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Effect of drought stress on the osmotic adjustment, cell wall elasticity and cell volume of six cultivars of common beans (*Phaseolus vulgaris* L.)

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Abstract

In the southern spring–summer season of 2001–2002 six cultivars (Orfeo, Arroz Tuscola, Barbucho, Coscorrón, Pinto and Tórtola) of common beans (*Phaseolus vulgaris* L.) were grown under two frequencies of irrigation in the central zone of Chile. Control plants were irrigated every 7 days and water stress plants were irrigated every 21 days. Leaf water potential (Ψ_W), leaf relative water content (RWC), turgid weight to dry weight ratio (TW/DW), osmotic potential at full turgor (ψ_S^{100}), osmotic adjustment (OA), elasticity module of the cell wall (ε) and cell size of the palisade and spongy tissue were evaluated at 74–76 days after sowing (DAS) in completely developed leaves. Water stressed plants showed lower Ψ_W than in control plants in all the varieties with values that averaged −1.4 and −0.9 MPa, respectively. According to the Drought Resistance Index (DRI) calculated from field measurements, among the cultivars studied, Orfeo was the most resistant to water stress and Arroz Tuscola, the most susceptible. A close negative relationship between leaf TW/DW and DRI under water stress conditions was observed ($r^2 = 0.63$). Leaf TW/DW decreased considerably with water stress in cultivar Orfeo (15%) but the decrease was higher in Tórtola (22%), and there was also a decrease (although smaller) in Pinto (11%). Arroz Tuscola under stress did not present an important change in TW/DW, but presented one of the highest values of TW/DW. There was a strong negative correlation between DRI and palisade cell size under water stress conditions ($r^2 = 0.85$) and a strong positive one between palisade cell size and TW/DW ($r^2 = 0.86$) thus higher DRI was associated with small palisade cell size and small TW/DW. The most resistant cultivar Orfeo did not show a decrease in ψ_S^{100} calculated from the pressure–RWC relationships and its resistance was not associated with maintenance of leaf TW/DW under water stress. ψ_S^{100} calculated from the pressure–RWC relationships decreased only in the cultivar Coscorrón. Cultivar Orfeo showed a strong decrease (35%) of ε, in association to a higher cell wall elasticity (CWE) and as consequence maintained better its cell turgescence but this was also the case of Tórtola (56%) and Pinto (34%) and to a lesser extent of Barbucho (18%). This was not the case for Arroz Tuscola where ε and CWE were not changed. These results suggest that CWE and to a lesser extent leaf TW/DW can be important components of the water stress adaptation mechanism in this specie that could contribute to the higher resistance to water stress of Orfeo compared to Arroz Tuscola.

Keywords: Beans cultivar; Cell wall elasticity; Osmotic adjustment; Water stress

1. Introduction

Drought is a major factor limiting crop productivity worldwide (Jones and Corlett, 1992) and crops with increased resistance to this stress appear to be crucial for keeping yield in areas where dry seasons are common. Therefore, improving the drought resistance in cultivated species has been, for a long time, a major objective for most of the breeding programs (Sánchez et al., 1998). Intensive studies have been carried out in order to identify physiological traits that can be used as criteria for selection for drought resistance (Blum et al., 1996; Lizana et al., 2006). Examples of criteria are osmotic adjustment (OA) (Bajji et al., 2001), water use efficiency (WUE) (Martínez et al., 2003; Condon et al., 2002) or cell wall elasticity (CWE) (Sinclair and Venables, 1983; Patakas and Noitsakis, 1997).

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About 60% of common beans produced world-wide are grown in regions subjected to water stress, making drought after diseases the second largest contributor to the yield reduction in this specie. This is one of the main reasons why in bean the average yield has remained for long time at low level (<900 kg ha^{-1}) (Thung and Rao, 1999; Singh, 2001; Lizana et al., 2006). In general, the progress in breeding for adaptation to drought stress in crop species has been successful (Blum et al., 1996; Condon et al., 2002). However, in the common bean this progress has been rather poor even if several selection criteria for resistance to drought have been used (White et al., 1994; Lizana et al., 2006). Some of the criteria used in bean screening programs for drought resistance are based on productivity parameters, but normally their measurements are time consuming and difficult. The screening for drought tolerance could be facilitated if physiological traits related to water stress are identified.

The maintenance of turgor has been reported to be essential for keeping a normal cell activity and contribute to growth under low water availability. Osmotic adjustment (OA) has been reported to contribute to maintain the turgor pressure (Chimenti et al., 2002; Martínez et al., 2004) and has drawn much attention during the last years. It has been hypothesized that these compounds benefit stressed cells in two ways: by acting as cytoplasmic osmolytes, thereby facilitating water uptake and retention, and by protecting and stabilizing macromolecules and structure (i.e. proteins, membranes, chloroplast, and liposomes) from damage induced by stress conditions (Martínez et al., 2004). Cell wall elasticity (CWE) is also considered one of the most important physiological mechanisms of adaptation to water stress (Patakas and Noitsakis, 1997). Increases in CWE might contribute to the maintenance of cell turgor or symplast volume and have been reported in several species as response to water stress (Kim and Lee-Stadelmann, 1984; Fan et al., 1994; Patakas and Noitsakis, 1997; Marshall and Dumbroff, 1999). Data also indicate that plants subjected to dehydration may avoid reduced water potential and maintain turgor by reduction of their turgor-loss volume via shrinkage associated with elastic adjustment of their cell walls (Fan et al., 1994; Marshall et al., 1999). Cell contraction means a reduction in cell size which seems to be a character associated with plant resistance to water stress (Cutler et al., 1977; Lecoeur et al., 1995). Cell size changes are known to occur in different species in response to abiotic stress. For example, a cell size reduction has been reported in cassava plants grown under water stress (Alves and Setter, 2004).

In common beans, a better understanding of the morpho-anatomical and physiological basis of such differences in water stress resistance could be used to select or create new varieties of crops to obtain a better productivity under water stress conditions. In this work, we show how the comparative analysis of differences in drought resistance index (Bidinger et al., 1987) between bean cultivars can be used as criterion for selection of cultivars differing in water stress resistance. Six cultivars of common bean used commercially in the central region of Chile were submitted to water stress at the reproductive stage in order to compare their morpho-anatomical and physiological behaviours and see if any relationships between characters like: OA, CWE, and cell size and water stress resistance exist.

2. Materials and methods

2.1. Plant material and growth conditions

Plants of common beans (*Phaseolus vulgaris* L.) (cultivars: Arroz Tuscola, Barbucho, Coscorrón, Orfeo, Pinto 114 and Tórtola) were grown from seeds under field conditions at the Antumapu Experimental Station of the University of Chile (33°40′S, 70°38′W; 605 m of altitude) during the southern spring–summer season. The six cultivars selected, among the most common cultivars used in the central zone of Chile and obtained by the Institute for Agriculture Research of Chile (INIA) were used. Seeds were sown directly into the soil and cultivated in rows 60 cm apart. The soil of the site was a typical xerochrepts soil, 80 cm deep, belonging to the coarse loamy over sandy family. Field experiment was according to a split-plot design, with two irrigation treatments as main plot treatments, six cultivars as sub-plot treatments and four replications as blocks. To facilitate agronomic management and irrigation, the water supply was applied by one gravity irrigation 6 days before sowing and additional irrigation depending on the treatment. The treatments were: (a) "Control" with the plants watered every week (b) and "Water stress" with the plants watered every 3 weeks. Main plots were14 m wide and 23 m long and main plot treatments were two irrigation frequencies. The 6.5 m long sub-plots, had five rows 0.6 m apart, and subplot treatment corresponded to cultivars. Each cultivar was cultivated at its recommended commercial density which was 21, 15, 13, 17, 21, and 18 plants per meter of row in cultivars Arroz Tuscola, Barbucho, Coscorrón, Orfeo, Pinto 114 and Tórtola, respectively. Plant samples were taken from the three central rows of each sub-plot. Fertilisers providing 30 kg of N (Nitrate of potassium) and 80 kg P_2O_5 (Triple Super Phosphate) per hectare were incorporated to the soil at sowing time. Pest and diseases were controlled using conventional chemical controls. At the beginning of grain filling, plants were sprayed with chlorpyriphos (Lorsban) to control *Epinotia aporema* W. Time of emergence, flowering and maturity were recorded as proposed by van Schoonhoven and Pastor-Corrales (1987). Emergence and flowering time were considered when cotyledons appeared at the soil level (8–9 DAS) and when the first flower was opened (55–69 DAS), respectively. Maturity time was considered when pods lost their pigmentation and when they started to dry (89–116 DAS).

2.2. Grain production

Grain production was determined harvesting plants from the central part of the rows of each sub-plot, excepting 25 cm as a border at each end of the row. Data recorded were: seed yield (g m^{-2} at 14% moisture), number of pod per plant, number of seed per pod and weight of 100-seed (g).

2.3. Drought resistance index

The drought resistance index (DRI) was calculated on the basis of a multiple regression of the grain yield of stressed and unstressed plants and the reproductive period (RP) for each cultivar (Bidinger et al., 1987). RP is the period of days between the beginning of the flowering and maturity. DRI for individual cultivars was computed as:

$$\mathrm{DRI} = \frac{\mathrm{YS} - \mathrm{YES}}{\mathrm{SES}}, \quad (1)$$

where YS is the observed value and YES the estimated value of grain yield obtained under stress per cultivar. YES was estimated according to Bidinger et al. (1987) by Eq. (2):

$$\mathrm{YES} = a + b\mathrm{YP} + c\mathrm{RP} \quad (2)$$

where the parameters a, b, and c are estimated by minimizing residuals and YP is the yield under unstressed conditions. SES is the standard error of predicted value YES calculated from the multiple regressions. Positive values of DRI (>1) denoted high resistance to drought. On the contrary, negative DRI values (<−1) denoted low resistance. DRI index is independent of the yield potential and days of the RP. These data were submitted to a significance test for response to drought (DRI) using a statistical program of Michigan State University (1988) specially adapted for this purpose (MSTAT-C).

2.4. Water relations

Leaf water potential (Ψ_W) and leaf relative water content (RWC) were measured at noon on 10 fully expanded leaves, sampled from plants located in the three central rows of each subplot. Measurements were done at 72 DAS. RWC was calculated as follows (Martínez et al., 2004):

$$\mathrm{RWC} = \left[\frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}}\right] \times 100, \quad (3)$$

where FW corresponds to the leaf fresh weight, TW to the leaf turgid weight measured after 12 h of incubation in deionised water at 4 °C in the dark (when leaf weight reached a plateau), and DW to the dry weight (48 h at 80 °C). Leaf water potential (Ψ_W) was measured using a pressure chamber (PMS Instruments Co., Corvallis, OR, USA) (Scholander et al., 1965). The pressure chamber was also used to obtain pressure–volume curves from leaves of each cultivar. Measurements were taken during 4 days period between 72 and 76 DAS. Fully expanded leaves were removed by cutting the petiole under distilled water, and re-hydrated overnight in a darkened humid chamber. Pressure–volume curves were generated using the repeated pressurisation technique, with leaves weighting on a precision balance between measurements of pressure (Tyree and Hammel, 1972). Osmotic potential at full turgor (ψ_S^{100}) was determined according to Sinclair and Venables (1983) using the linear regression of Ψ_S^{-1} versus 1 − RWC (Fig. 1). The cell wall elasticity (CWE) of leaf tissue was estimated through the determination of the elasticity module (ε) value. This parameter was determined after Schulte and Hinckley (1985) using the expression: $\varepsilon = \mathrm{d}P/\mathrm{dRWC}$ (MPa), where P is the turgor pressure.

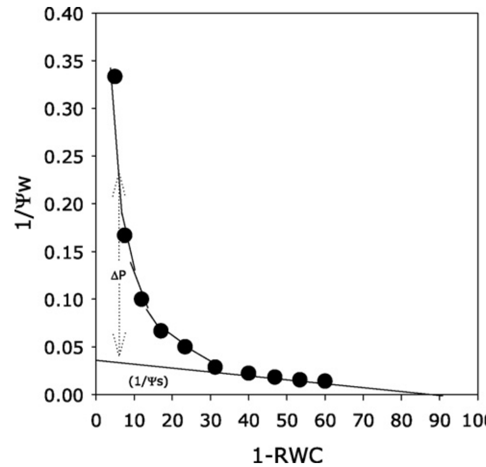

Fig. 1. Pressure–volume curves were generated from the relationship between the inverted value of water ($1/\Psi_W$) and the subtraction between 100 and relative water content (1 − RWC, %) for leaves of common bean plants (cultivar Orfeo). Relationship between the inverted value of water ($1/\Psi_S$) and 1 − RWC is represented by solid line. ΔP indicates magnitude of the turgor pressure which accounts for the difference between Ψ_W and Ψ_S (based on Sinclair and Venables, 1983).

2.5. Cell size of the palisade and spongy tissue

Leaf segments (10 mm^2) were placed in deionised water and illuminated with a lamp (Novilux, model 8103, Netherlands) during 4 h. Sections, 25 W m thick, cut using a new razor blade from deionised fresh material were made. The thickness of the palisade and spongy tissue layers were measured using a light microscope (Carl Zeiss, Standard 20, Germany). The cell size (palisade and spongy tissue volume) was determined after Nobel (1980). The measurements of cell volume were made at 76 DAS.

2.6. Statistical analysis

Data were analysed using a two-way analysis of variance (ANOVA) at a significance level of $^*P \le 0.05$. The model was defined as a split-plot design on the basis of fixed effects and hierarchical classification criterion. Effects of main factors corresponding to cultivar (sub-plot treatment) and irrigation regime (main plot treatment) and as well as their interaction were considered. When the ANOVA was significant at $P \le 0.05$, Duncan Multiple Range Test was used for comparison of means. The data were analysed by a MSTACT statistical package.

3. Results

3.1. Yield components and grain production

The level of water stress imposed in this experiment induced a significant reduction in the number of pods per plants in almost all the cultivars used (Table 1). The cultivar Arroz Tuscola was the most affected with 72% of reduction whereas the cultivar less affected was Orfeo with only 20% of reduction. The other cultivars showed 45–56% of reduction. In cultivars Orfeo, Bar-

Table 1
Number of pods per plant, number of seeds per plant and weight of seeds (g/100 seeds) in different cultivars of bean under water stress

Cultivars	Yield components					
	Number of pods/plant		Number of seeds/pod		Weight of seeds (g/100 seeds)	
	Control	Water stress	Control	Water stress	Control	Water stress
Arroz Tuscola	10.9 ± 0.43 a[a]A[b]	3.0 ± 0.15 cdB	3.8 ± 0.15 abcA	2.6 ± 0.15 bB	16.7 ± 0.33 dA	16.1 ± 0.40 eA
Barbucho	5.7 ± 0.22 cA	2.5 ± 0.12 dB	3.2 ± 0.13 cdA	2.7 ± 0.16 bA	34.0 ± 0.68 aA	34.2 ± 0.69 aA
Coscorrón	8.3 ± 0.41 abA	4.3 ± 0.22 cB	3.7 ± 0.14 bcA	2.6 ± 0.15 bB	33.0 ± 0.66 aA	29.3 ± 0.70 bB
Orfeo	9.2 ± 0.40 abA	7.3 ± 0.36 aA	4.5 ± 0.20 aA	4.0 ± 0.20 aA	20.3 ± 0.40 cA	18.7 ± 0.47 cdA
Pinto	8.0 ± 0.32 bcA	5.8 ± 0.30 bcB	3.7 ± 0.15 bcA	2.7 ± 0.16 bB	30.7 ± 0.61 bA	27.4 ± 0.69 bcA
Tórtola	7.0 ± 0.35 bcA	3.8 ± 0.20 cdB	3.4 ± 0.14 bcdA	2.9 ± 0.17 bA	29.6 ± 0.59 bA	25.8 ± 0.64 bcB
Average	8.1 ± 0.32 A	4.5 ± 0.23 B	3.7 ± 0.14 A	2.9 ± 0.17 B	27.4 ± 0.55 A	25.2 ± 0.63 B

Each value represents mean ± S.E. ($n = 4$).
[a] Lower case letters indicates differences between cultivars.
[b] Upper case letters indicate differences ($P \leq 0.05$) between treatments.

Table 2
Seed yield (g/m^2), during of reproductive period (days) and dry resistance index (DRI) in different cultivars of bean under water stress

Cultivars	Grain production		Reproductive stage		Dry resistance index (DRI)
	Seed yield (g/m^2)		Reproductive period (days)		
	Control	Water stress	Control	Water stress	
Arroz Tuscola	290 ± 15.0 a[a]A[b]	52 ± 4.2 dB	43 ± 2 cA	40 ± 1 cA	−1.28
Barbucho	180 ± 8.0 cA	65 ± 5.2 cdB	56 ± 1 abA	56 ± 1 abA	0.03
Coscorrón	204 ± 10.0 bcA	64 ± 6.0 cdB	64 ± 1 aA	64 ± 1 aA	−0.35
Orfeo	240 ± 12.0 abA	175 ± 14.0 abA	39 ± 2 cA	41 ± 1 cA	1.23
Pinto	302 ± 15.0 aA	151 ± 12.1 bB	37 ± 1 cA	37 ± 1 cA	0.28
Tórtola	230 ± 11.5 bA	85 ± 7.0 cB	63 ± 1 aA	57 ± 1 abB	0.29
Average	237 ± 12.0 A	97.5 ± 6.8 B	50 ± 1 A	49 ± 1 A	–

Each value represents mean ± S.E. ($n = 4$).
[a] Lower case letters indicates differences between cultivars.
[b] Upper case letters indicate differences ($P < 0.05$) between treatments.

bucho and Tórtola, the water stress did not affect the reduction of the number of seeds per pod (Table 1). This character was reduced in Arroz Tuscola, Coscorrón and Pinto (average reduction of 29%) but not in the other cultivars. Seed weight was little affected by water stress (Table 1). As a consequence of these reductions, the seed yield was diminished in average by 59% in all the cultivars (Table 2). The strongest decrease in seed yield was observed in cultivar Arroz Tuscola with 82% of decrease, whereas Orfeo showed only 27% of seed yield reduction.

3.2. *Relationship between reproductive period and seed yield*

The yield under control and water stress conditions was negatively correlated with the length of the reproductive period (RP) (control $r^2 = 0.57$; stress $r^2 = 0.41$; $P \leq 0.05$) (Fig. 2). Cultivars Pinto and Orfeo, which are the most productive under stress condition, had a short reproductive period with 37 and 39 days, respectively. In contrast Coscorrón and Tórtola, which under water stress had the lowest production, had the longest period between flowering and maturity: 64 and 63 days, respectively.

3.3. *Drought resistance index*

The drought resistance index (DRI) was calculated using Eq. (1). According to the DRI values, cultivars Orfeo and Arroz Tuscola were the most contrasting (Table 2) (Fig. 3). Orfeo which

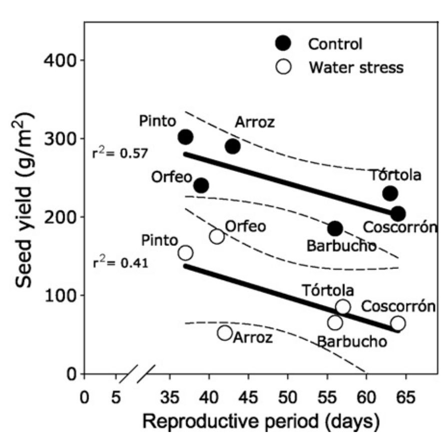

Fig. 2. Relationship between the seed yield (g/m^2) and reproductive period (days) in six cultivars of the common bean under control (●) and water stress (○) conditions. The broken lines around the regression line show the 5% confidence belt.

Table 3
Water potential (Ψ_W, MPa), relative water content (RWC) and turgor weight to dry weight ratio (TW/DW) in different cultivars of bean under water stress

Cultivars	Ψ_W (MPa)		RWC (%)		TW/DW	
	Control	Water stress	Control	Water stress	Control	Water stress
Arroz Tuscola	−0.95 ± 0.12 a[a]A[b]	−1.43 ± 0.18 aB	88.0 ± 1.0 aA	86.0 ± 0.9 Aa	6.5 ± 0.10 abA	6.3 ± 0.08 abA
Barbucho	−0.91 ± 0.11 aA	−1.45 ± 0.17 aB	86.9 ± 0.5 aA	87.2 ± 1.0 aA	6.5 ± 0.09 abA	6.4 ± 0.11 aA
Coscorrón	−0.95 ± 0.12 aA	−1.25 ± 0.10 aB	85.3 ± 1.5 aA	83.3 ± 0.8 aA	7.0 ± 0.11 abA	6.6 ± 0.10 aA
Orfeo	−0.98 ± 0.10 aA	−1.30 ± 1.5 aB	85.1 ± 1.5 aA	85.1 ± 2.0 aA	5.3 ± 0.07 cA	4.5 ± 0.05 cB
Pinto	−1.03 ± 0.12 aA	−1.41 ± 0.18 aB	90.8 ± 2.0 aA	85.6 ± 1.0 aA	6.1 ± 0.10 bcA	5.4 ± 0.08 bB
Tórtola	−0.95 ± 0.12 aA	−1.41 ± 0.20 aB	87.0 ± 3.0 aA	78.7 ± 3.5 aA	7.6 ± 0.11 aA	5.9 ± 0.08 bcB
Average	−0.96 ± 0.11 A	−1.38 ± 0.17 B	87.2 ± 1.5 A	84.3 A ± 2.0	6.5 ± 0.10 A	5.9 ± 0.10 B

Each value represents mean ± S.E. ($n = 4$).
[a] Lower case letters indicates differences between cultivars.
[b] Upper case letters indicate differences ($P \leq 0.05$) between treatments.

in terms of yield under stress was the most tolerant to water stress showed the highest positive DRI value (1.23) (Table 2). In contrast, Arroz Tuscola which had the lowest seed yield under stress had the most negative DRI value (−1.28). The other cultivars presented DRI values close to zero which indicate a tolerance to water stress intermediate between Orfeo and Arroz Tuscola.

3.4. Water potential and relative water content

Water potential (Ψ_W) and relative water content (RWC) values are shown in Table 3. The water stress treatment imposed in this case reduced significantly the Ψ_W in all the cultivars (44% average), but no significant differences were observed between the cultivars in any case (Table 3). Leaf RWC was measured on the same leaves as those used for Ψ_W measurements. In opposition to differences observed in Ψ_W between the treatments, the values of RWC obtained from stressed plants were not significantly different from the control and there were no differences between the cultivars either (Table 3). These results indicate that under water stress all the cultivars were able to keep RWC more or less constant despite a significant decrease in Ψ_W.

3.5. Turgor weight to dry weight ratio (TW/DW)

In response to the water stress treatment, leaf TW/DW decreased considerably in cultivars Tórtola (22%), Orfeo (15%) and Pinto (11%) (Table 3). Orfeo presented the lowest TW/DW value both under control and stress conditions whereas Arroz Tuscola which under stress did not present an important decrease in TW/DW, presented one of the highest values of TW/DW. On the other hand, the relationship between TW/DW and DRI under water stress presented a negative correlation ($r^2 = -0.63$; $P \leq 0.05$) (Fig. 4) indicating that a low TW/DW values are related with high DRI index.

3.6. Full turgor osmotic potential

According to the results obtained from the pressure–volume curves (Fig. 1), under well-watered conditions the full turgor osmotic potential (ψ_S^{100}) was similar between all the cultivars (Table 4), and it was not affected by the irrigation treatments. This indicates that when full turgor is re-established, the water

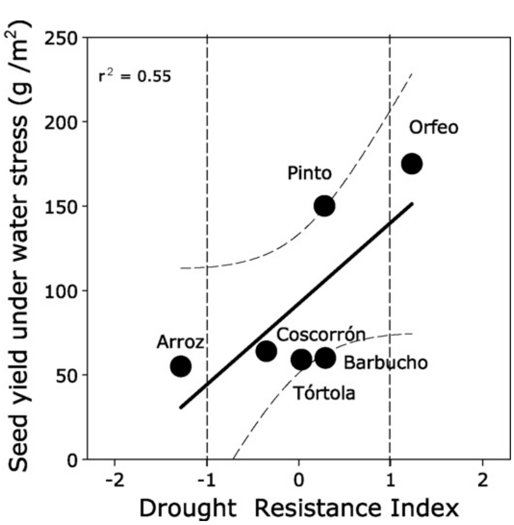

Fig. 3. Relationship between the seed yield (g/m^2) under water stress condition and the drought resistance index (DRI, units absolute) in six cultivars of the common bean. The broken lines around the regression line show the 5% confidence belt.

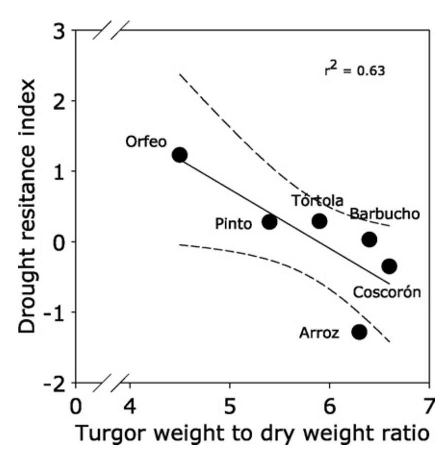

Fig. 4. Relationship between the drought resistance index (DRI) and the turgor weight to dry weight ratio (TW/DW) in six cultivars of the common bean under water stress condition. The broken lines around the regression line show the 5% confidence belt.

Table 4
Effect of water stress on the leaf full turgor osmotic potential ψ_S^{100} and the elasticity module (ε) in different cultivars of bean

Cultivar	ψ_S^{100} (MPa)		ε (MPa)	
	Control	Water stress	Control	Water stress
Arroz Tuscola	−1.3 ± 0.20 a[a]A[b]	−1.4 ± 0.20 abA	3.0 ± 0.10 bA	3.5 ± 0.18 abA
Barbucho	−1.6 ± 0.25 aA	−2.1 ± 0.30 cA	6.5 ± 0.15 aA	5.3 ± 0.16 aB
Coscorrón	−1.2 ± 0.15 aA	−1.9 ± 0.20 bcB	3.3 ± 0.10 bA	3.7 ± 0.11 abA
Orfeo	−1.6 ± 0.22 aA	−1.6 ± 0.32 abcA	3.7 ± 0.11 bA	2.4 ± 0.05 bB
Pinto	−1.6 ± 0.24 aA	−1.7 ± 0.30 abA	5.3 ± 0.13 abA	3.5 ± 0.10 abB
Tórtola	−1.4 ± 0.18 aA	−1.8 ± 0.27 abcA	4.6 ± 0.09 abA	2.0 ± 0.05 bB
Average	−1.5 ± 0.20 A	−1.7 ± 0.28 A	4.4 ± 0.10 A	3.4 ± 0.08 B

Each value represents mean ± S.E. ($n = 4$).
[a] Lower case letters indicates differences between cultivars.
[b] Upper case letters indicate differences ($P \leq 0.05$) between treatments.

potential was the same after water stress. Only the cultivar Coscorrón presented a significant reduction in ψ_S^{100}. This can indicate that osmotic adjustment occurs in this cultivar.

3.7. Volumetric elastic modulus (ε)

Under well-watered conditions, the high values of the volumetric elastic modulus (ε) observed in cultivars Barbucho, Pinto and Tórtola (Table 4), revealed that the wall of the leaf cells of these cultivars were less elastic than the walls of leaf cells of cultivars Arroz Tuscola, Coscorrón and Orfeo. In these last cultivars, ε presented values below 3.7. The contrast observed in other parameters between Orfeo and Arroz Tuscola was absent in this case under well-watered conditions. Particularly high was the ε value observed in cultivar Barbucho (6.5) which was significantly higher than in the rest of the cultivars. Most of the cultivars that under well-watered conditions presented high values of ε reduced significantly this parameter under water stress (Table 4). A contrasting behaviour of cultivars Orfeo and Arroz Tuscola was found under water stress conditions despite no difference in ε in controls. In fact Arroz Tuscola did not decrease ε under water stress and even it showed a small tendency to increase it. On the contrary, cultivar Orfeo which like Arroz Tuscola under well-watered conditions presented a low ε (3.7), showed a significant decrease in ε, by almost −35%. This decrease in ε showed as $\Delta\varepsilon$ in Table 4. A negative value of $\Delta\varepsilon$ in this case indicates an increase in the cell wall elasticity during acclimation to water stress. Thus, the highest and positive drought resistance index found in Orfeo (1.23) was associated with a negative value of $\Delta\varepsilon$ and therefore with an increase in cell wall elasticity. Whereas the most negative value of DRI found in Arroz Tuscola (−1.28) was associated to a small positive $\Delta\varepsilon$ or a slight decrease in elasticity. Plotting the DRI values of all cultivars against the $\Delta\varepsilon$ values expressed as % of controls a good correlation is obtained ($r^2 = 0.59$; $P < 0.05$) (Fig. 5). This indicates that under water stress, cultivars that decreased significantly their ε and therefore increased their cell wall elasticity, presented a positive DRI and better resistance to the water stress treatment. On the contrary cultivars that under stress their ε do not decrease like Arroz Tuscola have a negative DRI, indicating that they were more sensitive to the water stress treatment.

3.8. Size of the cells of palisade and spongy parenchyma

The size of the cells of the palisade and spongy parenchyma are shown in Table 5. In average, cells of the palisade parenchyma were bigger than those of the spongy parenchyma (Table 4). Under control conditions cells of spongy parenchyma were only 70% of the size of the palisade cells. This difference was larger under water stress treatment where the size of the spongy cell parenchyma represented only 40% of the size of the palisade cell. Under well-watered conditions, Orfeo presented the lowest palisade parenchyma volume: 12.4×10^3 μm^3 (followed by Barbucho with 13.3×10^3 μm^3) and Arroz Tuscola the highest one: 21.3 (followed by Pinto with 20.9×10^3 μm^3). In Orfeo, under water stress, the size of the parenchyma cells size was significantly reduced by almost 44% with respect to control plants (Table 5). Similar reductions were observed in cultivars Pinto (47%) and Tortola (37%). However in Arroz Tuscola, Barbucho and Coscorron the cell size was not significantly affected by water stress. Under water stress, reductions

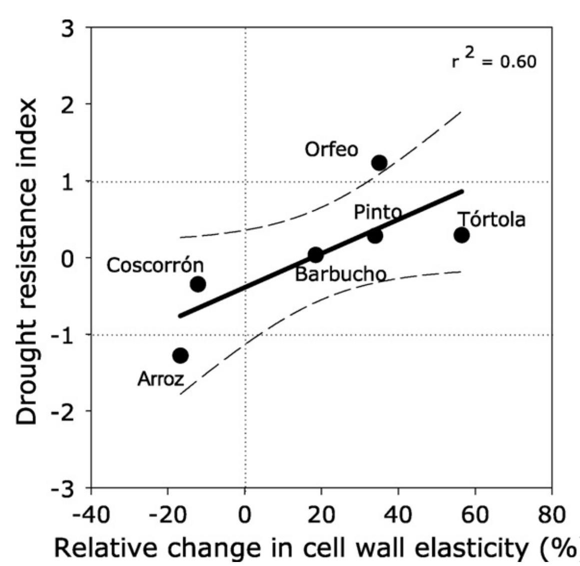

Fig. 5. Relationship between the drought resistance index (DRI) and the variation in cell wall elasticity (ΔCWE, %) in six cultivars of the common bean under water stress condition. The broken lines around the regression line show the 5% confidence belt.

Table 5
Effect of water stress on the cellular size (volume) of palisade and spongy parenchyma tissue in different cultivars of bean

Cultivars	Volume ($\mu m^3 \times 10^3$)			
	Palisade parenchyma		Spongy parenchyma	
	Control	Water stress	Control	Water stress
Arroz Tuscola	21.3 ± 0.42 a[a]A[b]	17.2 ± 0.70 abA	27.3 ± 0.40 aA	11.3 ± 0.39 aB
Barbucho	13.3 ± 0.26 bcA	14.0 ± 0.56 bcA	7.1 ± 0.10 dA	2.8 ± 0.08 bB
Coscorrón	19.3 ± 0.38 aA	17.3 ± 0.68 abA	6.9 ± 0.10 dA	4.0 ± 0.90 bA
Orfeo	12.4 ± 0.24 cA	6.9 ± 0.34 dB	12.3 ± 0.20 bA	5.5 ± 0.22 bB
Pinto	20.9 ± 0.41 aA	10.9 ± 0.30 cB	8.8 ± 0.11 cdA	4.2 ± 0.16 bB
Tórtola	18.0 ± 0.36 aA	11.3 ± 0.22 cB	11.5 ± 0.16 bcA	3.2 ± 0.02 bB
Average	17.5 ± 0.35 A	12.9 ± 0.64 B	12.3 ± 0.20 A	5.2 ± 0.3 B

Each value represents mean ± S.E. ($n = 4$).
[a] Lower case letters indicates differences between cultivars.
[b] Upper case letters indicate differences ($P \leq 0.05$) between treatments.

of spongy parenchyma cells were also observed. There was a good correlation between the size of cells from the palisade parenchyma and the TW/DW (Fig. 6A; $r^2 = 0.86$; $P < 0.05$) under water stress conditions. There was also under these conditions a close relationship between the DRI and palisade parenchyma size (Fig. 6B) with the correlation coefficient of 0.85 significant at $P \leq 0.05$.

4. Discussion

The yield component that was more affected by the water stress treatments was the number of pods per plant (Table 1). The reduction in this parameter was particularly important in cultivars Arroz Tuscola, and Barbucho where a 72 and 56% of reduction were observed, respectively. During the flowering and pod set periods, water stress exacerbation of the abortion of these organs is well documented (Graham and Ranalli, 1997; Terán and Singh, 2002). Pod retention is an important factor that determines yield and therefore it is a desirable characteristic for modern beans cultivars. Orfeo was the less affected by water stress since neither its number of pod per plant nor its seed yield were effected (Table 2). Moreover, it was the only one cultivar to have a DRI over 1. The numbers of seed per pod and weight per seeds have been reported to be more stable and less affected by environmental stress (Terán and Singh, 2002). In the present study, this was corroborated and therefore these yield components seem not to be much associated with drought resistance in bean. Water stress also produced significant reductions in the number of pods per plant and in the number of seed per pod (Table 1). These results are similar to those obtained by Lyon et al. (1995) and Nielsen and Nelson (1998). However both of these characters are affected by the duration of reproductive period (RP). This may mislead the interpretation of the resistance to water stress since a short RP can allow to a particular cultivar to be less exposed to water restrictions than other with a longer RP. Analysing the tolerance to water stress with the drought resistance index (DRI) allows to be independent of both yield potential (YP) and phenology (RP) effects (Bidinger et al., 1987). A high value of DRI indicates a high tolerance and a low value represents a high susceptibility to water stress. In this

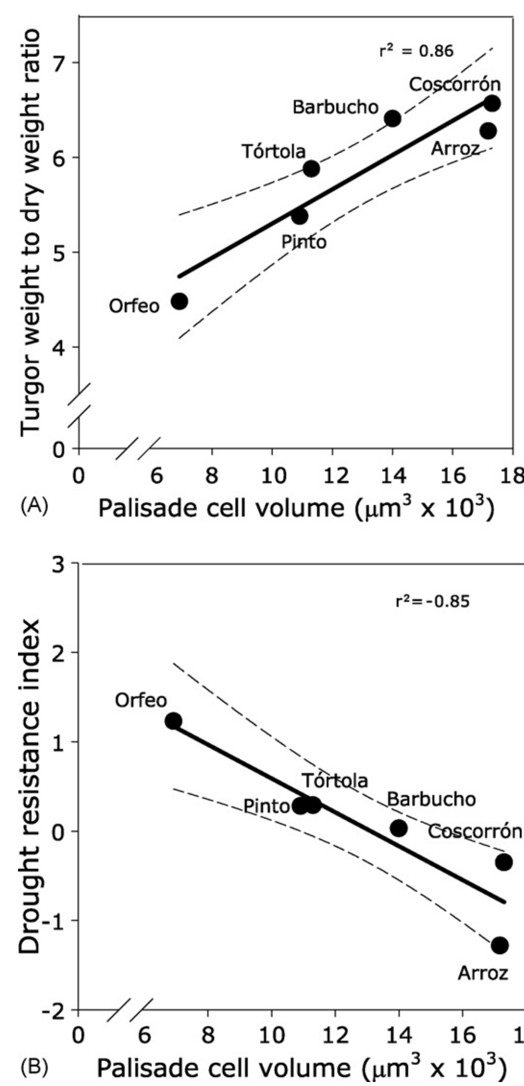

Fig. 6. (A) Relationship between the turgor weight to dry weight ratio (TW/DW) and the palisade cell volume ($\mu m^3 \times 10^3$) in six cultivars of the common bean under water stress condition. The broken lines around the regression line show the 5% confidence belt. (B) Relationship between the drought resistance index (DRI) and the palisade cell volume ($\mu m^3 \times 10^3$) in six cultivars of the common bean under water stress condition. The broken lines around the regression line show the 5% confidence belt.

study DRI values fluctuated between 1.23 and −1.28 among the six cultivars. After these values, Orfeo was again qualified as the cultivar most resistant to water stress and Arroz Tuscola as the most sensitive. These results are consistent with the contrasting effect of treatments on seed yield and the fact that factors others than RP were involved.

Although Ψ_W decreased in all cultivars under water stress condition, the leaf RWC did not present significant changes. This would mean that the decrease in Ψ_W was sufficient to avoid significant loss of water from the leaves in all the cultivars and that the decrease of Ψ_W was not associated directly with loss of water. Leaf TW/DW ratio was reduced by water stress in cultivars Orfeo, Pinto and Tórtola. Under stress Orfeo showed the lowest leaf TW/DW value and Arroz Tuscola presented one of the highest one (6.3). This reduction has been also observed in other species (Martínez et al., 2004; Clifford et al., 1998). This reduction in the leaf TW/DW could be the result of hemi-cellulose and cellulose accumulation in the cell wall (Wakabayashi et al., 1997). According to DRI and to the effect of treatment on yield Arroz Tuscola was the most susceptible to water deficit whereas Orfeo was the most resistant. There is therefore at least for these two most contrasted cultivars some consistency between water stress resistance and TW/DW. In the present work, decrease in cell size was observed in the palisade parenchyma of some cultivars such as the drought tolerant Orfeo but not in others such as the sensitive Arroz Tuscola. Moreover, there was a close relationship between the volume per cell of palisade parenchyma and the leaf TW/DW under water stress conditions (Fig. 6A). Thus, in these experiments a decrease in the leaf TW/DW (Table 3) indicated a decrease in cell size. A reduction in cell size is one of the most common anatomical changes observed in leaves affected by water stress (Lecoeur et al., 1995; Tardieu et al., 2000). Small cells can be advantageous to plants under water stress conditions and may contribute to an explanation of the resistance mechanism to drought. Our results are in agreement with this hypothesis, since we found a close relationship ($r^2 = 0.85$; $P \leq 0.05$) between DRI and the cell volume of palisade parenchyma in stressed plants. Observations on the behaviour of stress-hardened plants of cotton (Cutler et al., 1977) also support the hypothesis that plants or tissues with smaller cell size are more resistant to water stress.

In bean OA did not appear to be very important as a mechanism associated to resistance to water stress. In fact, only the cultivar Coscorrón presented some adjustment of this parameter under water stress.

Our results are consistent with a role of physiological adjustments in cell wall elasticity (CWE) as significant component of resistance to water stress. Cultivar Orfeo, decreased strongly the elasticity module (ε) of their cell walls (Table 4). In contrast, Arroz Tuscola did not show any statistically significant change in their ε (Table 4). The response of ε is consistent with the great tolerance of Orfeo to water stress and the high sensitivity of Arroz Tuscola (Tables 2 and 4). We found (Fig. 4) a positive correlation ($r^2 = 0.60$; $P \leq 0.05$) between the changes of CWE and DRI under water stress conditions, but this relation was due mainly to the contrasting behaviour and characters of Orfeo and Arroz Tuscola. The variations in CWE in response to water stress presumably reflect differences in wall structure. Chimenti and Hall (1994), Marshall and Dumbroff (1999) found in several species an increase of CWE in response to water stress. It would be of great interest to analyze the putative changes in the cell wall properties of sensitive and resistant bean cultivar. For example, in *Triticum aestivum*, increases in hemi-cellulose content of the cell wall have been reported under water stress (Wakabayashi et al., 1997). No single character taken alone however was sufficient to allow clearly pinpointing water resistant cultivars among the others. The study of factors and genes controlling cell size and cell wall elasticity may be useful for improving water stress resistance of this specie.

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