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SOME PHYSIOLOGICAL RESPONSES OF CANOLA *(BRASSICA NAPUS* L.*)* TO WATER DEFICIT STRESS UNDER LABORATORY CONDITIONS*

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Abstract – Drought is considered as one of the most important limiting factors for oil seed canola plant (*Brassica napus* L.) growth and productivity in Iran. On the basis of root and shoot dry weight as affected by water stress exerted by PEG 6000, out of 9 canola cultivars, a relatively tolerant (cv. Aghaii) and a sensitive cultivar (cv. PO4) were selected. Their responses to low water potential with respect to changes in activity of the antioxidant enzyme ascorbate peroxidase, K^+ uptake, and its translocation to the shoots and production of osmoprotectants proline and soluble sugars were analyzed and compared. Although low water potential did not influence ascorbate peroxidase activity, constitutively the enzyme activity was significantly higher in the tolerant cultivar. A significant increase in root proline content was observed with a decrease in external water potential up to -.48 MPa. The increase was 2 to 3 times higher in the tolerant Aghaii cultivar. Although the total soluble sugars tended to increase under low water potential, the amounts accumulated were mostly comparable in both cultivars. K^+ uptake by the roots and its translocation to the shoots decreased at low water potential, however the amount taken up and translocated was consistently higher in the tolerant cultivar. Constitutively higher ascorbate peroxidase activity along with the higher rates of proline accumulation and K^+ uptake are taken as part of the mechanisms which confer drought tolerance to the Aghaii cultivar.

Keywords – Water stress, canola plants, ascorbate peroxidase, proline, soluble sugars, K^+ uptake

1. INTRODUCTION

Water deficit stress due to drought, salinity or extremes in temperature is the main limiting factors for plant growth and productivity resulting in large economic losses in many regions of the world [1]. Plants respond to water stress through a number of biochemical, physiological and developmental changes [2, 3]. These include: decrease in photosynthetic carbon assimilation due to stomatal closure and losses in chloroplast activity [4], down-regulation of PS II activity [5], increase in O_2 consumption by Mehler-peroxidase reaction and photorespiration [6,7], increase in leaf ABA concentration and induction of many stress-responsive genes by ABA [3, 8], modification of the lipid matrix of the plasma membrane and the changes in the physical organization of the membrane [9], and accumulation of osmoprotectants such as sugar alcohols, amino acids and organic acids [10]. The type of responses observed depend on several factors such as severity and duration of the stress and the plant genotype.

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Oilseed canola plant (*Brassica napus* L.) is an important agricultural crop grown primarily for its edible oil. The meal that remains after oil extraction has value as a source of protein for the livestock feed industry [11]. In Iran, the production of the canola plant is limited by soil salinity and drought. Therefore, development of varieties or selections with increased salinity and drought tolerance is of prime importance for growing this economical plant in regions where water is limited. Understanding the biochemistry and physiology of canola adaptation to water stress will help develop varieties with enhanced stress tolerance. The observations that water deficiency causes the chloroplasts of wheat (*Triticum aestivum* L.) to reduce oxygen to superoxide [12] prompted McKersie *et al*. [9] to hypothesize that plants overexpressing antioxidants might have improved water deficit tolerance. In the present work, nine canola cultivars were tested for their tolerance to water stress exerted by polyethylene glycol 6000 (PEG 6000). On the basis of shoot and root dry weight, the relatively tolerant and sensitive cultivars were selected and were further analyzed for their responses to water stress by: 1) comparing the activity of antioxidant enzyme ascorbate peroxidase; 2) changes in membrane permeability to K^+ and 3) the extent of osmoprotectants production.

2. MATERIALS AND METHODS

a) Plant growth and stress application

Seeds of nine *Brassica napus* L. canola cultivars, Hayolla 401, Hayolla 408, Abshani, Okap, Aghaii, PO4, Tallai, SCM and Orient, were supplied by the Agricultural Research Experimental Station in Fars Province, Iran. Seeds were surface sterilized in 10% (v/v) sodium hypochlorite for 10 minutes, followed by several washes with distilled water. Seeds were germinated on wet filter papers in the dark at 25°C and uniform seedlings were transferred to plastic pots with roots immersed in 270 ml of aerated half-strength Hoagland nutrient solution. After six days, water stress was induced by adding PEG 6000 to the nutrient solutions.The Michel [13] equation was used to calculate the solutions water potential (Ψ). To minimize osmotic shock, PEG was raised to the desired levels by a stepwise addition of 6.4 gr PEG at 8-hr intervals. The seedlings were maintained in a growth room with 16:8 hr light-dark regime, and an illuminance of 1.5×10^4 lux at 25±3°C. Seedlings were harvested after 9 days and separated into roots and shoots. Where needed, plant materials were dried for at least 24 hrs in an oven set at 70˚C.

b) Enzyme extraction and assay

To determine ascorbate peroxidase activity, 250 mg of mature fresh leaf tissues were sampled from 16 day old plants grown in different external water potentials. Tissues were homogenized using an ice cold mortar and pestle in 5 ml of cold grinding buffer consisting of 100 mM potassium phosphate buffer pH=7, 2.2 mM ascorbate and 1.0 mM EDTA. The homogenate was centrifuged at 10,000g for 5 minutes and supernatant was assayed for ascorbate peroxidase activity as described by Chen and Asada [14]. The reaction mixture in a final volume of 1 ml consisted of 100 mM potassium phosphate buffer pH=7.0, 0.22 mM ascorbate, 0.3 mM H_2O_2 and enzyme extract. The decrease in A_{290} due to oxidation of the ascorbic acid by H_2O_2 was monitored using a Shimadzo double-beam spectrophotometer model UV-160A.

c) Determination of proline and total soluble sugars

For proline determination, 0.5 g of fresh root tissue was taken from plants grown as described above, and proline content was measured according to Bates *et al*. [15]. Total soluble sugar was

extracted from 0.5 g of mature fresh leaves with 70% (v/v) boiling ethanol treated with chloroform to separate chlorophyll, and the amount of soluble sugar was measured as described by Prakash and Prathapsenan [16].

d) K^+ uptake

Six-day-old seedlings were transferred to Erlenmeyer flasks containing 130 ml of nutrient solution. The nutrient solutions contained 5 mM KCl plus different amounts of PEG to give the desired solutions water potential. The seedlings were kept for 24 hrs in a growth room as before, and K^+ contents of shoots and roots were determined by a Jenway flamephotometer model PFP7 and subtracted from endogenous K^+ to give net K^+ taken up by the roots and translocated to the shoots. K^+ content was expressed in terms of mmole per Kg dry weight per hr. Each experiment was repeated three times and results were statistically analysed by SPSS 9.0.

3. RESULTS AND DISCUSSION

a) Screening cultivars for drought tolerance

Water stress stimulated root growth in most cultivars. When each cultivar was grown in solutions with different water potentials (-0.29, -0.4, -0.48, -0.71 and –1.17 MPa), the average root dry weight of Aghaii and Okap cultivars was increased by 24 and 26.6%, respectively, as compared with the control plants (Table 1). In contrast, in PO4 cultivar, the average root dry weight was decreased by 28.02%. Water stress reduced shoot growth in all cultivars. The effect on Abshani and Aghaii was the least, while the PO4 cultivar was the most affected. On the basis of these results, Aghaii and PO4 were chosen as relatively tolerant and sensitive cultivars, respectively, and their responses to water stress with respect to ascorbate peroxidase activity, proline and soluble sugar accumulation and K^+ uptake were studied.

Table 1. Effect of water stress on root and shoot dry weight of nine canola cultivars. Each value is percent dry weight relative to control. For details, see the text

Cultivars	Okap	Aghaii	Hayolla 401	Abshani	Tallai	Orient	Hayolla 408	SCM	PO4
Root	126.6 A	124.0 A	117.5 A	110.9 AB	106.9 ABC	87.2 BC	82.1 CD	77.5 D	72.0 D
Shoot	51.5 AB	70.1 AB	61.1 AB	75.0 A	48.6 AB	44.3 AB	54.0 AB	41.9 B	41.8 B

b) Effect of water stress on ascorbate peroxidase

Although in both tolerant and sensitive cultivars, the decrease in external water potential did not affect leaf ascorbate peroxidase activity significantly (Fig.1), indeed the enzyme activity in tolerant cultivar was constitutively higher in all treatments. Different types of environmental stresses exert at least part of their effects by causing oxidative damage. Consequently, the plant antioxidant defense systems and their roles in protecting plants against stresses have attracted considerable interest. SOD-transgenic alfalfa plants tended to show less injury from water-deficit stress [9]. Thus, these results seem to support the hypothesis that tolerance in oxidative stress plays an important role in adapting plants to adverse environmental conditions [9, 17]. In leaves of *Allium schoenoprasum* L., drought increased the specific activity of ascorbate peroxidase by 29% [18]. Similar findings were reported by

Tanaka *et al*. [19] for spinach leaves and by Castillo [20] for *Sedum album* leaves under mild water stress. In salt tolerant plant species, higher constitutive levels and a significant increase in ascorbate peroxidase and monodehydroascorbate reductase activities have been reported by several investigators [21-23]. Since water deficit stress restricts CO_2 assimilation, the formation of a superoxide radical by the transfer of electrons from PSI to molecular oxygen (Mehler reaction) is enhanced [7]. A Superoxide radical is rapidly scavenged by superoxide dismutases, thus producing H_2O_2. Increased superoxide dismutases and ascorbate peroxidase activities or higher constitutive levels of these enzymes promote the removal of reactive oxygen species (ROS), thus conferring higher drought resistance to plants.

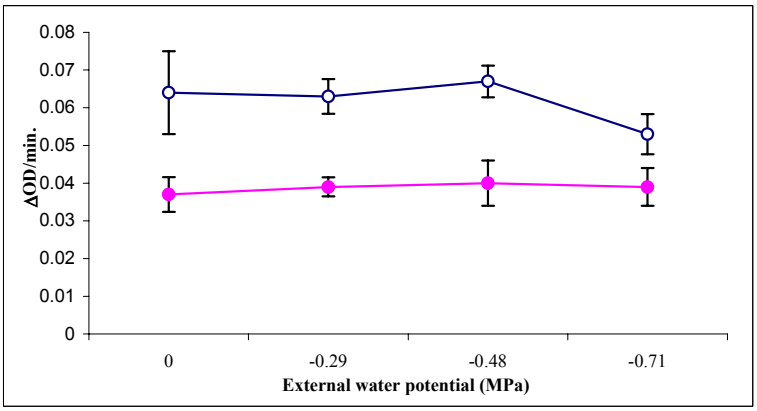

Fig. 1. Effects of external water potential on leaf acsorbate peroxidase in sensitive (●) and tolerant (○) canola plants. Each point is mean ± SE

c) Proline and total soluble sugars as affected by water stress

Drought stress up to –0.45 MPa resulted in a significant increase in root proline content of both cultivars, but the increase was more pronounced in tolerant rather than sensitive cultivar (Fig. 2). At –0.29 and –0.48 MPa water potentials, the proline content of sensitive cultivar increased by 90 and 160%, whereas the increase in resistant cultivar was 184 and 270%, respectively. At –0.71 MPa, proline accumulation was reduced in both cultivars. A common response to water deficit in plants is the accumulation of osmoprotectants such as proline and glycine betaine [17, 24]. Genotype variations in proline content have been reported in sorghum and in wheat under moisture stress [25].

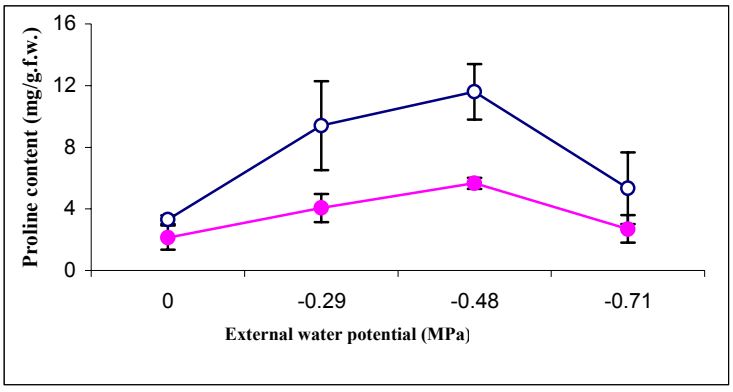

Fig. 2. Root proline extracted from sensitive (●) and tolerant (○) canola plants as affected by external water potential. Each point is mean ± SE

Total soluble sugar tends to increase, which is probably due to mobilization of reserved polysaccharides, by decreasing external water potential in both cultivars (Fig, 3). At -0.29 MPa, total soluble sugar was higher in resistant cultivar, whereas at –0.79 MPa sensitive cultivar accumulated more sugar. At all other water potentials no significant difference was observed between the two cultivars. An Increase in sucrose and hexose levels by water stress has been proposed in osmotic adjustment in sucrose-transporting species [26]. High resistance to several stresses has also been related to an increase in polyol synthesis [27]. Increased polyol transport, both in phloem and xylem occurs frequently as a result of salt and drought stresses. Engineering the synthesis of trehalose in tobacco greatly increased its drought tolerance [10]. This non-reducing disaccharide also efficiently stabilizes dehydrated enzymes and lipid membrane *in vitro*. In coleus plants, O-methylinositol was accumulated in response to water stress [2]. It is becoming clear that plants have developed several pathways to produce and accumulate a range of osmoprotectants as defense mechanisms against salinity and drought stresses. The occurrence of other compatible osmolytes and their degree of accumulation in canola needs further investigation.

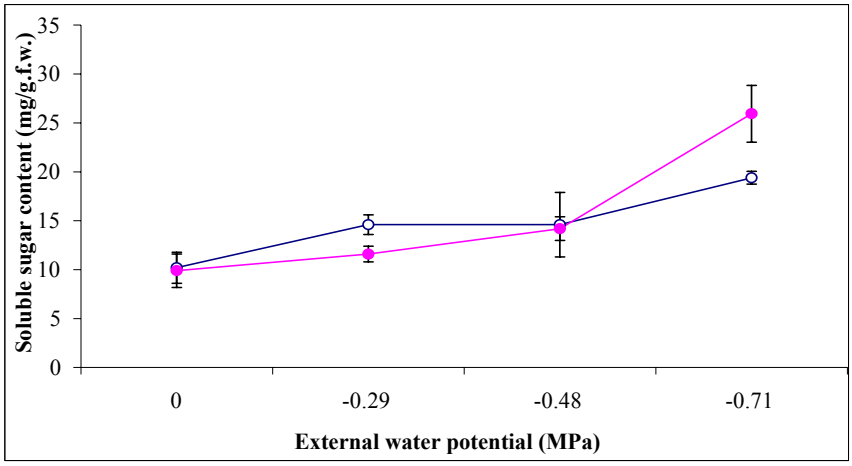

Fig. 3. Effects of water potential on leaf soluble sugar extracted from sensitive (●) and tolerant (○) canola plants. Each point is mean ± SE

d) K^+ uptake and translocation

Water stress at all levels reduced the rate of K^+ uptake by both cultivars (Fig. 4), however the reduction was more pronounced in the PO4 (sensitive) cultivar. Potassium translocation to the shoots followed the same pattern as K^+ uptake by the roots (Fig. 5). Water stress decreased the rate of K^+ translocation to the shoot much more in the sensitive cultivar than in the tolerant one, which may be due to a higher absorption of K^+ by tolerant cultivar. The observed differences in the K^+ uptake and translucation may be due to a lesser adverse effect of water stress on the membrane integrity of tolerant cultivar. In alfalfa, the disruption of membrane integrity by water deficit was estimated by leakage of cytoplasmic solutes from leaf discs [9]. After 5 days of water deficit, significant differences in electrolyte leakage was observed between SOD-transgenic and non-transgenic alfalfa plants, suggesting less injury to the membranes by drought-generated ROS in SOD-transgenic plants.A higher accumulation of K^+ has been reported in the roots and shoots of salt tolerant *Brassica* species by Ashraf *et al*. [28]. It has been suggested that K^+ accumulation is also a component of osmotic adjustment in salt- and water-stressed plants [24]. Continued root growth under water deficit stress observed in some canola cultivars facilitates water uptake from the soil and is considered to be

an adaptation of plants to drought [29-31]. In addition, since shoot growth is sensitive to low water potential, selection of cultivars with less sensitive shoots to growth inhibition by water stress is also of great significance for canola cultivation. It may be concluded that a higher costitutive level of antioxidant enzyme ascorbate peroxidase, higher accumulation of osmoprotectant proline, and probably a more organized cell membrane under water deficit stress are part of the mechanisms which confer drought resistance to Aghaii cultivar in this study.

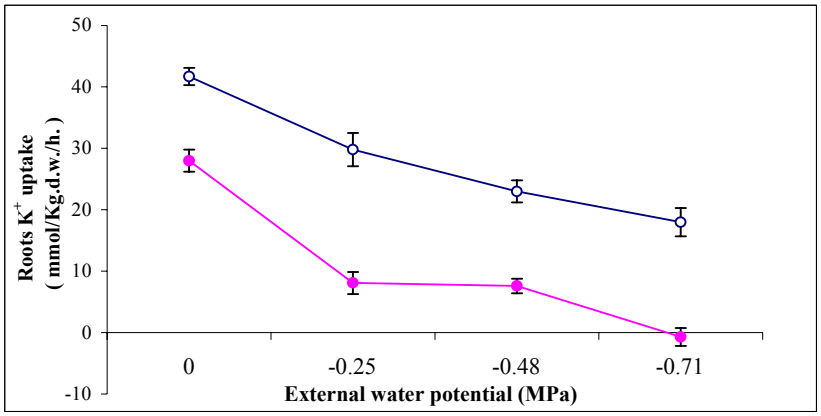

Fig. 4. K^+ uptake by the roots of sensitive (●) and tolerant (○) canola plants as affected by external water potential. Each point is mean ± SE

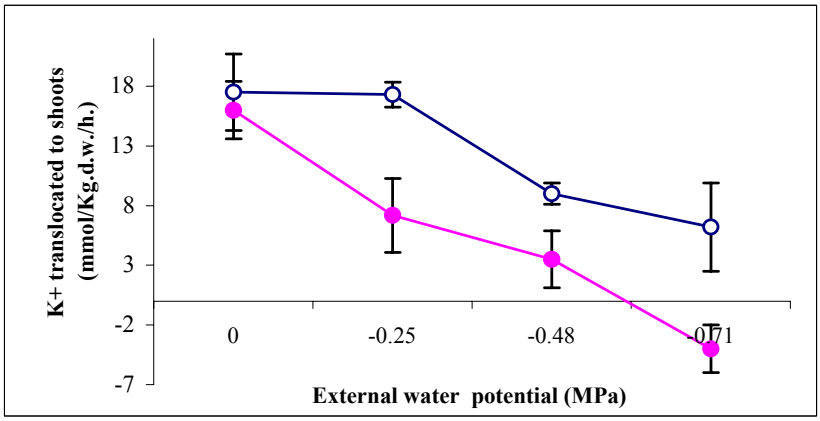

Fig. 5. Effects of external water potential on K^+ translocated to the shoots of sensitive (●) and tolerant (○) canola plants. Each point is mean ± SE

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