

Original Research Article

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Unveiling The Hidden Half: Root Phenotyping of Rice for Enhanced Drought Tolerance under Rainfed Conditions

Shambhavi Dubey^{ID}, Vidhya Bhushan Kuruwanshi^{ID}*, Arti Guhey and Sunil Verma

Indira Gandhi Agriculture University, Krishak Nagar, Raipur - 492012, India

*Corresponding author

ABSTRACT

Drought is a major abiotic stress that reduces crop productivity and weaken the global food” security. Rice (*Oryza sativa* L.) is a semi-aquatic plant and it requires an adequate amount of water but drought has a detrimental effect on crop growth factors and caused a 25.4% decreased in rice output. It is therefore, imperative to understand the mechanism of plant responses to water deficit conditions and finding the rice germplasm that can withstand intermittent water stress in order to support crop development initiatives for periods of intermittent drought. The present investigation entitled “Unveiling The Hidden Half: Root Phenotyping of Rice for Enhanced Drought Tolerance under Rainfed Conditions” was carried out with the objective to quantify the variations existed among the genotypes. The planting materials used in the experiment consisted of 28 genotypes in which 27 rice genotypes obtained from IIRR, Hyderabad under the AICRP on rice program and 01 check variety from IGKV, Raipur. To understand the root dynamics, the root phenotyping study was conducted in rainout shelter of IGKV research farm using glass mini-rhizotron and root morphology scanning was done in Department of Plant Molecular Biology and Biotechnology lab. The root studied was carried out following the completely randomized design with three replication and two water treatments (watering regularly and watering after 2 days of interval). The field experiment was laid out in randomized block design with three replications under two water regimes (irrigated and rainfed) for two years (2020 and 2021) during *kharif* season. The results of analysis of variance revealed that the presence of greater diversity among the genotypes for all the observed root traits. Based on mean performance, the highest root length was observed in DT-127, genotype high total root length (DT-127), total root surface area (DT-127), root average diameter (DT-108), root volume (DT-127) and root anatomy (DT-122) in root phenotyping. Similarly, the genotypes were identified for higher total root length (DT-122), total root surface area (DT-122), root average diameter (DT-114), root volume (DT-111), root anatomy (DT-122) under rainfed conditions. The most of genotypes were showed variability across the genotypes for various attributes and genotypes were decreased under rainfed condition as compared to irrigated conditions. The variations among the genotypes and checks *i.e.*, Indira Barani for each trait were noticed under irrigated and rainfed condition.

Keywords

Rice, Root Phenotyping, Rainfed, Abiotic Stress, water and nutrients

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Introduction

As the well-known "hidden half," the root system is crucial to the overall growth and development of the plant (Eshel and Beeckman, 2013). Through the intake of water and nutrients, roots have a direct role in the health, development, and survival of plants (Zhu *et al.*, 2011; Takehisa *et al.*, 2012; Sozzani and Iyer-Pascuzzi, 2014). They also affect on the hormonal control of the entire plant as the site of hormone synthesis and consumption (Zhang *et al.*, 2018; Atia *et al.*, 2018; Ramireddy *et al.*, 2018).

Addressing drought, the root system is in charge of the avoidance mechanism that keeps up water absorption and, consequently, a positive status for organic matter in circumstances of low soil water. The fundamental characteristics that propel plants' adaptive response to water scarcity are thought to be their root system's architecture and morphological flexibility during a drought (Henry, 2013; Bristiel *et al.*, 2019; Chaichi *et al.*, 2019), anticipating a direct influence on grain yield stability. However, before being directly applied in breeding programs, the complex relationship between

root traits and the persistence of grain yield during drought needs to be further explored (Dorlodot *et al.*, 2007; Kondo *et al.*, 2003; Gowda *et al.*, 2011; Comas *et al.*, 2013; Han *et al.*, 2016; Li *et al.*, 2017).

The initial information required when examining drought is related to the inter- and intra-annual chance of the occurrence of a water deficit, as well as its severity, length, and timing with respect to rice phenological phases (Heinemann *et al.*, 2008). The dynamics of root system growth and plant plasticity—the capacity to alter growth and structure in different environments—are important factors in plant adaptation to severe water deficits (Matthews *et al.*, 1991; Price *et al.*, 2002a).

By adopting a root system that is more suited for a given environment and has a higher ability to absorb nutrients, changes in the architecture of plant roots may enable increased output levels even in spite of harsh conditions (Lynch, 2007). For a considerable time, roots have been the major obstacle to phenotyping because of their belowground proliferation. Agronomists and breeders have been attempting to define the kinetics, morphology, and structure of the growth of roots in recent times (Henry, 2013; Paez-Garcia *et al.*, 2015; Cendrero-Mateo *et al.*, 2017; Bray and Topp, 2018). The link between anatomical functions, morphological traits, and root developmental processes has been the subject of certain investigations (Lynch, 2007; Lynch, 2014; Gu *et al.*, 2017; Passot *et al.*, 2018).

In rice breeding programs, the hunt for root traits that provide high efficiency in resource uptake—primarily in nutrient and water use efficiencies (NUE and WUE)—has intensified (Bernier *et al.*, 2008; Han *et al.*, 2016; Araus *et al.*, 2018; Mir *et al.*, 2019). This is especially true in upland environments where short dry spells are frequent.

Drought enhances the array of root morphological features in rice, according to findings by Kondo *et al.*, (2003) and Gowda *et al.*, (2011). A set of root traits, including root length, root thickness, root density, root branching, root length density, rooting depth, surface area, root diameter, and the distribution of root biomass in the soil profile, were established by breeders to be used in breeding programs for improving drought tolerance (Price *et al.*, 2002a; Matsui and Singh, 2003; Kondo *et al.*, 2003; Ganapathy *et al.*, 2010; Henry *et al.*, 2012; Kano-Nakata *et al.*, 2013; Kuijken *et al.*, 2015). However, these characteristics do not provide a comprehensive understanding of the root system and do

not enable the determination of the relative contributions of each attribute to the maintenance of grain output under drought (Gowda *et al.*, 2011; Mickelbart *et al.*, 2015; Kadam *et al.*, 2017).

Numerous QTLs and the related genes behind the genetic regulation of several root characteristics in rice have been identified as a result of extensive genetic research (Chen *et al.*, 2013; Uga *et al.*, 2015; Han *et al.*, 2018; Ramanathan *et al.*, 2018; Sandhu *et al.*, 2019). But the primary barrier to using genomics techniques is the lengthy and challenging process of phenotyping root structure characteristics. It is impractical to fully excavate a root system in a field or pot since root system removal is labor-intensive, disruptive, and fraught with the possibility of losing root structure (Masuka *et al.*, 2012; Armengaud *et al.*, 2009; Zhu *et al.*, 2011; Wasson *et al.*, 2012). A wide range of methods, including PVC pipes (Shashidhar *et al.*, 2012; Guimarães *et al.*, 2020), hydroponic systems (Courtois *et al.*, 2013), semi-hydroponic systems (Chen *et al.*, 2017), or rhizotrons (Shrestha *et al.*, 2014), have been employed in recent years to better access the entire root system. The scanning and analysis of plant pictures forms the basis of these phenotyping techniques. Thus, a high number of visuals may be obtained in a short amount of time, improving the capacity for phenotyping, enabling improved precision, and enhancing breeding efficiency (Pratap *et al.*, 2019).

This study examined the plastic response of the rice root system to a water deficit during the panicle initiation phase and evaluated the genetic diversity of the panel of 28 rice genotypes using a root phenotyping platform.

Materials and Methods

Plant Material

The planting materials used in the experiment consisted of 28 genotypes in which 27 rice genotypes obtained from IIRR, Hyderabad under the AICRP on rice program and 01 check variety from IGKV, Raipur (C.G.).

Experimental conditions

The study was conducted in rainout shelter of Research cum Instructional Farm and Department of Plant Physiology, Agricultural Biochemistry, Medicinal and Aromatic Plants, College of Agriculture, Indira Gandhi

Krishi Vishwavidyalaya, Raipur, Chhattisgarh (21°16'N latitude and 81° 26' E longitude with an altitude of 289.56 meters above the mean sea level) during *Kharif* 2020-21 using glass mini-rhizotron and root morphology scanning was done in Department of Plant Molecular Biology and Biotechnology lab.

Three sets of replicates and two water treatments were used in a completely randomized design to conduct the experiment. In order to get rid of the bigger particles, the soil was sieved (125 mm screen). Fertilizer was then administered two days prior to the sowing day, and no fertilization was done between plant cycles.

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 be 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 was opened. So it must be fixed.

Filled the soil in the rhizotron glass plates which is filtered by sieving to avoid any clod 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 had 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

For scanning, each genotype roots were used. Three plants from each genotype roots were utilized for the root

scanning process, which provided comprehensive data on all the characteristics related to the roots, such as their length, volume, diameter, and surface area.

The root scanning was carried out by using root scanner machine Epson Perfection V700/V750, 3.81 Version, Win Rhizo Reg 2009 (Fig. 1.2). The data for various root parameters, such as root length, average root diameter, root volume and root surface area were automatically stored in the computer. The process for root scanning was as follows:

Step 1. Acquiring washed Roots

Getting cleaned roots was the first step. If the plants are cultivated in a solid medium (such as soil), it might be the most challenging and time consuming stage in the experiment. The roots were maintained in the spirit solution (25%) in falcon tubes for root scanning after being carefully rinsed with tap water two or three times to prevent fine roots from damaging the soil. The extreme caution was used throughout the process to avoid further root damage and losses. The dead roots and undesired debris were removed from essential roots.

Step 2. Preparing Roots for Scanning

The three to four acrylic trays were thoroughly cleaned with water, dried and then the water was wiped up with tissue paper. The roots were placed in a tray and the tray was filled with water (about 3/4 of the tray, to allow for scans to be cleaned).

After that, the roots floated in water within the acrylic trays of the scanner; therefore, in order to achieve an accurate image and more information, we must need to organize the roots to minimize overlapping and crossing. The plastic forceps were utilized to position the roots in a precise way; this is a so delicate task that requires calm hands and enough illumination

Step 3. Scanning of Roots

To get optimal outcomes, utilize Win RhizoReg 2009 in conjunction with an authorized root scanning scanner that permits light to reach the roots from both above and below during the scanning process. The regent's literature refers to this feature which lessens shadows on the base image as "Dual Scan." It is not necessary to preview each scan because of the Reagent Positioning System, which makes it possible to arrange the trays consistently.

Scanner resolution at its best varies on the kind of material. Typically, roots are scanned in 10 x 15 cm trays at 600 dpi. The grayscale picture analysis was done for root length.

Step 4. The Right Threshold Value is Important

Results of analyses might vary depending on the threshold settings applied. Although, WinRhizo can configure these automatically, you can occasionally make manual adjustments. The colour traces on the root indicate where roots have been detected.

Step 5. Analyzing Scanned Images

The picture area, also known as the region of interest must be chosen before image analysis is begun. The software utilizes threshold to identify which picture is the root and which is not or other detritus, once the image has been scanned. A colored line in the picture indicated the roots located by WinRhizo, and the analysis was finished in a matter of seconds. The colors used for drawing them are coded according to root diameter. If any small picture alteration is needed, there are simple tools accessible and portions of the image can be removed from analysis.

Step 6. Saving of Measured Data

The data saving was the final stage of the root analysis process. WinRhizo is able to detect instances in which a variety of tools, such as Excel spreadsheets, might readily capture data. Additionally, the image and its analysis were saved to a file for reanalysis, validation or display in other software applications. The Rhizotron monitoring of root characteristics and plant output.

A WinRhizo root scanner was used to record the total root length, root diameter, root volume, and surface area 45 days after the seeds were sown.

Total Root Length: Using a root scanner, the total root length was measured in millimeters.

Total Root Diameter: Following root scanning, the root scanner measured the root diameter in millimeters.

Root Surface Area: Using a root scanner, the root surface area was calculated in centimeters.

Root Volume: Using a root scanner, the root volume was calculated in cm³.

Results and Discussion

Total Root length (mm)

The total root length of rice genotypes were varied from 121.9 mm to 1499.6 mm with mean value of 543.0 mm under irrigated condition while, 141.9 mm to 1400.9 mm with mean value of 532.9 mm under rainfed condition. It may be due the total root length tends to increase its length for water under rainfed condition and a deep root system allows the plant to extract deep soil moisture during moisture stress condition.

The maximum total root length was recorded in genotype DT-127 (1499.6 mm) followed by DT-106 (1322.5 mm), DT-116 (1146.7 and minimum root length was recorded in DT-117 (121.9 mm) followed by DT-118 (135.3 under irrigated condition. Whereas, genotype DT-112 (1400.9 mm) recorded maximum total root length followed by DT-125 (1255.0 mm), DT-122 (1208.1 mm) and minimum was found in DT-111 (141.9 mm) followed by DT-123 (143.7 mm) under rainfed condition.

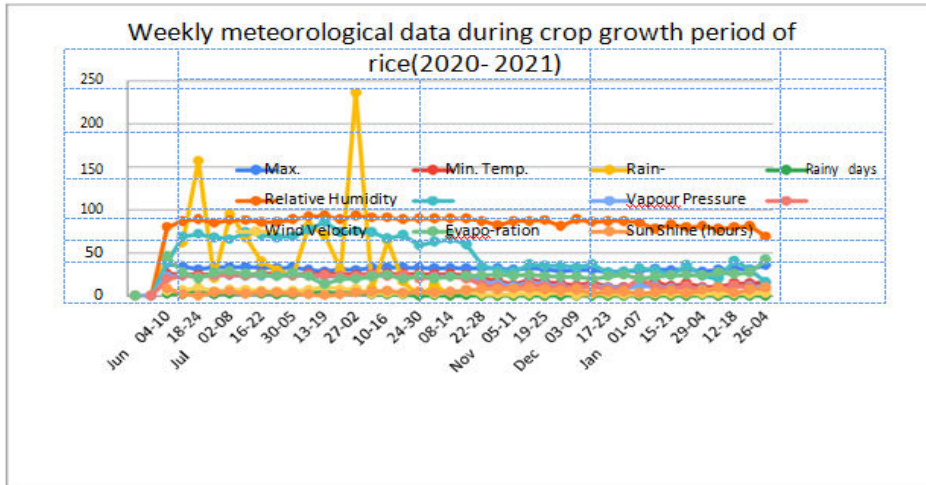
The overall total root length percent increased was recorded in DT-122 (80.0%) followed by DT-125 (71.3 %) and DT-103 (68.3 %), however there were some genotypes which reduced its length under rainfed condition as compared to irrigated condition.

The root length increased under drought condition due to involvement of phytohormones mainly auxin, cytokinin and ABA which plays an important role in drought-stress adaptation of plants. Whereas, the maximum total root length reduction was recorded in genotype DT-127 (78.5 %) followed by Indira Barani (69.5 %) and DT-116 (65.5). [Wullschleger and Hanson \(2006\)](#) also reported that ABA reduced transpiration and increased soil volume for water absorption, induction of stomata closure, leaf surface reduction, and a rise in the root : shoot ratio.

Total Root surface area (cm²)

The total root surface area ranged from 16.56 cm² to 140.92 cm² with mean value of 62.86 cm² and 12.28 cm² to 83.15 with average of 50.42 cm² under irrigated and rainfed condition respectively. The maximum root surface area was recorded in DT-127 (140.9 cm²) followed by DT-104 (118.1 cm²), DT-126 (116.1 cm²) and the minimum root surface area was recorded in DT-117 (16.5 cm²) followed by DT-111 (17.1 cm²) DT-119 (23.0 cm²) under irrigated condition.

Figure.1 Weekly meteorological data during crop growth period of rice (2020-2021)



(Source – Department of Agro meteorology, IGKV, Raipur)

Figure.2 Root Phenotyping study of rice genotypes under rhizotron condition

Rhizotron Glass Plates	Soil Filled Rhizotron
	
Seedling in rhizotron	Preparing Roots for Scanning
	
Analyzing scanned images	
	

Figure.3 Root Phenotyping study of rice genotypes under rhizotron condition



Figure.4 Mean performance of total root length (mm) of rice genotypes under irrigated and rainfed condition during year 2020

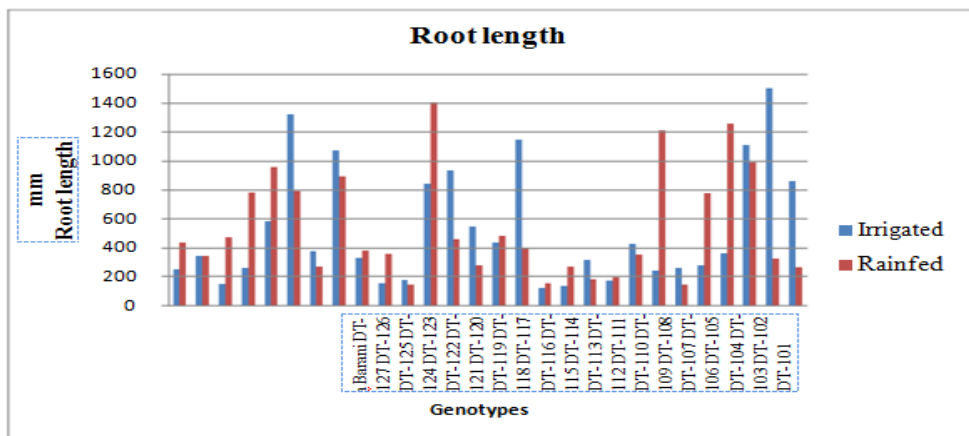


Figure.5 Mean performance of total root surface area (cm²) of rice genotypes under irrigated and rainfed condition during year 2020

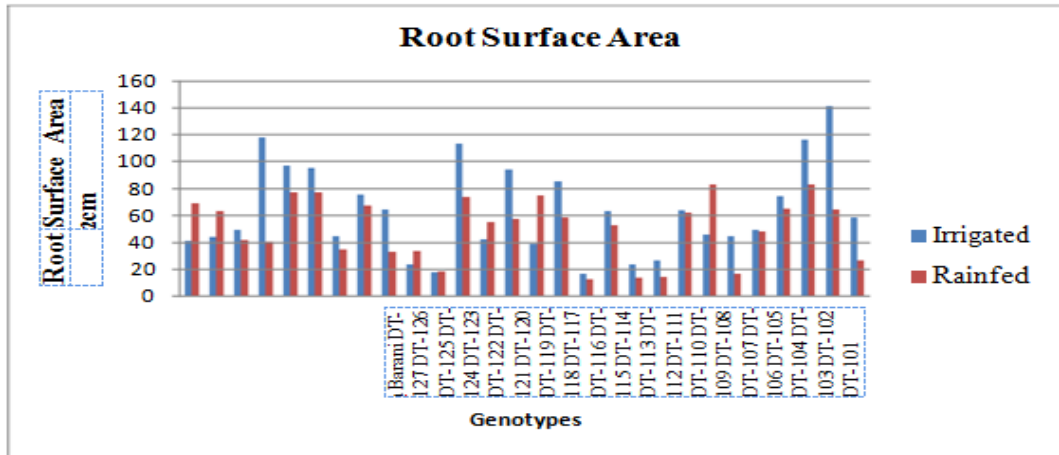


Figure.6 Mean performance of average root diameter (mm) of rice genotypes under irrigated and rainfed condition during year 2020

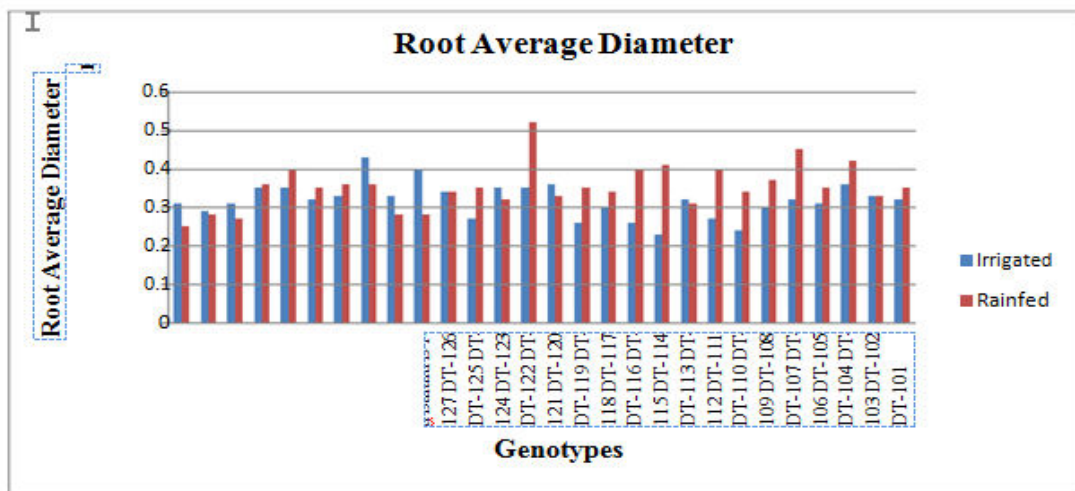
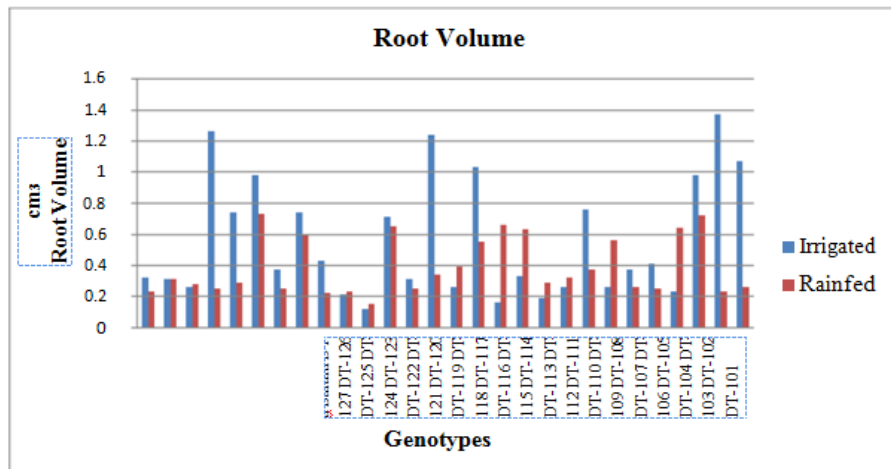


Figure.7 Mean performance of root volume (cm³) of rice genotypes under irrigated and rainfed condition during year 2020



Root average diameter (mm)

The root average diameter ranged from 0.23 mm to 0.43 mm with mean value of 0.31mm and 0.25 mm to 0.52 mm with mean value of 0.35 mm under irrigated and rainfed condition respectively. The maximum root average diameter was recorded in DT-108 (0.43 mm) followed by DT-110 (0.40 mm) and DT-115 (0.36 mm) and the minimum average diameter was recorded in DT-119 (0.23 mm) followed by DT-122 (0.24 mm) DT-118 (0.26 mm) under irrigated condition. While, rainfed condition, genotype DT-114 (0.52 mm) recorded maximum followed by DT-124 (0.45 mm) and DT-126 (0.42 mm). It might be due to the higher main root diameter plants are more capable of growing because they can better explore compact soil and have a direct correlation with water absorption. However, the minimum root average diameter was observed in DT-101(0.25 mm) followed by DT-103 (0.27 mm) and DT-110 (0.28 mm) as compared to rest of genotypes under rainfed condition.

The overall maximum root average diameter percent increased was found in genotype DT-119 (43.9%) followed by DT-128 (35.0 %) and DT-114 (32.7 %). It may be due to over expression of OsNAC5 (Jeong *et al.*, 2013) and OsNAC10 (Jeong *et al.*, 2010) in rice roots under drought condition.

However, genotype DT-110 (30.0 %) followed by DT-101 (19.3 %) and DT-108 (16.2%) were reduced maximum root average diameter under rainfed condition as compared to irrigated condition. A similar result was also reported by Wasaya (2018).

Root volume (cm³)

The amount of water that a plant can hold in its roots is directly influenced by its root volume. The root volume varies from 0.12 cc to 1.37 cm³ and mean value 0.56 cm³ and 0.15 cm³ to 0.73 cm³ with average of 0.38 cm³ under irrigated and rainfed condition respectively. Under irrigated condition, the maximum root volume was observed in DT-127 (1.37 cm³) followed by DT-104 (1.26 cm³), DT-114 (1.24 cm³) and minimum was recorded in DT-111 (0.12 cm³) followed by DT-117 (0.16 cm³) DT-119 (0.19 cm³) among the rice genotypes. While, genotype DT-106 (0.73 cm³) recorded maximum followed by DT-126 (0.72 cm³), DT-127 (0.66 cc) and minimum root volume was observed in DT-111 (0.15 cm³) followed by DT-109 (0.21 cm³) and DT-127 (0.23 cm³) under rainfed condition.

As results shown that the highest percent increased was recorded in DT-117 (75.8 %) followed by DT-125 (64.1 %) and DT-122 (53.6 %) because genotypes with more expansive root volumes are better able to withstand dry spells due to they have ability to drawn from reservoir of stored water.

However there were maximum reduction were found in genotype DT-127 (83.2 %) followed by DT-104 (80.1 %) and Indira Barani (75.7 %) under rainfed condition comparison with irrigated condition. These findings are in accordance with the results of Wasaya (2018) and Panda *et al.*, (2017).

The results from the analysis showed the existence of greater diversity between the genotypes for all the observed root traits. Based on mean performance, the highest total root length (DT-127), total root surface area (DT-127), root average diameter (DT-108), root volume (DT-under irrigated condition. Similarly, the genotypes were identified for higher total root length (DT-122), total root surface area (DT-122), root average diameter (DT-114), root volume (DT-111), and root anatomy (DT-122) under rainfed conditions.

Author Contributions

Shambhavi Dubey: Investigation, formal analysis, writing—original draft. V. B. Kuruwanshi: Validation, methodology, writing—reviewing. Arti Guhey:—Formal analysis, writing—review and editing. Sunil Verma: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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