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Research Article Assessing Root System Characteristics in a Subset of Rice Accessions Through Hydroponic Rhizotron Systems

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Abstract

Background and Objective: Rice is considered one of the most important cereal crops for human nutrition. Based on current predictions of the increasing global population, rice production needs to be increased by more than 50% to meet the future demand of the rice-eating countries. It has already been demonstrated that cereal crop yield could be increased by modifying root architectural traits for production under limited resources like water and nutrition. The main objective of the study is to assess the rooting behaviour of a panel of Boro and Amon rice cultivars collected from the Bangladesh Rice Research Institute (BRRI). **Materials and Methods:** Two established screening methods, hydroponic and soil-filled glass rhizotron system were used to assess the panel of Boro and Amon rice cultivars. Using these two methods, deep and shallow-rooted cultivars were identified. A panel of 16 rice cultivars was used in this study. **Results:** Assessing the 16 cultivars in a hydroponic system, 98% variation was observed by the genotype at day 46 (p = 0.001). Soil filled glass rhizotron was used to assess six of these accessions along with two check varieties revealing strong genotypic variation. Analysis of variance (ANOVA) showed a 73.37% variation explained by the genotype (F = 13.20, p = 0.001) at 46 days after sowing. **Conclusion:** Assessing all the traits, the rhizotron data revealed that BR 22, BR 60, BR 19 and BR 3 has the deepest rooting systems. The trait information could be useful to breed better cultivars, especially in drought-prone regions.

Key words: Rice, root, screening, traits, hydroponic, rhizotron, genotype, cereal crop, nutrition

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rice is one of the oldest domesticated crop species having fed more than 3.5 billion people¹ and offering 20% of their daily calories². To achieve the expected yield, a better understanding of the root systems will be required. Root systems play a vital role in the uptake of water and nutrients from the soil³. Root morphological characteristics are known to be important in the drought resistance of some rice (*Oryza sativa* L.) varieties. Several mechanisms of drought resistance are already been exploited by rice varieties and potentially the most promising one is the possessions of deep and thick root systems that allow access to water at depth⁴.

Root System Architecture (RSA) plays a pivotal role in crop performance, particularly for cultivation under non-optimal water and nutrient supply conditions⁵. Root System Architecture (RSA) made up of structural features- like root length, spread, number and length of lateral roots, among others, exhibits great plasticity in response to environmental changes and would be critical to developing crops with more efficient roots. With the changing climate and rainfall abrasions, there is a gradual shift in the system of rice cultivation from a traditional transplanted anaerobic to an aerobic system. To achieve sustainable agricultural production, it needs to be well studied of anatomy and root system architecture⁶.

A structured root system is necessary for favourable plant growth. The reason for adopting many cultivation practices is to improve the root environment like soil pH and the drainage system⁷. Crop growth, yield and product quality depend on the roots to a large extent⁸. Absorption of water and nutrients is the principle function of the root and drought is the foremost abiotic stress that affects more than twenty percent of the total rice-producing area in Asia9. Atmospheric water stress has been linked to root system characteristics¹⁰. The development of a deep and extensive root system is an adaptive strategy of plants for drought avoidance¹¹. Root characteristics such as thickness, depth of rooting and root length density have been associated with drought avoidance in rice^{12,13}. Root angle distribution, which is regulated by the growth angle of the crown roots, is one of the root morphological traits used to determine the area of soil over which root capture water and nutrients¹⁴. For example, deep rooting is useful for extracting more water from the soil and minimizing drought stress under upland and rainfed lowland conditions^{15,16}. In contrast, under waterlogged conditions, shallow rooting can help plants to absorb oxygen on the soil surface and helps avoid hypoxic conditions¹². Maximum root depth of rice and root length density increase with time until the flowering stage and after that, it decreases rapidly until grain maturity¹⁷. The structural shape of the root varies with soil physics; particle size, soil water status and soil compaction¹⁸. Rice root formation also depends upon cultivation methods¹⁹. In upland rice which is sewn directly, their roots grow deeper than in transplanted practices used in flooded low land conditions.

Nutrient uptake by roots in the soil is accomplished by root interception, mass flow and diffusion²⁰ where nutrient uptake occurs as a result of root surface contact with the nutrients in the soil. Root morphology (root length and radius) and architecture are the most important characteristics that influence plant resource acquisition²¹. Increasing root length density increases water uptake ability but gains in uptake afforded by root density are restricted to a critical root length density where all water in the soil is within approximately 1 cm of a root. Critical root length density is higher for nutrients with low movement in the soil, like phosphorus.

Rice having deep rooting potential may help plants to avoid drought-induced stress by extracting water from deep soil layers²². Lafitte *et al.*²³ showed that deep-rooted genotypes have lower susceptibility to drought and improved productivity under aerobic field conditions while genotypes with shallow rooting systems are more susceptible to stress thereby have lower productivity. Kanbar *et al.*²⁴ showed that maximum root depth, root shoot ratio and root dry weight conferred an advantage to grain yield under water stress. Root characteristics such as thickness, depth of rooting and root length density have been associated with drought avoidance in rice²⁵. Deep rooting is useful for extracting more water from the soil and minimizing drought stress under upland and rainfed lowland conditions²⁶.

To achieve a successful breeding programme, reliable and robust screening techniques is very important. A panel of 205 rice genotypes were screened through a hydroponic system²⁷. Several root traits, root volume and root length were assessed with this technique. A total of 140 recombinant inbred lines were screened through a rhizotron system developed by Price et al.28 and several root traits evaluated for root growth of the longest visible root, several root past at different depths, root thickness and root mass at different depths after 7 weeks growth. To gain a better understanding of the complex root traits, 134 lines from the Bala x Azucena parental population were grown in agar-filled Perspex chambers to identify Quantitative Trait Loci (QTLs) for both Seminal Root Morphology (SRM) and gravitropic response²⁹. In this experiment, a total of four and two main effect QTLs were detected for the SRM and gravitropic response respectively²⁹.

The main objective of this research is to assess the rooting behaviour of a panel of Boro and Amon rice cultivars collected from the Bangladesh Rice Research Institute (BRRI) and identify deep and shallow-rooted cultivars to facilitate the breeding of drought resistance in rice.

MATERIALS AND METHODS

Study area: The hydroponic experiment was conducted on 14 December, 2018 in the laboratory of the Department of Biochemistry and Molecular Biology, University of Chittagong, Bangladesh. The rhizotron experiment was set up in the Biological Faculty glasshouse on 8 April, 2019, University of Chittagong, Bangladesh.

Experimental design of hydroponic screening of root growth of 13 accessions: All 13 accessions collected from Bangladesh Rice Research Institute (BRRI) were tested in the hydroponic set-up described in the Price and Tomos²⁷. Three check varieties Azucena, Black Gora and IR 64 were included with these accessions, which were tested in hydroponics by Shrestha *et al.*³⁰.

Each screen was used a randomised complete block design with four replications. Diluted domestic bleach (1% sodium hypochlorite (NaClO₃) were used for surface sterilization of seeds and then washed with tap water three times. Seeds were then kept for germination at 30°C for three days. Germinated seedlings were placed in a black seedling tray which contains 84 wells with 55 mm deep and 40 mm and 12 mm diameter on the top and bottom respectively. Pieces of plastic mesh (15×5 mm) were used to hold germinated seedlings which were placed above the hole of the well. Each seedling tray was fitted in the 50 L plastic box 56 cm long, 36 cm wide and 29 cm deep. To prevent the entry of the light into the boxes, empty wells were then be covered with aluminium foil paper. The sides of the boxes were covered by black and white plastic paper. Aeration systems were set up in the box. About 50 L of half-strength Yoshida's Nutrient solution (pH 5.5) were used to fill up the box for the first three weeks and full strength for the final four weeks³¹. pH was adjusted daily and the nutrient solution was replaced on weekly basis. The experiment was conducted in a controlled environment, the humidity was maintained at 70% and the temperature was 25-28°C throughout the experiment. The supplementary lights 120 μ M m⁻² sec⁻¹ (PAR) hrs a day were supplied. The maximum root and shoot length of each plant was recorded weekly for five weeks.

Rhizotron screening: From 13 accessions were tested in hydroponic screening, 6 were selected for the rhizotron screening. Among six accessions four were Boro cultivars (BR 3, BR14, BR 19, BRRI 60) and two were Amon cultivars (BR 22 and BR 32). Two check varieties Azucena and IR 64 were included with these accessions.

A set of 32 glass-fronted rhizotrons was made as described by Price *et al.*⁴. Two sheets of 4 mm thick glass 120 cm long and 30 cm wide were used to make up each rhizotron. To hold the two sheets 15 mm apart two wooden strips and a coca-cola bottle cap were used. Duct tape was used to seal the base and two sides of the glass rhizotron. After that wooden strips were removed. Then the rhizotron was filled with 7 kg of soil which gave a total weight of 13 kg of each rhizotron. The rhizotron was grouped in a stack and leaned at an angle of 15 from vertical to encourage the root growth down the lower surface of the glass rhizotron. Polystyrene insulation board were used in front of the stacks to keep out light and maintain temperature.

Each chamber of rhizotron was saturated with full strength Yoshida's nutrient solution pH5.5 before sowing the seeds. Seeds were sown directly on rhizotron, after seedlings germination, the seedlings were thinned to one plant. Drip irrigation systems were connected to each rhizotron described as Price *et al.*⁴. Each rhizotron received last irrigation on the 45 DAS and watering was stopped until harvested on day 52.

Traits measured from rhizotron: Shoot growth was monitored as shoot length while the length of the longest visible root length was recorded on weekly basis until 52 DAS. On day 21 and 42 the angle of spread of the root system was measured with a protractor described as Price *et al.*⁴. On the evening of day 42, a digital image was taken of each rhizotron using 12 Megapixel cameras. At the end of experiment day 52, plant shoot was removed in one day, oven-dried at 80 for three days and dry weight was recorded. Over the week, rhizotron was opened, short sections of three of the thickest root are removed from each root system from the base of the root, gently washed with water, oven-dried and weighed.

Image analysis: The number of the main axes of nine angle classes representing each of the 100 subdivision of the protractor was counted. Roots in the division 0-100 and 170-1800 are counted together in the 0-100 class to give the number of roots in the most horizontal of the nine angle classes. A weighted average of the angle was calculated by

multiplying the number of roots in each class by the halfway angle of each class (e.g., 5 for the 0-10° class and 35 for the 30-40° class), summing across all classes and dividing that by the total number of axes.

Statistical analysis: Minitab version 17 was used to analyse the data. For both Experiment (Hydroponic and rhizotron screening) data were checked for block effect by analysis of variance (ANOVA) and corrected by taking the residual for further analysis if required. In each screen, each trait one way ANOVA was used to assess the existence of genotypic difference. Genotype means were used to observe the correlation between traits and screens.

RESULTS AND DISCUSSION

Hydroponic screening: The mean root length and shoot length increased linearly with time. Plants had a mean root length of 8.0 cm at day 26 which increased up to 9.0 cm at day 37 and up to 15 cm at day 52. Shoot growth showed a greater increase from 17 cm at days 26-21 cm at day 37 and increased up to 30 cm at day 52. All shoot and root length data collected in hydroponics screening were statistically analyzed for cultivars difference by one way ANOVA.

A total of 16 cultivars tested in the hydroponics showed a great difference in root length and shoot length throughout the experiment. The maximum root length and shoot length of all genotypes at days 26 is shown in Fig. 1.

In the root length, significant variation was observed at all days of measurement and the proportion of variation explained by the genotypes was increased from day 26-52 where it was highest at day 37. That means the highest discrimination of root length was observed on day 37. Azucena, Black Gora, BR 3, BR 19, BR 16, BRRI 60 had the longest root on that day while IR64, BRRI 51, BRRI 36, had the shortest (Fig. 2).

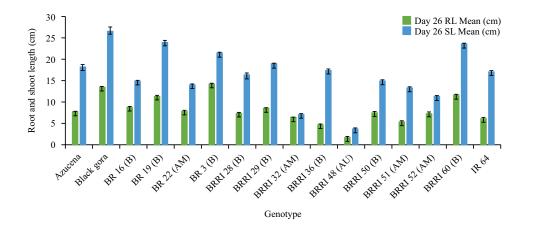
The maximum root length of days 37 ranges from 14-16 cm and Black Gora and BR3 were the highest and BRRI 36 and IR 64 were the lowest (Fig. 2) and at that time 58.27% variation explained by genotype (F = 5.27, p = 0.001).

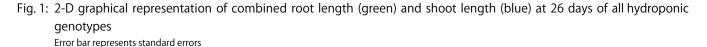
The data of root length and shoot length were recorded at 52 days of hydroponic experiment which showed significant result (p = 0.001). Black Gora, BRRI 60 and BR 3 had the longest root length and IR 64, BRRI 51 and BRRI 36 had the shortest root length (Fig. 3).

The maximum visible root and shoot length of the check varieties (Fig. 4 and 5) shows that Black Gora and Azucena had long root and shoot length and IR 64 had shallow rooted which follow the same pattern as previously detected in Al-Shugeairy *et al.*³².

Rhizotron screening: The mean values of all traits measured on the rhizotrons are presented in Table 1, which also summarises the one way ANOVA. All traits were highly significantly different between cultivars with cultivars generally explaining the majority of the variation.

The maximum visible root length measured in the rhizotron increased 109 cm on day 33-118 cm on day 45 (Fig. 6). The mean shoot length increased from 22-74 cm from day 14-45 respectively (Fig. 7). Among the accessions Azucena, BR14, BR32 had the highest shoot length where BRRI 60, BR 3 and IR 64 showed the shortest shoot length (Fig. 7 and 8). The





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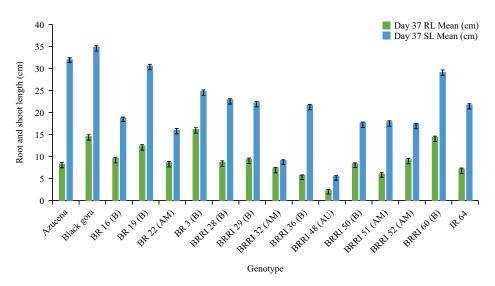
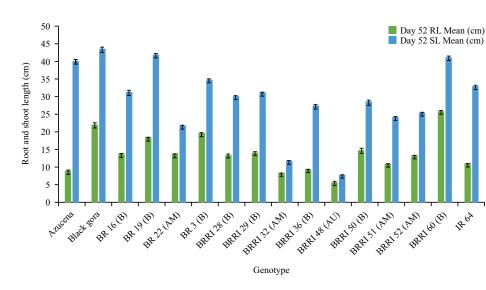


Fig. 2: 2-D graphical representation of combined root length (green) and shoot length (blue) at day 37 of all hydroponic genotypes



Error bar represents standard errors

Fig. 3: 2-D graphical representation of combined root length (green) and shoot length (blue) at day 52 of all hydroponic genotypes

Error bar represents standard errors

highest variation of the shoot length showed at day 42 where it explained 91.83% variation by accessions. The proportion variation explained by genotypes for the maximum visible root length showed an increasing pattern from day 24-45 respectively. Among the landraces genotype Azucena, BR 19, BR 3, BR 22 and BRRI 60 had the longest visible roots which were higher at all stages than the mean root length. IR 64, BR 14 and BR 32 had the shortest root systems (Fig. 8).

Comparing the genotypes with maximum visible root length and shoot length at day 46, the harvesting time BR 19, BR 22, BR 60, BR 3 and Azucena showed the highest root length where Azucena, BR 14 and BR 32 showed the highest shoot length. BR 32 and BR 14 showed almost similar shoot length at that time. IR 64 and BR 32 was the lowest on both traits (Fig. 8).

The root angle spread at day 46 was highly statistically different between genotype ($R^2 = 69.79\%$). BR 14 and BR 60 had a wide distributed root angle of approximately 139°. BR32 had the lower root angle which was measured at 118° at that time (Fig. 9).

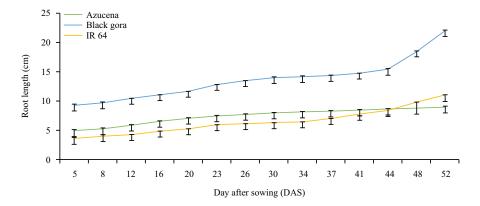


Fig. 4: 2-D line graph showing the increase in maximum root length from day 5-52 after sowing (DAS) of check cultivars Error bar represents standard errors

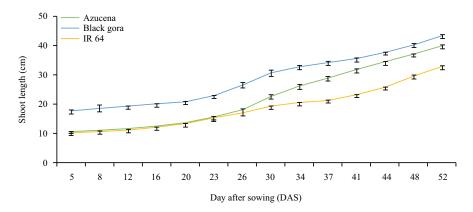


Fig. 5: 2-D line graph showing the increase in maximum shoot length from day 5-52 after sowing (DAS) of check cultivars Error bar represents standard errors

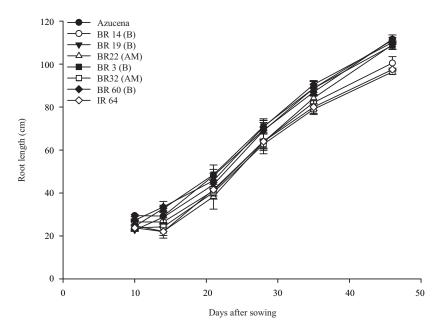


Fig. 6: Increase in maximum visible root length from 10-42 days after sowing for five cultivars Error bar represents standard errors

Trait	Mean	F	Р	R ² (adj)
Maximum visible root length day 10 (cm)	20.56	2.55	0.041	25.95
Maximum visible root length day 14 (cm)	27.39	3.12	0.017	32.37
Maximum visible root length day 21 (cm)	41.39	1.11	0.386	2.52
Maximum visible root length day 28 (cm)	66.81	0.81	0.589	0.00
Maximum visible root length day 35 (cm)	85.04	2.35	0.05	23.36
Maximum visible root length day 42 (cm)	98.93	15.38	0.00	76.45
Maximum visible root length day 46 (cm)	105.64	13.20	0.00	73.37
Shoot length day 10 (cm)	25.34	2.55	0.041	25.95
Shoot length day 14 (cm)	30.26	5.93	0.00	52.69
Shoot length day 21 (cm)	39.98	9.39	0.00	65.45
Shoot length day 28 (cm)	52.18	12.64	0.00	72.45
Shoot length day 35 (cm)	63.43	31.89	0.00	87.46
Shoot length day 42 (cm)	73.89	50.79	0.00	91.83
Shoot length day 46 (cm)	77.51	32.34	0.00	87.62
Root angle at day 46	135.78	11.23	0.00	69.79
Shoot dry weight (g)	5.69	182.20	0.00	97.61
Root mass (g)	8.50	54.27	0.00	92.32

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100 90 80 Shoot length (cm) 70 60 50 40 30 20 10 BR19(Am) BR22(Am) BR 32 Ann BRIA BR3(B) 0 BROOB Rok N Genotype

Fig. 7: Maximum shoot length at 42 DAS for the cultivars Error bar represents standard errors

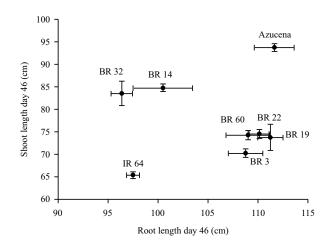


Fig. 8: Root and shoot length at 46 DAS of all rhizotron genotypes Error bars represent standard errors

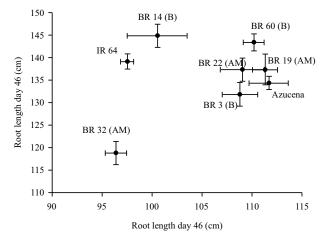
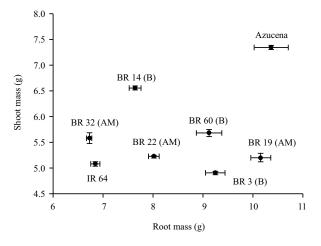
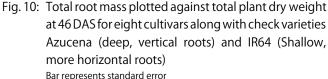


Fig. 9: Root length day 46 and root angle day 46 of all rhizotron cultivars Error bars represent standard errors

For the root, dry weight BR 19 and BR 3 had the highest root dry weight which was higher than that of BR 32 (Fig. 10). The root mass of the different genotype represented in Fig.10. Root mass showed great discrimination among the cultivars. Analysis of variance (ANOVA) revealed 92.32% variation explained by the accessions. Among the measured traits plant biomass showed the proportion of variation explained by the cultivars was 97.6%.

The maximum visible root length of the check varieties (Fig. 11) shows that Azucena long with vertical axes (Fig. 9) and IR 64 had shallow rooted which follows the same pattern as previously detected in Al-Shugeairy *et al.*³². The maximum visible shoot length from 21-45 Days After Sowing (DAS) of the check varieties (Fig. 12) Azucena and IR64 follows the same trends as described in Al-Shugeairy *et al.*³².





Correlation between rhizotron traits: The data collected from rhizotron root and shoot traits were closely related to each other. Many traits showed a strong correlation, the most significant correlations are described below.

Plant biomass had a very strong correlation with many of the root traits. It strongly correlated with maximum visible root length at all days, which was strongest at day10 (r = 0.55, p = 0.001).

Maximum visible root length correlated with other roots and shoot traits. At all days maximum visible root length correlated with shoot length at all days, the strongest correlation observed in maximum visible root length at day 21 and shoot length day 10 (r = 0.502, p = 0.003). Maximum visible root length did not correlate with root angle.

Comparing the maximum root length data in hydroponic screening, significant variation was observed within the accessions. But according to Shrestha *et al.*³⁰ hydroponic is probably not the most reliable method for testing rooting depth³⁰. The author suggests that rhizotrons are better though this rhizotron method requires much more effort. The genotypic variation observed in the hydroponic medium reflects the variation that also occurs in the soil. Shrestha *et al.*³⁰ reported that a very good agreement between root growth in a liquid medium and root growth in the soil pot. Evidence suggests that the correlation between herbicide score and root length in the hydroponic medium reflects that agreement³³.

Considering the rhizotron screening data, high heritability and significant trait variation suggest a clear and distinguishable difference within the accessions. The rhizotron data suggested that BR 19, BR 22 and BR 60 has the highest maximum visible root length among the cultivars tested. They had the highest root mass which indicated that the biggest rooting system (Fig. 10). Comparing the root and shoot mass, BR 14 had the highest shoot mass with low root mass revealing this as a cultivar with a remarkably low relative root mass. BR 19 and BR 3 had the highest root dry weight with low shoot mass. The highest root mass indicates well-developed root systems in addition to the shoot mass. The evidence suggested that in different planting practice Boro rice cultivars showed the best rooting behaviour like the number of roots, root length density than the other varieties³⁴. Another study also suggested that Boro rice cultivars showed better yield in strongly saline soils to all parameters studies³⁵. It is noted that cultivars identified deep-rooted here in this current study BR 3, BR 19, BR 60 is also from the Boro rice group.

Comparing the root length of the accessions tested in the rhizotron, they can be ranked as BR 19>BR 22>BR 60>BR 3. In the hydroponic screening system, comparing the root length data, ranked can be BR 60>BR 3>BR 19>BR 22 among the 16 accessions tested. Though in hydroponic screening systems and rhizotron screening system the ranked is a bit different, the rooting behaviour of these four cultivars is similar in these two methods.

The check varieties had similar rooting behaviour in the previously reported by Al-Shugeairy et al.³⁰ and Shrestha et al.³¹. The report suggested that Azucena had a long root with vertical axes whereas IR 64 had a shallowrooted lateral branching system. The traits information like root and shoot length, root and shoot biomass and root angle collected from the current study would be very valuable for future study. The accessions identified as deep and shallowrooted, field trial under drought and yield determination for these cultivars would be very important. At the same time, drought screening of these cultivars would be valuable to identify drought resistance cultivars to elucidate the natural genetic diversity within rice to breed better rice cultivars. The two methods hydroponic and rhizotron system, used in this study has the advantage that root and shoot growth can be continually assessed. The rank of the tested cultivars, based on these two experiments is a bit different due to the two systems, one is liquid media (hydroponic) and another is soilbased systems. In a hydroponic system though the root growth is monitored, however, there was an issue of maintaining the pH of the media on regular basis and the root of the accessions intertwine with each other. Like hydroponic, in the rhizotron method root growth and root angle can be continually measured, but due to 2D construction, the root growth is restricted in an all-natural direction as well as a very labour-intensive screening method.

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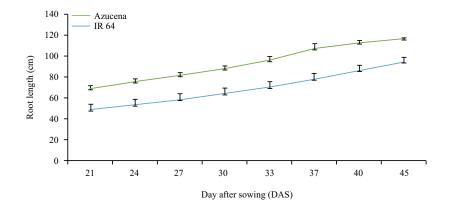


Fig. 11: Increase in maximum visible root length from 21-45 days after sowing for two check varieties (Azucena and IR64) Error bar represents standard errors

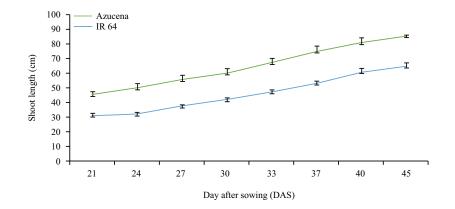


Fig. 12: Increase in maximum visible shoot length from 21-45 days after sowing for two check varieties (Azucena and IR64) Error bar represents standard errors

CONCLUSION

The hydroponic and rhizotron data suggested that BR 19 (Boro), BR 22 (Amon), BR 60 (Boro) and BR 3 (Boro) has the deepest rooting systems than the other accessions tested in these screening methods. The rhizotron data also suggested that BR19 and BR 3, both are Boro cultivars had surprisingly deep and shallow rooting systems with very low shoot mass among the cultivars. From the study, for example, BR 19, BR 22 and BR 3 is a very promising cultivar that needs to be assessed under field conditions. If these traits prove to be valuable under field conditions these cultivars have the scope for use in rice breeding programmes.

SIGNIFICANCE STATEMENT

Comparing the assessment of the screening methods, four accessions were considered as deeply rooted genotypes which may contribute in a greater way to the development of drought resistance variety in rice. The trait data which was collected from the study could be very helpful for the crop researcher to interpret the other drought data. It is, therefore, necessary to screen these cultivars at the field level under drought condition. The development of drought-tolerant cultivars and potential sources of tolerance will bring a direct impact on rice-eating countries and help to cope up with food insecurity.

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