

## MODULATION IN WATER RELATIONS, CHLOROPHYLL CONTENTS AND ANTIOXIDANTS ACTIVITY OF MAIZE BY FOLIAR PHOSPHORUS APPLICATION UNDER DROUGHT STRESS

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### Abstract

A pot experiment was conducted to evaluate the foliar applied phosphorous on physiological and biochemical processes of maize under optimum moisture and drought conditions. The experiment was conducted in wire house Department of Agronomy, University of Agriculture Faisalabad, Pakistan. Four maize hybrids viz., 6525, 32B33, 31P41 and Hycorn were used as to evaluate foliar effect of P @ 8 kg ha<sup>-1</sup> at 8<sup>th</sup> leaf stage under normal and drought condition. The foliar applied P performed better than control treatments under normal and stress conditions. The foliar application of phosphorous @ 8 kg ha<sup>-1</sup> at 8<sup>th</sup> leaf stage of maize increased the water relation, chlorophyll contents and antioxidant under well watered and stress conditions. The water stress decreased the physiological parameters such as water potential (24%), osmotic potential (65%) turgor potential (5.4%), total chlorophyll contents (36%) and total carotenoids (34.7%) as compared to non-stress. The biochemical parameters such as catalase, peroxidase and ascorbate peroxidase activity were increased by 68%, 79.7% and 57.3% respectively under water stress condition as compared to non-stress condition. The both drought tolerant hybrids of maize (6525 and 32B33) performed better than Hycorn and 31P41 under normal and water stress conditions. The study provides insight into that P regulate physiological and biochemical mechanisms responsible for improved drought tolerance in maize.

**Key words:** Drought stress, Foliar phosphorus, Water relations, Antioxidants activity, Maize.

### Introduction

Drought is the most important abiotic stress factor that limiting the crop growth and production in favorable condition (Ahmad *et al.*, 2015; Waraich *et al.*, 2010, 2011). The drought also reduced the uptake of water as well as nutrients to the plant (Du *et al.*, 2010). In many developing countries the water stress is the major constraint to agricultural production and also reduced the quality, growth and production of crops (Ahmad *et al.*, 2015; Golbashy *et al.*, 2010; Waraich *et al.*, 2010, 2011; Hongbo *et al.*, 2005) Worldwide, drought is considered to be the leading cause of crops yield losses which reduces yield up to 50% depending upon its severity (Wang *et al.*, 2003). It is a recognized fact that a variety of metabolic and physiological processes in plants are impaired by drought (Levitt, 1980). Significant reduction in growth, chlorophyll and water contents along with different fluorescence parameters are changed with drought (Ekmekçi *et al.*, 2005; Mohsenzadeh *et al.*, 2006; Yang *et al.*, 2006). Impaired active transport, reduced transpiration and membrane permeability caused by drought result in decreased nutrients uptake due to decreasing power of roots to absorb water (Tanguilig *et al.*, 1987). Hura *et al.* (2007) and Efeoglu *et al.* (2009) studied physiological response of maize cultivars under drought stress condition in south of Poland and Turkey respectively. He concluded that the chlorophyll *a*, *b*, total *a+b* and carotenoid contents of maize cultivars were significantly reduced under drought while Medici *et al.* (2003) reported that the drought stress in both vegetative and reproductive phases significantly decreased the relative leaf contents

and leaf osmotic potential of plants. Under water stress conditions the reactive oxygen species (ROS) are produced and these ROS cause the serious problems inside the plants (Waraich *et al.*, 2011). Antioxidants like catalase and peroxidase and proline, sugar act as scavenger and play an important role in ensuring the survival of plant by their stimulating actions.

Maize is the third important cereal crop in the world after wheat and rice on the basis of area and production (Tollenaar & Dwyer, 1999). In addition to use as food and feed for livestock and poultry, maize grains are also utilized in many other commercial and industrial products. For human consumption it is processed into a lot of products such as corn flour, pop corns, gruels, porridges, bread, beverages, snacks and pastes (Ortiz-Monasterio *et al.*, 2007; Menkir, 2008). The plants grow under well watered condition performed better however, the poor performance of reducing growth and yield was reported by many researchers under water stress conditions (Nezami *et al.*, 2008)

Phosphorus (P) is the 2<sup>nd</sup> major nutrient after nitrogen (N) and it is deficit in Pakistan soils. The growth and yield of many field crops are enhanced by the application of P and also increased the root growth in many crops under water stress condition (Yaseen & Malhi, 2009). The phosphorus application decreases the effect of drought in crops (Sing *et al.*, 1981). Phosphorus is an essential element for all living organisms and involved in nucleic acid and phospholipids synthesis. It also activates many enzymes (Lambers *et al.*, 2006). Phosphorus also plays a key role in energy transfer and is thus essential for photosynthesis under drought condition. The deficiency of P causes net photosynthesis reduction and decreased shoot and root biomass production in maize crop

(Wissuwa *et al.*, 2005). Leaf growth depression under P deficiency is well documented (Kavanova *et al.*, 2006). Phosphorus deficiency affects the rate of emergence and number of maize adventitious nodal roots (Pellerin *et al.*, 2000; Kavanova *et al.*, 2006). Foliar application of phosphorus could improve phosphorus use efficiency (PUE) by minimizing soil application in maize, when supply of nutrients either become deficient or needed the most under water stress condition (Girma *et al.*, 2007). A reasonable research work on improving efficiency of soil applied phosphorus under drought stress has been reported in the literature, however very little information is available on the interactive effect of foliar applied and soil applied phosphorus in alleviating the adverse effect of drought stress especially in maize. Keeping in view the food security and importance of phosphorus in maize, we performed a unique study on the response of foliar applied phosphorus on physiological and biochemical activity of maize plants under the drought stress.

### Material and Methods

A pot study was conducted to investigate the physicochemical responses to foliar applied phosphorus on the four maize hybrids (Hycorn, 32P33, 6525 and 31P41) under well watered and water stress (stress applied at 8<sup>th</sup> leaf stage) condition. Experiment was performed in the wire house of Department of Agronomy, University of Agriculture, Faisalabad, Pakistan during March 2014. The experiment was laid out in Randomized complete block design (RCBD) with factorial arrangement and with three replications. Four maize hybrids (Hycorn, 32B33, 6525 and 31P41) and two doses of foliar phosphorus (control and  $\text{KH}_2\text{PO}_4$  @ 8 kg ha<sup>-1</sup>) were applied under normal and water stress condition.

**Plant material and growth conditions:** The experiment was conducted in wire house and sand was used as a growth medium. The sand was initially sun dried, ground, sieved and mixed well in order to avoid any plant residues and 4 kg sand was filled carefully in each pot. Five seeds were sown in each pot and then watered with distilled water. In the beginning all pots were kept at field capacity level for obtaining good germination and emergence. Later on the water was applied according to the water stress levels specified for the experiment. Before imposing water stress the plants were thinned out and three healthy plants were kept in each pot. Recommended doses of NPK were applied in solution form at the time of planting, but N was applied after every 2 weeks.

At 8th leaf stage the pots were divided into two groups i.e., stress and non-stressed. The above following condition was maintained for one week there after applying the supplemental foliar P @ 8 kg ha<sup>-1</sup> at 8<sup>th</sup> leaf stage. For each treatment pots were weighed daily at about 9:00 am, calculated the amount of water consumed in evapotranspiration and watered until the pot weight reached to pre-determined weight. Plants were grown up to 40 days and data regarding various physiological and biochemical parameters were recorded using standard recommended methods.

**Water relations:** The third leaf from top (fully expanded youngest leaf) of plants from each treatment was used to determine the leaf water potential using Scholander type pressure chamber. The same leaf used for water potential, was frozen at -20°C for recoding osmotic potential using the method described by Nawaz *et al.* (2015a). Turgor potential was calculated as the difference between osmotic potential ( $\psi_s$ ) and water potential ( $\psi_w$ ) values.

$$(\psi_p) = (\psi_w) - (\psi_s)$$

Fully expand young leaf were taken from three plants of each treatment. Fresh weight (FW) of each sample was taken on digital electrical balance and dipped in test tube containing distilled water for 24 hours. Then it was wiped with the tissue paper and turgid weight (TW) was taken. The samples were dried at 65°C for 72 hrs and dry weight (DW) of each sample was taken. For each treatment, RWC was calculated by using the formula (Karrou & Maranville, 1995) given below:

$$\text{RWC} = [(\text{FW}-\text{DW}) / (\text{TW}-\text{DW})] \times 100$$

**Chlorophyll contents:** Chlorophyll contents were calculated by using the method of Arnon (1949) and Davies (1976). Fresh leaves of (0.5 g) were chopped into segments of 0.5 cm and extracted with 5 mL acetone (80%) at 10°C overnight. Centrifuge the material at 14000 rpm for 5 min. and measured the absorbance of the supernatant at 645, 652 and 663 nm on spectrophotometer. Calculated *a*, *b* and total chlorophyll.

$$\begin{aligned} \text{Chl } a &= [12.7 (\text{OD } 663) - 2.69 (\text{OD } 645)] \times V/1000 \times W \\ \text{Chl } b &= [22.9 (\text{OD } 645) - 4.68 (\text{OD } 663)] \times V/1000 \times W \\ \text{Total Chl} &= [20.2 (\text{OD } 645) + 8.02 (\text{OD } 663)] \times V/100 \times W \end{aligned}$$

$$\text{Carotenoids (g.mL}^{-1}\text{)} = \text{Acar}/\text{Emx}100$$

where V is the volume of sample extract and W is the weight of the sample

$$\begin{aligned} \text{Acar} &= (\text{OD}480) + 0.114 (\text{OD}663) - 0.638 (\text{OD}645); \\ \text{Emx}100 \text{ cm} &= 2500 \end{aligned}$$

**Assay of antioxidant:** Catalase activity (CAT) and peroxidase (POX) activity was determined according to the method of Chance & Maehly (1955), where-as the reports publish by Cakmak (2000) and Giannopolitis and Ries (1997) were used to record ascorbate peroxidase (APX) activity.

**Statistical analyses:** The recorded data were analyzed statically using analysis of variance technique by using MSTAT-C software. Least significant difference (LSD) test at 5% probability level was used to compare the significant mean.

### Results

**Water relations:** The leaf water potential ( $\Psi_w$ ), leaf osmotic potential ( $\Psi_s$ ), turgor potential ( $\Psi_p$ ) and relative water contents (RWC) were significantly

( $p < 0.01$ ) decreased by water stress in all four maize hybrids (Table 1). The decrease in leaf water potential, leaf osmotic potential, turgor potential and relative water contents were more pronounced in water stressed plants as compared to normal irrigated plants (Fig. 1 a, b, c, d). The water stress at 8<sup>th</sup> leaf stage of maize reduced leaf water potential (24%), leaf osmotic potential (65%), turgor potential (5.4%) and relative water contents (26.25%) as compared to non-stress plants. The folia applied P @ 8 kg ha<sup>-1</sup> at 8<sup>th</sup> leaf stage of maize significantly affect all hybrids. The 6525 and 32B33 hybrid performed better under stress as well non stress condition. The maximum value of  $\Psi_w$  (0.79, 1.12 & 0.83, 1.14 -MPa) and  $\Psi_s$  (0.34, 0.52 & 0.38, 0.56 -MPa) were recorded in 6525 and 32B33 where foliar applied P @ 8 kg ha<sup>-1</sup> at 8<sup>th</sup> leaf stage while the minimum value  $\Psi_w$  (1.10, 1.27 -MPa) and  $\Psi_s$  (0.46, 0.86 -MPa) were recorded in Hycorn under non stress and stress condition (Fig. 1a, b).

Similarly the highest value of  $\Psi_p$  and RWC were observed under well watered condition while lowest value of  $\Psi_p$  and RWC were recorded under stress condition. The 6525 and 32B33 hybrid performed better and recorded more  $\Psi_p$  (0.45, 0.60 & 0.45, 0.58 -MPa) and RWC (355.40, 243.34 & 335.47, 235.40%) where foliar applied P @ 8 kg ha<sup>-1</sup> at 8<sup>th</sup> leaf stage of maize as compared to other hybrids (Fig. 1c, d) under normal and stress conditions. Minimum values of  $\Psi_p$  (0.64, 0.41 -MPa) and RWC (188.69, 139.65%) were observed in control treatment where no foliar applied P under well watered and stress conditions (Fig. 1c, d).

All the interactions such as H x T and H x W x T were non-significant (Table 1).

**Chlorophyll contents:** Drought stress significantly reduced ( $p < 0.001$ ) the chlorophyll *a* and chlorophyll *b* contents (Table 2). The water deficit conditions at 8<sup>th</sup> leaf stage significantly decreased and chlorophyll *a* and chlorophyll *b* by 32.2% and 41.6% as compared to normally irrigated (control) plants (Fig. 2a, b). Maize hybrids 6525 and 32B33 performed better than 31P41 and Hycorn. The maximum chlorophyll *a* and chlorophyll *b* contents (1.13, 0.84 & 0.71, 0.47 mg g<sup>-1</sup> FW) were observed in hybrid 6525 where foliar applied P @ 8 kg ha<sup>-1</sup> at 8<sup>th</sup> leaf stage of maize while the minimum chlorophyll *a* and chlorophyll *b* contents (0.76, 0.50 & 0.50, 0.23 mg g<sup>-1</sup> FW) were observed in Hycorn under well watered and stress conditions (Fig. 2a, b).

The total chlorophyll contents and total carotenoids were significantly ( $p < 0.01$ ) decreased by water stress in all four maize hybrids (Table 2). The decrease in total chlorophyll contents and total carotenoids were more pronounced in water stressed plants as compared to normal irrigated plants (Fig. 2c, d). The water stress at 8<sup>th</sup> leaf stage of maize reduced total chlorophyll contents and total carotenoids by 36% and 34.7% as compared to normally irrigated plants (Fig. 2c, d). Maize hybrids 6525 and 32B33 performed better than 31P41 and Hycorn. The maximum total chlorophyll contents and total carotenoids (3.16, 2.00 & 0.32, 0.20 mg g<sup>-1</sup> FW) were observed in hybrid 6525 where foliar applied P @ 8 kg ha<sup>-1</sup> at 8<sup>th</sup> leaf stage of maize while the minimum total chlorophyll contents and total carotenoids (3.16, 2.00 & 0.32, 0.20 mg g<sup>-1</sup> FW) were observed in Hycorn in control treatment under well watered and stress conditions (Fig. 2c, d).

All the interactions were non-significant (Table 2).

**Table 1. Analysis of variance table for water potential, osmotic potential, turgor pressure and relative water contents of four maize hybrids in well-watered and water stress conditions with supplemental foliar applied phosphorus.**

SOV	Water potential (-MPa)	Osmotic potential (-MPa)	Turgor pressures (MPa)	Relative water contents (%)
Hybrids (H)	***	***	NS	***
Water levels (W)	***	***	NS	***
Treatments (T)	***	**	**	***
H x W	*	NS	NS	NS
H x T	NS	NS	NS	NS
W x T	**	*	*	NS
H x W x T	NS	NS	NS	NS

\*, \*\*, \*\*\* = Significant at 0.05, 0.01 and 0.001 level respectively; NS = Non significant

**Table 2. Analysis of variance table for chlorophyll *a*, chlorophyll *b*, total chlorophyll contents and total carotenoids of four maize hybrids in well-watered and water stress conditions with foliar applied phosphorus.**

SOV	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total chlorophyll contents	Total carotenoids
Hybrids (H)	***	NS	***	***
Water levels (W)	***	**	***	***
Treatments (T)	***	***	***	***
H x W	NS	NS	NS	NS
H x T	NS	NS	NS	NS
W x T	NS	NS	NS	NS
H x W x T	NS	NS	NS	NS

\*, \*\*, \*\*\* = Significant at 0.05, 0.01 and 0.001 level respectively; NS = Non significant

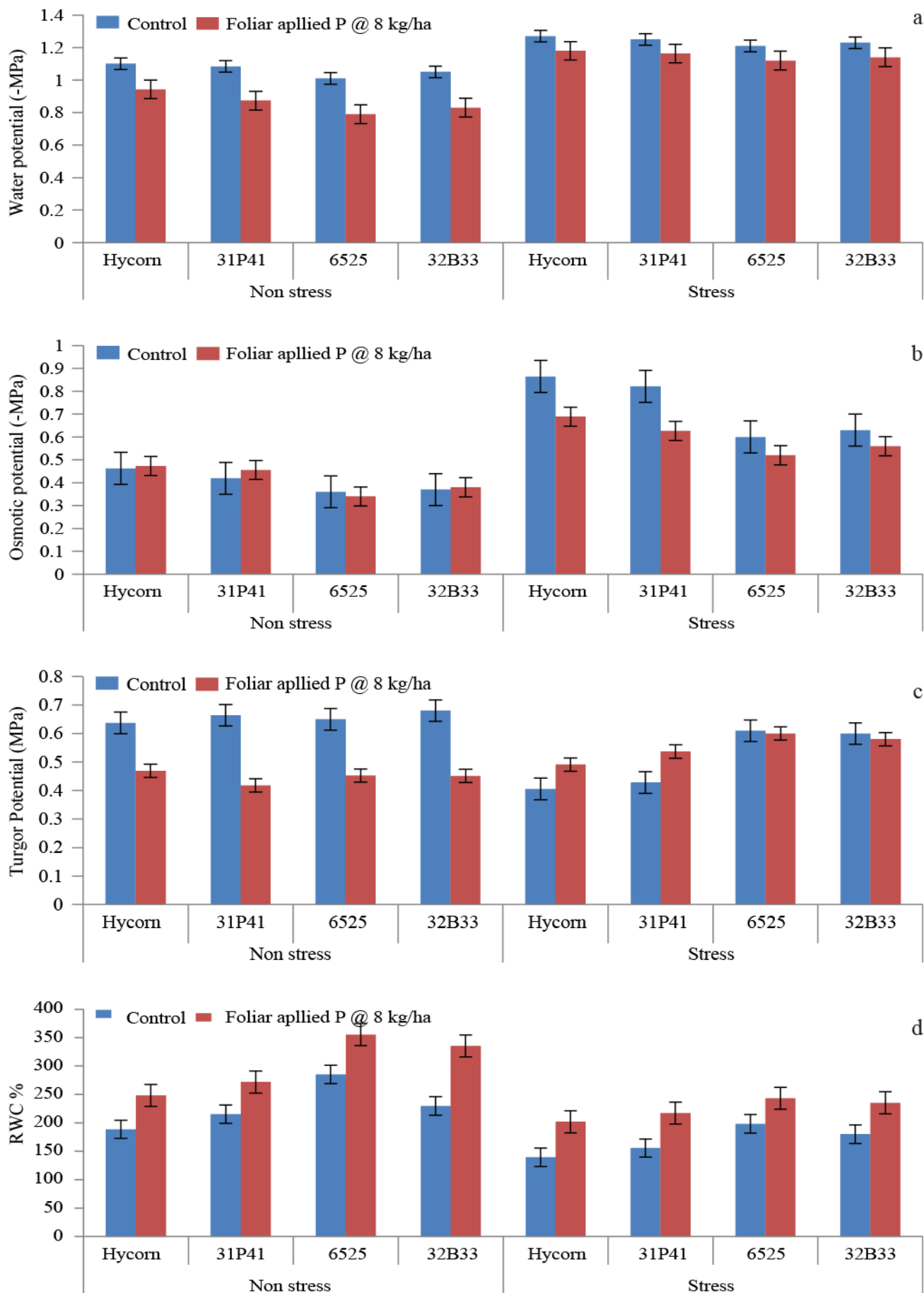


Fig. 1(a, b, c, d). Effect of supplemental foliar phosphorus application on leaf water potential, osmotic potential, turgor potential and relative water contents (%) of four maize hybrids grown under normal and water stress conditions

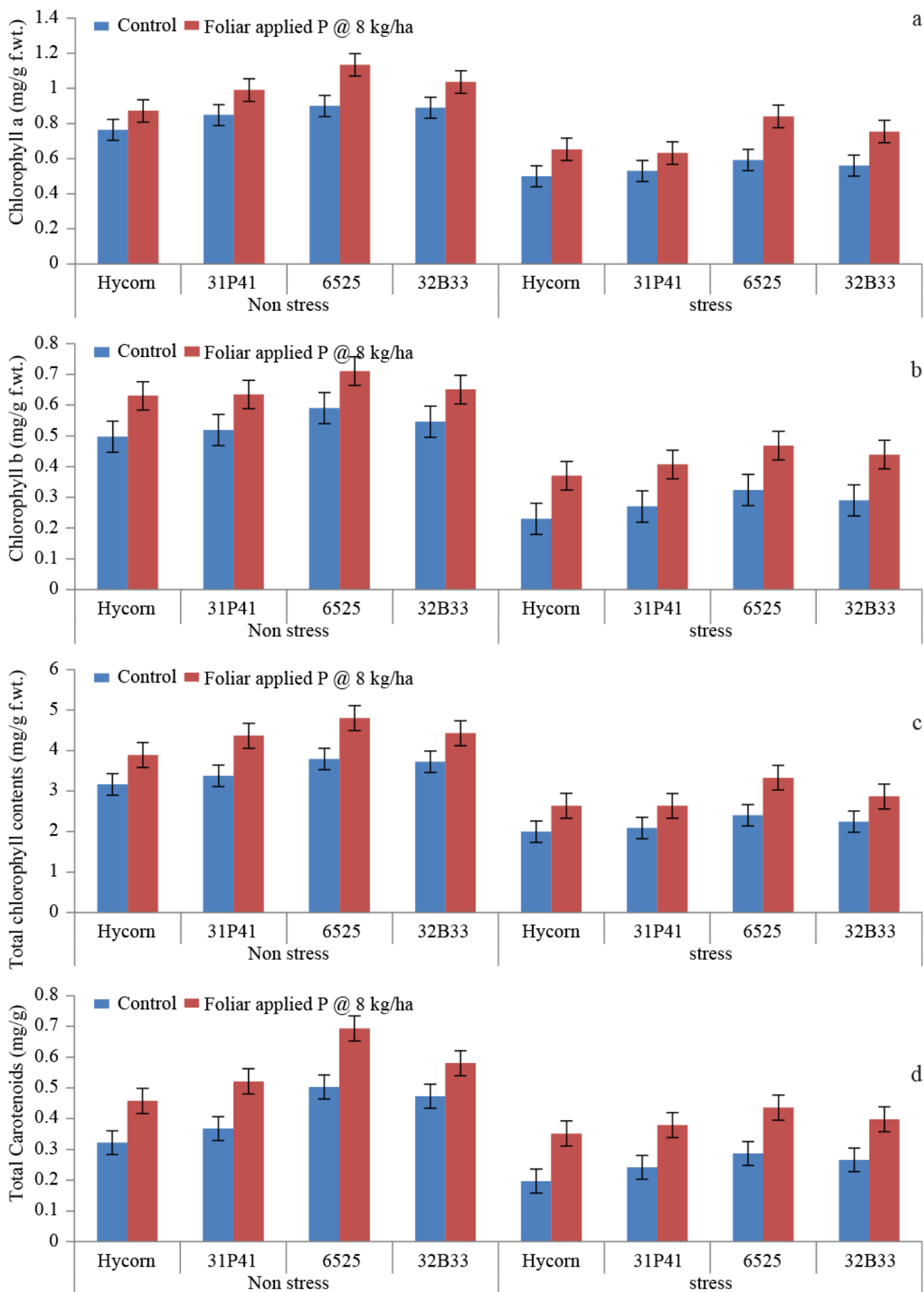


Fig. 2(a, b, c, d). Effect of supplemental foliar phosphorus application on chlorophyll a, chlorophyll b, total chlorophyll contents and total carotenoids of four maize hybrids grown under normal and water stress conditions.

**Table 3. Analysis of variance table for catalase activity, peroxidase activity and ascorbate peroxidase activity of four maize hybrids in well-watered and water stress conditions with foliar applied phosphorus.**

SOV	Catalase activity	Peroxidase activity	Ascorbate peroxidase activity
Hybrids (H)	***	***	***
Water levels (W)	***	***	***
Treatments (T)	***	***	***
H x W	NS	**	NS
H x T	NS	NS	NS
W x T	NS	*	NS
H x W x T	NS	NS	NS

\*, \*\*, \*\*\* = Significant at 0.05, 0.01 and 0.001 level respectively; NS = Non significant

**Antioxidant enzymes activity:** Drought stress significantly enhanced ( $p < 0.001$ ) the catalase, peroxidase and ascorbate peroxidase activity (Table 3). The water deficit conditions at 8<sup>th</sup> leaf stage significantly increased the catalase, peroxidase and ascorbate peroxidase activity by 68%, write value % and 57.3% as compared to normally irrigated (control) plants (3 a, b, c). Under both conditions (well watered and stress conditions) the hybrids 6525 and 32B33 performed better than 31P41 and Hycorn where foliar applied P @ 8 kg ha<sup>-1</sup> at 8<sup>th</sup> leaf stage of maize. The maximum catalase activity (192.13, 67.93 units min<sup>-1</sup> g<sup>-1</sup> FW), peroxidase activity (224.78, 47.53 units min<sup>-1</sup> g<sup>-1</sup> FW) and ascorbate peroxidase activity (4.99, 2.87 units' min<sup>-1</sup> g<sup>-1</sup> FW) were observed in hybrid 6525 where foliar P @ 8 kg ha<sup>-1</sup> at 8<sup>th</sup> leaf stage of maize was applied while the minimum catalase activity (145.37, 41.57 units min<sup>-1</sup> g<sup>-1</sup> FW), peroxidase activity (157.44, 28.86 units min<sup>-1</sup> g<sup>-1</sup> FW) and ascorbate peroxidase activity (2.91, 0.90 units' min<sup>-1</sup> g<sup>-1</sup> FW) was observed in Hycorn in control treatment under stress and well watered conditions (Fig. 3a, b, c).

All the interactions were non-significant (Table 3).

## Discussion

Foliar application of P @ 8 kg ha<sup>-1</sup> at 8<sup>th</sup> leaf stage of maize significantly improved the all water relation parameters such as water potential, osmotic potential, turgor pressures and relative water contents in all hybrids (6525, 32B33, Hycorn and 31P41) under well watered as well as water stress conditions. The foliar spray of P helped in maintaining the water status of plants possibly through osmotic adjustment, the accumulation of organic and inorganic ions such as free amino acids and proline (Shabala & Lana, 2011).

Osmotic adjustment helps the plants in the maintenance of water uptake under drought stress (Chen & Jiang, 2010; Abdelmalek & Khaled, 2011). It is reported that decrease in leaf water potential and increase in bulk modulus elasticity (a ratio of normal stress to a change in volume) together with decrease in osmotic potential maintain the plant turgor potential (Saito & Terashima, 2004). The maintenance of turgor by active lowering of osmotic potential ( $\Psi_s$ ) is generally considered as an adaptation of plants under water limited environment (Ludlow & Muchow, 1990). The plants exposed to drought stress had more negative leaf water potential ( $\Psi_w$ ) than the normal plants. The plants tend to

maintain favourable water relations that help to develop resistance against drought stress (Passioura & Fry, 1992; Kaldenhoff *et al.*, 2008). Available literature indicated variation between drought tolerant and susceptible genotypes which may be due to the maintenance of tissue turgor, physiological activities, water uptake from soil and reduction in water loss through stomata (Song *et al.*, 1995; Siddique *et al.*, 2000; Terzi & Kadioglu, 2006). The results of present experiment revealed that foliar applied P resulted in significantly advantageous response for all biochemical characteristics such as total chlorophyll content and phosphorus use efficiency in wheat. A significant increase in chlorophyll contents due to phosphorus application has, in fact, been observed by Thapar *et al.* (1990), Lopez- Cantarero *et al.* (1994) and Shubhra *et al.* (2003, 2004) in several plants suggesting that the biosynthesis of the pigment molecules was dependent on the uptake of P within optimum limits.

The antioxidant enzymes such as peroxidase (POD), catalase (CAT) and Ascorbate peroxidase (APX) were produced under water stress conditions (Li *et al.*, 2009). Ascorbate peroxidase (APX) is one of the most important antioxidant enzyme involved in the scavenging of ROS to protect cells of higher plants (Gill & Tuteja, 2010). In present study, foliar application of P increased the activity of antioxidants including catalase, peroxidase, and ascorbate peroxidase under stress conditions. Increase in antioxidant enzymes is the most efficient mechanism against oxidative stress (Farooq *et al.*, 2008). Drought tolerant maize genotypes produced high amount of ascorbate peroxidase and catalase under water stress condition as compared to drought sensitive ones (Sairam *et al.*, 1998). Nikolaeva *et al.* (2010) stated that APX activity varies in different wheat cultivars and depends on duration of drought and stage of leaf development. They observed that mild drought stress enhanced APX activity in leaves but prolonged drought reduced its activity due to increase in malonic dialdehyde (MDA) content. The present study showed that the supplemental foliar applied P @ 8 kg ha<sup>-1</sup> at 8<sup>th</sup> leaf stage enhanced the activity of different antioxidant's under stress condition as compared to well watered condition in maize. Both maize hybrids 6525 and 32B33 performed better than Hycorn and 31P41. CAT activity is enhanced in high light conditions under drought stress (Yang *et al.*, 2008). Pan *et al.* (2006) evaluated the combined effect of drought and salt stress and reported a decrease in CAT activity in *Glycyrrhiza uralensis* seedlings.

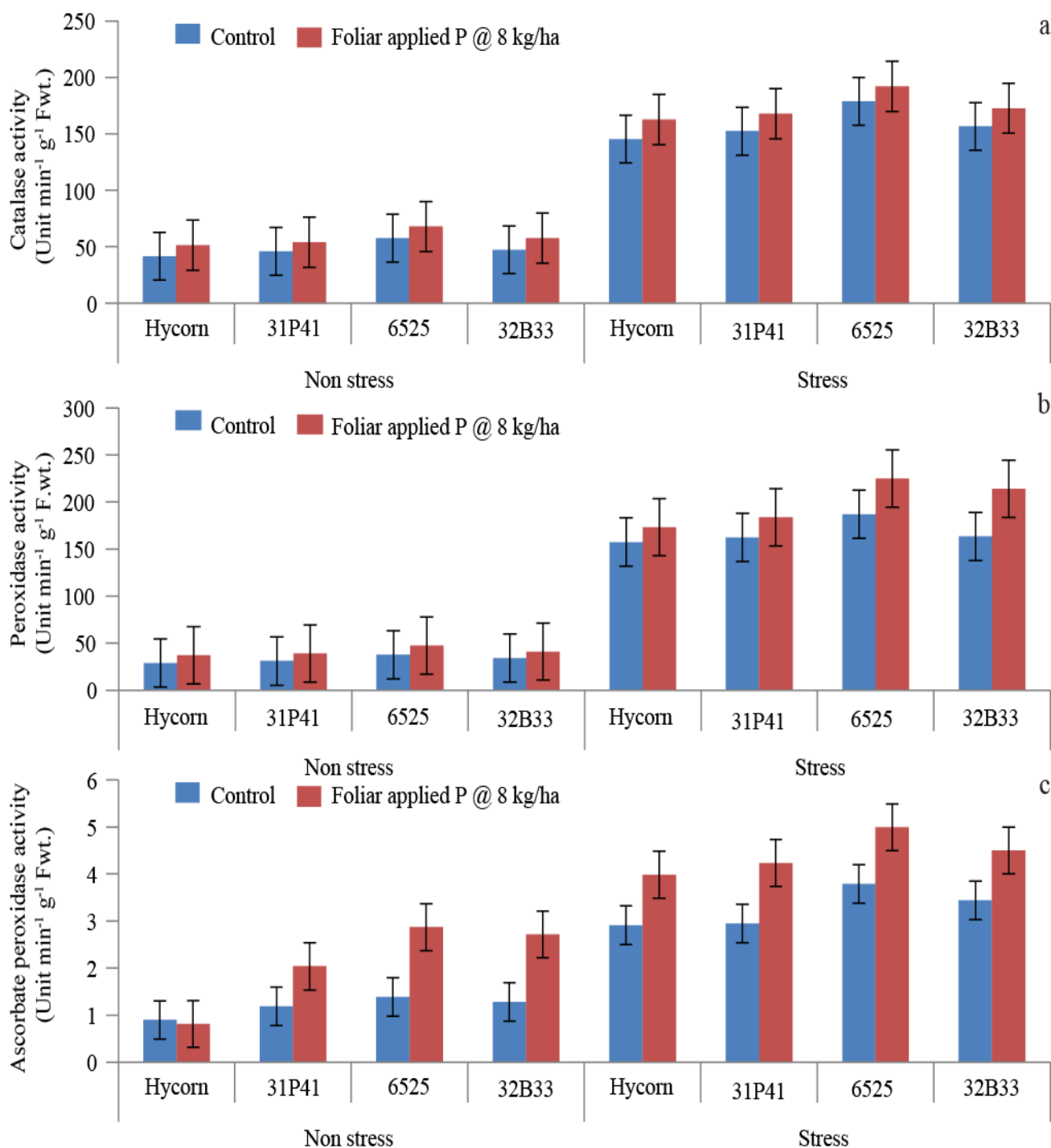


Fig. 3(a, b, c). Effect of supplemental foliar phosphorus application on catalase activity, peroxidase activity and ascorbate peroxidase activity of four maize hybrids grown under normal and water stress conditions.

**Conclusion**

In this study, foliar application of P @ 8 kg ha<sup>-1</sup> at 8<sup>th</sup> leaf stage of maize significantly improved the water relations, chlorophyll contents and antioxidants activity of the all maize hybrids under well watered and stress conditions. The both maize hybrids 6525 and 32B33 performed better than 31P41 and Hycorn under normal and water stress conditions. We concluded that the hybrid 6525 performed better when P @ 8 kg ha<sup>-1</sup> was applied at 8<sup>th</sup> leaf stage of maize under well watered and stress conditions.

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