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Analysis of root cross section for anatomical features governing root plasticity in *indica* rice genotypes

KG Kiran, B Akshara, Varanavasiappan, KK Kumar, K Iyanar, V Ravichandran, E Kokiladevi, J Ramalingam, D Sudhakar and L Arul

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

Abstract

This study focused on image-based analysis towards understanding root plasticity under drought and salt stress in rice. Plant high resolution imaging and visualization (PHIV-Root Cell) tool was used in this investigation to quantify root anatomical parameters from the cross section (s) of 14 *indica* rice genotypes which included land races and elite rice cultivars. Quantitative data on root anatomical parameters *viz*., total root area, exodermis area, cortex area, stellar area and stele lignification showed significant variations between the genotypes. Rice landraces notably *Cheriviruppu* and *Kallimadayan* showing larger root area, stelar and cortical area, are expected to have an increased water and nutrient absorption capacity paving the way for drought tolerance. On the other hand, smaller root area and lignified stele might be associated with salt tolerance in genotypes like *Pokkali, Mundan*, CSR27 and TRY3.

Keywords: Root anatomy, rice landraces, salinity and drought stress, image analysis, PHIV-Root Cell

Introduction

Rice (*Oryza sativa* L.) is one of the important cereal crops and staple food for more than half of world's population (Vinci *et al.*, 2023) [28]. However, rice production is highly constrained by biotic and abiotic stresses. Today's agriculture is challenged by unpredictable climatic conditions which necessitates improving crop's resilience against environmental stresses paving the way for improving agricultural productivity in terms of grain quality and quantity (Haque *et al.*, 2021) [12].

Roots play a critical role in plant anchorage, absorption of water and nutrients, storage, synthesis of hormones and metabolites etc., thus the overall functioning of the root system significantly impact the development of the shoot system as well the productivity in agricultural crops like rice (Koevoets *et al.*, 2016) [19]. Plant breeders, therefore explore roots primarily for boosting vegetative growth and crop productivity (Henry *et al.*, 2011; Kadam, *et al.*, 2017) [13, 16]. Apart from the above, root characteristics can enable plants resilience against various abiotic stresses particularly drought and salt. Root phenotypic plasticity is the ability of genotypes to adapt itself to challenging environments (O'Toole and Bland, 1987) [26]. It is the built-in genetic trait that confer the root's ability to sustain plant growth, development and productivity under biotic and abiotic stresses (Yamauchi *et al.*, 1994, Wang and Yamauchi, 2006) [32, 30]. However, the plasticity of root anatomical and architectural parameters to environmental cues is lesser understood. In this direction, understanding the anatomical and architectural characteristics of roots becomes relevant in the context of evolving breeding strategies for improving crops minimizing the impact of abiotic stresses (Gowda *et al.*, 2011) [11]. Earlier studies emphasized root anatomical features *viz.*, thickness of the cortical cells, diameter of the cortical cells, number of cortical cell layers and increased root area towards an improved absorption capacity. Specifically, stele diameter, xylem diameter and xylem area were associated with water and nutrient transport functions (Wang *et al.*, 2019) [29]. These findings shed light on the plasticity of the anatomical features towards improving root's overall performance even under environmentally challenging situations. Higher the root and stelar area manifests on improving nutrient and water absorption under drought stress (Kong *et al.*, 2017) [20]. Whereas, lesser root area was inturn associated with salt tolerance (Lupo *et al.*, 2021) [23].

Landraces are invaluable resources with an extensive genetic variability conferring an adaptive role under different environmental conditions (Azeez *et al.*, 2018) [2].

Rice is endowed with a strong genetic base with several genotypes adapted to varied agro-ecological situations propelling discovery of robust root specific structural traits towards improving the crop's ability to withstand abiotic stresses particularly, drought and salt. In this study, we analyzed 14 *Indica* rice genotypes for the root anatomical parameters *viz*., total root area, exodermis area, cortex area, stelar area and its lignification and their association with drought and salt stress using PHIV-RootCell (Lartaud *et al.*, 2015) [21]. The insight gained on the root anatomical features paves the way for identifying appropriate donor(s) in crop breeding programs.

Materials and Methods

Plant material and growing conditions

A total fourteen rice genotypes, comprising landraces (*Mundan*, *Kuthiru*, *Arikayama*, *Cheriviruppu*, *Odiyan*, *Orpandy*, *Pokkali*, *Kallimadayan*) and cultivars (CSR27, ASD16, CO52, FL478, IR64, TRY3) were included in this investigation. The experiment was carried out in a controlled greenhouse under hydroponics system using Yoshida nutrient solution (Yoshida *et al.*, 1976) [33]. Three replications of each genotype were raised in plastic net cups along with perlite as supporting medium for 28 days.

Root anatomy and fluorescent imaging

After 28 days, free-hand cross sections (CS) of the adventitious roots in all 14 genotypes were done at 7 to 8 mm above the root tip. The root cross section was stained using 0.1% berberine hemisulphate for 1 hour followed by 0.5% aniline blue counterstaining for additional 1 hour (Brundrett *et al.*, 1988) [4]. The samples were visualized and documented at 10 x magnification using epifluorescence microscope Nikon Eclipse-Vi-L fitted with blue filter (UV-2A excitation: 330 nm - 380 nm).

PHIV-Root Cell based quantitative analysis of root anatomical parameters

Root cross-sectional images were analyzed for root anatomical traits in all 14 rice genotypes using plant high resolution imaging and visualization (PHIV-RootCell) software (Lartaud *et al.*, 2015) [21]. Combined with an appropriate root staining technique for image acquisition, PHIV-RootCell program facilitates supervised measurements of rice root cross section for the following anatomical parameters *viz*., total root area, root exodermis area, cortex area and stelar area. To ensure precision and reproducibility, cross-sectional images in replicates were subjected to image analysis using PHIV-Root Cell. In addition, lignification of the of the stelar region was quantified based on the fluorescence intensity captured from the stelar area and analyzed with Image J software (Bankhead 2014) [3] with green filter option. The work methodology is presented as an illustration in Figure 1.

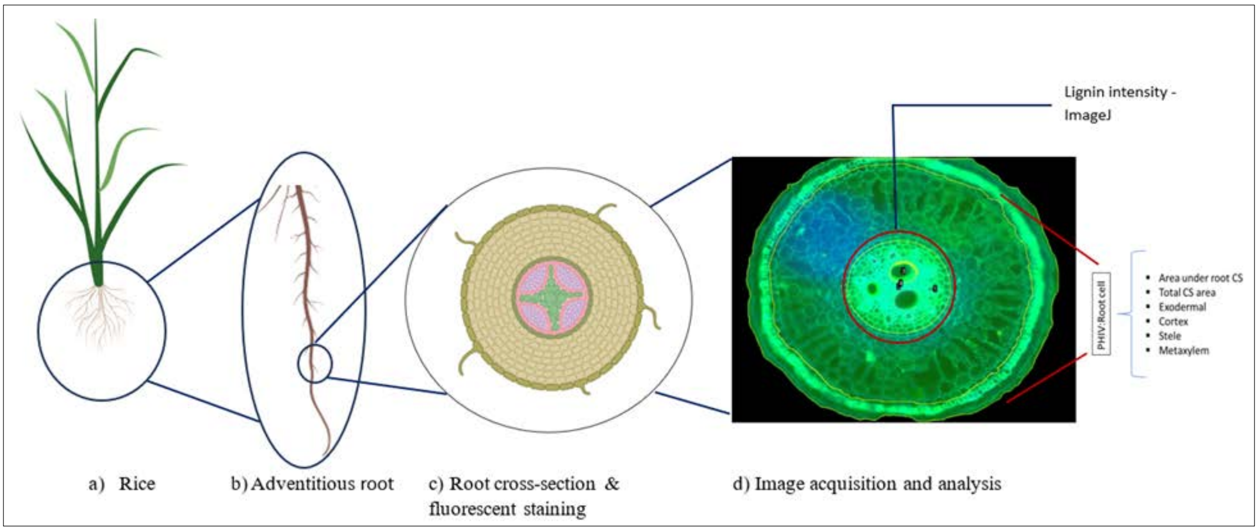

Fig 1: The schematic work flow includes, a) culturing rice in hydroponics b) selection of the adventitious root c) sectioning of root and fluorescent staining d) image acquisition and analysis using PHIV-RootCell and Image J software's

Statistical analysis

The Student's t-test and Ducan's multiple range test (DMRT) were performed for total root area, exodermis area, cortex area, stelar area and stele lignification using WASP-web Agri Stat Package.

Result and discussion

Developmental plasticity is tissue/organs adaptive response *via* functional, physiological and genetic changes under drought and salt stress in order to sustain plant growth, development and productivity. Landraces are locally adapted genotypes evolved with genetic capabilities to endure biotic and abiotic stresses. This study was into characterizing the root anatomical features among 14 *indica* rice genotypes, which included locally adapted landraces with varied responses to abiotic stresses and, high yielding rice cultivars. The primary interest was to appreciate the differences in anatomical parameters based on root cross sections *viz*., total root area and area explicitly under root exodermis, cortex and stele as well as stelar lignification. Here, we analyzed the differences in anatomical features between the landraces and cultivars looking for developmental cues that enabled their successful adaptation under drought and salt stresses. Among the 14 genotypes, *Mundan*, *Kuthiru*, *Arikayama*, *Cheriviruppu*, *Odiyan*, *Orpandy*, *Pokkali*, *Kallimadayan* belong to landraces and, elite rice cultivars include CSR27, ASD16, CO52, FL478, IR64, TRY3. Fluorescent stained root CS images in replicates were analyzed by PHIV-RootCell as well as ImageJ software (Fig 2a and 2b).

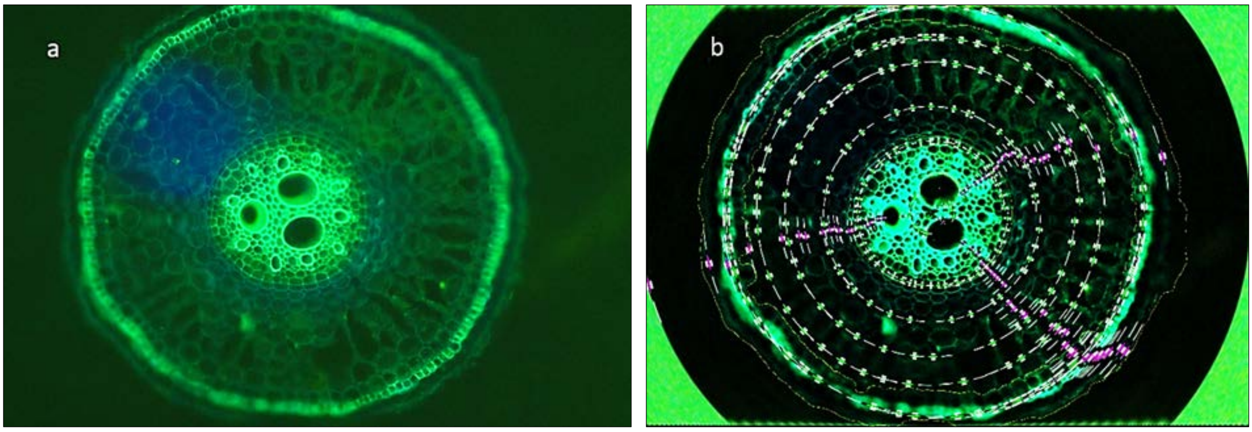

Fig 2: a) Representative image of rice root cross section stained with fluorescent dye and analyzed by Image J b) Fluorescent stained root cross section subjected to supervised image analysis by PHIV- Root Cell

Quantification of anatomical parameters of 14 rice genotypes using PHIV-Root Cell

Root diameter and area promotes root penetrance and drought tolerance

Root diameter is one of structural characteristics that determines plant's ability to access water and nutrients from the soil, especially under drought situations. Increased root diameter is associated with better root penetration through dense layers of soil (Clark *et al.*, 2008) [6]. The root size impacts it's ability to penetrate strong soil layers in turn how root diameter affects root growth pressure and the mechanism of soil deformation during penetration (Materechera *et al.*, 1992) [25]. Beyond root diameter, analyzing root cross-sectional area can be an important parameter in understanding roots efficiency of performance under abiotic stress.

In this direction, the total root cross-sectional area in 14 rice genotypes revealed statistically significant differences, *Cheriviruppu* and *Kallimadayan* registered highest root cross-sectional area whereas, rice cultivar TRY3 had the lowest root cross-sectional area (Fig 3a). This suggests that *Cheriviruppu* and *Kallimadayan* may possess a higher nutrient absorption ability and better survival under drought compared as compared to the rest of the genotypes.

To further analyze the root plasticity in response to salt stress, we identified seven genotypes well known for their salt tolerance and susceptibility. Accordingly, group (A) consisted of *Mundan*, *Pokkali*, CSR27 and TRY3 which fall under high and moderately salt tolerant category and group (B) consisted of ASD16, CO52, IR64 falling under salt susceptible category. Analysis of the root cross-sectional area within the subset of genotypes suggests that the tolerant group showed reduced root area as compared to the susceptible group (Fig 3b). Thus, a lesser cross-sectional area observed in group (A) is indicative of salt tolerance based on the study in grapes. Wherein, a narrow root area and decreased root density reduces water and nutrient absorption as well as sodium ions favoring salt exclusion thus enabling the plant salt tolerant (Lupo *et al.*, 2021) [23].

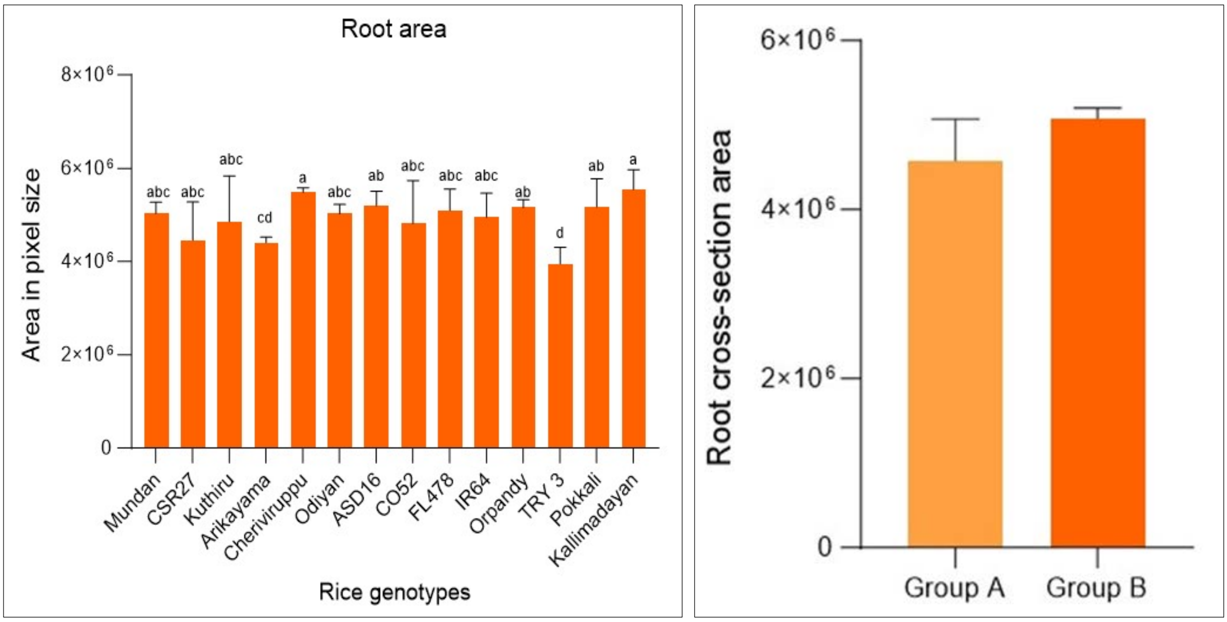

Fig 3: a) Quantification of total root area from the root cross section in all 14 rice genotypes. Data presents means ± SE (N=4). Different letter labels indicate that means are statistically significant ($p \leq 0.05$) as determined by Duncan's Multiple Range Test (DMRT). 3b) Comparison of total root area between salt tolerant (A) and susceptible (B) groups, data presents mean ± SE (N=3 or 4)

Cortex area in improving oxygenation under drought stress

In roots, cortex is predominantly composed of thin-walled, undifferentiated parenchyma cells located between the exodermis and endodermis regions and, primarily functions in storage and transport of food and nutrients (Lopez *et al.*, 2017) [22]. Besides, oxidative stress resulted in the growth of the cortex in response to abiotic stress (Cui *et al.*, 2015) [7]. Rice roots, with high cortex-to-stele ratio (CSR) and aerenchyma-to-cortex ratio (ACR) play an important role in efficient oxygen delivery, thus cortical enlargement protects against mild but not acute stresses. Overall, cortical expansion is considered an important for crops to deal with drought stress (Wang *et al.*, 2019) [31].

Significant changes in cortical area were recorded across the 14 genotypes with *Kallimadayan* having the largest area and TRY3 showing the smallest. Other genotypes with an increased area under cortex include *Mundan*, *Cheriviruppu, Pokkali* and ASD16. The above genotypes with highest cortex area, may benefit from efficient oxygen transport and protection against mild stressors, underscoring the role of cortex in plant resilience and adaptability (Fig. 4).

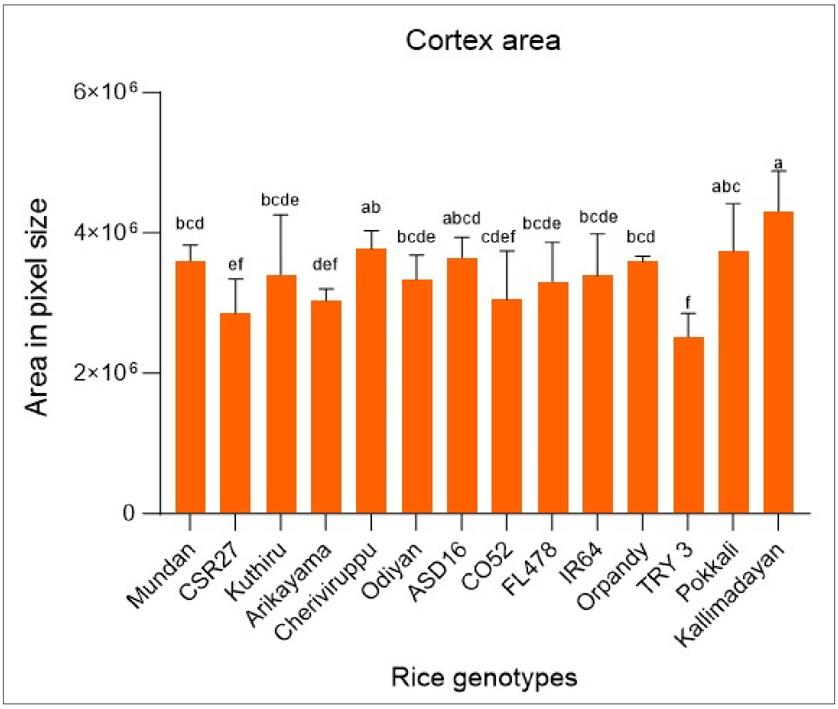

Fig 4: Quantification of cortex area from the root cross section in all 14 rice genotypes. Data presents means ± SE (N=4). Different letter labels indicate that means are statistically significant ($p \leq 0.05$) as determined by Duncan's Multiple Range Test (DMRT)

Stelar area improves the water retention ability under drought stress

The stele constitutes the inner core of the root system and plays an important function in absorption and transport of water and solutes from soil (Foster *et al.*, 1959) [10]. Roots characterized by a wider stelar area and suberized/lignified endodermis may hold more water in water-stressed conditions (Jeong *et al.*, 2013) [15]. The larger stelar area is accompanied by larger xylem vessels thus enhancing the water transport capacity (Burton *et al.*, 2012) [5]. Rice mutants with larger stele diameter and more of stelar cells were related to higher grain yield during drought stress conditions (Kadam *et al.*, 2015) [17].

Among the 14 genotypes investigated, highest stele area was recorded in *Kallimadayan* followed by *Mundan*, *Cheriviruppu* and FL478. The lowest stele area was observed in rice cultivar TRY3. *Kallimadayan* with a significant increase in stelar area might be having an improved water retention capacity leading to drought tolerance and better survival under stress. Thus, a larger stelar area appears to be a useful feature and can be considered important for the plant's ability to survive under water limiting environment (Fig. 5).

Stele lignification confers salt tolerance

The stelar region is made up of several structural compartments starting from the endodermis, pericycle, protoxylem, metaxylem, phloem, and parenchymatous stelar cells. According to Dixon and Barross (2019) [8], lignification adds mechanical strength, stiffness, and hydrophobicity to these cells. The root endodermis which surrounds the root vasculature, regulates the ionic balance via regulating the movement of water and solutes through the apoplastic route (Enstone *et al.*, 2003) [9]. Besides, lignification of xylem leads to strengthening of the xylem walls favoring high hydrolytic conductivity from the root to the shoot system. Lignification into the inner core of the stele confers resistance to the movement of water and solutes into the xylem vessels. The intensity of the stelar lignification measured in terms of pixels can be used to predict the lignin deposition within the stele. Among the 14 genotypes, a differential lignification pattern was observed, genotypes *viz.*, CSR27, *Orpandy*, *Kuthiru*, FL478, and *Kallimadayan* showed a significant lignin deposition, on the other hand rice cultivar CO52 was poorly lignified. The above genotypes demonstrating higher stelar lignification were known for their moderate level of salt tolerance whereas, elite rice cultivar CO52 was known for its sensitivity to salt stress. This goes well with the earlier report that lignin deposition, particularly on the endodermis and metaxylem were associated with the trait of salt tolerance (Aybeke 2016) [1] (Fig.6).

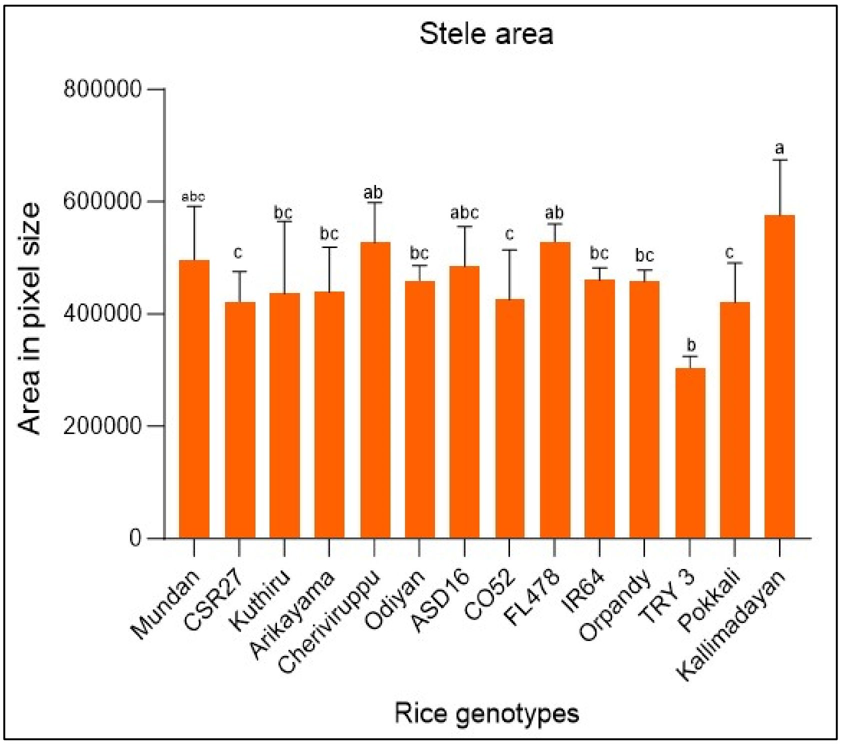

Fig 5: Quantification of total stelar area from the root cross section in all 14 rice genotypes. Data presents means ± SE (N=4). Different letter labels indicate that means are statistically significant ($p \leq 0.05$) as determined by Duncan's Multiple Range Test (DMRT).

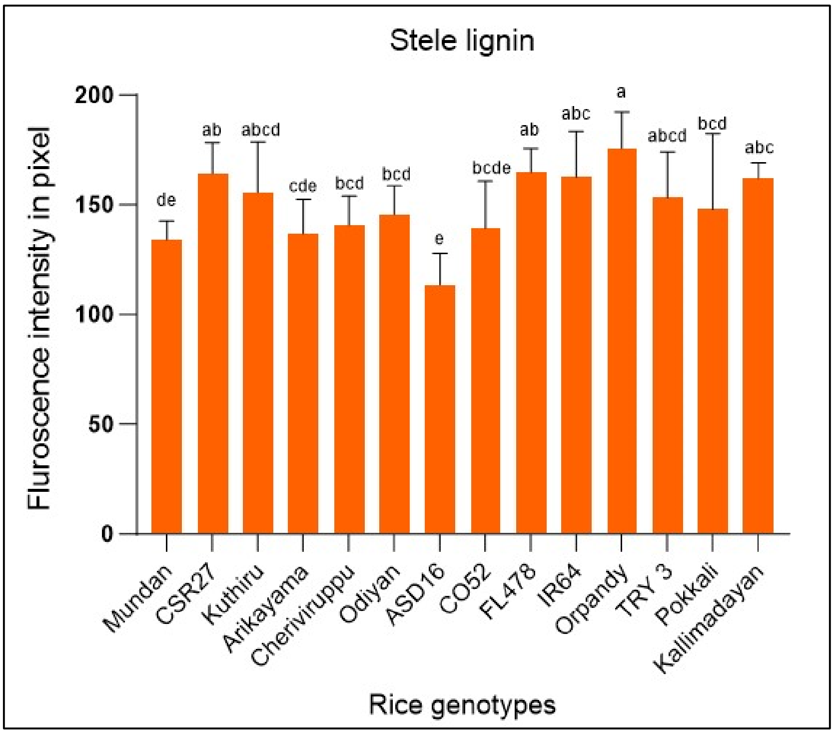

Fig 6: Quantification of stelar lignification from the root cross section in all 14 rice genotypes. Data presents means ± SE (N=4). Different letter labels indicate that means are statistically significant ($p \leq 0.05$) as determined by Duncan's Multiple Range Test (DMRT).

Exodermis in the interface of soil and roots

The external layer of roots comprises of the following, the epidermis, exodermis and sclerenchyma. The casparian strips on the cell walls of the exodermis, is a specialized root tissue and serves as an impermeable barrier (Hose *et al.*, 2001) [14]. It protects roots from infections and, regulates water, preventing excessive loss by diffusion while promoting water replenishment (Ma *et al.*, 2003; Soukup *et al.*, 2007) [24, 27]. Furthermore, the exodermis regulates the radial flow of water and nutrients as well as adaptation to external stressors (Enstone *et al.*, 2003) [9]. Thus, exodermis is important for root function, protection and adaptation to environmental challenges (Kamula *et al.*, 1994) [18]. Among the 14 rice genotypes, CO52, CSR27, *Cheriviruppu* and FL478 showed highest area under exodermis thus preventing excessive water loss conferring a barrier mechanism in the interface of soil and roots (Fig.7).

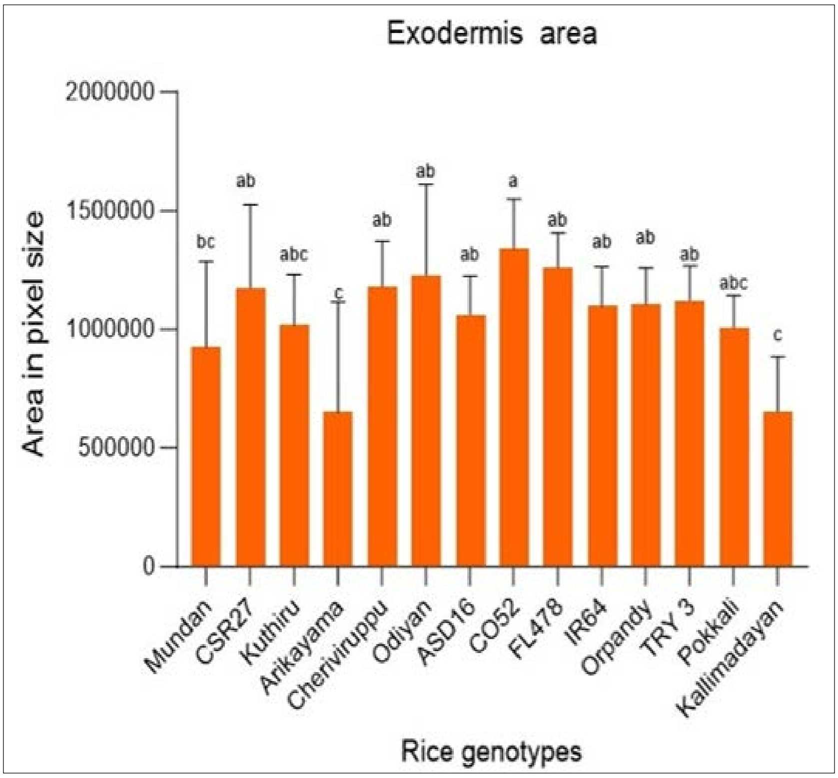

Fig 7: Quantification of exodermis area from root the cross section in all 14 rice genotypes. Data presents means ± SE (N=4). Different letter labels indicate that means are statistically significant ($p \leq 0.05$) as determined by Duncan's Multiple Range Test (DMRT).

Conclusion

This research explored the anatomical variations in root cross sections of 14 *indica* rice genotypes. The total root area and that of the individual anatomical parameters *viz*., exodermis, cortex and stele varied with individual rice genotypes as an adaptive response to abiotic stresses such as drought and salt. Screening rice genotypes based on the anatomical parameters can serve as a phenotyping tool for plant breeders and researchers to identify the right genotype of choice towards developing crop varieties better adapted to abiotic stress conditions. Landraces *Mundan, Cheriviruppu* and *Kallimadayan* with favorable anatomical traits *viz*., increased area under cortex and larger and lignified stelar region and as compared to others genotypes can be utilized as donors in crop improvement programs.

References

1. Aybeke M. Root anatomical plasticity in response to salt stress under real and full-season field conditions and determination of new anatomic selection characters for breeding salt resistant rice (*Oryza sativa* L.). Trakya University Journal of Natural Sciences. 2016;17(2):87-104.
2. Azeez MA, Adubi AO, Durodola FA. Landraces and Crop Genetic Improvement. In Tech; c2018.
3. Bankhead P. Analyzing fluorescence microscopy images with Image J Image J. 2014;1:10-1109.
4. Brundrett MC, Enstone DE, Peterson CA. A berberine-aniline blue fluorescent staining procedure for suberin, lignin, and callose in plant tissue. Protoplasma. 1988;146:133-142.
5. Burton AL, Brown KM, Lynch JP. Phenotypic diversity of root anatomical and architectural traits in Zea species. Crop Science. 2013;*53*(3):1042-1055.
6. Clark LJ, Price AH, Steele KA, Whalley WR. Evidence from near-isogenic lines that root penetration increases with root diameter and bending stiffness in rice. Functional Plant Biology. 2008;35(11):1163-1171.
7. Cui H. Cortex proliferation in the root is a protective mechanism against abiotic stress. Plant Signaling & Behavior. 2015;10(5):e1011949.
8. Dixon RA, Barros J. Lignin biosynthesis: Old roads revisited and new roads explored. Open Biology. 2019;9(12):190215.
9. Enstone DE, Peterson CA, Ma F. Root endodermis and exodermis: Structure, function, and responses to the environment. Journal of plant growth regulation. 2002;21:335-351.
10. Foster, Sherwood A, Ernest M. Gifford. Comparative morphology of vascular plants. Comparative Morphology of Vascular Plants; c1959.
11. Gowda VR, Henry A, Yamauchi A, Shashidhar HE, Serraj R. Root biology and genetic improvement for drought avoidance in rice. Field Crops Research. 2011;122(1):1-13.
12. Haque MA, Rafii MY, Yusoff MM, Ali NS, Yusuff O, Datta DR, *et al.* Recent advances in rice varietal development for durable resistance to biotic and abiotic stresses through marker-assisted gene pyramiding. Sustainability. 2021;13(19):10806.
13. Henry A, Gowda VR, Torres RO, McNally KL, Serraj R. Variation in root system architecture and drought response in rice (*Oryza sativa*): Phenotyping of the Oryza SNP panel in rainfed lowland fields. Field Crops Research. 2011;120(2):205-214.
14. Hose E, Clarkson DT, Steudle E, Schreiber L, Hartung W. The exodermis: A variable apoplastic barrier. Journal of Experimental Botany. 2001;52(365):2245-2264.
15. Jeong J S, Kim YS, Redillas MC, Jang G, Jung H, Bang SW, *et al.* OsNAC5 overexpression enlarges root

diameter in rice plants leading to enhanced drought tolerance and increased grain yield in the field. Plant Biotechnology Journal. 2013;11(1):101-114.
16. Kadam NN, Tamilselvan A, Lawas LM, Quinones C, Bahuguna RN, Thomson MJ. *et al.* Genetic control of plasticity in root morphology and anatomy of rice in response to water deficit. Plant Physiology. 2017;174(4):2302-2315.
17. Kadam NN, Yin X, Bindraban PS, Struik PC, Jagadish KS. Does morphological and anatomical plasticity during the vegetative stage make wheat more tolerant of water deficit stress than rice. Plant Physiology 2015;167(4): 1389-1401.
18. Kamula SA, Peterson CA, Mayfield CI. The plasma lemma surface area exposed to the soil solution is markedly reduced by maturation of the exodermis and death of the epidermis in onion roots. Plant, Cell & Environment. 1994;17(11):1183-1193.
19. Koevoets IT, Venema JH, Elzenga JTM, Testerink C. Roots withstanding their environment: Exploiting root system architecture responses to abiotic stress to improve crop tolerance. Frontiers in Plant Science. 2016;7:1335.
20. Kong D, Wang J, Zeng H, Liu M, Miao Y, Wu H, Kardol P. The nutrient absorption transportation hypothesis: optimizing structural traits in absorptive roots. New Phytologist. 2017;213(4):1569-1572.
21. Lartaud M, Perin C, Courtois B, Thomas E, Henry S, Bettembourg M, *et al.* PHIV-Root Cell: A supervised image analysis tool for rice root anatomical parameter quantification. Frontiers in Plant Science. 2015;5:790.
22. Lopez FB, Barclay GF. Plant anatomy and physiology. In Pharmacognosy. Academic Press; c2017. p. 45-60.
23. Yaniv L, Schlisser A, Dong S, Rachmilevitch S, Fait A, Lazarovitch N. Root Structure and Function of Grapevine Rootstocks (Vitis) in Response to Salinity; c2021.
24. Ma F, Peterson CA. Current insights into the development, structure, and chemistry of the endodermis and exodermis of roots. Canadian Journal of Botany. 2003;81(5);405-421.
25. Materechera SA, Alston AM, Kirby JM, Dexter AR. Influence of root diameter on the penetration of seminal roots into a compacted subsoil. Plant and Soil. 1992;144:297-303.
26. O'toole JC, Bland WL. Genotypic variation in crop plant root systems. Advances in Agronomy. 1987;41:91-145.
27. Soukup A, Armstrong W, Schreiber L, Franke R, Votrubová O. Apoplastic barriers to radial oxygen loss and solute penetration: a chemical and functional comparison of the exodermis of two wetland species, Phragmites australis and Glyceria maxima. New Phytologist. 2007;173(2):264-278.
28. Vinci G, Ruggieri R, Ruggeri M, Prencipe SA. Rice Production Chain: Environmental and Social Impact Assessment A Review. Agriculture. 2023;13(2):340.
29. Wang H, Wang Z, Dong X. Anatomical structures of fine roots of 91 vascular plant species from four groups in a temperate forest in Northeast China. PLOS One. 2019;14(5):e0215126.
30. Wang H, Yamauchi A. Growth and Function of Roots under Abiotic Stress in Soils. Third Edition, ch10 Plant-Environment Interactions; c2006. p. 271-319.
31. Wang T, Abe F, Tsutsumi N, Nakazono M. Root cortex provides a venue for gas-space formation and is essential for plant adaptation to waterlogging. Frontiers in Plant Science; c2019. p. 10:259.
32. Yamauchi A, Pardales Jr J R, Kono Y. Root system structure and its relation to stress tolerance. Roots and nitrogen in cropping systems of the semi-arid tropics. Patancheru, Andhra Pradesh, India; c1994. p. 211-233.
33. Yoshida S, Forno DA, Cock JH. Laboratory manual for physiological studies of rice. Laboratory manual for physiological studies of rice; c1971.

All Author's Names

KG Kiran
Department of Plant Biotechnology, Centre for Plant Molecular Biology & Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

B Akshara
Department of Plant Biotechnology, Centre for Plant Molecular Biology & Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

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

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

K Iyanar
Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

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

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

J Ramalingam
Department of Plant Biotechnology, Centre for Plant Molecular Biology & Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

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

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