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ORIGINAL ARTICLE

Analysis of Hydrophilic Antioxidant Enzymes in Invasive Alien Species *Parthenium hysterophrus* Under High Temperature Abiotic Stress Like Conditions

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In order to gain insight into the biochemical basis of invasiveness of invasive alien species, in the present study the expression of antioxidant boiling stable proteins (peroxidase, SOD and catalase) was studied in *Parthenium hysterophorus*. Parthenium an annual invasive weed of family *Asteraceae*, is native to tropical America while wide spread in north America, many parts of Africa, Australia and India. It tolerates a wide variety of abiotic conditions in the natural habitat. The analysis of boiling stable proteins was outlined by Native-PAGE analysis. Some barely detectable boiling stable proteins were detected. The Zymogram analysis revealed a substantial expression of BsPeroxi 60 and BsSOD 35/20 in the leaves and mature flowers of Parthenium. However, no substantial catalase activity was detected in the boiled protein samples of all the tissues examined. Based upon these results, a possible physiological role of BsPeroxi 60 and BsSOD 35/20 in parthenium tissues was discussed.

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At present time, water scarcity, high temperature due to global warming and high salt are the major problems worldwide which affect productivity of important crops (Bray 2002). So generation of stress tolerant high yielding cultivars through biotechnological approaches is the key issue for the research fraternity. To this end, it is important to identify the proteins and reveal the underlying physiological, biochemical mechanisms for high water efficiency in the crop species (Chaves *et al.*, 2003).

Over the past few years, in this stressful environmental conditions, Invasive alien species (IAS) are the such kind of plants which have encroached into many ecosystems through the world by sudden/accidental escape from their natural habitat (Henkel and Hofmann, 2009). An alien plant also refereed to exotic, introduced foreign, non-indigenous or non-native but has been introduced by humans accidently from the one

region to other. Only a few many plants introduced to novel habitat become invasive, suggesting the existence of a suite of characters that enables invasiveness. Many introduced species also become exotic after persisting for a long time at low abundances in the introduced habitats (Henkel and Hofmann, 2009), possibly after evolutionary adjustments. It is still a matter of discussion that why these IAS plants are so wide spread?. To date majority of studies on IAS plats have been conducted within their introduced ranges, and ecologists often know little about the ecology of such plants (Jung 2003).

It is generally presumed that plants inherently posses various molecular-biochemical mechanisms that are involved in stress tolerance (Ingram and Bartels 1996). Some of these stress-responses include regulatory proteins, soluble proteins, appearance of new isozymes; whereas others protect cells by causing the accumulation of metabolic proteins and cellular protectants including sugars (Ingram and Bartels 1996). These stress-induced responses enable the plant to adapt its physiology and survive. Stress induced proteins play a definite role in protecting plants from possible damage by these conditions. A growing body of evidence suggest that stress response involves synthesis of one set of proteins and degradation of the other (Chaves *et al.*, 2003). Antioxidant enzymes like SOD,CAT and Peroxidase are the major groups of stress-induced proteins which believed to contribute to the protection of cellular structures and metabolites during water stress (Asada 1992). The role of these enzyme systems for making plants tolerant to extreme environments has already been demonstrated. It is currently assumed that the negative effect of various environmental stresses is at least partially due to the generation of reactive oxygen species (ROS also called AOS (active oxygen species) and/or the inhibition of the system that defends against them. ROS include mainly the superoxide anion radical ($O_2^{-\bullet}$), H_2O_2, hydroxyl radical ($HO^\bullet$), perhydroxyl radical ($HO_2^\bullet$), and singlet oxygen ($_1O_2$). ROS are highly reactive and, in the absence of any protective mechanism, can seriously disrupt normal metabolism through oxidating membrane lipids, protein and nucleic acids (Lee *et al.*, 1999). However, to mitigate and repair damage initiated by AOS, plants evolved cellular adaptive responses such as upregulation of oxidative stress protectors and accumulation of protective solutes (Lee *et al.*, 1999). Antioxidant defense enzymes are systems that minimize the concentrations of ROS. Some stress-induced proteins (e.g. dehydrins, LEAs) are highly hydrophilic and remain soluble even after boiling, a characteristic that has been termed “boiling stability” (Jacobsen and Shaw 1989). Even some of the proteins detected in total protein extracts, under drought stress, are lost in boiling treated extracts. Earlier research also indicated that hydrophilins represent less than 0.2% of the total protein of a given genome (Arroyo *et al.*, 2000). Bioinformatic analyses of hydrophilins from several kingdoms including plant, bacteria and fungi have revealed the conservation of lysine-rich regions in these proteins, thus, suggesting an evolutionary role for these cellular boiling stable proteins during water-deficits (Jacobsen and Shaw 1989). Based on these observations, It is plausible that IAS plants may contain such kind of boiling stable proteins which maintains the survival of IAS plants under any abiotic condition like high temperature, drought, or high salt. Most of the available studies have been focused only on their invasiveness, habitat and ecology etc, however, the molecular mechanism

underlying their persistence in the different habitat is still not well known. Although some recent studies have documented that cellular concentration of HSPs can be directly related to wide range temperature tolerances (Gong *et al.* 2010; Henkel and Hofmann, 2009; Raghubanshi *et al.*, 2005), however, the role of boiling stable proteins (BSPs) is still not well documented. Therefore, the aim of the present study is to provide knowledge of the fascinating biochemical adaptations of IAS plant *Parthenium hysterophorus* in relation boiling stable proteins (BSPs) in general and antioxidant enzymes (Peroxidase, SOD and CAT) in particular.

Partheneium an annual invasive weed of family Asteraceae, is native to tropical America while wide spread in north America, many parts of Africa, Australia and India (Revero *et al.*, 2001). This weed was first reported in India in 1951. Since 1951, this weed has spread like wildfire throughout India (Raghubanshi *et al.*, 2005). Parthenium grows well in wastelands, forestlands, agricultural areas, overgrazed pastures and along roadsides. It tolerates a wide variety of soil types, has high reproductive potential, and extreme adaptability in hot and dry environments. It is considered as noxious weed because of its profile seed production, allopathic effect on other plants, loss of crop productivity, biodiversity depletion and health hazard to other animals and organisms (Davis and Swanson 2001). However, there has been little research on their physiological and biochemical mechanisms that confer different abiotic stress resistance. A better understanding of their mechanisms is very important even for controlling and predicting the potential distribution of parthenium species.

MATERIALS AND METHODS

Sampling Site

The samples of *Parthenium hysterophorus* were collected, growing under natural conditions**,** from Talhan sahb village near Jalandhar located at 71º31 east latitude and 30 º 33' north longitude. The area is characterized as dry whether belt at a distance of 15 km from the experimental lab at Jalandhar. The total area of village is approximately 2 km^2. The climate of the region represents extremes of heat and cold. The experiments were conducted in April, 2012 during hot period. The mean max and min temperature recorded was 38ºC and 19º C, respectively. The relative humidity was 45% with photoperiod of 9 hr. The study followed a random sampling method so that no bias is introduced. The 3 sites with in the sampling area were selected. The plant samples at reproductive stage (leave, immature un-bloomed flowers and fully mature bloomed flowers) were collected from plants and pooled together for further analysis.

Extraction of Boiling Stable Proteins

The boiling stable proteins were extracted as described previously (Rakhra *et al.*, 2011). Briefly, tissues were homogenized with chilled mortar and pestle in extraction buffer (50 mM Tris buffer (pH 7.0)). Crude extracts were centrifuged at 10,000 g for 10 min. The total extract as obtained was boiled for 15 min in order to get boiling stable protein fractions. The total soluble protein content in the supernatant was determined according to Bradford (1976) using BSA as a standard. Protein samples were resolved on NATIVE-PAGE on 12% (w/v) polyacryalamide gel and visualized by Coomassie brilliant blue as described in Sambrook *et al.* (1989). For molecular weight identification the markers used were as follows: catalase (240 kDa), bovine

serum albumin (67 kDa), ovalbumin (43 kDa), Trypsin (20.1 kDa) and lactoglobulin (18.4 kDa).

Zymogram analysis

Boling stable proteins were extracted as described above. The antioxidant activities were calculated as per Sharma *et al.*, 2013. The proteins were separated by a non-denaturing 12% polyacrylamide gel electrophoresis. When electrophoresis was complete, the gel was washed three times in 50 mM sodium acetate buffer (pH 5.0). Peroxidase activity was visualized by incubating the gel in 50 ml of solution having 50 mM sodium acetate buffer (pH 5.0), 330 ul of guaiacol (9M) and 1.5 ml of 6.6 % H_2O_2. The gel was incubated at room temperature in dark until reddish-brown bands appeared. The gel was washed in distilled water and used for further analysis. In order to detect SOD activity, the gel was first soaked in 25 ml of 1.23 mM NBT for 15 min, briefly washed, then soaked in the dark in 30 ml of 100 mM potassium phosphate buffer (pH 7.0) containing 28 mM TEMED and 2.8 x 10^{-2} mM riboflavin for another 15 min. The gel was briefly washed again and then illuminated on gel viewer having 20 w florescent tubes under white light to initiate photochemical reaction. All the procedures were carried out at room temperature. For Catalase activity, after electrophoresis the gel was incubated in 50 mM potassium phosphate buffer (pH 7.0) for 15 min and then in 0.03% H_2O_2solution for 10 min. The gel was rinsed twice with H_2O and then incubated in a mixture (1:1) of freshly prepared 2% potassium ferricyanide and 2% ferric chloride. The gel became greenish-blue while zones of catalase were yellow.

RESULTS AND DISCUSSION

Alternation in protein expression is an important part of the ability of the any plant to respond to the environmental conditions in any ecological system. Only a few many plants introduced to novel habitat become invasive, suggesting the existence of a suite of characters that enables invasiveness. Many introduced species also become exotic after persisting for a long time at low abundances in the introduced habitats, possibly after evolutionary/physiological/ biochemical adjustments. However, the role of boiling stable proteins in invasive alien plant species is still not well documented. Therefore, in the present study, in order to gain insight into the biochemical changes in *Parthenium hysterophorus*, the expression of boiling stable proteins (BSPs) in general and antioxidant enzymes (Peroxidase, SOD and catalase) in particular were studied. The expression of BSPs was outlined by NATIVE-PAGE followed by Zymogram (in-gel activity) analysis.

In Native-PAGE, many low and high mol weight protein bands were detected in the un-boiled samples (Fig 1). However, after boiling only a few barely detected heat stable protein bands were detected in leaves, mature flower (fully bloomed) and immature flower (un-bloomed), indicating boiling stable nature of existing proteins. The Zymogram analysis of Peroxidase (Fig 1A) revealed high expression of a 60-kDa protein band, designated as BsPeroxi 60 here and thereafter, in the un-boiled samples. The activity of peroxidases is thought to form an active barrier of defence against H_2O_2/ROS produced in the plant cells. Peroxidases are important enzymes of the antioxidative system for the reduction of H_2O_2 to water (Shigeoka *et al.*, 2002). Some studies on the response of peroxidases expression to some stress conditions and pathogen attacks have highlighted the importance of peroxidases activity in controlling H_2O_2 concentration in intracellular signaling (Lee *et al.*,

1999; Kacperska 2004). Surprisingly, BsPeroxi 60 was also detected in all the heat stable (HS) protein fractions, indicating that BsPeroxi 60 remained soluble after boiling. Earlier, Lu *et al.*, 2008 also reported the presence of peoxidases in Eupatorium species. In parthenium, the coordinated increase of oxygen detoxifying Peroxidase enzymes was effective in protecting the plant from the accumulation of AOS at high temperatures, thus averting cellular damage under unfavorable conditions. This study also supports the idea that higher Peroxidase activity being detected in all the tissues under adverse high temperature conditions, may be ascribed to high cellular H_2O_2 levels. It is well-documented that cellular level of H_2O_2 stay high in relation to stress situations and plays a central role in responses to both abiotic and biotic stresses in plants. Up to certain concentration (10^2–2×10^5 µM) (Lee *et al.*, 1999) this molecule seems to be a "master hormone" that controls a variety of stress responses and physiological adjustments, including the ROS/hormonal homeostasis in the cell.

Superoxide dismutase catalysis the dismutaion of O_2^- to H_2O_2 and O_2 (Azevedo *et al.*, 2005; Vaidyanathan *et al.*, 2003) and thus form a crucial part of the cellular antioxidant response system. In plants, H_2O_2 is scavenged by a number of enzymes, but catalases and peroxidases are considered the major H_2O_2 detoxifying enzymes in plants (Peltzer *et al.*, 2002). In this study, the zymogram analysis revealed the presence of two isoforms (BsSOD 35 and BsSOD 20) in all the un-boiled proteins samples (Fig 1 B). However, after boiling, activities of BsSOD 35 and BsSOD 20 were detected only in the fully mature bloomed flowers, indicating spatial expression of boiling stable SODs. Notably, as compared to BsSOD 35, the expression of BsSOD 20 was substantially higher in the boiled protein samples of mature flower. SOD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide, so substantial SOD activity suggested an increased production of H_2O_2. It has been suggested that hydrogen peroxide must be effectively scavenged in order to minimize cytotoxicity by activated oxygen in the presence of elevated SOD activity (Feierabend *et al.*, 1992). Catalase activity was substantially detected in the un-boiled proteins samples of all the three tissues. However, contrary to Peroxidase and SOD, no substantial activity of boiling stable catalase was detected in treated (boiled) samples of any tissue (Fig 1C). Similar alternations in SOD and catalase activities have been reported earlier (Jung 2003). Others have also found that catalase activity is photoinactivated under low and high temperature stresses and varied with plant species (Dietz et al, 1996). Further long term tissue-specific temporal studies are required to get insight into the biochemical relationship of antioxidant enzymes. Based upon these observations of high expression of BsPeroxi60, BsSOD 35 and BsSOD 20 in the heat stable protein fractions, It is plausible that parthenium contains such kind of proteins which may be ascribed to maintain the survival of these IAS plants under any unfavorable abiotic condition like high temperature, drought, or high salt. Previously, Dietz *et al.*, 1996 also concluded that some factors other than alleopathy might be operating in nature that favors rapid establishment and persistence of dense stands of alien species. Likewise, recently Zerebecki *et al.*,2011, reported the induction of hsp70 in the invasive population of *Diplosoma* and *Distaplia*. Similarly, Gong *et al.*, 2010 also have cloned, studied full characterization, and heterologous expression analysis of heat shock

protein genes (*hsp70* and *hsp90*) of the invasive alien weed, *Ageratina adenophora* (Asteraceae). This study emphasized that the relationship between, and regulation of, heat shock proteins might incorporate variable strengths to increase the adaptation of *A. adenophora*.

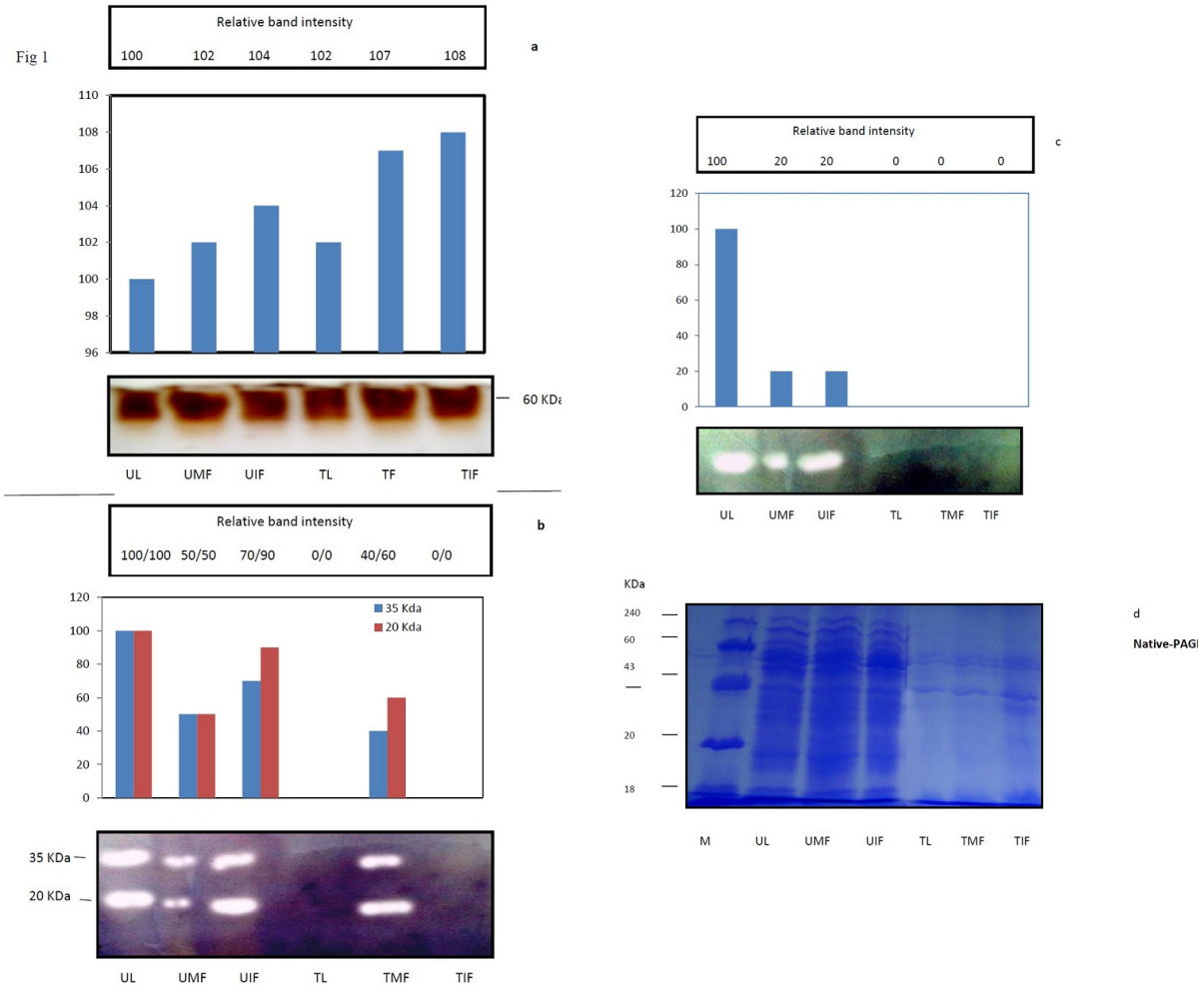

Figure 1(a-c): The Zymogram analysis (in-gel activity) of peroxidase **(a)**, SOD **(b)** and catalase **(c)** of un-treated (not boiled) and treated (boiled) proteins from different tissues of *Parthenium hysterophorus*. Numerical values and bar graphs as shown in the top of Panels, indicates relative band intensities, which were determined using Gel Visualization, Documentation and Analysis system (Bio-Rad, USA). Numerical comparisons are only valid within panels and cannot be made between panels.

Figure 1(d): NATIVE-PAGE analysis of un-treated (not boiled) and treated (boiled) proteins from different tissues of *Parthenium hysterophorus*. Each lane loaded with 60µg of boiling stable proteins was resolved on 12% Native-PAGE and analyzed for activity detection. Symbols used: UL: un-boiled leave protein sample; UMF: un-boiled mature flower protein sample (fully bloomed); UIF un-boiled immature flower protein sample (not bloomed); TL: boiled/treated leave protein sample; TMF: boiled/treated mature leave protein sample (fully bloomed); TIF: boiled/treated immature flower protein sample (not bloomed) : M: protein marker used.

Based on the present study findings, it can be postulated out that the interactions of peroxidases, catalases, and SOD with their involvement in scavenging AOS is very complex. The extent of oxidative stress causing membrane and cellular damage might possibly differ depending on the natural abiotic conditions imposed. Plants may respond in different ways, as shown in our experiments (Figures 1 A-C). Although several environmental stresses in plants are known to induce the expression and/or increasing levels of antioxidative enzymes and their mRNAs , the mechanism by which these activated oxygen-scavenging enzymes can work co-operatively, in response to abiotic conditions is not yet conditions known. Further analysis of the regulation of gene expression in these enzymes should elucidate the mechanism of different temperature tolerances. Identifying the genes that regulate some of the enzymes involved in stress and finding ways to interfere with gene regulation may prove useful to aiding an economically viable control of persistent perennial weeds in difficult-to-reach locations or along waterways where the use of herbicides is undesirable or illegal .

To conclude, higher activities of Peroxidase and SOD in boiling stable proteins samples were observed in leaves, and mature flowers of parthenium. This higher expression of boiling stable antioxidant enzymes may be ascribed to invasiveness of this alien plant under all the adverse abiotic conditions. These hydrophilins may be necessary to maintain protein function during this specific type of abiotic stress conditions. Further studies at immune-blot level using different antiseras and gene expression level during various long term natural environmental seasons is under way to find out any further correlation between BSPs and invasiveness of alien plants. Once these protein(s) will be identified, focus could be made to fish out the important genes from these IAS plants which is relevant to the ongoing efforts of agricultural biotechnologist to engineer crops (like wheat, rice, sorghum) that have improved resistance during drought, high temp, or salt by using both traditional plant breeding approaches and transgenic technology. Our results may provide entry point and a reference to future analysis of gene expression. In addition, our results can suggest possible targets for the enhancement of stress tolerance in crops by genetic engineering.

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REFERENCES

Arroyo, A.G., Flores, J.M.C, and Garciarrubio, A.C. (2000). Highly hydrophilic proteins in prokaryotes and eukaryotes are common during conditions of water deficit. *J. Biol. Chem*, **275**, 5666-5674.

Asada, K. (1992). Ascorbate peroxidase - a hydrogen peroxide-scavengingenzyme in plants. *Physiol Plant,* **85**, 235-241.

Azevedo, N.A.D., Prisco, J.T., Enéas-Filho, J., Medeiros, J.R. and Gomes-Filho. (200). Hydrogen peroxide pre-treatment induces salt-stress acclimation in maize plants. *J. Plant Physiol*., **162**, 1114-1122.

Battaglia, M., Olvera-Carrillo, Y., Garciarrubio, A., Campos, F. and Covarrubias, A. (2008). The Enigmatic LEA Proteins and Other Hydrophilins. *Plant Physiol,* **148**, 6-24.

Bradford, M.M. (1976). A rapid and sensitive method for quantitation of microorganism

quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**, 248-254.

Bray, E.A. (2002). Abscisic acid regulation of gene expression during water-deficit stress in the era of the Arabidopsis genome. *Plant Cell Environment*, **25**, 153-161.

Chaves, M.M., Maroco, J.P. and Pereira, J. (2003). Understanding plant responses to drought-from genes to the whole plant. *Functional Plant Biol*, **30**, 239-264.

Davis, D.G. and Swanson, H.R. (2001). Activity of stress-related enzymes in the perennial weed leafy spurge (*Euphorbia esula* L). *Environ Exp. Bot*. **46**, 95-108.

Dietz, H., Steinlein, T., Winterhalter, P. and Ullmann, I. (1996). Role of allelopathy as a possible factor associated with the rising of dominance of *Bunias orientlis* in some native plant assemblages. *J Chem Ecol*. **22**, 1797-1811.

Feierabend, J., Schaan, C. and Hertwig, B. (1992). Photoinactivation of catalase occurs under both high- and low-temperature stress conditionsand accompanies photoinhibition of photosystem II. *Plant Physiol,* **100**, 1554–1561.

Gong, W.N., Xie, Y.B., Wan, F.H. and Guo, J.Y. (2010). Molecular cloning, characterization, and heterologous expression analysis of heat shock protein genes (*hsp70* and *hsp90*) of the invasive alien weed, *Ageratina adenophora* (Asteraceae). *Weed Biol and Management*, **10**, 91–101,

Henkel, S.K. and Hofmann, G.E. (2009). Differing patterns of *hsp70* gene expression in invasive and native kelp species: evidence for acclimation-induced variation. *Developments in Applied Phycol*., **2**, 465-474,

Horling, F., Lamkemeyer, P., Konnig, J., Finkemeir, I., Kandlbinder, A. and Baier M. *et al.* (2003). Divergent light-, ascorbate-, and oxidative stress-dependent regulation of expression of the peroxiredoxin genefamily in *arabidopsis*. *Plant Physiol.,* **131**, 317–325.

Ingram, J. and Bartels, D. (1996). The molecular basis of dehydration tolerance in plants. *Annual Review of Plant Physiol. Plant Mol. Biol*.**, 47**, 377-403.

Jacobsen, J.V., and Shaw, D.C. (1989). Heat-stable proteins and Abscisic acid action in barley aleurone cells. *Plant Physiol*, **91**, 1520-1526.

Jung, S. (2003). The combined action of catalase (CAT) and superoxide dismutase (SOD) is critical in mitigating the effects of oxidative stress. *Pesticide Biochem Physiol*., **75**, 9-17.

Kacperska, A. (2004). Sensor types in signal transduction pathways in plant cells responding to abiotic stressors: do they depend on stress intensity? *Physiol Plant,* **122**, 159-168.

Lee, S.C., Kang, B.C. and Oh, S.E. (1999). Induction of ascorbate peroxidase by ethylene and hydrogen peroxide during growth of cultured soybean cells. *Mol. Cell*, **9**, 166-171.

Lu, P., Sang, W.G., and Ma, K.P. (2007). Differential responses of the activities of antioxidant enzymes to thermal streses between two invasive Eupatorium species in china. *J of Integrative Biol.*, Doi 10.111/j.1744-7909.2007.005383X.

Peltzer, D., Dreyer, E. and Polle, A. (2002). Differential temperature dependencies of antioxident enzymes in two contrasting species: *Fagus slvatica* and *Coleus blumei*.

Plant Physiol. Biochemistry, **40**, 141-150.

Raghubanshi, A.S., Rai L.C., Gaur J.P. and Singh J.S. (2005). Invasive alien species and biodiversity in India. *Current Science*, **88(4)**, 539- 540.

Rakhra, G., Sharma A.D., Singh, J., and Vyas, D. (2011). Expression analysis of boiling-stable protein (BsCyp) in response to drought, salt and osmotic treatments in drought tolerant and susceptible cultivars of *Triticum aestivum. J of Stress Physiology and Biochemistry,* **7**, 187-196**.**

Revero, R.M., Ruiz, J.M., Garcia, P.C., Lopez-Lefebe, L., Sanchez, E. and Romero, L. 2001. Resistance to cold and heat stresses: accumulation of phenolics compounds in tomato and watermelon plants. *Plant Sci.* **157**, 315-321.

Sambrook, J., Fritsch ,E.F. and Maniatis, T. (1989). Molecular cloning: a laboratory manual, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York. USA. p 18.64-18.75

Sharma, A.D., Rakhra G, Kaur S, and Kaur H. (2013). Expression of Boiling-stable Peroxidase (PRX) isoenzymes under combined effect of drought and heat in different tissues of *Triticum aestivum*. *J of Agricultural Technology*, **9**, 901-13

Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa, Y., Takeda, T., Yabuta, Y., and Yoshimura, Y. (2002) Regulation and function of ascorbate peroxidase isoenzymes. *J. Exp. Bot*., **53**, 1305-1319.

Vaidyanathan, H., Sivakumar, P., Chakrabarty, R. and Thomas, G. (2003). Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) – differential response in salt-tolerant and sensitive varieties. *Plant Sci*., **165**, 1411-1418.

Zerebecki, R.A. and Sorte, C.J.B. (2011). Temperature tolerance and stress proteins as mechanism of invasive species success. *PLOS one*, **6(4)**, e14806