Improvement Of Salt Tolerance In Some Varieties Of Rice By Ascorbic Acid Application

Wan Arfiani Barus, Abdul Rauf, Rosmayati, Chairani Hanum

ABSTRACT: Salinity stress is one of the serious abiotic stress and lead to reduced productivity and plant growth. The effect of salinity stress and its interaction with ascorbic acid was investigated on some morphological traits. Ascorbate is a strong antioxidant which has remarkable biological effects on plants growth, including an improvement in plants' tolerance under salinity stress conditions. The purpose of this study was to determine the effect of ascorbic acid application to improve salt tolerance of rice on saline soil. This research was down as a factorial based on randomized block design with 3 replications. In this research were tested eight varieties of Rice (Ciherang (V1), IR 64 (V2), Lambur (V4), Batanghari (V5), Banyuasin (V6), IR 42 (V8), Inpara 10 (V9) and Margasari (V10) and Ascorbic Acid is applied at concentrations of 0 (A0) , 500 ppm (A1) , 1000 ppm (A2) and 1500 ppm (A3). Ascorbic acid is applied in 4 times with at the age of 15, 35, 55 and 75 days after sowing. All concentrations of ascorbic acid generally has a positive effect on morphological characters. However, the best response was found at 1500 ppm (A3) concentration of ascorbic acid on Banyuasin variety.

Key words: Rice, Ascorbic Acid, Salt Tolerance, Morphological Characters

1. INTRODUCTION

Rice, most loved cereal of Asia and feeds the majority of indonesian people. More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's people and about two-thirds of the world's poor live (Khush and Virk, 2000). Green revolution helped to solve the world's demand for food, but is not enough to meet the 21st century's exploding population. Improved rice varieties and hybrids developed by institutes throughout the world including IRRI have helped to improve the quality and quantity of rice production. About 6.5% (831 million ha) of the world's total area (12.78 billion ha) is affected by salt in soils (FAO, 2008). Area under salt stress is on the increase due to many factors including climate change, rise in sea levels, excessive irrigation without proper drainage in inlands, underlying rocks rich in harmful salts etc.,Vast areas of land are not utilised due to salinity and alkalinity problems. Salinity appears to affect two plant processes water relations and ionic relations. During initial exposure to salinity, plants experience water stress, which in turn reduces leaf expansion. During long-term exposure to salinity, plants experience ionic stress, which can lead to premature senescence of adult leaves (Amirjani, 2011). Salinity has three potential effects on plants: a). Lowering of the water potential, b). Direct toxicity of any Na and Cl absorbed and c). Interference with the uptake of essential nutrients (Flowers and Flowers, 2005). In saline conditions, plant growth and development is hampered due to excessive accumulation of Na and Cl in the cytoplasm, causing changes in the cell metabolism. Enzyme activity inhibited by salt. These conditions also resulted in partial dehydration of cells and loss of cell turgor due to reduced water potential in the cell (Yuniati, 2004).

- *Department of Agrotechnology, Amir Hamzah, University, Medan Indonesia.* arfiani.barus@yahoo.co.id
- *Department of Agrotechnology, Sumatra Utara, University, Medan, Indonesia.*

Stomata play an important role as a tool for adaptation of plants to drought stress. In conditions of drought stress, the stomata will close in an effort to restrain the rate of transpiration. Environmental stress response triggers a wide variety of plants, ranging from changing the expression and cellular metabolism to accelerate aging and permanent wilting leaves, all leading to changes in growth rate and yield. Salt stress had a negative influence on the growth and productivity of plants (Reddy et al., 2004; Baek et al., 2005; Amor et al., 2005). Reactive Oxygen Species Through increased (ROS) that can cause oxidative stress causes cell damage by oxidation of lipids, proteins and nucleic acids (Pastori and Foyer, 2002; Apel and Hirt, 2004). To minimize the effects of oxidative salt stress, plant cells have evolved a complex antioxidant system formed which reduces the concentration of ROS (Beltagi, 2008). One of the approaches to stimulate oxidative stress tolerance will increase enzyme substrates at the cellular level is ascorbic acid. Ascorbic acid is an important primary metabolite in plants that act as antioxidants, enzyme cofactor and as a modulator of cell signaling in a variety of important physiological processes, including cell wall biosynthesis, secondary metabolites and phytohormones, stress tolerance, photoprotection, cell division and growth (Wolucka et al , 2005). Salinity imposes both ionic and osmotic stresses on plants (reviewed by Munns et al., 2006), and salt exclusion from photosynthetic tissues was considered an important mechanism associated with salt tolerance in monocots (Moradi et al., 2003, Davenport et al., 2005). Salinity is an ever increasing environmental problem and is a substantial restraint to agriculture. Plants that gripped salinity also suffer from oxidative stress resulting in inhibition of photosynthetic processes such as electron transport (Greenway and Munns, 1980). Oxidative stress induces ROS concentrations (Reactive Oxygen Species) higher / medium such as superoxide (O2), H2O2 and hydroxyl radicals, because the disrupted electron transport in chloroplasts, mitochondria, and photorespiration pathway. This situation resulted in an imbalance between Source and Sink in plant metabolism. Application of ascorbic acid, is expected to prevent / reduce ROS activity caused by stress so that the plants more tolerant of salt and as an indicator is the increased activity of SOD (superoxide dimustase) (Bohnert and Jensen, 1995), which acts to protect the function of chloroplasts (Orcutt and Nilsen, 2000).

2. MATERIALS AND METHODS

This experiment was conducted at Paluh Merbau, Percut Sei Tuan, Deli Serdang District, North Sumatra on May 2013 to August 2013 . The region studied is geographically located in latitude 98,74850 N and longitude 3,75150 E and 1,5 m altitude. Soil Chemistry characteristic in this location have salinity (EC) = 5,99 dS/m; pH.H_2O = 5,9; Total-N = 0,19%; P-Bray = 18,45 ppm; K.dd = 0,648 me%; Na.dd = 0,46 me%; dan Ca.dd = 1,028 me%. This research by using Randomized Block Design Factorial with three replications on a plot size of 1.5 m × 1.5 m, with two factors,e.i : Varity (V) and Ascorbic concentration (A). Eight Rice genotypes were used in this study,i.e.: Ciherang (V_1), IR 64 (V_2), Lambur (V_4), Batanghari (V_5), Banyuasin (V_6), IR 42 (V_8), Inpara 10 (V_9) dan Margasari (V_{10}). Ascorbic acid concentration ad 4 factors e.i. : A_0 = 0 ppm (control), A_1 = 500 ppm, A_2 = 1000 ppm, A_3 = 1500 ppm. Ascorbic acid is applied in 4 times with at the age of 15, 35, 55 and 75 days after sowing.

3. RESULTS AND DISCUSSION

Germination Percentages and Salt Injury Score

Table 1. *Modified Standard Evaluation Score (SES) of Visual Salt Injury at Seedling Stage*

Score	Observation	Germination (%)	Tolerance Grade
1	Normal Growth, no leaf symptoms	> 90%	Highly Tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	> 60%	Tolerant
5	Growth severely retarded, most leaves rolled, only a few are elongating	< 60%	Moderately Tolerant
7	Complete cessation of growth ; most leaves dry ; some plants dying	< 30%	Susceptible
9	Almost all plants dead or dying	< 10%	Highly susceptible

Source : *Gregorio et. al (1997)*

Salt injury score of some varieties are base on criteria in

Table 1.

Germination Percentages (%) and Salt Injury Score of Some Varieties of Rice was showed in Table 2.

Table 2. *Germination Percentages (%) and Salt Injury Score of Some Varieties of Rice*

Variety	Germination Percentage (%)	Salt Injury Score
Ciherang (V_1)	37.07	5
IR 64 (V_2)	36.93	3
Lambur (V_4)	30.47	5
Batanghari (V_5)	19.13	3
Banyuasin (V_6)	40.73	1
IR 42 (V_8)	20.93	3
Inpara 10 (V_9)	33.53	3
Margasari (V_{10})	21.87	3

A comparison of the responses of the different cultivars indicated that germination percentage was based on type of cultivar. In germination phase, Banyuasin had the highest germination percentage (40.73%). Sembiring and Gani (2005) and Hosseni.et al. (2012) reported that salt stress resulted in reduced germination rate. From Table 1, germination the lowest in Batanghari (V5) is equal to 19:13%. Based on salt injury score was obtained the highest scores on Banyuasin (V6) is 1 (very tolerant). Furthermore, the effect of salt stress on morphological characters after Ascorbic Acid application can be seen in Table 3.

Table 3. *Some Morphological Characteristics of Some Varieties of Rice with Ascorbic Acid Application*

Treatment	Parameters			
	Plant Height	Leaf Area (cm^2)	Number of Productive Tiller (pc)	Length of Panicle (cm)
V_1A_0	85.37 bc	18.50 b	15.11 b	18.46 b
V_1A_1	86.13 bc	19.92 ab	19.23ab	19.31 ab
V_1A_2	89.33 b	20.29 ab	20.05ab	20.46 ab
V_1A_3	93.95 a	21.86 a	20.15 a	21.33 a
V_2A_0	78.41 c	21.50 b	24.25 b	15.89 c
V_2A_1	83.08 ab	20.92 b	27.01 ab	17.49 b
V_2A_2	84.29 ab	22.92 b	28.13 a	19.18 ab
V_2A_3	85.08 a	25.58 a	27.99 ab	20.39 a
V_4A_0	75.60 d	19.84 bc	18.19 bc	18.38 b
V_4A_1	81.02 bc	22.32 b	20.19 b	17.88 bc
V_4A_2	83.73 b	21.40 b	23.09 ab	21.57 ab
V_4A_3	92.73 a	24.53 a	25.00 a	23.09 a
V_5A_0	91.09 b	22.61 b	23.11 cd	17.00 b
V_5A_1	91.82 b	24.14 b	24.61 c	21.63 ab
V_5A_2	93.24 b	24.65 b	28.09 b	24.10 a
V_5A_3	100.75 a	27.35 a	35.06 a	23.77 ab
V_6A_0	84.86 b	24.17 c	29.02 c	21.31 c
V_6A_1	86.00 ab	26.16 bc	30.01 bc	24.01 b
V_6A_2	86.04 ab	28.02 b	32.99 b	23.85 bc
V_6A_3	87.62 a	30.55 a	38.99 a	27.00 a
V_8A_0	69.18 c	18.46 b	16.70 d	19.00 bc
V_8A_1	74.51 b	19.93 b	19.99 c	21.11 b
V_8A_2	74.96 b	19.06 b	26.08 b	19.99 bc
V_8A_3	77.37 a	21.36 a	34.09 a	25.12 a
V_9A_0	73.79 c	21.00 bc	21.00cd	18.09 c
V_9A_1	75.01 c	21.74 bc	21.19c	19.77 c
V_9A_2	79.29 b	22.91 b	25.74b	23.98 a
V_9A_3	80.47 a	27.03 a	31.76a	22.80 ab
$V_{10}A_0$	83.92 d	22.93 cd	19.75 b	16.89 c
$V_{10}A_1$	102.36 c	25.57 c	21.98 b	19.05 bc
$V_{10}A_2$	107.34 b	28.93 b	25.16 ab	21.04 b
$V_{10}A_3$	125.36 a	31.96 a	27.18 a	23.17 a

Note: *The numbers that are not followed by the same letter in a same column or row, means significantly different according to DMRT at 5% level.*

Exogenous supply of ascorbic acid to rice plants improved their salt tolerance as indicated by studied morphological parameters. Among various concentrations of AA, 1500 ppm level resulted in improved growth of rice plant. Foliar application of AA at 1500 ppm counteracted the adverse effect of salinity that was accompanied by a significant increase in plant growth of eight varieties in Table 3. Table 3 shows that the application of ascorbic acid significantly affect the growth of plant height for all varieties tested. The increase in plant height growth was obtained after the application of ascorbic acid. This increase occurs because the effect of salinity stress immediately resolved with the availability of ascorbic acid as antioxidants (El-Hariri et al., 2010) and during the occurrence of stress. This is in line with those reported by Behary (2012) and Hossain et.al (2013), that the application of ascorbic acid through the leaves can prevent and cope with stress due to salinity stress. 64 IR varieties most responsive to ascorbic acid application. The results of this study are consistent with those reported by Dobermann and Fairhurst (2000) who concluded that the rice is relatively more tolerant to salinity when the germination, but plants can be affected when transplanting, young seedlings, and flowering. Further influence on the rice plant are: 1) Reduced speed of germination; 2) Reduced plant height and number of tillers; 3) poor root growth; 4) increased seed sterility; 5) Lack of 1000 grain weight and total protein content in the seeds due to excessive absorption of Na; and 6) Reduced biological N2 fixation and mineralization than soil.

4. CONCLUSION

The morphology characters had the positive effects of the application of ascorbic acid in improving salt tolerance on Rice and the best response was found at 1500 ppm (A_3) concentration of ascorbic acid on Banyuasin.

ACKNOWLEDGMENT

This research is a part of a dissertation for the doctoral program at North Sumatra University. Thank you for Directorate General of Higher Education that has funded this research.

REFERENCES

[1] Amirjani, Mohammad Reza. 2011. Effect of Salinity Stress on Growth, Sugar Content, Pigments and Enzyme Activity of Rice. International Journal of Botany. Vol. 7. Pp 73-81.

[2] Dobermann, A and T. Fairhurst. 2000. Rice. Nutrient disorders & nutrient management. International Rice Research Institute (IRRI). Potash & Phophate Institute/Potash & Phosphate Institute of Canada. p: 139-144.

[3] FAO. 2008. 20 Things on salinity. http://www.fao.com. [30 Januari 2009].

[4] Flowers T. J. and A.R. Yeo, Effects of salinity on plant growth and crop yield, in Environmental Stress in Plants, J.H. Cherry, Editor. 1989, Springer Verlag: Berlin. p. 101-119.

[5] Flowers, T.J. and Flowers, S.A., 2005. Why Does Salinity Pose such a Difficult Problem for Plant Breeders? Agr. Water Manage., 78: 15–24.

[6] Gregorio, Glenn B. : Dharmawansa Senadhira dan Rhulyx D. Mendoza. 1997. Screening Rice for Salinity Tolerance International Rice Research Instititute, Manila. Philippines.

[7] Hossein Sadeghi and Fatemeh Ansar shourijeh, 2012. Salinity Induced Effects on Growth Parameters, Chemical and Biochemical Characteristics of Two Forage Sorghum (Sorghum bicolor L.) Cultivars. Asian Journal of Plant Sciences, 11: 19-27.

[8] Khush, G.S and P.S. Virk. 2000. Rice breeding : Achievements and future strategies. Crop Improv., 27(2) : 115-144.

[9] Munns, R. 2002. Comparative Physiology of Salt and Water Stress. Plant Cell and Development. 25 239-250pp.

[10] Munns, R., R. A. James and A. Lauchli. 2006. Approaches to Increasing The Salt Tolerance of Wheat and Other Cereals. J. Exp.Bot., 57(5) 1025-1043.

[11] Orcutt, D. M. and Nilsen, E. T. (2000). Salinity. In : Physiology of Plants Under Stress, Soil and Biotic Factors. John Wiley & Sons, 177–237.

[12] Sembiring, Hasil dan A. Gani. 2005. Adaptasi varietas padi pada tanah terkena tsunami. ttp://io.ppi.jepang.org. [16 Nopember 2008].

[13] Yuniati, Ratna. 2004. Penapisan Galur Kedelai (Glycine max L. Merrill) Toleran Terhadap NaCl untuk Penanaman Di Lahan Salin. Makara Sains, Volume 8 No : 1, April. Halaman 21 – 24.