Exogenous abscisic acid enhances sugar accumulation in rice (Oryza sativa L.) under salinity

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Abstract

Sugar, a final product of photosynthesis, is reported to be involved in the defense mechanisms of plants against abiotic stresses such as salinity. The objective of this study was to determine the effects of exogenous ABA on carbohydrate metabolism in rice. We present here the comparative protective potentiality of exogenously applied abscisic acid (ABA) in mitigating NaCl toxicity and inducing short-term salinity tolerance in two indica rice varieties, namely IR29 (salt-sensitive) and FL485 (salt-tolerant). Salt stress may promote sugar accumulation, a high level of sugar were found in salt-tolerant rice. Meanwhile, the results indicated that exogenous abscisic acid can improve sugar accumulation under salinity. Increased glucose at the initial, and fructose at the end of experiment were observed in the flag leaf of both cultivars under salinity and to more extent by application of exogenous of ABA to unstressed plant. Salinity stress induced an accumulation of starch in cv. FL485. It is suggested that partitioning sugars into starch may involve in salinity tolerance by avoiding metabolic alteration.

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**Introduction**

Exposure to desiccation, salt stress and low temperature are generally accompanied by an increase in endogenous abscisic acid (ABA) levels prior to activation of a number of water- and salt-stress-induced genes (Chandler and Robertson, 1994), the products of which are thought to be involved in protection of the cell or in recovery from the stress-mediated physiological insult. Indeed, ABA is an important signal for triggering plant responses to adverse environmental conditions during vegetative growth (Leung and Giraudat, 1998; Nambara and Marion-Poll, 2005), and ABA coordinates many of these stress responses, including the immediate stomatal closure, osmolyte accumulation and the induction of the synthesis of stress-related proteins, such as late embryogenesis abundant and heat shock proteins, reactive oxygen scavengers, etc. However, whilst many abiotic-stress-inducible genes are controlled by ABA, some are not, which indicates that both ABA-dependent and ABA-independent regulatory systems are involved in stress-responsive gene expression (Bray et al., 2000; Zhu, 2002). In support of this, there are observations that the application of exogenous ABA to both whole plants, and in tissue culture explants or protoplasts, facilitated the adaptation to subsequent increased salinity (salt stress) in several phylogenetically diverse plants. ABA treatment prior to an increased salinity insult was reported to improve the growth of the common bean (*Phaseolus vulgaris*) (Khadri et al., 2007), to reduce leaf abscission and increase salt tolerance in citrus plants (Gómez-Cardenas et al., 2003), and to induce salt adaptation in jojoba (*Simmondsia chinensis*) shoots grown in vitro (Mills et al., 2001). Moreover, exogenous ABA application was shown to reduce the sodium concentration or its translocation to shoots, resulting in a salt-tolerant adaptation in both the cereal grass Sorghum (Amzallag et al., 1990) and the common bean (Khadri et al., 2007), whilst the increase in the K/Na ratio in rice following exogenous ABA application correlated with the increased salinity tolerance (Bohra et al., 1995). Adaptation by plants to stress involves morphological, physiological and biochemical changes, including the accumulation of osmolytes such as proline, sugar alcohols, soluble carbohydrates and glycine betaine in which may involve in stress tolerance (Bagniewska-Zadworna et al., 2007). Under salt-stress conditions, the elevated ABA levels inhibit Na and Cl transport to shoots in intact bean seedlings (Karmoker and Van Steveninck, 1979), and it has been suggested that the resultant changes in ion concentrations together with the increase in endogenous ABA levels generate the signal for the induction of stress-induced genes. If correct, ABA may play an important role in the tolerance of plants to increasing salinity. Carbohydrate metabolism is strongly affected by salinity. Salt stressed plants often accumulate sugars and their derivatives, such as polyols and raffinose family oligosaccharides (Valliyodan and Nguyen, 2006; Toldi et al., 2009). Accumulation of these osmolytes may help plants to tolerate dehydration by improving their ability to maintain osmotic balance within the cell (Choluj et al., 2008; Costa et al., 2008). Additional benefits of these solutes have been described, including buffering cellular redox potential, protecting the cell from dehydration by stabilizing membrane and protein structures and providing possible energy source under severe stress (Hasegawa et al., 2000). Furthermore, alteration in photoassimilate partitioning between source and sink tissues may also contribute to the accumulation of these solutes (Hare et al., 1998). In this research, two rice lines with a similar genetic background, but that differ in their salt-tolerant ability, the salt sensitive IR29 cultivar, and the salt-tolerant FL485 line were used. Here, we investigate if the optimal concentration range for exogenous ABA application prior to and during salt stress to induce salt tolerance in both rice lines can trigger sugars accumulation.

**Material and Methods**

**Plant materials, growth conditions and stress treatments**

Two rice cultivars contrasting in tolerance of salt stress during reproductive stages (Moradi et al., 2003) were selected for this investigation. FL485 is breeding line tolerant of salt stress at both the
seedling and reproductive stages, and IR29 is a cultivar sensitive to salt stress during both stages and is commonly used as a sensitive check in breeding nurseries. This experiment was conducted to evaluate the effects of salt stress and ABA spraying. Salt stress starting at about 10–7 d before panicle initiation and continuing through harvest. The experiment was carried out in a greenhouse with air temperature in the range of about 25 to 35 °C and light intensity in the range of about 600–1000 mmol m⁻² s⁻¹ and with 20 pots per cultivar in each replication. Pre-germinated seeds were sown in 1 L perforated plastic pots filled with fertilized (50 N, 25 P and 25 K mg kg⁻¹) Maahas clay soil (43 % clay, 44 % silt and 13 % sand; pH 5.9; Tirol-Padre and Ladha, 2004) and were kept in concrete tanks filled with tap water. The level of water was maintained at 3 cm below the soil surface for 2 d. Five seeds of each of the two cultivars were sown in each pot, thinned to one seedling 2 weeks later, and the water level was raised to about 1–2 cm above the soil surface. When the seedlings were 28 d old, water was siphoned out and the pots were drained for 12 h, then flooded with tap water (control) or with a saline solution with EC of 3 dS m⁻¹ using NaCl for 3 d, then increased progressively to 4 and 5 dS m⁻¹ at 3 d intervals, and finally stabilized at 6+0.3 dS m⁻¹ through harvesting. The pots were kept flooded thereafter for the duration of the experiment, and the EC of the water was monitored daily and adjusted when necessary using NaCl and tap water. From two days after anthesis, each pot was sprayed by ABA solution, at a concentration of 50 µM. Deionized water was sprayed as a control. Tween-20 (0.5%) was used as a wetting agent for each treatment, including the water as control. We sprayed the plants with solution until the leaves were completely wet and the solution ran off the leaves.

**Sampling**

All parameters were measured on flag leaves and panicle of the first two tillers that were tagged 25 d after sowing. Sampling of the flag leaves and panicles were removed from anthesis up to full grain maturity at seven day interval [7,14,21 and 28 days after spraying (DASP)] for the various biochemical analyses. For the biochemical assays, samples were cut into small pieces after measuring their fresh weight, frozen in liquid nitrogen, and stored at -80 °C. Three replicates were maintained for all measurements. The various plant parts were dried in an oven at 80 °C for dry matter analyses and various estimations.

**Concentration of starch and soluble sugars**

Carbohydrate concentrations in plant tissues were determined at intervals after treatment. A modified colorimetric method was used for analysis of starch and soluble sugar concentrations (Thakur and Sharma, 2005; Dkhil and Denden, 2010). For starch concentration, plant tissues were homogenized in an ice-cold mortar and pestle in a volume of 16 ml 80% (v/v) ethanol. The homogenates were centrifuged at 3000×g, for 10 min at 4°C, and then perchloric acid (HClO₄, 6 ml, 30%, v/v) was added to dissolve starch from the pellet. The slurry was left at room temperature for 6 h, and starch was detected with I₂-KI reagent prepared by diluting 0.1 ml stock solution (0.06 g I₂ and 0.60 g KI in 10 ml deionized water) with 0.05 M HCl just prior to the assay. Samples of 0.5 ml starch solution were mixed with 0.5 ml I₂-KI reagent, 1 ml 30% (v/v) perchloric acid and vortexed, then left standing at room temperature. The absorbance of the samples at 620 nm wavelength was then determined using a spectrophotometer, and the concentration determined using a standard curve. For soluble sugars, plant tissues were suspended in test tubes with 3 ml of 80% ethanol, the extract was evaporated to dryness and the residue was dissolved in 20 ml distilled water. Total soluble sugars were determined by the phenol-sulfuric acid method, using glucose as standard. The total NSC was determined as the sum of starch and soluble sugar concentrations.

**Statistical analysis**

The data were analyzed by analysis of variance (ANOVA) using IRRISTAT version 92 (IRRI, 1992). The crop data was arranged in split-plot factorial design with three replications. Cultivars in main plot, salt stress and foliar treatment as factorial in subplot. The comparison of treatment means was made by
least significant difference (LSD) at \( p = 0.05 \).

**Results**

**Changes in Soluble Sugars on leaves**

Irrespective of treatment, Leaves FL485 revealed higher soluble sugars content than IR29 throughout all stages sampling especially at the end of experiment, since the concentration of soluble sugars in tolerant varieties was 2.7 times more than susceptible one (Fig. 1B&D). Regardless of treatment the soluble sugars levels from day 21 onwards in IR29 was descending while ascending in FL485. Salinity stress caused a significant increase in leaf total soluble sugars of both cultivars (Fig. 1B&D). In comparison, ABA caused to elevate the flag leaf soluble sugars levels in both cultivars regardless to salinity stress. Since total soluble sugars concentration of ABA or ABA+Salinity in flag leaves increased from (7 to 21) DASP at both cultivars and reach to maximum value (45 mmol g⁻¹dw) by day 21, although a considerable differences were detected from day 21 onwards between cultivars, as substantial reduction occurred in IR29 and the value dropped to 27 and 23 mmol g⁻¹dw at the end of experiment for ABA and ABA+Salinity treatment respectively, as substantial reduction occurred in IR29 and the value dropped to 27 and 23 mmol g⁻¹dw at the end of experiment for ABA and ABA+Salinity treatment respectively, while a remarkable increment occurred for FL485 during this period as the value reached to nearly 58 and 45 mmol g⁻¹dw for ABA and ABA+Salinity treatment (Fig. 1B&D).

**Changes in flag leaf sucrose under different treatments**

Changes in flag leaf sucrose under different treatments were similar in both cultivars (Fig. 2B&D).

Salinity cause to substantial reduction in sucrose concentration of both cultivars in compare to their control treatment. In comparison, the highest sucrose concentration at all sampling stage was observed in the control treatment in both cultivars (Fig. 2B&D). Sucrose concentration further decreased from day 14 onwards in ABA+Salinity treatment compared to salinity treatment.

**Changes in Soluble Sugars on grains**

Irrespective to salinity, the lower soluble sugars levels were measured in grains of IR29 during all sampling

Fig. 1 (A-D). Effects of salinity and exogenous ABA on total soluble sugar in panicles (A and C) and flag leaves (B and D) in two rice cultivars (Salt-Sensitive cv. IR29 and Salt-Tolerant cv. FL485). Vertical bars represent ± SE of the mean (n=3).

Fig. 2 (A-D). Effects of salinity and exogenous ABA on sucrose in panicles (A and C) and flag leaves (B and D) in two rice cultivars (Salt-Sensitive cv. IR29 and Salt-Tolerant cv. FL485). Vertical bars represent ± SE of the mean (n=3).

Most changes in glucose concentration among treatments was observed in 7 to 21 days. From day 21 onwards glucose level was similar in all treatments (Fig. 3B&D). The highest level of glucose reached to 1575 and 1439 µmol g⁻¹dw on day 14 under ABA treatment in IR29 and FL485 respectively. No significant differences in glucose concentration was observed between control and salinity treatments from day 14 onwards in both cultivars (Fig. 3B&D). Fructose changes was almost identical under different treatments in susceptible cultivar while a marked elevation was observed under ABA and ABA+Salinity treatments from day 14 onwards in tolerant one (Fig. 4B&D).

Salinity cause to decrease the fructose concentration of FL485 flag leaf in compare to control treatment at the end of experiment while this reduction was not evident in IR29 at all sampling stage.

Changes in Soluble Sugars on grains

Irrespective to salinity, the lower soluble sugars levels were measured in grains of IR29 during all sampling
of experiment in compare to FL485 (Fig. 1A&C). In FL485 the initial concentration of soluble sugars was much more contrast to IR29 under control treatment (11 compare to 7 mmol g⁻¹ dw) and the value reached to 22 compare to 14 mmol g⁻¹ dw at the end of experiment. Salinity cause to more decrease in grains soluble sugars of both cultivars in respect to their control treatments (Fig. 1A&C). The magnitude of increase in grains soluble sugars, regardless to salinity appeared result in spraying of ABA followed by ABA+Salinity treatment in all sampling stages in both cultivars with a more extent in FL485.

Similar to that observed in the leaves of the cultivars, the sucrose concentration in grains sharply decreased under salinity (Fig. 2A&C). ABA spray could not improve the deleterious effect of salinity on sucrose concentration in compare to control treatment in both cultivars. However, the most sucrose concentration was found in application of exogenous ABA to the unstressed plants.

Regardless of treatments the grain glucose concentration increased over time in both cultivars (Fig. 3A&C). However the highest glucose level was observed in ABA spraying and then ABA+Salinity treatments at the end of experiment in both cultivars. No distinct differences were observed in grain glucose concentration of both cultivars result in applying salinity compared with the control treatment (Fig. 3A&C).

Irrespective to treatments the changes of FL485 grain fructose was very intense in compare to IR29 (Fig. 4A&C). Fructose trend in both cultivars was increased from 7 to 21 days, although this increase was not significant enough in IR29 variety, as the highest level of fructose reached to 94 and 244 µmol g⁻¹ dw on day 21 under control treatment in IR29 and FL485 respectively. Similar to that observed in grain glucose concentration, the most fructose level observed in ABA spraying and then ABA+Salinity treatment at all sampling stages in both cultivars, since the value reached to nearly 349 and 284 µmol g⁻¹ dw for ABA and ABA+Salinity treatments at day 21 in FL485 and 114 and 99 µmol g⁻¹ dw for same treatments in IR29. From day 21 onwards fructose concentration gradient in all treatments declined sharply in FL485 variety while there was no significant changes observed in sensitive cultivar (Fig. 4A&C).

Regardless of treatments the grain starch concentration increased over time in both cultivars (Fig. 5A&C). However the highest starch level was observed in ABA spraying and then ABA+Salinity treatments at the end of experiment in both cultivars. No distinct differences were observed in grain starch concentration of both cultivars result in applying salinity compared with the control treatment (Fig. 5A&C).
Changes in Starch in the leaves and grains

The starch contents in leaves of both cultivars were strongly affected by the salinity and the fluctuation pattern of starch was not similar in each cultivar by the time under different treatments (Fig. 5B&D). Opposite to flag leaf sucrose concentration, the leaf starch contents increased by salinity imposed in both cultivars, however, the increment was more pronounced in FL485 than IR29 during all samplings stage (Fig. 5B&D). In comparison, ABA spraying to the unstressed plants cause to decrease the starch levels significantly in respect to control treatment from day 7 to 21 in IR29, in addition, this reduction was not evident in tolerant cultivar during all sampling stages. In comparison the lowest leaf starch level was found in application of exogenous ABA to the IR29 stressed plant at the end of experiment. Irrespective to salinity, the higher starch level were measured in grains of the salt-sensitive, IR29 in control treatment in compare with FL485 at all sampling stages (Fig. 5A&C). Salt stress altered the starch concentration in the grains of the two rice genotypes as available starch concentration was suppressed due to salinity for IR29 but not for FL485 during all sampling stages (Fig. 5A&C). The reduction in starch content of stressed grains became much pronounced (30% of control) at 28 DASP in salt-sensitive compared with those of the control whereas, a marked elevation (31%) occurred during similar sampling stage in salt-tolerant compared with their respective controls (Fig. 5A&C). Furthermore, application of exogenous ABA to the stressed and unstressed plants, however, showed a substantial elevation just in tolerant cultivar compared to the control plants (Fig. 5A&C).

Discussion

Higher non-structural carbohydrate concentration in plant tissue under abiotic stresses was known to have positive effects on plant survival of stress and recovery afterwards (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). In rice, concentration of soluble sugars were induced by salinity stress (Dubey and Singh, 1999). Total soluble sugar content in the salt-stressed leaves and panicles was directly related to salt exposure times (Fig. 1). Our results indicated that soluble sugar content was also increased under salt stress in both cultivars and irrespective to salinity exogenous application of ABA can improve accumulation of soluble sugars in both cultivars. In addition, glucose and fructose accumulation in the flag leaf and panicle tissues of both cultivars grown under non-stress or salt stress was greater than that of plants grown under the control result in application of exogenous ABA. ABA-induced sugar accumulation may partly increase osmotic adjustment as well as protect enzymes and membrane against deleterious effects of destabilizing ions (Farooq et al., 2009) in which helps the plants to better survive under salinity condition.

The level of sugar accumulation in salt-stressed IR29 was lower than that in FL485. The sugar accumulation in the leaf and panicle tissues of FL485 rice may play an important role in the salt defense mechanism when exposed to salt stress conditions. Soluble sugars metabolism is pivotal in seed development and is particularly susceptible to hormone and salinity treatments. The decrease in seed sugars concentration due to salinity at all durations of stress in both cultivars (Fig. 1A&C), reflected the lower availability of the assimilate at source level as depicted in Figure 1. Direct relationship between sucrose availability and export rate at source level and the establishment of new sink organs has been shown for several crops (Setter et al., 2001; Liu et al., 2004). In line with these reports, we suppose that the higher decrease in sink size (data not shown) of the salinity-sensitive genotype due to stress is partly attributed to reduced availability of the assimilate at source level. Likewise, obvious differences observed for soluble sugars under exogenous ABA application between cultivars at the end of experiment (Fig. 1). Earlier studies indicated that ABA was implicated in the regulation of soluble sugar transport and metabolism (Kashem et al., 1998). It was found that both ABA and gibberellins...
(GA) help to regulate soluble sugar concentration (Bethke et al., 1997).

Salinity-induced reduction of sucrose in panicles and flag leaves of both cultivars. On the contrary, starch accumulation was noticed in flag-leaf of salt-tolerant cultivar exposed to stress. Starch maybe synthesized from simpler sugars. High starch accumulation in mature leaves of salt-tolerant cultivar of tomato was reported earlier (Balibrea et al., 2000.) Although starch may not play a crucial role in salt-tolerance mechanism, it was suggested that the ability of plants to partition sugars into starch may help to avoid metabolic alteration by lowering feedback inhibition caused by excess amount of sucrose in cytoplasm (Krapp and Stitt, 1995).

Furthermore, application of exogenous ABA even triggers more sugar accumulation, but decreases starch content. ABA also causes increases in sugar accumulation as well as decreased starch content in Polypodium vulgare (Bagniewska-Zadworna et al., 2007). Maintaining greater ability to convert soluble sugars into starch before and during salt stress could potentially play an important role in rice tolerance of salinity.

In conclusion, this study demonstrates that accumulation of sugars was associated with salt stress. Accumulation of sugar is considered to play an important role in salinity tolerance. Exogenous ABA was also shown to help the plants to enhanced sugar accumulation under salinity stress. On the other hand, starch concentration increased markedly in salt-tolerant cultivar when grown under salinity stress. It is possible that adjusted carbon partitioning and allocation could have an important on the overall plant growth under salinity.

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References

http://dx.doi.org/10.1093/jxb/41.12.1529

http://dx.doi.org/10.3923/jbs.2009.909.912

http://dx.doi.org/10.1007/s11738-006-0008-z

http://dx.doi.org/10.1111/j.1399-3054.2000.1100412.x

http://dx.doi.org/10.1093/jxb/48.7.1337

http://dx.doi.org/10.1111/j.1439-037X.1995.tb00197.x


http://dx.doi.org/10.1007/s11738-008-0166-2

http://dx.doi.org/10.3923/ja.2008.98.101


http://dx.doi.org/10.1023/A:1002160618700

http://dx.doi.org/10.1111/j.1439-037X.2009.00365.x

http://dx.doi.org/10.1007/s00344-002-0013-4

http://dx.doi.org/10.1046/j.1365-3040.1998.00309.x

http://dx.doi.org/10.1146/annurev.arplant.51.1.463

http://dx.doi.org/10.1111/j.1399-3054.1979.tb02613.x

http://dx.doi.org/10.1007/s004250050326

http://dx.doi.org/10.1016/j.envexpbot.2006.10.008

http://dx.doi.org/10.1016/j.plaphy.2007.05.003

http://dx.doi.org/10.1007/BF00202587

http://dx.doi.org/10.1146/annurev.arplant.49.1.199

http://dx.doi.org/10.1016/S0378-4290(03)00165-5

http://dx.doi.org/10.1078/0176-1617-00254


http://dx.doi.org/10.1146/annurev.arplant.56.032604.4444046


http://dx.doi.org/10.2135/cropsci2001.4153530X


http://dx.doi.org/10.1146/annurev.arplant.53.091401.143329