Phytohormone cross-talk and antioxidant gadgets tussles osmotic stress in indigenous rice landraces during seed germination

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Abstract

Seed germination plays a critical role in determining rice productivity under drought stress. We evaluated 100 traditional rice landraces originated from different agro-ecological zones of Tamil Nadu along with drought- susceptible (IR 64) and drought- tolerant (IR 64 DRT) checks. Moisture stress was induced using PEG 6000 and screening done over a range of osmotic potentials (-) 10 bars, (-) 12.5 bars and (-)15 bars for a period of 5 d. Physio-morphological traits such as germination rate, survival per cent, root and shoot length, vigor index, RS ratio and relative water content (RWC) were assessed during early drought stress. We observed significant changes in the seed macromolecules, phytohormone levels (GA and IAA), osmolytes and antioxidant responses (catalase and superoxide dismutase) between drought stress and control treatments. Kuliyadichan registered significantly higher IAA and GA (44% and 35% respectively over drought tolerant check IR 64 DRT) at drought stress, whereas all the landraces showed an elevated catalase activity. In PC analysis, first three PCs captured 88.93% of the total variation; significant differences were detected among genotypes with respect to the studied parameters. Six traditional landraces such as Kuliyadichan, Rajalakshmi, Sabhagidhan, Nootripathu, Chandaikar and Mallikar were selected and their inherent drought tolerance was associated with metabolic responses viz., triggered hydrolytic enzyme activities, hormonal cross-talk, ROS signaling and catalase under drought stress compared to drought sensitive IR64. Hence, these genotypes can be used as potential donor candidates towards genetic improvement of drought tolerance in rice.

Introduction

Rice (*Oryza sativa* L.) is the key staple in the daily diet of more than 70 percent of the Indian population. Annual rice production of rice during 2019-'20 is estimated to record 117.47 million tonnes in India (www.economictimes.com). By 2050, global demand for agricultural production is expected to increase by 70 percent due to the rising population (FAO 2011). Nevertheless, rice is grown in a wide range of ecosystems, including flood- and drought-prone environments; even a small reduction in rice production may pose a major threat to food security (Ahmadi et al. 2014). Drought is one of the most important constraints adversely affecting the rice productivity in Asia, as it can occur for varying lengths of time and intensity, at any stage of crop growth and development (Barnabás et al. 2008). Severe moisture stress during the reproductive and grain-filling stage reduces the economic yield to 48-94% and 60% respectively in rice (Basnayake et al. 2006). The current unpredictable climate changes causing frequent and severe droughts emphasize the need to understand the root phenomics in response to drought stress (Ghosh and Xu 2014; Kim et al. 2020).

Seed germination is very crucial in plant development during drought stress and it determines the plant productivity. The physiological and biochemical responses of seed during germination at moisture stress, impacts seedling survival rate and vegetative growth which inturn affects yield and quality (Munns 2002). Water stress drastically reduces seed germination rate and cause delay in the initiation of germination (Ali and Elozieri 2017). Biochemical constituents of seeds such as starch (70-80%), protein (15%), lipids (5%) and free aminoacid content are key molecules in mediating plant responses to abiotic stresses. Remobilization of starch and protein during moisture stress supports in maintaining the turgidity of cells. The released sugars and other derived metabolites support plant growth under stress, and maintain redox- homeostasis by acting as osmoprotectants (Krasensky and Jonak 2012).

Seed imbibition triggers the synthesis of various hydrolytic enzymes in the aleurone layer or scutellum in response to germination signals. Hydrolytic enzymes convert the storage reserve of seeds into available form for embryo uptake. The consumption of elevated oxygen level during hydrolysis activates mitochondrial enzymes involved in Krebs cycle and electron transport chain (Mayer 1989; Salisbury 1991). During seed germination, amylase synthesized by de novo biosynthetic pathways stimulates the stored starch mobilization. The amylase activity is high until the young plant initiates photosynthesis (Kohno and Nanmori 1991). Hydrolysis of stored protein in seeds involves peptidases such as cysteine, serine, aspartic and metalloproteases (Van der Hoorn 2008). The free amino acids produced activate protein synthesis in endosperm and embryo (40). Among the proteases, cysteine proteases are the most abundant group involved in the mobilization of seed proteins during germination in cereals, specifically rice (Martinez et al. 2009). Cysteine proteases are located in scutellum, aleurone and endosperm (Zhang et al. 1995), participating in protein maturation and elimination of unnecessary synthesized endogenous proteins (Cejudo et al. 2001). Lipases are concerned with lipid metabolism during seed germination, which catalyzes β-oxidation, releasing fatty acids
and organic alcohols. Previous studies reported an increase in α-amylase and protease activities under moisture stress in barley and rice respectively (Vijayakumar and Gowda 2012).

Endogenous phytohormones play an essential role in inducing plant acclimatization to environmental adversities via mediating growth, development, source/sink transitions, and nutrient allocation (Fahad et al. 2015). Phytohormones include auxin (IAA), abscisic acid (ABA), cytokinins (CKs), ethylene (ET), gibberellins (GAs), salicylic acid (SA), brassinosteroids (BRs), jasmonates (JAs) and strigolactones (SL). Moreover, phytohormones control a variety of cellular process and coordinates signal transduction pathways in response to abiotic stress (Vob et al. 2014). Seed imbition activates GA biosynthetic pathways which intum stimulates the genes encoding hydrolytic enzymes such as endo-β-1,3 glucanase (119) and β-1,4 mannann endohydrolase (Nonogaki et al. 2000). These hydrolytic enzymes acts on endosperm and curtails the inhibitory effects of ABA on embryo growth (Koornneef and Karssen 1994). The crosstalk between GA-ABA, is therefore a key mechanism to cope early drought stress. ABA inhibits water uptake by interfering in cell wall loosening, whereas GAs represses ABA effect by stimulating cell wall loosening enzymes such as α-expansins in early phase of germination (Debeaujon et al. 2000). Thus a rapid decrease in endogenous ABA during seed germination under water stress is one of many factors influencing seed germination rate (Weitbrecht et al. 2011). IAA plays a significant role in the defense against oxidative stress caused by drought (Lecube et al. 2014).

Several biochemical and cellular processes associated with germination such as metabolic reactivation, resumption of cellular respiration and mitochondrial biogenesis, translation and/or degradation of stored mRNAs, DNA repair, transcription and translation of new mRNAs, and the onset of reserve mobilization are triggered during seed imbition (Nonogaki et al., 2010). Accumulation of ROS (H$_2$O$_2$) as a result of water stress, acts as cellular messengers or signaling cues or toxic moieties causing seed vigor losses (Kumar et al. 2015). However, activation of antioxidant systems during the late phase of germination maintains ROS homeostasis. Recent advances in plant physiology shed light on the beneficial role of ROS in seed dormancy release and maintaining cell redox status. Interaction of ROS with ABA and GA transduction pathways regulates numerous transcription factors in response to stress (Bouteau and Bailly 2008; Liu et al. 2019). Comparing with the drought mitigating strategies of adult rice plant, the mechanisms of water stress tolerance in germinating phase are poorly interpreted due to various inherent factors such as genotypes and environment. We hypothesize that metabolic or physiological adaptions in traditional rice genotypes tolerant/resilient to cope up with early moisture stress is a key asset for developing drought tolerant high yielding rice varieties. The study envisages the morpho-physiological and metabolic responses of traditional landraces of Southern Tamil Nadu during seed germination in response to induced moisture stress.

**Materials And Methods**

**Plant materials and experimental design**

The present investigation was undertaken to study the physiological and metabolic factors contributing for the resilience/resistance and susceptibility in traditional rice genotypes against drought. Approximately 100 traditional rice landraces from different agro-climatic zones of Tamil Nadu, India (Table 1) were subjected to in vitro screening for drought tolerance along with respective checks (IR64 Drt1 and IR 64 as drought tolerant and susceptible controls respectively). The experiment was conducted at Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Killikulam, Vallanadu, Tuticorin Dt, Tamil Nadu, during the Rabi season (October – March) of 2019- 20. The experimental area is geographically situated at 8° 46’N latitude and 77° 42’E longitude in the southern part of Tamil Nadu, at an altitude of 40 m above MSL.

Pre-soaked seeds were surface sterilized with sodium hypochlorite (1.0%) for 3 mins and thoroughly rinsed 3 to 5 times in distilled water before introducing seeds on the top of moist absorbent paper placed inside the sterilized Petri plates. The seeds were then moistened with sterile water (non-stressed control -NS) or polyethylene glycol (PEG 6000) equivalent to osmotic potentials of (-) 1, (-) 1.25 and (-) 1.5 MPa (Michel and Kaufmann 1973). The experiment was conducted in factorial CRD with three replications. Fifty seeds were maintained for each treatment. The sealed Petri plates were incubated for 5d in a plant growth chamber for initiation of germination. The temperature of plant growth chamber maintained at 28°C, 60 % relative humidity and 12 h light intensity (200 moles.m$^{-2}$ s$^{-1}$). Seeds were considered to be germinated at the maximum stress intensity,
when the radicle length was at least 2 mm long. Then the seeds were transferred to hydroponics setup and osmotic stress was imposed accordingly.

The germinated seeds were placed in a fabricating seedling float (nylon mesh with holes to hold each seedling. These floats were placed in plastic tubes containing Yoshida nutrient medium (Yoshida et al., 1976). Subsequently, the seedlings were transferred to Yoshida nutrient medium on the 5th d, and grown under greenhouse conditions for 30 d. Artificial moisture tension imposed by using polyethylene glycol (PEG) 6000 MW maintained at -1.5 MPa (Swapna and Shylaraj, 2017). The pH of both control (NS) and treatment (S) nutrient hydroponic solution maintained at 5.0. However, the plants grown in a normal strength of Yoshida nutrient solution served as control (NS).

Germination rate (GR) and vigour index (VI)

The average number of seeds, germinated on 5 d after incubation (DAI) was accounted for calculating the germination rate.

\[
\text{Germination rate} = \frac{\text{Number of Germinated Seeds}}{\text{Number of Germination Days}}
\]

Vigour index was calculated on 5 DAI based as the product of germination per cent and seedling height (cm) (Abdul-Baki and Anderson 1973).

\[
\text{Vigour Index} = (\text{Average Shoot Length} + \text{Average Root length}) \times \text{Germination Percentage}
\]

Relative water content (RWC)

Fresh weight, turgid weight and dry weight of the seedlings measured under NS and DS conditions on 26th d were used to calculate Relative Water Content (Yamasaki and Dillenburg 1999) according to the equation,

\[
\text{Relative Water Content} = \frac{\text{Fresh Weight} - \text{Dry Weight}}{\text{Turgid Weight} - \text{Dry Weight}} \times 100
\]

Determination of seed carbohydrates and protein

Starch

Starch content in the germinated seeds was determined according to Clegg (1956). Fresh samples (0.5g per sample) were homogenized in hot 80% ethanol to remove sugars and dried over a water bath. Extraction buffer contained 5.0 mL water and 6.5 mL 52% fresh perchloric acid. The homogenate was centrifuged and supernatant was pooled after repeating the extraction. 5.0 ml assay mixture contained 0.2 ml of crude extract; 0.8 ml distilled water and 4 ml anthrone reagent and heated for 8 min in a boiling water bath. The intensity of green to dark green color was measured colorimetrically at 630nm against water as blank and expressed in g.100g\textsuperscript{-1} FW. Total soluble sugar content in the seed samples was determined according to the method of Yemm & Willis (1954).

Protein

Fresh samples (500 mg) were extracted in 5 - 10mL of ice cold phosphate buffer. The crude extract was centrifuged at 10,000 rpm for 10 min at 4°C and the supematant maintained at 0-4°C. To 0.2ml of the extract, alkaline copper solution (5ml) and 0.5ml of freshly prepared Folin's reagent were added followed by incubation in the dark for 30 min at room temperature. Absorbance was recorded at 660 nm by spectrophotometer against the blank. A standard curve representing 40-200 μg ml\textsuperscript{-1} of bovine serum albumin (BSA) was used to calculate the protein content and expressed in mg.g\textsuperscript{-1} FW (Lowry et al. 1951).

Total free amino Acids

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Total free amino acid was estimated by the Ninhydrin method (Moore and Stein 1954). Exactly 500 mg of the sample ground with small quantity of acid-washed sand followed by extraction in 10 mL of 80% ethanol. The supernatant extracted was used for the quantitative estimation of total free amino acids. To 1 ml of the extract equal volume of ninhydrin added and the volume made up to 2 ml with distilled water. Tubes were kept in boiling water bath for 20 min, added 5 ml of diluent (equal volume of water and n-propanol) and incubated at room temperature for 15 min. The absorbance was read in colorimeter at 570 nm against a reagent blank made with 0.1 ml of 80% ethanol. A standard curve representing 10-100 μg.ml⁻¹ of leucine was used to calculate the total free amino acid content and expressed in μg.g⁻¹ FW.

Quantitative assay of hydrolytic enzymes

Alpha amylase

The α-amylase activity in the germinated seeds (5 DAI), was determined according to Muscolo et al. (2014). Exactly 0.5 g of germinated seeds was homogenized in 1:4 w/v distilled water using chilled pestle and mortar. The extract was centrifuged at 14,000 rpm for 30 min at 4°C. The supernatant filtered through muslin cloth was used for the quantitative assay of α-amylase. One unit of amylase activity represents number of μmoles of reducing sugars formed min⁻¹ g⁻¹ FW.

Protease

Protease activity was determined according to Harvey and Oaks (1974). Fresh seed samples (1 g) homogenized in ice cold acetone were mixed with 10 mM Tris-HCl buffer at pH 8.0 and 2 M NaCl and incubated in an orbital shaker for 3 h. The extract collected after centrifugation at 10,000 rpm for 10 min at 4°C was used for protease assay at 660 nm. The reaction mixture contained 1 ml of crude enzyme extract, 3 ml phosphate buffer and 0.5% casein as substrate. In control tube, added 1 ml of distilled water and the tubes incubated at 30°C for 1 h. Standard graph using tyrosinase (0-100 µg) was used to calculate protease activity.

Lipase

Lipase activity assayed according to the titrimetric method of Maliks et al. (2000). Crude seed extract was prepared by homogenizing ground seed samples (1 g each) with twice the volume of ice-cold acetone followed by acetone: ether (1:1) wash. The air dried acetone powder was again extracted in ice-cold water and the supernatant served as enzyme source. The samples were titrated against 0.025 N NaOH using 1% phenolphthalein as indicator. The volume of titration used for calculating lipase activity.

\[
\text{Activity \ mg per min per g of sample} = \frac{\text{Volume of alkali consumed} \times \text{strength of alkali}}{\text{Weight of sample in g} \times \text{Time (min)}}
\]

Determination of endogenous phytohormones

Indole acetic acic (IAA)

Endogenous IAA of germinating seed on 5DAI was determined according to Andreae and Van Ysselstein (1956). 500 mg of fresh sample was homogenized with 5 ml of 95% ethanol and the supernatant was collected. The precipitate was washed with ethanol, centrifuged and the supernatant collected were pooled. One drop of strong ammonium acetate was added and the solution was evaporated. After evaporation, added one ml of 0.1 N NaHCO₃ solution and 4 ml of Salkowski reagent (2 % of 0.5 M FeCl₃ in 35 % perchloric acid) followed by incubation at dark for 30 min. The absorbance was measured at 530 nm using a spectrophotometer (Spectramax I3X). The IAA content was calculated against a standard curve prepared using Indole Acetic Acid and was expressed as μmol g⁻¹ FW.

Giberellic acid (GA)
GA extraction carried out based on the protocol described by Almeida Trapp et al. (2014) and Ghosh et al. (2019). Fresh germinating seed samples were ground to a fine powder in liquid nitrogen. The homogenized mixture in 1ml of 80% methanol was kept overnight at 4°C under shaking and centrifuged at 4 °C, 10,000 rpm for 15 min. The supernatant collected were dried in a vacuum concentrator. The residues suspended in 500 µl of 80% methanol were used for further analysis. 2.0 ml of zinc acetate solution (21.9 g zinc acetate in 80 ml distilled water and 1 ml glacial acetic acid; and volume made upto 100 ml) was added to the dissolved residue followed by 2.0 ml of potassium ferrocyanide solution (10.6 g in 100 ml water) and the mixture was centrifuged at 10,000 rpm for 10 min. Five ml of supernatant was added with 5 ml of 30% HCl and incubated at 20°C for 75 min. The absorbance was measured at spectrophotometrically at 254 nm.

**Estimation of seed proline content**

Seed proline content on 5DAI was detected based on the method described by Bates et al. (1973). Approximately 300 mg of germinating seed samples homogenized in 5ml of 3% sulfosalicylic acid were centrifuged at 3000xg for 20 min. The supernatant was mixed with 2 ml of glacial acetic acid and 2ml of ninhydrin. The mixture was boiled at 100 °C for 25 min and mixed with 4 ml of toluene. Absorbance of the extract was read spectrophotometrically at 535 nm.

**Determination of antioxidant enzymes**

A quantity of 500 mg plant sample at 26th d was homogenized for SOD, and CAT assays, with ice-cold pestle and mortar in a 5 ml ice-cold buffer containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA (ethylene diamine tetraacetic acid) and 1 % (w/v) PVP (polyvinyl pyrrolidone). The homogenate was centrifuged at 10,000 rpm for 30 mins at 4 °C. The supernatant obtained was used for the enzyme assay. Catalase (CAT) activity was estimated by the method described by Azevedo et al. (1998). The assay mixture contains 50 mM phosphate buffer (pH 7.0), 20 mM H₂O₂, and 0.1 ml enzyme extract. Reduction in absorbance of H₂O₂ was observed for 1 min at 240 nm on a microplate reader (Spectramax® i3X). The catalase activity was expressed as Units g⁻¹ FW min⁻¹.

Superoxide dismutase (SOD) activity assessed by the nitroblue tetrazolium (NBT) method described by Beauchamp and Fridovich (1971). About 100 µl of the enzyme mixture was added to the reaction mixture containing 3ml of sodium phosphate buffer (50 mM; pH 7.8) with 13 mM methionine, 75 µM NBT, 2 µM riboflavin and 0.1 mM EDTA. Then the reaction mixture incubated for 15-30 mins at 28 ± 2 °C. After incubation, the absorbance was measured at 560 nm, and activity was expressed as U. g⁻¹ FW min⁻¹.

**Statistical Analysis**

All data obtained through the experiment were subjected to the analysis of variance and Duncan's Multiple Range Test (DMRT) at p < 0.05 significance level to evaluate the significant difference in the traits among the genotypes was performed in R platform (R studio) using the package Agricolae.

**Results**

**Drought stress influences germination rate, seed vigor and RWC**

About 100 indigenous rice land races were screened for drought tolerance along with respective checks, (drought tolerant) IR 64 Drt1 and (drought susceptible) IR 64. The results showed that, only 52 per cent of landraces survived on maximum osmotic potential, (-) 15 bars or 1.5 MPa on 5 DAI. Germination percent calculated for the survived landraces revealed that Kuliyadichan and Rajalakshmi showed maximum survival percentage of 88.94 and 78.89 when compared to the drought tolerant check IR 64 Drt1 with the germination percent of 77.34, which is followed by the landraces Sabhagidhan (77.34%), Chandaikar (77.34%) Oheruchitteni (75.79%), Nootripattu (74.25%), Agulanthankodai (69.61%), Arikraavi (71.15%), Chenkayama (71.15%), Agulanthankodai (69.61%) and Mallikar (64.96%). The result on survival percentage revealed that the response of landraces significantly varied (P<0.05) under moisture stress (Fig 1a &b).
Germination rate of survived landraces varied from 1.10 to 12.70 under moisture stress condition (DS). Among the selected drought resilient landraces at -1.5MPa, Kuliyadichan (12.70) and Rajalakshmi (11.27) recorded the highest germination rate when compared to the drought tolerant check IR 64 DRT (11.05) followed by Chandaikar (11.04), Sabhagidhan (11.04), Oheruchitteni (10.82), Nootripathu (10.60), Chenkayama (10.16), Ariki raavi (10.16), Agulanthankodai (9.94) and Mallikar (9.28). (Table 2).

Among the selected drought resilient landraces, Rajalakshmi and Kuliyadichan showed maximum vigour index of 3568.5 and 3417.5 respectively, followed by Chenkayama (2849.8), Oheruchitteni (2550.2), Chandaikar (2476.5), Sabhagidhan (25.88), Mallikar (24.99), IR 64 DRT (24.52) and Chenkayama (24.12). The present investigation revealed that all the selected landraces had higher relative water content compared to the drought susceptible check IR 64 having the lowest relative water content of 10.93 (Table 2).

**Drought stress influences starch and protein remobilization during seed germination**

Starch content of selected rice landraces differed significantly \((p<0.05)\) under NS and DS conditions. Total starch content in the germinated seeds was negatively related to drought tolerance response. Among the landraces, Chandaikar (8.94 g. 100 g\(^{-1}\) FW) and Kuliyadichan (10.19 g. 100 g\(^{-1}\) FW), recorded lowest starch content compared with IR64 Drt1 (12.89 g. 100 g\(^{-1}\) FW) and are on par with each other. In case of DS, the results revealed that the all the ten selected landraces recorded lower starch content compared to the drought susceptible check IR 64 (34.16 g. 100g\(^{-1}\)FW) (Table 4;Fig 2a). The results showed relatively higher remobilizing response of starch under moisture stress while compared with non-stressed (NS) environment.

Similarly, protein content of selected rice landraces significantly varied \((p<0.05)\) under NS and DS conditions. Remobilization of protein reserve in germinating seeds of rice landraces under moisture stress showed enhanced drought tolerance capacity. Among the landraces, Rajalakshmi (0.51 mg. g\(^{-1}\) FW) and Nootripathu (0.51 mg. g\(^{-1}\)FW) recorded lowest mean values for protein and are in comparison with IR64 Drt1 (0.48 mg. g\(^{-1}\)FW). Protein content in all the landraces decreased in stress induced plants during seed germination, compared to the susceptible genotype IR64 (1006.73 mg. g\(^{-1}\)FW) (Table 4;Fig 2b).

Accumulation of total free amino acids content of selected rice landraces significantly varied \((p<0.05)\) under NS and DS conditions. In stress induced seeds, Oheruchitteni (0.472 μg. g\(^{-1}\)FW) recorded the highest free aminoacids content followed by Sabhagidhan (0.443 μg. g\(^{-1}\)FW), Nootripathu (0.394 μg. g\(^{-1}\)FW), Chenkayama (0.375 μg. g\(^{-1}\)FW) and Kuliyadichan (0.370 μg.g\(^{-1}\)FW) whereas, control seeds (NS) showed a considerable increase in the drought susceptible genotype IR64 (Fig 2c).

**Drought stress influences hydrolytic enzymes activities during seed germination**

\(\alpha\)-Amylase activity of selected rice landraces significantly varied \((p<0.05)\) under NS and DS conditions. The results implied that \(\alpha\)-amylase activity was significantly influenced by genotypic nature (Table 3&4 ; Fig 3). In stress induced treatments, Kuliyadichan (13.57 U.g\(^{-1}\) ) recorded highest \(\alpha\)-amylase activity and was on par with Chandaikar (12.95 U.g\(^{-1}\)), Rajalakshmi (12.3 U.g\(^{-1}\)) and IR 64 Drt1 (12.65 U.g\(^{-1}\)). The drought susceptible check IR 64 showed least \(\alpha\)-Amylase activity of 2.13 U.g\(^{-1}\).

Similarly, protease activity varied significantly \((p<0.05)\) both under NS and DS conditions. The osmotic stress induced seeds of Kuliyadichan (1.19 U.g\(^{-1}\)) and Rajalakshmi (1.09 U.g\(^{-1}\)) recorded the highest protease activity compared with IR64 Drt1 (1.03 U.g\(^{-1}\)) followed by Mallikar (0.97 U.g\(^{-1}\)), Chandaikar (0.88 U.g\(^{-1}\)) and Sabhagidhan (0.81 U.g\(^{-1}\)). However, no significant difference was observed among the genotypes under NS environment (Table 3&4; ;Fig 3).

Lipase activity in Kuliyadichan (29.21 U.g\(^{-1}\)) was significantly higher during seed germination compared to IR64 Drt1 (25.1 U.g\(^{-1}\)) during seed germination in stressed environment. Both the treatments NS and DS showed significant differences \((p<0.05)\) and
are highly influenced by genotypic nature. Here also, IR64 registered least lipase activity under DS (Table 3&4; Fig 3).

Drought stress influences seed endogenous hormone modulation during seed germination

The variance analysis showed that drought stress significantly influenced auxin homeostasis in seed during seed germination ($p < 0.05$) in all the genotypes. Our experimental results exhibited significant increase in seed endogenous IAA on 5 DAI under DS while compared to NS environment. Among the landraces, Kuliyadichan ($58.87 \mu\text{mol g}^{-1}\text{FW}$) and Agulanthankodai ($50.27 \mu\text{mol g}^{-1}\text{FW}$) recorded the highest IAA concentration compared to the drought susceptible check IR 64 ($40.79 \mu\text{mol g}^{-1}\text{FW}$). More precisely, the auxin level determined in terms of IAA content in emerging rice roots showed that DS pronounced 2 fold increase of IAA in most of the genotypes (Table 4;Fig 4).

Gibberellic acid (GA) in the seeds of all rice genotypes studied differed significantly ($p <0.05$) under control and stressed environment. The mean comparison values showed that the genotype Kuliyadichan ($3.18 \mu\text{mol g}^{-1}\text{FW}$) recorded the highest GA followed by IR 64 Drt1 ($2.350 \mu\text{mol g}^{-1}\text{FW}$) which are on par with Rajalakshmi ($2.28 \mu\text{mol g}^{-1}\text{FW}$), Sabhagidhan ($2.2 \mu\text{mol g}^{-1}\text{FW}$) and Nootripathu ($2.15 \mu\text{mol g}^{-1}\text{FW}$). The landraces Chenkayama ($0.75 \mu\text{mol g}^{-1}\text{FW}$) and Oheruchitteni ($0.70 \mu\text{mol g}^{-1}\text{FW}$) recorded the lowest GA concentration, However, GA level remarked an decreasing trend in stressed seeds in comparison with control, irrespective of genotypes (Table 4;Fig 4).

Drought stress influences seed antioxidant enzymes during seed germination

Catalase activity (CAT) in the germinating seeds of rice landraces differed significantly ($p <0.05$) under NS and DS treatments. Among the landraces, CAT activity in the genotype Rajalakshmi ($2.682 \text{mg/g}$) was significantly higher and was on par with Sabhagidhan ($2.58 \text{U.g}^{-1}\text{FW min}^{-1}$) compared to the drought tolerant check, IR 64 Drt1 ($2.29 \text{U.g}^{-1}\text{FW min}^{-1}$). CAT activity in Kuliyadichan was on par with IR 64 Drt1 and the landrace Agulanthankodai ($0.79 \text{U.g}^{-1}\text{FW min}^{-1}$) recorded the lowest CAT activity (Table 4;Fig 5).

Superoxide dismutase (SOD) activity showed a steady decline in stress induced seeds in all few landraces during seed germination ($p <0.05$). However, Oheruchitteni ($85.12 \text{U.g}^{-1}\text{FW min}^{-1}$), Ariki raavi ($83.79 \text{U.g}^{-1}\text{FW min}^{-1}$) and Nootripathu ($81.85 \text{U.g}^{-1}\text{FW min}^{-1}$) recorded the highest SOD activity. The drought tolerant check IR 64 Drt1 and the tolerant landrace kuliyyadichan showed 3.07 and 3.1 % reduction in SOD activity. On the contrary, Chandaikar, Nootripathu, Shabagaidhan responded by considerable increase in SOD activity (Table 4; Fig 5). The results also showed a differential response pattern for SOD activity during seed germination in stress induced seeds.

Influence on seed osmolytes during seed germination

Accumulation of osmolytes and compatible solutes helps the plants to cope from moisture stress, and maintaining the osmotic turgor in cells. Accordingly, our study showed a gradual increase in proline and total soluble sugars in emerging rice seeds under moisture deficit stress. Proline content of selected rice landraces significantly varied ($p <0.05$) under NS and DS conditions. Among the landraces, Kuliyyadichan ($88.21 \mu\text{g.g}^{-1}\text{FW}$) recorded the highest proline content compared with drought tolerant check IR64 (Drt1) ($85.800 \mu\text{g/g}$) followed by Rajalakshmi ($81.5 \mu\text{g.g}^{-1}\text{FW}$), Sabhagidhan ($79.2 \mu\text{g.g}^{-1}\text{FW}$), Nootripathu ($75.53 \mu\text{g.g}^{-1}\text{FW}$) and Mallikar ($72.01 \mu\text{g.g}^{-1}\text{FW}$) and the landrace Chenkayama ($46.2 \mu\text{g.g}^{-1}\text{FW}$) recorded the lowest proline content (Table 4; Fig 6). Total Soluble Sugar of selected rice landraces also significantly varied ($p <0.05$) among the treatments. Stress induced seeds of Kuliyadichan ($63.5 \text{mg.g}^{-1}\text{FW}$) recorded the highest TSS followed by Rajalakshmi ($61.00$), Sabhagidhan ($58.60$), Nootripathu ($53.5 \mu\text{g.g}^{-1}\text{FW}$), Mallikar ($46.2 \mu\text{g.g}^{-1}\text{FW}$), Chandaikar ($42.5 \mu\text{g.g}^{-1}\text{FW}$), Agulanthankodai ($35.2 \mu\text{g.g}^{-1}\text{FW}$) and Oheruchitteni ($32.5 \mu\text{g.g}^{-1}\text{FW}$) compared to susceptible genotype IR 64 (Table 4; Fig 6).

Principle component analysis for physiological and metabolic responses in rice landraces during seed germination influenced by moisture stress

In the present study, PCA was performed using RWC and other metabolic responses under DS in 10 selected rice landraces. The proportion of variance, cumulative proportion and eigen values are given in S1 (supplementary material). Out of eleven PCs, three
exhibited more than 1.0 eigen values and exhibited 88.93 % total variability among the variables studied. Among the three PCs, PC1 shared high proportion of total variation 66.61 % and PC2 and PC3 contributed 12.12 and 10.19 % of the total variance respectively. Eigen vectors of the PCs for physiological and metabolic responses during seed germination under DS are presented in S2. (supplementary material). In order to determine, the critical limit for the coefficients of the eigen vectors, coefficient values >0.3 were considered in the overall variation exhibited among selected landraces. The results showed that TSS, GA and hydrolytic enzymes had highest positive value (>0.9) followed by proline, CAT activity, RWC and IAA in PC1. Free aminoacids recorded highest positive value (0.91) followed by RWC (0.60) in PC2.

In the present investigation, the first two PCs were represented in a biplot to understand the relation among the landraces and their responses to DS (Fig 7). Traditional landraces viz., Nootripathu, Sabhagaidhan, Mallikar, Kuliyadichan and Rajalakshmi occupied the positive coordinates of the biplot showed positive values of the PCs. Hydrolytic enzymes, Phytohormones and CAT activity placed in the same quadrant influencing the inherent drought tolerance of these landraces.

Likewise, under non-stressed condition (NS) four PCs exhibited more than 1.0 eigen values and exhibited 88.61 % total variability. PC1 shared high proportion of total variation 41.87.61 %, whereas PC2, PC3 and PC4 contributed 23.9%, 13.1% and 7.7 % of the total variance respectively. Herein our study showed that lipase activity and GA had highest positive value of 0.96, followed by proline (0.92) and protease (0.85) in PC1. However in PC2, SOD recorded highest positive value followed by free aminoacids. Traditional rice genotypes viz., Mallikar, Sabhagidhan and Nootripathu formed a group in the right corner of the biplot showed positive values for both PCs. Unlike DS, free aminoacids, protease and lipase activity, RWC, proline and CAT activity influences the initial seed germination process. PCA results concluded amylase activity, IAA and GA modulation, osmolyte accumulation coupled with antioxidant gadgets are the possible mechanism for inherent drought tolerance and enhanced seed germination during DS in traditional rice landraces (Fig 7; S1. S2).

Discussion

Drought stress revamps morpho-physiological traits of rice landraces during seed germination

Development of drought-resistant crop plants remained to be a challenging task as drought tolerance is a quantitative trait with more environmental interactions. Identifying genetic variation is the first step towards development of drought-resistant crop plants (Basu et al. 2016). Landraces evolved under natural selection were the best source for improving traits controlling drought tolerance. In the present investigation, 100 landraces from various agro-climatic zones of Tamil Nadu, India were characterized and evaluated for their drought tolerance contributing morpho-physiological and biochemical traits during seed germination.

Seed germination is highly influenced by drought stress affecting the plant viability (Soleymani and Shahrajabian 2012). The germination percentage varied from 88.94 to 64.96% among the landraces with the highest germination percent registered in Kuliyadichan on 5 DAI compared to drought tolerant check (IR64 Drt1) and drought-suceptible check (IR 64). Only 52% of the tested rice landraces survived at lowest osmotic potential of -1.5 MPa. Germination percentage of survived landraces in our study showed a decreasing trend with increase in moisture stress which is in accordance with the previous studies done with fifteen rice landraces (Gampala et al. 2015). Furthermore, the germination rate of the survived landraces varied from 1.11 to 12.71 under induced moisture stress. Herealso, Kuliyadichan and Rajalakshmi performed far better than the drought tolerant check (IR64 Drt1). The results indicated that the induced moisture stress using PEG 6000 lowers the osmotic potential, affecting the water availability for germinating seeds. Seed hydration leads to the activation of key metabolic process. Elevated drought intensity reduce water uptake by seeds, thereby reducing seed germination rate and radicle development (Mishra and Panda, 2017). However, few rice landraces such as Kuliyadichan, Rajalakshmi exhibited considerable germination rate at -1.5MPa suggesting that the lower osmotic potential has no impact in the physical process of water uptake. Previous studies reported that the landraces are in a constant state of evolution by virtue of natural and artificial selection (Casanas et al., 2017). Besides, there was a significant G x T interaction for germination rate, highlighting that the landraces responded differentially to NS and DS.

Growth parameters such as root and shoot length of landraces were significantly reduced by early drought stress as compared to un-stressed seeds (NS). However, some of the landraces exhibited higher radicle and plumule length compared to the drought tolerant check (IR64 Drt1). More precisely, the susceptible check IR64 showed a sharp reduction in growth parameters. The vigor
index under DS ranged from 423.6 to 3417.5 across the landraces in the study. The reason for reduction in growth parameters under PEG-induced moisture stress is attributed to a reduction in turgor pressure which in turn influenced the cell elongation and expansion (Jaleel et al., 2009). Consequently, the RS ratio was reduced under DS and the differential responses of the landraces to drought were related to their inherent genetic potential. Thus the RS ratio and vigor index are considered as critical traits for identifying potential drought tolerant genotypes (Mishra and Panda, 2017). However, an increase in root growth under drought condition is a target trait and is considered as an adaptive strategy to increase water uptake (Basu et al., 2016). R/S is often found to be increased under harsh environmental conditions and has been reported as an essential trait for drought resilience (Xu et al., 2015, Govindaraj et al., 2010). Cultivars with a higher R/S ratio signify a good source-sink relationship and are the most preferred cultivars to screen for resilience to moisture stress. In the present investigation, Chenkayama, Oheruchitteni, Sabhagidhan, Kuliyadichan, Rajalakshmi, Chandaikar and Nootripathu showed significant RS ratio when compared to the drought tolerant check IR64 Drt1 which revealed that this landraces have better source-sink relationship.

RWC is the measure of dehydration level under PEG-induced moisture stress. The reduced osmotic potential of external microenvironment caused by PEG 6000 reversed the direction of water influx in the cell, thereby resulting in dehydration. In the present study, RWC of rice landraces declined under DS compared with NS. Few landraces registered higher RWC than the drought-tolerant check, IR64 Drt1 suggesting a wide spectrum of variation among the landraces for their sensitivity to drought. The data suggests that the traditional landraces possess improved cellular osmotic adjustment mechanisms to preserve membrane damage and sustain turgidity under DS (Swapna and Shyalaraj, 2017). Hence the landraces maintained more water in the cell in comparison to drought-sensitive IR64.

Drought stress remobilizes seed reserves during seed germination in rice landraces

Under moisture stress, drought resilient genotypes remobilize starch to provide energy and carbon at times when photosynthesis is potentially limited. Sugars and other derived metabolites released by starch remobilization support plant growth under stress, and function as osmoprotectants and compatible solutes to mitigate the negative effect of the stress (Krasensky and Jonak 2012). Starch degradation under stress is a common plant response and contributes to sugar accumulation under stress conditions (Thalmann et al. 2016). Sugars produced by starch remobilization act as signaling molecules, cross-talk with the ABA-dependent signaling pathway to activate downstream components involving stress response cascade (Rook et al. 2006). (Gonzalez-Cruz and Pastenes (2012) found that a drought-resistant variety of broad bean (Phaseolus vulgaris) degraded more starch than a drought-sensitive variety. Accordingly, our results showed that hydrolysis of starch had a positive relation with drought tolerance in landraces (Kuliyadichan, Chandaikar, Mallikar, Nootripathu, Rajalakshmi, Sabhagidhan, Agulanthankodai, Ariki raavi, Chenkayama and Oheruchitteni) compared to drought-tolerant check IR64 Drt1. Interestingly, the susceptible check, IR64 with maximum starch content under DS suggest that the remobilization of starch is slow, whereas it is vice versa under NS.

The quantitative and qualitative differences in seed reserves are highly related to the germination characteristics of species. Solutes produced by the degradation of storage proteins contribute to the germination of seed initiating radicle protrusion (Rosental et al. 2014). The synthesis and the accumulation of the compatible solutes like amino acids enhanced under osmotic stresses (Chinnusamy et al., 2005). Amino acids accumulation showed a characteristic linear increase and overall decrease in protein synthesis with the induction of drought stress in Brassica (Good and Zaplachinski 1994). Our study concluded that, the landraces Kuliyadichan, Chandaikar, Mallikar, Nootripathu, Rajalakshmi, Sabhagidhan, Agulanthankodai, Ariki raavi, Chenkayama and Oheruchitteni showed increased amino acid accumulation (13 to 116% increase over control) under moisture stress conditions. Similar results of increased total free amino acid accumulation in seven faba varieties under water stress were recorded (Ahmed et al. 2008). Increase in accumulation of aminoacids, acting as osmolyte and regulator of ion transport varied between the different Phaseolus species under osmotic stress (Parsons and Howe 1984).
Drought stress triggers hydrolytic enzymes during seed germination in rice landraces

Seed imbibition activates hydrolytic enzymes during germination process and mobilizes the seed macromolecules in the endosperm to simpler molecules. The activity of hydrolytic enzymes is associated with water activity. DS causes a significant reduction in water activity and thereby exerts a negative effect in activating hydrolases (Guzman-Ortiz et al., 2019). Like wise, the present study evidenced reduced amylase activity under DS when compared to NS in rice landraces on 5DAI. However, the genotype Kuliyadichan, Nootripathu, Chandaikar, Sabhagidhan, Rajalakshmi and Agulanthankodai performed on par with drought tolerant IR64 DRT, suggesting their genetic architect to withstand less water activity. The amylase activity in the cotyledons increased and reached maximum on fifth day of germination and depends on steeping factors, while the soluble sugars increased and starch decreased (Wang et al. 1988). Impaired amylase biosynthetic mechanism caused by DS prevents germination process as in the case of sensitive genotype IR64. On the contrary, studies in *Agropyron desertorum* seeds revealed that GAs alleviates the DS effects caused by α-amylase synthesis inhibition (Khondhare et al., 2015).

Storage protein mobilization is accomplished by *de nova* synthesized endopeptidases, of which proteases plays a crucial role during seed germination. Proteases are synthesized (as inactive or active precursors) and activated downstream, by other processing enzymes or autocatalytically (Grudkowska and Zagdanska, 2004). Cysteine proteases are the predominant protease group among rice and other cereals (Martinez et al. 2009). The results of the present study witnessed a reduction in protease activity under DS and significant GxT interaction noticed. The landraces Kuliyadichan and Rajalakshmi, recorded maximum proteolytic activity and further confirms efficient protein mobilization which supports further protein biosynthesis in embryo and endosperm (Tully and Beevers, 1978).

Lipase activity investigated under DS during seed germination showed significant G x T interaction, highlighting that the landraces responded differentially to NS and DS. Maximum lipase activity was observed under normal environment where the water activity is high. The triglycerides stored in oleosomes are hydrolyzed by lipases to energy which provides carbon backbones for embryonic growth (Quettier and Eastmond, 2009). DS impedes gluconeogenesis and sugar synthesis, thereby affecting carbon transport in gminating seeds. This intum inhibits seed germination. Our study identified few landraces with significant lipase activity such as Kuliyadichan, Nootripathu, Chandaikar, Sabhagidhan and Rajalakshmi, in contrast with IR64 Drt1, the drought-tolerant check. Nevertheless, the strategy to conquer reduced water activity and DS in traditional landraces is associated with their evolution and adaptive strategies (Casanaus et al., 2017).

Drought stress regulates phytohormone cross-talk during seed germination in rice landraces

Modifications in the synthesis, transport, and signaling of auxin profoundly affect drought resistance in rice. Increase in auxin level due to overexpression of auxin efflux carrier gene *OsGH3.2* (Du et al. 2012) resulted in improved drought tolerance. Increased level of endogenous indole-3-acetic acid (IAA) participate in the positive regulation of drought stress resistance, through regulation of root architecture, ABA-responsive genes expression, ROS metabolism, and metabolichomeostasis (Shi et al. 2014). Under drought stress condition, auxin response was significantly modulated. In the present study, the landraces Kuliyadichan, Chandaikar, Mallikar, Nootripathu, Rajalakshmi, Sabhagidhan, Agulanthankodai, Ariki raavi, Chenkayama and Oheruchitteni showed increased IAA concentration under moisture stress condition compared to that of the non-stress condition (NS). Auxins, more specifically IAA regulates seed germination process in a cross-talk with gibberellic acid (Gas) abscisic acid (ABA) and ethylene (ET) signaling pathways (Carrera et al., 2008; Wani et al., 2016).

Furthermore, there exists an antagonistic relationship between ABA and GA. Seed imbibition activates GA biosynthesis which intum elicits downstream endosperm hydrolyzing enzymes thereby alleviating the inhibitory effects of ABA on embryo development (Koomneef and Karssen, 1994). However, under DS, high ABA and low GA levels inhibits water uptake which intum prevents cell-wall loosening and reduces embryo growth (Schopfer and Plachy, 1985). Our study revealed that during DS, the landraces showed a remarkable increase in endogenous GA level over the tolerant check IR64 Drt1 which might be their inherent genetic makeup to adapt the unfavorable environment. The reason might be due to the repressive effect of GA on ABA during early phases of germination via eliciting the expression of genes encoding cell wall loosening enzymes such as α-expansins (Bhaskar et al., 2015). Thus GA exhibits an interplay between hydrolytic enzymes and cysteine proteases in plant-hormone mediated stress responses which is pronounced in the traditional rice landraces.
Drought stress resilience through enzymatic antioxidants during seed germination in rice landraces

Seed germination directly depends on ROS homeostasis that triggers associated cellular events. However, under stressed environment, ROS accumulation inhibits seed germination and aligns with the oxidative window for seed germination (Bailly et al., 2008). ROS moieties generated during seed germination are superoxide, hydrogen peroxide and free hydroxyl radicals and is regulated by enzymatic and non-enzymatic ROS scavenging mechanisms (Bailly, 2004). Significant changes of antioxidants such as superoxide dismutase, catalase and ascorbate-glutathione during seed germination were previously reported (Cembrowska-Lech et al., 2015; Anand et al., 2019). Accordingly the present study warrants activation of antioxidant gadgets, CAT and SOD in response to DS. The landraces exhibited increased catalase activity (2.4 to 54% increase over control), whereas SOD varied with land races. Interestingly, landraces with higher SOD activities suggest more oxidative stress and O$_2^-$ generation in response to DS. In order to maintain the ROS homeostasis, SOD would be activated in few landraces under study (Ahamad et al., 2018). Thus the landraces possess an inherent potential for early activation of antioxidant enzymes to prevent excess oxidative damage and ROS accumulation.

The O$_2^-$ generation in rest of the landraces might be below the critical limit and is more relevant in hormonal signaling (Finch-Savage, and Leubner-Metzger, 2006). Further, the correlation between ROS and phytohormones such as ABA and GA are well established by earlier studies. ROS accumulation remodels ABA synthesis which is a germination repressor and an antagonist to GA as discussed in the previous section. Increased CAT activity in the rice landraces indicates that H$_2$O$_2$ accumulation in germinating seeds degrade ABA via activation of ABA-8-hydroxylase and regulates its interaction with GA (Ishibashi et al., 2017; Anand et al., 2019). H$_2$O$_2$ stimulates GA/ABA balance in favour of GA which in turn triggers seed germination during DS (Kai et al., 2016; Liu et al., 2018).

Drought stress resuscitates osmolyte accumulation during seed germination in rice landraces

Osmolyte accumulation under DS for proline and sugar on 5DAI in germinating seeds registered a marked increase. Here also, the genotypes differed significantly (P < 0.01) with respect to these two parameters. The tolerant check IR64 Drt1 along with some indigenous landraces, such as Kuliya_idichan, Sabhadihan and Rajalakshmi showed higher proline and sugar accumulation under DS compared to the susceptible check (IR64). In general, drought tolerance mainly depends on the osmotic adjustment and maintenance of seed turgor (Sato and Yokoya 2008). The result of the present study indicates the prospects of rice landraces for regulation of redox potential and osmoprotection under DS (Singh et al., 2013). Hence the study authenticated that higher accumulation of osmolytes augments better protection from oxidative stress during seed germination, which is a feature of traditional rice landraces.

Relationship of morpho-physiological and biochemical traits under drought stress during seed germination

Correlation coefficients between morpho-physiological, biochemical traits, metabolic responses and germination rate during seed germination in different indigenous landraces under the DS (−1.5 Mpa) (Fig 8a; S3) revealed that the germination rate (GR) exhibited significant negative correlation with starch and SOD. However, positive correlations between GR and α-amylase, protease, lipase, catalase, TSS and RWC were observed. More precisely, GA showed positive correlation with hydrolytic enzymes and significant negative correlation with starch and protein content under DS. Likewise, IAA was strongly correlated with α-amylase, protease and indirectly proportionate to starch content. The results put forth that under DS conditions, the remobilization of seed reserves via hydrolytic enzymes is an intriguing factor in regulating phytohormone synthesis and ROS scavenging enzymes. However, under NS, GR was significantly correlated with hydrolytic enzymes, GA and osmolytes (sugar and proline) (Fig 8b; S4). Previous studies suggest that, early seedling vigor under drought stress was positively correlated with RWC, and accumulation of osmolytes (Swapna and Shyalaraj 2017; Mishra et al., 2019). Our findings suggested that the germination rate and seedling survival under DS was attributed by phytohormone modulation, antioxidants, triggered hydrolytic enzyme activities and osmoprotection.

Conclusion
The present investigation aimed at identifying potential local donor candidates towards genetic improvement of drought tolerance in rice. The study concluded 6 landraces viz., Kuliyadichan, Rajalakshmi, Sabhagidhan, Nootripathu, Chandaikar and Mallikar, selected among 100 traditional rice landraces germplasm from different agro-climatic regions of Tamil Nadu. Deciphering the possible mechanisms for drought tolerance revealed that germination rate and vigor index was governed by modulation of phytohormones antioxidants, hydrolytic enzymes and osmolyte accumulation. Further studies using omic approaches will give more understanding on the metabolic pathways and genes activated in these traditional landraces in contrast with drought tolerant IR64 Drt1.

**Abbreviations**

PEG - Poly Ethylene Glycol  
IAA - Indole Acetic Acid  
GA – Gibberellic acid  
ABA – Abscisic acid  
CAT – Catalase  
SOD – Superoxide dismutase  
NS - Non Stress  
DS - Drought Stress  
CRD - Completely Randomized Design

**Declarations**

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**Author contributions**

RA : Post graduate student executed most of the research work as a part of her M.Sc programme; ST: data curation and MS preparation; AS and DR guided and assisted in conducting the experiments; AKB: Principal Investigator and PG student supervisor, who has conceptualized the idea, designed the experiments and MS editing.

**Compliance with ethical standard**

**Conflict of interest**

The authors declare that they have no conflict of interest.

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Tables

Table 1. Details of collected rice landraces from different agro-ecological zones
| Agro-climatic Zone                      | Soil type               | Landraces                                                                 |
|----------------------------------------|-------------------------|---------------------------------------------------------------------------|
| North Eastern Zone                     | Red Sandy Loam, Clay Loam, Saline coastal Alluvium                       | Kar samba, Karudan samba, Karukot, Kadai kannan, Kattanoor, Krishna hemavathi, Sadhabahar, Kalanamak, Kalinga-3, Jai Sree Ram, Uma, Baskadam |
| North Western Zone                     | Non Calcareous Red, Non Calcareous Brown, Calcareous Black               | Maranelli, Kalaheri, Milagi, Molli karumbu, Vanapraba, Virendra, White sannam, Thuyamalli, China punchai |
| Cauvery Delta Zone                     | Red Loamy, Alluvium                                                  | Kuruvaikar, Seeraga samba, Kichadi samba, Kallinga, Kuruvi kalanchiyam, Meikuruvi, Surakuruvi, Kullakar, Kichali samba, Kattu samba, Thanga samba, Kothamalli samba, Milagu Samba, Aathur Kichadi Samba, Mapiillai samba, , Karuvalli, Eravi pondi |
| Southern Zone                          | Coastal Alluvium, Black, Red Sandy soil, Deep red soil                 | Kavuni, Chandaikar, Kuliadichan, Chithiraikar, Nootripathu, Poonkar, Mallikar, Rajalakshmi, Sivappu malli, Thamarai, Sivappukavuni, Norungan, Aanai Komban, Kattuyanam, Navarai, Karuppu Kavuni, Swama |
| High Rainfall Zone                     | Saline Coastal, Alluvium, Deep Red Loam                               | Mattaikar, Adukan, Anjali, Annada, Mulampunchan, Abya, Sabhagidhan, Pokkali, Bharathi, Jaya, Kayumma, Kottarasamba, Muttakaruva, Chenellu, Chembavu, Thondi, Aryan, Chenthadi, Kollam samba, Nochin samba, KunjuKurju Akshayponni, Chitteni, Chuvanna, Varakkura nellu, Chunjamkar nellu, Thavala Kannum, Vattan, Pattani, Ohenellu, Chakhae poirecton, Chakhaeamubi, Eluppai poo samba, Chemban, Chumala, Kerala kandhasala, Ariki raavi, Chenkayama, Oheruchitteni, Vandhana, , Varaputha, Veethirupa, Naatu ponni, Agulanthankodai |
| North Eastern part of India (Meghalaya)| Loamy to fine loamy                                                   | MBR (Meghalaya black rice)                                                |

*Seed materials are collected from farmers in the respective zones. The checks IR 64 and IR64 Drt1 obtained from DPB &G, A.C and R.I, Killikulam.

Table 2. Comparison of mean values between osmotic stress (-1.5MPa) and non-stressed (NS) traditional rice genotypes
| Genotypes            | Stress intensity | Germination rate | Root length | Shoot length | RS       | Vigour Index | RWC     |
|----------------------|------------------|------------------|-------------|--------------|----------|--------------|---------|
| Aanai Komban         | NS               | 3.08±0.03        | 4.66±0.08   | 24.02±0.44   | 0.194±0.001 | 2334.44±40.00 | 38.13±0.08 |
|                      | -1.5 MPa         | 2.76±0.11        | 4.00±0.07   | 17.66±0.32   | 0.227±0.001 | 423.56±18.59 | 23.15±0.10 |
| Aathur Kichadi Samba | NS               | 3.67±0.03        | 12.67±0.21  | 34.39±0.63   | 0.366±0.002 | 4554.98±78.05 | 33.54±0.07 |
|                      | -1.5 MPa         | 7.40±0.30        | 10.64±0.18  | 22.68±0.41   | 0.469±0.002 | 1743.52±76.52 | 22.64±0.11 |
| Adukan               | NS               | 3.62±0.03        | 8.31±0.14   | 24.22±0.45   | 0.343±0.002 | 3106.13±53.22 | 38.13±0.08 |
|                      | -1.5 MPa         | 6.41±0.26        | 5.47±0.09   | 14.85±0.27   | 0.369±0.001 | 921.09±40.42 | 23.15±0.10 |
| Agulanthankodai      | NS               | 3.63±0.03        | 7.60±0.13   | 16.25±0.30   | 0.468±0.002 | 1364.81±59.90 | 22.13±0.13 |
|                      | -1.5 MPa         | 9.94±0.41        | 6.18±0.10   | 11.04±0.20   | 0.560±0.002 | 1209.94±53.10 | 20.50±0.11 |
| Akshayaponni         | NS               | 3.67±0.03        | 10.13±0.17  | 25.02±0.46   | 0.405±0.002 | 3401.70±58.29 | 44.15±0.06 |
|                      | -1.5 MPa         | 6.85±0.28        | 7.30±0.12   | 20.87±0.38   | 0.350±0.001 | 1707.52±29.26 | 34.35±0.10 |
| Anjali               | NS               | 3.63±0.03        | 9.69±0.16   | 8.22±0.15    | 1.179±0.006 | 1707.52±29.26 | 34.35±0.10 |
|                      | -1.5 MPa         | 6.19±0.25        | 5.98±0.10   | 8.93±0.16    | 0.670±0.002 | 651.59±28.60  | 12.69±0.19 |
| Annada               | NS               | 3.67±0.03        | 14.06±0.24  | 12.14±0.22   | 1.159±0.006 | 2525.09±43.27 | 38.13±0.08 |
|                      | -1.5 MPa         | 7.40±0.30        | 5.67±0.10   | 12.64±0.23   | 0.449±0.002 | 958.67±42.07  | 23.15±0.10 |
| Ariki raavi          | NS               | 3.08±0.03        | 11.35±0.19  | 22.23±0.41   | 0.511±0.003 | 2724.87±46.69 | 34.14±0.09 |
|                      | -1.5 MPa         | 10.17±0.41       | 9.97±0.17   | 18.86±0.35   | 0.529±0.002 | 2071.50±90.91 | 14.59±0.12 |
| Aryan                | NS               | 3.08±0.03        | 8.71±0.15   | 43.06±0.79   | 0.202±0.001 | 4213.39±72.20 | 32.25±0.10 |
|                      | -1.5 MPa         | 4.42±0.18        | 4.76±0.08   | 27.99±0.51   | 0.170±0.001 | 1025.18±44.99 | 20.24±0.14 |
| Baskadam             | NS               | 3.67±0.03        | 5.07±0.09   | 24.92±0.46   | 0.203±0.001 | 2907.44±49.82 | 25.31±0.09 |
|                      | -1.5 MPa         | 4.97±0.20        | 3.55±0.06   | 17.76±0.32   | 0.200±0.001 | 750.02±32.92  | 10.22±0.09 |
| Bharathi             | NS               | 3.08±0.03        | 5.98±0.10   | 26.41±0.49   | 0.226±0.001 | 2635.40±45.16 | 38.13±0.08 |
|                      | -1.5 MPa         | 6.19±0.25        | 3.95±0.07   | 16.15±0.30   | 0.245±0.001 | 880.53±38.64  | 23.15±0.10 |
| Chakhae amubi        | NS               | 3.08±0.03        | 8.61±0.15   | 36.18±0.67   | 0.238±0.001 | 3644.01±62.44 | 33.78±0.10 |
|                      | -1.5 MPa         | 2.21±0.09        | 6.49±0.11   | 22.88±0.42   | 0.284±0.001 | 459.13±20.15  | 23.16±0.11 |
| Chakhae poirecton    | NS               | 3.08±0.03        | 8.31±0.14   | 34.78±0.64   | 0.239±0.001 | 3505.73±60.07 | 31.49±0.12 |
|                      | -1.5 MPa         | 2.21±0.09        | 6.69±0.11   | 23.98±0.44   | 0.279±0.001 | 479.57±21.05  | 21.86±0.12 |
| Chandaikar           | NS               | 3.68±0.03        | 16.52±0.28  | 25.22±0.46   | 0.655±0.003 | 4045.60±69.32 | 38.13±0.08 |
|                  |       |       |       |       |       |
|------------------|-------|-------|-------|-------|-------|
|                  | -1.5  | -1.5  |       |       |       |
|                  | MPa   | MPa   |       |       |       |
| Chemban          | 11.05±0.45 | 12.67±0.21 | 19.06±0.35 | 0.665±0.002 | 2476.48±108.69 | 23.15±0.10 |
|                  | 3.08±0.03 | 10.34±0.17 | 38.67±0.71 | 0.267±0.001 | 3985.64±68.29 | 34.50±0.08 |
|                  | 6.85±0.28 | 6.59±0.11 | 22.88±0.42 | 0.288±0.001 | 1428.18±62.68 | 20.50±0.11 |
| Chembavu         | 3.08±0.03 | 9.93±0.17 | 33.29±0.61 | 0.298±0.002 | 3513.87±60.21 | 33.54±0.07 |
|                  | 3.31±0.14 | 6.49±0.11 | 20.17±0.37 | 0.322±0.001 | 625.02±27.43 | 22.64±0.11 |
| Chenkayama       | 2.31±0.02 | 18.54±0.31 | 25.61±0.47 | 0.724±0.004 | 2684.20±45.99 | 54.55±0.67 |
|                  | 10.17±0.41 | 17.02±0.29 | 22.68±0.41 | 0.751±0.003 | 2849.76±125.07 | 24.13±0.66 |
| Chenthadi        | 3.08±0.03 | 8.51±0.14 | 37.08±0.68 | 0.230±0.001 | 3709.08±63.55 | 38.13±0.08 |
|                  | 6.74±0.27 | 7.50±0.13 | 19.97±0.37 | 0.376±0.001 | 1309.23±57.46 | 23.15±0.10 |
| Chumala          | 2.31±0.02 | 7.50±0.13 | 27.11±0.50 | 0.277±0.001 | 2110.76±36.17 | 30.18±0.07 |
|                  | 5.08±0.21 | 5.27±0.09 | 20.37±0.37 | 0.259±0.001 | 922.19±40.47 | 17.35±0.12 |
| Eluppai poo samba | 3.08±0.03 | 10.64±0.18 | 37.87±0.70 | 0.281±0.001 | 3944.97±67.60 | 28.69±0.07 |
|                  | 4.42±0.18 | 7.50±0.13 | 24.28±0.44 | 0.309±0.001 | 993.74±43.61 | 8.44±0.11 |
| Kar Samba        | 2.31±0.02 | 6.59±0.11 | 33.29±0.61 | 0.198±0.001 | 2434.08±41.71 | 46.99±0.09 |
|                  | 3.54±0.14 | 3.14±0.05 | 20.77±0.38 | 0.151±0.001 | 598.76±26.28 | 13.97±0.15 |
| Karuppu kavuni   | 3.77±0.03 | 9.83±0.17 | 41.86±0.77 | 0.235±0.001 | 5152.54±88.29 | 47.20±0.07 |
|                  | 6.74±0.27 | 7.19±0.12 | 28.90±0.53 | 0.249±0.001 | 1721.66±75.56 | 10.93±0.11 |
| Kattanoor        | 3.77±0.03 | 6.08±0.10 | 11.94±0.22 | 0.509±0.003 | 1788.61±30.65 | 47.20±0.07 |
|                  | 1.10±0.05 | 4.35±0.07 | 10.23±0.19 | 0.425±0.001 | 113.89±5.00 | 10.93±0.11 |
| Kattuyanam       | 3.77±0.03 | 5.17±0.09 | 19.83±0.37 | 0.261±0.001 | 2487.18±42.62 | 38.13±0.08 |
|                  | 7.51±0.31 | 3.45±0.06 | 16.86±0.31 | 0.204±0.001 | 1079.90±47.39 | 23.15±0.10 |
| Kerala kandhasala | 3.77±0.03 | 5.27±0.09 | 34.39±0.63 | 0.153±0.001 | 3956.59±67.80 | 24.84±0.09 |
|                  | 7.84±0.32 | 4.36±0.07 | 21.27±0.39 | 0.205±0.001 | 1423.38±62.47 | 12.28±0.08 |
| Kichadi samba    | 3.08±0.03 | 10.84±0.18 | 25.22±0.46 | 0.430±0.002 | 2928.22±50.17 | 38.13±0.08 |
|                  | 7.51±0.31 | 6.79±0.11 | 18.06±0.33 | 0.376±0.001 | 1320.47±57.95 | 23.15±0.10 |
| Kothamalli samba | 3.08±0.03 | 10.54±0.18 | 22.43±0.41 | 0.470±0.002 | 2676.07±45.85 | 24.12±0.10 |
|                  | 7.29±0.30 | 7.19±0.12 | 15.25±0.28 | 0.472±0.002 | 1157.10±50.78 | 10.96±0.14 |
| Kuliya dichan    | 3.82±0.01 | 18.75±0.32 | 25.42±0.47 | 0.738±0.004 | 4440.41±54.40 | 51.70±0.38 |
|                  | 12.71±0.52 | 16.01±0.27 | 22.07±0.40 | 0.725±0.003 | 3417.54±149.99 | 28.05±0.49 |
| Variety               | Type | Graininess | Protein (%) | Examination Date | Test Number | Test Method | Test Value | Test Value | Test Value | Test Value | Test Value |
|-----------------------|------|------------|-------------|------------------|-------------|-------------|------------|------------|------------|------------|------------|
| Kunju Kunju           | NS   | 3.78±0.03  | 10.74±0.18  | 40.46±0.75       | 0.266±0.001 | 5116.55±87.67 | 28.69±0.07 |
|                       | -1.5 MPa | 9.06±0.37  | 7.30±0.12   | 28.49±0.52       | 0.256±0.001 | 2295.03±100.72 | 8.44±0.11  |
| Mallikar              | NS   | 3.78±0.03  | 7.50±0.13   | 20.43±0.38       | 0.367±0.002 | 2744.17±26.88 | 47.20±0.07 |
|                       | -1.5 MPa | 9.28±0.38  | 6.59±0.11   | 13.04±0.24       | 0.505±0.002 | 1277.11±63.99 | 25.00±0.73 |
| Mapillai samba        | NS   | 3.58±0.03  | 10.44±0.18  | 35.38±0.65       | 0.295±0.002 | 4331.17±74.21 | 24.84±0.09 |
|                       | -1.5 MPa | 6.85±0.28  | 6.49±0.11   | 26.49±0.48       | 0.245±0.001 | 1598.78±70.17 | 12.28±0.08 |
| Mattaikar             | NS   | 3.74±0.03  | 7.50±0.13   | 27.01±0.50       | 0.278±0.001 | 3412.97±58.48 | 10.63±0.17 |
|                       | -1.5 MPa | 7.29±0.30  | 3.85±0.07   | 20.17±0.37       | 0.191±0.001 | 1240.13±54.43 | 8.06±0.08  |
| MBR                   | NS   | 3.58±0.03  | 9.22±0.16   | 32.69±0.60       | 0.282±0.001 | 3962.36±67.89 | 24.84±0.09 |
|                       | -1.5 MPa | 7.62±0.31  | 6.59±0.11   | 21.97±0.40       | 0.300±0.001 | 1540.60±67.61 | 12.28±0.08 |
| Milagu Samba          | NS   | 3.78±0.03  | 15.71±0.27  | 23.12±0.43       | 0.679±0.004 | 3867.39±66.27 | 25.98±0.07 |
|                       | -1.5 MPa | 8.62±0.35  | 8.01±0.14   | 21.97±0.40       | 0.142±0.001 | 578.26±9.91  | 28.46±0.06 |
| Mulam punchan         | NS   | 3.58±0.03  | 3.37±0.06   | 23.71±0.44       | 0.142±0.001 | 578.26±9.91  | 10.57±0.09 |
|                       | -1.5 MPa | 7.18±0.29  | 2.43±0.04   | 21.87±0.40       | 0.111±0.000 | 1236.67±54.27 | 10.57±0.09 |
| Muttakaruva           | NS   | 3.77±0.03  | 12.95±0.22  | 8.44±0.16        | 1.535±0.008 | 2113.39±36.21 | 24.84±0.09 |
|                       | -1.5 MPa | 4.86±0.20  | 6.89±0.12   | 6.82±0.12        | 1.011±0.004 | 470.23±20.64 | 12.28±0.08 |
| Nootripathu           | NS   | 3.67±0.03  | 5.88±0.10   | 21.63±0.40       | 0.272±0.001 | 2620.77±26.16 | 47.20±0.07 |
|                       | -1.5 MPa | 10.61±0.43 | 5.88±0.10   | 9.33±0.17        | 0.630±0.002 | 1130.82±56.72 | 25.90±0.21 |
| Norungan              | NS   | 3.77±0.03  | 4.76±0.08   | 31.40±0.58       | 0.152±0.001 | 3601.44±61.71 | 24.84±0.09 |
|                       | -1.5 MPa | 6.85±0.28  | 3.45±0.06   | 17.66±0.32       | 0.195±0.001 | 1023.61±44.92 | 12.28±0.08 |
| Ohennellu             | NS   | 3.77±0.03  | 4.97±0.08   | 31.69±0.58       | 0.157±0.001 | 3651.18±62.56 | 38.13±0.08 |
|                       | -1.5 MPa | 3.87±0.16  | 3.95±0.07   | 24.28±0.44       | 0.163±0.001 | 773.21±33.93 | 23.15±0.10 |
| Oheruchitteni         | NS   | 3.58±0.03  | 20.16±0.34  | 21.13±0.39       | 0.955±0.005 | 3886.71±66.60 | 45.44±0.62 |
|                       | -1.5 MPa | 10.83±0.44 | 14.09±0.24  | 9.26±0.35        | 0.731±0.003 | 2550.22±111.92 | 26.89±0.28 |
| Rajalakshmi           | NS   | 3.78±0.03  | 20.98±0.35  | 28.21±0.52       | 0.744±0.004 | 4896.70±83.90 | 49.67±2.04 |
|                       | -1.5 MPa | 11.27±0.46 | 18.75±0.32  | 26.09±0.48       | 0.719±0.003 | 3568.48±156.61 | 27.01±0.20 |
| Sabagaidhan           | NS   | 3.77±0.03  | 13.68±0.23  | 19.34±0.36       | 0.708±0.004 | 3273.13±56.08 | 41.61±1.33 |
|                       | -1.5 MPa | 11.05±0.45 | 11.65±0.20  | 16.05±0.29       | 0.726±0.003 | 2162.00±94.88 | 25.89±0.33 |
| Variety       | Enzyme | pH | Water Loss % | Enzyme Activity | Unit Activity | Enzyme Activity |
|---------------|--------|----|--------------|-----------------|---------------|-----------------|
| **Seeraka samba** | NS     | 3.58±0.03 | 3.55±0.06 | 2.29±0.04 | 1.548±0.008 | 548.49±9.40 | 35.73±0.07 |
|               | -1.5 MPa | 6.08±0.25 | 2.63±0.04 | 10.64±0.19 | 0.248±0.001 | 570.77±25.05 | 19.95±0.12 |
| **Sivappu malli** | NS     | 3.80±0.03 | 5.57±0.09 | 27.41±0.50 | 0.203±0.001 | 3314.27±56.79 | 39.07±0.06 |
|               | -1.5 MPa | 8.62±0.35 | 4.36±0.07 | 15.25±0.28 | 0.286±0.001 | 1195.78±52.48 | 12.94±0.09 |
| **Swarna**    | NS     | 3.77±0.03 | 9.22±0.16 | 27.41±0.50 | 0.316±0.002 | 3758.32±37.18 | 47.20±0.07 |
|               | -1.5 MPa | 3.98±0.16 | 8.31±0.14 | 22.07±0.40 | 0.377±0.001 | 847.11±42.38 | 10.93±0.11 |
| **Thuyamalli** | NS     | 3.77±0.03 | 6.99±0.12 | 20.43±0.38 | 0.342±0.002 | 2725.95±46.71 | 39.07±0.06 |
|               | -1.5 MPa | 6.41±0.26 | 4.36±0.07 | 16.25±0.30 | 0.268±0.001 | 934.77±41.02 | 12.94±0.09 |
| **Uma**       | NS     | 3.58±0.03 | 12.57±0.21 | 41.06±0.76 | 0.306±0.002 | 4986.09±49.42 | 41.26±0.09 |
|               | -1.5 MPa | 3.76±0.15 | 11.96±0.20 | 25.08±0.46 | 0.477±0.002 | 975.40±48.86 | 7.00±0.16 |
| **Vandhana**  | NS     | 3.77±0.03 | 6.18±0.10 | 18.84±0.35 | 0.328±0.002 | 2487.18±42.62 | 35.77±0.09 |
|               | -1.5 MPa | 5.19±0.21 | 4.46±0.08 | 13.55±0.25 | 0.329±0.001 | 661.42±29.03 | 14.91±0.13 |
| **Varaputha** | NS     | 2.31±0.02 | 4.66±0.08 | 39.37±0.73 | 0.118±0.001 | 2690.30±46.10 | 28.95±0.09 |
|               | -1.5 MPa | 6.96±0.28 | 3.65±0.06 | 26.49±0.48 | 0.138±0.000 | 1485.89±65.21 | 9.91±0.14 |
| **Veethirupa** | NS     | 3.77±0.03 | 7.60±0.13 | 23.42±0.43 | 0.325±0.002 | 3084.10±52.85 | 28.69±0.07 |
|               | -1.5 MPa | 4.20±0.17 | 4.15±0.07 | 19.46±0.36 | 0.213±0.001 | 702.06±30.81 | 8.44±0.11 |
| **Virendra**  | NS     | 3.55±0.03 | 15.61±0.26 | 29.00±0.53 | 0.538±0.003 | 4178.39±71.60 | 36.27±0.08 |
|               | -1.5 MPa | 6.85±0.28 | 8.51±0.14 | 19.26±0.35 | 0.442±0.002 | 1345.32±59.04 | 19.10±0.12 |
| **IR 64 Drt1** | NS     | 3.77±0.03 | 10.94±0.18 | 22.62±0.42 | 0.484±0.003 | 3332.80±57.11 | 50.68±2.35 |
|               | -1.5 MPa | 11.05±0.45 | 9.32±0.16 | 16.05±0.29 | 0.581±0.002 | 1981.18±86.95 | 24.52±0.53 |

Values represented are mean of three replications (±standard error).

**Table 3. Influence of drought stress on hydrolytic enzyme activities during seed germination in selected traditional landraces**
Germplasm  α-Amylase (U.g⁻¹ FW)  Protease (U.g⁻¹FW)  Lipase (U.g⁻¹FW)

|            | NS  | DS  | NS  | DS  | NS  | DS  | NS  | DS  |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|
| IR 64      | 19.55<sup>b</sup> | 2.13<sup>d</sup> | 1.72<sup>ab</sup> | 0.33<sup>f</sup> | 29.73<sup>de</sup> | 5.51<sup>d</sup> |
| IR 64 Drt1 | 23.72<sup>ab</sup> | 12.65<sup>a</sup> | 1.87<sup>a</sup> | 1.03<sup>abc</sup> | 40.07<sup>a</sup> | 25.10<sup>ab</sup> |
| Kuliyadichan | 24.51<sup>a</sup> | 13.57<sup>a</sup> | 1.71<sup>ab</sup> | 1.19<sup>a</sup> | 38.51<sup>ab</sup> | 29.21<sup>a</sup> |
| Mallikar    | 15.33<sup>e</sup> | 8.70<sup>abcd</sup> | 1.88<sup>a</sup> | 0.97<sup>abcd</sup> | 31.30<sup>abcd</sup> | 18.88<sup>abcd</sup> |
| Nootripathu | 20.97<sup>abcd</sup> | 11.50<sup>ab</sup> | 1.83<sup>a</sup> | 0.66<sup>b</sup> | 36.33<sup>abc</sup> | 23.42<sup>ab</sup> |
| Chandaikar  | 22.34<sup>abc</sup> | 12.95<sup>a</sup> | 1.67<sup>ab</sup> | 0.88<sup>b</sup> | 29.20<sup>abc</sup> | 15.20<sup>abcd</sup> |
| Sabhagidhan | 21.75<sup>abcd</sup> | 11.20<sup>ab</sup> | 1.80<sup>ab</sup> | 0.81<sup>b</sup> | 33.51<sup>abcd</sup> | 21.83<sup>abc</sup> |
| Rajalakshmi | 22.15<sup>abcd</sup> | 12.30<sup>ab</sup> | 1.75<sup>ab</sup> | 1.09<sup>abc</sup> | 37.88<sup>abcd</sup> | 26.51<sup>a</sup> |
| Agulanthankodai | 18.43<sup>cd</sup> | 10.53<sup>abc</sup> | 1.55<sup>bc</sup> | 0.55<sup>c</sup> | 28.42<sup>cdef</sup> | 11.20<sup>bc</sup> |
| Ariki raavi | 19.57<sup>b</sup> | 4.94<sup>d</sup> | 1.31<sup>d</sup> | 0.47<sup>c</sup> | 25.33<sup>cdef</sup> | 8.46<sup>cd</sup> |
| Chenkayama  | 17.53<sup>de</sup> | 3.88<sup>cd</sup> | 1.27<sup>d</sup> | 0.33<sup>f</sup> | 21.52<sup>f</sup> | 5.33<sup>d</sup> |
| Oheruchitteni | 18.26<sup>de</sup> | 4.10<sup>cd</sup> | 1.33<sup>cd</sup> | 0.45<sup>ef</sup> | 23.77<sup>ef</sup> | 5.88<sup>d</sup> |
| CD (0.05)   | 4.12 | 6.39 | 0.22 | 0.44 | 7.28 | 12.74 |

Values are mean (±standard error) (n=3) and values followed by the same letter in each column are not significantly different from each other as determined by DMRT (p < 0.05). NS – non-stressed; DS – drought stress at -1.5MPa

Table 4. Mean square of ANOVA analysis for morpho-physiological and metabolic parameters during seed germination

|         | RWC   | Proline  | TSS  | GA   | IAA   | CAT | SOD   | α-amylose | Protease | Lipase |
|---------|-------|----------|------|------|-------|-----|-------|-----------|----------|--------|
| Lnadraces | 1610.0 | 8355.7 *** | 5379.7 *** | 44.246 *** | 4211.1 *** | 12.6370 *** | 3908.9 *** | 648.40 *** | 3.8728 *** | 3569.6 *** |
| Treatment | 9005.9 *** | 28792.8 *** | 16172.1 *** | 38.500 *** | 3982.8 *** | 1.0015 *** | 8.4 *** | 2299.44 *** | 14.9331 *** | 4006.9 *** |
| GXT     | 835.1 *** | 2225.1 *** | 2565.0 *** | 1.001 *** | 1751.2 *** | 6.0718 *** | 54.4 *** | 158.35 *** | 0.8296 *** | 257.8 *** |
| Residuals | 268.1 | 75.1 | 29.1 | 0.187 | 13.9 | 0.1110 | 274.7 | 7.06 | 0.0480 | 16.0 |

G- Landraces; T- Treatment (drought stress and non-stressed environments)

Figures
Figure 1

Germination index and 1b. Survival per cent of traditional rice landraces under drought stress (-1.5 MPa). Data represented are mean (±standard error) (n=3) and values followed by the same lowercase letter are not significantly different from each other as determined by DMRT (p ≤ 0.05). IR 64 and IR 64 (Drt1) used as negative and positive checks respectively.
Figure 2

Influence of drought stress on a) starch b) protein and c) aminoacid content during seed germination on 5 DAI in selected rice landraces. Data represented are mean (±standard error) (n=3) and values followed by the same lowercase letter are not significantly different from each other as determined by DMRT (p ≤ 0.05). IR 64 and IR 64 (Drt1) used as negative and positive check respectively and stress imposed at -1.5 MPa.
Figure 3

Heatmap and clustering of selected traditional landraces based on hydrolytic enzyme activities viz., α-amylases, proteases and lipases. NS- non-stressed environment; DS- drought stress at -1.5 MPa in comparison to IR64 (Drt1).

Figure 4

Influence of drought stress on phytohormones, GA and IAA during seed germination on 5 DAI in selected rice landraces. Data represented are mean (±standard error) (n=3) and values followed by the same lowercase letter are not significantly different from each other as determined by DMRT (p ≤ 0.05). IR 64 and IR 64 (Drt1) used as negative and positive check respectively and stress imposed at -1.5 MPa.
Figure 5

Influence of drought stress on antioxidants, CAT and SOD in seedlings on 26th d in selected rice landraces. Data represented are mean (± standard error) (n=3) and values followed by the same lowercase letter are not significantly different from each other as determined by DMRT (p ≤ 0.05). IR 64 and IR 64 (Drt1) used as negative and positive check respectively and stress imposed at -1.5 MPa.

Figure 6
Influence of drought stress on osmolytes, proline and sugars during seed germination on 5 DAI in selected rice landraces. Data represented are mean (±standard error) (n=3) and values followed by the same lowercase letter are not significantly different from each other as determined by DMRT (p ≤ 0.05). IR 64 and IR 64 (Drt1) used as negative and positive check respectively and stress imposed at -1.5 MPa.

Figure 7

Biplots of principal component analysis under osmotic stress (-1.5 Mpa) for morpho-physiological, biochemical and metabolic responses in traditional landraces. DS- drought stress at -1.5 MPa in comparison to IR64 (Drt1); NS - non-stressed environment.

Figure 8

Correlation between morpho-physiological characteristics, phytohormone modulation and antioxidant enzymes in response to induced oxidative stress in selected traditional landraces. a) DS- drought stress at -1.5MPa. b) NS- non-stressed environment.

Supplementary Files

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