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Genetic Relationship and Structural Variation of Root Growth Angle for Deep Rooting in Rice (Oryza sativa L.)

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Authors' contributions

Author RA carried out all the field and laboratory works, statistical analysis and drafted the manuscript. Author AJ coordinated the research work and provided critical comments, time to time review and streamlining the research work and draft correction. Author KS helped in conducting the experiments and recording the observations. Author PB contributed for analysis of statistical data and interpretation of results. Author RS generated the basic material, financial assistance for field and laboratory works, supported for planning and execution of research work and draft correction.

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ABSTRACT

Breeding for drought tolerance revolves around selection of genotypes with desirable root characters. Root pattern studies in rice have shown significant relationship with drought tolerance. In order to understand the effects of deep rooting pattern and root growth angle in relation to water stress was assessed following basket method. The Backcross inbred lines (BILs) of ADT (R) 45*1/Apo and ADT (R) 45*1/Wayrarem with drought QTLs *viz.*, qDTY1.1, qDTY3.1, qDTY4.1 and qDTY12.1 were evaluated for various root traits. Out of 20 BILs and three parental lines studied four BILs showed high values for root growth at deep angle 65-90 °and yield under drought stress. Based on association studies among the root traits positive and significant correlation was observed between yield and root traits *viz.*, root dry weight, root growth at deep angle 65-90° (RA4), ratio of deep rooting and root length. Clustering of BILs and parents have grouped deep rooting BILs and

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drought tolerant donors into one cluster and drought susceptible ADT (R) 45 into a separate cluster II which clearly indicates, the importance of deep rooting and yield under drought stress. Strong association of root traits and drought tolerance clearly shows the importance in utilization of these traits as selection criteria for drought tolerance in rice.

Keywords: Back cross inbred lines; ratio of deep rooting; root growth angle; basket method.

1. INTRODUCTION

Rice (Oryza sativa L.) is the primary food source for more than half of the world population and provides livelihood security for millions of small and marginal farmers in South and South East Asia [1]. According to United Nations Department of Economic and Social Affairs, 2019 [2], present global population is 7.7 billion which is expected to touch 9 billion by 2050, in a changing global climatic order, rice varieties with higher yield potential and greater yield stability need to be developed. Globally water availability is becoming the primary limiting factor for crop productivity [3]. Current rice productions rely on ample water supply which is more vulnerable to drought stress [4] even in traditionally irrigated areas [5]. Drought is a major limitation in obtaining higher productivity from rainfed rice cultivation [6]. Consequently, in a point, water scarcity would translate into food scarcity. Water scarcity for rice cultivation has been serious and widespread, with approximately 18 million hectares of irrigated rice in Asia being projected to suffer from water scarcity by 2025 [7].

Water stress causes severe damage to plant, at different stages of its life cycle, *viz.* vegetative and reproductive stages [8,9], resulting in huge loss of productivity [10]. Plants respond to such external stimuli by various adaptative mechanism *viz.*, better root architecture [11], better osmotic adjustment, higher leaf water potential [12], so as to adapt to the stress environment. The term better root architecture includes deep root systems [13], root length density, seminal root angle [14], which has been shown to be a good proxy to determine the depth of roots in rice [15]. Such developmental response is known as root plasticity and is one of the key traits of plant adaption to various abiotic stresses.

Generally rice plant is a shallow rooting type relative to other cereal crops and hence is sensitive to moisture stress. Deep rooting may assist plants to avoid drought-induced stress by extracting water from deep soil layers [16,17]. The genetic control of deep rooting in rice was confirmed by Uga et al., (2011) [18].

Introducing deep- rooting trait into shallowrooting cultivars is considered one of the most promising breeding strategies to improve drought tolerance in rice. However, incorporating selection for root traits directly in a breeding program has been met with many challenges. Several studies have been reported for different root phenotyping methods including rhizotrons [19,20], soil coring [21], lysimeters [22], hydroponics [23] and rhizoboxes [24]. However, most of these techniques are either expensive or not precise enough and reproducible. Hence, the present study is undertaken to characterize the angle of mature roots in the "basket method" by Oyanagi et al., 1993 [25] with little modification. method highlights the importance of This identifying the inherent mechanism of root system that provide better exploration of soil layers to reach the residual moisture deep in the soil profile toward the end of the season to complete its reproductive cycle successfully.

Therefore, bearing in mind to evolve recombinants with deep rooting and subsequent utilization as donor in the superior background. this research was undertaken to study the root growth morphology and to assess the variation in ratio of deep rooting and root growth angle among two sets of BILs viz., ADT (R) 45*1/Apo with three drought QTLs viz., qDTY1.1, qDTY3.1 and qDTY 4.1 and ADT (R) 45*1/Wayrarem with one drought QTL gDTY12.1 contributing for yield under drought.

2. MATERIALS AND METHODS

The material for the study consisted two sets of BC_1F_4 generation back cross inbred lines (BILs) of ADT (R) 45 which were introgressed with three QTLs *viz.*, qDTY 1.1, qDTY 3.1, qDTY 4.1 from Apo and one QTL qDTY12.1 from Wayrarem for yield under stress (Table 1). A total of 9 BILs obtained from the cross ADT (R) 45 x Apo and 11 BILs from ADT (R) 45 x Wayrarem were utilized to assess the variation in deep rooting pattern and root growth angles by a modified basket method of Oyanagi et al., (1993) (Fig.1). The experiment was carried out in net house in a

randomized block design with two replication at Plant Breeding and Genetics unit of Tamil Nadu Rice Research Institute, Aduthurai during Summer, 2020.

2.1 Modified Basket Method

Open plastic mesh basket with top diameter of 25 cm, bottom diameter of 12 cm, height of 12 cm and slit length 1.5 cm and slit breadth 0.2 cm were taken. Large pots with top diameter of 30 cm and height 30 cm to hold the plastic baskets were selected. The pots were filled with clayey loam soil upto 15 cm height and plastic mesh baskets were placed upon and filled with clayey loam soil upto 10 cm. According to Richard et al., (2001) [26] in both pots and baskets 0 MPa soil compaction was maintained. Three seeds per genotype were placed in the middle of each plastic baskets representing one genotype. At four leaf stage of the seedling, thining was practised by removing two seedlings retaining the healthy plant.

To quantify the number of roots that penetrated the mesh, the sides of the baskets were divided into four groups of angles from horizontal. measured from the centre of the basket's top surface as RA1, RA2, RA3 and RA4 with the angle of 0 - 25°, 25°- 45°, 45° - 65° and 65° -90° respectively (Fig. 2). RA1 and RA2 represented shallow angle RA3 and RA4 represented deep angles of the growing roots. The traits days to 50% flowering, plant height, number of tillers, panicle length, root length, root volume, dry weight of root, total number of roots, ratio of deep rooting, root growth at different angles and single plant yield were measured after imposing stress by withholding water for fifteen days at maximum tillering stage (60 DAS).

The ratio of deep rooting (RDR) is defined as the number of roots that penetrated the lower part of the mesh basket divided by the total number of roots that penetrated the whole mesh basket.

Ratio of deep rooting (RDR) = N/T

N - Number of roots that penetrated the lower part of basket (RA 3 - 45-65° & RA 4 - 65°-90°) T - Total number of roots penetrated the whole mesh basket

Correlation matrix and Clustering of BILs and parental lines based on deep rooting in association with yield was done using statistical software STAR version 2.0.1.

3. RESULTS AND DISCUSSION

Mean performance and analysis of variance for the BILs and parents presented in Tables 2 and revealed that significant and wide 3 range of variability was observed for all the traits studied. Among them the following BILs viz., BIL W 40, BIL W 50, BIL A 127 and BIL A 129 were found to have high and stable yield with an average grain yield ranging from 8.08 to 10.45 g and RA4 (root growth at deep angle 65-90°) ranged between 37- 48.50. The knowledge on trait associations in breeding materials is essential for varied reasons: (i) to perceive the diversity of breeding material (ii) to identify the trait through which a crop is able to grow successfully in a given ecological condition with optimum productivity (iii) to avoid characters that have little or no breeding value; (iv) it also enables us to narrow down to a very few traits that not only account for large amount of variation but have a breeding value correlated with trait of interest. The knowledge of relationship between the trait of interest and other characters is desirable to choose the appropriate selection programme durina breeding. Correlation studies enable the breeder to determine the strength of relationship between various characters as well as the magnitude and direction of changes expected during selection.

3.1 Correlation Studies

The knowledge on understanding the relationship among drought related traits is very much useful while selecting best genotype for crop improvement. Estimation of simple correlation among thirteen traits (Table 4) revealed that correlation coefficient of single plant yield was highly significant and positively correlated with RA4 root angle at 65-90° (r: 0.802) and dry root weight (r: 0.801). The positive and strong association of dry root weight and RA4 with yield revealed the importance of trait in determining deep rooting this pattern to acquire moisture from deep layers during drought situation subsequently increasing yield. The drought adaptation of the rice root studies in the NIL population with a QTL for DEEPER ROOTING 1(DRO1) and IR 64 revealed similar results of association of total number of root and deep root angle towards yield under moisture stress [29]. Positive and significant association with yield was also observed in the traits viz., deep root ratio

SI. No.	Genotypes	Description	Reference
1.	Аро	Upland Indica genotype with qDTY1.1, qDTY2.1, qDTY3.1 and qDTY6.1 controlling grain yield	Venu Prasad et al.,2012 [27]
	·	under stress	
2.	Wayrarem	Major QTL qDTY 12.1 for grain yield under drought and root traits	Bernier <i>et al.,</i> 2009 [6]
3.	ADT (R) 45	High Yielding and popular variety	Priyadharshini et al., 2013 [28]
4.	BIL A 20	qDTY 1.1and 3.1	_
5.	BIL A 37	qDTY 1.1, 3.1 and 4.1	
6.	BIL A 47	qDTY 1.1, 3.1 and 4.1	Venu Prasad et al.,2012 [27]
7.	BIL A 62	qDTY 1.1, 3.1	
8.	BIL A 67	qDTY 1.1, 3.1	
9.	BIL A 68	qDTY 1.1 and 3.1	
10.	BIL A 106	qDTY 1.1, 3.1 and 4.1	
11.	BIL A 127	qDTY 1.1, 3.1 and 4.1	
12.	BIL A 129	qDTY 1.1 and 3.1	
13.	BIL W 4	qDTY 12.1	_
14.	BIL W 8	qDTY 12.1	
15.	BIL W 18	qDTY 12.1	
16.	BIL W 25	qDTY 12.1	Bernier <i>et al.,</i> 2009 [6]
17.	BILW 26	qDTY 12.1	
18.	BILW 37	qDTY 12.1	
19.	BIL W 40	qDTY 12.1	
20.	BILW 50	qDTY 12.1	
21.	BIL W 60	qDTY 12.1	_
22.	BILW 62	qDTY 12.1	_
23.	BIL W 75	qDTY 12.1	

Table 1. Experimental genotypes

(Source: TRRI, Aduthurai)

Source: Genotype 1-IRRI, Phillipines, : Genotype 2 - Indonesian rice variety and Genotype 3-23 TRRI, Aduthurai, Tamil Nadu BIL A - Back Cross Inbred Lines of ADT (R) 45 x Apo BIL W - Back Cross Inbred Lines of ADT (R) 45 x Wayrarem TRRI-Tamil Nadu Rice Research Station, Aduthurai



Fig. 1. Dimensions of basket for root angle measurements by basket method

Table 2. Analysis of	Variance among root	and its component traits
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Source	df	DFF	PH	NT	PL	RL	RV	DRW	RA 2	RA 3	RA 4	TR	RDR	SPY
Replication	1	1.065	0.001	0.087	3.289	0.611	2.174	19.958	0.196	0.087	0.783	0.543	0.001	0.196
Genotypes	22	39.265**	95.881**	1.806**	2.928**	71.995**	71.630**	225.344**	425.749**	158.678**	352.453**	167.737**	0.052**	5.921**
Error	22	1.565	5.176	0.632	1.388	4.354	3.219	6.998	5.105	5.041	5.283	5.816	0.001	0.370

DFF- Days to 50% Flowering, PH-Plant Height, NT-Number of Tillers, PL-Panicle length, RL-Root Length, RV-Root Volume, DRW-Dry Root Weight, , RA1-Root Angle 0-45°, RA2-Root Angle 45-65°, RA3-Root Angle 65-90°, TR- Total number of roots , RDR- Ratio of Deep Rooting and SPY-single plant yield * Significant at 5% level, ** significant at 1 % level







ADT (R) 45 Shallow root angle 0-45°

BIL W 50 Deep root angle 65-90°

Wayrarem Deep root angle 65-90°

Fig. 2. Variation in root angle among BILs and parents

SI. No.	Entry	DFF	PH	NT	PL	RL	RV	DRW	RA2	RA3	RA4	TR	RDR	SPY
1	Аро	87.50	86.50	7.50	18.15	35.55	25.50	40.91	18.50	33.00	45.50	97.00	0.81	8.92
2	Wayrarem	89.00	92.25	5.50	16.00	30.80	26.50	42.99	15.50	38.50	39.00	93.00	0.83	8.22
3	ADT (R)45	79.50	74.85	8.50	18.85	17.25	6.00	10.82	50.00	19.00	11.00	80.00	0.38	3.80
4	BILA 20	83.00	92.40	6.50	16.45	17.25	9.00	19.61	41.00	38.00*	12.50	91.50	0.55	5.12
5	BIL A 37	77.50	83.15	5.50	16.15	16.55	8.00	15.92	43.50	34.00	12.00	89.50	0.51	6.12
6	BIL A 47	89.50	91.90	5.50	19.00	17.80	18.00	36.14	63.00	33.50	8.50	105.00	0.40	6.68
7	BIL A 62	90.50	104.25	6.50	16.55	23.75	13.00	20.16	42.00	34.50	15.00	91.50	0.54	5.25
8	BIL A 67	88.00	102.25	5.00	16.50	23.95	16.50	17.41	38.00	22.50	21.00	81.50	0.53	6.02
9	BIL A 68	91.50	86.90	6.50	18.15	30.55	18.00	21.27	34.00	26.00	32.50	92.50	0.63	5.60
10	BIL A 106	90.00	81.35	5.50	16.00	30.80	28.50	30.19	56.50	34.50	11.00	102.00	0.45	6.27
11	BIL A 127	83.00	103.30	7.50	19.15	30.75	17.50	36.75	15.00	42.00	37.00	94.00	0.84	8.08
12	BIL A 129	89.50	94.25	8.00	19.95	35.25	23.50	45.59	13.50	36.50	48.50	98.50	0.86	10.45
13	BIL W 4	81.50	101.75	5.50	16.15	27.25	16.00	20.29	36.00	36.00	14.50	86.50	0.58	4.31
14	BIL W 8	80.00	94.40	5.00	16.50	21.95	21.00	25.69	47.50	24.00	13.50	85.00	0.44	5.42
15	BIL W 18	82.00	88.00	6.00	17.65	27.40	20.50	32.36	45.00	17.50	27.50	90.00	0.50	7.82
16	BIL W 25	79.50	102.85	6.50	16.50	23.95	19.00	34.58	34.50	27.50	14.00	75.50	0.54	3.88
17	BIL W 26	82.50	89.95	6.00	18.65	27.40	17.00	39.69	26.50	18.00	28.50	73.00	0.64	6.82
18	BIL W 37	80.50	94.35	6.00	16.50	29.95	19.00	42.44	27.00	27.00	25.50	79.50	0.66	8.22
19	BIL W 40	83.00	96.05	6.50	17.40	34.40	25.50	46.73	11.00	42.00	37.00	90.00	0.88	8.41
20	BIL W 50	84.00	85.00	7.50	19.00	35.30	27.50	43.94	12.00	44.50	47.00	103.50	0.88	8.56
21	BILW 60	80.00	97.00	6.00	17.80	21.95	17.50	32.34	25.00	22.50	42.00	89.50	0.72	6.97
22	BIL W 62	91.00	89.00	5.50	16.80	27.40	21.00	30.51	34.00	11.50	27.00	72.50	0.53	5.83
23	BIL W 75	86.00	86.50	6.50	18.00	27.25	17.00	36.02	30.50	27.50	37.00	94.50	0.68	7.46
MEAN		84.72	92.09	6.30	17.47	26.72	18.74	31.40	33.02	29.96	26.39	89.37	0.63	6.70
SED		0.88	1.61	0.56	0.83	1.48	1.27	1.87	1.60	1.59	1.63	1.71	0.02	0.43
CD (5%)		2.59	4.71	1.65	2.44	4.31	3.71	5.48	4.68	4.64	4.76	4.99	0.05	1.26
CD (1%)		3.53	6.41	2.24	3.32	5.88	5.06	7.46	6.37	6.33	6.48	6.80	0.07	1.71
CV		1.48	2.47	12.61	6.74	7.81	9.57	8.42	6.84	7.50	8.71	2.70	3.70	9.07

Table 3. Mean Performance of BILs and Parental Genotypes

Variable	DFF	PH	NT	PL	RL	RV	DRW	RA2	RA3	RA4	TR	RDR	SPY
DFF	1.000	-0.003	-0.043	0.111	0.376*	0.403*	0.145	0.071	0.077	0.204	0.373*	0.104	0.230
PH		1.000	-0.241	-0.207	0.048	0.019	0.103	-0.264	0.201	0.008	-0.173	0.210	-0.043
NT			1.000	0.698**	0.290	-0.073	0.175	-0.387	0.251	0.423*	0.210	0.406*	0.264
PL				1.0000	0.260	0.064	0.359*	-0.268	-0.056	0.507**	0.310	0.351*	0.457*
RL					1.0000	0.807**	0.715**	-0.674	0.327	0.755**	0.263	0.761**	0.696 **
RV						1.0000	0.757**	-0.426	0.260	0.547**	0.358**	0.515**	0.606**
DRW							1.0000	-0.681	0.305	0.689**	0.254*	0.715**	0.801**
RA2								1.0000	-0.379	-0.830	-0.014	-0.936	-0.667
RA3									1.0000	0.208	0.688**	0.537**	0.355*
RA4										1.0000	0.261	0.897**	0.802**
TR											1.000	0.290	0.465**
RDR												1.000	0.758**
SPY													1.000

Table 4. Estimates of Simple correlation coefficient between deep root ratio and component root traits with yield

DFF- Days to 50% Flowering, PH-Plant Height, NT-Number of Tillers, PL-Panicle length, RL-Root Length, DRW-Dry Root Weight, RV-Root Volume, RA2 - Root Angle 0-45°, RA3 - Root Angle 45-65°, RA4 - Root Angle 65-90°, TR- Total number of roots, RDR- Ratio of Deep Rooting and SPY- Single plant yield * Significant at 5% level, ** significant at 1 % level



Fig. 3. Dendrogram using agglomerative clustering method

(r: 0.758) root length (r: 0.696), root volume (r: 0.606), total number of roots (r: 0.465), panicle length (r: 0.457) and RA3 root angle at 45-65° (r: 0.355).

Similar studies on significant correlation between the DRO1 gene and root distribution in tolerant rice varieties with deep root system to avoid drought stress by absorbing more water stored in deep soil layers was reported by [30]. Hence, our results of positive correlation of dry root weight, RA4. RDR. root length and root volume with vield under water stress in rice is accordance with the earlier reports of different QTLs DRO 1, DRO 2, DRO 3, DRO 4 and DRO 5 for root growth angle[31,32,33]. DRO1, a QTL controlling root growth angle by negative regulation of auxin resulting in deep rooting to increase yield in shallow root cultivars [34]. Another promising QTL DRO2 to enhance drought avoidance of shallow rooting rice cultivars by controlling the root surface area was also reported by [32].

3.2 Cluster Analysis

The dendrogram graphed the 20 BILS and 3 parental lines into four clusters (Fig. 3). The four BILs with deep root angle at 65-90° RA4 *viz.*, BIL W 40, BIL W 50, BIL A 127 and BIL A 129 grouped in cluster –I along with the deep root donor parental lines Apo and Wayararem. Two genotypes BIL A 47 and BIL A 106 with more roots at RA3 angle 45-65° and higher total number of root were grouped in same Cluster III. Similar results of genotypes with highest value

for the root traits *viz.*, root volume, fresh root weight and dry root weight in a single cluster and genotypes characterized by drought susceptibility with intermediate and low root volume, root length and bottom root number grouped in another cluster was reported by [35].

The recipient parent and popular irrigated genotype ADT (R) 45 that had been taken as negative check was grouped alone in cluster II with shallow root and low yield under stress. Similar inference of irrigated genotypes IR64 and IR20 forming a distinct cluster from genotypes with QTLs DRO 1 and DRO 2 for root angle was reported by [36]. Other fourteen BILs with moderate yield and maximum rooting pattern at RA2 root angle 0-45°, RA3 root angle 45-65° were grouped in a major same cluster IV.

4. CONCLUSION

Drought is the most serious abiotic stress that hinders rice production globally. Breeding for deep rooting is a promising strategy to improve the root system architecture in shallow-rooting rice cultivars to avoid drought stress. Earlier studies in rice root architecture by adoption of different phenotyping methodologies suggest that one or more key factors controlling the deeper rooting in rice is root growth angle and root distribution. Similar findings in the present study revealed the strong association between root dry weight, RA4, RDR, RL and yield under drought among the promising BILs *viz.*, BIL W 40, BIL W 50, BIL A 127 and BIL A 129. Based on positive association between root traits yield under stress we conclude that selection based on root traits *viz.*, root dry weight RA4, RDR and RL would be ideal for rapid genetic gain towards improving the yield under drought stress in rice.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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