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Role of Zn nutrition in membrane stability, leaf hydration status, and growth of common bean grown under soil moisture stress

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Abstract

Under controlled conditions four common bean genotypes (KS21486, D81083, Akhtar, and COS16) were exposed to drought by reducing soil moisture content from 100±5 % FC to 55±5 % FC, which supplied whether with 4.5 mg Zn kg^{-1} soil, or did not receive Zn. Superoxide dismutase activity; SOD, malodialdehyde content; MDA, membrane stability index; MSI, relative water content; RWC, stomatal conductance, gs; and growth parameters (fresh weigh; FW, and dry weight; DW of shoot, and root DW) were measured. Dehydration caused a small change in SOD activity. RWC and gs fell down due to water stress which was in accordance with lipid peroxidation and solute leakage. MDA content and hydration status of Zn- fed leaves tend to be less adversely affected by water shortage than those grown at adequate Zn. Under Zn deficiency, decreased SOD activity and RWC, as well as, partial stomata closure contributed to damages on membranes lipids and integrity. Furthermore, such plants exhibited remarkable stunted growth. With the exception of root biomass, other growth-related traits were hampered by soil dryness. Drought- induced reductions in growth were associated with increments in MDA content and consequently MSI and in parallel to decrease of RWC. Enhanced the extent of lipid peroxidation and solute leakage in COS16 was in accordance with lower SOD activity and gs, due to greater water loss from leaf tissues caused by reduced available water. In conclusion, Zn application would help the crop to cope with drought during early growth stages, through mitigating of drought- induced oxidative stress and maintenance of leaf water status.

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Introduction

Drought stress is a major abiotic stress constraining common bean (*Phaseolus vulgaris* L.) productivity in agricultural systems, because about its 60% production is obtained from regions subjected to water shortage. Comparing to other legumes, common bean sensitivity to water deficit is average (Cruz de Carvalho *et al.*, 1998). Zinc (Zn) is a transition metal with atomic number 30 and is the 23rd most abundant element on earth (Broadley *et al.*, 2007). In solution, Zn exists in the +II oxidation state and unlike Fe^{2+} or Cu^{2+}, is redux-stable under physiological conditions as a result of a complete d-shell of electrons (Auld, 2001). It is essential element for living organisms and an integral component of thousands of proteins (Broadley *et al.*, 2007). Zinc deficiency is a widespread micronutrient disorder in different agro-climatic regions of the world, particularly Zn disorders of plants might be prone to occur in arid and semi- arid regions, where topsoil is often deficient in water, and so Zn transport from soil solution to roots mostly by diffusion is slight (Cakmak *et al.*, 1996).

Increasing evidence has indicated that much of the injury to plants due to abiotic stresses is associated with oxidative damage at the cellular level through direct of indirect formation of reactive oxygen species (ROS) like super oxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\cdot}$). Under normal physiological conditions, the production and destruction of ROS is regulated well in the cell metabolism. However, drought (Sairam and Saxena, 2000) or nutrient disorders (Yu and Rengel, 1999; Tewari *et al.*, 2004) are believes to result an imbalance between ROS formation and their detoxification (Mittler, 2002). The ROS attack lipids, proteins, and nucleic acids causing lipid peroxidation, protein denaturation, and DNA mutation (Bowler *et al.*, 1992). The degree of cell membrane injury induced by water stress may be easily estimated through measurements of electrolyte leakage from the cells. It has been demonstrated recently that electrolyte leakage measurements may be correlated with several physiological and biochemical parameters conditioning the plant responses to environmental conditions such as antioxidative enzyme synthesis (Liu and Huang 2000; Sreenivasulu *et al.*, 2000), membrane acyl lipid concentrations (Lauriano *et al.* 2000), water use efficiency (Franca *et al.*, 2000; Saelim and Zwiazek, 2000), stomatal resistance and osmotic potential (Premachandra *et al.*, 1989). It is therefore not surprising that electrolyte leakage has been recommended as a valuable criterion for identification of stress resistant cultivars in several crop species (Stevanovic *et al.*, 1997). In plants, there are many potential places for generation of ROS, such as chloroplasts (Foyer and Noctor, 2000), mitochondria and peroxisomes (Del Roi *et al.*, 2002). In order to avoid an overproduction of reactive molecules, plants have developed their antioxidant systems to ensure a control of the cellular redox state (Foyer and Noctor, 2003).

Drought results in the increased generation of ROS due to energy accumulation in stressed plant which absorbs more light energy than consumed through photosynthetic carbon fixation (Asada, 1999). Water deficit stress inhibits or slows down photosynthetic carbon fixation mainly through limiting the entry of CO_2 into the leaf or directly hampering metabolism (Apel and Hirt, 2004). Zn deficiency also recognized to cause higher levels of ROS in plants and relevant damagers (Cakmak, 2000). It has been reported that in Zn- deficient plant $O_2^{\cdot-}$ generation enhances in two ways: (1) by promoting NADP- dependant oxidase activity, as Zn is reported to inhibit NADPH- oxidase (Pinton *et al.*, 1994) and (2) by decreasing NADP- to-NADPH ratio as a result of decreased uptake and photosynthetic CO_2 fixation (Sharma *et al.*, 1995). Moreover, being an integral constitute of Cu/Zn-superoxide dismutase (Cu/Zn SOD), Zn plays an important role in the detoxification of $O_2^{\cdot-}$ to H_2O_2 (Apel and Hirt, 2004). Indeed, SOD activity, particularly Cu/Zn SOD that has Zn as its constitute, negatively affect by low Zn supply (Yu and Rengel, 1999; Sharma *et al.*, 2004). An adequate Zn nutrition also has protective effects on photoxidative damage

catalyzed by ROS in chloroplasts (Wang and Jin, 2005). Considering that drought represents as an oxidative stress, it is therefore, likely that drought-stress related ROS production and plants sensitivity to photoxidative damage in chloroplasts are additionally accentuated when plants would simultaneously suffer from Zn deficiency stress. This experiment aims to clear whether a sufficient supply of Zn can improve protect common bean from the harms of high ROS levels resulting from water shortage. The changes in leaf RWC and stomatal conductance, as well as, growth parameters were studied in condition of water stress and Zn application.

Materials and methods

Experimental setup

Plastic pots measuring 190 mm diameter × 210 mm deep were filled with 6.7 kg of air dried and sieved soil with sandy loam texture, collected from Agricultural Research Farms of University of Tabriz, Iran. Some properties of this soil are represented in Table 1.

Table 1. Some physic-chemical properties of the soil used in the experiment.

Field capacity (%)	pH	EC (dS m^{-1})	$CaCO_3$ (%)	Organic matter (%)	Total N (%)	Available (mg kg^{-1})					
						P	K	Fe	Cu	Mn	B
20	7.65	0.94	7.27	0.63	0.054	18.7	324	3.32	1.1	1.92	0.76

The concentration of DTPA- Zn prior to sowing was 0.6 mg kg^{-1} (below the critical level of available soil Zn in arable lands of Iran). Two Zn treatments were set: no applied Zn (-Zn) and 4.5 mg Zn kg^{-1} soil (+Zn) applied as $ZnSO_4.7H_2O$ solution before potting. A basal dressing of 64 mg CO $(NH_2)_2$, 64 mg FeEDDHA, and 103 mg $MnSO_4.H_2O$ with Zn treatments was used for each pot. At flowering 64 mg CO $(NH_2)_2$ was also added to all plots by fertigation. Seeds of each genotype (KS21486, D81083, Akhtar, and COS16) having similar weight were surface-sterilized by soaking in 70 % ethanol (60 s) and then in sodium hypochlorite (6 min) followed by washing three times with distilled water. Thereafter, eight seeds were buried at depth of 5 cm, followed by adding distilled water to bring soil moisture content to FC. When the first trifoliate emerged, seedlings were thinned down to three in each pot. Soil water was kept at FC until plants establishment. To induce drought-stress watering was decreased to achieve 55±5% FC and continued till harvest. Pots were weighed daily to replenish the water lost through evapotranspiration. Dependent on the genotypes timing of drought imposition was not same. In the greenhouse average day/night temperatures, photoperiod and relative air humidity were 30/17±2 °C, 12/12 h and 60-70 %, respectively. D*ichlorvos* (DDVP) was used as a fumigant to control thrips (*Thrips tabaci*) as needed.

Measurements and sampling procedures

Lipid peroxidation

Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) formed using the thiobarbituric acid reactive substances (TBARS) method described by Cakmak and Horst (1991). Frozen leaf samples (0.2 g) were homogenized in 0.1% thrichloroacetic acid (TCA) at 4 °C and the homogenate was centrifuged at 12,000 rpm for 5 min. To a 1.0 ml aliquot of the supernatant, 4.0 ml of 0.5 thiobarbitutic acid (TBA) in 20% TCA was added. The mixture was then heated at 95 °C for 30 min, and then cooled in an ice bath. After centrifugation in 10, 000 × g for 5 min, the absorbance of supernatant was recorded at 532 and 600 nm. The MDA content (n mol g^{-1} FW) was calculated using an extinct coefficient of 155 mM cm^{-1} after subtracting the non- specific at 600 nm.

Extraction and enzyme assay

Tested plant material (0.2 g of frozen samples) was ground in 3 ml HEPES-KOH buffer; containing 50 mM sodium phosphate and pH 7.8. The homogenates were centrifuged at 2-4 °C for 15 min at 12000 × g.

Supernatant was used to measure the enzyme activity and protein content assays. The specific activity for enzyme was expressed as absorption changes (ΔA) mg^{-1} protein min^{-1}. Total soluble protein content of the enzyme extracts were determined according to Bradford (1976), using bovine serum albumin as the standard. Superoxide dismutase (EC; 1.15.1.1) activity was assayed by monitoring the inhibition of the photochemical reduction of nitro blue tetrazolium, according to the method of Giannopotitis and Ries (1977). One unit of superoxide dismutase activity is defined as the amount of enzyme required to cause 50% inhibition of nitroblue tetrazolium reduction, which was monitored at 560 nm.

Spectrophotometric analyses were conducted on a spectrophotometer (WPA- biowave, S 2100).

Cell membrane stability index

The percentage of electrolyte leakage from fresh leaf samples was determined using electrical conductivity meter and was used to assess changes in cell membrane permeability. The procedure was base on the method of Sairam *et al* (2002). Fully developed leaf samples from each treatment were cut into 0.1 g segments; after rinsing 3 times with distilled water to remove surface contamination they were then placed in individual stoppered vials containing 10 ml of distilled water. The vials were kept at 40 °C for 30 min. Electrical conductivity of the bathing solution (EC_1) was read after this time. Another vial containing leaf samples from the same treatment were boiled in a water bath for 10 min and after the bathing solution was cooled to room temperature a second reading (EC_2) was taken. Cell membrane stability index (MSI) was calculated as: $[1- (EC_1/EC_2)]\times100$.

Stomatal conductance

Measurements of stomatal conductance to water vapor (gs, m mol m^{-2} s^{-1}) were made on the adaxial surface of five new fully developed leaves, using a portable gas exchange system (HCM-1000,Walz, Germany) at saturating light of 800 μ mol m^{-2} s^{-1} of photosynthetic active radiation.

Leaf hydration status

Using the method outlined by Barrs and Weatherley (1962), leaf moisture status expressed as relative water content (RWC), was determined and computed according to the formula of (Fw- Dw) / (Tw- Dw)×100 , where Fw is the fresh leaf weight at the sampling time, Tw is the full turgid weight recorded after 24 h immersion in distilled water at 4 °C, and Dw is the weight of the discs after being dried at 75°C for 18 h.

Leaf RWC and gs parameters were measured between 12:00 and 14:00 hours.

Growth parameters

At the flowering, three plants were harvested and their fresh weight (FW)s were recorded immediately. After being dried at 75 °C for 48 h, mean dry weight (DW) per plant was recorded. Roots of plants removed from gently the culture medium, under running tap and then, were washed thoroughly of soil. Drying was performed as described above.

Experimental design and data analysis

There were 192 pots altogether (2 FC levels × 2 Zn treatments × 4 genotypes × 3 replications × 4 pots per replication). In order to facilitate pots weighing and control watering, experimental units were distributed over a split plot design (based on RCB). All data were subjected to analysis of variance appropriate to the experimental design by SAS software. Duncan's Multiple Range Test (DMRT) was used to compare significant differences of means for each individual trait.

Results

The water shortage- mediated changes in total SOD activity was negligible, while low soil Zn concentration induced considerable reduction the activity of SOD. As in leaves did not supplied with SOD activity was founds to be 37 % less than those received Zn (Table 2). There were substantial variations in SOD activity among the genotypes at deficient soil moisture. With dropping soil water content to 55±5% FC, SOD activity increased in leaves

of Akhtar and D81083, that occurred sharply and greater for D81083. Under the control treatment no major changes were detected with respect to the enzyme activity in the genotypes (Fig. 1). Data concerning oxidative damage to membranes as MDA content are represents in Table 2 . Water or Zn deficiencies severely affected membranes, and result in higher concentrations of MDA in the leaf. They showed a significantly positive interaction, as well. It was demonstrated that the extent of lipid peroxidation caused by drought, was more accentuated under condition of no- applied Zn (-Zn) was more accentuated, compared to that respective controls (+Zn), implying the role Zn in protecting membranes from the harms of drought stress-mediated increment in high ROS. We also find a consistent difference in lipid peroxidation amongst the test genotypes. Comparing to others, COS16 had the highest MDA concentration (Table 2).

Table 2. Mean values of measured traits in the experiment treatments**.**

Treatment	SOD activity (unit g^{-1} FW)	MDA content (nmol g^{-1} FW)	MSI (%)	RWC (%)	g_s (m mol m^{-2} s^{-1})	Shoot FW (g plant $^{-1}$)	Shoot DW (g plant $^{-1}$)	Root DW (g plant $^{-1}$)
Soil moisture								
100±5% FC	5.00 ^{a}	4.93 ^{a}	77.96 ^{a}	77.94 ^{a}	112.9 ^{a}	16.26 ^{a}	2.82 ^{a}	1.17 ^{a}
55±5% FC	5.77 ^{a}	17.87b	71.93 ^{b}	70.15 ^{b}	35.28 ^{b}	8.81 ^{b}	1.78 ^{b}	0.98 ^{a}
Zinc level								
- Zn	4.17 ^{b}	11.86 ^{b}	73.54 ^{b}	72.98 ^{a}	69.26 ^{a}	12.04 ^{b}	2.08 ^{b}	1.02 ^{b}
+ Zn	6.59 ^{a}	10.94 ^{a}	76.35 ^{a}	75.11 ^{a}	78.92 ^{a}	13.31 ^{a}	2.51 ^{a}	1.65 ^{a}
Genotypes								
KS21486	5.30 ^{b}	11.5 ^{a}	80.62 ^{a}	75.9 ^{a}	89.86 ^{a}	7.09 ^{c}	1.84 ^{a}	0.56 ^{c}
D81083	6.02 ^{a}	10.78 ^{a}	77.28 ^{ab}	75.35 ^{a}	93.52 ^{a}	13.68 ^{b}	2.52 ^{a}	0.91 ^{b}
Akhtar	5.41 ^{b}	11.24 ^{a}	74.31 ^{b}	72.74 ^{a}	54.36 ^{bc}	16.12 ^{a}	2.53 ^{a}	1.46 ^{a}
COS16	4.81 ^{c}	12.10 ^{b}	67.59 ^{5}	72.17 ^{a}	61.62 ^{b}	13.46 ^{b}	2.30 ^{a}	1.38 ^{a}

Means not assigned by the same letters are significantly different at $p<0.05$ by Duncan's test. FC, field capacity; -Zn; low Zn supply; +Zn, adequate Zn supply; SOD, superoxide dismutase; MDA, malodialdehyde; MSI, membrane stability index; RWC, relative water content; g_s, stomatal conductance; FW, fresh weight; DW, dry weight.

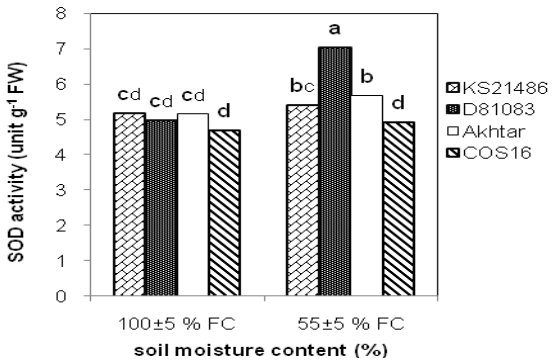

Fig. 1. SOD activity changes in leaves of common bean genotypes under well-watered (100±5% field capacity, FC) or water-deficit (55±5% FC) conditions.

Limited soil water content, impaired greatly cell membrane permeability by decreasing MSI, up to 7.7 % over the control (100±5 % FC). The electrical conductivity of tissue external solution was reduced approximately 3.7 % in non- treated plants comparing Zn-treated ones (Table 2). The results also showed the existence of a considerable difference among the genotypes with respect to membranes intactness. KS21486 and COS16 had the highest and lowest MSI, respectively.

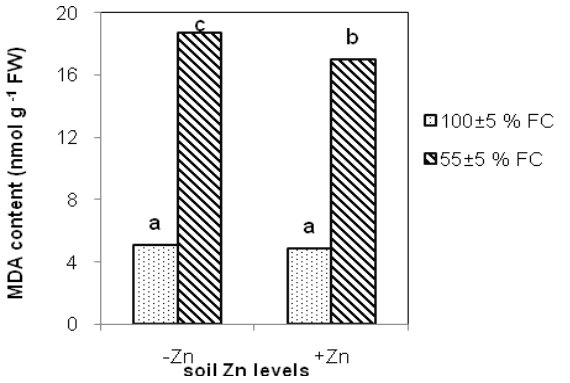

Fig. 2. The amount of lipid peroxidation in common bean leaves as affected by Zn nutritional status under well-watered (100±5% field capacity, FC) or water-deficit (55±5% FC) conditions.
-Zn and +Zn shows: low (no- applied) and adequate (application 4.5 mg Zn kg^{-1} soil), respectively. Bars with the same letters are not significantly different according to Duncan's test at 5% level.

Responses of leaf RWC in the genotypes to FC levels are shown in Fig. 3. Generally, RWC decline as influenced by water stress up to 10 % (Table 2), however, the genotypes behaved distinctly against different watering regimes. Leaf RWC showed small and insignificant changes among the genotypes under well-watering condition, thought the lowest value for this parameter were found to belong to D81083. When soil dryness condition was imposed, hydration status in D81083 was least affected, followed by KS21486. They also retained leaves water much better than did Akhtar and COS16 (Fig. 3). Addition of Zn into the soil, affected RWC depending on the soil FC. In sufficient moisture, Zn could hardly affect water relation of common bean leaves. However, in plants subjected to water deficiency Zn improved RWC to a great extent, made to reach from 67.94 % in –Zn to 72.38 % in +Zn treatments (Fig. 4).

The stomatal conductance (gs) showed an appreciable decline under the applied water stress, but it was a genotype-dependent response (Fig. 5). In well-hydrated leaves of KS21486 and D81083 stomata were drastically more open. A large reduction in gs (around 71 %) was accrued by imposing water stress. KS21486, D81083 and Akhtar had decreases varying between 60 and 70 % as compared to their respective controls (Fig. 5). Nevertheless KS21486 and D81083 did not close their stomata as much as did Akhtar and COS16. Zinc providing improved stomata aperture by 14 % over the nil Zn treatment (-Zn). Though it was not statistically significant (Table 2).

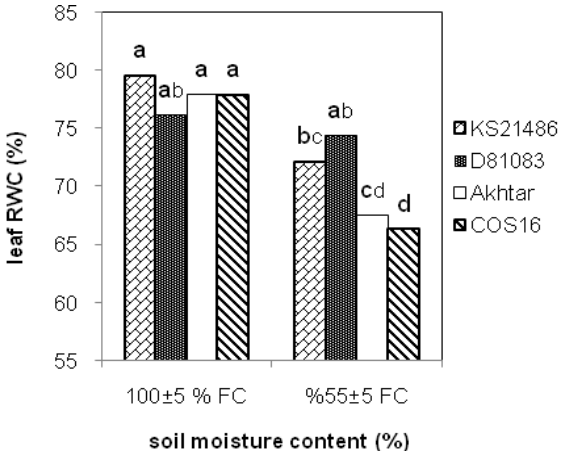

Fig. 3. Leaf RWC changes in leaves of common bean genotypes under well-watered (100±5% field capacity, FC) or water-deficit (55±5% FC) conditions.

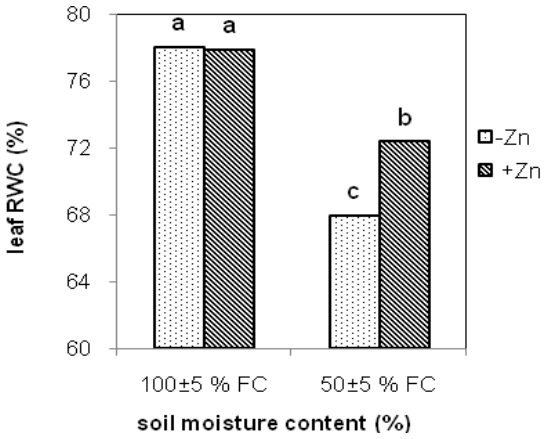

Fig. 4. Leaf RWC in common bean leaves as affected by Zn nutritional status under well-watered (100±5% field capacity, FC) or water-deficit (55±5% FC) conditions.

Deficient-water treatment cause retardation in plant growth, as, the reductions observed for FW and DW were more than 46 and 36%, respectively. Impaired Zn status of plant inhibited production of fresh and dry masses about 9.5 and 17 % over the condition at which plants received 4.5 mg Zn kg^{-1} soil, respectively

(Table 2). Alterations observed for root dry mass were not important between FC treatments. However, the decline in root DW driven by Zn deficiency conditions was notable (Table 2). The genotypes responded differently regarding their root growth to varying soil Zn levels (Fig. 6). In case of D81083 and COS16, root biomass was increased to a great extent by Zn addition. The best root biomass performing genotype at nil Zn treatment was Akhtar (Fig. 6). Substantial differences were also observed among the genotypes. In terms of the growth parameters, lowest values were recorded for KS21486 (Table 2).

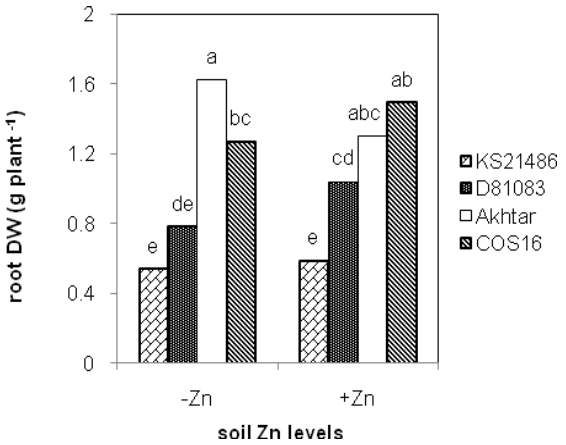

Fig. 5. Root growth of common bean genotypes as affected by Zn nutritional status.

-Zn and +Zn shows: low (no- applied) and adequate (application 4.5 mg Zn kg^{-1} soil), respectively. Bars with the same letters are not significantly different according to Duncan's test at 5% level.

Discussion

Water stress at 55±5 % FC and Zn deficiency represented conditions which induced detectable biochemical and physiological changes in common bean. The occurrence of malodialdehyde (MDA), a secondary end product of the oxidation of poly unsaturated fatty acids, is considered a useful index of general lipid peroxidation (Smirnoff, 1993). It is already known that free radical caused peroxidation of lipid membranes is both a reflection and measure of stress- induced damage at the cellular level (Logani and Davies, 1980). By catalyzing of $O_2^{\cdot-}$ to O_2 and H_2O_2 and blocking $O_2^{\cdot-}$ - driven cell damage, SODs are a major component of the antioxidative defense system of plant cells (Bowler *et al.*, 1992). Reduced SOD activity provides OH· formation through over-accumulation of $O_2^{\cdot-}$. Hydroxyl radicals attack bio molecules within cells and lead to strong metabolic disruptions (Mittler, 2000). In our experiment a 3/6 fold raise in MDA content induced by drought stress appeared to be derived from decreased SOD activity (Table 2) which was not sufficient to handle to scavenge $O_2^{\cdot-}$ and therefore, maintenance of structural integrity of biomembranes. On the other hand, strong stomatal closure in plants exposed to drier condition contributed to increased lipid peroxidation, resulting in a decline in leaf CO_2 concentration. This, in turn, might cause a decrease in the concentration of NADP+ available to accept electrons from photosystem I/II and then initiate CO_2 decline with the concomitant generation of free radicals (Hernandez *et al.*, 2000). It is well established that modulation of several cellular antioxidant enzymes contribute to complete inhibition of ROS generation and oxidative stress (Vaidyanathan *et al.*, 2003). Comparing to KS21486 and Akhtar, SOD activity in leaf was much higher in D81083. However, the values of the lipid peroxidation in D81083 were comparable to those in KS21486 and Akhtar. It is possible that in D81083 there was not a balance between SOD activity and other efficient antioxidant enzymes including ascorbate peroxidase and hence, MDA content increased in its leaves. Indeed, the water shortage- mediated oxidative stress in D81083 did not be control well. It has been reported that different common bean cultivars had different photochemical efficiencies and used various anitioxidant enzymes to scavenge ROS (Terzi *et al.*, 2010). Amounts of MDA in COS16, might be related to lower SOD activity that disturbed cell balance and protection against ROS- mediated damage to membrane lipids. Since that increasing in total SOD activity did not correspond to stress intensity in this genotype. In the previous studies, variations in SOD activities have been shown depending on drought severity and duration, and plant species (Badiani *et*

al., 1990; Quartacci and Nacarri- Izzo, 1992; Turkan *et al*., 2005).

It is evident from the present study that SOD activity reduction was accompanied by remarkable increment in MDA content (Table 2), denoting ROS injury to cell membranes in Zn- starved leaves. In higher plants, Cu/Zn SOD is the most abundant SOD, while Mn-SOD and Fe-SOD form a smaller proportion of total SOD (Alscher *et al*., 1997). Being an integral constituent of Cu/Zn SOD, deficient supply of Zn reduces SOD activity in leaves of several crops (Cakmak *et al*., 1997; Yu and Rengel, 1999; Yu *et al*., 1998). As SODs play critical roles in the antioxidative defense systems of all biological tissues (Scandalios, 1993), it can be suggested that plant with reduced SOD acitivity (i.e. marginal to severe Zn deficiency) should be highly sensitive to oxidative stress factors. As we observed here, under drought membranes lipid were more vulnerable to lipid peroxidation in leaves suffering from concomitant Zn deficiency, compared to those received Zn (Fig. 2). This implicates that Zn represents an excellent protective agent against the oxidation of several vital cell components like membrane lipids under hostile conditions (Cakmak, 2000), which in turn might enhance the ability of the crop to cope with water stress during early growth stages. More damages due to nutrient disorders are believed to result, when the generation of reactive O_2 species is enhanced to a level in excess of the capacity of cells to detoxify them under unfavorable environments (Apel and Hirt, 2004).

Cell membrane is one of the first targets of plants (Levitt, 1972). Drought- induced lipid peroxidation means that essential solutes leak out from the organelles and from the cell and cause the damage of membrane function and metabolic processes (Blokhina *et al*., 2003). Therefore, the ability to control ion movement rate in and out of cells is used as a test of damage to tissues. Zn has a unique property of existing in a divalent state, without any redox cycling, and is thereby stable in biological medium in which oxidoreductive potential is subjected to continuous flux (Vallee and Falchuk, 1993). As a result of these properties, membrane lipid packing is protected from ROS peroxidation, which in turn prevents ion leakage from ion channels (Bray and Bettger, 1990). Similarly, the present study's results showed that the leaves experienced drought or Zn deficiency had high stress injury, assessed as cell membrane stability index (Table 2). This denoted that both stressors could damage severely the integrity of cellular membranes, as well as, cellular component such as lipids as was indicated by higher MDA content in the bean leaf tissues. Grewel and Wiliams (2000) cautioned that plant of alfalfa grown at low Zn supply developed Zn deficiency symptoms, and there was a severe solute leakage from the leaves of Zn-deficient plants. Loss of cell membrane integrity under these stresses was also in parallel to decline in leaf RWC (Table 2). Soil dry conditions, caused water loss from leaf tissues which induces disorganizations on both membranes structure and function (Buchanan *et al*., 2000), especially those of thylakoids and consequently, decreased their intactness. This makes biomembranes more permeable. Electrolytes leakage is generally proportional to the stress damage (Valentovic *et al*., 2006; Simova- Stoilova *et al*., 2008). The greatest concentration of MDA and membrane intactness (more ion leakage) was measured in COS16 (Table 2). The possible reason might be *lower SOD activities observed in condition of limited watering (Fig. 1). Moreover, enhanced injury on cellular membranes in this genotype might be linked with greater water loss from leaf (Fig. 3) which corresponded to remarkable decreased in gs (Fig. 5),* as a consequence of soil moisture stress. Similar dependence is discussed in barley (Kocheva *et al*., 2003), common bean (Turkan *et al*., 2005) and wheat (Tas and Tas, 2007) genotypes.

Leaf RWC considered to be an informative parameter for cellular water status and metabolism relationship (Lawlor and Cornic, 2002), usually depends on the turgor of plant cells (Lawlor, 1995). It was reported that the maintenance of leaf water status or capacity

of leaf turgescence retention is a result of proper control via guard cells in any environmental conditions, supporting growth activities and enhance the ability of species in the stressful conditions (Steudle, 2000). From this viewpoint, improved RWC in drought- stressed plants through Zn application would provide advantages toward adaption of the crop to water shortage. Confirming our results Gadallah (2000) showed that supplementary Zn improved leaf relative water content of soybeans, particularly under water stress. Such an effect could be due to the role of Zn in improvement of vascular tissue formation and prevention of their destruction under unfavorable stress conditions (Gadallah and Ramadan, 1997), as well as, osmotically active solutes accumulation in roots (Gadallah, 2000).

The treatments of common bean Zn amended significantly common bean growth. Reactive O_2 species is the basis of plant growth disturbance caused by Zn deficiency (Calkmak, 2000). Accordingly, low activity of SOD exhibited in Zn-deficient plants make largely take part in stunted growth of common bean grown in condition of low level of Zn in the soil. Because such plants would have high levels of superoxide radicals (Cakmak and Marschner, 1988). Impaired growth which was in accordance with perturbations in membranes structural integrity, was also associated with decreased leaf RWC at deficient supply of Zn. Much more sensitivity of shoot growth in relation to root to drought stress (Table 2), might attribute to higher capacity of root in osmotic adjustment and so, turgor maintenance at low soil water potentials than the shoot (Hsiao, 2000).

Conclusion

A conclusion could be drawn of the present study findings is that Zn supplementation is indispensable for common bean grown in condition of water shortage to keep cell membranes functional and intact. This might be partly attained by preservation leaf water stats which in turns, participates in improved crop performance under water- limited situations.

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