

Full Length Research Paper

Is salinity tolerance of rice lines concerned to endogenous ABA content or to the cellular ability for ABA synthesis under stress?

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In this work we tested its putative relationship of Abscisic acid with the degree of tolerance to this abiotic stress. For this purpose, we have examined the responses of sensitive (IR29) and tolerant (IR651) varieties of indica rice (*Oryza sativa* L.) to a range of salinity (0 (control) and 90 mM NaCl). Shoot and root dry weight reduced and leaf Na concentration increased in response to salinity for both cultivars with a higher extent of sensitivity. Tolerance of IR29 to saline stress was generally improved by ABA treatment and leaves Na content reduced to the level of their respective control treatment. This ABA effect was evident in IR29 with low tolerance, as their ability to recover from stress increased up to seven fold. Independence of the saline treatment and the absolute endogenous leaf ABA content in sensitive variety was significantly more than that of the tolerant one. However, with stress, the increase in endogenous ABA synthesis was higher in tolerant than in sensitive varieties. These data together with those obtained by using fluridone, an inhibitor of ABA synthesis, suggest first of all, that there was differential sensitivity to ABA in the tolerant and sensitive leaves cultivars and enhanced concentrations at tolerant levels acted primarily to maintain root and shoot growth and secondly, the differences in the level of tolerance to saline stress is related to their different capacity of ABA synthesis under stress conditions.

Key words: Abscisic acid, fluridone, *Oryza sativa* L., salinity.

INTRODUCTION

Salinization of arable land is increasing, and could possibly have devastating global effects. Over 6% of the world's land and 20% of the world's irrigated land are currently affected by salinity (Rhoades et al., 1992; Munns, 2005). In such soils, NaCl concentrations typically exceed 40 mM, and much higher values are frequently found (Munns, 2005). Enhancing membrane permeability in salt stressed plants has been well documented (Hasegawa et al., 2000), this results in Na⁺ accumulation and increasing Na⁺/K⁺, conversely reducing K⁺ in many plant species e.g. rice, green bean (Yasar et al., 2006), winter wheat (Zheng et al., 2008) and umbu plant. From some previous studies, the results showed that rice growth and development are reduced

when rice is exposed to salinity stress (Morsy et al., 2007; Cha-um et al., 2007; Shannon et al., 1998). However, the response of rice to salt stress depends on its salt tolerance abilities. During the last two decades, it has been well established that abscisic acid (ABA) is a vital cellular signal that mediates the expression of a number of salt and water deficit-responsive genes. Direct relation between stress tolerance and increased levels of ABA does not always exist. Thus, in barley and wheat, changes in endogenous ABA levels have been reported to be unrelated to freezing tolerance or to cold hardening (Kadlecová et al., 2000; Faltusová-Kadlecová et al., 2002). Among the comparisons between stress-tolerant and stress sensitive cultivars within the same species, some reports have shown higher ABA levels in tolerant cultivars, although the differences appeared in either unstressed, non-acclimated cultivars (Bravo et al., 1998) or following the stress (Chen et al., 2002; Zheng and Li,

2000). In rice, ABA contents increased in a semi salt-tolerant variety but only marginal changes were measured in salt-tolerant and salt sensitive ones (Bohra et al., 1995). Exogenous ABA treatments prior to subjecting the plants or tissues to adverse conditions have been reported to improve tolerance to cold temperatures (Bornman and Jansson, 1980; Duncan and Widholm, 1987), osmotic stress (Nayyar and Kaushal, 2002; Nayyar and Walia, 2003), soil salinity and air humidity (Asch et al., 1995). In the current study, we tested the important role of ABA in tolerance to abiotic stresses, and also provided new insights into the mechanism of improving salt tolerance by the application of exogenous ABA on crops.

MATERIALS AND METHODS

Plant material and growth conditions

Two rice cultivars different in tolerance to salt stress during the vegetative stages (Moradi et al., 2003) were selected for this investigation. IR65192-4B-10-3 (abbreviated as IR651) is breeding lines tolerant to 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.

The experiment was carried out in the Agricultural Biotechnology Research Institute of Iran (48°20'N; 31°41'E; 20 m above sea level), in the growing season of 2010 to 2011. This experiment was conducted in a growth cabinet with light intensity of about 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 12 h duration, 70% relative humidity and 29/22°C day/night temperature. Pre-germinated seeds of each cultivar were sown in holes made on Styrofoam sheets with a nylon net at bottom (Gregorio et al., 1997). Two seeds were sown per hole, with 50 holes per entry. The sheets were first floated on distilled water in 11 L plastic trays for three day, after which a nutrient solution (Yoshida et al., 1976) was used until the plants were 28 days old. A control treatment (nutrient solution) and the level of salt stress, 10 dms^{-1} (90 mM) using NaCl was introduced thereafter. Four hours after adding the salt treatment, half of the plants from both cultivars were exposed to spraying of fluridone (30 μM) or ABA (10 μM). The youngest fully expanded leaf (leaves that had completed petioles), were harvested at 0 (immediately before starting the salinity), 4, 12, 24, 48, 96 and 168 h after the start of the salt treatments for ABA measurements. Shoot and root dry matter, K^+ and Na^+ concentration of youngest fully expanded leaf were measured at the end of this experiment.

The experiment was designed as 2 × 2 (cultivars × salinity) factorials in completely randomized design (CRD) with three replicates. The mean in each treatment was compared by Duncan's new multiple range test (DMRT) at $p < 0.01$ and analyzed by SPSS software (SPSS for Windows, SPSS Inc., Chicago, Illinois, USA). The culture solution was renewed once with the pH adjusted daily to 5.5 by adding either NaOH or HCl.

Sodium and potassium measurements

Dried samples were ground to a fine powder. About 0.2 g was transferred to a test tube containing 10 ml 0.2 N acetic acid. Then, test tubes were heated in a water bath at 80°C for 2 h. The extracted tissue was cooled at room temperature and left overnight and then filtered by using Whitman filter paper no. 42. Sodium and

potassium concentrations were determined by using an atomic absorption spectrometer (Perkins Elmer, Norwalk, CT, USA).

Leaf gas exchange measurement

Stomatal conductance (g_s) was made on fully expanded youngest leaf of each plant using an open system LCA-4ADS portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). Measurements were performed with the following specifications/adjustment: molar flow of air per unit leaf area 403.3 $\text{mmol m}^{-2} \text{s}^{-1}$, atmospheric pressure 99.9 kPa, water vapor pressure into chamber ranged from 600 to 890 Pa, Par at leaf surface was maximum up to 1711 $\mu\text{mol m}^{-2} \text{s}^{-1}$, temperature of leaf ranged from 28.4 to 32.4°C, ambient temperature ranged from 22.4 to 27.9°C and ambient CO_2 concentration 352 $\mu\text{mol mol}^{-1}$.

Measurement of endogenous ABA levels

Endogenous ABA analyses were performed as described by Gómez-Cadenas et al. (2002). Frozen rice leaves of 1.5 g were finely ground in liquid nitrogen and 10 ml of 80% methanol was added together with 0.01 g of ascorbic acid and 0.01 g polyvinylpyrrolidone (PVP) to prevent oxidation reactions during extraction. The homogenate was stirred overnight at 4°C. After centrifugation (4000 $\times g$, 15 min), the supernatant was recovered and adjusted to pH 8.0. The aqueous methanol was evaporated under reduced pressure at 35°C. The residue was dissolved in 5 ml of water. Then, it was frozen and thawed for three cycles. After centrifugation (4000 $\times g$, 15 min), the supernatant was recovered and adjusted to pH 2.5 and partitioned against ethyl acetate. Then, the solution with the free ABA in ethyl acetate was collected. This process was repeated thrice. After that, the collection was adjusted to pH 8.0 and dried. The resulting dried precipitate was dissolved in 1 ml of 3% methanol containing 0.1 M acetic acid, and was filtered through a 0.45 mm membrane filter. The extract (100 μl) was automatically injected and processed by high performance liquid chromatography (HPLC) (Agilent 1100 Series, USA) equipped with a reverse phase column (4.6 × 250 mm Diamonsic C18, 5 μm). It was eluted with a linear gradient of methanol (3 to 97%), containing 0.01% acetic acid, at a flow rate of 4 ml/min. The detection was run at 260 nm with a diode array detector. The retention time of ABA was 36.4 min and shifted to 0.1 to 0.5 min. Quantification was obtained by comparing the peak areas with those of known amounts of ABA.

RESULTS

Effect of stress on K and Na concentration with impaired ability for ABA synthesis

A different approach was used to study the implication of ABA in the mechanism of stress-tolerance. This experiment was performed with fluridone treatment, an inhibitor of ABA biosynthesis. Irrespective of fluridone application, no statistically significant differences were measured between tolerance and sensitive cultivars in Na concentration in control treatment of both cultivars investigated (Figures 2A and B). Salinity treatment increased by two fold the Na level in IR29 but became unchanged in IR651 with their respective control (Figure 2A). The Na accumulation in young leaves of both

cultivars became more pronounced upon application of fluridone in salinity treatment (Figures 2A and B) and caused rapid increase in Na; 7.1 and 2.6 fold in sensitive and tolerant cultivars, respectively, (in contrast to control treatments). Fluridone had a dramatic effect on response to stress in both cultivars.

Thus, when plants of both cultivars were sprayed with fluridone, they showed higher sensitivity to a subsequent salinity stress than stressed plants that had not been sprayed with the inhibitor (Figures 2A and B). Salt stress induced approximately 30% increase in endogenous leaf ABA content of tolerant cultivar, while spraying with 50 μM fluridone during the salinity treatment not only suppressed this increase but decreased it by more than 30% (Figure 3).

In a further experiment, plants from tolerant and sensitive cultivars were treated with exogenous ABA and subjected to salinity (Figures 2A and B). When plants were treated with ABA (10 μM), leaf of tolerant cultivar did not change significantly their Na level response to the subsequent stress. In comparison, in IR29 plants, application of exogenous ABA led to dramatic reduction of the Na level and reduced the value to that of normal treatment (Figure 2B).

It has to be pointed out that the effect of ABA was totally counteracted with the inhibitor. Regardless of the salinity, ABA or fluridone treatments, a very similar changing pattern was observed for K concentration in leaves of both cultivars and no significant difference was observed (Figures 2C and D).

Effect of stress on endogenous ABA levels

Endogenous ABA levels of both tolerant and sensitive cultivars were determined in order to check whether different degree of tolerance to stress correlated with hormone contents. Irrespective of salinity treatment, leaves ABA concentration in IR29 was higher than that of IR651. Endogenous ABA levels of leaves under non stress conditions varied in the range of 12.2 to 13.50 $\mu\text{g g}^{-1}$ Fwt in IR29. Compared to sensitive cultivar, the tolerant cultivar had a lower ABA concentration, and the values varied in the range of 6 to 9.5 $\mu\text{g g}^{-1}$ Fwt in the control treatment. Leaves ABA content of stressed IR651 plants was significantly higher than that of control plant throughout the sampling time and the differences became more pronounced and achieved a maximum value by the end of the experiment (Figure 3). Contrary to tolerant cultivar, leaves ABA levels in sensitive cultivar increased slightly but did not reach the level of statistical significance at the end of experiment compared to their respective control treatment (Figure 3).

Biomass accumulation

Result regarding plant biomass shows inverse relation-

ship with salinity. A general trend of decrease in dry weight of plants with salinity was noted in both cultivars. However, shoot and root weight reduction was highest in the susceptible variety compared to tolerant variety (Figures 1B and D). Fluridone application led to drastical decline in root and shoot dry weight of both cultivars (Figures 1A and C). Regardless of salinity, shoot and root dry weight in IR651 was twofold of IR29.

DISCUSSION

The plant hormone, abscisic acid has been implicated in plant responses to abiotic factors, causing water stress. In most studies, elevated ABA levels prior to stress, or as a first step in the response to adverse conditions, are involved in tolerance mechanisms. Under this rationale, it is expected that increased ABA in leaves of tolerant variety have a positive role in alleviating stress effects. In the present work, differences in ABA contents were observed between sensitive and tolerant varieties studied, and we can ascribe their different response to stress to a different endogenous hormone level under control and non-stress conditions (Figure 2) when plants were subjected to stress and we could detect differences in ABA synthesis in varieties with respect to non-stressed controls (Figure 3). Tolerant variety seems to have a higher ability for ABA synthesis than sensitive variety. This agrees with results comparing sensitive/tolerant cultivars of different species (Chen et al., 2002; Lee et al., 1993). Although salt-tolerant rice cultivars showed higher increases of ABA than the sensitive cultivars, there was no direct correlation between absolute ABA content and degree of tolerance. Therefore, they pointed out the importance of both the rate of ABA increase as well as the absolute ABA levels. The fact that IR29 produce less ABA under stress, could explain their sensitivity response to stress (Figure 3). Further experiments, using fluridone, confirm the involvement of ABA synthesis in the mechanism of response to stress. Irrespective of cultivar used, spraying 30 μM fluridone, drastically increased leaf Na concentration in the tolerance and sensitive varieties when exposed to saline stress (Figure 2). However, the extent of this increase varied as a function of the tolerance level of the varieties used. Thus, in tolerant variety, the increase was of three fold, while in sensitive variety, it reached eight fold with respect to the control treatments (Figure 2). Thus, when plants of both cultivars were sprayed with fluridone, they showed higher sensitivity to a subsequent saline stress than stressed plants that had not been sprayed with the inhibitor. This protective role of ABA is confirmed in the present work (Popova, 1998). While stress induced dramatically increased endogenous ABA, spraying with 50 μM fluridone during the salinity treatment suppressed this increase (Figure 3). We observed that in ABA treatment during saline stress, there was about seven times

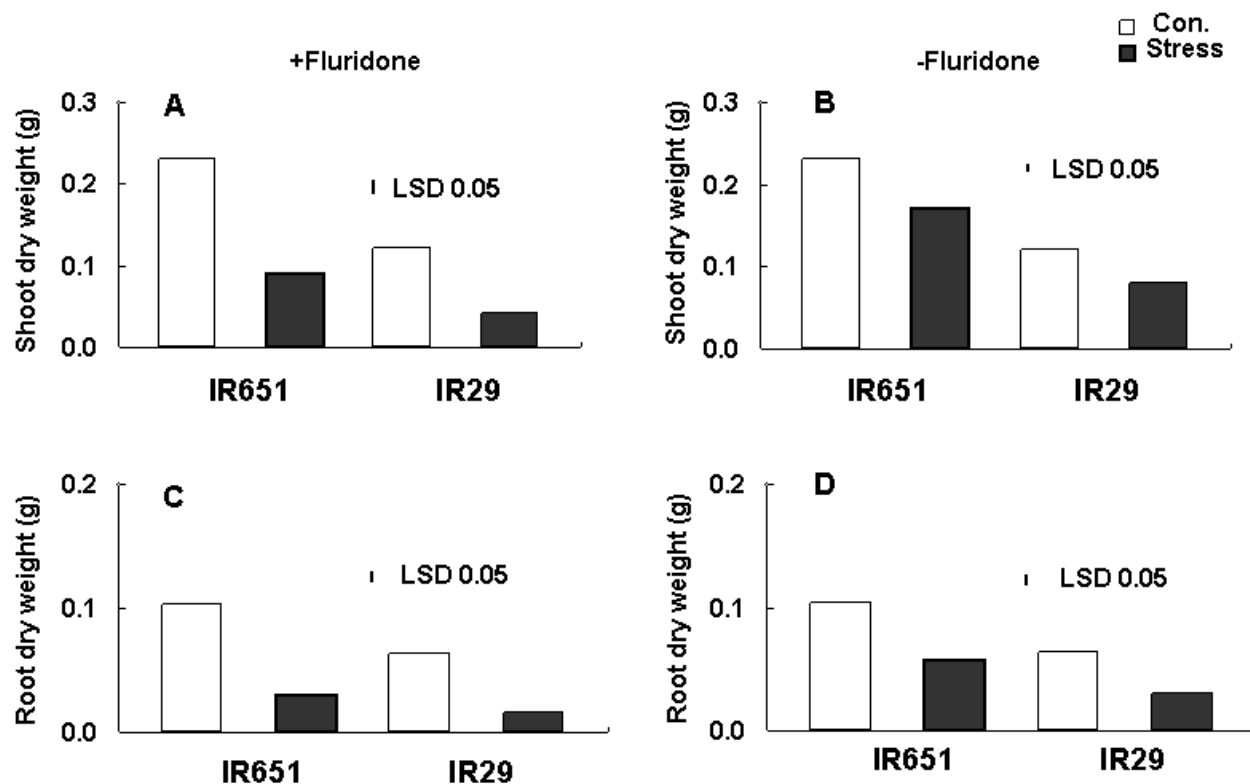


Figure 1. Shoot and root dry weight of two rice cultivars during the seedling stage under control and salt stress of 12 dsm^{-1} , with (A and C) or without (B and D) spraying fluridone ($50 \mu\text{M}$). The data are mean values of three replications with three subsamples per replication, and vertical bars are $\text{LSD}_{0.05}$.

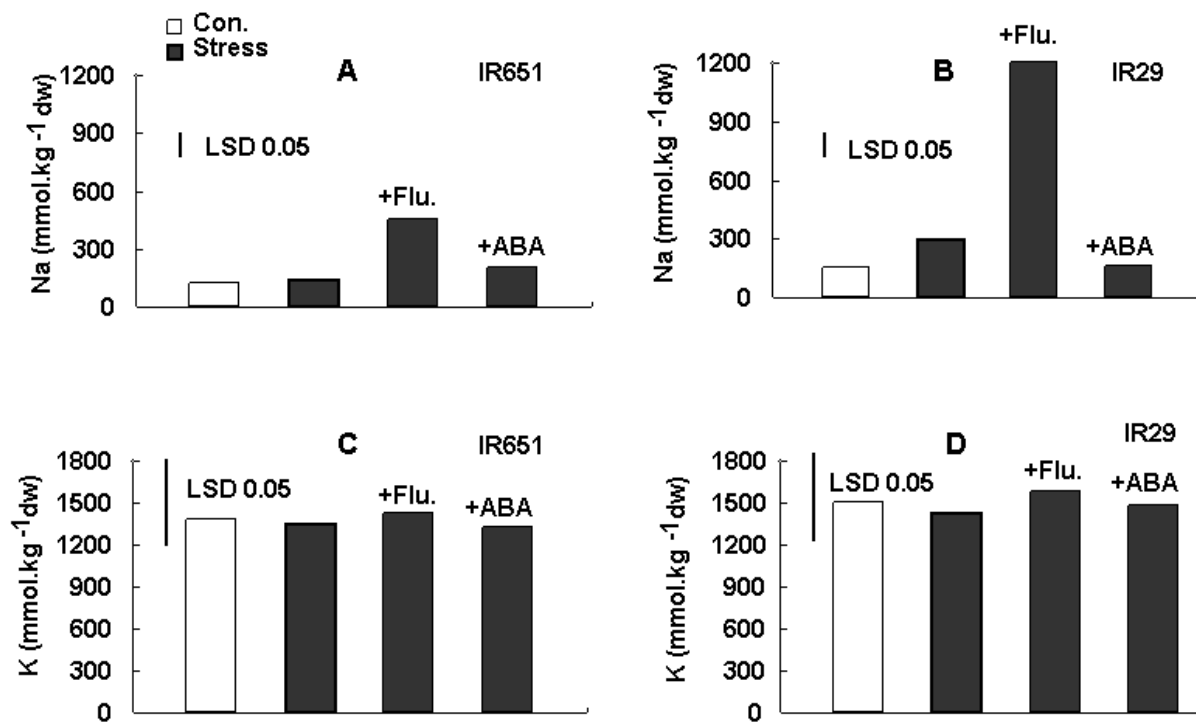


Figure 2. Na and K concentration of youngest full expanded leaf of two rice cultivars during the seedling stage under control and salt stress of 12 dsm^{-1} , with spraying fluridone ($50 \mu\text{M}$) or ABA ($20 \mu\text{M}$). The data are mean values of three replications with three subsamples per replication, and vertical bars are $\text{LSD}_{0.05}$.

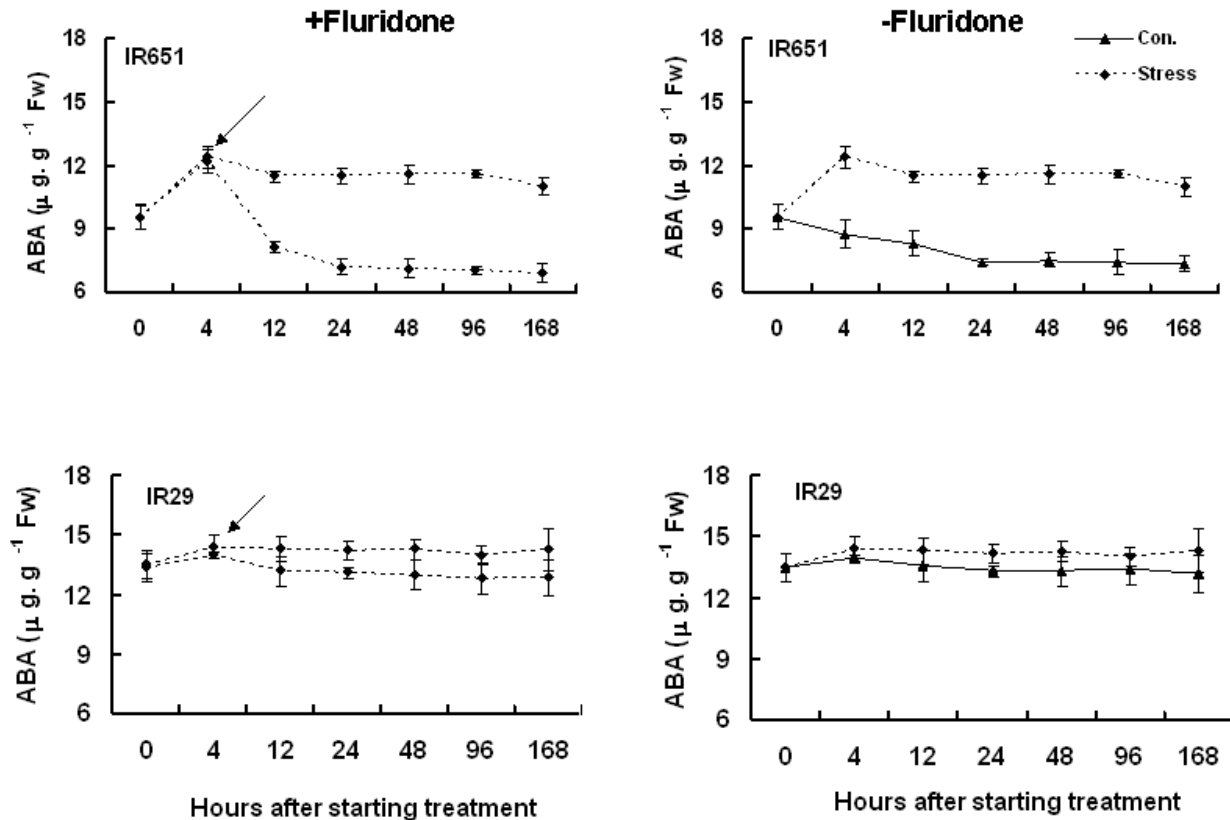


Figure 3. ABA measured on the youngest fully expanded leaf of two rice cultivars during the seedling stage under control and salt stress of 12 dsm^{-1} . The data are mean values of three replications with three measurements per replication, and vertical bars represent \pm SE of the mean ($n = 3$) where these exceeded the size of the symbol. The values on the x-axis represent hours after the start of salt stress treatment of 21-day-old seedlings. The arrows indicate the spraying of fluridone.

reduction of the leaf Na content of sensitive cultivar to recover from stress in comparison to untreated plants subjected to the same stress conditions (Figure 2). In contrast, tolerant cultivar was not affected by the hormonal treatment. In agreement with this result, Bohra et al. (1995) have also showed that exogenous ABA treatments improved tolerance to salinity in sensitive but not in tolerant rice cultivars. It is interesting to note that non-stressed barley varieties with lower ABA contents were more sensitive to freezing and responded better to exogenous ABA treatments than other varieties with higher hormone levels (Bravo et al., 1998). In fact, growth rate and leaf Na concentration of plants were significantly affected by this inhibitor (Figures 1 and 2), which interfere with ABA action. Similar results have been obtained by Bianco-Trinchant and Le Page-Degivry (1998) in protoplasts from different plant origin.

Our results demonstrate the importance of ABA synthesis in the tolerance to stress. Tolerant variety has a higher capacity of ABA synthesis (Figure 3).

In summary, our study led to the following conclusions:

1. Salts tolerance of a given variety does not appear to be related to endogenous ABA levels under control

conditions, but to the different rate of ABA synthesis under stress.

2. The fact that cells from IR651 produce more ABA when subjected to stress, could explain their better response to salinity condition.

3. There was a different threshold irritability for ABA content, because contradictory to the higher leave ABA level in sensitive variety, more deleterious effect was observed in this cultivar.

4. Regardless of the presence or absence of salinity treatment, IR651 showed a higher growth rate and low accumulation of Na with fluridone application in contrast to IR29, suggesting that improved growth probably alleviated Na^+ toxicity by dilution, this finding is consistent with that of Nooden (1988).

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