# Respons of Giberellic Acid and Salicylic Acid Under Salt Stress on Roselle (*Hibiscus sabdarifa* L.)

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## Abstract

Salinity in the soil have a devastating effect on plant metabolism, disrupting cellular homeostasis and uncoupling major physiological and biochemical processes. This research was conducted in a greenhouse of Agriculture Faculty, North Sumatra University, Medan, from February until June 2014. The research used completely randomized design with three factors. The first factor was giberellic acid were giberellic acid (5 mg $L^{-1}$) and without giberellic acid. The second factor was salicylic acid were 0 mM, 0,5 mM and 1 mM. The third factor was salinity were 0 $dsm^{-1}$ and 4-5 $dsm^{-1}$. The interaction of giberellic acid and salicylic acid indecate significant effect on parameter such as chlorophyll a and b and betacarotene. Interaction between giberellic acid and salt stress indicated significant effect on chlorophyll a and b and betacarotene. Interaction between salicylic acid and salt stress indicated significant effect on growth and production such as chlorophyll a and b and betacarotene. Interaction three factors of giberellic acid, salicylic acid and salt stress indicated significant effect on chlorophyll a and b and betacarotene. Thus, evaluate the respons of giberellic acid and salicylic acid under salt stress on roselle (*Hibiscus sabdariffa* L) helped in the tolerance of plants to salt stress.

***Key words*** : salt stress, giberellic acid, salicylic acid, *Hibiscus sabdariffa*.

## Abstrak

Salinitas pada tanah memiliki dampak buruk pada metabolisme tanaman, mengganggu homeostasis seluler dan proses fisiologis dan biokimia yang tidak terkoordinasi. Penelitian ini dilakukan di rumah kaca Fakultas Pertanian, Universitas Sumatera Utara, Medan, mulai Februari sampai Juni 2014. Penelitian ini menggunakan rancangan acak lengkap dengan tiga faktor. Faktor pertama adalah giberellin terdiri dari giberellin (5 mg $L^{-1}$) dan tanpa giberellin (0 mg $L^{-1}$). Faktor kedua adalah asam salisilat terdiri dari 0 mM, 0,5 mM dan 1 mM. Faktor ketiga adalah salinitas terdiri dari 0 $dsm^{-1}$ dan 4-5 $dsm^{-1}$. Interaksi antara giberellin dan asam salisilat berpengaruh sangat nyata terhadap peubah amatan yaitu klorofil a dan b dan betakaroten. Interaksi antara giberellin dan salinitas menunjukkan pengaruh nyata terhadap peubah amatan klorofil a dan b dan betakaroten. Interaksi antara asam salisilat dan salinitas menunjukkan pengaruh nyata terhadap peubah amatan klorofil a dan b dan betakaroten. Interaksi tiga faktor antara giberellin, asam salisilat dan salinitas menunjukkan pengaruh nyata terhadap klorofil a dan b dan betakaroten. Jadi, evaluasi terhadap respon giberellin dan asam salisilat di bawah tekanan garam pada tanaman rosela (*Hibiscus sabdariffa* L.) membantu toleransi tanaman terhadap salinitas.

***Kata kunci*** : cekaman garam, giberellin, asam salisilat, *Hibiscus sabdariffa*.

## Introduction

Roselle (*Hibiscus sabdariffa*) is mostly planted in India, Africa, Mexico and tropical zone and hasa special position in traditional medicine. It is used for controlling blood pressure in Senegal. Also, it has been reported that it is sexual stimulator, appetizer, restorative, cathartic, cancer-protective, anti-cough and refrigerant (Lin *et al*. 2007; Chen *et al*. 2002; Morton. 1987). Previous studies had significantly shown that roselle seeds contained high amounts of protein, dietary fiber, and minerals such as phosphorus, magnesium and calcium (Ismail *et al*. 2008).

High salt concentration in the soil or in the irrigation water can also have a devastating effect on plant metabolism, disrupting cellular homeostasis and uncoupling major physiological and biochemical processes. Biochemical and molecular studies of salt stress responses in plants have revealed significant increases of reactive oxygen species(ROS), including singlet oxygen ($^1O_2$), superoxide ($O^{2-}$), hydroxyl radical (OH•) and hydrogen peroxide ($H_2O_2$) (Tanou *et al*. 2009; Ahmad *et al*. 2010; Ahmad *and* Umar 2011). However, the effect of salt stress on plants depends on the concentration and time of exposure of salt, plant genotypes and environmental factors.

In order to alleviate deleterious effects of salinity, different types of phytohormones have been used. Among them, $GA_3$ have been the main focus of some plant scientists. Innumerable works have confirmed the potential of $GA_3$to synergistically improve crop performance under normal conditions. In recent decades, light has been thrown on the influence of $GA_3$ during salt stress (Kaya *et al*. 2009). The application of $GA_3$ reduced the inhibitory effect of NaCl on growth attributes and photosynthetic pigments in *Hibiscus sabdariffa* by inducing the enzyme activity and enhancing RWC and thus $GA_3$ helped in the tolerance of plants to salt stress (Ali *et al*. 2011) .

In the process of germination, Gibberellic Acid ($GA_3$) is the most important growth regulator, which breaks seed dormancy, promotes germination, internodal length, hypocotyl growth and cell division in cambial zone and increases the size of leaves. GA stimulates hydrolytic enzymes that are needed for the degradation of the cells surrounding the radicle and thus speeds germination by promoting seedling elongation growth of cereal seeds (Rood *et al*. 1990).

Protective SA action includes the development of antistress programs and acceleration of normalization of growth processes after removal of stress factors (Sakhabutdinova *et al*. 2003). Some studies demonstrate that application of SA (0.5 mM) may promote the formation of ROS in the photosynthetic tissues and increase oxidative damage during salt and osmotic stresses (Barba-Espin *et al*. 2011). Thus, evaluate the respons of giberellic acid and salicylic acid .) under salt stress on roselle (*Hibiscus sabdariffa* L) helped in the tolerance of plants to salt stress.

## Materials and Methods

In order to study the effects of giberellic acid and salicylic acid on growth of roselle (*Hibiscus sabdariffa* L. under salt stress) an experiment was conducted in a greenhouse of Agricuture Faculty, North Sumatra University, Medan, from February until June 2014. The experiment was a completely randomized design with three factors and with three replications. The first factor was giberellic acid were giberellic acid (5 mg $L^{-1}$) and without giberellic acid. The second factor was salicylic acid were 0 mM, 0,5 mM and 1 mM. The third factor was salinity were 0 $dsm^{-1}$ and 4-5 $dsm^{-1}$, then gained 36 treatment combinations. The number of plants percombination was entirely as much as 3 number of plants 108 plants. If the effect of different treatments on the real variance, then tested further by Duncan's multiplerange test.

**Land preparation**. Land located in the green house area cleaned. Size of 10 kg polybags filled withtop soil.

**Seed preparation**. The seeds are used in this study comes from Darmaga, Bogor, West Java. The varieties used are sabdariffa. The seeds were disinfected by alcohol 70% (for 10 seconds), sodium hypochlorite 10% (for 60 seconds) and benomyl 2:1000 (for one

minute) (Seghatoleslami, 2010). Afterwards ,the seeds were rinsed with distilled water twice.

**Gibberellic acid treatment.** Seeds were soaked in 500 ml of distilled water for 12 hours (without $GA_3$ treatment), and the seeds were soaked in 500 ml of a solution of$GA_3$ treatment for 12 hours and then redried to original weight with forced air under shade (Sundstrom *et al.*, 1987).

**Salinity treatment**. To apply the salinity treatment, given any treatment by the EC salinity levels at 0 and 4-5 ds/m NaCl in each polybag according to the treatment, then at the level of salinity measurements done 3 times a week using DHL meters.

**Planting**. The seeds were germinated directly on the polybag according to each treatment.

**Salicylic acid treatment**. Salicylic acid were given each treatment in polybag according to the privilege level 0, 50 and 100 mg $L^{-1}$(Hasanuzzaman *et al*. 2013). Given as much as 2 times. The application by using handsprayer at weeks 2 and 4 after planting.

**Parameters observed:**

**Chlorophyll a and Chlorophyll b.** Analysis of chlorophyll a and chlorophyll b was conducted in Laboratorium Centra, Faculty of Agriculture, University of Sumatra Utara, Medan.

**Harvest Time**. Observations harvesting is done by calculating the age of the plant started from seed planting until the plants are ready for harvest after physiologically mature.

**Beta-carotene Content**. Analysis of beta—carotene content performed at the Laboratorium Pangan, AgricultureFaculty, University ofNorth Sumatra, Medan.

## Results and discussion

### 1. Effects of Interaction Giberellin and Salt StressOn Growth Of Roselle

Giberellin on salinity stress conditions showed significant effect on the growth and production variables such as chlorophyll a and b, beta-carotene. Giberellin markedly better effect on salt stress. Giberellic acid (5 mg $L^{-1}$) under salt stress on roselle maintain germination success although chlorophyll a and b decreased and was not formed flowers (generative phase) to be harvested. This is because gibberellin given during seed soaking focused to sustain the germination success, so do not increase levels of chlorophyll a and b. In this study the majority of the roselle are getting treatment salinity stress thus lowering the average thick cuticle, but when viewed from the flats of plants that survived produced an increase in the thickest of the thick cuticle contained in roselle that are subjected to salinity stress. Magome *et al*. (2004) that gibberellic acid (GA) promotes germination, stem/hypocotyl elongation, and leaf expansion during seedling development in arabidopsis. Significant cuticular thickness increase was observed under high salinity. Salinity not only affects leaf morphology and transpiration rate but also leads to reduction of total chlorophyll content as the salt concentration increases.

Ali *et al* (2011), the application of GA 3 reduced the inhibitory effect of NaCl on growth attributes and photosynthetic pigments by inducing the enzymes CA activity and enhancing RWC. Therefore, it is concluded that GA 3 might help in the tolerance of plants to salt stress. It is also supported by the statement of Davies (1995), which states that the effect of gibberellin onstem growth, $GA_1$ causes hyperelongation of stems by stimulating both cell division and cell elongation. This produces tall, as opposed to dwarf plants. Bolting in long day plants, GAs cause stem elongation in response to long days.Induction of seed germination, GAs can cause seed germination in some seeds that normally require cold (stratification) or light to induce germination.Enzyme production during germination, GA stimulates the production of numerous enzymes, notably a-amylase, in germinating cereal grains. Gibberellin regulates the growth of the fruit, which is caused by the exogenous application of several pieces (eg. wine).

### 2. Effects of Interaction Salicylic Acid and Salt Stresson Growth of Roselle

The interaction salicylic acid andshowed the effect of lowering the salt stress thick cuticle, chlorophyll a and b, that is the provision of salicylic acid (0.5 mM) under salt stress on plants decrease chlorophyll a and chlorophyll b. Although the roselle unable to survive in salt stress to the generative phase. This is because in this

reseach the majority of the roselle are getting salt stress experienced death so that lowering the average chlorophyll a and b, but when viewed from the flats of plants that can survive the resulting increase the chlorophyll a and b, if viewed from the flats of plants that survived produced an increase in chlorophyll a and b.

El Tayeb (2005) found that SA application to barley induced a pre-adaptive response to salt stress, enhanced the synthesis of Chl a, Chl b, and Car, and maintained membrane integrity, leading to improvement of plant growth.Protective SA action includes the development of antistress programs and acceleration of normalization of growth processes after removal of stress factors (Sakhabutdinova *et al*. 2003 )

Table. Average Chlorophyll A, Chlorophyll B, Beta-carotene, Harvest Time and Beta-carotene on Interaction of giberellic Acid, Salicylic Acid and Salinity Stress

| Treatment | Chlorophyll A | Chlorophyll B | Harvest Time | Beta-carotene |
|---|---|---|---|---|
| **Interaction Giberellic Acid and Salicylic Acid** | | | | |
| A0B0 | 1462.500 d | 586.500 f | 45.5 | 1.777 c |
| A0B1 | 1478.500 c | 810.000 c | 45.5 | 1.852 b |
| A0B2 | 1010.500 f | 626.000 d | 44 | 1.607 e |
| A1B0 | 1759.000 b | 1188.000 b | 44 | 1.692 d |
| A1B1 | 2265.000 a | 1337.500 a | 43.667 | 1.937 a |
| A1B2 | 1241.500 e | 608.500 e | 42.833 | 1.691 d |
| **Interaction Giberellic Acid and Salt Stress** | | | | |
| A0C0 | 2634.333 a | 1348.333 a | 90 | 3.491 b |
| A0C1 | - | - | - | - |
| A1C0 | 2350.667 b | 1265.333 b | 87 | 3.547 a |
| A1C1 | 1159.667 c | 824.000 c | - | - |
| **Interaction Salicylic Acid and Salt Stress** | | | | |
| B0C0 | 2555.500 b | 1205.000 c | 89.5 | 3.469 b |
| B0C1 | 666.000 e | 569.500 e | - | - |
| B1C0 | 2670.000 a | 1481.000 a | 89.167 | 3.789 a |
| B1C1 | 1073.500 d | 666.500 d | - | - |
| B2C0 | 2252.000 c | 1234.500 b | 86.833 | 3.298 c |
| B2C1 | - | - | - | - |
| **Interaction Giberellic Acid, Salicylic Acid and Salt Stress** | | | | |
| A0B0C0 | 2925.000 b | 1173.000 g | 91 | 3.554 c |
| A0B0C1 | - | - | - | - |
| A0B1C0 | 2957.000 a | 1620.000 a | 91 | 3.704 b |
| A0B1C1 | - | - | - | - |
| A0B2C0 | 2021.000 g | 1252.000 d | 88 | 3.214 f |
| A0B2C1 | - | - | - | - |
| A1B0C0 | 2186.000 e | 1237.000 e | 88 | 3.384 d |
| A1B0C1 | 1332.000 h | 1139.000 h | - | - |
| A1B1C0 | 2383.000 d | 1342.000 b | 87.333 | 3.874 a |
| A1B1C1 | 2147.000 f | 1333.000 c | - | - |
| A1B2C0 | 2483.000 c | 1217.000 f | 85.667 | 3.381 e |
| A1B2C1 | - | - | - | - |

Means values in a column and row followed by unlike letter (s) are significantly different at 5% level using DMRT (Duncan Multiple Range Test).

However, some studies demonstrate that application of SA (0.5 mM) may promote the formation of ROS in the photosynthetic tissues and increase oxidativedamage during salt and osmotic stresses (Hasanuzzaman *et al*. 2013). For instance, Barba-Espin *et al*. ( 2011 ) studied the effect of SA treatment on the response of P. sativum plants to salinity. NaCl-induced damage to leaves was increased by SA, which was correlated with a reduction in plant growth.

**3. Interaction Effect of ThreeFactorsgiberellic Acid, Salicylic Acid and Salinity Stress on Growth of Rosella**

In this research the interaction of three factors giberellic acid, salicylic acid and salt stress increase cuticle thickness, chlorophyll a and b. Interaction giberellic acid (5 mg L-1) and salicylic acid (0.5 mM) in salt stress increase chlorophyll a and b when compared without giving gibberellic acid and salicylic acid.

Salinity not only affects leaf morphology and transpiration rate but also leads to reduction of total chlorophyll content as the salt concentration increases. According to Davies (1995), the effect of gibberellin onstem growth, $GA_1$ causes hyperelongation of stems by stimulating both cell division and cell elongation. This produces tall, as opposed to dwarf plants. According to Ali *et al* (2011), the application of $GA_3$ reduced the inhibitory effect of NaCl on growth attributes and photosynthetic pigments in Hibiscus sabdariffa by inducing the enzyme activity and enhancing RWC and thus $GA_3$ helped in the tolerance of plants to salt stress. ( El Tayeb (2005), found that SA application to barley induced a pre-adaptive response to salt stress, enhanced the synthesis of Chl a, Chl band Car, and maintained membrane integrity, leading to improvement of plant growth. Erdal *et al.* (2011) investigated the effects of foliar-application of SA on salt sensitivity of *T. aestivum*. They observed that salt-induced deleterious effects in wheat seedlings were significantly alleviated by the SA treatment. SA can be used as a signaling molecule to investigate plant defense against abiotic stress. SA can be used as a signal molecule to investigate plant defense to abiotic stress. After the applications of SA, increasing tolerance of wheat seedlings to salt stress may be related to increasesin antioxidative enzyme activity. Exogenous SA treatment significantly increased the fresh and dry weights in both root and shoots of wheat plants under salt stress. In parallel to increasing antioxidant activity, SA treatment decreased $H_2O_2$ content when compared to plants growing under salt stress without SA. In *Brassica juncea*, Yusuf *et al*. (2012) reported that SA enhanced the level of antioxidant system (SOD, CAT and POX) both under stress and stress-free conditions.

**Conclusion**

Giberellic acid (5 mg L-1) under salt stress (4-5 dsm-1) on growth of roselle help maintain germination success although chlorophyll a and b decreased and was not formed flowers (generative phase) to be harvested.

Salicylic acid (0.5 mM) under salt stress (4-5 dsm-1) on growth of roselle has not been able to increase the chlorophyll a and chlorophyll b.

Interaction giberellic acid (5 mg L-1) and salicylic acid (0.5 mM) on salt stress (4-5 dsm-1) increase chlorophyll a and b when compared without giving gibberellic acid and salicylic acid.

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