African Journal of Biotechnology Vol. 11(86), pp. 15231-15235, 25 October, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2264
ISSN 1684-5315 ©2012 Academic Journals

Full Length Research Paper

Effect of soil salinity on the growth, amino acids and ion contents of rice transgenic lines

Muhammad Jamil[1]*, Muhammad Anees[2], Shafiq Ur Rehman[3], Muhammad Daud Khan[1], Chang-Hyu Bae[4], Sheong Chun Lee[4] and Eui Shik Rha[4]

[1]Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology (KUST), Kohat 26000, Pakistan.
[2]Department of Microbiology, Kohat University of Science and Technology (KUST), Kohat 26000, Pakistan.
[3]Department of Plant Sciences, Kohat University of Science and Technology (KUST), Kohat 26000, Pakistan.
[4]Department of Well-being Resources, College of Agriculture and Life Science, Sunchon National University, Suncheon, Korea.

Accepted 10 August, 2012

Rice seedling of different transgenic lines (T-99, T-112, T-115 and T-121) were grown in sand culture with salt concentration of 0, 50, 100 and 150 mM to determine the effect of salinity on growth, amino acid, and ion contents. It was observed that all the lines could tolerate concentration of up to 50 mM of salt solution. The lines (T-99, T-112 and T-115) were more salt tolerant even at a concentration of 100 mM of NaCl with their relative growth rate (RGR), and net assimilation rate (NAR) were unaffected as compared to the control. With the increase in salt concentrations, the proline contents increased for all the transgenic lines. On the other hand, a gradual decrease in the contents of glycine and arginine were observed with the increase in salinity treatments. A massive increase in the Na^+ contents was measured in all the transgenic lines by application of the saline solutions. The K^+ and Ca^{++} contents decreased with the increase in salt concentrations. The present study shows that although the plants accumulated Na, the lines T-99, T-112 and T-115 could tolerate concentrations of up to 100 mM.

Key words: Salt stress, proline, glycine, Na, K, Ca, *Oryza sativa*.

INTRODUCTION

Salinity is one of the common problems of agricultural farming around the globe. Soils are generally considered as saline when the ECe is more than or equal to 4 ds/m equivalent to 40 mM NaCl (Munns and Tester, 2008). Plants may adapt to the saline conditions by excluding Na^+ or Cl^- and take up water from the soil (Munns, 2005). Plants which grow naturally in saline soils (hallophytes) are able to maintain exclusion even at high salinity. For instance, sea barley grass excludes both Na^+ and Cl^- even until 450 mM (Garthwaite et al., 2005). As salinity is a common feature of arable lands, the plants may have evolved mechanisms to tolerate it.

Different plants have varying degrees of tolerance against soil salinity. Generally, rice is the most sensitive and on the other hand, barley is the most tolerant cultivated plant (Aslam et al., 1993; Colmer et al., 2005).

The response of plants to salt stress occurs in two ways, 1st is the rapid response towards increased external osmotic pressure and a slower response due to deposition of Na^+ in leaves. As soon as the external osmotic pressure increases, the rate of shoot growth falls significantly. Overall, there is decrease in the rate of leaf expansion, and new leaves and buds emerge slower. Generally, the shoot dry weight is reduced (Munns and Termaat, 1986). Hence, the reduction in shoot weight is an important indicator/symptom of the salt tolerance in plants. Another important feature is the increased root to shoot growth ratio, although the roots are the first to face the stress (Cheeseman, 1988).

If the salinity sustains in the soil, the concentration of Na^+ may become toxic and leaves start dying. If the rate

*Corresponding author. E-mail: m.jamil@kohat.edu.pk.

of dying leaves or buds is more that the rate at which they emerge, the plant wilts (Munns et al., 1995). The genetic variations occur within the same species of the plant for the salinity tolerance. A number of candidate genes have been reported to be involved in the salt tolerance mechanisms (Zhu, 2002; Bartels and Sunkar, 2005; Munns, 2005).

Plant response to salinity is not that simple to understand. A number of amino acids, principally proline, have been reported to accumulate in the salt stressed plant tissues. proline is a proteinogenic amino acid, and is vital for primary metabolism. A number of studies have shown that its contents increase in response to environmental stresses, principally the salt stress (Kemble and Macpherson, 1954; Verbruggen and Hermans, 2008). arginine is one of the essential amino acid which is considered the main precursor of polyamines (Bouchereau et al., 1999). Polyamines are involved in many processes including cell cycle, cell division, morphogenesis in phytochrome and plant hormone mediated process and control of plant senescence (Walters, 2000). Arginine application has been reported to counter the salt stress on growth of plants (Qados, 2010).

Ion imbalance is another important consequence of the salinity induced responses in the plant. The imbalance mainly includes the increased accumulation of Na that may replace other ions such as K^+ and Ca^{++} (Yahya, 1998; Munns et al., 2006) and thus impairment of the general physiological activity of plants. Rice is an important staple crop of the people across the world. Because of increase in the degree of saline soils, there is need to search for the salinity tolerant rice varieties. Therefore, present study was designed to evaluate the salt tolerance level of different transgenic lines, that is, lines 99, 112, 115, and 121 on the basis of plant growth, amino acids and ion contents.

MATERIALS AND METHODS

Rice seeds of four transgenic lines (T-99, T-112, T-115 and T-121) were obtained through crossing between Shanghaixiangrenou and transgenic line. F-13 generation's seeds were used in this experiment.

Ten days seedlings of different transgenic lines were grown in plastic pots filled with sand and irrigated with full-strength Hoagland (Hoagland and Arnon, 1950) nutrient solution. The seedlings were subjected to 0 (control), 50, 100 and 150 mM NaCl stresses after 2 weeks. All measurements were made after 4 weeks, when plants had achieved a steady state.

Growth measurements

The relative growth rates (RGR) and net assimilation rate (NAR) were calculated by using the following formulas (Hunt, 1990):

1. $RGR = 1/W * \Delta W/\Delta T$
2. $NAR = 1/L_A * \Delta W/\Delta T$

Where, W, T and L_A are plant dry weight (g), time (day) and leaf area (cm^2), respectively.

Ion analysis

Leaf samples (25 mg) were digested by a mixture of concentrated sulphuric acid and hydrogen peroxide mixed in 2:1 (v/v) ratio. Na^+, K^+, and Ca^{2+} ion contents were estimated with a flame emission spectrophotometer by using the method of Awan and Salim (1997) with little modification.

Amino acid analysis

For proline extraction, leaf samples were extracted with 3% sulfosalicylic acid and then centrifuged at 3,000 *g* for 20 min. The supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 h and then absorbance was estimated at 520 nm by using the method of Bates et al. (1973). For arginine and glycine, leaf samples were homogenised with 2% sulfosalicylic acid and then homogenate was centrifuged at 15,000 *g* for 20 min. The supernatant was directly used for amino acid analysis by an amino acid analyzer. Contents of amino acids are expressed as nmol g^{-1} FW or nmol g^{-1} FW.

Statistical analysis

Analysis of variance was performed by using SAS statistical package while means were separated in column by Duncan's multiple range tests at 5 % level.

RESULTS

Effect of salinity on plant growth

All the transgenic lines were affected by salinity in terms of plant growth. There was a general trend of gradual decrease in the relative growth rate (RGR) as well as the net assimilation rate (NAR) of different transgenic lines in response to increase in the concentration of NaCl (Table 1). The different transgenic lines seemed to tolerate up to 100 mM of NaCl except line121 where there was significant decrease in RGR at 100 mM as compared to 50 mM and the control. At the salt concentration (150 mM), the RGR and NAR decreased significantly in all the lines. However, no significant difference was observed among the transgenic lines 99, 112, 115 for the impact of salinity on them.

Effect of salinity on the amino acids contents

With the increase in salinity, the proline contents increase for all the transgenic lines (Table 2). In the control conditions, the proline contents were highest in line 121, and same was true for line 121 also at concentration of 50 mM of salt. Highest levels of proline contents were observed in all the transgenic lines at the highest level of salinity.

On the other hand, a general depression in the contents

Table 1. The effect of salinity on the relative growth rate (RGR) and the net assimilation rate (NAR) of the different transgenic lines of rice.

Transgenic lines	NaCl (mM)	RGR* (g/g/day)	NAR** (g/m^2/day)
99	0	13.29[a]	14.20[a]
	50	11.31[a]	13.11[a]
	100	10.02[a]	12.02[a]
	150	5.60[b]	8.03[b]
112	0	16.01[a]	15.16[a]
	50	14.80[a]	14.29[a]
	100	12.74[a]	13.62[a]
	150	7.04[b]	9.01[b]
115	0	14.11[a]	13.29[a]
	50	12.06[a]	12.44[a]
	100	10.55[a]	11.01[a]
	150	4.99[b]	8.19[b]
121	0	15.29[a]	12.90[a]
	50	13.17[a]	11.35[a]
	100	6.05[b]	9.50[a]
	150	4.92[b]	6.88[b]

Means are separated by Duncan's multiple range test where the different small letters indicate significant difference (5% level).

Table 2. The effect of salinity on proline, glycine and arginine contents of the different transgenic lines of rice.

Transgenic lines	NaCl (mM)	Proline	Glycine	Arginine
99	0	4.15 ± 0.09	3.97 ± 0.17	4.02 ± 0.19
	50	6.08 ± 0.11	3.00 ± 0.04	4.00 ± 0.05
	100	10.11 ± 0.04	2.81 ± 0.07	1.07 ± 0.11
	150	17.01 ± 0.07	2.10 ± 0.19	0.99 ± 0.14
112	0	3.77 ± 0.01	5.00 ± 0.10	3.20 ± 0.11
	50	5.51 ± 0.06	4.33 ± 0.20	2.24 ± 0.03
	100	8.89 ± 0.13	3.12 ± 0.01	0.97 ± 0.10
	150	14.90 ± 0.09	2.03 ± 0.11	0.81 ± 0.26
115	0	4.00 ± 0.10	3.72 ± 0.14	4.40 ± 0.29
	50	6.88 ± 0.01	3.22 ± 0.14	3.63 ± 0.21
	100	11.58 ± 0.08	2.26 ± 0.02	1.11 ± 0.01
	150	13.76 ± 0.04	1.94 ± 0.10	1.00 ± 0.17
121	0	5.02 ± 0.05	4.77 ± 0.15	2.95 ± 0.01
	50	7.77 ± 0.01	3.87 ± 0.22	2.80 ± 0.01
	100	9.41 ± 0.14	2.66 ± 0.17	1.55 ± 0.24
	150	16.05 ± 0.08	2.20 ± 0.13	0.30 ± 0.04

Each value is the mean ± SE.

of glycine was observed with the increase in salinity treatments in all the transgenic lines (Table 2). This depression in glycine contents was more pronounced in the higher NaCl concentrations. Similarly, the contents

Table 3. The effect of salinity on potassium (K), sodium (Na), and calcium (Ca) contents of the different transgenic lines of rice.

Transgenic lines	NaCl (mM)	K (mg/g dry weight)	Na (mg/g dry weight)	Ca (mg/g dry weight)
99	0	2.22 ± 0.13	0.99 ± 0.01	4.40 ± 0.04
	50	1.80 ± 0.24	4.32 ± 0.08	2.33 ± 0.01
	100	1.82 ± 0.16	5.98 ± 0.05	2.28 ± 0.05
	150	1.01 ± 0.19	7.01 ± 0.05	1.93 ± 0.04
112	0	4.04 ± 0.01	0.58 ± 0.02	5.16 ± 0.01
	50	3.63 ± 0.22	3.90 ± 0.03	2.99 ± 0.08
	100	3.62 ± 0.18	6.87 ± 0.09	2.70 ± 0.06
	150	1.76 ± 0.05	8.55 ± 0.04	2.00 ± 0.05
115	0	2.37 ± 0.17	1.46 ± 0.01	3.94 ± 0.07
	50	2.49 ± 0.26	4.09 ± 0.14	1.95 ± 0.06
	100	1.45 ± 0.06	7.72 ± 0.06	1.82 ± 0.11
	150	0.63 ± 0.29	11.91 ± 0.10	1.06 ± 0.04
121	0	3.59 ± 0.08	0.79 ± 0.13	4.88 ± 0.06
	50	3.60 ± 0.18	3.99 ± 0.18	2.03 ± 0.15
	100	1.70 ± 0.21	7.40 ± 0.06	2.11 ± 0.10
	150	0.99 ± 0.24	9.01 ± 0.05	1.06 ± 0.09

Each value is the mean ± SE.

of arginine were considerably reduced by the different treatments of the salinity especially at the 100 and 150 mM concentrations (Table 2).

Effect of salinity on the ion contents

An enormous increase in the Na^+-contents was observed in all the transgenic lines by application of the saline solutions (Table 3). Na^+ contents were highest at the highest concentration of NaCl solution. The highest Na^+ contents were observed in line 115 among all the lines during all the salinity treatments.

K^+ contents decreased with the application of saline solution (Table 3). There was no considerable change in K+ contents between 50 and 100 mM application of NaCl solution for the transgenic line 99. Similar was the case of the line 112. However, there was no decrease in the K^+ contents between 0 and 50 mM NaCl application for the transgenic line 121. The lowest contents of K^+ were measured in line 115 at the two higher doses of the salt solutions. Similar kind of results was noticed for Ca^{++} ions with the increasing level of salt concentrations.

DISCUSSION

Rice is one of the most susceptible cultivated crops grown around the world. The crop is usually cultivated in heavy texture soil with high water holding capacity. As the crop requires extensive water for its growth and development, it is generally cultivated in the irrigated areas. Irrigation water generally augments the amount of salt in the soil leading to increased salinity. Furthermore, the increased salinity may cause decrease in yield by damaging the crop growth and development. Therefore, it is of immense importance to improve the salt tolerance/resistance of the rice cultivars. The present study is based on screening the four transgenic lines for their resistance towards the salt stress. All the lines tested so far showed some level of salt resistance at least to salt stress ≤ 50 mM of salt. Three of the lines (99, 112 and 115) were highly salt tolerant avoiding stress even at 100 mM of salt in terms of their growth. The ability to tolerate or resist salt stress may make them suitable to study further for the genetic basis of the salt resistance which is an important perspective of the study. Salt tolerance of these varieties may be due to additive gene effects. However, the basic molecular mechanism of salt tolerance has hardly been experimentally investigated.

Generally, free amino acids accumulate in plants exposed to salt stress (Ashraf and Tufail, 1995). The present study showed accumulation of proline contents in the plant tissues of the various transgenic rice lines confronted with salt stress. Although, the exact role of proline in salt metabolism is not known, a number of explanations can be done. The early studies have proposed that proline may be a reactive oxygen species (ROS) scavenger (Smirnoff and Cumbes, 1989). Proline accumulation may provide a way to buffer cystolic pH and to balance cell redox status (Verbruggen and Hermans,

2008) because salinity and drought are known to induce the oxidative stress. Another explanation can be part of the signal influencing adaptive responses (Maggio et al., 2002). In anyway, the proline accumulation is due to increased synthesis and reduced degradation. In overall, the role of proline during stress is poorly understood (Rentsch et al., 1996). However, the present study shows a depression in the contents of some of the amino acids. The contents of glycine and arginine reduced significantly in all the transgenic lines specifically at 100 mM of salt and at the concentrations mentioned earlier. This depression seems to be related to the artificial salt stress imposed on the plants which needs further investigation.

The present study shows that the different lines show accumulation of Na^+ ion which increases with increase in the salt stress. In fact, the salt damage on plants may be depending on both ionic as well as osmotic stresses (Tester and Davenport, 2003). Accumulation of Na^+ may damage the plant tissues. Higher amount of Na^+ may hamper the uptake of other nutrients by interfering with transporters in the root plasma membrane, such as K^+-selective ion channels (Tester and Davenport, 2003). This may be why the contents of K^+ ions and Ca^{++} ions are reduced at higher salt treatments above 50 mM in the present study. The K^+ ion contents are crucial for growth through activation of multiple enzymes in the plants (Blaha et al., 2000).

In conclusion, the transgenic lines studied here present a moderate level of salt tolerance which is effective at least up to 50 mM of the salt concentration on the basis of plant growth, amino acids and ion contents.

ACKNOWLEDGEMENT

This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (Grant Number: KRF-2007-331-F00004)

REFERENCES

Ashraf M, Tufail M (1995). Variation in salinity tolerance in sunflower (*Helianthus annum* L.). J. Agron. Crop Sci. 174:351-362.

Aslam M, Qureshi RH, Ahmed N (1993). A rapid screening technique for salt tolerance in rice (*Oryza sativa* L.). Plant Soil 150:99-107.

Awan JA, Salim UR (1997). Food analysis manual. Unitech Communication, Faisalabad, Pakistan.

Bartels D, Sunkar R (2005). Drought and salt tolerance in plants. Crit. Rev. Plant Sci. 24:23-58.

Bates LS, Waldren SP, Teare ID (1973). Rapid determination of free proline for water-stress studies. Plant Soil 39:205-207.

Blaha G, Stelzl U, Spahn CMT, Agrawal RK, Frank J, Nierhaus KH (2000). Preparation of functional ribosomal complexes and effect of buffer conditions on tRNA positions observed by cryoelectron microscopy. Methods Enzymol. 317:292-309.

Bouchereau A, Aziz A, Larher F, Martin-Tanguy J (1999). Polyamines and environmental challenges: recent development. Plant Sci. 140:103-125.

Cheeseman JM (1988). Mechanisms of salinity tolerance in plants. Plant Physiol. 87:547.

Colmer TD, Munns R, Flowers TJ (2005). Improving salt tolerance of wheat and barley: future prospects. Aust. J. Exp. Agric. 45:1425-1444.

Garthwaite AJ, Von Bothmer R, Colmer TD (2005). Salt tolerance in wild *Hordeum* species is associated with restricted entry of Na^+ and Cl^- into the shoots. J. Exp. Bot. 56:2365.

Hoagland DR, Arnon DI (1950). The water-culture method of growing plants without soil. California Agr. Exp. Stat. Circ. 347:1-32.

Kemble AR, Macpherson HT (1954). Liberation of amino acids in perennial rye grass during wilting. Biochem. J. 58:46.

Maggio A, Miyazaki S, Veronese P, Fujita T, Ibeas JI, Damsz B, Narasimhan ML, Hasegawa PM, Joly RJ, Bressan RA (2002). Does proline accumulation play an active role in stress induced growth reduction? Plant J. 31:699-712.

Munns R (2005). Genes and salt tolerance: bringing them together. New Phytol. 167:645-663.

Munns R, James RA, Läuchli A (2006). Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Bot. 57:1025.

Munns R, Schachtman DP, Condon AG (1995). The significance of a two-phase growth response to salinity in wheat and barley. Funct. Plant Biol. 22:561-569.

Munns R, Termaat A (1986). Whole-plant responses to salinity. Funct. Plant Biol. 13:143-160.

Munns R, Tester M (2008). Mechanisms of salinity tolerance. Ann. Rev. Plant Biol. 59:651-681.

Qados AMSA (2010). Effect of arginine on growth, nutrient composition, yield and nutritional value of mung bean plants grown under salinity stress. Nat. Sci. 8:30-42.

Rentsch D, Hirner B, Schmelzer E, Frommer WB (1996). Salt stress-induced proline transporters and salt stress-repressed broad specificity amino acid permeases identified by suppression of a yeast amino acid permease-targeting mutant. Plant Cell Online 8:1437.

Smirnoff N, Cumbes QJ (1989). Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 28:1057-1060.

Tester M, Davenport R (2003). Na^+ tolerance and Na^+ transport in higher plants. Ann. Bot. 91:503.

Verbruggen N, Hermans C (2008). Proline accumulation in plants: a review. Amino Acids 35:753-759.

Walters DR (2000). Polyamines in plant-microbe interactions. Physiol. Mol. Plant Pathol. 57:137-146.

Yahya A (1998). Salinity effects on growth and on uptake and distribution of sodium and some essential mineral nutrients in sesame. J. Plant Nutr. 21:1439-1451.

Zhu JK (2002). Salt and drought stress signal transduction in plants. Ann. Rev. Plant Biol. 53:247-273.