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Rooting traits of rice genotypes as influenced by different water regimes and biofertilizer

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Abstract
The study was undertaken at Integrated Farming System Research Station (IFSRS), Karamana, Thiruvananthapuram, Kerala during the summer season of 2020-'21. Three rice genotypes, *viz*., Prathyasa, KAU Manu Rathna and Sharada were grown under three different water regimes (flooded, saturated and aerobic condition), with and without biofertilizer, *Azospirillum lipoferum*. The root parameters of these varieties were recorded at harvest. It was observed that the rooting depth, root dry weight, root volume and root shoot ratio were superior in the variety, Sharada while number of roots at crown region was more under Prathyasa. Similarly, these parameters, except number of roots at crown region, were the highest under aerobic condition in the presence of *Azospirillum lipoferum*. In contrast, the root mass density and root length density were the highest under flooded and saturated condition.

Keywords: Azospirillum lipoferum, KAU Manu Rathna, Prathyasa, root mass density, root length density

Introduction

Rice (*Oryza sativa* L.) is considered as the world's most important staple crop which serves more than three billion people across the globe (Vengatesh and Govindarasu, 2017) [38]. Kerala lies in the southernmost tip of India surrounded by Arabian Sea on the west and Western Ghats in the east with diverse geographical features. Kerala has a rich culture and tradition of rice cultivation (Athira and Kumar, 2016) [4]. Paddy is cultivated in all the three seasons, *viz*., autumn, winter and summer except in Wayanad district. During 2019-'20, paddy was cultivated in an area of 1,982 km^2 with a production of 5,87,078 tonnes and productivity of 3,073 kg ha^{-1}, including dry paddy cultivation (GoK, 2021) [12]. However, it has been estimated that the rice requirement is around 3.5 - 4.0 million tonnes per hectare in Kerala but achieves only one-fifth of this requirement (Krishnankutty *et al*., 2021) [19].

With the emerging scenario of climate change, we should shift from single to diverse rice production technologies to adapt with the changing environment. Rice, being a water-intensive crop, has resulted in the depletion of water table. The irrigation water is declining day-by-day and this water scarce condition has threatened the sustainability of irrigated rice ecosystem (Tuong *et al*., 2004) [44]. Under such situation, rice production with less water input is significant. This investigation is thus carried out to ascertain the performance of rice genotypes under water limited condition and to identify genotypes with better root growth which can help in better foraging of soil resources to sustain the yield under such situation.

The most important part of rice under water limitation is its root. Root is an integral part that aids in the absorption and uptake of water and nutrients from the soil. Enhancement of root growth and development under water deficit condition will eventually help in the acquisition of water and nutrients from the maximum volume of soil. Plant growth promoting rhizobacteria (PGPRs) are known to magnify the root growth and proliferation by synthesizing growth regulators like IAA and GA (Tejaswini *et al*., 2017) [36] thereby mediating tolerance under water limited condition. Inoculating rice with *Azospirillum* has increased the root biomass (Kannan and Ponmurugan, 2010). Thus, in this study, *Azospirillum lipoferum* was used to determine its effect on rice roots under water limited condition.

2. Materials and Methods

The field experiment was carried out at Integrated Farming System Research Station (IFSRS), Karamana, Thiruvananthapuram, Kerala during the summer season of 2020-'21. PVC pipe of 8" diameter and 4 kg cm^{-3} density was used for this experiment. The soil of the experimental site was sandy clay loam in texture, strongly acidic in reaction (pH of 5.61), medium in

Available nitrogen (248.53 kg ha^{-1}) and available potassium (169.82 kg ha^{-1}), high in organic carbon (1.54 and available phosphorus status (25.68 kg ha^{-1}). One meter deep pit was taken in the field and the 100 cm-long PVC pipes were arranged vertically in this pit. These pipes were then filled with the same soil as in the surrounding area and three seeds were dibbled in each pipe. Three varieties, *viz*., Prathyasa (MO 21), KAU Manu Rathna (HS 16) and Sharada (MAS 946-1) were used for this experiment. These three varieties were subjected to three different water regimes, W_1 - flooded condition (water column of 5 cm was maintained throughout the crop growth), W_2 - saturated condition (irrigating to 1 cm depth one day after the disappearance of standing water column) and W_3 - aerobic condition (irrigation to obtain 2.5 cm depth of irrigation and subsequent irrigation once in five days), with and without biofertilizer, *Azospirillum lipoferum* KAU isolate. The plants were harvested carefully and the following root parameters were recorded as per the procedures given by Misra and Ahmed (1989) [22] and the recorded data were then subjected to statistical analysis as described by Gomez and Gomez (1984) [13].

a. Rooting depth: The plants were uprooted carefully, root portion was separated, cleaned and the length was measured from base of the plant to the tip of the longest root. The mean value was calculated and expressed in centimeters (cm).

b. Number of roots at crown region: The total number of roots at crown region were counted and recorded.

c. Root dry weight: The root portion was separated and dried in a hot air oven at 70 °C till constant weights were recorded and expressed in grams (g).

d. Root volume: Root volume per plant was found out by water displacement method and expressed in cm^3 per plant.

e. Root density: Root density was calculated both in terms of root mass density (RMD) and root length density (RLD) per unit of soil volume and expressed in g cm^{-3} and cm cm^{-3}, respectively.

f. Root shoot ratio: The root and shoot portion were separated and dried in a hot air oven at 70 °C till constant weights were recorded. It was expressed as the ratio of root dry weight and shoot dry weight.

3. Results

a. Rooting depth: The Table 1 shows that the aerobic rice variety, Sharada (34.22 cm) recorded 54.35 per cent and 45.80 per cent deeper roots than Prathyasa (22.17 cm) and KAU Manu Rathna (23.47 cm), respectively. Among the water regimes, aerobic condition (32.30 cm) resulted in 57.72 per cent and 19.67 per cent more deeper roots over flooded and saturated condition, respectively. Similar results were also reported by Nguyen *et al*. (2009) [25], Grewal (2011) [14] and Phule *et al*. (2019) [45]. *Azospirillum lipoferum* increased the rooting depth by 14.09 per cent over no biofertilizer application. This result was also reported by Purushothaman *et al*. (1987) [28] and Muthukumasraswamy *et al*. (2006).

The interaction effect showed that Sharada under aerobic (43.41 cm) and saturated condition (33.56 cm) recorded the deepest roots over other varieties under different water regimes. Similarly, Sharada with the application of biofertilizer produced the deeper roots (35.94 cm), presented in Table 1b. The rooting depth, in general, increased under aerobic condition with biofertilizer application (35.52 cm) over saturated and flooded condition. Among all the treatment combinations, the variety, Sharada under aerobic condition with *Azospirillum lipoferum* (46.41 cm) application resulted in significantly deeper roots (Table 1c).

b. Number of roots at crown region: The number of roots at crown region was higher by 30.53 per cent and 8.71 per cent in Prathyasa, as compared to Sharada and KAU Manu Rathna, respectively. In the case of water regimes, aerobic condition reduced the number of crown roots by 33.56 per cent and 20.07 per cent over flooded and saturated condition, respectively (Table 1a). This result was in accordance with the findings of Gao and Lynch, 2016 and Sandar *et al*., 2022. [11, 31] The application of biofertilizer had no significance with respect to crown root number. The interactions between and among the treatments showed no significance with respect to number of roots at crown region.

c. Root dry weight: Significant variations were noticed in the varieties, water regimes and biofertilizer application with respect to root dry weight (Table 1a). Sharada produced 35.14 per cent and 33.22 per cent more root dry weight than Prathyasa and KAU Manu Rathna, respectively. Among the water regimes, aerobic condition increased the root dry weight by 14.91 to 31.05 per cent over flooded and saturated condition. The results are in conformity with those of Grewal (2011) [14] and Wang *et al*. (2018) [39]. An increase of 10.30 per cent in root dry weight was recorded in the presence of biofertilizer. Similar results were also reported by El-Khawas and Adachi (1999) [8] and Santhosh *et al*. (2018) [33]. Among the interaction effects, the variety, Sharada under aerobic condition resulted in the highest root dry weight (9.15 g/plant) followed by Sharada under saturated condition (7.50 g/plant). The other two varieties produced comparatively similar root dry weight under different water regimes with a slight increase under aerobic condition (Table 1b).

d. Root volume: The root volume of Sharada enhanced by 28.63 per cent and 17.45 per cent than Prathyasa and KAU Manu Rathna, respectively. Among the water regimes, an increase in the root volume by 34.98 per cent and 16.90 per cent was observed under aerobic condition over flooded and saturated condition, respectively (Table 1). The results corroborated with the findings of Mitchell *et al*. (2012) [23] and Jinsy (2014). A reduction of 8.90 per cent in root volume was noticed in the absence of *Azospirillum lipoferum* (Table 1). This result is in accordance with the findings of Quadros (2009) [29] and Zeffa *et al*. (2019) [42] in maize. No significant interactions were reported with respect to root volume.

e. Root density: Root density was measured in terms of mass and length at the top 15 cm of soil layer. The response of varieties had no significance in terms of root density. However, the RMD (11.02 and 5.22 and RLD (6.60 and 1.35 under flooded condition were significantly higher than aerobic and saturated condition, respectively (Table 1). This result was in accordance with the findings of Kato and Okami (2010) [18] and Mitchell *et al*. (2012) [23]. The interaction effects had no statistical significance.

f. Root shoot ratio: Significant variations were observed among the varieties and water regimes with respect to root shoot ratio (Table 1). Higher root shoot ratio was observed in Sharada (52 higher than Prathyasa and 22.58 higher than KAU Manu Rathna). Among the three water regimes, aerobic condition resulted in an increase in root shoot ratio by 33.33 per cent and 16.13 per cent over flooded and saturated condition, respectively. Similar result was reported by Nguyen *et al*. (2015) [26] and Phule *et al*. (2019) [45]. Application of biofertilizer resulted in an increment of root shoot ratio by 10 per cent. Battistus *et al.* (2014) also reported similar result in maize. The treatment combinations were not significant with respect to root shoot ratio.

4. Discussion

Rice has been developed from a semi-aquatic predecessor. It has a unique root morphology and anatomy such as the development of aerenchyma with comparatively shallow and compact root system than other crops (Yoshida and Hasegawa, 1982; Angus *et al*., 1983 and Fukai and Inthappan, 1988) [41, 1, 9]. Root architecture in rice is a function of adventitious roots and lateral roots emerging from the adventitious roots (Rebouilla *et al*., 2009) [30]. The survival of a plant under water deficit condition largely depends on its rooting behavior and how they acquire water and nutrients to tolerate the stressed condition.

The rooting traits such as rooting depth, root dry weight, root volume and root shoot ratio were greater under Sharada (MAS 946-1) than Prathyasa and KAU Manu Rathna. Sharada, being an aerobic rice variety, is known for producing better root system as an adaptation strategy under water deficit situation. It was revealed that deeper roots and higher root biomass are the features of rice genotypes that are suitable for cultivation under aerobic condition (Bing-Song *et al*., 2006; Martin *et al*., 2007; Sandhu *et al*., 2012 and Phule *et al*., 2019) [7, 21, 32, 45]. The partitioning of assimilates more into the roots favours deeper roots under aerobic condition (Kato and Okami, 2010) [18]. The number of crown roots reduced under aerobic condition to facilitate deeper penetration of roots (Sandar *et al*., 2022) [31]. The number of roots at crown region is a crucial factor that improves acquisition of resources like water and nutrients under water deficit condition by penetrating into deeper soil strata and enhances root penetration and reduces lateral root branching (Zhan and Lynch, 2015 and Gao and Lynch, 2016) [43, 11]. An ideal number of crown roots are required to avoid lodging under too low crown root number which results in dispersed axial roots and are insufficient to acquire required water and nutrients. If the number of roots at crown region is too high, the roots will start competing with each other for soil resources thereby reducing the root penetration (Lynch, 2013) [20]. The RMD and RLD at top 15 cm were higher under flooded and saturated condition because of the unavailability or less availability of oxygen that decreases the tendency of roots to penetrate deeper and thus, concentrates on the surface layer with the development of aerenchyma (Suralta and Yamauchi, 2008) [35]. However, under aerobic condition, root elongates into deeper layer for the acquisition of soil resources thus reduces its density at the top layer.

Azospirillum lipoferum enhanced the root parameters of rice than in its absence. Inoculation of *Azospirillum* changes the growth and morphology of roots. *Azospirillum* fixes atmospheric nitrogen and synthesizes phytohormones like IAA (indole acetic acid) and GA (gibberellic acid) which favours better root growth (Tejaswini *et al*., 2017) [36], increases root proliferation and root biomass (Tien *et al.*, 1979) [37] thereby augmenting the acquisition of water and nutrients by plants (Sumner, 1989 and Ardakani and Mafakheri, 2011) [34, 2]. *Azospirillum* also confers tolerance to plants under limited moisture supply due to increased root growth (Ilyas *et al*., 2012; Arzanesh *et al*., 2011 and Bano *et al*., 2013) [15, 3, 46]. The phytohormones synthesized by *Azospirillum* acts as signaling molecules (Fukami *et al*., 2018) [10] and also triggers induced systemic tolerance (IST) and confers tolerance under water deficit condition (Yang *et al*., 2009) [40].

Table 1: Effect of varieties, water regimes and *Azospirillum lipoferum* on root parameters of rice

Treatments	Rooting depth (cm)	Number of roots at crown region	Root dry weight (g/plant)	Root volume (cc)	Root density		Root shoot ratio
					Root mass density (g cm^{-3})	Root length density (cm cm^{-3})	
Varieties (V)							
v_1 (Prathyasa)	22.17^c	7.61^a	5.55^b	21.45^b	1.35	2.24	0.25^c
v_2 (KAU Manu Rathna)	23.47^b	7.00^b	5.63^b	23.49^b	1.36	2.24	0.31^b
v_3 (Sharada)	34.22^a	5.83^c	7.50^a	27.59^a	1.30	2.13	0.38^a
SE m(+)	0.394	0.213	0.215	0.807	0.035	0.039	0.011
CD (0.05)	1.129	0.610	0.616	2.313	NS	NS	0.031
Water regimes (W)							
w_1 (Flooded)	20.48^c	7.72^a	5.41^c	20.70^c	1.41^a	2.26^a	0.27^c
w_2 (Saturated)	26.99^b	6.94^b	6.17^b	23.90^b	1.34^{ab}	2.23^{ab}	0.31^b
w_3 (Aerobic)	32.30^a	5.78^c	7.09^a	27.94^a	1.27^b	2.12^b	0.36^a
SE m(+)	0.394	0.213	0.215	0.807	0.035	0.039	0.011
CD (0.05)	1.129	0.610	0.616	2.313	0.100	0.113	0.031
Biofertilizer *Azospirillum lipoferum* (A)							
a_0 (Without A)	24.84^b	7.00	5.92^b	23.15^b	1.33	2.19	0.30^b
a_1 (With A)	28.34^a	6.63	6.53^a	25.21^a	1.35	2.22	0.33^a
SE m(+)	0.321	0.174	0.175	0.659	0.028	0.032	0.009
CD (0.05)	0.922	NS	0.503	1.889	NS	NS	0.026

Table 2: Interaction effect of varieties, water regimes and *Azospirillum lipoferum* on root parameters of rice

Treatments	Rooting depth (cm)	No. of roots at crown region	RDW (g/plant)	Root volume (cc)	Root density		Root shoot ratio
					Root mass density (g cm^{-3})	Root length density (cm cm^{-3})	
Interaction (V × W)							
v_1w_1	17.09^{e}	8.50	5.17^{c}	18.55	1.42	2.30	0.23
v_1w_2	21.71^{d}	7.83	5.53^{c}	20.94	1.35	2.27	0.25
v_1w_3	27.42^{c}	6.50	5.94^{c}	24.87	1.30	2.16	0.28
v_2w_1	18.64^{e}	8.17	5.21^{c}	20.27	1.46	2.28	0.28
v_2w_2	25.72^{c}	7.50	5.49^{c}	23.32	1.36	2.27	0.29
v_2w_3	26.06^{c}	5.33	6.19^{c}	26.90	1.27	2.17	0.35
v_3w_1	25.70^{c}	6.50	5.85^{c}	23.30	1.35	2.19	0.30
v_3w_2	33.56^{b}	5.50	7.50^{b}	27.44	1.32	2.17	0.38
v_3w_3	43.41^{a}	5.50	9.15^{a}	32.05	1.24	2.04	0.44
S.Em (+)	0.682	0.369	0.372	1.397	0.060	0.068	0.019
CD (0.05)	1.956	NS	1.067	NS	NS	NS	NS
Interaction (V × A)							
v_1a_0	21.16^{e}	7.89	5.43	20.45	1.35	2.23	0.25
v_1a_1	22.99^{d}	7.33	5.66	22.46	1.36	2.25	0.26
v_2a_0	20.86^{e}	7.11	5.44	22.51	1.35	2.23	0.29
v_2a_1	26.08^{c}	6.89	5.82	24.47	1.37	2.26	0.32
v_3a_0	32.50^{b}	6.00	6.88	26.49	1.30	2.12	0.36
v_3a_1	35.94^{a}	5.67	8.12	28.70	1.31	2.14	0.40
S.Em (+)	0.557	0.301	0.304	1.141	0.049	0.056	0.015
CD (0.05)	1.597	NS	NS	NS	NS	NS	NS
Interaction (W × A)							
w_1a_0	19.16^{e}	7.89	5.25	19.64	1.41	2.24	0.25
w_1a_1	21.79^{d}	7.56	5.57	21.77	1.40	2.28	0.29
w_2a_0	26.29^{c}	7.11	5.92	23.21	1.33	2.23	0.29
w_2a_1	27.70^{bc}	6.78	6.42	24.58	1.36	2.24	0.32
w_3a_0	29.07^{b}	6.00	6.58	26.60	1.26	2.11	0.35
w_3a_1	35.52^{a}	5.56	7.60	29.27	1.28	2.13	0.36
S.Em (+)	0.557	0.301	0.304	1.141	0.049	0.056	0.015
CD (0.05)	1.597	NS	NS	NS	NS	NS	NS

Table 3: Interaction effect of varieties, water regimes and *Azospirillum lipoferum* on root parameters of rice

Treatments	Rooting depth (cm)	No. of roots at crown region	Root dry weight (g/plant)	Root volume (cc)	Root density		Root shoot ratio
					Root mass density (g cm^{-3})	Root length density(cm cm^{-3})	
Interaction (V × W × A)							
$v_1w_1a_0$	17.04^{j}	8.67	5.13	17.61	1.42	2.28	0.22
$v_1w_1a_1$	17.14^{j}	8.33	5.21	19.48	1.41	2.32	0.24
$v_1w_2a_0$	20.13^{i}	8.00	5.41	20.42	1.33	2.26	0.22
$v_1w_2a_1$	23.29^{gh}	7.67	5.65	21.46	1.36	2.27	0.28
$v_1w_3a_0$	26.31^{def}	7.00	5.76	23.30	1.29	2.16	0.30
$v_1w_3a_1$	28.54^{d}	6.00	6.12	26.43	1.30	2.16	0.26
$v_2w_1a_0$	16.24^{j}	8.33	5.06	19.79	1.46	2.25	0.26
$v_2w_1a_1$	21.03^{hi}	8.00	5.36	20.74	1.45	2.32	0.29
$v_2w_2a_0$	25.83^{defg}	7.67	5.41	22.31	1.34	2.26	0.28
$v_2w_2a_1$	25.60^{efg}	7.33	5.57	24.32	1.37	2.28	0.30
$v_2w_3a_0$	20.51^{i}	5.33	5.85	25.44	1.27	2.17	0.32
$v_2w_3a_1$	31.62^{c}	5.33	6.53	28.35	1.28	2.18	0.37
$v_3w_1a_0$	24.20^{fg}	6.67	5.55	21.50	1.34	2.19	0.27
$v_3w_1a_1$	27.21^{de}	6.33	6.14	25.09	1.35	2.20	0.34
$v_3w_2a_0$	32.91^{c}	5.67	6.95	26.90	1.31	2.16	0.38
$v_3w_2a_1$	34.21^{c}	5.33	8.05	27.97	1.33	2.18	0.39
$v_3w_3a_0$	40.41^{b}	5.67	8.14	31.06	1.23	2.01	0.43
$v_3w_3a_1$	46.41^{a}	5.33	10.16	33.03	1.26	2.06	0.46
SE m(+)	0.964	0.521	0.526	1.976	0.085	0.096	0.027
CD (0.05)	2.766	NS	NS	NS	NS	NS	NS

5. Conclusion

This investigation revealed that the root traits, *viz*., rooting depth, number of roots at crown region, root dry weight, root volume and root shoot ratio are critically associated with aerobic adaptation along with the application of *Azospirillum lipoferum*. Among the three genotypes, Sharada (MAS 946-1) performed better with respect to rooting traits under aerobic condition. However, the root growth of Prathyasa and KAU Manu Rathna increased to a limited extent under aerobic condition indicating that both these varieties tried to evade water deficit situation. All the three varieties produced deeper roots under aerobic condition to acquire water and nutrients from the deeper layers. These morphological characteristics can form the preliminary requirement of root phenotyping for developing genotypes suitable for cultivation under aerobic condition.

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