



Study of Protein Accumulation in Some Native Rice Genotypes Under Salinity Stress

KEYWORDS

Rice, *Oryza sativa*, salt tolerance, total protein, salinity stress

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ABSTRACT *Salinity is one of the most important abiotic stresses that limit growth, development, productivity and yield in crop plants. Rice plant is glycophytic in nature but some genotypes have developed adaptations to tolerate moderate levels of salt stress. Salt stress is expressed with the help of both structural and physiological adaptations. Production of stress proteins is one such adaptation thus increasing total protein content in salt stressed plants. A pot experiment was carried out in the first crop season of 2013 to examine the variation in total protein content under salt stress in certain native rice cultivars of Kerala state of India. The rice plants were exposed to increasing salinity concentrations (0, 10, 30, 50, 70, 100 and 200 mM NaCl) progressively. The present study showed that there was a significant increase in protein accumulation in all the rice genotypes studied, in relation to progressive salt stress. The percentage of increase was significantly higher in salt sensitive genotypes.*

INTRODUCTION

A wide range of environmental stresses like high or low temperature, drought, alkalinity, salinity, UV stress and pathogen infection are potentially harmful to plants (Van Breusegem et al., 2001). Among those, salt affected soil or water is one of the major and serious abiotic stresses that affect agricultural land and resulting in significant loss of crop yield by reduced plant growth, development and productivity worldwide (Bhandal and Malik, 1988; Allakhverdiev et al., 2000; Blaha et al., 2000; Tester and Davenport, 2003; Munns et al., 2006; Koca et al., 2007; Tuteja, 2007; Munns and Tester 2008; Siringam et al., 2011). It is estimated that about 20% of the earth's land mass and nearly half of all irrigated land are affected severely by salinity. Increased salinization of arable land is expected to have devastating global effects, with predictions of 30% land loss within the next 25 years, and up to 50% by the year 2050 (Wang et al., 2003). Rice is a major cereal crop plant of the world, especially Asia, providing carbohydrate food source for more than half of the world's population (Ma et al., 2007). It has been predicted that the demand for rice in the world will increase from 560 million tons to 780 million tons by the year 2020 (Shabbir et al., 2001). It is considered as a salt sensitive crop plant and salinity affects almost all stages of the growth and development of rice plant (Maas and Hoffman, 1977; Shannon et al., 1998). The crop response to salinity varies with growth stages, concentration and duration of exposure to salt stress. In almost all the commonly cultivated rice types, young seedlings were very sensitive to salinity (Lutts et al., 1996).

In general, a high concentration of salt causes ion imbalance, hyperosmotic stress and oxidative damages to the plants (Zhu, 2002). The deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution, nutritional imbalance, specific ion effect or a combination of any of these factors (Ashraf, 1994). During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis and energy and lipid metabolisms are affected. The earliest response is a reduction in the rate of leaf surface expansion followed by cessation of expansion as the stress intensifies but growth resumes when the stress is relieved (Parida and Das 2005).

Salinity is generally detrimental to plant growth that adversely affects the metabolism of plants and causes important modifications in gene expression in plants to survive in the stress conditions. Such modifications may lead to the accumulation or exhaustion of certain metabolites resulting in an imbalance in the levels of a relatively small set of cellular proteins, which could increase, decrease, appear or disappear after salt treatment. Over the past few years, much attention has been concentrated on resolving the identity of salt stress proteins, in order to identify and understand the role of proteins in rice salt tolerance and the actual mechanism of salt tolerance. A number of proteins induced by salt stress, reflecting the complexity of biochemical and physiological responses have been identified. There are many reports showing that these protein changes are accompanied with the biological changes of the adaptation process, which make the organism more fit in the altered environment (Singh et al., 1985; Hurkman et al., 1988). Several proteins have been characterized to play prominent roles in the regulation of K^+ and/or Na^+ fluxes (Maathuis and Ammtmann, 1999). However, most of them do not always confer tolerance to salinity (de Souza Filho et al., 2003). Changes in protein profiles also depend on the plant parts studied and the nature of the plant species (Apse et al., 1999; Shi et al. 2000; Ashraf and Harris, 2004).

In the case of rice there is an accumulation of several stress proteins, such as the RAB family (Mundy and Chua, 1988), the dehydrin family (Bradford and Chandler, 1992) and the LEA family of proteins (Mundy and Chua, 1988; Moons et al., 1998) and their transcripts during exposure to salt stress. Proteins that accumulate in plants under saline conditions may also provide a storage form of nitrogen that is re-utilized later (Singh et al., 1987) and may play a role in osmotic adjustment. They may be synthesized de novo in response to salt stress or may be present constitutively at low concentration (Pareek et al., 1997). In higher plants, osmotic stress induces several proteins in vegetative tissues, which are related to late-embryogenesis-abundant (LEA) proteins. The correlation between LEA protein accumulation in vegetative tissues and stress tolerance indicates its protective role under dehydration stress (Ingram and Bartels, 1996). Engineered rice plants overex-

pressing a barley LEA gene, HVA1, under the control of rice actin 1 promoter showed better stress tolerance than the wild type (Xu et al., 1996). It has been reported that a number of proteins induced by salinity are cytoplasmic which can cause alterations in cytoplasmic viscosity of the cells (Hasegawa et al., 2000). A higher content of soluble proteins has been observed in salt tolerant cultivars of barley, sunflower, finger millet and rice also (Ashraf and Harris, 2004). Several salt induced proteins have been identified in plant species and it is suggested that stress proteins could be used as important molecular markers for improvement of salt tolerance using genetic engineering (Ashraf and Harris, 2004; Pareek et al., 1997).

Other consequences of salt stress include disturbances in the integrity of cell membrane and organelles, resulting in plant growth reduction and abnormal development prior to plant death and changes in the levels of growth regulators, alterations in metabolic activities including photosynthesis and increased production of reactive oxygen species (Zhu, 2002; Davenport et al., 2005; Quintero et al., 2007; Siringam et al., 2011). Additional biochemical changes that have been observed in plants grown under salt stress include altered concentrations of total soluble carbohydrates, total phenols, glycine betaine, proline (Lacerda et al., 2001; Ashraf and Foolad, 2007), chlorophyll (Netono et al., 2004) and total proteins (Lunde et al., 2007). Salinity stress triggers the expression of several osmoreponsive genes and proteins in rice tissues (Chourey et al., 2003). The present experiment is intended to study the effect of salinity stress in total protein content in some native rice cultivars of North Kerala, India.

MATERIALS AND METHODS

Germination of seedlings, planting and experimental treatment

The experiment was conducted in the experimental rainout poly house of Department of Botany, University of Calicut, Kerala, India located at 11°35'N latitude and 75°48'E longitude in the first crop season of 2013. Seven native cultivars of rice including five cultivars namely Orthadian, Orkazhama, Kuthiru, Kuttusan and Chowarian collected from one of the saline rice habitats of Kerala and two native rice cultivars namely Kunhutty and Veliyan collected from one of the non-saline rice habitats of Kerala were used for the study. Enough number of good caryopses were taken from single plants and washed in running tap water to remove infected and unfilled grains and dust particles. The seeds were soaked in distilled water and allowed to germinate in 10cm diameter Petri dishes covered with lid under room temperature. The water was changed every day. The seeds started to germinate from the third day. On 10th day, required numbers of the germinated seedlings were transferred to coloured plastic pots of 25cm diameter filled with paddy soil mixed with enriched compost in 3:1 ratio. Two seedlings were initially planted per pot and after establishment of the seedlings the smaller among the two were removed. The plants were maintained in the experimental poly house under wetland conditions in Randomized Block Design with three replications always maintaining 3cm of water above the soil level. The soil was fertilized with 1g N: P: K = 18: 18: 18 per pot at fortnightly intervals starting from the 30th day. Weeding was done manually whenever required. The experimental treatment was started from the 45th day onwards starting from 10mM (0.91dSm⁻¹) to 200mM (18.26 dSm⁻¹) aqueous solution of sodium chloride as detailed in Table 1. The experimental treatment was applied at par with the variation in salinity in the saline rice tracts of the study area where rice cultivation starts in the

paddy fields flooded with water by the beginning of the south west monsoon in the month of June and with almost negligible salinity and the salinity level gradually increases as the crop progresses due to the retreat of the monsoon.

Table 1. Salinity treatment details

Sl. No.	Treatment
T1	Control
T2	10mM (0.91dSm ⁻¹) on 45 th day
T3	10mM (0.91dSm ⁻¹) on 45 th day & 30mM (2.74 dSm ⁻¹) on 53 rd day
T4	10mM (0.91dSm ⁻¹) on 45 th day, 30mM (2.74 dSm ⁻¹) on 53 rd day & 50mM (4.57 dSm ⁻¹) on 61 st day
T5	10mM (0.91dSm ⁻¹) on 45 th day, 30mM (2.74 dSm ⁻¹) on 53 rd day, 50mM (4.57 dSm ⁻¹) on 61 st day & 70mM (6.39 dSm ⁻¹) on 69 th day
T6	10mM (0.91dSm ⁻¹) on 45 th day, 30mM (2.74 dSm ⁻¹) on 53 rd day, 50mM (4.57 dSm ⁻¹) on 61 st day, 70mM (6.39 dSm ⁻¹) on 69 th day & 100mM (9.13 dSm ⁻¹) on 77 th day
T7	10mM (0.91dSm ⁻¹) on 45 th day, 30mM (2.74 dSm ⁻¹) on 53 rd day, 50mM (4.57 dSm ⁻¹) on 61 st day, 70mM (6.39 dSm ⁻¹) on 69 th day, 100mM (9.13 dSm ⁻¹) on 77 th day & 200mM (18.26 dSm ⁻¹) on 85 th day

Protein extraction

100mg each of rice leaves from the randomized samples of each treatment and control were weighed separately using an electronic balance (Sartorius, Germany). The weighed tissues were ground to fine powder using liquid nitrogen in a clean pre-chilled mortar and pestle. The tissue was homogenised in ice-cold extraction buffer (50mM Tris-HCl (w/v) (Himedia, India)- pH 8, 10mM NaCl (w/v) (Himedia, India), 1% SDS (w/v) (Himedia, India), 5% 2-mercaptoethanol (v/v) (Himedia, India), 0.1mM PMSF, 0.1mM DTT, mixed well and kept undisturbed in a refrigerator. Equal volume of 10% trichloro acetic acid (TCA 10%, w/v, Himedia, India) was added, mixed well and followed by centrifugation at 10,000g at 4°C for 15minutes (Sigma, Germany). Supernatant was decanted off and 2% TCA (w/v) was added to the residue and centrifuged again in the same conditions and supernatant was decanted off. The precipitate was washed with 80% acetone (v/v, Qualigens, India) to remove the pigments. Two washes were carried out in 80% acetone and final washing in anhydrous acetone. 5ml of 0.1N sodium hydroxide (0.1N NaOH, w/v, Himedia, India) was added to the pellet in each centrifuge tube and boiled for 5minutes in water bath, cooled and centrifuged. The supernatant was then transferred to test tubes and used for protein estimation with BSA as the standard. Extracts were stored at -20°C.

Determination of total soluble protein

Reagents used: A- 2% Sodium carbonate in 0.1N Sodium hydroxide; B- 0.5% Copper sulphate in 1% Potassium sodium tartarate, C- Alkaline Copper sulphate solution: Made by mixing 50 ml A and 1 ml of B prior to use; D - Folin-Ciocalteu reagent.

Procedure: Suitable aliquots were taken in duplicates from each preparation. Volume was made up to 1ml with double distilled water. Then 5ml of reagent C was added to each tube, mixed well and kept at room temperature for 10 minutes and 0.5ml 1N Folin-Ciocalteu reagent was added with immediate mixing. The tubes were kept for 30 minutes for colour development. Absorbance (Optical Density) was read at 700nm using a UV-Visible spectrophotometer (Thermo Scientific, USA).

Statistical analysis

The data were subjected to analysis of variance and the means were compared at $P \geq 0.05$. Data presented are mean \pm standard error.

RESULT AND DISCUSSION

The result shows that total protein content in all the rice genotypes is increased significantly with the increase of salinity stress (Table 2 & Fig. 1). Highest amount of total protein content was noticed in the cultivar Orthadian followed by Kuthiru, Chovvarian, Orkazhama, Kuttusan, Veliyan and Kunhutty. The genotypes Veliyan and Kunhutty were collected from one of the traditional non saline rice tracts of north Kerala, while the others were collected from one of the saline rice tracts of north Kerala. It shows that the rice cultivars collected from the saline rice tract showed higher quantum of total protein accumulation under salt stress. The cultivars Kuttusan and Veliyan showed significant increase in total protein content from 30 mM NaCl concentration, Orthadian, Chovvarian and Kuthiru from 50 mM concentration and Orkazhama and Kunhutty showed significant increase in protein content from 70 mM NaCl treatment onwards. The percentage of increase in total protein content was the highest in Chovvarian (20.72%) followed by Veliyan (19.82%) and Kunhutty (19.54%) (Table 2 & Fig. 2). The lowest percentage of increase in total protein content was shown by the cultivar Kuthiru.

Table 2. Variation in total protein content in response to progressive salt stress in the rice cultivars studied

Cultivars/ Treatments	Mean \pm SE	CD @5%	Percentage of increase
ORTHADIAN			
0mM	104.3 \pm 0.27	2.73	0.00
10mM	104.4 \pm 0.13		0.10
30mM	105.7 \pm 0.23		1.34
50mM	108.0 \pm 0.30*		3.55
70mM	111.8 \pm 0.26*		7.19
100mM	116.5 \pm 0.59*		11.70
200mM	121.4 \pm 0.39*		16.40
CHOVVARIAN			
0mM	91.2 \pm 0.36	2.63	0.00
10mM	92.2 \pm 0.33		1.10
30mM	93.1 \pm 0.26		2.08
50mM	94.1 \pm 0.26*		3.18
70mM	96.4 \pm 0.36*		5.70
100mM	102.1 \pm 0.27*		11.95
200mM	110.1 \pm 0.52*		20.72
KUTTUSAN			
0mM	89.5 \pm 0.46	2.71	0.00
10mM	91.0 \pm 0.40		1.68
30mM	93.4 \pm 0.30*		4.36
50mM	95.5 \pm 0.16*		6.70
70mM	97.4 \pm 0.30*		8.83
100mM	100.0 \pm 0.34*		11.73
200mM	106.3 \pm 0.33*		18.77
KUTHIRU			
0mM	107.5 \pm 0.46	3.16	0.00
10mM	107.9 \pm 0.20		0.37
30mM	109.5 \pm 0.36		1.86
50mM	111.7 \pm 0.30*		3.91
70mM	115.9 \pm 0.56*		7.81
100mM	119.2 \pm 0.30*		10.88
200mM	120.9 \pm 0.46*		12.47
ORKAZHAMA			
0mM	92.5 \pm 0.36	2.83	0.00
10mM	93.6 \pm 0.26		1.19
30mM	94.1 \pm 0.23		1.73
50mM	95.3 \pm 0.34		3.03
70mM	97.8 \pm 0.39*		5.73
100mM	100.5 \pm 0.47*		8.65
200mM	107.3 \pm 0.36*		16.00

KUNHUTTY			
0mM	81.9 \pm 0.26	3.67	0.00
10mM	82.5 \pm 0.33		0.73
30mM	83.3 \pm 0.26		1.71
50mM	84.7 \pm 0.39		3.42
70mM	86.1 \pm 0.30*		5.13
100mM	90.8 \pm 0.36*		10.87
200mM	97.9 \pm 0.92*		19.54
VELIYAN			
0mM	88.3 \pm 0.33	1.72	0.00
10mM	89.4 \pm 0.41		1.25
30mM	91.1 \pm 0.40*		3.17
50mM	93.4 \pm 0.26*		5.78
70mM	95.6 \pm 0.30*		8.27
100mM	100.6 \pm 0.43*		13.93
200mM	105.8 \pm 0.39*		19.82

Fig. 1. Variation in total protein content in response to progressive salt stress in the rice cultivars studied

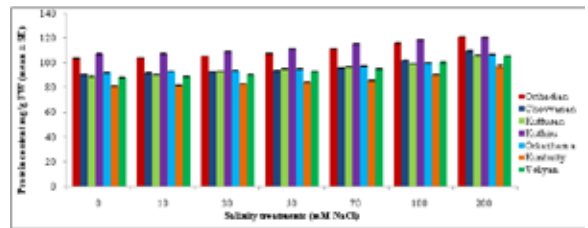
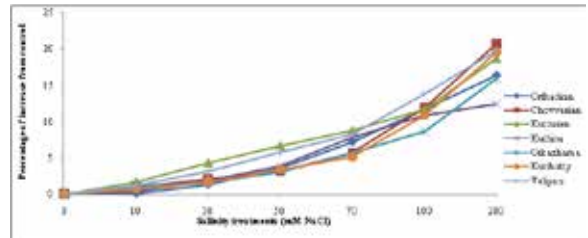


Fig. 2. Percentages of increase in total protein content in response to progressive salt stress in the rice cultivars studied



Rice plants are relatively susceptible to soil salinity as an abiotic stress (Flowers & Yeo, 1989; Gao et al., 2007). The change in the protein content is due to the influence of abiotic stresses and is relatively genotype dependent (Marschner, 1995). In the present experiment significant and progressive accumulation of soluble proteins in the plant cells in relation to increase in salt stress has been observed. The total protein content was estimated in order to find out genotypic variation in the accumulation of salt stress induced proteins. The present study showed that there was a significant increase in protein accumulation in all the rice genotypes studied, in relation to progressive salt stress (Table 2). The percentage of increase was significantly higher in salt sensitive genotypes. Earlier workers have also reported increase in total soluble protein content in rice under salinity stress. According to Chitteti and Peng (2007) the increase in protein content was due to reduction in proteolysis caused by salinity leading to slower depletion of reserve protein rather than enhancement in protein synthesis. Experiments with model plants like Arabidopsis and rice by earlier workers showed that salt stress was associated with stress responses and the regulation of post translational modifications (Yan et al., 2005; Jiang et al., 2007).

The accumulation of proteins in plants growing under salinity stress condition may provide a storage form of nitrogen that is re-utilized when stress is over and may play a role in osmotic adjustment. Increase in soluble protein

content may be due to synthesis of osmotin like proteins or structural proteins, in particular synthesis of proteins involved in the modification of cell wall (Amini and Ehsanpour, 2005).

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