, b and total chlorophyll contents. The chlorophyll content reduction under water deficit stress was also reported in barley (Fayez, Bazaid, 2014) and sweet basil (Kordi et al., 2013). Under stress condition, decrease in photosynthetic pigment due to



stress is related to the increase of ROS. These free radicals cause peroxidation, disintegration and reduction of chlorophyll content in plant (Schutz, Fangmir, 2001). In this study, it was observed that exogenous spraying of SA slightly increased total chlorophyll (chlorophyll a + b) content. Kordi et al. (2013) concluded that impact of SA on photosynthetic pigments may be related with its influence on the scavenging of ROS, including antioxidative enzyme activities and hydrogen peroxide metabolism.

Turgor maintenance by osmotic adjustment is an important physiological effect of water deficit stress. Osmotic adjustment is the decrease of osmotic potential by the active accumulation of organic and inorganic solutes within plant cells (Sanchez et al., 1998). Our data suggested that to alleviate the effects of drought stress by osmotic adjustment, the low concentration of SA slightly induced sugar accumulations, including fructose and sucrose accumulations. In the previous study, under salinity stress, SA could induce a significant accumulation of soluble sugar in leaves and roots of cucumber seedlings (Dong et al., 2011).

Following re-watering after drought led to recoveries of plant growth by the increasing of root fresh weight, shoot fresh weight and shoot dry weight. Moreover, RWC and sucrose content were increased and EL was decreased. Other study suggested that the ratio of chlorophyll a/b, carbonic anhydrase activity and net photosynthetic rate were increased, but the leaf area and electrolyte leakage from the cut leaf pieces were decreased (Xu, Zhou, 2007). The recoveries of plants under re-watering were due to co-occurrence in stomatal opening and repairing of photosynthetic function, including rubisco activity, CO_2 availability in the chloroplast and PSII photochemistry efficiency (Xu et al., 2010). In addition, we observed that the low concentrations of SA affected plant response to re-watering as shown by the increases in root length, RWC and total chlorophyll content and the decrease in EL as compared to re-watering without SA application.

Conclusions

Our results implied that SA improved drought tolerance in rice cv. KDML 105 by turgor maintenance, the increasing of photosynthetic pigments, the alleviation of oxidative damage and consequent increase in plant growth. SA repaired the subversion of plants performance under water deficit condition as shown by the increases in plant growth and RWC and the decrease in EL. Re-watering led to the recoveries of plants as shown by the increases in plant growth, RWC and sucrose accumulation, and the decrease in EL. The recoveries after re-watering of low concentrations of SA were more apparent compared to re-watering without SA treatment. The results suggested that SA can improve some physiological characteristics of rice under drought and re-watered conditions.

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