

The effect of priming duration with salicylic acid under salinity stress on growth and leaf anatomy of sweet corn (*Zea mays* L.)**

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ABSTRACT

Salinity stress is known for adverse effect on plants. Priming with salicylic acid was able to improve plant performance under salinity stress. This study aimed to determine the effect of priming duration with salicylic acid on growth, leaf anatomy and the optimal priming duration for sweet corn seedlings (*Zea mays* L.) under salinity stress. The experiment was based on Completely Randomized Design with two factors and five replications. The first factor was priming duration with salicylic acid (2 mM) with four different durations (0, 12, 18 and 24 h). The second factor was the level of salinity (NaCl 0% and 3%). Parameters observed were germination percentage, plant height, root length, fresh weight, dry weight, chlorophyll content, leaf proline content, leaf anatomy and stomatal density. Data were analyzed with t-test, ANOVA and followed by Duncan's test at 95% confidence level. The results showed that 18-h priming duration observed as the highest germination percentage which was 7% higher than control. Priming for 24 h showed phytotoxic effect for sweet corn on the germination phase by reducing the percentage of germination. The application of salicylic acid mitigated the toxic effects of NaCl stress on maize seedlings and considerably improved root and shoot growth, photosynthetic pigments, fresh weight, dry weight, proline content, and stomatal density, as well as could maintaining the leaf anatomy. The optimal priming duration with salicylic acid for sweet corn seedlings under 3 % salinity was 18 h.

Keywords: Sweet corn; salicylic acid; salinity; priming duration.

INTRODUCTION

Seed priming is one of the physiological approaches that can increase the resistance of plants in stress conditions (Nawaz *et al.* 2013). Priming techniques are being used to improve seed germination under both optimal and adverse condition (Jisha *et al.*, 2013). After priming, seeds can be dried and stored for long periods and the growth of seedlings will be faster and uniform (Imran, 2012).

Hormonal priming is a priming treatment with phytohormones, such as gibberellin, kinetin or salicylic acid (Nawaz *et al.* 2013). Salicylic acid is a phenolic compound that plays a role in plant defense mechanisms against both biotic and abiotic stress, including salinity stress (Zahra *et al.*, 2010). Salicylic acid can prevent the release of K⁺ ions and the membrane potential recovery that impacts on the increase of plant tolerance

to salinity stress (Jayakannam *et al.*, 2013). In *Torreya grandis*, salicylic acid can increase chlorophyll and biomass (Li *et al.*, 2014). Together with gibberellin, salicylic acid elevated levels of chlorophyll a and b on *Trigonella foenum-graecum* when subjected to salt stress (Farahmandfar *et al.*, 2013). The success of hormonal priming itself is influenced by a number of factors such as duration, concentration and type of hormone given (Sedghiet *et al.*, 2014).

Priming with salicylic acid in salinity-treated maize significantly increased dry weight, leaf area, leaf number and stem height with optimum salicylic acid concentration was 2 mM (Zadehbagheri, 2014). Salicylic acid can also induce specific changes in leaf anatomy (Khanet *et al.*, 2013). Maize varieties and salinity level differences also affect the tolerance level of the plant to the saline field. For example, a lower initial salinity treatment may increase corn tolerance in

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the vegetative phase when subsequent salinity conditions are increased (Suwignyo *et al.*, 2010).

In Indonesia, projected demand for sweet corn in 2010-2050 tends to increase by 0.65% per year based on population growth, food preferences, income, price changes, arable land areas and diversification trends in the community (Sudaryanto, 2010). With the increasing transition of agricultural land into residential and industrial areas, causing the area of agriculture to decline. To increase agricultural land, marginal areas that usually have a high salt content, can be an alternative. The soil called saline contains excess salt which generally inhibit the growth of various non-tolerant species to salinity (Dachlan *et al.*, 2013). Salinity is one of the obstacles of sweet corn cultivation in Indonesia (Palungkun and Asiani, 2004).

The anatomical structure of the leaves can also undergo changes when subjected to stress and induction of certain hormones. The thickness of the adaxial and abaxial epidermis of maize leaves treated with salinity was thinner than that of controls or plants given salicylic acid (Carcamo *et al.*, 2012). The others anatomical structure changes included Kranz anatomy and mesophyll cell. Salicylic acid also has inhibitory effect on the entry of Na⁺ and Cl⁻ ions at the membrane transport level so that this hormone is responsible for the anatomical changes of plants (Romero-Aranda *et al.*, 2001). The ionic competition between salicylic acid with Na⁺ and Cl⁻ will prevent ionic phytotoxicity so that the tissue in the leaves can still thrive under salinity. Salinity stress increased stomata number in maize leaves (Carcamo *et al.*, 2012). Meanwhile, the reduction of stomatal density and its conductance in tomato leaves resulting from a decrease in plant transpiration rates during salt stress (Romero-Aranda *et al.*, 2001).

The purpose of this study was to evaluate the effect of priming duration with salicylic acid on leaf anatomical structure and sweet corn plant growth (*Zea mays* L.) and to determine the optimal priming duration for salted maize (*Zea mays* L.) maize.

MATERIALS AND METHODS

The research was conducted in February–May 2017 at Greenhouse, Plant Structure and Development Laboratory, and in Plant Physiology Laboratory, Faculty of Biology UGM, Yogyakarta.

1. Experiment design

Experiment used Completely Randomized Design (CRD) with two factors. The first factor was priming duration with 2mM salicylic acid (Zadehbagheri,

2014) with for different priming time (0 h (P0), 12 h (P1), 18 h (P2) and 24 h (P3) (Arif *et al.*, 2010). The second factor was NaCl (0% = K0 and 3% = K1). The 3% was NaCl able to inhibit maize germination up to 50% (Zadehbagheri, 2014).

2. Germination and seedling growth

Sweet corn seeds were soaked in 2 mM salicylic acid solution each for 0, 12, 18 and 24 h. The weight ratio of seeds per volume of salicylic acid solution during submersion was 1: 5 (gmL⁻¹) (Mukhtar *et al.*, 2013). After each priming treatment, the seeds were rinsed 3 times with aquadest and dried for 1 day at room temperature (27 ° C) (Herlina and Aziz, 2016). Seeds with priming treatment were planted on polybags with size 15 cm x 7.5 cm filled with planting medium in the form of husk: compost (2: 1) with a depth of 1 cm. Watering with 3% NaCl solution is done on day 0 and every seven days, while the control treatment is doused with water (Adillah, 2016). The watering volume is adjusted to the field capacity for growing media. During the period of one week, the seed germination was observed. The germination rate was determined by the seeds that germinate,

$$GP (\%) = \frac{\text{The number of seeds germinated}}{\text{The number of seeds tested}} \times 100 \%$$

marked as by the appearance of radicles. The germination percentage (GP) was calculated by the formula (Wawo, 2008) as follows:

The height of the stem (cm) was measured on a weekly basis. Plants were harvested when they were five weeks old, to measure root length (cm), total fresh weight (g), total dry weight (g) and canopy ratios.

3. Analysis of Chlorophyll and proline content

Total chlorophyll content was measured using spectrophotometric method at 646 nm and 663 nm wavelength (Harborne, 1998). Chlorophyll was extracted in acetone 80%. Meanwhile, leaf proline content was measured using a spectrophotometrically method (Bates, 1973) with pure proline as standard. To prepare proline extract, about 0.5 g leaves was ground in 10 mL sulphosalicylic acid 3%. Filtrate was added with 2 mL acetic acid and 2 mL ninhydrine acid. Sample solution was then extracted with 4 mL toluene and homogenized for 20s. Supernatant absorbance was then measured spectrophotometrically at wavelength of 520 nm.

4. Anatomical slides preparation

The fourth leaf from the bud was sliced across the paraffin cuffing method (GTAC, 2016). The anatomical structure of the leaf is described, then measured by thickness of the mesophyll tissue (μm), the thickness of the upper epidermal layer (μm), the thickness of the lower epidermal layer (μm), the distance between the vascular bundle sheath (μm), and the Kranz anatomy diameter (μm).

The calculation of stomatal density was performed on leaf impression slide. Clear nail polish was applied to the abaxial leaf and left it to dry. The clear sticky tape was placed on top of dried nail polish, removed with tweezers, placed on top of an object glass, dropped by water and covered by a glass cover. The field diameter of microscope view was measured using a micrometer to determine the area of view. The density of stomata (stomata per mm^2) was identified at 10 x 40 microscope magnification. The length of stomata (μm) and stomata width (μm) was also measured.

5. Data analysis

Quantitative data were analyzed using t-test, ANOVA, and followed by DMRT test $\alpha=5\%$.

RESULT AND DISCUSSION

1. Germination and root length

The duration of priming with salicylic acid showed different results between the 0% and 3% salinity treatment (Table 1). At 24 h priming yielded the lowest percentage of germination with 70%, and 60% of 0% and 3% salinity respectively. These results indicate that the 24-h priming duration with salicylic acid may be phytotoxic in sweet corn by decreasing the percentage of germination. Salicylic acid in excess doses may inhibit the expression of alpha-amylase in aleurone cells, an enzyme for food reserves

(starch) degradation in seeds that begin to germinate (Xie *et al.*, 2007).

A priming treatment for 12 and 18 h at 3% salinity can maintain a normal germination percentage (as in 0 h and 0% NaCl treatment), while 24 h decreased it. These results indicate that inhibition of germination on sweet corn can be prevented by a priming treatment using salicylic acid (2 mM) with a duration of 12 h or 18 h. Similar results was shown in cucumber (Rehman *et al.*, 2011) that the administration of salicylic acid may increase the percentage of germination. During seed priming, supply water to seed is controlled, so that the seed moisture at a level below needed for real germination. This level is enough to start many physiological processes related to early germination but prevent the seed to full germination (Jisha *et al.*, 2013; Paparella *et al.*, 2015).

Salinity is known to cause two main effects in plants, namely osmotic stress and phytotoxic ions (Djukri, 2009). Plants are generally able to absorb water and minerals under normal conditions due to the osmotic pressure in the cells is higher than the osmotic pressure of the soil. When subjected to salinity treatment, the osmotic pressure on the soil becomes higher than that of the plant cell. This causes the ability of plants to absorb water was disturbed (Boudsocq and Laurière, 2005). In germinated seeds, water has an important role to stimulate the activation of hydrolytic enzymes that play a role in degrading food reserves. If the ability of water absorption in seeds is disrupted by salinity treatment, then the activation of the enzyme for germination will also be inhibited. This will certainly have a negative impact on the percentage of seed germination. Priming salicylic acid 2 mM with a duration of 12 h or 18 h can reduce the negative impacts above (Table 1).

Table 1 also shows that priming duration has no

Table 1. The effect of priming duration with salicylic acid on germination percentage (%) and root length (cm) on sweet corn seedlings grown under different salinity conditions

Salinity	Priming duration (h)			
	0	12	18	24
	Germination percentage (%)			
0%	90.00 \pm 6.13 ^{aA}	93.30 \pm 6.13 ^{aA}	96.60 \pm 5.46 ^{aA}	70.00 \pm 3.94 ^{bA}
3%	66.65 \pm 3.78 ^{bB}	90.00 \pm 5.10 ^{aA}	93.32 \pm 5.13 ^{aA}	60.00 \pm 4.12 ^{bA}
	Root length (cm)			
0%	31.30 \pm 5.16 ^{aA}	29.62 \pm 4.62 ^{aA}	31.73 \pm 1.31 ^{aA}	29.30 \pm 8.15 ^{aA}
3%	20.50 \pm 3.28 ^{bB}	29.10 \pm 3.50 ^{aA}	28.80 \pm 1.71 ^{aA}	26.53 \pm 3.68 ^{aA}

Remarks: The number followed by the lowercase letter on the same row was not significantly different according to the DMRT test at $\alpha=5\%$. The numbers followed by the same capital letters in the column was not significantly different according to the t test at $\alpha=5\%$.

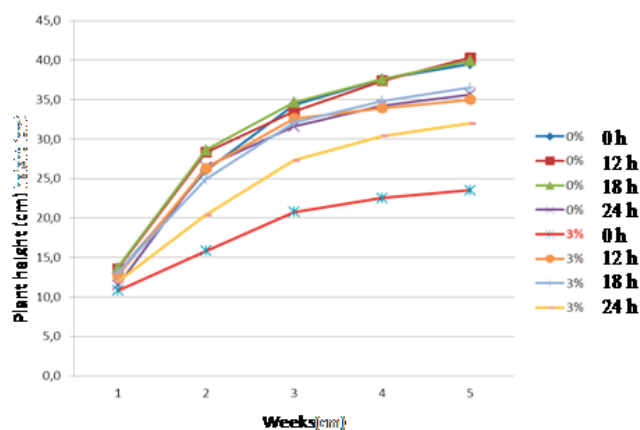


Figure 1. The effect of priming duration with salicylic acid on the plant height of sweet corn seedlings on different salinity

significant effect on root length of sweet corn seedlings at 0% salinity condition. However, plants with 3% salinity without priming treatment had shorter roots than priming treatments of 12 h, 18 h and 24 h. The 3% salinity treatment without priming inhibits root elongation of sweet corn seedlings, which was similar on *Crotalaria juncea* L. (Nugraheni *et al.*, 2003), meanwhile, priming treatment of 12, 18 and 24 h tends to recover the elongation of root.

In addition to inhibiting root growth, salinity was also shown to inhibit the growth of sweet corn stem. From Figure 1, it was seen that the 3% salinity without priming yielded the shortest stem. This also occurred in soybean (Kasanah and Maryani, 2017) and in maize under 120 mM NaCl (Agami, 2013) which the shoot length was back to normal growth as control when seeds were previously primed with 0.1mM salicylic acid. High salt concentration around the roots increases to a threshold level making it harder for the roots to extract water, the rate of shoot growth falls considerably (Batool *et al.*, 2014). The above negative effects can be minimized by salicylic acid,

where under 3% salinity, all priming treatments resulted in higher plants stems compared to that without priming (Figure 1). Salicylic acid is known to play an important role in inhibiting the effects of ionic toxicity due to its ability to compete with Na⁺ and Cl⁻ ions at membrane transport levels (Gunes *et al.*, 2005).

2. Fresh and dry weight

At 0% salinity, there was no significant difference on fresh and dry weights between treatments of priming duration (Table 2). In contrast, when 3 % salinity was applied, there was a decrease in fresh and dry weight compared to control (0 h priming and 0% priming duration) in all treatments, except for 18 h on fresh weight. Priming treatment with salicylic acid for 12 h, 18 h and 24 h increased fresh and dry weight of seedlings at 3% salinity treatment compared with control (0 h).

Salinity treatment decreased fresh and dry weight in *Ceriops tagal* (Ramayani *et al.*, 2012) and maize (Agami, 2013) due to Na⁺ and Cl⁻ ions absorbed into the tissues inhibit metabolism and growth. Reduced

Table 2. The effect of priming duration with salicylic acid on fresh and dry weight (g) on sweet corn seedlings grown under different salinity conditions

Salinity	Priming duration (h)			
	0	12	18	24
Fresh weight (g)				
0%	4.24±0.01 ^{aA}	4.21±0.08 ^{aA}	4.26±0.23 ^{aA}	4.16±0.14 ^{aA}
3%	2.80±0.08 ^{cB}	3.23±0.44 ^{bB}	4.27±0.14 ^{aA}	3.18±0.06 ^{bB}
Dry weight (g)				
0%	0.71±0.09 ^{aA}	0.74±0.07 ^{aA}	0.72±0.05 ^{aA}	0.68±0.05 ^{aA}
3%	0.34±0.03 ^{bB}	0.42±0.10 ^{aB}	0.46±0.05 ^{aB}	0.39±0.02 ^{aB}

Remarks: The number followed by the lowercase letter on the same row was not significantly different according to the DMRT test at $\alpha=5\%$. The numbers followed by the same capital letters in the column was not significantly different according to the t test at $\alpha=5\%$.

Table 3. The effect of priming duration with salicylic acid on total chlorophyll and leaf proline content on sweet corn seedlings grown under different salinity conditions

Salinity	Priming duration (h)			
	0	12	18	24
Total chlorophyll (mg/L)				
0%	6.79±0.09 ^{dA}	11.08±0.03 ^{aA}	9.85±0.06 ^{bA}	9.42±0.75 ^{cA}
3%	3.33±0.11 ^{dB}	12.87±0.07 ^{aA}	8.94±0.09 ^{cA}	10.03±0.06 ^{bA}
Proline content in leaves (µmol g ⁻¹ fresh weight)				
0%	0.64±0.02 ^{cB}	0.77±0.05 ^{bB}	1.14±0.10 ^{aA}	1.17±0.03 ^{aA}
3%	1.08±0.06 ^{bA}	1.11±0.06 ^{bA}	1.28±0.05 ^{aA}	1.30±0.08 ^{aA}

Remarks: The number followed by the lowercase letter on the same row was not significantly different according to the DMRT test at $\alpha=5\%$. The numbers followed by the same capital letters in the column was not significantly different according to the t test at $\alpha=5\%$.

moisture levels in plant tissues due to salt stress will be followed by a decrease in stomatal closure, cell turgor, thus inhibiting the rate of photosynthesis and the low amount of assimilate produced (Pangaribuan, 2001).

High salinity can cause hyperosmotic pressure in plants (Boudsocq and Laurière, 2005). This results in reduced turgor pressure and loss of water from the tissues. Excess Na⁺ ion in the plant tissue will increase the level of membrane leakage (Anthraper and Dubois, 2003). Priming treatment with salicylic acid for 12 h, 18 h and 24 h were able to raise fresh and dry weight of sweet corn seedlings when received 3% NaCl treatment compared to control. Salicylic acid in a dose of 0.1 mM was able to increase the activity of antioxidant enzymes such as catalase and peroxidase that reduce the effects of oxidative stress due to stress (Agami, 2013). In this study application of salicylic acid, as seed soaking in absence or presence of NaCl stress stimulated the growth compared to the control. These positive results were obtained as a result of salicylic acid overcoming the harmful effects of NaCl stress by the induction photosynthetic pigments and proline content (Table 3) which consequently delayed leaf senescence which is induced by salt stress.

3. Total chlorophyll content and proline

At 0% and 3% salinity, the priming duration of 12 h yields the highest total chlorophyll compared with other priming durations (Table 3). Salinity 3% significantly reduced chlorophyll content, when applied without priming. Salinity suppresses the enzyme responsible for the chlorophyll synthesis process (Munns and Tester, 2008). In addition, high salinity will reduce the ability of plants to absorb nutrient minerals such as magnesium (Mg) required by plants for photosynthetic pigment biosynthesis

such as chlorophyll (Anthraper and Dubois, 2003). In this study, the reduction of chlorophyll content was recovered by salicylic acid application, in all priming durations. Under 0% salinity condition, salicylic acid significantly increased chlorophyll content. Salicylic acid in low doses can stimulate CO₂ assimilation in *Brassica juncea* seedlings (Fariduddin, 2003), Rubisco activity in corn (Khodary, 2004), antioxidant enzyme activity in corn (Agami, 2013) and in *Torreyia grandis* (Li *et al.*, 2014) followed by increased chlorophyll content. In excessive doses, salicylic acid increased in ethylene production leading to a decrease in chlorophyll in plants (Arfan *et al.*, 2007).

On a priming duration of 0 h, a 3% salinity treatment increased the accumulation of proline in leaves (Table 2). Priming treatment with salicylic acid 2 mM can increase the level of proline under normal conditions or 3% salinity treatment. The longer the duration of priming, the accumulation of proline increases. In general, in the priming treatment, in the 3% NaCl condition, the proline level was higher than the 0% salinity treatment, although significant results were only in the priming duration of 0 h and 12 h. In the treatment without priming, the proline level increased significantly from 0.64 µmol g⁻¹ to 1.08 µmol g⁻¹ under 3% salinity treatment. In the 18 h and 24 h priming treatments, although there was an increase, there was no significant difference between the proline level when conditions were recorded and the salinity was not recorded.

The same results were reported by Khan *et al.* (2013) on wheat (*Triticum aestivum* L.) treated with salicylic acid 0.5 mM and in *Crotalaria juncea* L. (Djukri, 2009). The accumulation of proline in stressful plants will increase as salicylic acid is known to interact with proline metabolism (Khan *et al.*, 2013).

Table 4. The effect of priming duration with salicylic acid on leaf anatomy and stomatal density on sweet corn seedlings grown under different salinity conditions

Salinity	Priming duration (h)			
	0	12	18	24
Adaxial Epidermal cell thickness (μm)				
0%	19.7 \pm 1.68 ^{aA}	19.7 \pm 1.68 ^{aA}	19.7 \pm 1.68 ^{aA}	19.7 \pm 1.68 ^{aA}
3%	16.89 \pm 0.78 ^{bB}	16.89 \pm 0.78 ^{bB}	16.89 \pm 0.78 ^{bB}	16.89 \pm 0.78 ^{bB}
Abaxial Epidermal cell thickness (μm)				
0%	17.85 \pm 1.07 ^{aA}	17.67 \pm 1.33 ^{aA}	18.11 \pm 1.31 ^{aA}	17.52 \pm 1.22 ^{aA}
3%	15.67 \pm 0.71 ^{bB}	17.85 \pm 1.07 ^{aA}	17.68 \pm 0.89 ^{aA}	17.96 \pm 1.09 ^{aA}
Mesophyll cell thickness (μm)				
0%	68.78 \pm 4.65 ^{aA}	69.36 \pm 5.69 ^{aA}	67.06 \pm 4.27 ^{aA}	66.89 \pm 7.13 ^{aA}
3%	52.16 \pm 6.64 ^{bB}	67.39 \pm 4.94 ^{aA}	68.42 \pm 4.71 ^{aA}	66.36 \pm 6.44 ^{aA}
Leaf thickness (μm)				
0%	106.33 \pm 4.84 ^{aA}	107.53 \pm 5.68 ^{aA}	106.18 \pm 4.45 ^{aA}	104.48 \pm 6.28 ^{aA}
3%	84.72 \pm 7.31 ^{bB}	104.88 \pm 6.23 ^{aA}	105.43 \pm 4.70 ^{aA}	103.31 \pm 6.31 ^{aA}
Distance between vascular sheets (μm)				
0%	40.61 \pm 3.05 ^{aA}	43.5 \pm 4.02 ^{aA}	43.24 \pm 4.59 ^{aA}	43.65 \pm 5.13 ^{aA}
3%	41.65 \pm 3.64 ^{aA}	42.25 \pm 4.86 ^{aA}	42.19 \pm 4.25 ^{aA}	43.37 \pm 4.82 ^{aA}
Diameter of Kranz anatomy (μm)				
0%	56.32 \pm 4.97 ^{aA}	58.83 \pm 6.39 ^{aA}	56.14 \pm 6.28 ^{aA}	56.59 \pm 9.34 ^{aA}
3%	52.87 \pm 5.22 ^{bA}	57.31 \pm 4.86 ^{abA}	58.76 \pm 4.98 ^{aA}	55.89 \pm 5.36 ^{abA}
Stomata length (μm)				
0%	26.62 \pm 1.33 ^{aA}	25.87 \pm 0.88 ^{aA}	26.41 \pm 1.37 ^{aA}	25.78 \pm 1.40 ^{aA}
3%	25.36 \pm 0.79 ^{aA}	26.84 \pm 1.40 ^{aA}	26.12 \pm 1.69 ^{aA}	26.47 \pm 1.56 ^{aA}
Stomata width (μm)				
0%	20.23 \pm 1.13 ^{aA}	20.26 \pm 1.55 ^{aA}	20.17 \pm 1.41 ^{aA}	20.34 \pm 1.47 ^{aA}
3%	18.37 \pm 0.66 ^{bB}	20.13 \pm 1.01 ^{aA}	20.64 \pm 1.61 ^{aA}	19.98 \pm 0.94 ^{abA}
Stomatal density (number of stomata per mm^2)				
0%	45.88 \pm 5.17 ^{aA}	44.00 \pm 5.13 ^{aA}	45.25 \pm 7.73 ^{aA}	43.99 \pm 5.93 ^{aA}
3%	38.34 \pm 4.64 ^{bB}	44.62 \pm 6.92 ^{aA}	43.37 \pm 4.64 ^{abA}	44.52 \pm 6.92 ^{aA}

Remarks: The number followed by the lowercase letter on the same row was not significantly different according to the DMRT test at $\alpha=5\%$. The numbers followed by the same capital letters in the column was not significantly different according to the t test at $\alpha=5\%$.

4. Leaf anatomical structure

The duration of priming with salicylic acid did not affect all anatomical characters of sweet corn seedling leaves at 0% salinity condition (Table 4). In contrast, the 3% salinity treatment showed the increasing thickness of the upper and lower epidermal, mesophyll, leaves and Kranz anatomy as well as stomata width at the priming, but the vascular intercellular distance and stomatal length did not differ significantly between all treatments of priming duration. Similar results were also reported in maize plants treated with salinity and salicylic acid application through roots (Carcamo *et al.*, 2012). Salinity negatively affects the anatomical structure of maize leaves so that the size is smaller, but the application of salicylic acid in the growing medium

will prevent the change of mesophyll, epidermal, anatomic, and xylem tissue.

Anatomical changes are one of the responses of plants when under stress to maintain water efficiency and carbon uptake (Hernandez, 2001). Figure 2 shows that sweet corn seedlings with 3% salinity treatment without priming (E) have reduced leaf thickness, and have bulliform cells and stomata of smaller size than other treatments. This reduction is not caused by a reduction in the number of mesophyll layers or epidermis, but rather because the cell size changes from the tissue become smaller (Table 4). The decrease in mesophyll thickness of the NaCl stressed leaves may arise from a reduction in mesophyll cells and the decrease in lamina thickness can also

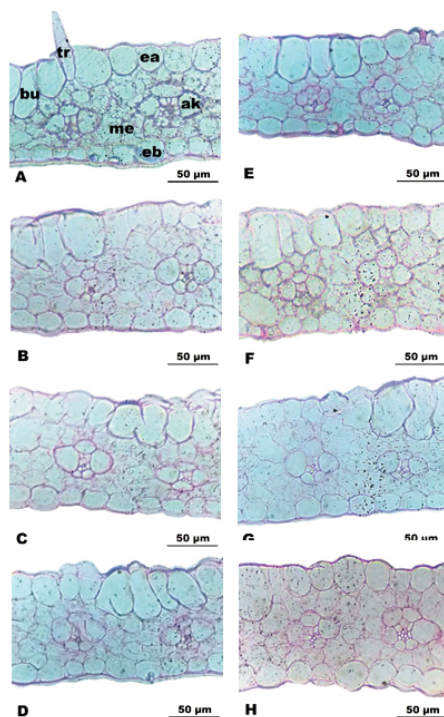


Figure 2. Cross section of sweet corn (*Zea mays* L.) leaf with salinity treatment 0% (left) and 3% (right, and duration priming 0 h (A, E), 12 h (B, F), 18 h (C, G) and 24 h (D, H). ea = adaxial epidermis; eb = abaxial epidermis; me = mesophyll; ak = Kranz anatomy; bu = bulliform cells; tr = trichome

be related to smaller volumes of mesophyll cells in salinity stressed leaves (Agami, 2013). The reduced leaf thickness indicates that the growth of leaf cells is hampered by salinity treatment. Cell growth itself is closely related to the osmotic balance in tissues. If the osmotic balance is disrupted, the rate of cell division and elongation will also decrease (Bastias *et al.*, 2004).

The inhibition of leaf growth may also be associated with the toxicity of Na^+ and Cl^- ions that are accumulated by plants to increase the osmotic value of the tissues (Munns and Tester, 2008). Salicylic acid is known to have an important role to inhibit the removal of certain ions such as Na^+ and Cl^- at the transport level of the membrane (Gunes *et al.*, 2005).

5. Stomatal density

Duration of priming with salicylic acid had no effect on stomatal density on sweet corn seedlings at 0% salinity (Table 4). In contrast, in the 3% salinity treatment, there was a significant difference between stomata density of sweet corn seedlings treated without priming with priming treatment of 12 h and 24 h. At different salinity levels, the density of stomata only differed significantly in treatment without priming. Meanwhile, in the treatment of priming duration 12 h, 18 h and 24 h, salinity level did not seem to have real effect. Similar results were found in tomato

plants where stomata density was reduced after was given salt stress (Romero-Aranda, 2001). These results may be related to the disruption of growth of stomatal cells in the leaves due to osmotic disturbances as well as toxicity of Na^+ and Cl^- ions (Munns and Tester, 2008). Negative effects can be prevented by the administration of salicylic acid that is able to reduce ionic phytotoxicity due to the excessive entry of Na^+ and Cl^- ions in plant tissues (Gunes *et al.*, 2005).

CONCLUSIONS

Without priming, salinity 3% significantly decreased the percentage of germination, root length, plant height, fresh and dry weight, chlorophyll content, the thickness of adaxial epidermis, mesophyll and leaf, stomatal density and width, but increased proline content. Priming seeds with salicylic acid for 12 and 18 h, significantly maintained the germination percentage, while 24 h priming tended to inhibit germination both in 0 % and 3% salinity. Priming with salicylic acid for 12, 18 or 24 h recovered the root and stem growth, increased fresh and dry weight, chlorophyll and proline content as well as maintained the leaf anatomical structure from the 3 % NaCl effect. The optimal duration of priming with salicylic acid on sweet corn (*Zea mays* L.) with salinity

treatment was 18 h.

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