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RESEARCH ARTICLE

VARIATION IN TOTAL SOLUBLE SUGAR CONTENT DUE TO SALINITY STRESS IN SOME NATIVE RICE CULTIVARS OF NORTH KERALA, INDIA

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ABSTRACT

In order to estimate soluble carbohydrate levels in rice leaves under different salinity stress levels, rice genotypes from both saline and non-saline rice tracts of North Kerala, India were exposed to 0, 10, 30, 50, 70, 100 and 200 mM of NaCl progressively starting from the 45th day after germination. The plants were maintained at the corresponding salinity levels during their further growth periods. Leaf samples were collected separately from the plants of each treatment on 90th day of growth and total soluble sugar content was estimated by phenol sulphuric acid method. Salinity caused accumulation of soluble sugar content in all the cultivars. High total soluble sugar concentration in the shoot of these rice cultivars is probably for adjusting osmotic potential and for better water uptake under salinity. These mechanisms help the plants to avoid tissue death and enable to continue their growth and development under saline conditions.

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INTRODUCTION

The explosive increase in world population, along with the continuing deterioration of arable land, scarcity of fresh water and increasing environmental stress pose serious threats to global agricultural production and food security. Soil salinity is one of the major a biotic stresses affecting plant growth and development leading to the reduction of crop productivity especially in rice, world-wide (Munns, 2002; Sahin *et al.*, 2002; Wang *et al.*, 2003). Soil salinity is one of the major abiotic stresses that affect plant productivity. In saltaffected soil, there are many salt contaminants, especially NaCl which readily dissolves in water to yield the toxic ions, sodium (Na^+) and chloride (Cl^-). Also, the water available in the salt contaminated soil is restricted, inducing strong osmotic stress (Castillo *et al.*, 2007; Pagter *et al.*, 2009). Na^+ is a small molecule that is easily absorbed into root tissues of higher plants and transported throughout plant organs, leading to toxic ion damage, osmotic stress and nutritional imbalance (Cha-um *et al.*, 2007; Siringam *et al.*, 2009). Root tissues are the first barrier which not only select nutrient ions but also guard against toxic ions. Excess of Na^+ in plant cells directly damages membrane systems and organelles, resulting in plant growth reduction and abnormal development prior to plant death (Essah *et al.*, 2003; Tester and Davenport, 2003; Davenport *et al.*, 2005; Quintero *et al.*, 2007). Rice has been classified as a glycophytic species, which is very sensitive to saline stress at both seedling and reproductive developmental stages, but tolerant at the vegetative phase (Abdullah *et al.*, 2001; Zeng *et al.*, 2001). Increasing salinity of agricultural irrigation water together with progressive salinization of agricultural land is of increasing importance to agriculture because it limits the distribution of plants in certain natural habitats and induces a wide range of adverse metabolic responses in higher plants. Rice is one of the most extensively grown crops of India in almost all parts. This crop is grown under diverse agro-ecological conditions as irrigated rice, upland rice, lowland rice and deep water rice. Sterility of rice panicles is very common in plants growing in a saline environment, thus reducing the yield of rice significantly. Salinity appears to affect two plant processes: water and ionic relations. During initial exposure to salinity, plants experience water stress, which in turn reduces leaf expansion. Due to longstanding exposure to salinity, plants will experience ionic stress, which can lead to premature senescence of adult leaves resulting in reduction in the photosynthetic area available to sustain continued growth and development and also in growth reduction of young leaves (Munns, 2002). Osmotic adjustment is effected by the accumulation of free amino acids, betaines, organic acids,

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proline and sugars in the roots and shoots and also in leaves and other parts of the plant (Hasegawa *et al*., 2000; Izanloo *et al*., 2008). The accumulation of sugars may play an important role in the plant defensive mechanisms of osmoregulation and energy preservation and these compounds serve as osmoprotectants and in some cases, stabilize biomolecules under stress conditions (Norwood *et al*., 2003; Minorsky, 2003; Morsy *et al*., 2007; Pattanagul and Thitisaksakul, 2008; Cha-um *et al*., 2009; Siringam *et al*., 2012). Interestingly, soluble sugars derived from photosynthesis and starch metabolism in '*Pokkali*', a salt tolerant rice cultivar have been reported as novel targets relating to salt tolerancedefence mechanisms (Theerawitaya *et al*., 2012). These osmolytes may also contribute to the stabilization of protein molecules and membranes (Hamilton and Heckathorn, 2001) or may serve as reserve for plant metabolism (Serrano and Gaxiola, 1994). The objective of the present study was to estimate the variation in the total soluble sugar content in relation to variation in NaCl induced salt stress in some native rice cultivars of North Kerala, India.

MATERIALSAND METHODS

Germination of seeds and transfer to the experiment site

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 *Chovvarian* 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 healthy and mature caryopses collected from a single plant in the case of each cultivar were taken and washed in running tap water to remove infected and unfilled grains and dust particles. The seeds were soaked in distilled water, 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, 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. Plants were grown in Randomized Block Design with three replications.

Experimental treatments and observations

The experimental treatment was started from the 45th day onwards starting from 10 mM to 200 mM aqueous solution of sodium chloride as detailed in Table 1.

Estimation of total soluble sugar content

Total soluble sugar content was determined by phenol sulphuric acid method (Dubois et al. 1956) using5% phenol (Qualigens, India) solution, standard D-glucose (1mg/ml) (Himedia, India) and 96% sulphuric acid (Merck, India).100mg of the fresh leaf tissue samples were cleaned properly, weighed separately using an electronic balance (Sartorius, Germany) and putinto a boiling tube. It was then hydrolysed by keeping the tubes in a boiling water bath for 3hours with 5ml of 2.5N HCl (Merck, India) and cooled to room temperature. It was then neutralised with solid sodium carbonate (Himedia, India) until the effervescence ceased. The volume was made up to 10mlwith distilled water and centrifuged at 5500 rpm for 5 minutes at 4°C using a refrigerated centrifuge (Sigma, Germany).Appropriate volume of the sample was taken in a separate test tube and made up the volume to 1ml with distilled water. 1ml of distilled water was also set as blank. 1ml of phenol solution was added to it followed by 5ml of 96% sulphuric acid. The mixture was thoroughly shaken using a vortex mixer and kept for 10minutes at room temperature. The contents in the tubes were shaken and placed in a water bath at 25-30°C for 20 minutes and the optical density was read at 490nm using a spectrophotometer (Thermo Scientific, USA). D-glucose (Himedia, India) (1mg/ml) was used as the standard. Amount of total soluble carbohydrates present in the sample solution was calculated using the standard graph.

Statistical analysis

The experimental design was randomized block design with three replicates per treatment. The data were subjected to analysis of variance (ANOVA) using *Microsoft Excel* statistical software package and the significance of variation compared at $P \geq 0.05$. Data are presented as mean ± standard error.

RESULTS AND DISCUSSION

Upon raising the salt concentration from 0 to 200 mM, the soluble sugar content in the fresh leaves of all the rice cultivars collected from saline and non- saline tracts increased significantly (Table 2 & Figure 1). However, the extent of increase was cultivar specific. The cultivar *Veliyan* showed the highest concentration of total soluble carbohydrate content at the highest salinity content applied followed by *Kuttusan* and *Orkazhama*. Among these, *Veliyan* is a rice cultivar collected from the non saline rice tract and the others collected from saline rice tract. The observation indicates that the potential to respond to salt stress in terms of production of soluble sugars is seen both in the cultivars collected from the saline and non saline rice tracts. In terms of percentage of increase in response to salt stress also, *Veliyan* showed the highest increase (44.27%) followed by *Orkazhama* (41.03%) and *Kuttusan* (37.76%). There is a tremendous hike in the percentage of increase in soluble sugar in all the genotypes. Genotypes such as *Kuttusan*, *Orkazhama* and *Veliyan* showed significant raise in soluble sugar concentration starting from 30 mM salinity treatment, while the other genotypes showed significant variation starting from 50 mM salinity level. From the data it is clear that there is a direct relationship between the increase in salinity concentration and the total soluble sugar content in the different rice cultivars studied. Carbohydrates produced by photosynthetic tissues are either transported to other organs as soluble sugars, or accumulated in leaves as soluble sugars and starch during different growth stages of plants. Under most types of abiotic stresses, the ability of plants to recover from stress normally increases with increasing concentrations of photosynthetic assimilates in

Table 1. Salinity treatment in Detail

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

Table 2. Concentration and percentage increase in the total sugar content of rice genotypes under different salinity levels

Cultivars/ Treatments	Mean ± SE (mg/g FW)	CD@5%	Percentage of increase
ORTHADIAN			
0mM	96.93±0.22		0.00
10mM	97.73±0.39		0.83
30mM	97.87±0.35		0.97
50mM	104.13±0.41*	2.88	7.43
70mM	108.27±0.41*		11.70
100mM	117.87±0.35*		21.60
200mM	122.13±0.33*		26.00
CHOVVARIAN			
0mM	94.00±0.17		0.00
10mM	94.53±0.13		0.56
30mM	95.07±0.33		1.14
50mM	104.40±0.46*	3.87	11.06
70mM	113.60±0.23*		20.85
100mM	120.80±0.91*		28.51
200mM	128.67±0.61*		36.88
KUTTUSAN			
0mM	97.47±0.18		0.00
10mM	97.60±0.17		0.13
30mM	100.40±0.09*		3.00
50mM	111.33±0.27*	1.85	14.21
70mM	121.60±0.26*		24.76
100mM	128.40±0.26*		31.73
200mM	134.27±0.31*		37.76
KUTHIRU			
0mM	94.27±0.18		0.00
10mM	94.67±0.13		0.42
30mM	94.93±0.13		0.70
50mM	103.20±0.31*	1.98	9.47
70mM	110.67±0.18*		17.40
100mM	117.47±0.44*		24.61
200mM	125.73±0.18*		33.37
ORKAZHAMA			
0mM	93.60±0.23		0.00
10mM	94.13±0.31		0.57
30mM	97.87±0.13*		4.56
50mM	109.20±0.17*	2.07	16.67
70mM	118.40±0.17*		26.50
100mM	126.67±0.43*		35.36
200mM	132.00±0.13*		41.03
KUNHUTTY			
0mM	96.00±0.15		0.00
10mM	96.00±0.09		0.00
30mM	97.73±0.27		1.80
50mM	107.47±0.22*	1.76	11.95
70mM	115.47±0.22*		20.28
100mM	122.00±0.26*		27.08
200mM	129.47±0.27*		34.86
VELIYAN			
0mM	96.67±0.05		0.00
10mM	96.93±0.18		0.27
30mM	98.53±0.13*		1.92
50mM	110.93±0.18*	1.72	14.75
70mM	121.47±0.18*		25.65
100mM	130.13±0.33*		34.61
200mM	139.47±0.31*		44.27

*shows significant variation at 5%

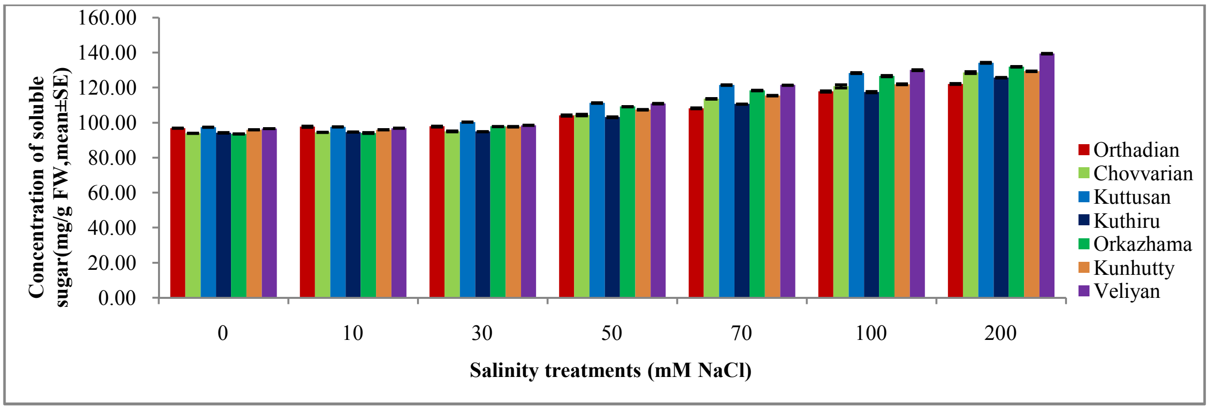

Figure 1. Variation in of total soluble sugar concentration under different salt concentration

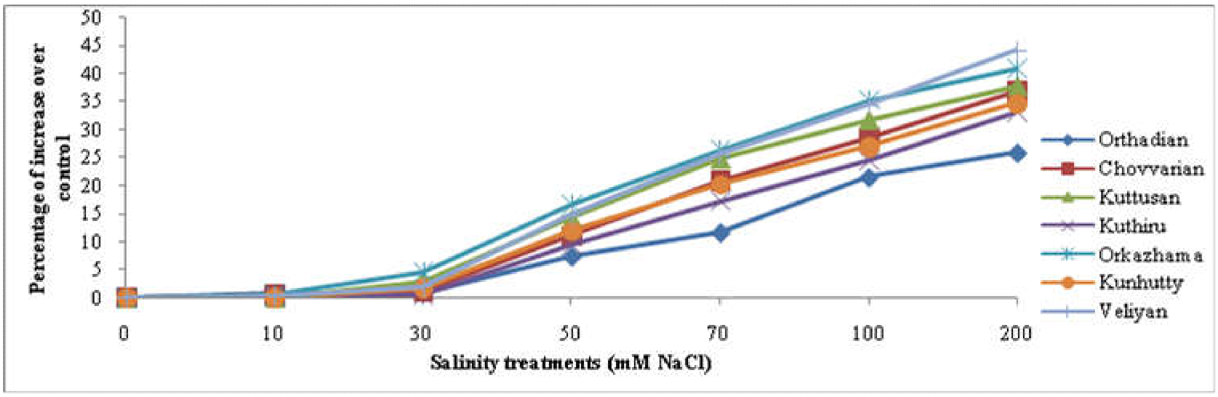

Figure 2. Percentage of increase in total soluble sugar content in the rice genotypes studied under different salt concentration

plant tissues during orafter stress. Higher accumulation of soluble carbohydrates in plant tissue under abiotic stresses has got a positive effect on plant survival (Bagheri and Sadeghipour, 2009; Naureen and Naqvi, 2010). Our present results show a remarkable increase in the total soluble carbohydrate content in the rice cultivars studied, in response to salt stress. In the present investigation, *Veliyan,* a cultivar from a traditional non saline rice tract showed the highest content of soluble sugars followed by *Kuttusan* and *Orkazhama* from a saline rice tract. The primary effect of salinity on plants is a drought effect or water deficit since it reduces the availability of free water to the plant. Plants try to reduce their osmotic potential via increasing mineral ion content and synthesis of compatible solutes to ensure better water uptake under salinity. Soluble carbohydrates are one of the important solutes that are synthesized and accumulated in cytosol under salt stress in plant cells. In the present experiment, the quantity of total soluble carbohydrates present was higher under saline conditions when compared to control in the case of all the genotypes. Increase of total soluble carbohydrate content probably resulted in better osmotic adjustment and maintained turgor for growth under salinity stress. Kerepsi and Galiba (2000) reported that carbohydrate changes were important because of their relationship with such physiological processes as photosynthesis, translocation and respiration. Soluble sugars have a complex essential role in plant metabolism. They act as the substrates for various biosynthetic processes, energy production and sensing and signalling systems. It has been reported that sugar flux may be the signal for metabolic regulation under abiotic stresses (Kishor *et al*., 2005). Soluble carbohydrates and starch, which accumulate under normal conditions before the stress commonly constitute the main resources for plants to supply energy during stress condition, as well as during Recovery (Khelil *et al*., 2007). Therefore, a higher concentration of carbohydrates in plant tissue is one of the important adaptive mechanisms (Dkhil and Denden, 2010). Reduction in plant biomass is also sometimes observed under severe salt stress, and this is possibly because of the decrease in carbohydrate accumulation caused by reduction in carbon assimilation (Moradi and Ismail, 2007; Pattanagul and Thitisaksakul, 2008). Soluble sugars, like hormones, can act as primary messengers and regulate signals that control the expression of different genes involved in plant growth and metabolism (Rolland *et al*., 2006; Chen, 2007). They regulate growth and metabolism by modulation of gene expression and enzyme activities in both sugar exporting (source) and importing (sink) tissues. The metabolic pathways for the synthesis of osmoprotectants like soluble sugars are very important in abiotic stresses.

They are up-regulated during various abiotic stresses (Umezawa *et al*., 2006). The leaf sheath would most likely be the primary site for an adaptive response such as osmotic adjustment. Osmotic adjustment can therefore be seen as a strategy to protect the meristematic tissues during water and osmotic stress. In rice and other grasses the meristematic tissue is enclosed by the leaf sheath and it is expected that the leaf sheath would exhibit a relatively higher rate of osmotic adjustment than the leaf blade or the root (Volaire, 2003). The accumulation of soluble sugars more in the leaf sheath can be attributed to the highest expression of the salt responsive genes in the leaf sheath in response to salt stress (Claes *et al*., 1990). There are reports that the accumulation of soluble sugar and other osmoprotectants is found to be more in young leaves than older leaves and this suggests that young leaves have higher ability to adapt to the change in soil moisture (Watanabe *et al*., 2000; Cechin *et al*., 2006).

Higher non-structural carbohydrate concentration in plant tissue under abiotic stresses was known to have positive effects on plant survival of stress and recovery (Bagheri and Sadeghipour, 2009; Naureen and Naqvi, 2010). These carbohydrates could provide important resources for energy supply under abiotic stresses when carbon assimilation is reduced (Khelil *et al*., 2007) and is considered as important adaptation strategies in rice (Das *et al*., 2005; Dkhil and Denden, 2010). In this study we observed that soluble sugar content varied significantly with the rise in the salinity levels. Since the sugars are osmo-protectants, the more the sugar concentration the more the plant is resistant to the abiotic stress. Higher total soluble sugar levels are essential for osmotic adjustment in the shoot of salt tolerant plants and therefore the genotypes with higher accumulation of soluble sugars are capable of suppressing the osmotic and toxic effects of salinity and can show better growth performance under salinity stress and such genotypes can be used as planting material for saline tracts.

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