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Response to Salinity in Rice: Comparative Effects of Osmotic and Ionic Stresses

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Abstract : The effects of the osmotic component of salt stress on rice cultivar IR64 were examined. Treatments were four combinations of two levels of osmotic stress at two developmental stages: medium- and high-level stress applied at the vegetative and reproductive stages using salt (NaCl) and polyethylene glycol-6000 (PEG) as sources of osmotic stress. Both PEG and NaCl reduced the total above ground biomass and delayed flowering and maturity, with a longer delay observed with the high-level stress. The reduction in number of filled spikelets, 1,000-grain weight, and hence grain yield was significantly greater when they were applied during the reproductive stage than during the vegetative stage. The sodium concentration in plant tissues also increased in plants treated with NaCl, indicating that besides osmotic stress, plants were also subjected to ionic stress. Treatment with NaCl decreased the potassium concentration in plant tissues but did not cause significant differences in phenology, biomass accumulation, yield or N uptake compared with PEG. We concluded that the response of IR64 to NaCl was attributed to the osmotic component. These findings may be specific to IR64, which has a medium tolerance to salinity stress. Further studies are needed with longer stress durations to achieve a higher Na^+ concentration in plant tissues in several varieties with contrasting tolerance to salt stress to further establish the relative importance of osmotic versus ionic components of salt stress in rice.

Key words : Drought, Ionic stress, NaCl, Polyethylene glycol, Salinity.

Salinity is an important environmental factor that limits growth and yield of rice. It currently affects millions of hectares of soils otherwise suitable for rice cultivation in South and Southeast Asia (Ponnamperuma and Bandyopadhya, 1979). In saline soil, rice plants experience osmotic stress as a consequence of reduced osmotic potential of the soil solution. This results in reduced uptake of water by the plant, and generates effects similar to those of water stress caused by drought stress. Exposure to salinity also causes accumulation of salts into the plant tissues. These salts will eventually rise to toxic levels, especially in older leaves (Mitsuya et al., 2002), and may cause Na^+ toxicity (ionic stress) thereby reducing nutrient acquisition or causing nutritional imbalances (Munns and Termaat, 1986).

Since both osmotic and ionic damages are interrelated and co-exist under saline conditions, separating the two components is an important step in understanding the basis of tolerance. Previous researchers used salt (NaCl) and polyethylene glycol (PEG) to compare the effects of drought and salinity stress on the growth and ion absorption in different crops. PEG is a neutral polymer (nonionic) available in a range of molecular weights, with high solubility in water and low toxicity to mammals. Because it cannot readily penetrate the cell membrane, it does not affect the composition of the cell (Claes et al., 1990). PEG has been shown to closely mimic specific levels of water stress due to drought (Attree and Fowke, 1993). However, Storey and Wyn Jones (1978) showed that, at the same osmotic pressure, barley grew better in PEG than in NaCl, although in tomato growth was the same or worse under PEG than under NaCl stress (Perez-Alfocea et al., 1993). Murillo-Amador et al. (2002) showed that NaCl-induced damage during germination, emergence, and early seedling growth is attributed to osmotic effects rather than to specific ion effects in cowpea.

Adverse effects of salinity on rice have been reported (Yeo and Flowers, 1986; Munns and Termaat, 1986; Castillo et al., 2003; Mitsuya et al., 2003; Moradi et al., 2003); however, systematic studies on the comparative damage caused by osmotic versus ionic stresses when rice is subjected to salinity are limited and produced contrasting results. Matoh et al. (1986) suggested that the growth reduction in the rice plants subjected to salt stress was mainly due to excess of ions, while Castillo et al. (2004) suggested that the effect of salt on rice is mainly caused by the osmotic component. However, Castillo et al. (2004) did not examine in details the effects of salt stress on plant growth, yield components and cation uptake. To further quantify the importance of the osmotic component of the salinity stress in rice,

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Table 1. Effects of the source of osmotic stress on phenological development of the main tillers of IR64 in experiment 2 (high-level stress during vegetative stage).

Stress level-timing	Source of stress	Flowering date	Harvest Date
Control		25 Jul	20 Aug
High-vegetative	NaCl	6 Aug	9 Sep
High-vegetative	PEG	6 Aug	9 Sep

we compared the effects of various levels of osmotic stress caused by PEG and NaCl imposed at different timing on the development, growth, and yield of rice cultivar IR64. Furthermore, we tested whether the inhibitory effect on nitrogen and cation uptake by the rice plant is greater under NaCl or PEG treatment.

Materials and Methods

1. Experimental site

Two pot experiments were conducted in a controlled-environment glasshouse at the International Rice Research Institute, Los Baños, Laguna, Philippines (14°30´N, 121°1´E), during March-August 2002. The maximum and minimum temperatures during the experiments were maintained at 29°C and 21°C, respectively, and the relative humidity was about 86%.

2. Experimental design

Details of experiment 1 were described in Castillo et al. (2004). In summary, a 2-factor randomized complete block design with 3 replications was used. The first factor consisted of 3 level combinations of osmotic stress and timings: medium-level osmotic stress applied at the vegetative stage (OS_{mvg}) and medium- (OS_{mpi}) and high-level osmotic stress (OS_{hpi}) applied approximately 0-2 d before the panicle initiation stage (PI). The osmotic potentials of the medium- and high-level osmotic stress treatments were −0.25 MPa and −0.52 MPa, respectively. The second factor consisted of two sources of osmotic stress: NaCl (S_{NaCl}) and PEG (S_{PEG}). To obtain the above osmotic stress levels, the electrical conductivity (EC) of the NaCl solution was adjusted to 6 dS m^{-1} (3.38 g NaCl L^{-1} water) and 12 dS m^{-1} (6.75 g NaCl L^{-1}), while the concentration of PEG to 108 g L^{-1} and 178 g L^{-1}, respectively.

Experiment 2 was conducted 2 months after experiment 1, in order to evaluate the effects of a high-level osmotic stress applied during the vegetative stage (OS_{hvg}). All conditions were the same as those in experiment 1 except that only the high-level osmotic stress (−0.52 MPa) was applied during the vegetative stage with both NaCl and PEG. Apart from the stress treatments, one control treatment with no stress (NS) was included in each of the two experiments.

In both experiments, the stress treatment was terminated when the tip of the youngest leaf of the stressed plants turned whitish in color. PEG with high molecular weight (Mw = 6000) was used in both experiments because it is considered to be better than that with lower molecular weights and it has been shown to have an effect on plant tissue similar to that of natural water stress (Kauffman and Eckard, 1971; Michel, 1971).

3. Planting material and nutrient solution

Four 14-day-old seedlings of IR64 were transplanted into 25 cm high × 20 cm diameter PVC pots containing approximately 8.5 kg of 0.5-1.0 mm sand, and later thinned to 2 plants per pot. One hole was cored at the bottom of each pot for drainage and was covered with a fine-screen cloth to avoid loss of sand particles. Macro- and micro-elements were supplied to the growing plants using Yoshida solution (Yoshida et al., 1976), with pH maintained at 5.5 using 1 N HCl or 1 N NaOH. For further details of the preparation and management of the planting and nutrient solution, see Castillo et al. (2004).

4. Stress imposition and relief

NaCl solution was prepared by adding table salt to the nutrient solution to achieve the desired EC level. PEG solution was prepared by dissolving pre-weighed PEG in water. After complete dissolution, the PEG solution was purified by passing it through an ion exchange column (Universal II cartridge) to remove any impurities. The PEG was filtered using Miracloth (22-25 μm) and the filtrate was mixed thoroughly with the nutrient solution. The osmotic potential was read using a vapor pressure osmometer (WESCOR, Model VAPRO 5520, Wescor Inc., Utah, USA) and the solution was adjusted to the desired osmotic potential using filtered PEG solution or nutrient solution. The pH of the PEG-nutrient solution was adjusted to 5.5 before adding it to the respective pots.

At the start of the stress period, the nutrient solutions were drained from pots and replaced with the respective NaCl or PEG solution. EC (using a WTW-LF330 Conductivity Meter, Wissenchaftlich-Technische Werkstatten GmbH, Germany) of NaCl-nutrient solution and osmotic potentials of both NaCl-nutrient and PEG-nutrient solutions were monitored at the water surface and in the middle (5-8 cm from sand surface) and at the bottom of the pot (15-18 cm from sand surface). The nutrient solutions were drained and replaced a few times until the desired EC or osmotic potentials were attained throughout the sand profile. Normally, addition and leaching of NaCl or PEG nutrient solutions repeatedly three times was found adequate to reach the targeted stress level. The soil solutions in the stressed pots were also changed at the same intervals as those of the control.

After the completion of the stress period, stressed

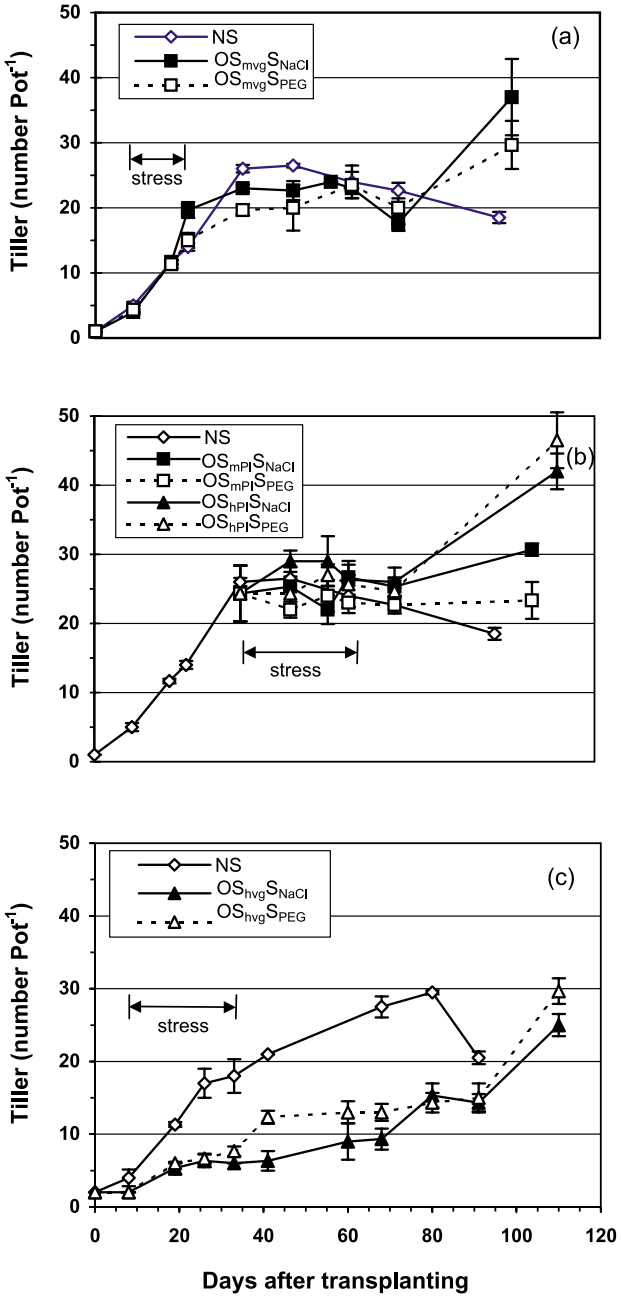

Fig. 1. Tiller number of IR64 as affected by the level, timing, and source of osmotic stress given at a) vegetative stage in experiment 1, b) reproductive stage in experiment 1, and c) vegetative stage in experiment 2. Values are means of 3 replications and vertical bars indicate ± SE.

pots were drained and re-watered with the normal nutrient solution. This was repeated several times until the EC and osmotic potential of the solution became equivalent to those of the non-stressed pots.

5. Plant growth, yield, and cation uptake measurements

Days at each developmental stage were recorded in all treatments. Above ground biomass (shoot weight), tiller number, and leaf area index, using total area per pot (calculated based on 20 cm × 20 cm spacing), were determined before stress, during and after stress treatment, and at designated growth stages [transplanting, active tillering, PI, flowering (FL), grain filling (GF), and physiological maturity (PM)]. At PM, straw and grain weights and yield components were determined. The grain weight was adjusted to 14% moisture content. Plant parts were ground to a fine powder and analyzed for the concentrations of nitrogen (N) using the Kjeldahl method, (Varley, 1966), K^+, and Na^+ using an atomic absorption spectrometer (Perkin-Elmer 3300) after extraction in HCl (Yoshida et al., 1976). The amount of the above cations in straw and panicles was calculated by multiplying the cation concentration by weight of respective organs.

6. Data analysis

Concentrations and uptake by plants (amount per plant) of various elements and all agronomic parameters measured in this study were analyzed using SAS Proc Mixed model for a 2-way randomized complete block design (Littell et al., 1996). The main effects and interaction means were separated by Tukey's multiple range test. When there were no interactions between level/timing and source of stress, means of the main effects were presented.

Results

Some preliminary results on the effect of stress on the aboveground biomass (shoot weight), grain yield, and plant Na^+ concentration measured in experiment 1 were presented in a *short paper* by Castillo et al. (2004). For the sake of comparison and discussion, some of these results are reproduced here. However, this paper focuses on the new results of the two experiments presented here.

1. Phenological development

Table 1 shows the effect of the stress treatment on the phenology of IR64 in experiment 2. High-level stress at the vegetative stage delayed flowering of IR64 by 11 days and maturity by 19 days. There was no significant difference between the delay due to NaCl and PEG. The delay in experiment 2 was longer than that in experiment 1 (3-4 days, Castillo et al., 2004). The difference was attributed to the higher level of osmotic stress imposed during the vegetative stage in experiment 2 than in experiment 1, suggesting that the higher the osmotic stress, the larger the delay in crop development.

2. Crop growth and aboveground biomass

At most sampling days, there was no significant difference in tiller number between NaCl and PEG treatments (Fig. 1). Treatments with NaCl and PEG

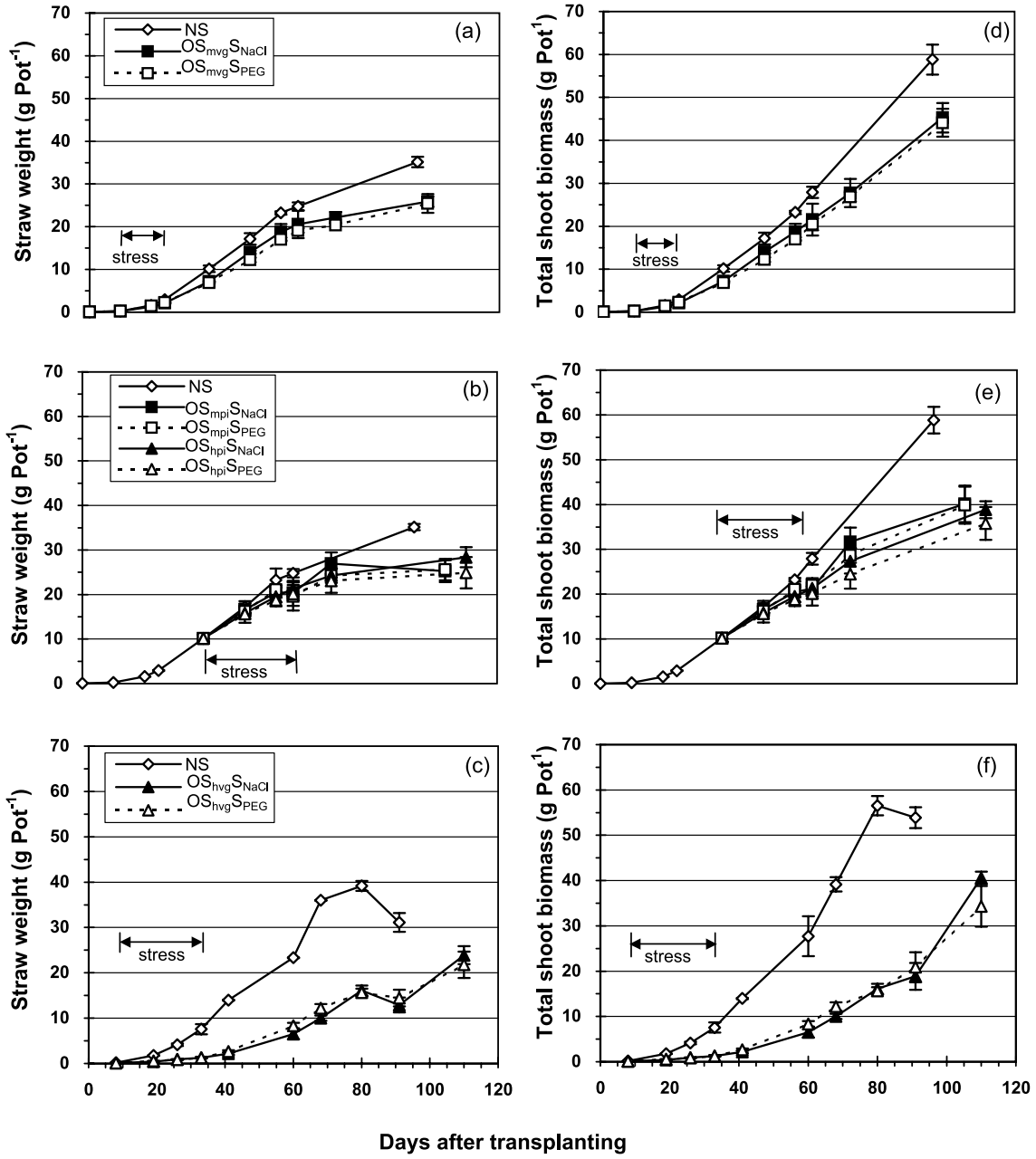

Fig. 2. Straw and shoot biomass per pot in IR64 as affected by the level, timing, and source of osmotic stress: a, d) medium-level stress during vegetative stage in experiment 1; b, e) medium- and high-level stress during reproductive stage in experiment 1, and c, f) high-level stress during vegetative stage in experiment 2. Values are means of 3 replications and vertical bars indicate ± SE. Data in d) and e) are from Castillo et al. (2004) with permission from Soil Science and Plant Nutrition Journal.

at the medium stress level at the vegetative stage and at the PI stage did not significantly affect the tiller number during and a few days after the stress periods (Figs. 1a, b). However, the high-level stress imposed at the vegetative stage reduced tiller number compared with the control (Fig. 1c). Tiller number at maturity was significantly higher in the stressed plants than in the control under all stress treatments (Fig. 1).

Stress with either NaCl or PEG significantly decreased straw as well as aboveground biomass compared with the control in both experiments (Fig. 2). For all stress timing and levels, there were no significant differences in straw and above ground biomass between the stresses with NaCl and PEG.

The effect of stress on leaf area index (LAI) varied with the timing of stress imposition and the osmotic stress level (Fig. 3). At the medium stress level, lower LAI values were observed when stress was applied during PI than during the vegetative stage because the plants stressed at the vegetative stage were able

to recover after the stress, but plants stressed at the PI stage were not. Plants subjected to high-level stress during the vegetative stage had lower LAI (Fig. 3c), whereas plants subjected to the same stress level during PI were less affected, probably because the canopy has almost fully developed before the exposure to the stress. There was an interactive effect of the level and source of osmotic stress on LAI. When a high-level osmotic stress was applied during the vegetative stage, LAI was slightly higher under PEG treatment than under NaCl (Fig. 3c), whereas, at other combinations of osmotic stress and timing, LAI under PEG treatment was lower than that under NaCl treatment. Nonetheless, the symptom of salt stress such as drying of leaf tips, and an increase in the number of dead leaves (data not shown) was clearer when NaCl was applied than when PEG was applied.

3. Grain yield and yield components

Table 2 summarizes the effects of stress treatments on grain yield and yield components in the two experiments. Except for one case with panicle number per pot (PEG > NaCl in OS_{hpi}, experiment 1) and two cases with filled spikelet number per panicle (PEG > NaCl in OS_{mpi}, experiment 1; NaCl > PEG in OS_{hvg}, experiment 2), at the same stress timing and stress level, there were no significant differences between the effects of the two sources of osmotic stress on the parameters studied. When imposed during the vegetative stage, medium osmotic stress level did not significantly affect yield and yield components (Table 2, experiment 1) compared with the control treatment. However, a high-level of PEG osmotic stress imposed at the vegetative stage reduced the number of filled spikelets per panicle and yield significantly (Table 2, experiment 2). Stress at PI significantly reduced the number of filled spikelets per panicle, 1000-grain weight, and percentage of filled spikelets, leading to lower grain yield than the control treatment. Among the yield components, the percentage of filled spikelets was significantly affected by the level of osmotic stress at the PI stage. Under high-level stress, it was only about half of that under medium-level stress, leading to lower yield and harvest index. Yield and harvest index in the treatments with high-level osmotic stress at PI were significantly lower than that in all other treatments (Table 2, experiment 1).

At harvest, the final tiller numbers in the stress treatments (Fig. 1) were much higher than the panicle numbers (Table 2). This implies that osmotic stress increased the number of non-productive tillers. The very low harvest index in the treatments with high-level osmotic stress during PI was a result of low grain yield and the late non-productive tillers, which were formed after the stress was relieved. Some of the late tillers also produced panicles, as evidenced by the high number of panicles when the stress was imposed at the PI stage

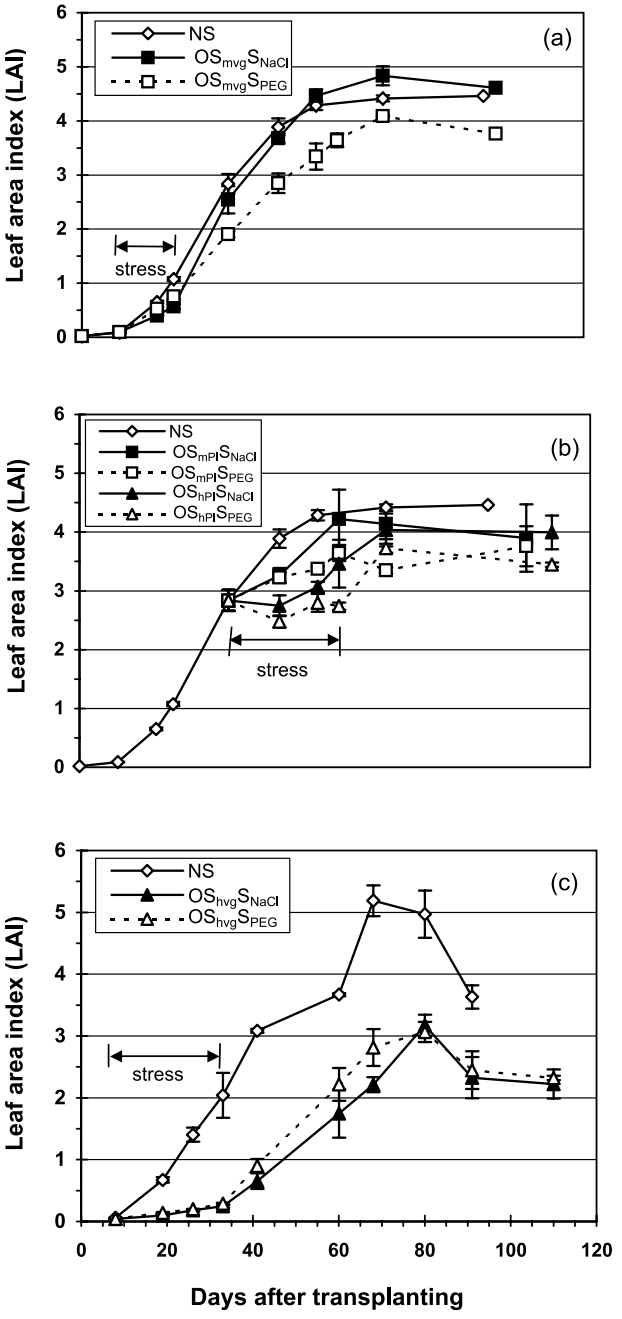

Fig. 3. Leaf area index of IR64 as affected by the level, timing, and source of osmotic stress: a) medium-level stress during vegetative stage in experiment 1, b) medium- and high-level stress during reproductive stage in experiment 1, and c) high-level stress during vegetative stage in experiment 2. Values are means of 3 replications and vertical bars indicate ± SE.

(Table 2). These panicles, however, contained mainly unfilled spikelets, as reflected in the low percentage of filled spikelets in Table 2.

4. Ion uptake

(1) Nitrogen

Nitrogen is the limiting nutrient for crop

Table 2. Yield and yield components of IR64 as affected by osmotic levels, timing, and sources of stress in experiments 1 and 2 .

Stress timing/level	Source	Panicles	Filled Spikelets	1000-grain wt.	Filled spikelet	Grain weight	Harvest index
		(no. pot^{-1})	(no. panicle^{-1})	(g)	(%)	(g pot^{-1})	
Experiment 1							
control[1]		18[2]b[3]	57a	22.1a	83.0a	21.7a	0.35a
OS_{mvg}	NaCl	18b	45ab	21.7a	82.0a	17.6ab	0.36a
OS_{mvg}	PEG	17b	46ab	21.5a	74.0ab	16.6ab	0.38a
OS_{mpi}	NaCl	22ab	27c	18.4b	58.5b	12.9bc	0.32a
OS_{mpi}	PEG	18b	39b	18.5b	70.0ab	12.3bc	0.31a
OS_{hpi}	NaCl	18b	20cd	17.5b	37.0c	6.1c	0.16b
OS_{hpi}	PEG	27a	13d	18.1b	39.0c	6.2c	0.17b
Experiment 2							
control		21a	46a	20.8a	79.7a	19.8a	0.37a
OS_{hvg}	NaCl	17a	48a	20.5a	86.3a	16.5ab	0.41a
OS_{hvg}	PEG	17a	35b	21.2a	80.4a	12.6b	0.37a

[1] Plants grown in optimum conditions.

[2] Means are average over 3 replications.

[3] In each column, means followed by the same letter are not significantly different at the 5% level using Turkey.

OS_{mvg} = medium osmotic stress at vegetative stage. OS_{mpi} = medium osmotic stress at panicle initiation stage.
OS_{hpi} = high osmotic stress at panicle initiation stage. OS_{hvg} = high osmotic stress at vegetative stage.

production. To test whether osmotic stress with NaCl or PEG exert similar or different effects on N uptake, we monitored the concentration and the amount of total N in the straw and panicles per plant (straw N/plant and panicle N/plant, respectively) of IR64 under different treatments. N concentration in shoot (shoot N concentration) was higher under NaCl treatment than under PEG treatment during stress applied at both vegetative (Fig. 4a, c) and PI stages (Fig. 4b), as well as after the stress treatment. This suggests that N uptake was not hindered by higher levels of NaCl in the nutrient solution. After stress recovery and towards maturity, N concentrations became similar under both NaCl and PEG treatments.

Despite higher plant N concentration, stress treatments resulted in a smaller amount of N per plant than the control (Fig. 4d, e, f). This relates to the reduced above ground biomass by the stress treatments (Fig. 2), and suggests that the stress affected above ground biomass more than shoot N concentration. This concurs with the observation that total N/ plant under the PEG and NaCl treatments did not differ significantly, though the plant N concentration under NaCl treatment was higher than that under PEG treatment.

Table 3 shows the straw N/plant and panicle N/plant at maturity. In both experiments and at the same osmotic stress level and timing, straw N and panicle N did not vary significantly with the source of stress (PEG or NaCl). In experiment 1, straw N in the plants stressed at PI was lower than that in the control, though not significant ($P > 0.05$), and comparable with that in the plants treated at the vegetative stage. On the other hand, stress at PI significantly reduced the panicle N compared with the control. The reduction became progressively greater with increasing osmotic stress level. This implies that poor partitioning of N into grains occurred when the stress treatments were imposed during the PI stage.

(2) Potassium

Potassium concentration in shoot (shoot K^+ concentration) decreased with time in all treatments up to the maturity stage (Figs. 5a, b, and c). At most sampling times, there was no significant difference between the shoot K^+ concentration in the control and the PEG treatments, however, NaCl treatments reduced the shoot K^+ concentration significantly compared with the control, especially when stress was applied at the vegetative stage (Figs. 5a, c). However, the shoot K^+ concentration increased rapidly after the stress relief and, at maturity, it was similar to that in the control (Fig. 5).

Osmotic stress reduced the amount of K^+ per plant regardless of the source, level, and time of stress when compared with the control. This reduction in plant K^+ uptake in the stress treatments was progressively more apparent until maturity (Figs. 5d-f). This reduction is due to the effect of stress on shoot biomass production, which was irreversible, rather than on shoot K^+ concentration, which was able to recover to the control level after the stress.

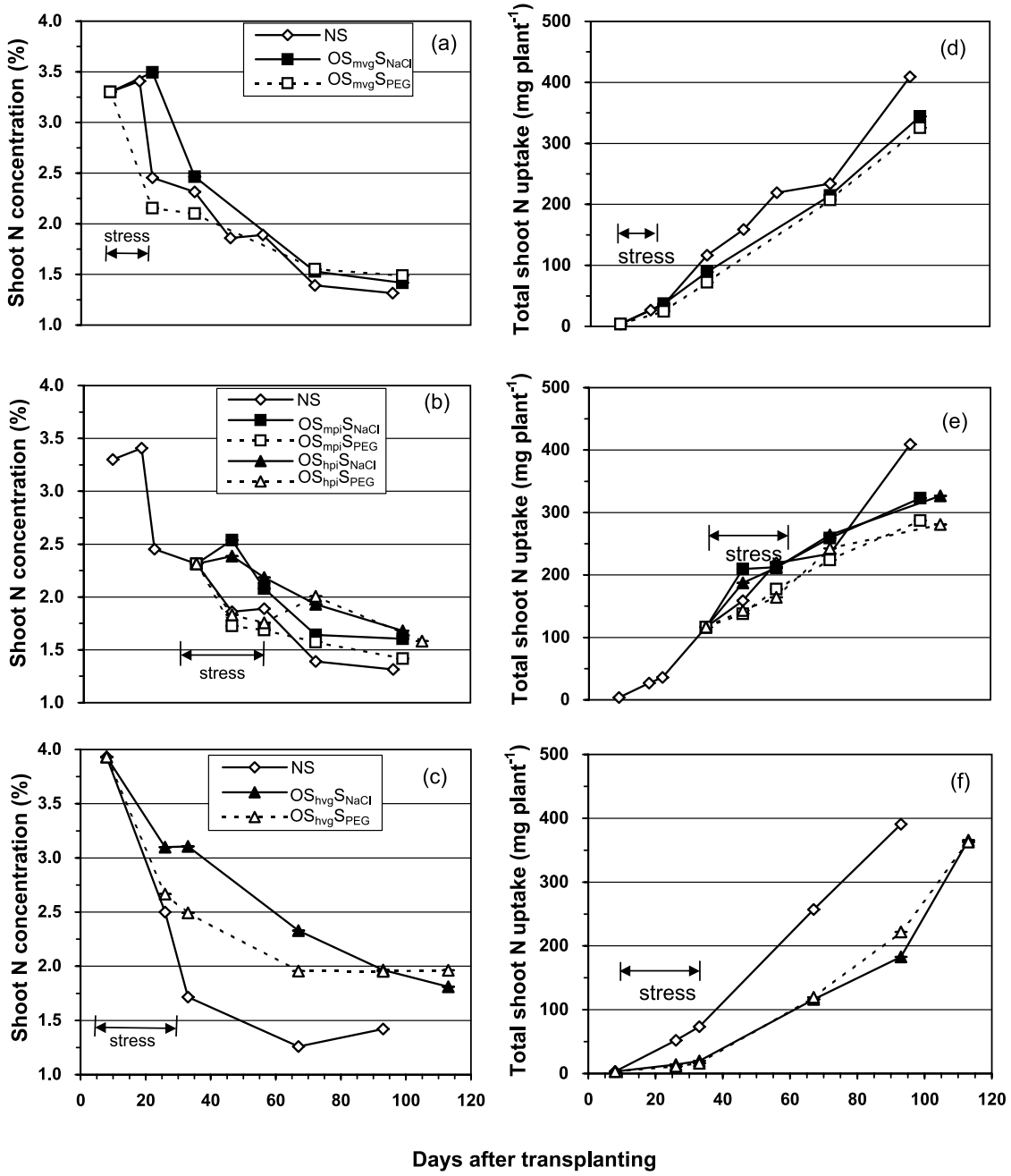

Fig. 4. Nitrogen concentration and uptake of IR64 as affected by the level, timing, and source of osmotic stress: a, d) medium-level stress during vegetative stage in experiment 1; b, e) medium- and high-level stress during reproductive stage in experiment 1; and c, f) high-level stress during vegetative stage in experiment 2. Values are means of 3 replications and vertical bars indicate ± SE.

Table 3 shows the partitioning of K^+ into the straw K^+ and panicle K^+ at maturity. In both experiments, at the same osmotic stress level and timing, straw K^+/plant and panicle K^+/plant in the PEG treatment did not differ significantly from those in the NaCl treatment. Stress reduced total straw K^+/plant compared with the control in experiment 2. Medium-level osmotic stress (experiment 1) and high-level osmotic stress (experiment 2) at the vegetative stage did not significantly affect the panicle K^+ /plant. The panicle K^+/plant in the PI stress treatment was smaller than that in the control, but the difference in panicle K^+/plant in the control and that in the stress treatment was significant only when the osmotic stress level was high.

(3) Sodium uptake

The dynamics of Na^+ concentration in shoot in experiment 1 was reported by Castillo et al. (2004) and also shown in Figs. 6a and b for comparison.

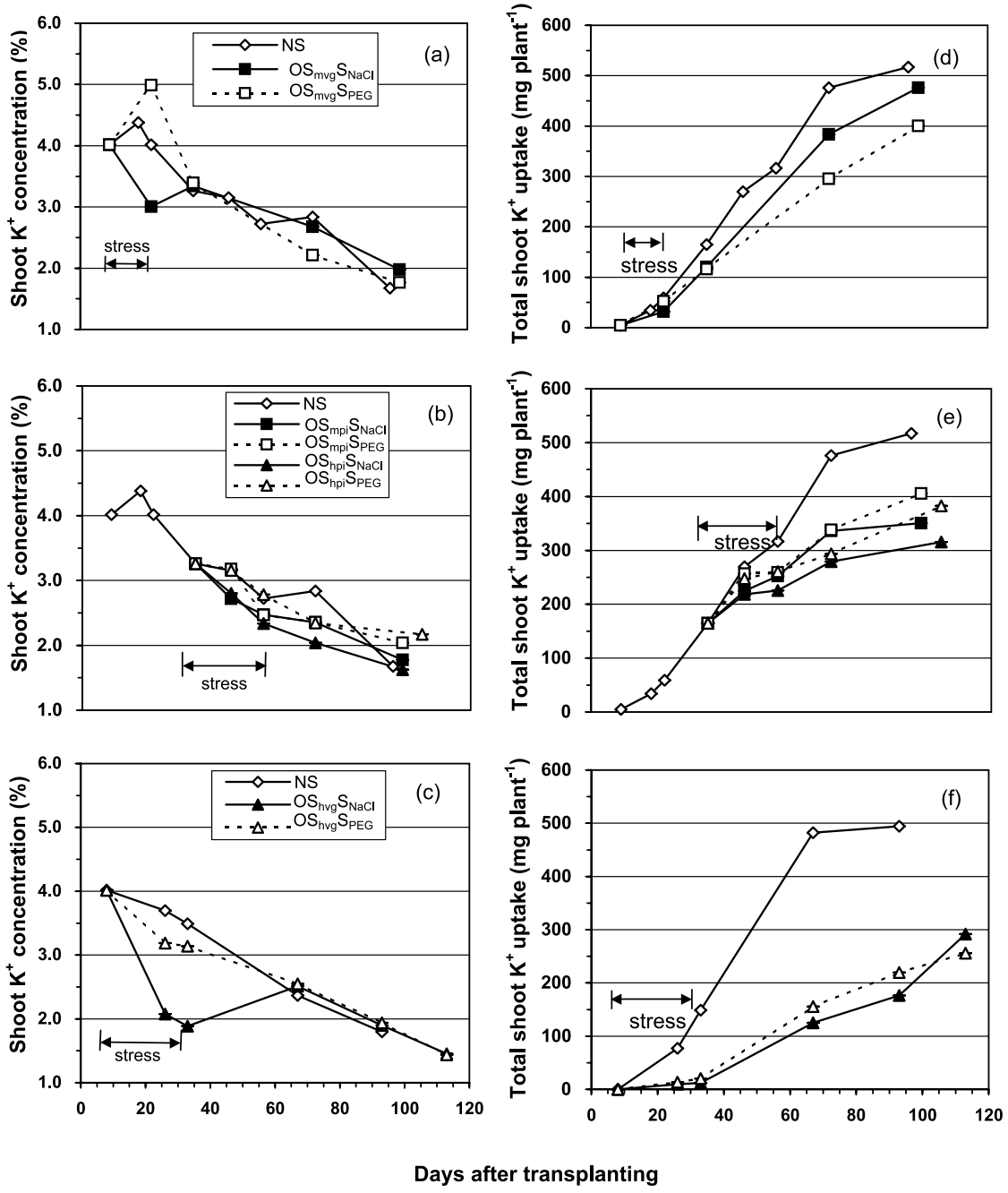

Fig. 5. Shoot K^+ concentration and shoot K^+ /plant in IR64 as affected by level, timing, and sources of osmotic stress: a, d) medium-level stress during vegetative stage in experiment 1; b, e) medium- and high-level stress during reproductive stage in experiment 1; and c, f) high-level stress during vegetative stage in experiment 2. Values are means of 3 replications and vertical bars indicate ± SE.

In experiment 2, NaCl stress at the vegetative stage significantly increased shoot Na^+ concentration compared with PEG stress and the control treatment. The shoot Na^+ concentration in this treatment reached the maximum at the end of the stress treatment, but declined rapidly thereafter and became similar to that in other treatments at about 40 days after the stress relief (Fig. 6c).

At all osmotic stress levels and timing, PEG treatments had no effect on the amount of Na^+/plant (Na^+ uptake). The treatments with NaCl invariably increased the Na^+ uptake/plant during the stress period (Figs. 6d-f). The higher the stress level, the greater the increase in shoot Na^+ concentration. Similar to the shoot Na^+ concentration, the shoot Na^+ /plant in the NaCl treatments increased only during the stress period and decreased rapidly thereafter. When NaCl stress at the vegetative stage was relieved,

Table 3. The amounts of N, K^+, and Na^+ in straw and panicle per plant in IR64 at maturity as affected by level, time, and source of stress in experiments 1 and 2.

Stress timing/ level	Source	N (mg plant^{-1})		K^+ (mg plant^{-1})		Na^+ (mg plant^{-1})	
		straw	panicle	straw	panicle	straw	panicle
Experiment 1							
Control[1]		257[2]a[3]	152ab	456a	61a	3.7c	0.34a
OS_{mvg}	NaCl	168a	176a	419a	57ab	5.8c	0.17a
OS_{mvg}	PEG	180a	146ab	355a	46abc	4.7c	0.17a
OS_{mpi}	NaCl	197a	126bc	314a	37abc	26.9b	0.15a
OS_{mpi}	PEG	175a	113bcd	370a	36abc	4.1c	0.11a
OS_{hpi}	NaCl	254a	73d	290a	25c	78.4a	0.14a
OS_{hpi}	PEG	193a	88cd	352a	31bc	6.1c	0.17a
Experiment 2							
Control		214a	177a	421a	73a	6.4a	0.38a
OS_{hvg}	NaCl	222a	144a	245b	47a	7.3a	0.35a
OS_{hvg}	PEG	219a	144a	215b	41a	5.0a	0.28a

[1] Plants grown in optimum conditions.

[2] Means are average over 3 replications.

[3] In each column, means followed by the same letter are not significantly different at the 5% level using Turkey.

OS_{mvg} = medium osmotic stress at vegetative stage.

OS_{mpi} = medium osmotic stress at panicle initiation stage.

OS_{hpi} = high osmotic stress at panicle initiation stage.

OS_{hvg} = high osmotic stress at vegetative stage.

shoot Na^+ /plant dropped to the level of the control treatment (Fig. 6a, d). However, when NaCl stress was applied during the PI stage, shoot Na^+ /plant also decreased after the stress relief, although it remained significantly higher than that of the control and the PEG treatments at maturity (Fig. 6e).

At harvest, most of the sodium taken up by plants seems to be accumulated in the straw and only a small amount was allocated to the grains (Table 3). In both experiments, there were no significant differences in panicle Na^+/plant with the treatment. Straw Na^+/plant in the treatments with NaCl at the PI stage was significantly larger than that in other treatments, and it increased with increasing osmotic stress level (Table 3). The large straw Na^+/plant in the treatments with NaCl at PI was attributed mainly to higher straw Na^+ concentration, because there was no significant difference in straw weight at harvest among the stress treatments (Fig 2).

Discussion

In this study, PEG and NaCl were used to create osmotic stress in soil solution. The results were similar to those reported by previous investigators who demonstrated that drought stress applied at vegetative and PI stages delayed crop development, and the delay was greater at higher stress levels (Wopereis et al., 1996; Tuong et al., 2002). Osmotic stress with PEG and NaCl during PI was more detrimental to rice yield than that at the vegetative stage. Yield reduction caused by osmotic stress was mainly attributed to the decrease in percentage of filled spikelets and the number of filled grains per panicle. Stress at PI must have affected the grain formation process since rice is highly sensitive to drought and salt stress during reproduction (Wopereis et al., 1996; Moradi et al., 2003).

Though water-related parameters to quantify the osmotic stress were not measured, we assumed that the effect of osmotic stress could adequately mimic that of drought at a similar magnitude of osmotic potentials, as concluded by previous authors (e.g., Kauffman and Eckard, 1971; Michel, 1971; Attree and Fowke, 1993). This assumption was supported by the similarity between findings in this study and those of previous authors (Wopereis et al., 1996; Tuong et al., 2002; Castillo et al., 2006). The higher N concentration in the stressed plants suggests that osmotic stress limited growth more than N uptake by the plants.

Under the NaCl treatment, rice roots absorb Na^+ from the soil solution, subjecting the plants to both ionic and osmotic stresses. The high concentration of Na^+ in the plant treated with NaCl (Fig. 6) suggests that the culture in NaCl solution imposed ionic stress on the plant, in addition to osmotic stress. This is

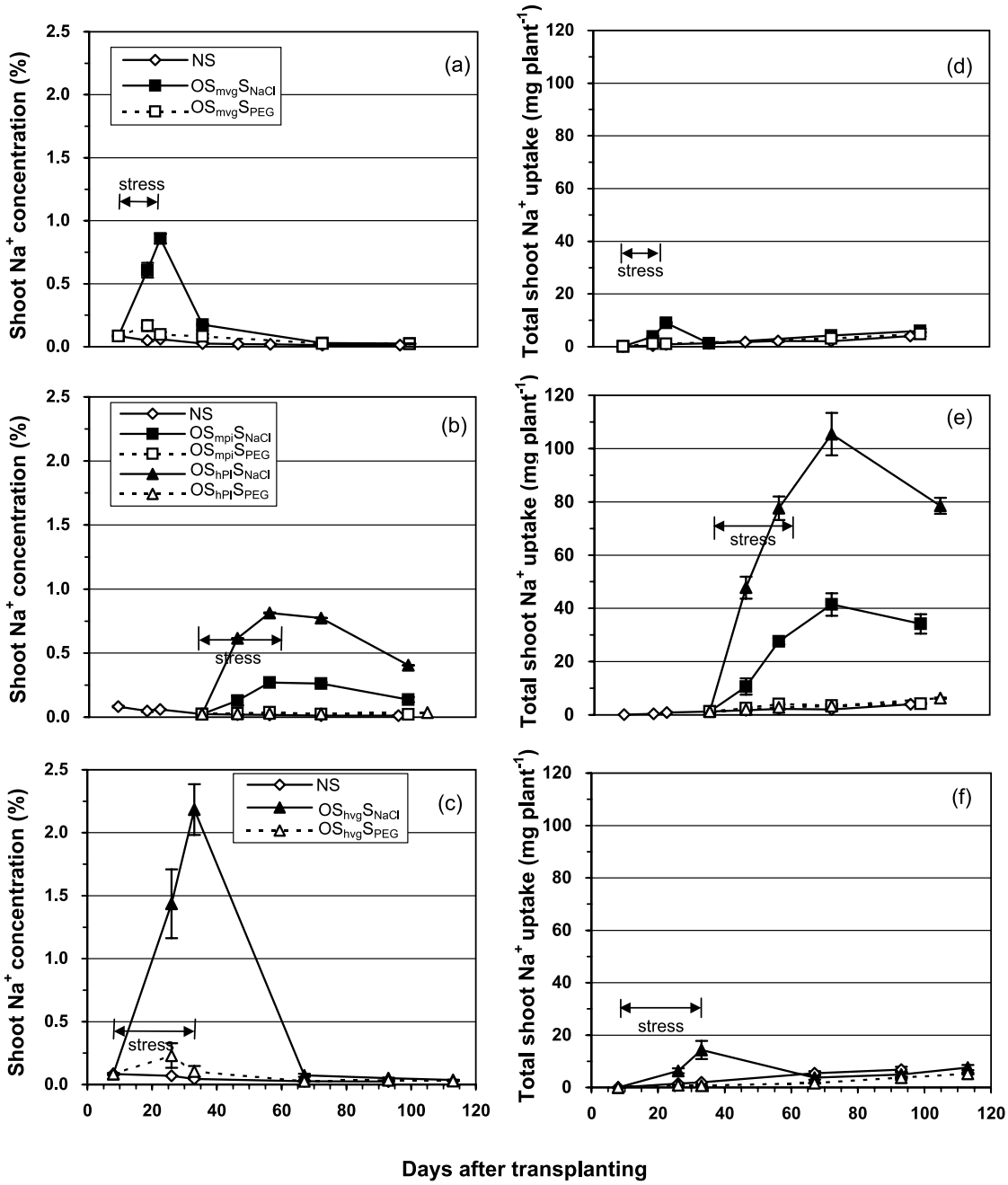

Fig. 6. Shoot Na^+ concentration and shoot Na^+/plant in IR64 as affected by the level, timing, and source of osmotic stress: a, d) medium-level stress during vegetative stage in experiment 1; b, e) medium and high stress during reproductive stage in experiment 1; and c, f) high stress during vegetative stage in experiment 2. Values are means of 3 replications and vertical bars indicate ± SE. Data in a) and b) are from Castillo et al. (2004) with permission from Soil Science and Plant Nutrition Journal.

further evidenced by the antagonistic effect of higher levels of Na^+ on K^+ uptake (Figs. 5, 6), which has been shown to occur in previous studies with high concentrations of Na^+ in nutrient or soil solution (Yeo and Flowers, 1989; Romero et al., 1994; Lutts et al., 1996; Benes et al., 1996). It can be argued that excess NaCl could lead to the loss of K^+ due to membrane depolarization and displacement by Na^+ ions (Cramer et al., 1991). The two cations also have similar atoms and the K^+ transporters are known to be less specific at toxic levels of Na^+.

There were no significant differences between the effects of the two sources of osmotic stress, PEG and NaCl, on plant development, growth and yield parameters. This suggests that the observed effects of salinity stress on the rice cv. IR64, were mainly caused

by osmotic stress. Despite the larger shoot Na^+ /plant in NaCl treatments, the effects of the ionic stress were not apparent. This was in contrast to the findings of Matoh et al. (1986), who argued that growth reduction in rice plants (cv. Kinmaze) was mainly due to excess ions.

There are several possible explanations of the difference in the effects of ionic stress between this and previous studies. The highest Na^+ concentration observed in this study (2.2% in the $OS_{hvg}S_{NaCl}$ treatment, Fig. 6c) was lower than the values reported earlier (3.5% at 100 mM NaCl by Matoh et al. 1986 and 3.2% at 50 mol m^{-3} by Yeo and Flowers, 1986). The stress periods were short and the shoot Na^+ concentration in this study might not be high enough to reach the toxic level. Another possibility is that, although higher shoot Na^+ concentrations were observed in the NaCl-treated plants, the salts have accumulated in the vacuoles or other apoplastic compartments, maintaining the cytoplasm at a relatively lower salt concentration (Flowers et al., 1977; Wyn Jones et al., 1977). The compartmentation of Na^+ into the vacuoles may efficiently avert the deleterious effects of Na^+ on the cytosol keeping the cell's osmotic potential, and alleviating the water deficit in a saline environment. The variety IR64 used in this study might be more efficient in this compartmentation than the varieties used by previous investigators. This is further evidenced by the substantially lower level of sodium in the panicles of IR64 compared with that in the straw under NaCl stress, suggesting that IR64 has the ability to exclude salt from the panicles. This could be attributed to more selective uptake since the uptake of ions into reproductive organs is mainly symplastic. Our studies (Ismail, unpublished) also showed that salt-tolerant cultivars have greater ability to exclude salt from developing panicles during salt stress, which is similar to previous findings (e.g. Asch et al., 1999; Tsuda et al., 2001).

Another possible explanation for insignificant effects of ionic stress on plant development and growth is that, Na^+ ions may have been re-translocated back into the medium (Lessani and Marchner, 1978; Berthomieu et al., 2003), or compartmented to other less sensitive organs within the plant such as older leaves and roots (Ismail, unpublished; Yamanouchi et al., 1997; Mitsuya et al., 2002). The observed decline in shoot K^+/plant after the NaCl stress period (Fig. 6) supports the postulation of re-translocation of sodium back to the medium. This was particularly true when stress was applied during the vegetative stage since shedding of plant leaves at this stage and in such a short period may not be possible to explain the reduction in shoot Na^+/plant. Some reduction in shoot Na^+/plant observed at maturity when NaCl stress was applied at PI (Fig. 6e) may be attributed to gradual senescence and shedding of older leaves, which are known to contain higher concentration of sodium. The fact that sodium concentration in plant tissue decreased to the ground level only when NaCl treatment was imposed during the vegetative stage may also suggest that the proposed recirculation might not occur during reproduction.

Finally, under NaCl stress, the root of IR64 may experience less osmotic stress than under PEG stress. This is because partial uptake of NaCl by root decreases the osmotic gradient between roots and soil solution. In addition, some other specific effects of PEG might have contributed to the insignificant differences in growth and yield between the plants stressed with PEG and NaCl. For instance, the high viscosity of the PEG-nutrient solution might affect the N uptake. The insignificant effects of ionic stress could also be attributed to inadequate accumulation of Na^+ in the plant tissue, which may not have reached a toxic level for IR64 due to the relatively shorter duration of stress.

Conclusions

The lack of significant differences between the effects of NaCl and PEG treatments on phenological development, plant growth, grain yield, yield components, and N uptake indicated that the response of IR64 to salinity stress could mostly be attributed to the osmotic component of the salt stress. The ionic stress (toxicity) effect was reflected only in the reduced uptake of K^+ under NaCl treatment, compared with that under PEG treatment. The findings of this study may be specific to IR64 in which the Na^+ concentration in the plant tissues was at a relatively low or intermediate level. Further studies are needed with longer stress durations to create higher Na^+ concentration in the plant tissues using several varieties contrasting in their salinity tolerance, to further affirm the relative importance of osmotic and ionic components of salt stress in rice.

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