Original Research Article

Hydroponics studies to screen the root characters of rice landraces (*Oryza sativa* L.) under drought stress

ABSTRACT

Drought is one of the most abiotic stress factor that limit the rice production worldwide. Drought stress induces the osmotic stress to plants. The studies are 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. Two treatments were given at 30 days old seedlings. Control with another treatment is PEG 6000 maintained at -7 bar. Drought stress was imposed on 10 days old seedlings. Observations were recorded after imposing the drought stress. In this study, rice landrace 337 was identified as a tolerant and 101 was identified as a moderately tolerant. The 224 was a susceptible when compared to check variety Anna (R) 4. The findings on root length, root fresh weight, root dry weight, root volume, root tissue density and lateral root numbers were increased and shown the development of drought tolerance of rice under drought stress. The results reveals that these are the indicators which expressed the vigour and growth potential of rice in 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.

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 forecasts that the global food production will need to increase by over 40 % by 2030 and 70 % by 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 and physiological 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]. The screening of rice genotypes using Poly Ethylene Glycol (PEG) is a simple, rapid and preliminary bioassay that can be used in mass screening for evaluating seedling of rice genotypes under drought stress [8]. PEG 6000 is induced the positive adaptive trait responses of rice varieties towards drought stress [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]. Plants have evolved efficient defense mechanisms by manipulating their tolerance potential through integrated molecular and cellular responses to counteract the adverse effects of environmental insults [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 and remedy 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 the drought induced nutrient solution. Hydroponics screening is done under controlled conditions has an advantage of precision. The rice genotypes were reported to exhibit tolerance under abiotic stresses because the traditional rice landraces are less productive [16]. Thus, the aim of the present study was to screen the rice genotypes under hydroponics to identify the root based traits for the drought tolerance.

2. MATERIALS AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

The hydroponics experiments were conducted in the glass house of 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 imbibed in water for overnight and followed by surface sterilization by using fungicides. The intact seeds were incubated at 28^{0}C for 48 hours to get uniform seed germination. The germinated seeds were placed in the fabricating seedling float with a thermacoal sheet covered by wire mesh were floating on the solution. The seedlings were kept in the holes of floats was placed on the plastic trays containing nutrient solution. The two sets of floats namely control and the treated (PEG 6000 at -7 bar) treatment. This setup were kept in nutrient solution for 30 days with a pH at 4.5 to 5.5 was maintained by adding conc. HCl.

2.2 Initial screening

The screening studies was fitted with the experimental design was Factorial Completely Randomized Design (FCRD) contain five replications and two treatments (Control, PEG 6000:-7bar). Drought stress was imposed at 10 days old seedling grown in the Yoshida nutrient solution [17]. Under this treatments, PEG 6000 -7 bar were sustained for 20 days. The non-stressed plants were grown in a normal strength of the hydroponic nutrient solution.

2.3 Nutrient solution

The nutrient solution contains an appropriate proportion of both the macro and micro nutrients. Macro nutrients were stored in jam bottles and prepared separately. Each element contain a micronutrient solution was dissolved separately in 50 ml distilled water and then by using a magnetic stirrer to mix everything together. The volume is 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 treatment which were maintained at the pH of 5.0. pH is monitored regularly by using portable pH meter by using conc. HCl and

NaOH, the deviation in pH from 4.5 to 5.3, were also adjusted. Components of nutrient solution are given in Table 1. The following composition of nutrients are used as a nutrient solution [18].

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	

2.4 Physiological performances of seedlings under drought stress

Observation on seedling parameters

Observations were taken after 10 days imposing drought stress. Root length was measured from the collar region to the longer root tip. Seedling weight was measured manually. Root fresh weights were also measured separately. Dry weight of the seedling was taken by drying the plants in a hot air oven at 50^0C for 3 days. Dry root are also measured.

Seed germination, seedling growth and vigour index

Seed germination percentage (GP) was recorded at 7 days after sowing in hydroponics. Seedling vigour characteristics of 30 days old seedlings i.e. root length, root fresh weight, root dry weight, root volume, root tissue density and lateral root numbers of four plants with five replication were measured.

2.5. Root development parameters by WinRHIZO analysis

The root samples of control and drought induced plants were collected root image analyzer. The washed root samples were placed in the tray floated with water and arranged the roots without overlapping. The tray was placed in the dual scan optical scanner Root image acquisition is the rapid technique to evaluate the root architecture and it is easy and rapid at early seedling stage. The instrument WinRHIZO optical scanner (version 5.0) software were used to acquire the roots for all rice genotypes and the image was taken at 400 dpi resolution with color scale [19]. The major root growth parameters like total root length (TRL), Root volume (RV), root dry matter (RDM), root tissue density (RTD) and lateral root numbers (LRN).

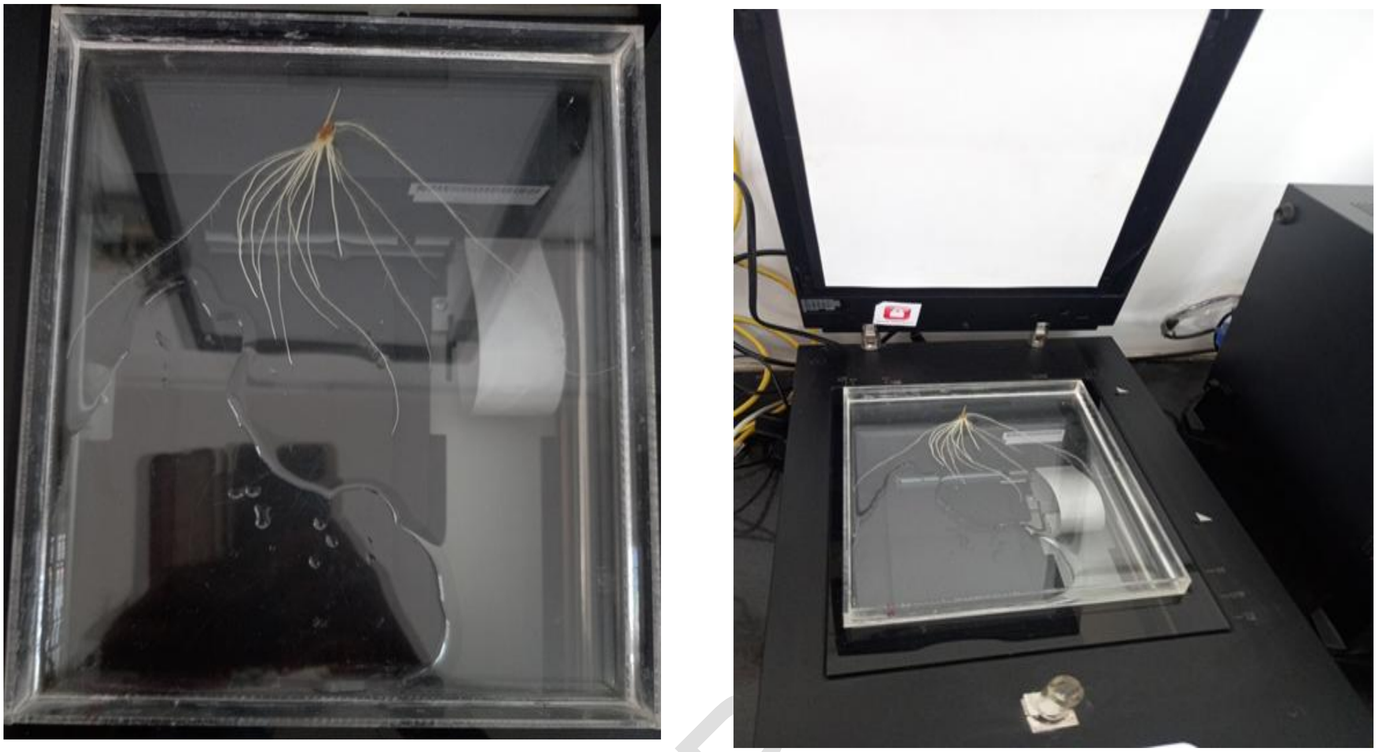

Fig. 1. WinRHIZO - Root image analyzer

2.6 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).

3. RESULTS AND DISCUSSION

Physiological responses under drought stress

Based on the observed data, drought stress imposition after 10 days in hydroponics condition, the rice landraces 337- IC 116006, 224- IC 463809, 101- IC 464685 were showed significant increase ($P < 0.05$) when compared to the check tolerant variety Anna (R) 4. The three rice landraces were classified as tolerant, moderately tolerant and susceptible. Under drought stress, the significantly maximum root traits was expressed by the landrace 337- IC 116006 perform as a tolerant landrace.

3.1 Root length (cm)

Data pertaining to root length was furnished in the Table 2 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-IC 116006 (18.41 cm and 14.75cm) followed by moderately tolerant landrace 101-IC464685 (16.13cm and 13.63cm) respectively. However, minimum root length was observed in susceptible landrace 224- IC463809 (15.68 cm and 12.64 cm) when compared to the check variety Anna (R) 4 (19.71 cm and 17.61 cm) under control and PEG 6000 (-7 bar). 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. Maximum PEG 6000 makes water unavailable to seeds affecting the imbibition process of seed which is a fundamental process for germination [20]. Under stress condition, PEG 6000 induce the drought. Root length influences water and nutrient uptake, root growth maintenance during drought was importance for drought tolerance. Root length is an important trait under drought stress condition because longer root growth has a resistant ability under drought condition [21].

Table 2. Effect of drought stress (PEG 6000: -7 bar) on rice root length (cm)

Genotypes	Root length (cm)		
	Control	PEG 6000 (-7 bar)	Mean
Anna(R) 4	19.71	17.61	**18.65**
337- IC116006	18.41	14.75	**16.57**
101- IC464685	16.13	13.63	**14.88**
224- IC463809	15.68	12.63	**14.16**
Mean	**17.48**	**14.65**	**16.07**
	G	**T**	**G x T**
SEd	0.138	0.098	0.196
CD (P=0.05)	0.282**	0.201**	0.401**

P≥0.05* **P≥0.01**

3.2 Root fresh weight (g)

The root fresh weight was significantly decreased under drought conditions when compared to control. Data pertaining to root fresh weight was furnished in the Table 4. Among the four genotypes, under control and drought, significantly maximum root fresh weight was recorded in tolerant rice landrace 337 (0.38 g and 0.28 g) under control and PEG 6000 respectively. However, minimum fresh weight was recorded in susceptible rice landrace 224 (0.28g, 0.17 g) along with check variety Anna (R) 4 (0.45 g and 0.37 g) under control and PEG 6000: -7 bar. Drought stress caused the reduction in root fresh weight in the present study. The concentration of cell wall materials in a plant increases with maturity and increased cellulose content leading to the increased plant size affects the total fresh weight [22]. Water deficit leads to a decline in cell size, cell division, cell wall composition and decrease in cell growth. Extreme water deficit limit the root growth and development because of the low water availability [23]. It seems that the reason for such differences depends on the penetration in deeper depths as well as nutrient uptake, which leads to the increase in weight of root decreased under drought stress condition. [24].

Table 3. Effect of drought stress (PEG 6000:-7 bar) on rice root fresh weight (g)

Genotypes	Root fresh weight(g)		
	Control	PEG 6000 (-7 bar)	Mean

Anna(R) 4	0.45	0.37	**0.41**
337- IC116006	0.38	0.28	**0.33**
101- IC464685	0.33	0.23	**0.28**
224- IC463809	0.28	0.17	**0.225**
Mean	**0.36**	**0.26**	**0.31**
	G	**T**	**G x T**
SEd	0.005	0.003	0.007
CD (P=0.05)	0.011**	0.007**	0.014**

***P≥0.05** p≥0.01**

3.3 Root dry weight (g)

The root dry weight were positive and had significantly decreased under drought stress. Data pertaining to root dry weight was represented in the Table 6. Under control and PEG 6000, significant the root dry weight was observed in tolerant landrace 337 (0.28g and 0.23 g). However, minimum dry weight was recorded in susceptible landrace 224 (0.12 g and 0.05 g) when compared to check variety Anna (R) 4 (0.37g and 0.28 g) respectively. 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, water deficit decreases nutrient uptake, transfer and consumption lead leading low dry matter [25]. Drought resistance is considered by a small reduction of dry weights under water deficit conditions [26]. The decreasing trend in root dry weight or root dry mass was also reported by other researchers [27] who found that drought stress had a significant effect on dry matter production.

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

Genotypes	Root dry weight(g)

	Control	PEG6000 (-7 bar)	Mean
Anna(R) 4	0.37	0.28	**0.28**
337- IC116006	0.23	0.17	**0.20**
101- IC464685	0.18	0.10	**0.17**
224- IC463809	0.12	0.05	**0.11**
Mean	**0.23**	**0.15**	**0.19**
	G	**T**	**G x T**
SEd	0.007	0.005	0.011
CD (P=0.05)	0.015**	0.011**	0.215*

***P≥0.05** p≥0.01**

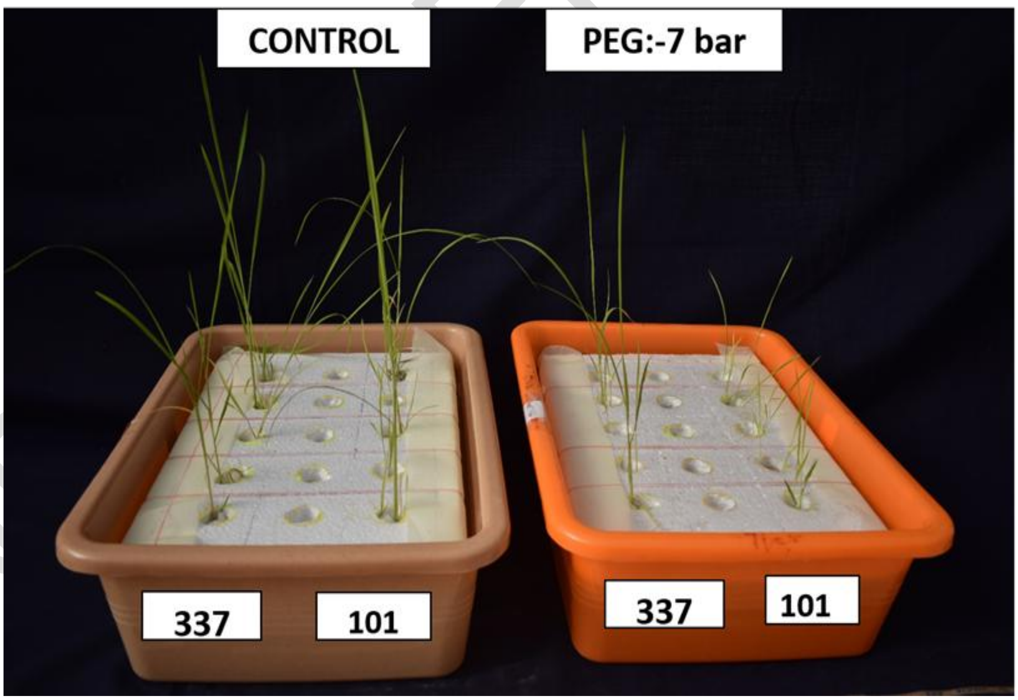

Fig. 2. Effect of control and PEG on rice genotypes 337 –IC 116006 and 101 –IC 463809

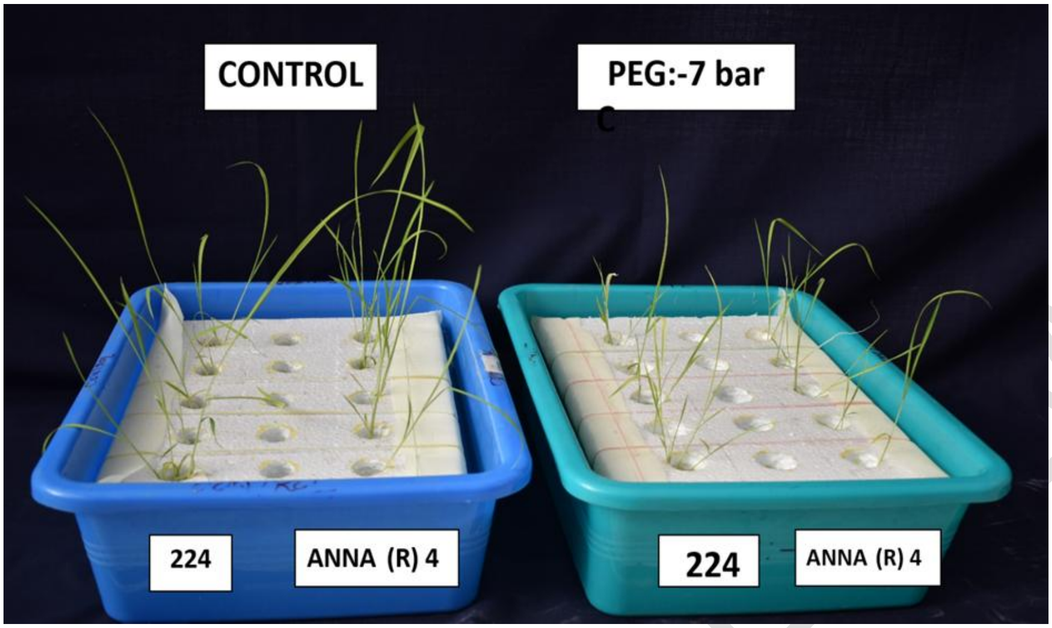

Fig. 3. Effect of control and PEG on rice genotypes 224- IC 464685 and Anna (R) 4

3.4 Root volume (cm^3)

A significant variation was observed in root volume under drought conditions compared to control. Data pertaining to root volume was represented in Table 5. Among two treatments, under control and PEG 6000, significantly highest root volume was observed in tolerant landrace 337 (0.228 cm^3 and 0.141 $cm^{3)}$ respectively. The susceptible landrace 224 (0.07 cm^3 and 0.047 cm^3) was revealed as a significantly lowest root volume as compared to check variety Anna (R) 4 (0.341 cm^3 and 0.179 cm^3). The root volume was measured by placing the rots in measuring cylinder. The increase in water level after placing the root is equal to root volume [28]. Root volume can be measured by the average root diameter and root length. The volume of soil occupied by the root system of the plant. Root volume is increased under drought condition by altering cell wall permeability and influence the growth and architecture of root system [29].

Table 5. Effect of drought stress (PEG -7 bar) on rice Root volume (cm^3)

Genotypes	Root volume (cm^3)		
	Control	PEG 6000: -7 bar	Mean
Anna(R) 4	0.341	0.179	**0.260**
337- IC116006	0.228	0.141	**0.185**
101- IC464685	0.179	0.096	**0.138**
224- IC463809	0.070	0.047	**0.059**
Mean	**0.205**	**0.116**	**0.160**

	G	T	G x T
SEd	0.004	0.003	0.006
CD (P=0.05)	0.008**	0.006**	0.012**

***P≥0.05** p≥0.01**

3.5 Root tissue density (g cm^{-3})

There is significant differences between treatments. Data pertaining to Root tissue density was furnished in Table 6. Root tissue density revealed that a decreased trend was observed under drought stress when compared to control. Under control and PEG 6000, the significantly highest root tissue density (34.66 g cm^{-3} and 31.91 g cm^{-3}) was recorded in landrace 337, whereas the significantly lowest root tissue density was recorded in landrace 224 (17.33 g cm^{-3} and 16.18 g cm^{-3}) as compared to check variety Anna (R) 4 (37.33 g cm^{-3} and 31.72 g cm^{-3}). Root tissue density is the ratio of root dry mass to root volume. Denser root tissue is arranged either thick or thin in diameter. The root and leaf tissue density are generally positively correlated, which suggested that root tissue density aligns with leaf economic traits. Root with high tissue density are commonly long- lived and have been associated with the slow growth strategy [29]. It controls specific root length and specific surface area, increase water uptake under drought condition [30].

Table 6. Effect of drought stress (PEG -7 bar) on rice Root tissue density (g cm^{-3})

Genotypes	Root tissue density (g cm^{-3})		
	Control	**PEG 6000: -7 bar**	**Mean**
Anna(R) 4	37.33	31.72	**34.53**
337- IC116006	34.66	31.91	**33.28**
101- IC464685	18.57	17.35	**17.96**
224- IC463809	17.33	16.18	**16.76**
Mean	**26.97**	**24.29**	**25.63**
	G	**T**	**G x T**
SEd	0.078	0.056	0.111
CD (P=0.05)	0.161**	0.113**	0.226**

***P≥0.05** p≥0.01**

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

A significant reduced number of lateral roots was found under drought conditions when compared to control. Lateral root numbers are significantly decreased under drought conditions. Data pertaining to lateral root numbers are represented in Table 7. The mean values of lateral root number were 294.71 and 238.70 under control and drought conditions. The lateral root number was higher in the tolerant rice landrace 337 (307.26 and 240.86 plant^{-1}), followed by moderately tolerant landrace101 (280.69 and 234.46 plant^{-1}). However lesser root number was observed in the susceptible landrace of (231.70 and 202.19 plant^{-1}) when compared to the check variety Anna (R) 4 (359.19 and 277.28 plant^{-1}) under control and PEG 6000:-7 bar condition.The formation of lateral roots or root branches is a significant determinant of overall root surface area. Lateral roots contribute to total root biomass, total root length and root surface area. A root system with lesser lateral roots reduces the carbon reserves utilized in the metabolic activity to maintain an elaborate root architecture and allocate more resources to the deeper roots to access N [31]. Lateral roots develop more root hairs when the plants are exposed to drought stress [32]. The absorption of more water from the root zone might influence the increase of lateral roots [33].

Table 7. Effect of drought stress (PEG -7 bar) on rice lateral root numbers (Plant-1)

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Genotypes	Lateral root numbers (Plant^{-1})		
	Control	PEG 6000: -7 bar	Mean
Anna(R) 4	359.18	277.28	**318.23**
337- IC116006	307.26	240.86	**274.06**
101- IC464685	280.69	234.46	**257.58**
224- IC463809	231.70	202.19	**216.95**
Mean	**294.71**	**238.70**	**266.70**
	G	**T**	**G x T**
SEd	2.857	2.020	4.041
CD (P=0.05)	5.820**	4.115**	8.231**

***P≥0.05** p≥0.01**

4. CONCLUSION

The morphological and physiological parameters on 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 showed greater drought tolerance followed by moderately tolerant (101- IC 464685). The rice landrace 224 –IC 463809 is susceptible in terms of root characters such as root length (RL), root fresh weight, root dry weight, root volume, root tissue density and lateral root numbers when compared with check variety Anna (R) 4 were found to inferior root performance under drought stress. The results on the root characters of different rice landraces during seedling development being a starter indicators of vigour 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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