

Effect of NaCl-Stress on Metabolism of NO_3^- , NH_4^+ and NO_2^- at Several Rice Varieties

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Received 3 March 2009 / accepted 3 September 2010

ABSTRACT

Effect of NaCl-Stress on Metabolism of NO_3^- , NH_4^+ and NO_2^- at Several Rice Varieties (MZH Utama): This study was conducted to evaluate the effect of NaCl-stress on metabolism of NO_3^- , NH_4^+ and NO_2^- at several rice varieties. The results showed that an addition of NaCl had lesser effect on NaCl-tolerant varieties as compared to NaCl-sensitive in term of reduction in NO_3^- , NH_4^+ , and NO_2^- uptake. Rice adaptation ability to NaCl stress occurred through the mechanism of NO_3^- , NH_4^+ , and NO_2^- metabolism physiology. It was indicated by the difference concentration of NO_3^- , NH_4^+ and NO_2^- between the tolerant (Cisadane), moderate (Batang Lembang, Rendah Kuning, and Batang Piaman) and sensitive (IR 66) varieties. Concentration of NH_4^+ and NO_2^- of tolerant rice (Cisadane) at NaCl treatment were about 1.16 and 2.6 times higher than that at control, respectively, while concentration of NO_3^- was only 0.03 times lower than control. In contrast, concentration of NO_3^- , NH_4^+ , and NO_2^- of sensitive rice (IR 66), were about 0.09, 0.27, and 0.41 times lower than that in control respecting at NaCl treatment, respectively.

Keywords: NaCl-tolerant, NH_4^+ , NO_2^- , NO_3^- , rice varieties

INTRODUCTION

Increased productivity of rice has been attempted in Indonesia since the 1970s, in order to increase income and welfare of the community and enhance national food security (Amang and Sawit 1999). Increasing rice production in the future, will have too many more complex challenges, related to nutrient stress, climate, weeds, pests and diseases (Bilman 2008; Sunadi 2008). No less important problem is the lack of tolerant varieties on environmental stress (Utama 2008), especially salinity stress.

One of the potential land that can be used to increase rice production is swamp land (Djafar 2002). Extensive swamps in Sumatra, which can be utilized for agriculture reached 10.80 million hectares. One area of them are swamps in the Pesisir Selatan district of West Sumatra, a coastal swamp land that affected by tidal sea water or sea water intrusion. Marsh area in the Pesisir Selatan District reach 42,000 hectares,

where from the land area is utilized only 3,251 hectares or only 8% (Anonymous 2004).

Serious problem in cultivation in the coastal marsh land is toxicity of Na^+ and Cl^- that causes crops cell damage, water deficit (Marschner 1995) so that cause inhibition of plant growth. Growth constraints on the soil increased especially in high water conditions and the dry season (Harjadi and Yahya 1988; Utama *et al.* 2009). This condition cause Na^+ toxic to plants and the low solubility of essential nutrients, causing nutrient deficiency.

Plant tolerance to Na^+ and Cl^- is an important factor to adapt to the soil saline. Identification of obstacles to the growth of plants due to increased concentrations of Na^+ and Cl^- in the nutrient solution is a parameter for selection of plant genotypes, according to the level of tolerance of salinity stress (Utama *et al.* 2009).

In high salt conditions, Na^+ and Cl^- are the dominant ions, while Cl^- is an micro-nutrient for plants and high levels of Na^+ is an nutrient for plants, but on

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the condition of copy number far exceeds the needs of causing toxicity to plants is not tolerant. Plants which are tolerant to environmental stress has the ability to adapt to the morphology and physiology (Marschner 1995; Pellet *et al.* 1995; Ma, 2000; Utama *et al.* 2005).

Plant tolerance to environmental stress can occur internally or externally, as a mechanism to be able to adapt to the environment. Both mechanisms can occur simultaneously, although in different degrees depend on types of plants and their ability to adapt. The main difference of these two mechanisms is the area where the binding occurred, whether in simplast (internal) or apoplast (exclusion) (Delhaize and Ryan 1995; Pellet *et al.* 1995; Sopandie 1999; Ma 2000).

The method which is often used to observe the tolerance of plants to fight stress or environmental stress as follows (1) Looking at the ability of plants with symbiotic soil microorganisms (Smith and Read 1997; Utama and Yahya 2003), (2) Seeing the growth of roots plants (Marschner 1995; Kochian 1995; Ma *et al.* 2000), and (3) Looking at the mechanism of Al detoxification by organic acids, either by accumulation or exudation (Pellet *et al.* 1995; Ma, 2000; Utama *et al.* 2007).

One method to overcome the problem of marginal land is to take advantage of the tolerant plant to environmental stress (Marschner 1995; Ma 2000; Utama *et al.* 2004). An effort to improve plant growth and neutralize the bad effect of Na^+ becomes increasingly important to increase plant growth, especially rice cultivation in the swamp lands with high salinity.

The low use of the land is because of the limited technology and tolerant rice varieties to salinity (Sabiham and Ismangun 1996; Russnetty 2000; Munir *et al.* 2004). To take advantage of these swamp lands required a program of selection of some rice varieties to produce tolerant plant on environmental stress such as high salinity, particularly for the South Coastal District, West Sumatra.

The purpose of this study was to determine metabolic activity of Nitrate (NO_3^-), ammonium (NH_4^+), and Nitrite (NO_2^-), in tolerant and sensitive rice varieties to high salinity of environment stress.

MATERIALS AND METHOD

Research was conducted at the Laboratory of "Kopertis" Region X, from February to October 2008. Selected rice varieties used in this experiment

consisted of 5 varieties (Utama *et al.* 2009), namely: three of tolerant varieties (Cisadane, Batang Lembang, and Rendah Kuning), and two of sensitive varieties (Batang Piaman and IR 66). Chemicals were used as a nutrient solution and the main tool for analysis was Nova Spectroquan Thermoreactor TR 400 (Merck, Germany).

Experimental Setup

This experiment used two-factors of a factorial design in a Completely Randomize Design with three replications. The first factor was selected rice varieties (3 tolerant and 2 sensitive varieties to high salinity), namely: V_1 = Cisadane, V_2 = Batang Lembang, V_3 = Rendah Kuning, V_4 = Batang Piaman, and V_5 = IR 66. The second factor was the level of NaCl, namely: G_0 = 0.0 mg kg^{-1} and G_1 = 4,000 mg kg^{-1} .

Before germinated, rice seeds were firstly soaked in a solution of fungicide Dithane M-45 and the insecticide Decis, each with a concentration of 3 g L^{-1} and 1 ml L^{-1} for 20 minutes. Then the seeds were thoroughly rinsed and soaked for 24 hours. Seedlings were grown for 10 days in a perforated plastic tub, at a dark room, and at room temperature. Sprouts which were used in this experiment had a length of 5 cm root.

Nutrient solution for water culture medium used a composition consisting of: 1.5 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; 1.0 mM NH_4NO_3 ; 1.0 mM KCl; 0.4 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 1.0 mM KH_2PO_4 ; 0:50 mg kg^{-1} $\text{MnSO}_4 \cdot \text{H}_2\text{O}$; 0:02 mg kg^{-1} $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; 0:05 mg kg^{-1} $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; 0:50 mg kg^{-1} H_3BO_3 ; 0:01 mg kg^{-1} $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$; 0068 mM Fe-EDTA, and NaCl (Sopandie 1999; Utama 2008). Sprouts were grown in a plastic tub filled with 2.5-liter nutrient solution without NaCl at pH 4.0. Media adaptation used 1/5 the concentration of nutrient solution without NaCl at pH 4.0 for 7 days, after which the seeds were grown in nutrient solution using 1/3 concentration at pH 4.0 with NaCl stress treatment in accordance with the established treatment. The nutrient solution replacements were conducted every 7 days during the experiment and the nutrient solution flowing through the air with aerator. After 21 days treatment, the plants were harvested for analysis of nutrient uptake in the laboratory. The analysis included an analysis of nutrient uptake levels of NO_3^- , NH_4^+ and NO_2^- in plant tissue.

Analysis of Plant NO_3^- , NH_4^+ , and NO_2^- Uptake

Plant samples were weighed, crushed until smooth, then heated until all the smoke (SO_3) exit.

The samples were cooled for 24 hours, then followed by adding H₂O₂ until the solution became homogen. The heating was repeated until the solution volume was about 2-3 ml. After that, the solution was transferred into 50 ml flask and fitted with aquades.

Analysis of concentration of NO₃⁻, NH₄⁺, and NO₂⁻ in solution used a Nova Spectroquant Thermoreactor TR 420. Barcode standard solutions were put in Nova Spectroquant Thermoreactor TR 420. Kid test solutions (NO₃⁻, NH₄⁺ and NO₂⁻) were prepared, 2 ml of solution was added to each kid test solution, and let it for 10 minutes. After that, a mixture of test kid was incorporated into the crystal cuvet sizing 10 ml and immediately read with Nova Spectroquant Thermoreactor TR 420 (Anonymous 2007).

RESULTS AND DISCUSSION

The concentration of nitrate (NO₃⁻), ammonium (NH₄⁺) and nitrite (NO₂⁻) of rice varieties of Cisadane,

Batang Lembang, Rendah Kuning, Batang Piaman and IR 66 at salinity treatment, are presented in Tables 1, 2, and 3, respectively. Tables 1, 2, and 3 indicated that the tested five rice varieties were able to absorb NO₃⁻, NH₄⁺ and NO₂⁻. This showed that there were no obstacles in the absorption of NO₃⁻, NH₄⁺ and NO₂⁻ in the five rice varieties although under NaCl treatment.

Concentration in of NO₃⁻ Batang Lembang and Batang Piaman under NaCl treatments was higher than in control, which increased 1.31 and 3.36 times respectively. This was very different with Cisadane, Rendah Kuning and IR 66 varieties where the concentration of NO₃⁻ declined 0.03, 0.77, and 0.09 times respectively. The highest increasing of NO₃⁻ by NaCl treatment occurred in stem of Batang Piaman variety (3.36 time), while the highest decreasing in Rendah Kuning variety (0.77 times).

Table 2 showed that concentration of NH₄⁺ in Cisadane, Rendah Kuning and Batang Piaman varieties treated with NaCl were higher than in control

Table 1. Concentration of Nitrate (NO₃⁻) in some varieties of rice treated with NaCl at three weeks after planting.

Concentration of NaCl (mg kg ⁻¹)	Concentration of NO ₃ ⁻				
	Cisadane	Batang Lembang	Rendah Kuning	Batang Piaman	IR 66
 mg kg ⁻¹				
0	144.31 cd	114.81 a	92.16 f	69.02 g	150.78 c
4,000	140.77 d	265.34 e	20.55 h	231.94 b	136.66 d
CV (%) = 3.72					

Numbers followed by different letters in the same row and column showed significantly different at 5% level by LSD test.

Table 2. Concentration of ammonium (NH₄⁺) in some varieties of rice treated with NaCl at three weeks after planting.

Concentration of NaCl (mg kg ⁻¹)	Concentration of NH ₄ ⁺				
	Cisadane	Batang Lembang	Rendah Kuning	Batang Piaman	IR 66
 (mg kg ⁻¹)				
0	2.26 f	4.77 b	3.42 d	3.43 d	3.72 d
4000	4.88 b	3.71 d	4.23 c	9.63 a	2.73 e
CV (%) = 4.36					

Numbers followed by different letters in the same row and column showed significantly different at 5% level by LSD test

Table 3. Concentration of Nitrite (NO_2^-) in some varieties of rice treated with NaCl at three weeks after planting.

Concentration of NaCl (mg kg ⁻¹)	Concentration of NO_2^-				
	Cisadane	Batang Lembang	Rendah Kuning	Batang Piaman	IR 66
 (mg kg ⁻¹).....				
0	4.27 b	8.57 b	5.27 b	53.26 a	11.17 b
4,000	15.38 b	7.83 b	8.73 b	17.34 ab	6.58 b
CV (%) = 15.77					

Numbers followed by different letters in the same row and column showed significantly different at 5% level by LSD test.

which increased about 1.16, 0.24, and 1.81 times, respectively. This was very different with Batang Lembang and IR 66 varieties, where the concentration of NH_4^+ at NaCl treatment was lower about 0.22 and 0.27 times respectively. The highest increasing with NaCl treatment occurred in Batang Piaman variety (1.81 times) while the highest decreasing occurred in the IR 66 variety (0.27 time).

Concentrations of NO_2^- in Cisadane and Rendah Kuning varieties treated with NaCl were higher than control, which increased 2.6 and 0.66 times respectively. While the concentration of NO_2^- in Batang Lembang, Batang Piaman and IR 66 varieties were lower than in control, which decreased about 0.1, 0.67, and 0.41 times respectively. The highest increasing by NaCl treatment occurred in Cisadane variety while the highest decreasing in Batang Lembang variety (Table 3).

The results of analysis of rice field soil taken from Pulau Karam Village, Tarusan Sub District, Pesisir Selatan District were presented in Table 4. The table showed that the electrical conductivity

Table 4. Initial paddy soil of swamp land analysis of Pulau Karam Village, Tarusan Sub-District, Pesisir Selatan District.

Soil Properties	Unit	Results
EC	Mmhos cm ⁻¹	2.98
pH		4.90
N	mg kg ⁻¹	71.25
P	mg kg ⁻¹	0.08
Exch. Na	me 100g ⁻¹	12.00
Cl ⁻	mg kg ⁻¹	5,750.00
NO_3^-	mg kg ⁻¹	50.00

(EC), excheangable Na and Cl⁻ levels were so high, thus they were potentially toxic to plants.

NaCl toxicity symptoms may cause disturbances of growth such as the reduction in the number of saplings. EC values between 2-4 would cause plant growth interference, especially at sensitive varieties. Similarly, high concentration of Cl⁻ could lead to toxicity (Harjadi and Yahya 1988; Marschner 1995; Rengel 2000). Element of Cl⁻ was a micro-nutrient needed in small amounts for photosynthesis activities related to oxygen production.

Experimental results of physiological characteristics on salinity stress in some varieties of rice in tolerant (Cisadane); moderate (Batang Lembang, Rendah Kuning, and Batang Piaman) and sensitive (IR 66) rice varieties showed a difference in metabolic response to NO_3^- , NH_4^+ , and NO_2^- (Table 1-3). This indicated a difference of tolerance among rice varieties to NaCl stress. Physiological factors associated with the tolerance to Al stress in soybeans and legumes cover crops include the ability of preferences to NO_3^- , NH_4^+ and NO_2^- (Sopandie 1999; Utama 2008).

Tolerant rice variety (Cisadane) could absorb NH_4^+ 1.16 time and NO_2^- 2.6 times NaCl stress concentration than without stress, while the uptake of NO_3^- only the 0.03 time decreased. Conversely, sensitive rice cultivar (IR 66) showed a decreasing of absorption to NH_4^+ , NO_3^- , and NO_2^- , each about 0.27, 0.09, and 0.41 times, respectively.

The tolerance to NaCl stress at tolerant varieties, presumably because a greater ability to absorb anions and cations at NaCl stress condition was compared to sensitive varieties. Concentration of NH_4^+ in Cisadane variety about 0.8 times higher than the

IR 66 variety, concentration of NO_3^- was only the 0.03 times higher, while the concentration of NO_2^- was 1.3 times higher at NaCl stress condition. The most important mechanisms of tolerance of Na^+ and Cl^- stress was the selectivity of ion transport. Tolerant rice plant has a remarkable ability to acquire essential nutrient from a saline solution, although non-essential elements (toxic ions) were much greater than the essential elements.

CONCLUSIONS

Rice adaptation ability to NaCl stress occurred through the mechanism of nitrate (NO_3^-), ammonium (NH_4^+), and nitrite (NO_2^-) metabolism physiology. It was indicated by the difference concentration of NO_3^- , NH_4^+ and NO_2^- between the tolerant (Cisadane), moderate (Batang Lembang, Rendah Kuning, and Batang Piaman) and sensitive (IR 66) varieties.

Concentration of NH_4^+ and NO_2^- of tolerant rice (Cisadane) at NaCl treatment were about 1.16 and 2.6 times higher respectively than at control, while concentration of NO_3^- was only 0.03 times lower. In contrast, concentration of NO_3^- , NH_4^+ , and NO_2^- of sensitive rice (IR 66), were about 0.09, 0.27, and 0.41 times lower aspect only at NaCl treatment than in control.

ACKNOWLEDGEMENTS

Thanks to DP2M, DIKTI, Ministry of National Education who funded this research by DIPA Number: 0145.0/023-04.0/-/2008, December 31, 2007, Letter of Agreement, Implementation Research Number: 306/SP2H/PP/DP2M / III/2008, March 6, 2008. Thanks also to Ir. Widodo Haryoko, MP for his kind help in this research implementation.

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