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Exploiting Genetic Variability for Root Traits in Traits Pyramided Doubled Haploid Rice Lines and Examining The Relevance of Root Traits under Phenomics Facility

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Abstract

Pyramiding of relevant drought adaptive traits on to an agronomically superior cultivar and accelerating the development of stress tolerant lines in a shortest period of time through doubled haploid technology can form as an important step in crop improvement programmes. With the aim of improving the drought adaptation in rice with sustained productivity under water-limited conditions, drought adaptive traits such as root, water use efficiency (WUE) and epicuticular wax were pyramided. Pyramiding of traits was done by crossing the trait introgressed line (TIL-14) which has root and WUE traits with epicuticular wax genotype (AC35310) and accelerating the breeding cycle through doubled haploid technology. In the present study, the developed doubled rice lines were phenotyped for pyramided traits and more specifically, the root traits both in root study structures and in pots. Accordingly, a significant variability in root traits such as root length, root volume and root weight were observed among the DH lines in both the experiments to suggest that, the DH lines are diverse for root traits. Although each of the DH lines are homozygous and homogenous within but, throws out a large variation across the DH lines. Further, the root trait was found to be stable across the seasons and growing conditions as the Spearman's correlation coefficient analysis showed a strong correlation between the experiments to suggest that the root trait is more stable and can be a useful trait in crop improvement programmes. Based on the diversity, the contrasting DH lines differing in root traits was identified and selected. Further, when the relevance of root traits was examined under phenomics facility with the imposed moisture stress, the high root type DH lines performed well over the low root type DH lines suggesting the importance of root traits under water limited conditions.

Keywords : Doubled haploids, Drought adoptive traits, Traits pyramiding, Phenotyping, Root trait, Phenomics facility

Rice is the most important staple food crop feeding more than half of the world's population. However, its production is progressively being limited by several environmental stresses with about 30 per cent of the area affected in rainfed lowlands of Asia alone (Dar *et al.,* 2014). Among the various environmental stresses, drought stress serves as the serious constraint for rice production and it has been estimated that, more than 50 per cent of the global rice area is affected by drought (Bouman *et al.,* 2005 and Fukao *et al.,* 2011). Drought stress during booting, flowering and terminal stages can interrupt floret initiation, cause spikelet sterility resulting in lower grain weight and hence, poor grain yield (Kamoshita *et al.,* 2004; Sheshshayee *et al.*, 2018 and Dharmappa *et al.*, 2019). The extent of grain yield loss depends on the intensity of the stress, the crop growth stage and the duration of water scarcity (Kumar *et al.,* 2014). The varieties exhibiting drought tolerance along with better grain yield and desirable quality can serve as the most logical approach to cope up with the menaces inflicted by drought (Singh *et al*., 2013).

Drought tolerance is a highly complex process regulated by many drought adaptive traits. Among the several drought adaptive traits, water acquisition through roots, WUE, cellular level tolerance (CLT) and water conservation associated with epicuticular wax load found to have relevance under water limited conditions (Sheshshayee *et al*., 2013; Raju *et al*., 2016). Introgression of these traits on to agronomically superior genotypes appears to improve the yield under aerobic conditions (Sheshshayee e*t al*., 2013; Pooja and Sheshshayee, 2017; Sheshshayee *et al*., 2018 and Dharmappa *et al*., 2019). Therefore, the major goal of this investigation was to pyramid the drought adaptive traits onto a single elite genetic background with an objective of improving the drought tolerance of rice and further validating the pyramided traits for their relevance. Pyramiding of traits through conventional breeding takes a very long time while, the doubled haploid (DH) technology accelerates the breeding cycle and therefore, fix the traits of interest in a short time (Herawati *et al.*, 2010). *In vitro* androgenesis is an attractive biotechnological technique that provides rapid transformation of heterozygous hybrids into homozygous lines and thus, significantly reducing the duration of breeding cycle (Debina *et al*., 2016 & 2019; Ghalagi & Mohan Raju, 2022 and Kavita *et al.*, 2022).

In the present study, the relevant drought adaptive traits were pyramided and the doubled haploid rice lines were developed through anther culture. Here, one of the best TIL's (Trait introgressed line-14) which is having good root and high WUE traits (Dharmappa *et al.,* 2019) was pyramided with one more drought adaptive trait such as epicuticular waxes to improve the drought adaptation of rice cultivar further (Kavita *et al*., 2022). Among the three drought adoptive traits pyramided, root trait seems to be the most important trait for drought adaptation as it facilitates the plants to mine water from a larger soil volume (Sheshshayee *et al*., 2011; Raju *et al.,* 2014 and Koevoets *et al*., 2016). Deeper root system is highly relevant for mining water from a deeper soil profile to meet the transpirational demand and hence, helps to maintain tissue water status especially under water limited environments. Therefore, the main objective of this study was to capture the genetic variability for root traits and to examine the stability of the traits in the doubled haploid rice lines with an overall goal of using the most stable lines for further crop improvement programmes. The other objective of the study was to examine the relevance of root traits under water limited condition.

MATERIAL AND METHODS

Development of Plant Material

Previously, with an objective of pyramiding the relevant drought adaptive traits together, one of the traits introgressed rice lines (TIL-14) having root and water use efficiency (WUE) traits was crossed with a epicuticular wax trait donor line (AC35310) and produced the F1 seeds. From these F1 seeds, plants were raised and anthers were collected and cultured in vitro to develop haploids and doubled haploids (Kavita *et al*., 2022). The doubled haploids so generated were phenotyped for pyramided traits and more specifically, the root traits. In order to study the stability of the root traits over seasons and growing conditions, besides phenotyping the developed DH lines in root study structures, they were also phenotyped in pots.

Details on Root Study Structure Establishment and Phenotyping for Root Traits

The traits pyramided doubled haploid rice lines were phenotyped for root traits in root study structures constructed at the experimental field, Department of Crop Physiology, University of Agricultural Sciences, Bangalore, India. The root study structure (150 cm x 300 cm x 1800 cm) was built above ground using solid cement blocks. After curing of structure, it was filled with fertile top soil and compacted sufficient enough to mimic the real field conditions. Later, 21 days old nursery grown seedlings of all the DH lines of rice with their parents and F1 including check varieties were transplanted onto the root study structure in an augmented block design with a spacing of 15 × 20 cm as per the package of practice as recommended for land grown plants. Regular watering was given almost on a daily basis and other agronomic practices including fertilizers applications, weeding and other operations were carried at the appropriate time. At flowering stage (110 DAS), the side walls of the root study structures were dismantled and the plants with their roots were extracted carefully with a jet of water to wash away the adhered soil from the roots. Roots were separated from the shoot and several root traits such as root length, root volume and root dry weight were recorded. While the root length was measured with a simple graduated scale and expressed in centimeters (cm), the root volume was determined by water displacement method and expressed in cubic centimeter (cc). Further, the roots were air dried and placed in hot air oven (70^{o}C) for 48 hrs and root dry weight was measured and expressed in grams (g).

Pot Experiment to Phenotype the DH Lines for Root Traits

Pots of uniform size were taken and filled with a known amount of potting mixture (10 kg) and was brought to 100 per cent field capacity by adding known amount of water to each of the pots. Later, 21 days old seedlings of each of the DH lines and the checks were planted separately. For each DH line, 5 pots were maintained. At the time of anthesis, the plants were harvested and the root parameters were recorded. The important root parameters such as root length, root volume and root dry weight were measured as explained above.

Spearman's rank correlation: Any breeding material which can be taken forward in the breeding programme requires higher heritability with low G×E interaction. In other words, the traits must be stable across seasons, locations, growing conditions and over generations. In this context, in order to examine how strongly the root traits are correlating, either between the experiments or between seasons, the data of both the experiments were subjected for Spearman's rank correlation analysis. Accordingly, to check the correlation for the root trait, the root trait data (root dry weight) of all the DH lines of rice in root study structure experiment was compared and correlated with the root data of pot experiment. The root data (root dry weight) of individual DH lines from both the experiments were ranked from those with highest root dry weight (Rank 1) to the lowest root dry weight (Last rank).

$$r_s = 1 - \frac{6\sum d_i^2}{n\,(n^2-1)}$$

Spearman's rank correlation measures association between two variables. This correlation values ranges from -1 to 1 ($-1 \text{ d} \leq r_s \text{d} \leq +1$) with values near 1 indicating similarity in ranks for the two variables and values near -1 indicating ranks are dissimilar for the two variables. Spearman's correlation coefficient is a statistical measure of the strength of a relationship between the paired data. The Spearman's rank correlation was worked out as per the formula given below.

Where n = Number of samples

d = Different between ranks

d^2 = Different squared

Examining the Relevance of Root Traits under Phenomics Facility

Root has been shown to be an important organ of the plant wherein, the amount of water absorption and mineral acquisition depends on how effectively root functions. Plants with bigger and deeper root system has been shown to mine water from a deeper layer of the soil and hence, survive and yield better under water limited conditions (Sheshshayee *et al*., 2011., Dharmappa *et al*., 2019). Therefore, in the present

Plate 1 : Phenotyping of contrasting DH lines of rice differing in root traits under naturally ventilated phenomics facility

study, the relevance of root trait was examined by taking the contrasting DH lines differing in root traits. Here, 2 DH lines of rice with high root and 2 lines with low root were selected and grown them in phenomics facility (Plate 1). The phenomics facility established at UAS, Bangalore has a unique option to impose and regulate the precise moisture stress at any developmental stages of the crop growth. Using this facility, the contrasting DH lines were imposed with moisture stress (40% moisture stress) for 15 days at reproductive stage and examined the stress effect on growth and yield of DH rice.

Statistical Analysis

The data generated in all the experiments mentioned above were subjected for statistical analysis. For root study structure experiment, augmented block design was followed and data were analyzed by using INDOSTAT software. Stability of the root traits over the two experiment was analyzed (Genotype and environment interaction) by Spearman's rank correlation. However, for examining the relevance of root traits, student's 't" test was adopted.

Results and Discussion

In recent years, a few relevant component traits that are essential for improving the drought adaptation have been enumerated and deciphered (Vadez *et al*., 2013). For a comprehensive improvement in saving water and sustaining productivity, relevant drought adaptive traits need to be introgressed on to a single elite genetic background (Raju *et al*., 2014; Sheshshayee *et al*., 2018 and Dharmappa *et al.,* 2019). Therefore, the study was majorly focused on pyramiding drought adoptive traits such as root, WUE and epicuticular wax to improve the stress tolerance of rice. To pyramid the traits of interest, one of the traits introgressed rice lines (TIL-14; with IR-64 background) for 'root and water use efficiency' was crossed with a 'epicuticular wax' trait donor line (AC35310). The resultant F_1 plants were used as anther donor line for the development of doubled haploids. The developed DH lines were characterized for root traits under root study structure and in pots.

Phenotyping of Root Parameters of the Doubled Haploid Lines in the Root Study Structure

Roots play a vital role in resource acquisition and plant growth regulation by being the primary interface for water and nutrient capture (Sheshshayee *et al*., 2011). In addition, roots also provide anchorage and interact with symbiotic organisms in the soil (Rebouillat *et al*., 2009). Regardless of the ecosystem where rice breeding is aimed at, researchers look forward understanding the role of roots for improving nutrient and water acquisition and increasing grain yield. In fact, various screening methods are used to identify root traits associated with drought tolerance in rice germplasm. Type of root system is a good selection criterion for selecting the drought tolerant lines or varieties. Drought stress caused pronounced changes in root structure such as increased branching and density. Here, DH lines showed significant diversity for root traits (Table 1).

The DH line, DH 52 was found to have the longest root (39.0 cm) followed by DH 33A (36.5 cm). The lowest root length was observed in DH 26B (26.5 cm). Root volume was highest in DH 33A (96.25 cc) followed by F1 hybrid (87.5 cc) and DH 30.1 (78.33 cc) and the lowest root volume was found in DH 26B (10.0 cc). Further, the root dry weight was significantly high in F1 hybrid (13.14 g) followed by DH 33A (13.08 g) and DH 30.1 (12.5 g) (Table 1). These results indicate that, the pyramided root trait has been passed onto the next generation and the DH lines developed from anthers of traits pyramided rice indeed thrown out lot of variability with some of them having root traits similar to F1 hybrid (having root, WUE and epicuticular wax traits) and better than the parent (TIL 14).

Plant height was observed to be more in AC 39020 (96.9 cm) followed by DH 47 (96.3 cm) and lowest in DH 26B (41.5 cm). Similarly, tiller number was more in DH 33A and lowest in DH 26B. These results showed a significant variability for several parameters associated with plant growth to indicate that the doubled haploid lines are different from each other (Table 1). Such variations for several parameters/ traits

TABLE 1

Variations in root traits among DH lines of rice assessing under root study structure

DH lines	Plant height (cm)	Tiller number	Root length (cm)	Root volume (cc)	Root dry weight (g)
DH1C	63.50	15.75	27.25	41.25	6.55
DH2A	52.00	15.67	22.00	36.67	7.80
DH3	49.50	17.50	21.25	52.50	8.23
DH7C	72.00	11.50	29.25	43.75	7.48
DH8C	88.50	12.50	25.00	31.25	4.75
DH11	63.88	27.50	29.63	66.25	8.85
DH13.2	82.88	12.50	29.88	47.50	7.06
DH14	81.63	11.50	36.50	42.50	6.30
DH14A	72.00	18.50	26.00	47.50	6.55
DH15A	89.00	16.33	27.83	36.67	6.77
DH16	83.25	18.00	26.88	50.00	8.33
DH16A	75.00	15.00	25.50	43.75	7.10
DH18	86.00	14.67	29.00	73.33	9.17
DH18B	68.67	13.00	28.67	36.67	5.70
DH21	51.20	16.40	27.00	48.00	8.14
DH22	75.25	18.00	27.88	70.00	8.25
DH26B	41.50	10.50	18.50	10.00	4.82
DH29	70.25	20.75	24.75	45.00	6.00
DH30A	74.33	19.33	30.33	50.00	8.47
DH30.1	75.67	16.33	29.00	78.33	12.47
DH31B	74.33	15.67	30.00	48.33	6.53
DH33A	78.75	28.25	35.75	96.25	13.08
DH34	83.80	15.20	25.00	49.00	8.22
DH35A	52.20	17.40	22.20	47.00	7.48
DH39	74.00	24.20	29.00	66.00	8.80
DH39B	70.60	14.60	20.69	38.00	6.25
DH40A	86.60	13.20	27.00	41.00	5.46
DH41	72.80	18.20	31.60	68.00	10.79
DH47	96.25	12.25	23.75	47.50	8.68
DH52	69.25	18.00	39.00	62.50	8.98
DH56B	71.50	16.00	25.63	45.00	5.80
DH70B	69.00	11.67	25.33	38.33	6.10
DH79	76.00	14.50	27.00	45.00	8.70
TIL14	70.43	13.64	31.32	61.70	9.27
AC35310	81.42	13.38	25.73	40.04	6.68
F1	82.25	24.00	31.75	87.50	13.14
IR64	66.39	16.70	26.96	42.33	4.95
IET16348	71.55	18.47	25.67	47.33	6.67
AC39020	96.90	7.70	24.84	87.00	10.46
Mean	73.33	16.26	27.44	51.51	7.82
CD@5%	8.33	4.09	12.19	26.24	5.73
CV (%)	3.39	8.05	13.63	15.11	22.05

TABLE 2

Variations in root traits among DH lines of rice assessing under pot experiment

DH lines	Plant height (cm)	Tiller number	Root length (cm)	Root volume (cc)	Root dry weight (g)
DH1C	36.13	9.75	26.75	23.75	2.04
DH2A	39.06	10.25	37.50	46.25	6.49
DH3	41.25	9.00	28.50	56.25	5.52
DH7C	46.50	10.00	34.63	67.50	6.35
DH8C	45.50	9.25	36.00	40.00	2.62
DH11A	37.53	6.63	23.94	37.50	7.80
DH13.2	38.50	7.50	28.75	32.50	3.21
DH14	34.13	8.75	31.63	56.25	5.49
DH14A	41.75	10.50	36.00	65.00	6.97
DH15A	39.25	8.25	33.00	56.25	6.37
DH16	44.13	7.25	33.00	35.00	3.15
DH16A	33.25	8.25	38.38	56.25	6.74
DH18	31.81	9.25	26.81	20.00	2.07
DH18B	45.00	8.25	27.38	21.88	1.86
DH21	31.63	7.75	39.25	38.75	3.27
DH22	39.56	7.50	31.25	32.50	2.34
DH26B	23.50	7.75	24.25	50.00	4.21
DH29	31.13	12.25	31.69	51.25	4.61
DH30A	35.63	9.00	30.38	42.50	4.72
DH30.1	40.00	9.25	33.75	55.00	6.80
DH31B	31.56	7.50	31.38	42.50	4.58
DH33A	41.75	9.00	37.75	72.50	8.62
DH34	49.25	11.00	33.25	50.00	5.17
DH35A	29.38	5.25	27.75	25.00	1.56
DH39	46.38	8.25	23.63	42.50	4.07
DH39B	31.38	10.50	32.50	27.50	2.64
DH40A	40.50	8.00	36.75	32.50	2.41
DH41	43.88	10.75	39.75	41.25	5.42
DH47	30.97	7.25	31.19	39.38	4.85
DH52	29.00	7.50	36.88	57.50	5.20
DH56B	32.94	9.75	41.00	22.50	2.54
DH70B	39.00	7.25	30.25	32.50	2.19
DH79	33.50	7.75	39.38	62.50	5.60
TIL14	32.50	11.25	43.38	62.50	7.36
AC35310	31.13	10.00	37.13	41.25	3.05
F1	42.00	9.50	36.25	53.75	6.65
IR64	33.50	6.25	26.25	15.00	1.20
IET16348	32.13	9.75	29.50	40.00	3.62
AC39020	37.56	4.00	28.00	50.00	8.99
Mean	37.01	8.64	33.20	43.46	4.27
CD @ 5%	2.23	1.32	1.97	5.34	0.39
CV (%)	4.31	11.03	4.23	8.78	6.45

have been reported by a large number of workers in different crop species and more specifically has been reported among the DH lines (Naik *et al.,* 2016; Chaikam *et al*., 2019; Debina, 2019; Debina *et al*., 2019; Roopa, 2019 and Harif, 2020).

Phenotyping for Root Traits in the Doubled Haploid Rice Lines in Pots

Here also, DH lines showed wide variations for root traits. In pot experiment, root length was more in DH 56B while, lower root length was found in DH 39. Root volume was more in DH 33A and lower in DH 18. Further, root dry weight was more in DH 33A and lower in DH 35A (Table 2). Being completely homozygous, the DH lines having higher root dry weight can directly serve as trait donor lines in hybridization programmes or in any other crop improvement programmes. Such genotypes for root traits have been identified in rice (Sumanth Kumar, 2014), sunflower (Shashidhara, 2009), mulberry (Sudhakar, 2005), finger millet (Mohankumar *et al*., 2012). High root type DH lines could extract water from deeper layer and can sustain water requirement for transpiration of plants and thereby, enhancing biomass production (Li *et al*., 2005 and Sheshshayee *et al*., 2011). These studies emphasize the relevance of breeding to improve root traits to achieve better productivity under water limited condition (Reynolds and Tuberosa, 2008). Deep root system is considered to be a good character for improving stress tolerance as it facilitates mining of water from deeper profile of the soil (Fageria *et al*., 2013; Uga *et al*., 2013; Chen *et al*., 2015). Among the root traits, the root biomass is often used as a direct indicator to predict rice yield under stress (Fageria and Moreira, 2011). Further, it was also shown that the deep rooted plants are productive under water deficit conditions than the shallow rooted plants (Reynolds & Tuberosa 2008 and Dharmappa *et al*., 2019).

Correlation studies for root traits: The correlation was drawn between root dry weight and total dry matter and accordingly, a significant positive correlation

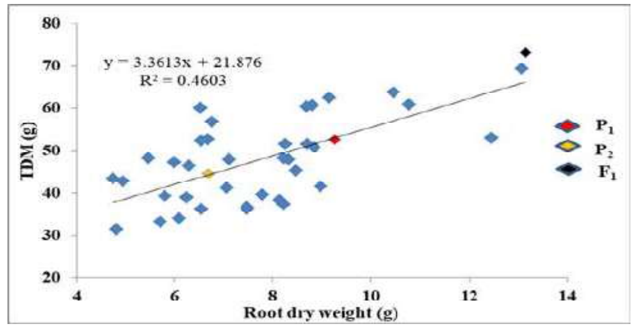

Fig. 1 : Relationship between root dry weight (g) and TDM in DH lines of rice
P_1:TIL14, P_2:AC35310, F_1: TIL14 x AC35310

was observed between root dry weight and biomass (Fig. 1) suggesting the relevance/ importance of root traits in productivity. Similarly, the root dry weight under root study structure experiment found to exhibit a significant and positive correlation with root dry weight of pot experiment with a r^2 value of 0.3611 suggesting that the root trait is stable across the experiments and seasons (Fig. 2). Therefore, if the crop productivity

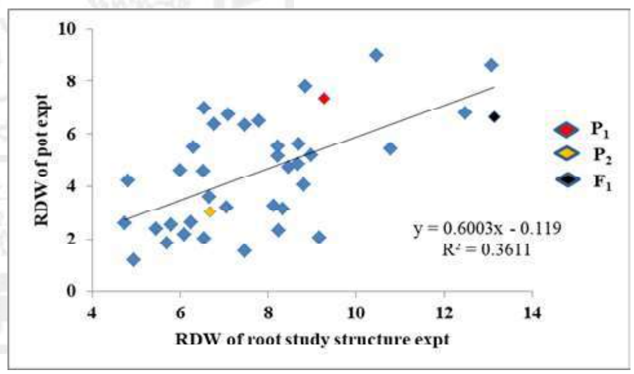

Fig. 2 : Relationship between root dry weight (g/plant) of root study structure and pot experiment
P_1:TIL14, P_2:AC35310, F_1: TIL14 x AC35310

has to be improved under stress, one has to improve the root traits so that, the biomass and yield could be increased. These correlations studies therefore, suggest the importance of root traits on productivity in DH rice. Such similar correlations were observed by Harif (2020) while working with doubled haploid rice lines for root traits. Among the various root related traits, the root biomass is often used as a direct evidence to predict rice yield under drought stress condition (Fageria and Moreira, 2011).

TABLE 3

Spearman's rank correlation analysis to assess the extent of correlation for root trait between two experiments and season

DH lines	Root dry weight (g)					
	Root study structure experiment	Rank	Pot experiment	Rank	d	d^2
DH1C	6.55	26	2.04	37	-11	121
DH2A	7.80	19	6.49	8	11	121
DH3	8.23	16	5.52	12	4	16
DH7C	7.48	20	5.35	14	6	36
DH8C	4.75	39	2.62	31	8	64
DH11	8.85	9	4.56	22	-13	169
DH13.2	7.06	22	3.21	27	-5	25
DH14	6.30	29	5.49	13	16	256
DH14A	6.55	26	6.97	5	21	441
DH15A	6.77	23	6.37	9	14	196
DH16	7.10	21	3.15	28	-7	49
DH16A	8.33	14	6.74	7	7	49
DH18	5.70	34	2.07	36	-2	4
DH18B	8.98	8	5.20	15	-7	49
DH21	8.14	18	3.27	26	-8	64
DH22	8.25	15	2.34	34	-19	361
DH26B	4.82	38	4.21	23	15	225
DH29	6.00	32	4.61	20	12	144
DH30A	8.47	13	4.72	19	-6	36
DH30.1	12.47	3	7.80	2	1	1
DH31B	6.53	28	4.58	21	7	49
DH33A	13.08	2	8.62	1	1	1
DH34	8.22	17	5.17	17	0	0
DH35A	5.30	36	1.56	38	-2	4
DH39	8.80	10	4.07	24	-14	196
DH39B	6.25	30	2.64	30	0	0
DH40A	5.46	35	2.41	33	2	2
DH41	10.79	4	6.80	6	-2	2
DH47	8.68	12	4.85	18	-6	36
DH52	9.17	7	5.20	15	-8	64
DH56B	5.80	33	2.54	32	1	1
DH70B	6.10	31	2.19	35	-4	16
DH79	8.70	11	5.60	11	0	0
TIL14	9.27	6	7.36	4	2	4
AC35310	6.68	24	3.05	29	-5	25
F1	13.14	1	7.65	3	-2	4
IR64	4.95	37	1.20	39	-2	4
IET16348	6.67	25	3.62	25	0	0
AC39020	10.46	5	6.00	10	-5	25
					∑d²	**2860**
					r	**0.71**

Assessing the Traits Rank Value of DH Rice Lines through Spearman's Rank Correlation

Spearman's correlation measures the linear correlation between two sets of data. Therefore, Spearman's rank correlation analysis was adopted to examine whether two data sets are correlating with each other or not. Accordingly, the root trait data of root study structure experiment when correlated with pot experiment; a strong positive correlation was observed with an r value of 0.71 (Table 3) suggesting that the root trait is also stable over seasons and growth conditions. The stability of root traits was also noticed by others in different crops (Naik *et al*., 2016 and Debina *et al*., 2019) to suggest that these traits are more stable and can be used in breeding programmes.

Relevance of Root Traits under Water Limited Condition

Upon examining the relevance of root traits under phenomics facility with precise imposition of moisture stress during reproductive stage, it was observed that, both high and low root type DH lines did not show any significant difference in plant height, tiller number, total leaf area and even total dry matter not only between themselves but even between the treatment (Fig. 3 & 4). Although expected to have difference between the treatments, it was not observed in the present study as the moisture stress was imposed during reproductive phase and by which time, the plants have put on the vegetative growth and therefore, significant difference was not seen for many of the growth parametrs. However, a significant difference in root length, root dry weight and grain yield was observed between high and low root type DH lines (Fig. 3 & 4). Interestingly, under stress condition, the low root type DH lines yielded significantly low compared to high root type DH lines. Further, low root type DH lines although could yield almost similar to that of high root type DH lines under controlled condition, they performed dismally low under stress condition (Fig. 4). This reiterates the importance of root traits under water limited conditions. In fact, the present results corroborate with that of earlier studies where they showed sustained productivity in high root genotypes/ varieties under moisture stress conditions compared to low root type genotypes (Sheshshayee *et al.,* 2011 and 2018; Dharmappa *et al.,* 2019). The study further confirms the importance of root traits for sustained productivity under water limited conditions.

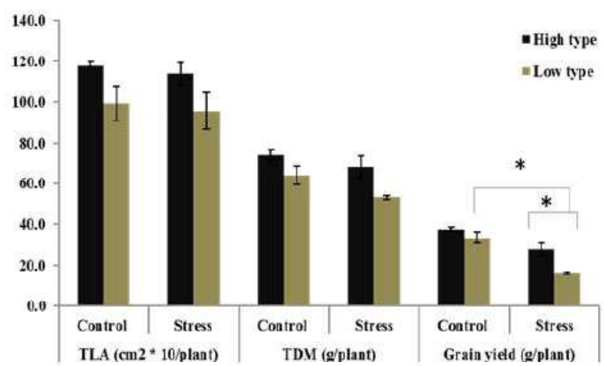

Fig. 4: Effect of moisture stress on total leaf area, TDM and grain yield in high and low root type DH lines

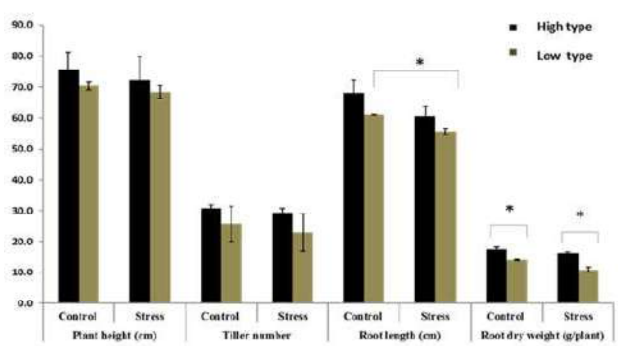

Fig. 3: Effect of moisture stress on root traits and other growth parameters in high and low root type DH lines

Improved stress tolerance coupled with sustained productivity can be best achieved by pyramiding relevant drought adaptive traits. In this regard, the doubled haploid rice lines derived from F1 plants of a cross between root and WUE trait donor line with epicuticular wax genotype were phenotyped for root traits and found significant variability among the DH lines. Further, the root trait was found to be stable across seasons and growing conditions suggesting that the root trait is a breedable trait and can be used in crop improvement programmes. In addition, root trait was found to be highly relevant under water limited condition as the high root type DH lines sustained productivity while, the low root types did not. Finally, the DH technology followed in the present study, serves as an important tool in fixing the traits of interest and generating completely homozygous lines that are stable across seasons, environments and over generations.

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