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ORIGINAL ARTICLE

# Physiological introspection into differential drought tolerance in rice cultivars of North East India

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## Abstract

Drought is a vice to world crop production, exponentially enhanced by global climate change. Rice, a basic food crop for a major chunk of world populace, is largely affected by environmental challenges such as drought, salinity and heavy metal. This study brings to limelight differential drought tolerance capacity of rice varieties indigenous to North East India, a hot bed of *indica* rice diversity. Initial screening of rice varieties were performed through physiological dose-dependent studies under PEG (0%, 10%, and 20% which is equal to osmotic potential values of 0.001, 0.54 and 1.09 MPa, respectively), induced drought stress for three time intervals of 1, 3 and 5 days. Hierarchical clustering of the parameters on which the cultivars were analysed revealed Tampha and KMJ 1-12-3 to be relatively more tolerant whereas Chandan and Ketaki Joha as the sensitive ones. Biochemical studies for hydrogen peroxide ($H_2O_2$), proline, lipid peroxidation (MDA), lipoxygenase (LOX), superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and glutathione reductase (GR) were then performed on two cultivars (Tampha and Chandan) to elucidate the differential tolerance to drought, focusing on anti-oxidative response mechanism. The biochemical fingerprint showed significantly lower accumulation of $H_2O_2$ and MDA; higher accumulation of proline; and higher activity of SOD, CAT, DHAR, MDHAR and GR in the tolerant variety Tampha when compared to Chandan. In addition, alteration of chlorophyll fluorescence due to stress was also monitored to ascertain the variation in photosynthetic efficiency between the tolerant and sensitive cultivars. Tampha showed better photosynthetic activity in comparison to Chandan as quantified by measuring chlorophyll fluorescence. This manuscript thus throws new light into the drought stress response of the varieties from North East India with global implications.

**Keywords** *Oryza sativa* · Drought · Abiotic stress · Tolerance · Physiology

## Introduction

Rice [*Oryza sativa* L. spp. *indica*] is the basic food grain for most of the world populace and 80% of the world's undernourished (IRRI 2016). But its production is highly compromised by abiotic stressors such as drought and salinity (Omisun et al. 2018). To be specific, 66% of the world area is struck by drought (McCabe and Wolock 2015). To fulfil UN global sustainable goals and thus to provide food security for the ever increasing global population, it is very much essential to enhance rice production through mitigation of constraints (Griggs et al. 2013). Sustainability in a particular stress depends on very specific response, which in turn is tailored by multiple factors. Limited knowledge on how plants respond to drought severity and time duration impeded works on improving tolerance in crops. Thus it is

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very much necessary to elucidate in detail drought-mediated responses in plants.

Drought stress itself is complex and more complex is the plants' response to drought. Plants adopt diverse response mechanisms to drought varying upon species, and also severity of drought. Drought basically causes water deficiency (osmotic stress) which leads to alteration in growth and metabolism. Root and shoot, growth rate and biomass are important markers for water deficiency (Nguyen et al. 2004). Relative Water Content (RWC) which is a sign of the state of water balance of a plant (Todaka et al. 2017) is also an important indicator of level of drought stress. Water being the universal solvent, reduction in RWC disrupts cellular homeostasis (Sun et al. 2015). Though downregulation of shoot growth caused by dehydration stress can be beneficial for plant in times of drought by limiting transpiration, but such alteration also decreases crop yield and productivity (Bhaskara et al. 2017).

The physiological signatures due to osmotic stress are the outcome of change in internal metabolic activities such as loss of reactive oxygen species (ROS) homeostasis, excessive production of antioxidant enzymes and metabolites. The excessive production of ROS is due to osmotic stress-induced inhibition of carbon dioxide assimilation, malfunction in electron transport chain, enhancement in Mehler reaction, etc. (Munne-Bosch and Pinto-Marijuan 2016). Significant loss of ROS homeostasis leads to higher build-up of ROS like $H_2O_2$, $O_2^-$; lipid peroxidation that leads to membrane instability. Magnitude of lipid peroxidation can be measured by scoring malondialdehyde (MDA) and thus also acts as an indicator of oxidative stress (Saha et al. 2016a). Under mild drought stress, these ROS moieties act as signalling molecules regulating stress avoidance responses but in adverse condition excessive ROS overwhelm the anti-oxidative defence mechanism resulting in extensive cellular damage (Saha et al. 2016b; Cruz de Carvalho 2008). Excess ROS yield also leads to chlorophyll breakdown (Aken and Breusegem 2015). The light energy absorbed by chlorophyll pigments during day time is either used to drive photosynthetic reactions or dissipated as heat or emitted as fluorescence. The fluorescence hence emitted can be measured to indirectly ascertain the efficiency of the light absorbed by pigments to drive photosynthesis under stress condition (Sheng et al. 2008).

The antioxidant enzymes especially those which are responsible for Ascorbate–Glutathione (AsA–GSH) cycle are important players responsible for mitigating the plant stress response whose components are found in cytosol, mitochondria, chloroplast and peroxisomes (Kang et al. 2013). AsA–GSH cycle helps in maintaining balance between ascorbate (AsA) and glutathione (GSH), in the process detoxifying ROS. Antioxidant enzymes of the Ascorbate–Glutathione (AsA–GSH) cycle, i.e. ascorbate peroxidase (APX), monodehydroascorbate reductase cycle (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR) are important players in detoxification of excess ROS molecules (Wu et al. 2015). Beside these catalase (CAT), proline, carotenoids, and tocopherols have also major role to play in ROS detoxification (Gill and Tuteja 2010).

North East India is a hotspot of *Indica* rice diversity, which grow in varied ecologies such as upland, lowland, rainfed, irrigated, and flood-prone areas. Though North East India is not prone to scarce rainfall, low soil moisture during post-monsoon season is a serious problem (Das et al. 2017). The different ecologies in which these varieties are cultivated indicate for existence of unexplored diverse gene pool. Evaluation of these varieties might lead to fishing out a relatively tolerant rice variety which can later be used for 'omic' studies to better understand stress responses. Hence, a study on differential tolerance of the native varieties, with an ultimate goal of improving rice crop production in drought-affected domains of the world for sustainable agriculture is essential.

## Materials and methods

### Plant material collection and growth regime

Twenty-one rice genotypes were procured from Regional Agriculture Research Station (RARS), Akbarpur, Karimganj, Silchar, Assam, India and Regional Rainfed Lowland Rice Research Station (RRLRRS) Gerua, Guwahati, Assam, India (Supplementary 1). Required quantity of viable rice seeds were directed for surface sterilisation (0.01% $HgCl_2$ w/v) for 3–5 min. The treated seeds were rinsed properly with distilled water for 5–6 times, soaked in water for 6 h, then placed for germination in petri plates with cotton bed at 30 °C. Three-day old healthy equally sprouted seeds were transplanted into plastic container holding Hoagland nutrient solution (pH 6.2; Hoagland and Arnon 1950). The germinated seedlings were then allowed to grow in growth chamber for 7 days under white fluorescent tube light (Philips 20W TLD, India) for a photoperiod of 16 h. The nutrient medium was changed every 2 days. On the 7th day, Polyethylene Glycol 6000 (PEG; 10% and 20%) treatment was given. Plants are harvested on first, third and fifth days after the treatments for various physiological and biochemical analyses.

### Physiological analysis

Physiological analysis of 21 varieties, i.e. root length, shoot length, fresh weight, dry weight of both root and shoot and relative water content (RWC) of shoot were carried out at 1,

3 and 5 days for 10% and 20% PEG. Five plants were randomly selected for the measurement of both root and shoot in centimetre scale. Fresh weight of these five plants was also scored. Shoots were then kept submerged in water for at constant temperature in diffused light for 6 h and turgid weight of shoot was recorded. Both root and shoots were oven-dried at 72 °C for 3 days and dry weight measured.

### Grading of varieties for relative osmotic tolerance

Drought-tolerant indices were calculated to grade the 21 varieties according to their differential drought tolerance capability by dividing the scores obtained from measuring the physiological parameters at 1-, 3- and 5-day stress durations, for different stress doses of PEG (10% and 20%) to their respective controls. Hierarchical cluster analysis (HCA) was performed using Euclidean distance algorithm (Ali et al. 2014). Cluster group gradings were obtained and the genotypes were ranked on the basis of the sums of the cluster group rankings at 1, 3 and 5 days. The smallest and the largest sum designated the most tolerant and sensitive varieties, respectively.

### Measurement of chlorophyll fluorescence

Junior PAM (Heinz, Walz, Germany) was used to measure the maximum photochemical quantum yield of photosystem II (Fv/Fm) and non-photochemical quenching (NPQ) of tolerant and sensitive rice leaves after a 20 min of acclimation in dark at room temperature in the laboratory condition (Cheng et al. 2016).

### Hydrogen peroxide content

$H_2O_2$ estimation was done following Sagisaka (1976).

### Proline content

Proline was conducted using Bates et al. (1973) protocol.

### Estimation of lipid peroxidation

The extent of lipid peroxidation was quantitated in terms of MDA content (Heath and Packer 1968).

### Lipoxygenase (LOX) activity estimation

LOX (EC. 1.13.11.12) assay was carried out following the protocol of Axelrod et al. (1981). Absorbance was scored at 234 nm.

### Histology of $O_2^-$, $H_2O_2$, lipid peroxidation, and membrane damage

Detection of superoxide radical ($O_2^-$) was performed by nitro-blue tetrazolium (NBT) staining with a slight modification of Rao and Davis (1999) both in root and leaf sections under stressed as well as control conditions. Hydrogen peroxide ($H_2O_2$) was determined by 3,3-diaminobenzidine (DAB) staining (Ramel et al. 2009). Histological detection of lipid peroxidation was performed following the method of Awasthi et al. (2018). The loss of plasma membrane wholeness was checked employing Evans blue staining following the procedure of Schutzendubel et al. (2001).

### Glutathione assay (estimation of total, reduced and oxidised glutathione)

Glutathione assay was performed following the method of Sahoo et al. (2017).

### Measurement of antioxidant enzymes

To prepare enzyme extract, tissue sample (root and shoot) was grounded with 0.1 M phosphate buffer (pH 6.8) containing 0.1 mM EDTA and 1% PVP in a chilled mortar and pestle. The homogenate was centrifuged at 15,000 rpm for 20 min at 4 °C. Then supernatant was used as enzyme extracts for estimation of CAT, POX, SOD, POX, GR, DHAR, and MDHAR. For APX extraction, tissue sections were homogenised with 0.1 M phosphate buffer of pH 6.8 containing 0.1 mM EDTA, 1% PVP and 1 mM ascorbate.

#### Superoxide dismutase (SOD)

SOD (EC 1.15.1.1) assay was performed following the protocol of Giannopolitis and Ries (1977).

#### Catalase (CAT)

CAT (EC 1.11.1.6) assay was done by the method of Chance and Maehly (1955).

#### Peroxidase (POX)

POX (EC 1.11.1.7) activity was assayed according to Chance and Maehly (1955) procedure.

#### Ascorbate peroxidase (APX)

APX (EC 1.11.1.11) assay was conducted using Nakano and Asada (1981) protocol.

#### Dehydroascorbate reductase (DHAR)

DHAR (EC 1.8.5.1) assay was conducted following the method of Ma and Cheng (2004).

#### Monodehydroascorbate reductase (MDHAR)

MDHAR (EC 1.6.5.4) assay was done according to the method of Miyake and Asada (1992).

#### Glutathione reductase (GR)

GR (EC 1.6.4.2) was assayed by following Smith et al. (1988).

### Statistical analysis

All experiments were performed thrice. Statistical comparison between variance was ascertained by ANOVA (Analysis of Variance) and significant difference ($p < 0.5$) between mean values, where $n = 3$ ($n$ is number of times experiments done) was indicated. All statistical analyses were carried out using SPSS 18 software package and Excel 2013. Data were represented as mean ± S.E. Hierarchical cluster analysis was performed and heat maps were generated using MeV version 4.9.0 (http://mev.tm4.org/). Graphical representations were made using OriginLab (Version 8.5) software package.

Correlation analysis was performed to determine the relationships among SOD, CAT, DHAR, GPX, GR, MDHAR, POX, APX, LOX, MDA, $H_2O_2$ and proline at 20% PEG stress for 1, 3 and 5 days for root and shoot separately.

## Results

### Measurement of relative root and shoot length

Induction of drought stress through PEG treatment on rice seedling caused physio-morphological alterations directing towards growth suppression. Growth in indigenous rice cultivars as expressed by root lengths was found to be significantly reduced. The decrement in root elongation was more prominent in Chandan where 24.76%, 29.29%, and 40.54% root reduction was documented while in Tampha variety only 4.49%, 6.08%, and 7.25% reduction was observed on 1, 3 and 5 days, respectively, when compared to control (Table 1, supplementary 2). The root length was less reduced in Tampha, CR Dhan 601, Aghoni bora, and KMJ 1-12-3, while was more profound in Chandan, Keteki Joha, Joymati, and Anjali, respectively on PEG stress. In shoot, similar observations were made among the 21 varieties screened. In this study, reduction in relative shoot length (%) of Tampha was observed to be 4.14%, 5.31%, and 9.20%; while in Chandan it was 31.71%, 39.05%, and 44.87% on 1, 3 and 5 day PEG stress, respectively, on exposure to 0% 10%, and 20% PEG dose (Table 1, supplementary 2). The percentage of root length reduction in Chandan was significantly greater than all other genotypes. These results indicate that Tampha shows higher tolerance than other genotypes (Fig. 1).

### Root relative fresh and dry weight

The presence of PEG stress in the hydroponic nutrient medium significantly ($p < 0.05$) lessened the relative root fresh weight and dry weight at different time points. Root fresh and dry weight were observed to be drastically reduced on higher PEG dose. It is evident that there was prominent deviation in dry matter assemblage within the varieties. Chandan, Keteki Joha and Joymati were found to be very susceptible to PEG stress, since their fresh and dry matter decrement was very prominent (40.22%, 37.78%, 33.9% and 43.15%, 41.18%, 39.53%) at 5 days. Of all genotypes, only Tampha, CR Dhan 601 and KMJ 1-12-3 had less diminution in root fresh and dry matter aggregation when revealed to stress (9.25%, 10.99%, 11.55% and 11.77%, 14.52%, 18.69%, respectively) at 5 days. Drought stress significantly decreased relative root length in all genotypes of rice (Table 1, Supplementary 2). On exposure to PEG stress gradual decline in growth was recorded at higher concentration on long-term exposure.

### Shoot fresh, dry biomass and RWC

The presence of PEG in the liquid nutrient medium significantly ($p < 0.05$) reduced the shoot fresh weight and dry weight at 1, 3 and 5 days (Table 1, Supplementary 2). Shoot fresh and dry weight were observed to be significantly reduced on stress and it was quiet evident that there was prominent divergence in dry matter reduction among the cultivars. The cultivars Chandan, Keteki Joha, and Joymati were susceptible to PEG stress, and their fresh and dry matter decrement was found to be pronounced. At 5 days, the fresh and dry matter decrease of Chandan, Keteki Joha and Joymati were observed to be 46.32%, 43.34%, 41.93% and 42.86%, 43.94%, 41.41%, respectively, at 20% of PEG. Of all genotypes, only Tampha, CR DHAN 601, and KMJ 1-12-3 had less diminution in shoot fresh and dry matter aggregation when revealed to PEG stress (10.40%, 19.22%, 24.16% and 11.31%, 15.01%, 12.86%, respectively) at 5 days. RWC is a sign of the state of water balance of a plant. Among the plants which were subjected to PEG stress, significant

**Table 1** Effect of PEG stress on relative length, relative fresh weight and relative dry weight in root and shoot of 21 rice genotypes after 5 days in 20% PEG containing hydroponic solution

| Parameters | Root | | | Shoot | | | |
|---|---|---|---|---|---|---|---|
| | %RRL | %RFW | %RDW | %RRL | %RFW | %RDW | %RRWC |
| Cultivars | | | | | | | |
| Tampha | 7.25 ± 3.99 | 9.25 ± 3.81 | 11.77 ± 3.50* | 9.20 ± 3.34* | 10.40 ± 1.07* | 11.31 ± 2.76 | 9.08 ± 0.30 |
| CR Dhan 601 | 7.52 ± 2.00 | 10.99 ± 2.22 | 14.52 ± 5.79 | 11.82 ± 2.66 | 19.22 ± 2.53* | 15.01 ± 5.78 | 10.54 ± 0.36 |
| KMJ 1-12-3 | 7.18 ± 3.38 | 11.55 ± 2.05* | 18.69 ± 4.99* | 12.16 ± 4.09* | 24.16 ± 2.45* | 12.86 ± 2.62 | 11.08 ± 1.49 |
| Gopinath | 10.15 ± 2.44 | 13.1 ± 2.22* | 21.53 ± 2.32* | 15.27 ± 3.36 | 21.32 ± 0.94* | 17.97 ± 4.99* | 13.00 ± 2.19* |
| Aghoni bora | 7.99 ± 0.73 | 15.04 ± 0.96* | 15.14 ± 8.12* | 12.82 ± 3.90* | 23.48 ± 6.01* | 16.25 ± 0.98* | 13.36 ± 1.37* |
| Gandhi biroin | 14.42 ± 4.40* | 17.12 ± 5.60* | 20.74 ± 5.89* | 20.94 ± 1.53* | 26.10 ± 2.92* | 19.80 ± 0.90* | 14.31 ± 1.52 |
| Pooja | 17.19 ± 2.82* | 18.75 ± 5.34* | 21.96 ± 4.68* | 21.91 ± 2.84* | 28.06 ± 0.74* | 22.32 ± 1.99* | 15.31 ± 2.16* |
| Luhit | 10.42 ± 2.36* | 17.99 ± 3.03* | 23.89 ± 3.89* | 20.93 ± 1.30* | 27.12 ± 1.99* | 20.75 ± 0.51* | 15.06 ± 5.08 |
| Disang | 18.42 ± 1.62* | 24.83 ± 3.52* | 23.84 ± 4.76* | 23.62 ± 4.61* | 29.30 ± 2.76* | 26.51 ± 3.57* | 17.25 ± 3.72* |
| Naveen | 18.15 ± 2.79* | 22.90 ± 3.04* | 22.40 ± 6.77* | 21.30 ± 1.69* | 28.15 ± 3.59* | 23.43 ± 5.26* | 15.66 ± 1.09* |
| Khamti lahi | 22.32 ± 3.75* | 25.23 ± 1.84* | 28.15 ± 4.15* | 25.39 ± 1.01* | 30.40 ± 0.88* | 28.76 ± 2.74 | 18.12 ± 0.82* |
| Abhisek | 20.12 ± 2.46* | 27.23 ± 3.92* | 24.64 ± 2.43* | 25.43 ± 1.62* | 30.88 ± 2.16* | 30.05 ± 1.89* | 17.67 ± 1.89* |
| Sabita | 26.18 ± 1.96* | 27.82 ± 3.82* | 36.51 ± 1.73* | 30.57 ± 3.33* | 35.68 ± 2.19* | 39.82 ± 0.93* | 21.72 ± 1.62* |
| Bakul Joha | 23.52 ± 2.51* | 29.00 ± 5.01* | 34.19 ± 3.14* | 27.27 ± 1.64* | 31.97 ± 6.11* | 36.40 ± 0.24* | 20.94 ± 6.73 |
| Anjali | 29.34 ± 1.9* | 32.65 ± 1.76* | 37.35 ± 6.21* | 31.46 ± 4.34* | 39.67 ± 5.49* | 40.73 ± 1.85* | 23.69 ± 5.20 |
| Kunkuni Joha | 22.76 ± 1.16* | 31.46 ± 2.03* | 31.94 ± 3.68* | 25.62 ± 0.37* | 32.32 ± 2.44* | 31.53 ± 2.66* | 18.29 ± 0.62 |
| Kola Joha | 28.96 ± 2.34* | 33.00 ± 2.92* | 31.82 ± 4.25 | 27.21 ± 2.98* | 33.30 ± 2.40* | 34.36 ± 2.04* | 18.42 ± 0.58* |
| Joymati | 30.56 ± 2.53* | 33.92 ± 3.47* | 39.53 ± 4.57* | 32.32 ± 2.44* | 41.93 ± 2.36* | 41.41 ± 1.72* | 23.20 ± 1.37 |
| Swarna | 24.74 ± 3.26* | 35.17 ± 3.29* | 36.67 ± 3.33* | 25.73 ± 1.56* | 33.88 ± 5.58* | 39.11 ± 2.27* | 20.22 ± 1.93* |
| Keteki Joha | 31.17 ± 4.66* | 37.78 ± 2.23* | 41.18 ± 2.43* | 36.25 ± 1.30* | 43.34 ± 1.02* | 43.94 ± 1.14* | 37.15 ± 3.34* |
| Chandan | 40.54 ± 4.19* | 40.22 ± 2.18* | 43.15 ± 3.69* | 44.87 ± 1.92* | 46.32 ± 3.00* | 42.86 ± 1.79* | 47.98 ± 1.75* |

Data presented are mean ± SE ($n = 3$)

Significant mean difference between control and stress plants was significant at $p < 0.05$ (*) by LSD test

*%RRL* percentage reduction in relative length, *%RFW* percentage reduction in relative fresh weight, *%RDW* percentage reduction in relative dry weight, *%RRWC* percentage reduction in relative water content

decline in RWC was observed in higher doses (Table 1, Supplementary 2). In shoot, relatively high decline in RWC was observed in Chandan, Keteki Joha and Joymati (47.98%, 37.15% and 23.19%, respectively) while less decline in Tampha, CR Dhan 601 and KMJ 1-12-3 (9.08%, 10.54%, and 11.08%, respectively) at 5 days' interval when compared to control.

## Grading of varieties for relative drought tolerance

To group the 21 varieties in accordance to their drought tolerance capability, hierarchical clustering using Euclidean distance algorithm was done. For 1-, 3- and 5-day stress durations, three, two and six clusters were formed, respectively (Fig. 2, Supplementary 3). The ranking of the cultivars on the basis of cluster analysis depicted Tampha and KMJ 1-12-3 to be most tolerant, whereas Ketaki Joha and Chandan to be the most sensitive ones (Table 2). Based on this analysis Tampha and Chandan were chosen for further biochemical analysis.

## Chlorophyll fluorescence

Chlorophyll fluorescence was measured in terms of Fv/Fm and NPQ which depicts the extent of alteration in PSII efficiency in response to environmental cues. Fv/Fm was measured in both Tampha and Chandan varieties in control and stressed conditions. Chandan under stress condition showed more retardation from the stable range of 0.8 depicting the impact of drought on photosynthetic apparatus from that of Tampha. Whereas under control condition both the varieties showed their Fv/Fm values to be almost 0.8 (Fig. 3).

## Hydrogen peroxide measurement

$H_2O_2$ generally accumulate in all living cells, it is a ROS molecule which had been used as an indicator of levels of ROS and loss of ROS homeostasis. As can be seen, $H_2O_2$ contents increased in both varieties 1, 3 and 5 days after PEG treatment. $H_2O_2$ increased in all the stressed plants when

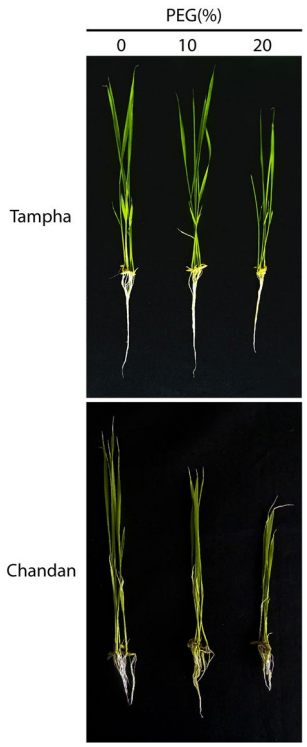


**Fig. 1** Physiological difference between Tampha and Chandan at 0, 10, and 20% PEG stress

compared to control (Fig. 4a, b) but was found to be more heightened in Chandan.

## Measurement of proline content

Proline generally acts as an osmo-protectant and non-enzymatic antioxidant. The proline content was found to be more eminent in shoot than in root after stress in both Tampha and Chandan (Fig. 4c, d). In response to PEG stress, Tampha cultivar showed a significant increased proline content when compared to Chandan, under stressed condition both in shoot and root tissues (Fig. 4c, d).

## Lipid peroxidation

MDA, a product of lipid peroxidation, is broadly employed as a marker. In this study, MDA content increased in root and shoot samples of both rice varieties at 1, 3 and 5 days of PEG treatment. MDA increased in all the stressed plants. Significant ($p < 0.05$) increment in MDA content was scored in Chandan while least in Tampha, at higher dose of PEG in shoot (Fig. 4a). MDA content in shoot and root showed gradual increment with respect to PEG concentrations (Fig. 5a, b).

## LOX measurement

In present investigation, lipoxygenase (LOX) enzyme activity was evaluated both in root and shoot which was observed to be elevated in higher dose of drought stress in both cultivars (Fig. 5c, d). In shoot, LOX activity was increased significantly when exposed to higher dose at 1, 3 and 5 days (Fig. 5c). In root also, increased LOX activity was observed in both cultivars, at 5 days (Fig. 5d).

## Histochemical assay of root and leaf samples

The root and leaf samples of Tampha and Chandan were subjected to qualitative estimation of ROS molecules through NBT and DAB; lipid peroxidation through Schiff's staining assay whereas for membrane damage Evans blue staining was conducted. Tampha showed comparatively lesser accumulation of ROS molecules and less membrane damage based on the stain absorbed by the tissues when compared to Chandan (Figs. 6, 7).

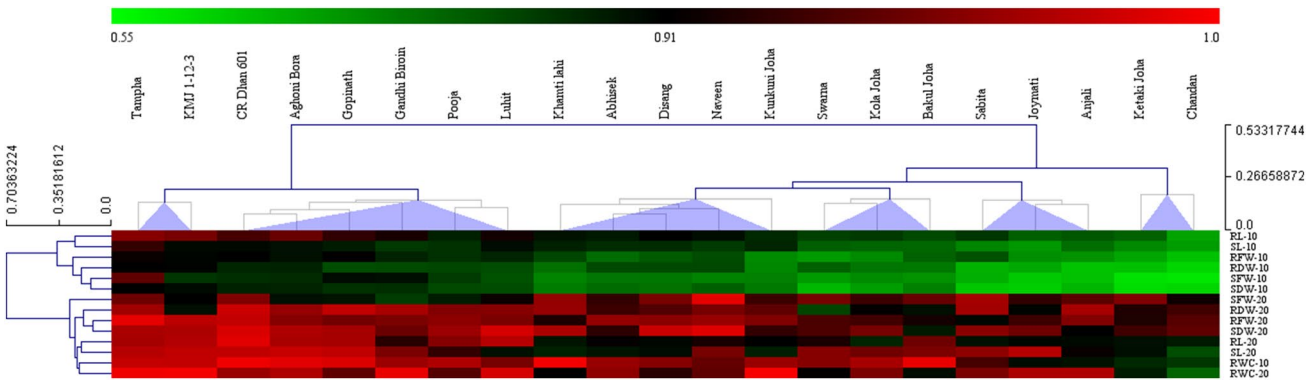


**Fig. 2** Hierarchical clustering of drought tolerance indices scored on the basis of physiological parameters at 5 days for 10 and 20% PEG stress

**Table 2** Cluster analysis for ranking *indica* rice cultivars according to their drought tolerance index from physiological parameters (shoot length, shoot fresh weight, shoot dry weight, root length, root dry weight, root fresh weight, and relative water content)

| Genotypes | Drought duration (days) | | | Sum[a] | Genotype ranking[b] | Tolerance degree[c] |
|---|---|---|---|---|---|---|
| | 1 | 3 | 5 | | | |
| Tampha | 1 | 1 | 1 | 3 | 1 | Tolerant |
| CR Dhan 601 | 1 | 1 | 3 | 5 | 2 | Tolerant |
| KMJ 1-12-3 | 1 | 1 | 1 | 3 | 1 | Tolerant |
| Gopinath | 1 | 1 | 3 | 5 | 2 | Tolerant |
| Aghoni bora | 1 | 1 | 3 | 5 | 2 | Tolerant |
| Gandhi biroin | 1 | 1 | 3 | 5 | 2 | Tolerant |
| Pooja | 1 | 1 | 3 | 5 | 2 | Tolerant |
| Luhit | 1 | 1 | 3 | 5 | 2 | Tolerant |
| Disang | 1 | 1 | 5 | 7 | 4 | Moderate |
| Naveen | 1 | 1 | 5 | 7 | 4 | Moderate |
| Khamti lahi | 1 | 1 | 5 | 7 | 4 | Moderate |
| Abhisek | 2 | 2 | 5 | 9 | 6 | Susceptible |
| Sabita | 2 | 2 | 2 | 6 | 3 | Moderate |
| Bakul Joha | 2 | 2 | 2 | 6 | 3 | Moderate |
| Anjali | 3 | 2 | 2 | 7 | 4 | Moderate |
| Kunkuni Joha | 2 | 2 | 5 | 9 | 6 | Susceptible |
| Kola Joha | 2 | 2 | 4 | 8 | 5 | Moderate |
| Joymati | 3 | 2 | 2 | 7 | 4 | Moderate |
| Swarna | 2 | 2 | 4 | 8 | 5 | Moderate |
| Ketaki Joha | 3 | 2 | 6 | 11 | 7 | Susceptible |
| Chandan | 3 | 2 | 6 | 11 | 7 | Susceptible |

[a]Sum of the cluster group rankings on addition of rank numbers for three different time duration in each genotype

[b]Genotypes were ranked with the smallest sum being the most tolerant

[c]1, 2; 3, 4, 5; and 6, 7 indicate tolerant, moderate and susceptible genotypes

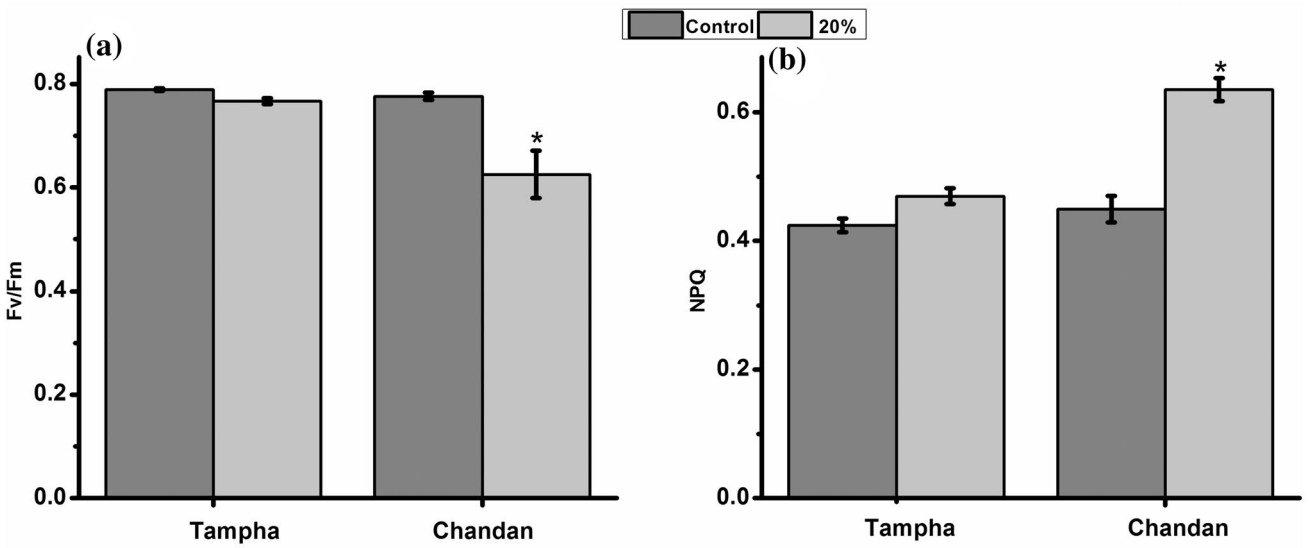


**Fig. 3** Chlorophyll fluorescence in Tampha and Chandan under control and 20% PEG stress, **a** Fv/Fm, **b** NPQ. The values are represented as the means ± SE ($n = 3$) of at least three independent experiments. Differences between control and treated plants were significant at $p < 0.05$ (*)

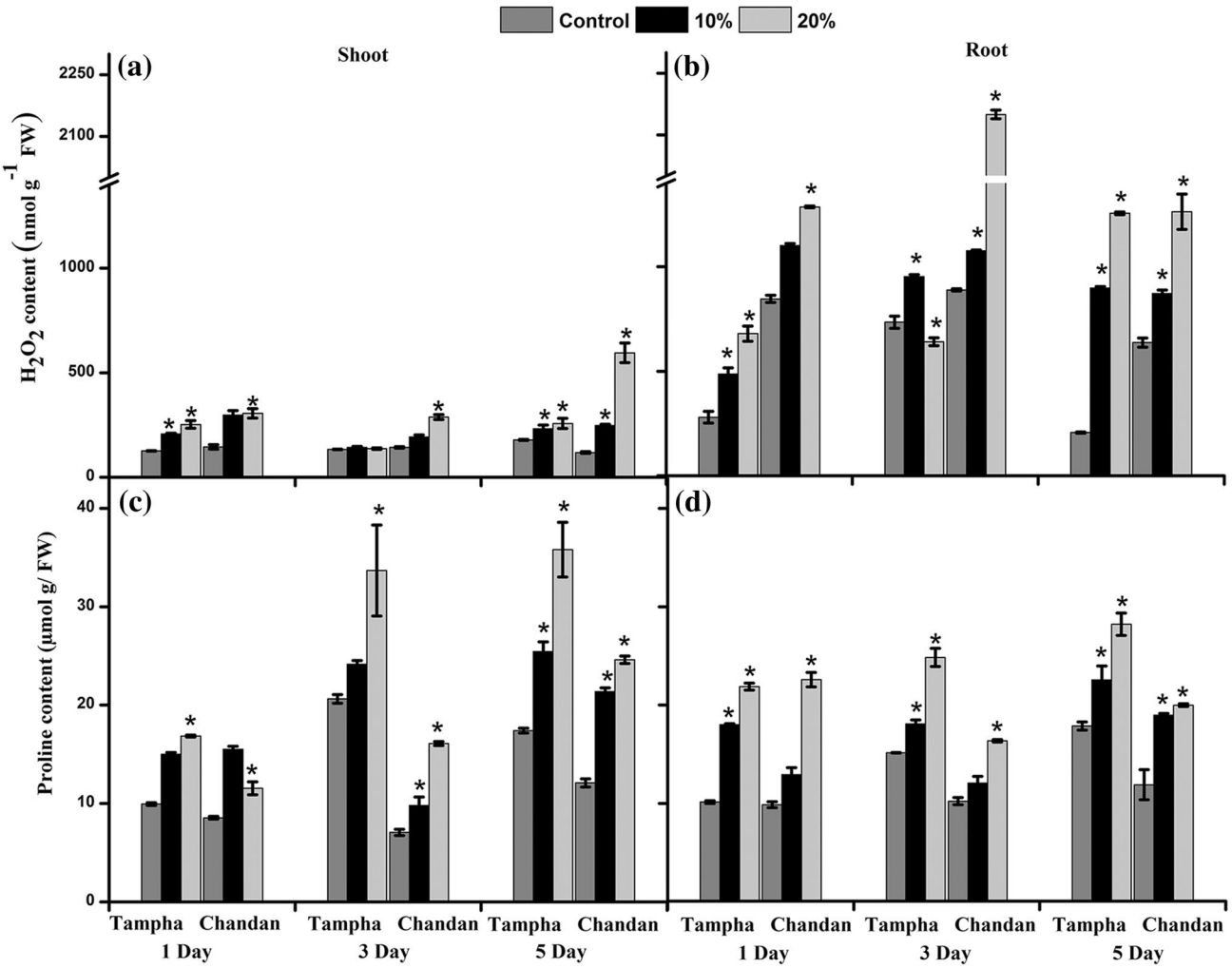


**Fig. 4** Effect of PEG-induced drought stress on rice plants. **a, b** Hydrogen peroxide ($H_2O_2$); **c, d** proline content under different concentrations of PEG (0, 10, and 20%) stress in shoot and root samples. The values are represented as the means ± SE ($n = 3$) of at least three independent experiments. Differences between control and treated plants were significant at $p < 0.05$ (*)

## Glutathione assay (estimation of total, reduced and oxidised glutathione)

GSH and GSSG were estimated in the two *indica* rice varieties. Drought stress significantly increased glutathione (GSH) levels, compared to control (Table 3). Decrement in GSH/GSSG ratio was observed in rice seedlings for both cultivars on exposure to PEG stress.

## Measurement of antioxidant enzymes

This study revealed that drought stress condition leads to significant increment in the activities of SOD, CAT and POX in root and shoot tissues of rice. In shoot, higher SOD activity was observed at higher concentration of PEG stress. In Chandan variety, SOD activity was higher than Tampha after 5 days (Fig. 8a). In root, increased SOD activity was observed as the dose of PEG stress increased at 1, 3 and 5 days (Fig. 8b). After 1, 3 and 5 days' treatment CAT activity was observed to increase in time-dependent manner in shoot (Fig. 8c) with the increase in drought stress, interestingly decreased CAT activity was noticed in Chandan variety. However, the CAT activity in treated root was elevated as compared to control among all the concentration in both the varieties. CAT activity was less elevated in Chandan while in Tampha, more activity was observed both in root and shoot (Fig. 8c, d). POX activity was also analysed in both root and shoot tissues. Gradual significant increase of POX activity was observed with the increase in PEG dose. In Tampha variety, higher activity was observed at 1- and 3-day intervals compared to Chandan variety (Fig. 8e). However, in root sample for Tampha variety, POX activity was observed to be lower when compared to Chandan variety after 5 days (Fig. 8f).

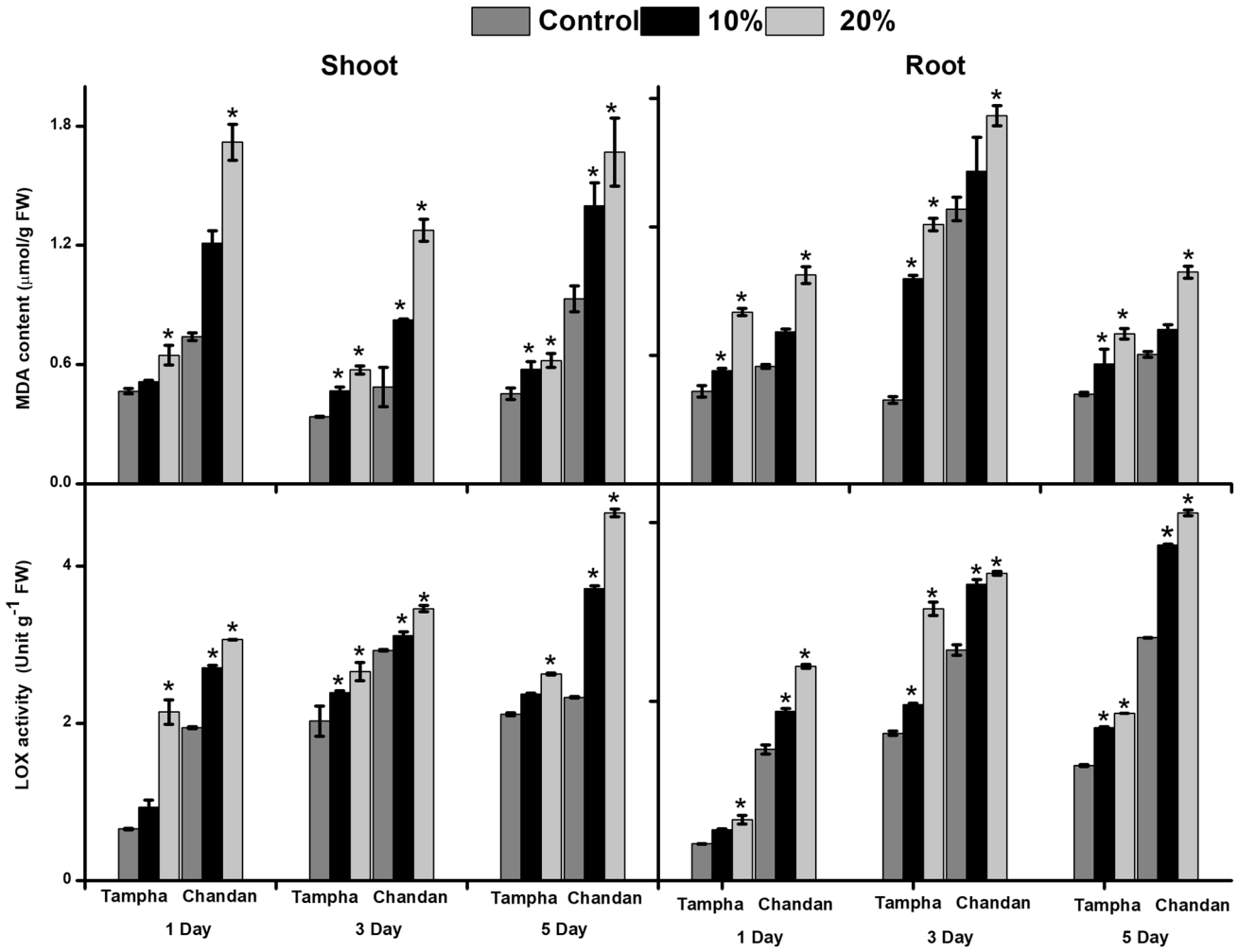


**Fig. 5** Effect of PEG-induced drought stress on rice plants. **a, b** MDA; **c, d** lipoxygenase (LOX) activity under different concentrations of PEG (0, 10, and 20%) stress in shoot and root samples. The values are represented as the means ± SE ($n = 3$) of at least three independent experiments. Differences between control and treated plants were significant at $p < 0.05$ (*)

Ascorbate–Glutathione cycle is an crucial cycle of the ROS scavenging system which detoxify $H_2O_2$ to $H_2O$. Our study revealed that the APX activity elevated on PEG stress in shoot and root tissues. Under stress condition more APX content was observed as equated to control. In both shoot and root (Fig. 9a, b), APX content was found to be elevated and more significantly increased after 1, 3 and 5 days. DHAR activity was also determined, and its activity was increased for treated samples as compared to control (Fig. 9c, d). MDHAR content was found to be elevated significantly after 1, 3 and 5 days of stress in shoot and root in Tampha variety compared to Chandan (Fig. 9e, f). GR activity increased significantly with time and concentration of PEG stress (Fig. 9g, h). Thus, in Tampha, variety the activity of AsA-GSH cycle enzymes was more when compared to Chandan.

## Correlation analysis among the biochemical parameters

The interaction between 12 biochemical parameters stands clearly elucidated from correlation analysis to better understand the stress response (Tables 4, 5). The difference among most of the parameter was found to be statistically significant at $p < 0.05$ level for both root and shoot tissues.

## Discussions

Rice [*Oryza sativa* L. spp. *indica*] caters to the food requirement of major chunk of the world population but its production is highly compromised by environmental challenges. This work evaluates the variability in

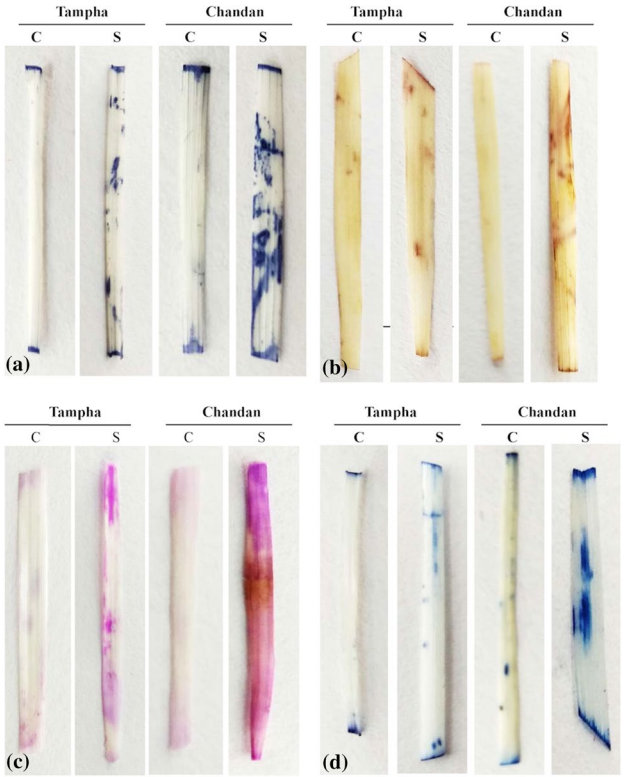


**Fig. 6** Histochemical assay of leaf under drought stress (20% PEG and control condition). **a** NBT-stained leaf segments depicting $O_2^-$ accumulation; **b** DAB-stained leaf segments depicting $H_2O_2$ accumulation; **c** Schiff's staining of leaf segments to detect lipid peroxidation; **d** Evans blue staining of leaf to ascertain cell membrane integrity. *C* control, *S* stressed

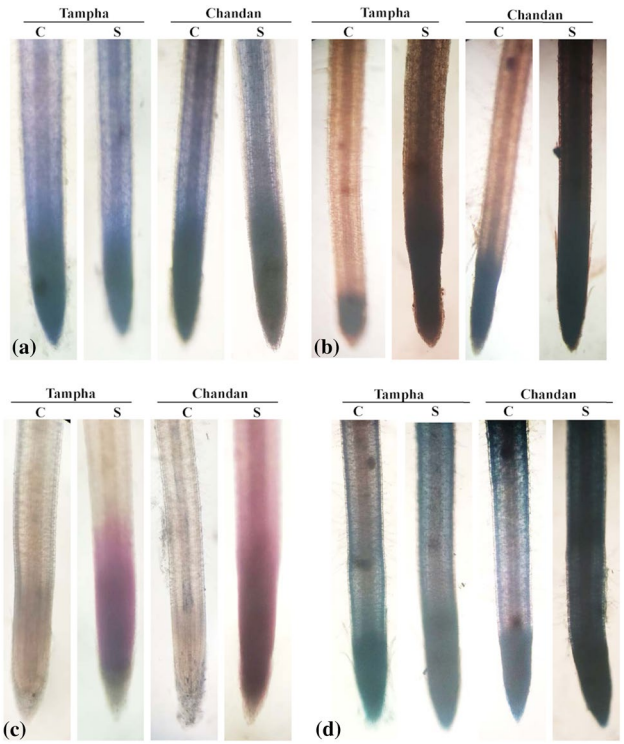


**Fig. 7** Histochemical assay of root under drought stress (20% PEG and control condition). **a** NBT-stained root segments depicting $O_2^-$ accumulation; **b** DAB-stained root segments depicting $H_2O_2$ accumulation; **c** Schiff's staining of leaf segments to detect lipid peroxidation; **d** Evans blue staining of roots to ascertain cell membrane integrity. *C* control, *S* stressed

drought stress response among various cultivars of North East India, a hot spot of *indica* rice biodiversity, which might latter be useful for 'omic' studies to enhance the knowledge on drought responsive players in rice. Use of PEG 6000 as an osmoticum is a very common option to judge the performance of plants under laboratory conditions (Yang et al. 2017; Huang et al. 2018; Hellal et al. 2018). Preliminary physiological analysis showed time and dose-dependent decrease in shoot length, root length, and biomass in all the 21 varieties on exposure to PEG when compared to those grown under control condition (Table 1, Supplementary 2). These parameters have been chosen for initial analysis based on the fact that they are important markers for drought tolerance (Joshi et al. 2017). Among these varieties, Tampha showed better acclimatisation to drought with higher root-shoot length and biomass whereas Chandan the least. This was further confirmed with ranking of cultivars on the basis of cluster numbers of HCA for three stress durations (1, 3 and 5 days) under 10% and 20% PEG stress (Fig. 2; Table 2, Supplementary 3). The cultivars were divided into three categories tolerant, moderate and sensitive; Tampha and KMJ 1-12-3 being the most tolerant whereas Chandan and Ketaki Joha being the sensitive ones. HCA has been previously employed to deduce and rank the sensitive and tolerant varieties in rice and wheat for salt tolerance (Siddiqui et al. 2017; Ali et al. 2014). A recent study from our group showed the exceptional ability of Tampha to counter salt stress (Omisun et al. 2018). The capability of a salt-tolerant cultivar to also perform better under drought stress might be due to similar ramifications and the intensive signalling cross-talk of both the stresses. Not only abiotic stress, Tampha has been found to be resilient to rice root nematodes (Devi et al. 2014). RWC in shoot decreased with stress dose and duration in all the cultivars. It is the measure of difference in ability of plant to absorb water from soil and loss of water due to transpiration from shoot (Sun et al. 2015). Tampha showed the least decrease in shoot RWC at 9.09%, a signal of better acclimatisation to osmotic stress (Table 1). Though less biomass and shoot length are indicative of avoidance mechanism of plants to counter excessive loss of water, this reduction have detrimental effect towards crop yield and productivity in the long run (Fang and Xiong 2015; Kooyers 2015). Taking into consideration the established physiological markers and based on HCA

**Table 3** Glutathione content in two rice varieties under PEG stress

| Varieties | Treatment | Total Glutathione (nmol $g^{-1}$ FW) | GSSG (nmol $g^{-1}$ FW) | GSH content (nmol $g^{-1}$ FW) | GSH/GSSG ratio |
|---|---|---|---|---|---|
| Tampha | Control | 409.53 ± 39.86 | 19.17 ± 2.21 | 390.35 ± 37.66 | 21.51 ± 1.03 |
| | 20% PEG | 484.72 ± 42.80* | 26.38 ± 3.25* | 458.34 ± 40.02* | 17.82 ± 1.93 |
| Chandan | Control | 359.59 ± 41.88 | 18.36 ± 2.84 | 341.22 ± 39.06 | 20.82 ± 1.63 |
| | 20% PEG | 430.59 ± 47.40* | 20.77 ± 2.72 | 409.82 ± 44.73* | 18.91 ± 0.94 |

The effects of PEG on the levels of non-enzymatic antioxidant glutathione (GSH), as well as its redox states (GSH/GSSG) in the leaves of rice plants for 5 day stress condition. Values are means ± SD of three independent replications ($n = 3$). Differences between control and stress plants were significant at $P < 0.05$ (*) by LSD test

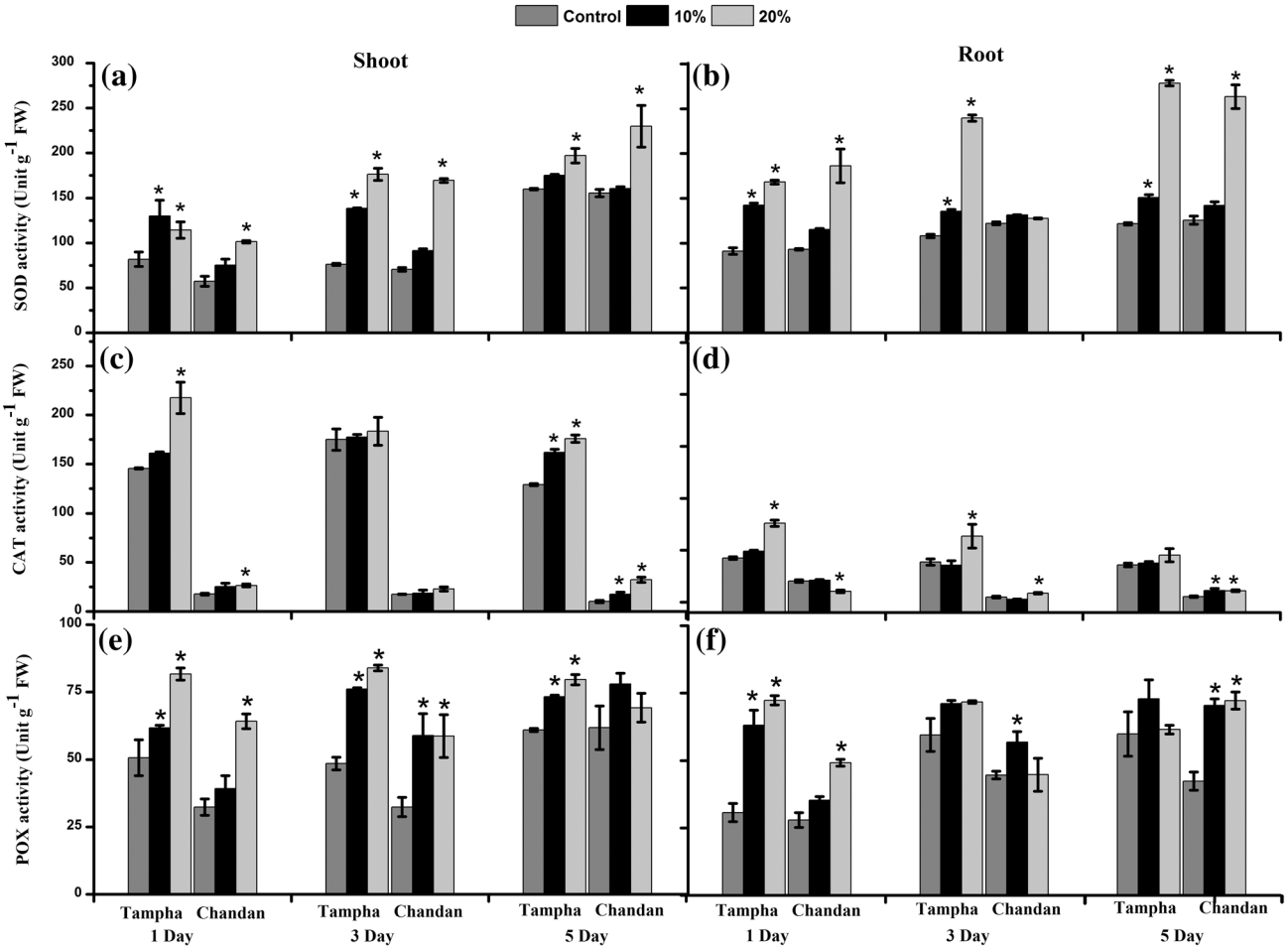


**Fig. 8** Biochemical analysis of rice seedling under drought conditions. **a, b** Superoxide dismutase (SOD) activity; **c, d** catalase (CAT) activity; **e, f** peroxidase (POX) activity in two rice plants under different PEG (0, 10, and 20%) treatment in shoot and root samples. The values are represented as the means ± SE ($n = 3$) of at least three independent experiments. Differences between control and treated plants were significant at $p < 0.05$ (*)

ranking system, only the tolerant (Tampha) and sensitive cultivar (Chandan) were chosen for in-depth biochemical studies.

Chlorophyll fluorescence is often utilised to analyse photosynthesis in plants under abiotic and biotic stress treatments (Liu et al. 2015; Lu et al. 2015). In this study also, the photosynthetic efficiency has been estimated by scoring the fluorescence in the form of Fv/Fm ratio and NPQ in both stressed and control condition in the two varieties, Tampha and Chandan. Here, Tampha was found to have greater photosynthetic efficiency in comparison to Chandan under stressed condition (Fig. 3) showing less damage of the

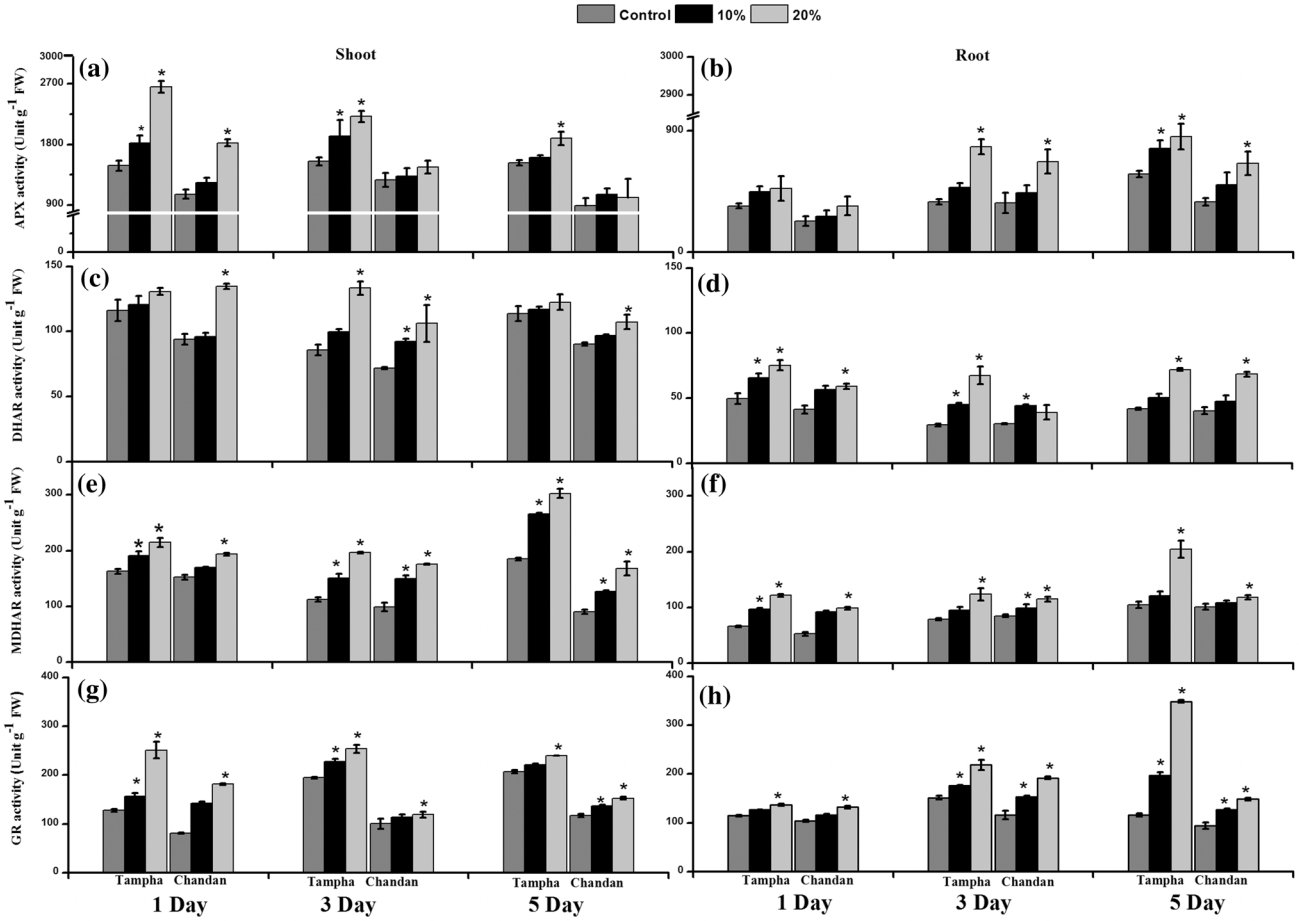


**Fig. 9** Biochemical analysis of rice seedling under drought conditions. **a, b** Analysis of ascorbate (APX) activity; **c, d** dehydroascorbate reductase(DHAR) activity; **e, f** monodehydroascorbate reductase (MDHAR) activity; **g, h** glutathione reductase (GR) activity in two rice varieties under different PEG (0, 10, and 20%) treatments in shoot and root samples. The values are represented as the means ± SE ($n = 3$) of at least three independent experiments. Differences between control and treated plants were significant at $p < 0.05$ (*)

**Table 4** Correlation between different biochemical parameters estimated from shoot tissue samples of Tampha and Chandan at 20% PEG stress for 1, 3 and 5 days

| | SOD | CAT | DHAR | GPX | GR | MDHAR | POX | APX | LOX | MDA | $H_2O_2$ | Proline |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SOD | 1.00 | | | | | | | | | | | |
| CAT | 0.06 | 1.00 | | | | | | | | | | |
| DHAR | 0.36 | 0.49 | 1.00 | | | | | | | | | |
| GPX | 0.14 | 0.98* | 0.59* | 1.00 | | | | | | | | |
| GR | 0.38 | 0.83* | 0.70* | 0.84* | 1.00 | | | | | | | |
| MDHAR | 0.45 | 0.51 | 0.77* | 0.55 | 0.66* | 1.00 | | | | | | |
| POX | 0.71* | 0.54 | 0.79* | 0.64* | 0.82* | 0.67* | 1.00 | | | | | |
| APX | 0.03 | 0.77* | 0.72* | 0.80* | 0.83* | 0.62* | 0.65* | 1.00 | | | | |
| LOX | 0.60* | 0.63* | 0.43 | 0.64* | 0.78* | 0.66* | 0.76* | 0.57 | 1.00 | | | |
| MDA | 0.39 | −0.60* | 0.25 | −0.53 | −0.20 | 0.07 | 0.19 | −0.22 | −0.12 | 1.00 | | |
| $H_2O_2$ | 0.61* | −0.23 | 0.25 | −0.20 | 0.08 | 0.30 | 0.35 | −0.08 | 0.19 | 0.77* | 1.00 | |
| Proline | 0.71* | 0.56 | 0.46 | 0.61* | 0.74* | 0.65* | 0.75* | 0.42 | 0.86* | −0.06 | 0.27 | 1.00 |

The asterisk marks indicate statistically significant difference at $p < 0.05$ according to Duncan's multiple range test

**Table 5** Correlation between different biochemical parameters estimated from root tissue samples of Tampha and Chandan at 20% PEG stress for 1, 3 and 5 days

| | SOD | CAT | DHAR | GPX | GR | MDHAR | POX | APX | LOX | MDA | $H_2O_2$ | Proline |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SOD | 1.00 | | | | | | | | | | | |
| CAT | 0.23 | 1.00 | | | | | | | | | | |
| DHAR | 0.81* | 0.55 | 1.00 | | | | | | | | | |
| GPX | 0.51 | 0.85* | 0.68* | 1.00 | | | | | | | | |
| GR | 0.37 | 0.47 | 0.29 | 0.72* | 1.00 | | | | | | | |
| MDHAR | 0.81* | 0.29 | 0.64* | 0.69* | 0.63* | 1.00 | | | | | | |
| POX | 0.71* | 0.51 | 0.61* | 0.50 | 0.20 | 0.60* | 1.00 | | | | | |
| APX | 0.77* | 0.32 | 0.55 | 0.60* | 0.57 | 0.85* | 0.66* | 1.00 | | | | |
| LOX | 0.50 | 0.51 | 0.32 | 0.70* | 0.72* | 0.76* | 0.62* | 0.88* | 1.00 | | | |
| MDA | 0.27 | −0.28 | 0.08 | −0.19 | 0.17 | 0.22 | 0.14 | 0.38 | 0.21 | 1.00 | | |
| $H_2O_2$ | 0.31 | −0.44 | 0.07 | −0.23 | 0.25 | 0.34 | 0.02 | 0.33 | 0.16 | 0.74* | 1.00 | |
| Proline | 0.86* | 0.47 | 0.79* | 0.69* | 0.59* | 0.83* | 0.77* | 0.76* | 0.64* | 0.19 | 0.25 | 1.00 |

The asterisk marks indicate statistically significant difference at $p < 0.05$ according to Duncan's multiple range test

photosynthetic apparatus in Tampha. Better chlorophyll and water content is an indication of better tolerance capability (Ma et al. 2018).

Imposition of environmental stress leads to metabolic malfunction and hence overproduction of ROS molecules such as $H_2O_2$, $O_2^-$, and $OH^-$ in cellular compartments (chloroplast, peroxisomes, and mitochondria; Shigeoka et al. 2002). Analysis for $H_2O_2$ content in the two contrasting varieties showed higher production of the same in root tissues when compared to shoots and similar to other discussed parameters, showed stress dose, and duration-dependent accumulation. Tampha showed less $H_2O_2$ content in comparison to Chandan depicting the better tolerance capacity of Tampha (Fig. 4a, b), suggesting the presence of a better antioxidant system in the former.

Proline besides being an important osmolyte also acts as a singlet oxygen quencher and scavenger of hydroxyl radicals in response to stress (Rejeb et al. 2014). Tampha was found to accumulate more proline in response to drought stress thus providing an advantage for better physiological appearance when compared to Chandan (Fig. 4c, d; Sofo et al. 2004). This higher accumulation of proline in Tampha seems to justify the fact why it was showing less ROS accumulation and photosynthetic damage. MDA level and LOX activity, both indicative of oxidative damage caused due to oxidative stress were found to be enhanced in Chandan variety as compared to Tampha (Fig. 5). This might be indicative of much better repair mechanism with lesser oxidative damage in Tampha in response to drought stress which led to its comparatively better performance (Basu et al. 2010).

Histochemical staining with NBT, DAB and Schiff's solution showed increased intensity of staining in stressed leaf and root tissues especially in Chandan when compared to control seedlings. Whereas Tampha depicted lesser ROS ($H_2O_2$, $O_2^-$) production or increased scavenging through lesser absorption of stains (Figs. 6, 7). Previous reports on *Brassica juncea* (Saha et al. 2016a), *Oryza sativa* (Awasthi et al. 2017; Omisun et al. 2018), etc., showed better stress tolerance in plants depicting lesser staining and hence lesser accumulation of ROS molecules. Similar result was observed for Evans blue staining to depict membrane damage (Figs. 6, 7).

The AsA-GSH pathway acts as an integrator of metabolic information to environmental cues (Foyer and Noctor 2011). The enzymes analysed for their activity under stressed condition in both Tampha and Chandan, viz, APX, DHAR, MDHAR, GR, SOD, CAT, POX were found to be increased under stressed condition (Figs. 8, 9). Increased activity means greater ROS scavenging activity and hence greater oxidative stress tolerance capacity. Tampha showed much more increase in activity when compared to Chandan, which is in consistency with results from experiments performed for other plant species (Anjum et al. 2011).

PEG stress led to decrement in GSH/GSSG ratio but significant increase in GSH and GSSG contents in both varieties, leading the whole system to a more reduced state. Hence, thereby impairing the ROS detoxification mechanism and consequently increased oxidative stress (Mostofa et al. 2015). Increased GSH content has been scored in extracts of several plants subjected to diverse stresses (Kocsy et al. 1996). Previous studies reported the presence of increased GSH and GSSG level under drought stress in citrus and mungbean (Zandalinas et al. 2017; Hasanuzzaman et al. 2018). Glutathione is often regarded as the most crucial intracellular antioxidant, especially the GSH/GSSG ratio have got huge impact in determining redox status and stress signalling processes. The results comprehensively suggest that Tampha has higher levels of GSH and GSSG content, in comparison to sensitive Chandan varieties (Table 3).

Thus, through this study it can be concluded that the presence of strong anti-oxidative defence mechanism, depicted by enhanced activity of APX, DHAR, MDHAR, GR, SOD, CAT, POX, higher accumulation of proline and better GSH/GSSG ratio, leading to reduced ROS accumulation and increased photosynthetic activity renders Tampha to be more tolerant under PEG-induced drought stress when equated to Chandan. These two varieties Tampha and Chandan may be analysed in future to unravel the drought stress responsive molecular mechanisms and for development of newer varieties with better drought/osmotic stress tolerance traits.

**Author contribution statement** SKP and SS conceived and designed the experiment. Data curation was done by SS and JPA. SS and JPA conducted the experiment. Formal analysis of the work was done by SS, BS and JPA. SS and PB investigated the work. SH and TO procured the seeds for the experiment. Methodology was written by SS, JPA, and SKP. Project administration was done by SKP and JP. The whole work was supervised by SKP and JP. Manuscript was prepared by BS, SS, and SKP.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interest.

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