Alleviation of oxidative stress induced by drought stress through priming by β-aminobutyric acid (BABA) in Rapeseed (*Brassica napus* L.) plants

Neda Mohamadi[1,2], Amin Baghizadeh[3*], Sara Saadatmand[1], Zahra Asrar[4]

1. Department of Plant Biology, Faculty of Basic Science, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Young Researchers and Elites Club, Kerman Branch, Islamic Azad University, Kerman, Iran.
3.Department of Biotechnology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman-Iran
4.Biology Department, Faculty of Science, Shahid Bahonar University of Kerman, Kerman, Iran.

Abstract

The objective of this study was to investigate the role of β-aminobutyric acid (BABA) (0, 300µM) in reduction of oxidative damages in leaves and roots of *Brassica napus* L. under drought stress (0, -0.2, -0.4 MPa). β-aminobutyric acid was investigated as an internal regulator hormone and its role in defense mechanisms against biotic and abiotic stresses. In this study, BABA pretreatment prevented drought induced decrease in K^+ content and increase in lipid peroxidation and H_2O_2 content. Mild and severe drought stress increased leaf and root MDA and other aldehydes content. In BABA pretreated plants under stress, leaf and root MDA and other aldehydes content significantly decreased as compare to drought condition. In addition, in drought stressed plants non-enzymatic antioxidants [GSH] were elevated over the control and BABA pretreatment increased GSH content. The Na^+ and Ca^{++} concentration increased under mild and severe drought stress. On the basis of the results, BABA-treated plants exhibited enhanced drought tolerance.

Keywords: non-enzymatic antioxidants, β-aminobutyric acid, drought stress, oxidative stress, *Brassica napus.*

Mohamadi, N., A. Baghizadeh, S. Saadatmand, Z. Asrar .2017. 'Alleviation of oxidative stress induced by drought stress through priming by β-aminobutyric acid (BABA) in Rapeseed (*Brassica napus* L.) plants'. *Iranian Journal of Plant Physiology* 7 (4), 2203-2210.

Introduction

Water stress due to drought can lead to major physiological and biochemical disruptions such as reduced photosynthesis (Lowlor and Cornic, 2002) and marked changes in gene expression (Romo et al. 2001).

*Corresponding author
E-mail address: *amin_4156@yahoo.com*
Received: March, 2016
Accepted: May, 2017

In humid and temperature areas, drought periods are a major factor impeding plant growth and development. Plants have different mechanisms to avoid water deficit. One of these responses is production of abscisic acid (ABA) that, in turn, elevates cytosolic Ca^{2+} concentrations in guard cells leading to stomatal closure. Closure of stomata as result of water deficit and consequent decrease in CO_2 concentration in the leaf mesophyll results in the accumulation of NADPH in the chloroplasts under such conditions, where

NADP is limiting, O_2 acts as an alternative electron acceptor resulting in the formation of superoxide radical (Baisak 1994). The O_2, through a series of univalent reduction reaction, produces hydrogen peroxide (H_2O_2) hydroxyl radical ($HO^{\bullet}$) and singlet oxygen ($^{\bullet}O_2$). This reactive oxygen species (ROS) are highly toxic and can damage important cellular biomolecules such as lipids, proteins, nucleic acids and chlorophyll (Baisak 1994). Maintaining a high level of enzymatic antioxidants such as catalase (CAT), peroxidase (POD) and other compounds such as Ascorbate (Asc), Malondialdehyde (MDA), α-tocoferol and carotenoids activity may contribute to drought tolerance by increasing the capacity a better protection mechanism against oxidative damage (Sharma and dubay 2005).

Intracellular homeostasis of sodium and potassium for cytosol enzyme action, maintenance of membrane potential and regulation of cell volume. Under salt stress, maintenance of K^+ and Na^+ homeostasis is even more severe, so regulation of ion transfer by signs of salinity stress provides a model for understanding the complete regulation of ion homeostasis in plant cells. In addition to understanding how plants cope with excess Na^+ in the environment, in terms of agriculture is important. Na^+ stress is disrupted K^+ uptake by the root cells, when Na^+ enters the cell, it accumulates in high levels and will be toxic for plant. To prevent of growth, stop or cell death, excess Na^+ should be omitted or enter the vacuoles (hasegava et al 2000).

A common theme underlying responses to arrange of biotic and abiotic stresses is the phenomenon of priming (Wu et al. 2010). Priming refers to a phenomenon where plants are sensitized to stress (Conrath et al. 2002; Van der Ent et al. 2009). Over the past years, priming-inducing activity in plants has been reported for many natural and synthetic compounds that include the non-protein amino acid β-amino butyric acid (BABA) (Beckers 2007).

BABA is a non-protein amino acid which occurs rarely in nature. The only report in connection to plants describes its presence in root exudates of tomato plants grown in solarized soil (Jacab et al. 2001). It was shown that, the BABA increases *Arabidopsis* resistance to different, unrelated stress such as microbial pathogens, salt, drought and heat shock (Zimerly et al. 2000; 2001; 2008; Ton and mauch-mani 2004; Jacab et al. 2005). Recently Cao et al (2009) showed that BABA enhanced heavy metal cadmium resistance through a glutathione-dependent pathway in *Arabidopsis*. BABA acts through potentiation of ABA-dependent signaling pathway and salicylic acid dependent defence mechanism (Zimerly et al. 2000). However, little is known about the role of BABA in antioxidant metabolisms under drought stress.

Therefore, the objective of this study was to investigate the accumulation of ROS and antioxidant system and Changes in ion levels in leaves and roots of *Brassica napus* L. under drought stress and after priming by BABA.

Materials and Methods

Plant material and treatment

Seeds of *Brassica napus* L. Cv. Madonna were collected from Karaj Agricultural Centre. Seeds were sterilized using 0.1% sodium hypochlorite solution, washed with distilled water and planted in pot filled with perlite. Pots were transferred to growth chamber with day/night temperature of 25/20 °C and a 16 h light/8 h dark photoperiod, with a relative humidity of 70%. During the first week of seed sowing, seedlings were irrigated with distilled water. For the rest of experiment, half strength Hoagland nutrient solution was used to irrigate plants every other day. Drought was induced by stopping to water 4 week-old plants 1 day after BABA (Fluka) (0, 300µM) treatment by soil drench. Three level of soil moisture were applied.

(1) Well-watered (C): the soil water potential was controlled at filed capacity.

(2) Mild drought stress (D1): the soil water potential was -0.2MPa.

(3) Severe drought stress (D2): the soil water potential was -0.4Mpa.

At the end of experiment, the leaves and roots of plants were harvested and immediately were frozen in liquid nitrogen and stored at -80ºC for the future analysis.

Lipid peroxidation

For each sample, 0.2 g of the leaf tissue was homogenized in 10 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10000 ×g for 5 min. For every 1ml of aliquot, 4ml of 20% TCA containing 0.5% thiobarbiturice acid was added. Mixture was heated at 90 °C for 30 min. Samples were cooled on ice for 5 min and then re-centrifuged at 10000 ×g for 10 min and absorbance of the supernatant was recorded at 532 and 600nm. For MDA measurement, the non–specific absorbance of supernatant at 600 nm was subtracted from the maximum absorbance at 532nm and an extinction coefficient of 1.55×10^5/ M Cm and was used for determination of MDA concentration (Heath and Packer, 1969). For other aldehydes, an extinction coefficient (ε) of 0.457×10^5/ M Cm was used at 455 nm at the average of e obtained for other aldehydes (propanal, butanal, hexanal, heptanal and propanalheptanal and propanal dimethylacetal). Results were expressed as nM g^{-1} FW.

Hydrogen peroxide content

H_2O_2 content was measured spectrophotometrically after reaction with potassium iodide (KI) according to the method of (Alexieva et al. 2001). Leaf tissues (500 mg) were homogenized in ice bath with5 ml 0.1% TCA. The homogenate was centrifuged at 12000 g for 15min. The reaction mixture consisted of 0.5 ml of supernatant, 0.5 ml of 100mM potassium phosphate buffer (pH 7.0) and 2 ml reagent (1M KI in fresh double-distilled water). The blank probe consisted of 0.1% TCA in the absence of leaf extract. The reaction was carried out for 1 h in darkness and absorbance was measured at 390 nm. The amount of hydrogen peroxide was calculated using a standard curve prepared with known concentrations of H_2O_2.

Mineral content

K^+, Na^+ and Ca^{2+} contents were determined using an atomic absorption spectrophotometer (Shimadzu, model 610, Japan) after wet digestion of the ash with nitric acid and expressed as mg g^{-1} DW.

Determination of total glutathione

The level of total acid-soluble SH compound (glutathione GSH) was determined with Ellman's reagent (1959). Samples of 0.5 g were homogenized in 15% m-phosphoric acid. The homogenate was centrifuged at 10000 g for 30min. The reaction mixture consisted of 200 µl of supernatant, 2.6 ml of 100mM sodium phosphate buffer (pH 7.7) and 200 µl DTNB (6 M). The absorbance at 412 nm was measured after 30 min. The GSH concentration was determined from a standard curve.

Statistical analysis

Data for each parameter were subjected to ANOVA and significant differences between treatment means were determined by Duncan Multiple Range test using the SPSS software. Data are shown as means with three replicates and significance was determined at the 95% confidence ($p \leq 0.05$) limits.

Table 1.

Effects of BABA (0,300 µM) and drought stress (0, -0.2,-0.4 MPa) on H2O2, MDA and Other aldehydes content in the leaves and root of *B. napus*.

Treatment	H_2O_2 (µMg^{-1} FW)/leaf	H2O2 (µM g^{-1} FW)root	MDA(nMg^{-1} FW) leaf	MDA(nMg^{-1} FW) root	Other aldehydes (nMg^{-1} FW) leaf	Other aldehydes (nMg-1 FW) root
Control	22.2±2.1^{d}	4.47±0.2^{d}	0.66±0.037^{d}	131.7±1.68^{c}	1.3±0.3^{e}	1.9±0.076^{c}
D1	67.3±2.38^{b}	8.3±0.15^{b}	1.8±0.259^{b}	148.06±1.18^{b}	5.2±0.22^{c}	3.07±0.037^{b}
D1+BABA	44.6±5.03^{c}	3.4±0.22^{e}	1.04±0.033^{cd}	136.9±3.06^{c}	3.6±0.12^{d}	2.3±0.25^{c}
D2	142.18±5.03^{a}	15.6±0.72^{a}	2.4±0.16^{a}	205.06±2.8^{a}	11.6±0.87^{a}	4.5±0.07^{a}
D2+BABA	60.9±2^{b}	5.2±0.21^{c}	1.4±0.1^{bc}	145.5±1.86^{b}	8.2±0.52^{b}	3.31±0.216^{b}

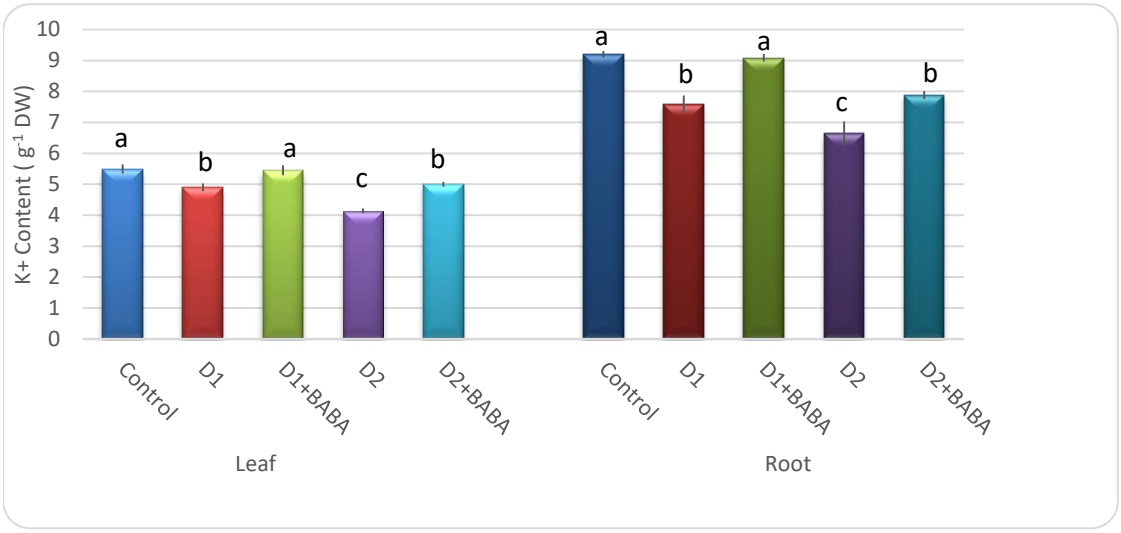

Fig. I. Effects of mild and severe drought stress and pretreatment of BABA on K^+ content in *B. napus* under drought stress.

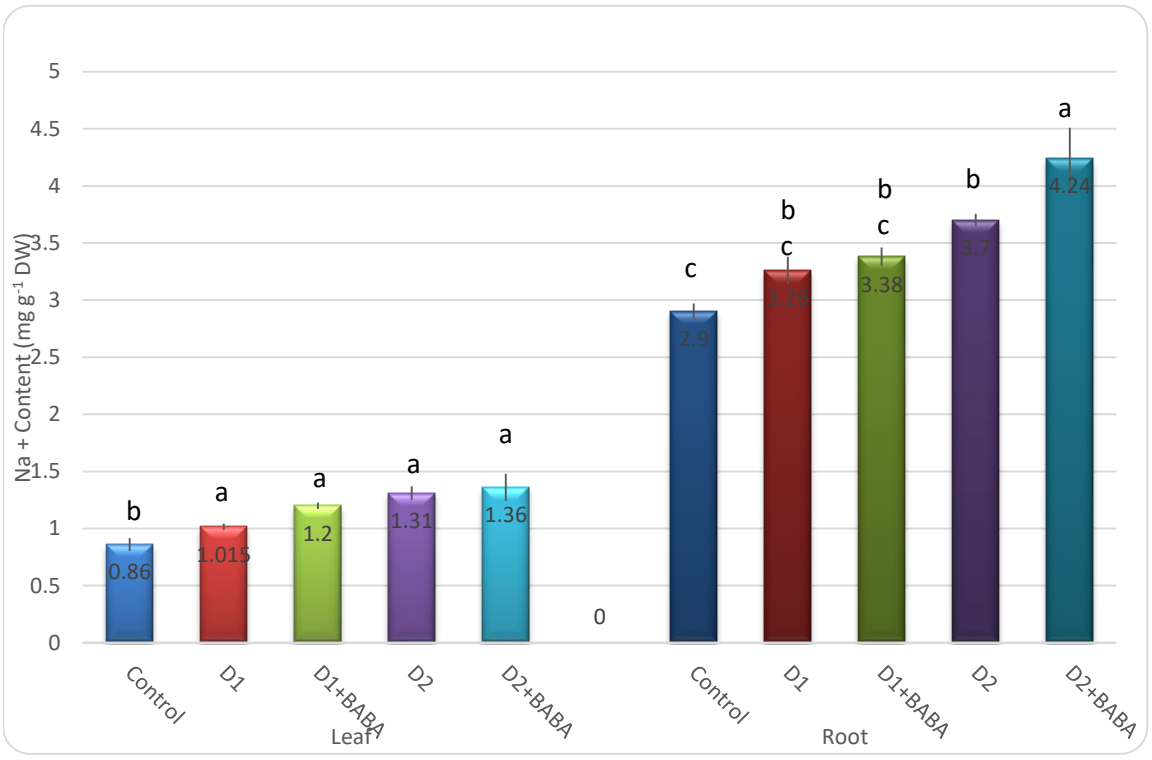

Fig.II. Effects of mild and severe drought stress and pretreatment of BABA on Na+ content in B. napus under drought stress.

Results

The leaf H_2O_2 content increased 3-fold and 6.3-fold under mild and severe drought stress, respectively, relative to the control. However, BABA-treated plants showed significant reduction in H_2O_2 content to 66% and 43% as compare to non-treated plants under mild and severe drought stress (Table 1). Mild and severe drought stress increased 1.8-fold and 3.5-fold root H_2O_2 content. In BABA pre-treated plants under stress, root H_2O_2

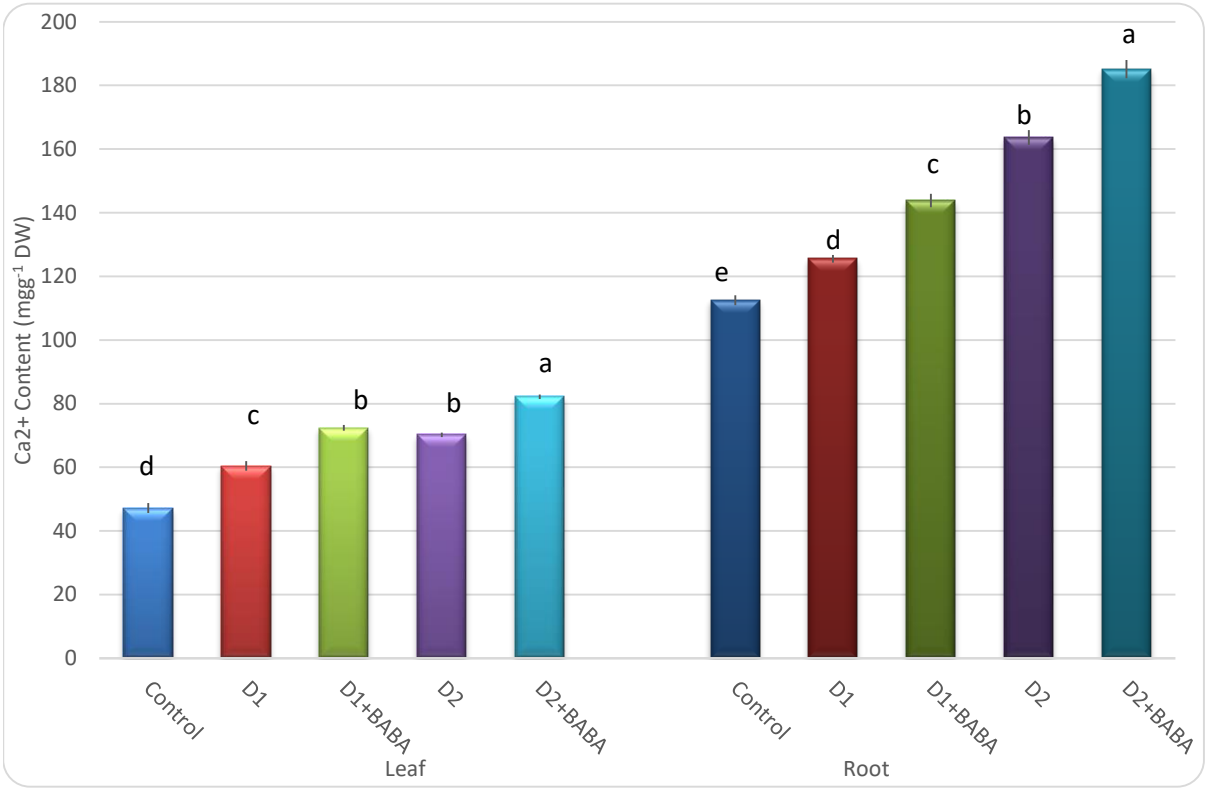

Fig. III. Effects of mild and severe drought stress and pretreatment of BABA on Ca2+ content in B. napus under drought stress

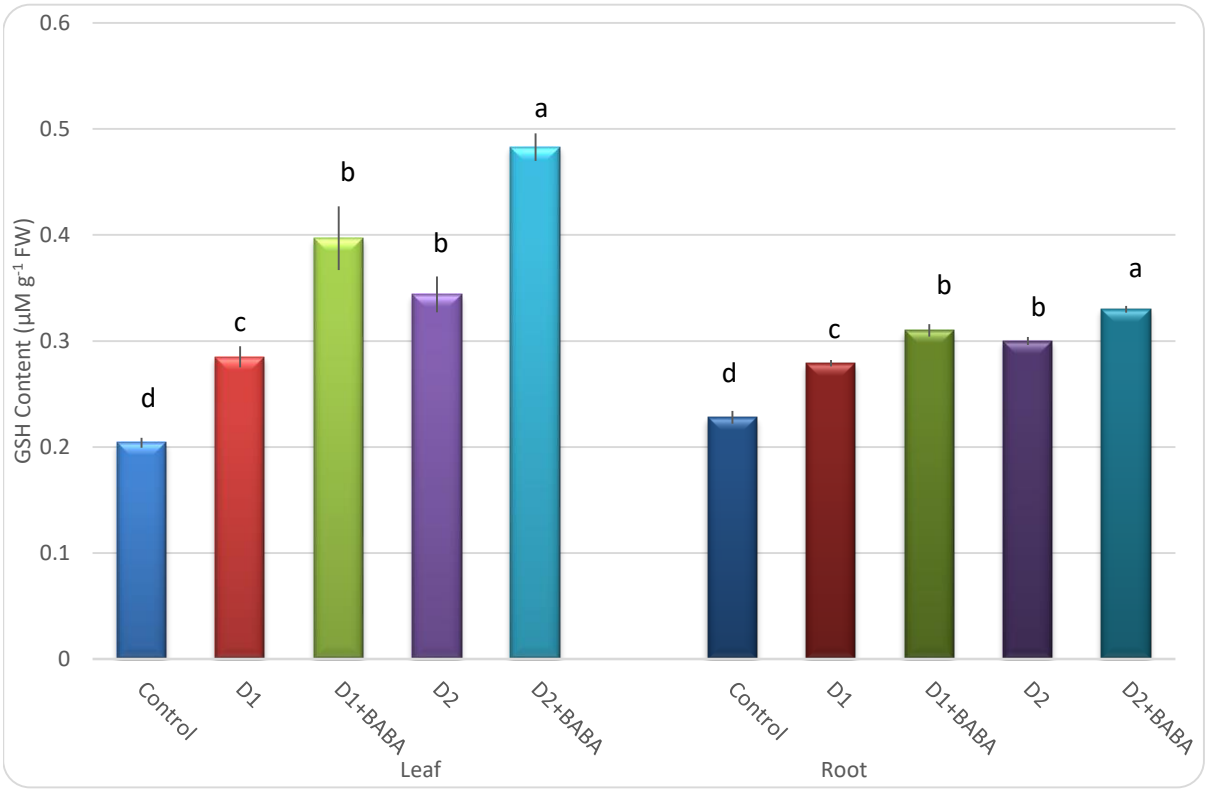

Fig.IV. Effects of mild and severe drought stress and pretreatment of BABA on GSH content in B. napus under drought stress.

compare to drought condition (Table 1). Mild and severe drought stress increased 2.7-fold and 3.6-fold leaf MDA content, 1.12fold and 1.56-fold root MDA content, 4-fold and 9-fold leaf other aldehydes content, 1.6-fold and 2.7-fold root other aldehydes content. In BABA pre-treated plants under stress, leaf and root MDA and other aldehydes content significantly decreased to 57.7% and 58.3% for leaf MDA, 92% and 71% for root MDA, 69% and 70.6% for leaf other aldehydes and 75% , 74% for root other aldehydes content as compare to drought condition (Table 1).

As shown in Fig. I, K^+ content was significantly decreased to 11%, 25.4% for leaf and 17.4%, 55% for root under mild and severe drought stress respectively. A significant increscent K^+ content about 1.2-fold for root and 1.1-fold for leaf BABA-treated plants than those of water treated plants under stress. The leaf Na^+ concentration increased to 1.3-fold and 1.5-fold under mild and severe drought stress, respectively. Root Na^+ concentration significantly increased to 1.1-fold and 1.3-fold in mild and severe drought stress. In contrast Na^+ content was not significantly affected by BABA (Fig. II).

Mild and severe drought stress caused a significant increase in Ca^{2+} content to 1.3-fold, 1.5-fold in leaf and 1.1-fold, 1.45-fold in root respectively. BABA increased the Ca^{2+} content, up to 1.16-fold, 1.17- fold relative to mild and severe drought condition (Fig. III). The leaf GSH content increased significantly 1.4-fold and 1.7-fold and the root GSH content increased 1.2-fold, 1.4-fold under mild and severe drought stress relative to control (Fig. IV). As shown in Fig. IV, GSH content increased by BABA pretreatment in plants under drought condition.

Discussion

On the basis of the results, BABA-treated plants exhibited enhanced drought tolerance. Jacab have shown that pretreatment of Arabidopsis plants with BABA, reduced the wilting rate by 50% (Jacab et al. 2005). The ion leakage is an indicator of cell membrane stability and integrity, which is commonly considered as one of the best physiological components of drought tolerance (Liu et al. 2011). The increasing of MDA and other aldehydes, index of lipid peroxidation, was accompanied by an increase of ion leakage in drought condition. Our finding illustrated that, BABA lead to reduction in lipid peroxidation. Maintaining the integrity of cellular membrane under stress is considered a part of drought tolerance mechanisms (Korkmaz 2007).

K^+ is a major cation in cell organization and it was reported to be a major contributor to osmotic adjustment under stress condition in several species (Santos-Diaz and Alejo-Ochoa 1994). Reduction in K^+ and increase in Na^+ has also been reported in wheat plant under drought condition (Munns and King 1988). Cao et al found that higher K^+ content was detected in shoot and root of BABA-treated seedlings than those of water treated seedlings. They showed that the transcript level of *AtHAK5* and *LKS1* involved in K^+ uptake in BABA-treated plants was significant higher than that in water treated. This result suggest that BABA play a role in increasing K^+ uptake, at least in part, via modulation of *AtHAK5* and *LKS1* (Cao et al. 2008).

The accumulation of O_2^- occurred in the leaves did not go along with the changes in total SOD activity (Carvalho 2008). The increased APX in the leaves and increase GR activity in roots and unchanged APX activity in the root of plants may help maintain levels of ASA and GSH (Foyer 1993). Soil drench treatment with the chemical BABA primes appropriate defence mechanisms and provides long-term protection against biotrophic bacteria (Zimerly 2000), necrotrophic fungi (Zimmerli et al. 2001) and abiotic stresses (Jacab et al., 2005). Zhang (2000) suggests that BABA is able to induce drought tolerance in Arabidopsis plant dependent of functional ABA signalling. The earlier and faster ABA production, stomatal closure and expression of ABA-regulated genes lead to an enhanced water use efficiency of the plants (Jacab et al. 2005). Pre-treatment with BABA reduced the level of lipid peroxidation and enhanced the survival in *Brassica napus* exposed to drought stress. Priming is usually defined as a sensitization to stress responsiveness. As a result, priming boosts the plant's defence response and primed plants are more resistance to biotic and abiotic stress. In Wu et al. study was shown that BABA acts as a chemical stress and therefore induces the stress-induced morphogenic response (SIMR) in Arabidopsis.

Consequently, BABA may act as a stressing agent in Arabidopsis (Wu et al. 2010). This hypothesis has been further confirmed as BABA-treated Arabidopsis activate ABA and ethylene stress signaling concomitantly with an accumulation of stress-induced transcripts (Zimmerli et al. 2008).

References

Alexieva, V., I. Sergiev., S. Mapelli and **E. Karanov**. 2001.'The effect of drought and ultraviolet radiation growth and stress markers in pea and wheat'. *Plant Cell Environ*, 24: 1337-1344.

Baisak, R., D. Rana, P. Acharya and **M. Kar**. 1994. 'Alterations in the activities of active oxygen scavenging enzymes of wheat leaves subjected to water stress'. *Plant Cell Physiol*, 35: 489-495.

Bakalova, S., A. Nikolova and **D. Wedera**.2004. 'Isoenzyme profiles of peroxidase, catalase and superoxide dismutase as affected by dehydration stress and ABA during germination of wheat seeds'. *Journal of Plant physiology*, 30: 64-77.

Beckers, J.M.G and **U. Conrath**.2007. 'Priming for stress resistance: from the lab to the field'. *Current Opinion in Plant Biology*, 10:425–431.

Ben Hamed, K., A. Castagna., E. Salem., A. Ranieri and **C. Abdelly**.2007. 'Sea fennel (*Crithmum maritimum* L.) under salinity conditions: a comparison of leaf and root antioxidant responses'. *Plant Growth Regulation*, 53: 185-194.

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 Biochem*, 72: 248-254.

Cao, S., L. Jiang., H. Yuan., J. Hongyong., G. Ren., X. Bian., J. Zou and **Z. Chen**.2008. '*b*-Amino-butyric acid protects Arabidopsis against low potassium stress'. *Acta Physiol Plant*, 30:309–314.

Carvalho, M.H.2008. 'Drought stress and reactive oxygen species'. *Plant signaling & Behavior*, 3(3): 156-165.

Conrath, U., C.M. Pieterse and **B. Mauch-Mani**. 2002. 'Priming in plant– pathogen interactions'. *Trends in Plant Science*, 7: 210–216.

Dhindsa, R.S., P. Plumb–Dhindsa and **T.A. Thrope**.1981. 'Leaf Senescence: correlated with increased levels of membrane permeability and lipid per oxidation, and decreased levels of superoxide dismutase and catalase'. *J. Exp. Bot,* 32: 43-101.

Ellman, G.I.1959. 'Tissue sulphydryl groups'. *Arch Biochem Biophys*, 82:70-77.

Foyer, C.H and **B. Halliwell**.1976. 'The presence of glutation and glutationreductase in chloroplast: A proposed role in ascorbic acid metabolism'. *Planta*, 133: 21-25.

Foyer, C.H and G. Noctor. 2005. 'Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context'. *Plant Cell Environ*, 28: 1056–1071.

Giannopolitis, C.N and **S.K. Ries**.1977. 'Superoxide dismutase I. Occurrence higher plants'. *Plant Physiol*, 59: 309-314.

Hasegawa, P.M., R.A. Bressan., J.K. Zhu and **H.J. Bohnert**. 2000. 'Plant cellular and molecular responses to high salinity'. *Annu Rev Plant Physiol,* 51:463-499.

Heath, R.L and **L.packer.** 1969.' Photoperoxidation in isolated chloroplast. I. kinetics and stoichiometry of fatty acid peroxidation'. *Archive of Biochem Biophys*, 125: 189-198.

Jacab, G., V. Cottier., V. Toquin., G. GRigoli ., L. Zimmerli., J.P. Metraux and **B. Mauch-Mani.**2001. 'b-Amino butyric acid-induced resistance in plants'. *Eur J Plant Pathol*, 107:29–37.

Jacab, G., J.Ton.,V. Flors., L. Zimmerli., J.P. Me´ traux and **B. Mauch-Mani**. 2005.'Enhancing Arabidopsis salt and drought stress tolerance by chemical priming for its abscisic acid responses'. *Plant Physiology*, 139: 264–174.

Korkmaz, A., M.Uzunlu and **A.R. Demirkairan**. 2007.'Treatment with acetylsalicylic acid protects muskmelon seedlings against drought stress'. *Acta Physiologiae Plantarum*, 29: 503-508.

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

Liu, C., Y.Liu., K. Guo., D.Fan., G.Li., Y.Zheng., L. Yu and **R. Yang**.2011.'Effect of drought on

pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China'. *Environmental and Experimental Botany*, 71: 174–183.

MC de Pinto, D., L.Francis and **D.Gara**.1999. 'The redox state of ascorbate-dehydroascorbate pair as a specific sensor of cell division in tobacco BY-2 cells. Protoplasma', 209: 90-95.

Munns, R and **R.W.King**. 1988. 'Abscisic acid in not the only inhibitor in the transpiration stream of wheat plant'. *Plant Physiology*, 88: 703-708.

Nakano, V and **K. Asada**.1981. 'Hydrogen Peroxide is scavenged by ascorbate- specific Peroxidase in Spinach chloroplasts'. *Plant Cell Physiol*, 22: 867-880.

Romo, S., E. Labrador and **B. Dopico**.2001. 'Water stress-regulated gene expression in *Cicer arietinum* seedlings and plants'. *Plant Physiol. Biochem*, 39: 1017-1026.

Santos-Diaz, M.S and **N.Ochoa-alejo**. 1994. 'PEG-tolerant cell clones of chilli pepper *(Capsicum annuum* L.): Growth, osmotic potentials and solute accumulation'. *Plant Cell Tiss. Org. Cult*, 37: 1-8.

Sharma, P and **R.S. Duby**.2005. 'Lead toxicity in plants'. *Braz. J. Plant Physiol*, 17 (1): 35-52.

Ton, J and **B. Mauch-Mani**. 2004. 'Beta-amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA dependent priming for callose'. *The Plant Journal*, 38: 119–130.

Van der Ent, S., M.Van Hulten.,M.J. Pozo., T. Czechowski., M.K. Udvardi., C.M. Pieterse and **J. Ton**.2009. 'Priming of plant innate immunity by rhizobacteria and beta-aminobutyric acid: differences and similarities in regulation'. *New Phytologist*, 183: 419–431.

Wu, C.C., P.Singh., M.C. Chen and **L. Zimmerli**. 2010.' L-Glutamine inhibits beta-aminobutyric acid-induced stress resistance and priming in Arabidopsis' *Journal of Experimental Botany*, 61(4): 995-1002.

Zimmerli, L., B.H. Hou., C.H.Tsai., G. Jacab., B.Mauch-Mani and **S. Somerville.** 2008.'The xenobiotic beta-aminobutyric acid enhances *Arabidopsis thermotolerance*'. *The Plant Journal*, 53: 144–156.

Zimmerli, L., G. Jacab., J.P. Metraux and B. **Mauch-Mani**. 2000.' Potentiation of pathogen-specific defence mechanisms in Arabidopsis by beta-aminobutyric acid'. Proceedings of the National Academy of Sciences, USA. 97: 12920–12925.

Zimmerli, L., J.P. Me´ traux and **B. Mauch-Mani**. 2001. 'beta-Aminobutyric acid-induced protection of Arabidopsis against the necrotrophic fungus *Botrytis cinerea*'. *Plant Physiology*, 126: 517–523.