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Sodium Chloride (NaCl) and Hydrogen Peroxide (H_2O_2) Induced Changes in Antioxidant Enzymes (SOD, CAT, and POX) of Contrasting Wheat Cultivars under Favourable Growth Conditions

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A B S T R A C T

Differential response of CAT1, CAT2 and CAT3 indicated the positive correlation of catalase and H_2O_2 accumulation in C306 under control, H_2O_2, NaCl and NaCl+H_2O_2 treatments accompanied with enhancement of SOD activity in flag leaves of the drought tolerant wheat cultivar. SOD activities provide extra protection in combination with catalase in wheats under oxidative stresses. The POX and SOD activity enhancement in flag leaves was positively associated with plant height and leaf size reduction under salt stress in wheats. The differential accumulation of H_2O_2 is cultivar specific and associated with SOD isoforms (CuZnSOD) that is involved in lignin biosynthesis. CuZnSOD or CAT were not inhibited under salt stress suggest that it is superoxide rather than other forms of ROS mediating proline oxidase induced apoptosis. Proline accumulation/ oxidation alter the intracellular redox status by proline oxidase inhibition by MnSOD and superoxide dismutation to H_2O_2 which is utilised by POX for lignin biosynthesis.

Introduction

Plants adjust their antioxidant enzymes to avoid cellular injury due to oxidative stress triggered by reactive oxygen species. Superoxide dismutase (MnSOD, FeSOD and CuZnSOD) a group of metal-enzymes dismutase the superoxide radical to hydrogen peroxide. Catalase detoxifies H_2O_2 to water and oxygen. Peroxidases catalyze lignin polymerization using monolignols (coniferyl, sinapyl p-coumaryl alcohols) and H_2O_2 in the apoplastic space, modify cell walls incorporating suberin. Lignin is present in xylem vessels and xylem fibers in high concentrations. Peroxidases play role in the cell wall loosening and cell elongation via generation of hydroxyl radicals (OH radical) with the ability to chop cell wall polysaccharides. Peroxidases stiffening of cell wall by cross linking cell wall protein and ferulic acid residues in polysaccharides and cessation of cell elongation.

Superoxide, hydrogen peroxide and hydroxyl radicals are continuously generated in

respiration and photosynthesis. ROS serve as signalling molecules similar to phytohormones. ROS accumulation modifies cell walls, root elongation, leaf expansion, biomass accumulation plant growth and development, therefore, plant productivity.

Drought and salinity affect plant molecular, biochemical and physiological processes via ROS (Smirnoff, 1993, Hasegawa *et al.,* 2000). Plants modulate the antioxidant enzymes to alleviate the cellular injury caused by ROS (Foyer and Noctor, 2005).

The production of both ABA and H_2O_2 is induced by water stress and drought due to salt stress; can act as signals under stress conditions. Therefore, we investigated the relationships between ABA, salt stress, H_2O_2, accumulation and changes in antioxidants enzymes under favourable growth conditions. Wheat is an important staple food crop worldwide. Therefore, the study was undertaken to identify the marker antioxidant enzymes of drought sensitive and drought tolerant wheat cultivars those may be helpful in plant breeding for salt tolerance.

Materials and Methods

Drought sensitive wheat cultivar, HD2428 and drought tolerant wheat cultivar- C306 were grown under normal environment for growth and development (November 15, 2018) to expose them to normal and oxidative stress environment under late sown conditions. Plants were grown in a greenhouse green house in earthen pots (size 30x30 cm) filled with sandy loam soil and farmyard manure in 3:1 under natural environment. Each pot was fertilized corresponding to 120, 90 and 60 kg ha^{-1} of N, P and K, respectively. Plants were kept free from diseases. Twenty pots were used for H_2O_2 (10 mM) spray treatment, NaCl (200 mM) soil application and H_2O_2 (10 mM) spray treatment after five days of NaCl treatment. Twenty pots were used for seeds harvested from 15 January 2017 grown plants and sown in the normal season (November 15, 2018) for epigenetic phenotypes characterization.

Fresh flag leaves samples were ground in liquid nitrogen and homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing 2 mM EDTA and 4% (w/v) PVP-40 and centrifuged at 10000 g for 20 min at 4^0C.

The supernatant was used for protein estimation (Bradford 1976). Antioxidant enzymes (SOD, CAT and POX) activity staining was performed following (Beauchamp and Fridovich, 1971; Seevers *et al.,* 1971; Woodbury *et al.,* 1971) using equal amount of protein. Isozymes pattern of wheats were compared with epigenetic phenotypes to analyse the changes due to H_2O_2 or ABA accumulation under favorable growth conditions. Kharchia DW1278 a salt tolerant cultivar was used as a check under salinity. Proline was extracted following Bates *et al.,* (1973).

Results and Discussion

Drought tolerant wheat cultivar C306 exhibited more SOD isoforms and activity staining in flag leaves of control and in roots under salt treatment than drought sensitive wheat cultivar HD2428. NaCl and a combination of salt with H_2O_2 spray induced CuZnSOD5 in roots of both cultivars. H_2O_2 spray treatment induced CuZnSOD4 in flag leaves of C306 that suggest higher levels of superoxide radicals in C306 control flag leaves than HD2428. Further induction of SOD4 could have increased H_2O_2 accumulation by dismutation of superoxide radicals under this treatment. Salt tolerant wheat cultivar already had higher levels of CuZnSOD3 & 4, therefore, higher H_2O_2

accumulation in flag leaves of control than HD2428 and C306. These data suggested that HD2428 maintained low levels of H_2O_2 in the cytosol by antioxidant enzyme other than CuZnSOD. C306 maintained higher SOD isoforms in leaves and roots of epigenetic phenotype than HD2428 epigenetic phenotype under same growth environment. The pattern was similar in both epigenetic phenotypes when compared with both wheat cultivars under NaCl stress. Both cultivars showed inhibited root and shoot growth and asymmetrical leaf growth (visual observation) in these phenotypes. The data clearly indicated the rise in H_2O_2 accumulation in flag leaves of HD2428 & C306 phenotypes than flag leaves of control plants under favourable growth conditions. SOD isoform pattern in flag leaves and roots of both phenotypes were similar to that of salt tolerant cultivar Kharchia, drought tolerant cultivar C306 and drought sensitive wheat cultivar HD2428 under + H_2O_2 treatment. Reduced proline accumulation in these phenotypes indicated reduced injury and senescence (Plate1A). Therefore, role of inherited ABA cannot be ruled out in the delayed senescence of these phenotypes of both wheat cultivars under normal growth environment. Exogenous ABA has been shown to increase wheat plant biomass under drought and related with stomatal closure reduced transpiration and solute uptake (Kirkham 1983). Growth retardants, growth inhibitors and plant growth promoters alter the plant growth under water stress either by changing the rate of water uptake/ water loss from the plant or changing the osmolyte/factors affecting the water within the plant cells. A shift in osmotic pressure due to stomatal closure or osmolyte accumulation could have metabolic consequences.

The higher levels of proline and SOD isoforms indicating more oxidative stress accompanied with proline accumulation in salt tolerant Kharchia than epigenetic phenotypes. Further, the delayed senescence and the lowest levels of proline in flag leaves of epigenetic phenotypes are associated with changes in gene expression for SOD, CAT and POX when compared with control flag leaves of wheats.

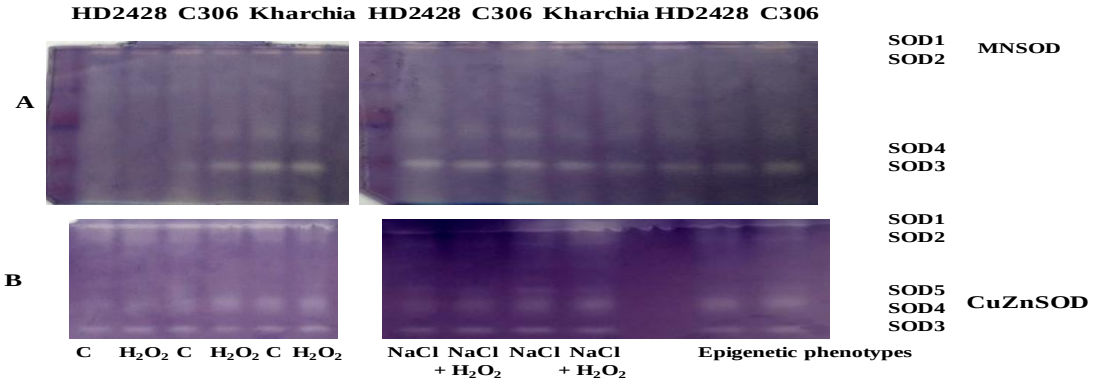

Plate1. Superoxide dismutase (SOD) in leaves (A) and roots (B) of contrasting wheat cultivars under sodium chloride (NaCl) and hydrogen peroxide (H_2O_2) treatments and favourable growth conditions

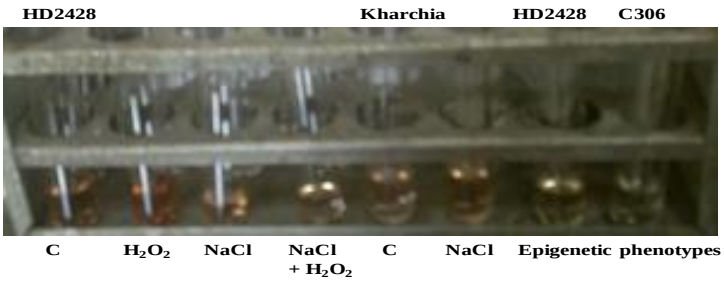

Plate1A. Proline accumulation in flag leaves of drought sensitive wheat cultivar HD2428, salt tolerant wheat cultivar Kharchia and Epigenetic phenotypes of HD2428 and C306 under oxidative stress conditions (NaCl) and hydrogen peroxide (H_2O_2) treatments under favourable growth conditions

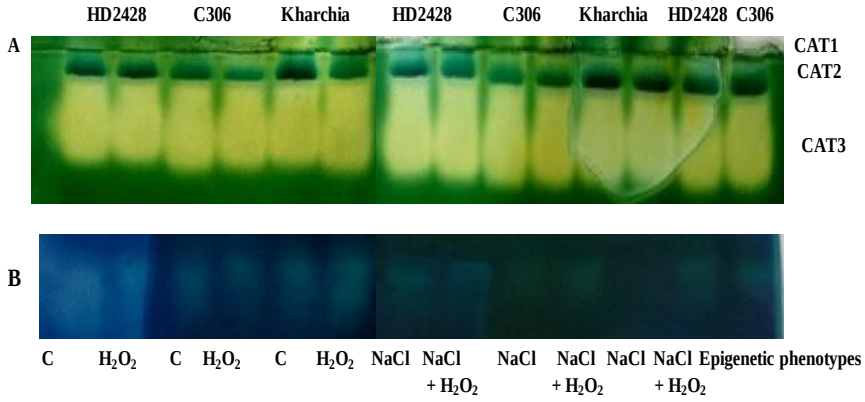

Plate2. Catalase (CAT) in leaves (A) and roots (B) of contrasting wheat cultivars under sodium chloride (NaCl) and hydrogen peroxide (H_2O_2) treatments and favourable growth conditions

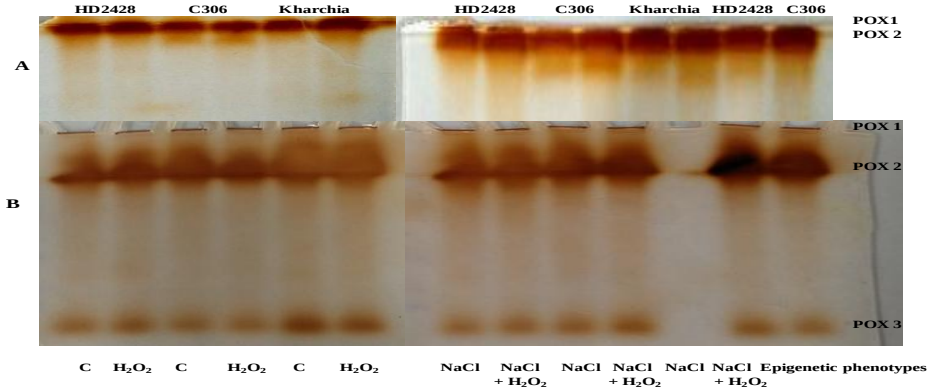

Plate3. Peroxidase (POX) in leaves (A) and roots (B) of contrasting wheat cultivars under sodium chloride (NaCl) and hydrogen peroxide (H_2O_2) treatments and favourable growth conditions

Kharchia showed higher activity staining of SOD isoforms in flag leaves of control and H_2O_2 treated plants. The lowest proline accumulation was exhibited in epigenetic phenotypes of HD2428 and C306 with delayed senescence under favourable growth conditions.

H_2O_2 scavenging antioxidant enzyme CAT activity was very low in roots of wheats under all treatments and favourable growth conditions. Differential response of CAT1, CAT2 and CAT3 indicated the positive correlation of catalase and H_2O_2 accumulation in C306 under control, H_2O_2, NaCl and

$NaCl+H_2O_2$ treatments accompanied with enhancement of SOD activity in flag leaves of the drought tolerant wheat cultivar.

Overall CAT staining activity (CAT1, 2&3) was higher in both epigenetic phenotypes than control flag leaves of wheats. SOD activities provide extra protection in combination with catalase in wheats under oxidative stresses. Expression of SOD isoforms depends on the nature of the elicitor triggering oxidative stress and intracellular pH changes (Abele et al.1998).

Overall POX activity was increased in flag leaves and roots of the contrasting wheats under H_2O_2 treatment. NaCl treatment enhanced these POX isozymes in wheats. POX1was not altered in flag leaves and roots under any treatment. POX2 exhibited considerable enhancement in flag leaves under NaCl and $NaCl+H_2O_2$ treatment. The POX and SOD activity enhancement in flag leaves was positively associated with plant height and leaf size reduction under salt stress in wheats. Data clearly indicate the rise in levels of H_2O_2 accumulation and its utilisation by POX in lignin/suberin biosynthesis in leaves and roots.

The differential accumulation of H_2O_2 is cultivar specific and associated with SOD isoforms (CuZnSOD) that is involved in lignin biosynthesis. The highest activities of POX2 in flag leaves and roots of wheat phenotypes were associated with asymmetric leaf growth due to restricted mechanical stretching caused by lignin deposition and reduced root growth in both phenotypes under favourable growth conditions. Further the development of mechanical strength in higher number of xylem vessels in C306 (unpublished) correlate with the differential response of POX activity under all treatments. POX activity also indicates the effect of salt stress on the degree of root extension in contrasting wheats. The highest POX2 activity and delay in the onset of senescence in the drought sensitive semi dwarf wheat cultivar HD2428 followed by drought tolerant tall wheat cultivar C306 accompanied reduced root biomass than roots of their controls. Plant growth response to salt stress and ABA biosynthesis in epigenetic phenotypes of wheats involves POX activity enhancement in roots and leaves to utilise H_2O_2.. However, CuZnSOD in C306 control flag leaves indicates its association with lignin biosynthesis supporting tall stem under favourable growth conditions.

MnSOD inhibits proline oxidase that led to proline accumulation in drought sensitive cultivar of wheat HD2428. CuZnSOD or CAT were not inhibited under salt stress suggest that it is superoxide rather than other forms of ROS mediating proline oxidase induced apoptosis. Accompanying the decrease in CuZnSOD isoforms in the flag leaves, increased proline oxidation with generation of superoxide radicals markedly increased the level of H_2O_2 by MnSOD that is reflected in POX activity enhancement in epigenetic phenotypes. MnSOD generated a higher concentration of H_2O_2 owing to dismutation of superoxide radicals which was elevated by proline oxidase (Yongmin *et al.,* 2005) in both phenotypes of wheats. Excess H_2O_2 can participate in protein oxidations via hydroxyl radical generation.

Since proline accumulation level is the lowest in C306 epigenetic phenotype, the increased level of superoxide radicals is exhibited as increased activity of CuZnSOD in flag leaves and roots than HD2428 under favourable growth conditions.

Therefore, proline accumulation/ oxidation alter the intracellular redox status by proline oxidase inhibition by MnSOD and superoxide dismutation to H_2O_2 which is utilised by POX for lignin biosynthesis.

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