Available online at http://www.journalajst.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 5, Issue, 06, pp.1463-1467, June, 2013

ISSN: 0975-833X

RESEARCH ARTICLE

CHANGES IN PEROXIDASE ACTIVITY DURING NATURAL AND OXIDATIVE STRESS-INDUCED SENESCENCE OF *Azadirachta indica* A. juss DETACHED LEAVES

[1,*]Prashanth B. Goud, [1]Sanjay N. Harke and [2]Manvendra S. Kachole

[1]MGM's Institute of Biosciences and Technology, Aurangabad - 431 001 Maharashtra, India
[2]Department of Biochemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad - 431 004 Maharashtra, India

ARTICLE INFO

Article History:
Received 04th March, 2013
Received in revised form
25th April, 2013
Accepted 18th May, 2013
Published online 15th June, 2013

Key words:
Hydrogen peroxide, Leaf senescence, Oxidative stress, Peroxidase.

ABSTRACT

The present study tries to examine the influence of natural and oxidative stress [hydrogen peroxide (H_2O_2) treatment]-induced senescence on peroxidase (POD) activity in neem (*Azadirachta indica* A. juss) leaves. Data indicated that incubation of detached leaves in presence of H_2O_2 has induced POD(s), that enzyme activity is also enhanced in natural senescing leaves. Changes in POD activity and protein loss during H_2O_2-promoted senescence has shown similarity with natural senescence suggests that the underlying regulatory mechanisms might be the same in both stresses, at least in neem. Initial induction of POD activity under H_2O_2 stress suggests that PODs play a very important role during the early phases of leaf senescence. Reduction in POD activity along with the increase in protein loss at latter stages suggests that there was no correlation between PODs and senescence, at least in neem.

INTRODUCTION

Leaf senescence refers to the final developmental stage of leaves by which cells undergo programmed changes which results in hydrolysis of macromolecules such as proteins, lipids, polysaccharides, and DNA, which leads to cell death. Yellowing of the leaves due to chlorophyll breakdown is the most obvious visible characteristic (Smart, 1994, Gan and Amasino, 1997; Gepstein, 2004). Numerous environmental stimuli such as extremes of temperature, drought, ozone, nutrient deficiency, pathogen infection, wounding and shading, whereas the autonomous factors include age, reproductive development and phytochrome levels can induce leaf senescence (Gan and Amasino, 1997). The question of whether natural senescence and oxidative stress-induced senescence are same or different phenomena is still under discussion. At the physiological level natural senescence and oxidative stress-induced senescence have shown very similar features such as suc starvation, breakdown of macromolecules etc., but could be different at molecular level. For example it was reported that two cDNAs were isolated from wound and drought induced barley leaves, but are not detected in natural senescing leaves, while a novel cDNA is expressed during natural senescence, but not under stress conditions (Smart, 1994). In contrast it was reported that genes involved in the oxidative stress response are also induced during natural senescence including the genes for Fe^{2+}-ascorbate oxidase, anionic POD, glutathione s-transferase and a blue copper-binding protein (Nam, 1997). Recently the mRNA expression profiles of 402 major transcription factors were monitored at different developmental phases and stress conditions. Among the 43 transcription factor genes that were induced during senescence, 28 were also induced by stress treatments (Lim *et al.*, 2003) suggesting that the underlying regulatory mechanisms might be similar in both conditions. Incubation of detached leaves under hydrogen peroxiode (H_2O_2) treatment is an ideal system for the rapid induction of leaf senescence (EI-Shora, 2003; Hung and Kao, 2005; Upadhyaya *et al*., 2007). It has shown previously that H_2O_2 treatment induces leaf senescence in various plant species such as *Cucurbita pepo* (EI-Shora, 2003), rice (Hung and Kao, 2007; Lin and Kao, 2007), *Ramonda serbika* (Veljovic-Jovanovic, 2006), and pigeonpea (Goud and Kachole, 2011a). Detection of lipid peroxidation and protein loss in H_2O_2-promoted senescing leaves (Veljovic-Jovanovic, 2006; Lin and Kao, 2007; Nam, 1997) suggests that H_2O_2-promoted senescence is mediated through oxidative stress. H_2O_2 is the most stable form of the ROS and is capable of rapid diffusion across cell membrane (Upadhyaya *et al*., 2007). It can also react with superoxide radicals to form more toxic hydroxyl radicals in the presence of transition metals (Hung and Kao, 2005). H_2O_2 is not only a harmful reactive oxygen species (ROS) but also has a role as a signling molecule in pathways of stress signal transduction (Liu *et al*., 2010). It alters the redox status of surrounding cells where it induces an antioxidative response by acting as a signal of oxidative stress (Upadhyaya *et al*., 2007). In this work, we have studied the effect of H_2O_2 treatment on one of the major antioxidant enzyme, named peroxidase (POD, EC 1.11.1.7) in neem (*Azadirachta indica* A. juss) leaves. We have also investigated the similarities and differences in POD activity and isoforms between natural and H_2O_2-promoted senescing leaves of neem.

POD is a heam protein, which is a member of oxidoreductases and catalyses the oxidation of a wide variety of organic and inorganic substances such as phenolics, cytochrome C and nitrite in the presence of H_2O_2 (Chen *et al*., 1992; Alokail and Ismael, 2005, Sat, 2008). POD has been implicated in a variety of physiological processes, such as plant growth and development, auxin catabolism, the oxidation of phenolics to form lignin, the cross-linking of hydroxyproline-rich glycoproteins in plant cell walls, as well as biotic and abiotic stress responses (Jr. Cipolini, 1998; Fang and Kao, 2000). The involvement of PODs in stress-related physiological processes as well as in plant pathogen interactions was demonstrated (Luhova *et al*., 2003). POD was cited as a screening parameter for different physiological stresses. POD activity was also used as a

Corresponding author: Prashanth B. Goud
MGM's Institute of Biosciences and Technology, Aurangabad - 431 001 Maharashtra, India

biochemical marker for different types of pollution (Mohamed and Rangappa, 1993). The enzymatic scavenging of hydrogen peroxide includes several more or less specific enzymes that can convert ROS to water or maintain the pool of antioxidants in their reduced state (Lepedus *et al*., 2005). An elevated POD level is induced by cold, drought, hypoxia and salt stress (Mohamed and Rangappa, 1993). Pretreatment with low concentrations of the molecule increases the accumulation of GSH (Yu *et al*., 2002) and antioxidant enzyme activity (de-Azevado *et al*., 2005) in plants, thereby alleviating harm from ROS (Yu *et al*., 2002; Wahid *et al*., 2007). It has not been previously reported the effect of H_2O_2 treatment on peroxidase activity in neem leaves. Neem, a meliacea family tree, commonly found in South Asia and part of Africa as one of the most versatile medicinal plants having wide spectrum of biological activity, neem has been extensively used in ayurveda, unani, and homeopathic medicine (Biswas *et al*., 2002). Literature survey revealed that at present, there are very few medicinal plant universal models for the study of the process of leaf senescence. In this study, the medicinal plant neem (*Azadirachta indica* A. Juss) was chosen as a model for the study of senescence as it is easily available almost all around the year, there was not much variation in the plant population, young/mature and old leaves are available simultaneously and neem leaves in open show very low incidences of pathogen attack.

MATERIALS AND METHODS

Site description

Neem trees grown in the farms of Biochemistry Department, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad in India were used. The site is located near about 10 km away from the center of Aurangabad, and the above trees are not under any specific air pollution. The site of the sample area is surrounded by hills on all directions, and characterized by a semiarid climate, with annual temperature in range from 5-46°C and mean annual precipitation of 734 mm. The main climatic characteristics of the sample site are shown in Table 1.

Table 1. Main characteristics of the sample site

Parameter	Value
Annual temperature range	5-46°c
Average annual precipitation	734 mm
Altitude	513 m
Latitude	19° 32' N
Longitude	75° 14' E

Plant material

A sample of dark green and thick mature leaves and yellow and old senescing leaves at equal distance from twig tip was collected from three neem trees.

Incubation conditions

After through washing in running tap water and then in distilled water, mature green leaves were excised into small pieces. Excised mature leaves (about 15 g fresh weight) were submerged in 100 ml of 1 mM H_2O_2 and incubated at 27°C in the dark. Experimented leaves were harvested at different intervals.

Preparation of leaf protein powder

Leaf protein powder of mature, natural senescing and H_2O_2-promoted senescing leaves of neem were prepared by using acetone precipitation method essentially as described in Fang *et al.* (1998). Excised leaf segments were homogenized using a mortar pestle in the presence of -20°C cold acetone. The homogenate was filtered through sintered glass funnel and washed with cold acetone until the residue was color less. The residue designated as leaf protein powder and was dried in vacuo and stored at -20°C.

Determination of protein

For protein determination the protein powder obtained from mature green, natural senescing and H_2O_2-promoted senescing leaves of neem were suspended in 200 mM Tris-HCl buffer (pH 8.0) just before use as described by Jones (1968). Protein content was determined by Lowry's method (1951) using bovine serum albumin as standard.

POD assay

The activity of POD was assayed as described in Rao *et al*. (1999) with slight modifications[56]. The reaction mixture in a total volume of 2 ml contained 100 mM potassium phosphate buffer (pH 6.5), 200 µl of 16 mM guaiacol, 20 µl of 6% H_2O_2 and 100 µl of leaf extract. Leaf extract was the last component to be added and the increase in absorbance was recorded at 470 nm (extinction coefficient 25.2 mM^{-1} cm^{-1}) using a UV-Vis spectrophotometer (Jasco-V500, Japan) at 10 s intervals up to 1 min. One unit of POD activity represents the amount of enzyme catalyzing the oxidation of 1 µmol of guaiacol in 1 min.

Electrophoresis and POD zymograms

Electrophoretic separation was performed on non denaturing poly acrylamide gel electrophoresis (PAGE) as described by Luhova *et al.* (2003) with slight modifications using 3% stacking gel and 10% separating gel with 0.25 M Tris-2M glycine buffer (pH 8.3), at 20 mA for 3 hours. Samples containing about 10 µg of protein as determined by the Lowry's method (1951) were loaded in to each well. After electrophoresis, determination of POD activity on the gels was performed as described in EI-Dougdoug *et al*. (2007). The gel was immersed for 2-4 h under the dark at 25°C in 50 mM sodium phosphate buffer (pH 5.5) containing 250 mM H_2O_2 and 2 mM guaiacol and photographed immediately.

Statistical analysis

Each experiment was repeated two times with three replicates each and the data presented are mean values of independent experiments.

RESULTS

Protein content in natural and H_2O_2-promoted senescing leaves

Changes in protein content have long been considered one of the principal criterions of leaf development, especially during the final phase leaf development i.e., senescence period. Senescence of neem leaves in the present study was followed by measuring the decrease in protein content, an indicator of leaf senescence. The changes in protein content in natural and H_2O_2-promoted senescing leaves were shown in (Fig. 1). The decrease in protein content was evident in senescing leaves compared to mature leaves. The protein content in natural senescing leaves was 85% of mature leaves, whereas in H_2O_2-promoted senescing leaves it was 73% of mature leaves.

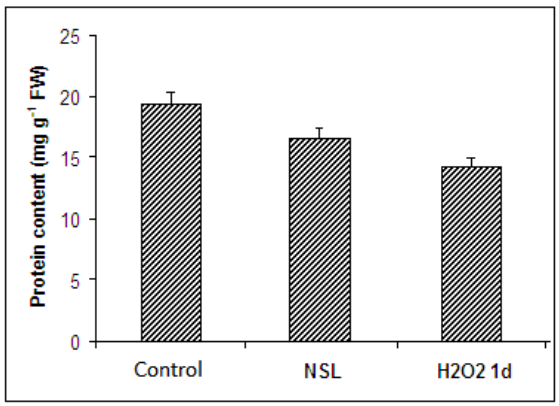

Fig.1. Changes in Protein content in natural and H_2O_2-promoted senescing leaves

POD activity and isoforms in natural and H_2O_2-promoted senescing leaves

Induction of PODs has been reported from the beginning to the final stages in natural senescing leaves of *Ramonda serbica* (Veljovic-Jovanovic, 2006) and H_2O_2-promoted senescing leaves of rice (Hung and Kao, 2005). To determine the correlation between POD activity and senescence, an attempt was made to detect PODs in natural and senescing leaves by spectrophotometric method using guaiacol as substrate and H_2O_2 as a co-substrate. In this study, increased POD activity was detected in both natural and H_2O_2-promoted senescing leaves as compared to mature green control leaves (Fig. 2A). In natural senescing leaves the total POD activity was 2.33-fold higher than the control leaves, whereas in 1 day treated H_2O_2-promoted senescing leaves PODs showed 2-fold increase in their activity. Isoform analysis of POD activity by native PAGE revealed the similar expression with a single band in all the control and experimented leaves, but differing only by the intensity of the bands (Fig. 2B). Marked increase in POD intensity was observed in natural senescing leaves as compared to control and 1 d treated H_2O_2-promoted senescing leaves. Changes in intensity of the POD bands further validated the earlier results found by spectrophotometric method.

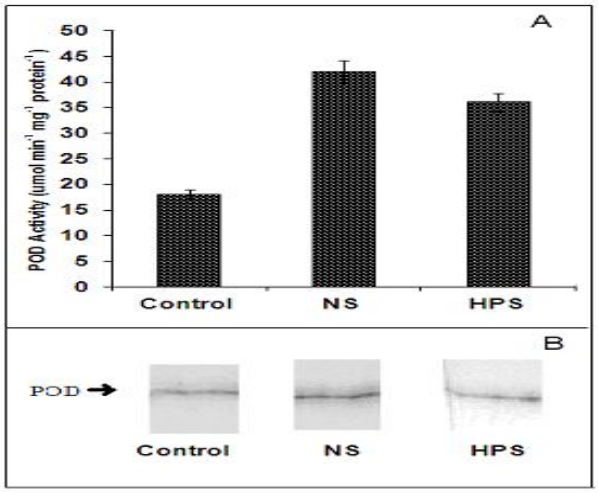

Fig.2. Changes in peroxidase (POD) activity and isoforms in natural and H_2O_2-promoted senescing leaves

Effect of H_2O_2 on protein content in neem leaves

The changes in protein content in neem leaves were measured in H_2O_2 treated and untreated control leaves in order to assess the effect of H_2O_2 in promoting oxidative stress. The protein content showed significant changes in its activity in H_2O_2 treated leaves as compared to H_2O_2 untreated control leaves (Fig. 3). The gradual decrease in protein content was evident upto 4 days under H_2O_2 treatment. The protein content in H_2O_2 treated leaves at day 1 was 73% of H_2O_2 untreated mature green leaves, whereas 46% at day 4. Clearly, H_2O_2 is effective in promoting the senescence of neem leaves.

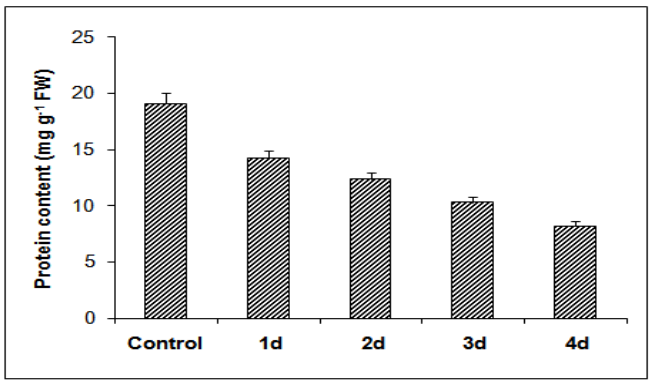

Fig.3. Effect of H_2O_2 on protein content in detached neem leaves

Effect of H_2O_2 on POD activity and isoforms in neem leaves

Induction of PODs constitutes the critical point during various biotic and abiotic stresses could be indicative of oxidative stress in plant tissues (Jr. Cipolini, 1998; Lamikarna and Watson, 2001). The increased POD activity has been documented under a variety of stressful conditions such as water deficit (Lee *et al*., 2009), salinity (Lin and Kao, 2000; Garg and Noor, 2009), ozone (Mohamed and Rangappa, 1993), UV light (Mahdavian *et al*., 2008), and chilling (Raimbault *et al*., 2011). It was also reported that H_2O_2 treatment induces POD activity in rice (Hung and Kao, 2005), cucumber (Liu *et al*., 2010), and pigeonpea leaves (Goud and Kachole, 2011b). In this study, changes in activity of POD were measured in detached neem leaves subjected to H_2O_2 treatment and untreated control leaves, in order to access the role of PODs in H_2O_2-induced stress tolerance. When the neem detached leaves were subjected to H_2O_2 treatment in dark the POD activity significantly increased up to 2 days, but than significantly decreased until 4 days (Fig. 4A). After 1, 2 and 3 days the POD activities in H_2O_2 treated leaves were 2-, 2.2- and 1.4-fold higher than control leaves, respectively. The value of the POD activity was 18 umolmin-1g-if higher than the control leaves. However, after 4 days the activity was 88% of control leaves. Our results are in line with the previous results found in pigeonpea leaves that H_2O_2 treatment induces POD activity at the initial stage and then reduces at latter stages of senescence (Goud and Kachole, 2011c). Decline in POD activity at latter stages may be due to destruction of these proteins along with other proteins. Changes in intensity of the POD bands further validated the earlier results found by spectrophotometric method (Fig. 4B).

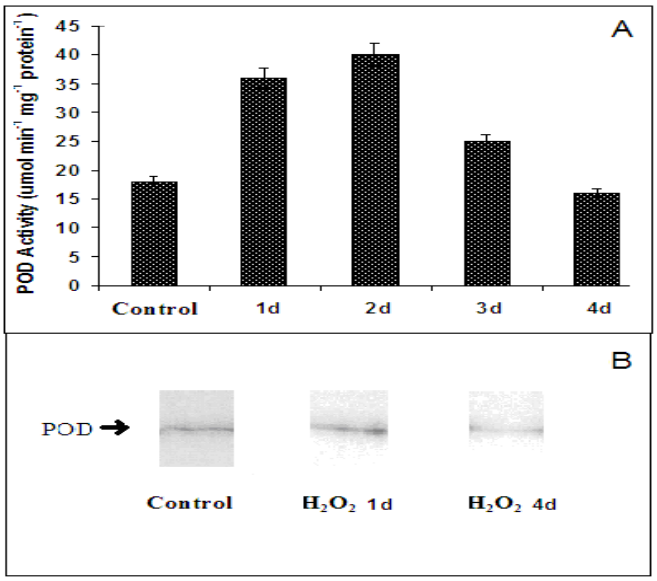

Fig.4. Effect of H_2O_2 on peroxidase (POD) activity and isoforms in detached neem leaves

DISCUSSION

Although leaf senescence can generally be defined as a late developmental process leading to cell death, the primary molecular pathway of this program is not known (Gepstein, 2004). A number of reports are available regarding the increase and/or decrease in senescence associated genes (SAGs) during leaf senescence (Gan and Amacino, 1997). PODs constitute the front line of defense against ROS and most consistently associated with senescence of leaves (Veljovic-Jovanovic, 2006). However, the question of whether natural and oxidative stress-induced senescence are same or different phenomena is still under discussion. In this study, we detected an increase in POD activity during natural and H_2O_2-promoted senescence of neem leaves. It suggests that the underlying regulatory mechanisms might be similar in both conditions in plant species, at least in neem. However, induction of POD activity was strongly

abundant in natural senescing leaves as compared to H_2O_2-promoted senescing leaves. In contrast, we have shown previously that marked increase in POD activity in H_2O_2-promoted senescent leaf chloroplasts of neem as compared to natural senescing leaf chloroplasts (Goud and Kachole, 2011c). It suggests that H_2O_2 treatment primarily alters the molecular mechanism of chloroplasts and majority of H_2O_2-induced PODs from cytoplasm were chloroplastidial. In contrast to the changes observed in POD isoforms during oxidative stress (drought)-induced senescence of *Ramonda serbica* leaves (Veljovic-Jovanovic, 2006), neem leaves showed similar expression in both natural and H_2O_2-promoted senescent leaves, but differing only by the intensity of the bands. Intensity of the bands further confirmed the earlier results found by spectorphotometric method by showing proportionality with the specific activities. However, an important question remains unsolved: whether this increased POD activity is simply related to H_2O_2 scavenge or it is involved in H_2O_2-dependent cell wall lignification and leaf growth reduction. The transgenic tobacco plants with suppressed expression of PODs are over responsive to pathogen-mediated oxidative stress (Mitler *et al*., 1999), whereas plants with overexpressing PODs had increased resistance to it (Aono *et al*., 1991; Yun *et al*., 2000). It suggests that PODs play a very important role against various biotic and abiotic stresses. On the other hand, decline in POD activity at latter stages of H_2O_2 treatment suggests the destruction of these proteins along with other proteins.

In plant systems it was shown previously that H_2O_2 treatment induces leaf senescence in various plant species (EI-Shora, 2003; Hung and Kao, 2005; Veljovic-Jovanovic, 2006; Upadhyaya *et al*., 2007). Effect of H_2O_2 on leaf senescence is intensively studied in many plant species, however, is a new area in neem. We observed that there was a significant decrease in the content of protein during the H_2O_2 treatment in neem leaves. The protein degradation during senescence may be due to a cytotoxic effect of H_2O_2 on protein degradation could have resulted from the effects of free radicals observed in H_2O_2-promoted senescing leaves (Upadhyaya *et al*., 2007). Plant cells are equipped with several ROS detoxifying enzymes to protect them against oxidative damage (Hung and Kao, 2005). In order to clarify the protective mechanism of the antioxidant enzymes against H_2O_2 stress, we determined the changes in POD activity in the neem leaves subjected to H_2O_2 stress. Induction of POD activity at the initial phase of senescence suggesting that PODs play a very important role during neem leaf senescence. However, reduction in POD activity along with increase in protein loss suggesting that there was no correlation between PODs and senescence, at least in neem. On the other hand, decline in POD activity after initial induction indicating its destruction along with other proteins. One more question regarding the mechanism of leaf senescence is whether the gene expression of SAGs is the same regardless of all the environmental factors that induce the condition. By clarifying the question in some extent changes in POD activity during H_2O_2-promoted senescence shown similarity with the earlier reports found in dark-induced senescence of corn leaves (Yeh and Kao, 1994), suggesting that the underlying regulatory mechanisms might be the same in both stresses. In contrast to the changes in POD isoforms during oxidative stress-induced senescence (drought) of *Ramonda serbica* leaves (Veljovic-Jovanovic *et al*., 2006), neem leaves shown similar expression in H_2O_2 treated and untreated leaf and leaf-chloroplasts. However, the changes in intensity of the POD bands confirmed the earlier results found by spectrophotometric method. In summary our results show that (i) neem leaves constitutes single POD isoform at mature and senescing stages (ii) neem-leaf-H_2O_2 model could be used for biochemical and physiological studies on senescence (iii) underlying regulatory mechanisms might be the same in both natural and oxidative stress induced senescence in neem leaves, atleast in case of POD. Because oxidative stress and leaf senescence have been shown to accompany many of the environmental stresses, such as drought, light, ozone, and pathogen attack, our results may be important in the future engineering of plant resistance to different environmental stresses by modulating the activity of PODs.

REFERENCES

Alokail MS, Ismael MA. Thermostable Characteristics of Peroxidase from Leaves Arabian Palm Date *(Phoenix dacty lifera* L.). *Soudi J Biol Sci* 2005; 12:25-32.

Aono M, Kubo A, Saji H, Natori T, Tanaka K, Kondo N. Resistance to active oxygen toxicity of transgenic *Nicotiana tabacum* that express the gene for glutathione reductase from *Escherichia coli*. *Plant Cell Physiol* 1991; 32:691-697.

Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (*Azadirachta indica*). *Curr Sci* 2002; 82:1336-45.

Chen LO, Lo H, Chen T, Lee L. Peroxidase zymograms of sweet potato [*Ipomea batatas* (L.) Lam] grown under hydroponic culture. *Bot Bull Acda Sinika* 1992; 33:247-52.

De-Azevedo Neto AD, Prisco JT, Ene´ as-Filho J, Medeiros JVR, Gomes-Filho E. Hydrogen peroxide pre-treatment induces salt-stress acclimation in maize plants. *J Plant Physiol* 2005; 162:1114–22.

EI-Dougdoug KhA, EI-Harthi HMS, Korkar HM, Taha RM. Detection of somaclonal variations in banana tissue culture using isozyme and DNA fingerprinting analysis. *J Appl Sci Res* 2007; 3:622-27.

EI-Shora HM. Activities of antioxidative enzymes and senescence in detached *Cucurbita pepo* under Cu- and oxidative stress by H_2O_2. Becth. Mock. Yh-Ta 2003; 44:66-71.

Fang W, Kao CH. Enhanced peroxidase activity in rice leaves in response to excess iron, copper, and zinc. *Plant Sci* 2000;158:71-76.

Fang Z, Bouwkamp JC, Solomos T. Chlorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild type of *phaseolus vulgaris L. J Exp Botany* 1998; 49:503-510.

Gan S, Amasino RM. Making sense of senescence. *Plant Physiol* 1997; 113:313-19.

Garg N, Noor Z. Genotypic differences in plant growth, osmotic and antioxidative defense of *Cajanus cajan* (L.) Millsp. modulated by salt stress. *Arch of Agron Soil Sci* 2009; 55: 3-33.

Gepstein S. Leaf senescence - not just a wear and tear phenomenon. *Genome Biol* 2004; 5:212.

Goud PB, Kachole MS. Effect of exogenous hydrogen peroxide on peroxidase and polyphenol oxidase activities in *Cajanus cajan* (L.) Millsp. leaves. *Inter J Curr Res* 2011a; 3: 061-65.

Goud PB, Kachole MS. Role of chloroplastidial proteases in leaf senescence. *Plant Sign Behav* 2011b; 6: 1371-76.

Goud PB, Kachole MS. Effect of hydrogen peroxide on protein content and catalase activity in *Cajanus cajan* (L.) Millsp. leaves. *Inter J Curr Res* 2011c; 3:268-71.

Hung KT, Kao CH. Hydrogen peroxide, calcium, and leaf senescence in rice. *Crop Environ Bioinformatics* 2005;4:145-50.

Hung KT, Kao CH. Nitric oxide counteracts the senescence of rice leaves induced by hydrogen peroxide. *Bot Bull Acad Sinika* 2007; 46:21-28.

Jones OTG. Ferrochetalase of spinach chloroplasts. Biochem J 1968; 107:113-119.

Jr. Cipolini DF. The induction of soluble peroxidase activity in bean leaves by wind-induced mechanical perturbation. *Amer J Bot* 1998; 85:1586-91.

Lamikarna O, Watson MA. Effects of ascorbic acid on peroxidase and polyphenoloxidase activities in fresh-cut cantaloupe melon. J Food Sci 2001; 66:1283-86.

Lee BR, Li LS, Jung WJ, Jin YL, Avice JC, Ourry A, Kim TH. Water deficit-induced oxidative stress and the activation of antioxidant enzymes in white clover leaves. *Biol Plant* 2009; 53:505-10.

Lepedus H, Jozi M, Stolfa I, Pavicic N, Hackenberger BK, Cesar V. Changes in peroxidase activity in the peel of unshiu mandarin (*Citrus unshiu* Marc.) fruit with different storage treatments. *Food Technol Biotechnol* 2005; 43:71–77.

Lim PO, Woo HR, Nam HG. Molecular genetics of leaf senescence in *Arabidopsis*. Trends Plant Sci 2003; 8:272-78.

Lin CC, Kao CH. Effect of NaCl stress on H_2O_2 metabolism in rice leaves. *Plant Growth Regul* 2000; 30:151-55.

Lin YC, Kao CH. Proline accumulation induced by excess nickel in detached rice leaves. *Biol Plant* 2007; 51:351-54.

Liu Z, Guo Y, Bai J. Exogenous hydrogen peroxide changes antioxidant enzyme activity and protects ultra structure in leaves of two cucumber ecotypes under osmotic stress. *Journal of Plant Growth Regulation* 2010; 29:171-83.

Lowry OH, Roserbrough N, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193:265-275.

Luhova L, Lebeda D, Hedererova D, Pec P. Activities of amine oxidase, peroxidase and catalase in seedlings of *Pisum sativum* L. under different light conditions. *Plant Soil Environn* 2003; 49:151-57.

Mahdavian K, Ghorbanli M, Kalantari KhM. The effects of ultraviolet radiation on some antioxidant compounds and enzymes in *Capsicum annuum* L. Turk J Bot 2008; 32: 129-134.

Mitler R, Herr EH, Orvar BL, Camp W, Willikens H, Inze D, Ellis BE. Transgenic tobacco plants with reduced capability to detoxify reactive oxygen Intermediates are hypersensitive to pathogen infection. PNAS 1999; 96:14165-70.

Mohamed AI, Rangappa M. The role of peroxidase in tolerance to ozone in bean. *Vigrinia J Sci* 1993; 44: 279-85.

Mudd JB, Vliet HHDMV, Deenen LLMV. Biosynthesis of galactolipids by enzyme preparations from spinach leaves. *J Lipid Res* 1969; 10:623-630.

Nam HG. The molecular genetic analysis of leaf senescence. *Curr Opin in Biotechnol* 1997; 200-07.

Prasad TK, Anderson MD, Stewart CR. Localization and characterization of peoxidase in the mitochondria of chilling-acclimated maize seedlings. Plant Physiol 1995;108:1597-1605.

Raimbault A, Marie-Alphonsine P, Horry J, Francois-Haugrin M, Romuald K, Soler A. Polyphenol oxidase and peroxidase expression in four pineapple varieties (*Anana comosus* L.) after a chilling injury. *J Agri Food Chem* 2011; 59: 342-348.

Rao MV, Paliath G, Ormrod DP. Ultraviolet-B-and ozone induced changes in antioxidant enzymes of *Arabidopsis thaliana. Plant Physiol* 1996; 110:125-136.

Sat IG. The effect of heavy metals on peroxidase from Jerusalem artichoke (*Helianthus tuberosus L.*) tubers. *Afr J Biotechnol* 2008; 7:2248-53

Scotti N, Cardi T, Marechaldrouard L. Mitochondrial DNA and RNA isolation from small amounts of potato tissue. *Plant Mol Biol Rep* 2001; 19:67a-h.

Smart CM. Gene expression during leaf senescence. *New Phytol* 1994; 126:419-48.

Upadhyaya H, Khan MH, Panda SK. Hydrogen peroxide induces oxidative stress in detached leaves of *Oryza sativa* L. Gen and *Appl Plant Physiol* 2007; 33:83-95.

Veljovic-Jovanovic S, Kukavica B, Stevanovic B, Navar-Izzo F. Senescence and drought-related changes in peroxidase and superoxide dismutase isoforms in leaves of *Ramonda serbica. J Exp Bot* 2006; 57:1759-68.

Wahid A, Perveen M, Gelani S, Basra SMA. Pretreatment of seed with H_2O_2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *J Plant Physiol* 2007; 164:283–94.

Yeh J, Kao CH. Lack of evidence for casual relationship between peroxidase activity and dark-induced senescence of detached corn leaves. *Bot Bull Acad Sinika* 1994; 35:147-151.

Yu C, Murphy TM, Sung W, Lin C. H_2O_2 treatment induces glutathione accumulation and chilling tolerance in mung bean. *Funct Plant Biol* 2002; 29:1081-87.

Yun BW, Huh GH, Lee HS, Kwon SY, Jo JK, Kim JS, Cho KY, Kwak SS. Differential resistance to methyl viologen in transgenic tobacco plants that express sweet potato peroxidases. *J Plant Physiol* 2000; 156:504-509.

****