Physiological, Biochemical and Molecular Responses to Oxidative Stress in *Saccharum spontaneum*

Jini Narayanan[1] · R. Manimekalai[1] · A. Selvi[1] · R. Arun Kumar[2]

Abstract The oxidative stress response was studied in five different clones of *Saccharum spontaneum* (IMP-564, IS76-216, Pamba, SES-90 and Taiwan) by analyzing the physiological and biochemical parameters and gene expression pattern. The sixty days-old plants were sprayed with different concentrations of 30% H_2O_2 (300 ppm, 500 ppm and 1000 ppm) for consecutive three days in glass house. Adaptive response was recorded in terms of chlorophyll fluorescence, chlorophyll stability index (CSI), activities of peroxidase (POX) and super oxide (SOD), lipid peroxidation (LPO), protein and proline content at 48 h and 72 h of H_2O_2 treatment. The IMP -564 clone recorded high SOD and lower lipid peroxidation levels. Based on chlorophyll fluorescence, CSI, peroxidase, SOD and lipid peroxidation values, and the clone SES-90 recorded positive trend for stress tolerance. CSI showed significant difference between genotypes and different concentrations of H_2O_2. As the genotypes IMP-564 and SES-90 showed positive response to oxidative stress tolerance, the *S. spontaneum* genotype SES-90 was selected for comparative gene expression studies with *Erianthus* sp clones IJ76-389 and IK76-91. Different concentrations of H_2O_2 and different time intervals (control, 500 ppm and 1000 ppm for 48 h and 72 h) were used for gene expression studies. The stress responsive genes viz. Ascorbate peroxidase gene (*Apx*) Nam, Ataf1 and Cuc2 gene (*NAC*), Ethylene response factor gene (*Erf*), Glutathione S-transferases gene (*Gst*), Myeloblastosis antisense gene (*Mybas*) and Catalase gene (*Cat*) showed differential expression among the genotypes of *S. spontaneum* and *Erianthus* sp. Among the genotypes, SES 90 and IK76-91 showed better tolerance against oxidative stress and the expression levels of stress responsive genes in the genotype IK76-91 was higher than the genotype SES 90.

Keywords Sugarcane · Reactive oxygen species · Quantitative PCR · Gene expression · Chlorophyll fluorescence · Chlorophyll stability index · Superoxide dismutase · Peroxidase

Abbreviations
ROS	Reactive oxygen species
SOD	Superoxide dismutase
POX	Peroxidase
LPO	Lipid peroxidation
CF	Chlorophyll fluorescence
Fv/Fm	Variable fluorescence/Maximum fluorescence
CSI	Chlorophyll stability index
TFs	Transcription factors
qRT-PCR	Quantitative Reverse Transcriptase—Polymerase Chain Reaction
H_2O_2	Hydrogen peroxide

✉ R. Manimekalai
r.manimekalai@icar.gov.in; rmanimekalaiicar@gmail.com

1 Crop Improvement Division, ICAR- Sugarcane Breeding Institute, Indian Council of Agricultural Research (ICAR), Coimbatore, Tamil Nadu 641 007, India

2 Crop Production Division, ICAR- Sugarcane Breeding Institute, Indian Council of Agricultural Research (ICAR), Coimbatore, Tamil Nadu 641 007, India

Introduction

Sugarcane is a commercial crop of India and accounts for more than 70 percent of world sugar production. India is the second largest producer of sugar in the world market,

closely following Brazil. Dramatically changing environment poses serious direct and indirect threat to the sustainable agricultural production leading to global food insecurity. The fate of sugarcane productivity is of no difference from other crops. Environmental stresses such as drought, salinity, chilling, metal toxicity, and UV-B (short Ultra Violet) radiation as well as pathogens attack lead to enhanced generation of reactive oxygen species (ROS) in plants due to disruption of cellular homeostasis (Sharma and Dubey 2007; Hu et al. 2008; Tanou et al. 2009; Maheswari and Dubey 2009; Han et al. 2009; Srivastava and Dubey 2011; Mishra et al. 2011; Hamim et al. 2017; Ferreira et al. 2017). Environmental stresses, such as salinity, drought and extreme temperature increase the ROS levels in plant cells. Over production of ROS in the system will ultimately result in oxidative stress. This can lead to the peroxidation of lipids, damage to nucleic acid, oxidation of proteins, enzyme inhibition, DNA mutation and finally to cell death (Sharma and Dubey ; Gill and Tuteja 2010; Mitler et al. 2011; Kumari et al. 2007,2015). ROS like H_2O_2 is more stable and can diffuse directly from chloroplasts to the nuclei (Exposito-Rodriguez et al. 2017), thus influencing gene expression in the nuclei both directly and indirectly.

To adapt to the environmental stresses, plants undergo various physiological and metabolic processes. The major molecular response is the activation of stress-responsive genes and synthesis of diverse functional proteins through a complex signal transduction network (Shinozaki et al. 2003; Hirayama and Shinozaki 2010). Some of the most common responses for abiotic stress tolerance in plants are overproduction of osmo-protectants or osmolytes (such as sucrose, betaines, and proline), cellular metabolic changes (defense-related secondary metabolite production, proteolytic activity, adenylate energy charge, ionic homeostasis, redox state regulation, and antioxidant enzymes activation), anatomical and morphological changes in plant tissues.

Various enzymes like superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX), catalase (CAT) and non-enzymatic antioxidants such as tocopherols, ascorbic acid (AsA), glutathione (GSH) and genes like *Erf* and *NAC* are involved in regulation of signal transduction and gene expression during stress response and play different roles in detoxifying ROS (Manimekalai et al. 2018a; b). Another group which regulate stress response are a number of transcription factors (Shinozaki et al. 2003; Yamaguchi-Shinozaki and Shinozaki, 2006; Cattivelli et al. 2008), which regulate various stress-inducible genes cooperatively or separately, and may constitute gene networks. Stress induced transcription factors belong to DREB (Dehydration Response Elements Binding) protein family, zinc finger protein family, MYB (Myeloblastosis) family, bZlP (basic leucine zipper domain) family, WRKY family (named after the conserved WRKYGQK amino acid sequence), and NAC family. These transcription factors regulate stress-inducible genes thereby initiating stress responses and establish plant's response to tolerate the stress. Over expression of these transcription factors genes enhanced the drought, salt, and cold tolerance in many crops (Ito et al. 2006; Nakashima and Shinozaki 2006; Zeng et al. 2008; Ambawat et al. 2013; Hong et al. 2016; Roy 2015).

Saccharum spontaneum, a species involved in hybridization program with sugarcane show tolerance to biotic/abiotic stresses. Since past decades, the wild species has contributed the tolerant genes to cultivated sugarcane through introgressive breeding. Several reports explained the use of hydrogen peroxide and methyl viologen in simulating oxidative stress in plants (Upadhyaya et al. 2007; Vijayalakshmi et al. 2014). Vijayalakshmi et al. (2014) used different concentrations of H_2O_2 (0, 0.05, 0.1, 0.15, 0.2 mM) to induce oxidative stress in rice. Deng et al. (2012) induced the oxidative stress in sweet potato by exogenous application of H_2O_2 (up to 2.5 mM) into the adventitious root culture. In the present study, *Saccharum spontaneum* genotypes were screened for the adaptive physiological, biochemical traits and expression of stress responsive genes. The study helped in identifying the stress responsive genotypes of *S. spontaneum* under oxidative stress conditions and uses them for higher level of gene to gene interaction studies.

Materials and Methods

A pot culture experiment was designed to measure the physiological parameters in *S.spontaneum* genotypes. The potting mixture was prepared with sand, loam and farmyard manure in the ratio 2:1:1. The clones were selected from the already available germplasm present in Sugarcane Breeding Institute, Coimbatore, based on the earlier screening reports, viability of buds and tillering capacity. Sixty days old plants of five genotypes (IMP-564, IS76-216, Pamba, SES-90 and Taiwan) were used for the experiment. Three different concentrations (300 ppm, 500 ppm and 1000 ppm) of 30% hydrogen peroxide (commercially available in the market) were used as treatments. Three replications were maintained for different treatment. One set from each genotype was retained as control by spraying with normal water. The spraying was done consecutively for three days (24 h, 48 h and 72 h) at 8 am. Physiological parameters like photochemical efficiency (CF), chlorophyll stability index (CSI), lipid peroxidation, protein and proline content and enzyme assays for peroxidase and super oxide dismutase (SOD) for 48 h

and 72 h of H_2O_2 treatment were followed as per the protocol described by Manimekalai et al. 2018a). The data were analyzed using the software JMP 9.0.

For gene expression studies, data mining through literature survey was performed and seven multiple stress responsive genes *Hsp* (Heat Shock Proteins gene), *Apx* (Ascorbate peroxidase gene), *NAC* (defined by Nam, Ataf1 and Cuc2 genes), *Erf* (Ethylene response factor gene), *Gst* (Glutathione S-transferases gene), *Mybas* (Myeloblastosis antisense gene) and *Cat* (Catalase) were selected. The experiment conducted in a randomized complete block design with four treatments and two replications, consisting of 0 ppm (control), 300, 500 and 1000 ppm of 30% hydrogen peroxide. The plants were maintained in a glass house in pots. Plant samples were collected on 2nd (48 h) and 3rd (72 h) days of treatment and were further processed for expression studies.

RNA isolation was done using TRIzol reagent (Invitrogen), cDNA conversion with cDNA synthesis kit (Thermofisher scientific), and gene expression analysis with SYBR green assay (Applied Biosystems) as per the protocol (Manimekalai et al. 2018b). The fold change of expression was calculated in terms of relative quantification (Rq) using Livak's method and the values were analyzed using statistical methods (Livak and Schmittgen 2001).

Results and Discussion

Abiotic stress factors like drought, salinity, water-logging, and heavy metal toxicity lead to oxidative stress in plants (You and Chang 2015). Generally the wild species respond to such factors in different ways to make it adaptable. At the molecular level, differential expression of certain stress responsive contributes to the tolerance (Li et al. 2019). Exogenous application of hydrogen peroxide was proven to induce oxidative stress in rice and sweet potato (Vijayalakshmi et al. 2014; Deng et al. 2012), and we used it in the present study to induce the oxidative stress in *S. spontaneum* and *Erianthus* sp.

Morphologically, though not much remarkable variation was seen at lower concentration of hydrogen peroxide (300 ppm), but slight yellowing of leaves (Fig. 1b and e) and leaf tip drying and rolling at higher concentrations (Fig. 2c) were observed. The physiological and biochemical assays were performed to screen the clones of *Saccharum spontaneum*. Gene expression studies offered the opportunity to compare the physiological and biochemical responses with molecular response.
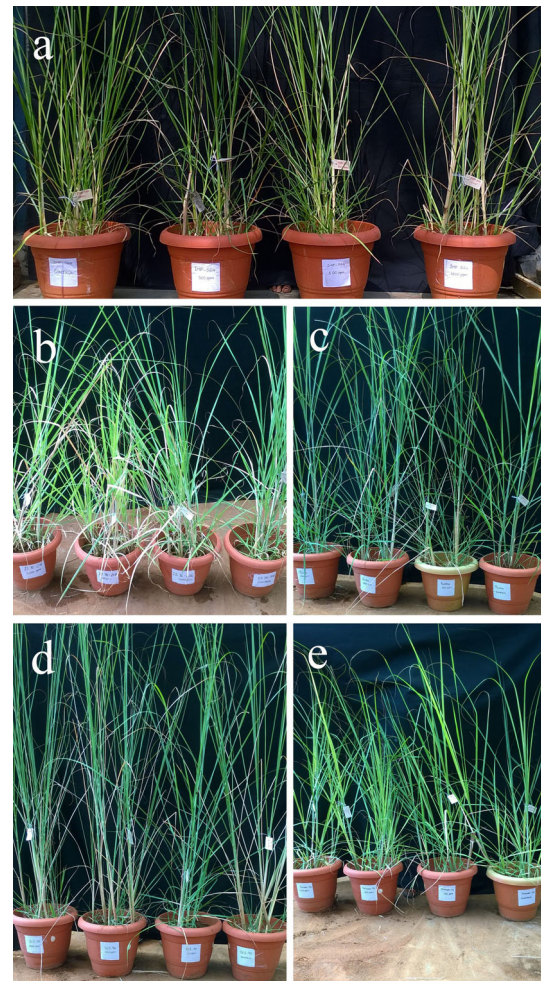
Fig. 1 Clones of *S. spontaneum* sprayed with different concentrations (Control, 300,500 and 1000 ppm) of 30% H_2O_2. IMP 564 (a), IS 76 286(b), Pamba (c), SES 90(d) and Taiwan (e)

Fig. 2 Clones of Erianthus (IJ76-389, IK76-91) and *S. spontaneum* (SES-90) sprayed with different concentrations of 30% H_2O_2— Control (a), 500 ppm (b), 1000 ppm (c)

Physiological and Biochemical Responses to the Oxidative Stress

The three-way ANOVA on physiological and biochemical parameters (chlorophyll fluorescence, peroxidase, superoxide dismutase, lipid peroxidation, protein and proline) showed highly significant differences among the genotypes ($p < 0.001$) for SOD, POX, LPO, while changes in proline was significant at $p < 0.05$. The change in the soluble protein content was non-significant among the genotypes. The effect of different concentrations of hydrogen peroxide on levels of SOD was significant ($p < 0.001$) and POX ($p < 0.05$), while other traits were non-significant. The duration of stress treatment (T) showed significant effect on protein and LPO ($p < 0.05$). The interaction effect of VxS and VxT had significant effect on LPO and SOD, POX, proline, LPO, respectively. The SxT and the higher order of interaction VxSxT had no significant effect on the studied parameters. Thus, genotypes, concentration of hydrogen peroxide and duration of treatment and their interactions on the various biochemical traits were showed different levels of response (Table 1). The main effect of genotypes, concentrations of hydrogen peroxide and the interaction effect (VxS) were significant ($p < 0.001$) for CSI (Table 2).

Enzyme Activities—SOD and POX

Super Oxide Dismutase (SOD) is a key anti-oxidative enzyme neutralizes the activated species of oxygen (Van Toai and Bolles 1991). It is the first defense against reactive oxygen species which converts super oxides to H_2O_2. Increased SOD activity of certain genotypes can be taken as an indirect indication of enhanced stress tolerance (Arisi

et al. 1998). Lots of other studies corroborate the role of SOD during stress. Specific activity of SOD increased, under prolonged oxidative stress induced by methyl viologen (Chagas et al. 2008; Bowler et al. 1994) and in the present study too, SOD showed high activity in clones IMP-564 and SES-90 (Fig. 3a). IMP 564 showed increased SOD activity with increase in concentration at both 48 and 72 h. But the activity was found to decrease on prolonged treatment (1000 ppm) 72 h of H_2O_2 treatment with 500 ppm in IMP 564 showed 24% increase with respect to control. At 48 h, 1000 ppm of H_2O_2 it showed 18% increase over its control. IS76-216 showed an increase in SOD activity at 300 ppm but failed to accumulate at higher concentration of H_2O_2. ANOVA showed that the genotypes (V) and concentration of H_2O_2 (S) were highly significant on superoxide dismutase (SOD), but exposure time was non-significant. However, the interaction between VxT was significant with SOD. The other interaction viz, V × S, S × T, V × S × T were non-significant.

Peroxidase (POX) activity increases as a protective response in plants during environmental stresses (Pujari and Chanda 2002). All clones in this study showed an increased POX activity at 500 ppm H_2O_2 for 48 h (Fig. 3 b). Except for the clone SES-90, there was a decrease in POX activity with higher concentration of H_2O_2 (1000 ppm). SES-90 showed an increase of 23% activity at 72 h treatment. IMP 564 showed a drastic decrease in POX activity on high concentration of H_2O_2 (1000 ppm) with respect to its 500 ppm (25% reduction). Pamba and Taiwan did not show any significant SOD activity, rather there was decreased enzyme activity. Statistical analysis revealed that the main effect V, S and the interaction V × T showed significance on POX, while the other level of interactions were not significant.

Table 1 Analysis of variance on biochemical parameters

Factor	dF	F ratio and probability					
		SOD	POX	Proline	Protein	LPO	CF
S. spontaneum clones (V)	4	18.96***	3.86***	3.67*	0.24[ns]	5***	5.773***
H_2O_2 spray(S)	3	6.06***	4.12*	1.8[ns]	0.12[ns]	0.97[ns]	2.300[ns]
Time (T)	1	3.42[ns]	0.08[ns]	0.02[ns]	7.08*	4.66*	3.246**
V × S	12	1.75[ns]	1.31[ns]	1.42[ns]	0.04[ns]	2.34*	27.28***
V × T	4	3.28*	5.6***	3.18*	0.003[ns]	3.19*	1.314[ns]
S × T	3	1.45[ns]	1.7[ns]	0.61[ns]	0.19[ns]	1.26[ns]	1.187[ns]
V × S × T	12	0.76[ns]	1.24[ns]	1.03[ns]	0.04[ns]	1.12[ns]	0.984[ns]
Error	40	–	–	–	–	–	–
Total	79	–	–	–	–	–	–

Three-way ANOVA shows the clone (Varieties-V) effects with physiological parameters (enlisted) during different time interval (T) with different concentration (Spray-S) of H_2O_2. Note: Levels of significance: ***, **, * and ns indicate significant difference at $p < 0.001$, $p < 0.01$, $p < 0.05$ and non- significant, respectively

Table 2 Analysis of variance for chlorophyll stability index

Factor	dF	F ratio and probability of CSI
S. spontaneum clones (V)	4	23.23***
H_2O_2 spray (S)	3	4.82*
V × S	12	5.30***
Error	20	–
Total	39	–

The effects of clones (Varieties-V) toward CSI with different concentration (Spray-S) of H_2O_2. Note: Levels of significance: ***, **, * and ns indicate significant difference at $p < 0.001$, $p < 0.01$, $p < 0.05$ and non- significant, respectively

Lipid Peroxidation

Lipid peroxidation is directly proportional to the damage to the plants. A low LPO value signifies tolerance response of the plants. Higher levels of H_2O_2 accompanied by an increase in lipid peroxidation were observed in young sugarcane plants during the initial growth phase under severe water stress (Boaretto et al. 2014). Within the clone SES-90, the plants that were treated with 500 ppm of H_2O_2 for 48 h and 1000 ppm of H_2O_2 for 48 and 72 h showed

lower LPO values than with 300 ppm (Fig. 4 a). This denotes the induction of stress tolerance upon prolonged exposure to H_2O_2. During 72 h of H_2O_2 exposure, the clones IS76-216, Pamba, and Taiwan were found to be the most affected, demonstrating their susceptibility. Pamba showed a higher lipid peroxidation (50% increase over control) on prolonged exposure (72 h with 1000 ppm). Similar enhancement on LPO was observed in maize (Xu et al. 2011), *Leymus chinensis* and *Stipa grandis* (Xu and Zhou. 2006; Xu et al. 2009 and Song et al. 2015).

Soluble Protein Content

Higher protein content is correlated with higher RuBisCo activity and high photosynthetic efficiency under stress conditions. The abiotic stress results in enzyme inhibition and disruption of pathways in protein synthesis (Paramjeet et al. 2014). In the present study, all genotypes showed a marked decrease in the protein content with prolonged exposure to H_2O_2. All genotypes showed increased soluble protein content at 1000 ppm except the clone Taiwan (Fig. 4b). However, the variation is non-significant among the genotypes and interaction effect except time (T).

Fig. 3 Effect of oxidative stress on the ROS scavenging enzymes activity of—a) Superoxide dismutase and b) Peroxidase in five different clones of *S spontaneum*. Stress was induced using different concentration (control, 300, 500, 1000 ppm) of 30% H_2O_2 for 48 and 72 h
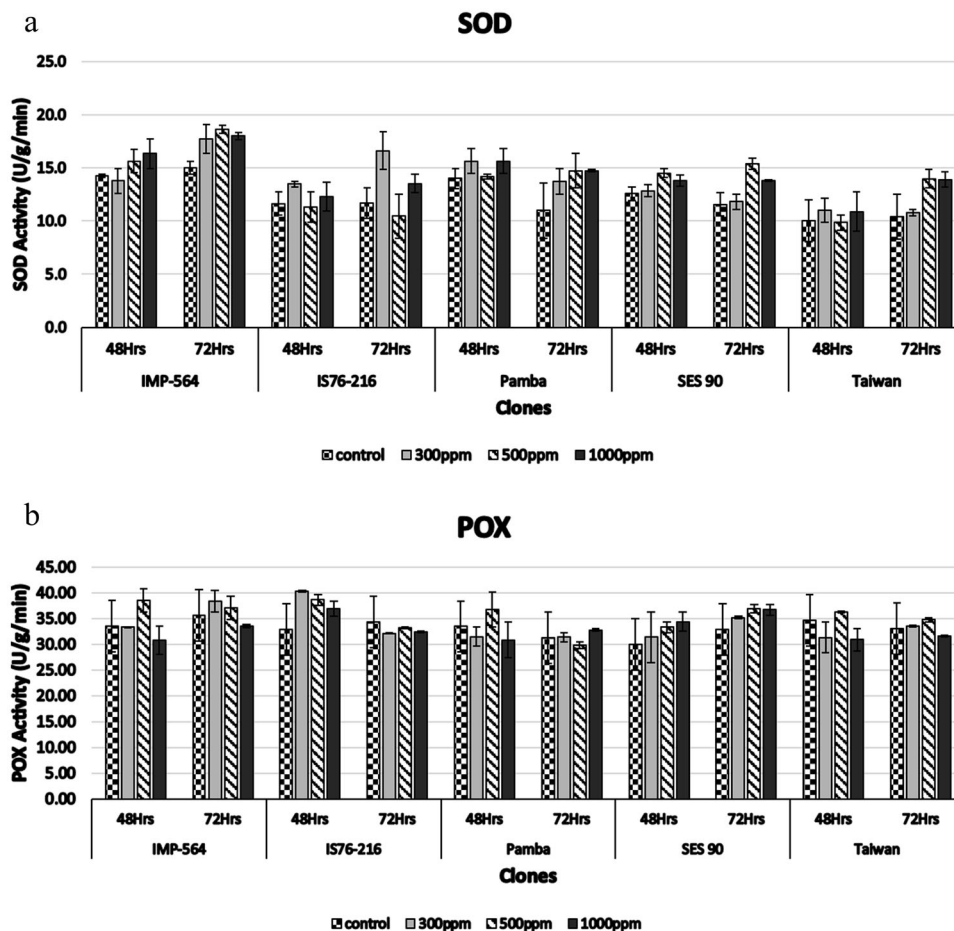
Fig. 4 Effect of oxidative stress on non-enzymatic parameters – a) lipid peroxidation, b) Protein and c) Proline estimation five clones of *S. spontaneum*. Stress was induced using different concentration (control, 300, 500, 1000 ppm) of 30% H_2O_2 for 48 and 72 h
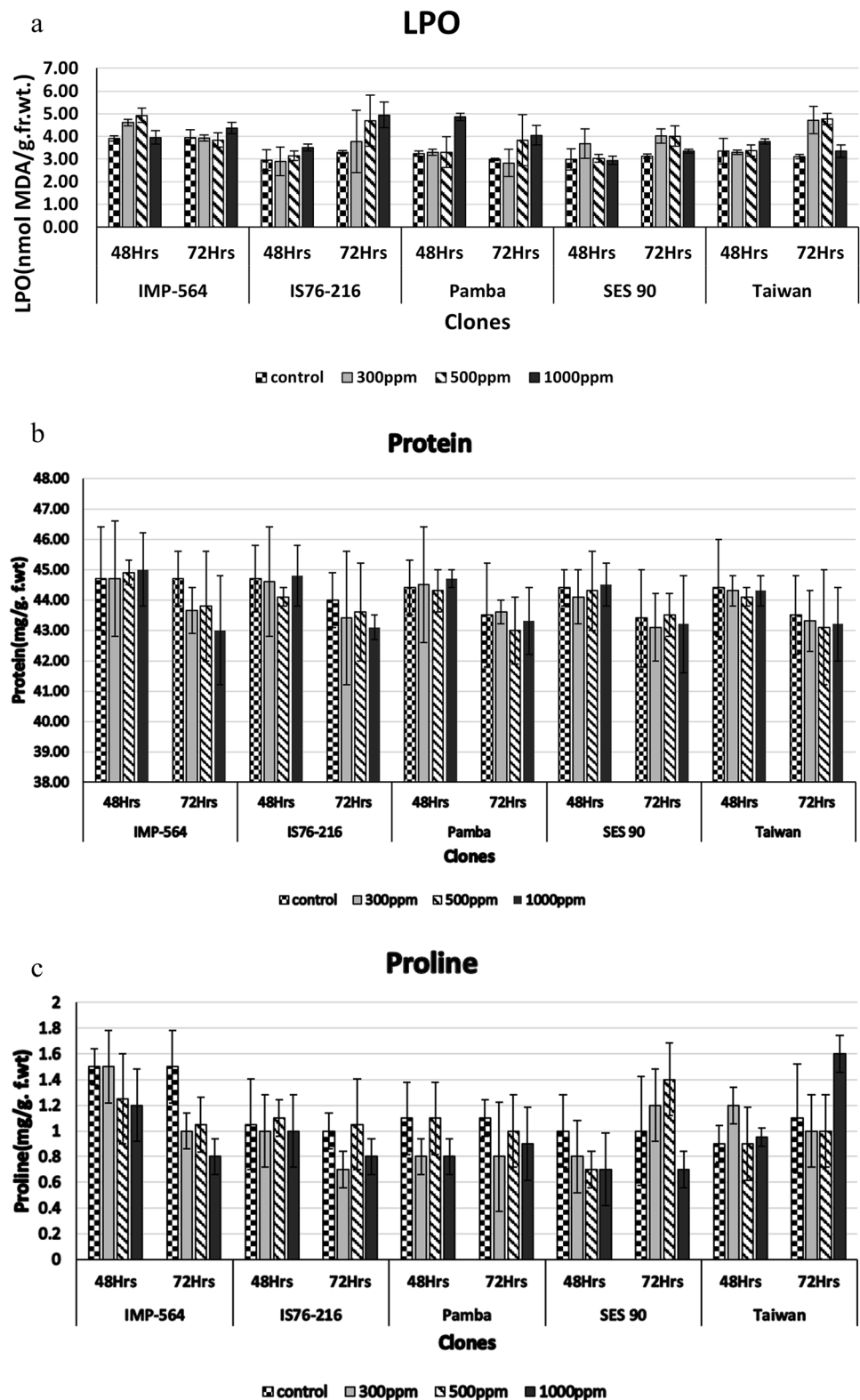
Proline Content

Proline content increases substantially under abiotic stress conditions. Organisms acquire oxidative stress tolerance by accumulating proline. Proline accumulates in the cytosol and vacuole during stress and was shown to protect plant cells against damages caused by $^1O_2$ or $HO^·$ (Hong et al. 2016; Matysik et al. 2002). It acts as an osmolyte, a

chemical chaperon and as a direct scavenger of ROS (Liang et al. 2013). In our study, SES-90 showed higher accumulation of proline 500 ppm H_2O_2, and had 40% increase in proline content at 1000 ppm (Fig. 4c). The main effect V and the interaction VxT are significant for proline content, while the other level of interactions were non-significant.

Chlorophyll Fluorescence

Chlorophyll fluorescence is determined using the Fv/Fm (Variable fluorescence/Maximum fluorescence) ratio. A lower Fv/Fm value indicates the plants are under stress (Maxwell and Johnson 2000). There was no much difference with respect to chlorophyll fluorescence. However, the genotype Pamba showed increase (8.0% in chlorophyll fluorescence at 500 ppm over its control. IMP 564 too showed a moderate increase in fluorescence (Fig. 5).

Chlorophyll Stability Index

CSI is an indicator of maintenance of photosynthetic pigments. Reactive oxygen species causes damage to chloroplast thereby reduces the chlorophyll stability. Higher CSI value indicates higher stress tolerance. A study in rice by Mohan et al. (2000) indicated that chlorophyll content was more affected in susceptible varieties than in tolerant varieties under salt stress. In the present study, IS76-216 and Pamba showed a relatively low index when compared to the control (Fig. 6). SES-90 showed higher chlorophyll stability index at 500 ppm of H_2O_2 than other clones (31.8% increase over control). Similarly, higher CSI was observed in tolerant varieties of sugarcane under heat stress (Kohila and Gomathi 2018) and drought stress (Sanghera

and Kumar 2018). ANOVA for CSI showed significant difference between genotypes and concentrations of H_2O_2 and hence, this parameter is particularly of use in screening larger number genotypes. Overall analysis showed that the clones IMP-564 and SES-90 exhibited desirable levels of physiological and biochemical parameters which may contribute the tolerance.

Relative Gene Expression under Oxidative Stress

Expression pattern of seven multiple stress responsive genes was analyzed under different hydrogen peroxide concentrations and at various time intervals. The expression of genes under stress was analyzed with respective to its control. The expression of key genes implicated in stress was reported here.

NAC Gene

NAC (NAM (No apical meristem), ATAF 1/2 (*Arabidopsis* transcription activator factor 1/2) and CUC2 (cup shaped cotyledon)) family consists of several genes (> 100), and they are reported to be involved in diverse functions in plants especially biotic and abiotic stress tolerance. Fang et al. (2015) reported that the NAC transcription factor gene, *SNAC3* functions as a positive regulator under high temperature and drought stress in rice. The gene enhanced the abiotic stress tolerance by modulating H_2O_2 homeostasis state through controlling the expression of ROS-associated genes. Phylogenetic analysis of NAC transcription factor genes by Manimekalai et al. (2016) revealed the presence of multiple stress tolerant NAC genes in sugarcane orthologous to sorghum, wheat and maize. Current study showed an increased expression at
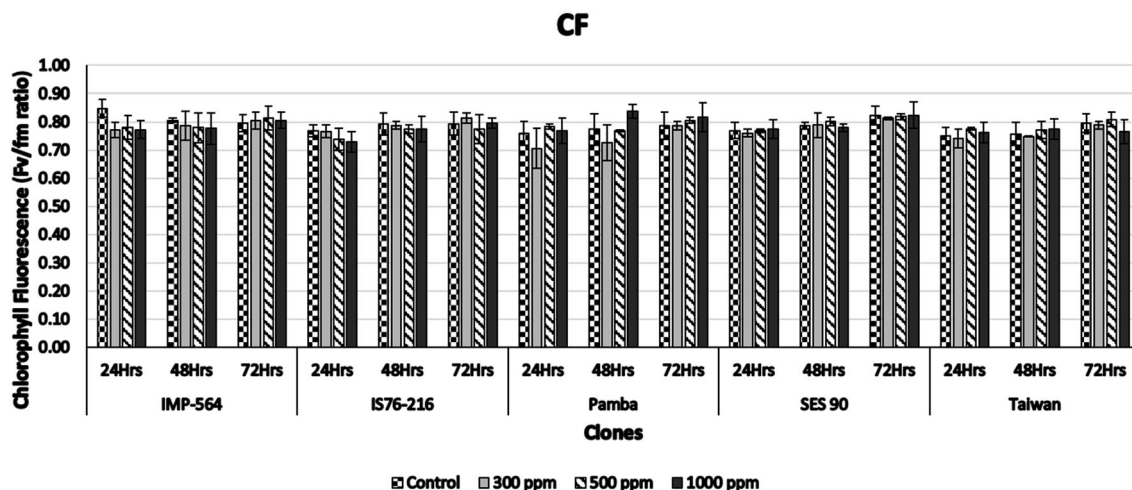
Fig. 5 Effect on chlorophyll fluorescence in five clones of *S. spontaneum* subjected to different concentration of 30% H_2O_2 (control, 300, 500, 1000 ppm) for 24, 48 and 72 h

Fig. 6 Effect of different concentration (control, 300, 500, 1000 ppm) of 30% H_2O_2 on Chlorophyll stability Index in five different clones of S.spontaneum with 72 h of treatment
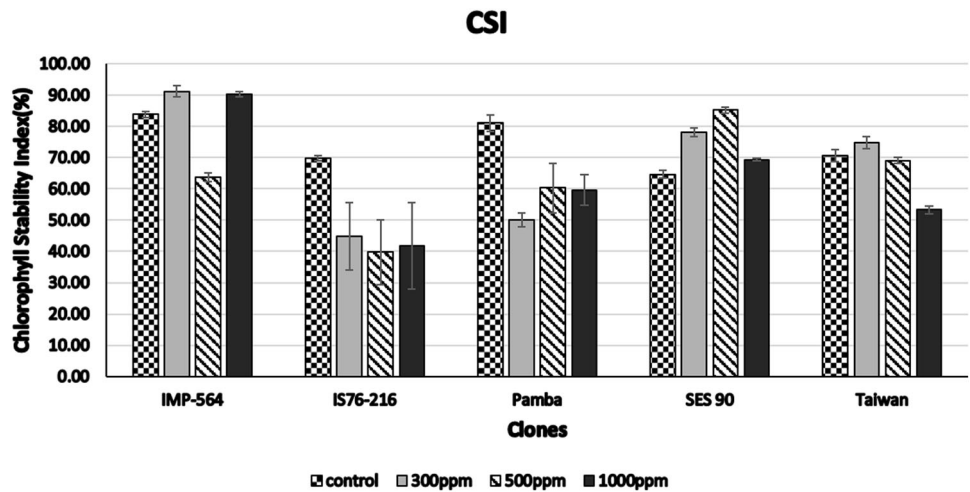
500 ppm H_2O_2 in *Erianthus* sp. genotypes (IJ76-389 and IK76-91) and *S. spontaneum* under both 48 h and 72 h exposure (Fig. 7); however, the level of expression is comparatively lesser in *S.spontaneum*.
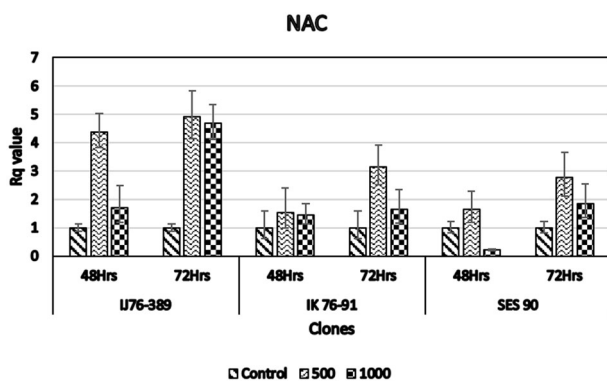
Myb Transcription Factor Gene

Most MYB proteins function as transcription factors with varying numbers of MYB domain repeats conferring their ability to bind DNA. They are widely distributed in plants and implicated in the ABA response (Ambavat et al. 2013). In the present study, the expression levels were higher at 500 ppm (Fig. 8). This was in congruence with the studies by Roy (2015) where *Myb* genes showed higher expression under the epigenetic regulation of stress in Arabidopsis. The genotype IJ76-389 had higher expression levels upon prolonged exposure (72 h) and at higher concentration of H_2O_2 (1000 ppm). Our previous study (Manimekalai et al. 2018b) also indicated an increase in gene expression for *Mybas* under higher concentration of H_2O_2 stress.

Relatively, the expression levels of *Mybas* genes were higher in *Erianthus* sp. than *S. spontaneum*.

Erf (Ethylene Response Factors) Gene

The expression of *Erf* genes is responsive to several abiotic stress factors. The up-regulation of *Erf* gene was reported against environmental stress conditions (Mizoi et al. 2012; Cui et al. 2021). Quantitative PCR study by Wang et al. (2015) revealed over expression of *JcERF2* gene by multiple stresses like abscisic acid, drought and salt stress in tobacco. Gene expression varies with different genotypes (Fig. 9). SES-90 showed a dramatic increase in gene expression at 500 ppm at 48 h, further the expression ceases after 72 h which could be implicated its involvement in signalling pathway leading to tolerance.
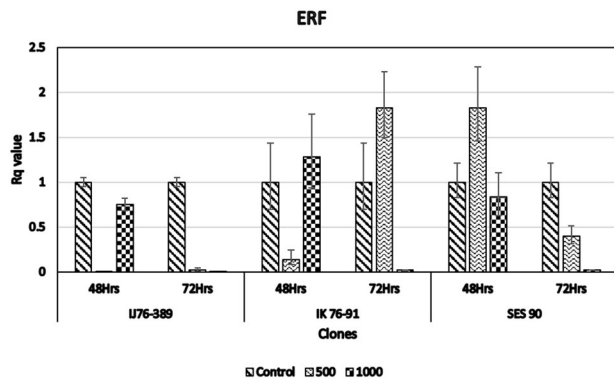

Fig. 7 The graphical representation of Rq values for NAC transcription factor gene expression at different concentration of H_2O_2 induction in *Erianthus* sp. (IJ76-389, IK76-91) and. *S spontaneum* (SES-90) at 48 h and 72 h
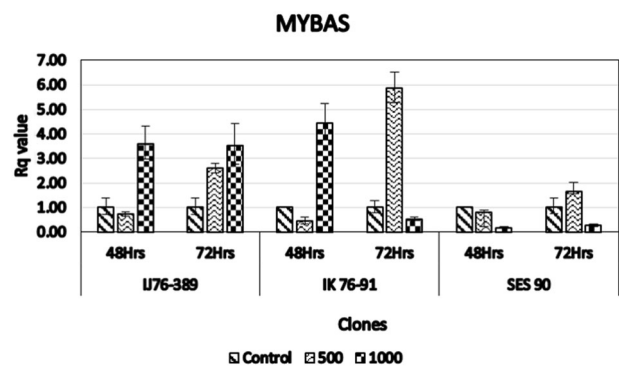
Fig. 8 The Graphical representation of Rq values for MYB transcription factor with different concentration of H_2O_2 induction in *Erianthus* sp. (IJ76-389, IK76-91) and *S. spontaneum* (SES-90) at 48 h and 72 h
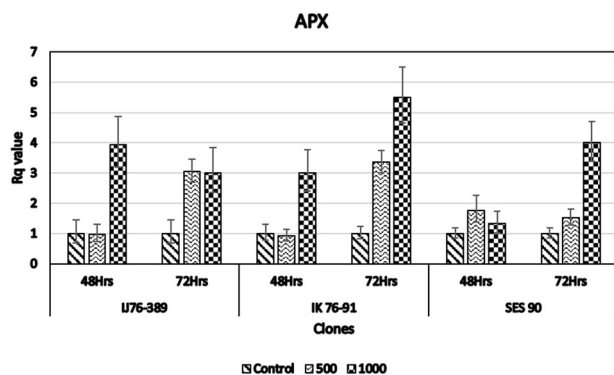
Fig. 9 The Graphical representation of Rq values for ethylene responsive factor (ERF) gene expression at different concentration of H_2O_2 induction in *Erianthus* sp. (IJ76-389, IK76-91) and *S. spontaneum* (SES-90) at 48 h and 72 h
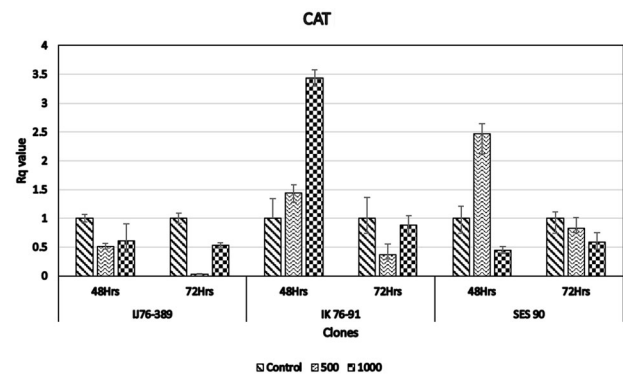
Apx (Ascorbate Peroxidase) Gene

Apx gene, known for its scavenging nature, seems to express on prolonged exposure to stress. Our study showed the over expression of *Apx* gene during 72 h of H_2O_2 treatment (Fig. 10). At 1000 ppm, both the species showed an increased expression under stress conditions. Likewise, studies in barley leaves (Harb et al. 2016) revealed higher expression of *Apx* gene upon prolonged stress conditions.

Cat (Catalase) Gene

CAT is an enzymatic antioxidant that catalyses the conversion of H_2O_2 into H_2O and O_2, thereby detoxifying the ROS present in plant cells (Mhamdi et al. 2010). Harb et al. (2016) reported that the catalase gene was up-regulated after 9 days of drought treatment. The scavenging nature of catalase gene is well established through different studies. In the current study, both *E. arundinaceus* and *S. spontaneum* showed an increase in expression at 500 ppm
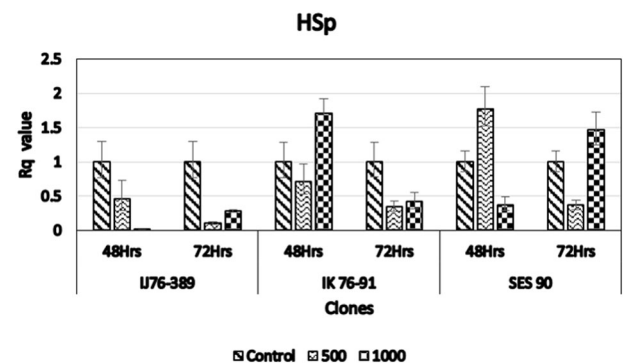

Fig. 11 The Graphical representation of Rq values for catalase gene expression at different concentration of H_2O_2 induction in *Erianthus sp.* (IJ76-389, IK76-91) and *S spontaneum* (SES-90) at 48 h and 72 h

treatment at 48 h exposure but during 72 h the expression was reduced (Fig. 11). The lower *Cat* gene levels lead to reduced CAT enzyme activities (Cavalcanti et al. 2004). Chakraborty and Bhattacharjee (2015) have also observed a decreased CAT activity in rice under cold stress and CAT activity declined in cowpea under salinity stress (Cavalcanti et al. 2004).

Hsp (Heat Shock Proteins) Genes

HSPs are molecular chaperones involved in membrane and protein stability during stress conditions. In Sugarcane, the *Hsp* gene from *E. arundinaceus* conferred enhanced water and salinity stress tolerance (Augustine et al. 2015). In the present study, 48 h of 500 ppm H_2O_2 showed an increase in expression of *Hsp* gene in the genotypes IK 76–91 and SES-90 than at 72 h (Fig. 12).

Fig. 10 The Graphical representation of Rq values for Ascorbate peroxidase (APX) gene expression at different concentration of H_2O_2 induction in *Erianthus* sp. (IJ76-389, IK76-91) and *S spontaneum* (SES-90) at 48 h and 72 h

Fig. 12 The Graphical representation of Rq values for heat shock protein (HSP) gene expression at different concentrations of H_2O_2 induction in *Erianthus* sp.(IJ76-389, IK76-91) and *S spontaneum* (SES-90) at 48 h and 72 h
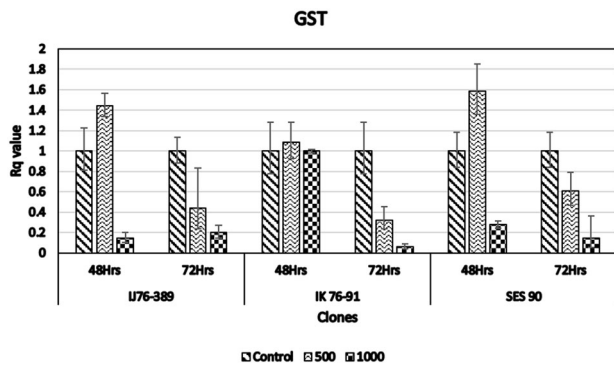
Fig. 13 The Graphical representation of Rq values for glutathione-s-transferase gene expression at different concentration of H_2O_2 induction in *Erianthus* sp. (IJ76-389, IK76-91) and *S. spontaneum* (SES-90) at 48 h and 72 h

Gst (Glutathione S-transferases) Gene

Glutathione S-transferases are ubiquitous enzymes that respond to xenobiotic and oxidative compounds. Studies suggest that expression of GSTs is induced by various abiotic stresses such as drought (Xu et al. 2015), oxidative stress (Levine et al. 1994; Chen et al. 2012), salt stress (Jia et al. 2016) and multiple abiotic stresses (Ding et al. 2017). In our study, all the genotypes showed higher *Gst* expression when exposed to 500 ppm after 48 h (Fig. 13). But on prolonged exposure (72 h), the expression levels receded.

Conclusion

Physiological parameters (chlorophyll stability index and lipid peroxidation) and biochemical parameters (superoxide dismutase, peroxidase and proline content) are significantly different among the *S.spontaneum* genotypes. Based on these parameters, the genotypes IMP-564 and SES-90 are considered as tolerant to oxidative stress. The genes such as *NAC* and *Apx* are actively transcribed during oxidative stress which contributes to tolerance under stress conditions either by acting as signal transduction or by efficiently scavenging reactive oxygen species. The comparison of expression pattern of multiple stress responsive genes showed higher levels of expression in *Erianthus* sp. clone IK76-91 than the *S. spontaneum* clone SES 90 under oxidative stress.

Author Contributions RM planned and executed the work; JN conducted the experiments and prepared the MS; AS edited the MS, and AK analyzed the data.

Declarations

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