The Pharma Innovation Journal 2023; 12(3): 1973-1976

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(3): 1973-1976

www.thepharmajournal.com
Received: 24-01-2023
Accepted: 27-02-2023

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Biochemical and molecular behaviour of clusterbean and mungbean against fluoride stress

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Abstract
Fluoride affects plants in negative manner through increasing the level of ROS species and reducing the plant growth. In this study Guar (*Cyamopsis tetragonoloba* L. Taub) and Moong (*Vigna radiata* L.) was exposed to fluoride stress in a half-diluted Hoagland solution. A concentration- dependent analysis (*Cyamopsis tetragonoloba* L. 0, 10, 20, 30, 40, 50, and 60) and (*Vigna radiata* L. 0, 2.5, 5, 7.5, 10, 12.5, and 15 mM NaF). The study revealed, as a consequence of the high stress produced by fluoride, we observed that both and SOD and CAT gene showed changes in the expression in Guar (*Cyamopsis tetragonoloba* L.) and Moong (*Vigna radiata* L.) root treated samples in comparison to the control. However, the up-regulation changes observed in treated root sample is more evident for Catalase generate 30 mM concentration Guar (*Cyamopsis tetragonoloba* L. Taub.) and for 7.5 mM concentration for Moong (*Vigna radiata* L.). Contrary to it SOD gene showed down- regulation for either Guar (*Cyamopsis tetragonoloba* L.) or Moong (*Vigna radiata* L.) samples at 30 mM and 7.5 mM concentrations respectively. In the fluoride concentration-based analysis, the highest expression of SOD and CAT was observed in the roots compared to the control.

Keywords: Antioxidant enzymes, abiotic stress, *Cyamopsis tetragonoloba* L., gene expression RTPCR, fluoride stress (NaF) and *Vigna radiata* (L.)

Introduction
Agroecological disturbances due to abiotic stresses has not been made, it is clear that such stresses affect large tracts of land and significantly impact the loss. Qualitative and quantitative in crop production (Cramer *et al*., 2011) [3]. Fluoride stress is one among the majority of significant abiotic stresses. Fluoride is a common environmental pollutant and Fluoride rich soil is a potential source of its contamination in the groundwater, in the food chain and ultimately in the human body. (Meenakshi and Maheshwari 2006) [10]. Fluoride toxicity affects the most morphological, physiological and biochemical parameters in the plant due to germination and early seedling growth. Fluoride affects a wide variety of physiological processes, such as germination, growth, mineral nutrition, photosynthesis, respiration, carbohydrate metabolism, and protein synthesis and lipid metabolism. Fluoride also interferes with the metabolism of proteins, lipids and carbohydrates. Fluoride usually inhibits enzymes that require cofactors such as Ca^{2+}, Mg^{2+}, and Mn^{2+} ions. Inhibition is partially attributed to the elimination of Ca^{2+} cofactor. Changes in enzyme activity and intermediate metabolism caused by chronic fluoride exposure may cause the organism to grow, develop and multiply (McCune and Weinstein, 1971). To prevent oxidative damage due to ROS, plants have a molecular response that allows them to manage and adapt to oxidative stress conditions (Baxter *et al*., 2007) [2]. Plants have developed a complex network of antioxidant enzymes. This network includes enzymes such as "Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX), Peroxidase (POD), Catalase (CAT), Glutathione Reductase (GR), Glutathione Peroxidase (GPX), and Glutathione-S-transferases" (GST). Superoxide Dismutase (SOD) is a family of metal enzymes, which "catalyzes the disproportionation of Superoxide O^{-2} in molecular oxygen and H_2O_2. Three isozymes of SOD, Mn-SOD, Cu / Zn-SOD and Fe have been identified‖. SOD in a variety of nine plant species these isoforms are located in various compartments of the cell, the breakdown of H_2O_2 by the enzymes Ascorbate Peroxidase (APX), Peroxidase (POD) and Catalase (CAT), these enzymes. Decompose H_2O_2 into H_2O and O_2. They differ strongly in their affinity for substrates and ensure tight control of H_2O_2 concentrations at very low levels, APX, POD present in most compartments of the cell, whereas CAT is located exclusively in Peroxisomes and not. Found in chloroplasts (Nakano and Asada, 1980) [11] Glutathione (GSH) is a well-known antioxidant molecule that plays an

important role in stressful conditions. GSH is oxidized to glutathione disulfide (GSSG). A reductase (GR) catalyzes a NADPH-dependent reaction in which GR converts GSSG to GSH. This enzyme maintains the effective amount of GSH under stressful conditions. GR is located in most cell compartments, including chloroplast, mitochondria, and cytosolic fractions (Creissen *et al*., 1996 [4]; Hernandez *et al*., 2000) [7]. Other "enzymes such as glutathione peroxidase (GPX) and glutathione-S" -transferases (GST) also make an effort for redox in the cell. By understanding the role of SOD and CAT as antioxidants relative to other antioxidant enzymes at the biochemical level and by analyzing SOD and CAT at the genetic level, it will be possible to form future legume plants that can easily survive under various environmental pressures including fluoride stress. Therefore, a large number of fluoride-tolerant plants can be developed to be sustainable and increase productivity in the future.

Material and methods

Guar (*Cyamopsis tetragonoloba* L.) Var. RGC-1038 and Mung bean (*V. radiate* L.) var. RMG-492 were obtained from Rajasthan Agricultural Research Institute (Sri Karan Narendra Agriculture University Jobner), Rajasthan, India, for experimental purposes developed for the present study. The seeds of Gwar were cleaned with 0.5% sodium hypochlorite, used for 15 minutes, and then washed with distilled water. The sterilized seeds are germinated in a Petri dish with filter paper dipped in distilled water after 24 hours in the dark. 3 days after the seedling appeared, ten seedlings were placed in each plastic pot containing a medium-strength Hoagland nutrient solution, production tolerance maintained at 28±2 °C in a thermostatically prohibited indoor culture and 500 °C photoperiod at 16 hours photoperiod at μmol^{-2} h^{-1}. Seedlings (*Cyamopsis tetragonoloba* L.) acclimated to the 15 days climate were grown with different concentrations of NaF (0, 10, 20, 30, 40, 50 and 60 for 5 days mM). Control plant maintained in a strong Hoagland environment without fluoride treatment. Seeds of mungbean were surface cleaned by 0.5 per cent sodium hypochlorite used for 15 min, furthermore, washed in the distilled water. After that, sterilized seed germinates into a Petri plate contain filter paper pre-soaked by the distilled water at 24 h in the dark condition. After 3 days, the appearance of the planula, ten seedlings were transferred to each plastic pot contain half-strength Hoagland nutrient solution with tolerable to produce up and about in a thermostatically proscribed room culture maintained by 28±2 °C and at 500 μmol^{-2} s^{-1} expose to 16 h photo-period. Seedlings of (*V. radiate* L.) 15 days old acclimated were treated by different concentrations of NaF (0, 2.5, 5, 7.5, 10, 12.5, and 15 mM) for 5 days. The control plant was maintained in a half strength Hoagland medium without fluoride treatment.

DNA isolation

DNA isolation was carried out using cetyltrimethyl ammonium bromide (CTAB) according to the method Doyle and Doyle (1990) [6].

RNA isolation

Total RNA was isolated from (*Cyamopsis tetragonoloba* L. Taub.) and (*Vigna*. *Radiata* L.) Root samples using Trizol method of RNA isolation (Rio DC *et al*. 2010) [12] followed by DNase treatment to remove contaminations of DNA molecules.

cDNA Synthesis RT-qPCR

The relative expression levels of selected genes i.e., SOD and CAT was examined using primers, giving an amplicon of 150-200 bp and anneals at 60 °C temperature. The quality of purified RNA samples was analyzed on 1.2% denaturing agarose gel and quantified using Nano Drop 8000 Spectrophotometer (Thermo Scientific). A total of 1 µg of total RNA was used in the cDNA preparation reaction using the iScript (Ref.) kit (BioRad) following the manufacturer's protocol. Total cDNA was diluted up to 20 ng/µl, and a total 80 ng was used in a 10 µl reaction mixture using Power SYBR® Green PCR Master Mix (Life Technologies), and the reaction was performed on StepOnePlus™ Real-time PCR system.

Quantitative Real-Time PCR (qRT-PCR) analysis

Total RNA was isolated from (*Cyamopsis tetragonoloba* L. Taub.) and (*Vigna*. *Radiata* L.) Root samples using Trizol method of DNA isolation followed by DNase treatment to remove contaminations of DNA molecules. On column DNAse-I (Qiagen) treatment was given t remove contaminating DNA in the RNA preparations. The relative expression levels of selected genes i.e., SOD and Catalase was examined using primers, giving an amplicon of 150-200 bp and anneals at 60 °C temperature. The quality of purified RNA samples was analyzed on 1.2% denaturing agarose gel and quantified using Nano Drop 8000 Spectrophotometer (Thermo Scientific). A total of 1 µg of total RNA was used in the cDNA preparation reaction using the iScript kit (BioRad) following the manufacturer's protocol. Total cDNA was diluted up to 20 ng/µl, and a total 80 ng was used in a 10 µl reaction mixture using Power SYBR® Green PCR Master Mix (Life Technologies), and the reaction was performed on StepOnePlus™ Real-time PCR system (Life Technologies). For each reaction, three technical replicates and no template control (NTC) were kept to check for contaminants. The following thermal cycling program was used: 10 min at 95 °C (enzyme activation), 10 sec at 95 °C (cyclic denaturation), and 30 sec at 60 °C (annealing/extension) for 40-45 cycles, which includes data acquisition. Finally, a dissociation curve (Melting Curve) analysis was performed from 55 °C-95 °C in increments of 0.1 °C, each lasting for 5 sec, to ensure the presence of a specific product. The RNA concentration in different samples was normalized using *Actin* gene transcript abundance for both (*Cyamopsis tetragonoloba* L. Taub.) and (*Vigna*. *Radiata* L.) samples (Livak and Schmittgen 2001) [8].

Results and Discussion

In clusterbean, the isolated RNA from root tissues were examined over 1.2 per cent MOPS containing denaturing Agarose gels. We observed two distinct bands, 28S, and 18S rRNA molecules are present in each of the RNA samples which ensure that the quality of RNA is of good quality to proceed with cDNA synthesis and relative transcript estimation. As the isolated RNA has been given treatment with DNase to remove the residual DNA, we checked for the presence of DNA contamination using Actin gene-specific primers. We do not observe the presence of an amplicon in all the samples analyzed. The RNA concentration was equalized in each sample to 1.0 µg/10 µl concentration after analyzing on Nanodrop using nuclease-free DEPC treated water. This

RNA was converted in cDNA, followed by analysis for the presence of the Actin transcript. In all the samples, we found that a transcript of 200 bp specific sizes has been observed in all the samples indicating that cDNA is of good quality and could be used for an abundance estimation of selected genes. Followed by confirmation of the quality cDNA synthesis, we analyzed the presence of the transcripts for different genes in cDNA samples of root tissue. We observed that a specific size of amplicon was present for each of the transcripts. The gel picture shows presence of a single size amplicons of expected sizes and completes absence of any amplicon in no template control (NTC). The RT-qPCR assay was performed using three technical replicates of each sample for which cDNA synthesis has been performed separately and independently. Post PCR, both the amplification plot and melting curve plot was examined for all the genes. It has been observed that most of the genes are yielding a single melting curve and those who are giving multiple peaks for the melting curve have been excluded from the analysis. We observed that Actin is giving a single peak at ~80 °C, which ensures that cDNA is of good quality. Similarly, the sigmoidal curve obtained in the amplification plot indicates that PCR amplification efficiency is good. After calculating the log (base 2) expression values using 2-ddCT method (Livak and Schmittgen 2001) [8], we observed that both SOD and CAT gene showed changes in the expression in guar root treated samples in comparison to the control. However the up-regulation changes observed in treated sample is more evident for Catalase gene at 30 mM concentration for *Cyamopsis tetragonoloba* (L.) Taub root samples. Contrary to it SOD gene showed down-regulation for either *Cyamopsis tetragonoloba* (L.) Taub. Samples at 30mM concentrations. The use of 18S rRNA as an internal standard could be a valuable alternative to quantify genes of interest, considering that it could reduce variations in expression. Quantification of hsp 20.2 expression using ef1a as a single household gene resulted in results similar to those obtained using two household cleanse genes. These results were in agreement with (Dean *et al*. 2002) [5] and with (Sturzenbaum and Kille 2001) [14] who stipulated that the elongation factor-1 at was a good invariable control. Several studies have shown that Hsps plays a crucial role in protecting plants against abiotic strains (Visioli *et al*., 1997 [16]; Wang *et al*., 2004) [17]. Among the five conserved families of Hsps (Hsp70, Hsp60, Hsp90, Hsp100, and small Hsp), small Hsp are the most predominant in plants (Visioli *et al*., 1997 [16]; Wang *et al*., 2004) [17]. Many mechanisms explaining the phenomenon of cross-tolerance have suggested that specific proteins have been induced in various types of stress (Sabehat *et al*., 1998) [13]. (Avrova *et al*. 2003) [1] showed the involvement, at an early stage, of several heat shock proteins in Phytophthora infestans infection, but no small Hsp. In moong bean observed that both SOD and CAT and the gene showed changes in the semi-quantitative RT PCR expression performed. In contrast, the SOD gene showed down regulation for guar samples at concentrations of 7.5 mM. Through biochemical and molecular procedures, SOD and CAT are found to be highly induced at 7.5mM stress fluoride (NaF). Genetic body methylation can improve splice accuracy and promoter expression and may be functionally important than unmethylated genes (Takuno and Gaut, 2012) [15]. Gene body methylation reported for all MSAP loci suggests that it may play an important role in regulating gene expression in organs and genotypes specifically under salinity stress, which is consistent with previous studies (Zhang *et al*., 2011, Wang *et al*., 2011) [19, 18].

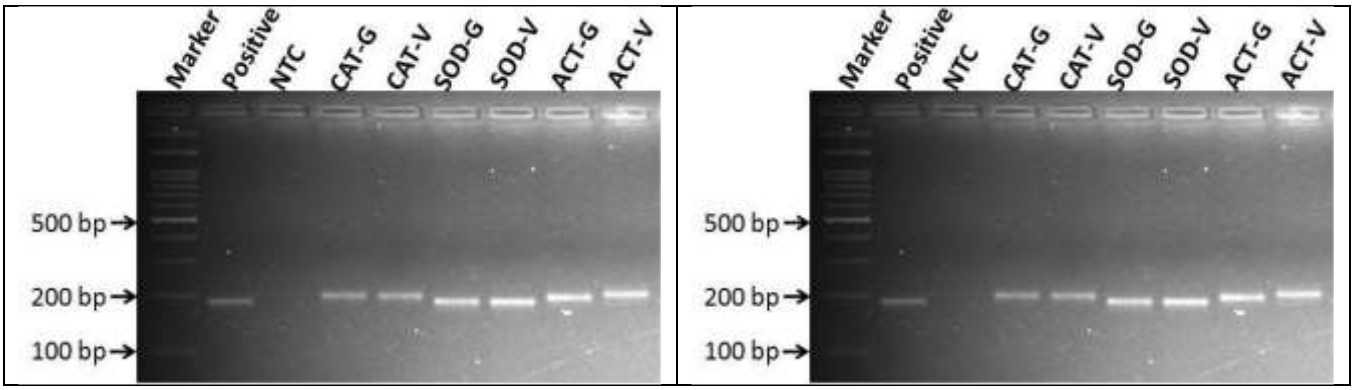

Fig 1: Gel photograph showing amplification of different sizes of amplicons corresponding to Superoxide Dismutase (SOD), Catalase (CAT), and Actin (ACT) genes in Guar (*Cyamopsis tetragonoloba* L. Taub.) and Moong (*Vigna radiata* L.) of root cDNA samples.

Conclusion

In the present study, proline was found to be highly induced at high concentrations of fluoride (NaF) in both plants. CAT, SOD, GR, APX and POD actively induced during the fluoride (NaF) stress condition in both plants. These three enzymes are highly induced to 30 nM NaF in Guar (*Cyamopsis tetragonoloba* L. Taub.) and 7.5 mM NaF in Moong (*Vigna radiata* L. The current study reveals, as a consequence of the high stress produced by fluoride, we observed that both and SOD and CAT gene showed changes in the expression in Guar (*Cyamopsis tetragonoloba* L. Taub.) and Moong (*Vigna radiata* L.) Root treated samples in comparison to the control. However, the up-regulation changes observed in treated root sample is more evident for Catalase gene at 30 mM concentration Guar (*Cyamopsis tetragonoloba* L. Taub.) and for 7.5mm concentration for Moong (*Vigna radiata* L.). Contrary to it SOD gene showed down-regulation for either Guar (*Cyamopsis tetragonoloba* L. Taub.) or Moong (*Vigna radiata* L.) samples at 30mM and 7.5mM concentrations respectively. In the fluoride concentration-based analysis, the highest expression of SOD and CAT was observed in the roots compared to the control.

Acknowledgment

Authors are thankful to Hon'ble Chairperson JV'n Vidushi Garg and Hon'ble Founder and Advisor JV'n Dr. Panckaj Garg, Jayoti Vidyapeeth Women's University, Jaipur (Rajasthan) for their kind cooperation, encouragement, and

providing the facilities of University Innovation Center and other laboratories.

References

1. Avrova AO, Venter E, Birch PRJ, Whisson SC, *et al*. Profiling and quantifying differential gene transcription in Phytophthora infestans prior to and during the early stages of potato infection. Fungal Genetics and Biology. 2003;40:4--14.
2. Baxter CJ, Redestig H, Schauer N, Repsilber D, Patile KR, Nielsen J, *et al*. The metabolic response to heterotrophic Arabidopsis cells to oxidative stress. Plant Physiology. 2007;143:312-325.
3. Cramer GR, Urano K, Delrot S, Pezzotti M, Shinozaki K *et al*. Effects of abiotic stress on plants: A systems biology perspective. BMC Plant Biol. 2011;11:163.
4. Creissen G, Broadbent P, Stevens R, Wellburn AR, Mullineaux P, *et al*. Manipulation of glutathione metabolism in transgenic plants. Biochemical Society Transactions. 1996;24:465-469.
5. Dean JD, Goodwin PH, Hsiang T, *et al*. Comparison of relative RT-PCR and northern blot analyses to measure expression of b-1, 3-glucanase in *Nicotiana benthamiana* infected with *Colletotrichum destructivum*. Plant Molecular Biology Reporter. 2002;20:347-356.
6. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus. 1990;12:13-15.
7. Hernandez JA, Jimenez A, Mullineaux P, Sevilla F. *et al*. Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defences. Plant Cell and Environment. 2000;23:853-862.
8. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real time quantitative PCR and the 2(-Delta Delta C(T) Method. 2001;25(4):402-406.
9. McCune DC, Weinstein LH. Metabolic Effects of Atmospheric Fluorides on Plants. Environmental Pollution. 1971;1:169-14.
10. Meenakshi RC, Maeshwari RC. Fluoride in drinking water and its removal. Journal of Hazardous Material. 2006;137:456-463.
11. Nakano Y, Asada K. Spinach chloroplasts scavenge hydrogen peroxide on illumination Plant and Cell Physiology. 1980;21:1295-1307.
12. Rio DC, Ares M, Gregory JR, Hannon J, Timothy W. Nilsen Purification of RNA using TRIzol (TRI reagent). Cold Spring 2010; Harb Pro PDB. Prot 5439. DOI: 10.1101/pdb.prot 5439.
13. Sabehat A, Weiss D, Lurie S. Heat-shock proteins and cross tolerance in plants. Physiologia Plantarum. 1998;103:437-441.
14. Sturzenbaum SR, Kille P. Control genes in quantitative molecular biological techniques: the variability of invariance. Comparative Biochemistry and Physiology Part B. 2001;130:281-289.
15. Takuno S, Gaut BS. Body-methylated genes in *Arabidopsis thaliana* are functionally important and evolve slowly. Mol Biol Evol. 2012;29:219-227.
16. Visioli G, Maestri E, Marmiroli N. Differential displaymediated isolation of a genomic sequence for a putative mitochondrial LMW HSP specifically expressed in condition of induced thermotolerance in *Arabidopsis thaliana* (L) Heynh. Plant Molecular Biology. 1997;34:517-527.
17. Wang W, Vinocur B, Shoseyov O, Altman A. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends in Plant Science. 2004;9:244-252.
18. Wang WS, Zhao X, Pan Y, Zhu L, Fu B. DNA methylation changes detected by methylaton-sensitive amplified polymorphism in two contrasting rice genotypes under salt stress. J Genet Genomics. 2011;38:419-424.
19. Zhang M, Xu C, Wettstein DV, Liu B. Tissue-specific differences in cytosine methylation and their association with differential gene expression in *Sorghum*. Plant Physiol. 2011;156:1955-1966.