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RESEARCH ARTICLE

GENOTYPIC VARIATION FOR ROOT GROWTH ORIENTATION IN RICE (*ORYZA SATIVA* L.) UNDER AEROBIC AND WELL-WATERED CONDITION

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ABSTRACT

Deep root system study is an important component of drought resistant rice (*Oryza sativa* L.) variety. Root morphology is a complex trait combining various components. Drought acts as major limitation for growing rice in rainfed ecosystems. Therefore, there is a need to screen and breeding rice genotypes for drought resistance. The objective of this study is to elucidate the genotypic variation among rice genotypes in root distribution and root angle including deep-root development. The experiment was conducted with eight genotypes using basket method in aerobic and well-watered conditions. The results from genotype × year interactions for the S-R (Shoot-root) ratio, with Devamallige, AM65, and BJ21 responded more to water stress as compared to other genotypes. Genotypes azucena had highest root diameter (1.5 mm) followed by devamallige (1.4 mm) and AM65 (1.4 mm) under both aerobic and well-watered conditions. IR20 showed lowest root diameter/thickness (0.7 mm) in both the conditions. Increase in number of roots at 0^0-45^0 under aerobic condition indirectly measures the increase in deep root length. Correlation coefficients between total root diameter and deep root length were also high in both aerobic ($r = 0.724$ and $r = 0.924$) and well- watered ($r = 0.605$ and $r = 0.842$) conditions which subsequently evolved into greater deep root length.

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INTRODUCTION

The root system of plants are said to be important which anchors uptake of water and minerals. Investigating the morphology of roots is very important as plants are highly dependent on root function. Although studying the root system is quite tedious and time consuming, there has been several research reports available for the same. Deep roots during drought, is considered as a key trait for improving drought resistance in upland rice as they contribute to water uptake from deeper soil layers (Yoshida and Hasegawa, 1982; Arakiand Iijima, 2005; Kato *et al*., 2006a). Deeper and thick root development allows plants to access water from deep soil profile (Ekanayake *et al*., 1985; Fukai and Cooper, 1995).

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Soil condition largely affects deep root development (Cairns *et al*., 2004; Kato *et al*., 2006), and interaction between genotype x environment have been previously recognized (Kondo *et al*., 2003). Among environmental factors, the effect of water regime on deep root development of rice is also well understood (Kato *et al*., 2006). Root growth in flooded condition is generally shallow due to its adaptation to submerged condition, but in rainfed and or aerobic condition root length is usually longer than flooded condition (Nicou *et al*., 1970).

Aerobic rice genotypes have traits with improved lodging resistance, input responsiveness and tolerance to occasional flooding (Bouman, 2001; Shashidhar *et al*., 2007). These genotypes have characteristics of drought-resistant of upland varieties and high-yielding characteristics of lowland varieties (Lafitte *et al*., 2002). Thus, they have a better yield performance under both favorable and drought-stressed upland conditions. Genotypes grown under aerobic conditions traditional and improved upland and high-yielding irrigated lowland genotypes have the ability to retain both high biomass production and harvest index (Atlin *et al*., 2006).

The genetic differences exhibited by germplasm in water uptake may provide important insights into drought-resistant germplasm that may be overlooked with root length measurements alone. Several studies on upland rice with genetic differences in water uptake as measured with a neutron probe have been reported (Puckridge and O'Toole, 1981; Lilley and Fukai, 1994; Gowda *et al*., 2012). Lafitte *et al*., (2001) reported indica genotypes characteristically showed thin, shallow roots; japonica genotypes had deeper, coarse roots. Genotypic variation for root growth was also being observed in different water regimes (Asch *et al*., 2004; Sigari *et al*., 2000; Gowda *et al*., 2011). As such, genotype × water regimes should be precisely quantified.

There are several methods available to study root morphology in rice. The wax layer system was first applied for screening root penetration ability by Taylor and Gardner (1960) which was used to simulate the hardpan present in lowland fields (Yu *et al*., 1995; Ray *et al*., 1996; Ali *et al*., 2000; Babu *et al*., 2001; Clark *et al*., 2002, 2008). However, Clark *et al*., (2002) found that cultivars with good penetration under the wax layer screen did not consistently show superior performance in the field. The PVC cylinder system is an improved root study method over pot culture, where root development is restricted (Upchurch and Taylor, 1990; Toorchi *et al*., 2002; Kanbar *et al*., 2010; Shashidhar *et al*., 2012).

Among several methods of studying root morphology, growth of root systems in baskets has been used to predict rooting depth indirectly according to growth angle (Oyanagi *et al*., 1993). Using the basket method, Kato *et al*. (2006) demonstrated the relationship between high root growth angle and root depth in rice, and Uga *et al*. (2009) observed variation in root growth angle in rice accessions under upland field conditions. Therefore, to support root angle studies (Oyanagi *et al*., 1993) basket method was developed. This method allows us to easily count angle of (0^0-45^0 and 45^0-90^0) roots which penetrate from each part of the mesh for classification of deep root growth. Using the basket method, Kato *et al*., (2006) and Uga *et al*., (2009) investigated the root angle frequency. The root growth angle may also be related to the deep root development of upland rice, although negligible studies have been reported on genotypic variation due to root growth angles. Uga *et al*., (2011) reported Dro1 gene can potentially be used to improve drought avoidance of rice by changing its rooting pattern from a shallow to a deep system. Hence, in this context, the present investigation aims at study the improvised method of main morphological root traits that contribute to the root development in drought resistance rice variety. Special attention was paid to root growth angles as a possible morphological indicator for deep root development.

MATERIALS AND METHODS

Experimental site

The Present investigation was conducted at experimental blocks at department of plant biotechnology, University of Agricultural Sciences, Bengaluru, India located at the latitude of $12^0$58' North; $77^0$35' East and altitude of 930 meters above mean sea level (MSL).

Plant materials

The experimental material consists of eight genotypes comprising both *japonica* and *indica* group with different origins. The characteristics of genotypes and their origin are mentioned in Table 1.

Experimental design

The experiment was conducted using basket method as previously described (Oyanagi *et al*., 1993; Uga *et al*., 2009) in completely randomized design (CRD) with three replications during summer and *Kharif* 2014 under both aerobic and well-watered conditions. Open plastic mesh basket with top diameter of 15 cm, bottom diameter of 8.5 cm, height of 15 cm, and mesh size of 2 mm were used for the experiments (Fig.1). The basket were filled with soil that had been mixed evenly with inorganic fertilizer NPK at the rate of 60, 39, 67 kg ha^{-1}, and soil was compacted by watering regularly at the center of the each basket. In each basket three to five seeds were sown. These baskets were placed in a pot with diameter of 16 cm and height 30cm. Under aerobic condition, pots carrying baskets were placed in a pit to maintain ground level and plants were irrigated every alternate day. Under well watered condition, baskets were placed in a water tank and water level was maintained till the edges of pot.

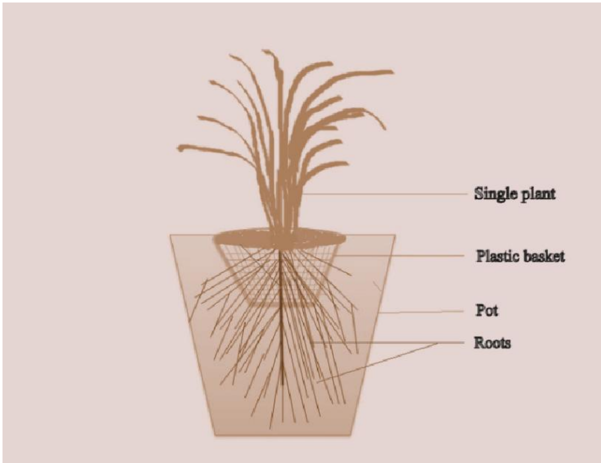

Fig.1. System for evaluating root angle in rice

Collection of data

Plants were sampled for recording observations at 40 DAS for shoot length in cm (SL), number of tillers (NT), shoot dry weight in g (SDW) and number of leaves (NOL). Roots were sampled by soaking pots carrying basket into water tank for 24-48 hours. The next day roots were thoroughly washed with fine jet of water. The morphological traits such as maximum root length in cm (RL), total number of roots (RN), number of roots at 0^0-45^0 (RN 0^0-45^0), number of roots at 45^0-90^0 (RN 45^0-90^0), root dry weight in g (RDW) and root diameter in mm (RD) were recorded. Further, shoot to root ratio (SR ratio) was computed as ratio of shoot dry weight and root dry weight.

Table 1a. Analysis of variance (mean sum of squares) for quantitative traits in rice under aerobic condition during summer and *Kharif* 14

Source of variation	df	Tillers number	Shoot length (cm)	Number of leaves	S-R ratio	Root angle 0^0-45^0	Root angle 45^0-90^0	Total no of roots	Deep root length	Root diameter(mm)
Replication	2	1.271	54.276	15.896	0.061	2.083	57.583	74.812	2.461	0.001
Years (Y)	1	2.083	68.402	0.333	0.145	8.333	682.521**	468.750**	404.550**	0.021*
Genotypes (G)	7	80.524**	364.836**	378.464**	0.941**	136.464**	804.664**	1281.702**	102.107**	0.467**
G × Y	7	0.226	37.194	22.333	0.182*	10.619*	123.092**	124.655*	19.215	0.001
Error	30	2.026	26.451	11.34	0.072	4.728	37.717	39.013	13.981	0.003

*, ** Indicate significance at 0.05 and 0.01 levels respectively

Table 1b. Analysis of variance (mean sum of squares) for quantitative traits in rice under well-watered condition during summer and *Kharif* 14

Source of variation	df	Tillers number	Shoot length (cm)	Number of leaves	S-R ratio	Root angle 0^0-45^0	Root angle 45^0-90^0	Total no of roots	Deep root length	Root diameter(mm)
Replication	2	0.333	7.701	3.423	0.257	15.25	903.521	1135.27	41.19	0.006
Years (Y)	1	4.083	647.535**	131.672	1.833**	1.688	713.021	645.333	291.560**	0.002
Genotypes (G)	7	50.286**	832.496**	297.339**	0.447**	129.759**	4214.830**	5428.702**	346.675**	0.329**
G × Y	7	7.083	23.886	39.267	0.099	14.402	127.164	191.667	20.654	0.002
Error	30	5.311	19.88	31.578	0.09	10.983	173.299	245.515	22.332	0.004

*, ** Indicate significance at 0.05 and 0.01 levels respectively

Statistical analysis

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

RESULTS AND DISCUSSION

Analysis of variance

Significant mean squares due to genotypes for all the characters studied under both aerobic (Table 2a) and well-watered (Table 2b) conditions indicate genotypes differed in their expression and hence adequacy of material being used. However, magnitude of variation due to genotypes was higher in well watered condition for all the traits except SR ratio, no. of roots at 0^0-45^0 and root diameter. In aerobic condition, contribution of genotypes was slightly higher to total variation for drought resistance, traits such as SR ratio, no. of roots at 0^0-45^0 and root diameter when compared to that of well-watered condition.

The results clearly indicated that genotypes responded better under drought condition than the well-watered condition for these traits. On the other hand, non-significant mean squares due to years under aerobic condition for drought resistance traits such as SR ratio and number of roots at 0^0-45^0 indicate absence of seasonal fluctuations in the expression of these traits. However, significant mean squares due to genotype (G) × year (Y) interaction for SR ratio, no. of roots at 0^0-45^0, no. of roots at 45^0-90^0 and total number of roots suggest differential response of genotypes in both years under aerobic condition. On the other hand, under well-watered condition G × Y interaction was non- significant for all the traits studied (Kondo *et al*., 2003).

In both the conditions mean squares due to root length is highly significant with root diameter. The genotypes with longer deep root length will became thicker at the nodal root point as root grew deeper in search of water (Nguyen *et al*., 1997; Yoshida and Hasegawa, 1982). In well watered condition, water was easily available to plants making them to develop more number of roots at 45^o-90^o angle. The present study showed that genotypes with thicker root diameter tend to develop a deep root system (Sigari *et al*., 2000).

Performance of genotypes for root morphological traits

During summer, all the genotypes exhibited higher SR ratio and no. of roots at 0^0-45^0compared to *Kharif* season under both aerobic (Tables 3a and 3b) and well-watered condition (Tables 4a and 4b). On the other hand, all the genotypes showed high mean for traits such as root length, total no. roots, root diameter, shoot length, no. of leaves and no. of tillers in *Kharif* compared to summer in both aerobic and well-watered condition.

Azucena had highest root diameter (1.5 mm) followed by Devamallige (1.4 mm) and AM65 (1.4 mm) under both the condition. Whereas, IR20 showed lowest root thickness (0.7mm) in both the conditions. While, BI33 showed highest no. of tillers, Azucena and Burma black showed lowest no. of tillers in aerobic and well-watered condition, respectively. Azucena followed by AM65 showed more number of roots at 45^0-90^0 in both the seasons under aerobic condition. Whereas, under well-watered condition, Devamallige followed by azucena and AM65 had more number of roots at 0^0-45^0 in both seasons.

Highest root length was recorded in BJ21 followed by Burma black and Devamallige, while lowest in IR20 in both seasons under well-watered condition. Interestingly, Devamallige and BI33 showed more total number of roots in both the seasons. The results indicate azucena, devamallige and AM65 were best genotypes for root morphological traits under aerobic conditions whereas, devamallige and burma black were best under well-watered conditions.

Further, the results showed that intermittent water stress (aerobic condition) throughout growth period increased root shoot ratio by 61% and number of roots at 0^0-45^0 by 53.57% but reduced root growth under well-watered condition during summer. Sigari *et al*. (2000) under pot condition reported that intermittent water stress decrease in SR ratio with increase in deep root length.

Table 3b. Well-watered condition means values summer and *kharif* 2014

Genotypes	Tillers number		Shoot length (cm)		Lo of leaves		S-R ratio	
	Summer-14	*Kharif*-14	Summer-14	*Kharif*-14	Summer-14	*Kharif*-14	Summer-14	*Kharif*-14
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Am65	11±1.00	10.3±2.08	53.9±3.70	56.1±3.10	24±3.00	31.7±4.04	0.63±0.45	1.2±0.13
Azucena	9±1.00	12.3±1.15	57.97±1.10	66.5±5.29	29.67±1.15	34.3±2.52	1.1±0.71	1.6±0.55
BI33	15.67±1.15	16.3±1.53	48.7±3.86	54.8±4.09	33±5.00	45±2.65	0.62±0.24	0.7±0.28
BJ21	14±1.73	11.3±2.31	38.67±5.08	51.7±7.41	38±2.00	33.3±2.52	0.59±0.13	0.8±0.41
Burma black	8.67±1.53	8±0.00	46.27±1.87	59±1.02	22±1.73	24.7±1.15	0.34±0.07	0.8±0.14
Devamallige	11.67±0.58	11.3±0.58	76.6±2.74	79.5±4.62	33.83±2.40	36±1.00	0.57±0.05	1.1±0.10
IR20	17±2.00	13±7.21	34.97±1.36	41.8±4.51	38±7.94	36.7±17.79	0.66±0.21	0.6±0.28
Jeeige sanna	8±1.00	7.7±5.39	51.2±1.10	57.6±21.79	17.67±1.53	21±15.18	0.78±0.05	1.5±0.58

Cont/-

Root angle 0^0-45^0		Root no 450-900		Total no of roots		Root length		Root diameter (mm)	
Kharif-14	Summer-14	*Kharif*-14	Summer-14	*Kharif*-14	Summer-14	*Kharif*-14	Summer-14	*Kharif*-14	Summer-14
Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
20.3±0.58	20.33±1.15	35±8.19	36.33±10.02	55.3±8.08	56.67±10.02	36.9±3.16	38.4±2.54	1.4±0.10	1.4±0.10
27±1.00	23.67±6.29	34.7±5.86	60.33±10.25	61.7±5.77	84±16.41	38.7±7.78	41.33±1.74	1.5±0.06	1.47±0.07
16.7±1.53	11.67±2.08	35±7.94	41±5.00	55.3±10.60	52.67±7.02	29.1±5.36	39.95±3.45	1.3±0.00	1.37±0.06
14±2.65	14±5.00	37.3±6.66	54.33±10.97	51.3±5.13	68.33±7.09	33.5±3.10	36.5±1.00	0.9±0.06	1±0.00
17.7±2.52	17.33±2.08	54.3±5.03	54±5.57	72±2.65	71.33±5.13	30.9±6.15	36.8±2.14	1.3±0.06	1.37±0.06
18±1.00	21±1.00	51±3.00	56±7.55	69±3.61	77±8.54	31.1±0.64	41.17±2.52	1.4±0.06	1.43±0.06
15±1.73	16.67±2.52	23.7±0.58	27.67±2.08	38.7±1.53	44.33±4.51	22.5±2.51	31±3.48	0.7±0.00	0.77±0.06
11±2.00	8.33±7.68	23.7±4.51	25.33±17.58	34.7±6.51	33.67±25.20	28.5±2.33	32.5±4.92	0.9±0.00	0.9±0.28

Table 4. Correlation coefficients among the plant characters at 40 days under both aerobic and well watered condition in summer – 2014

Traits	Condition	Tillers number	Shoot length	SR ratio (g g-1)	Root angle 0^0-45^0	Root no 45^0-90^0	Total no of roots	Deep root length	Root diameter (mm)
Tillers number	Aerobic	1	-0.30	0.35	-0.22	0.03	0.00	-0.67	-0.08
	Well-watered	1	-0.43	-0.19	-0.12	0.14	0.10	-0.14	-0.33
Shoot length	Aerobic	-0.01	1	0.33	0.30	0.73^{*}	0.70	0.60	0.71^{*}
	Well-watered	-0.16	1	0.16	0.79^{*}	0.41	0.50	0.26	0.54
Rs ratio (g g-1)	Aerobic	0.30	0.51	1	-0.49	0.43	0.17	-0.40	-0.13
	Well-watered	-0.40	0.52	1	0.03	-0.22	-0.19	-0.39	-0.01
Root angle 0^0-45^0	Aerobic	-0.12	0.48	0.11	1	0.28	0.59	0.71	0.77^{*}
	Well-watered	0.28	0.73^{*}	0.29	1	0.67	0.75^{*}	0.57	0.84^{**}
Root no 45^0-90^0	Aerobic	-0.23	0.59	0.03	0.59	1	0.93^{**}	0.31	0.60
	Well-watered	0.52	0.51	-0.22	0.76^{*}	1	0.99^{**}	0.78^{*}	0.53
Total no of roots	Aerobic	-0.22	0.61	0.06	0.76^{*}	0.97^{**}	1	0.50	0.79^{*}
	Well-watered	0.50	0.56	-0.15	0.82^{*}	0.99^{**}	1	0.79^{*}	0.61
Deep root length	Aerobic	0.02	0.79^{*}	0.28	0.56	0.75^{*}	0.76^{*}	1	0.72^{*}
	Well-watered	0.00	0.52	0.10	0.55	0.65	0.65	1	0.61
Root diameter (mm)	Aerobic	0.04	0.90^{**}	0.41	0.60	0.66	0.70	0.92^{**}	1
	Well-watered	0.23	0.55	0.19	0.80^{*}	0.69	0.72^{*}	0.85^{**}	1

**. Correlation is significant at the 0.01 level (2-tailed).*. Correlation is significant at the 0.05 level (2tailed).Above half diagonal values on the table indicates correlation coefficients in summer -2014, below half diagonal values on the table indicates correlation coefficients in *kharif* - 2014.
Note:

Previously several workers in rice reported an average >30% increased SR ratio under water stress condition (Singh *et al*., 2000). However, Kato *et al*., (2006) in upland rice reported 66% increase in root to shoot ratio with decrease in deep root length.

Apparent conflicts of present study with earlier reports indicate that the effect of water regime on deep root development largely depend on the timing and severity of water stress, growth conditions such as pot vs field, upland vs lowland and genotypes. The effect of increase in SR ratio under water-limiting upland conditions as in this study would differ if the water stress were very severe (Asch *et al*., 2004). On the other hand, SR ratio might decrease under well-watered conditions (Sigari *et al*., 2000)

The present study also showed the Genotype × year interactions for the SR ratio. The genotypes Devamallige, AM65 and BJ21 responding more to water stress than the other genotypes. Similar report of Genotype × year interactions for the SR ratio was documented by Kondo *et al*., (2003) and Kato *et al*., (2006) in rice. On the other hand, increase in number of roots at 0^0-45^0 under aerobic condition indirectly measure increase in deep root length. There are several studies indicating that deep root ratio tend to increase as water stress progress in pot experiments (Asch *et al*., 2004; Sigari *et al*., 2000; Price *et al*., 2002). The genotypes, Azucena and AM65 had highest root angle under aerobic condition in both years indicating that these genotypes are highly drought responsive. Hence, genotypic differences were also important in deep root ratio development rather than mechanical operations. On contradictory, in upland rice, several reports indicate that deep root ratio are only slightly affected by drought (Kondo *et al*., 2003; Bouman *et al*., 2006) and observed shallower root distribution (Kando *et al*., 2003). However, in the penetration of nodal roots in deeper soil layers, lateral root development and distribution at depth also important for deep root development of rice (Banoc *et al*., 2000)

In this study deep root length in deeper soil layers did not increase under aerobic conditions for all the genotypes indicating that the lateral root development in the deeper soil layer was not promoted by water stress. The similar report of decrease in specific root length of deeper roots under water stress compared with that of shallow roots in upland rice was also recognized by Kato *et al*. (2006) in upland rice.

The verdict root distribution of the root system would be determined by nodal root morphology such as nodal root system length and root growth angle (Abe and Morita, 1994). Significance genotypic variation and G × E interaction exists in nodal growth angle (nodal deep root number). Thick roots have been empirically equated with deep roots in rice (Nguyen *et al*., 1997; Yoshida and Hasegawa, 1982). This study showed that genotypes with thicker roots may not only take advantage of deep root development due to longer nodal root lengths (Champoux *et al*., 1995; Ekanayake *et al*., 1985) and higher penetration ability (Babu *et al*., 2001; Cairns *et al*., 2004; Yu *et al*., 1995), but also due to higher nodal root growth angles. Abe and Morita (1994) and Araki *et al*. (2002) showed that thicker nodal roots had higher growth angles (more vertical direction) due to a wider columella with larger amyloplasts as a gravisensing organ, and had more assimilates supplied for nodal root growth after root emergence.

Correlation among different root morphological traits

The number of roots was moderately associated with deep root length under both aerobic and well-watered conditions in both years. This indicates vertical root distribution more constitutively affecting deep root length. Correlation coefficients between total root diameter and deep root length were also high in both aerobic (0.724 and 0.924) and well-watered (0.605 and 0.842) conditions (Table 5) and hence consequently developed greater deep root length. The similar results were also observed by Sigari *et al*. (2000) and Kato *et al*. (2006) in rice.

Conclusion

Among deep root traits, significant genotype and G × E variation exists for number of roots at 0^0-45^0 (number of deep roots) and S-R ratio under both aerobic and well-watered conditions. Among all genotypes, Azucena, Devamallige and AM65 found superior for deep root traits and root length under both aerobic and well watered conditions suggesting that vertical root distribution constitutively affects deep root length. It was suggested that root diameter/thickness was associated with vertical distribution through root growth angles, as well as by root length and penetration ability. Hence, root growth angle will be useful for rough estimations of genotype variation in the vertical root distribution in aerobic rice.

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