Proline Content Variation in Some Rice Varieties under Salinity Stress

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ABSTRACT

Proline content is one indicator of plant resistance to salinity stress. This proline content varies based on the level of resistance of each variety. This study aims to determine the variation of leaf proline content from eight rice varieties under salinity stress. This research was carried out in Paluh Merbau, Percut Sei Tuan, Deli Serdang North Sumatra, Indonesia. This research location has saline soil and it was so close to the beach (1 km). This study used a non-factorial randomized block design. There were eight rice varieties tested namely Ciherang (V_1), IR 64 (V_2), Lambur (V_3), Batanghari (V_4), Banyuasin (V_5), IR 42 (V_6), Inpara 10 (V_7) and Margasari (V_8). The results showed that the leaf proline content of the eight varieties was very varied. Varieties that were resistant to salinity stress tend to have a higher proline content than susceptible varieties. Banyuasin has the highest proline content than the other varieties.

Keywords: Proline Content, Rice, Salinity Stress, Varieties

A. INTRODUCTION

The ability of plants to face salinity stress can be indicated by the accumulation of proline compounds. Proline is one of the amino acids produced by plants when experiencing abiotic stress. Poustini, Siosemardeh and Ranjbar (2006) found that there was an increase in proline accumulation in wheat plants that experienced salinity stress.

There is a relationship between plants experiencing stress with proline accumulation, thus, proline can be used as a marker of wheat resistance against drought and salinity stress conditions. Stress in plants caused by stress salinity, drought, temperature, oxygen and heavy metal poisoning are the main reasons for the decline in agricultural yields (Rai and Takabe, 2005).

Salinity can be caused due to continuous accumulation of NaCl in the soil, for example on land along the coast which when tide is inundated. When the water recedes, NaCl from sea water will be left on the ground so that NaCl will accumulate and cause the soil to become saline. Another cause of saline soil is low rainfall in an area. Causing the NaCl content of the soil can not be dissolved and washed out and eventually accumulates on the soil.

Proline is an amino acid synthesized from the results of glutamate phosphorylation. The trajectory of glutamine is the primary route for proline biosynthesis under conditions of seismic salinity. Proline accumulation is a result of an increase in free amino acids when plants are in a stressful environment, such as drought, high salinity, and temperatures that are too low or too high. Active plants produce various kinds of metabolites and defense systems to survive, for example osmoprotectants such as proline, glycine betaine, mannitol, and sugar as tolerance compounds to stress and drought salinity. Proline accumulates in meristem tissue which is part of a plant that actively divides in plants that experience stress. When cell division occurs, much energy will be needed for respiration so that the enzyme activity there will be very high including the enzyme that synthesizes proline.

Proline is a biochemical characteristic compound or osmotic metabolite which is widely synthesized and accumulated in various plant tissues, especially in leaves when the plants face drought stress. Plants that accumulate proline in gripped conditions generally have better morphological features and have higher survival than plants that do not accumulate them (Hamim *et al.*, 2008). Proline diversity in plants and the amount of proline accumulation produced by plants will differ according to their genetic traits as indicated by differences in varieties, types and duration of stresses experienced and plant age will also affect the amount of proline accumulation. In corn plants (Turan *et al*, 2009), it can be seen that the salinity stresses given will increase the accumulated proline concentration. The salt stress was related with drought stress. Salt stress made drought stress and the effect was similar. Based on observations made by Mafakheri *et al.* (2010), showed that beans with 3 varieties without the salt stress of the proline amount produced were 0.67; 1.26; 0.42 mol / gram fresh weight, while the provision of drought stress increases proline accumulation to 8.28; 9.45; 8.4 µ mol / gram fresh weight in the vegetative phase and 7.36; 8; 29; 7; 30 µ mol / gram fresh weight in the flowering phase.

This shows that plants that experience stress will accumulate more proline in the vegetative phase, where cell division is actively taking place. The three varieties of beans also showed a different amount of proline, so the level of plant resistance was different.

In the same plant species as the stress experienced, the amount of proline accumulation produced will differ according to the variety. In the research of Changhai *et al*. (2010), wheat plants with 4 different varieties showed an increase in the number of proline produced different from the effect of the same stress, which increased 174.7%, 175.0%, 249.2% and 242.9%. In the research conducted by Turan *et al*. (2009), regarding the effect of salinity stress on corn plants on proline concentration, analysis of proline content was obtained from young leaf extract and apical shoots. Proline testing of wheat plants that experience drought stress is extracted from flag leaves, in the heading or flowering phase shows very high proline accumulation (Maralian *et al*., 2010). The two studies above show that proline is extracted from the organs of plants that are still young and which is definitely an active cell division.

B. 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.H2O = 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.

C. RESULTS AND DISCUSSION

Analysis of the variety of leaf proline content of eight rice varieties under salinity stress can be seen in Table 1. Analysis of variance showed that the varieties had significant effect on the proline content of eight varieties under salinity stress and based on the average different test, leaf proline content among eight rice varieties is real different. The highest Proline content is found in Banyuasin (V_6) varieties, namely: 214.53 µmol/g and significantly different from other varieties, while the lowest Proline content is found in IR 64 (V_2) varieties which is 86.50 µmol / g and also significantly different from other varieties

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Table 1. Proline content of eight rice varieties below stressed salinity

Ciherang (V_1)	IR 64 (V_1)	Lambur (V_1)	Batanghari (V_1)	Banyuasin (V_1)	IR 42 (V_1)	Inpari 10 (V_1)	Margasari (V_1)
µmol/g							
176,47 c	86,50 g	137,54 e	149,87 d	214,53 a	148,50 d	119,38 f	184,10 b

ased on Table 1, it can be seen that the Banyuasin variety has the highest proline content, this indicates that the Banyuasin variety has the highest resistance to salinity stress when compared with other varieties. Plant reactions to salinity stress differ depending on the nature of the tolerance of the plant. Markers that can be used to show a plant's resistance to alinity stress are increased proline accumulation. Proline is an amino acid synthesized from the results of glutamate phosphorylation. The trajectory of glutamine is the primary route for proline biosynthesis under conditions of drought stress (Madan *et.al*., 1995). Proline accumulation is a result of an increase in free amino acids when plants are in a stressful environment, such as high salinity. Active plants produce various kinds of metabolites and defense systems to survive, for example osmoprotectants such as proline, glycine betaine, mannitol, and sugar as tolerance compounds to stress and drought salinity. In plants that are tolerant of abiotic stress conditions such as drought or salinity, there is a mechanism to maintain turgor to remain above zero, to keep the water potential in the cell low compared to its external water potential so that plasmolysis does not occur. From the proline amount produced by plants, it can be a marker of how much tolerance it is to drought and salinity stress.

Proline accumulates in meristem tissue which is part of a plant that actively divides in plants that experience stress. When cell division occurs, much energy will be needed for respiration so that the enzyme activity there will be very high including enzymes that synthesize proline. Plant adaptability to salinity can be indicated by the amount of proline compounds synthesized when experiencing stress. Proline is one of the amino acids produced by plants when experiencing abiotic stress. Abiotic stress is meant when plants experience high salinity stress. Proline is synthesized as a compound that can keep plants from retaining cell turgor. Many researchers have found that plants that experience salinity stress will accumulate a certain amount of proline. Proline accumulation increase in wheat plants that experienced salinity stress was also observed by Poustini, Siosemardeh and Ranjbar (2006). With the discovery of lowland tropical wheat plants that are resistant to abiotic stress, it can be the beginning of improvement in domestic food security. Stress salinity can cause osmotic stress as well as dryness and can also cause toxicity. In wheat plants, the symptoms of salinity stress are indicated by the appearance of white on the tips of leaves, leaflets rolling up and drying, chlorosis on leaves and seed production will be very low. The amount of proline accumulation produced by plants will differ according to their genetic traits as indicated by differences in varieties, types and duration of stresses experienced and the age of plants will also affect the amount of proline accumulation. In corn plants (Turan *et al*. 2009), it can be seen that the higher salinity stresses given will increase the accumulated proline concentration, namely at low NaCl concentrations the proline amount produced by plants is 18.6 μ mol / gram fresh weight and at concentration High NaCl amount of proline accumulation increased to 44.16 μ mol / gram fresh weight. Based on observations made by Mafakheri, *et al*. (2010), showed that beans with 3 varieties without the stress of the proline amount produced were 0.67; 1.26; 0.42 mol / gram fresh weight, while the provision of drought stress increases proline accumulation to 8.28; 9.45; 8.4 μ mol / gram fw in the vegetative phase and 7.36; 8; 29; 7; 30 μ mol / gram fw in the flowering phase. This shows that plants that experience stress will accumulate more proline in the vegetative phase, where cell division is actively taking place. The three varieties of beans also showed a different amount of proline, so the level of plant resistance was different. In the same plant species as the stress experienced, the amount of proline accumulation produced will differ according to the variety. In the Changhai *et al* (2010) study, wheat plants with 4 different varieties showed an increase in the number of proline produced different from the effect of the same stress, which increased 174.7%, 175.0%, 249.2% and 242.9%. Turan *et. al* (2009) reported the effect of salinity on corn plants on proline concentration, analysis of content that was obtained from young leaf extracts and apical shoots. Proline testing of experience wheat plants through stress is extracted from flag leaves, in the heading or flowering phase shows very high proline accumulation (Maralian *et al*., 2010). The proline is extracted from an active cell division which is definitely active cell division. Adaptation of plants to stress both salinity stress and drought stress can be seen as an appearance of an anatomy plant. In plants that are drought-resistant, they can be used to produce maximum dry weight of plants. Changhai *et al*. (2010), also conducted observations on dry weight plants seen from the efficiency of its transportation. 10.2% and 5.8% while the low efficiency, dry weight dropped to 15.1% and 26.6% respectively. Here the role of proline as osmoprotectant is seen as a stomatal cell guard in preventing too high transpiration.

In a study conducted by Hamim, Sopandie and Jusuf (1996) regarding observational morphological and physiological characteristics of soybean tolerant and sensitive to stress, the results showed that when stress was stressed, plants that were tolerant to proline testing showed an increase of up to 7 times normal conditions. Morphological appearance of narrow leaf size due to developmental obstruction (Nofyangtri, 2011 and El-Hendawy *et al*., 2005), root growth rate, root / leaf ratio (El-Hendawy, *et al.* 2005), total dry weight (Pessarakli and Huber, 1991; El-Hendawy *et al*., 2005) are exposed physiological activities of plant stress that can be observed. Plants with small, thick leaves indicate that this plant is tolerant of stress (Nofyangtri, 2011).

D. CONCLUSIONS

The presence of salinity stress increases proline levels, and there are differences in the amount of proline accumulation by various rice varieties when experiencing stress at different growth phases. So that it can be seen that the presence of proline can be a marker of rice plant resistance to salinity stress.

REFERENCES

Changhai S., Baodi1 D., Yunzhou Q., Yuxin L., Lei S., Mengyu L., Haipei. 2010. Physiological regulation of high transpiration efficiency in winter wheat under drought conditions. Plant Soil Environ. Vol. 56, 2010 (7): 340–347.

Chaves M.M. 2002. How plant cope with water stress in the field. Photosynthesis and growth. Annual of Botany 89, 907-916.

El-Hendawy Salah E.,Yuncai Hu, Yakout Gamal M., Ahmed M. Awad, Hafiz Salah E., Urs Schmidhalter. 2005. Evaluating salt tolerance of wheat genotypes using multiple parameters. Europe. J. Agronomy 22 (2005) 243–253.

Hamim, Sopandie Didi dan Jusuf Muhammad. 1996. Beberapa karakteristik morfologi dan fisiologi kedelai toleran dan peka terhadap cekaman kekeringan. Hayati, Juni 1996, hal 30-34.

Madan S, Nainawatee HS, Jain RK, Chowdhury JB. 1995. Proline and proline metabolizing enzymes in *in-vitro* selected NaCl-tolerant *Brassica juncea* L. under salt stress. Ann. Bot. 76: 51-57.

Mafakheri A., Siosemardeh A., Bahramnejad B., Struik P.C., dan Sohrabi Y. 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian Journal of Crop Science. 4(8):580-585.

Maralian H., Ebadi A., Didar T. R dan Eghrari B. 2010. Influence of water deficit stress on wheat grain yield and proline accumulation rate. African Journal of Agricultural Research, Vol. 5 (4) pp. 286-289, 18 February, 2010.

Nofyangtri Sahera. 2011. Pengaruh cekaman kkeringan dan aplikasi mikoriza terhadap morfo-fisiologis dan kualitas bahan organik rumput dan legum pakan. Tesis Institute Teknologi Bandung. Bandung.

Pessarakli M. dan Huber J.T. 1991**.** Biomass production and protein synthesis by alfalfa under salt stress. J. Plant. Nutr. 14: 283-293.

Poustini K., Siosemardeh A., dan Ranjbar M. 2006. Proline accumulation as a respone to salt stress ini 30 wheat cultivars differing in salt tolerance. Genet Resour Crop Evol (2007) 54:925-934.

Rai Ashwani K., Takabe Teruhiro. 2005. Abiotic Stress Tolerance in Plants Toward the Improvement of Global Environment and Food. Published by Springer. Netherlands.

Turan Murat Ali, Elkarim Abdelkarim Hassan Awad, Taban Nilgun dan Taban Suleyman. 2009. Effect of salt stress on growth, stomatal resistance, proline and chlorophyll concentrations on maize plant. African Journal of Agricultural Research. Vol. 4 (9), pp. 893 – 897.