The Pharma Innovation Journal 2021; 10(10): 2337-2343

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(10): 2337-2343

www.thepharmajournal.com
Received: 10-08-2021
Accepted: 16-09-2021

Shahina Perween
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

E Kokiladevi
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

KK Kumar
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

S Varanavasiappan
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

V Ravichandran
Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

D Sudhakar
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

L Arul
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author:
L Arul
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Analysis of root traits and their relationship to salt tolerance in rice

Shahina Perween, E Kokiladevi, KK Kumar, S Varanavasiappan, V Ravichandran, D Sudhakar and L Arul

Abstract
Soil salinity is a great challenge in rice cultivation which reduces the plant growth and yield. A successful breeding programme requires an appropriate screening strategy to identify the best genotypes/recombinants possessing improved salt tolerance capabilities. In the present study, we have screened the performance of 6 selected rice genotypes (*Pokkali*, *Kuthir*, *Mundan*, *Odiyan*, ASD16, IR29) for selected root parameters *viz*., morphological, architectural, physiological and anatomical under salinity stress (75 mM NaCl) in hydroponics set up. Phenotyping for salt tolerance was scored based on IRRIs Standard Evaluation Score (SES). The salt tolerant rice genotypes (*Pokkali* and *Kuthir*) with low SES values had maintained a low Na^+/K^+ ratio as against the salt susceptible genotypes (ASD16 and IR29). The moderately tolerant genotypes (*Mundan* and *Odiyan*) were found to have an intermediary Na^+/ K^+ ratio. There was a strong positive correlation between the SES and root Na^+/K^+ ratio, a strong negative correlation between the shoot length and root Na^+/ K^+ ratio and, between the root length and root Na^+/K^+ ratio. Root architectural traits *viz*., the maximum number of roots and root network length were significantly affected under salt stress as compared to normal condition invariably in all the rice genotypes.

Keywords: Salinity, Na^+/ K^+ ratio, SES, root architecture, hydroponics

Introduction
Rice is the staple food for over half of the world's population. India, China together contributes about 55% of total rice production, globally (Milovanovic and Smutka, 2017) [7]. However, the present rate of rice production has slowed down due to the various biotic and abiotic stresses. Among the abiotic stresses, salinity and drought are the primary cause of yield loss in rice. Globally, 953 m ha of land is affected by salinity and sodicity, while in India, the salt-affected land area is about 6.73 m ha. Salinity is the consequence of an excess of dissolved salts like chlorides and sulphates of sodium, magnesium and calcium in soil and irrigation water and severely impairs plant growth and development. The dominant salts among them are NaCl and Na_2SO_4. The manifestation of salinity in plants occurs in two phases *viz*., osmotic stress which involves an ion-independent growth reduction, this takes place within minutes to days and leads to stomatal closure along with inhibition of cell expansion mostly in the shoot (Munns and Passioura, 1984; Munns and Termaat, 1986; Rajendran *et al.*, 2009) [8, 9, 11]. The second phase is due to ionic stress which takes place over days or even weeks and leads to the build-up of ion to cytotoxic levels, causing a slowdown of metabolic processes, premature senescence, and ultimately cell death (Munns and Tester, 2008) [10].
Rice crop shows variable sensitivity towards salinity at different stages of growth, it is more sensitive to salt stress at early seedling stages than at tillering stage (Grattan *et al.* 2002; Shereen *et al.* 2005, Rekha *et al.* 2018) [2, 13, 12]. Being a glycophyte, rice is relatively sensitive to salt stress with a low threshold 3 dS/m and 12% reduction in the yield per dS/m beyond the threshold. Reduction in seedling growth and fresh weight is observed with increased salt stress from 5.0 to 7.5 dS/m (Kazemi and Eskandari 2011) [4]. Significant symptoms of salt injury include white leaf tip, stunted plant growth, change in flowering duration, leaf rolling and patchy growth in fields. This study was conducted to understand the usefulness of the root morphological (shoot/root length), physiological (Na^+/K^+), root anatomical and architectural traits and their association with salt tolerance in rice.

Material and Methods

In vitro screening of rice genotypes for seedling stress tolerance

Six genotypes which included salt tolerant and susceptible landraces and cultivars *viz*., *Pokkali* and *Kuthir* (salt tolerant), *Mundan* and *Odiyan* (moderately tolerant), ASD16 and IR29 (salt susceptible) were used in this investigation. The experimental set up comprised of six plastic trays each of six litres volume meant for culturing rice using Yoshida medium (Yoshida *et al.*, 1971) [15]. In each tray, six plastic net cups of one inch diameter filled with perlite were fixed on an appropriately sized thermocol support. Similarly, six trays were assembled, three each for control and stress treatments. The six rice genotypes were directly germinated on the perlite imbibed in Yoshida medium at pH 4.5. Salt stress was imposed by spiking NaCl at 75 mM NaCl in Yoshida culture solution at three weeks old seedling stage. The stress was imposed for additional two weeks. Parallelly, three unstressed trays served as control. The Yoshida culture solution was renewed after every five days until the experiment was terminated.

Phenotyping of rice genotypes for salt tolerance

At the completion of two weeks of salt stress treatment, the impact of salt stress was assessed based on the visual salt injury in shoots. Leaf chlorosis, leaf tip drying, total complete drying of the leaf formed the basis for scoring. Numerical scoring was as per the IRRIs modified standard evaluation score (SES).

Morphological scoring for salt stress injury

The shoot lengths and root lengths were measured. The root length was measured from the collar region to the tip of the primary root and expressed in cm. Similarly, the shoot length was measured from the collar region to the tip of the primary leaf and expressed in cm.

The percentage change in shoot length and root length (Table 2) were calculated by using given formula

$$\Delta SL = \frac{Stressed\ mean\ shoot\ length - control\ mean\ shoot\ length}{control\ mean\ shoot\ length} \times 100$$

$$\Delta RL = \frac{Stressed\ mean\ root\ length - control\ mean\ root\ length}{control\ mean\ root\ length} \times 100$$

Analysis of Na^+/K^+ ratio

After two weeks of salt stress, the roots were kept for drying under sun followed by complete desiccation in hot air oven (65^0 C) for 48 hours. The dried roots were powdered with the help of liquid nitrogen using pestle and mortar. Exactly 0.5 g from each sample was used for estimation of sodium and potassium contents. First the samples were digested with triple acid (HNO_3, H_2SO_4 and $HClO_4$ in ratio of 9:2:1). And, these digested products were used for estimation of Na^+ and K^+ analysis by using Flame Photometer (Hald, 1947) [3].

Characterization of root architectural parameters using GiA Roots software

Selected parameters governing root architecture were recorded at the end of two weeks of salt stress. The roots were cut at the collar region and placed in a water filled petri dish (Fig. 1). Then the roots were spread out in an inverted cone arrangement and imaged. Analysis of the following root parameters were performed with GiA Roots software.

Fig 1: Representative Root images meant for GiA Roots analysis from a) control b) salt stressed genotype

Analysis of rice root anatomy

Cross-sections were cut free hand at 10 mm intervals from the root tip. Sections were stained with 0.1% Berberine hemisulfate (SIGMA) for 1 hour then counter-stained with 0.5% percent Aniline blue (SIGMA) for an additional hour to study the lignin deposition. The sections were stained and visualized at 20 X magnification under fluorescence microscope (NIKON) with ultraviolet filter (Cai *et al*., 2011) [1].

Statistical analysis

The correlation studies primarily involving SES and Na^+/ K^+ ratio was performed using SPSS (Statistical Package for the Social Sciences) and, student 't' test was performed to study the significant differences between the genotypes under salt stress and control conditions.

Results and Discussion

Phenotyping of rice genotypes for salt tolerance

The present study explored the relationship between selected root traits and salt tolerance in rice. Phenotyping genotypes for salt tolerance based on IRRIs modified standard evaluation score (SES) (Table 1) was as follows, *Pokkali* and *Kuthir* were assigned the score "3" for salinity tolerance, *Mundan* and *Odiyan* scored "5", and ASD16 and IR29 was assigned "7" after two weeks of salt stress at 75mM NaCl (Fig 2). Among the salt tolerant genotypes, *Pokkali* is an internationally popular salt tolerant genotype from the state of Kerala, India. The widely introgressed SALTOL QTL implicated in the exclusion of Na^+ from root xylem was originally mapped in *Pokkali* (Thomson *et al*., 2010, Kiruthikadevi *et al*., 2020) [14, 5]. SALTOL QTL has been reported to be a major effect QTL influencing much on the trait of Na/K homeostasis leading to salt tolerance in the land race *Pokkali*. However, *Kuthir* is a lesser explored land race from the state of Kerala. Landraces, *Mundan* and *Odiyan* again from the state of Kerala were known for their ability to withstand salinity at moderate level. On the other hand, rice cultivars ASD16 and IR29 were included in this investigation as they were known to be highly susceptible to salt stress. Among them, IR29 is an internationally popular susceptible check cultivar.

Table 1: Modified IRRI Standard Evaluation Score (SES) of visual salt injury

Score	Observation	Tolerance level
1	Normal growth	Highly Tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

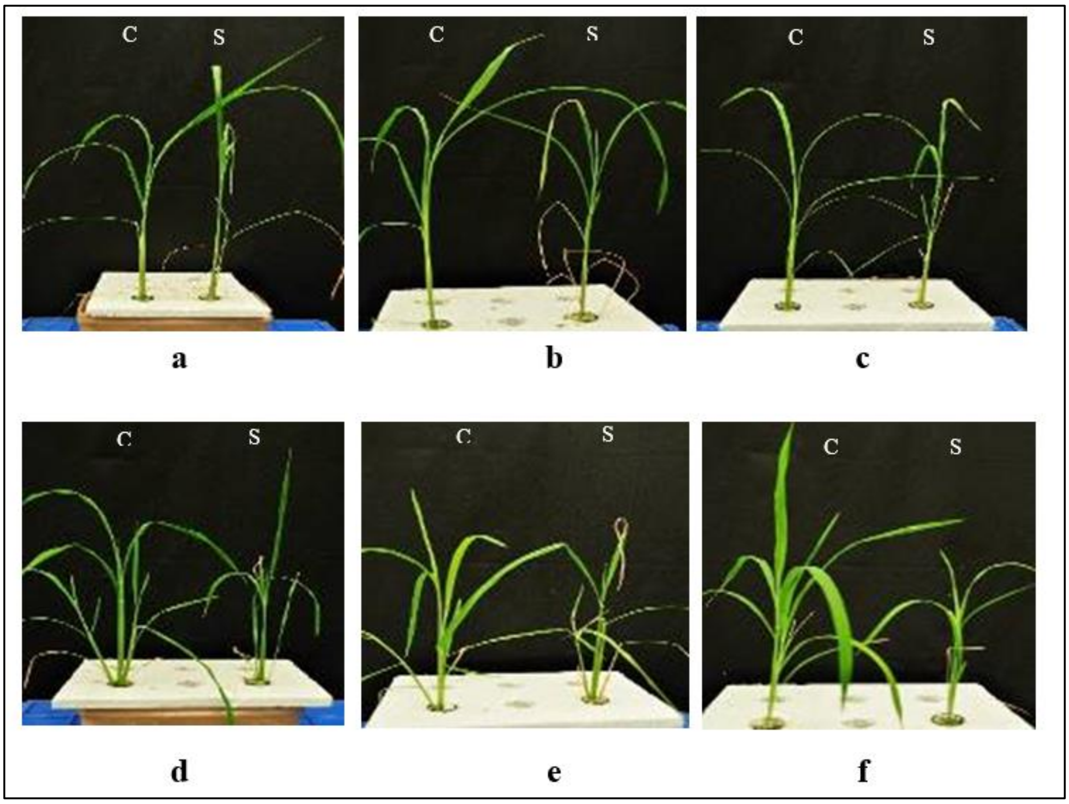


Fig 2: Rice seedlings after two weeks of salt stress a) *Pokkali*, b) *Kuthir*, c) *Mundan*, d) *Odiyan*, e) ASD16, and f) IR29. Phenotyping for salt stress tolerance was based on the IRRIs Standard Evaluation Score (SES). Towards better comparison, a single plant each representing the unstressed control (c) and salt stressed (s) genotype(s) were assembled side by side in the same tray and photographed

Morphological scoring for salt stress injury

Salinity had varying effect on the shoot length and root length among the rice genotypes which were used in this study. Difference in mean shoot/root length between the treatments for each of the genotypes based on ΔSL (percentage change in shoot length) and ΔRL (percentage change in root length) throws light on the resilient nature of the genotypes to sustain the growth and development under salt stress. It was found that, the percentage change in shoot length was on the minus side for all the genotypes. However, the ΔSL was lowest for salt tolerant genotypes *Pokkali* (-0.78), *Kuthir* (-8.08) and *Odiyan* (-6.48). As expected, the salt susceptible genotypes *viz*., cv ASD16 (-22.335) and IR29 (-33.333) showed a higher ΔSL. The percentage change in root length (ΔRL) was as follows *Pokkali* (13.186), *Kuthir* (9.615), *Odiyan* (-1.923), *Mundan* (0.0), ASD16 (-53.706) and IR29 (-42.857).

Unlike the ΔSL, the percentage change in root length (ΔRL) was effective in delineating the highly salt tolerant genotypes *viz*., *Pokkali* and *Kuthir* from the other genotypes. The above two genotypes had clearly demonstrated a gain in root length under salt stress. On the other hand, the highly susceptible rice cultivars ASD16 and IR29 had suffered a drastic reduction in root growth of about 50%, the ΔRL values remained nearly unchanged for the moderately salt tolerant genotypes *Odiyan* and *Mundan* (Table 2)

Table 2: The percentage change in shoot length and root length

	MSL (cm) (control)	MSL (cm) (stressed)	MRL (cm) (control)	MRL (cm) (stressed)	ΔSL	ΔRL
Pokkali	85.333 ± 1.202	84.666 ± 3.180	15.166 ± 0.726	17.166 ± 0.928	-0.7812	13.186
ASD16	65.666± 2.186	51 ± 2.309	18 ± 0.764	8.333 ± 0.882	-22.335	-53.703
Kuthir	66 ± 2.517	60.666 ± 0.882	17.333 ± 0.441	19 ± 1.528	-8.080	9.615
Odiyan	61.666 ± 1.764	57.666 ± 0.333	17.333 ± 0.882	17 ± 0.601	-6.486	-1.923
Mundan	75.666 ± 2.028	60.666 ± 0.667	17 ± 0.764	17 ± 0.764	-19.823	0
IR29	59 ± 1.528	39.333 ± 1.202	15.166 ± 0.667	8.666 ± 1.202	-33.333	-42.857

MSL (Mean shoot length); MRL (Mean root length); ΔSL (Percentage change in shoot length); ΔRL (Percentage change in root length)

Root Na^+/K^+ ratio

Significant difference for root Na^+/K^+ ratio was observed in all the genotypes under salt stress as compared to the control conditions **(**Fig 3). Here it was observed that salt tolerant rice genotypes maintained low Na^+/ K^+ ratio as compared to salt susceptible rice cultivars. The Na^+/ K^+ ratio under salt stress was low for *Pokkali* and *Kuthir* (1.875 and 1.733 respectively) as compared to the salt susceptible genotypes, ASD16 (2.29) and IR29 (2.40) with higher ratios. Interestingly, the moderately salt tolerant genotypes *viz.*, *Odiyan* and *Mundan* was to found to have intermediate values for Na^+/ K^+ at (2.00 and 2.15, respectively (Table 3). The Na^+/ K^+ homeostasis is an important aspect governing the outcome the salt stress response in genotypes. Lower the values for Na^+/ K^+ favours salt tolerance in genotypes and vice-versa.

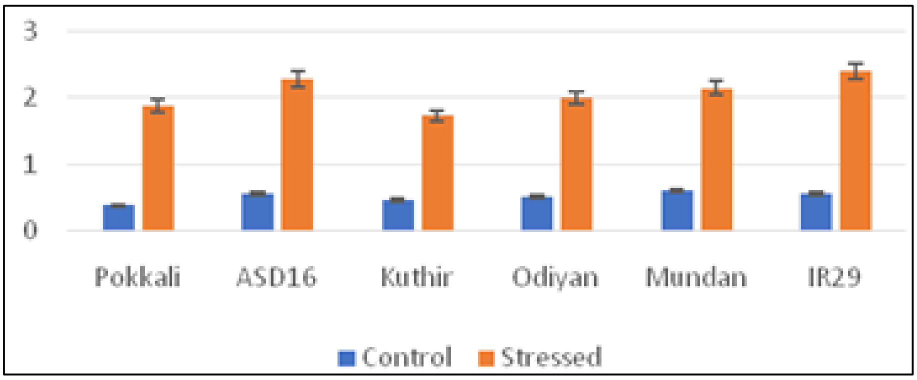


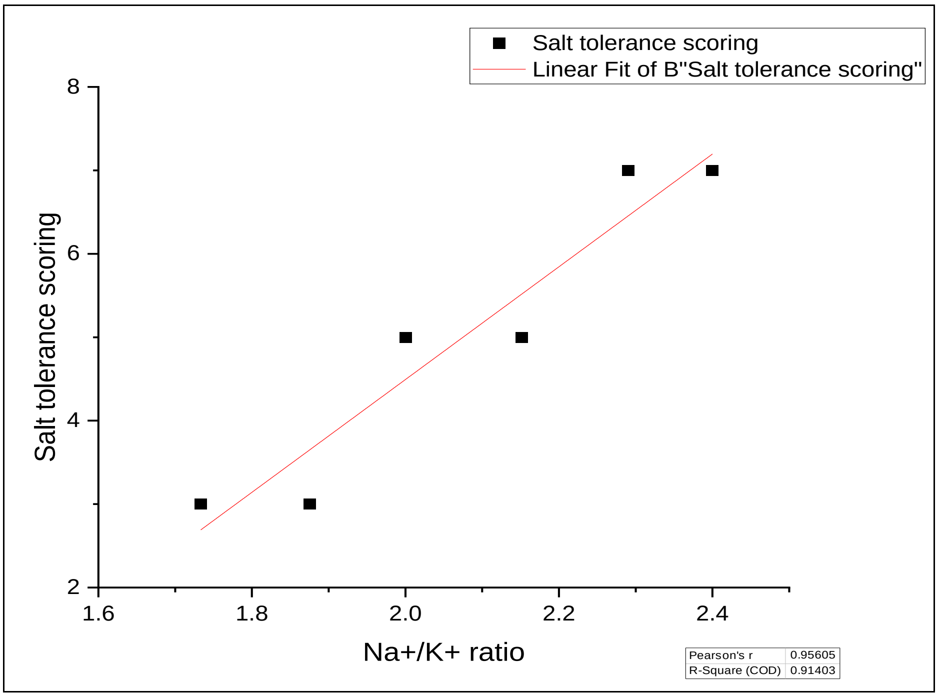
Fig 3: Analysis of root Na^+/ K^+ ratio under salt stress and control

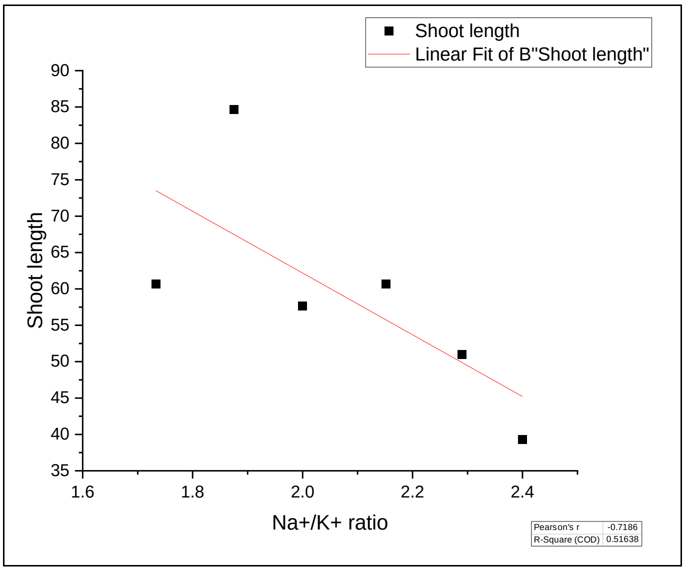
Table 3: Phenotype score for salt tolerance (SES) and root Na^+/ K^+ ratio for the rice genotypes under control and salt stress conditions

	Salt stress			Unstressed control	
Genotypes	**Salt tolerance score**	**Salt tolerance group**	**Root Na^+/ K^+ ratio**	**Salt tolerance score**	**Root Na^+/ K^+ ratio**
Pokkali	3	T	1.875	1	0.371528
ASD16	7	S	2.290323	1	0.542484
Kuthir	3	T	1.733333	1	0.463768
Odiyan	5	MT	2.0	1	0.509091
Mundan	5	MT	2.151515	1	0.591837
IR29	7	S	2.4	1	0.547059

(T: tolerant; S: sensitive; MT: moderately tolerant)

Besides, a strong positive correlation was observed (r = 0.956) between the salt tolerance score (SES) and Na^+/K^+ ratio (Fig. 4a) under salt stress. However, a strong negative correlation (r = -0.718) exists between shoot length and Na^+/K^+ ratio (Fig. 4b) similarly, a strong negative correlation (r = -0.886) was observed between root length and Na^+/K^+ ratio (Fig. 4c). Under salt stress, the sodium ions enter through the root apoplast in an increasing manner leading to an enhanced accumulation of salts in the shoots as a consequence the shoot shows salt injuries. Thus, a strong correlation between the Na^+/K^+ ratio and salt tolerant phenotype enabled grouping of the six genotypes distinctly into three categories *viz.*, i) tolerant (*Pokkali*, *Kuthir*), ii) moderately tolerant (*Odiyan*, *Mundan*) and iii) susceptible cultivars (ASD16, IR29).





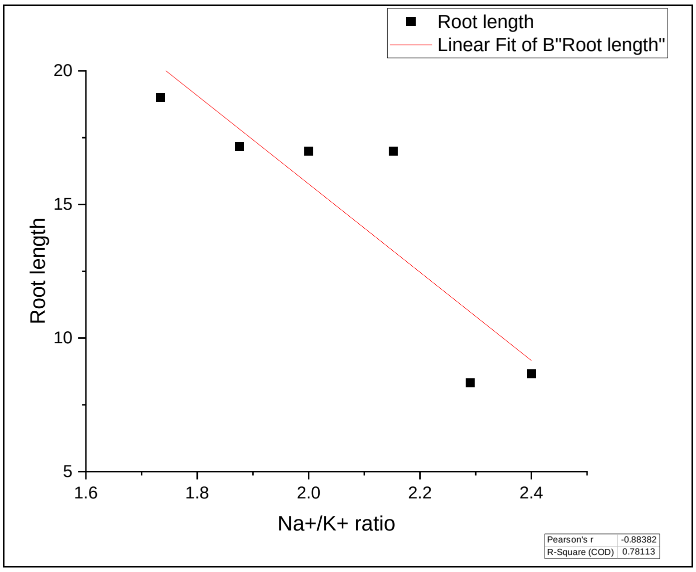


Fig 4: Correlation of salt tolerance (SES), Na^+/ K^+ ratio and shoot/root lengths

a) A positive correlation (0.956) between salt tolerance score (SES) and Na^+/ K^+ ratio
b) A negative correlation (-0.718) between shoot length and Na^+/K^+ ratio
c) A negative correlation (-0.886) between root length and Na^+/K^+ ratio

Root architectural parameters

Root architectural parameters (Table 4) which were analysed using the GiA Roots software. It was observed that in all the rice genotypes there was a significant difference ($p< 0.05$) in parameters like maximum number of roots and network length between control and salt stressed rice roots. The above two parameters were highly affected in all the genotypes. Significant difference was observed for the parameter root network width to depth ratio in all the genotypes except *Mundan* ($p< 0.05$). In salt sensitive genotypes (i.e) ASD16 and IR29, it was observed that all the root parameters were having significant difference ($p< 0.05$) except specific root length in IR29 (Table 5). The remaining four parameters *viz.*, network bushiness, network depth, network surface area and network volume were found to have no defined relationship with the phenotype of salt tolerance. Based on the above, all the root architectural parameters did not have relevance to the function of salt tolerance

Table 4: Parameters used in GiA Roots software

Traits	Units	Description
Maximum no of roots	n	After sorting the number of roots crossing a horizontal line from smallest to largest, the maximum number is considered to be the 84th-percentile value (one standard deviation).
Network bushiness	n/n	The ratio of the maximum to the median number of roots
Network depth	cm	The number of pixels in the vertical direction from the upper-most network pixel to the lower-most network pixel
Network length	cm	The total number of pixels in the network skeleton.
Network surface area	cm^2	The sum of the local surface area at each pixel of the network skeleton, as approximated by a tubular shape whose radius is estimated from the image.
Network volume	cm^3	The sum of the local volume at each pixel of the network skeleton, as approximated by a tubular shape whose radius is estimated from the image
Network width to depth ratio	cm/cm	The value of network width divided by the value of network depth.
Specific root length	cm/cm^3	Total network length divided by network volume. Volume is estimated as the sum of cross- sectional areas for all pixels of the medial axis of the root system.

Table 5: Analysis of root architectural parameters using GiA Roots software

Parameters	Network Bushiness		Network Depth		Maximum Number of Roots		Specific Root Length		Network Surface Area		Network Length		Network Volume		Network Width to Depth Ratio	
	T stat	P	T stat	P	T stat	P	T stat	P	T stat	P	T stat	P	T stat	P	T stat	P
Pokkali	1.45	0.28	3.88	0.06	3.21	0.03^*	3.13	0.03^*	4.9	0.016^*	4.05	0.015^*	-0.8	0.42	-3.35	0.07
Asd16	-5.29	0.004^*	9.61	0.01^*	5.39	0.005^*	7.55	0.004^*	6.79	0.02^*	4.45	0.02^*	-3.1	0.03^*	-5.7	0.029^*
Kuthir	-2.2	0.092	5.34	0.005^*	2.79	0.049^*	2.65	0.11	3.5	0.07	6.09	0.003^*	-5.2	0.006^*	-5.2	0.006^*
Odiyan	-3.2	0.08	-1.2	0.31	12.22	0.0002^*	4.32	0.02^*	-1.7	0.23	6.13	0.02^*	-6.8	0.54	-6.7	0.006^*
Mundan	-2.2	0.15	2.3	0.14	39.46	0.00003^*	16.44	0.0004^*	6.48	0.02^*	29.42	0.000008^*	-5	0.03^*	-2.3	0.144
IR29	-5.3	0.03^*	-3.2	0.03^*	3.06	0.05^*	-2.6	0.11	4.2	0.01^*	6.64	0.021^*	10.35	0.0019^*	5.79	0.004^*

(*Significant difference at 5% level)

Root anatomy

Lignin deposition pattern and intensity of fluorescence differed between the salt tolerant and susceptible genotypes. The salt tolerant genotypes *Pokkali* and *Kuthir* were distinct from the salt susceptible genotypes cv. ASD16 and IR29 for the lignin distribution and content. In case of *Pokkali* and *Kuthir*, the distribution pattern of lignin was observed touching the inner borders of the pericycle encompassing the entire stele involving both the vascular and parenchymatous cells, the fluorescence intensity was as well as pronounced.

On the other hand, in *cv*. ASD16 and IR29, the lignin distribution pattern was very much restricted to the inner zone of the vascular bundle confined to the surroundings of the xylem vessels and accompanied by a lesser fluorescence intensity. While in *Mundan* and *Odiyan*, a moderate distribution of lignin was observed, the fluorescence intensity was comparatively lower to genotypes (Fig. 5).

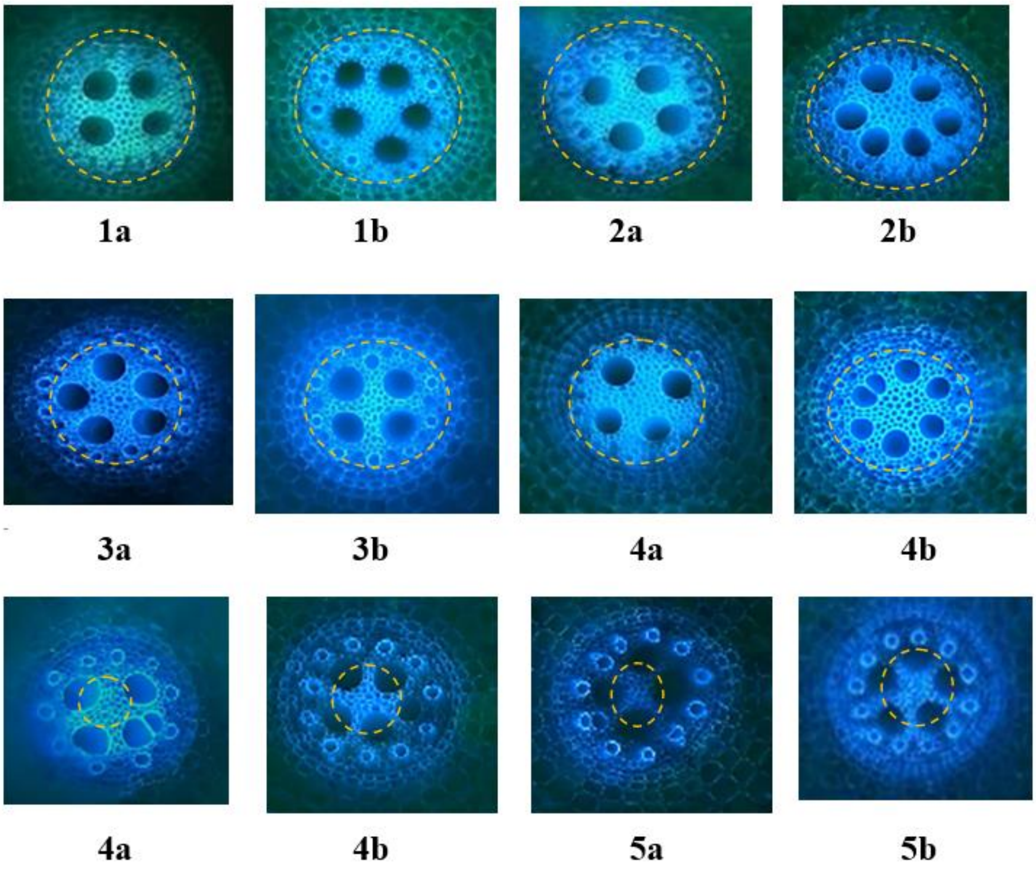


Fig 5: Cross sections of rice root 1-*Pokkali* (a) control (b) stressed; 2-*Kuthir* (a) control (b) stressed; 3-*Mundan* (a) control (b) stressed; 4-*Odiyan* (a) control (b) stressed; 5-ASD16 (a) control (b) stressed; 6-IR29 (a) control (b) stressed. Qualitative assessment of the extent of lignin deposition inside the stele (encircled) and degree of lignification as an indirect measure of the fluorescent intensity were used for comparing the genotypes under salt stress and control conditions.

Conclusion

The understanding on the lignin content in roots and their relationship to salt stress is an emerging concept, a few works have been published in this direction to establish the importance of lignification especially on the epidermis and endodermis and their role in salt tolerant function in rice (Krishnamurthy *et al*., 2009; Cai *et al*., 2011) [6, 1]. The strong correlation of root Na^+/K^+ ratio with the salt phenotype based on (SES) pave way for a simple and practical way to characterize genotypes for the function of salt tolerance. Besides, the percentage change in root length (ΔRL) appears to be an effective parameter to screen the salt tolerant genotypes. The percentage change in root length is very informative than merely looking at the mean of the root length.

The lignin deposition and intensity of fluorescence appears to be directly related to the salt tolerance level of rice. Root anatomy for lignin deposition/intensity has the potential to identify salt tolerant genotypes. The use of different morphological, physiological and anatomical trait analysis for screening of salt tolerant genotypes in rice has advantages, as they are simple, reproducible and less time-consuming techniques having the scope for high throughput screening. This investigation concludes that root Na^+/K^+, ΔRL and lignification of stele serve as root-based screening traits for characterizing rice for salt tolerance.

Acknowledgement

The authors would like to thank Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology TNAU and Department of Crop Physiology, TNAU for their support and guidance for doing this work. SP would like to thank DBT for fellowship.

References

1. Cai X, Chen T, Zhou Q, Xu L, Qu L, Hua X, *et al*. Development of Casparian strip in rice cultivars. Plant signaling & behavior 2011;6(1):59-65.
2. Grattan STEPHEN, Zeng L, Shannon M, Roberts S. Rice is more sensitive to salinity than previously thought. California agriculture 2002;56(6):189-198.
3. Hald PM. The flame photometer for the measurement of sodium and potassiumin biological materials. Journal of Biological Chemistry 1947;167(2):499-510.
4. Kazemi K, Eskandari H. Effects of salt stress on germination and early seedling growth of rice (Oryza sativa) cultivars in Iran. African Journal of Biotechnology 2011;10(77):17789-17792.
5. Kiruthikadevi U, Banumathy S, Arunachalam P, Renuka R, Thirumurugan T. Correlation, path analysis and stress indices studies of Saltol introgressed lines of rice for salinity tolerance. Electronic Journal of Plant

Breeding 2020;11(01):230-237.
6. Krishnamurthy P, Ranathunge K, Franke R, Prakash HS, Schreiber L, Mathew MK. The role of root apoplastic transport barriers in salt tolerance of rice (Oryza sativa L.). Planta 2009;230(1):119-134.
7. Milovanovic V, Smutka L. Asian countries in the global rice market. ACTA Universitatis agriculturae et silviculturae mendelianae Brunensis 2017;65(2):679-688.
8. Munns R, Passioura JB. Effect of prolonged exposure to NaCl on the osmotic pressure of leaf xylem sap from intact, transpiring barley plants. Functional Plant Biology 2017;11(6):497-507.
9. Munns R, Termaat A. Whole-plant responses to salinity. Functional Plant Biology 1986;13(1):143-160.
10. Munns R, Tester M. Mechanisms of salinity tolerance. Annual Review of Plant Biology 2008;59:651-681.
11. Rajendran K, Tester M, Roy SJ. Quantifying the three main components of salinity tolerance in cereals. Plant, cell and environment 2009;32(3):237-249.
12. Rekha G, Padmavathi G, Abhilash V, Kousik MBVN, Balachandran SM, Sundaram RM, *et al*. A protocol for rapid screening of rice lines for seedling stage salinity tolerance. Electronic Journal of Plant Breeding 2018;9(3):993-1001.
13. Shereen A, Mumtaz S, Raza S, Khan MA, Solangi S. Salinity effects on seedling growth and yield components of different inbred rice lines. Pakistan Journal Botany 2005;37(1):131-139.
14. Thomson MJ, de Ocampo M, Egdane J, Rahman MA, Sajise AG, Adorada DL, *et al*. *et al*. Characterizing the Saltol quantitative trait locus for salinity tolerance in rice. Rice 2010;3(2-3):148-160.
15. Yoshida S, Forno DA, Cock JH. Laboratory manual for physiological studies of rice. Laboratory manual for physiological studies of rice 1971.