

MECHANISMS OF DROUGHT RESISTANCE IN GRAIN II: STOMATAL REGULATION AND ROOT GROWTH

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ABSTRACT: Earlier research works conducted to identify mechanisms of drought resistance in grain legumes under soil water stress of -0.6 MPa showed that drought resistance (maintenance of turgor) in chick pea was due to a significant decrease in osmotic potential (osmotic adjustment) while in common bean it was due to maintenance of high leaf water potential, but not due to osmotic adjustment. Green house experiments were conducted in the University of Hohenheim, Germany to determine whether maintenance of high plant water potential in common bean under stress was the function of stomatal regulation and/or root growth. Seven days mild drought (-0.15 MPa) decreased the water potential to -0.35 and -0.89 MPa, accompanied by a dry matter decrease of 25 and 15% in common bean and chick pea, respectively. Higher dry matter decrease in common bean was due to reduced CO₂-fixation, whereby photosynthesis was reduced by 75% in common bean but only by 20% in chickpea. Significant decrease in the rate of photosynthesis decreased the sugar reserve (glucose, fructose and sucrose) of common bean significantly, which could be responsible for the reduced biomass synthesis. Decrease in the rate of photosynthesis was attributed to significant decrease in stomatal conductance. However, water use efficiency was significantly higher in common bean than in chick pea regardless of water regimes. A rhizotrone experiment showed that root length density of common bean was higher than that of chick pea (by a factor of two), accompanied by higher root weight. It was concluded that high plant water potential of common beans under stress was not due to osmotic adjustment but it was the function of effective stomatal regulation and robust root system.

Key words/phrases: Common bean, chickpea, drought resistance, root density, stomata regulation

INTRODUCTION

Grain legumes became part of the Ethiopian farming system from the time of immemorial, and the land allocated for growing legumes has been increasing in the recent years. This is mainly due to an increased demand for legumes as protein sources and soil fertility restorers. Most legumes, however, are commonly grown in the drought-prone regions of the country, like the common bean in the maize/sorghum systems and the chickpea in the teff/wheat dominate systems. Both species are commonly exposed to end-of-season drought as chickpea is grown using residual moisture, while common bean is grown in intercropping or relay cropping in association with high water demanding companions (maize and sorghum).

Legumes respond to drought differently, and presumably possess various drought resistance strategies (Tilahun Amede *et al.*, 1999). Comparison

of four species under soil water stress of -0.64 MPa showed that faba bean and pea lost turgor while common bean and chickpea maintained turgor (Tilahun Amede and Schubert, 2003). Turgor maintenance (drought resistance) in chickpea was strongly associated with osmotic adjustment. However, in contrast to chickpea, osmotic adjustment was not the principal mechanism of turgor maintenance in common bean.

Common bean was found to maintain high tissue water content even when exposed to severe soil water stress (Sangakkara, 1994; Tilahun Amede and Schubert, 2003). This water-conserving attribute seemed to be accompanied by stomatal regulation. Stomatal closure is one of the first steps of defence against drought since it is a more rapid and flexible process than alternative mechanisms such as changes in life cycle, root growth, leaf area (Chaves, 1991), or osmotic adjustment. Under drought conditions, stomatal closure could be induced by both changes in chemical signalling

and/or hydraulic status of the plant (Tardieu and Davies, 1992). It may allow the plant to avoid dehydration and maintain higher water status until more favourable conditions prevail. Alternatively, stomatal closure may also be a disadvantage since it will reduce CO₂ fixation and hence may cause an assimilate shortage. We hypothesize that Haricot beans failed to adjust osmotically due to shortage of active osmotica (e.g. water soluble sugars) as a result of drought-induced stomatal closure, thereby a decrease in the rate of photosynthesis. In earlier investigations, chickpea plants exhibited a significantly higher (by about 72%) transpirational demand than haricot bean plants. We hypothesize that this luxurious water use in chickpea plants is supplemented by either effective solute accumulation that may create a strong osmotic gradient and/or more efficient water acquisition via the root system than is the case to common bean plants.

The research was conducted to test the hypotheses whether (i) turgor maintenance in haricot bean under low soil water potentials was associated with stomatal regulation (ii) chickpea, with a higher water demand, possesses a more robust root system than common bean plants, and (iii) to assess the accompanying effect of stomatal regulation on dry matter synthesis and sugar reserve in common bean and chickpea plants.

MATERIALS AND METHODS

Experiment on photosynthesis

The experiment was carried out in a greenhouse at the University of Hohenheim in 1995 and 1996 from March to May. Seeds of common bean cv. Brilliant and chick pea cv Gab-3 were sown into Mitscherlich pots filled with 7 kg of soil and sand mixture (5:3 w/w). The soil was fertilized by a complete fertilizer 'Blaukorn'. The experiment had a randomised complete block design with three harvesting dates (0, 7 and 15 days after the onset of stress) and four replications per treatment for both well-watered control and stressed plants. Soil water capacity was maintained at 70–80% until the imposition of water stress treatment. Soil moisture was controlled gravimetrically by re-watering to the given weight at least twice daily. Water stress was imposed 50 days after planting, shortly before the initiation of flowering, and was maintained for 7 or 15 days at soil water content of 40% (-0.15 MPa SWP). There was no difference in physiological development between species. Flowering of both crops was completed during the experimental

period. Harvesting was conducted at 0, 7 and 15 days after the onset of water stress. Shortly before each harvest, leaf water potential (ψ_w) of the youngest fully expanded leaf was measured (Scholander probe). Evapotranspiration was measured gravimetrically starting from emergence.

Measurements of photosynthesis were conducted in a growth chamber on day 0 after the onset of stress, then after 3, 6 and 12 days of stress in both well-watered and stressed treatments. The net CO₂ exchange between a leaf and the atmosphere was measured using an infrared gas analyser (type Binos 100, Walz, Germany). Net photosynthesis and transpiration rates were calculated from differences between inlet and outlet air, and air humidity and CO₂ partial pressure. Additional parameters for calculation were the airflow through the system, enclosed leaf area, temperature and air pressure. Photosynthesis, transpiration rates and stomatal conductance were calculated according to the model of Farquhar *et al.* (1980).

On each day of measurements, a newly emerged young leaf was used. After equilibrium state had been reached (commonly after 15 minutes) values were considered for determination. Measurements on legumes specie were handled in a randomized manner. During measurements the growth chamber were kept constant with a light intensity of 650 $\mu\text{E m}^{-2} \text{s}^{-1}$, temperature of 25 °C and relative humidity of 60–65%. The area of the leaf used for photosynthesis measurements was determined immediately using a leaf area meter (LI-COR Model 3100).

Experiment on root growth

The experiment was conducted in greenhouse in 1996. On the 7th July, seeds were planted in rhizotrones using three replications. Each rhizotrone had two vertically divided compartments of 130 cm x 11 cm x 5 cm volume. Each compartment was filled with 18 kg well-fertilized loess soil with a soil density of 1.4 g soil/cm³. The treatments were well-watered control chickpea (80% water capacity), stressed chickpea (40% water capacity), well-watered control common bean (80% water capacity), and stressed common bean (40% water capacity). Stress was induced slowly shortly after emergence and maintained until harvest (50 days after plantation). Root growth was followed by tracing roots appearing at the front panel of the compartment on a transparency every week with various colours. Root length was measured from the marked

transparency with a rolling counter designed for determination of highway distances on maps. At the end of the experiment, root weight was determined after washing and drying the roots to constant weight.

RESULTS

There were striking visual differences between common bean and chickpea plants in response to mild water stress (-0.15 MPa SWP). When plants were exposed to extended drought, common bean oriented the leaves towards sunlight, showed wilting symptoms in the warmest part of the day but recovered in cooler evening hours. Chickpea responded to low soil water potential by earlier flowering of 7 days, thus facilitating earlier pod formation than in the adjacent well-watered treatments. After prolonged drought, chickpea shed the oldest leaves possibly to minimize transpiration. There was no senescence in common bean, not even at severe level of water stress.

Water relations

The per pot evapo-transpiration of both species was adjusted to be comparable (about 400 ml water d^{-1} for control pots) by planting five plants of common bean and four plants of chick pea per pot.

In well-watered control plants, the initial water potential varied between species, in that ψ_w in beans was 0.2 MPa higher than in chickpea. When exposed to stress, the initial response of both species was similar (Fig. 1) in that water potential of plants under stress treatments was reduced. After the third day of stress, there was an exponential decrease in ψ_w in chickpea, while the level of ψ_w in common bean did not change much with drought, not even 15 days after the onset of stress. On the seventh day of stress, water potential in stressed plants of chick pea was highly reduced ($p < 0.001$), which may indicate that chick pea had no substantial control over transpiration. In contrast, even on the 15th day of stress in common bean, there was no significant difference in ψ_w between stressed and well-watered plants (Fig. 1).

Biomass

Biomass yield was significantly higher for common bean than for chickpea, regardless of water regimes (Fig. 2). Drought decreased fresh weight significantly more than dry weight, and yield reduction was higher in common bean than in chickpea plants (Fig. 2). When the total vegetative growth period was considered, drought caused a reduction in dry matter yield of

25% and 15% in plants of common bean and chickpea, respectively. The effect of drought on biomass reduction within the first seven days was similar to the effect in the second seven days. This indicated that soil water potential was maintained at similar levels throughout the experimental period.

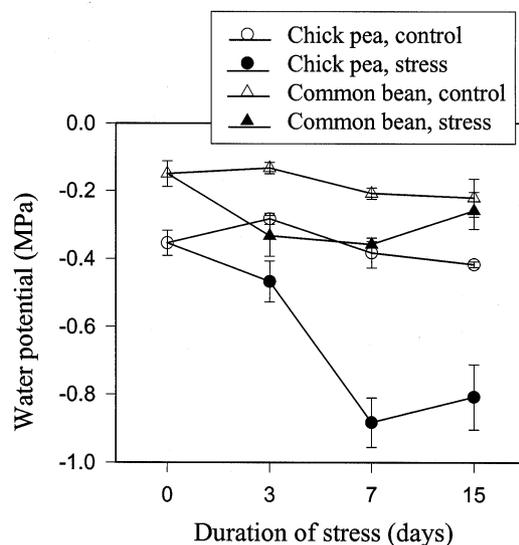


Fig. 1. Leaf water potential of common bean and chickpea under drought stress conditions. Bars indicate \pm SE, $n = 4$.

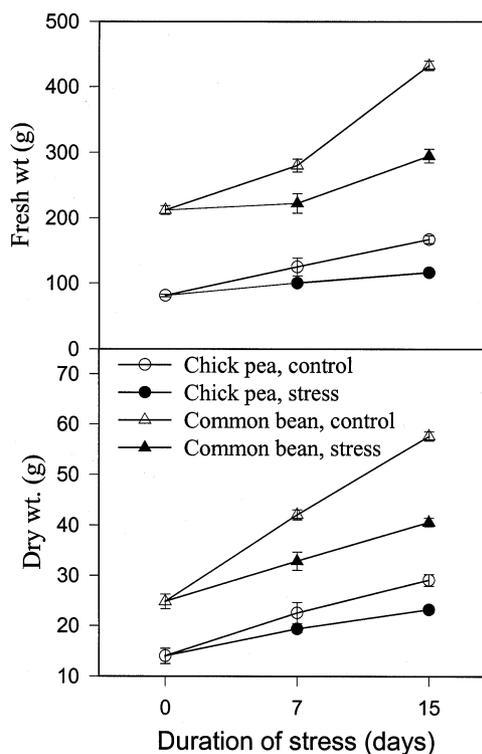


Fig. 2. Dry weight and fresh weight of common bean and chickpea under drought stress conditions. Bars indicate \pm SE $n = 4$.

Photosynthesis and transpiration

CO₂ fixation values were comparable to the values reported by Castogeny and Markhart (1992). In well-watered plants, CO₂ fixation during the experimental period increased with time (Fig. 3). Well-watered plants of common bean assimilated four to five times more carbon than those of well-watered chickpea plants. After the third day of drought stress, CO₂ fixation in stressed plants of common bean dropped significantly to about 20% of the control and did not recover with time (Fig. 3A). In contrast, chickpea continued to assimilate for the whole stress period with only a slight decline after the six day of stress. After an adaptation period of 12 days, the photosynthetic rate of chickpea recovered to about 85% of the control, a level comparable to that of the third day stress. Besides very high assimilate demand in beans at flowering (Fraser and Bidwell, 1974), the possible reason for the increase in assimilation rate

could be leaf position since continual measurements were not conducted on the same leaf but on the newly emerged fully expanded leaves.

A reduction in photosynthesis was strongly accompanied by reduced transpiration rates regardless of species (Fig. 3B). Under water stress conditions, the decrease in transpiration rate was much more substantial in common bean than in chickpea. This may be attributed to the decreased stomatal conductance in haricot bean, which sharply dropped within the first three days of stress (Fig. 4A). Recovery in transpiration rate of chickpea plants was presumably due to a recovery in stomatal conductance (Fig. 4A). This was not the case in common bean plants. Stomatal conductance of well-watered plants at later growing stages increased more in beans than in chickpea plants. However, in stressed plants the reverse occurred (Fig. 4A).

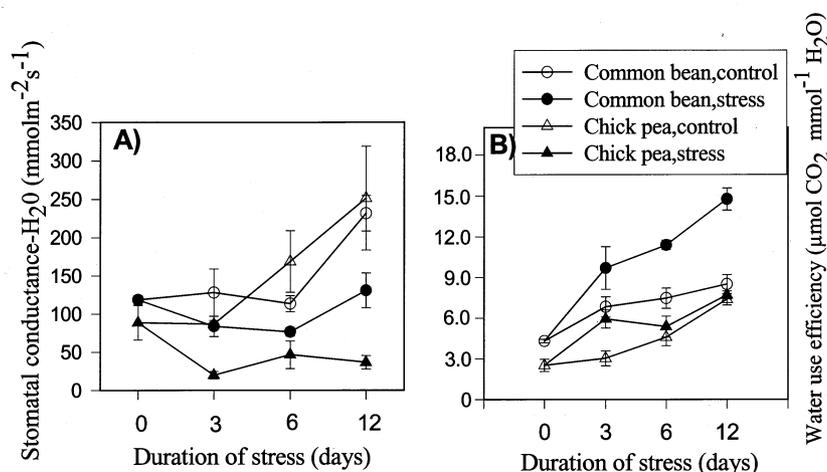


Fig. 3. Effect of drought stress on the rates of CO₂ fixation (A) and transpiration (B) in common bean and chickpea plants. Bars indicate ± SE, n = 3.

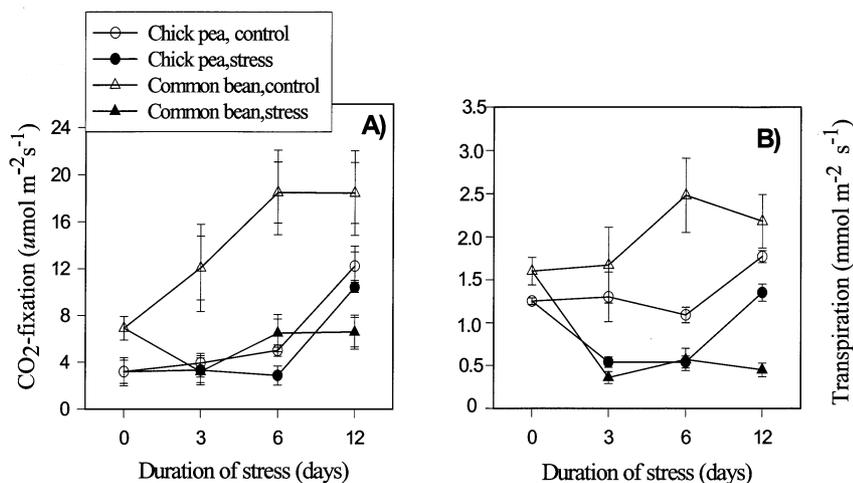


Fig. 4. Stomatal conductance for H₂O (A) and water use efficiency (B) in common bean and chickpea under drought stress conditions. Bars indicate ± SE, n = 3.

Water use efficiency

With increasing drought, both species reduced the amount of water needed to fix a specific amount of CO_2 (water use efficiency, WUE: Fig. 4B). Water use efficiency was significantly higher in common bean than in chickpea, irrespective of water regimes. Drought increased WUE in both species mainly due to drought-induced decrease in transpiration rate. Common bean was more responsive to drought, since WUE in stressed common bean plants was at least two-fold higher than that of chickpea. This result was supported by higher dry matter yield reduction in bean plants (Fig. 2).

Water use efficiency in stressed plants of chickpea was higher than in control plants only shortly after drought imposition. In beans, WUE was highest at only -0.6 MPa of ψ_w , while in chickpea; ψ_w was reduced to -1.0 MPa (Fig 1) without having any substantial effect on WUE.

Sugar reserve

Drought significantly reduced glucose and fructose of common bean while it significantly increased these monosaccharides in chickpea plants (Fig. 5A&B). The level of sucrose in beans was very low regardless of water regimes and did not change so much with drought. On the other hand the sucrose concentration tripled

with drought in chickpea plants (Fig. 5C), which could be explained by continually high photosynthesis rate (Fig. 3) but reduced plant growth (Fig. 2).

Root growth

The aboveground biomass was significantly higher in beans than in chickpea (Fig. 2), while water demand per plant was significantly higher in chickpea than in bean plants. Long-term drought did not affect the root weight of haricot bean plants, but significantly decreased the root weight of chickpea plants (Fig. 6A). Root length density of bean plants was significantly higher (more than factor two) than that of chickpea plants regardless of water treatments (Fig. 6B). Similarly to root weight, drought did not affect root density in beans while it significantly reduced the root density in chickpea. Under favourable moisture conditions (control), root growth rate of chickpea was, however, consistently higher than in bean plants, particularly up to the fourth week of the experiment (data not shown). The roots of bean were morphologically thicker than chickpea, and a drought effect on root dry weight was not observed (Fig. 6).

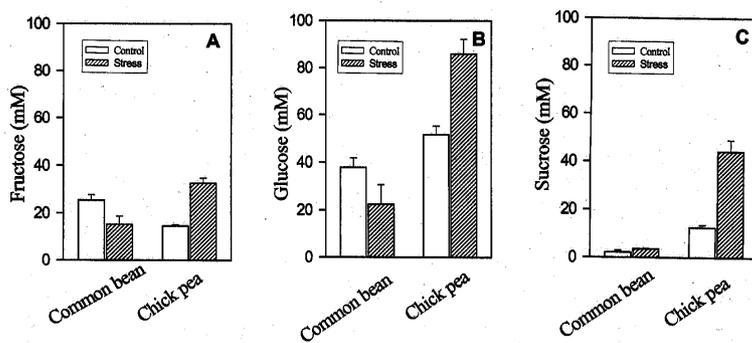


Fig. 5. Effect of drought on fructose (A), glucose (B) and sucrose (C) concentration (mM) in the press-sap of young leaves of common bean and chickpea. Bars indicate \pm SE, $n = 4$.

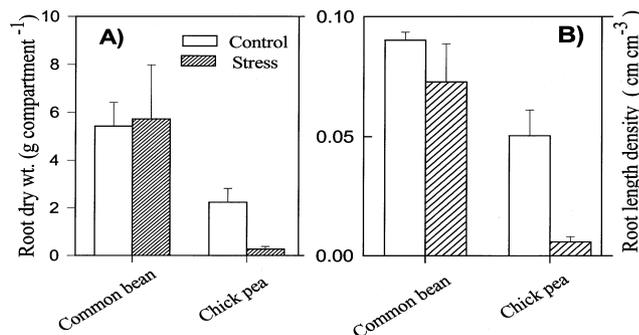


Fig. 6. Effect of long-term drought on root dry weight (g) (A) and root length density (cm cm^{-3}) (B) of common bean and chickpea plants in a rhizotron experiment. Bars indicate \pm SE, $n = 3$.

DISCUSSION

Effect of drought on CO₂ fixation and dry matter synthesis

Earlier findings indicated that common bean was a relatively drought-resistant species, though mechanisms other than osmotic adjustment were responsible for the resistance (Tilahun Amede and Schubert, 2003). The results of the present experiment indicate that drought resistance in beans is achieved through effective stomatal regulation (Figs 3&4). Stomatal regulation enabled the plant to maintain higher leaf water potential even under extended drought conditions (Fig. 1). Drought-induced stomatal regulation in beans has also been reported earlier (O'Toole *et al.*, 1977; Moldau *et al.*, 1993). The parallel decrease of CO₂ fixation and transpiration rate (Fig. 3) also strongly indicate stomatal closure as the major factor in reducing photosynthesis (O'Toole *et al.*, 1977; Chaves, 1991). Stomatal conductance of common bean was reduced by about the same proportion as CO₂ fixation, supporting the results of Wong *et al.*, (1985). In beans, CO₂ fixation decreased by about 75% after the first 3 days and remained at lower level (Fig. 3) while relative growth rate inclined towards zero (data not shown). This could explain that under this growth condition, assimilates were available only for maintenance requirements of the plant. On the other hand, CO₂ fixation in chickpea was not affected by drought except for a moderate reduction in assimilation on the sixth day of stress (Fig. 3). Hence, at the given stress intensity, drought had no substantial effect on stomatal opening in chickpea plants. Continual CO₂ fixation in chickpea under water stress conditions was confirmed by a significant amount of sugar accumulation in the sink leaves under drought stress (Figs 5A-C). In contrast, drought-induced stomatal closure in common bean caused assimilate shortage, whereby the level of sugars (glucose and fructose) was significantly reduced.

In control plants, about 52% and 55% of the total dry matter synthesis during the vegetative stage in chickpea and beans, respectively, was accumulated within the two weeks of the experimental period. A similar growth rate was also observed in faba beans (Tilahun Amede *et al.*, 1999). Hence, an increase in photosynthetic rate in control plants (Fig. 3) is to be expected. An increase in assimi-

lation rate could be induced by higher sink demand during flower initiation (Fraser and Bidwell, 1974). Good and Bell (1980) indicated that the rate of photosynthesis is modified by the availability of sinks for the assimilated products. Higher dry matter synthesis in beans in comparison to chickpea (Fig. 2) was obtained, not only because of differences in the number of plants/pot but also because of the higher photosynthetic rate of bean plants (Fig. 3). Dry matter synthesis could be inhibited also by drought, despite a higher rate of CO₂ fixation, mainly because of increased photorespiration. Effective osmotic adjustment in chickpea (Tilahun Amede and Schubert, 2003) may have assisted the plant to maintain open stomata for optimum gas exchange. As a result, drought caused a growth depression of only 15% in chickpea in comparison to 25% in bean. Ten percent more yield loss in common bean in comparison to chick pea was presumably because of frequent stomatal closure in common bean plants at periods of maximum sunlight (midday). This advantage of C gain in chickpea ceased when plants were exposed to severe drought for extended periods (data not shown). McCree and Richardson (1987) did not find differences in C gain between osmotically adjusting sugar beet and stomatal closing cow pea because of rapidly induced drought, leading to complete consumption of available water in the small pots and resulting in sugar beet dying earlier than cow pea.

There was a significant difference in WUE between the stomatal regulating crop (common bean) and the osmotically adjusting crop (chick pea), which is in agreement with McCree and Richardson (1987). The results showed that WUE was higher in beans than in chickpea even under higher leaf water potentials (Fig. 1). These physiological traits, which indicate the tissue water relation of a species, suggest different adaptation strategies of crops to drought stress. Chickpea, which had the lowest ψ_w (Fig. 1), lowest relative water content (data not shown) and lowest WUE (Fig. 4B) tolerated drought through osmotic adjustment (Tilahun Amede and Schubert, 2003). In contrast, haricot bean, which had the highest ψ_w , highest relative water content, and also highest WUE, tolerated drought through stomatal control (Fig. 3).

Decreased stomatal conductance in beans may improve yield stability under intermittent water stress conditions by minimizing water loss, thereby reducing the probability of exhausting soil water before maturity. Alternatively, stomatal conductance could also reduce productivity (Fig. 4A), presumably by reducing leaf area, duration and/or rate of photosynthesis. Chickpea, which does not regulate water loss either through stomatal regulation or growth adjustment, may require a higher supply of soil water to cope with the atmospheric demand.

Drought and root growth

The average transpiration rate of a chickpea plant was about 72% higher than that of bean plants (data not shown). To meet such high water demand, besides osmotic adjustment, chickpea plants may possess an extensive root system that could explore water from deeper and/or wider soil horizons. On the other hand, extensive root system is a condition for common beans to grow under dry soils as they lack the trait of osmotic adjustment.

Although plants of both species were exposed to water stress after emergence, drought did not affect root growth in bean plants while root growth of chickpea plants was strongly inhibited. Drought-stressed chickpea plant roots ceased to grow after the third week of stress (data not shown). However, drought-induced effects on root growth of chickpea plants may not be associated with matrix potential since chickpea was tolerant to drought stress mainly through osmotic adjustment, while osmotic potential changes are also expected to occur in the root. Besides root density and depth, root hydraulic resistance (the radial resistance from soil to root xylem) may have played a decisive role in water uptake of chickpea plants, so as to cover luxurious water demand. Likewise, root length density of wheat was substantially greater than that of lupines, while water uptake per root surface was higher in lupines (Hamblin and Tennant, 1987). Similar results were also reported for chick pea and barley, whereby chick pea plants were found to have lower root length density than barley, but absorbed water more efficiently than barley plants (Thomas *et al.*, 1995). The difference in water use between these species was a function of root

hydraulic conductivity, which is governed by the diameter and distribution of the meta-xylem vessels (Hamblin and Tennant, 1987), rather than by root density.

It was concluded that drought resistance in chickpea was the effect of osmotic adjustment while in common bean it was the function of effective stomatal regulation and high root density.

ACKNOWLEDGEMENTS

The first author would like to thank the German Academic Exchange Service (DAAD) for a scholarship grant, and Drs Christoph Stober, Reiner Fortmeier, Helge Fortmeier, Robert Feurle and Yan Feng for their technical support during the course of the study. The research work was conducted in the Institute of Plant Nutrition, University of Hohenheim, Germany.

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