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Full Length Research Paper

Identification of proteins involved in excess boron stress in roots of carrot (*Daucus carota* L.) and role of niacin in the protein profiles

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Plants are constantly challenged by various biotic and abiotic stresses in nature. Boron toxicity have become one of the important abiotic stress factor for plants. Boron toxicity responses of plants is reflected by alterations in protein expression level, activity, location and concentration. In this study, we identified the proteins which respond to the boron excess stress in *Daucus carota* root cells, and examined the effects of niacin (nicotinamide) on the protein profiles of boron excess. Protein profile changes in responce to boron and niacin were compared with the control using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) gel electrophoresis. Protein **bands of** interest were **excised from gel and digested by trypsin. The trypsin-digested peptides were analyzed on nanoLC-ESI-MS/MS. Six different proteins involved in plant defense system were identified in total [CR16 (Major allergen Dau c1), glutathion peroxidase, glyoxylase I, ısocitrate dehydrogenase (NADP$^+$), adenosyl homocysteinase, EDPG precursor)]. Our results clearly demonstrated the presence of boron and niacin in growth medium stimulated expression and synthesis of proteins role in plant defence mechanism.**

Key words: Boron, niacin, *Daucus carota*, plant defence enzymes.

INTRODUCTION

Drought, cold and freezing, heat, salinity, nutrient deficiency, toxic heavy metals, oxidative stress as well as oxygen shortage and mechanical stress are the diverse forms of abiotic stresses (Poethig, 2001; Somerville and Dangl, 2000; Arnhold-Schmitt, 2004). Excess boron stress is one of the most significant abiotic stress that affects every aspect of plant physiology and metabolism. The physiological effects of boron toxicity include reduced root cell division (Liu et al., 2000), decreased shoot and root growth (Lowatt and Bates, 1984; Nable et al., 1990), decrease in leaf chlorophyll, inhibition of photosynthesis, lower stomatal conductance (Lowatt and Bates, 1984), deposition of lignin and suberin (Ghanati et al., 2002), reduced proton extrusion from roots (Roldan, 1992), increased membrane leakiness peroxidation of lipids and altered activities of antioxidation pathways (Karabal et al., 2003). The mechanisms of the toxicity revolve around the possible disruption of cell wall development due to the excess binding to apiose (furanoid sugar in the rhamnogalakturonan II complex), or metabolic disruption due to binding to ribose, either as the free sugar or as a component of key compounds such as RNA, ATP, NADP or NADH. During excess boron stress, high concentrations of boron enters the cells and accumulates to a concentration that induces ionic and osmotic stress in plants (Reid et al., 2004).

Tolerance is associated with the ability to restrict boron uptake into the plant (Nable, 1988; Paull et al., 1988). It was so surprising that permeability to boron were similar in both sensitive and tolerant genotypes (Hayes and Reid, 2004). The difference was that although a steady state internal boron concentration in the tolerant cultivar was maintained at approximately half that of the sensitive variety, and at equilibrium boron concentration in the sensitive cultivar was similar to that in the solution, while

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in the tolerant cultivar, the internal concentration was much lower. This difference in the concentration clearly must require an input of energy in order to maintain the concentration gradient. Boron is actively pumped from the cells in the tolerant varietes like carrot. Understanding the signalling pathway of plant boron resistance is important for improving plant boron tolerance, especially for improving agricultural productivity in irrigated land. Plant cells respond and adapt to these adverse conditions through signalling networks by linking glycoprotein and glycolipids (Parr and Loughman, 1983) and altering the homogenity of the membrane (Linden et al., 1973). Membrane rafts are thought to have a specific physiological role in membrane signal transduction and to serve as the sites for glucosylphosphatidyl-inositol (GPI) protein association which would provide a significant number of B complexing sites. BOR 1 is a transporter protein which catalyses the loading of boron from xylem parenchyma cells into the xylem of *Arabidopsis* at toxic boron concentrations (Takano et al., 2002). But at high boron concentrations, BOR 1 protein is degraded by nonparticipation in a boron tolerance mechanism, and a number of BOR 1-like genes appear in other plant species with different tissue expression patterns (Takano et al., 2005).

In this study, we aimed to identify the proteins that resulted from excess boron stress in carrot (*Daucus carota* L.) root cells and examine the effects of niacin, added to the excess boron medium to make a balance in the uptake of ribose attached NAD^+ content together with excess boron, on the expression of excess boron proteins for the first time.

MATERIALS AND METHODS

Plant material and culture conditions

Roots of carrot (*D. carota* L.) were surface sterilized with hypochloride solution (7.5%) and shake for 20 min before sowing to media. After sterilization, roots were washed with sterile distilled water for three times in sterile cabinet.

Callus induction

MS basal medium (Murashige and Skoog, 1962) at full strength was used for callus induction from root explants of carrot with the addition of 0.1 mg/l N^6benzyl adenine and 1 mg/l naphtalene acetic acid. A number of combinations of different media were assessed for callus induction. Media were prepared with: 6.2 mg/l boron and 0.5 mg/l niacin (control = MS); 31 mg/l (5 folds) boron (5B), 31 mg/l boron; addion of 2.5 mg/l niacin (5B/5N).

In this study, the jars containing five explants each were placed on Fitotron and regulated to 16 h/8 h light/dark photoperiod and 25 ± 2°C temperature condition.

Protein extraction

Proteins were extracted from *D. carota* callus following the method of Hurkman and Tanaka (1986) with some modifications. Briefly, 2 g of callus was homogenized first in liguid nitrogen. The homogenate was mixed thorougly in the presence of 2 ml of extraction buffer (100 mM Tris/HCl pH 8.0, containing 1 mM EDTA, 1 mM PMSF (phenlymethanesulfonly fluoride), 2% (v/v) β-mercaptaethanol). The homogenate was centrifuged for 30 min at 5000 rpm at 4°C. The supernatant was mixed with 4 vol of 0.1 M ammonium acetate in methanol and incubated overnight at -20°C. Proteins were pelleted by centrifugation at 5000 rpm for 30 min at 4°C. The pellet was washed twice with ammonium acetate in methanol, once with cold acetone (80% v/v), and dried at room temperature. The dried residue was resuspended directly in sample buffer (25 mM Tris pH 6.8, 1% w/v SDS, 10% (v/v) glycerol, 5 % (v/v) β-mercaptoethanol and 0.002% (w/v) bromophenol blue) and boiled for 2 min before eletrophoresis.

Protein determination

Protein contents were determined by the method of Bradford (1976) using bovine serum albumin as the standard protein.

SDS-polyacrylamide gel electrophoresis

D. corata root proteins were separated by SDS-polyacrylamide gel electrophoresis according to the method of Laemmli. SDS-PAGE was carried out using a Mini Protean II apparatus (Biorad, Richmond CA, USA). Polyacrylamide gel (12%) was prepared as described in the Biorad Mini Protean II Dual slab cell instruction manual. Protein bands was determined by molecular mass standards (sigma SDS7): bovine albumin (66 kDa), egg albumin (45 kDa), rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (36 kDa), bovine carbonic anhydrase (29 kDa), bovine pancreas trypsinogen, (24 kDa), soybean trypsin ınhibitor (20 kDa), bovine milk a-lactalbumin (14 kDa). Proteins in the gel were stained with Coomassie Brillant Blue R-250.

Protein identification using nanoLC-ESI-MSMS

Protein identification using Nano LC–ESI-MS/MS was performed by Proteome Factory (Proteome Factory AG, Berlin, Germany). Firstly, protein bands of interest were excised from the gel stained with Coomassie blue using sterile scalpel blades and then the excised gel pieces were washed three times with MQ water, destained twice with 50 mM NH_4HCO_3 in 50% acetonitrile, reduced with 10 mM DTT in 50 mM NH_4HCO_3, alkylated with 40 mM iodoacetamide in 50 mM NH_4HCO_3, dried twice with 100% acetonitrile and finally digested by trypsin (Promega, Mannheim, Germany) in 50 mM NH_4HCO_3. The trypsin-digested peptides were analyzed on nanoLC system. Peptide and MS/MS mass tolerance were set to ± 0.5 Da. For identification, proteins were searched against the NCBInr protein database using Mascot software (Matrix Science Ltd., London, UK).

RESULTS

Proteins extracted from *D. carota* callus were separated by SDS-PAGE and identificated by nanoLC-ESI-MS/MS. The names of identificated proteins were: [CR16(Major allergen Dau c1), glutathion peroxidase, glyoxylase I, ısocitrate dehydrogenase ($NADP^+$), adenosyl homo-cysteinase, EDPG precursor)] (Table 1). The differences between protein bands in the control and treated plants with 5B and 5B/5N were evaluated. Glutathion pero-xidase and glyoxylase I did not express in the control.

Table 1. Identification of proteins which respond to the boron excess stress in *D. carota* root cells using nanoLC-ESI-MSMS.

Protein number♣	Accession number	Best match protein	Species	Sequences of MS/MS matched peptides	emPAI	Mascot protein Score	Predicted molecular mass (kDa)	Expect value
1	gi|1663522	CR 16 (Major allergen Dau c1)	*Daucus corata*	KAIEAYLIAN KGDAVVPEENIKF KFADAQNTALFKA KIFSGIVLDVDTVIPKA RIITLPEGSPITSMTVRT MGAQSHSLEITSSVSAEKI	1.15	300	16	2.2e-06
2	gi|82581134	Glutathion peroxidase	*Plantago major*	KGGLFGDGIKW KAEYPIFDKV RYAPTTSPLSIEKD	0.18	103	19	0.00089
3	gi|4127862	Glyoxylase I	*Glycine max*	RVLGMSLLKR + Oxidation (M) KGYIMQQTMFRI RGFGHIGVTVDDTYKA + Methyl (DE)	0.16	78	21	0.038
4	gi|2623962	isocitrate dehydrogenase (NADP+)	*Apium graveolens*	KLVFVPEGKE KYFDLGLPHRD KTIEAEAAHGTVTRH	0.07	105	46	0.078
5	gi|417744	Adenosyl homocysteinase	*Lupinus luteus*	KVYVLPKH RATDVMIAGKV RGIIILAEGRL RWVFPDTGRG KDMSLADFGRL RHSLPDGLMRA RITIKPQTDRW KDIIMVSDMRK RTEFGPSQPFKG KVALIAGYGDVGKG RLVGVSEETTTGVKR RLVGVSEETTTGVKRL KDQADYISVPVEGPYKPAHYRY KSGAIPDPASTDNAEFQIVLSIIRD RLYQMQQNGTLLFPAINVNDSVTKS	0.94	696	53	3.3e-05

Table 1. Contd.

6	gi|285741	EDPG precursor	*Daucus corata*	KAVTEAFIKE RVGFSGTLLGSRT RIALPSQFASAFSFKR RTPLVSENLVVDLGGRF KDASTLQYVTTINQRT KISTINPYTVLETSIYKA RFLWVDCDQNYVSSTYRPVRC + Pro RTSIVIGGHQLEDNLVQFDLATSRV	0.74	427	46	3.5e-05

♣Protein number as indicated on the SDS-PAGE gel image.

Expression levels of CR16 (Major allergen Dau c1) and ısocitrate dehydrogenase (NADP$^+$), showed a significant increase as compared to the control. There was no significant difference between 5N and 5B/5N treatment. The same protein bands were identified after 5N and 5B/5N treatment. There was only difference in expression levels of CR16 (Major allergen Dau c1), ısocitrate dehydrogenase (NADP$^+$), adenosyl homocysteinase and EDPG precursor.

DISCUSSION

Under various stress conditions such as heat, cold, salt, drought and heavy metals, higher plants synthesize proteins that are involved in stress tolerance mechanism. Today, studies of plant species among genotypes of the same species which show susceptibility to boron toxicity and that these differences cause large differences in the plants due to boron toxicity are expressed equally, and are affected by the physiological and morphological mechanisms (Baykal et al., 2006). One of the several approaches towards understanding the physiological, biochemical and molecular mechanisms of boron tolerance may be the study of changes in polypeptide composition of root tissues during stress. An apoplastic function of boron was improved in recent years (Kobayashi et al., 1996). Sugar moieties such as mannose, apiase or galactose and other hydroxylated ligands such as serines and threonine acting as specific acceptor molecules form ester-like complexing with B (Ralston and Hunt, 2000). These B-binding membrane structures are known to be surface proteins named hydoxyproline-rich proteins which are found in lower contents in cell walls of *Phaseolus vulgaris* (Goldback and Wimmer, 2007). Mahboobi et al. (2000), used ten-day-old seedlings of barley {*Hordeum vulgare* L. Cultivar Anadolu [boron (B)-tolerant] and Hamidiye (B-sensitive)} in their studies. Total protein patterns were obtained by analysis of total protein extract from root and leaf tissues of control and B-treated plants using two-dimensional gel electrophoresis followed by silver staining. Silver-stained gels showed that B stress caused increases or decreases in a number of proteins in root and leaf tissues. Moreover, as a result of B treatment, one newly synthesized protein with relative molecular weight (Mr) of 35.0 kDa was detected in root profile of the tolerant cultivar. This protein failed to show up in root profile of the B treated sensitive cultivar. Three proteins were quantitatively increased in B treated root profile of both cultivars. Following B treatment, three proteins were increased in root profile of the tolerant cultivar, but were not changed in the sensitive one. In leaf tissues, however, there were remarkable changes in total protein profiles after B treatment, relative to the control. Following B treatment, in leaf tissues, at least seven proteins increased in amount in tolerant cultivar but were unchanged in the susceptible one. In tolerant and sensitive cultivars, amounts of two proteins were increased in B-treated plants, relative to control seedlings. In addition, four proteins (Mr:29, 58, 58 and 22 kDa) were unchanged in the control and B-treated seedlings of the tolerant cultivar. In the susceptible cultivar, however, among these four proteins, the first one (Mr:29) was very much reduced and the others (Mr: 58, 58 and 22 kDa) were completely lost in B-treated seedlings. Moreover, following B treatment, a set of high-molecular-weight proteins was quantitatively decreased in the susceptible cultivar but was unchanged in the tolerant cultivar. These results indicate that in barley, certain proteins may be involved in tolerance to B toxicity. In their studies,

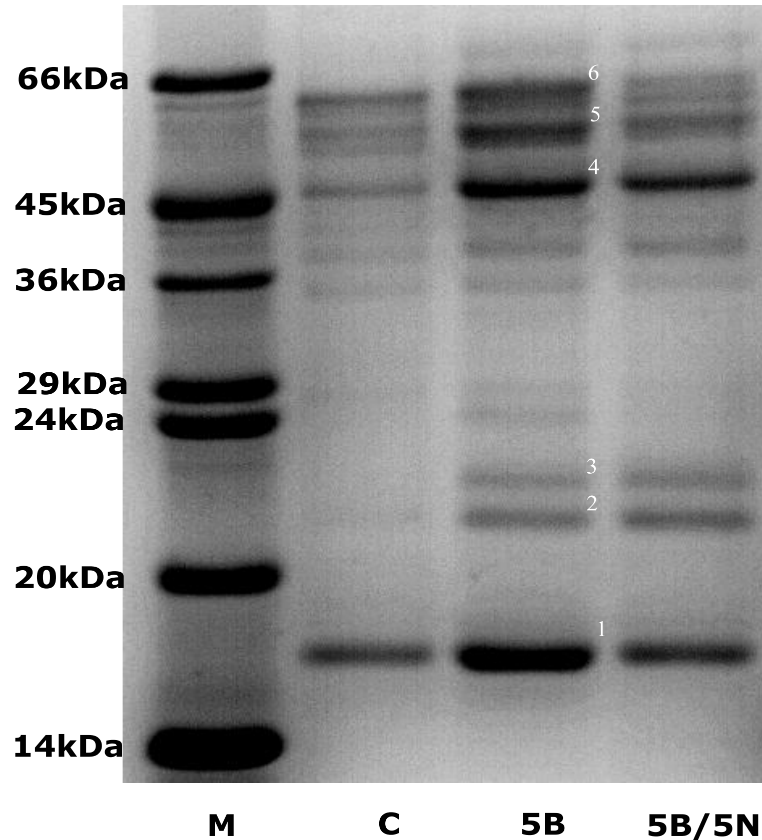

Figure 1. The SDS-PAGE profiles of *D. corata* callus proteins: Line 1 (marker), line 2 (control), line 3 [5 folds boron (5B)], line 4 [5 folds boron and niacin (5B/5N)]. 1- CR16 (Major allergen Dau c1), 2- glutathion peroxidase, 3- glyoxylase I, 4- isocitrate dehydrogenase ($NADP^+$), 5- adenosyl homocysteinase, 6- EDPG precursor)].

changes in polypeptide composition as a result of B toxic concentration in leaf tissues were more abundant than in roots. Therefore, it is suggested that these changes, especially at shoot level may form the basis of the tolerance mechanism to B toxicity.

Two pigeonpea [*Cajanus cajan* (L.) Millsp.] genotypes, a salt tolerant Manak and a salt sensitive ICPL 88039 were subjected to stress treatment of 3 mM boron, 60 mM NaCl and boron + NaCl at the seedling stage. Boron treatment were found to increase 28.3 kDa proteins in plumule and 38.3 and 51.9 kDa proteins in radicle of

Manak, however, there was no specific protein in ICPL 88039 either in plumule or in radicle. In NaCl treatment, 95.6 kDa proteins appeared in plumule and 67.5 kDa proteins in radicle of Manak. Conversely, content of some proteins decreased by boron treatment alone or in combination with NaCl although they were present in the controls. Thus, 54.3 kDa protein disappeared in ICPL 88039 plumule, 68.4 kDa in Manak radicle and 28.1 kDa in ICPL 88039 radicle (Bishnoi et al., 2006). Baykal et al. (2006) in their studies, investigated the responses of two genotypes belonging to two wheat species (*Triticum aestivum* L. cv. Kıraç 66 and *Triticum durum* Desf. cv. Kunduru 1149 to boron toxicity. The boron toxicity treatments was carried out with the addition of 0, 15, 30, 45, 60 and 75 mg kg^{-1} B in soil. At the end of the study, it was determined that seedling length and amount of percent dry matter were decreased, boron amount increased and relative water content did not changed significantly in boron exposed plants. Soluble protein amount were increased in Kiraç 66 genotypes at 60 mg kg^{-1} boron concentration, but were decreased at 45 and 75 mg kg^{-1} boron concentration in Kunduru 1149 genotypes. Results indicate that there were important differences between two studied genotypes in tolerance to boron toxicity.

In this study, six different proteins involved in plant defense system were identified [CR16 (Major allergen Dau c1), glutathion peroxidase, glyoxylase I, ısocitrate dehydrogenase ($NADP^+$), adenosyl homocysteinase, EDPG precursor (Figure 1)]. CR16 protein was a major root protein of carrot with high homology to intracellular pathogenesis-related proteins, stress induced proteins and polen allergens (Yamamato et al., 1997). Actin

binding and proline rich motifs of CR 16 proteins belong to the verprolin family of proteins which is the first member of verprolin identified from *Saccharomyces cerevisiae* (Donnelly, 1993). Pathogenesis related proteins are commonly induced in resistant plants, expressing a hypersensitive necrotic response (HR) to pathogens of viral, fungal and bacterial origin. Later, however, it turned out that B-proteins are induced not only in resistant, but also in susceptible plant- pathogen interactions, as well as in plants, subjected to abiotic stress factors (Edreva, 2005). Induction of pathogenesis-related proteins was investigated in legume root nodules under boron deficiency during the legume-rhizobia interaction (Reguera et al., 2010).

Boron is an abiotic stress, its toxicity triggers the formation of reactive oxygen species (ROS) in plant tissues. For protection against ROS, plants contain antioxidant enzymes such as superoxide dismutase (SOD) (EC 1.15.1.1), catalase (CAT) (EC 1.11.1.6) or ascorbate peroxidase as well as a wide array of non-enzymatic antioxidants (Mitler, 2002). The ascorbate/ glutathione cycle is known to play an essential role in the oxygen toxic species detoxification mechanisms and cells of roots and leaves under B-deficit conditions both ascorbate and glutathione levels have been shown to decrease (Çakmak and Römheld, 1997).

Under excess boron grown roots, glutathion peroxidase activity overexpression provided increased glutathione-dependent peroxide scavenging and ascorbate metabolism that lead to reduced oxidative damage (Virginia et al., 2000) in the same manner with the sunflower plants subjected to aluminium stress (Ruiz et al., 2006). This protein did not express in the control. Two important functions such as redox transducer and a scavenger in abscisic acid and drought stress responses of glutathione peroxidase were predicted in *Arabidopsis thaliana* (Yuchen et al., 2006). It has been demonstrated that B toxicity impedes the conversion of cysteine to glutathione. In addition, external application of cysteine, and especially of glutathione, significantly reduces B toxicity, these plants reflecting a foliar biomass similar to that of control (Ruiz et al., 2003). The concentrations of S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH) and methylated DNA were determined in embryos at different developmental stages and were found to increase during somatic embryogenesis of carrot (Munksgaard et al., 1995). Excess boron stress were found to induce adenosyl homocysteinase protein in the carrot root cells, a boron tolerant plant, showing a similarity with these studies.

Protein level of glyoxalase I was identified in excess boron grown carrot root cells and also in our experiments, we used the explants of carrot (*D. carota* L.) root and in band 4 which was obtained in 5B application was identificated as glyoxalase I. This protein did not express in the control. Glyoxalase I is mainly a detoxifiying enzyme in defense mechanism, and the levels were moderate in radish and carrot, while highest in onion (Hossain et al., 2007), also most of the stresses were found to increase the glyoxalase I activity, white light causing the highest induction followed by salinity, chemical, drought and heavy metal stresses in pumpkin seedlings (Hossain et al., 2009). Also, the activity of glyoxalase I was increased in the yeast *Schizosaccharomyces pombe* by osmotic stress (Takatsume et al., 2005).

One of the proteins found in excess-boron grown root cells of carrot is EDGP precursor, known as an extracellular dermal glycoprotein to be expressed in response to wounding (Satok et al., 1992).

Isocitrate dehydrogenase ($NADP^+$) is known to be involved in the supply of 2-oxoglutarate for ammonia assimilation and glutamate synthesis in higher plants and pine (*Pinus* spp.) seedlings in which the cytosolic localization was detected in their green cotyledones (Palomo et al., 1998). Because of the strong binding properties of pyridine nucleotide coenzymes (NAD+) (Ralston and Hunt, 2000), boron was found to reduce the available concentration of important metabolic intermediates, particularly NAD^+ and to a lesser extent NADH and NADPH (Reid et al., 2004). The reduction in the concentration of these compounds leads to an inhibition of metabolism. There was an increase in $NADP^+$-isocitrate dehydrogenase protein in carrot root cells which was grown under excess boron-niacin according to only excess boron grown root cells. Plants contain three additional forms like as: $NADP^+$ specific isoform in the cytosol chloroplast and mitochondria. In addition to NAD^+-specific mitochondrial isoform associated with the tricarboxylic acid cycle (McIntosh and Oliver, 1992), $NADP^+$- isocitrate dehydrogenase protein in carrot root cells grown under excess boron-niacin caused the mobilization of excess boron by binding to Mg^{++} or Mn^{++} as cofactors (Brown et al., 2002; Yasutake et al., 2003).

Conclusions

Stress proteins are critical for maintaining homeostasis under abiotic stresses such as drought, cold and freezing, heat, salinity, nutrient deficiency, toxic heavy metals and oxidative stress. Excess boron stress is one of the most significant abiotic stress. The up regulation of stress proteins, which occurs against a background of depressive changes in polypeptide formation, relative to normal environmental conditions, is one of the main components of the adaptive response. In our study, comparing the protein profiles in boron sufficient (control), boron excess and boron/niacin excess using SDS-PAGE revealed that the boron excess induced significant changes in the pattern of proteins. Glutathion peroxidase and glyoxylase I did not express in the control. CR16 (Major allergen Dau c1) and isocitratede hydrogenase proteins, which also exist in the control roots, were

specifically increased and clearly observed in boron and boron/niacin excessment. We identified the proteins which respond to the excess boron and boron-niacin stress proteins for the first time and found the boron mobilization effect of $NADP^+$-isocitrate dehydrogenase protein.

REFERENCES

Arnholdt-Schmitt B (2004). Stress- induced cell reprogramming. A role for global genome regulation. Plant Physiol. 136: 2579-2586.

Baykal AŞ, Öncel I (2006). Changes of Soluble Phenolic and Soluble Protein Amounts on the Tolerance of Boron Toxicity in Wheat Seedlings. Faculty of Science and Arts, Celal Bayar University. Sci. 27: p. 1.

Brown PH, Bellaloui L, Wimmer MA, Basil ES, Ruiz J, Hu H, Pfeffer H, Dannel F, Römheld V (2002). Boron in plant biology. Plant Biol. 4: 205-223.

Bishnoi SK, Kumar B, Rani C, Data KS, Kumari P, Sheoran IS, Angrish R (2006). Changes in protein profile of pigeonpea genotypes in response to NaCl and boron stress. Biol. Plant. 50(1): 135-137.

Bradford MM (1976). Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Çakmak I, Römheld V (1997). Boron deficiency-induced impairments of cellular functions in plants. Plant Soil. 193: 71-83.

Donnelly SF, Pocklington MJ, Pallotta D, Orr E (1993). A proline rich protein, verprolin, involved in cytoskeletal organization and cellular growth in the yeast *Saccharomyces cerevisiae.* Mol. Microbiol. 10: 585-596.

Edreva A (2005). Pathogenesis-related proteins: Research progress in the last 15 years. Genn. Appl. Plant Physiol. 31(1-2): 105-124.

Ghanati F, Morita A, Yokota H (2002). Induction of suberin and increase of lignin content by excess boron in tobacco cells. Soil Sci. Plant Nutr. 48: 357-364.

Goldback HE, Wimmer MA (2007). Boron in plants and animals: is there a role beyond cell-wall structure? J. Plant Nutr. Soil Sci. 170: 39-48.

Hayes JE, Reid RJ (2004). Boron tolerance in barley is mediated by efflux of boron from the roots. Plant Physiol. 136: 3376-3382.

Hossain MD, Rohman MM, Fujita M (2007). Comperative investigation of glutathione S- Transferases, glyoxalase I and Alliinase activities in different vegetable crops. J. Crop Sci. Biotechnol. 10: 21-28.

Hossain MA, Hossain MZ, Fujita M (2009). Stress-induced changes of methylglyoxal level and glyoxalase I activity in pumpkin seedlings and cDNA cloning of glyoxalase I gene. Aust. J. Crop Sci. 3(2): 53-64.

Hurkman W, Tanaka C (1986). Solubilization of plant membrane proteins for analysis by two –dimensional gel elektrophoresis. Plant Physiol. 81: 802-806.

Karabal E, Yücel M, Ökte HA (2003). Antioxidant responses of tolerant and sensitive barley cultivars to boron toxicity. Plant Sci. 164: 925-933.

Kobayashi M, Matoh T, Azuma JI (1996). Two chains of rhamno-galacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. Plant Physiol. 110: 1017-1020.

Linden CD, Wright KL, McConnel HM, Fox CF (1973). Lateral phase separations in membrane lipids and the mechanism of sugar transport in *Escherichia coli.* Proc. Natl. Acad. Sci. USA. 70: 2271-2275.

Liu D, Jiang W, Zhang L, Li L (2000). Effects of boron ions on root growth and cell division of broadbean (*Vicia faba* L.). Isr. J. Plant Sci. 48: 47-51

Lowatt CJ, Bates LM (1984). Early effects of excess boron on photosynthesis and growth of *Cucurbita pepo.* J. Exp. Bot. 35(152): 297-305.

Mahboobi H, Yücel M, Öktem H (2000). Changes in total protein profiles of barley cultivars in responses to toxic boron concentration. J. Plant Nutr. 23(3): 391-399.

McIntosh CA, Oliver DJ (1992). NAD^+-linked isocitrate dehydrogenase: isolation, purification, and characterization of the protein from pea mitochondria. Plant Physiol. 100: 69-75.

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

Munksgaard D, Mattsson O, Okkels FT (1995). Somatic embryo development in carrot is associated with an increase in levels of S-adenosylmethionine, S-adenosylhomocysteine and DNA methylation. Physiol. Plantarum. 93(1): 5-10.http://dx.doi.org/10.1034/j.1399-3054.1995.930102.x

Murashige T, Skoog F (1962). A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Nable RO, Cartwright B, Rathjen A (1988). Responses of wheat and barley genotypes to toxic concentrations of soil boron. Euphytica, 39:137-144.

Nable RO, Cartwright B, Lance RC (1990). Genotypic differences in boron accumulation in barley: Relative susceptibilities to boron deficiency and toxicity. Genetic Aspects of Plant Mineral Nutrition eds El. Bassam N, Dambroth M, and Loughman B. Dordrecht Kluwer Academic Publishers: pp. 243-251.

Palomo J, Gallardo F, Suárez MF, Cánovas FM (1998). Purification and characterization of $NADP^+$ -linked isocitrate dehydrogenase from Scots Pine. Plant Physiol. 118: 617-626.

Parr AJ, Loughman BC (1983). Boron and membrane function in plants. In metals and micronutriens uptake and utilization by plants eds Robb DA and Pierpoint WSA. New York; Academic Press, pp. 87-107.

Paull JG, Cartwright B, Rathjen A (1988). Responses of wheat and barley genotypes to toxic concentrations of soil boron. Euphytica, 39: 137-144.

Poethig RS (2001). Life with 25.000 genes. Genome Res. 11(3): 313-316.

Ralston NVC, Hunt CD (2000). Biological boron interactions: charge and structure characteristics required for boroester formation with biomolecules. FASEB J. 14: A 538.

Reid RJ, Hayes JE, Post A, Stangoulis JCR, Graham RD (2004). A critical analysis of the causes of boron toxicity in plants. Plant Cell Environ. 25: 1405-1414.

Reguera M, Bonilla I, Bolaños L (2010). Boron deficiency results in induction of pathogenesis-related proteins from the PR-10 family during the legume-rhizobia interaction. J. Plant Physiol. 167: 625-632.

Roldan M, Belver A, Rodriguez-Rosales P, Ferrol N, Donaire JP (1992). *In vivo* and *in vitro* effects of boron on the plasma membrane proton pump of sunflower roots. Physiol. Plantarum, 84: 49-54.

Ruiz JM, Rivero RM, Romero L (2003). Preliminary studies on the involvement of biosynthesis of cysteine and glutathione concentration in the resistance to B toxicity in sunflower plants. Plant Sci. 165(7): 811-817.http://dx.doi.org/10.1016/S0168-9452(03)00276-0

Ruiz JM, Rivero RM, Romero L (2006). Boron increases synthesis of glutathione in sunflower plants subjected to aluminium stress. Plant Soil, 279: 25-30.

Satok SA, Sturm T, Fujii MJ, Chrispeels MJ (1992). cDNA cloning of an extracellular dermal glycoprotein of carrot and its expression in response to wounding. Planta, 188: 432-438.

Somerville C, Dangl J (2000). Plant Biology in 2010. Sci. 290(4599): 2077-2078.

Takano J, Noguchi K, Yasumori M, Kobayashi M, Gajdos Z, Miwa K, Hayashi H, Yoneyama T, Fujiwara T (2002). *Arabidopsis* boron transporter for xylem loading. Nature, 420: 337-339.

Takano J, Miwa K, Yuan LX, von Wiren N, Fujiwara T (2005). Endocytosis and degradation of BOR1, a boron transporter of *Arabidopsis thaliana,* regulated by boron availability. Proc. Natl. Acad. Sci. USA., 102: 12276-81.

Takatsume Y, Izawa S, Inoue Y (2005). Unique regulation of glyoxalase I activity during osmotic stress response in the fission yeast *Schizosaccharomyces pombe*: neither the mRNA nor the protein level of glyoxalase I increase under conditions that enhance its activity. Arch. Microbiol. 183: 224-227.

Yamamato M, Torikai S, Oeda K (1997). A major root protein of carrots with high homology to intracellular pathogenesis-related (PR) proteins and polen allergens. Plant Cell Physiol. 38(9): 1080-1086.

Yasutake Y, Watanabe S, Yao M, Takada Y, Fukunaga N, Tanaka I (2003) Crystal Structure of the Monomeric Isocitrate Dehydrogenase in the Presence of NADP+. J. Biol. Chem. 278(38): 36897-36904.