



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

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### Abstract

The objective of this study was to investigate the role of  $\beta$ -aminobutyric acid (BABA) (0, 300 $\mu$ M) in reduction of oxidative damages in leaves and roots of *Brassica napus* L. under drought stress (0, -0.2, -0.4 MPa).  $\beta$ -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,  $\beta$ -aminobutyric acid, drought stress, oxidative stress, *Brassica napus*.

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### 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).

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

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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 ( $^1O_2$ ). 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),  $\alpha$ -tocopherol 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  $\beta$ -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, 300 $\mu$ M) 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 -80°C 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% thiobarbituric 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  $1.55 \times 10^5 / \text{M Cm}$  and was used for determination of MDA concentration (Heath and Packer, 1969). For other aldehydes, an extinction coefficient ( $\epsilon$ ) of  $0.457 \times 10^5 / \text{M Cm}$  was used at 455 nm at the average of  $\epsilon$  obtained for other aldehydes (propanal, butanal, hexanal, heptanal and propanalheptanal and propanal dimethylacetal). Results were expressed as  $\text{nM g}^{-1}$  FW.

#### Hydrogen peroxide content

$\text{H}_2\text{O}_2$  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 with 5 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  $\text{H}_2\text{O}_2$ .

#### Mineral content

$\text{K}^+$ ,  $\text{Na}^+$  and  $\text{Ca}^{2+}$  contents were determined using an atomic absorption spectrophotometer (Shimadzu, model 610, Japan) after wet digestion of the ash with nitric acid and expressed as  $\text{mg g}^{-1}$  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  $\mu\text{l}$  of supernatant, 2.6 ml of 100mM sodium phosphate buffer (pH 7.7) and 200  $\mu\text{l}$  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  $\mu\text{M}$ ) and drought stress (0, -0.2,-0.4 MPa) on  $\text{H}_2\text{O}_2$ , MDA and Other aldehydes content in the leaves and root of *B. napus*.

Treatment	$\text{H}_2\text{O}_2$ ( $\mu\text{Mg}^{-1}$ FW)/leaf	$\text{H}_2\text{O}_2$ ( $\mu\text{M g}^{-1}$ FW)root	MDA( $\text{nMg}^{-1}$ FW) leaf	MDA( $\text{nMg}^{-1}$ FW) root	Other aldehydes ( $\text{nMg}^{-1}$ FW) leaf	Other aldehydes ( $\text{nMg}^{-1}$ FW) root
Control	22.2±2.1 <sup>d</sup>	4.47±0.2 <sup>d</sup>	0.66±0.037 <sup>d</sup>	131.7±1.68 <sup>c</sup>	1.3±0.3 <sup>e</sup>	1.9±0.076 <sup>c</sup>
D1	67.3±2.38 <sup>b</sup>	8.3±0.15 <sup>b</sup>	1.8±0.259 <sup>b</sup>	148.06±1.18 <sup>b</sup>	5.2±0.22 <sup>c</sup>	3.07±0.037 <sup>b</sup>
D1+BABA	44.6±5.03 <sup>c</sup>	3.4±0.22 <sup>e</sup>	1.04±0.033 <sup>cd</sup>	136.9±3.06 <sup>c</sup>	3.6±0.12 <sup>d</sup>	2.3±0.25 <sup>c</sup>
D2	142.18±5.03 <sup>a</sup>	15.6±0.72 <sup>a</sup>	2.4±0.16 <sup>a</sup>	205.06±2.8 <sup>a</sup>	11.6±0.87 <sup>a</sup>	4.5±0.07 <sup>a</sup>
D2+BABA	60.9±2 <sup>b</sup>	5.2±0.21 <sup>c</sup>	1.4±0.1 <sup>bc</sup>	145.5±1.86 <sup>b</sup>	8.2±0.52 <sup>b</sup>	3.31±0.216 <sup>b</sup>

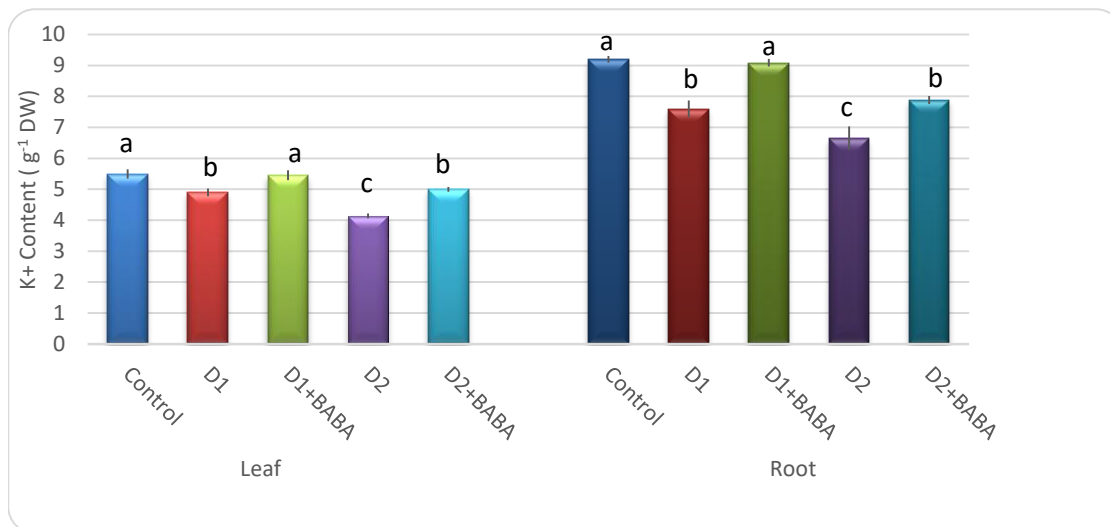


Fig. I. Effects of mild and severe drought stress and pretreatment of BABA on K<sup>+</sup> content in *B. napus* under drought stress.

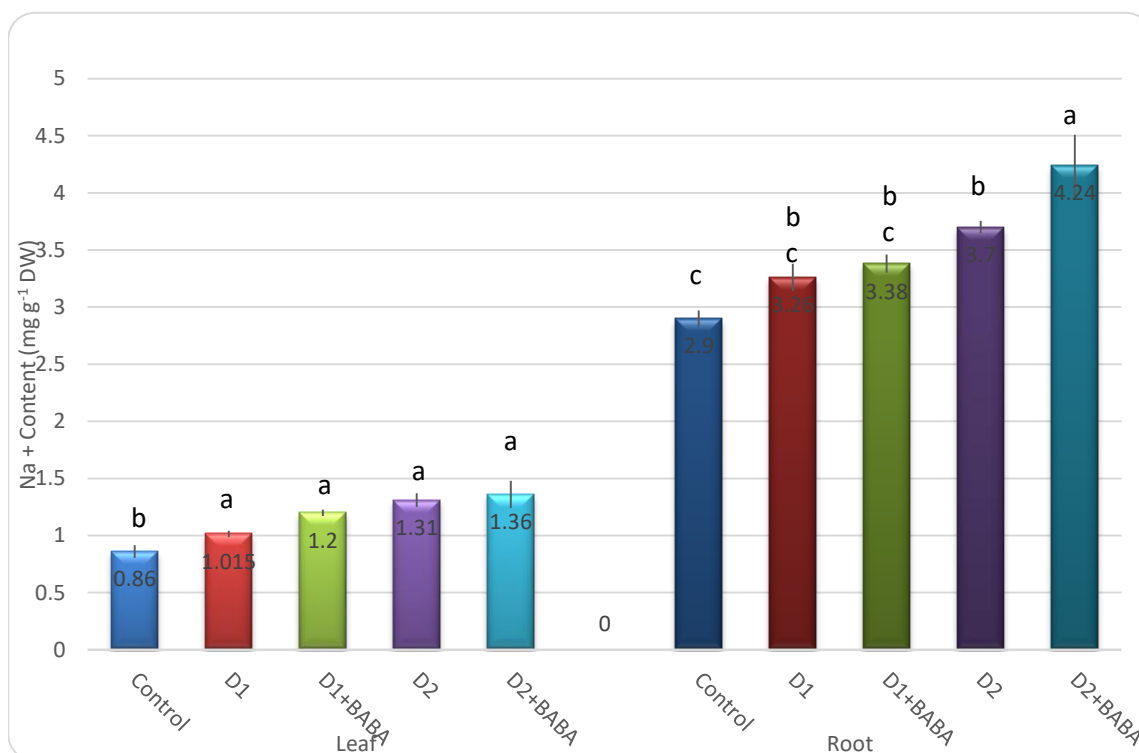


Fig.II. Effects of mild and severe drought stress and pretreatment of BABA on Na<sup>+</sup> content in *B. napus* under drought stress.

## Results

The leaf H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> content. In BABA pre-treated plants under stress, root H<sub>2</sub>O<sub>2</sub>

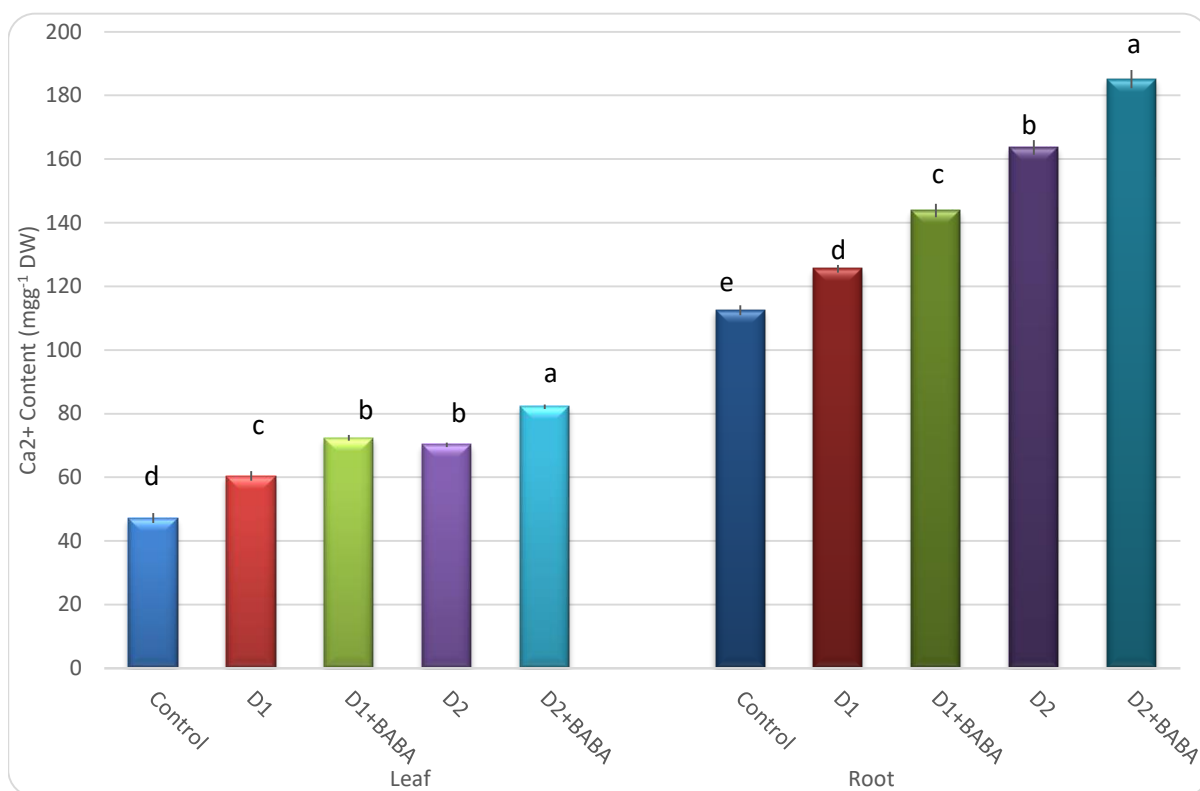


Fig. III. Effects of mild and severe drought stress and pretreatment of BABA on Ca<sup>2+</sup> content in *B. napus* under drought stress

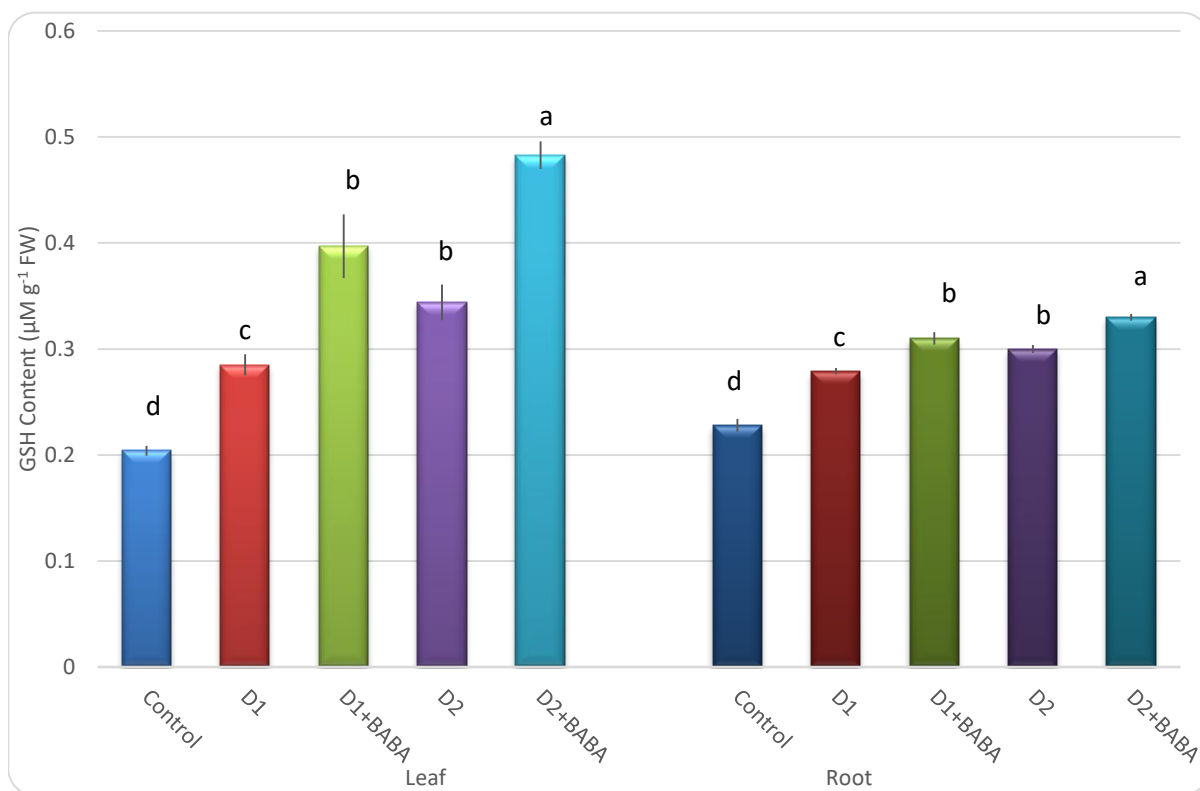


Fig. IV. Effects of mild and severe drought stress and pretreatment of BABA on GSH content in *B. napus* under drought stress.

compare to drought condition (Table 1). Mild and severe drought stress increased 2.7-fold and 3.6-fold leaf MDA content, 1.12-fold 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 increment  $K^+$  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. Jacob have shown that pretreatment of Arabidopsis plants with BABA, reduced the wilting rate by 50% (Jacob 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 (Jacob 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 (Jacob 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).

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## کاهش تنش اکسیداتیو ناشی از تنش خشکی از طریق پرایمینگ با اسید $\beta$ -آمینو بوتیریک در گیاه کلزا (*Brassica napus* L.)

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### چکیده فارسی

هدف از این مطالعه بررسی نقش اسید  $\beta$ -آمینو بوتیریک (صفر و ۳۰۰ میکرومولار) در کاهش آسیب های اکسیداتیو در برگ و ریشه های گیاه کلزا، تحت شرایط تنش خشکی (صفر، ۰/۲ و ۰/۴ - مگا پاسکال) بود. اسید  $\beta$ -آمینو بوتیریک و نقش آن به عنوان یک هورمون تنظیم کننده داخلی در مکانیسم های دفاعی در برابر تنش های زیستی و غیرزیستی مورد بررسی قرار گرفت. در این مطالعه، پیش تیمار  $\beta$ -آمینو بوتیریک اسید مانع از کاهش محتوای یون پتاسیم و افزایش پراکسیداسیون لیپیدها و محتوای پراکسید هیدروژن در اثر تنش خشکی شد. تنش خشکی ملایم و شدید مقدار مالون دآلدئید و سایر آلدئیدها را در بافت های برگ و ریشه افزایش داد. تحت شرایط تنش، در گیاهان تیمار شده با اسید  $\beta$ -آمینو بوتیریک، محتوای مالون دآلدئید و سایر آلدئید های برگ و ریشه به طور معنی داری نسبت به گیاهان شاهد کاهش می یابد. علاوه بر این، در گیاهان تحت تنش خشکی، مقدار ترکیبات آنتی اکسیدانی غیر آنزیمی نسبت به گیاهان شاهد بیشتر بود و پیش تیمار  $\beta$ -آمینو بوتیریک اسید مقدار ترکیبات آنتی اکسیدانی غیر آنزیمی را افزایش داد. غلظت یون های سدیم و کلسیم تحت تنش خشکی ملایم و شدید افزایش یافت. بر اساس نتایج حاصله، گیاهان تحت تیمار اسید  $\beta$ -آمینو بوتیریک، افزایش تحمل به خشکی را نشان دادند.

**کلمات کلیدی:** آنتی اکسیدان های غیر آنزیمی، اسید بتا آمینو بوتیریک، تنش خشکی، تنش اکسیداتیو، کلزا