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# Effects of sugar metabolism on NaCl stress in rice

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**Abstract**

Tow rice genotypes, salt-tolerant FL478 and salt sensitive IR29 from the IRRI (International Rice Research Institute) were used in this study. For the greenhouse trial, rice plants were grown in a hydroponic culture at ABRII (Agricultural Biotechnology Research Institute of Iran). The average greenhouse temperature and humidity over the growth season was 28 ºC and 57%, respectively. A complete randomized design was used, with 4 replicates for each treatment. All samples were collected for metabolite measurements at IPK (Leibniz Institute of Plant Genetics and Crop Plant Research-Germany). Mean comparisons indicated that $Na^+$ accumulation was more in blade of IR29 in salinity condition which showed that this genotype was not able to salt exclusion. The salt stress caused to reduce glucose in root of salt sensitive genotype (IR29) but in Fl478 it increased in salt stress. Glucose can play an important role as an osmotic solution in mitigation of salt effects in tolerant genotype. Fructose variations and its partitioning over the plant organs were similar to glucose in every genotype at stress and control condition. Total sucrose content in above ground organs was too more than root. Sucrose content in stress and control conditions were similar in IR478. It could be related to more tolerance of this genotype but this variation in IR29 was more accelerate. Starch content didn't have significant difference in blade and root in control and stress conditions. Its content was very low in different organs and was less than glucose, fructose and sucrose content.

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## Introduction

Salinity limits plants distribution throughout natural habitats and is a severe agricultural problem in many regions on the Earth. Salt stress involves osmotic and ionic components as a consequence of soil osmotic pressure increase and a higher concentration of potentially toxic ions. Generally, plant responses to salinity are evaluated by their growth, ion balance, compatible organic solutes synthesis and osmotic adjustment (Sanchez-Blanco *et al.,* 1991). Salinity may cause disturbances in plant water balance, including reduction in pressure potential, growth inhibition, stomatal closure and photosynthesis reduction (Poljakoff-Mayber, 1982).

Salinity is one of the major abiotic factors limiting global agricultural productivity, rendering an estimated one-third of the world's irrigated land unsuitable for crops (Frommer *et al.*, 1999). Salt stress in plant cells is primarily caused by a combination of osmotic and ionic stress resulting from high Na+ concentration in the soil (Hasegawa *et al.*, 2000). Metabolic acclimation via the accumulation of compatible solutes is often regarded as a basic strategy for the protection and survival of plants under abiotic stress (Bohnert and Jensen, 1996; Hanson and Hitz, 1982; Sakamoto and Murata, 2000; Shabala and Cuin, 2006). About 20% of irrigated agricultural land is considered to be saline (Flowers and Yeo, 1995). Salt-affected soils in arid and semi-arid regions of Asia, Africa, and South America cause considerable agronomic problems. In Asia alone, 21.5 million ha of land area is thought to be salt affected (Sahi *et al.*, 2006). Different varieties of a particular plant species exhibit a high degree of variation in salt tolerance (Chen *et al.*, 2007; Epstein *et al.*, 1980).

Rice is an important food crop for the entire world population. While active efforts are being made to increase rice productivity, a considerable amount of rice biomass for which genetic potential exists in the present-day cultivars is not harvested under field conditions, primarily because of the sensitivity of this crop to various stresses (Widawsky and O'Toole, 1990; Shimamoto, 1999; Minhas and Grover, 1999). Rice is a salt-sensitive crop (Grover and Pental, 2003). This research discusses the effect of salinity on sugar metabolism in two salt sensitive and tolerant rice genotypes in perspective of how soluble and insoluble sugars accumulate in different organs and the role of proline in salinity stress as a metabolite in mitigation of stress effect or as a symptom of stress.

## Materials and methods

*Plant materials and growth conditions*

Tow rice genotypes: salt-tolerant FL478 and salt sensitive IR29 from the IRRI (International Rice Research Institute) were used in this study. For the greenhouse trial, rice plants were grown in a hydroponic culture as described before (Chen *et al.,* 2005) at ABRII (Agricultural Biotechnology Research Institute of Iran). The average greenhouse temperature and humidity over the growth season was 28 °C and 57%, respectively. A complete randomized design was used, with 4 replicates for each treatment. Ushida's solution was used in both control and salt-treated plants. Salt treatment was applied at 80 mM NaCl commencing 1 month after transferring the seedling to greenhouse.

Genotypes fresh and dry weight was measured on 1, 2, 3, 4, 5, 7, 10, 15 days after salt treatment to record significant difference between control and stress conditions. After 15 days of salt treatment and recording significant difference between control and salt treatments in sensitive genotype, leaf blade, leaf sheath, shoot and root samples were collected for metabolite measurements at IPK (Leibniz Institute of Plant Genetics and Crop Plant Research-Germany). Plants were harvested, fresh mass weighed and dry mass determined after 72 h at 65 °C in a Dryer. All data was analyzed by SAS software.

*$Na^+$ and $K^+$ content*

Plants samples was dried in 70 °C for 3 days and 0.1 g of them add to 15 ml falcon and 10 ml acetic acid 0.1 M also was added and put them in Ban Mari for 4 hour in 80 °C. After that they centrifuged in 10000 rpm and supernatant was read by Flame photometer.

*Determination of soluble and insoluble sugars*

Sugars were determined photometrically in enzyme-coupled assay. Samples were freeze-dried and ground to a fine powder. Samples (50 mg) of plant material were incubated for 60 min at 80 °C in 0.7 ml 80% ethanol. After centrifugation of homogenate for 10 min at 14,000 rpm at 4 °C, the supernatant was collected. The extracts were dried under reduced pressure at 40 °C for around 60-90 min and resolved in 250 µl distilled water. Produced glucose, fructose, sucrose were measured as described in (Hajirezaei *et al.,* 2000).

*$Na^+$ and $K^+$ content in blades and roots*

Analysis of variance showed that the effect of genotypes and salinity and their interaction effects on $Na^+$ content of blade and roots had significant different at the %5 level of probability (Table 1 & 2). Mean comparisons indicated that $Na^+$ accumulation was more in blade of IR29 in salinity condition (Table 3 & 5) which showed that this genotype was not able to salt exclusion. Therefore, it is more sensitive to salt stress than another genotype that able to reduce $Na^+$ content in its blade.

**Results and discussion**

The most amount of $Na^+$ has seen in IR29 roots in salinity condition about the threefold more than FL478 that indicate this genotype couldn't prevent $Na^+$ uptake whereas there was not significant difference between tow genotypes in $K^+$ uptake characteristics (Table 4 & 6).

*Soluble and insoluble sugars*

*Glucose*

The leaf blade glucose content was decreased significantly in IR29 in stress condition and it was similar to glucose variation in root (Table 3 & 4). Results showed that there was higher content of glucose in root both in control and stress conditions in comparison with above ground tissue. It might be related to glucose transfer from photosynthesis organ to underground tissue. The relative difference of glucose content in root was higher than blade in IR29 between control and stress conditions.

**Table 1.** Analysis of variance for sugars, $Na^+$ and $K^+$ content in rice blades.

| S.O.V. | D.F. | Mean of Square | | | | | |
|---|---|---|---|---|---|---|---|
| | | Blade Glucose | Blade Fructose | Blade Sucrose | Blade Starch | Blade $Na^+$ | Blade $K^+$ |
| Genotype(G) | 1 | 28.409$^{**}$ | 28.112$^{**}$ | 74.235$^{n.s.}$ | 0.386$^{*}$ | 94.603$^{**}$ | 167.733$^{n.s.}$ |
| Salinity (S) | 1 | 8.775$^{**}$ | 7.211$^{**}$ | 238.844$^{**}$ | 0.076$^{n.s.}$ | 250.131$^{**}$ | 167.733$^{n.s.}$ |
| G × S | 1 | 17.745$^{**}$ | 13.917$^{**}$ | 4.974$^{n.s.}$ | 0.087$^{n.s.}$ | 115.568$^{**}$ | 531.530$^{n.s.}$ |
| Error | 8 | 0.166 | 0.374 | 17.413 | 0.056 | 3.346 | 375.034 |
| C.V. (%) | - | 18.593 | 29.944 | 16.083 | 25.884 | 13.347 | 5.289 |

n.s., * and **: Non-Significant, Significant at the 5% and 1% probability levels, respectively.

**Table 2.** Analysis of variance for sugars, $Na^+$ and $K^+$ content in rice roots.

| S.O.V. | D.F. | Mean of Square | | | | |
|---|---|---|---|---|---|---|
| | | Root Glucose | Root Fructose | Root Sucrose | Root $Na^+$ | Root $K^+$ |
| Genotype(G) | 1 | 113.561$^{**}$ | 106.301$^{**}$ | 6.144$^{**}$ | 1403.721$^{**}$ | 14.182$^{n.s.}$ |
| Salinity (S) | 1 | 37.844$^{*}$ | 94.412$^{**}$ | 4.157$^{**}$ | 4864.225$^{**}$ | 57781.375$^{**}$ |
| G × S | 1 | 491.705$^{**}$ | 666.408$^{**}$ | 5.410$^{**}$ | 1477.342$^{**}$ | 514.723$^{n.s.}$ |
| Error | 8 | 3.524 | 3.779 | 0.170 | 66.164 | 497.580 |
| C.V. (%) | -- | 17.653 | 13.179 | 20.286 | 28.686 | 9.338 |

n.s., * and **: Non-Significant, Significant at the 5% and 1% probability levels, respectively.

**Table 3.** Main effects of genotype and salinity on sugars, $Na^+$ and $K^+$ content in rice blades.

| Traits / Treatment | Blade Glucose (µmol/ gFW) | Blade Fructose (µmol /gFW) | Blade Sucrose (µmol/ gFW) | Blade Starch (µmol/ gFW) | Blade $Na^+$ (mg/lit) | Blade $K^+$ (mg/lit) |
|---|---|---|---|---|---|---|
| Genotype | | | | | | |
| IR29 | 3.732 a | 3.573 a | 28.433 a | 1.094 a | 16.513 a | 369.88 a |
| FL478 | 0.655 b | 0.512 b | 23.459 a | 0.735 b | 10.898 b | 362.40 a |
| Salinity | | | | | | |
| Normal | 3.049 a | 2.818 a | 21.485 b | 0.994 a | 9.140 a | 362.40 a |
| Stress | 1.338 b | 1.268 b | 30.407 a | 0.835 a | 18.271 b | 369.88 a |

Means within a column followed by the same letters are not significantly different at the %5 level according to Fisher's Least Significant Difference Test.

**Table 4.** Main effects of genotype and salinity on sugars, $Na^+$ and $K^+$ content in rice roots.

| Traits / Treatment | Root Glucose (µmol/gDW) | Root Fructose (µmol/gDW) | Root Sucrose (µmol/gDW) | Root $Na^+$ (mg/lit) | Root $K^+$ (mg/lit) |
|---|---|---|---|---|---|
| Genotype | | | | | |
| IR29 | 13.711 a | 17.727 a | 1.317 b | 39.171 a | 237.78 a |
| FL478 | 7.559 b | 11.774 b | 2.748 a | 17.540 b | 239.95 a |
| Salinity | | | | | |
| Normal | 12.411 a | 17.555 a | 1.444 b | 8.222 b | 308.26 a |
| Stress | 8.859 b | 11.946 b | 2.621 a | 48.489 a | 169.47 b |

Means within a column followed by the same letters are not significantly different at the %5 level according to Fisher's Least Significant Difference Test.

The salt stress caused to reduce glucose in root of salt sensitive genotype (IR29) but in Fl478 it increased in salt stress. Glucose can play an important role as an osmotic solution in mitigation of salt effects in tolerant genotype.

Glucose content in IR478 didn't have significant difference in leaf blade, sheath and was a little more in shoot at stress condition. This difference was seen in root too. There was more glucose in IR478 at stress. So glucose could be as an osmotic solute contributes to salt tolerance in rice. Plants respond to salt stress by restricting the uptake of salt and adjust their osmotic pressure by the synthesis of compatible solutes (proline, glycinebetaine, sugars, etc.) (Greenway and Munns 1980).

*Fructose*

Fructose variations and its partitioning over the plant organs were similar to glucose in every genotype at stress and control condition. This means that these two metabolites originated from same resources and consume in same ways. Variation in invertase specially cytosolic invertase was according to glucose and fructose variations. This enzyme could produce them from sucrose as the same amount.

**Table 5.** Mutual effects of genotype and salinity on sugars, $Na^+$ and $K^+$ content in rice blades.

| Treatment: Genotype | Salinity | Blade Glucose (µmol/ gFW) | Blade Fructose (µmol /gFW) | Blade Sucrose (µmol/ gFW) | Blade Starch (µmol/ gFW) | Blade $Na^+$ (mg/lit) | Blade $K^+$ (mg/lit) |
|---|---|---|---|---|---|---|---|
| IR29 | Normal | 5.803 a | 5.426 a | 23.328 b | 1.259 a | 8.844 c | 372.79 a |
| | Stress | 1.661 b | 1.721 b | 33.538 a | 0.929 ab | 24.182 a | 366.96 a |
| FL478 | Normal | 0.294 c | 0.211 c | 19.641 b | 0.729 b | 9.436 bc | 352.01 a |
| | Stress | 1.016 bc | 0.814 bc | 27.276 ab | 0.741 b | 12.36 b | 372.79 a |

Means within a column followed by the same letters are not significantly different at the %5 level according to Fisher's Least Significant Difference Test.

**Table 6.** Mutual effects of genotype and salinity on sugars, $Na^+$ and $K^+$ content in rice roots.

| Treatment: Genotype | Salinity | Root Glucose (µmol/ gDW) | Root Fructose (µmol/ gDW) | Root Sucrose (µmol/ gDW) | Root $Na^+$ (mg/lit) | Root $K^+$ (mg/lit) |
|---|---|---|---|---|---|---|
| IR29 | Normal | 21.888 a | 27.984 a | 0.057 b | 7.942 c | 313.72 a |
| | Stress | 5.534 c | 7.470 c | 2.577 a | 70.400 a | 161.84 b |
| FL478 | Normal | 2.933 c | 7.127 c | 2.831 a | 8.502 c | 302.79 a |
| | Stress | 12.184 b | 16.421 b | 2.665 a | 26.578 b | 177.11 b |

Means within a column followed by the same letters are not significantly different at the %5 level according to Fisher's Least Significant Difference Test.

*Sucrose*

Total sucrose content in above ground organs was too more than root. Also its content in above ground organs was more than glucose and fructose but in root was vice versa. So it can be resulted that sucrose was the transfer form of sugar that produce in shoot and transferred to roots and analyzed there to glucose and fructose.

Shoot sucrose variation in IR478 was more than IR29 at stress and control condition. This variation between control and stress condition in leaf sheath was more than leaf blade in IR478 in related to IR29. However this variation in blade was similar. It could be related to more transfer of sucrose into sheath in control condition by IR29. Sucrose content in stress and control conditions were similar in IR478. It could be related to more tolerance of this genotype but this variation in IR29 was more accelerate.

*Starch*

Starch content didn't have significant difference in blade and root in control and stress conditions. Its content was very low in different organs and was less than glucose, fructose and sucrose content. Two genotypes also didn't have significant difference. The young rice genotypes under each condition in this experiment had efficient soluble sugar transfer and photosynthesis and use them as an osmotic soluble and growth substrate. This means that there isn't any requirement to produce of more starch. Also photosynthetic system might be reduce at any unfavorable condition and starch couldn't be produced and reserved in chloroplasts and transfer to other organs as well as control condition that prefer to produce and transfer simple and soluble sugars.

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