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Physiological Characterization of Rice (*Oryza sativa* L.) Genotypes

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ABSTRACT

Drought is one of the most serious production constraints for world agriculture and is projected to worsen with anticipated climate change. From past few decades many scientists have been trying to understand and dissect the mechanisms of plant tolerance to drought stress using different approaches, but success is not up to the mark. Recently trait based breeding plays a major role in unravelling the problem. Trait based extensive phenotyping is the base for marker assisted breeding. Trait based mapping population was selected along with the parental lines and were screened for root traits. Roots were phenotyped using root structure which mimics the field condition. Based on the results, best recombinant inbred lines were selected which can be considered as trait donor lines.

Keywords: *Drought, Phenotyping, Root structure, Roots traits.*

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for nearly half of the world's population. With rapid population growth in the coming decades, particularly in Asian nations; production of more rice is extremely important (Uga et al., 2013). Several technologies were developed to investigate the possibility of growing rice that reduces the requirement of water. Promising techniques include intermittent irrigation, saturated soil culture and the system of rice intensification (SRI). These techniques however, still use extended flooding periods so that water loss stays high. Therefore essentially the distinct strategy would be to grow rice on non-flooded aerobic soil like an upland crop like wheat to save considerable amount of water. A new strategy known as aerobic approach has shown excellent potential to save water but with a serious yield compromise (Bouman et al., 2000). Thus, it is imminent that rice cultivars must be genetically improved to suit aerobic practice for stabilizing productivity.

Drought is the most important constraint to realize the potential yields of our crops. Moisture stress during the crop growth period, accounts for about 30 to 70 per cent loss in productivity in the country.

Out of the total gross cultivated area of the country, 56 mha is subjected to inadequate and highly variable rainfall. With increasing demand for water, even the irrigated agro-ecosystem might experiences moisture stress. Hence, the emphasis must be to increase the adaptation of crop plants to water limited conditions and to develop crop genotypes that would produce more with less irrigation.

Plants have evolved diverse adaptive strategies to cope with water-limited environments. However, from the agronomic perspective, mechanisms that can sustain growth under water limited environment need greater emphasis. Furthermore, the drought tolerance mechanisms and their relevance at specific target environment depend on intensity, magnitude, and frequency of occurrence. From this context, water mining traits associated with roots, water use efficiency (WUE), water conservation strategies have great relevance.

Breeding for these physiological traits is complex, as these traits have polygenic inheritance and are also difficult to quantify. In this direction, much progress has been made in understanding the genetic basis of complex traits through Quantitative Trait Loci (QTL) mapping strategy. Through traditional bi parental linkage and association mapping approaches, there has been phenomenal success in defining QTLs for several such traits. Successful quantitative trait identification of QTLs relies on the choice of contrasting parents and the level of complex trait segregation in the recombinants.

In the present study, phenotyping of the bi-parental mapping population along with contrasting lines was performed for different drought adaptive traits especially roots traits.

MATERIALS AND METHODS

Plant material:
Kallurundaikar is a traditional medium durated tall variety in Tamil Nadu; bold grains possess high root biomass. IR 64 is a lowland indica cultivar, long slender semi dwarf short duration variety possess low root biomass. RIL population (F8) consisting of 160 lines were obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore.

The recombinant inbred lines along with parental lines were sown in the raised bed nursery during July 2015, kharif season. Twenty five days old seedlings were transplanted in the structures developed to study root parameters with the spacing of 15 cm between plants and 20 cm between rows. On the 70th day after sowing (DAS), the side walls of root structures were dismantled, and then the roots were extracted carefully using a powerful jet of water to wash away the soil from the roots.

Observations recorded in mapping population:
Root length: In the inter-nodal place, the root was separated from each plant. The root length was reported in a graduated scale in centimeters.

Root weight: Roots collected from each plant were oven dried at 80°C for four to five days in a hot air oven and the weight was recorded in grams.

Total dry matter: During the experimental era, the biomass collected was calculated by summing up of leaf, stem dry weights, and root dry weights.

Comparison of means and variances
In individual experiments, mean, range and variances were calculated for the whole set including (parental lines and RIL population). For means comparisons throughout the study were based on Newman-Keuls test (Newman, 1939; & Keuls, 1952) and the homogeneity of variances was tested using SAS / SAST2 ® 9.3 by the Levene (1960) test.

Phenotypic and Genotypic coefficient of variation
The genotypic and phenotypic coefficient of variation were estimated by the formula given by Burton (1953) and expressed in percentage.
Phenotypic coefficient of variation (PCV, %) = (Phenotypic variance) $^{1/2}$/ General mean *100
Genotypic coefficient of variation (GCV, %) = (Genotypic variance) $^{1/2}$/ General mean *100
The estimates of PCV and GCV were categorized on the scale given below (Sivasubramanian & Menon, 1973).

Low   : less than 10 per cent
Moderate   : 11 - 20 per cent
High   : more than 20 per cent

Heritability

Heritability in broad sense was calculated according to Lush (1940) and expressed in percentage.

Heritability in broad sense (h^2) = Genotypic variance (σ^2_g)/ Phenotypic variance (σ^2_p) * 100

The range of heritability was categorized as according to Johnson et al. (1955)

Low   0 - 30 per cent
Medium   31 - 60 per cent
High   61 per cent and above

Genetic advance

The genetic advance was worked out based on the formula given by Johnson et al. (1955).

Genetic advance (GA) = Genotypic variance (σ^2_g)/ Phenotypic variance $((\sigma^2_p)^{1/2})$* K

Where, K = 2.06 (selection differential at 5 per cent selection intensity)

GA as per cent of mean = Genetic advance/ Grand mean*100

The range of GA as per cent of mean was classified into low (1-10 per cent), medium (11-20 per cent) and high (21 per cent and above) as suggested by Johnson et al. (1955).

RESULTS AND DISCUSSION

Phenotypic evaluation of RIL population derived from IR 64 × Kallurundaikar

A set of 160 RILs were assessed in root systems together with their parents (Fig 1). On each line under semi-irrigated condition, phenotypic data on multiple drought adaptive characteristics such as root and WUE and other morpho-physiological characteristics were reported.

Mean performance of the parents (IR 64 and Kallurundaikar) and RILs for the measured traits under aerobic condition in root structures are shown in Table 1.

Roots, the hidden half of plants, facilitate numerous functions including water and nutrient uptake that make it difficult to overlook their importance to plant productivity. The constitutive traits like root depth, thickness and root system architecture have been positively correlated with drought

tolerance by enhanced water harvesting through deep and fast growing root system (Tradieu, 2011).

The parental lines differed significantly for all the traits especially for root traits. Kallurundaikar had greater root traits compared to IR 64 and hence the mapping population developed by using these genotypes would represent excellent segregating lines for mapping. Roots and related traits were significantly varied between the parents where, Kallurundaikar, which is a high root type, showed higher root biomass compared with the low root type IR 64 (Table 1). The average value of RILs for root biomass was 3.13 g pl^{-1} with the range of 0.71 to 9.54 g pl^{-1}. Similar trend was followed for RL, RV in parents with the significant variation between the parents and among the RILs.

Total dry matter showed a significant variability between the parents (Table 1). The mean performance of the RILs for total dry matter ranged from 14.3 to 87.4 g pl^{-1} with the average value of 39.23 g pl^{-1}.

Estimation of coefficient of variation:

The estimation of genotypic coefficient of variation (GCV) for the traits was lesser compared to the phenotypic coefficient variation (PCV) (Table 2), Indicates the existence of substantial environmental (E) impact on physiological character expression.

GCV for the traits of RILs ranged from 3.05% (DFF) to 37.54 % (RW) with a mean of 23.81 % and it was found to be more for drought tolerance trait (RW) followed by R/S, RV and NT (Table 2). Similar trend was observed for PCV also. Results indicate that, these traits might be governed by additive gene action.

Estimation of heritable variations for all traits provides initial options for selection of lines based on phenotypic performance. The heritability estimates ranged from 5.86 to 78.93 % for R/S and DFF respectively (Table 2). Study exhibited higher heritability (>50%) for four traits (RL, RW, RV and DFF), other traits showed less than 50%. High GCV coupled with high heritability is indicative of high genetic gain while selection. Similar

results were observed for RW and RV and NT. It confirms the existence of G X E interaction. The genetic advance as percent mean ranged from 5.59 in DFF to 57.89 in RW. Higher values of h^2 and moderate GA were observed for RL. DFF showed higher h^2. This could be because of complexity of trait and growing environment (soil structure, soil texture, bulk density, nutrient status etc.). RW, RV and NT had higher h^2 and high GA. Over all, traits which were phenotyped under semi irrigated condition provides indicative information regarding GCV, h^2 and GA and these indices will be useful for selection of promising lines suitable for cultivation under aerobic condition.

Test of normality:

All traits measured in RILs, with the exception of RL, failed the normality test (Table 1, Figure 2). Table 2 presents the outcomes of skewness and kurtosis of the 7 traits collected. For all the traits, the kurtosis values were less than three except for R/S, suggesting that the curves of distribution appear to be platykurtic. The distribution with negative excess kurtosis was seen for DFF, while other traits showed positive kurtosis, which indicates the production of fewer and less extreme outliers than does the normal distribution. The population had large number of lines more than the value of parents for root biomass, which can infer that these traits are dominant in nature by having donor line genome or high root parent Kallurundaikar (Figure 2). The kurtosis value of R/S is more than three suggest the distribution curve as leptokurtic. The RILs were negatively skewed for DFF, while other traits were positively skewed showed the distribution pattern of these RILs, indicates which direction and a relative magnitude of how far a distribution deviates from normal. The distribution pattern of the RILs for drought adaptive traits is shown in Figure 2.

Correlation of root traits

Study of correlation of component traits for drought is important for effective selection. In the present study, correlation among traits was analyzed under root structure and most of the traits showed significant correlation.

There were a total of 15 pair-wise combinations of 6 traits, 5 of which were identified to be significant (Fig. 3). Root related traits were correlated with each other except R/S with RL. Root weight showed significant association with RL, RV and R/S. Similarly root length showed positive significant association with RV, RW. This suggests that, most of the water mining traits are positively correlated and demonstrated prospective opportunity to exploit deeper layer water to boost drought yield. RV showed important favorable connection for all correlated characteristics.

Out of 5 significant correlations, single pair (RV with RW) explained the biologically meaningful ($-0.71 \leq$ r $\geq +0.71$) correlation as suggested by Skinner, et al. 1999. A meaningful correlation explains, more than 50% of the variation in one trait can be predicated by the other trait (Snedecor & Cochran, 1980). That is, selection of a specific trait leads to an effective selection of the other correlated trait. However, identification of causative correlation would significantly enhance the effective selection of a relevant dependent trait that can lead to improving drought adoption.

Several surrogates such as SLA and Δ^{13}C are used frequently to assess the variability of WUE. There was, however, no discernible connection between Δ^{13}C and root characteristics. During photosynthesis, plants discriminate against the heavy carbon isotope (^{13}CO$_2$). This discrimination is facilitated by the fractionation of heavy and light isotopes that occur during any biophysical and/or biochemical processes. During diffusion of CO$_2$ and during the carboxylation process ^{13}C is actively excluded to an extent of 4.4 ‰ and 29 ‰, respectively (O'leary, 1981, & Farquhar et al., 1989). These two physiological processes namely stomatal diffusivity and carboxylation capacity of the chloroplast also determine transpiration and photosynthetic rate respectively. Since the same factors determine the differences in WUE and carbon isotope discrimination, a strong relationship between these parameters exists. However, these two

complex physiological processes display significant genetic variability and therefore differential influence on the variation in WUE.

Based on the results obtained from the experiment of root structure especially root biomass, five lines were considered as best promising lines. RIL-286, RIL- 51, RIL-266, RIL-109-2 and RIL-60 are the lines which have better root biomass compared with high

root parent, Kallurundaikar (Fig 4 and Table 3). The mean value of the promising lines were more for root weight and TDM was also high for promising lines comparing with parental lines. These lines can be used as trait donor lines to develop mapping population and can be used in marker assisted breeding program.

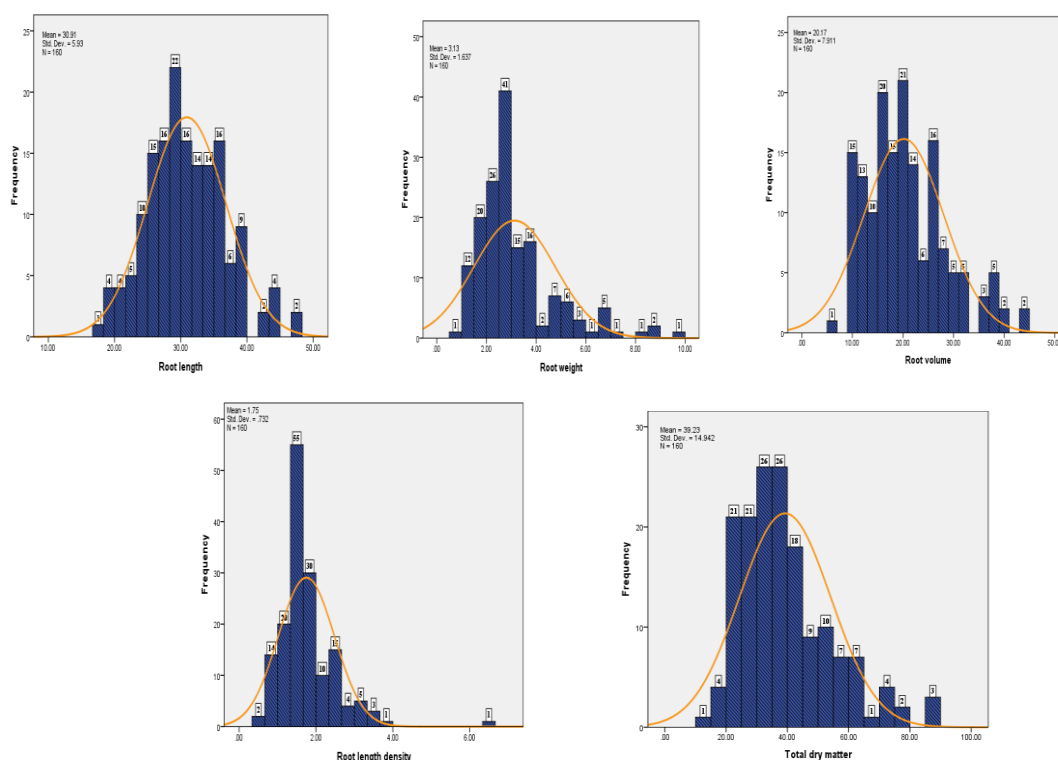**Figure 1: Details of root phenotyping using root structures**

Figure 2: Frequency distribution of RILs derived from IR 64 × Kallurundaikar for roots and other morpho-physiological traits in semi irrigated condition in root structure
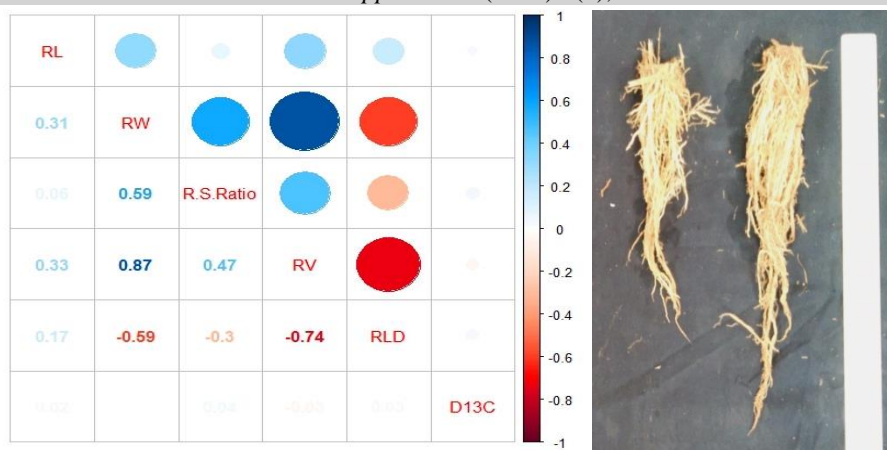
Figure 3: Correlation of root traits of recombinant inbred lines.

RL: Root length (cm), RW: Root weight (g), RV: Root volume (cm^3), RLD: Root length density.
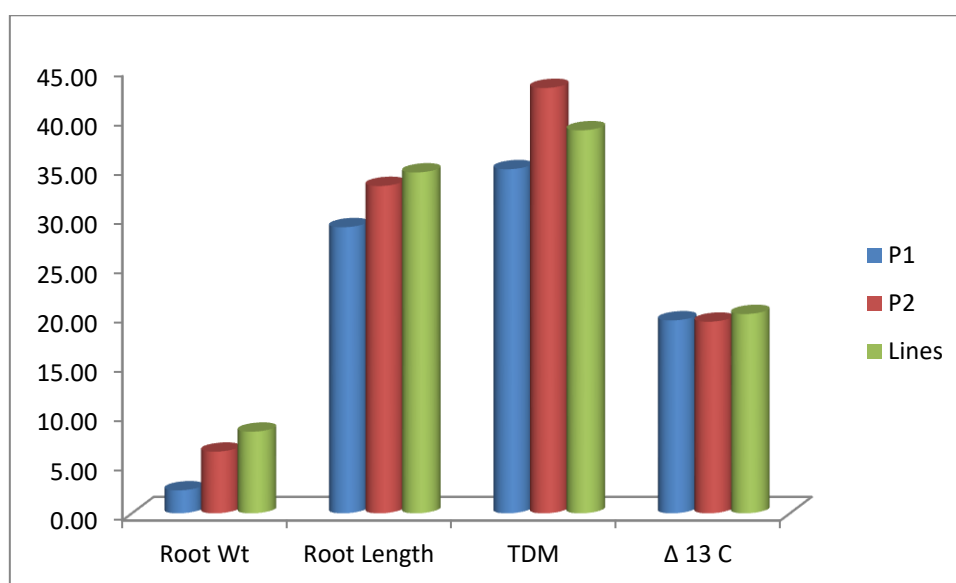

Figure 4: Differences in mean values of best lines in recombinant inbred lines for drought adaptive traits along with parents P1- IR-64 and P2- Kallurundaikar.

Table 1: Performance of parents (IR 64 and Kallurundaikar) and RILs for roots and other morpho-physiological traits under semi irrigated condition in root structure.

Traits	Parents			RILs						
	P1	**P2**		**Range**		**Mean**	**SD**	**SE**	**CD**	**P Value**
	IR 64	**Kallurundaikar**	**T test**	**Min**	**Max**					**T test**
RL	29	33.17	*	18.33	48	30.91	5.93	0.47	8.87	ns
RW	2.34	6.27	**	0.71	9.54	3.13	1.64	0.13	2.57	**
RV	20	35	**	6	44	20	7.89	0.62	11.34	**
R:S Ratio	0.1	0.24	*	0.03	2.25	0.15	0.21	0.02	0.49	**
TDM	34.86	43.07	**	14.33	87.4	39.23	14.94	1.18	28.5	**
NT	20	18	*	3	46	21	8.51	0.67	14.37	**
DFF	104	105	ns	94	109	102.73	3.74	0.3	4	**

* and ** significant P < 0.05, and P < 0.01 respectively. ns: is non- significant. RL: Root length (cm), RW: Root weight (g), RV: Root volume (cm^3), TDM: Total dry matter (g plant $^{-1}$), NT: No. of Tillers and DFF: Days to fifty percent flowering.

Table 2: Estimation of genetic variability in RILs derived from IR 64 × Kallurundaikar for roots and other morpho-physiological traits under semi irrigated condition.

Traits	Mean	Min	Max	GCV (%)	PCV (%)	h^2 (%)	GA	Skewness	Kurtosis
RL	30.91	18.33	48	14.19	18.34	59.85	22.61	0.32	0.05
RW	3.13	0.71	9.54	37.54	50.14	56.04	57.89	1.57	2.83
RV	20	6	44	29.68	37.42	62.93	48.5	0.78	0.27
R:S Ratio	0.15	0.03	2.25	32.28	33.34	5.86	16.1	7.41	6.56
TDM	39.23	14.33	87.4	22.47	37.03	36.81	28.08	1.03	0.98
NT	21	3	46	27.59	39.18	49.62	40.04	0.68	0.58
DFF	102.73	94	109	3.05	3.44	78.93	5.59	-0.65	-0.71

RL: Root length (cm), RW: Root weight (g), RV: Root volume (cm^3), TDM: Total dry matter (g plant $^{-1}$),
NT: No. of Tillers and DFF: Days to fifty percent flowering.

Table 3: Mean values of promising lines can be used as trait donor lines for drought adaptive traits along with parents P$_1$-IR 64 and P$_2$- Kallurundaikar

Line	Root Wt (g)	Root Length (cm)	TDM	Δ 13 C
286	9.54	29.67	33.42	20.552
51	8.96	36.33	32.19	19.341
266	6.96	45	25.19	20.388
109-2	8.86	28	25.19	20.388
60	7.17	33.67	77.96	20.482
Mean	8.3	34.53	38.79	20.23
P1	2.34	29	34.86	19.585
P2	6.27	33.17	43.07	19.451

CONCLUSION

Trait specific mapping population was extensively phenotype for drought adaptive traits. The best recombinant inbred lines were selected specific to root traits and these lines can be used as trait donor line in marker assisted selection. These results suggested that extensive phenotyping of root traits can be done using special structures designed specifically to study root parameters.

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