

## ROOT-TO-SHOOT SIGNAL TRANSDUCTION IN RICE UNDER SALT STRESS

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### Abstract

This paper describes the impact of salt stress on changes in the level of Absciscic acid (ABA) and cytokinins as signal molecules communicated through root-to-shoot in rice. The study focus to investigate the time related changes in the salt induced ABA and cytokinins accumulation concomitant with the changes in water potential and stomatal conductance of salt stressed plants. Seeds of 3 rice varieties were grown in plastic pots in phytotron. The changes in the level of abscisic acid (ABA), transzeatin riboside (t-zr) and 2-isopentyl adenine (2-ipa) were monitored in xylem sap and leaves of three rice varieties viz. BAS-385 (salt-sensitive), BG-402 (moderately tolerant) and NIAB-6 (tolerant). The salt solution ( $\text{NaCl}$ ,  $1.2 \text{ dSm}^{-1}$ ) was added to the rooting medium after transplanting when plants were 50 d old.

There was delay in response of stomata to salt treatment in BAS-385 as opposed to earlier increase in leaf resistance in BG-402 and NIAB-6. The stem water potential increased sharply in all the varieties following salt treatment but the decrease in stomatal conductance of leaves preceded the decrease in stem water potential. The concentration of xylem ABA increased significantly greatly reaching a peak in BAS-385 much earlier (24 h of salt treatment) than that of other varieties. The ABA accumulation was delayed and the magnitude of ABA accumulation was greater in BG-402 and NIAB-6. The xylem flux of ABA followed a similar pattern. The concentration of xylem t-zr showed a short-term increase in all the varieties but the magnitude of increase was greater in BAS-385 at all the measurements till 96h of salt treatment. The concentration of xylem 2-ipa was higher in BAS-385 till 48 h of salt treatment. The flux of both the t-zr and 2ipa was greater in the tolerant variety 96h after salt treatment.

The basal level of ABA and cytokinin appears to play important role in determining the response of a variety to salt stress. The xylem flux of ABA and cytokinin (2-ipa and t-zr) in response to salt stress determines the sensitivity of the tissue to stomatal resistance. Salt induced modulation in the level of ABA and CK has been discussed.

### Introduction

One of the most important environmental factors limiting rice yields are soil salinization and sodification, during seedling and reproductive phases. The magnitude of the salt effect in rice depends on the duration of salt treatment and age of plant. ABA content increase in leaves of plants exposed to water and osmotic stresses (Lachno & Baker 1986; Parry *et al.*, 1990) and also in xylem sap of salt stressed plants (Munns & Cramer, 1996). Evidences have accumulated indicating that application of exogenous ABA can improve salt tolerance (La Rosa *et al.*, 1987; Khadri *et al.*, 1990). Noreen & Ashraf (2008) reported that foliar spray of SA alleviated the adverse effects of salt stress on sunflower but did not change sub stomatal  $\text{CO}_2$ . Mutlu & Bozcuk (2007) demonstrated positive role of polyamines in salt tolerance of sunflower.

Studies indicate that changes in ABA levels do not always accompany osmoticum-induced physiological responses and strongly suggest that responses to osmotic stress can not be explained solely by changes in ABA levels (Xu & Bewley, 1991) as signal originating from roots (Dodd, 2005). Reduction in cytokinin concentration in roots and shoot tissue in response to salt stress was noted (Kuiper *et al.*, 1990; Walker & Dumberoff, 1980; Fricke *et al.*, 2006).

Time related changes in ABA and Cytokinins level under salt stress in rice is lacking. The present investigation focuses to investigate the critical period when the salt induces modulation in ABA and cytokinin level concomitant with the changes in the non hydraulic signals viz. stomatal conductance and water potential in 3 varieties of rice differing in salt tolerance.

## Materials and Methods

Seeds of rice (*Oryza sativa* L.) cv. BAS-385 (salt sensitive), NIAB-6 & BG 402 (salt tolerant) were obtained from Faisalabad, Pakistan. Three weeks after sowing (21-25DAS) these seedlings were transferred to plastic pots (330 ml polystyrol) filled with 400g soil (compost & 5 g/l Ascorbic acid). Nutrient solution containing  $(\text{NH}_4)_2\text{SO}_4$  (100 mg / Kg),  $\text{K}_2\text{SO}_4$  (60 mg/kg) and Flory 5 (50 mg/Kg) was added to the soil at the time of transplantation.

The growth chambers were maintained at 30/20°C day / night temperature and 12 h photoperiod with light intensity of 250  $\mu\text{mol}/\text{m}^2/\text{s}$  (xenon lamps, osram), 75-80% relative humidity. The plants were treated with salt at 45-50 d after sowing.

**Hormone analyses:** The concentrations of ABA in the xylem sap and leaves were determined by the method of ELISA according to Walker-Simons (1987). The ABA-4-RSA conjugate was produced according to Weiler (1982). The cross reactivity was tested in xylem sap for ABA, 2ip and t-zr. The cytokinins, 2ip and t-zr were measured in the xylem sap by ELISA according to Schwarzenberg *et al.*, (1988).

**Salinity treatments:** Salt solution (1.2  $\text{dS m}^{-1}$  NaCl) was added to soil using glass rod that had been installed prior to plantation. The salt was added daily as required to maintain level of salt in each pot. Soil electrical conductivity was determined with a conductivity meter.

**Collection of xylem sap:** For measurement of bulk water potential (stem) and collection of xylem sap the plants were detopped. The detopped plants with their entire root system were inserted into a Scholander bomb. After determining the root-shoot water potential, sap was collected with a microliter syringe for 5 minutes at 0.3 Mpa above water potential the xylem sap was stored in eppendorf tubes at 20°C till further analyses.

Stomatal resistance on the abaxial surface of the youngest fully expanded leaf was measured at different periods by the Delta -T Devices.

**Preparation of leaves extract:** The total leaves of plants were harvested and lyophilized for 5 d, grinded / powdered in a mill and extracted overnight in distilled water at 4°C with gentle shaking. The aqueous extract was centrifuged twice at 10000 rpm for 25 min and the aliquot was used for analysis of ABA and CK.

## Results and Discussion

The results indicated that increasing salt concentration increased the stomatal resistance of leaves (Figs. 1&2) with no significant variation in stem water potential (Fig. 3) till 24h of salt treatment. However, the magnitude of increase in leaf resistance was significantly less in BAS-385 till 48 h of salt treatment. The stem water potential remained less negative in the sensitive variety BAS-385 as compared to the other 2 varieties.

The xylem ABA (Figs. 4 & 5) increased shortly in BAS-385 forming a peak at 24 h of salt treatment thereafter it remained steady with no significant change. In contrast, BG-402 and NIAB-6 showed no significant change in xylem ABA content till 48h of salt treatment but marked accumulation of ABA was recorded for the measurement made 96h after of treatment. The magnitude of ABA accumulation in BG-402 and NIAB-6 was greater than that of BAS-385. The ABA flux in xylem sap followed similar pattern in response to salt treatment.

The xylem t-zr content (Figs. 6 & 7) was significantly less in unstressed control of BAS-385 as compared to that of BG-402 & NIAB-6. In response to salt treatment the xylem t-zr content of all the varieties showed a transient increase. The magnitude of increase was relatively greater in BAS-385, the xylem flux of t-zr showed a transient increase, 24h of salt treatment in BAS-385. The BG-402 showed small transient increase in response to salt but significant decrease was observed with longer duration of salt treatment. However, in the tolerant variety NIAB-6 there was gradual increase in t-zr till 48 h of salt treatment which remained steady thereafter.

Under unstressed condition the cv. NIAB-6 and BG-402 have relatively low xylem 2ipa than that of BAS-385, but on induction of salt stress xylem 2ipa increased markedly greatly in BG-402 and NIAB-6 (Figs. 8 & 9). The xylem flux of 2ipa was reduced significantly and remained low throughout the salt treatment in BAS-385 but not in BG-402 and NIAB-6. Kuiper *et al.*, (1990) observed decrease in the CK concentration in root and shoot tissue of salt resistant plants of barley and wheat. Walker & Dumberoff (1980) reported rise in t-zr of tomato under salt stress. The cytokinin production may form the built in trigger for tuning growth and are important in salt resistance.

**Leaf ABA:** The basal level of free ABA content (Table 1) of sensitive variety, BAS-385 was significantly lower than that of that of semi-tolerant variety BG-402 and the tolerant variety NIAB-6. On exposure to salt stress the free ABA content of leaves was increased in all the varieties but the magnitude of increase was greater (about 2 fold of the basal level) in BAS-385, 48h after salt treatment. The conjugated ABA of the tolerant variety NIAB-6 was also much higher than that of the sensitive variety BAS-385 under unstressed condition but salt induced increase was greater in BAS-385 though the value was still less than that of NIAB-6 for the measurement made 48 h after salt stress.

**Leaf t-zr:** The basal level of leaf t-zr (Fig. 10) was greater in BAS-385 as compared to BG-402 and NIAB-6. On induction of salt stress a marked decrease in leaf t-zr occurred in BAS-385, 6 h after treatment thereafter it started to increase till 48 h; on contrary the leaf t-zr of tolerant variety showed a marked decrease in response to salt.

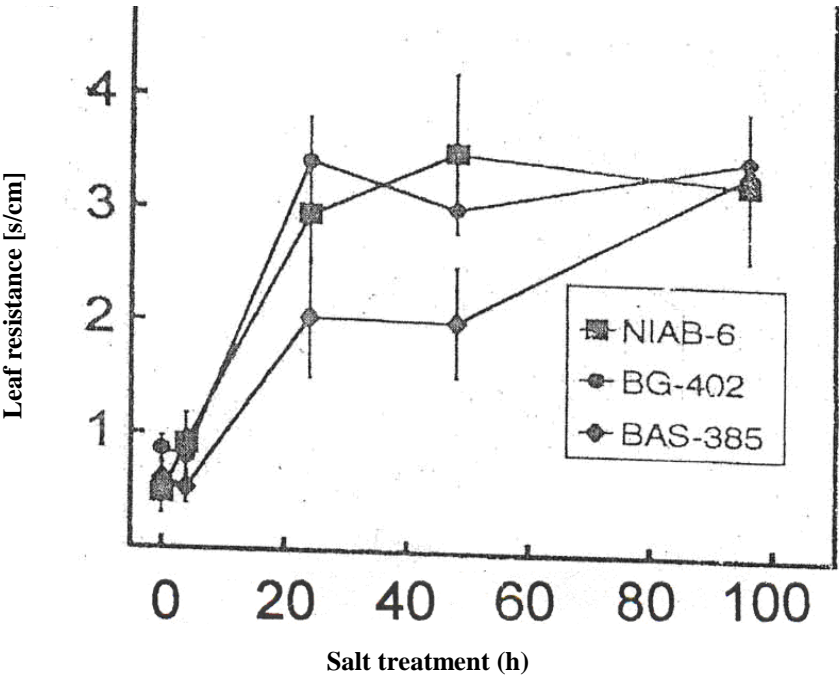


Fig. 1. Changes in the stomatal resistance of leaves of rice plants under salt stress.

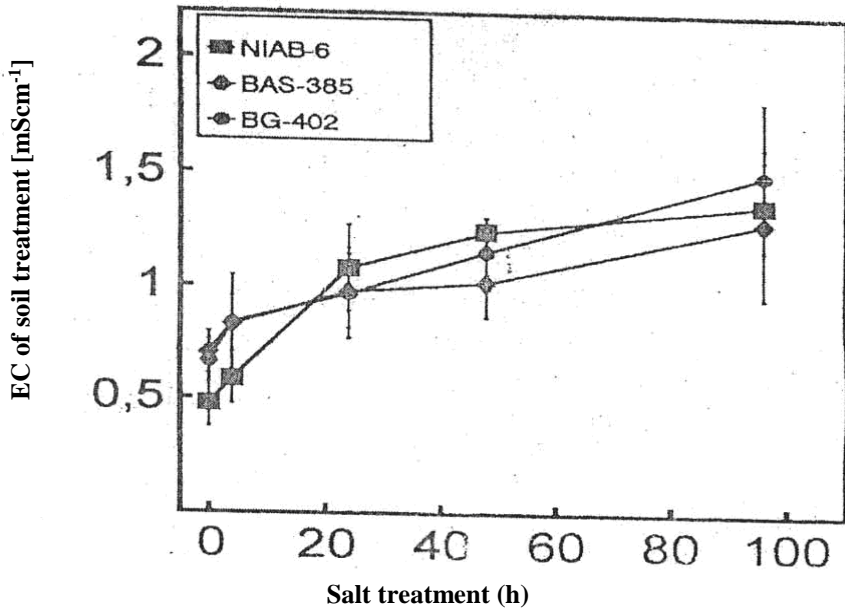


Fig. 2. Changes in the EC of soil treated with 1.2dS/m NaCl.

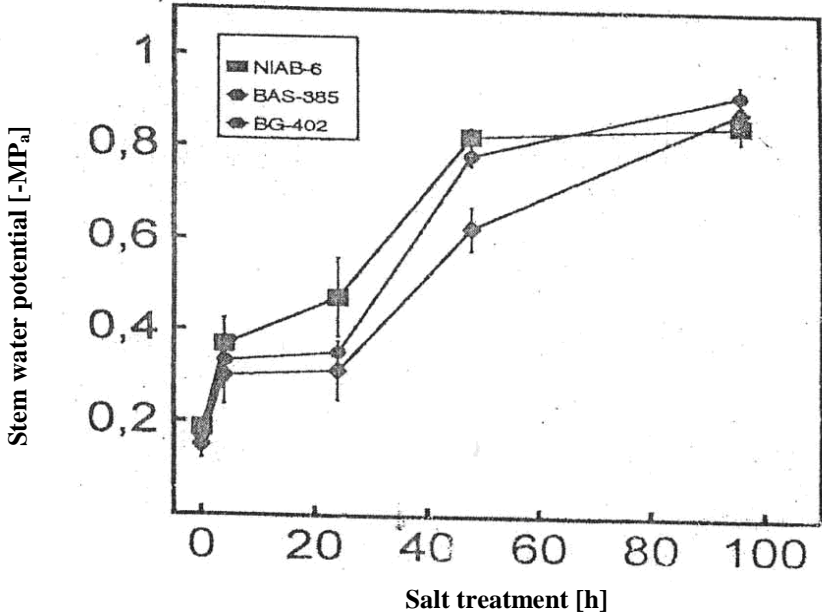


Fig. 3. Changes in the Stem water potential of rice plants treated with 1.2d S/m.

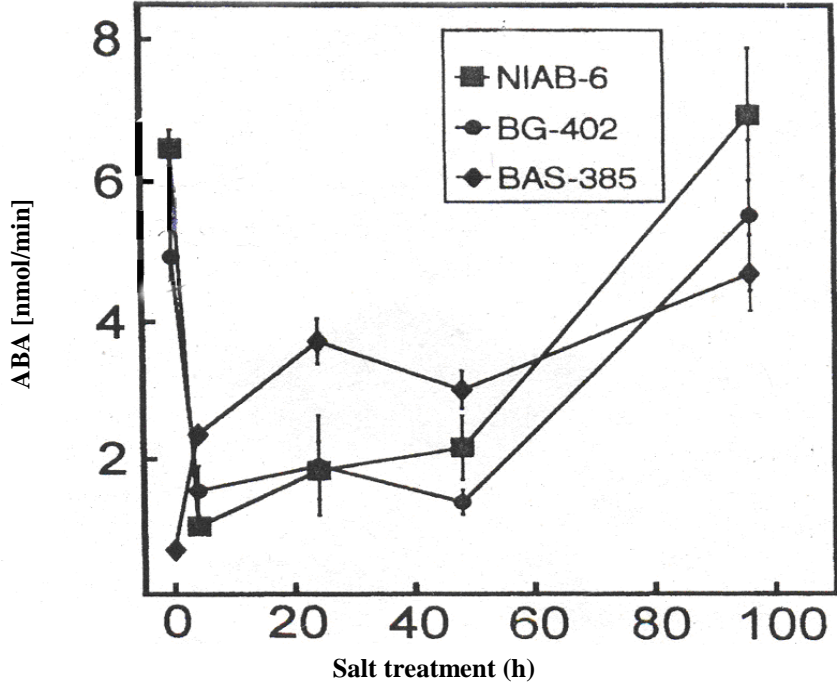


Fig. 4. Changes in the ABA flux in xylem sap of rice plant treated with 1.2 dS/m NaCl.

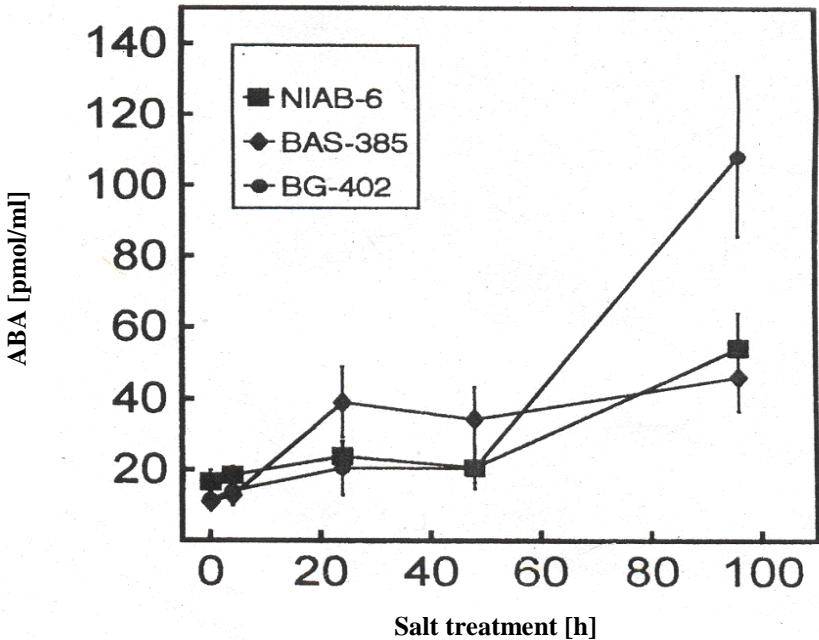


Fig. 5. Changes in the ABA concentration in xylem sap of rice plant treated with 1.2 dS/m NaCl.

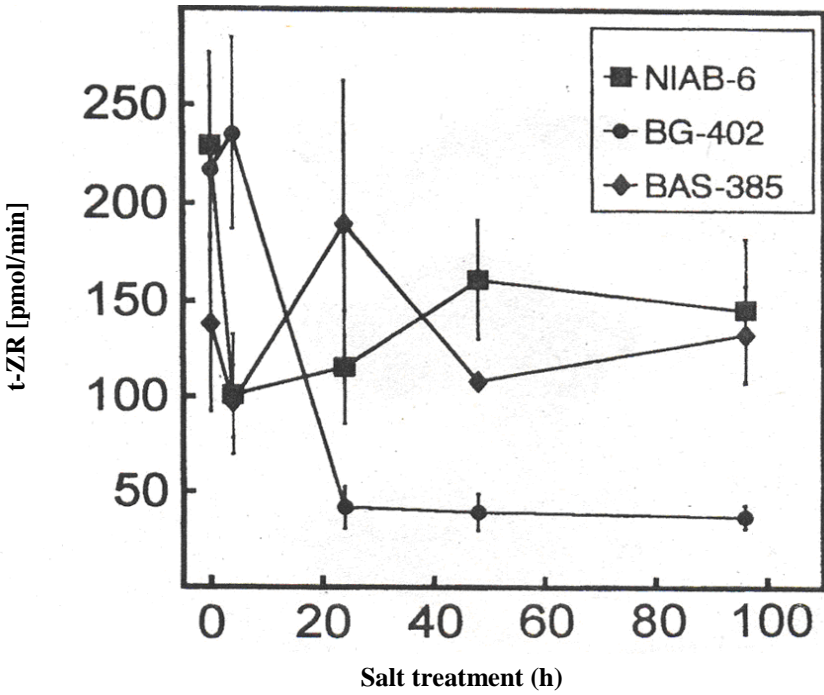


Fig. 6. Changes in the t-zr flux in xylem sap of rice treated with 1.2 dS/m NaCl.

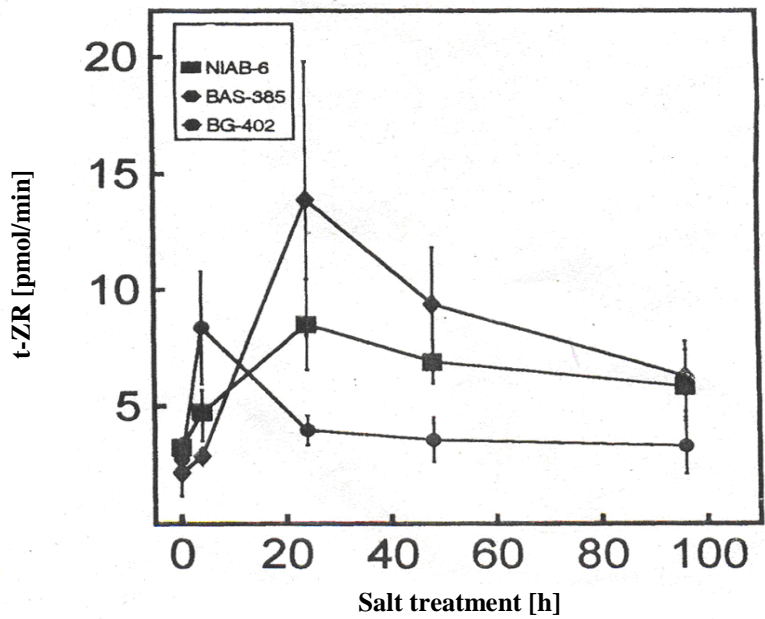


Fig. 7. Changes in the t-zr concentration in xylem sap of rice treated with 1.2dS/m NaCl.

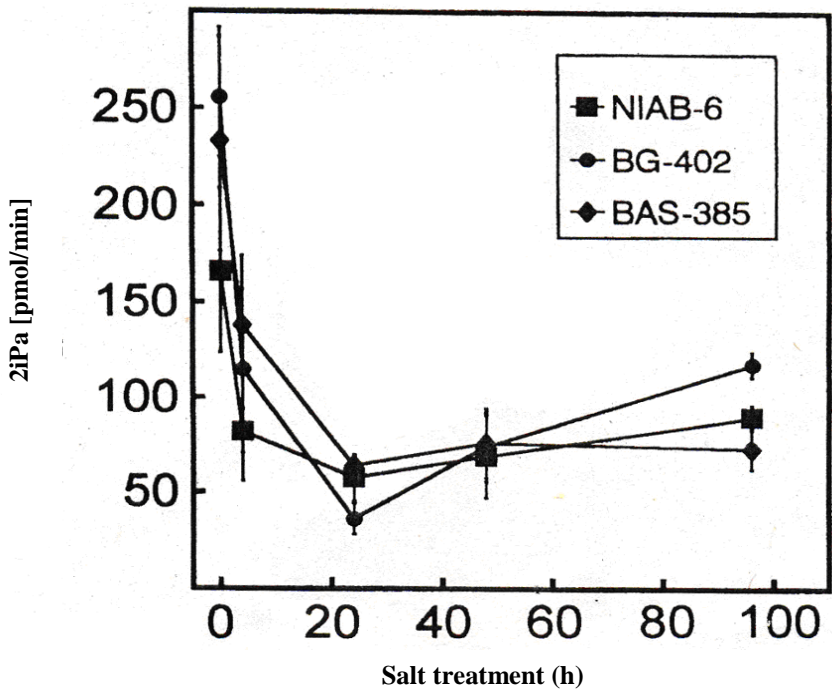


Fig. 8. Changes in 2-iPa flux in xylem sap of rice plant treated with 1.2dS/m NaCl.

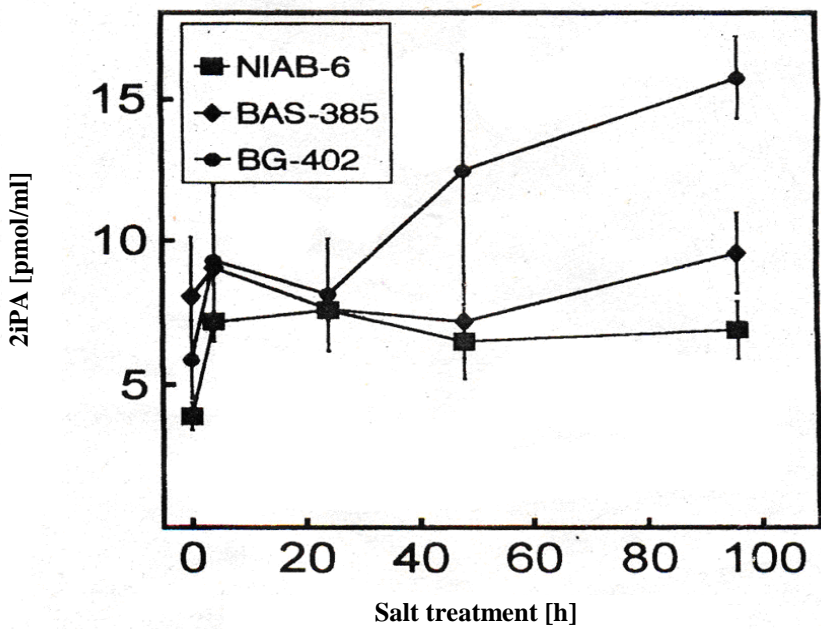


Fig. 9. Changes in 2-iPa concentration in xylem sap of rice treated with 1.20 mM NaCl.

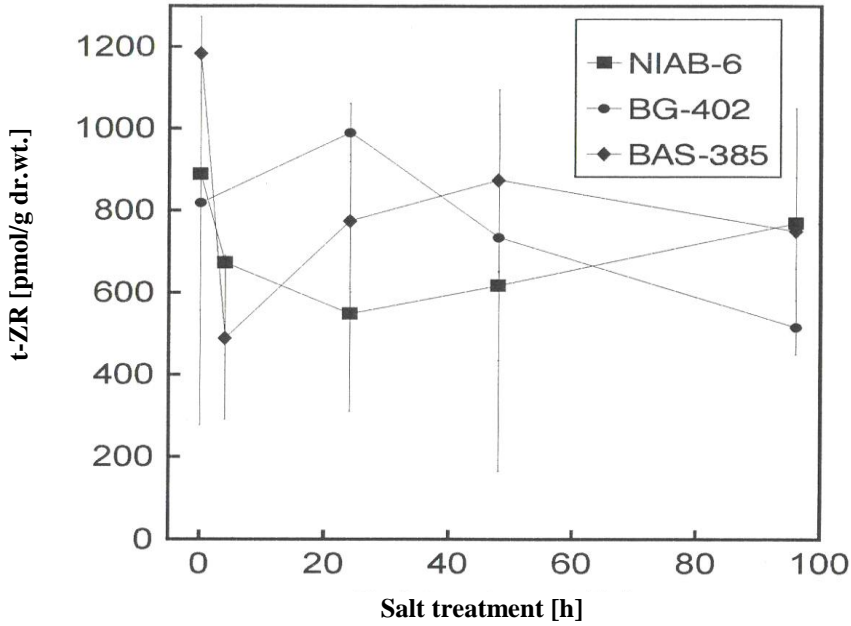


Fig. 10. Changes in the t-zr content of leaves of rice plants treated with 120 mM NaCl (EC 1.2 dS/m).



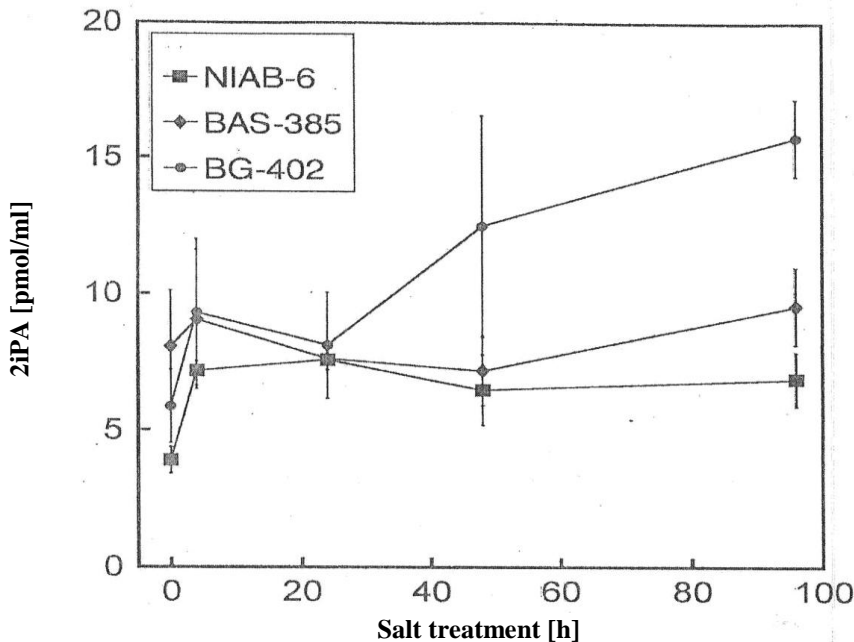


Fig. 11. Changes in the 2-iPa content of rice plants treated with 120 mM NaCl.

Table 1. Changes in the free and conjugated ABA (n mole g<sup>-1</sup>dwt.) in leaf of rice plants treated with 120 mM NaCl. Data were recorded 48 h after treatment. Figures in parantheses represent std. error value for mean of 3 independent experiments.

Variety	Free ABA		Conjugated ABA	
	Control	Salt stressed	Control	Salt stressed
BAS-385	2613 (±239)	5069 (±117)	1453 (±263)	3604 (±727)
BG-402	3742 (±36)	3995 (±604)	1466 (±432)	1422 (±315)
NIAB-6	4307 (±600)	4685 (±981)	2259 (±146)	4059 (±2183)

**Leaf 2-ipa:** The short term effect of salt treatment indicated marked increase in 2 ipa content of leaves (Fig. 11) of BAS-385 which decreased thereafter and remained low and insignificantly different from that of basal level. In contrast the 2 ipa content of tolerant var. NIAB-6 declined 24h after salt treatment, remained low till 48 h and increased thereafter. Similar pattern was followed by BG-402.

Although the salt stress reduces the total cytokinins (2 ipa and t-zr) fluxes in xylem sap as compared to their respective controls, but in the tolerant variety the flux was greater than the sensitive variety even after 96 h of treatment.

The BAS-385 responded to salt treatment quickly by accumulating ABA but the changes in stomatal conductance was delayed. In BAS-385 significantly greater increase occurred in t-zr, the most active transported form of cytokinin in xylem sap but the xylem ABA flux was low 48h after salt treatment. The concentration of plant hormones in xylem sap has been shown to be the most important signal controlling stomatal resistance

(Borel *et al.*, 1997). The regulation of stomatal resistance is possibly controlled by the interactive effects of ABA and CK in xylem. Perhaps the physiological response to ABA is controlled by the levels of ABA as well as the sensitivity of tissue that are competent to respond (Xu & Bewley, 1991). It appears that certain critical level of ABA is necessary to coordinate stomatal function and maintenance of water budget in plants under salt stress. The EC of soil where the plants were grown and treated with NaCl (Figs. 2) was also much lower in BAS-385 (throughout the measurement periods after salt treatments) than that of NIAB-6 and BG-402. Dodd (2003) reported that interaction between soil environmental factors and the hormonal factors modulate stomatal behaviour. Walker & Dumberoff (1980) reported rise in t-zr in tomato under salt stress, Kuiper *et al.*, (1990) observed decrease in the CK concentration in root and shoot tissue of salt resistant plants of barley and wheat but not of salt sensitive plants.

In BAS-385, the increase in salt induced ABA was initially faster but 24 h after treatment it became steady whereas in the moderately tolerant variety, BG-402 and the tolerant variety, NIAB-6, ABA increase was rather slow and needed longer duration of salt treatment for appreciable increase in xylem ABA.

The observed transient increase in the xylem ABA 24 h after salt treatment in BAS-385 may indicate the salt shock in the sensitive variety which may account for the redistribution of ABA within the plant. Munns (2002) postulated that initial reduction in shoot growth is due to hormonal signals generated by the roots. Noaman *et al.*, (2002) demonstrated role of ABA in acclimation to salt stress. A pretreatment with ABA increases tolerance to salt shock (Parida & Das, 2005). The results also indicate that basal level of free ABA is important in determining the response of plant to salt stress. It is also indicated from the result that 48h of salt stress is the critical period for the modulation of ABA and cytokinin level in xylem. The role of 2-ipa as parent cytokinin need to be investigated as there was increase in the level of 2-ipa in xylem sap and leaves of tolerant variety 48 h of salt stress, possibly the xylem 2ipa may have positive role in salt tolerance. Different forms of cytokinins have different functions and are related to the specific developmental stage of rice plant. Kuiper *et al.*, (1995) suggested that a number of specific molecular mechanisms are responsible and that initial exposure to salt will activate part of these processes with enhanced energy demand which limits growth during adaptation.

The basal level of high free ABA in xylem sap and leaves and maintenance of the large pool of the conjugated ABA in leaves concomitant with low level of t-zr in salt tolerant cultivar NIAB-6 appears to determine the response of plant to salt stress. A time course study involving changes in free and conjugated ABA in leaves and its relation with other physiological parameter accompanied by molecular characterization might demonstrate better the mechanism of salt tolerance.

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