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Discerning the Response of Rice (*Oryza sativa* L.) Cultivars to Salinity under Contrasting Moisture Regimes

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ABSTRACT

Rice is an important cereal grown under different cultivation systems. The impact of different cultivation system on salinity is a concern. A study using six genotypes (having varied level of salt tolerance) with five saline treatments under aerobic and wetland conditions imposed at reproductive stage showed different behavior for different genotypes. Salinity tolerance at cellular level was understood by quantifying apoplastic, intracellular sodium (Na^+) and potassium (K^+). Pokkali varieties exhibited lower Na^+ in the apoplastic space reflecting their saline tolerance character. ARB6 recorded higher apoplastic Na^+, indicating a possible trade-off between drought resistance (aerobic condition) and saline tolerance. Same trend was also seen in intracellular Na^+. There was no significant difference for K^+ / Na^+ ratio between aerobic and wetland conditions. T test indicated the significant difference for apoplastic Na^+ accumulation and intracellular K^+ accumulation between two cultivation systems of rice. Tiller number and root volume manifested significant difference between two conditions studied. Salt accumulation under aerobic condition was significantly higher than wetland conditions in all varieties, indicating different mechanism of saline tolerance among cultivation systems.

Keywords: Apoplastic, intracellular, potassium, rice, salinity, sodium

SALT stress challenges productivity and the area under salinity is progressively increasing. Apart from natural salinity, a significant proportion of recently cultivated agricultural land has become saline owing to land clearing or irrigation, both of which cause water tables to rise and concentrate the salts in the root zone (Munns and Tester, 2008). Moreover, soil salinization due to irrigation is becoming increasingly detrimental to agriculture (Kader and Lindberg, 2005).

One of the principal adverse effects of high salinity in non-tolerant plants is growth inhibition by toxicity to Na+. Maintenance of a high cytosolic [K^+]: [Na^+] ratio is critical for the function of cells. Short term salinity leads to physiological drought and its persistence causes ionic stress (Zhu., 2002) leading to altered K^+/ Na^+ ratios, which affects all aspects of plant growth. Plant responses to salinity occur in two phases: a rapid, osmotic phase that inhibits growth of young leaves, and a slower, ionic phase that accelerates senescence of mature leaves.

In saline conditions, Na^+ competes with K+ for uptake through common transport systems and this happens often, since in the environment [Na^+] is usually higher than [K^+]. Thus, elevated levels of cytosolic Na^+, or in another way a high [Na^+]:[K^+] ratio, exerts metabolic toxicity by competition between Na^+ and K^+ for the binding sites of many enzymes (Tester and Davenport, 2003). Protection of this Na^+ sensitive metabolic mechanism under saline conditions partly depends on the ability to keep cytosolic Na^+ levels low.

Maintaining low levels of Na^+ in the cytoplasm is critical for survival under saline stress, and plants have been shown to maintain low cytoplasmic Na^+ by intracellular (Fukuda *et al*., 2004 and Anil *et al.,* 2007) and extracellular (Anil *et al*., 2005) compartmentalization. Excessive Na^+ in the apoplast has been shown to correlate with poor survival of plants (Flowers *et al*., 1991 and Krishnamurthy *et al.,* 2009). The mechanisms by which Na+ enters the shoots of plants are still ambiguous (Kronzucker and Britto, 2011), but apoplastic transpirational bypass flow of water and solutes has been shown to play a significant role in rice (Ochiai and Matoh, 2002).

Rice is an important cereal and is relatively tolerant to salinity at the germination stage but it is highly sensitive at seedling and panicle initiation stage. Panicle initiation stage is directly related to crop yield. Stress at panicle initiation stage causes spikelet sterility that ultimately leads to yield loss. On the other hand, it also exhibits enormous varietal differences with respect to salt sensitivity, with some of these cultivars growing in very high salt concentrations (Munns and Tester, 2008). This availability of cultivars with widely differing sensitivity to salinity can be exploited to explore mechanisms of salt tolerance.

Aerobic rice is a method of rice cultivation of rice under non-flooded conditions in non puddled and unsaturated soil. Absence of transplanting and puddling saves water. It is responsive to high inputs, can be rainfed or irrigated, and tolerates occasional flooding. Recent findings also suggest more number of beneficial micro-organisms dwell in aerobic condition (Shashidhar, 2007). However, change in the cultivation system might expose aerobic rice varieties to salinity which is unlike the conditions present under puddled conditions (Kirk, 2004). Initial experiment to understand the behavior of aerobic rice varieties under saline conditions has to be done. In the present study, responses of six rice varieties to salinity are investigated under both aerobic and puddled conditions in terms of accumulation of Na^+ and K^+ in intra and intercellular space.

MATERIAL AND METHODS

Plant material, growth condition and salinity treatments

Six cultivars of rice *viz*., Pokkali cv. Chittiviruppu (Pokkali C), Pokkali (Pokkali L), Ezhome-1, Ezhome-2, IR-64 and ARB-6 (an aerobic rice variety) were used. Plants were grown in PVC pipes of diameter 16cm and length 60cm. Field soil with pH of 5.7 was mixed with organic vermicompost (Organic carbon 9-17 per cent, Nitrogen0.5-1.5 per cent, Phosphorous 0.1-0.3 per cent, Potassium 0.15-0.56 per cent, Sodium 0.06-0.3 per cent and micronutrients) in the ratio 2:1 (soil: manure), and used to fill the pipes. Further, compaction of the soil was achieved by watering and pressing the soil at intervals in order to mimic the compaction of soil infield conditions as described in Shashidhar *et al*. (2012).

Seeds were sown in pipes for both aerobic as well as wetland condition. One plant per pipe was maintained and grown for 90 days. A total of 180 pipes (90 pipes each for both the conditions) were used with three replications. Pipes layout followed factorial randomized complete block design. Pipes were regularly irrigated for wetland condition whereas for aerobic condition irrigation was done thrice a week till 90th day. From 91st day, saline stress was imposed with different level of NaCl, 0mM NaCl (T1-Control) 100mM NaCl (T2), 150mM NaCl (T3), 200mM NaCl (T4) and 250mM NaCl (T5) for one week. 300ml of NaCl solution was added to each pipe with different concentration, after two hours water was added to the pipe in order to maintain uniform distribution of the salt solution within the pipe (Chowdery *et al*., 2016).

Plant morphology and biomass: After one week of stress, soil column along with whole plant was taken out of pipe. Roots were carefully washed free of soil. The numbers of lateral roots, tillers were counted manually, shoot and root lengths were measured. Root volume and fresh weight of the plants were recorded.

Estimation of apoplastic and intracellular Na^+ *and* K^+ *in shoot:* Plants exposed to different level of salinity were harvested, washed thoroughly 2-3 times with distilled water to remove surface Na^+. Apoplastic and intracellular Na^+ and K^+ from shoot were estimated using the method described by Anil *et al.,* (2005). Freshly harvested shoots were taken, cut into 1 cm pieces, kept in double-distilled water for two hours. For apoplastic Na^+ and K^+ content, filtered fluid was taken. For the intracellular Na^+ and K^+, soaked 1cm pieces were dried and crushed to powder with liquid nitrogen and kept in double distilled water for two hours and then filtered fluid was taken. The Na^+ and K^+in these fluids were estimated by flame photometry.

Statistical analyses: Paired t test was used to estimate the difference between aerobic and wetland condition. ANOVA was used to detect the significant differences in each genotypes under five treatments between two factors (aerobic and wetland). Differences between genotypes across treatments were analyzed by Duncans' Multiple Range Test (DMRT) (Duncan, 1955).

RESULTS AND DISCUSSION

Morphological analysis: Different level of stress resulted in differences in plant growth pattern. Leaves of stressed plants showed brownish red discoloration. Shoot length, root length, root volume root number and fresh weight of the plant traits exhibited significant variation for different treatments and conditions.

Results of ANOVA for all traits are summarized in Table I.

(i) *Shoot length:* Shoot growth exhibited sensitivity to saline stress than root growth, a phenomenon that also occurs in drying soils and for which there is as yet no mechanistic 1 explanation. Shoot length ranged from 35 to 71cm in aerobic condition whereas in wetland, it varied from 22 to 75 cm. There was significant difference between treatments for shoot length. There was a significant interaction between genotype and treatment as well as between genotype and condition for this trait.

(ii) *Leaf area:* In cereals, the major effect of salinity on total leaf area was reduction in the number of tillers (Sala *et al.,* 2016). Hence, the number of tillers which directly influenced on biomass was calculated. The tiller number exhibited significant variation between two conditions irrespective of genotypes. The maximum tiller number was 16 in aerobic condition where as it was 22 in wetland condition. This trait was influenced more by wetland condition than by aerobic condition. Combinations of treatments in these conditions were also influencing the tiller number significantly.

(iii) *Root length:* Significant variation in root length was observed among the genotypes as well as among both conditions. This trait was significantly influenced by the combined effect of condition and treatments. Not much difference was seen in Pokkali (Chittivirupppu) and ARB-6, small but significant increase was observed for Ezhome 2 and Pokkai L, whereas IR-64, Ezhome 1 showed significant decrease in root length under wetland condition under salinity.

(iv) *Root volume:* Root volume was influenced significantly by the two moisture regimes (conditions) and significant variation was observed between the genotypes, however, no

TABLE I

Analysis of variance for plant morphological traits under aerobic and wetland condition

Source of variation	Mean Sum of Squares						
	df	SL	TN	RL	RV	RN	FW
Condition (Wetland, Aerobic)	1	0.968	121.68 *	996.39 *	7488.45 *	268.87	64.76
Genotype	5	1386.20	19.06	960.98 *	991.44 *	2966.63 *	237.45 *
Treatment (Five levels)	4	273.28 *	13.39	200.20	121.83	333.54	151.51 *
Condition x Genotype	5	114.86 *	16.76	53.18	581.75 *	111.92	123.24
Condition x Treatment	4	196.82	44.86 *	369.81 *	387.14 *	2472.95 *	495.14 *
Genotype x Treatment	20	50.19 *	10.84	180.54	164.51	835.66	70.47
Condition x Genotype x Treatment	20	120.30	12.49	153.30	122.05	1217.98	113.33 *
Error	118	79.34	9.15	121.14	147.27	769.59	58.21
Total	179	123.97	11.94	163.88	229.62	896.70	83.84
SE		8.91	3.03	11.01	12.14	27.74	7.63
CV		17.23	44.72	29.04	67.19	44.47	42.48
CD @5%		14.40	4.89	17.79	19.62	44.84	12.33

SL: Shoot Length, TN: Tiller Number, RL: Root Length, RV: Root Volume, RN: Root Number, FW: Fresh Weight significant variation was observed between treatments. Interaction of conditions with genotypes and conditions with treatments exhibited significant variation for root volume. Root volume was higher in Ezhome 1 wetland condition than aerobic condition.

(v) *Fresh weight:* Fresh weight of shoot was significantly varied among the genotypes as well as among treatments. The combination of treatments, conditions and genotypes had superior influence on fresh weight.

Sodium and potassium uptake by rice plants

In rice, it has been suggested that K^+ and Na^+ enter shoot by distinct mechanisms which are genetically regulated. Significant differences in apoplastic and intracellular Na^+ and K^+ levels along with K+ and Na+ ratios were observed and are presented in Table II.

Na+ content varies under salt stress: For most species, Na^+ appears to reach a toxic concentration before Cl– does and so most studies have concentrated on Na^+ exclusion and the control of Na^+ transport within the plant.

Apoplastic transpirational bypass flow of water and solutes has been shown to play a significant role in rice (Ochiai and Matoh, 2002). The apoplastic Na^+ shown to be correlated well with survival under salt stress (Chowdery *et al*., 2016). The Apoplastic Na^+ showed highest significant variation among the genotypes studied. It was also found to be varied significantly between conditions used. It was also influenced significantly by different level of treatments. Significant change in the apoplastic Na^+ was observed in combination of interactions. In the combination of genotype and conditions apoplastic Na^+ was found to have no interaction.

Intracellular Na^+ was not influenced significantly by the condition alone but significant variation was observed among the genotypes and treatments irrespective of the conditions. Effect of the treatment did not change with change in conditions. Genotype and treatment interactions were present. Genotype and treatment interaction; genotype and condition; genotype, condition and treatment interactions were found to have significant interaction.

K^+ transport: The concentration of K^+ in the cytoplasm relative to that of Na^+ may be a contributing factor to salinity tolerance. Significant variation was observed for apoplastic K^+ between the conditions, between the treatments and also among the genotypes. But, non- significant variations were found between the interactions, between condition and genotype, between condition and treatments, between the genotype and treatment interaction. Only under combination of condition, treatment and genotype had significant effects.

Intracellular K^+ was also had significant differences between the genotypes, between the conditions, and also between the treatments. Interaction of treatments and genotypes on the condition differ significantly. Like apoplastic K^+, here also condition did not influence on genotype and treatments.

K^+/Na^+ ratio maintenance is critical for the plant growth under stress. In case of intracellular fluid, K / Na ratio was significantly varied between treatments irrespective of the condition and also it was found to be varied significantly among the genotypes. Only genotype and treatment interaction is present. As the treatment changed, K^+/Na^+ ratio in cytosol was also changed among the genotype. In case of apoplastic fluid, K^+/Na^+ ratio had significant variation between genotypes and between treatments. There was significant interaction between genotype and treatment as well as genotype, treatment and condition.

Total K^+ and Na^+ have less influence on the tolerance than apoplastic Na^+. But total Na^+ and K^+ per gram of the shoot is influenced by the conditions, genotypes and treatments. No interaction was observed for these two traits between condition and genotype interaction. It was previously reported that survival is best correlated not with total shoot Na^+ content, but with the Na^+ content of apoplast fraction of the shoot (Anil *et al*., 2005).

t-test: t-test was used to understand the differences between the conditions for each trait and results of t-test are presented in Table III. Significant difference between aerobic and wetland condition was observed for tiller number, root volume, apoplastic Na^+ and intracellular K^+, while other traits did not vary significantly.

TABLE II

ANOVA for Apoplastic and intracellular Na+ and K+ in the shoots

Source of variation	df	Mean Sum of Squares							
		Na^+ Ap o	K^+ Apo	Na^+ Sym	K^+ Sym	K^+/Na^+ Apo	K^+/Na^+ Sym	Total Na^+	Total k^+
Condition (wetland aerobic)	1	3723.35 *	21173.75 *	27.16	3773.18 *	320.31	125.00	4352.33 *	45477.00 *
Genotype	5	909.21 *	15091.65 *	68.15 *	722.32 *	1067.54 *	142.93 *	1299.31 *	20338.95 *
Treatment	4	3425.62 *	14759.43 *	46.12 *	348.18 *	1466.92 *	103.42 *	3819.2 *	20280.94 *
Condition X Genotype	5	158.07	2296.45	35.07 *	289.22	73.01	12.46	152.53	3093.45
Condition X Treatment	4	1250.57 *	5096.81	22.87	315.69	149.25	111.43 *	969.97 *	6687.75
Genotype X Treatment	20	442.92 *	4353.56	42.16 *	300.35 *	285.70 *	27.26	503.00 *	6102.99 *
Condition X GenotypeX Treatment	20	454.77 *	7453.54 *	33.58 *	224.73	239.30 *	27.34	548.74 *	6363.49 *
Error	118	203.43	3291.80	11.53	138.77	91.87	36.58	231.88	3197.12
Total	179	401.46	4573.69	20.79	218.76	189.04	40.46	455.12	5020.3
SE		14.26	57.37	3.40	11.78	9.58	6.05	15.23	56.54
CV		86.91	51.00	59.03	59.55	68.25	118	68.78	43.11
CD @5%		23.06	92.74	5.49	19.04	15.49	9.78	24.61	91.4

Na^+ Apo - Apoplastic Na^+, K+ Apo - Apoplastic K^+, Na^+ Sym -intracellular Na^+, K^+Sym – intracellular K^+ K^+/Na^+ Apo-Apoplastic K^+/Na^+ ratio, K^+/Na^+ Sym- Intracellular K^+/Na^+

TABLE III

T test values for the selected traits under aerobic and wetland condition

Trait	t - value	Significant / non significant
Shoot length	0.94821	NS
Tiller number	0.006326	S
Root length	0.952101	NS
Root volume	8.74E-07	S
Root number	0.638011	NS
Fresh weight	0.728646	NS
Apoplastic Na^+	0.027094	S
Apoplastic K^+	0.061114	NS
Apoplastic K^+/Na^+ ratio	0.376884	NS
Intracellular Na^+	0.425436	NS
Intracellular K^+	0.001	S
Intracellular K^+/Na^+ ratio	0.097963	NS

DMRT: DMRT is a popular test to compare treatment means using different or multiple ranges. The present data showed that Na^+ and K^+ content in shoot varied across all the conditions imposed in this study for all the treatments (Fig. 1-4). It is well documented that genetic variation exists in the rate of accumulation of Na^+ in leaves, as well as in the degree to which these ions can be tolerated (Mannus and Tester, 2008).

(i) *Apoplastic tissue*: Na^+ of apoplastic tissue varied between the treatments for all the genotypes. Under the control condition, apoplastic Na^+ was highest in ARB-6, Ezhome 1 and IR64 whereas Pokkai C, Pokkali L and Ezhome 2 had the lower apoplastic Na^+. Under wetland condition, only IR-64 showed significant increase in apoplastic Na^+, other genotypes maintained least apoplastic Na^+ (Fig. 1a).

K^+ in the apoplast was significantly higher in Pokkali C (228.3), Ezhome 1(176.3), whereas, significant decrease in apoplastic K^+ was observed in ARB-6, IR-64, Ezhome 2 and Pokkali L under aerobic condition. In wetland condition, no significant differences between the genotypes were observed, all the genotypes were found to be statistically same (Fig. 1b).

Apoplastic K^+/ Na^+ ratio was found to be increased significantly in Pokkali C under aerobic condition, others had significant decreased ratio in aerobic condition but under wetland condition there was no significant difference between genotypes. Variation in the trait was observed in aerobic condition for apoplastic Na^+, K^+ and its ratio, whereas, under wetland condition only for apoplastic Na^+ variation was found (Fig. 1c).

When the plants were treated with100 mM of NaCl, there was no change in the apoplastic Na^+ content observed for ARB-6 under aerobic condition compare to the control. Whereas Ezhome1, Ezhome 2 and IR64 had significantly less Na+ in the cytoplasm, Pokkali C and Pokkali L had least Na^+ which is good for the plant to tolerate the stress. Under wetland condition, there was no significant difference found among the genotypes (Fig. 1d).

Even though ARB-6 manifested highest apoplastic Na^+ in the cytoplasm; it was earlier reported to be moderately salt tolerant because of high suberin accumulation at the root so that stress was decreased (Chowdery *et al*., 2016).

Significant increase in the apoplastic K^+ was found after the saline treatment in all the genotype except IR-64 under aerobic condition. Increase in apoplastic K^+ is desirable for the tolerance as this phenomenon may be related to root K^+ status, although a strong relationship between leaf K^+ concentrations and salinity tolerance has not been found. No significant change in K^+ and K^+/Na^+ ratio seen in any variety under wetland condition. The K^+/Na^+ ratio was maintained in all the genotypes, and it was less in IR-64 under aerobic condition (Fig. 1e and 1f).

Under the treatment of 150 mM NaCl, no significant change in any variety observed for Na^+ under aerobic condition. But it was reduced compare to control and first treatment in ARB-6. This is may be by the Na^+ exclusion from leaf blades Na^+ exclusion by roots ensures that Na does not accumulate to toxic concentrations within leaves (Fig. 1g).

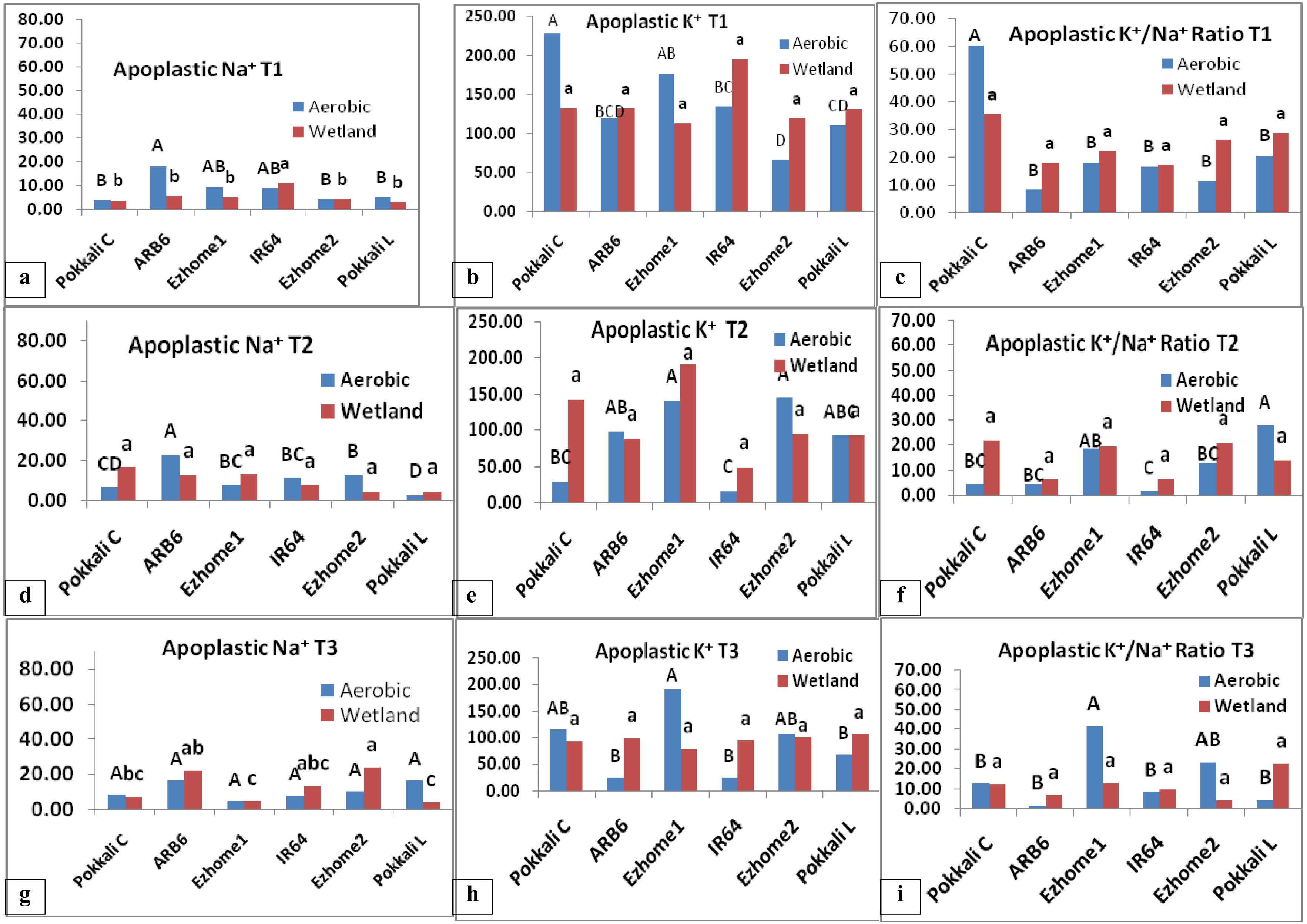

Fig.1: Variation on apoplastic Na+ and K+ uptake,K^+/Na^+ ratiosin rice cultivars (a,b,c): Control (d, e f): 100mM NaCl (g,h,i): 200mMNaCl

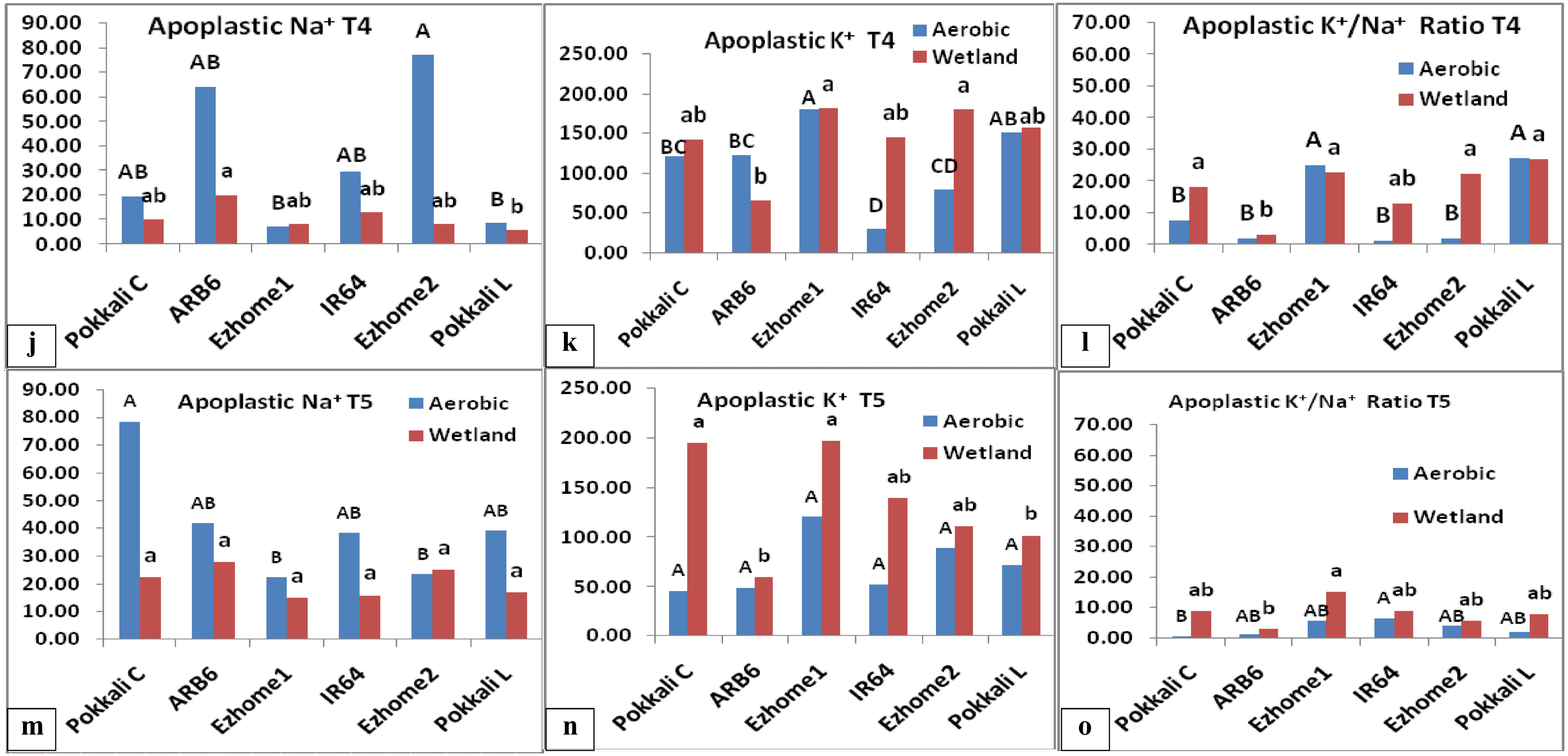

Fig. 2: Variation in apoplastic, Na+, K+ uptake and K⁺/ Na⁺ ratios in rice cultivars under (j, k, l): 200mM and(m, n, o): 250mM NaCl
T4- 200mM NaCl and T5- 250MM NaCl

Wetland condition showed significant increase in Na^+ in Ezhome 2 and ARB-6 followed by IR-64 and Pokkali C, and it was decreased significantly in Pokkali L and Ezhome 1. K^+ in the apoplast was significantly highest for Ezhome1, Pokkali C and Ezhome 2, it was decreased significantly in rest of the varieties. Even the ratio was maintained in these genotypes under aerobic condition, wetland condition had not shown any significant difference in the ratio for any varieties (Fig.1 h and Fig. 1i).

The treatment of 200mM NaCl showed significantly higher Na^+ in apoplast under aerobic condition for all the varieties except Ezhome1 and Pokkali C, this could be because the concentration of salt was more and plants were not allowed for recovery of the stress, after imposing the treatment for a week plants were taken and analyzed. In wetland condition it was significantly lower in all the varieties (Fig. 2j).

Pokkali L had got minimum Na^+ in cytoplasm which is good for the growth of the plant, it has also got high K^+ in the cytoplasm under both the condition, and this trend was followed by Ezhome 1. Even the ratio of K^+/ Na^+ was maintained significantly higher in these two genotypes under both conditions so these two are found to be highly tolerant under both the condition (Fig. 2 k and Fig. 2 l).

When the plants were subjected to 250 mM of NaCl, decrease in apoplastic Na+ was observed in all varieties under aerobic condition compared to 200mM NaCl, but within the treatment, Pokkali C had showed highest apoplastic content which was much lower in rest of the treatments (Fig. 2m).

ARB-6, Ezhome 2 had maintained lower Na^+ compared to other treatments, Ezhome 1and Pokkali L showed significant difference in Na^+ but it was increased from 200mM NaCl, this is may be due to a failure in Na^+ exclusion manifests its toxic effect after days or weeks, depending on the species, and causes premature death of older leaves (Sala *et al.,* 2016). So drastic change in the apoplastic Na^+ may be because of taking the older leaves where the salt will be compartmentised as a tolerant mechanism (Fig. 2m, n and figure 2o).

In IR-64 no change in Na^+ under aerobic condition was observed from 200mM NaCl to 250mM NaCl, tolerance level of this variety is 200mM NaCl since the growth of the plant was decreased and the old leaves were started dying (Fig. 2 k and Fig. 2 m).

Apoplastic K^+ had showed no significant difference under wetland condition and aerobic, whereas, it was less in aerobic condition, than wetland. The ratio was also maintained in all the genotypes except ARB-6 under wetland condition, this may be due to, the adaptability of the plant to aerobic condition since it is an aerobic variety where as under aerobic condition it was significantly same as that of other four varieties which had higher ratio. Pokkali C a wetland rice, showed significant decrease in the ratio under aerobic condition.

Only at high salinity levels, or in sensitive species that lack the ability to control Na^+ transport, does the ionic effect dominate the osmotic effect. The time of exposure to salinity and the severity of the salt treatment determine the physiological and molecular changes that are observed.

(ii) *Intracellular tissue:* Not much variation was observed in intracellular Na^+ compared to apoplastic sodium under control condition. Under aerobic, Pokkali C and Ezhome 1 had highest content than others, under wetland condition all the same amount except Pokkali L which has got decreased intercellular Na^+ under both the condition. No significant changes were found in the K^+/Na^+ ratio for any of the varieties under two conditions (Figure 3a and 3c).

Intracellular Na^+ was significantly lesser than control in 100 and 150mM NaCl treatments, whereas, in 200 and 250mM of NaCl treatment it increased significantly. ARB-6 has showed increase in intercellular Na^+ from control to all the treatment under both the conditions (Fig. 4k and 4m). ARB6 has also got highest Na^+ in the intracellular area under higher level of stress, which could be used by the plant for compartmentalizing to reduce the Na^+ toxicity (Krishnamurthy *et al*., 2011).

No significant change in Na^+ in 250mM NaCl was observed under aerobic condition. Wetland

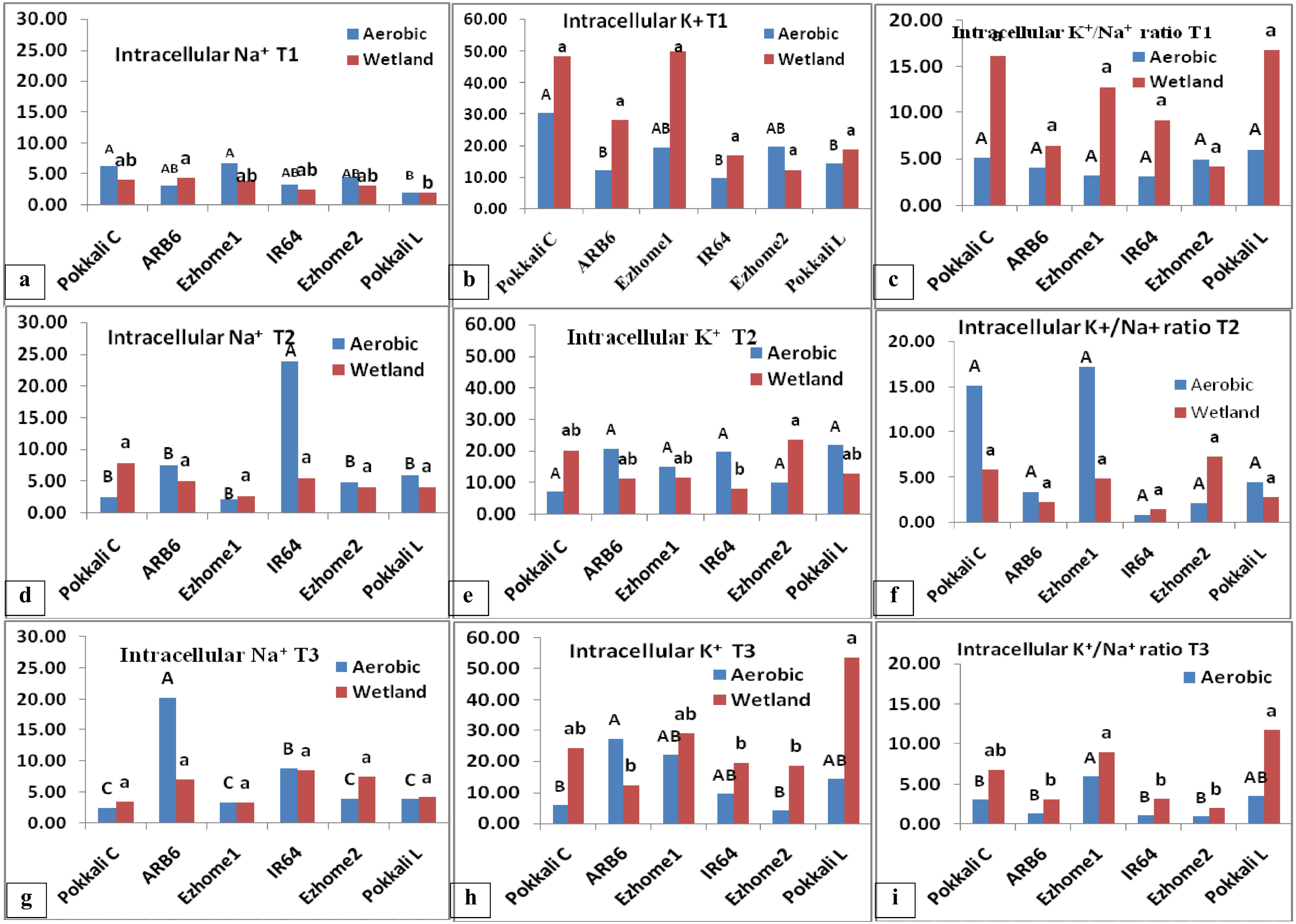

Fig. 3: Variation in intracellular Na$^+$ uptake in rice cultivars (a,b,c): Control (d, e f): 100mM NaCl (g,h,i): 200mMNaCl T1-Control, T2-100mM NaCl, T3- 150mM NaCl,

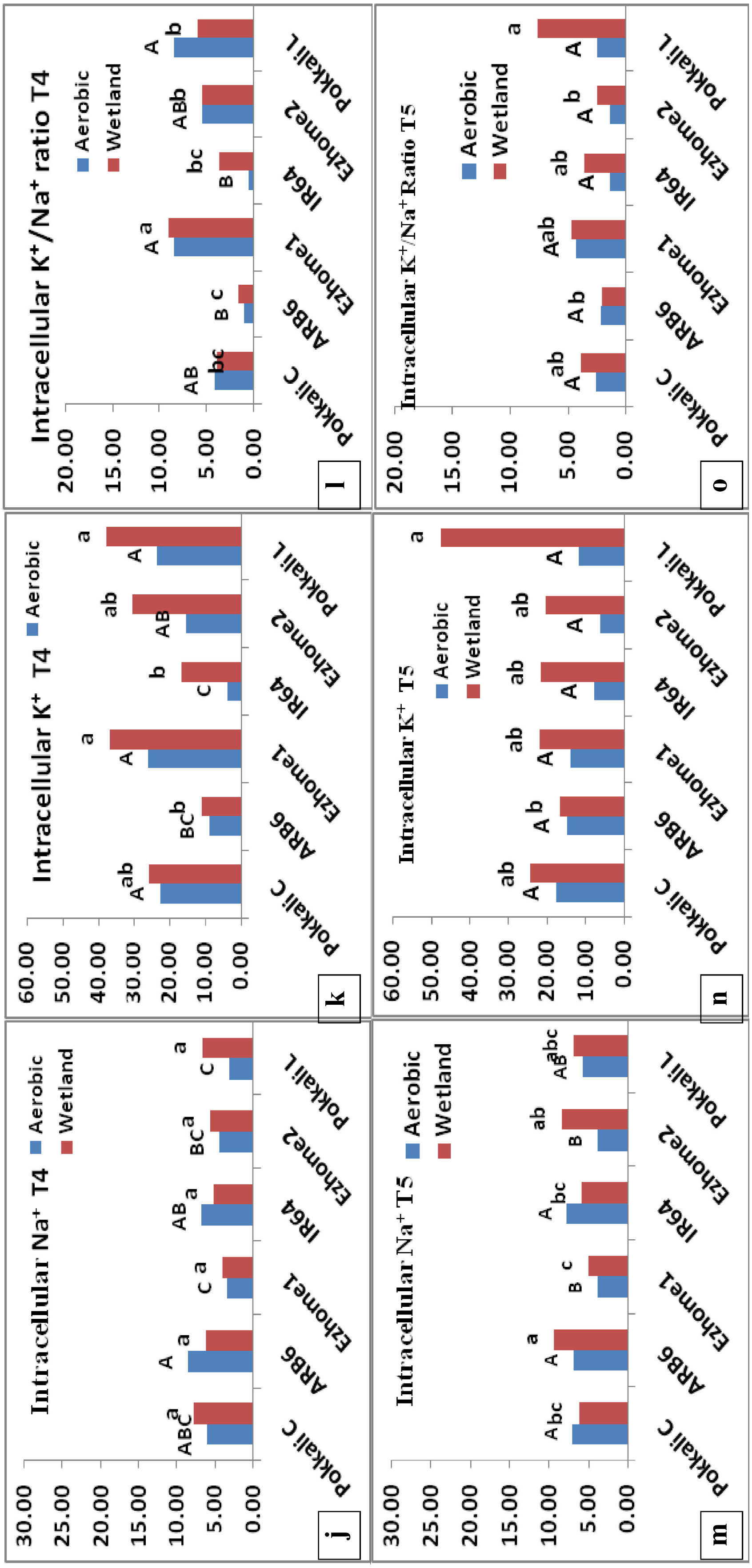

Fig. 4: Variation in intracellular, Na+, $K^+$and K^+/Na^+ ratio uptake in rice cultivars under different treatments (j, k, l): 200mM and (m, n, o): 250mM NaCl
T4- 200mM NaCl and T5- 250MM NaCl

condition had also showed the same results except for Pokkali L which has got highest intercellular Na, this Na could be used for compartmentalization, which will be moved to older leaves and older leaves will die (Fukuda *et al.*, 2004; Anil *et al.,* 2007). Tolerance requires compartmentalization of Na^+ and Cl– at the cellular and intracellular level to avoid toxic concentrations within the cytoplasm, especially in mesophyll cells in the leaf. Toxicity occurs with time, after leaf Na^+ increases to high concentrations in the older leaves (Mannus and Tester, 2008). Hence, Na^+ extrusion mechanism also plays a role.

No significant changes were observed for Na^+ and K^+ ratio maintenance in all the genotypes under two conditions. The ratio was higher in 250mM NaCl treatment compared to other four treatments that means under high level of stress also plants of all the genotypes have maintained Na^+ / K^+ ratio inside the plant tissue for their survival (Anil *et al.*, 2005).

Accumulation of Na^+ and K^+ has impact on the survival of the plants especially under saline stress. Higher absorption of Na^+ under saline stress enforces plant to either exclude the Na^+ from entering the cell or compartmentalize absorbed Na^+. Maintaining healthy K^+/ Na^+ ratio within and outside the cell is important for plant survival. Lower the Na^+ accumulated in the apoplastic space, higher will be the tolerance to salinity. In the present study, Pokkali varieties exhibited lower Na^+ in the apoplastic space reflecting on their well-documented saline tolerance character. ARB6 recorded higher apoplastic Na^+, indicating a possible trade-off between drought resistance (aerobic condition) and saline tolerance. Intracellular Na^+ should also be lesser as higher cytoplasmic Na^+ is detrimental to plants survival. Results from the present study also indicate the same pattern.

The study revealed that, t test indicated the significant difference for apoplastic Na^+ accumulation and intracellular K^+ accumulation between two cultivation systems of rice. Salt accumulation under aerobic condition was significantly higher than wetland conditions in all varieties, indicating different mechanism of saline tolerance among cultivation systems. In case of varieties bred for saline tolerance, this can be justified as they were meant to be cultivated under wetland condition and immediate change in the cultivation system might have contributed to the increased accumulation of salts under aerobic condition (drought). In case of aerobic bred varieties, as explained earlier, it could be because of the possible trade-off between resistance pathways of two abiotic stresses, as reported by earlier studies. However, crossing aerobic variety like ARB6 with salt tolerant varieties like Pokkali, would develop a population which can accommodate enough variation to select for aerobic, salt tolerant lines. Further, a detailed molecular analysis will help in understanding the differential regulation of genes responsible for trade-offs in these varieties under different moisture regimes.

REFERENCES

ANIL, V. S., KRISHNAMURTHY, H. AND MATHEW, M. K., 2007, Limiting cytosolic Na+ confers salt tolerance to rice cells in culture: a two-photon microscopy study of SBFI loaded cells. *Physiol. Plant.,* **129** : 607 - 621.

ANIL, V. S., KRISHNAMURTHY, P., KURUVILLA. S., SUCHARITHA, K., THOMAS, G. AND MATHEW, M. K., 2005, Regulation of the uptake and distribution of Na+ in shoots of rice (*Oryza sativa*) variety Pokkali: role of Ca2+ in salt tolerance response. *Physiol. Plant*., **124** : 451 - 464.

CHOWDERY, R. A., SHASHIDHAR, H. E. AND MATHEW, M. K., 2016, Analysis of Morphological and physiological responses to drought and salinity in four rice (*Oryza sativa* L.) varieties. *Agrotechnology*, **5** : 3.

DUNCAN, D. B., 1955, Multiple range and multiple F-tests, *Biometrics*, **11** (1) : 1 - 42.

FUKUDA, A., NAKAMURA, A., TAGIRI, A., TANAKA, H., MIYAO, A., HIROCHICA, H. AND TANAKA, Y., 2004, Function, intracellular localization and the importance in salt tolerance of a vacuolar Na+/H+ antiporter from rice. *Pl. and Cell Phy.,* **45** : 146 - 159.

KADER, A. AND LINDBERG, S., 2005, Uptake of sodium in protoplasts of salt-sensitive and salt-tolerant cultivars of rice, *Oryza sativa* L. determined by the fluorescent dye SBFI. *J. Experimental Botany*, **56** (422) : 3149 - 3158.

KRISHNAMURTHY, P., RANATHUNGE, K., FRANKE, R., PRAKASH, H. S., SCHREIBER, L. AND MATHEW, M. K., 2009, The role of root apoplastictransport barriers in salt tolerance of rice (*Oryza sativa* L.). *Planta,* **230** : 119 - 134.

KRISHNAMURTHY, P., RANATHUNGE, K., NAYAK, S., SCHREIBER, L. AND MATHEW, M. K., 2011, Root apoplastic barriers block Na+ transport to shoots in rice (*Oryza sativa* L.). *J. Experimental Botany,* **62** (12) : 4215 - 4228.

KRONZUKER, H. J. AND BRITTO, D. T., 2011, Sodium transport in plants:a critical review. *New Phytologist,* **189** : 54 - 81.

MUNNS, R. AND TESTER, M., 2008, Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.,* **59** : 651 - 81.

OCHIAI, K. AND MATOH, T., 2002, Characterization of the Na+ delivery from roots to shoots in rice under saline stress: excessive salt enhances apoplastic transport in rice plants. *Soil Sci. and Plant Nutrition,* **48** : 371 - 378.

SALA, M., SHANTHI, P., SELVI, B. AND RAVI, V., 2016, Marker based Genetic Diversity of Rice Genotypes for Salinity Tolerance at Panicle Initiation Stage. *Vegetos,* **29** : 1.

SHASHIDHAR, H. E., 2007, Aerobic rice- an efficient water management strategy for rice production. In Ashwathnrayana (ed) Food and water security in developing countries. Taylor and Francis, Leiden.

SHASHIDHAR, H. E., VIMARSH, H. S., RAVEEDRA, G. M., PAVAN, J. K. AND KUMAR, G. N., 2012, PVC tubes to characterize roots and shoots to complement field plant productivity studies. In Methodologies for Root Drought Studies in Rice. AGKB.

TESTER, M. AND DAVENPORT, R. J., 2003, Na+ transport and Na+ tolerance in higher plants. *Ann. Bot.,* **91** : 503 - 27.

ZHU, J. K., 2002, Salt and drought signal transduction in plants. *Annu. Rev. Plant Biol.,* **53** : 247 - 73.

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