Effects of Plant Hormones Interaction Under Salt Stress on Growth of Roselle (*Hibiscus Sabdarifa* L.)

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ABSTRACT

Salinity has reached a level of 19.5% of all irrigated land agriculture worldwide. One of the most important abiotic factors limiting plant germination and early seedling stages is water stress brought about by salinity. The aim of this study was to evaluate effects of plant hormones interaction under salt stress on growth of roselle. This study was conducted in a greenhouse, University of Sumatra Utara, Medan. The study used completely randomized design with three factors. The first factor was GA_3 were GA_3 (5 mg L^{-1}) and no GA_3. The second factor was SA were 0 mM, 0,5mM and 1 mM. The third factor was salinity were 0 dsm^{-1} and 4-5 dsm^{-1}. The interaction of GA3 and Salt Stress indicated significant effect on percentage of germination and cuticle thickness. The interaction of three factors of GA_3, SA and salt stress indicated significant effect on thick cuticle where GA_{3+}no SA+no salinity treatment was the highest then followed no GA3+0,5mMSA+no salinity treatment and no GA3 + 1 mMSA+no salinity was the lowest. The application of GA_3 and SA helped in the tolerance of plants to salt stress. Thus, plant hormones have been found effective in mitigating the salt induced damage in plant.

Keywords *:GA_3, Salicylic Acid, Roselle.*

Introduction

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). Moosavi *et al*. (2013) reported that salt stress adversely affected the germination rate, germination percentage, seedling length, shoot length and seed viability index of roselle (*Hibiscus sabdariffa*). In Indonesia, it is estimated to have 40-43 million ha and 13.2 million ha problematic of the land affected by salinity (Departemen Pekerjaan Umum. 1997).

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) (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.
Attempts to improve yield under stress conditions by plant improvement have been largely unsuccessful, primarilydue to the multigenic origin of the adaptive responses.

Therefore, a well focused approach combining the molecular, physiological, biochemical and metabolic aspects of salt tolerance is essential to develop salt tolerant crop varieties. Exploring suitable ameliorants or stress alleviant is one of the tasks of plant biologists. In recent decades exogenous protectant such as osmoprotectants (proline, glycinebetaine, trehalose, etc.), plant hormone (gibberellic acids, jasmonic acids, brassinosterioids, salicylic acid, etc.), antioxidants (ascorbic acid, glutathione, tocopherol, etc.), signaling molecules (nitric oxide, hydrogen peroxide, etc.), polyamines (spermidine, spermine, putrescine), trace elements (selenium, silicon, etc.) have been found effective in mitigating the salt induced damage in plant (Azzedine *et al*. 2011; Yusuf *et al*. 2012). These protectants showed the capacity to enhance the plant's growth, yield as well as stress tolerance under salinity. 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 ofgermination, 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. 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).

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. However, the influence of SA on antioxidant system was more pronounced under stressful condition, therefore, suggesting that the elevated level of antioxidant system might be responsible for increased tolerance of *B. Juncea* plants to NaCl 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.

The aim of this study was to evaluate effects of plant hormones interaction under salt stress on growth of roselle (*Hibiscus sabdariffa*L.).

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, University of Sumatera Utara, 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, 0,5mM 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 multiple range test.

Land preparation. Land located in the greenhouse area cleaned. Size of 10 kg polybags filled with top 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.

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**:**

Percentage of Germination**.** Germination percentage is calculated up to 7 days. Germination (DB) is calculated based on the percentage of normal seedling compared to the number of seeds that germinated. Germination was calculated by the following formula (Copeland and McDonald, 2004): DB=(Σ normal seedling)/(Σ seeds were germinated) x100%.

Specific Leaf Area. Specific leaf area is the leaf are aper unit leaf dry weight. SLA measurements performed on stage before harvest. SLA value is calculated as the ratio between leaf area (L) and the weight of dry material (Leaf DM); so, SLA=L : Leaf DM (Suwarto. 2013).

Number of Stomata. Observations carried out by using the number of stomata microscop at 6 WAP and 10 WAP in Disease Laboratory, Faculty of Agriculture, University of Sumatra Utara, Medan.

Cuticle thickness. Observations carried out by using a thick cuticle compound microscop Carl Zeiss Primo Star, at the age of 9 Week after planting and 10 week after planting at Integrated Laboratory, Faculty of Medicine, University of Sumatra Utara, Medan.

Results and Discussion

1. Effects of Interaction Giberellin and Salicylic Acid On Growth Of Roselle

Giberellin and salicylic acid showed significant effect on the growth and production variables such as thick cuticle (Table). The differential effects of responses to Gibberellin Acid and Salicylic Acid on growth of roselle were clearly observed on cuticle thickness effect on their growth. Giberellin (5 mg L^{-1}) and Salicylic Acid on growth of roselle increased cuticle thickness (generative phase) to be harvested. A comprehensive survey of SA levels in the leaves and reproductive structures of agronomicaly important species and in foods derived from plants has confirmed the ubiquitous distribution of this compound in plants. Most people learn of the effects of Salicylic Acid on flowering. However, some indications of the mechanisms by which SA inhibits ethylene biosynthesis. It is unlikely that the endogenous levels of SA present in the tissue. In this study SA was not formed flowers (generative phase) to be harvested. In this study the majority of the roselleare getting treatment salinity stress thus lowering the average harvest time and harvest index, but when viewed from the flats of plants that survived produced an increase in the flower of the harvest time and harvest index. Davies (1995),

All growing, differentiated tissues are potential sites of GA biosytesis. There is incontrovertible evidence that developing fruits and seeds are sites of GA biosynthesis.

Gibberellic acid (GA) promotes germination, stem/hypocotyl elongation, and leaf expansion during seedling development in arabidopsis.

This observation suggest environmental conditions during roselle development can influence the eventual hormone responsiveness of aleurone. Of all the plant hormones that have been applied to plants under strictly noninductive condition, only GA_3 have been shown to effectively cause flower formation in a wide variety of species. In views of the large number of species in which exogenous GA_3 cause vegetative plants to flower under noninductive conditions, it is logical to conclude that GA_3 has a critical role in the regulation of flower formation. As yet, however , such a role cannot be defined. This is partly due to the analytical difficulties encountered when investigating GA physiology.

2. Effects of Interaction Giberellin and Salt Stress On Growth Of Roselle

Giberellin on salinity stress conditions showed significant effect on the growth and production variables such as thick cuticle, germination percentage (Table). Giberellin markedly better effect on salt stress. Giberellin (5 mg L^{-1}) under salt stress on roselle maintain germination percentage success although cuticle thickness 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.

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. Gibberellic acid (GA) promotes germination, stem/hypocotyl elongation, and leaf expansion during seedling development in arabidopsis. Unfortunately, this study different from the results of research Hajibagheri *et al*. (1983), salinity leads alteration of leaves morphology such as leaf size, area and thickness. Various studies have reported decrease in leaves size and increase in cuticle thickness of leaves. 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.The growth regulator pretreatments mostly increased the stem diameter, epidermis cell width, cortex zone thickness, vascular bundle width, xylem width, trachea diameter and phloem width in comparison with the control seedlings grown in saline medium. In addition, they generally decreased the cuticle thickness, epidermis cell length and cambium thickness. These anatomical changes indicate that salt stress on the stems of radish may be reduced by growth regulators. Actually, in their work with radish, observed that the growth regulators alleviated the salt induced inhibition on seed germination, percentage of hypocotyl and water uptake. Growth regulators such as GA_3 and 24-epibrassinolide (EBR) may not require an increase in cuticle thickness or a reduction in stem diameter, epidermis cell size and other anatomical parameters studied by alleviating the growth inhibitive effect of ABA increase induced by salinity. It is also supported by the statement of Davies (1995), which states that the effect of gibberellin on stem 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).

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3. Effects of Interaction Salicylic Acid and Salt Stress on Growth of Roselle

Various types of soil can be planted roselle, particularly structure in structured light and well drained. Rosella tolerant of acid soils and alkaline, but not suitable to be planted in saline soils or high salinity. Results of research Jalilimarandi *et al.* (2011), the usage of salicylic acid resulted in the increase of cuticle thickness of the leaf and 2 mM density of salicylic acid had the most effect in increasing cuticle thickness of the leaf.

In this research resulted in the reduction of cuticle thickness, this is because salt stress causes death in plants, thereby reducing the average yield on the plants. The interaction salicylic acid and showed the effect of lowering the salt stress thick cuticle, that is the provision of salicylic acid (0.5 mM) under salt stress on plants decrease thick cuticle. 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 thick, but when viewed from the flats of plants that can survive the resulting increase in the thickest thick cuticle contained in the roselle plant treated salt stress.

Table Average Percentage of Germination, Specific Leaf Area, Number of Stomata and Cuticle Thickness on Interaction of giberellic Acid, Salicylic Acid and Salinity Stress

Treatment	Percentage of germination	Specific Leaf Area	Number of Stomata		Cuticle thickness	
			6 MST	**10 MST**	**9 MST**	**10 MST**
Interaction of Giberellic Acidand Salicylic Acid						
A0B0		18.651	59.667	70.833	29,345 d	33,675 d
A0B1		19.786	65.667	75.167	32,885 c	31,570 e
A0B2		16.825	77.833	74.667	28,905 e	37,435 c
A1B0		38.971	107.5	131.167	66,530 a	69,960 b
A1B1		33.883	102.667	108.333	58,850 b	70,555 a
A1B2		27.078	109	96.5	21,115 f	23,780 f
Interaction of Giberellic Acid and Salt Stress						
A0C0	100,000 a	36.842	105.667	147.111	60,757 a	68,453 a
A0C1	88,890 b	-	29.778	-	-	-
A1C0	100,000 a	39.481	136.556	150.778	42,643 c	51,897 c
A1C1	100,000 a	27.141	76.222	73.222	55,020 b	57,633 b
Interaction of Salicylic Acid and Salt Stress						
B0C0		36.913	111.667	140.833	50,250 b	57,085 c
B0C1		20.71	55.5	61.167	45,625 d	46,550 d
B1C0		40.399	125	153.167	54,830 a	62,225 a

B1C1	13.271	43.333	30.333	36,905 e	39,900 e
B2C0	37.172	126.667	152.833	50,020 c	61,215 b
B2C1	6.731	60.167	18.333	-	-
Interaction of Giberellic Acid, Salicylic Acid and Salt Stress					
A0B0C0	37.302	99.333	141.667	58,690d	67,350 d
A0B0C1	-	20	-	-	-
A0B1C0	39.573	107.333	150.333	65,770c	63,140 e
A0B1C1	-	24	-	-	-
A0B2C0	33.65	110.333	149.333	57,810e	74,870 c
A0B2C1	-	45.333	-	-	-
A1B0C0	36.523	124	140	41,810 h	46,820 h
A1B0C1	41.419	91	122.333	91,250a	93,100 a
A1B1C0	41.225	142.667	156	43,890 f	61,310 f
A1B1C1	26.541	62.667	60.667	73,810b	79,800 b
A1B2C0	40.694	143	156.333	42,230 g	47,560 g
A1B2C1	13.462	75	36.667	-	-

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

Being thick of cuticle, is an important factor in maintaining the relative water content of leaves, And varieties that, they showed a greater thickness of the cuticle of leaves, in the dry conditions, retains a higher relative water content in their leaves and are more resistant against drought.The increase of drought stress results in stomata density (the numbers of stomata in unite of leaf area). The number of stomata in Rashe cv. is more than BidaneSefid cv. Likely one cause of increased stomatal density, during drought stress is getting smaller cell size that causes will be placed more stomata per unit area. By decreasing the number of stomata in unit area and length of stomata, plant's resistance to dehydration becomes more (Hussain *et al*. 2008).

The thickness of the leaf cuticle, directly correlates with drought tolerance and increases with increasing water stress and can be used as a marker for the identification of resistant varieties. However, 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 (Hasanuzzaman *et al*. 2013).

4. Interaction Effect of Three Factors giberellic 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. Interaction giberellic acid (5 mg L-1) and salicylic acid (0.5 mM) in salt stress increase cuticle thickness when compared without giving gibberellic acid and salicylic acid.

Salinity leads alteration of leaves morphology such as thickness. Various studies have reported decrease in leaves size and increase in cuticle thickness of leaves. A similar sentiment was stated by Hajibagheri *et al*. (1983), which states that 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. According to Davies (1995), the effect of gibberellin on stem 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.

Conclusions

The interaction of three factors of GA_3, SA and salt stress indicated significant effect on thick cuticle. Plant hormones have been found effective in mitigating the salt induced damage in plant. Thus, the application of GA_3 and SA helped in the tolerance of plants to salt stress.

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