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## Research Article

# Rapid screening for drought tolerance in traditional landraces of rice (*Oryza sativa* L.) at seedling stage under hydroponics

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### Abstract

In the present investigation, a total of 50 indigenous rice genotypes collected from rice growing regions of Tamil Nadu were screened for their drought tolerance ability. The experimental setup comprises of hydroponic treatments with different levels of osmotic potential, upto (-) 15 bars in a progressive manner. Phenotypic responses showed that, 30% of the landraces tested were highly susceptible and only 12% were highly tolerant to moisture stress on 26 DAS. The germination percent (GP) was significantly varied between 0.83 to 1.14 for stress (S) and control treatments respectively. The genotypes *viz.*, kuliyadichan and rajalakshmi recorded high germination percent (1.18) compared to other land races under moisture stress condition. The significant mean root length varied from 10.65cm to 4.05cm and 13.65cm to 5.60cm at an OP of (-) 15 bars and control respectively. Similarly, the significant mean shoot length varied from 24.15 cm to 7.35cm under moisture stress ((-) 15 bars) and 38.25cm to 17.15cm under control. The highest R/S ratio was observed in kuliyadichan (0.87) while the lowest ratio was recorded by the genotype annada (0.18) at OP of (-) 15 bars. In addition, high SHR ratio (120.26) was also recorded for kuliyadichan and low SHR (8.99) for virendra. These results suggest that the genotypes kuliyadichan, chandaikar, mallikar, mattaikar, rajalakshmi and nootripattu represent better source-sink relationship. It is concluded that these genotypes can be used as donor candidates towards genetic improvement of drought tolerance (DT) in rice.

### Key words

Rice landraces, drought tolerance, hydroponics, PEG 6000, source-sink relationship

### Introduction

Rice is a staple food for over half of the world's population especially in Asian region, of which India is the second largest producer, next to China. The UN/FAO forecasts that global food production will need to increase by over 40% by 2030 and 70% by 2050. It is estimated that an additional amount of 381.4 metric tonnes of rice is required by 2030, (FAO, 2018). Despite the growing demand, rice production is constantly dictated by the global climatic changes including both biotic and abiotic stresses. Drought is one of the major limiting factor in rainfed rice production. In Asia alone, nearly 23 million hectares of rainfed rice cultivating area is drought-prone and affecting more than 50% of rice yield (Bin Rahman and Zhang 2016). With erratic rainfall patterns and constant climatic anomaly exhibited in India, especially in Tamil Nadu, the identification of more stable and drought tolerant (DT) rice genotypes are of high importance.

Drought influences the growth and development of rice by causing numerous changes at the physiological, metabolic and molecular levels (Zu *et al.*, 2017). Some of the mechanisms for drought

avoidance are, maximization of water uptake by deep, dense root systems, the minimization of water loss by stomatal closure and a reduction in leaf area, osmotic adjustment (OA) or changes in cell wall elasticity as well as other essential processes for maintaining physiological activities throughout extended periods of drought (Saha *et al.*, 2016). It affects crop growth by negatively affecting germination, seedling vigor, photosynthesis, leaf membrane stability and osmolyte content (Pandey and Shukla, 2015). The complex and multigenic nature of drought tolerance, makes the selection process quite difficult (Tirado and Cotter, 2010). Due to the heterogeneity in the rainfed ecosystem, different types of traditional rice landraces are being cultivated by the farmers. These landraces could be the sources of genetic variation, and used in drought breeding programs (Pandey *et al.* 2007; Vikram *et al.* 2016). ). On the other hand, the progress of drought tolerance breeding is trailing due to lack of true drought tolerant genotypes for the target environment and lack of suitable screening methods (Pandey and Shukla 2015; Bin Rahman and Zhang 2016).

In upland rice ecosystem, drought appears in severe form in 7-10 d after withdrawing irrigation, whereas in the lowlands, it takes 18-21 d. Hence the drought intensity in screening for drought tolerances should be very close to the drought situation prevailing in that ecological zone (Swamy *et al.*, 2012). Southern Tamil Nadu is the treasure home for large number of traditional rice landraces and majority is untapped. Though the traditional rice landraces are less productive, these genotypes are reported to exhibit tolerance to biotic and abiotic stresses (Sarkar and Bhattacharjee, 2011). There is a dearth of phenotypic knowledge of indigenous rice landraces of Tamil Nadu with respect to drought tolerance. Thus, the present investigation is aimed to characterize the rice landraces in Southern Tamil Nadu under induced moisture stress and to select the best drought resistant donor for the future drought tolerance breeding program.

### Materials and Methods

A total of 50 indigenous landraces collected from various rice growing regions of Tamil Nadu were screened and compared with a susceptible check, IR 64.

To screen for drought tolerance (DT) under hydroponics, the dormancy broken viable seeds were imbibed in water for overnight, followed by surface sterilization with sodium hypochlorite. The intact seeds were incubated at 28°C for 48 h and the germinated seeds were placed in the fabricating seedling float with a nylon mesh. These floats with holes to hold each seedling were placed in plastic tubs containing Yoshida nutrient medium comprised of both macro and micronutrients (Yoshida *et al.*, 1976).

The seedlings of each line were arranged in two sets of floats (stress (S) and control). The seedlings were kept in water at 28 °C for 3d. On the 4<sup>th</sup> d, the seedlings were transferred to Yoshida nutrient medium and grown under greenhouse conditions for 3 d. The experimental setup is represented in plate 1. Under hydroponics screening moisture stress is induced artificially by using poly ethylene glycol (PEG) 6000 MW in various strengths (Swapna and Shylaraj, 2017). The nutrient solution was applied in a progressive pattern. Under treatments, the plants were grown primarily in half strength of Yoshida nutrient solution, with an osmotic potential (OP) of (-) 7.5 bars for 5 d at (28 ± 2) °C. Consequently, the OP level was increased at the rate of (-) 5 bars upto (-) 12.5 bars and sustained for 14 d. Then, the seedlings were triggered to further high stress intensity with OP of (-) 15 bars for 4 d. However, the non-stressed/control, plants were grown in a normal

strength of Yoshida nutrient solution. The pH of both control and treatment (S) were maintained at 5.0. The screening experiment was done in glass house conditions with day/night temperature of 32/22°C and relative humidity of 60% during the day. Observations on root length, shoot length, germination percentage and root shoot ratio (R/S) were recorded on 30 DAS.

The plants were scored for drought scoring at 18 d and 26 d after stress imposition in the score between 0-9 (Table 1) according to the Standard Evaluation System (SES) for rice (IRRI, 1996). Based on the visual symptoms like leaf tip drying and leaf rolling, the seedlings were categorized as highly tolerant, tolerant, moderately tolerant and sensitive.

### Results and Discussion

The rice land races were categorized based on the standard evaluation scoring system (SES) of IRRI (1976). Under moisture stressed condition, the land races exhibited significant variations in visual symptoms with a score range between 0-9 (Table 2). Among the 50 land races, only 14 land races could withstand extreme moisture stress on 18 DAS with a score of 0 and 1; 8 were moderately resistant (score 3), and 16 genotypes showed susceptible and highly susceptible response (score 7 & 9). Similarly, on 26 DAS, five genotypes namely, kuliyadichan, chandaikar, mallikar, nootripathu and rajalakshmi were highly resistant, 8 land races were resistant (score 3) and 16 were registered under highly susceptible category (score 9). Drought score is treated as an alternative approach to determinate plant drought tolerance (Fen *et al.*, 2015). Visual scoring is a reliable measure of tolerance for the estimation of oxidative damages in crop plants which reflects the dehydration of plant tissue (Cabuslay *et al.*, 2002). When moisture stress develops, the plant naturally evolved a defensive mechanism for abbreviating the energy load on the leaf (Chaturvedi *et al.*, 2012) and experienced leaf rolling and drying to reduce net radiation load on the leaf. The highly susceptible genotypes are abya, jaya, kadaikannan, chithiraikar, kalheri, thamarai, vanaprabha, varaisamba, karudan samba, kavuni, kayumma, kichali sambi, milagi, swarnamalli, white sannam and IR64. The results suggests that among the genotypes tested, 30% were highly susceptible and 12% were highly tolerant to moisture stress on 26 DAS (Fig 1). The 5 moisture stress tolerant genotypes will be further exploited as candidate genotypes for pyramiding drought tolerant (DT) traits in popular cultivars.

Moisture stress induced by PEG6000 had significant effect on the seed germination and the

data represents the germination per cent (GP) on 30 DAS. Among 50 land races, 13 land races were able to germinate at (-) 15 bars whereas, the other genotypes showed initial germination and later exhibited stunted growth. The germination capacity significantly varied (0.83 in treatment to 1.14 in control) among the genotypes. The two genotypes, kuliyadichan and rajalakshmi recorded high germination percent (1.18) compared to other land races under moisture stress condition (Fig 2). On contrary, all the genotypes performed well with 100 per cent germination under normal condition. The decline in GP with decreasing water potential might be due to low hydraulic conductivity of the environment. Results of the current study were in agreement with earlier reports of Radhouane, (2007), Govindaraj (2010) and Basha *et al.*, (2015). PEG 6000 makes water unavailable to seeds, affecting the imbibition processes of the seed which is fundamental for germination (Lobato, 2009). The lowest germination percentage at (-) 15.0 bars could be attributed to high viscosity, where solubility and diffusion of oxygen were reduced compared to control. Among 13 genotypes, six genotypes namely, kuliyadichan, chandaikar, mallikar, mattaikar, rajalakshmi, sivappumalli, recorded significant germination percentage. Higher rate of germination even under water deficit conditions is considered as a desirable feature for DT (Lobato, 2009) and (Basha *et al.*, 2015).

Early and rapid elongation of root is an important indication for DT. Deep root length is useful for extracting available soil moisture beyond field capacity (FC). In the present study, significant reduction in root length was observed with increase in moisture stress intensity. All land races registered decreased root length under moisture stress when compared to control. Out of 13 promising land races, significant mean root length varied from 10.65cm to 4.05cm and 13.65cm to 5.60cm at an OP of (-) 15 bars and control respectively. Similarly, the significant mean shoot length varied 24.15 cm to 7.35cm under moisture stress ((-) 15 bars) and 38.25cm to 17.15cm under control (Fig 3). The land race annada showed maximum shoot length (24.15cm) both under stress and control. Long roots were reported as a component trait for DT as they play a direct role with high penetration ability and have large xylem vessel radii and lower axial resistance to water flux aiding in greater water acquisition (Govindaraj, 2010) and (Piwowarczyk, 2014). These results corroborates with the findings of (Basha *et al.*, 2015) and (Pandey and Shukla, 2015) who had also observed the retardation in growth of shoot and root length in response to increasing moisture stress under field as well as laboratory condition.

In addition to root and shoot lengths, R/S also plays a major role in selecting DT lines. Genotypes with high R/S ratio under moisture stress are much preferred. The present study revealed significant variations for the R/S ratio among the genotypes studied. The R/S ratio ranged from 0.26 for adukan to 0.71 for kuliyadichan in control. Among the treatments, the highest R/S ratio was observed in kuliyadichan (0.87) while the lowest ratio was recorded by the genotype annada (0.18) at OP of (-) 15 bars. In control, high R/S ratio was exhibited by several genotypes which failed to record the same under drought stress. However, DT genotypes recorded significant root/shoot ratio under moisture stressed condition. High R/S has been reported as a component trait for drought avoidance (Govindaraj *et al.*, 2010; Radhouane, 2007 and Xu *et al.*, 2015). The results indicate that the plants have an inherent potential to diversify nutrients and recruits activities of different metabolic pathways for producing biomass in both shoots and roots. Further, the studies of Xu *et al.*, 2015, revealed that under drought conditions the plasticity of the plants allow increased allocation of several primary metabolites to roots, and reduced allocation to shoots. It needs to be augmented by field evaluation methods to validate drought resistant genotypes identified by *in vitro* screening. In general, the findings of this study revealed that PEG-induced moisture stress reduced germination percentage, shoot and root lengths while increased the R/S in DT genotypes when compared to susceptible ones. This corroborates with the findings of Govindaraj *et al.*, (2010) and Kim (2011). Though many factors influence the drought tolerance of crops, these factors are not independent, but interact with one another. In this study, all the genotypes showed significant differences in R/S ratio among themselves at various levels of OP. (Fig 3).

Seed vigour (SV) is sum total of those properties of seed which determine the performance level of seed during germination and seedling emergence. Seed vigour index declined with the increase in drought intensity. In the study, highest SV was recorded at control and lowest at extreme OP of (-) 15 bars (Fig 2). Among the significant genotypes seeraga samba recorded highest seed vigour (28.47) under control and nootripattu (15.31) under extreme osmotic stress. On contrary, kuliyadichan (13.02) exhibited lowest SV under control and mattaikar (6.61) at (-) 15 bars. Moisture content of seedlings plays an important role in various physiological processes including growth. It has also been reported that drought suppresses the uptake of essential nutrients like P and K, which could adversely affect seedlings growth and vigor. This results in physiological and biochemical





changes in both anabolic and catabolic organs of the seeds and seedlings (Nasim, 2008).

Relative seedling height (RSH) also decreased as the OP increased and the seedling height of different rice landraces were significantly affected by moisture stress. At high OP level of (-) 15 bars, the maximum seedling height were observed in adukan, anjali, annada, chandaikar, kuliyadichan, mallikar, mattaikar, nootripathu, mulam punchan, kallurandaikar, rajalakshmi, seeraga-samba and virendra. The highest RSH (91.73%) was recorded in virendra, whereas lowest RSH (46.39%) in kuliyadichan (Fig 4). Under moisture stress, it has been shown that the inhibition of radicle emergence is mainly because of decrease in water potential gradient between the external environment and the seed and consequently impairs seedling height (Sokoto and Muhammad, 2014).

The seedling height reduction (SHR) under moisture stress was compared with control. From the results it is evident that the seedling height decreased as the PEG concentration increased. The seedling height of different land races were significantly affected by moisture stress. The high SHR ratio (120.26) was recorded in kuliyadichan and low SHR (8.99) was recorded in virendra (Fig 4). The reason might be due to the impairment of metabolic activities of seeds caused by decreased water potential between the external environment and seeds. The results are in accordance with the findings of Islam *et al.*, 2018. Therefore, it can be inferred that the moisture stress tolerance mechanisms employed by these tolerant rice land races are significantly sufficient to maintain normal growth under drought.

The present study suggests that the growth parameters such as germination percentage, root and shoot length, R/S ratio and SHR are few drought tolerant indices under *invitro* condition. Hence it can be concluded that the genotypes kuliyadichan, chandaikar, mallikar, mattaikar, rajalakshmi and nootripattu possessing better source-sink relationship, are potential donor candidates towards genetic improvement of drought tolerance in rice. The future scope on pyramiding these desirable traits of landraces in popular rice cultivars for sustaining high yield under water deficit condition is more apparent.

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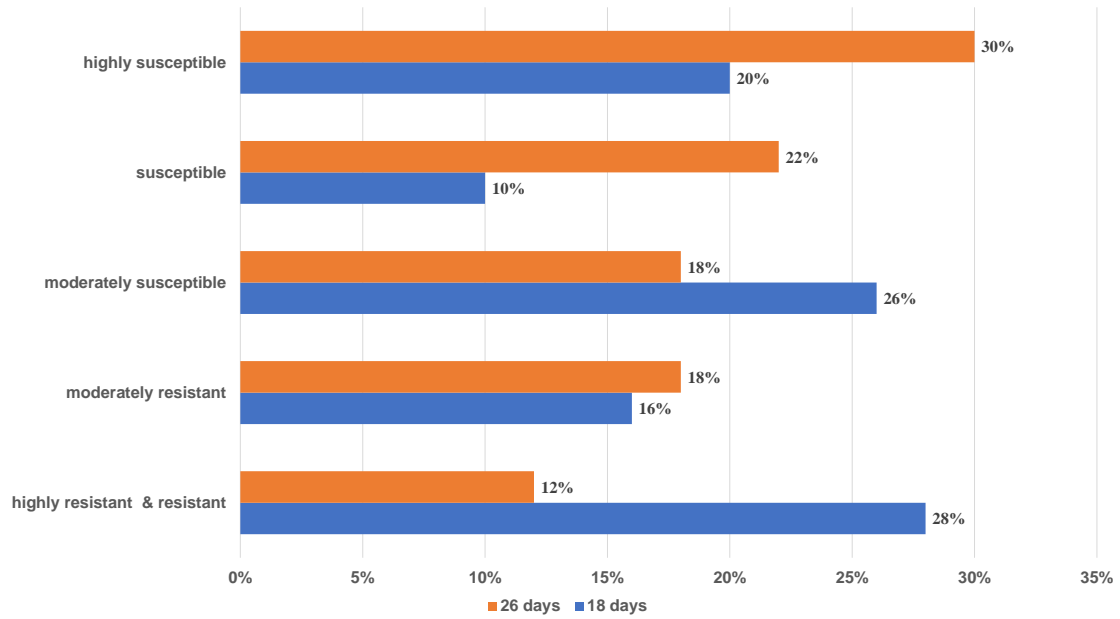
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**Table 1. Drought scoring system in rice SES of IRRI, 1996**

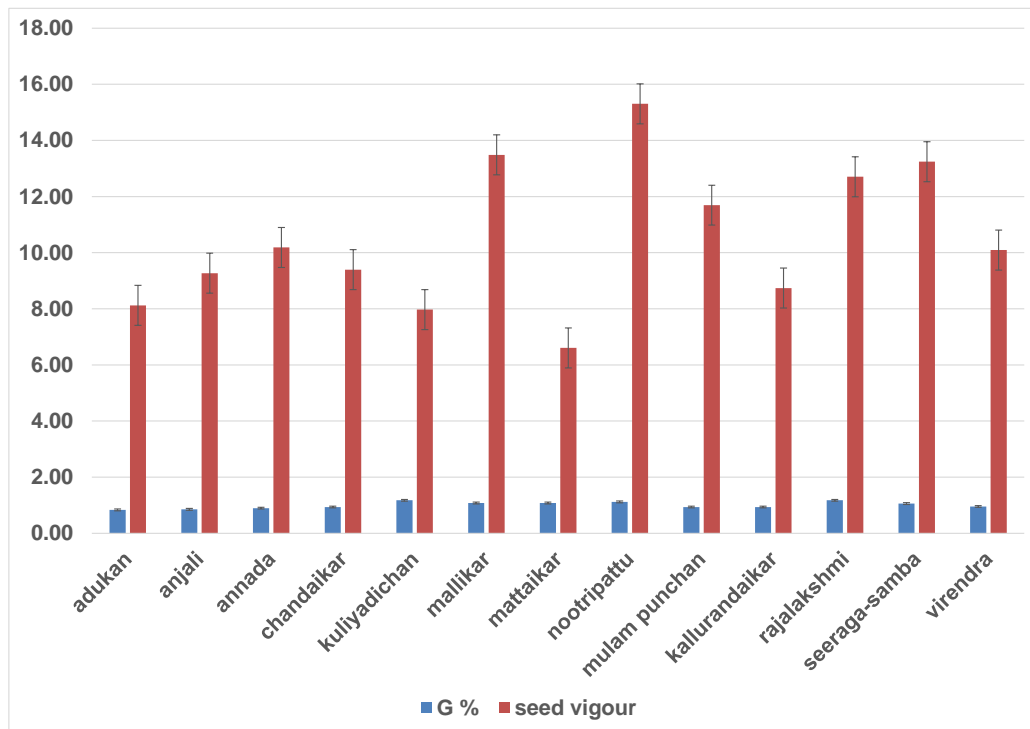
Decimal score	Drought scoring
0	Highly resistant: no symptoms
1	Resistant: light tip drying
3	Moderately resistant: tip drying to ¼ length in most leaves
5	Moderately susceptible: ¼ to ½ of leaves fully dried
7	Susceptible: more than 2/3 of leaves fully dried
9	Highly susceptible: all plants apparently dead

**Table 2 Categorization of rice landraces for drought tolerance based on SES of IRRI 1996**

Drought tolerant categories	Phenotype scoring of rice landraces at 18 days after sowing	Phenotype scoring of rice landraces at 26 days after sowing
Highly resistant & resistant (score 0 & 1)	adukan, anjali, chandaikar, annada, kuliyadichan, mallikar, mattaikar, nootripattu, mulampunchan, molikarumbu, muttakuruva, rajalakshmi, seeraga-samba, sabagaidhan,	kuliyadichan, chandaikar, mallikar, mattaikar, nootripattu, rajalakshmi,
Moderately resistant (score 3)	virendra, kallurandaikar, dhalahaera, karsamba, kullakar, kuruvaikar, kuruvai kalanchiyam,	adukan, anjali, annada, mulampunchan, kallurandaikar, seeraga-samba, virendra, sivappumalli, sabagaidhan.
Moderately susceptible (score 5)	bharathi, kalinga-3, karukot, kattanoor, kottarasamba, krishna hemavathi, maranellu, meikuruvai, Navara, Pattani, pokkali, sadhabhar, surakuruvai,	kattanoor, sadhabhar, krishna hemavathi, kullakar, molikarumbu, muttakuruva, kuruvaikar, dhalahaera, kuruvaikalanchiyam,
Susceptible (score 7)	chithiraikar, kalaheri, thamarai, vanaprabha, varaisamba,	bharathi, kalinga-3, karukot, kottarasamba, maranellu, meikuruvai, navara, pattani, pokkali, sadhabhar, surakuruvai,
Highly susceptible (score 9)	abya, jaya, kadaikannan, karudan samba, kavuni, kayumma, kichali samba, milagi, swarnamalli, white sannam, IR64	abya, jaya, kadaikannan, chithiraikar, kalaheri, thamarai, vanaprabha, varaisamba, karudan samba, kavuni, kayumma, kichali samba, milagi, swarnamalli, white sannam, IR64

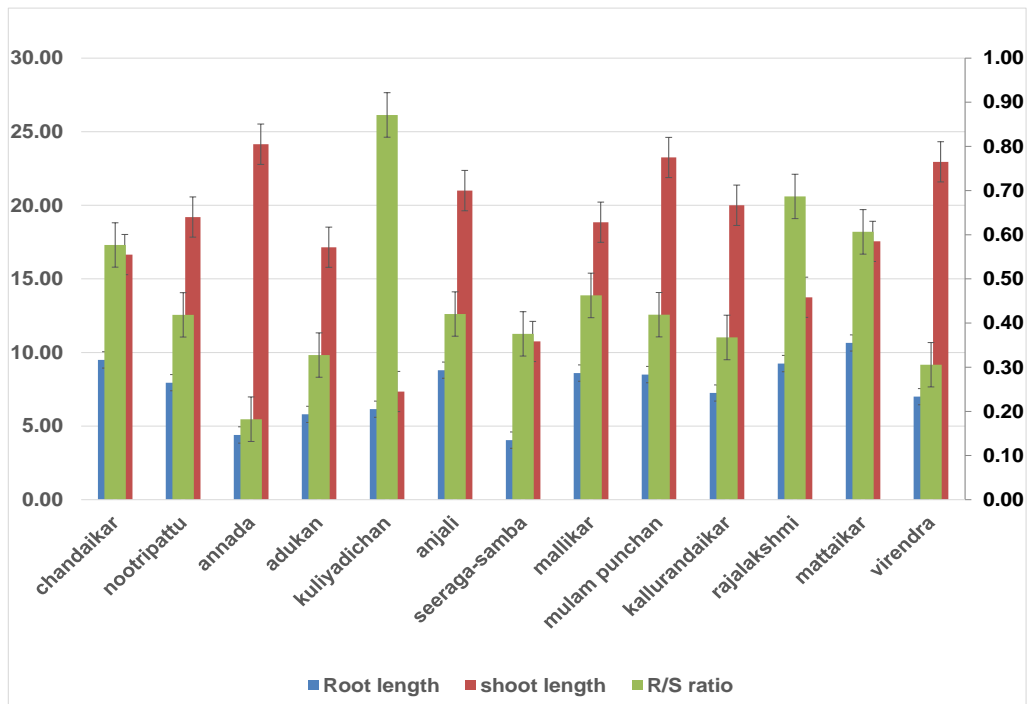


**Fig. 1. SES scoring of 50 indigenous rice landraces for moisture stress tolerance upto (-) 15 bars on 18 and 26 DAS under hydroponics**

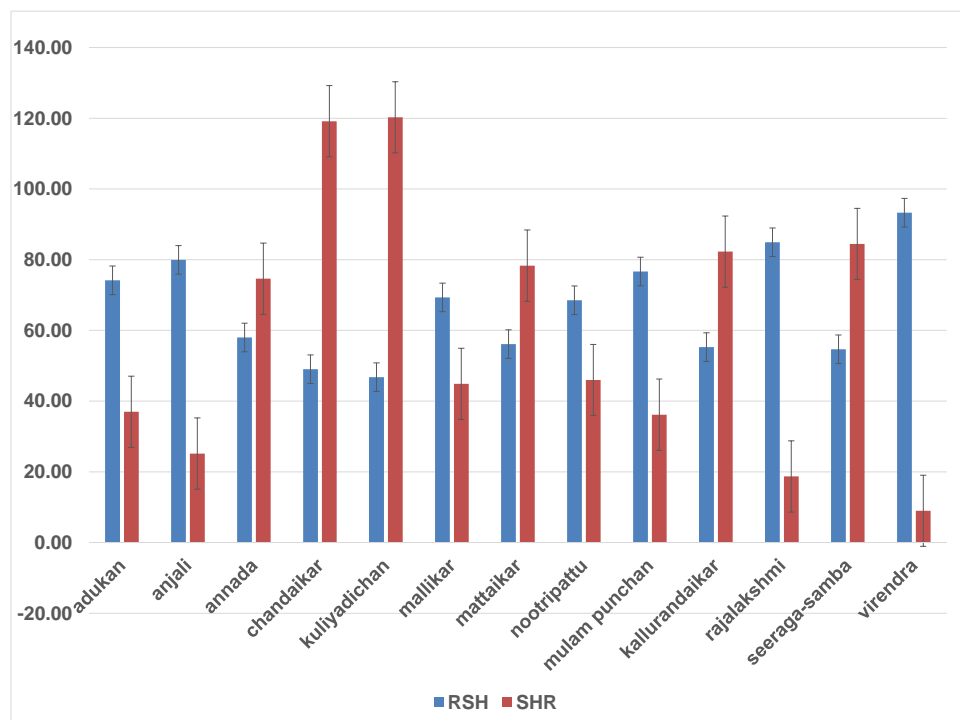


**Fig. 2. Germination percentage (arcsine transformed values) and seed vigour of 13 drought tolerant landraces (score 0-3) under hydroponics at an osmotic potential of (-)15 bars**

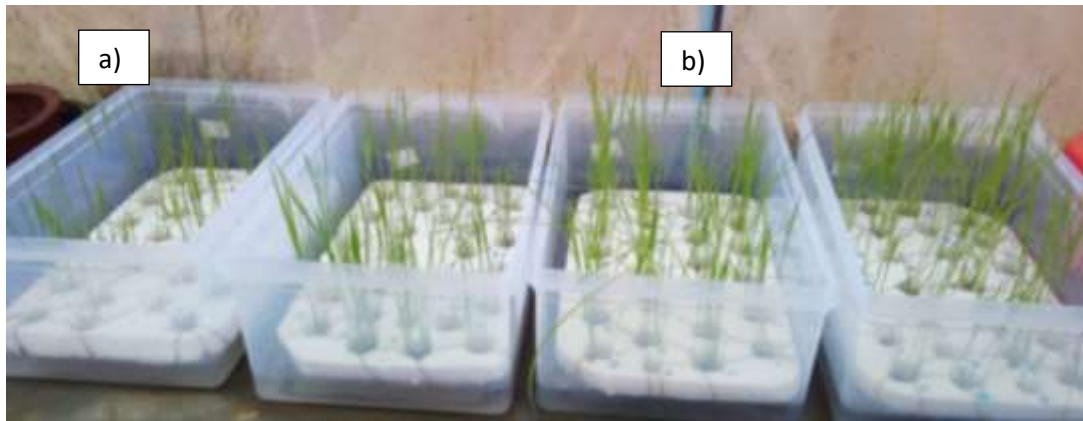




**Fig. 3.** Root and shoot length, root shoot ratio (R/S) of 13 drought tolerant landraces (score 0-3) under hydroponics at an osmotic potential of (-)15 bars. Highest R/S ratio represents better source-sink relationship



**Fig. 4.** Relative seedling height (RSH) and seedling height reduction (SHR) of 13 drought tolerant landraces (score 0-3) under hydroponics at an osmotic potential of (-)15 bars.



**Plate 1. Experimental setup for screening drought tolerant rice landraces under hydroponics.**  
a) Moisture stress upto (-) 15 bars; b) Control

