



RESEARCH PAPER

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Physiological changes associated with soil drought stress in common bean (*Phaseolus vulgaris* L.) as influenced by zinc supply

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Abstract

Physiological responses of four common bean genotypes (*KS21486*, *D81083*, *Akhtar*, and *COS16*) to drought stress and zinc (Zn) nutrition were evaluated under pot conditions. Plants were grown under deficient (no applied Zn) or adequate (4.5 mg Zn kg⁻¹ soil) Zn levels and two soil moisture regimes (100±5% field capacity (FC) and 55±5% FC). Water saturation deficit, gas exchange (net photosynthetic rate- A; transpiration rate- E; stomatal conductance- g_s), instantaneous water use efficiency- iWUE, photosynthetic pigments content (chlorophylls and carotenoid) and chlorophyll fluorescence parameters (electron transport capacity- F_v/F_m and activity- F_v/F_o of PSII), were measured. Zinc improved leaf moisture status of plants exposed to water stress, which was accompanied by higher E. Pigments content and fluorescence parameters did not change by drought and a drastic reduction in A (57%) was associated with severe stomatal closure (69%). Drought-induced increase in iWUE was the consequence of greater inhibition of transpiration rather than of photosynthesis. Unstressed plants supplied with Zn used available water more efficiently. Though Zn increased stomatal opening and pigments content, this did not improve significantly carbon assimilation. *KS21486* and *D81083* appeared to be the most drought tolerant genotypes. That was underlined by the equilibrium between physiological parameters (WSD, E, g_s, iWUE) ensuring their growth and productivity. As a whole, Zn application is recommended for beans grown either under sufficient or limited soil water supplies in order to maximize the resource use efficiency or to ameliorate drought effects on the crop.

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Introduction

Arid and semi-arid regions are generally noted for their low primary productivity which is due to a combination of low and unpredictable water supply and impaired soil fertility (Peek and Forseth, 2003). As much as 60% production of common bean (*Phaseolus vulgaris* L.) as a basic pulse crop in developing world, takes place under conditions of water shortage (Graham and Ranalli, 1997).

At the whole-plant level, the effect of water stress is usually perceived as a decrease in photosynthesis (Cornic and Massacci, 1996). The capacity of a plant to maintain functional photosynthetic activity under drought is typically determined by leaf water relations data (White *et al.*, 2000). The rate of CO₂ assimilation in leaves was reduced at moderate water deficits (Lawlor and Uperty, 1993) or even before any changes in leaf water status in response to a drop in air humidity (Bunce, 1981) or in soil water potential (Socias *et al.*, 1997). Even a mild drought can reduce the net photosynthetic rate in stress-sensitive plants such as common bean (dos Santos *et al.*, 2006) in field and greenhouse (Cruz de Carvalho *et al.*, 1998), especially at the pollination stage (Pimentel *et al.*, 1999).

Among the micronutrients, Zn deficiency is recognized as the most widespread nutritional disorder in crop plants grown under a wide range of soils (Graham *et al.*, 1992). Soils in arid and semi-arid regions are often poor in plant-available Zn associated with high CaCO₃ content and alkaline pH (Takkar and Walker, 1993). Common bean is very sensitive to Zn deficiency particularly under high light intensity (Marschner and Cakmak, 1989). Consequently, bean plant growth and development may be challenged by Zn disorders and soil moisture deficits coincidentally during the growing season. Zinc participates in photosynthetic carbon turnover (Marschner and Cakmak, 1989) and the synthesis and activity of O₂⁻ and H₂O₂⁻ scavenging enzymes (Cakmak and Marschner, 1993). Hence, impairment of the Zn nutritional status of plants can exacerbate photo-oxidative damage and in turn limit plant

performance under stressful environmental conditions (Marschner, 1995). For example, yield losses due to Zn deficiency in a number of cereals were greater under rainfed comparing irrigation conditions (Ekiz *et al.*, 1998).

Although drought and Zn deficiency may occur together, these stresses have generally been examined separately and there have been very little works on how they may interact in common bean. The present research aimed to investigate the responses of common bean leaf water status to water shortage under varied soil Zn levels, to quantify whether leaf gas exchange and stomatal conductance to water vapor are modified by zinc application, and to elucidate the sensitivity of photosynthesis light dependant stage to drought stress and Zn deprivation in common bean genotypes.

Materials and methods

Soil preparation, plant material and growth conditions

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.

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 applied as ZnSO₄·7H₂O solution before potting. A basal dressing of 64 mg CO (NH₂)₂, 64 mg FeEDDHA, and 103 mg MnSO₄·H₂O with Zn treatments was used for each pot. At flowering 64 mg CO (NH₂)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 trifoliolate emerged, seedlings were thinned down

to three in each pot. Soil water was kept at FC until plants establishment. To induce drought-stress (DS) 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. *Dichlorvos* (DDVP) was used as a fumigant to control thrips (*Thrips tabaci*) as needed.

Measurements and sampling procedures

Water status of leaves

Using the method outlined by Barrs and Weatherley (1962), leaf moisture status expressed as water saturation deficit (WSD), was determined and computed according to the formula of $(T_w - F_w) / (T_w - D_w)$, where F_w is the fresh leaf weight at the sampling time, T_w is the full turgid weight recorded after 24 h immersion in distilled water at 4 °C and D_w is the weight of the discs after being dried at 75°C for 18 h.

Gas and water vapor exchange characteristics

Measurements of gas exchange parameters including net CO₂ fixation (A , $\mu\text{ mol m}^{-2} \text{ s}^{-1}$), transpiration rate (E , $\text{m mol m}^{-2} \text{ s}^{-1}$) and stomatal conductance to water vapor (g_s , $\text{m mol m}^{-2} \text{ s}^{-1}$) were made in a portable gas exchange system (HCM-1000, Walz, Germany) at saturating light of 800 $\mu\text{ mol m}^{-2} \text{ s}^{-1}$ of photosynthetic active radiation. Water use efficiency at the leaf level was determined as the ratio of A/E (Passioura, 1982).

Photosynthetic pigments content

Chlorophylls (Chl a and Chl b), and carotenoid (Car) content of fresh leaf samples were determined spectrophotometrically following Arnon's (1949) method. The pigments concentration ($\text{mg g}^{-1} \text{ FW}$) was calculated as described by Kirk and Allen (1965).

Chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters (photochemical efficiency of PSII, F_v/F_m ; and activity of PSII, F_v/F_o) were measured by a portable fluorometer (OS-

30, Opti-sciences, U.S.A) after acclimating the leaves to dark for 30 min and in parallel to gas exchange measurements. The irradiance was set as 990 μE .

All the traits were evaluated at pod formation stage between 11:00 and 14:00. Gas exchange and fluorescence parameters were measured on five selected uppermost fully expanded leaves per replication.

Experimental design and data analysis

There were 192 pots altogether (2 FC levels \times 2 Zn treatments \times 4 genotypes \times 3 replications \times 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

Water saturation deficit

Both water and Zn deficiency stresses caused great increase in WSD of bean leaves (Table 2). Zinc supply improved leaf water status depending on the soil water content. In control plants WSD was hardly affected by Zn, while stressed leaves which received Zn, had about 15.5% higher water content (Fig 1a). There was also a substantial genotypic variation in leaf moisture retention capacity (Table 2), but differences were more apparent in plants exposed to DS, 55±5% FC. KS21486 and D81083 retained leaves water much better than did Akhtar and COS16, showed an insignificant increase or no change in WSD comparing with control, 100±5% FC (Fig 1b).

Gas and water vapor exchange characteristics

Unlike iWUE, photosynthetic gas and water vapor exchange were considerably lower in stressed leaves (Table 2). Genotypes exhibited significant differences in the traits (Table 2), but their response was modified with changing FC levels (Figs 2a, b and c) or soil Zn concentration (Fig 2d) except photosynthesis.

As could be foreseen from g_s response, in well-watered condition stomata were drastically more open in KS21486 and D81083 (Fig 2b). When soil moisture dropped to 55% FC strong stomatal closure

was occurred in KS21486 and D81083, nevertheless they did not close their stomata as much as did Akhtar and COS16 (Fig 2b) and thereby they could obviously kept higher E (Fig 2a).

Table 1. Some physical and 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	7.65	0.63	0.054	18.7	324	3.32	1.1	1.92	0.76

For each genotype, WUE behaved distinctly against watering regimes. Akhtar and KS21486 were superior in control and DS treatments, respectively (Fig 2c). Non-significant increase in A via Zn application occurred by improved g_s (Table 2) and Chl content (Table 3). The severity of the effect for iWUE on Zn-fed plants differed among the genotypes (Fig 2d) and soil moisture contents (Fig 3b). In Akhtar WUE

improved to a great extent through Zn, whereas in -Zn treatment KS21486 used transpired water more efficiently (Fig 2d). In the latter genotype, photosynthetic capacity per unit leaf area was also higher (Table 2). Zinc providing improved WUE in irrigated beans more than in those were under stress (Fig 3b). Water shortage exacerbated transpiration reductions in plants which did not receive Zn (Fig 3a).

Table 2. Mean values of leaf water status and gas exchange in the experiment treatments.

Treatment	WSD (%)	A (μ mol m ⁻² s ⁻¹)	E (m mol m ⁻² s ⁻¹)	g_s (m mol m ⁻² s ⁻¹)	iWUE (A/E \times 10 ⁻³)
Soil moisture					
100 \pm 5% FC	21.92 ^B	7.00 ^A	3.13 ^A	112.9 ^A	2.45 ^B
55 \pm 5% FC	29.82 ^A	2.97 ^B	1.21 ^B	35.28 ^B	2.79 ^A
Zinc					
- Zn	26.83 ^A	4.55 ^A	2.19 ^A	69.26 ^A	2.53 ^A
+ Zn	24.88 ^B	5.51 ^A	2.33 ^A	78.92 ^A	2.91 ^A
Genotypes					
KS21486	23.15 ^B	5.90 ^A	2.53 ^B	89.86 ^A	2.90 ^{AB}
D81083	24.64 ^{AB}	4.71 ^{AB}	3.07 ^A	93.52 ^A	1.97 ^C
Akhtar	27.48 ^A	4.35 ^B	1.40 ^D	54.36 ^{BC}	3.24 ^A
COS16	27.82 ^A	4.95 ^{AB}	2.05 ^{BC}	61.62 ^B	2.38 ^{BC}

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; WSD, water saturation deficit; A, net photosynthetic rate, E, transpiration rate; iWUE, instantaneous water use efficiency; g_s , stomatal conductance.

Photosynthetic pigments content and chlorophyll fluorescence parameters Water stress caused only a very small change in leaf pigments content (Table 3). However, the differences were statistically proved ($p < 0.01$) between Zn levels and among the genotypes (Table 3). Due to Zn starvation Chl biosynthesis was largely impaired. The plants grown without Zn contain about 13, 16 and 14% lower amounts of Chl a, Chl b and total Chl, respectively. Conversely, the

carotenoid content was higher in -Zn treatment. The suppressive effect of soil dryness or Zn deficiency was not determined on F_v/F_m and F_v/F_o ratios. Great variations were seen among the genotypes for photosynthetic pigments and Chl fluorescence, which were significantly higher in Akhtar (Table 3).

Table 3. Mean values of photosynthetic pigments content and chlorophyll fluorescence parameters in the experiment treatments.

Treatment	Chl a (mg g ⁻¹ FW)	Chl b (mg g ⁻¹ FW)	Total Chl (mg g ⁻¹ FW)	Car (mg ⁻¹ FW)	F _v /F _m	F _v /F _o
Soil moisture						
100±5% FC	0.93 ^A	0.42 ^A	1.36 ^A	0.062 ^A	0.78 ^A	3.93 ^A
55±5% FC	0.83 ^A	0.39 ^A	1.22 ^A	0.065 ^A	0.77 ^A	3.63 ^A
Zinc						
- Zn	0.82 ^B	0.37 ^B	1.19 ^B	0.067 ^A	0.78 ^A	3.77 ^A
+ Zn	0.94 ^A	0.44 ^A	1.39 ^A	0.051 ^B	0.78 ^A	3.78 ^A
Genotypes						
KS21486	0.84 ^C	0.38 ^{BC}	1.22 ^C	0.055 ^C	0.77 ^C	3.51 ^C
D81083	0.75 ^D	0.36 ^C	1.10 ^C	0.068 ^B	0.76 ^C	3.42 ^C
Akhtar	1.02 ^A	0.47 ^A	1.50 ^A	0.084 ^A	0.81 ^A	4.27 ^A
COS16	0.93 ^B	0.42 ^{AB}	1.45 ^B	0.054 ^C	0.79 ^B	3.91 ^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; Chl, chlorophyll; Car, carotenoid; F_v/F_m, maximum quantum yield of PS II photochemistry; F_v/F_o, PS II activity.

Discussion

Leaf hydration status is a useful indicator of plant water balance (Ashraf *et al.*, 1994). We evaluated water relations of bean plants using leaf WSD determination. In Zn-deficient leaves increased WSD (Table 1) indicates that such plants are water-stressed (Sharma *et al.*, 1995). 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). More perturbations in leaves water balance related to drought with concomitant Zn deficiency (Fig 1a) were accompanied by a severe decline in E, that were about 76% in -Zn, and 45% in +Zn (Fig 3a). Decreases in g_s and transpirational water loss from Zn-deficient cabbage plants were due to low leaf water potential (Sharma and Sharma, 1987). Among the genotypes, KS21486 and D81083 would be able to grow and yield better in water-deficit conditions by maintaining hydration status of leaf tissues (Fig 1b). The capacity of leaf turgor maintenance is a result of proper

control via guard cells, supporting growth and enhances species ability in any stressful situations (Steudle, 2000).

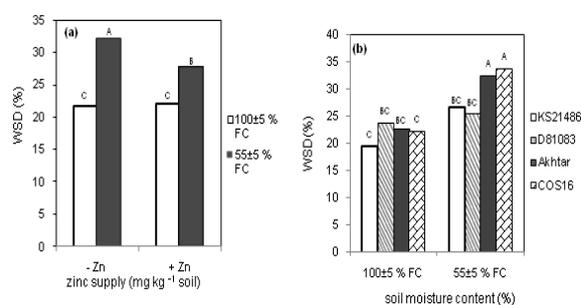


Fig. 1 . Water saturation deficit (WSD) changes as affected by soil zinc, low (-Zn) or adequate, (+Zn) levels (a) and genotypes (b) under well-watered (100±5% field capacity, FC) or water-deficit (55±5% FC) conditions. Bars with the same letters are not significantly different according to Duncan's test at 5% level.

The decline of CO₂ uptake caused by DS is supposed to be dependent on leaf stomatal closure and non-stomatal components. It is well established that in moderate water stresses the photosynthetic apparatus is not damaged. It continued to function, thanks to coupling with photorespiration (Brestic *et al.*, 1995), as attested by photochemical (dos Santos *et al.*, 2006)

and substomatal CO₂ concentration, C_i (Donovan and Ehleringer, 1994) measurements. On the other hand, when water potentials of plant fell below a certain threshold (variable according to the species) under severe drought, the frequently increase in C_i (Brodribb, 1996) and decline in Rubisco activity (Vu *et al.*, 1998) indicating the predominance of non-stomatal limitations to photosynthesis. In *P.vulgaris* it is difficult to have an accurate measure of C_i during drought, due to stomatal patchiness which usually leads to an over estimation of C_i values under ambient CO₂ concentration (Chaves, 1991). In our trial, drastic reduction in leaf photosynthetic capacity (57%) of bean under DS was linked to severe stomatal closure (Table 2) and so reduced C_i. Consequently, there may be a close relationship between g_s and A reductions in the genotypes under study. Not-changed leaf Chl content or electron transport capacity (Table 3) could partly support this hypothesis. In sunflower, photosynthetic CO₂ fixation was suppressed by drought-induced impairments in metabolic processes, mainly through losses of leaf pigments content (Reddy *et al.*, 2004).

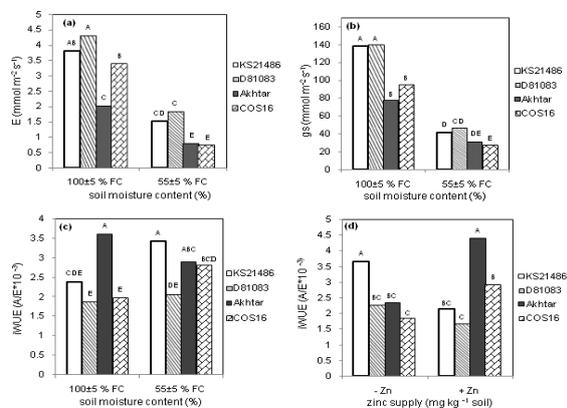


Fig. 2. Transpiration rate (E), stomatal conductance to water vapor (g_s), and instantaneous water use efficiency (iWUE) of the genotypes at differential watering regimes (a, b, and c) and soil Zn levels (d). Well-watered (100±5% field capacity, FC) and drought-stressed (55±5% FC) plants were grown in a Zn- deficient soil without (-Zn) and with (+Zn) zinc supplied. Bars with the same letters are not significantly different according to Duncan's test at 5% level.

Contradictory claims have been made on the impact

of soil (Tardieu *et al.*, 1992; Davies and Zhang, 1991) or leaf (Costa- França *et al.*, 2000) moisture on stomatal responses in common bean cultivars. In current study, the extent to which shoot water status was coupled with g_s is clearly dependant on the genotype (Figs 1b and 2b). In KS21486 and D81083 early stomatal responses to substrate water depletion were not triggered by considerable changes in leaf water content. Meanwhile, these genotypes had higher rates of transpiration under DS (Fig 2a), because they were able to keep their stomata more open relative to Akhtar and COS16 (Fig 2b) which might be assumed as a desirable physiological trait in mitigating water stress damages in drought tolerance (Cabuslay *et al.*, 2002). In short periods of drought, stomatal closure is an efficient adaptive response against desiccation (Cornic and Briantais, 1991), while in prolonged lacks of soil moisture tolerant genotypes at the cellular level are often able to maintain stomata open (Cruz de Carvalho *et al.*, 1998).

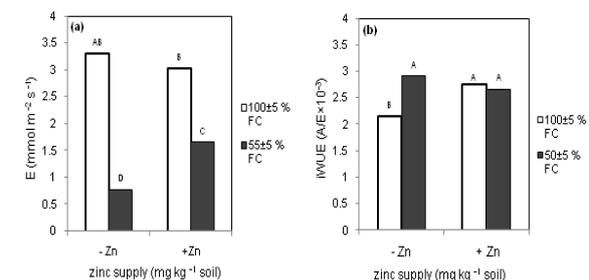


Fig. 3. Transpiration rate, E (a) and instantaneous water use efficiency, iWUE (b) changes in response to soil Zn supply and watering regimes. Well-watered (100±5% field capacity, FC) and drought- stressed (55±5% FC) bean plants were grown in a Zn- deficient soil without (-Zn) and with (+Zn) zinc supplied. Bars with the same letters are not significantly different according to Duncan's test at 5% level.

Water use efficiency improvement, increases crops productivity under both well-watered and rainfed conditions (Turner, 1993). Leaf gas exchange techniques may provide an assessment of the transpiration efficiency of whole plant (Heitholt, 1989). It has been reported that a higher WUE can be achieved either by lower stomatal conductance (Ashraf and Bashir, 2003), higher photosynthetic

capacity, or a combination of both them (Condon *et al.*, 2002; Morgan and Le Cain, 1991). In the control, high iWUE observed for Akhtar (Fig 2c) corresponded to its lower rates of transpiration (Fig 2a). While under DS, more efficient use of water by KS21486 might be due to a minimum effect of g_s on CO_2 uptake, since that its stomata closed noticeably (Fig 2b) while photosynthesis remained greater (Table 2). These findings are in accordance with reports of dos Santos *et al.* (2006). Keeping the mind that pathways of A versus E are different within a leaf, stomatal closure under water shortage will not affect the two processes to the same degree (Cornish and Radin, 1990). This could give rise to the differences in WUE of different common bean genotypes under water stress. Considering some test genotypes, Zn deficiency was associated with lower stomatal conductance (data are not shown) and lower WUE (Fig 2d). Hence, it appears that any potential benefits of limited stomatal aperture on iWUE might be negated by a reduction in net carbon fixation, as has been noted by Tavallali *et al.* (2009).

Decreased or unchanged chlorophyll content has been reported depending on the duration and severity of drought (Mohsenzadeh *et al.*, 2006). Although soil dryness condition impaired photosynthesis (Table 2), it did not promote photoinhibition, since that Fv/Fm remained constant (Table 3). This indicates that thylacoid membranes did not damage and hence, chloroplastic Chl concentration did not reduce as a consequence (Mohsenzadeh *et al.*, 2006). In contrary to chlorophylls, total carotenoid content in plants grown without Zn was greater (Table 3). This is analogous with that of reported by Kösesakal and Ünal (2009) in tomato. They speculated that these changes could be resulted from physiological response caused by Zn deficiency. Zinc is involved in pigments biosynthesis by taking part in the regulation of cytoplasmic concentrations of nutrients (Fischer, 1997), ultimately proving beneficial for photosynthetic machinery of plant system (Arvind and Prasad, 2004). Strong differences among the genotypes for pigments content and fluorescence parameters might in part link with varietal

characteristics.

Conclusions

All the above elements underline the necessity of improved Zn nutrition in common bean crop. Although Zn fertilization provided a non-significant increase in CO_2 assimilation, it could have the capacity to protect the photosynthetic apparatus against photodamages. Water relations of Zn-fed beans tended to be less adversely affected by drought that was along with keeping high rates of transpiration. This facilitates the dissipation of excess heat during periods of water stress. Therefore, incorporation of a properly controlled fertilization regime involving sufficient levels of Zn would become more important under conditions where water availability is limited, too. Significant genotypic variation observed for WUE can give some indications of superiority when comparing common bean genotypes for agronomic fitness under drought or Zn deficiency stresses.

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