



Differential response of antioxidant enzymes to water deficit stress in maize (*Zea mays*) hybrids during two leaf stage

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ABSTRACT

Experiment was conducted to analyse maize (*Zea mays* L.) hybrids for antioxidant enzymes and non-enzyme compound at two leaf stages, under normal irrigation (IRR) and water deficit stress (WDS) condition. WDS was induced by withholding irrigation water for three days in seven-day old seedlings grown in pots. The hybrids exhibited wide variability in their antioxidant pools combined with activities of enzymes involved in defence against oxidative stress. The results showed that antioxidant activity was higher in tolerant hybrids than the susceptible hybrids. Superoxide dismutase activity was higher in susceptible hybrids under IRR, whereas it decreased significantly under WDS. On the other hand, peroxidase activity was increased almost two folds in all the tolerant hybrids. Under WDS, glutathione reductase activity increased in all the hybrids except VIVEKHYD-9, whereas the increase was highest, i.e. 37.4% in tolerant than the susceptible hybrids. Catalase activity increased 56% in tolerant hybrid, whereas a decrease of about 30% was recorded in susceptible under WDS. Also, ascorbate peroxidase activity increased in tolerant hybrids and decreased in susceptible hybrids under WDS. In response to WDS, glutathione content (GSH) decreased 11% in tolerant while increased 55% in susceptible hybrids. Increase in GSH content was highest (100%) in HQPM-7 followed by NK-6240 (56%). In general, ascorbic acid content increased under WDS in both tolerant and susceptible hybrids, however the increase was higher in tolerant (80%) than susceptible (45%). Among the hybrids, highest increase was recorded in PRAKASH (99%) and lowest in VIVEKHYD-9 (29%), thus PRAKASH seems to be drought tolerant while VIVEKHYD-9 is drought susceptible. Thus status of antioxidant enzymes in maize hybrids could be evaluated with the drought tolerance during different stages of development and growth and could be used in developing climate resilient maize hybrids.

Key words: Antioxidant defence system, Climate resilient crop, Crop improvement, Drought tolerance

Maize (*Zea mays* L.) is cultivated in the semi-arid tropics worldwide. It is one of the most important crops grown in these regions, whereas water scarcity environment remains the major limitation to crop productivity (Blum 2005). In addition, due to global warming the available water for irrigation may further deplete because weather expected to become generally drier and warmer. This may lead to intensify the competition for water between human being and crop plants. It is estimated that by 2050 food production will be needed to increase by at least 50% to match the projected population growth (Godfray *et al.* 2014). Moreover, drought tolerance (DT) during one growth stage may not correlate with another, while WDS during vegetative stage may induce some adaptation in plant and may improve water use efficiency and the ultimate productivity as reported in groundnut (Nautiyal *et al.* 2002). Whereas, in

case of maize the time period between tasseling and silking indicates about the nature of DT, for example, lesser is the time period between tasseling and silking higher is the degree of DT (Abrokwah 2015). If DT at one stage is related with DT at another stage, such as, reproductive stage and seedling stage, it would be much easier to screen large number of genotypes for DT. It is well known that at the molecular level reactive oxygen species are generated under WDS which damages the cellular membrane and its components (Bartwal *et al.* 2013). Plants have the potential to defend itself from oxidative stress by making use of array of antioxidant enzymes (Chug *et al.* 2011). It was also reported that superoxide radicals originates in electron transport chains and hydrogen peroxide are detoxified by ascorbate peroxidase and catalase (Nehnevajova *et al.* 2012). Several non-enzymatic compounds such as cysteine, reduced glutathione, carotenoids, ascorbate, α -tocopherol are also known to play an important role in antioxidation mechanism. Further damage to fatty acid could produce small hydrocarbon fragments including malondialdehyde. In maize antioxidative response is well correlated with DT

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and increased activities of antioxidant enzymes during the pre and post-flowering stages were reported (Farooq *et al.* 2009). In addition, during seedlings stage DT maize genotypes exhibited enhanced photosynthetic rate (Moussa and Abdel-Aziz 2008). But overall DT during seedling stage has received little attention. This study was aimed to evaluate maize hybrids for DT during seedling stage following the assay of various antioxidant enzymes and non-enzyme chemicals.

MATERIALS AND METHODS

Maize hybrids were procured from the Indian Institute of Maize Research, New Delhi, and experiment was conducted at Water Technology Centre, Indian Agricultural Research Institute, New Delhi (77° 12'E, 28° 40'N; 228.6 MSL) in July 2013 in green house in pot. Oxidative stress tolerance was studied in the seedlings grown in greenhouse during 2013 in pots filled with farmyard manure and soil in 1:4 ratio. Pots were arranged in two sets each set having 24 pots, replicated six times. Three seeds after surface sterilized with 0.1 % (w/v) sodium dodecyl sulphate solution followed by thorough rinse with sterile deionised water were sown in each pot in case of each genotype and treatment. After emergence two seedlings were maintained per pot. Seven days after emergence, one set was treated as control by providing irrigation water regularly while another set was exposed to WDS for three days by withholding irrigation water. Plant sampling was performed after 3 days of WDS treatment when second leaf was fully expanded and used for the extraction and assay of enzymes.

Sampling was performed from the individual pots of each genotype and treatment and leaf samples were analysed for various enzyme assays. One gram leaf samples were grind with the help of motor and pestle at 4°C in cold room. Superoxide dismutase (SOD), peroxidase (POX) and reduced glutathione (GR) were extracted by homogenizing the sample in 0.1M phosphate buffer (pH 7.5) containing 1% polyvinylpyrrolidone (PVP), 1mM EDTA and 10mM β -mercaptoethanol. While catalase (CAT) and ascorbate peroxidase (APX) were extracted with 0.05 M phosphate buffer (pH 7.5) containing 1% PVP. In each case homogenate were centrifuged at 10 000g for 20 min and the supernatant was used for assaying the enzymes activity at 30°C.

Superoxide dismutase (SOD) (*E.C.1.15.1.1*) activity was determined following the method described by Marklund and Marklund (1974). The reaction mixture contained 1.4 ml of 100mM Tris HCL buffer (pH 8.2), 0.5 ml of 6mM EDTA, 1 ml of 6mM pyrogallol solution and 0.1 ml of enzyme extract. Changes in absorbance were recorded at 420nm after an interval of 30 s up to 3 min. A unit of enzyme activity was expressed as the amount of enzyme causing 50% inhibition of auto-oxidation of pyrogallol observed in blank.

Peroxidase (POX) (*E.C.1.11.1.7*) activity was determined by following the method described by Shannon *et al.* (1966). The reaction mixture contained 3 ml of 0.05 M guaiacol in 100 mM phosphate buffer (pH 6.5), 0.1 ml of

enzymes extract and 0.1 ml of 0.8 M H₂O₂. The reaction mixture without H₂O₂ was taken as a blank. The reaction was initiated by adding H₂O₂ and rate of changes in absorbance was recorded at 470nm for 3 min at an interval of 30 s. POX activity was expressed as change in absorbance/min/g of fresh weight. Protein content of all enzyme extract was determined by the method of Lowry *et al.* (1951).

Glutathione reductase (GR) (*E.C.1.8.1.7*) activity was determined following the method described by Esterbauer and Grill (1978). The reaction mixture contained 0.2 ml of 200 mM potassium phosphate buffer (pH 7.5), 0.1 ml MgCl₂ (1.5 mM), 0.1 ml EDTA (0.2 mM), 0.2 ml NADPH (0.025 mM), 0.2 ml of enzymes extract, followed by 0.2 ml of oxidized glutathione (0.25 mM). Decrease in absorbance at 340 nm after an interval of 30 s up to 3 min was recorded. The molar extinction coefficient for NADPH was 6.22/mM/cm. GR activity was expressed as n moles of NADP⁺ formed/min/g of fresh weight.

Catalase (CAT) (*E.C.1.11.1.6*) activity was determined by taking 1.8 ml of 50mM sodium phosphate buffer (pH 7.5) to which 0.2 ml of enzyme extract was added. The reaction was initiated by adding 1 ml H₂O₂ and utilization of H₂O₂ was recorded at an interval of 30 s for 3 min by measuring the decrease in absorbance at 240nm following the method of Chance and Machly (1955). Extinction coefficient for H₂O₂ was 0.0394/mM/cm. CAT activity was expressed as μ moles of H₂O₂ decomposed/min/g of fresh weight.

Ascorbate peroxidase (APX) (*E.C.1.11.1.11*) activity was measured following the method described by Nakano and Asada (1987), it catalyses the reduction of H₂O₂ by ascorbate. The reaction mixture of 3 ml containing 1 ml of 50mM sodium phosphate buffer (pH 7.0), 0.8 ml of 0.5mM ascorbic acid, 0.2 ml of enzyme extract and 1 ml of H₂O₂ solution was made. Absorbance was recorded at 290 nm at an interval of 30 s up to 3 min. Extinction coefficient of monodehydroascorbic acid (MDAA) was 2.8/mM/cm. APX activity was expressed as n moles of MDAA formed min/g of fresh weight.

For the estimation of antioxidant compounds tissue sample (0.39 g) was homogenized with 2 ml of ice cold 50 mM sodium phosphate buffer (pH 7.0) using liquid N₂. Homogenate was centrifuged at 10 000 g for 20 min and supernatant was collected and H₂O₂ content was estimated following the method of Sinha (1972). Ascorbic acid was estimated in the supernatant following the method of Law *et al.* (1983). For estimation of reduced ascorbic acid, 0.1g of tissue sample was crushed in 1.5 ml of 5% ice-cold metaphosphoric acid and then centrifuged at 10 000 g for 10 min. For glutathione estimation, 0.1 g of tissue sample was ground and homogenized in 2 ml of 5% sulphosalicylic acid and mixture was centrifuged at 10 000 g for 15 min. Glutathione reductase (GR) was estimated using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), NADPH and glutathione reductase (Smith *et al.* 1985). Malondialdehyde (MDA) was extracted and estimated by using a

thio-barbituric acid reaction following Heath and Packer (1968).

For statistical validity each treatment was made in six replicates and the data was expressed as \pm standard error (SE) analysed following one way ANOVA. Statistical significance was checked at $P=0.05$.

RESULTS AND DISCUSSION

Antioxidant enzymes

In this study, maize hybrids exhibited differential

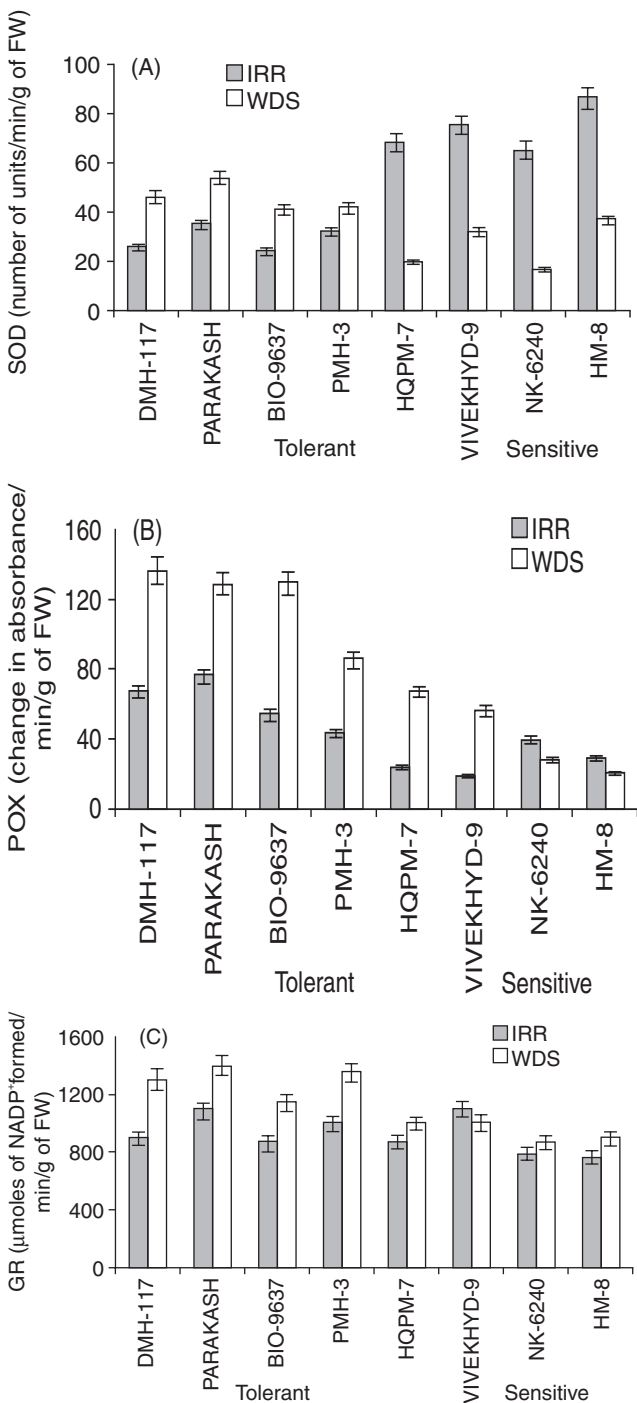


Fig 1 Changes in SOD (A), POX (B), GR (C), CAT (D) and APX (E) activities. Vertical bars show SE from mean of three replicates

response in their antioxidative properties. These results clearly indicated that drought tolerant hybrids are well equipped with a strong antioxidant mechanism than the susceptible hybrids. Antioxidant activity, in general was higher in tolerant hybrids than the susceptible hybrids. Similar findings were also reported by Hirayama and Shinozaki (2010). In addition, water stress limits the oxygen supply and elevates reactive oxygen species at any of the crop growth stage (Licausi *et al.* 2013) and activation of antioxidative defence mechanisms correlates with drought-induced oxidative stress tolerance (Chugh *et al.* 2011). It is known that plants have a well-organized defense system against ROS under stress conditions and SOD constitutes the first line of defence via detoxification of superoxide radicals (Sairam *et al.* 2000). Under IRR, SOD activity was higher in susceptible hybrid but decreased significantly under WDS (Fig 1A). Thus tolerant and susceptible hybrids exhibited wide genetic variability in their response to WDS. The higher increment in the SOD activity in all four tolerant hybrids might have decreased the possible toxic concentration of O₂⁻ radicals more efficiently than the four susceptible in which the SOD activity decreased under WDS.

Peroxidase, another important H₂O₂ scavenging enzyme showed enhanced activity in tolerant hybrids under WDS. The per cent increment in its activity was higher in tolerant hybrids and lower in susceptible hybrids especially in NK-6240 and HM-8 under WDS (Fig 1B). Enhancement

in POX activity under various stress conditions was associated with protection from oxidative damage, lignifications and cross-linking of cell wall to protect from such adverse conditions (Dalal and Khanna-Chopra 2001). Water deficit stress induced increase in POX activities was also reported higher in drought tolerant sorghum cultivars (Gill and Tuteja 2010).

GR activity increased in all the hybrids except VIVEKHYD-9, whereas the increase was more (37.4%) in tolerant than the susceptible hybrids (8.5%) under WDS. In addition, GR activity remained more or less similar in both tolerant and susceptible hybrids under IRR (Fig 1C).

Catalase activity increased under WDS only in tolerant hybrids though the percentage increase was higher in PRAKASH which has been identified to be drought tolerant (Nautiyal PC, unpublished data, Chugh *et al.* 2011; Fig 1D). On the other hand, CAT activity increased 56.5% in tolerant hybrids and decreased 30% in susceptible under WDS. Among the hybrids CAT activity was higher in HQPM-7 under IRR and in PRAKASH under WDS. Role

of POX is well defined in scavenging free radicals induced by WDS. The APX activity also showed increment in tolerant hybrids under WDS while a decrease was recorded in susceptible (Fig 1E). Again the increase in APX activity was highest in PRAKASH (33.3%) followed by BIO-9637 and lower in NK-6240 (10%) under WDS. Such increase or decrease in APX activity in maize seedlings of differential DT was reported (Chugh *et al.* 2011). The APX activity has also been reported to improve DT in tobacco (Lim *et al.* 2007), sweetpotato (Wang *et al.* 2005) and tomato (Sreenivasulu *et al.* 2007). Thus increase in APX activity of tolerant hybrids might be an adaptive response in maize seedlings though the activity of APX was higher in tolerant hybrids than susceptible under normal irrigation. This indicated that both inherent and acquired potential in APX activity were higher in tolerant than susceptible hybrids.

H₂O₂ and MDA contents

The H₂O₂ content increased in susceptible while decreased in tolerant hybrids under WDS (Fig 2A). The

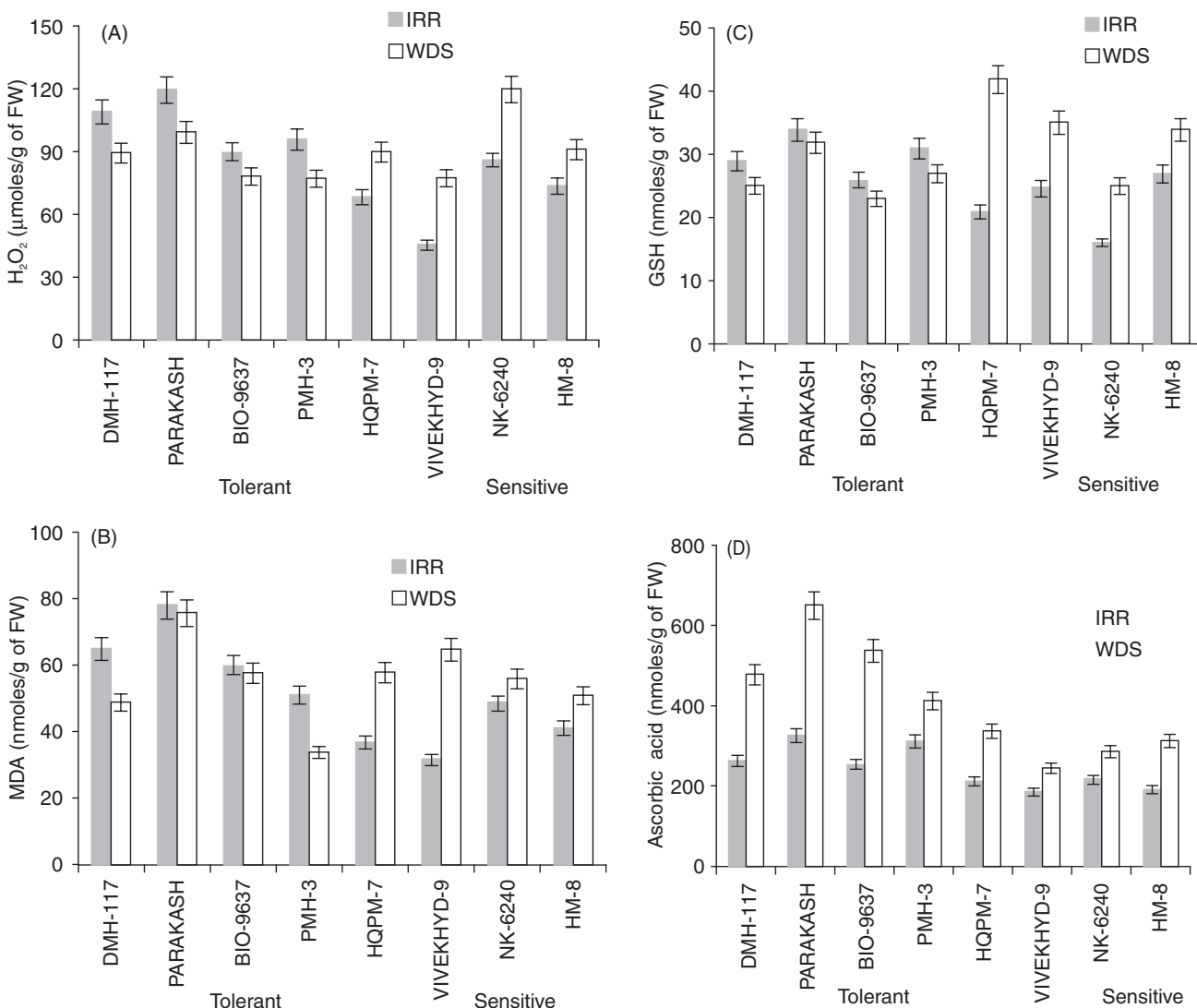


Fig 2 Changes in H₂O₂ (A), MDA (B), GSH (C) and ASA (D) content. Vertical bars show SE from mean of three replicates.

decrease or increase in H₂O₂ content however was higher in PRAKASH and NK-6240 hybrids, respectively, indicating less severe oxidative damage in tolerant hybrids. Higher H₂O₂ content in the leaves of susceptible maize seedlings under drought as compared to tolerant one has also been reported by Moussa and Abdel-Aziz (2008). It is known that H₂O₂ is a toxic compound that is produced as a result of scavenging of superoxide radicals. Its higher concentration is injurious to plant via lipid peroxidation and membrane injury.

Under WDS, MDA content increased in susceptible and decreased in tolerant hybrids though the level of decrease varied among the hybrids (Fig 2B). Thus it is clear that genetic response in decrease or increase in MDA content among tolerant and susceptible hybrids is distinct however, the level of increase or decrease varied. The content of MDA is often used as an indicator of lipid peroxidation in plant tissues, resulting from an oxidative stress induced by various abiotic stresses. Thus even a slight decrease in MDA content in tolerant hybrids suggests that these are better adapted at the cellular level with efficient free radical quenching system than susceptible.

GSH and ASA contents

In response to WDS, glutathione content (GSH) decreased (11%) in tolerant while increased (55%) in susceptible hybrids. Increase in GSH content however, it was highest (100%) in HQPM-7 followed by NK-6240 (56%) (Fig 2C). In general, ascorbic acid content (ASA) increased under WDS in both tolerant and susceptible hybrids however the increase was higher in tolerant (80.0%) than susceptible (45%) (Fig 2D). Among the hybrids highest increase was recorded in PRAKASH (99%) and lowest in VIVEKHYD-9 (29%), thus PRAKASH seems to be the drought tolerant while VIVEKHYD-9 the susceptible. Ascorbic acid plays an important role in protecting against oxidative stress by eliminating ROS through multiple mechanisms and also maintains membrane-bound antioxidant β -tocopherol in the reduced state. In addition, it indirectly eliminates H₂O₂ through activity of APX. Our results are in agreement with previous workers but these reports are on a limited number of genotypes such as one genotype from each tolerant and susceptible and two from tolerant and four from susceptible groups (Chug *et al.* 2011).

In conclusion, an experiment was conducted to analyse maize hybrids for antioxidant enzymes and non-enzyme compound at two leaf stages, under normal irrigation and water deficit stress. Water deficit stress was induced by withholding irrigation water for three days in seven day old seedlings grown in pots. The hybrids exhibited wide variability in their antioxidant pools combined with activities of enzymes involved in defence against oxidative stress. The distinct variation in the response of maize hybrid classifies them into two distinct groups, i.e. the tolerant and susceptible. The results showed that antioxidant activity was higher in tolerant hybrids than the susceptible hybrids. Thus the status of antioxidant enzymes could be

very useful tool for depicting DT in crops which could be useful to plant breeders for developing drought tolerant genotypes.

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