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Full Length Research Paper

Enhanced antioxidant defense after exogenous application of Ca^{2+} and K^+ in *Brassica napus* seedlings under water deficit stress

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Supply of adequate moisture is one of the most important factors limiting agricultural extension and productivity. Ca^{2+} and K^+ are both essential plant nutrients required in a number of developmental and metabolic processes. Both of these nutrients play an important role in ameliorating drought stress in crop plants. This experiment was designed to study whether exogenous application of Ca^{2+} and K^+ before the drought could enhance the potential of plants to survive under limiting water conditions. *Brassica napus* L. cv Bulbul-98 seedlings were exposed to drought stress for 10 days after exogenous application of different concentrations of Ca^{2+}, K^+ and N through irrigation water. Exogenous Ca^{2+} and K^+ application was associated with increased relative water content, membrane stability and chlorophyll content in seedlings exposed to water deficit. Though the quantity of Pro and activities of APX (EC: 1.11.1.11) and CAT (EC: 1.11.1.6) were increased after K^+ application, they were strongly induced in seedlings supplemented with Ca^{2+} in a dose dependent manner. Increasing quantity of N application, however, had a negative effect on these parameters.

Key words: Drought, Ca^{2+}, K^+, AOS enzymes, proline, chlorophyll.

INTRODUCTION

Plants are usually exposed to several types of environmental factors that have adverse effects on metabolism, growth and yield. Among the different environmental factors, water availability and salinity, are of major importance in determining the productivity and plants community composition of an ecosystem (Boyer, 1982). According to FAO report, about 45% of the agricultural land of the world is exposed to water deficit stress (Bot et al., 2000). Drought stress usually causes the inhibition of growth leading to the production of a variety of modifications in physiological, biochemical and molecular characteristics of plants (Bajji et al., 2001; Smirnoff, 1993). The water deficit stress increases theformation of active oxygen species (AOS) (Karpinski et al., 1997; Smirnoff, 1993) and alters water relationships within the plant. An increase in the quantity of abscisic acid (ABA), the major messenger, is the first response of plants stimulating the closure of the guard cells of the stomata to reduce water loss (Smirnoff, 1993). This process decreases CO_2 availability for photosynthesis, resulting in an imbalance between the generation and the use of electrons, provoking the overproduction of AOS. The increase in steady state level of the AOS results in damage to all the major biological molecules (Asada, 1999; Karpinski et al., 1997; Smirnoff, 1993). AOS also plays role in intracellular redox sensing and activation of antioxidant resistance mechanisms, among other

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Abbreviations: AOS, Active oxygen species; **APX,** ascorbate peroxidase; **CAT,** catalase; **Chl,** chlorophyll; **MSI,** membrane stability index; **Pro,** proline; **RWC,** relative water content; **RWL,** rate of water loss from excised leaves.

adaptive processes (Karpinski et al., 1997). The extent to which plants can minimize increase in AOS steady state and water relation determines the degree of resistance to water stress.

Ca^{2+} is the universal second messenger molecule in plant signaling processes. Ca^{2+} addition under drought stress enhances water conservation and improves the hydrophobicity of cellular membrane while lowering its permeability through connecting to the phosphates and carboxyl of phosphatides and proteins in cellular membranes, thus strengthening their stability (Bush, 1995; Shao et al., 1995; Shao et al., 2008a). Ca^{2+} can alter the hydration degrees of membrane and improve the cohesion of cell walls, thus enhancing the viscosity of protoplasm and cells capacity of dehydration resistance. Thus Ca^{2+} can stabilize plant cells through its direct effects as a structural basis for plant drought-resistance (Ma et al., 2009; Shao et al., 2008b). Furthermore, in plant cells, Ca^{2+} plays roles as a second messenger coupling a wide range of extra-cellular stimuli with intracellular responses. Different extra-cellular stimuli trigger specific Ca^{2+} signatures. Dynamics, amplitude and duration of Ca^{2+} transients specify the nature, implication and intensity of stimuli. Ca^{2+}-binding proteins (sensors) play a critical role in decoding calcium signatures and transducing signals by activating specific targets and corresponding metabolic pathways (Shao et al., 2008b).

K^+ is the most prominent inorganic plant solute and inadequate levels enhance drought resistance, water-use efficiency and plant growth under drought conditions. It lowers the osmotic potential for solute transport in xylem and the water balance of plants. Adequate K^+ fertilization of crop plants may facilitate osmotic adjustment, which maintains turgor pressure at lower leaf water potentials and can improve the ability of plants to tolerate drought stress (Egilla et al., 2001; Mengel and Arneke, 1982). In cereal crops, higher tissue K^+ concentrations reduce the rate of transpiration (Anderson et al., 1992; Mengal and Arneke, 1982). Similarly, it has been reported that the application of extra K^+ to the nutrient solution mitigates the negative effects of salt on vegetative growth and fruit yield due to the restoration of the tissue K^+ levels (Kaya and Higgs, 2003). However, addition of ABA inhibits the loading of K^+ into the xylem of roots, though it has no effect, at least in the short term, on the influx from the soil into the roots (Roberts, 1998). Furthermore, ABA and Ca^{2+} signaling are also involved in modulation of K^+ channels. Thus, application of calcium had increased membrane associated Ca^{2+}, resulting in tightening of plasmalemma and reduction of K^+ efflux. Enhanced Ca^{2+} was found to protect *Chlorella* cells and reduced K^+ efflux in case of cell dehydration (Basset and Issa, 1994).

Thus, this experiment was designed to study whether external supplies of the Ca^{2+} and K^+, before the onset of drought, could ameliorate the adverse effects on *Brassica napus* seedlings during early growth stage. Plant water relations, disintegration of cellular membranes, chlorophyll (Chl) and proline (Pro) content and the activities of two AOS scavenging enzymes, ascorbic acid peroxidase (APX) and catalase (Cat), were taken into consideration to access the response of the seedlings to water scarcity during this experiment.

MATERIALS AND METHODS

Plant material and experimental plan

The experiment was conducted at the Institute of Biotechnology and Genetic Engineering, Agricultural University Peshawar, Khyber Pakhtunkhwa, Pakistan during February 2009 to March 2010. Seeds of *B. napus* cv Bulbul-98 were sown in plastic pots filled with 5.5 kg of silty clay loam soil and farm yard manure (FYM) (2:1). After germination, five plants of uniform size were maintained in each pot. Plants were supplemented with Ca^{2+} (30, 60 and 90 mM $Ca(NO_3)_2.4H_2O$) and K^+ (50, 100 and 150 mM KNO_3), respectively. Similarly, because Ca^{2+} and K^+ were provided in the form of nitrates, three supplementations of 30, 60 and 100 mM NH_4NO_3 were also provided to check changes due to the supplemental nitrogen. After supplementation, the pots were irrigated with equal amount of water for one week, after which drought stress was imposed by withholding water from half the pots. Analyses were made after 10 days of imposition of drought stress. Each treatment was replicated at least three times and five pots were maintained in each treatment.

Determination of relative water content (RWC)

Leaf samples (5 cm^2 diameter) were obtained from control and drought stressed seedlings in pre-weighed 15 ml falcon tubes and immediately weighed with analytical balance to obtain fresh weight (W_f). The leaf samples were then completely immersed in double distilled water and were placed at 4°C for 24 h in the dark. After 24 h, the samples were blotted dry on filter paper and weighed again to obtain the turgid weight (W_t). The samples were then oven dried at 70°C for 48 h and dry weights were obtained (W_d). Relative water content was calculated using the following formula.

$$RWC\ (\%) = [(W_f - W_d) / (W_t - W_d)] \times 100$$

Membrane stability index (MSI)

The membrane stability index (%) was calculated by determining the electrolyte leakage from the leaf disks with a conductivity meter (Consort C-931, USA). The initial conductivity (C_i) was measured after subjecting the samples from controlled and drought stressed seedlings to incubation at 25°C in 5 ml de-ionized water for about 3 h with continuous shaking. The samples were then autoclaved at 121°C for 20 min at 120 psi. Final conductivity (C_f) was measured after the samples had cooled down to 25°C. The MSI in each sample was determined as follows:

$$MSI\ (\%) = [1 - (C_i / Cf)] \times 100$$

Determination of chlorophyll content

Arnon (1949) method was used to determine total chlorophyll content. A known weight (usually ~100 mg) of leaf samples were immediately frozen in liquid nitrogen and homogenized in 3 ml of 80% acetone. The homogenate was centrifuged at 15,000 rpm for

10 min at 4°C. The supernatant was collected and the chlorophyll content was determined by noting the absorbance using a spectrophotometer (BioRad SmartSpec™ Plus, USA).

Determination of proline

500 mg of frozen plant material was homogenized in 1 ml of sterilized ion-free water; the debris was removed by centrifugation at 5,000 rpm. Proline was measured as described by Bates et al. (1973) with minor modifications. 100 µl of the extract was reacted with acid ninhydrin for 1 h at 100°C, and the reaction was then terminated in an ice bath. The reaction mixture was mixed with toluene and its optical density was measured at 520 nm. The amount of proline was determined from a standard curve in the range of 0 to 20 $\mu g.ml^{-1}$ of L-proline.

Protein isolation and enzymes assay

Leaf samples were collected from the well irrigated and stressed plants 10 days after imposition of drought and stored at -80°C. Approximately 0.5 g of leaf sample was homogenized in pre-chilled mortar and pestle using 50 mM potassium phosphate buffer (pH 7.8) containing 0.4 mM $EDTA_2Na$, 1 mM ascorbate, 5% (v/v) glycerol and 2% (w/v) PVP, and centrifuged at 15,000 g for 20 min. The supernatant was used as the crude enzyme solution. Protein content was determined according to the method of Bradford (1976). Ascorbate peroxidase (APX) activity was determined by the procedure of Nakano and Asada (1981) with some modifications using the crude enzyme and a reaction mixture containing 25 mM potassium phosphate buffer, 0.25 mM ascorbate, 0.1 mM EDTA, and 0.1 mM H_2O_2. The oxidation of ascorbate was monitored at 290 nm with a UV/Vis spectrometer. Catalase (Cat) activity was assayed according to Chandlee and Scandalios (1984) in a 2.0 ml reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 10mM H_2O_2 and the crude enzyme solution. The reaction was initiated by the addition of H_2O_2 solution and the activity was determined by monitoring the decrease of absorbance at 240 nm, as a result of the consumption of H_2O_2.

RESULTS

Ca^{2+} and K^+ application enhances RWC when water is limited

RWC is the relation between the actual and fully turgid water contents of leaf tissue and it is closely associated with the developmental and physiological activities. RWC is an appropriate measurement of plant hydration status in terms of the physiological consequence of cellular water deficit (Araghi and Assad, 1998). No significant differences were noted in the RWC of the non supplemented and seedlings supplemented with Ca^{2+}, K^+ or N under well watered conditions. Withholding water reduced RWC of the non supplemented *B. napus* L. seedlings to approximately 59 ± 6.8% (Figure 1). However, supplementation with Ca^{2+} under the same conditions gradually increased the RWC when compared with non supplemented seedlings. The RWC of the seedlings was 64 ± 7.0, 70 ± 3.0 and 74.0 ± 0.01% when exposed to drought stress for 10 days after supplementation of 30, 60 and 90 mM $Ca(NO_3)_2.4H_2O$, respectively. K^+ supplementation also gradually increased the RWC of the seedlings under drought stress conditions. RWC of 76 ± 3.1 and 83 ± 4.1% of the *B. napus* L. seedlings supplemented with 100 and 150 mM KNO_3 was significantly higher than that of the non supplemented seedlings. However, RWC of Ca^{2+} and K^+ supplemented and non supplemented seedlings under well watered conditions were statistically similar. Furthermore, RWC of the seedlings exposed to drought after N supplementation was not significantly different from non supplemented controls.

Ca^{2+} and K^+ supplementation enhances MSI

The degree of injury to cell membranes was estimated through measuring the electrolyte leakage from leaf tissues. Data recorded for MSI during this experiment revealed that the seedlings had suffered damage to the membranes, irrespective of supplementation under drought stress conditions. With no supplementation, the MSI decreased from 92.59 ± 1.57% in well watered seedlings to 55.62 ± 3.07% in drought stress seedlings. Partial protection of the membranes, shown by MSI of 80.14 ± 3.23 and 75.51 ± 5.34% in seedlings after 10 days drought, was noted after supplementation with 90 mM $Ca(NO_3)_2.4H_2O$ and 150 mM KNO_3, respectively. Application of N, on the other hand, further enhanced damage to cellular membranes shown by MSI of 44.94 ± 6.74% after 100 mM NH_4NO_3 supplementation, indicating significantly more damage when compared with control seedlings (Figure 2A).

Protection of Chl with Ca^{2+} and K^+ application under drought

An increase, though statistically non-significant, in chlorophyll content was noted in the seedlings supplemented with Ca^{2+}, K^+ and N when compared with the control plants under well watered conditions. However, significant reduction was noted in the seedlings after 10 days of water deficit, irrespective of supplementation or no supplementation (Figure 2B). Chlorophyll content in the well watered seedlings under control conditions was 1199.67 ± 19.66 $\mu g.g^{-1}$ FW which decreased to 696.67 ± 17.62 $\mu g.g^{-1}$ FW or 58% of irrigation after exposure to drought. After supplementation with 30 mM $Ca(NO_3)_2.4H_2O$, the chlorophyll content was 1216.00 ± 14.00 $\mu g.g^{-1}$ FW in well watered and 946.33 ± 23.71 $\mu g.g^{-1}$ FW (78% of irrigated control) in seedlings exposed to drought stress. Addition of more Ca^{2+} resulted in further increase in Chl content. After 90 mM $Ca(NO_3)_2.4H_2O$ supplementation, chlorophyll content under well watered conditions was 1198.67 ± 26.63 $\mu g.g^{-1}$ FW while it was 1088.67 ± 10.79 $\mu g.g^{-1}$ FW (91% of well

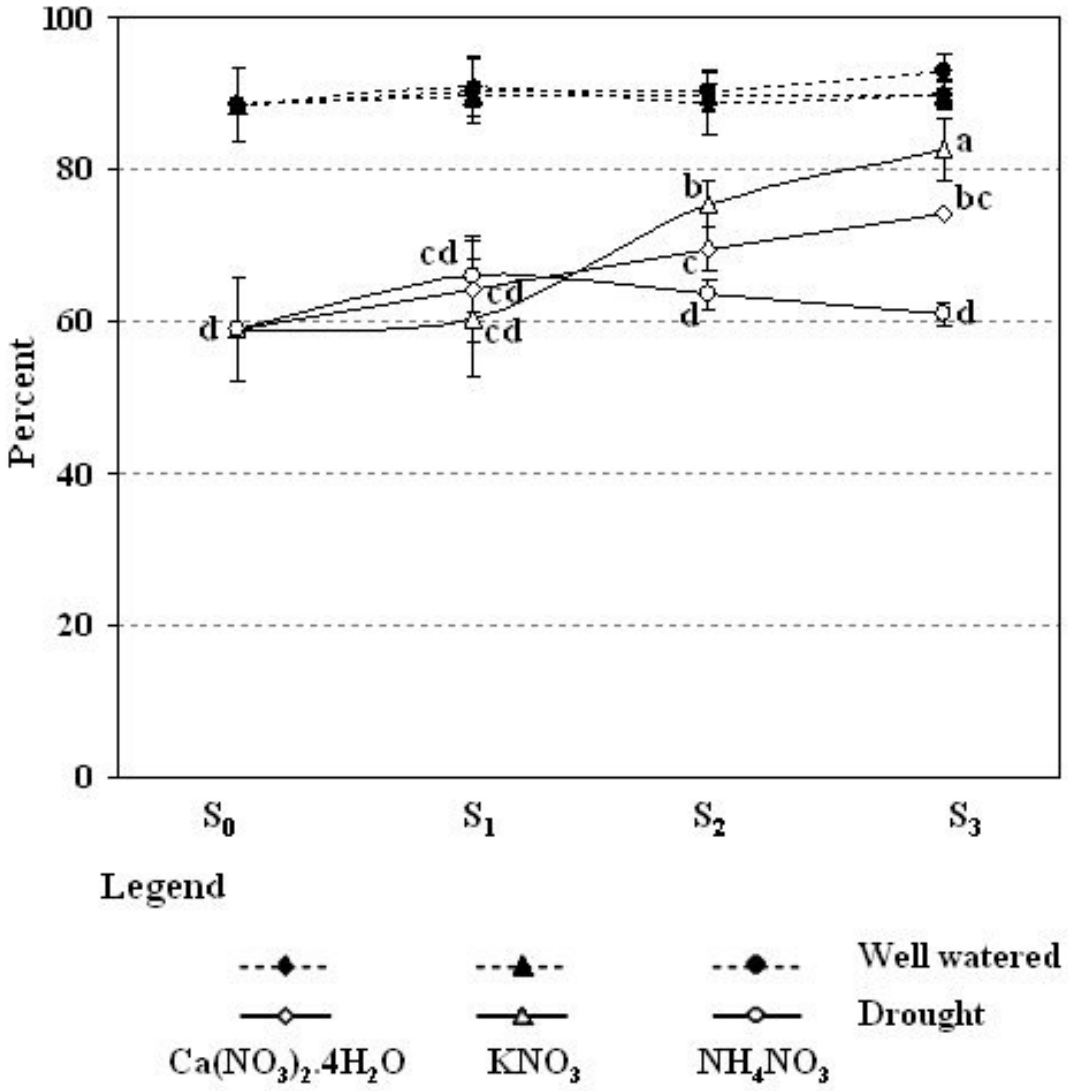

Figure 1. RWC under well water and drought stress condition of *B. napus* seedlings induced by increasing concentration of Ca, K and N. Data are shown as mean of five seedlings from each replication. S_0 represent no supplementation and S_1, S_2 and S_3 represent $Ca(NO_3)_2.4H_2O$, KNO_3 and NH_4NO_3 application in increasing order of quantity. Data with same letter are not significantly different ($P > 0.05$) according to LSD test.

watered control) in seedling exposed to water deficit for 10 days. K^+ application also resulted in increase in Chl content and maximum of 1033.33 ± 8.02 µg.g^{-1} FW (84% of well watered control) Chl was obtained after 150 mM KNO_3 supplementation. N supplementation, however, is negatively correlated with Chl content under drought stress conditions. After 30 mM NH_4NO_3 supplementation, Chl content was 1204.00 ± 24.43 µg.g^{-1} FW which decreased to 927.33 ± 10.79 µg.g^{-1} FW (77% of irrigated control) after withholding water for 10 days. At the maximum 100 mM NH_4NO_3 supplementation, Chl content in well watered seedlings was 1230.67 ± 17.04 µg.g^{-1} FW. However, the maximum decrease in Chl content of the seedlings exposed to water stress (620.00 ± 16.09 µg.g^{-1} FW; 50% of control) was obtained with the same supplementation.

AOS scavenging capacity after Ca^{2+} and K^+ application under drought

In order to study the physiological state of the non supplemented seedlings and those supplemented with Ca^{2+}, K^+ or N in irrigated and drought stress conditions, the content of the osmoprotectant and AOS scavenger Pro were also measured in the leaves. Drought stress stimulated Pro accumulation in the *brassica* seedlings (Figure 3A). The Pro content increased from 2.00 ± 0.18

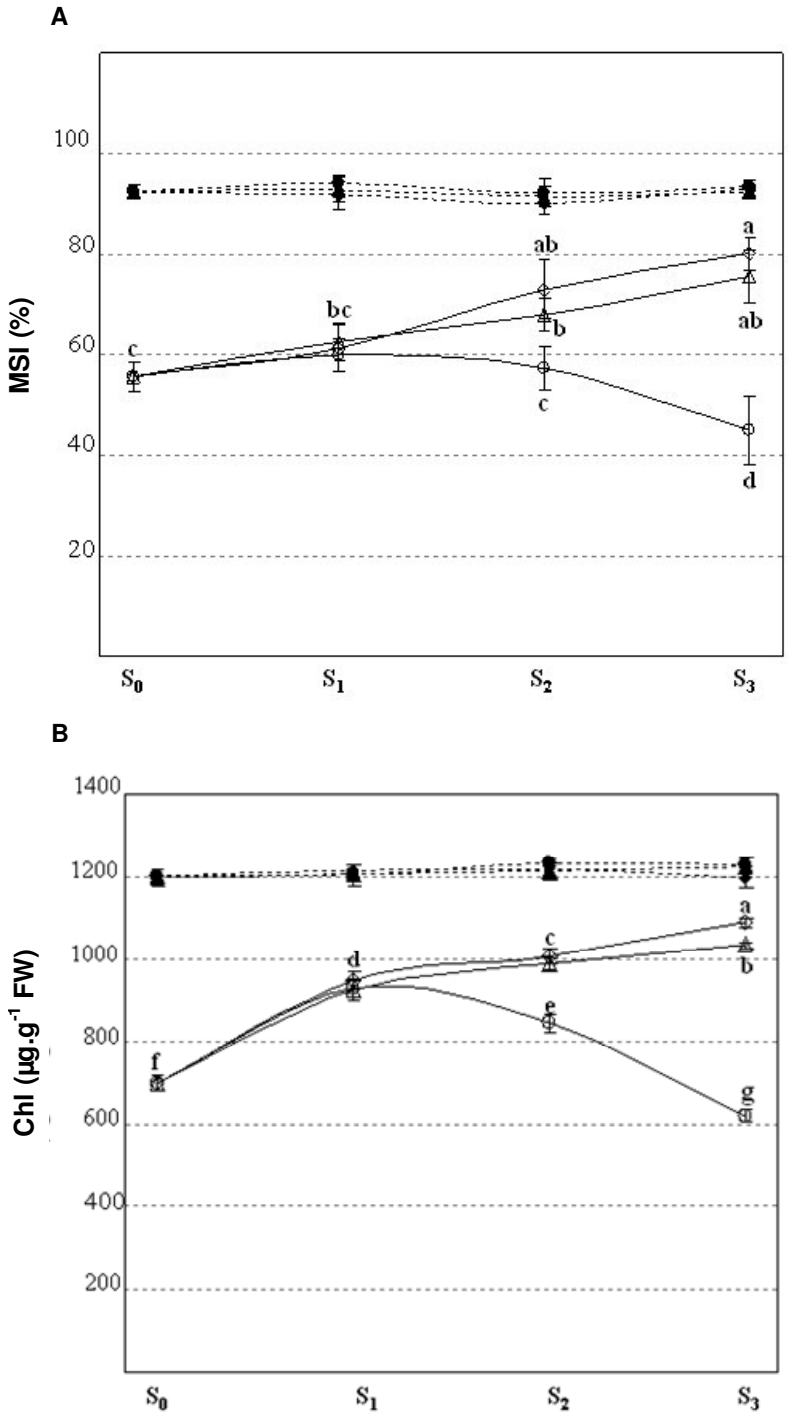

Figure 2. Dose dependent enhancement of MSI **(A)** and Chl protection **(B)** under drought stress after supplementation with Ca or K. Result represents means ± SD of at least five seedlings from each replication. S_0 represent no supplementation and S_1, S_2 and S_3 represent $Ca(NO_3)_2.4H_2O$, KNO_3 and NH_4NO_3 application in increasing order of quantity. Data with same letter are not significantly different ($P > 0.05$) according to LSD test.

to 9.16 ± 0.75 µmol.g^{-1} FW 10 days after exposure to drought. The supplementation with Ca^{2+} strongly induced Pro accumulation under drought stress conditions. The Pro content was 14.16 ± 1.51, 17.79 ± 1.91 and 23.93 ± 1.55 µmol.g^{-1} FW in the seedlings exposed to drought stress after supplementation with 30, 60 and 90 mM

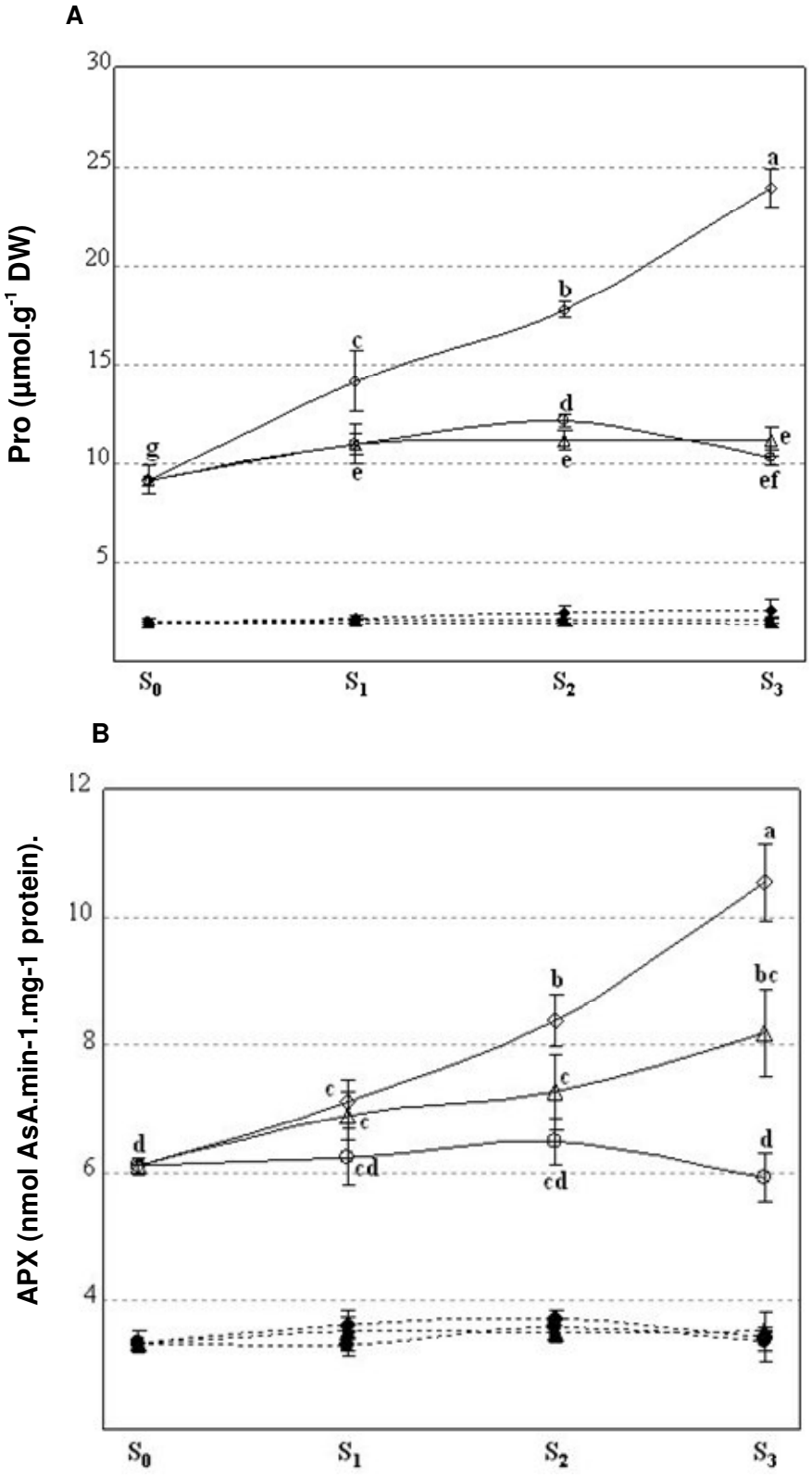

Figure 3. Induction of Pro **(A)**, APX **(B)** and CAT **(C)** activities after exogenous application of Ca. Result represents means ± SD of at least five seedlings from each replication. S_0 represent no supplementation and S_1, S_2 and S_3 represent $Ca(NO_3)_2.4H_2O$, KNO_3 and NH_4NO_3 application in increasing order of quantity. Data with same letter are not significantly different ($P > 0.05$) according to LSD test.

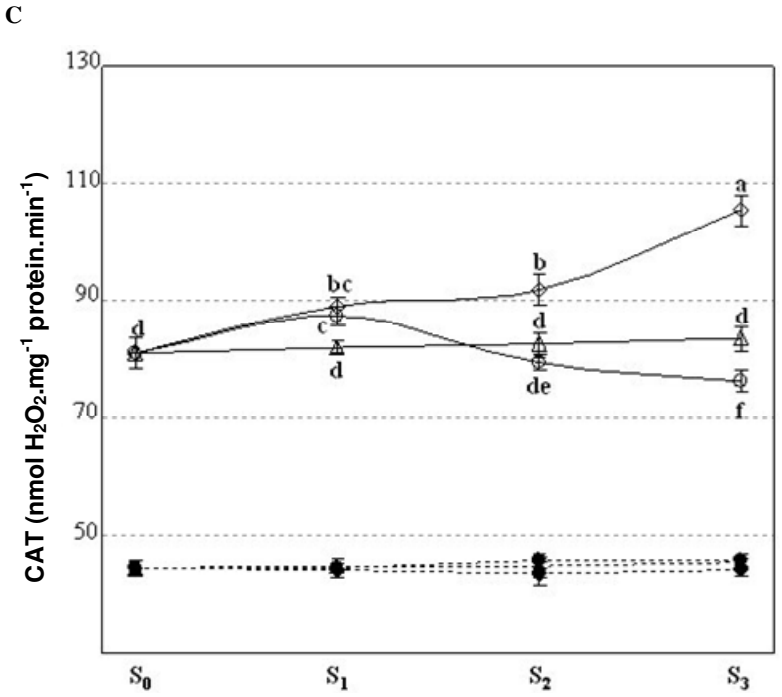

Figure 3. Contd.

$Ca(NO_3)_2.4H_2O$. K^+ and N supplemented seedlings also had higher content of Pro when compared with non supplemented seedlings under drought stress conditions. However, supplementation of Ca^{2+}, K^+ or N has no significant effect on Pro content in the well watered seedlings.

The activities of two antioxidant enzymes, APX and Cat involved in AOS scavenging were also determined. The activities of APX and Cat were 3.36 nmol AsA.mg^{-1} protein.$min^{-1}$and 44.50 nmol H_2O_2.mg^{-1} protein.min^{-1}, respectively in well watered conditions (Figure 3B and 3C, respectively). Under similar conditions, supplementation with Ca^{2+}, K^+ or N had no significant effect on the activities of these enzymes. The activity of Cat was 43.57 to 45.83 nmol H_2O_2.mg^{-1} protein.min^{-1}, whereas, that of APX was 3.33 to 3.74 nmol AsA.mg^{-1} protein.min^{-1}, respectively in well watered seedlings. However, an increase in the activities of both Cat and APX was noted seven days after exposure of seedlings to water deficit. Similar to the Pro content, an increase in induction of both the AOS scavenging enzymes was noted after application of Ca^{2+}, however, the activities of these enzymes did not differ significantly between the non supplemented and seedlings exposed to drought stress after supplementation with K^+ and N. Maximum Cat and APX activities of 105.47 nmol H_2O_2.mg^{-1} protein.$min^{-1}$and 10.55 nmol AsA.mg^{-1} protein.min^{-1} (2.39 and 3.04 fold increase over control) were obtained when seedlings were exposed to drought stress after supplementation with 90 mM of $Ca(NO_3)_2.4H_2O$.

DISCUSSION

Different climatic models predict more extreme weather conditions, with more frequent droughts and torrential rains, in the future. Under these conditions, research into crop management practices that enhance drought resistance, plant water use efficiency and growth when water is limiting has become increasingly important. Water deficit led to diminished plant uptake of mineral nutrients and to greater recalcitrance of minerals in soil, leading to a reduction of mineral nutrients in the ecosystem due to losses through leaching and erosion. However different studies indicate that when limited, plant can remobilize the mineral nutrients from older tissues and cellular stores (Sardans and Penuelas, 2007). Thus, it was hypothesized that replenishing the cellular stores with excess of Ca^{2+} or K^+ will improve drought tolerance potential of the plants.

The first response of plants to water deficiency is the closing of stomata. During this experiment, an increase in RWC was observed under drought stress conditions with the supplementation of both Ca^{2+} and K^+ in a dose dependent manner. Stomatal movement is affected by changes in osmotic potential and turgor of the guard cells through modulation of ion movement across the membranes and accumulation of osmotically active solutes (Gilroy et al., 1991). A Ca^{2+}-dependent and Ca^{2+}-independent transduction pathway linking abscisic acid perception to stomatal closure is in operation in plants (Cousson and Vavasseur, 1998). However, ABA is less

effective at regulating water transport in K-deficient sunflower plants (Fournier et al., 2005). Studies in *Arabidopsis* have found that K^+ starvation induces ethylene synthesis, and that ethylene inhibits ABA action on stomatal closure (Tanaka et al., 2005). Thus, the improved water relations in the seedlings supplemented with K^+ could be due to the osmotic adjustment as well as more regulated stomatal opening. Our results for heavy water losses from seedlings provided exogenous N is in close agreement with the previous findings of reduction in root growth, increase in certain diseases and high water use associated with excessive use of N fertilizers under dry conditions (Heitholt, 1990). Furthermore, the net fluxes and tissue concentration of NH_4 and K^+ are negatively correlated (Hoopen et al., 2010), thus the enhanced susceptibility of NH_4NO_3 fed seedlings to drought could partly be due to K^+ efflux and low tissue concentration.

Production of AOS is one of the major secondary responses of stress. Genotypes able to maintain a low steady state of the AOS are better adapted to tolerate stress conditions. Damage to cellular membranes and Chl can be used as reliable indicators to determine the extent of damage suffered by plants due to the AOS. Maintaining the integrity and stability of cell membranes under stress conditions is a major component of drought tolerance in plants. Removal of water from the membrane disrupts the normal bilayer structure and displaces membrane proteins, leading to loss of membrane integrity, disruption of cellular compartmentalization and loss of enzymes activity, which are primarily membrane based (Bajji et al., 2001). Chloroplast membranes are in particular sensitive to oxidation stress damage caused by the generation of excessive amount of AOS in these membranes (Asada, 1999; Karpinski et al., 1997). Our results indicate that water deficiency resulted in damage to the cellular membranes irrespective of the supplementation. Partial protection of the membranes was observed after supplementation with Ca^{2+} and K^+, however, application of N further increased membrane damage. Similarly, Chl content could estimate influence of the environmental stress on growth and yield, since it is closely correlated with the rate of carbon exchange (Araus et al., 1998). Seedlings supplemented with Ca^{2+} and K^+ suffered lesser reduction in the Chl content, indicating better adoption to water deficit. N application, on the other hand, resulted in the higher reduction of Chl content. K^+ has been implicated in the maintenance of photosynthetic CO_2 fixation. There is increasing evidence that plants suffering from environmental stresses such as drought have higher requirement for K^+ to maintain photosynthesis and protect chloroplast membranes from oxidative damage. Furthermore, the activity of NADPH (nicotinamide adenine dinucleotide phosphate-oxidase) oxidase decreases with increase in K^+ concentration, resulting in lower production of AOS and protection of membranes (Cakmak, 2005). Similarly, an increase in the cytosolic Ca^{2+}, as a signaling molecule, regulates genes expression as well as different physiological and biochemical processes. Ca^{2+} treatment has been previously reported to increase antioxidant enzyme activities, resulting in protection of membranes and enhanced resistance to abiotic stresses (Jiang and Huang, 2001).

Drought tolerance or sensitivity of plants is closely correlated with the activation of antioxidant response (Smirnoff, 1993). During this experiment, we measured the content of osmoprotectant and AOS scavenger Pro as well as the activities of CAT and APX, the two most important enzymes for detoxification of H_2O_2. An increase in the Pro accumulation is correlated with enhanced abiotic stress tolerance (Kumar et al., 2008; Zhu et al., 1998). In our case, maximum increase in Pro content was noted with exogenous Ca^{2+} application, though seedlings supplied with K^+ and N before drought exposure also had more Pro as compared to non supplemented controls. In agreement with our results, previous reports found an increase in Pro content in rice under salt stress conditions that was proportional to tissue Ca^{2+} concentration (Kumar et al., 2008). However, unlike this study, the previous reports concluded that the increase in Pro content was also proportional to tissue K^+ concentration.

In limited CO_2 supply under drought stress conditions, the rate of photorespiration increases which enhances H_2O_2 production through the glycolate oxidase reaction (Noctor et al., 2002). Beside H_2O_2 production, photorespiration provides metabolites for other metabolic processes, e.g. glycine for the synthesis of glutathione, which is also involved in stress protection (Wingler et al., 2000). CAT is essential to prevent H_2O_2 accumulation coupled to the photorespiration pathway, and represents one of the primary enzymatic defenses against oxidative stress induced by senescence, chilling, dehydration, osmotic stress, wounding, paraquat, ozone and heavy metals (Apel and Hirt. 2004). However, in the photosynthetic organisms, APX is the most important enzyme for scavenging H_2O_2. During this study, an increase in the activity of CAT and APX was noted after the seedlings were exposed to drought. Though minor increase in CAT and APX activities were noted in seedlings exposed to drought after supplementation of K^+, maximum induction of the activities of these enzymes were noted after application of Ca^{2+}. The induction of antioxidant enzyme activities are in agreement with previous reports wherein Ca^{2+} has been identified to be involved in ABA-induced APX and SOD activities under chilling (Zhou and Guo., 2009) and salt stress (Amor et al., 2010). Thus, taken together with the Pro content, this study show an increase in the AOS scavenging capacity of plants treated with Ca^{2+}. Though exogenous K^+ application had little effect on the quantity of Pro and induction of CAT and APX activities, it protected the membranes and improved water relations of the seedlings mainly through

functioning as an osmoticum and decreasing the degradation of proteins.

In conclusion, this study demonstrate that exogenous addition of both Ca^{2+} and K^+ significantly benefited *B. napus* seedlings, improving the water relations and ameliorating the damage after exposure to drought stress. Further investigation into the metabolites, transcripts and proteins of the seedlings exposed to drought stress conditions after supplementation with different combinations of these elements would provide more insights into adaptation mechanism and help in designing an integrated strategy for improving stress tolerance in crop plants.

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