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ORIGINAL ARTICLE

Influence of salicylic acid on rubisco and rubisco activase in tobacco plant grown under sodium chloride *in vitro*

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Abstract The present study was designed to evaluate the influence of salicylic acid (SA) on the growth of salt stress (sodium chloride) induced in tobacco plants. In addition, quantification of rubisco and rubisco activase contents of the plants was also determined in treatments with the control, 10^{-4} mM SA, 50 mM NaCl, 100 mM NaCl, 150 mM NaCl, SA + 50 mM NaCl, SA + 100 mM NaCl and SA + 150 mM NaCl, respectively after *in vitro* culture for 5 weeks. The growth of the tobacco plant decreased in 50 mM and 100 mM NaCl when not treated with SA. However, the growth was accelerated by SA, and the growth retardation caused by NaCl was improved by SA. The content of rubisco was improved by SA only in plants treated with 50 mM NaCl, and the activity of rubisco was increased by SA resulting in the decreased effect of NaCl, but only in 50 mM NaCl treated plants. The content of rubisco activase decreased due to NaCl, and SA did not improve the effect caused by NaCl. The activity of rubisco activase was increased by SA resulting in decreased activity caused by NaCl, but increased effect by SA was not recovered to the level of NaCl untreated plants. The activity of rubisco and rubisco activase, which decreased due to denaturing agents, did not demonstrate significant improvement when compared to the control.

1. Introduction

Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) occupying about more than 50% of water-soluble protein existing in leaves of higher plants consists of two subunits and the larger subunit is coded in plastid genome, and is synthesized in the plastid ribosome of stroma. This constitutes the entire structure in eight subunits in total by chaperone. Small subunits are coded in the nuclear genome, and are synthesized on cytoplasmic ribosome, constituting the entire structure in 8 subunits in total (Gatenby and Ellis, 1990). In general, rubisco like this has a high affinity with CO_2 for the carboxylation among dark reactions to proceed smoothly depending on low atmospheric concentration of CO_2 (Roh et al., 1996). In addition, the activation of rusbisco *in vivo* is catalyzed by rubisco

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activase which is a water-soluble chloroplast enzyme (Salvucci et al., 1985).

Rubisco activase expedites the dissociation of ribulose-1,5-bisphosphate (RuBP) by binding to deactivated rubisco-RuBP complex, thereby increases the activation of rubisco. ATP is required at this time of binding to rubisco. Rubisco activase similar to this started to be refined in chloroplasts of spinach, and is also found in higher plants and green algae (Wang and Portis, 1992).

Salicylic acid (SA) belongs to secondary metabolites found in plants, and serves as a plant growth regulator in phenolic compounds, which is biosynthesized through a phenylpropanoid pathway (Durner et al., 1997). SA is an elicitor to create resistive materials against plant pathogens, and contributes to systemic acquired resistance (Yalpani et al., 1993). In tobacco plants, SA induces the gene expression of pathogenesis-related, accumulating the Pathogenesis related (PR) proteins (Klessig and Malamy, 1994). SA also controls the biosynthesis of ethylene and K^+ absorption in plants (Leslie and Romani, 1986), stimulates the chemical parameters for interaction among organisms (Raskin, 1992), and activates nitrogen reduction (Jain and Stivastra, 2006). At the time of blooming of arisaema like a taro, it also works as an endogenous regulator for heat generation, and secretion of insect pheromones (Raskin et al., 1987). It also affects the closing of stomata, production of fruits and seed germination (Cutt and Klessig, 1992).

Plants on the globe are affected by biotic stresses like fungi, bacteria, and viruses, and by abiotic stresses like moisture, temperature, ions, and salts (Sticher et al., 1997). SA induces the protective action against loss due to environmental stresses like these (Horvath et al., 2007), and also induces the stress-resistance unique to the species (Kogel and Langen, 2005).

In general, plants are affected by salt stress, but cotton, barley, and spinach are known to show a relatively strong salt tolerance (Greenway and Munns, 1980). Salt stress is caused simply by occurrence of metabolic disorders due to deterioration of moisture content in the soil (Poljakoff-Mayber and Lerner, 1994) or due to excessive accumulation of Na^+ and Cl^- in plants (Flowers et al., 1977). Salt stress does damage to the photosynthesis of plants at a variety of levels, such as in plastids, gas exchange in stomata, structure and function of the thylakoid membrane, and the electron transport system (Sudhira and Murthy, 2004).

Currently, there are lots of reports stating that salt stress affects the moisture content (Binzel et al., 1985) and the content of proline, glycinebetaine in plants (Meloni et al., 2004), and that it also affects the activation of antioxidative enzymes like catalase, peroxidase and superoxide dismutase, nitrate reductase, and carbonic anhydrase (Mittler, 2002; Meloni et al., 2004; Yusuf et al., 2008).

Reports showed that SA improved the growth, photosynthesis, and content of chlorophyll affected by salt stress through researches on seedlings of maize (Khodary, 2004), barley (El-Tayeb, 2005), *Brassica juncea* (Yusuf et al., 2008) and wheat seedlings (Kang et al., 2012). In addition, there is a report stating that in seedlings of rice affected by salt stress, the activation of SA photosynthesis enzymes was induced, causing the inner level of SA to increase (Sawada et al., 2006). Likewise, the study with regard to growth and enzymes related to salt stress and SA in plants has been reported independently, and the study on rubisco and rubisco activase related to NaCl and SA is not yet known. Influence of SA on the effect of NaCl affecting growth, rubisco and rubisco activase was studied in tobacco plants.

2. Materials and methods

2.1. Chemicals and apparatus

Murashige and Skoog (MS) media used in this study was obtained from Duchefa Biochemie (Haarlem, Netherland). SA, sodium chloride, enzymes, and other reagents were purchased from Sigma Chemical Co. (St. Louis, MO). For isolation and determination of content and activity of enzymes, a refrigerator centrifuge (Kontron T-324), a fraction collector (Bio-Rad 2110), a UV–VIS spectrophotometer (GeneQuant 100), and a ELISA microplate reader (Bio-Rad 680) were used.

2.2. Growth of tobacco plant

Seeds of tobacco (*Nicotiana tabacum* L.) were germinated and grown aseptically in a cell culture vessel containing MS agar medium (Murashige and Skoog, 1962). Shoots were cut into 3 cm segments and used as explants. 2 explants were placed on an induction MS medium of 8 groups, respectively. Explants were separated in 8 groups of control (not treated with SA and NaCl), SA, 50 mM NaCl, 100 mM NaCl, 150 mM NaCl, SA + 50 mM NaCl, SA + 100 mM NaCl, and SA + 150 mM NaCl. These explants were maintained on these media at 27 ± 2 °C under a 16-h light (800 μM/m^2/s PFD) and 8-h dark photoperiod (Roh et al., 1996). Plant growth of each experiment was measured by total fresh weight and leaves weight, and then compared. Fully expanded leaves from 5 week old mature plants were used as material for rubisco and rubisco activase studies. All experiments were independently triplicated.

2.3. Isolation of rubisco

Rubisco was isolated from tobacco leaves using a modified method of Wang et al. (1992). Leaf tissue was ground to a fine powder with a pre-cooled mortar and a pestle in liquid nitrogen and then extracted in the extraction buffer containing 50 mM 1,3-bis(tris(hydroxymethyl) methylamino)propane (BTP) (pH 7.0), 10 mM $NaHCO_3$, 10 mM $MgCl_2$, 1 mM EDTA, 0.5 mM ATP, 10 mM DTT, 1 mM phenylmethylsulfonylfluoride (PMSF), 1 mM benzamidine, 0.01 mM leupeptin, 1.5% PVPP and 3 mM mercaptobenzothiazole (MBT). The leaf slurry was filtered through four layers of cheesecloth and one layer of Miracloth. Filtered solution was centrifuged at 30,000*g* for 40 min. $(NH_4)_2SO_4$ powder was slowly added into the supernatant to 35% saturation and stirred for 30 min. The supernatant and pellet were collected by centrifugation at 8000*g* for 10 min. The supernatant contains rubisco and the resuspended pellet contains rubisco activase. The supernatant collected was brought to 55% saturation of $(NH_4)_2SO_4$ by the addition of powder. The pellet collected by centrifugation at 8000*g* for 10 min was resuspended in 5 ml of 20 mM BTP (pH 7.0) containing 0.2 mM ATP, 10 mM $MgCl_2$ and 2 mM MBT (buffer A), and 50% PEG-10 K was added to a final concentration of 18%. The resulting precipitate was collected by centrifugation at 8000*g* for 10 min and resuspended in buffer

A. Resuspended solution was loaded onto a Q-Sepharose column equilibrated with 20 mM Tris (pH 7.5), 10 mM $MgCl_2$, and 10 mM $NaHCO_3$. The column was washed with the same buffer containing 0.1 M NaCl before starting elution with a linear gradient from 0.1 to 0.5 M NaCl at a flow rate of 1 ml/min. 3 ml fractions were pooled, and assayed for rubisco content and activity.

2.4. Measurement of rubisco content

Rubisco content was measured at 280 nm and calculated by the following equation according to Wishnick and Lane (1971).

$$\text{Content (mg/ml)} = A_{280} \times 0.61.$$

2.5. Activity assays of rubisco

Rubisco activity was determined spectrophotometrically by monitoring NADH oxidation at 340 nm (Racker, 1962). The assay medium contained 1 M Tris (pH 7.8), 0.006 M NADH, 0.1 M GSH, 0.5% glyceraldehyde-3-phosphate dehydrogenase, 0.025 M 3-phosphoglycerate kinase, 0.05% α-glycerophosphate dehydrogenase-triose phosphate isomerase, 0.025 M RuBP, 0.2 M ATP, 0.5 M $MgCl_2$, 0.5 M $KHCO_3$, and isolated rubisco solution in a final volume of 1 ml. One unit of enzyme was defined as the amount of enzyme producing 1 μM of RuBP per min.

2.6. Isolation of rubisco activase

To isolate rubisco activase in the resuspended pellet obtained above, 50% (w/v) PEG-10 K was added to buffer A to a final concentration to 18%, and centrifuged at 8000*g* for 10 min. The pellet was dissolved in buffer A. This solution was cleared by spinning at 20,000*g* for 10 min. Afterward, the pellet was resuspended in buffer A, and the solution was cleared again. The collected supernatants were loaded onto a 20 ml Q-Sepharose column equilibrated with 20 mM BTP (pH 7.0). The column was eluted with 20 mM BTP (pH 7.0) at a flow rate of 1 ml/min before continuing with a linear gradient from 0 to 0.5 M NaCl in 20 mM BTP (pH 7.0). 3 ml fractions were pooled, and assayed for rubisco activase content and activity. All purification processings were done at 4 °C except as indicated.

2.7. Measurement of rubisco activase content

Rubisco activase content was measured at 595 nm according to a Bradford method (1976) using a microplate reader with bovine serum albumin as standard.

2.8. Assay of rubisco activase activity

Rubisco activase activity was assayed as the ability to produce ADP in an ATP-dependent reaction in absorption at 340 nm by procedure of Robinson and Portis (1989). The isolated rubisco activase solution was added to a total volume of 0.4 ml of the activation reaction mixture containing 50 mM Tricine (pH 8.0), 20 mM KCl, 10 mM $MgCl_2$, 1 mM ATP, 1 mM phosphoenolpyruvate, 0.3 mM NADH, 40 units/ml pyruvate kinase, and 40 units/ml lactate dehydrogenase. One unit was defined as 1 μM ATP hydrolyzed per min.

2.9. Assay of rubisco and rubisco activase activity by denaturing agents

10 mM of L-cysteine, EDTA, urea, thiourea, guanidine-HCl, and β-mercaptoethanol as denaturing agents used to measure the effect of denaturing agents on rubisco and rubisco activase activity.

2.10. Statistical analysis

Treatment means were compared by the analysis of variance using SPSS (SPSS ver. 21). Standard error between replicates was also calculated.

3. Results

3.1. Effect of SA and NaCl on growth of tobacco plant

To investigate the effect of SA and NaCl affecting growth, explants were placed on MS media consisting of control (without SA and NaCl treatment), 10^{-4} mM SA, 50 mM NaCl, 100 mM NaCl, 150 mM NaCl, SA + 50 mM NaCl, SA + 100 mM NaCl, and SA + 150 mM NaCl, respectively. Growth was best in SA + 100 mM NaCl, and was worst in 50 mM NaCl (Fig. 1). Total fresh weight was measured to confirm the results shown in Fig. 1. Total fresh weight was measured as 7.28 g in control, 11.25 g in SA, 5.14 g in 50 mM NaCl, 6.59 g in 100 mM NaCl, 12.79 g in 150 mM NaCl, 9.18 g in SA + 50 mM NaCl, 16.59 g in SA + 100 mM NaCl, and 15.92 g in SA + 150 mM NaCl, respectively. In SA untreated group, the growth in 50 mM and 100 mM NaCl was lower than that in the control, but the growth in 150 mM NaCl was higher. In SA treated group, the growth in 50 mM NaCl was low compared to the growth in NaCl untreated group, but the growth in 100 mM and 150 mM was higher. Compared to the control, the growth treated with SA was higher in all concentrations of NaCl (Fig. 2). As a result of measuring the weight of leaves, control, SA, 50 mM NaCl, 100 mM NaCl, 150 mM NaCl, SA + 50 mM NaCl, SA + 100 mM NaCl, and SA + 150 mM NaCl are represented as 4.96 g, 7.0 g, 1.42 g, 3.46 g, 6.59 g, 4.77 g, 10.55 g, and 8.41 g, respectively (Fig. 3). These results showed the same patterns as the results of total fresh weight.

3.2. Effect of SA and NaCl on content and activity of rubisco

In the present study, the content of rubisco in control, SA, 50 mM NaCl, 100 mM NaCl, 150 mM NaCl, SA + 50 mM NaCl, SA + 100 mM NaCl, and SA + 150 mM NaCl were 0.023 mg/ml, 0.196 mg/ml, 0.161 mg/ml, 0.065 mg/ml, 0.014 mg/ml, 0.170 mg/ml, 0.024 mg/ml, and 0.011 mg/ml, respectively. In SA untreated group, the concentration of rubisco in 50 mM and 100 mM NaCl was higher than in the control, but in 150 mM NaCl treatment, the content decreased rapidly, and became lower than that in the control. In SA treated group, the rubisco content decreased in all

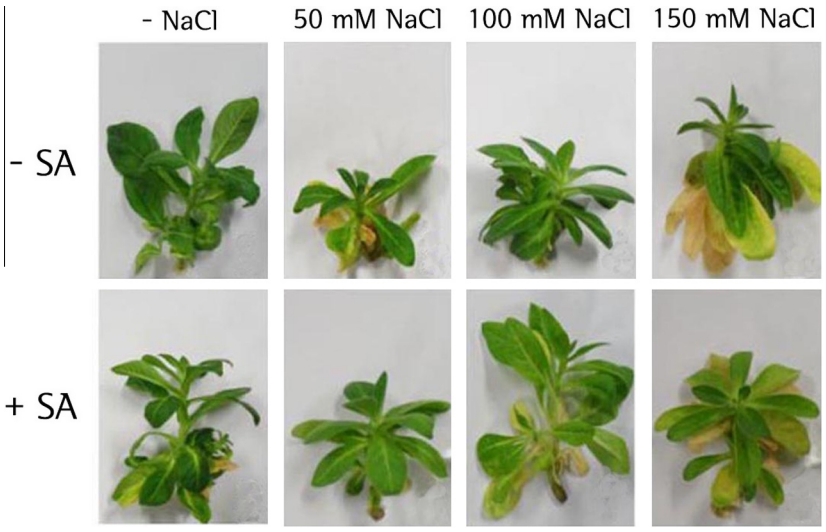

Figure 1 *In vitro* induction of tobacco plant grown on MS medium for 5 weeks. Control: without SA + NaCl.

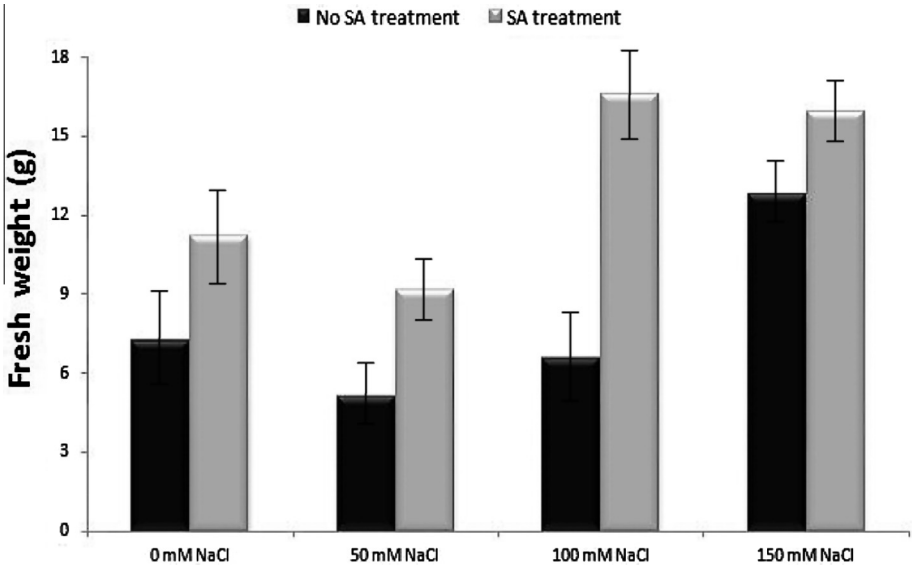

Figure 2 Effect of SA and NaCl on growth of tobacco plants. The total fresh weight of tobacco plant was determined. Control: without SA + NaCl.

concentrations of NaCl compared to the NaCl untreated group. Comparing SA untreated and treated groups, the content was high in the SA group and 50 mM NaCl + SA, but was low in 100 mM and 150 mM (Fig. 4). The activity of rubisco in control, SA, 50 mM NaCl, 100 mM NaCl, 150 mM NaCl, SA + 50 mM NaCl, SA + 100 mM NaCl, and SA + 150 mM NaCl were 1.132 unit/ml, 1.208 unit/ml, 0.545 unit/ml, 0.774 unit/ml, 0.844 unit/ml, 1.568 unit/ml, 0.968 unit/ml, and 0.920 unit/ml, respectively. In the SA untreated group, the activity was lower than that in control in all concentrations of NaCl, but in the SA treated group, compared to the NaCl untreated group, the activity increased in 50 mM NaCl + SA, but decreased in 100 mM and 150 mM NaCl. Compared to SA untreated groups, the activity was higher in the SA treated group (Fig. 5).

3.3. Effect of SA and NaCl on content and activity of rubisco activase

In the present study, the content of rubisco activase in control, SA, 50 mM NaCl, 100 mM NaCl, 150 mM NaCl, SA + 50 mM NaCl, SA + 100 mM NaCl, and SA + 150 mM NaCl was 0.183 mg/ml, 0.171 mg/ml, 0.166 mg/ml, 0.157 mg/ml, 0.086 mg/ml, 0.075 mg/ml, 0.045 mg/ml, and 0.045 mg/ml, respectively. In the SA untreated group, the rubisco activase content was low compared to the control in all concentrations of NaCl, and was the same in the SA treated group. In comparison of both SA untreated and treated groups, the content of rubisco was lower in the SA treated group in all concentrations of NaCl (Fig. 6). The activity of rubisco activase in control, SA, 50 mM NaCl, 100 mM NaCl, 150 mM NaCl,

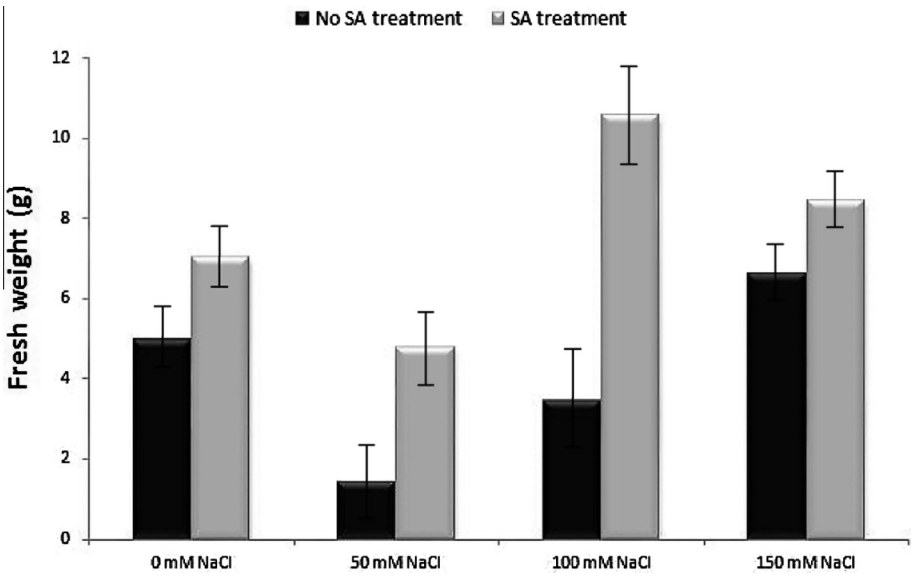

Figure 3 Effect of SA and NaCl on growth of tobacco leaves. The total fresh weight of tobacco leaves was determined. Control: without SA + NaCl.

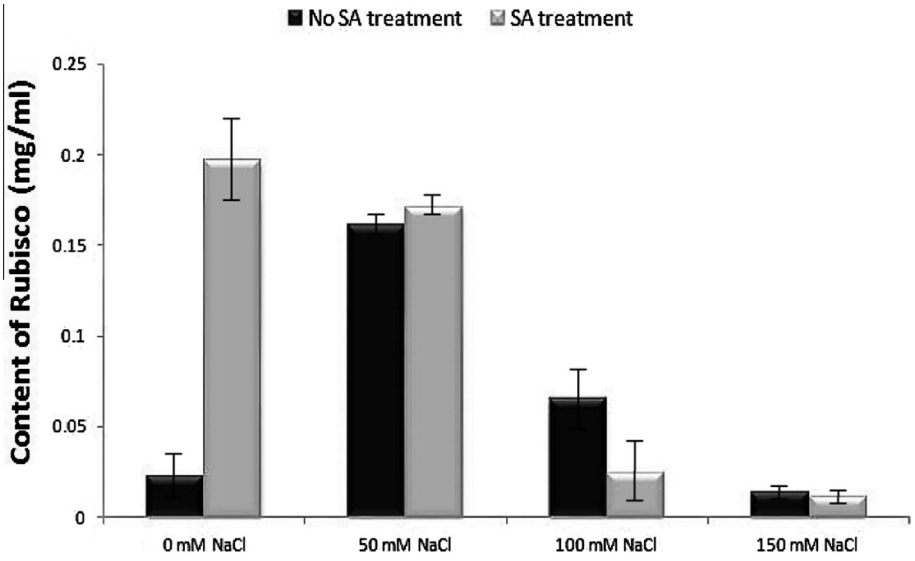

Figure 4 Effect of SA and NaCl on content of rubisco in tobacco leaves. Plants were grown on MS medium without SA + NaCl (control), with SA, NaCl, and SA + NaCl, respectively. 10^{-4} mM SA was used in this study.

SA + 50 mM NaCl, SA + 100 mM NaCl, and SA + 150 mM NaCl was 0.127 unit/ml, 0.132 unit/ml, 0.086 unit/ml, 0.097 unit/ml, 0.107 unit/ml, 0.092 unit/ml, 0.102 unit/ml, and 0.114 unit/ml, respectively. In the SA untreated group, the activity was lower than the control in all concentrations of NaCl, and increased in concentrations more than 50 mM NaCl. These results were the same with the results of the SA treated group. The activity of SA treated groups was higher than SA untreated groups in all concentrations of NaCl (Fig. 7).

3.4. Effect of denaturing agents on activity of rubisco

In the present study, the value of denaturing agents was represented under the condition that the activity of the control without SA, NaCl and denaturing agents was set as 100%. From denaturing agent treatment, it was confirmed that the activity was inhibited in denaturing agent treatment groups compared to the groups not treated with denaturing agents. In NaCl treatments, the activity was inhibited by denaturing agents compared to the control, but thiourea did not inhibit the activation greatly compared to 150 mM NaCl. L-cysteine and thiourea showed a lower inhibition ratio of hindrance in the SA group compared to the control. When SA + NaCl was treated with EDTA and β-mercaptoethanol, the activity was expedited compared to SA, and the activity was inhibited by the other four denaturing agents compared to SA. As a result of comparing SA + 50 mM NaCl with 50 mM NaCl, the activity of SA + 50 mM NaCl was expedited by urea, guanidine-HCl and β-mercaptoethanol. While comparing

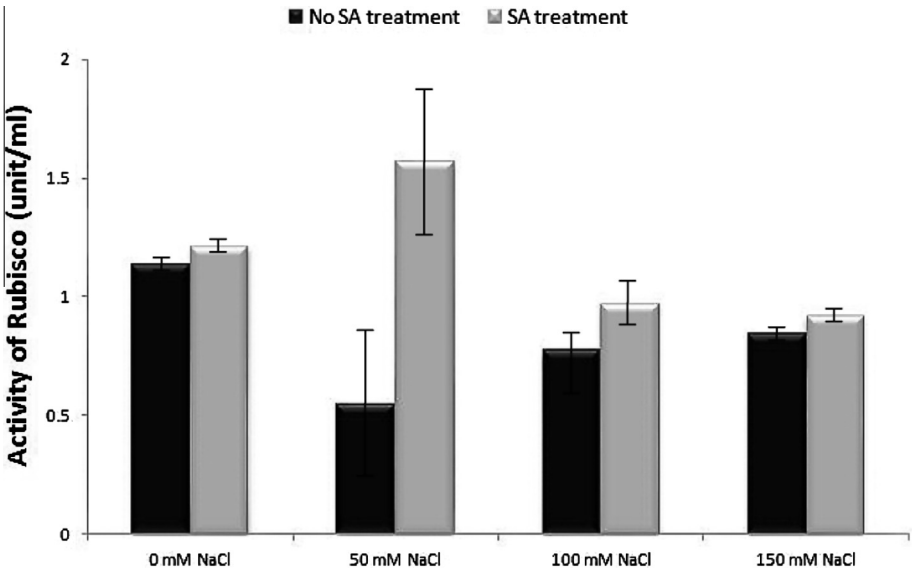

Figure 5 Effect of SA and NaCl on activity of rubisco in tobacco leaves. Plants were grown on MS medium without SA + NaCl (control), with SA, NaCl, and SA + NaCl, respectively. 10^{-4} mM SA was used in this study.

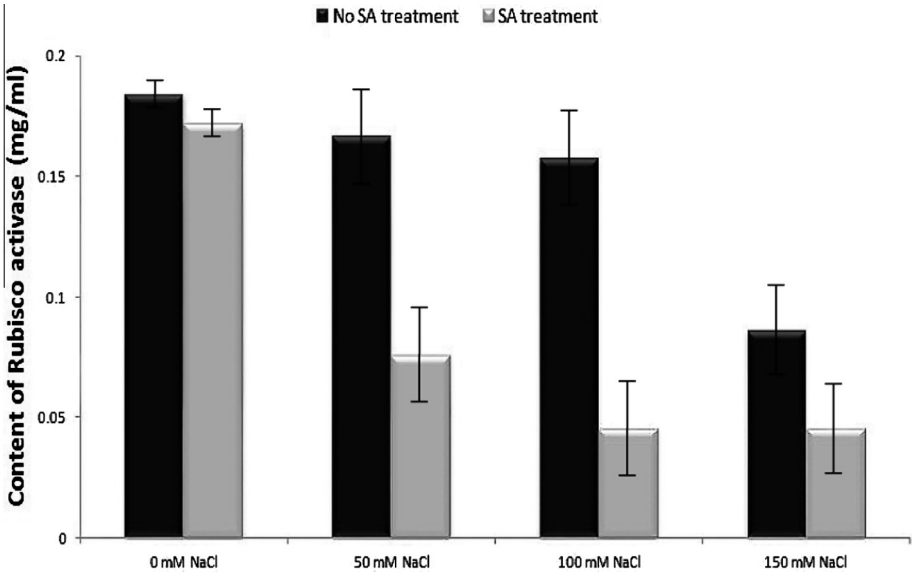

Figure 6 Effect of SA and NaCl on content of rubisco activase in tobacco leaves. Plants were grown on MS medium without SA + NaCl (control), with SA, NaCl, and SA + NaCl, respectively. 10^{-4} mM SA was used in this study.

SA + 100 mM NaCl with 100 mM NaCl, the activity of SA + 100 mM NaCl was facilitated more by L-cysteine, EDTA, urea and β-mercaptoethanol. As a result of comparing SA + 150 mM NaCl with 150 mM NaCl, the activity of SA + 150 mM NaCl was inhibited by urea, thiourea, guanidine-HCl, and β-mercaptoethanol (Table 1).

3.5. Effect of denaturing agents on activity of rubisco activase

On treating with denaturing agents, the activity was inhibited in denaturing agent treatment groups compared to groups not treated with denaturing agents. In particular, the inhibition ratio of activity was very high in the control, and β-mercaptoethanol showed a ratio of 91%. In NaCl treatment, the activity was expedited compared to the control, but when L-cysteine and EDTA were used, as the concentration of NaCl increases, the activity was inhibited. Urea, thiourea and guanidine-HCl showed the lowest ratio of hindrance in 100 mM NaCl. In SA treatment, the activity was expedited most, and was least hindered by thiourea.

In SA + NaCl treatment, the activity was inhibited compared to SA, and as a result of comparing SA + 50 mM NaCl with 50 mM NaCl treatments, the activity of SA + 50 mM NaCl was hindered by L-cysteine, EDTA, urea, thiourea and β-mercaptoethanol. Comparing SA + 100 mM NaCl with 100 mM NaCl treatments, the activity of SA + 100 mM NaCl

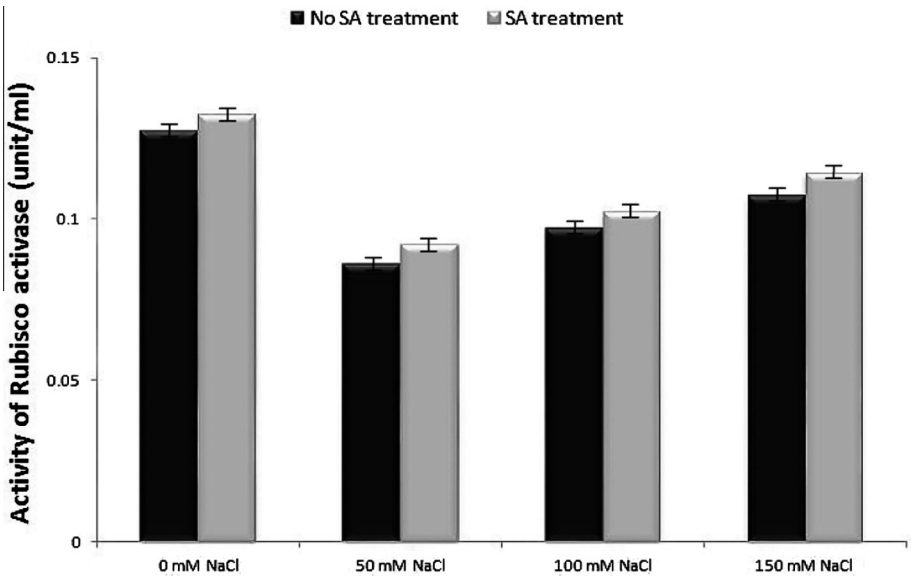

Figure 7 Effect of SA and NaCl on activity of rubisco activase in tobacco leaves. Plants were grown on MS medium without SA + NaCl (control), with SA, NaCl, and SA + NaCl, respectively. 10^{-4} mM SA was used in this study.

Table 1 Effect of denaturing agents on rubisco activity.

	Rubisco activity (%)							
	Control	SA*	NaCl (mM)			SA* NaCl (mM)		
			50	100	150	50	100	150
Control	100 ± 2.0	107 ± 1.0	48 ± 1.5	68 ± 1.5	75 ± 1.0	139 ± 2.0	86 ± 1.0	81 ± 1.0
L-Cysteine	68 ± 2.5	75 ± 1.5	64 ± 1.0	71 ± 0.5	61 ± 1.5	61 ± 1.0	73 ± 1.5	63 ± 2.0
EDTA	76 ± 1.0	52 ± 2.0	65 ± 1.5	51 ± 1.0	55 ± 2.0	63 ± 1.5	59 ± 1.0	74 ± 1.0
Urea	78 ± 1.5	74 ± 1.0	63 ± 2.0	49 ± 2.0	68 ± 1.5	68 ± 1.0	59 ± 2.0	65 ± 1.5
Thiourea	78 ± 1.5	83 ± 1.0	63 ± 1.0	68 ± 1.5	93 ± 1.0	62 ± 2.0	57 ± 1.5	64 ± 1.0
Guanidine-HCl	70 ± 1.5	66 ± 2.0	66 ± 1.0	76 ± 1.0	71 ± 2.0	71 ± 1.5	61 ± 1.0	63 ± 2.0
β-Mercaptoethanol	66 ± 2.0	49 ± 2.5	61 ± 2.0	53 ± 1.0	74 ± 1.5	74 ± 1.0	78 ± 1.5	68 ± 1.0

* 10^{-4} mM SA was used in this study.

was hindered by L-cysteine, urea, thiourea and guanidine-HCl. For SA + 150 mM NaCl and 150 mM NaCl treatments, the activity of SA + 150 mM NaCl was expedited by L-cysteine, EDTA, urea and guanidine-HCl (Table 2).

4. Discussion

Salt stress expedites the creation of reactive oxygen species (ROS) like superoxide, hydrogen peroxide, and singlet oxygen in plants due to toxicity of sodium (Mittler, 2002). In addition, as the concentration of NaCl increases, the activation of carbonic anhydrase, nitrate reductase is controlled, and lots of physiological processes including photosynthesis are disturbed, thereby diminishing the growth of the plant (Gouia et al., 1994).

It has been reported that SA affects various kinds of physiological and biochemical activation, growth and production of plants (Arberg, 1981). In addition, there was a report stating that SA increased its resistance against moisture deficiency in wheat seedlings (Bezrukova et al., 2001), impact of high and low temperature in tomato and bean (Senaratna et al., 2000), and damage to rice due to heavy metals (Mishra and Choudhuri, 1999). SA represented the effect of resistance against diverse abiotic stress (Horvath et al., 2007), so in this study, the researcher made a study of the impact of SA on salt stress which belongs to abiotic stress in tobacco plants, cultured *in vitro*.

On investigating the influence of SA on the effect of NaCl affecting the growth of tobacco plants, total fresh weight and weight of leaves showed a similar trend, and as the concentration of NaCl increases, the growth of tobacco plant increased. In *B. juncea* (Yusuf et al., 2008) and maize (Khodary, 2004), when the concentration of NaCl increased, the growth was retarded, which was different from the present study.

In this study, when comparing the growth between the control and the SA group, the growth increased due to SA. This is in accordance with the report that when SA was treated to *Brassica napus* (Ghai et al., 2002), bean (Gutierrez-Coronado et al., 1998) and wheat seedlings (Shakirova et al., 2003), the growth increased, but this showed results different from the

Table 2 Effect of denaturing agents on rubisco activase activity.

	Rubisco activase activity (%)							
	Control	SA*	NaCl (mM)			SA* NaCl (mM)		
			50	100	150	50	100	150
Control	100 ± 2.0.	104 ± 1.0	68 ± 1.5	76 ± 1.0	84 ± 1.5	72 ± 1.0	80 ± 2.0	90 ± 2.0
L-Cysteine	31 ± 2.0	80 ± 2.0	78 ± 1.0	67 ± 1.5	62 ± 1.0	59 ± 2.0	13 ± 1.0	93 ± 1.0
EDTA	39 ± 3.0	80 ± 2.0	83 ± 0.5	72 ± 2.0	47 ± 2.0	68 ± 1.5	74 ± 1.5	86 ± 1.5
Urea	31 ± 1.5	72 ± 1.5	61 ± 1.0	81 ± 1.0	66 ± 0.5	15 ± 1.0	69 ± 1.0	71 ± 1.0
Thiourea	21 ± 1.5	97 ± 2.0	70 ± 1.0	97 ± 1.5	43 ± 1.0	39 ± 0.5	70 ± 0.5	16 ± 0.5
Guanidine-HCl	15 ± 2.0	68 ± 1.5	46 ± 1.5	84 ± 1.0	50 ± 1.5	65 ± 1.0	13 ± 1.0	81 ± 1.0
β-Mercaptoethanol	9 ± 1.0	81 ± 1.0	76 ± 1.0	74 ± 1.0	78 ± 2.0	71 ± 2.0	80 ± 2.0	75 ± 1.0

* 10^{-4} mM SA was used in this study.

report that the growth of common dayflower treated with SA was retarded (Lee, 1999). This is considered due to the fact that there are differences depending on plant species and concentration of SA treated groups. In addition, in this study, the growth retarded by NaCl was improved by SA, and improved to the maximum by SA in 100 mM NaCl. This SA is considered as related to the growth of plant hindered due to stress, and prevents the decrease of the contents of Indole acetic acid (IAA) and cytokinin (Sakhabutdinova et al., 2003). This accorded with the report that when barley (El-Tayeb, 2005), wheat seedlings (Kang et al., 2012) and sunflower (Noreen and Adhraf, 2008) were treated with SA, growth increased. Likewise, the effect of improvement by SA on growth of plant suffering from salt stress is considered to be related to hydrophilicity (Barkosky and Einhelling, 1993), regulation of stomata (Arfan et al., 2007), nutrient absorption (Glass, 1974), and photosynthesis (Khan et al., 2003).

In relation to the report that SA improves not only the growth of plants suffering from salt stress, but also the ratio of photosynthesis reduced due to salt stress (Shakirova and Bezrukova, 1997; Stevens et al., 2006), the influence of SA on the effect of NaCl affecting the content and activity of rubisco catalyzing the carbon dioxide fixation of photosynthesis was studied. The content of rubisco decreased at concentrations above 50 mM NaCl, and was increased considerably by SA. In 50 mM NaCl, the content of rubisco was increased by SA, but in more than 100 mM NaCl, the content was decreased on the contrary by SA. It is considered that SA expedites the effect of decrease by NaCl. According to Pancheva and Popova (1997), when the content was measured after 1 mM SA higher than 10^{-4} mM applied to this study was treated to rubisco separated from leaves of barley, the content was rather decreased, and the concentration of 500 μM, 100 μM became lower, the content increased. In addition, the content of protein decreased in the leaves of *Salvia officianlis* L. affected by salt, but the content of protein was increased by SA (Sahar et al., 2011).

The activity of rubisco was decreased by NaCl treatment, and increased by SA. In 50 mM NaCl, the activity was improved by SA, and in 100 mM and 150 mM NaCl, the activity was increased by SA, but compared to the control, it was not improved. Like this, the result of this study accorded with the report by *B. juncea* that the ratio of photosynthesis decreased by 50 mM NaCl was improved due to 10^{-5} M SA treatment (Yusuf et al., 2008). In addition, there is the report stating that the activity of rubisco of maize decreased by 50 mM and 100 mM NaCl was improved by SA 10^{-2} M, causing the increase of the photosynthesis ratio (Khodary, 2004), and the photosynthesis ratio of tomatoes decreased by 150 mM NaCl but was increased by SA 0.1 mM (Stevens et al., 2006). It is considered that there is species specificity depending on the concentration of SA and NaCl affecting rubisco.

In addition, there is the report stating that the carboxylase activation and total content of chlorophyll decreased due to drought was increased by SA (Singh and Usha, 2003), and also a report stating that in arabidopsis (Williams et al., 1994), tomato (Bartholomew et al., 1991) and rice (Vu et al., 1999), the quantity of small subunits of rusbisco decreased due to drought stress.

Since rubisco activase is activated by rubisco (Robinson and Portis, 1989), the influence of SA on the effect of NaCl by measuring the content and activity of rubisco activase was studied in order to find out whether or not the result of NaCl and SA on rubisco is related to rubisco activase. As the concentration of NaCl increased, the content of rubisco activase decreased, which was opposed to the report that the content of rubisco activase in leaves of rice increased by 50 mM NaCl (Parker et al., 2006). In addition, in this study, the content was also decreased by SA, and the content was decreased by NaCl and was decreased even more by SA. It is considered that SA expedites the effect of decrease by NaCl .

The activity of rubisco activase was decreased by NaCl, and in more than 50 mM NaCl, the activity increased. The activity was increased by SA, and decreased by NaCl and was increased by SA, but was not improved. Unlike the report stating that the content and activity of rubisco activase in tobacco plants affected by Cd was improved by SA (Wang and Roh, 2012), it was not improved in the results of this study, so compared to Cd, it is considered that there is no effect of improvement by SA affecting the rubisco activase influenced by NaCl. In the report of Wang and Roh (2012) on the effect of SA on rubisco induced by Cd, the results of rubisco and rubisco activase were similar, which shows that rubisco and rubisco activase acts in connection, which was interpreted differently from this report. So, it is considered that rubisco is caused by rubisco activase, but SA works independently at various levels of rubisco and rubisco activase.

Denaturalization occurs in protein like enzymes, but denaturing agents like urea, thiourea, guanidine-HCl, destroy the hydrogen bonds of protein or hydrophobic interactions (Dhuna et al., 2005), and increased the solubility of proteins

(Vanzi et al., 1998), and L-cysteine and β-mercaptoethanol break up the disulfide bond existing in proteins in the presence of the SH group, causing the denaturalization (Wang and Roh, 2012). Study on the effect of denaturing agents on the activity of rubisco shows that, the activity of rubisco affected by the influence of NaCl was inhibited by denaturing agents, and it is considered that denaturing agents expedite the inhibition of activity. The inhibition of L-cysteine and thiourea to activity of rubisco affected by SA was lowest, and the inhibition of β-mercaptoethanol to the activity of rubisco affected by SA was highest. This accorded with the results by Wang and Roh (2012) stating that the activity of rubisco affected by SA was increased by denaturing agents, but was not the same as in β-mercaptoethanol. The activity of rubisco affected by SA in 50 mM and 100 mM NaCl was expedited in most cases by treatment of denaturing agents, but in 150 mM NaCl, the activity of rubisco affected by SA was inhibited in most cases by treatment of denaturing agents. This is to be considered as the denaturing agents were acting differently on SA.

Regarding the effect of denaturing agents on the activity of rubisco activase, the activity of the control due to denaturing agents was hindered to the most, and the activity of rubisco activase affected by NaCl was higher than that in the control treated with denaturing agents. Compared to the control that belongs to non-denaturing agents group, the activity was inhibited due to denaturing agents treatment, and the denaturing agents expedited the inhibition to activity, which showed the same aspect with rubisco. The activity of rubisco activase affected by SA was least inhibited, and among them, the inhibition by thiourea was lowest. Since it was not improved by SA, this was opposed to the result that the activity was improved due to SA impact on rubisco activase affected by Cd and SA was expedited by denaturing agent treatment (Wang and Roh, 2012). In addition, in this study, the activity of rubisco activase affected by SA in 50 mM and 100 mM NaCl was inhibited in most cases due to denaturing agent treatment, but the activity of rubisco activase affected by SA in 150 mM NaCl was expedited in most cases due to denaturing agent treatment. This was opposed to the result represented in rubisco. We could see that the activity of rubisco activase affected by SA for NaCl was inhibited by thiourea. Son et al. (2014) reported that the activity of rubisco activase decreased as a result of six denaturing agents, and the effect caused by EDTA and guanidine-HCl was the greatest, while the effect caused by L-cysteine and urea was minimal.

5. Conclusion

SA improved the retardation of growth caused by NaCl, and the content of rubisco was improved by SA only in 50 mM NaCl treatment. There was no improvement on rubisco activase in NaCl treated plants. The activities of rubisco and rubisco activase were not improved by denaturing agents.

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