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Screening of root related above ground traits for early reproductive drought stress in rice genotypes

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Abstract

The present work was carried out in fourteen rice genotypes of same maturity group and showing partial tolerance to drought stress at early flowering stage. Besides, variations in root morphological traits, genotypic specificity cannot be underestimated. RL (root length) got increased in all the genotypes under drought stress as compared to control. RDM (root dry matter), root:shoot ratio, SRL (specific root length), RMF (root mass fraction) determined genotypic specificity in the response towards drought. Root RWC (Relative water content) of genotypes BRR-0028, BRR-0026 and Sabour Ardhjal showed maximum with 88%, 87% and 86% respectively. Moreover, the RWC percent is greater in leaves from that of root revealing either translocation capacity of water from roots to shoots or alteration of leaf stomatal conductance during drought situation. This had been encouraged by higher accumulation of proline content in the genotypes with potentials of osmotic adjustment whereby genotypes BRR-0028 was found to be maximum with 54.32 μ g g⁻¹dry wt. Drought induced in decreasing photosynthetic rate and stomatal conductance thereby increasing the rate of transpiration. However, the results also lighted on better performances of genotypes in drought than that of control. Correlation study showed the root traits had command over physiological parameters of above ground part of the plant. For instance, strong positive correlation of RL and SRL with stomatal conductance at p < 0.01 whereby further interrelating with shoot length, and then to photosynthesis rate and transpiration rate at p < 0.05. The root mass study showed a non significant negative correlation with photosynthesis rate and stomatal conductance which probably an indication of increasing availability of assimilates for aboveground parts especially leaves during early reproductive phase drought. Findings were the evidence for root tempering changes in above ground shoot physiological traits under drought.

Keywords: Root, shoot ratio, root dry mass, photosynthetic rate, transpiration rate, stomatal conductance

Introduction

Root systems are of decisive importance for uptake of nutrients and water thus playing an important role in the development of aboveground organs and yield formation ^[1-2]. Root affects the behaviour and growth of the whole plant especially where resistance to extreme stress are concerned. They also participate in the whole vegetative development effecting the growth and whole plant metabolism.

Among various abiotic stresses drought is considered as one of the most important factors for restricting crop production upto 50% ^[3-4] due to significant reduction in plant growth and development ^[5]. Drought tolerance is one of the most challenging facts due to the lack of fast, reproducible screening techniques and inability to routinely create defined and repeatable water stress conditions where a large amount of genotypes can be evaluated efficiently ^[6]. Plants usually change the distribution of their roots and grow them deeper to absorb water and minerals as a mechanism of drought tolerance ^[7-8]. However, drought stress generally reduces the ability of roots to absorb water and nutrients from the soil and it has been demonstrated that plants with vigorous and extensive root systems can only develop the ability to cope with drought and become water deficit-tolerant ^[7-9]. Franco and co-worker ^[10] also reported that root growth was usually less affected by drought stress than shoot growth. Thus, any alteration in root traits could be expected to change in the above ground traits especially during stress conditions. A common observation on decrease in shoot: root ratio under drought-stress may possibly results either from an increase in root growth or from a relatively larger decrease in shoot growth than in root growth.

Based on this hypothesis several components of root morphology contributing to drought tolerance have been identified ^[11]. Amongst which maximum root length and root dry weight were taken as good indicators of drought avoidance in upland rice. The previous literature further supported that root characters are considered to be a vital component of dehydration

Corresponding Author: Sareeta Nahakpam Department of Plant Physiology and Biochemistry, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India postponement mechanism since they contribute to regulation of plant growth and extraction of water and nutrients from deeper layers ^[12]. Hormonal regulation of root to shoot signalling pathways as sense and response of roots towards abiotic and biotic stresses, which was rather regulated by hormones like ABA (abscisic acid), ethylene and auxin were further extrapolated in drought tolerant rice ^[13-14].

Although, the root system has long been noticed and studied, progress remains slow and limited as compared with aboveground organs due to a massive volume of work and limitations of research methods. As discussed above many researchers reported that roots are a major sink for assimilates, reducing root mass increases the availability of assimilates for aboveground parts including grain yield ^[15]. Roots can regulate above ground physiological parameters, not only stomatal conductance, and also affect the posture of leaf blade and photosynthesis rate under soil impedance, nutrient, drought and salt stresses. Various report also support and have cross talked about root-to-shoot signalling promoted by soil drying and resulted to sharp decrease in their photosynthetic rates, leaf water potentials, starch concentration, and leaf sucrose contents [16-19].

Therefore, the present study was designed to determine relationship between root morphological traits with most studied above ground physiological parameters that contribute to yield such as RWC, photosynthetic rate, transpiration, stomatal conductance under drought when exposed to the particular growth stage. This investigation was also an attempt to exaggerate non-destructive and effortless screening of root related above ground traits for rice genotypes tolerant to drought stress.

Materials and Methods

Plant materials and treatments

Fourteen genotypes of rice (Oryza sativa L.) with partially drought tolerance seeds of same maturity group were used for the present investigation. Seeds were sown on plastic pots (20 x 30 x 40 cm) filled with soil mixture containing garden soil, sand and vermi-compost in 1:1:1 ratio. Thinning was done on 15th day after sowing (DAS) and 5 plants were retained in the pot. The pots were laid out in a Randomized Block Design (RBD) with three replications of each genotype and experiment was carried out under rain out shelter. Plants were subjected to drought by withholding irrigation from 6 days ahead adjusting the soil moisture content (SMC) upto 30% (gravimetric method) and recovery by re-watering. Soil moisture content was calculated using the weight fraction: SMC $\% = [(FW-DW)/DW] \times 100$, where FW was the fresh weight of soil portion taken from the depth of 10 cm of each pot and DW was the dry weight of the soil after drying in hot air oven at 85°C for 2 days. For the control, plants were well watered throughout the study as required. And leaf length was measured using centimetre scale. All the data were collected at early reproductive stage (60 days) of growth.

Root trait measurement

Plants were carefully dug out from the root level and washed properly in running tap water to separate roots from soil and any debris. Root traits were determined for root length (cm), root dry mass (g), specific root length (root length/root dry mass), root:shoot ratio (root dry weight/ shoot dry weight), root mass fraction (root dry mass/total dry mass) root relative water content at 60 days of growth for control and drought stress. Relative water content was measured and calculated using formula [(fresh weight-dry weight/turgid weight-dry weight) x 100].

Above ground trait measurement

Above ground traits were determined and data was recorded at 60 days of growth for shoot length, leaf relative water content (measurement and calculation was done as mentioned above for the root RWC). Physiological parameters such as photosynthetic rate (µmol CO₂ m⁻² s⁻¹), transpiration rate (mmol H₂O m⁻² s⁻¹) and stomatal conductance (gs) (mmol m⁻² s⁻²) were determined using Infra red gas analyser (IRGA), the photosynthesis portable system LI-6400, Biosciences.

Statistical analysis

The experiments were performed in triplicates. Data collected was analyzed statistically using Analysis of variance for the variables measured during the study period to test for significant differences between the treatments and the genotypes. The correlation analysis between root traits and above ground physiological parameters was performed with simple coefficient matrix at $P \le 0.05$ and $P \le 0.01$.

Results and Discussion

Changes in root traits of fourteen genotypes studied under drought stress

Drought stress caused variations in response and root traits specificity of genotypes (Table 1). There was significant increase in root length for all genotypes studied under drought condition as compared with controls whereby genotypes BRR-0029 showed maximum root length (18.2 cm). Deep rooting is a critical factor under water deficit stress influencing the ability of the plant to absorb water from the deeper layers of the soil ^[10]. SRL ranges from 20.11 cm g⁻¹ to 110.48 cm g⁻¹ whereby genotypes R Bhagwati and Sabour Surbhit showed the maximum SRL under drought situation. Increased RDM and RMF in genotypes Sabour Surbhit, BRR-0031 and BRR-0015 respectively with almost 50% were noticed under drought stress than the same genotypes grown under control condition.

Drought stress led to concomitant decrease in root:shoot ratio as compared to control except in genotypes BRR-0028 followed by BRR-0018, BRR-0014, BRR-0031, Sabour Surbhit, MAS 946. This increased in root:shoot ratio of above mentioned genotypes may be an indication of roots less sensitivity than shoots to growth inhibition by low water potentials ^[20]. Franco and co-workers ^[10] also reported about less affected of root growth by drought stress and further suggested a possibility of decreasing in root: shoot ratio results either from an increased in root growth or from a relatively larger decreased in shoot growth than root growth. Interestingly, genotype Sabour Ardhjal showed a peculiar response on root morphological trait, there was hardly any change under drought condition, which indicated being more tolerant to water stress.

Root relative water content (root RWC) measured the potentiality of water holding capacity by the roots. Root RWC was comparatively lower than the leaf relative water content, but decrease percent in the content of drought exposed to control remain similar in both roots and leaves, suggesting its association in moulding crops adaptability to withstand stress.

Changes in above ground physiological parameters of fourteen genotypes under drought stress

At early reproductive stage (60 days) of growth, drought

generally affects the shoot length. Besides, variation amongst genotypes ranging from 39.8 cm to 67.9 cm in shoot length R Bhagwati, BRR-0029, BRR-0019, BRR-0026 revealed the maximum length with 67.1cm, 66.2cm, 65.9cm, 63.2cm respectively (Table 2). However, BRR-0012 was recorded to be minimum length of 39.8 cm under drought as compared with control. The result is an agreement with the report of Nonami ^[21], who described reduction in growth due to impaired mitosis; cell elongation and expansion which generally inhibited by interruption of water flow from the xylem to the surrounding elongating cells under severe water stress conditions.

Leaf Relative water content

Relative water content was studied as a measurement of plant water stress leaves of rice genotypes at 60 days of growth under control and drought condition. A significant reduction in leaf water content performances of fourteen genotypes was observed in the study (Table 2). A sharp decline in RWC % was noted in genotypes Sabour Surbhit (37%) and BRR-0014 (34.2%) under drought condition as compared to the control. Moreover, the results revealed genotypic variations by increasing with almost two folds in genotypes BRR-0021, BRR-0023, BRR-0031 and 15 folds in genotype BRR-0019 under drought than those in controls. These variations might be attributed in drought tolerance mechanism at specific stage of growth period, with consideration of maintaining high relative water content in leaves thus controlling other physiological parameters like stomatal conductance and transpiration ^[22], water extraction ability ^[23], and variation in the canopy size at the onset of stress ^[24].

Physiological parametes

Photosynthesis rate, Transpiration rate and stomatal conductance

Wider variation in physiological respond amongst genotypes was noticed under drought stress condition affecting significantly amongst the studied physiological as compared to the control plant (Table 2). Among the fourteen genotypes studied, 7 genotypes showed an increased percentage of photosynthetic rates under drought stress condition in respect to control whereby, maximum increased of 41.61% and 30.75 % were recorded in genotype Sabour Surbhit and Sabour Ardhjal respectively. This increased in photosynthetic rate under drought stress may probably due to the higher chlorophyll stability which control oxidative stress that resulting to photo-oxidation rather than chlorophyll degradation ^[25]. The result was also supported by the report of high photosynthesis rate in winter wheat drought stress ^[26]. However, 41.78% reduction in photosynthetic rate was recorded in genotype BRR-0021 which was followed by BRR-0014 (39.92%). Chlorophyll degradation is considered as one of the consequences of drought stress which has resulted from sustained photo-inhibition and photo breeding leading to dropped in the rate of photosynthesis [27-28]. Throughout the study revealed genotypic specificity in terms of stress responses. For these genotypes showing less or decreased in photosynthetic rate is generally due to less chlorophyll stability of the genotypes when and in exposure to stress^[22].

Drought stress adversely affected stomatal conductance and transpiration rate at early reproductive stress (60 days) of growth stages in all the genotypes (Table 2). The result is an indication that low relative water content due to drought stress inhibited growth and plant function which were reflected in

lower total dry mass, decreased photosynthetic rate and lower stomatal conductance. Similar reports in different crops like wheat, peanut, sugarcane etc are also available in previous literature ^[29-31]. However, genotype BRR-0018 exhibited significantly higher of 28.39% followed by genotype BRR-0015 with 22.01% in transpiration rate under drought stress as compared to the same genotypes grown under control condition. Minimum decreased in transpiration rate were recorded in genotypes BRR-0014, BRR-0019 and BRR-0012 with 26.18%, 25.19% and 25.11% respectively showing more tolerance ability under stress situation. The maximum reduction in stomatal conductance was observed in genotype BRR-0023 and BRR-0021 with 39.1% and 35.1% respectively but genotypes BRR-0015 and Sabour Surbhit showed less reduction percentage with only 5.77% and 7.32% respectively showing better performance under drought stress.

Correlation analysis of root traits and above ground physiological parameters

At the 60 days of growth period, the root traits (RL, RDM, RMF, SRL R:S) were significantly correlated with above ground physiological traits (SL, Photosynthesis rate, transpiration rate, Stomatal conductance). The finding is also supported by the reports of Shi et al. [32]. RL and SRL showed strong positive correlation with stomatal conductance at p < 0.01 and correlated with shoot length, photosynthesis rate and transpiration rate at p < 0.05 (Table 3). However, a non significant negative correlation was observed between root mass related traits with photosynthesis rate, stomatal conductance. Root: shoot ratio showed both significant and non significant positive relation with leaf RWC, Photosynthesis rate, and transpiration, stomatal conductance respectively but gave negative correlation with SL. RMF and RDM has negative correlation with photosynthesis rate. The results also revealed a positive correlation amongst above ground physiological parameters. Photosynthetic rate was positively correlated with transpiration rate and stomatal conductance. Shoot lengths showed strong positive correlation with leaf RWC and transpiration rate.

As revealed in the correlation study, a strong positive correlation among the above ground physiological trait may be due to control of photosynthetic activity by stomatal and non stomatal mechanisms ^[17-19]. Stomata are the entrance of water loss and CO₂ absorbability and stomatal closure is one of the first responses to drought stress which result in declined rate of photosynthesis and vice versa. Stomatal closure deprives the leaves of CO₂ and photosynthetic carbon assimilation is decreased in favour of photorespiration. Considering the past literature as well as the current information on drought-induced photosynthetic responses, it is evident that stomata close progressively with increased drought stress. It is well known that leaf water status always interacts with stomatal conductance and a good correlation between leaf water potential and stomatal conductance always exists, even under drought stress. This is the one reasons of how leaf RWC maintained in some genotypes even under stress condition. Moreover, root association in controlling the leaf activities such as RWC has become more cleared. The report is also supported by the findings that drought-induced root to leaf signalling, which is promoted by soil drying through the transpiration stream, resulting in stomatal closure [33]

Root system plays an important role in the development of aboveground organs and yield formation ^[1-2]. There is also increasing evidence suggesting the close relationship of root

morpho-physiological traits with growth and development of above ground parts of plants and formation of grain yield ^[23,34-35]. It is been reported that under drought stress conditions roots induce a signal cascade to the shoots via xylem causing physiological changes eventually determining the level of adaptation to the stress. This drought induced root to leaf responses may signify the feasible non destructive way of analysing effectual traits in rice. Therefore, the present study has been carried out to develop an understanding towards a correlation of root traits with common most studied physiological traits and differences in genotypic responses of drought tolerant rice at early reproductive drought stress (60

days). Results indicate root morphological trait has direct or indirect relation with plant above ground physiological traits. Reviewing the above results it can be concluded that strong association between root system and above ground physiological traits under drought stress should be regarded as an important key point to reduce a massive volume of work and limitations of root research methods. Moreover, this correlation study will give an insight to breeders and investigation will exaggerate screening for more desirable root traits that associate positively above ground physiological traits and to grain yield of rice genotypes for drought situation.

Table 1: Root morphological traits of fourteen genotypes in response to control and drought stress condition at early reproductive stage (60 days) of growth. Values with different alphabets are significantly different at $P \le 0.05$.

Construnce	RL (cm)		SRL (cm g ⁻¹)		Root: Shoot		RDM		TDM		RMF		Root RWC (%)		Proline (µg g-1 dry wt)	
Genotypes	Cont	Dro	Cont	Dro	Cont	Dro	Cont	Dro	Cont	Dro	Cont	Dro	Cont	Dro	Cont	Dro
R. Bhagwati	11.5 ^c	15.0 ^b	42.18 ^c	110.48 ^a	0.098 ^d	0.056 ^e	0.068d ^e	0.045^{f}	1.27 ^e	0.94 ^f	0.087 ^d	0.041 ^e	87	73	26.68 ^b	35.62 ^c
Sabour Surbhit	10.8 ^d	11.16 ^c	43.47°	104.57 ^a	0.084^{d}	0.115°	0.037 ^e	0.075^{d}	1.88 ^{ab}	1.26 ^d	0.069 ^e	0.094 ^e	79	73	21.64 ^{cd}	36.14 ^c
Sabour Ardhjal	13.3ª	15.3 ^b	56.35 ^b	56.30 ^c	0.099 ^{cd}	0.095 ^d	0.090 ^c	0.086°	1.09 ^e	1.62 ^b	0.126 ^{cd}	0.119 ^d	89	86	25.14 ^b	43.88 ^b
BRR-0012	10.9 ^d	13.8°	29.29 ^e	51.28°	0.100°	0.081^{d}	0.091°	0.065 ^e	1.42 ^d	1.19 ^e	0.165°	0.130 ^c	84	79	23.16 ^c	38.78 ^c
BRR-0014	11.9 ^c	18.0 ^a	21.17 ^e	55.03 ^c	0.108 ^c	0.147 ^b	0.081 ^c	0.091 ^c	1.63°	2.02^{a}	0.137°	0.106^{d}	77	69	21.66 ^{cd}	33.98 ^d
BRR-0015	10.3 ^d	15.1 ^b	37.54 ^d	34.98 ^e	0.085^{d}	0.089 ^d	0.078 ^c	0.151 ^a	2.59ª	1.45 ^c	0.111 ^d	0.182^{b}	85	77	20.51 ^{cd}	39.74 ^{bc}
BRR-0018	11.7 ^c	14.6 ^b	25.86 ^e	30.30 ^e	0.108 ^c	0.118 ^c	0.106 ^c	0.122^{b}	1.82 ^{ab}	1.99 ^a	0.116^{d}	0.198 ^b	86	81	23.40 ^c	40.19 ^{bc}
BRR-0019	11.1 ^c	13.2°	36.77 ^d	38.25 ^e	0.104 ^c	0.092 ^{cd}	0.059 ^e	0.084^{c}	2.33ª	2.15 ^a	0.155°	0.122 ^c	88	79	18.8 ^e	35.15 ^c
BRR- 0021	9.4 ^d	12.4 ^c	65.85 ^a	32.39 ^e	0.148^{b}	0.095 ^d	0.051 ^e	0.087^{c}	1.55°	1.24 ^d	0.082 ^d	0.176 ^b	90	81	28.17 ^a	47.03 ^b
BRR-0023	9.1 ^d	13.8°	49.62 ^b	53.03°	0.161^{b}	0.102 ^c	0.073 ^c	0.093 ^c	1.42 ^d	1.21 ^d	0.131°	0.110^{d}	77	72	19.06 ^e	33.82 ^d
BRR-0026	12.2 ^b	19.3ª	14.93^{f}	28.03 ^c	0.148^{b}	0.094 ^d	0.129bc	0.086°	1.34 ^d	1.32 ^c	0.201 ^{bc}	0.107 ^d	91	87	18.5 ^e	38.41°
BRR-0028	10.6 ^d	15.1 ^b	18.03^{f}	20.11^{f}	0.198 ^{ab}	0.268 ^{ab}	0.179 ^a	0.113 ^{bc}	1.39 ^d	1.38 ^c	0.386 ^{ab}	0.242^{ab}	88	88	29.65 ^a	54.32ª
BRR-0029	12.6 ^b	18.2 ^a	61.85 ^a	47.95 ^d	0.068 ^e	0.065 ^e	0.058 ^e	0.061 ^e	1.69 ^c	1.17 ^e	0.083 ^d	0.073 ^e	79	73	21.91 ^{cd}	39.77 ^{bc}
BRR-0031	9.9 ^d	14.0 ^b	42.69°	29.89 ^f	0.099 ^{cd}	0.122 ^b	0.090°	0.109°	1.80 ^{ab}	1.18 ^e	0.121 ^{cd}	0.145°	84	80	19.99 ^{de}	34.41 ^{cd}

 Table 2: Changes in shoot physiological traits of fourteen genotypes in response to control and drought stress condition. The values are mean of three replicate ± SD.

Genotypes	Shoot length (cm)	Leaf RWC (%)		Photosynt (µmol CC	thesis rate D ₂ m ⁻² s ⁻¹)	Tanspira (mmol H ₂	tion rate O m ⁻² s ⁻¹)	Stomatal Conductance (mmol m ⁻² s ⁻²)	
	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
R. Bhagwati	67.1	97	81	16.20 ± 0.81	13.43±0.67	4.44±0.22	4.08±0.20	36.48 ± 1.8	28.34±1.4
Sabour Surbhit	47.2	89	82	10.75±0.53	18.41 ± 0.92	4.68 ± 0.23	3.91±0.19	34.39±1.7	31.87±1.5
Sabour Ardhjal	59.2	96	89	13.17±0.65	19.02±0.95	4.95±0.24	4.12±0.20	36.09 ± 1.8	30.18 ± 1.5
BRR-0012	39.8	92	86	16.22±0.81	12.88 ± 0.64	4.46±0.22	3.34±0.16	33.45±1.6	23.45±1.1
BRR-0014	61.2	92	81	14.23±0.71	8.55±0.42	4.24±0.21	3.13±0.15	29.36 ± 1.4	24.63 ±1.2
BRR-0015	51.1	91	80	11.16±0.55	11.02±0.55	3.33±0.166	4.27±0.21	31.71 ±1.5	29.88±1.4
BRR-0018	47.8	89	82	12.26±0.61	17.10±0.85	2.85 ± 0.14	3.98±0.19	32.65 ± 1.6	29.27 ±1.4
BRR-0019	65.9	90	84	16.59±0.82	16.44 ± 0.82	6.35±0.31	4.75±0.23	32.46 ± 1.6	28.67 ± 1.4
BRR-0021	59.1	89	76	10.89±0.54	6.34±0.31	2.39±0.11	3.09±0.15	29.90±1.4	19.39 ±0.96
BRR-0023	56.8	83	77	12.68±0.63	9.57±0.47	4.24±0.21	3.50±0.17	32.48 ±1.6	22.44 ±1.1
BRR- 0026	63.2	81	74	9.74±0.48	13.77±0.68	3.42±0.17	3.87±0.19	37.38 ±1.8	29.01 ±1.4
BRR-0028	65.9	91	84	12.97±0.64	17.09±0.85	3.82±0.19	4.44 ± 0.22	32.18 ± 1.6	26.09±1.3
BRR-0029	66.2	88	82	13.32±0.66	14.07±0.70	3.93±0.19	4.90±0.24	31.57±1.5	28.55 ±1.4
BRR-0031	58.3	89	83	11.22±0.56	15.45±0.77	3.96±0.19	4.00±0.20	38.15±1.9	29.39±1.4

 Table 3: Correlation coefficient of root morphological traits and shoot physiological parameters under drought stress at early reproductive stage

 (60 days) of growth

Parameters	RL	SRL	RDM	RMF	Root:Shoot	SL	RWC	Photo. rate	Transp. rate	Stomatal Cond.
RL	-	0.143	-0.298	-0.062	-0.349	0.497*	0.283	0.598*	0.496*	0.838**
SRL		-	0.764**	0.678*	-0.413	-0.083	0.521*	0.431	0.347	0.738**
RDM			-	0.743**	0.196	-0.246	-0.161	-0.116	0.157	-0.154
RMF				-	0.012	-0.099	0.352	-0.019	0.511*	0.269
R:S					-	-0.159	0.502*	0.541*	0.382	0.264
SL						-	0.728**	0.214	0.655**	0.451
RWC							-	0.479*	-0.238	0.352
Photo. rate								-	0.539*	0.573*
Trans. rate									-	0.511*
Stomatal Cond.										-

*Significant (*p*< 0.05); **Significant (*p*< 0.01)

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