



Detection of biochemical and molecular changes in *Oryza sativa* L during drought stress



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ABSTRACT

Drought stress is an important abiotic stress to current agriculture around the world, due to its implacable damage to agriculture crops. Plants adapt these climatic changes by various physiological, biochemical, cellular and molecular alterations. In present study, we analyzed the physiological, biochemical and cellular responses of rice seedlings during drought stress. The study emphasized the expression of different genes regulated during the drought stress. Drought stress revealed a significant reduction in the growth, photosynthetic pigments, proteins, enhanced proline and antioxidant enzymes activity. Genomic DNA and nuclear fragmentation in leaf tissues confirm the pronounced overproduction of reactive oxygen species (ROS) during drought stress. Further, the expression pattern of the antioxidant, transcription factor, programmed cell death and autophagy genes revealed their role in drought tolerance. The study concludes that drought stress alters the different attributes of the plants. To avoid stress plants response with enhanced tolerance mechanism with the activation of multiple factors. Finally, autophagy and PCD both mechanisms co-regulates and increase tolerance against the drought stress in rice.

1. Introduction

To fulfil the food requirement of a rapidly growing population in developing countries and overcome the changing environmental burden on crops for doubling the crop yields in coming years is a prerequisite (Tilman et al., 2002). An estimate of the Food and Agriculture Organization (FAO), the world food production should be increased by 70% till the year 2050 to feed the growing population (Vinet and Zhedanov, 2010). Rice (*Oryza sativa* L.) is the primary food source in developing countries and cultivated in mostly all parts of the world in different environmental conditions. It is open to a wide range of environmental stresses, such as salinity, drought, flooding, cold and high temperature, causing global crop losses every year (Khan et al., 2018; Das et al., 2018). Drought stress, a manifestation of global climate change, is the most important and challenging environmental stress affecting crop productivity in all the crop plants including rice (Daryanto et al., 2017). Drought stress resulted in inhibition or reduction of normal physiological process and metabolism due to the absence or insufficient water availability which eventually cause the death of the plant. The diverse plant species across climatic regions with severe drought environment adopted the different morphological, biochemical and physiological mechanisms to cope up with drought stress (Aroca, 2013; Quan et al., 2016). To combat with drought stress plants

undergoes various metabolic adjustments to regulate physiological and morphological adaptation (Poonam et al., 2017; Hemalatha and Francis, 2000). Some important feature of drought stress like reduced water uptake, higher transpiration rate, stomata closer and excessive generation of reactive oxygen species (ROS), leads to cell injury with increased cellular temperature, protein deposition and denaturation (Tuteja, 2010). Drought enhanced the metabolite production like proline, glycine betaine, soluble carbohydrates and Gamma-aminobutyric acid (GABA) (Sinha et al., 2018). This causes toxicity and impaired enzyme activity with reduced photosynthesis, bleaching, curling, wilting of leaves and ultimately death of the plant.

The physiological and biochemical responses during water stress are also regulated by a complex gene network (Nautiyal et al., 2013). Therefore, it is vital to identify the regulatory marker genes of drought. Several genes involved in cellular metabolism, transport and signal transduction and cell wall alterations (Lawton and Hemalatha, 2011) during drought stress (Sinha et al., 2018). Climate change will impact the fortune of each crop in the coming decades and water consumption will be one of the main reasons. Since rice is the most water-intensive commodity, the research community need to develop climate-smart varieties and water-efficient production technologies that consume less water. Autophagy or self-eating is an intracellular protein degradation process. It occurs in almost all eukaryotes where cytoplasmic

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constituents transported to vacuoles (yeast and plants). Autophagy mechanism occurs during normal growth conditions, however markedly induced during nutritional starvation, biotic or abiotic stresses (Avin-Wittenberg, 2018). Luo et al. (2017) demonstrated that autophagy is rapidly induced by salt stress and required for salt tolerance in *Arabidopsis*. Earlier finding clearly reported that ATG18 overexpressing plants showed enhanced drought tolerance in apple (Sun et al., 2018).

The mechanism of autophagy is well reported in yeast (*Saccharomyces cerevisiae*) and extensively studied in mammals and humans. In recent years significant progress has been made in understanding the plant autophagy mechanism and their responses to biotic and abiotic stresses. Although, the critical role of plant autophagy has been well established in a wide spectrum of biological processes, including stress responses. Our understanding of the regulation and action mechanisms of plant autophagy is extremely limited. Liu and Bassham (2010) showed that silencing of TOR gene leads to constitutive formation of autophagosomes in *Arabidopsis*. This suggested that TOR is a negative regulator of autophagy in plants. Furthermore, a NADPH oxidase inhibitor blocks autophagy induction upon nutrient starvation and salt stress but not in osmotic stress (Liu et al., 2009). Thus, ROS may mediate the induction of autophagy during some, but not all, stress conditions. Zhou et al. (2014) confirmed silencing of tomato *WRKY33* genes compromised tomato heat tolerance and reduced heat-induced *ATG* gene expression and autophagosome accumulation. However, little information is available concerning the transcriptional regulation, programmed cell death and plant autophagy genes in different stress conditions in rice.

Hence, the current study was undertaken to explore the physiological, biochemical, cell biological and molecular responses during drought stress in rice. In addition, we have analysed the expression of key genes of programmed cell death, autophagy and transcription factors involved in molecular mechanism of drought tolerance in rice.

2. Materials and methods

2.1. Plant materials and growth conditions

The seeds of rice (*Oryza sativa* var. MTU 1010) were procured from National Seeds Corporation (NSC), Chennai, Tamilnadu, India. The seeds were washed with sterile distilled water to remove contaminants and surface sterilized with 1% sodium hypochlorite solution. Sterilized seeds were soaked in ultra-purified water for 2 days at room temperature. The germinated seeds were transplanted in 15 cm diameter x 7 cm height, plastic pots filled with 500 g of red sandy soil. The sterilized (121 lbs, pressure for 20 min) soil (P^H 8.25, EC ($ds\ m^{-1}$) 0.55, Texture-sandy Clay, Organic Carbon - 2.8 kg/ha, N- 24.9 kg/ha, P- 12.5 kg/ha, K- 6.2 kg/ha; Reed and Martens, 1996) were used for the experiment. Rice seedlings were irrigated with water for fourteen days and then drought stress was imposed for holding irrigation for 7 days. While control seedlings were irrigated continuously. After twenty one days of sowing fresh leaf tissues from control and drought treatments were collected, frozen in liquid nitrogen before store in $-80\ ^\circ C$ and further used for the experiments.

2.2. Physiological and biochemical analysis

The root and shoot lengths were determined from control and drought treatment twenty-four days after sowing (DAS). Ten plants from each replicate were uprooted and measured through a meter scale.

2.3. Total chlorophyll and carotenoids contents

The fresh leaves of rice seedlings (100 mg) from the control and drought treatment were harvested and washed with sterile distilled water. Leaves were dried and ground in a mortar and pestle by using 80% acetone (Maclachlan and Zalik, 1963). The absorbance of the leaf

extract was measured at A_{645} and A_{663} nm for chlorophyll estimation and at A_{480} and A_{510} nm for carotenoid estimation using a bio-spectrophotometer (Khan et al., 2019a) (Shimadzu, Tokyo, Japan).

2.4. Total protein content

The protein content was estimated according to the method described by Lowry et al. (1951) in control and drought treatment. The 100 mg leaves were ground in 1 ml of sterilized distilled water and filtrate was centrifuged at $5000 \times g$ for 10 min at $4\ ^\circ C$. In supernatant equal volume of 10% trichloroacetic acid (TCA) was added and kept for 30 min. Further, the pellet was clarified in 1 ml of 0.1 N sodium hydroxide. The absorbance of the solution was estimated at A_{740} nm. Bovine serum albumin (1 mg/ml) was used as a standard. The total protein content was expressed in $mg\ g^{-1}$ fresh weight.

2.5. Total proline content

Proline content was quantified according to (Bates et al., 1973). Leaves tissues were homogenized with 3% sulfosalicylic acid and centrifuged ($11500 \times g$). The obtained supernatant was mixed with acid ninhydrin, phosphoric acid and glacial acetic acid. The mixture was incubated at $100\ ^\circ C$ for 1 h and allowed to cool. Finally, toluene was added and absorbance was read at 520 nm.

2.6. Antioxidant enzyme assay

The fresh leaves (100 mg) were ground in 50 mM phosphate buffer (pH 7.0), in a pre-chilled mortar and pestle, and centrifuged at $12,000 \times g$ for 20 min at $4\ ^\circ C$. The supernatant was used for antioxidant enzyme assay (Akther and Hemalatha, 2019).

2.7. Catalase (CAT) activity

Catalase (EC 1.11.1.6) enzyme activity was measured to analyse the rate of disappearance of H_2O_2 (Chandee and Scandalios, 1984). The 3.0 ml reaction mixture contained 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 and 0.1 ml enzyme extract. The decomposition of hydrogen peroxide was recorded at absorbance A_{240} nm. The enzyme activity was expressed in $U\ min^{-1}\ g^{-1}$ fresh weight (Khan et al., 2019b).

2.8. Superoxide dismutase (SOD) activity

SOD (EC 1.15.1.1) activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction method of (Beauchamp and Fridovich, 1971). The reaction mixture (50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 13 mM methionine, 63 mM nitroblue tetrazolium (NBT), 1.3 mM riboflavin, and 0.1 ml enzyme extract) were taken in 5.0 mL test tubes and incubated for 15 min under 15 W fluorescent lamp at $25\ ^\circ C$. The blank tubes containing a similar amount of reaction mixture were covered with black cloth and not illuminated to light. Following illumination treatment, absorbance was recorded at A_{560} nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of the photoreduction of NBT to blue formazan.

2.9. Peroxidase (POX) activity

Peroxidase (EC 1.11.1.7) activity was analysed by method given by Kumar and Khan (1982) and Sánchez et al. (1995). The 2 ml reaction mixture (0.1 M phosphate buffer (pH 6.8), 1 mL of 0.01 M pyrogallol, 1 mL of 0.5 mM H_2O_2 and 0.5 mL of the enzyme extract) were utilized to estimating the peroxidase activity. After incubation of 10 min at $25\ ^\circ C$ the termination was achieved by adding 2.5 N H_2SO_4 . The optical density of purpurogallin formation was analysed at A_{420} nm. The unit

activity was expressed in U mg⁻¹ fresh weight. Where, one unit of the enzyme activity corresponded to an amount of the enzyme that makes changes in the absorbance by 0.1 min⁻¹ mg⁻¹ protein.

2.10. Cell biological analysis

To analyse the saline stress on nuclear morphology in *O. sativa* the leaves were stained with DAPI (4', 6-diamidino-2-phenylindole) (Gadjev et al., 2004). Firstly, leaves were washed thoroughly, cut into small pieces and stained with 0.1 mg⁻¹ ml DAPI and incubated for 5 min in dark. After incubation leaves were destained in distilled water and visualized under UV light (Carl Zeiss Axio1 inverted fluorescence microscope, Germany) by using DAPI filters. To determine the Reactive Oxygen Species (ROS) generation at cellular level, leaves were stained with DAB (3, 3'-diaminobenzidine). Leaves were infiltrated with DAB for 30 min followed by ethanol fixation for 1 h and visualized under visible light using microscope (Carl Zeiss Axio1 microscope, Germany).

2.11. Molecular analysis

2.11.1. DNA, RNA isolation and real-time PCR expression analysis

Genomic DNA was isolated from leaf tissues using a modified CTAB (cetyltrimethylammonium bromide) and the size of the DNA was analysed by agarose gel stained with ethidium bromide.

(Khan et al., 2007). Total RNA was extracted by Trizol reagent (Invitrogen, CA, USA) following the manufacturer's instructions. Purity of RNA was estimated at A₂₆₀/A₂₈₀. The RNA was reverse transcribed into cDNA by reverse transcription PCR using high capacity reverse transcription kit (Applied biosystems by Thermo Fisher Scientific, USA).

Advanced Universal SYBR Green Supermix (Biorad) was used for reaction and PCR was performed in CFX 96 Touch Real-Time Detection System (Bio-Rad, CA, USA) with the programme recommended by manufacturer. The normalized gene expression was calculated with the $\Delta\Delta$ CT method (Livak and Schmittgen, 2001) and *O. sativa* *ACTIN1* gene was used as internal control. The primer sequences used in this study are given in supplemental table 1.

3. Results

3.1. Drought stress reduced the shoot and root growth

The drought stress showed a significant decrease in growth parameters when compared to control plants. Water limitations in rice seedlings showed a decrease in growth, yellowing and curling of leaves. Growth parameters including shoot and root lengths showed a significant decrease of 55% and 43% respectively as compared to control (Fig. 1a–b).

3.2. Drought stress affects the pigment system, protein and proline accumulation

The pigment system such as Chlorophyll *a*, *b*, total chlorophyll and total carotenoids content were reduced in drought condition as compared to control plants (Fig. 2a). Similarly, anthocyanin and total protein content were also significantly decreased in stressed plants (Fig. 2b–c). Drought stress severely affects metabolites production. We observed a significant increase in proline content in drought treatment as compared to control (Fig. 2d).

3.3. Drought enhanced the activity of antioxidant enzymes

The antioxidant enzymes such as catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) showed the significant increase in enzyme activity during drought stress as compared to control (Fig. 3a–c).

3.4. Hallmarks of apoptosis observed under drought stress

The staining of rice leaves with DAB showed the excessive generation of ROS in drought treated leaves. The DAB forms a brown precipitate with ROS and deposited in the leaves grown under drought stress but it did not show any precipitate in control leaves (Fig. 4a–b). Drought stress also caused cell injury due to leakage of ions and osmotic stress and induced the PCD in plants. The DAPI staining of leaves showed the nuclear fragmentation in drought condition and the control leaves showed the intact nuclei with DAPI staining (Fig. 4c–d).

Further, we isolated the genomic DNA from leaf tissues of control and drought treated rice seedlings. Fig. 5 showed the gel electrophoresis of genomic DNA in drought and control plants. The agarose gel did not show any fragmentation or DNA laddering in leaves grown under drought condition.

3.5. Gene expression analysis

The Real-Time PCR analysis showed the differential gene expression in various genes tested in the study. The genes encoding the antioxidant enzymes including CAT-1, GPX and SOD have different locations in cell and showed differential expression. The CAT-1 and SOD showed no change in drought treatments as compared to control while, GPX showed significant fold increase during drought (Fig. 6a–c).

The gene BAX Inhibitor-1 (BI-1) responsible for inhibition of Bax induced cell death showed significant fold increase in expression during drought stress as compared to control. Similarly, MAPK1 and transcription factor WRKY53 gene also showed enhanced fold changes during drought stress treatment (Fig. 7a). The ATG genes encoding the autophagy-associated protein and involved in autophagy mechanism during the stress condition showed a differential gene expression. The autophagy-related genes ATG1, ATG4, ATG6, ATG7, ATG8, ATG9 and ATG10, showed significant fold increase while ATG3 and ATG13 showed no change in the expression during drought as compared to control (Fig. 7b).

4. Discussion

Drought or water deficit in the soil causes low water potential; inhibit the plant growth and development. Roots play an important role in the regulation of water uptake. The complex structures of roots mediate the transport of water and different solutes in plants. The results of the present study suggested that under limited water availability the root versus shoot ratio showed more enhancement (Fig. 1a–b). Roots are less sensitive than the shoots by lower water deficit (Wu and Cosgrove, 2000). The photosynthetic pigments, viz. Chlorophyll *a*, *b*, total chlorophyll, carotenoids and anthocyanin contents showed significant decline under water deficit (Fig. 2a–b). These are the major pigments played important role in photosynthesis. The reduction of chlorophyll pigments under drought stress may lead to oxidative damage that could be due to photo-oxidation and chlorophyll degradation. Khaleghi et al. (2012) reported a significant decline in chlorophyll *a*, *b* total chlorophyll content during drought. Thus the limited chlorophyll and other pigment contents may directly affect the photosynthetic potential of plants (Akther et al., 2017). Water stress directly affects protein synthesis by inhibiting the protein translation process in plant tissues. The present study showed a significant reduction in total protein content (Fig. 2c). Earlier studies showed that high protein accumulation in drought tolerant genotypes than in the drought susceptible under water-limited condition (Serraj and Sinclair, 2002).

Proline is an important solute to maintain the osmotic adjustment and higher proline level is an indicator of drought induced stress (Alam et al., 2013). The cell maintains itself during dehydration through the turgor pressure by lowering osmotic potential of cytoplasm through the accumulation of different inorganic and organic solutes in plants. Our results showed significant increase in proline level in drought grown

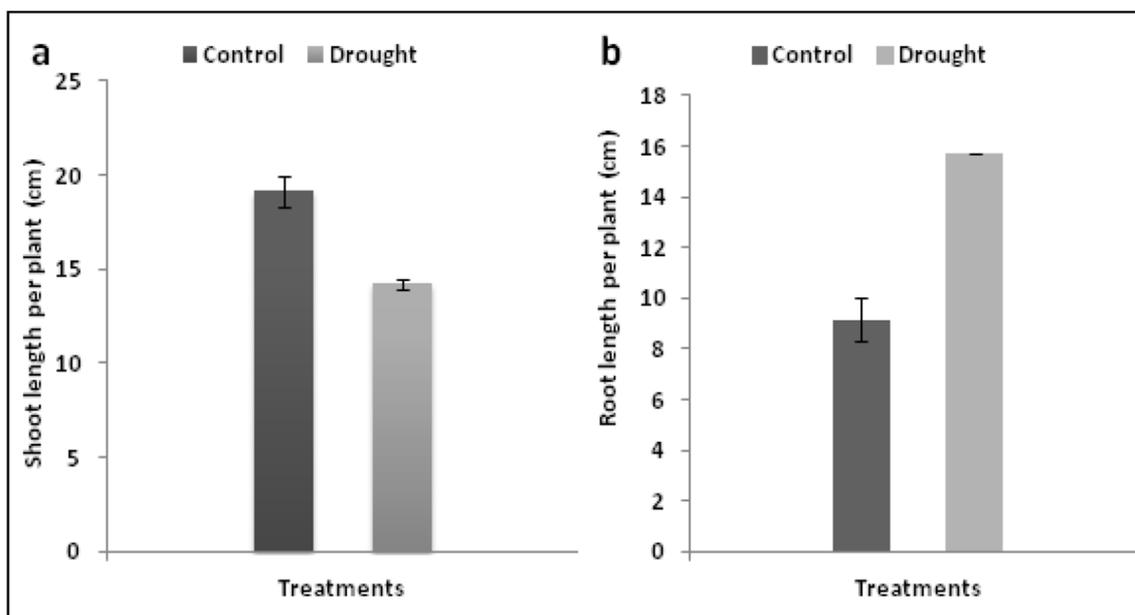


Fig. 1. Effect of drought stress on (a) shoot length and (b) root length of *O. sativa* seedlings. Standard errors are mean of three independent experiments.

rice seedlings (Fig. 2d). Earlier studies depicted the critical role of proline as an osmoprotectant, which maintains the osmoregulation by preventing water loss or leaf relative water content (Hasegawa et al., 2000; Ben Ahmed et al., 2011). Earlier it has been reported that proline accumulation was higher under water deficit condition and enhanced the tolerance in wheat (Kagarlitskii et al., 2003). Our findings suggested that proline may function as a stress signalling molecule of drought, a

strong scavenger, source of carbon and nitrogen to cope up with stress condition.

Further, to get deeper insight of drought induced oxidative stress we performed the biochemical analysis of antioxidant enzymes. The antioxidant enzymes (CAT, POX and SOD) showed increased enzyme activity in drought treatment (Fig. 3a–c). This suggested plants activate their antioxidant system to scavenge the oxidative stress induced ROS

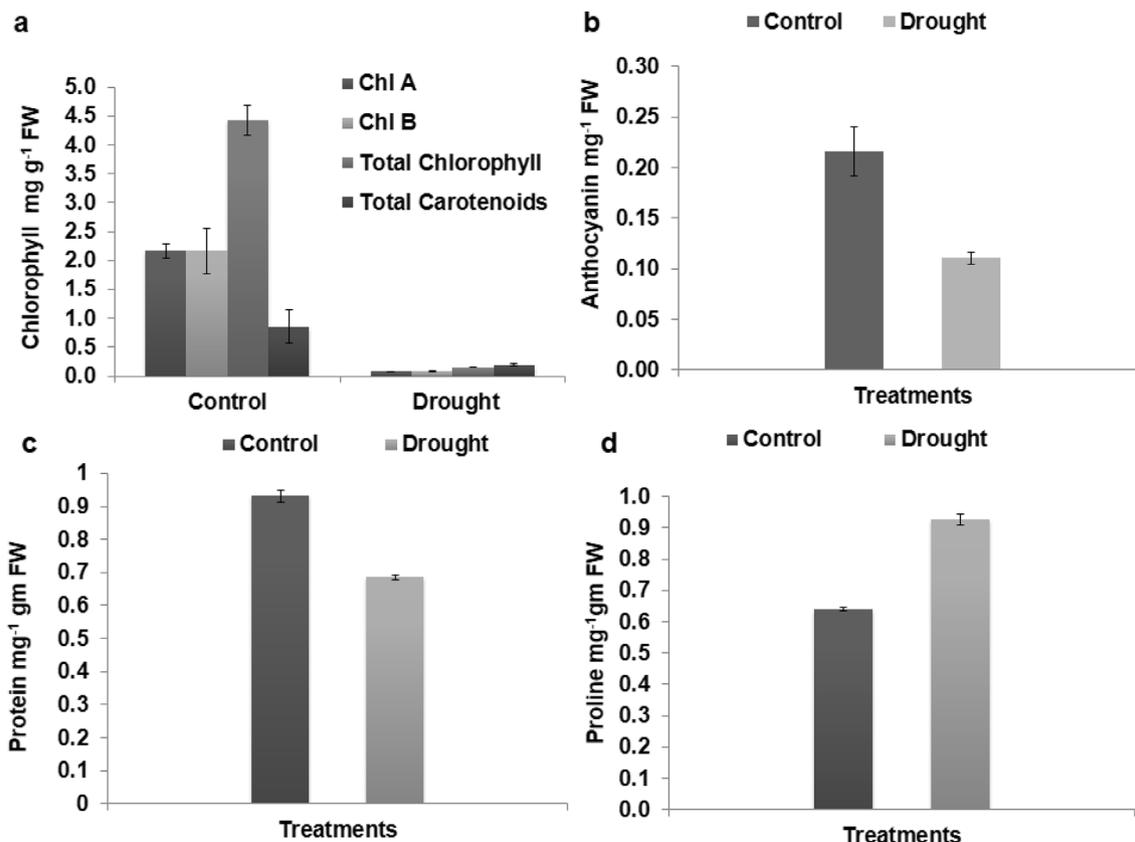


Fig. 2. Effect of drought stress on (a) Chlorophyll *a*, *b*, total chlorophyll and carotenoids (b) total anthocyanin (c)total protein and (d)proline content of *O. sativa* seedlings. Standard errors are mean of three independent experiments.

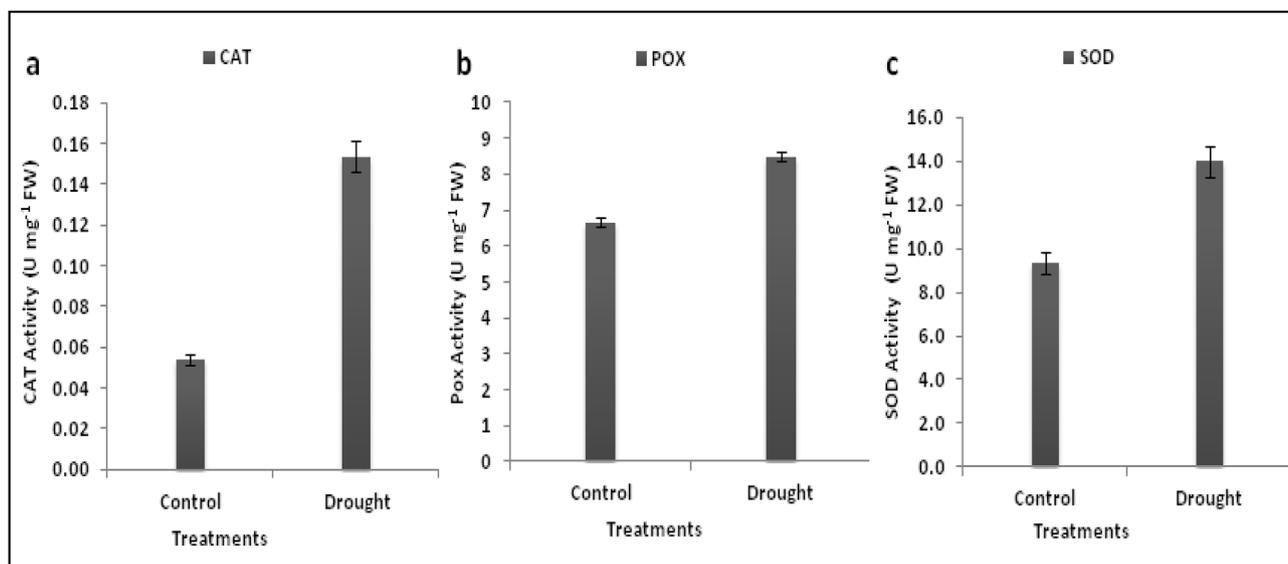


Fig. 3. Effect of drought stress on antioxidant enzyme (a) CAT (b) POX and (c) SOD activity in *O. sativa* seedlings. Standard errors are mean of three independent experiments.

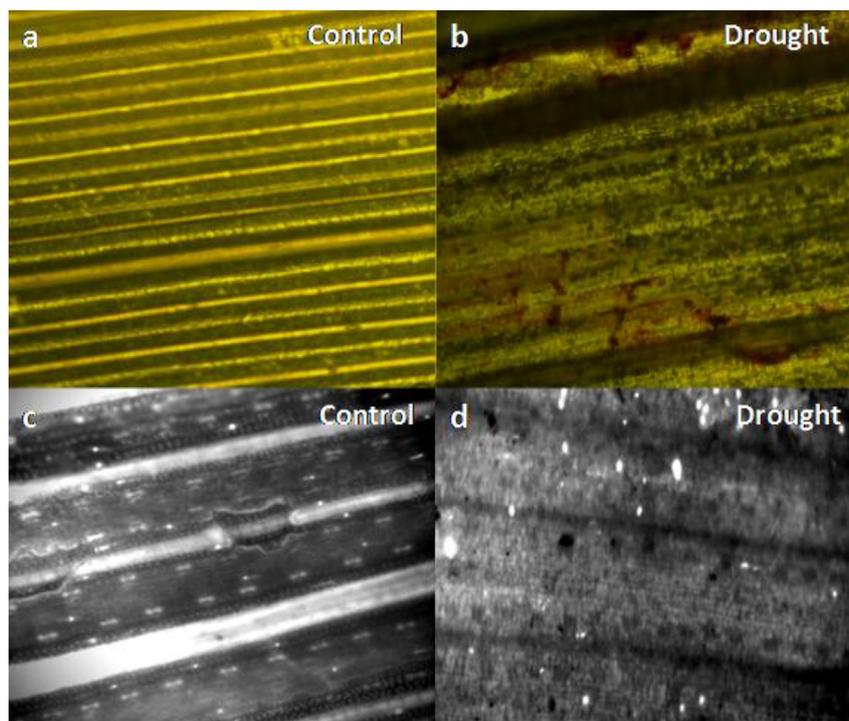


Fig. 4. Leaves of *O. sativa* were stained with (a–b) DAB to detect accumulation of ROS and (c–d) with DAPI to analyse the nuclear degradation.

during drought. Earlier catalase activity during drought stress showed a significant increase in drought-sensitive varieties of wheat as compared to tolerant (Simova-Stoilova et al., 2010). SOD activity was also reported higher in *Phaseolus vulgaris* and *Oryza sativa* in response to drought stress (Zlatev et al., 2006). POD activity in wheat genotypes was recorded increased 50% in tolerant genotypes of wheat as compared to sensitive during water deficient condition (Devi et al., 2012).

Enhanced ROS level induces the oxidative burst of cellular components and impaired the redox potential of cellular machinery. Most common kind of ROS reported in plants is hydrogen peroxide (H_2O_2). To analyse the cellular changes induced by ROS during drought, we stained the cells with DAB that forms brown precipitate with free radicals such as H_2O_2 (Fig. 4a–b). Leaves showed darker brown

precipitate at the site of ROS generation in drought leaves. This suggested that drought stress induces more H_2O_2 production. Results-agreed with the earlier studies showed the formation of brown precipitate at the site of H_2O_2 production in *Arabidopsis* leaves (Gechev et al., 2012). Nuclear fragmentation and DNA laddering is a key feature of PCD in animals and plants both. Results of the present study showed more fragmented nuclei as compared to control during drought with DAPI staining (Fig. 4c–d). This suggested that drought stress induces the nuclear fragmentation as well as PCD. However, genomic DNA fragmentation analysis by gel electrophoresis in control and drought treatment did not show any significant fragmentation (Fig. 5). This suggested possibly, at initial stages drought stress may not degrade the nucleic acid (DNA/RNA) but severe drought or prolonged exposure may

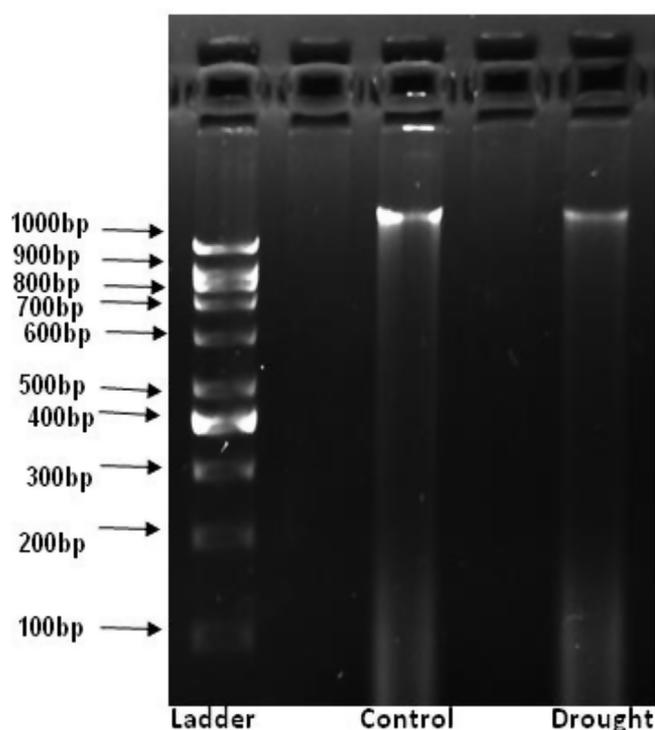


Fig. 5. Agarose gel electrophoresis of genomic DNA isolated from control and drought-treated leaves of *O. Sativa* seedlings.

cause the nucleic acid as well as nuclear fragmentation.

Furthermore, to elaborate the understanding of drought tolerance we analysed the expression of different genes during drought in rice. The expression of antioxidant genes of rice CAT1, SOD and GPX showed a differential expression pattern (Fig. 6a–c). CAT1 and SOD was downregulated while, GPX showed the significant upregulation during drought (Fig. 6b). This is possibly, due to different subcellular localization of these enzymatic antioxidant. A number of earlier studies depicted the response of these antioxidant gene family to various abiotic stresses including cold, drought, heat and salinity (Islam et al., 2015;

Yang et al., 2014; Lum et al., 2014).

The mechanism of PCD is a fundamental, active, genetically organized process to eliminate the cells selectively. In general, PCD and cell proliferation/elongation regulate the growth and development of plant tissues. PCD also considered as a defence response against the environmental stimuli including biotic and abiotic stresses. Drought stress greatly affects the productivity and yield which restrict the energy supply and impaired the ROS balance. The prolonged drought leads to ROS generation, due to reduced CO₂ fixation and electrolyte leakage which eventually leads to PCD (Gechev et al., 2012). It has been studied that drought induced oxidative stress in rice anthers leading to the PCD and pollen sterility (Nguyen et al., 2009). To confirm the ROS generated PCD in drought and correlates it with cellular data we analysed the expression of BAX inhibitor- 1 (BI-1) gene. Earlier studies highlighted that BI-1 located in the endoplasmic reticulum (ER) and plays important role in regulation of cell death (Khan and Hemalatha, 2016) In our findings we reported that BI-1 genes exhibited upregulation in drought treatment (Fig. 7a). We suggested BI-1 plays an important role in drought tolerance by restricting the progression of cell death by inhibiting the ROS induced oxidative damage to cells.

Earlier reports also suggested the upregulation of BI-1 gene during salinity, drought and high temperature in tobacco and displayed markedly improved tolerance to stresses (Isbat et al., 2009). Similarly, BI-1 overexpression in *Arabidopsis* showed the suppression of cell death induced by BAX, H₂O₂ and salicylic acid (Kawai-Yamada et al., 2004). We analysed the expression of MAPK1 gene that showed the significant up-regulation in drought treated seedlings (Fig. 7a). This suggested that kinase family have a positive correlation with drought as well PCD and autophagy induction during drought stress. Earlier findings suggested that MAPKs play a pivotal role in signal transduction, developmental processes and in response to various biotic and abiotic stresses (Asai et al., 2002). The activation of MAPK in response to cold, drought, salt and heat has been reported earlier in different plant systems (Ichimura et al., 2002). This highlighted the specific role of kinase family in drought tolerance.

Further, the transcription factors (TFs) have a prominent role in abiotic stress tolerance. To investigate the role of TFs during drought tolerance we investigated the expression of WRKY53 by Real-time PCR. The WRKY transcription factors (TF) are a family of regulatory proteins contains 74 and 109 members in *Arabidopsis* and rice respectively

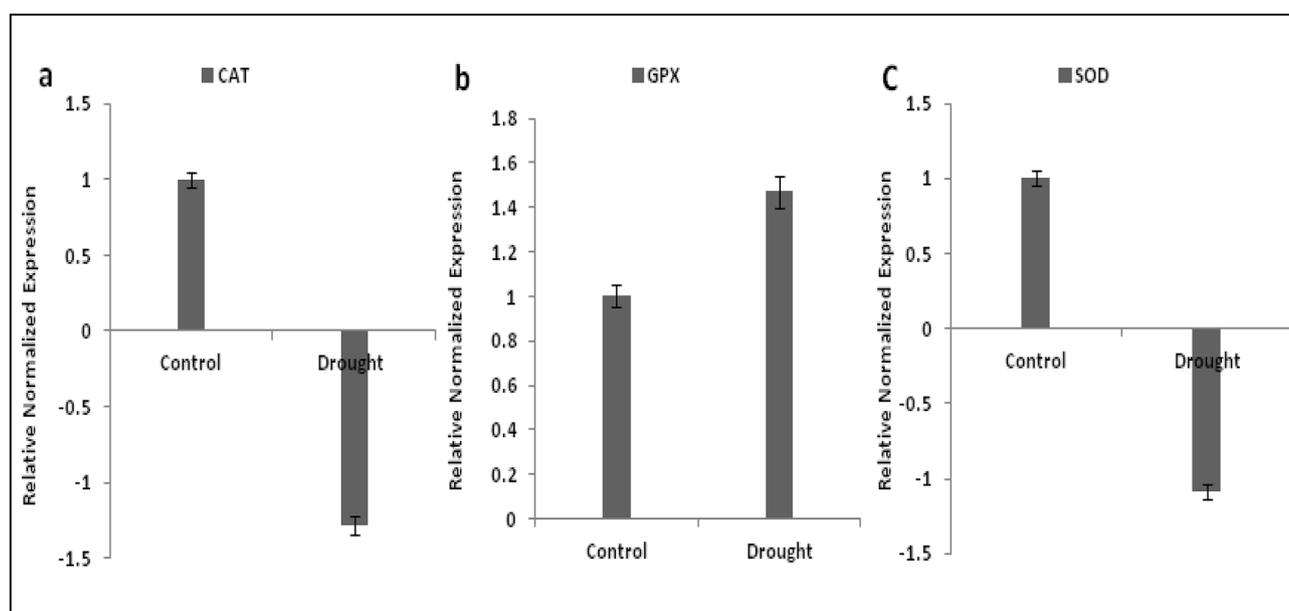


Fig. 6. Effect of drought stress on (a) CAT-1, (b) GPX and (c) SOD genes showing the relative normalized expression in *O. sativa* seedlings. *ACTIN1* used as an internal control to normalize the expression.

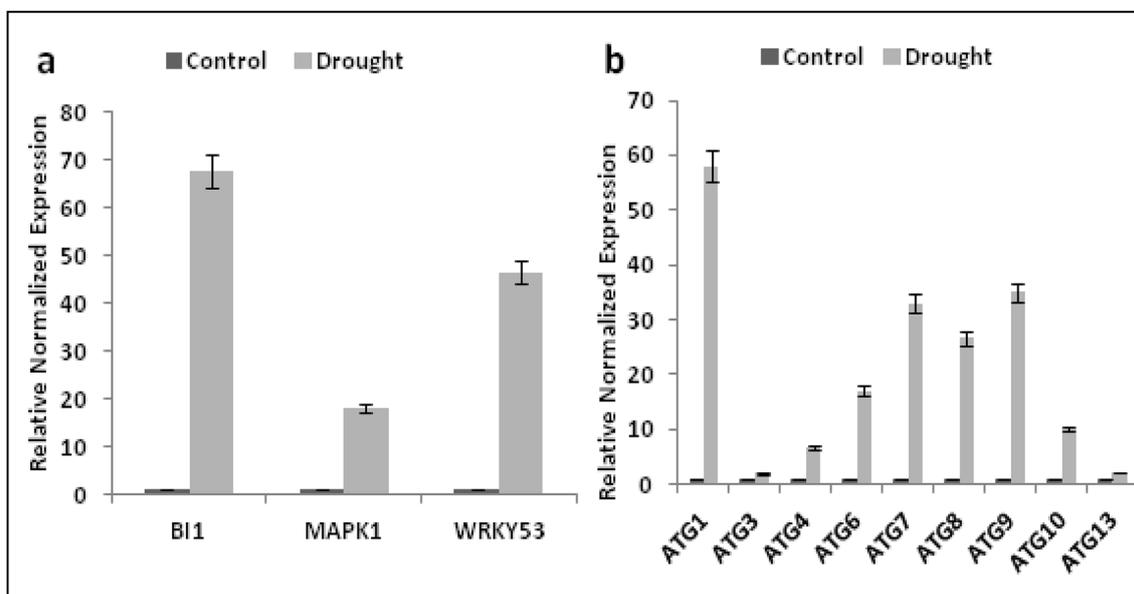


Fig. 7. Effect of drought stress on the expression of (a) BI-1, MAPK1 and WRKY53 and (b) ATG genes showing the relative normalized expression in *O. sativa* seedlings. *ACTIN1* used as an internal control to normalize the expression.

(Eulgem and Somssich, 2007). The role of WRKY genes during abiotic stress has been studied in different plant species. The *AtWRKY53* has a major role in senescence induced cell death (Zentgraf et al., 2010). The present study showed the higher expression of WRKY53 in drought treatment (Fig. 7a) and suggesting that WRKY53 has a prominent role in drought tolerance by senescence induced cell death. Further, to explore the molecular mechanism of autophagy in depth relationship with drought tolerance we characterized the various key genes of autophagy pathway (Khan and Hemalatha, 2015) In the present study, seven autophagy genes showed the up-regulation during the drought stress (Fig. 7b) while two genes were down regulated. The study suggested that autophagy genes regulate the drought tolerance differentially. Earlier studies revealed the specific roles of autophagy genes during various stresses. The *Arabidopsis* ATG8f exhibited induced autophagy in mild salt and osmotic stress. Similarly, *AtATG18a* defective plants showed more sensitivity to salt and drought condition as compared to wild type suggested the role of autophagy in response to these stresses (Liu et al., 2009). In a recent study has been demonstrated that Heat Shock Factor A1 (HsfA1a) induces drought tolerance by activating ATG (ATG10 and ATG18f) genes and inducing autophagy, which may promote plant survival by degrading ubiquitinated protein aggregates under drought stress in tomato (Wang et al., 2015).

5. Conclusion

Our study elucidated the effect of drought stress on physiological, cellular and molecular attributes in rice. Drought stress negatively affected the growth and metabolism of the rice. The higher accumulation of proline content, generation of ROS through DAB analysis, DNA and nuclear fragmentation represented the hallmark features of PCD. The biochemical and molecular studies of antioxidant enzymes showed high enzymatic activity and differential gene expression suggested that plants alleviate the drought induced oxidative stress through a strong scavenging system. The gene expression of MAPK1 depicts the role of stress responsive signal kinase possibly participate in regulation of PCD and autophagy pathways during drought stress. The expression of WRKY53 genes suggested that WRKY gene family or other TFs may be positive regulators of autophagy and PCD genes during drought tolerance. Furthermore, the expression pattern of autophagy genes evidenced the role of autophagy in drought tolerance mechanism. Thus, we proposed that autophagy, is co-regulated with programmed cell

death controlled by strong scavenging system, signalling cascade and TFs. Furthermore, the study can be elaborated for future research to identify the PCD and autophagy targets in different plants.

Conflicts of interest

Authors declare they have no actual or potential conflict of interest.

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Abbreviations

ATG	Autophagy-related gene
BI-1	BAX Inhibitor-1
BSA	Bovine Serum Albumin
CAT-1	Catalase-1
DAB	3, 3'-diaminobenzidine
DAPI	4', 6-diamidino-2-phenylindole
GPX	Glutathione Superoxide Dismutase
HsfA1	Heat Shock Factor A1
MAPK1	Mitogen-Activated Protein Kinase-1
Mn-SOD	Manganese-Superoxide Dismutase
PCD	Programmed Cell Death
POX	Peroxidase
ROS	Reactive Oxygen Species

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bcab.2019.101150>.

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