INT. J. BIOL. BIOTECH., 13 (4): 561-570, 2016.

EVALUATION OF MAIZE VARIETIES BASED ON ANTIOXIDANT SYSTEM IN RESPONSE TO DROUGHT STRESS

Ahmad Nawaz Khan[1], Samran Durrani[1], Muhammad Daud Khan[2], Abdullah Jalal[3] and Nisar Ahmad*[1]

[1]*Department of Biotechnology, University of Science & Technology Bannu, Pakistan*
[2]*Department of Biotechnology, Kohat University of Science & Technology, Pakistan*
[3]*Institute of Biotechnology & Genetic Engineering, University of Agriculture Peshawar, Pakistan*
*corresponding author

ABSTRACT

Maize is the most important crop plant that provides food for human consumption. With increasing temperature due to global climate changes, the availability of water in soil declines and is considered as a major threat to crop plants. Thus, the development of drought tolerant varieties is pre-requisite to cope with the water shortage situation. In this study, we analyzed the response of two maize varieties (Azam and Baber) to drought stress using antioxidant system. The imposition of drought for 7 days resulted in decrease in fresh weight of leaf and total proteins. ROS such as H_2O_2 were strongly accumulated whereas MDA level was also increased indicating the ROS based lipid peroxidation under drought stress. With the increase in the level of H_2O_2, antioxidant enzymes such as APX, CAT, SOD and POD also showed significant increase as compared to the control leaves. Thus, the tested maize varieties responds to drought with enhanced enzymatic activities of the defense mechanism to cope with the elevated level of ROS and can be considered as drought tolerant based on possession of well established antioxidant system.

Key-words: *Zea mays*, drought, stress markers, reactive oxygen species, antioxidant enzymes.

Abbreviations: ROS, reactive oxygen species, H_2O_2, hydrogen peroxide, MDA, Malondialdehyde, APX, ascorbate peroxidase, CAT, catalase, SOD, superoxide dismutase, GR, glutathione reductase, GSH, reduced glutathione, GSSG, oxidized glutathione, RWC, relative water content, ABA, abscisic acid,

INTRODUCTION

Most of the crop plants are exposed to abiotic stress such as drought that has detrimental effect on growth. Drought results from climate change, water shortage and much water use (Mittler and Blumwald, 2010; Hu and Xiong, 2014). Drought frequency and severness increases as the global warming and climate may increase and carbon dioxide (CO_2) increase day by day (IPCC, 2013). As a result, mean earth's temperature is increasing (Rahmstorf *et al.*, 2007), with heat waves in different regions of world especially in Pakistan (Meehl and Tebaldi, 2004). Drought is considered as a severe threat for plants. It is the deficiency of sufficient moisture essential for a plant to grow habitually and complete its life cycle (Zhu, 2002). Plants are more susceptible to drought and climate change conditions especially C_3 and C_4 plants in which the photosynthetic carbon rapidly decreases as a result of stomatal closure (Hsiao, 1973; Lawlor and Cornic, 2002), ultimately leading to decrease in plant productivity. Root system play important role to identify water shortage at first glance and accordingly passes signal to the leaves via xylem sap. One of the major root-to-shoot signals are mediated by phytohormone abscisic acid (Brunner and Godbold, 2007). In response to these signals, leaves are able to minimize the water loss by reducing the transpiration flux through stomata closure and the entrance of CO_2 is also limited. As a result, the net photosynthesis is reduced leading to accumulation of reactive oxygen species (ROS) by plants due to metabolic imbalance during drought stress (Mittler, 2002).

Various complex mechanisms are involved in drought tolerance (Beck *et al*., 2007; Anjum *et al*., 2011). Abscisic acid (ABA) plays a vital role to cope with different stresses by promoting closure of stomata (Shinozaki and Yamaguchi-Shinozaki, 2007). In addition, water hydraulic conductivity and damages to chlorophyll pigment is enhanced that ultimately leads to leaf senescence (Lim *et al*., 2007). Photosynthetic pigments are crucial for plant and a substantial damage has been observed in numerous reports upon drought (Din *et al*., 2011). During water shortage, it has been observed that the reduction of Chl b is more than that of Chl a (Jaleel *et al*., 2009).

ROS are the chemical species that are formed upon partial reduction of oxygen and includes the superoxide anion (O_2-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (HO•). These free radicals have very short half-life, high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids (Kokate *et al.*, 2004).

ROS are expected to arbitrate the toxicity of oxygen because of their greater chemical reactivity with respect to oxygen (Nathan, 2003).

Plants possess very efficient antioxidant defense system including non-enzymatic and enzymatic components to detoxify or minimize the detrimental effect of ROS (Pang and Wang, 2008). These antioxidants protect the plant growth and development by reducing the toxic effect of free radicals (De Pinto and De Gara, 2004). They combine with different cellular components and in addition play an important role in ROS scavenging.

Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR). SODs remove O_2 by catalyzing its dismutation, one O_2 being reduced to H_2O_2 and another oxidized to O_2 (Mittler *et al*., 2004; Singh *et al*., 2008). CAT is important in the removal of H_2O_2 generated in peroxisomes by oxidases involved in b-oxidation of fatty acids, photorespiration and purine catabolism (Polidoros and Scandalios, 1999). GR catalyses the NADPH dependent reaction of disulphide bond of GSSG and is thus important for maintaining the glutathione (GSH) pool (Reddy and Raghavendra, 2006; Chalapathi and Rao, 2008).These enzymes are present in different subcellular organelles of the cell and activates in response to oxidative stress (Noctor and Foyer, 1998).

In this study, we investigated the response of antioxidant system to prolonged drought stress in leaf of maize.

MATERIAL AND METHODS

Seeds Collection

Healthy seeds of two maize varieties i.e. Azam and Baber were obtained from Cereal Crop Research Institute (CCRI) Pirsabak, Nowshera Khyber Pakhtunkhwa, Pakistan for this study.

Germination and growth conditions

The current study was conducted in the Green House of University of Science and Technology Bannu. The seeds of the maize cultivars were grown in pots containing clay and sand soil (1:1 ratio). Pots were watered uniformly from germination to the three-leaf stage to maintain near full soil water capacity. After two weeks of sowing, the maize seedlings were exposed to water stress conditions. Control plants were applied with water regularly whereas drought stressed plants were without water for 7 days.

Determination of Relative water content (RWC)

RWC was determined by taking the fresh weight (FW) of leaves of control and drought-stressed plants of both varieties. Afterwards, the leaves were kept in water at 4^oC for 4 hrs to record the turgid weight (TW). To further determine the dry weight (DW), the rehydrated leaves were kept in oven for 2 days at 80^oC. Relative water content was determined by using formula;

RWC (%)= (FW-DW)/(TW –DW)*100

Determination of Photosynthetic pigments

Approximately 25 mg of leaf material of control and drought-stressed plants was taken and then ground to a fine powder. Magnesium oxide (MgO) was added to the grinded material in order to prevent the formation of pheophytin and neutralize acid. After the addition of 5 ml of methanol to the tube containing sample and MgO, the sample was then homogenized on a shaker for 2 h followed by centrifugation at 4000 rpm for 5 min at room temperature. The supernatant was transferred to cuvette and absorbance readings at three different wavelengths (653 and 666 nm) were taken against a solvent blank using spectrophotometer. Chlorophyll a and b were calculated according to Lichtenthaler and Wellburn (1983) by a formula as stated below.

Chlorophyll a= 15.65 A_{666}– 7.340 A_{653}

Chlorophyll b= 27.05 A_{653}– 11.21 A_{666}

Determination of Sodium (Na^+), Potassium (K^+) and Calcium (Ca^{+2})

Approximately, 25 mg powdered dry materials of leaf samples were digested continuously with a mixture of hydrogen peroxide (H_2O_2) and sulphuric acid (H_2SO_4) with a ratio of 2:1in a 50 mL$_{beaker}$. The mixture was then heated until the small oily drops were obtained. 20 mL of distilled water was then added to each sample along with shaking of beakers to digest completely. Samples were then filtered with Whatman filter paper. The prepared samples were subjected to flame photometer for determination of different ions according to Rudge *et al*. (2009).

Assays for determination of ROS scavenging enzymes

Various antioxidant activities were determined by taking 0.5g of grinded leaf material and homogenized in an ice cold 50mM phosphate buffer (pH7.8) with mortar and pestle. After vertexing, the crude enzyme extract was twicely centrifuged at 4°C at 12000 rpm for 15 minutes. Afterwards the supernatant was analyzed for various antioxidant enzymes.

Determination of oxidative stress markers (MDA and H_2O_2)

H_2O_2 was quantified as described by Velikova *et al*. (2000). A mixture containing 1ml of enzyme extract, 1mL of 10mM potassium phosphate buffer (pH 7.0) and 2 mL of 1M KI. H_2O_2 contents were calculated based on the absorbance at 390nm. Amount of H_2O_2 produced was expressed as µmol/g FW.

Lipid peroxidation in terms of malondialdehyde (MDA) was determined by the method of Daud *et al*. (2013). Briefly, 1 mL enzyme extract was added to 2 mL reaction mixture containing 0.5 % (v/v) thiobarbituricand 10 % (v/v) trichloroacetic acid. The mixture was then placed at 95°C in water bath for 30 minutes and then immediately kept on ice for 15 minutes. Afterwards, samples were centrifuged at 4000 rpm for 15 minutes followed by the measurement of absorbance at 532 and 600 nm.

Determination of Ascorbate peroxidase (APX) activity

APX activity was determined according to Nakano and Asada 1981. First of all a reaction mixture of 3 mL was prepared containing 2.7 mL of 50 mM potassium phosphate (pH7.0), 0.1 mL of 0.5 mM ascorbic acid, 0.1 mL of 2 % H_2O_2, and 0.1 mL enzyme extract. Afterwards, absorbance was recorded at 290 nm for 1 minute.

Determination of catalase (CAT)activity

Catalase activity was determined according to Daud *et al.* (2013). Briefly, the disappearance of H_2O_2 was monitored by measuring the absorbance at 240 nm (E=0.036 $mM^{-1}cm^{-1}$) of a reaction mixture consisting of 25 uL of 10 mM H_2O_2, 100 uL enzyme extract, and 2.7 mL of 25 mM potassium phosphate buffer. The final activity was expressed as mM/g FW.

Determination of superoxide dismutase (SOD) activity

SOD activity was determined by spectrophotometer assay according to Zhou *et al*.(1997). First of all, a reaction mixture of 3 ml was prepared in beaker containing 2.725 mL reaction substrate (NBT 15.5 mg, Riboflavin 0.02 mg, Na EDTA 10 mg and methionine 485 mg in 250 mL), 25 µL enzyme extract and 25 µL $H_20_2$and then were placed under light condition at 4000 lux for 20 minutes, control sample was placed at both under dark and light condition. In control samples 25 µL distilled water was used instead of enzyme extract. Absorbance was measured at 560 nm.

Determination of peroxidase (POD) activity

POD activity was measured by the method as described by Zhou *et al*. (1997). The reaction mixture contained 100 µL enzyme extract, 2.7 mL of 50mM potassium phosphate buffer (pH 6.1), 100 µL of 1.5% guaiacol (used as a substrate), 100 µLof 0.4 % H_2O_2. Increase in the absorbance was measured at 470 nm. Enzyme activity was calculated at 470 nm absorbance.

Determination of total soluble proteins

Proteins were determined according to Bradford (1976). Fresh leaves (100 mg) were homogenized in 1mL phosphate buffer (pH 7.0) by using mortar and pestle. The crude homogenate was centrifuged for 15 minutes at 4000 rpm. In a reaction mixture, 2 mL distilled water, 20 µL enzyme extract and 0.5ml Bradford reagent was added. Absorbance was noted at 595 nm by spectrophotometer (UV-2600) using bovine serum albumin (BSA) as a standard.

Statistical analyses

Statistical analysis was performed using unpaired t-test. Sigma Stat 12.0 was used for checking the constant variance and normal distribution of data. Moreover, the Mann-Whitney rank sum test was used to analyze samples that did not follow normal Gaussian distribution. Asterisks in all figures indicate the significance: *, $0.05 \geq p > 0.01$; **, $0.01 \geq p > 0.001$; ***, $p \leq 0.001$.

RESULTS AND DISCUSSION

Due to sessile in nature, plants cope with different environmental conditions during their life. These conditions effect plant growth and development. Water shortage is the leading factor that reduces the plant productivity more than any other stress condition. Plant tolerates drought by responding at biochemical and molecular level to minimize the detrimental effect of stress by altering the metabolism. The present study was conducted to explore the antioxidant mechanism in drought stressed maize leaves.

Two maize varieties were obtained from Cereal Crop Research Institute Pirsabak, KPK for drought stress experiment. All plants were provided with the optimum growth conditions and grown for two weeks under well watered condition. After that, both the varieties were exposed to drought stress for 7 days whereas control plants were applied with water regularly (Fig. 1A).

Effect of Drought on physiological parameters

Relative water content is the gross estimate of indirect changes in the water status in leaves (Canny and Huang 2006). The exposure of plants to water stress drastically decreased relative water content in both varieties. After 7 days of drought stress, the RWC was reduced to 56 and 51% in leaves of Azam and Baber, respectively, whereas control plants remained at full water capacity (90 and 95%)(Fig. 1B).This decrease in RWC by almost 50% indicating the proper onset of drought.

This decrease in RWC also affected the fresh weight of the leaves. More than 50 % reduction was observed in the FW of both varieties under drought stress (Fig. 1C). Chlorophyll is the green photosynthetic pigment that helps the plants to get energy from light and its determination is important in order to know the effect of drought on the chlorophyll contents. As expected, the total chlorophyll contents were decreased under drought stress condition. An average reduction of about 19% was observed in Azam variety whereas 23% decrease was recorded in Baber under water stress in comparison to well-watered plants (Fig. 1D). In addition, total soluble proteins were also quantified. A decrease was observed in the protein level in both varieties during drought (Fig. 1E). This decrease was significant and even more pronounced in Baber variety compared to Azam.

Relative water content (RWC) is an important indicator for determination of water status of plant, due to which the metabolic activity are greatly affected and ultimately leading to tissues death. As a loss of turgor that results in limited cell expansion and so reduced crop growth (Lu *et al*., 2010; Ashraf *et al*., 2010). In our results water contents were decreased upto 45% in drought stressed leaves while control leaves were showed normal growth. Our results are in agreement with Tiantian *et al*., 2011 who also observed more or less the same decrease in RWC upon exposure of maize under water stress condition. We found a decrease in chlorophyll contents in drought stressed leaves indicating that photosynthesis is greatly affected by water shortage reported in many studies (Chaves, 1991; Flexas *et al.,* 2004; Chaves *et al.,* 2008). Drought has direct effect on the photosynthetic apparatus leading to a decrease in photosynthesis. Moreover Mohammad Khani (2007) also found same decrease in chlorophyll level in maize during water stressed study. Total soluble proteins were also found to be decreased under drought stressed. Jha and Dubey (2004) also reported a decrease in TSP level in rice seedling upon drought stress which is quite similar to our results.

Impact of drought on ions concentration

In cytosol, intracellular osmotic homeostasis is attained by the accumulation of compatible osmolytes. Osmotic homeostasis and ion balance are crucial for plant growth and development (Zeng *et al*., 2003). Potassium and sodium are important minerals for plant growth that regulates leaf osmotic potential affecting the water use efficiency (Ashley *et al*., 2006). Calcium acts as a secondary messenger to ameliorate water stress (Nayyar and Kaushal, 2002). Calcium interacts strongly with ROS and important in most cellular signaling processes (Sanders *et al*. 2002). In our study, a decline in ions concentration was observed under drought although this decrease was not significant (Fig. 2A, B & C)

Drought effect on oxidative stress markers (MDA and H_2O2)

H_2O_2 level was determined in order to find out the extent of oxidative stress. In leaves of both varieties, the H_2O_2 level was increased relative to control. Almost 2-fold increase in H_2O_2 content was observed in Azam variety under drought stress whereas Baber showed 1.3-fold accumulation compared to unstressed plants (Fig. 3A). This high accumulation in H_2O_2 level points towards the formation of ROS under drought stress condition. Malondialdehydeis the lipid peroxidation product and an important indicator of drought based oxidative stress. Elevated level of MDA contents determines the severity of oxidative stress and cell wall damage (Reddy *et al*., 2004). The high accumulation of H_2O_2 further leads us to determine the oxidative stress based damages under drought stress.Both the varieties showed an elevated level of MDA contents in drought stressed leaves compared to control. Taking together, an average increase of 1.5-fold was observed in the level of MDA upon the onset of

drought although this increase was not significant. This trend of increase in MDA level indicates to the oxidative based lipid peroxidation (Fig. 3B).

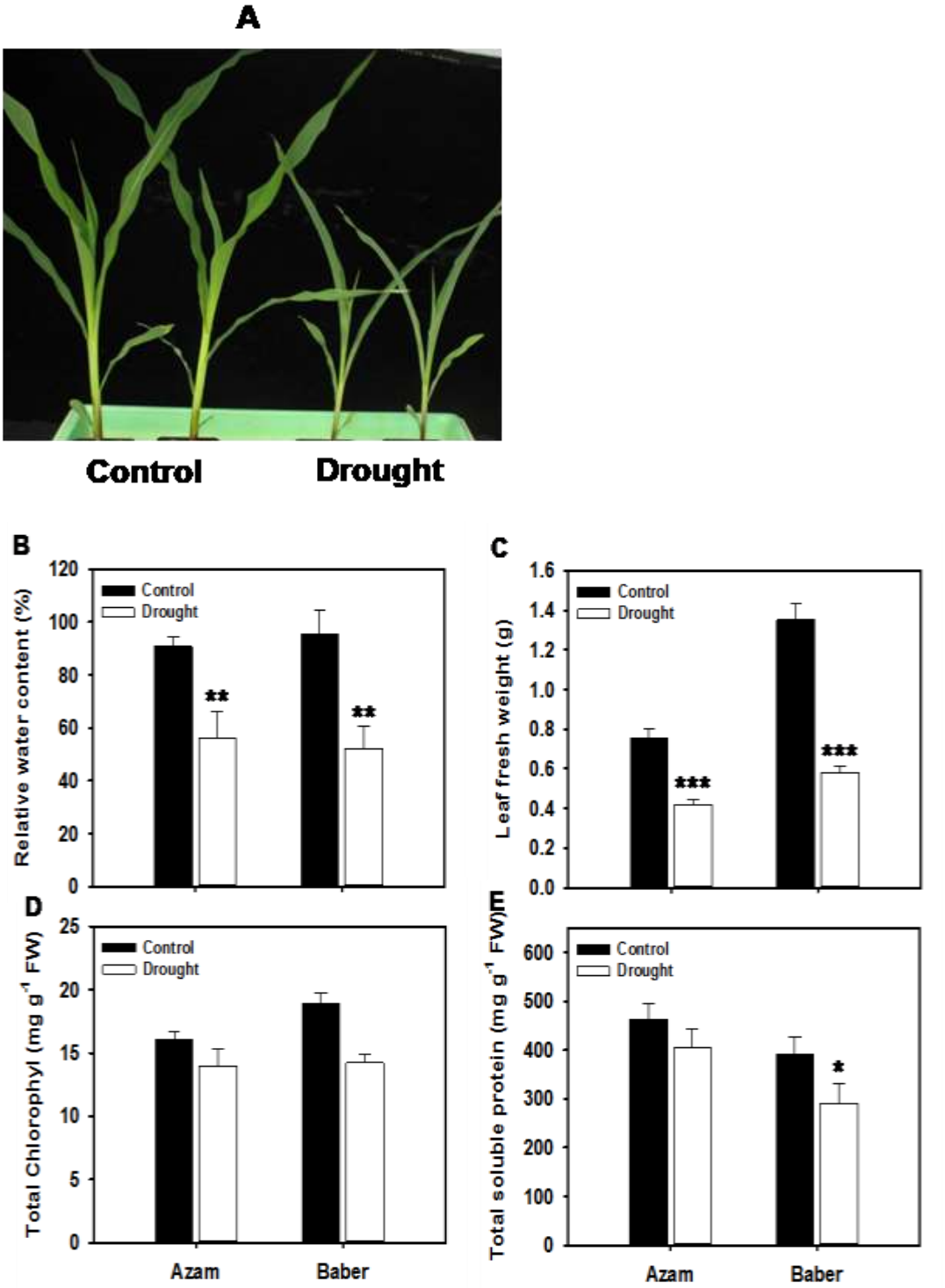

Fig. 1. Impact of drought on growth of maize plants: Maize plants grown on soil for 2 weeks and then subjected to drought stress for 7 days, (A) Phenotype of control and drought stressed plants, (B) Relative water content, (C) Leaf fresh weight (D) Total chlorophyll contents and (E) Total soluble proteins. Asterisks in all figures indicate the significance using the unpaired t-test: *, $0.05 \geq p > 0.01$; **, $0.01 \geq p > 0.001$; ***, $p \leq 0.001$. Mean ± standard deviations are shown.

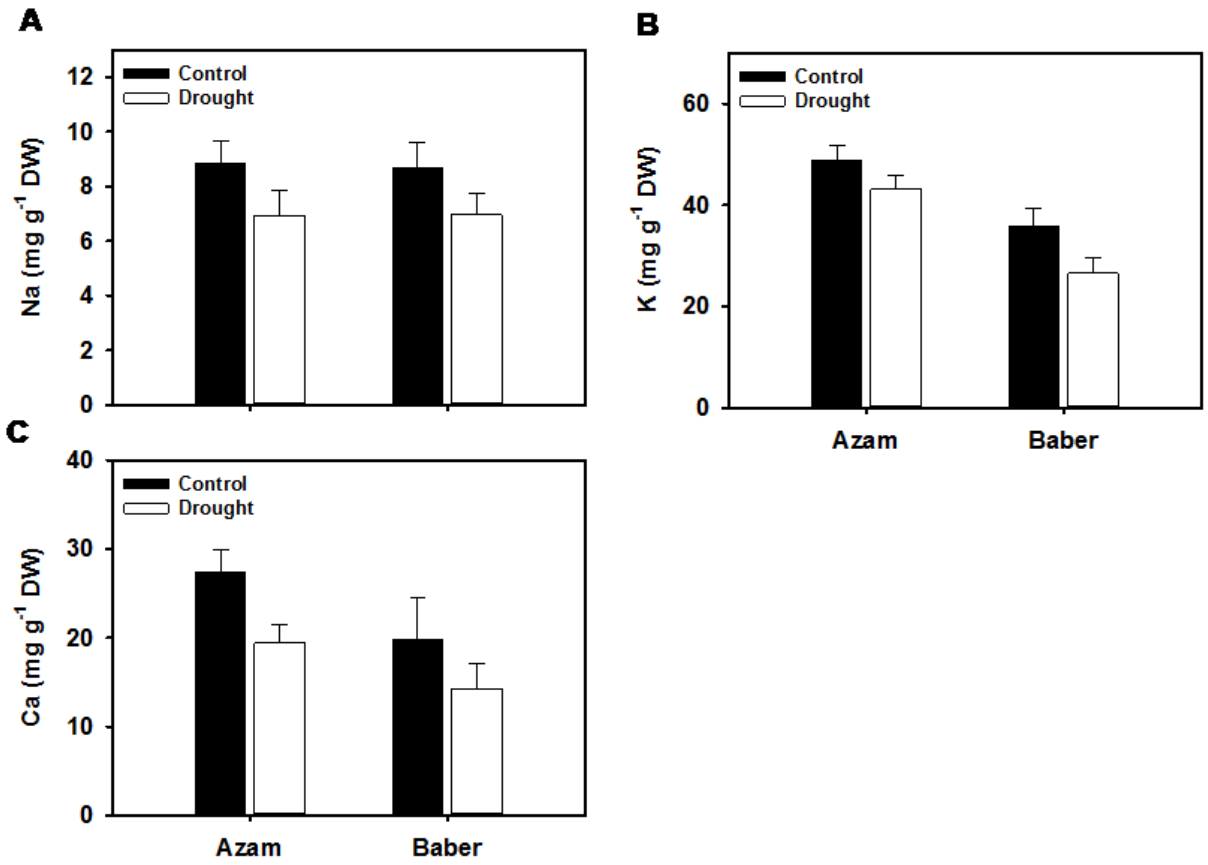

Fig. 2. Impact of drought on the ions concentration: Maize plants grown on soil for 2 weeks and then subjected to drought stress for 7 days, (A) Na level (B) K level (C) Ca level. Asterisks in all figures indicate the significance using the unpaired t-test: *, $0.05 \geq p > 0.01$; **, $0.01 \geq p > 0.001$; ***, $p \leq 0.001$. Mean ± standard deviations are shown.

H_2O_2 is the most stable ROS that is produced in plants under various stress conditions. Our data showed a significant increase in the level of H_2O_2. This high accumulation reflects that drought leads to formation of ROS due to metabolic imbalance leading to lipid peroxidation. In agreement with our results, most of the reports showed the accumulation of ROS (Jiang and Zhang, 2002) in maize and other species (Cruz-De-Carvalho, 2008). As the ROS in excessive amount can have a harmful effect and cause oxidative damage to plants. Malondialdehyde is an important indicator of lipids degradation due to which oxidative stress and membrane disruptions have been reported under drought condition (Sairam *et al.*, 2000; Wang *et al.*, 2014). Our data showed that MDA level was higher in drought stressed leaves as compared to control indicating ROS based lipid peroxidation which is in agreement with Wang *et al.*, 2014 who also reported an increase in the MDA level upon the onset of drought in wheat.

Impact of drought on the enzymes of the antioxidant system

The increase level of both H_2O_2 and MDA under drought stress in both varieties prompted us to determine the enzymatic activities of the antioxidant system. APX are ROS scavenging enzyme located at different sites of plant cells that reduce H_2O_2 to water at the expanse of AsA and/or GSH (Dietz *et al*., 2006; Meyer *et al*., 2012; Noctor *et al*., 2014). Both the varieties (Azam and Baber) showed elevated level of the APX activity under drought relative to control. An average increase of about 1.2-fold was observed after 7 days of drought stress in comparison to unstressed plants (Fig. 4A). Taking together, this hints towards the accumulation of H_2O_2 caused an increase in the APX activity to keep the ROS level at minimum. CAT directly converts H_2O_2 into H_2O and 1/2 O_2. Several H_2O_2-degrading enzymes are present in plants. However, CATs are unique that works in the absence of any cellular reducing agent (Del Rio *et al*., 2006). As expected, Azam showed almost 2-fold increase in CAT level under drought whereas 1.3-fold was observed for Baber variety (Fig. 4B). This huge increase in the CAT activity indicating that both the varieties have a strong antioxidant system to counteract with the ROS. In addition, SOD activity was also determined that converts $O_2^{\bullet-}$ into H_2O_2 and is important that acts as first line of defense. SOD has a protective effect against oxygen toxicity by regulating concentration of superoxide anionic radical (Noctor *et al*.,

2014). Like other enzymes of the defense mechanism, SOD also showed an increase under drought stress. Both the varieties showed more than 1.5-fold accumulation in the activity of SOD when exposed to stress for 7 days as compared to control (Fig. 4C). Peroxidase are also key enzymes in ROS detoxification and appear to be more active when coordinated with SOD in coping with the elevated level of ROS (Sobrino-Plata *et al.*, 2009). An approximately 2-fold increase was showed by both varieties in the activity of POD under water stress in comparison to control (Fig. 4D).

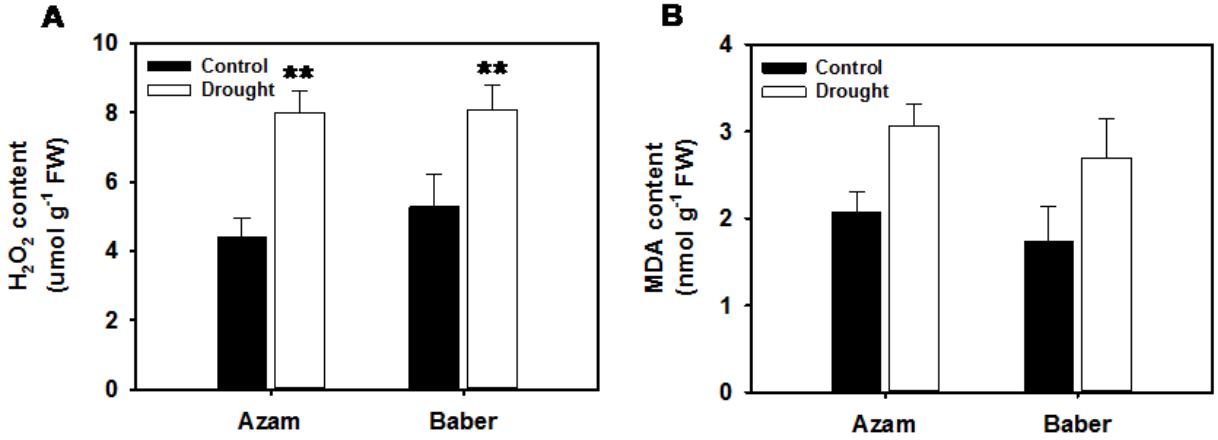

Fig. 3. Impact of drought on the oxidative stress markers: Maize plants grown on soil for 2 weeks and then subjected to drought stress for 7 days, (A) Hydrogen peroxide level (B) MDA level. Asterisks in all figures indicate the significance using the unpaired t-test: *, $0.05 \geq p > 0.01$; **, $0.01 \geq p > 0.001$; ***, $p \leq 0.001$. Mean ± standard deviations are shown.

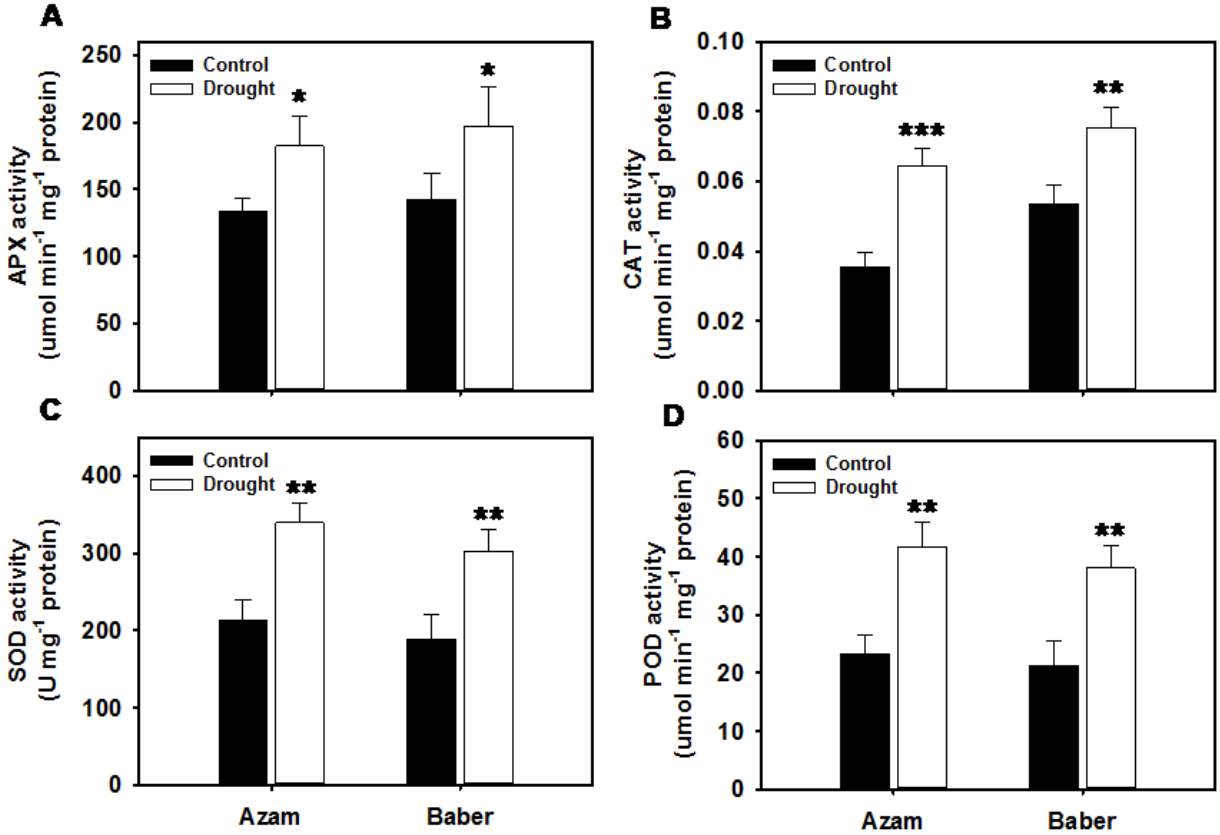

Fig. 4. Impact of drought on the enzymes of the defense mechanism: Maize plants grown on soil for 2 weeks and then subjected to drought stress for 7 days, (A) APX (B) Catalase (C) SOD and (D) POD activities. Asterisks in all figures indicate the significance using the unpaired t-test: *, $0.05 \geq p > 0.01$; **, $0.01 \geq p > 0.001$; ***, $p \leq 0.001$. Mean ± standard deviations are shown.

This ROS can be detoxified by a well-established antioxidant mechanism present in different cellular compartments. SOD, POD, APX and CAT plays important role scavenging the toxic effect of ROS. SOD,s are the mainstream enzyme and acts as first action by catalyzing the dismutation of superoxide radicals to H_2O_2 and O_2. We found an increase in the activities of all these enzymes indicating that both the varieties have the ability to keep the level of ROS at minimum level due to strong scavenging capability to cope with oxidative stress in leaves. As these enzymes have greater potential in removing ROS so can play critical role in drought tolerance. The activity of SOD has been reported to increase under drought stress in maize (Pastori *et al*., 2000). Same increase of antioxidant enzymes were also found by Avramova *et al*. (2015) in maize because these enzymes help the plants to detoxify against ROS and minimize the toxic effect of stress.

It is concluded from the study that the tested maize varieties (Azam and Baber) have the capability to cope with ROS and thus can be considered as drought tolerance based on the well established antioxidant system.

REFERENCES

Anjum, S.A., X.Y. Xie, L.C. Wang, M.F. Saleem, C. Man and W. Lei (2011). Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Resources*, 6: 2026-2032.

Ashley, M.K., M. Grant and A. Grabov (2006) Plant responses to potassium deficiencies, a role for potassium transport proteins. *Journal of Experimental Botany*, 57: 425-436.

Ashraf, M. (2010). Inducing drought tolerance in plants, some recent advances. *Biotechnology Advances*,28: 169-183.

Avramova, V., H. Abd Elgawad, Z. Zhang, B. Fotschki, R. Casadevall, L. Vergauwen, D. Knapen, E. Taleisnik,Y. Guisez, H. Asard and T.S.Gerrit (2015). Drought induces distinct growth response, protection and recovery mechanisms in the maize leaf growth zone. *Plant Physiology*, 169: 1382-1396.

Beck, E.H., S. Fettig, C. Knake, K. Hartig and T. Bhattarai (2007). Specific and unspecific responses of plants to cold and drought stress. *Journal of Biosciences*, 32: 501-510.

Bradford, M.M. (1976.) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*,72: 248-254.

Brunner, I. and D.L. Godbold (2007). Tree roots in a changing world. *Journal of Forest Research*, 12: 78-82.

Canny, M.J.and C.X. Huang (2006).Leaf water content and palisade cell size. *New Phytology*, 170: 75-85.

Chalapathi-Rao, A.S.V. and A.R.Reddy (2008).Glutathione reductase, a putative redox regulatory system in plant cells. In: *Sulfur Assimilation and Abiotic Stresses in Plants.*(N.A. Khan, S. Singh and S. Umar Eds.), Springer, The Netherlands.

Chaves, M. (1991.) Effects of water deficits on carbon assimilation. *Journal of Experimental Botany* 42: 1-16.

Chaves, M.M., J. Flexas and C.Pinheiro (2008) Photosynthesis under drought and salt stress, regulation mechanisms from whole plant to cell. *Annals of Botany*, 103: 551-560.

Cruz-de-Carvalho, M.H. (2008) Drought stress and reactive oxygen species. Production, scavenging and signaling.*Plant Signal Behaviour* 3: 156-165.

Daud, M.K., S. Ali, M.T. Varaith and S.J. Zhu (2013). Differential physiological, ultra morphological and metabolic responses of cotton cultivars under cadmium stress. *Chemosphere*,93: 2593-2602.

Del Rio, L.A., L.M. Sandalio, F.J. Corpas, J.M. Palma and J.B. Barroso (2006).Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. *Plant Physiology*, 141: 330-335.

De-Pinto, M.C. and L. De-Gara (2004). Changes in the ascorbate metabolism of apoplastic and symplastic spaces are associated with cell differentiation. *Journal of Experimental Botany*, 55: 2559-2569.

Dietz, K.J., S. Jacob, M.L. Oelze, M. Laxa, V. Tognetti and S.M. De-Miranda (2006). The function of peroxiredoxins in plant organelle redox metabolism. *Journal of Experimental Botany*, 57: 1697-1709.

Din, J., S.U. Khan,I. Ali and Gurmani (2011). Physiological and agronomic response of canola varieties to drought stress. *Journal of Animal Plant Sciences* 21: 78-82.

Flexas, J., J. Bota, F. Loreto, G. Cornic and T. Sharkey (2004). Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biology* 6: 269-279.

Hsiao, T.C. (1973) Plant responses to water stress. *Annual Review of Plant Physiology*, 24: 519-570.

Hu H, Xiong L (2014) Genetic engineering and breeding of drought-resistant crops. *Annual Review of Plant Biology*, 65: 715-741.

IPCC (2013). *Climate change 2013, the physical science basis*. Cambridge University Press.

Jaleel, C.A., P. Manivannan and A. Wahid (2009). Drought stress in plants: a review on morphological characteristics and pigments composition. *International Journal of Agricultural Biology*, 11: 100-105.

Jha, A.B. and R.S. Dubey (2004) Carbohydrate metabolism in growing rice seedlings under arsenic toxicity. *Journal of Plant Physiology*,161: 867-872.

Jiang, M. and J. Zhang (2002). Involvement of plasma-membrane NADPH oxidase in abscisic acid and water stress-induced antioxidant defense in leaves of maize seedlings. *Planta*, 215: 1022-1030.

Kokate, C.K., A.P. Purohit and S.B. Gokhale (2004).*Text book of Pharmacognosy*, Nirali Prakashan, India.Pp.542.

Lawlor, D.W. and G. Cornic (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, Cell and Environment*, 25: 275-294.

Lichtenthaler, H.K. and A.R. Wellburn (1983) Determination of total caroteonids and chlorophyll a and b of leaves and different solvents. *Biological Society*,11: 591.

Lim, P.O., H.J. Kim and H.G. Nam (2007). Leaf senescence. *Annual Review of Plant Biology*,58: 115-136.

Lu, G.H., D.L. Ren, X.Q. Wang, J.K. Wu and M.S. Zhao (2010).Evaluation on drought tolerance of maize hybrids in China. *Journal of Maize Science*,3: 20-24.

Meehl, G.A. and C. Tebaldi (2004). More intense, more frequent, and longer lasting heat waves in the 21st century. *Science*, 305: 994-997.

Meyer, Y., C. Belin, V. Delorme-Hinoux, J.P. Reichheld and C. Riondet (2012). Thioredoxin and glutaredoxin systems in plants, molecular mechanisms, crosstalks, and functional significance. *Antioxidant Redox Signal*, 17: 1124-1160.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Science*,7: 405-410.

Mittler, R. and E. Blumwald (2010). Genetic engineering for modern agriculture: challenges and perspectives. *Annual Review of Plant Biology*, 61: 443-462.

Mittler, R., S. Vanderauwera, M. Gollery and F. Van-Breusegem (2004).Reactive oxygen gene network of plants.*Trends Plant Science*, 490-498.

Mohammadkhani, N. and R.Heidari (2007). Effects of water stress on respiration photosynthesis pigments and water content in two maize cultivars. *Pakistan Journal of Biological Sciences*,10: 4022-4028.

Nakano, Y. and K. Asada (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology*,22: 867-880.

Nathan, C. (2003) Specificity of a third kind, reactive oxygen and nitrogen intermediates in cell signaling. *Journal of Clinical Investigations*, 111: 769-778.

Nayyar, H. and S.K. Kaushal (2002). Chilling induced oxidative stress in germinating wheat grains as affected by water stress and calcium. *Biologia Plantarum*, 45: 601-604.

Noctor, G. and C.H. Foyer (1998). Ascorbate and glutathione, keeping active oxygen under control. *Annual Review of Plant Biology*, 49: 249-279.

Noctor, G., A. Mhamdi and C.H. Foyer (2014). The roles of reactive oxygen metabolism in drought, not so cut and dried. *Plant Physiology*, 164: 1636-1648.

Pang, C.H. and B.S. Wang (2008).Oxidative stress and salt tolerance in plants. In: *Progress in Botany* (Luttge, U., Beyschlag, W. and Murataeds). Springer, Germany.pp.231-245.

Pastori, G.,C. Foyer and P. Mullineaux (2000). Low temperature induced changes in the distribution of H_2O_2 and antioxidants between the bundle sheath and mesophyll cells of maize leaves. *Journal of Experimental Botany*, 51: 107-113.

Polidoros, N.A. and J.G. Scandalios (1999). Role of hydrogen peroxide and different classes of antioxidants in the regulation of catalase and glutathione S-transferase gene expression in maize (*Zea mays* L.). *Physiology of Plants*, 106: 112-120.

Rahmstorf, S., A. Cazenave, J.A. Church, J.E. Hansen, R.F. Keeling, D.E. Parker and R.C.J. Somerville (2007). Recent climate observations compared to projections. *Science*, 316: 709.

Reddy, M.A., O.A. Bateman, C. Chakarova, J., Ferris, V. Berry, E. Lomas, R. Sarra, M.A. Smith, A.T. Moore, S.S. Bhattacharya and C. Slingsby (2004). Characterization of the G91del CRYBA1/3-crystallin protein, a cause of human inherited cataract.*Human Molecular Genetics*, 13: 945-953.

Reddy, R. and A.S. Raghavendra (2006). Photo oxidative stress. In: *Physiology and Molecular Biology of Stress Tolerance in Plants.* (K.V. MadhavaRao, A.S. Raghavendra, K.J. Reddy Eds.). Springer Netherland, 157-186.

Rudge, C.V., H.B. Rollin, C.M. Nogueira, Y. Thomassen, M.C. Rudge and J.A. Odland (2009). The placenta as a barrier for toxic and essential elements in paired maternal and cord blood samples of South African delivering women. *Journal of Environmental Monitoring*, 11: 1322-1330.

Sairam, R., G. Srivastava and D.Saxena (2000). Increased antioxidant activity under elevated temperatures: a mechanism of heat stress tolerance in wheat genotypes. *Biologia Plantarum*, 43: 245-251.

Sanders, D., J. Pelloux, C. Brownlee and J.F. Harper (2002).Calcium at the Cross roads of Signaling. *The Plant Cell*, 14: 401-417.

Shinozaki, K. and K. Yamaguchi-Shinozaki (2007). Gene networks involved in drought stress response and tolerance. *Journal of Experimental Botany*, 58: 221-227.

Singh, N.A., N.A. Anjum, Khan and R.Nazar (2008). Metal binding peptides and antioxidant defence system in plants, significance in cadmium tolerance. In: *Abiotic Stress and Plant Responses* (N.A. Khan, S. Singh Eds.). IK International, New Delhi, 159-189.

Sobrino-Plata, J., C. Ortega-Villasante, M.L. Flores-Caceres, C. Escobar, F.F. Del Campo and L.E. Hernandez (2009). Differential alterations of antioxidant defenses as bioindicators of mercury and cadmium toxicity in alfalfa. *Chemosphere*, 77: 946-954.

Tiantian, H., K. Shaozhong, L. Fusheng and Z. Jianhua (2011). Effects of partial root-zone irrigation on hydraulic conductivity in the soil–root system of maize plants. *Journal of Experimental Botany*, 62: 4163-4172.

Velikova, V., I. Yordanov and A. Edreva (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants, protective role of exogenous polyamines. *Plant Science*, 151: 59-66.

Wang, X., J. Cai, F. Liu, T. Dai, W. Cao, B. Wollenweber and D. Jiang (2014). Multiple heat priming enhances thermo-tolerance to a later high temperature stress via improving subcellular antioxidant activities in wheat seedlings. *Plant Physiology and Biochemistry*, 74: 185-192.

Zeng, L., J.A. Poss, C. Wilson, A.E. Draz,, G.B. Gregorio and C.M. Grieve (2003). Evaluation of salt tolerance in rice genotypes by physiological characters. *Euphytica*, 129: 281-292.

Zhu, J.K. (2002). Salt and drought stress signal transduction in plants. *Annual Review of Plant Biology*, 53: 247-258.

Zhou, W., D. Zhao and X. Lin (1997). Effects of water logging on nitrogen accumulation and alleviation of water logging damage by application of nitrogen fertilizer and mixtalol in winter rape (*Brassica napus* L.). *Journal of Plant Growth Regulation*, 16: 47-53.

(*Accepted for publication September 2016*)