General Impact Factor (GIF): 0.875

e- ISSN: 2394-5532
p- ISSN: 2394-823X

International Journal of Applied And Pure Science and Agriculture

www.ijapsa.com

Study of Rice (*Oryza sativa* L.) Root Anatomy under Aerobic and Waterlogged Conditions.

PAVAN J KUNDUR[1, 2, 3], VIMARSHA H S[1, 4], RAM SANJAY[1], KRISHNAMURTHY K.V[1], HARISH B.G[2, 3] and SHASHIDHAR H. E[1]

[1]Department of Plant Biotechnology, UAS, GKVK, Bangalore-560065, INDIA
[2]MS Ramaiah Institute of technology, Bangalore-560054, INDIA
[3]Visveshwarya Technological University, Belgaum-590018, INDIA
[4] Liveon Biolabs Pvt. Ltd. KIADB Phase II, Tumkur – 572106, INDIA

Abstract:

Rice has served as a prime model plant species for studies of constitutive root aerenchyma. In present investigation, 20 days old seedling root anatomy were studies in eight diverse and phenotypically contrasting rice genotypes. Studies were carried out in contrasting water regimes i.e. aerobic and water-logging situations. Higher number of aerenchyma formation were observed in water logging treatment compared to aerobic condition. Thus, development of aerenchyma in response to water logged condition would help supply O_2 to root cells. Aerenchyma cells in aerobic roots ranged from 11 (BJ21) to 23 (Devamallige). However, under water logged condition, Higher numbers were observed ranging from 19 (Jeerigesanna) to 28 (BI33). Aerenchyma number showed highly significant positive correlation with pith area (r = 0.57) and negatively correlation with xylem numbers (r = -0.46) and phloem number (r = -0.46). On the other hand, cross section area radius positively correlated with pith area (r = 0.41) and xylem number (r = 0.70).

Key words: Aerenchyma, aerobic, root anatomy, water logged

I. Introduction

Plants are aerobic organisms and lack of oxygen poses a severe stress. Soil waterlogging and flooding are environmental conditions that are frequently encountered by plants growing in aquatic or semi-aquatic habitats.(Bianka Steffens *et al*., 2010).

Plant roots often experience anoxia, as gas exchange is strongly decreased in waterlogged soils. However, when whole plant is submerged under flooding, shoots also experience oxygen shortage. The problem of oxygen supply becomes more severe in turbid waters or with deep floods, which cause low light conditions that prevent photosynthetic oxygen production. Plants, such as rice (Oryza sativa), endure frequent flooding by adaptations found not only in roots, but also in stems. One basic strategy to cope with flooding is to improve gas exchange.

Roots, rhizomes and other plant organs usually obtain sufficient oxygen for aerobic respiration directly from gaseous spaces in the soil. However, the diffusion of O_2 from the air into the soil can be effectively blocked when soil becomes flooded or waterlogged (Urška videmšek et al., 2006).

Studying root anatomy will help associating root traits that impact yield of plant. Aerenchyma in roots provide a transport and other essential gases from above ground parts to roots. (Armstrong and Drew, 2002; Colmer 2003). The number and size of aerenchyma have shown association with drought tolerance and also yield of plant in over expression studies of OsNAC genes (Redillas *et al*., 2012; Jeong *et al*., 2013).

Rice and also corn have respectively served as model species in studies of constitutive and of inducible root aerenchyma. Similarities and differences exist between root aerenchyma developments in both of these species (Armstrong and webb, 1985). In rice roots, the amount of aerenchyma gas space and the position of aerenchyma formation along the root have been shown to be influenced by flooding and/or hypoxia. However, some studies indicate that these variables do not appreciably affect constitutive amounts of rice root aerenchyma (Jackson *et al.,* 1985). Though it is believed that aerenchyma is formed mostly in water logged conditions, recent reports in maize show significant higher aerenchyma formation in water-stressed conditions and in nitrate treatments (Yang *et al*., 2012).

Aerobic rice is a new method of cultivating rice in less water than traditional flooded condition. Aerobic rice varieties are developed by crossing lowland varieties with upland varieties and cultivated in irrigated but non-flooded and non-puddle soils (Bouman *et al.,* 2002; 2005; Shashidhar H.E, 2007) and water logged conditions with three replications.

Studies on aerenchyma characters are usually done in hydroponic solutions. However, phenotype exhibited by roots when grown in hydroponic conditions may not mimic as that of field grown condition. Studies on aerenchyma characters under field grown conditions are limited and less reported. Therefore, constituting the root experiments matching the field conditions are more desired.

Present investigation is an approach to characterize and compare rice genotypes with contrasting genetic basis in regard to their ability to form aerenchyma in aerobic and water-logged conditions.

II. Materials and Methods

The plant materials used for the present investigation comprised of eight genotypes that were selected for their ability to grow in water-logged and aerobic conditions **(Table 1)**

Table 1: List of genotypes used for root anatomical studies.

Genotype Code	Genotype Name	Rice type	Origin	Ecotype
1.	AM65	Japonica	Bengaluru, India	High Iron content upland
2.	Azucena	Trop. Japonica	Philippines	Traditional upland
3.	BI-33	Indica	Bengaluru, India	Aerobic upland
4.	BJ21	Indica	Bengaluru, India	High Iron content upalnd
5.	Burma black	Indica	Myanmar	Traditional rainfed
6.	Devamallige	Indica	India	traditional
7.	IR-20	Indica	Philippines	Coastal
8.	Jeerige sanna	Indica	India	Irrigated Medium

Experimental site:

The experiment was carried out during *Kharif* season of 2014 at aerobic rice research laboratory of Department of Plant Biotechnology, University of Agricultural Sciences, GKVK Campus, Bengaluru, India. Experimental site is located at an latitude of 12^{o} 58’ North, longitude of 77^{o} 35′ East and altitude of 930 meters above mean sea level (MSL).

Aerobic and water-logged condition treatment:

The experiment was conducted in Randomized Complete Block Design (RCBD) with three replications both in aerobic and waterlogged treatments. Poly Vinyl Chloride (PVC) pipes of 3.0 inch diameter and length of 1.5 feet were used for growing the seedlings. Uniform soil mixture was prepared with the mix of manure and basal dose of NPK fertilizers and compacted into PVC pipes. Soil was compacted by frequent watering.

Direct sowing was done and seeds were germinated in aerobic condition for five days. Thereafter, one set of replicated PVC pipes were submerged to create the water-logged treatment. Seedlings were then grown for 20 days and anatomical studies were done on roots of these seedlings. The roots that were selected for anatomical studies with length more than 15 cm were classified under long roots category.

The cross sections thus made using fine blades and immersed in 2% glycerol solution were observed in a stereo microscope (Nikon Progress CS2). The objective lens magnification of 10X was sufficient enough for recording the standard anatomical features of root cross sections **(Fig 1**).

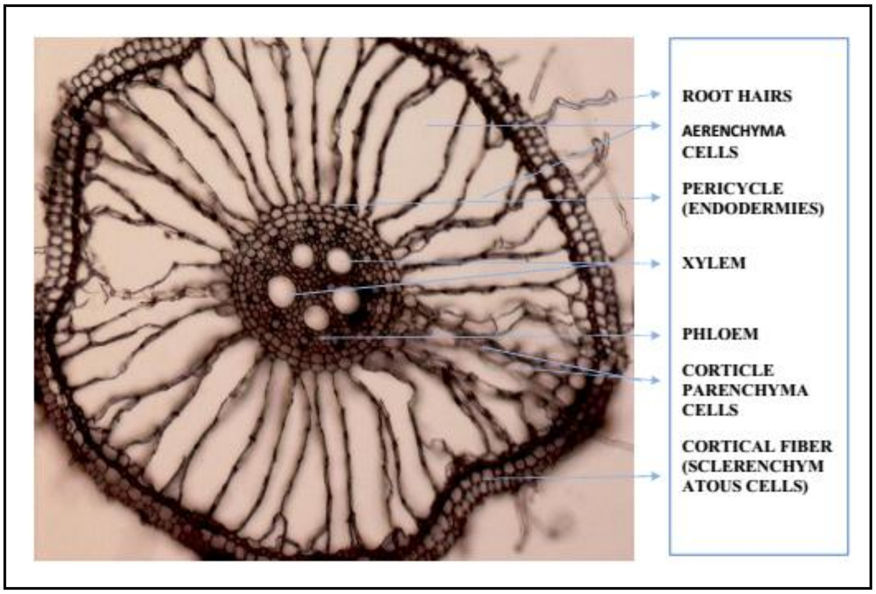

Fig1: Anatomical features studied under stereo microscope

The measurements that were made in microscope were obtained as default standards with picture pixels. The microscope was calibrated using stage micrometer (0.01µm unit length) for 10x magnification. The calibrated value of 1214 pixels was equivalent to 1µm. These values were substituted for converting the pixelate picture to micro meter readings. The area measurements were automated by camera software Progress CS2, as base values of pixels were calibrated and stored. The area measurements of round and oval shapes were used for recording data.

Statistical analysis:

Two way analysis of variance (ANOVA) was performed using IBM SPSS statistical package for both aerobic and well-watered conditions. Pearson correlation co-efficient among different root morphological traits was estimated for both growth conditions.

III. Result and discussion

In the present study, analysis of variance revealed highly significant variance among all genotypes for aerenchyma numbers, Cross section area, Pith area, Aerenchyma area, Xylem Cross section area except for total cross section area of root. **(Table 2 & 3).**

Table 2: ANOVA for anatomical traits of roots (20 days old) under aerobic condition.

Source of Variations	df	AN	CSR	PA	AA	XCA	CSA
Year	2	1.29	3640.95*	362.56**	158.40*	171.82001	8148.11
Genotypes	7	56.95**	9768.81**	180.22**	1556.04**	687.84*	8919.07
Error	14	3.43	564.82	12.32	30.03	210.87	2368.28
Total	23	19.53	3633.52	93.87	505.63	352.64	4864.59
C.V.%		10.1	6.3	5.21	5.987	62.3	14.6
S.E.Diff.Mean		1.51	19.4	2.86	4.475	11.85	39.73

Table 3: ANOVA for anatomical traits of roots (20 days old) under water logged condition.

Source of Variations	df	AN	CSR	PA	AA	XCA	CSA
Year	2	11.37	2123.83	12.43	36.25	2.01	4055.65*
Genotypes	7	32.89**	4027.23**	369.49**	972.97**	45.59*	7277.01**
Error	14	3.32	848.94	38.15	61.82	2.78	695.02
Total	23	13.02	1927.11	136.75	336.90	15.74	2990.46
C.V.%		7.48	8.67	7.63	7.84	9.49	8.91
S.E.Diff.Mean		1.48	23.79	5.04	6.42	1.36	21.52

Note- AN - Aerenchyma numbers, CSR - Cross section radius, PA - Pith area, XN- Xylem numbers AA - Arenchyma area, XCA - Xylem Area, CSA - Cross section area

***Genetic variability study for aerenchyma cell numbers*:**

Cross sections depicting the anatomical differences between aerobic and water-logged genotypes shown in **Fig 2**. Aerenchyma cell numbers are more in water logged condition than in aerobic condition. The aerenchyma cells under aerobic condition ranged from 11 (BJ21) to 23 (Devamallige). On the other hand, under water logged condition, number of aerenchyma cells ranged from 19 (Jeerigesanna) to 28 (BI33). This is because of anatomical and morphological changes in plant in response to environmental i.e., plants phenotypic plasticity in response to environment conditions to survive (Sultan, 2000). Similarly, De Souza *et al.* (2010) reported that in maize flooding caused increase in porosity of roots through formation of aerenchyma. **(Table 4 & 5)**

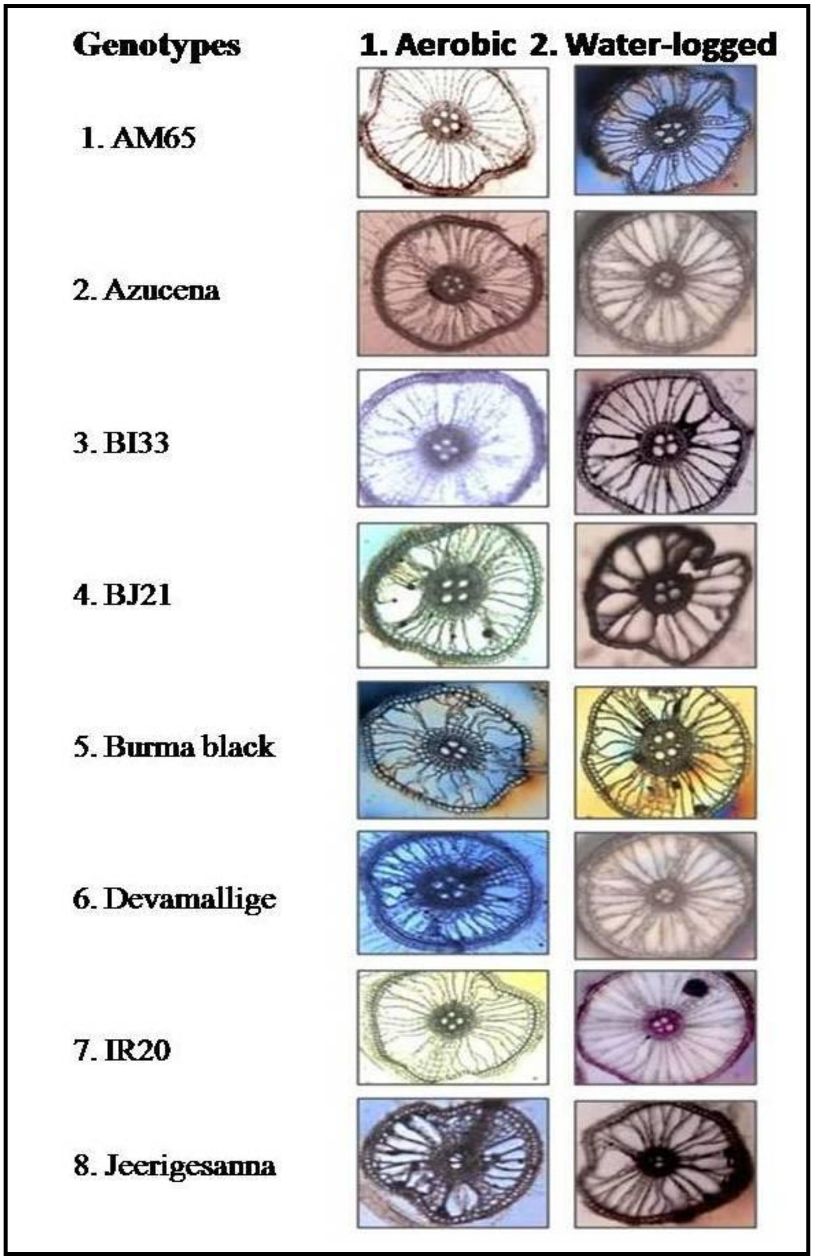

Fig2: Cross sections depicting the anatomical differences between aerobic and water-logged genotypes

Whereas, Xylem cell numbers in aerobic condition ranged from 3 (Jeerigesanna) to 6 (Azucena) under water logged condition it was least ranging 3 (Jeerigesanna) to 5 (Azucena and BI33) in numbers. Graphical representations of different root anatomical traits in aerobic and well-watered conditions **(Fig 3)**

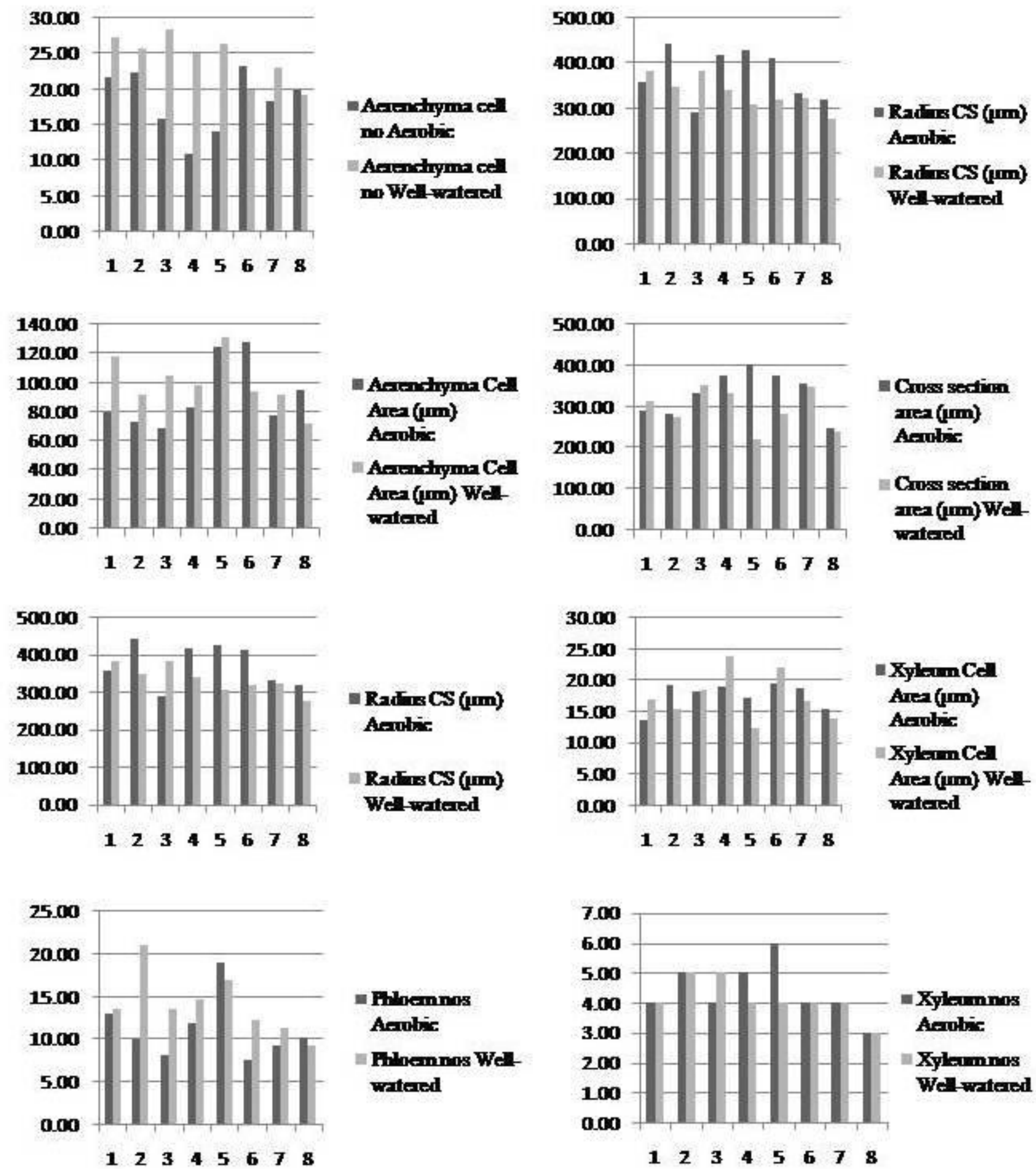

Fig.3. Graphical representations of different root anatomical traits in two treatments (aerobic and well- watered) Note: Entries from 1 to 8 such as 1. AM65, 2.Azucena, 3.BI33, 4.BJ21, 5.Burmabalck, 6.Devamallige, 7.IR20 and 8.Jeerigesanna.

Table 4: Anatomical traits of rice roots mean values under aerobic condition

SL No.	Genotypes	Aerenchyma cell number	Radius CS (µm)	Pith Area (rds)(µm)	Xylem numbers	Phloem numbers	Aerenchyma Cell Area (µm)	Xylem Cell Area (µm)	Root Thickness (µm
1	AM65	21.67±2.0	360.33± 34.2	81.30±55.7	4.00±0.0	13.00±1	80.00±2.0	13.71±0.6	292.63±55.4
2	Azucena	22.33±2.0	445.00±15.0	76.22±6.3	5.00±0.0	10.00±1.0	73.53±8.6	19.40±2.0	283.00±95.8
3	BI33	16.00±1.0	292.53±42.3	60.05±12.2	4.00±1.0	8.30±1.0	69.33±8.3	18.23±1.6	333.60±53.3
4	BJ21	11.00±1.0	419.40±42.3	63.64±38.3	5.00±0.0	12.00±1.0	83.34±8.3	18.94±1.7	374.87±53.8
5	Burma Black	14.00±1.0	429.37±6.4	64.69±5.6	6.00±0.0	19.00±1.7	124.87±10.9	17.37±0.8	404.93±66.8
6	Devamallige	23.33±2.5	412.53±56.6	69.55±9.4	4.00±0.0	7.60±0.5	127.67±7.3	19.55±3.2	375.77±53.9
7	IR20	18.33±0.5	335.47±15.28	60.66±5.6	4.00±0.0	9.30±1.5	78.08±4.2	18.77±0.6	354.87±9.8
8	Jeerigesanna	20.00±2.6	320.17±8.6	62.52±7.5	3.00±0.0	10.30±2.0	95.50±5.6	60.49±2.3	246.70±44.2

Table 5: Anatomical traits of rice roots mean values under water-logged condition

Sl.No.	Genotypes	Aerenchyma cell numbers	Radius CS (µm)	Pith Area (rds)(µm)	Xylem numbers	Phloem numbers	Aerenchyma Cell Area (µm)	Xylem Cell Area (µm)	Root Thickness (µm)
1	AM65	27.33±3.0	384.67±10.9	82.23±10.9	4.00±0.0	13.67±2.0	118.67±4.5	17.13±0.6	314.43±45.7
2	Azucena	25.67±2.0	349.17±33.9	84.50±0.8	5.00±0.0	21.00±1.0	91.33±0.9	15.46±2.3	274.37±34.9
3	BI33	28.33±1.5	383.73±4.3	100.10±6.4	5.00±0.0	13.67±0.5	104.80±14.4	18.50±0.7	352.80±38.7
4	BJ21	25.00±2.0	339.81±59.6	88.49±6.5	4.00±0.0	14.67±1.0	98.10±8.5	24.00±0.6	334.27±6.6
5	Burma Black	26.33±2.6	309.07±20.6	73.65±9.8	4.00±0.0	17.00±1.5	130.87±1.2	12.50±2.3	222.40±54.3
6	Devamallige	20.00±2.0	319.61±12.3	83.37±7.6	4.00±0.0	12.33±3.2	94.34±4.4	22.17±2.6	282.70±34.3
7	IR20	23.00±2.0	323.13±27.1	67.92±3.1	4.00±0.0	11.33±1.5	91.35±8.1	16.83±1.8	347.03±28.4
8	Jeerigesanna	19.33±1.5	277.67±44.4	66.97±2.5	3.00±0.0	9.33±4.1	72.42±1.2	14.09±0.1	238.70±12.1

Correlation studies

Aerenchyma number showed highly significant positive correlation with pith area (0.57) and negatively correlated with xylem numbers (-0.46) and phloem number (-0.46). On the other hand, root thickness positively correlate with pith area (0.41) and xylem numbers (0.70). However, xylem number showed positive correlation with phloem numbers (0.67) and highly negative correlation with xylem cross section area (-0.46) under aerobic condition.

In the same way, under water logged condition aerenchyma number showed highly significant positive correlation with cross section area radius (0.58), pith area (0.52), xylem number (0.63), phloem number (0.55) and aerenchyma area (0.65). For Cross section area radius showed highly significant correlation with pith area (0.68) and xylem number (0.60). Pith area was highly correlated with xylem numbers (0.67), cross section area (0.51) and xylem cross section area (0.47).(**Table 5 & 6)**

Table 5: Correlation coefficients among the plant anatomical traits under aerobic condition

	AN	CSR	PA	XN	PN	AA	CSA	XCA
AN	1	-0.01	0.57 **	-0.46*	-0.42*	0.07	-0.41	0.09
CSR		1.00	0.41 *	0.70 **	0.35	0.40	0.30	-0.22
PA			1.00	0.09	0.13	0.00	-0.15	-0.18
XN				1.00	0.67**	0.25	0.51 *	-0.46**
PN					1.00	0.37	0.31	-0.04
AA						1.00	0.47 *	0.10
CSA							1.00	-0.40**
XCA								1.00

**. Correlation is significant at the 0.01, *Correlation is significant at the 0.05 level

Table 6: Correlation coefficients among the plant anatomical traits under water-logged condition.

	AN	CSR	PA	XN	PN	AA	CSA	XCA
AN	1	0.58 **	0.52**	0.63 **	0.55 **	0.65**	0.37	-0.06
CSR		1	0.68 **	0.60 **	0.27	0.41 *	0.40 *	0.21
PA			1	0.67 **	0.29	0.21	0.51 **	0.47**
XN				1	0.63 **	0.27	0.382	0.12
PN					1	0.37	-0.11	-0.11
AA						1	-0.03	-0.12
CSA							1	0.58
XCA								1

**. Correlation is significant at the 0.01, *Correlation is significant at the 0.05 level
Note- AN - Aerenchyma numbers, CSR - Cross section radius, PA - Pith area, XN- Xylem numbers AA - Arenchyma area, XCA - Xylem Area, CSA - Cross section area

IV. Conclusion

Variation for the aerenchyma and anatomical traits were significant across genotypes. Non-significant variation in replications is a direct indication of trait integrity of the genotypes. Waterlogged roots had higher number of aerenchyma as compared to aerobic condition. Also, mean individual aerenchyma area was highest over the length of the roots.

Present study also observed formation of the aerenchyma being a constitutive process, speculate to relate exact role of aerenchyma formation in rice. It is possible that, it may be a strategy of the plant to reduce its metabolic energy by killing the non-Photosynthesizing cells of roots. Also, the oval structure of Aerenchyma, with regular order of suberization in exterior walls of roots may provide additional physical strength to roots.

ACKNOWLEDGEMENTS

Authors thankful to Dr. Shashidhar H. E Aerobic rice laboratory Department of Plant Biotechnology, UAS, GKVK, Bengaluru for providing facilities to execute work, Limagrain France for grant support under Maize Root Architecture Project (Ab/Ac: 5108), M.S Ramaiah Institute of Technology, Bangalore and Visveshwarya Technological University, Belgaum for their constant support during the research period.

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