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Screening Root Traits of Rice Landraces Seedlings (*Oryza sativa* L.) Under Induced Drought Stress using Hydroponic System

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Authors' contributions

This work was carried out in collaboration among all authors. Author SJP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SV and AJJ managed the analyses of the study. Author NS managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Drought is one of the most abiotic stress factor that limit the rice production worldwide. Drought stress induces the osmotic stress to plants. This study was conducted under hydroponics system with the three rice landraces 337 – IC116006, 101 – IC464685 and 224 – IC463809 along with drought tolerant check variety Anna (R) 4 under drought stress during seedling stage. The treatments were applied at 20 days old seedlings by imposing stress with PEG 6000 (-7 bar) along with control. The root traits *viz.,* root length, root fresh weight, root dry weight, root volume, root tissue density and lateral root numbers were measured ten days after stress. In this study, the landrace 337 – IC116006 was observed as a tolerant, 101- IC464685 was observed as a moderately tolerant whereas landrace 224- IC463809 was observed as a susceptible. The root traits are the indicators which could contribute for the vigour and growth of the rice landraces at their later stage. The positive adaptive drought tolerance of rice landraces during seedling stage can be utilized as a trait in the breeding programs development for the drought tolerant cultivar.

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Keywords: Rice landraces; hydroponics; Polyethylene glycol; root; drought tolerance; susceptible.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the main staple cereal crop, feeding the daily calories for ever increasing population around the world, particularly in Asia [1]. The FAO estimates that the worldwide food production ought to increase over 40 % by 2030 and 70 % 2050. It is estimated that a rice is required an additional amount of 381.4 million tonnes by 2030 [2]. Southern Tamil Nadu is the treasure home for huge number of traditional rice landraces and majority is undiscovered. Drought is one of the most significant factors limiting rice yield, as it occurs for variable periods of time and severity [3]. Drought influences the growth and development of rice by causing various changes at the morphological levels [4].

Water is necessary for the growth and development of rice plants [5, 6]. The moisture absorption from deeper soil layers has an advantage over roots with an increasing penetration rate [7]. For screening of rice genotypes, Poly ethylene Glycol (PEG) is used. PEG could be a basic, fast and preparatory bioassay that can be utilized in mass screening for assessing seedling of rice genotypes under drought stress condition [8]. PEG 6000 induced drought have the positive adaptive trait responses of rice genotypes [9]. Screening of rice genotypes under hydroponics could be an efficient mutual and increase the precision, as it free from soil associated stress problems [10].

Effective selection of high-yielding rice genotypes under drought stress is required and stringent drought screening, distinguished drought-susceptible lines from drought tolerant lines [11]. A decrease in root xylem diameter can lead an increase in hydraulic conductivity under adequate accessibility of moisture under drought tolerance [12]. To counteract the adverse effects of environmental insults, plants have evolved efficient defense mechanisms by manipulating their tolerance potential through integrated cellular and molecular responses [13]. If drought stress develops after panicle initiation, the number of spikelets were reduced and might resulted in the reduction of yield [14, 15].

The important management strategy to overcome the yield loss under drought condition is to develop the drought tolerant cultivars. Hydroponic screening of rice genotypes is depending on the ability of the seedling to flourish in nutrient solution. Hydroponics screening is done under controlled conditions has an advantage of precision. The rice landraces were reported to exhibit high tolerance under abiotic stresses [16]. Hence, the present study has advantage of precision to identify the root based traits for the drought tolerance of rice landraces.

2. MATERIALS AND METHODS

The hydroponics experiments were conducted in the glass house at Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore during 2021. The four rice genotypes namely Anna (R) 4, 337- IC116006, 101-IC464685 and 224- IC463809 were used for screening under hydroponics by inducing drought stress.

2.1 Experimental Setup for Drought Treatment under Hydroponics

The dormancy broken viable rice seeds were soaked in water and kept overnight. The seeds were surface sterilization by using fungicides. To induce uniform seed germination, the intact seeds were incubated at 28^0C for 48 hours. The germinated seeds were placed in the fabricated thermacoal sheet covered by wire mesh were floated on the solution. Two sets of floats namely control and the treated (PEG 6000 at -7 bar) were maintained as a treatment. This setup was kept in nutrient solution for 30 days with a pH at 4.5 to 5.5 was maintained by adding concentrated HCl. The seedlings of rice landraces 337- IC116006 and 101-IC464685 were shown in the Fig. 1. and the rice genotype Anna (R) 4 and the rice landrace 224- IC463809 were depicted in the Fig. 2. The screening studies was fitted with the experimental design Factorial Completely Randomized Design (FCRD) with five replications and two treatments (Control, PEG 6000:-7bar). Drought stress was imposed at 20 days old seedling grown in the Yoshida nutrient solution [17]. Under this treatments, PEG 6000 -7 bar were sustained for 10 days. The non-stressed plants were also maintained in a hydroponic nutrient solution.

2.2 Nutrient Solution Preparation

The nutrient solution contains an appropriate proportion of both the macro and micro nutrients.

Macro nutrients and micro nutrients were stored in bottles and prepared separately. The prepared nutrient solution were dissolved separately in 50 ml distilled water and then by using a magnetic stirrer for proper mixing of everything together. The volume was made up to 1000 ml by using the distilled water and mixture is prepared. The distilled water contains pH less than 5.5. The nutrient solution's pH is measured for both control and stress treatment which were maintained at the pH of 5.0. [18]. pH was monitored regularly by using portable pH meter. The deviation in pH from 4.5 to 5.3 was adjusted by using concentrated HCl and NaOH. Components of nutrient solution are provided in Table 1.

2.3 Root Performances of Seedlings under Drought Stress

Observation on seedling traits: Observations were taken after 10 days imposing drought stress. Four plants with five replication of root length was measured from the collar region to the longer root tip. The root fresh and dry weight was measured manually. Root fresh weight (RFW) were also measured separately. Root dry weight (RDW) was taken by drying the plants in a hot air oven at 50^0C for 3 days. Dry root are also measured. By using WinRHIZO software, root volume, root tissue density and lateral root numbers were measured.

Reduction percentage was calculated based on this conversion for all root traits.

$$\%\ \text{Reduction} = \frac{\text{Value of PEG} - \text{Value of Control}}{\text{Value of Control}} \text{x } 100$$

2.4 Root traits measured by WinRHIZO Software

The root samples of control and drought induced plants were collected for root image analysis. The roots are placed in the tray which contains water to avoid the roots overlapping [Image 1]. The setup was placed in the dual scan optical image analyzer. Root image acquisition is the rapid technique to evaluate the root architecture and it is easy and rapid at early seedling stage [Image 2.].The instrument WinRHIZO optical scanner (version 5.0) software was used to acquire the roots for all rice genotypes and the image were taken at 400 dpi resolution with color scale [19]. The major root growth traits like total root length (TRL), Root volume (RV), root fresh weight (RFW), root dry weight (RDW), root tissue density (RTD) and lateral root numbers (LRN).

2.5 Statistical Analysis

The collected data on different root parameters were analyzed and subjected to an Analysis of Variance (ANOVA) under Factorial Completely Randomized Design (FCRD) by using AGRES software. Least significance difference was calculated at 5% level of significance.

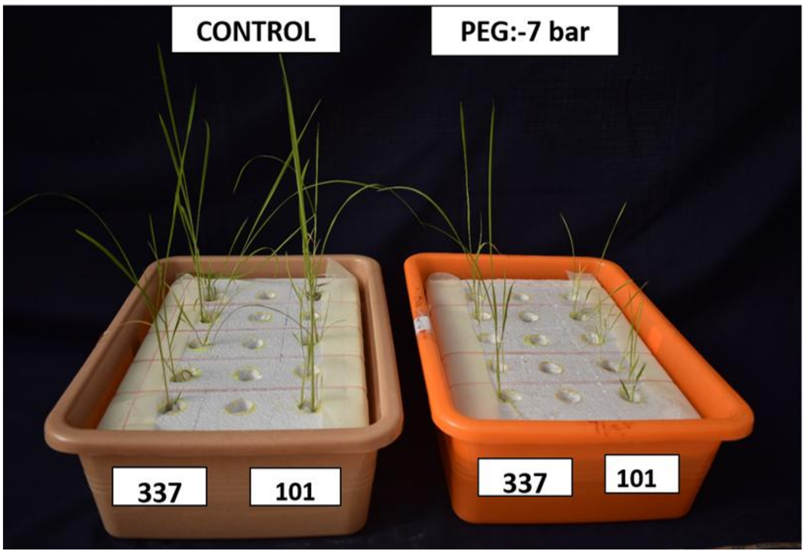

Fig. 1. Effect of control and PEG 6000 (-7 bar) on rice genotypes 337 –IC 116006 and 101 –IC 463809

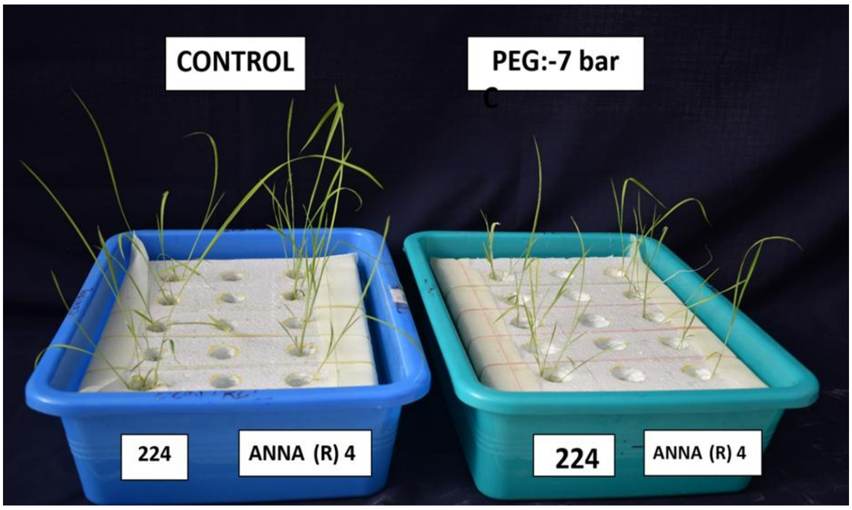

Fig. 2. Effect of control and PEG 6000 (-7 bar) on rice genotypes 224- IC 464685 and Anna (R) 4

Table 1. Components of Yoshida nutrient solution

Stock No.	Reagent (AR grade)	Preparation (g/L)	Concentration of stock / L of nutrient solution
Stock I	NH_4NO_3	91.4	1.25
Stock II	$NaH_2PO_4.2H_2O$	35.6	1.25
Stock III	K_2SO_4	71.4	1.25
Stock IV	$CaCl_2.2H_2O$	117.35	1.25
Stock V	$MgSO_4.7H_2O$	324	1.25
Stock VI	$MnCl_2.2H_2O$	1.5	1.25
	$(NH_4)_6Mo_7O_{24}.2H_2O$	0.074	
	$ZnSO_4.7H_2O$	0.035	
	H_3BO_3	0.934	
	$CuSO_4$ $.5H_2O$	0.031	
	$FeCl_3.6H_2O$	7.7	
	$C_6H_8O_7.H_2O$	11.9	

Image 1. Roots without overlapping

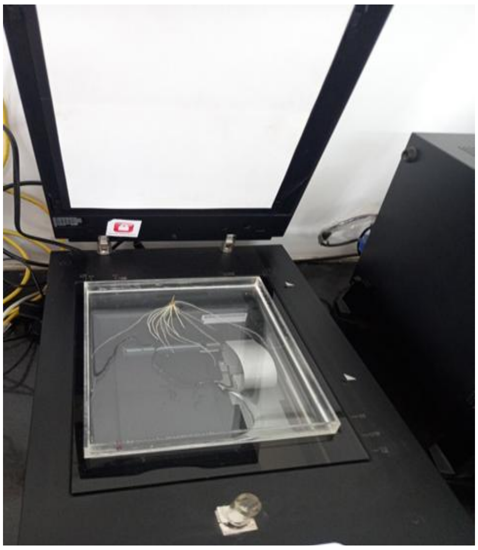

Image 2. WinRHIZO - Root image analyzer

3. RESULTS AND DISCUSSION

3.1 Root Length (cm)

Root length is an important trait under drought stress condition because longer root growth has a resistant ability under drought condition [20]. Root length influences water and nutrient uptake, root growth maintenance during drought was importance for drought tolerance. Data pertaining to root length was represented in the Fig.3. Showed that root length was significantly increased under drought conditions when compared to control. Significant increase in root length was observed in rice landrace 337-IC116006 under control (18.41 cm) and drought (14.75cm) followed by moderately tolerant landrace 101-IC464685 under control (16.13cm) and drought (13.63cm). However, minimum root length was expressed in susceptible landrace 224- IC463809 under control (15.68 cm) and drought (12.64 cm) when compared to the check variety Anna (R) 4 under control (19.71 cm) and drought (17.61 cm). The other rice landrace 101-IC 464685 have a root length lesser than the tolerant genotypes and had higher root length than the susceptible genotypes. There is a lesser reduction of root length in Anna (R) 4. The capacity of plants to absorb water from the deeper layers of soil and is produced by better root penetrability is root length. Maximum PEG 6000 (-7 bar) makes water unavailable to seeds affecting the imbibition process of seed which is a fundamental process for germination and hence, the root length was affected under stress [21].

3.2 Root Fresh Weight (g)

Data pertaining to root fresh weight was represented in Fig.4. The recorded data showed that root fresh weight was significantly decreased under drought conditions (0.26g) compared to control (0.36g). Among the four genotypes, the rice genotype Anna (R) 4 showed significantly higher root fresh weight under both control (0.45g) and drought (0.37g) conditions compared to other landraces followed by rice landrace 337-IC116006 under both control (0.38g) and drought (0.28g) conditions. The rice landrace 224-IC463809 recorded lower root fresh under control (0.28g) and drought (0.17g) conditions followed by moderately tolerant landrace 101-IC464685.Significant interactions were observed between the genotypes and treatments. The reduction in root fresh weight under moisture deficit conditions is due to decline in cell size and reduction in the rate of cell division and also reduction in depositions of cell wall materials in the cell wall under drought conditions [22]. Under the influence of drought, the availability of moisture to the root cells was reduced which leads to decrease in root growth and development [23]. It seems that the reason for such differences depends on the penetration in deeper depths as well as nutrient uptake, which influences the root weight under drought stress condition [24].

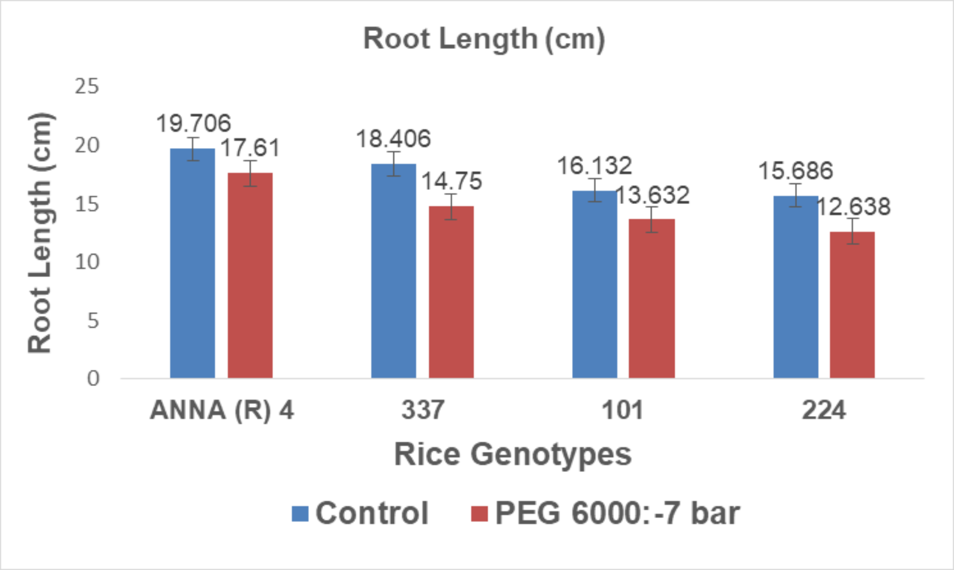

Fig. 3. Effect of drought stress (PEG 6000: -7 bar) on rice root length (cm)

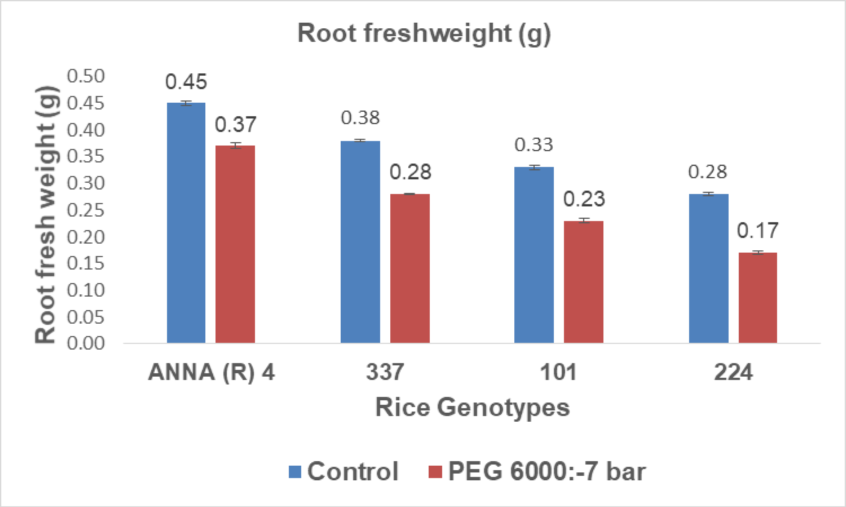

Fig. 4. Effect of drought stress (PEG 6000:-7 bar) on rice root fresh weight (g)

3.3 Root dry Weight (g)

Plant root traits is directly depended on the water availability in the soil. The root dry weight were positive and had significantly decreased under drought stress. Data pertaining to root dry weight was represented in Fig 5. The root dry weight was observed higher in tolerant landrace 337-IC116006 under control (0.23 g) and drought (0.17 g) followed by moderately tolerant landrace 101-IC464685 under control (0.18 g) and drought (0.10 g). However, minimum dry weight was expressed in susceptible landrace 224-IC463809 under control (0.12 g) and drought (0.05 g) when compared to check variety Anna (R) 4 under control (0.37g) and drought (0.28 g).

There is a lesser reduction of root dry weight in Anna (R) 4 as compared to other genotypes. The possible reasons of increased root weight of the rice seedling under drought due to the component trait for drought tolerant has a resistant to water flux aiding in more water acquisition. The effect of drought stress on reduction in a dry matter of plants and water deficit decreases nutrient uptake, transfer and consumption lead leading low dry matter [25]. Many studies have reported that, drought stress causes reduction in the plant root weight [26] and the drought resistance is considered by a small reduction of dry weights under water deficit conditions. The decreasing trend in root dry weight or root dry mass was reported by many researchers [27] found that the drought stress had a significant effect on dry matter production.

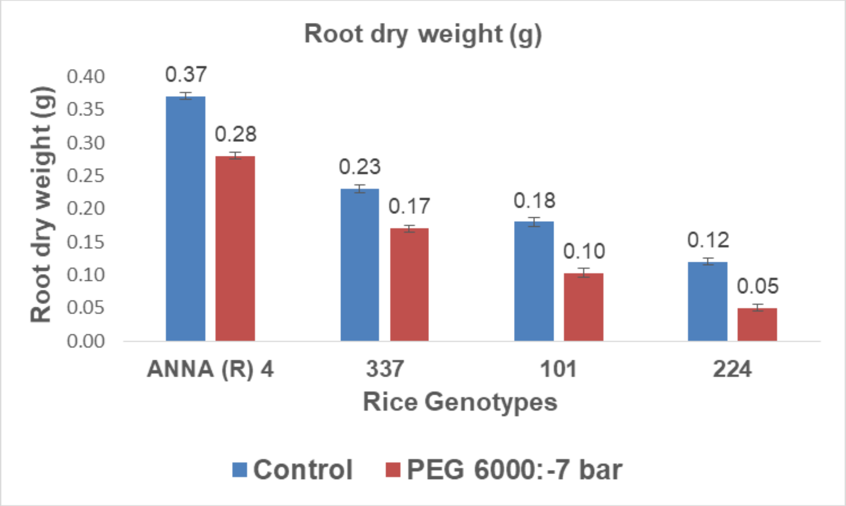

Fig. 5. Effect of drought stress (PEG 6000:-7 bar) on rice root dry weight (g)

3.4 Root Volume (cm^3)

A significant variation was observed in root volume under drought condition compared to control. Data pertaining to root volume was provided in Table 2. Significantly higher root volume was observed in tolerant landrace 337-IC116006 under control (0.228 cm^3) and drought (0.141 cm^3) followed by moderately tolerant landrace 101-IC464685. The susceptible landrace 224 - IC463809 exhibited reduced root volume as compared to check variety Anna (R) 4) and there was a lesser reduction percentage of root volume in 224 - IC463809 (-32.86) than other landraces except Anna (R) 4. Higher root volume could improve the underground hydraulic function under drought stress conditions. The increase in root volume under drought condition is due to altering the cell wall permeability which influences the growth and architecture of root system [28].

3.5 Root Tissue Density (g cm^{-3})

Root tissue density is the ratio of root dry mass to root volume. There was a significant difference between treatments. Data pertaining to root tissue density was provided in Table 3. Root tissue density revealed that a declining trend was observed under drought stress when compared to control. The mean values of root tissue density were 26.97 and 24.29 under control and drought conditions. respectively Significantly higher root tissue density under control (34.66 g cm^{-3}) and drought (31.91 g cm^{-3}) was noticed in landrace 337-IC116006, whereas lowest root tissue density was expressed in landrace 224 - IC463809 under control (17.33 g cm^{-3}) and drought (16.18 g cm^{-3}) followed by moderately tolerant landrace 101-IC464685. There was a lesser reduction percentage of root tissue density was observed in 101-IC464685 (-6.57). Root with high tissue density are generally durable and have been associated with the slow growth strategy [29]. Deep rooting is also a critical factor of root tissue density that influence the capacity of the plant to absorb more water from the deeper layer as well as it controls the specific root length and specific surface area under drought condition [30].

3.6 Lateral Root Numbers ($Plant^{-1}$)

The formation of lateral roots or root branches is a significant determinant of root surface area. Lateral roots contribute to total root biomass, total root length and root surface area. Reduced number of lateral roots was found under drought conditions when compared to control. Data pertaining to lateral root numbers are provided in Table 4. The lateral root number was higher in the tolerant rice landrace 337-IC116006 under control (307.26 $plant^{-1}$) and drought (240.86 $plant^{-1}$), followed by moderately tolerant landrace 101-IC464685. There was a lesser reduction percentage of lateral root numbers in 224 - IC463809 (-12.74). However, lesser root number was expressed in the susceptible landrace of 224 - IC463809 when compared to the check variety Anna (R) 4. A root system with lesser lateral roots reduced the carbon reserves utilized in the metabolic activity to maintain an elaborate root architecture and allocate more resources to the deeper roots to access nutrients [31]. In general, lateral roots develop more root hairs when the plants are exposed to drought stress [32] so as to absorb more water from the root zone that might influences the increase of lateral roots [33].

Table 2. Effect of drought stress (PEG6000: -7 bar) on rice Root volume (cm^3)

Genotypes	Root volume (cm^3)		
	Control	**PEG 6000: -7 bar**	**% Reduction**
Anna(R) 4	0.341	0.179	-47.51
337- IC116006	0.228	0.141	-38.16
101- IC464685	0.179	0.096	-46.37
224- IC463809	0.070	0.047	-32.86
Mean	**0.205**	**0.116**	-43.41
	G	**T**	**G x T**
SEd	0.004	0.003	0.006
CD (P=0.05)	0.008**	0.006**	0.012**

SEd –Standard Error of the difference CD- Critical Difference
P≥0.05 p≥0.01*

Table 3. Effect of drought stress (PEG6000: -7 bar) on rice Root tissue density (g cm^{-3})

Genotypes	Root tissue density (g cm^{-3})		
	Control	**PEG 6000: -7 bar**	**% Reduction**
Anna(R) 4	37.33	31.72	-15.03
337- IC116006	34.66	31.91	-7.93
101- IC464685	18.57	17.35	-6.57
224- IC463809	17.33	16.18	-6.64
Mean	**26.97**	**24.29**	-9.94
	G	**T**	**G x T**
SEd	0.078	0.056	0.111
CD (P=0.05)	0.161**	0.113**	0.226**

SEd –Standard Error of the difference CD- Critical Difference
P≥0.05 p≥0.01*

Table 4. Effect of drought stress (PEG6000: -7 bar) on rice lateral root numbers (Plant^{-1})

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Genotypes	Lateral root numbers (Plant^{-1})		
	Control	**PEG 6000: -7 bar**	**% Reduction**
Anna(R) 4	359.18	277.28	-22.80
337- IC116006	307.26	240.86	-21.61
101- IC464685	280.69	234.46	-16.47
224- IC463809	231.70	202.19	-12.74
Mean	**294.71**	**238.70**	-19.01
	G	**T**	**G x T**
SEd	2.857	2.020	4.041
CD (P=0.05)	5.820**	4.115**	8.231**

SEd –Standard Error of the difference CD- Critical Difference
P≥0.05 p≥0.01*

4. CONCLUSION

The root traits of three rice landraces under drought stress revealed that, the capacity of drought tolerance was found to have high positive correlation with the landraces 337- IC 116006 which showed greater drought tolerance followed by the land race 101- IC 464685 (moderately tolerant). The rice landrace 224 –IC 463809 is susceptible in terms of root characters such as root length, root fresh weight, root dry weight, root volume, root tissue density and lateral root numbers when compared with check variety Anna (R) 4. The results on the root characters of different rice landraces during seedling development being a starter indicators of vigor and growth potential of rice could be expressed during their later stages of growth. Hence, the positive adaptive root traits of rice landraces can be considered for the development of drought tolerant cultivar in the breeding programs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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