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Root phenotypic study of different rice genotype for drought tolerance using mini-rhizotron

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Abstract
Drought is the major concern of Indian agriculture under changing scenario of climate to sustain the productivity and to fulfill the food requirement of ever increasing population. Hence, the present study was carried out at Research cum Instructional Farm and Department of Plant Physiology, Agricultural Biochemistry, Medicinal and Aromatic Plants, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during *Kharif* during 2018-19 and 2019-20. The planting materials for experiment consisted of 30 rice genotypes from IIRR, Hyderabad under AICRP on rice programme and 01 local check (Poornima) from IGKV, Raipur (C.G.). The root Phenotyping study was done in mini-rhizotron and scanning was carried out by using root scanner machine Epson Perfection V700/V750, 3.81 Version, Win Rhizo Reg 2009. The image base root Phenotyping of different rice genotypes using in mini-rhizotron the local check Poornima was registered relatively better in traits like root length, fresh and dry root weight, root diameter, root surface area, root volume followed by RFU 329, RFU 327, RFU 304 due to root parameters determines the hydraulic conductance of root that can potentially increase water uptake and contribute to improved water use efficiency in rice under water limited conditions. Thus, these genotypes may further used under farmers' field in large scale to sustain the productivity under rainfed ecosystem of India.

Keywords: Phenotypic, rice, genotype, tolerance, mini-rhizotron

Introduction

Rice (*Oryza sativa* L.) is the staple food of more than half of the world population. In world rice is cultivated over an area of 156.1 m ha with a production of 680 mt. In world, rice has occupied an area of 154 million hectares, with a total production of 476 million tonnes and productivity 2948.82 kg ha-1 (Anonymous, 2012) [1]. In India, the total area under irrigated, rainfed lowland and upland rice is 22.0, 14.4, and 6.3 million ha, respectively. India is the second largest producer of rice after china which has an area of over 45.5 million hectares and production 105.31 million tonnes with productivity 2393 kg ha^{-1} (Anonymous, 2013a) [2].

Chhattisgarh state is popularly known as "rice bowl of India" because maximum area is covered under rice during *kharif* and contributing major share in national rice production. Rice is cultivated in an area around 3.67 million hectares with the production of 7.49 million tonnes and productivity 2041 kg ha-1 (Anonymous, 2014) [3]. The prime causes of low productivity of rice in Chhattisgarh are inappropriate adoption of agronomical practices, limited irrigation (28.0%), lack of improved drought tolerant varieties with high yielding characteristics suitable to different ecosystems and extension services. Chhattisgarh farmers are mainly depending on climate for rice cultivation. Since the rainfed ecosystem of Eastern India is highly variable and unpredictable, which can range from normal situation to severe drought condition, therefore identification of a stable genotype performing well under water deficit conditions is required. In Eastern India, water deficit condition after flowering is the most frequent type severely affecting the yield (Pandey *et al.* 2005) [10, 11]. In upland areas, drought is an important environmental factor adversely affecting rice production, wherein the rainfall distribution is erratic and uncertain (McWilliam, 1986) [8]. A well defined research programme based on physiological evaluation of secondary traits is needed for the improvement of rainfed rice. The low moisture in rainfed condition is the most important limiting factor for crop production and it is becoming severe problem in many regions of the world.

Drought resistance is a complicated process that involves interaction of multiple pathways, genes and traits. Crop plants follow different drought resistance mechanisms against drought stress that are categorized as drought tolerance, drought escape and drought avoidance (Levitt J, 1980) [7].

Drought tolerance involves the ability of plants to develop low osmotic potential, cellular elasticity and increase membrane resistance. Drought escape involve the modulation of vegetative and reproductive growth to avoid drought stress by completing the life cycle before onset by either rapid plant growth or little growth during dry season but tremendous growth during wet season. Drought avoidance involves maintenance of water potential during low soil matric potential through minimal water loss through reduction in transpiration or optimize water uptake through hydraulic conductance. Major challenge for plant breeder is to improve the drought resistance of crop plants for stabilizing the yield. Therefore, there is need to exploit the natural variation of crop plant in drought adaptation as the drought adaption strategy of genotypes within species differ and can provide better in-sight to improve yield and resistance under drought conditions. Plant breeding and generation of transgenic plants through gene manipulation of drought related genes has opened up remarkable opportunities to accelerate the production drought tolerant varieties. Thus, generation and breeding of drought tolerant genotypes is a key strategy to improve the crop productivity.

In vitro plant regeneration through somatic embryogenesis is considered the common mode of regeneration in rice and other monocotyledonous species (Rueb *et al.* 1994) [12]. Selection for important agronomic characters such as plant height, tiller number, maturity, tolerance against herbicides, drought stress and various physiological and biochemical characters can be done *in vitro* through selection of somaclones by imposing stress conditions. These traits are pre-dominantly associated with drought condition and assist stress tolerant varieties to combat drought. Since, yield is affected by many factors such as plant phenotype and external environment; it cannot serve as sole selection criterion for breeding programs. Previously, selection of high-yielding wheat lines on the trait-based selection such as xylem diameter has achieved success. Definite root phenes related to drought tolerant and sensitive indica rice varieties may be more useful for selection however; it remains an uncharacterized criteria (Anupama *et al.* 2018) [4]. Keeping all these points in mind the investigation was undertaken with the objectives to assess the image base root Phenotyping for drought tolerance genotypes in mini-rhizotron.

Materials and Methods

Study area

The present study was carried out carried out at Research cum Instructional Farm and Department of Plant Physiology, Agricultural Biochemistry, Medicinal and Aromatic Plants, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during *Kharif* during 2018-19 and 2019-20.

Climatic conditions

The climate of this region is dry, sub-humid to semi-arid. The average annual rainfall ranges from 1200-1400 mm out of which 80-85 percent is received from middle of June to end of September and very little during October and February. The maximum and minimum temperature goes to 42.6 ^{0}C and 9.6^{0}C, respectively in the months of May and December or January. During crop growth period, the maximum temperature varied between 32.1 ^{0}C to 35.5 ^{0}C, respectively. The minimum temperature ranged between 24.9 ^{0}C in the first week of July to 16 ^{0}C in the fourth week of November. Crop received sunshine hours of 2.5 to 9.6 per day. The maximum and minimum humidity during the crop growth period was 92 and 36 per cent, respectively. A total of 738.6 mm rainfall was received during the crop period.

Source of Rice planting materials

The planting materials for experiment work was consist of 30 rice genotypes taken from IIRR, Hyderabad under AICRP on rice programme and 01 check variety from IGKV, Raipur (C.G.). The 30 rice genotypes taken for present study are following –

Table 1: Details of rice genotypes used in present study

RFU 301	RFU 306	RFU 311	RFU 316	RFU 321	RFU 326
RFU 302	RFU 307	RFU 312	RFU 317	RFU 322	RFU 327
RFU 303	RFU 308	RFU 313	RFU 318	RFU 323	RFU 328
RFU 304	RFU 309	RFU 314	RFU 319	RFU 324	RFU 329
RFU 305	RFU 310	RFU 315	RFU 320	RFU 325	Poornima (LC)

Experimental details

Root phenotype study

To identify the drought tolerance capacity of 30 different rice genotypes, the root phenotype study was carried out. The standard protocol of root phenotypic study was elaborated here –

Thirty glass plates, two types of tapes one with broad width and another with narrow width, Scissor, Spacer (plastic tube pieces of 3cm height), Soil and water etc.

Method

- Wash the glass plates with water carefully and kept for air drying.
- After the glass plates has dried then arrange the two plates in a specific manner and placed spacers in between these two plates two spacer at the top and one at the bottom of glass plate.
- Fix these plates with the help of tape (with broad width) wrapped around the edge of the plates to seal the three sides and one side should opened (to fill the soil and for growing seeds) so they would not displace. Take care of the spacer would not disturb and plates in a proper orientation in vertical manner.
- Wrapped the tape (with narrow width) above the open edge which is opened so it must be fixed.
- Filled the soil in the rhizotron glass plates which is filtered by sieving to avoid any cloud then apply water kept it for one day.
- Soil was settled down at the bottom again filled the rhizotron plates with soil and then apply water kept it for one day.
- Sowing was done in the next day and we can sow 3-5 seeds in one rhizotron plate. I have grown 3 seeds in one rhizotron plate.
- After sowing apply water and then germination was found within 7 days after sowing.
- Watering should be done after 2 days of interval and let the plants grown for 45 days
- After 45 days glass plate filled with soil was washed with water and separate root for root scanning.

Root scanning of selected genotypes of rice

The roots of each genotype were used for scanning. The roots of three plants from each genotypes line/variety were used for root scanning which given the detailed information about all root parameters including root length, root volume, root diameter and root surface area etc. The root scanning was carried out by using root scanner machine Epson Perfection V700/V750, 3.81 Version, Win Rhizo Reg 2009 (Fig 1) the data was recorded automatically in the computer for different root parameters including root length, average root diameter, root volume, root surface area etc. Following procedure was used for root scanning:

Acquiring washed Roots

The first step is acquiring washed roots. This can be the most difficult and laborious step in the experiment if plants are grown in solid medium (e.g. Soil). For root scanning the roots washed with tap water for 2-3 times gently so that fine roots would not damage to remove soil completely and the roots were preserved in the spirit solution (25%) in Falcun Tubes for root scanning. The procedure was conducted very cautiously to prevent supplementary root damage and losses. Unwanted Debris and dead roots were removed from vital roots.

Preparing Roots for Scanning

The 3-4 acrylic trays were washed with water completely, then by using tissue paper wipe out water, clean properly and dried completely. The tray filled with water (3/4th portion of the tray so that scanning will be cleared) and the roots were placed in a tray. Then the roots were floated in water in the acrylic trays in the scanner, so we need to Arranged these roots to reduce the overlapping and crossing of roots for obtaining accurate image and other details. Plastic forceps were used to arrange the roots in specific manner, this is so delicate work, enough lighting and steady hands are helpful for this work.

Scanning of Roots: For obtaining best results, Win Rhizo Reg 2009 with an approved scanner used for root scanning, which allow the roots to be light from above and below while root being scanned. This is an important feature (called “Dual Scan” in Regent’s documentation), which reduces shadows on the root image. The reagent Positioning System allows the trays to be consistently placed, thus obviating the need to preview each scan. Optimum scanning resolution depends on the type of samples. Generally, roots scanned at 600 dpi in 10×15 cm trays. Root length analysis was carried out with grayscale images.

The Right Threshold Value is Important

Analysis results can be sensitive to the threshold parameters used. Win Rhizo can automatically set these; one you may manually tweak them from time to time. The colour traces on the root indicate where roots have been detected.

Analyzing Scanned Images

For image analysis we need to select the image area or region of interest, and it is analyzed. After image have been scanned, the software uses threshold to determine to determine which one is the root and which is not root or other debris. After few seconds, the analysis was complete and roots found by Win Rhizo were identified by coloured line in image. The colors used for drawing them are coded according to root diameter. Portions of the image can be excluded from analysis if necessary, and there are basic tools available if any minor image editing is required.

Saving of Measured Data

The last step of root analysis was data saving Win Rhizo knows when data was easily recordable by many programs including spreadsheet style like Excel. Image and their analysis were also saved to file for later validation, reanalysis, or for visualization in other software programs. Rhizotron observation for plant production and root trait.

The data was recorded after 45 days after sowing in which Total root length, root diameter, root volume and surface area will be recorded by using root scanner.

- **Root Length:** The total root length was recorded in cm unit from the point of attachment to shoot to the tip of root using scale.
- **Total Root Length:** The total root length was measured by root scanner in millimetre.
- **Root Weight (Fresh):** The fresh root weight was measured by using the weighing balance in grams.
- **Root Weight (Dry):** The dry root weight was measured on weighing balance after oven drying for 24 hrs.
- **Total Root Diameter:** The root diameter was measured by root scanner after root scanning in mm.
- **Root Surface Area:** The root surface area was measured by root scanner in cm^2.
- **Root Volume:** The root volume was measured by root scanner in cm^3.

A B

Fig 1: (a) Study of root trait of rice genotypes under rhizotron condition. (b) Root scanners (EPSON PERFECTION V700)

Results and Discussion

Root Length (cm)

Root length is the length from the base of the root to the end of the root tip it may include the length of the single long root. A perusal of data illustrated in Table 2 indicated that root length of rice genotypes was significantly affected under the root phenotypic studied by using mini rhizotron. The root length of different rice genotypes was varied from 22.00 to 52.00 cm. The maximum root length was observed in Poornima (LC) followed by RFU 329, RFU 327 and RFU 304 indicating that these entire genotypes were performed better under water stress condition by avoiding drought stress. Whereas, the minimum root length was observed in RFU 316 and RFU 317 genotypes were sensitive for various drought conditions. Since, deeper roots offer plants with improved access to stored water and nutrient uptake in the deeper layers of the soil substratum (Vejchasarn *et al.* 2016) [14].

Total Root Length (mm)

The total root length is the addition of length of the entire roots which includes the main root length and length of lateral root with their branching. Analysis of variations (Table 2) showed that total root length was varying significantly among rice genotypes under the root phenotypic studied and the total root length of different rice genotypes was ranged from 2105.00 to 3083.43 mm. The maximum total root length was observed in Poornima (LC) (3083.43 mm) followed by RFU 329, RFU 327, RFU 304 were performed better and the minimum total root length was observed in RFU 306(2105.00 mm) followed by RFU 313,314 under drought stress conditions (Lanna *et al.* 2019) [6].

Fresh and dry Root Weight (gm)

The fresh and dry root weight of rice genotypes under the root phenotypic study was presented in Table 2. A wide range of fresh and dry root weight was observed among thirty genotypes, which is helpful to identify the water stress rice genotypes. The fresh and dry root weight of different rice genotypes were ranged from 18.00 gm to 32.00 gm and from 6.66 gm to 11.56 gm respectively. The maximum fresh and dry root weight (32.00 gm and 11.56 gm) was observed in Poornima (LC) followed by RFU 329, RFU 327, RFU 304 genotypes which were performed as tolerance to drought stress. Whereas, the minimum fresh and dry root weight (18.00 gm and 6.66 gm) was observed in RFU323followed by RFU 322,306 and indicated as sensitive genotypes for water stress condition (Tiwari *et al.* 2019) [13].

Total Root Diameter (mm)

Data presented in Fig 2 indicated that total root diameter of rice genotypes was found varying non significantly under the root phenotypic study by using mini rhizotron. The total root diameter of different rice genotypes was varied from 0.24 to 0.46 cm. The maximum total root diameter (0.46 mm) was observed in Poornima (LC) followed by RFU 329, RFU 327, RFU 304, indicating that these entire genotypes can perform better under drought stress conditions by avoiding drought stress. Whereas the minimum total root diameter (0.24 mm) was observed in RFU 315 and RFU 317, indicating that these genotypes are sensitive for various drought conditions. The higher root diameter offer plants with improved access to stored water and nutrient uptake in the deeper layers of the soil substratum (Clarck 2011; Wasaya 2018) [5, 15].

Table 2: Phenotyping study of different root traits of rice genotypes by using mini-rhizotron

Rice genotype	Root Length (cm)	Total Root Length (mm)	Fresh Root Weight (gm)	Dry Root Weight (gm)
RFU 301	36.00	4076.00	23.34	8.48
RFU 302	40.00	3515.00	23.00	8.41
RFU 303	42.00	2518.00	21.00	7.71
RFU 304	45.00	4529.00	27.00	9.81
RFU 305	36.00	4166.00	24.00	8.76
RFU 306	37.00	2105.00	19.00	7.01
RFU 307	38.00	2515.00	24.00	8.76
RFU 308	33.00	2402.00	23.00	8.41
RFU 309	31.00	4212.00	21.00	7.71
RFU 310	30.00	3492.00	23.00	8.41
RFU 311	28.00	3353.00	24.00	8.76
RFU 312	29.00	2605.00	22.00	8.06
RFU 313	30.00	2409.00	24.00	8.76
RFU 314	36.00	2409.00	21.00	7.71
RFU 315	38.00	3164.71	21.00	7.71
RFU 316	22.00	3099.62	22.00	8.06
RFU 317	26.00	3069.95	23.00	8.41
RFU 318	30.00	3109.37	20.00	7.36
RFU 319	32.00	3007.97	26.00	9.46
RFU 320	36.00	2925.26	23.00	8.41
RFU 321	27.00	2983.85	25.00	9.11
RFU 322	28.00	3017.34	19.00	7.01
RFU 323	30.00	3061.29	18.00	6.66
RFU 324	28.00	2979.10	20.00	7.36
RFU 325	32.00	2942.46	24.00	8.76
RFU 326	33.00	2913.14	23.00	8.41
RFU 327	48.00	2935.15	28.00	10.16
RFU 328	42.00	2972.73	21.00	7.71
RFU 329	49.00	3012.99	30.00	10.86
Poornima (LC)	52.00	3002.16	32.00	11.56
Mean	34.80	3083.43	23.15	8.46
CD	15.24	637.25	NS	NS
S.Em±	5.26	224.53	3.51	2.45

Root Surface Area (cm^2)

A perusal of data summarized in Fig 2 indicated that the root surface area was significantly differed among rice genotypes under the root phenotypic study. The root surface area of different rice genotypes was ranged from 15.67 to 60.00 cm^2. The maximum root surface area (60.00 cm^2) was observed in Poornima (LC) followed by RFU 329, RFU 327, RFU 304 and the minimum (15.67 cm^2) was observed in RFU 308 followed by RFU 307 indicated as drought tolerance and drought sensitive genotypes respectively (Lanna *et al.* 2019; Tiwari *et al.* 2019) [6, 13].

Root Volume (cm^2)

The root volume among rice genotypes was found varying significantly under the root phenotypic study by using mini rhizotron illustrated in Fig 2 the root volume of different rice genotypes was ranged from 0.32 to 2.62 cm^3. Among the genotypes the maximum root volume was observed in Poornima (LC) (2.62 cm^3) followed by RFU 329, RFU 327, RFU 304 genotypes were avoiding drought stress. Whereas, the minimum root volume was observed in RFU 311and 318 (0.32 cm^3) followed by RFU 319 genotypes were sensitive to drought condition (Wasaya 2018; Panda *et al.* 2017) [15, 19].

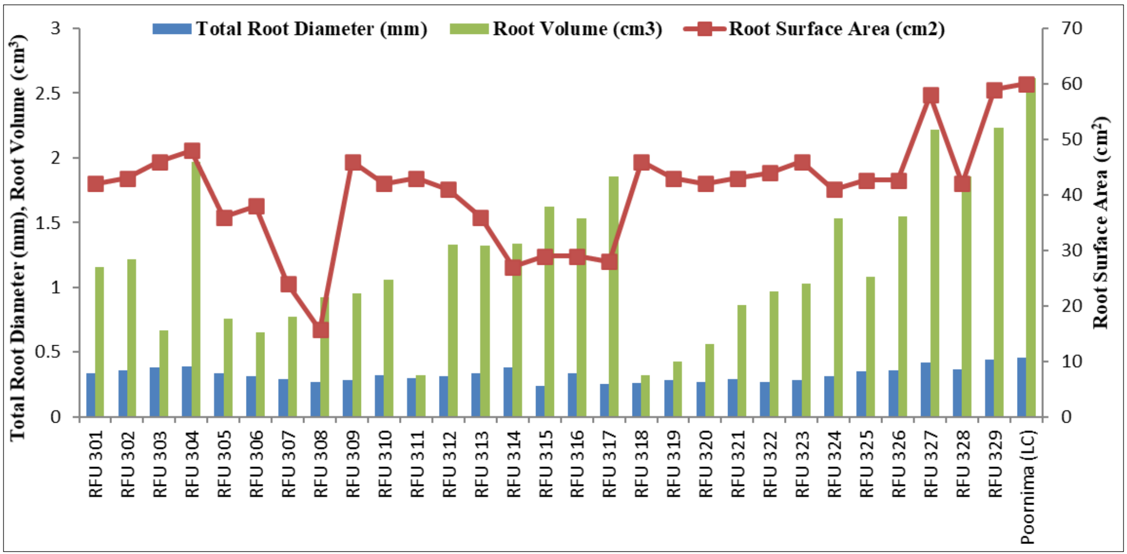

Fig 2: Phenotyping of different root traits of rice genotypes by using rhizotron glass plates

Conclusion

The image base root Phenotyping of different rice genotypes using in mini-rhizotron the local check Poornima was registered relatively better in traits in terms of root length, fresh and dry root weight, root diameter, root surface area, root volume followed by RFU 329, RFU 327, RFU 304 due to root parameters determines the hydraulic conductance of root that can potentially increase water uptake and contribute to improved water use efficiency in rice under water limited conditions.

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