



RESEARCH PAPER

# Differential adaptation of two varieties of common bean to abiotic stress

## I. Effects of drought on yield and photosynthesis

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### Abstract

The yield of 24 commercial varieties and accessions of common bean (*Phaseolus vulgaris*) has been determined at different sites in Chile and Bolivia. Statistical analysis was performed in order to characterize whether a particular variety was more or less stable in yield under different environmental conditions. Amongst these, two varieties have been identified for more detailed study: one variety has a higher than average yield under unstressed conditions but is strongly affected by stress, and another has a reduced yield under unstressed conditions but is less affected by stress. The contrasting rate of abscission of the reproductive organs under drought stress was clearly consistent with these differences. The more tolerant genotype shows a great deal of plasticity at the biochemical and cellular level when exposed to drought stress, in terms of stomatal conductance, photosynthetic rate, abscisic acid synthesis, and resistance to photoinhibition. By contrast, the former lacks such plasticity, but shows an enhanced tendency for a morphological response, the movement of leaves, which appears to be its principal response to drought stress.

Key words: Abscission, common bean (*Phaseolus vulgaris* L.), drought stress, photoinhibition, stomatal conductance, yield stability.

### Introduction

Maintaining crop yields under adverse 'stress' environmental conditions is probably the major challenge facing modern agriculture. To meet this challenge, it is necessary to understand the contrasting adaptations of plants to growth in stressed and unstressed conditions, and the compromises and trade-offs between them. Drought is perhaps the major factor limiting crop production worldwide (Jones and Corlett, 1992). Management practices can contribute to a decrease in yield loss in water-deficient environments, but major progress can also be achieved through genetic improvement (White *et al.*, 1994; Singh, 2001). The availability of crops with increased drought resistance is then crucial for maintaining yield in areas where dry seasons are common. Thus, improvement in the drought resistance of cultivated species is a major objective of many breeding programmes. Intensive studies have been carried out in cereals (Fisher and Maurer, 1978; Acevedo and Ceccarelli, 1989); pea (Sánchez *et al.*, 1998), and many other crops (Blum *et al.*, 1996) in order to identify physiological traits that can be used as criteria for selection for drought resistance. Plant responses to water stress include morphological and biochemical changes that lead first to acclimation and later, as water stress become more severe, to functional damage and the loss of plant parts (Chaves *et al.*, 2003). During the acclimation phase, water stress typically results in slower growth rates because of the inhibition of cell expansion, the reduction in carbon assimilation (Osorio

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*et al.*, 1998) and the resultant effect on carbon partitioning (Hsiao and Xu, 2000). In crops such as common bean these reductions can impact directly on the abscission rate of flowers, a major determinant of yield (Osborne, 1989; Clements and Atkins, 2001).

Drought is one of the most significant problems affecting bean production because about 60% is obtained from regions subjected to water shortage. Despite the identification of several selection criteria for resistance to drought (White *et al.*, 1994) and the great effort made in bean breeding during the latter half of the twentieth century, the average global yield of bean remains low (<900 kg ha<sup>-1</sup>) (Thung and Rao, 1999; Singh, 2001). This reveals that progress in transferring morphological, physiological, and biochemical traits with potential impact in drought tolerance to bean cultivars has been rather poor. Furthermore, resistance to drought seems to have been inadvertently reduced in modern bean varieties (Singh, 2001), probably because the emphasis of breeding has been mainly in introducing better resistance to biotic (insect pests and diseases) rather than to abiotic stresses.

In crops such as common bean, in which seed yield is the important economic trait, the main selection criteria for drought resistance are parameters of plant growth and grain production (Acosta-Gallegos and Adams, 1991). However, even though these parameters may reflect plant performance under stress, most are difficult and time-consuming to measure. Therefore, the screening of more cultivars for drought tolerance would be accelerated if the physiological traits related to water stress could be identified. Therefore, new strategies are needed to identify close relationships between yield parameters and specific physiological traits. In this work, it is shown how the comparative analysis of the yield stability between varieties (Fisher and Maurer, 1978) can be used as criteria for selecting contrasting

varieties of bean. Evidence is then presented of how differences in this yield parameter between two contrasting bean varieties can be explained by their contrasting physiological and cellular responses to water stress.

## Materials and methods

### Plant material

Collections of bean were assembled from different cropping areas of South America. Twenty-four varieties were selected, comprising the most common varieties used in Bolivia and Chile and some new lines coming from the International Centre for Tropical Agriculture (CIAT) in Colombia. Two varieties were selected for more detailed study: Orfeo and Arroz. Orfeo is a type II growth habit variety obtained by the National Institute for Agriculture Research of Chile (INIA) by crossing Negro Argel with Great Northern varieties. Arroz is a white type I growth habit variety also obtained by INIA by selection of Chilean white type 1 accessions.

### Field sites

Five different sites in Chile and Bolivia were used. Table 1 shows a summary of the soil and environmental characteristics of each site. In Chile, experiments were conducted under irrigation because almost no rainfall occurs during the growing season. In Bolivia, the sites had an average rainfall of 300 mm and irrigation was not necessary.

### Yield analysis

Ten yield experiments were conducted under four replicate randomized blocks between 2000 and 2003 at the five different sites. The performance and the yield stability of each of the 24 varieties were determined using the statistical technique described by Finlay and Wilkinson (1963). This method allows the comparison of the yield average and the regression coefficient of a particular variety, grown at different sites and seasons, with the site mean yield of the all varieties tested. Using both indices, it is then possible to characterize whether a particular variety is more or less stable in yield under different environmental conditions.

**Table 1.** Characteristics of five field sites used for yield determinations

Shown are the data for field sites in Chile and Bolivia.

	Field site				
	Antumapu (Chile)	Sacta Valley (Bolivia)	Mayra (Bolivia)	La Violeta (Bolivia)	Chinguri (Bolivia)
Location	33° 40' S, 70° 38' W	17° 06' S, 64° 45' W	17° 30' S, 65° 45' W	17° 20' S, 66° 13' W	18° 23' S, 65° 13' W
Altitude (m)	605	219	2025	2680	2135
Soil depth (cm)	80	200	70	170	70
Soil texture	Sandy loam	Silty loam	Loam	Silty loam	Silty loam
Soil bulk density (g cm <sup>-3</sup> )	1.48	1.42	1.43	1.34	1.37
Rainfall year average (mm)	341	3850	500	544	450
Rainfall 2001 season (mm)	0	248	–	334	–
Rainfall 2002 season (mm)	10	310	208	381	–
Rainfall 2003 season (mm)	0	290	–	353	200
Temp av. (°C)	18	25	21	16.3	22
Temp min. (°C)	4.4	20	14	7.1	14
Temp max. (°C)	28.2	30	28	25.6	30
Planting time	October	May–June	Nov–Dec	Nov–Dec	Nov–Dec
Harvesting time	March	Aug–Sept	March–April	April–May	March–April

### Drought treatment under field conditions

Plants of varieties Arroz and Orfeo were grown from seed under field conditions at the Antumapu Experimental Station (Table 1) during the summer season. Soil was a typical xerochrepts soil, with alluvial and sedimentary origin, 80 cm deep and belonging to the coarse-loamy-over-sandy family. Seeds were sown directly into the soil and cultivated in rows 60 cm apart. A split-plot design was used, with two irrigation treatments, two subtreatments (cultivars), and four replications. Water was supplied by gravity 6 d before sowing and then again after germination. The treatments used were: (i) 'control' with the plants watered every week; and (ii) 'water stress' with the plants watered every week until the fourth trifoliate leaf was fully expanded and from then every 15 d until the beginning of grain filling. Two plots (14 m wide and 23 m long) were used for the two irrigation treatments. The subplots, with five rows of 6.5 m long and 0.6 m apart, corresponded to each cultivar. The density of planting was 21 and 17 plants  $m^{-1}$  in Arroz and Orfeo, respectively. Plant samples were taken from the three central rows of each subplot. Fertilizers providing 30 kg of N and 83 kg of K (nitrate of potassium) and 35 kg of P (triple superphosphate)  $ha^{-1}$  were incorporated to the soil at sowing time. Pest and diseases were controlled using conventional chemical controls. Times of flowering and maturity were recorded as proposed by van Schoonhoven and Pastor-Canales (1987): flowering time was considered when the first flower was opened in each variety; maturity time was considered when pods lost their pigmentation and started the drying process. Abscission of the reproductive organs (flowers plus pods) was recorded from flowering until maturity by collecting them periodically from a plastic net located beneath the plants. Grain production was determined by sampling five plants from the central part of each of the three central rows of each subplot, omitting the two lateral rows and the 25 cm from the border at each extreme of the row. In total, 15 plants were harvested per replication (subplot). Data recorded were: seed yield ( $g\ m^{-2}$  at 14% moisture), number of pods per plant, number of seeds per pod, and 100-seed weight (g). Data were analysed using a two-way analysis of variance (ANOVA) at a significance level of  $P \leq 0.05$ . The model was defined on the basis of fixed effects and hierarchical classification criterion. Main effects were considered to be due to cultivars and treatments as well as their interactions. When the ANOVA was significant at  $P \leq 0.05$ , means comparison was done using the Duncan multiple range test.

### Greenhouse experiments

Plants were grown from seed in plastic pots using a vermiculite/perlite mixture (3:1 v/v) as substrate. Plants were watered every three days with 0.5 l of water. For the 30 d following germination plants were grown under natural light in the greenhouse (approximately  $300\ \mu\text{mol}\ \text{PAR}\ \text{m}^{-2}\ \text{s}^{-1}$ ) with a 16 h photoperiod. At this stage drought was imposed by allowing the plants to desiccate for over 17 d. Two light intensity regimes were used during the desiccation period: 300 and  $600\ \mu\text{mol}\ \text{m}^{-2}\ \text{s}^{-1}$  provided by 400 W metal halide lamps during a 12 h photoperiod. The leaf relative water content (RWC) was measured periodically by sampling lateral leaflets of mature trifoliate leaves during the first hour of the photoperiod and then drying in an oven ( $70\ ^\circ\text{C}$ ) until constant weight. The relative growth rate (RGR) was determined in four plants per treatment using the equation:

$$RGR = (FW_{130} - FW_{147}) / FW_{147} \times 17$$

where  $FW_{130}$  and  $FW_{147}$  are the fresh weights of plants after 30 d and 47 d of growth, respectively.

### Plant growth room experiments

Plants were grown from seed as for greenhouse experiments in a controlled environment room with a 12 h photoperiod, illumination

provided by 400 W metal halide lamps with an intensity of either  $1000\ \mu\text{mol}\ \text{PAR}\ \text{m}^{-2}\ \text{s}^{-1}$  (high light, HL) or  $300\ \mu\text{mol}\ \text{PAR}\ \text{m}^{-2}\ \text{s}^{-1}$  (low light, LL). In HL the temperature of the room was  $32\text{--}35\ ^\circ\text{C}$  during the light period and  $25\ ^\circ\text{C}$  in the dark. In LL the temperature was  $25\ ^\circ\text{C}$  day and night. Plants were watered on alternate days. All experiments were carried out on the lateral leaflets from the third fully expanded (mature) trifoliate leaves, approximately 4–6 weeks following germination. To induce drought, watering was stopped. For detached leaf experiments, plants were maintained under the growth conditions for at least 2 h before the start of the experiment. Leaves to be detached were then dark-adapted for 15 min, removed from the plant, and placed abaxial face up on a wooden surface under the growth lights (light intensity  $1000\ \mu\text{mol}\ \text{m}^{-2}\ \text{s}^{-1}$  at  $35\ ^\circ\text{C}$ ). Measurements were made every 30 min during the treatment.

### Photosynthesis measurements

Carbon dioxide fixation and stomatal conductance were measured on plants grown in the plant growth room, using a Li-Cor 6400 portable IRGA (Lincoln, Nebraska). For  $P_{\text{max}}$  measurements a light intensity of  $2000\ \mu\text{mol}\ \text{m}^{-2}\ \text{s}^{-1}$  and a temperature of  $35\ ^\circ\text{C}$  were used. Stomatal conductance was measured at 350 ppm  $\text{CO}_2$ , block temperature  $35\ ^\circ\text{C}$ , and a light intensity of  $2000\ \mu\text{mol}\ \text{m}^{-2}\ \text{s}^{-1}$ . For LL plants, the temperature was  $25\ ^\circ\text{C}$ . Chlorophyll fluorescence measurements were carried out using the integrated Li-Cor fluorimeter or a Walz PAM 2000 portable fluorimeter (Effeltrich, Germany).  $F_v/F_m$  was measured after a period of 15 min dark adaptation.

### Measurement of ABA-induced stomatal closure

To measure the effect of ABA on stomatal closure the central leaflets from the second and third fully expanded mature leaves were sprayed with different concentrations of ABA (0, 1, 10 or  $100\ \mu\text{M}$ ). Three leaflets from each variety were sprayed three times with MES buffer (20 mM MES, pH 6.18, 0.1% Triton X100) containing different concentrations of ABA, the excess allowed to run off, and then left to dry for 5 min. Control plants were sprayed with a placebo containing only the MES buffer. This process was then repeated and the plants placed back under the growth lights for a further 2 h before stomatal conductance was measured using a Li-Cor IRGA. The treatment was repeated at each concentration of ABA on different plants.

### Assay of ABA

Leaf discs were subjected to an osmotic shock by incubating at  $35\ ^\circ\text{C}$  in mannitol ( $\Psi\pi = -1.6\ \text{MPa}$ ). After 2 h and 4 h of incubation, samples were collected and immediately frozen in liquid nitrogen. Samples were thawed and extracted in distilled water for 16 h at  $4\ ^\circ\text{C}$  in the dark. Quantitative analysis was performed on crude aqueous extracts using solid-phase radioimmunoassay using a monoclonal antibody raised against free (S)-ABA (Vernieri *et al.*, 1989). Each replicate sample was assayed in duplicate.

### Leaf movement

Leaf movement was measured in the flanking leaflets of fully mature trifoliate leaves in HL-grown plants 30 min after the onset of illumination, and 30 min before the end of the photoperiod. Orientation was also checked at midday. Leaf angle ( $\theta$ ) was measured by taking photographs of the plants and measured as the rotation of the leaf about the pulvinus, with the angles ( $\theta$ ) for fully open and fully closed leaves being  $0^\circ$  and  $90^\circ$ , respectively. Samples were taken from triplicate batches of plants containing six plants of each variety.

### Assay of anthocyanin content

Anthocyanin content was assayed essentially according to the method of Laby *et al.* (2001). Plants from each variety were grown in either low or high light conditions. Three 5 cm leaf discs taken from each

leaflet of the third fully expanded trifoliate leaf were pooled and extracted with acidified methanol at 4 °C for 48 h until the leaf discs were completely bleached. The OD was then measured at 530 and 657 nm and the concentration of anthocyanin calculated. Results represent the means  $\pm$ SE for at least 12 leaves from six plants taken from at least two separate growth experiments.

#### Assay of lipid peroxidation

The malondialdehyde (MDA) assay for estimating lipid peroxidation was carried out on leaf tissue as described in Hodges *et al.* (1999). Three 5 cm leaf discs were taken from each leaflet of the third fully expanded trifoliate leaf and homogenized in 80% ethanol with inert sand. The samples were pooled and MDA assayed. Results represent the means  $\pm$ SE for at least 12 leaves from six plants taken from at least two separate growth experiments.

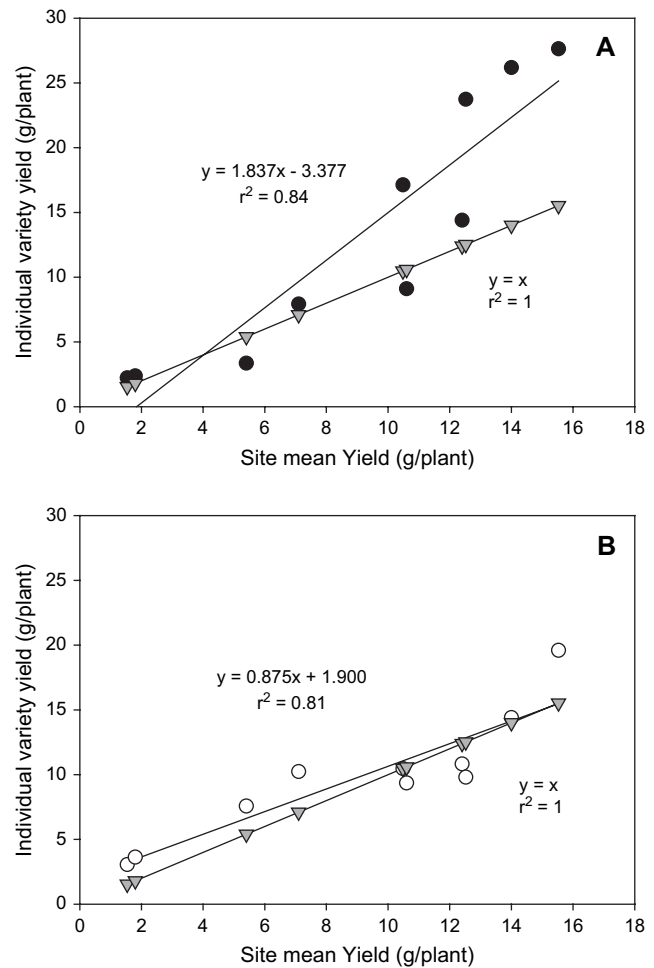
## Results

### Yield stability over different seasons and different sites

Figure 1 shows the relationship between the individual variety yields ( $\text{g plant}^{-1}$ ) and the site population mean of 24 varieties grown at different sites and seasons. Because the individual variety yields are plotted against the mean of all the varieties yields, the mean of the population (24 varieties) has a regression coefficient of 1.0. The regression coefficient of Arroz was 1.84, which was significantly higher than 1 indicating that it has a lower stability than the mean of the population (Fig. 1A). On the other hand, Orfeo had a regression coefficient of 0.88, slightly lower than the mean of the population. This indicates that this variety has a high yield stability under different conditions (Fig. 1B). In both varieties the correlation between individual yield and site mean yield was significant ( $P > 0.05\%$ ) and  $r^2$  values were 0.84 and 0.81 for Arroz and Orfeo, respectively. It can be seen that the yield of Arroz is higher than the population mean in high-yielding environments, but lower than the mean in low-yielding environments. By contrast, the yield of Orfeo is similar to the mean in high-yielding environments, but higher than the mean in low-yielding environments.

### Effects of water stress on yield

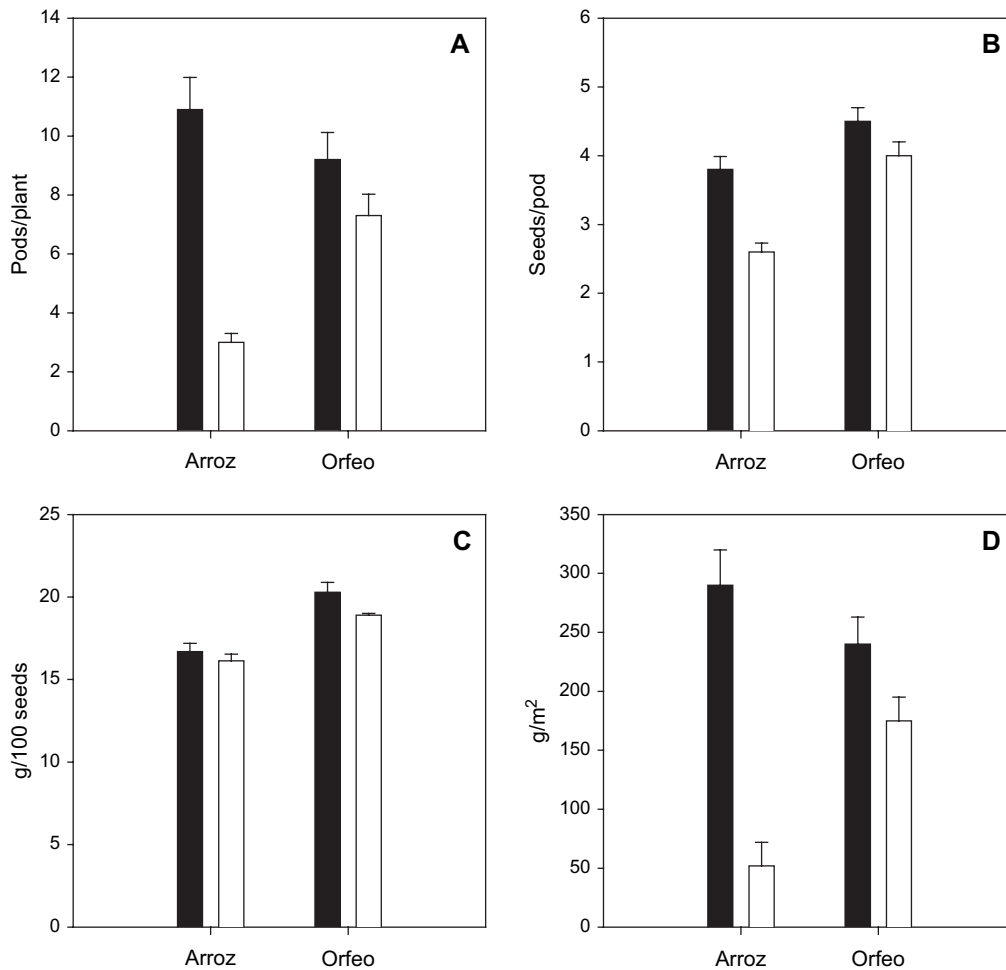
The water stress treatment in field experiments carried out in Chile resulted in a decrease in water potentials in both Arroz and Orfeo. In Arroz, water potentials measured for the fourth trifoliate leaf at noon at the beginning of the grain-filling period were  $-0.87 \pm 0.11$  MPa in control plants and  $-1.55 \pm 0.14$  MPa in water-stressed plants. In Orfeo, water potential values were a little more positive,  $-0.58 \pm 0.08$  and  $-1.42 \pm 0.23$  MPa, respectively, but not statistically different under water stress conditions. Figure 2 shows the yield response for Orfeo and Arroz to water stress. Under well-watered conditions, Arroz had on average 10.9 pods  $\text{plant}^{-1}$ , which is almost 18% more than the value found for Orfeo (9.2 pods  $\text{plant}^{-1}$ ). This largely determines the better grain yield observed for Arroz under well-watered conditions, which was 21% higher than



**Fig. 1.** Relationship between individual variety yield ( $\text{g plant}^{-1}$ ) and site mean yield in two varieties of bean: (A) Arroz (closed circles) and (B) Orfeo (open circles) compared with the data average from 24 genotypes (grey arrowhead) obtained from ten experiments realized at five different locations in Chile and Bolivia between 2002 and 2003. The equations of the linear regression are shown.

Orfeo (Fig. 2D). However, under water stress, Arroz exhibited a 72% reduction in the number of pods per plant compared with well-watered conditions (Fig. 2A) and a 32% decrease in the number of seeds  $\text{pod}^{-1}$  (Fig. 2B). In the case of Orfeo, there was no significant reduction in these parameters. This was also the case for the harvest index where Arroz showed a reduction of 41%, going from 0.44 under well-watered conditions to 0.26 under stress. In Orfeo, the reduction in harvest index was not significant, going from 0.39 to 0.34 under water stress.

Water stress did not significantly modify the time of flowering and, in the case of the harvesting time, this was advanced by 7 d in Arroz and by 5 d in Orfeo (data not shown). Water stress also did not modify the weight of the seeds in either variety (Fig. 2C). Therefore it was the significant reductions in the number of pods  $\text{plant}^{-1}$  and seeds  $\text{pod}^{-1}$  that gave rise to the 83% reduction in the grain yield caused by water stress in Arroz. By contrast, the smaller changes in



**Fig. 2.** Yield components of two bean varieties grown under well-watered conditions (black bars) and water-stress conditions (white bars) in the field. (A) Pods plant<sup>-1</sup>; (B) seeds pod<sup>-1</sup>; (C) 100-seed weight; and (D) grain yield in g m<sup>-2</sup>. Results represent the mean  $\pm$ SE of four replications ( $n=15$ ) per treatment.

these two parameters in Orfeo resulted in the much smaller reduction in grain yield (27%) in this variety (Fig. 2D).

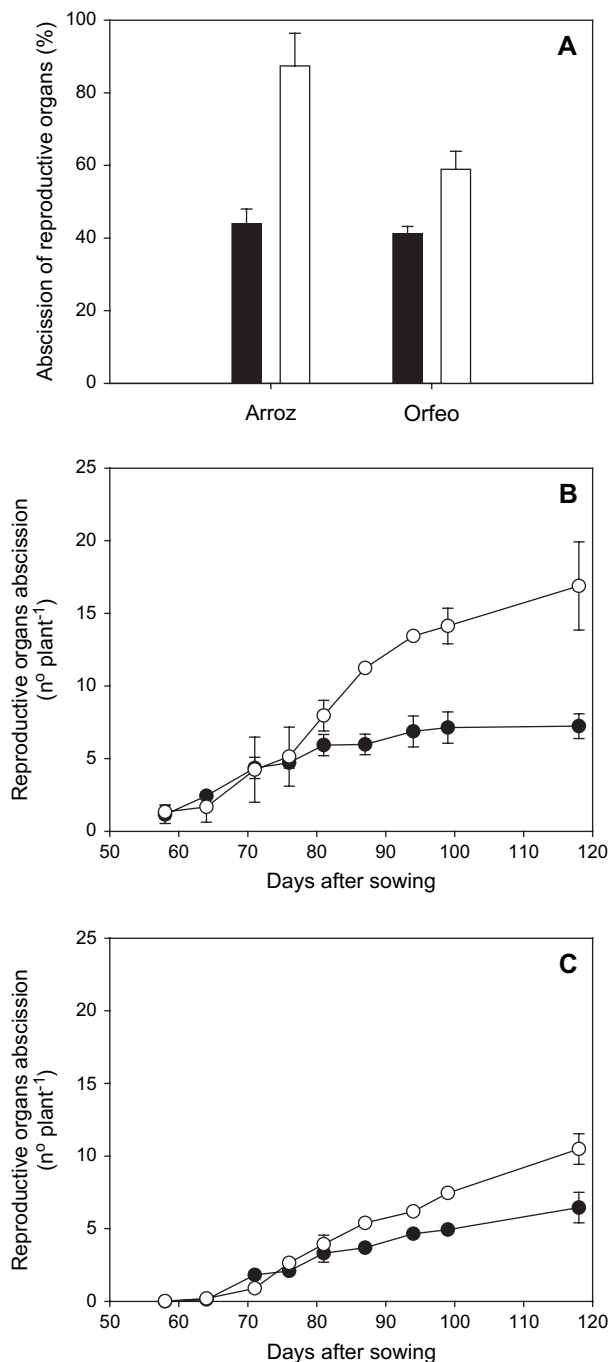
The reduction in the final number of pods plant<sup>-1</sup> was due to the significant increase in abscission of flower and pods caused by water stress in both varieties (Fig. 3A). Abscission was more frequent in Arroz where, from a value close to 43% under well-watered conditions, abscission increased more than 2-fold under water stress, reaching 85% by harvesting time. In Orfeo, abscission in well-watered plants was also about 40%, very similar to Arroz, but under water stress, abscission increased to only 55%, much less than observed in Arroz. The development of abscission was also very different between both varieties. While in the case of Arroz, the rate of abscission increased abruptly after withholding water (Fig. 3B), in Orfeo it increased steadily until harvesting time (Fig. 3C).

#### Effects of water stress on RGR and RWC

The relative growth rate (*RGR*) was determined following the withdrawal of water in greenhouse conditions (Fig. 4).

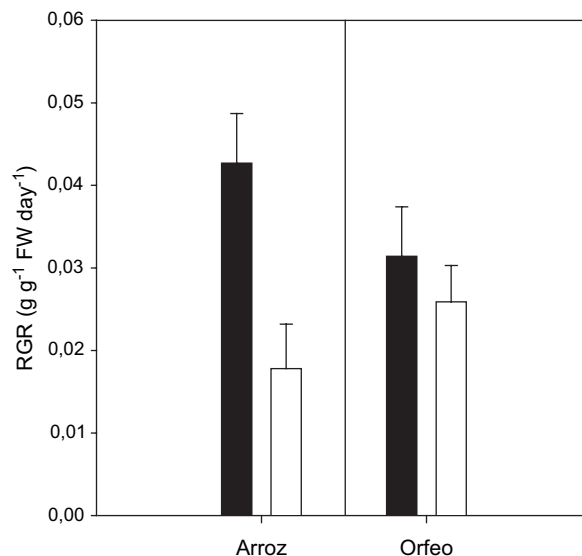
After 15 d of desiccation, the *RGR* of Arroz was reduced by over 60%. In Orfeo, *RGR* was almost unaffected, with a reduction of only 17.5%. Figure 5 shows the kinetics of the change in the relative water content (*RWC*) of the leaves during the desiccation treatment. Independent of the light conditions, in well-watered plants the *RWC* remained almost constant at around 95% throughout the experimental period. However, when water shortage was imposed in Arroz, the *RWC* started to decline significantly after 8–10 d. This decline was more evident under high light conditions (Fig. 5A, C). However, in the case of Orfeo at both light intensities, the withholding of water had a negligible effect on the *RWC* of leaves (Fig. 5B, D).

Similar results were obtained from experiments using detached leaves taken from plants grown in the growth room. Leaves were left to desiccate at 35 °C and 1000  $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ . For a given period of time, Arroz clearly lost more water than Orfeo (Fig. 6A). Under these conditions, at the very beginning of the desiccation period stomata were almost closed, showing a very low conductance in



**Fig. 3.** Effect of the water stress on abscission of reproductive organs in two bean varieties. (A) Total percentage of reproductive organs abscised; (B, C) number of reproductive organs abscised in Arroz (B) and Orfeo (C) as a function of days after sowing. Control, black bars/symbols; water stress, white bars/symbols. Results are the mean  $\pm$ SE of field experiments from two growth seasons (2001/2002 and 2002/2003) with four replications ( $n=15$ ) per treatment per season.

both varieties (Fig. 6B). Continued exposure to desiccation in both cases caused further reductions in stomatal conductance, but Arroz always had the higher values. Transpiration rates were also higher in Arroz during the desiccation period (Fig. 6C).

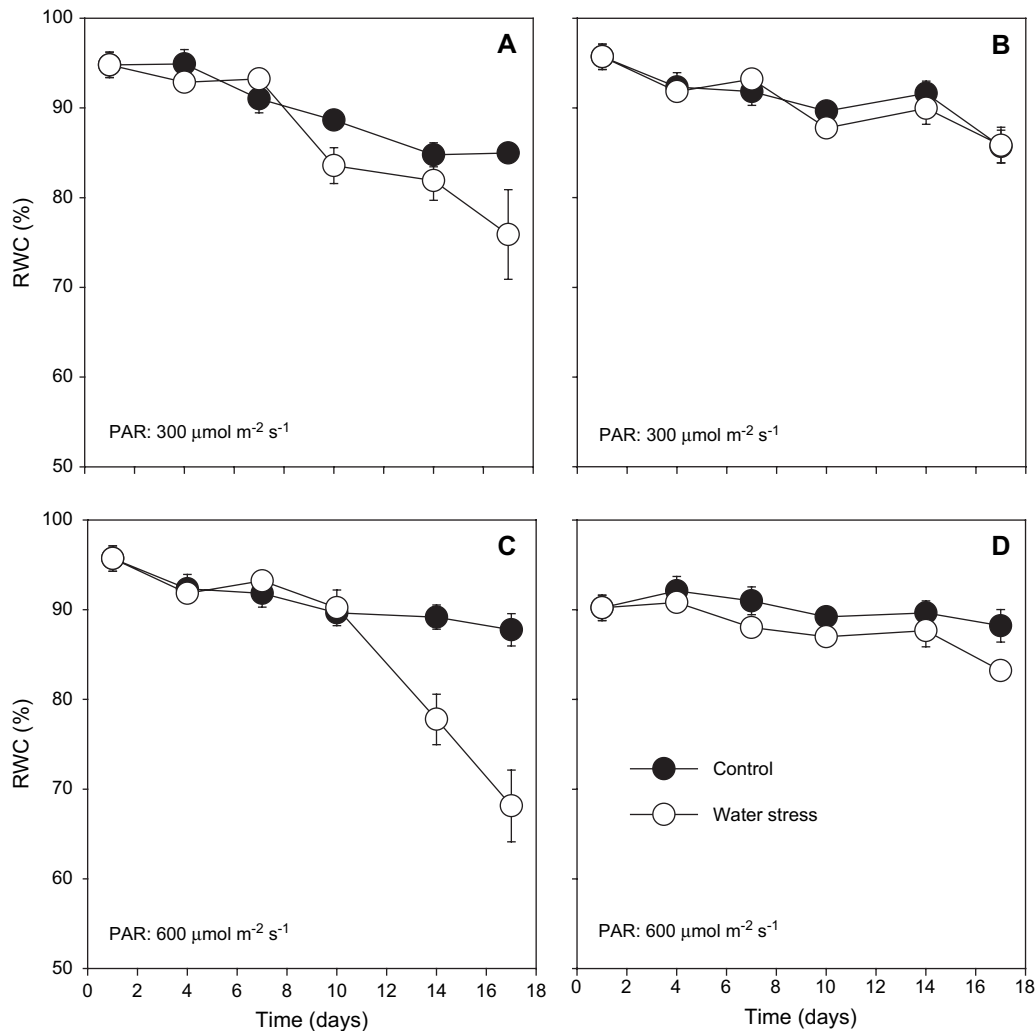


**Fig. 4.** Relative growth rates (RGR) of two varieties after 17 d of desiccation treatment under greenhouse conditions. Control; black bars; water stress; white bars. Results represent the mean  $\pm$ SE of four plants per treatment.

#### Photosynthetic rates

Figure 7 shows the photosynthetic capacities of Arroz and Orfeo grown under controlled conditions. When grown under low light ( $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and at  $25^\circ\text{C}$  they had exactly the same  $P_{\text{max}}$ . However, when grown under high irradiance and  $35^\circ\text{C}$ , the  $P_{\text{max}}$  for Orfeo was approximately 35% higher than Arroz. This difference in  $P_{\text{max}}$  was increased further if the plants were subjected to drought; after 2 d of drought, the  $P_{\text{max}}$  of both varieties declined, but Arroz was much more sensitive (Fig. 7A).  $P_{\text{max}}$  of Orfeo was almost three times higher than Arroz under these conditions. Continued exposure to drought caused further reductions in photosynthesis in both varieties, but Orfeo always kept higher values. As expected, drought also caused a decrease in stomatal conductance (Fig. 7B). Interestingly, after 4 d and 7 d of drought the stomatal conductance of Arroz was higher than that of Orfeo, even though its  $P_{\text{max}}$  was lower. Figure 7c clearly shows how Arroz always maintains higher conductances than Orfeo for a given  $P_{\text{max}}$  value, indicating higher water use efficiency in Orfeo.

The characteristics of the stomata in these two varieties are shown in Table 2. Under high light in Arroz ' $g_{\text{max}}$ ' was  $0.22 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ; which is almost 50% lower than the ' $g_{\text{max}}$ ' value of Orfeo,  $0.32 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ . In dark-adapted leaves, the stomatal conductance ( $g_{\text{min}}$ ) of Arroz and Orfeo were also different. Under both well-watered and droughted conditions and after 30 min of dark adaptation, the conductance of Arroz was nearly three times higher than that of Orfeo. Another interesting aspect is that the stomata of Orfeo show a greater dynamic range (64.5) than those of Arroz (13.5), with a lower minimum conductance both



**Fig. 5.** Relative water content (RWC) of two varieties plants (Arroz, A and C; Orfeo B and D) grown under desiccation treatment (2 weeks) under low light (A, B;  $300 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$ ) and high light (C, D;  $600 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$ ) under greenhouse conditions. Water stress, open circles; control, closed circles. Results represent the mean  $\pm$ SE of four leaves per plant obtained from four plants per treatment.

in the dark and under drought, but also with a higher maximum conductance. Times for stomata to become full opened or full closed were also different between both varieties. Upon illumination, the conductance increases almost two times faster in Arroz, but decreases more slowly upon darkening.

#### Differential response to abscisic acid

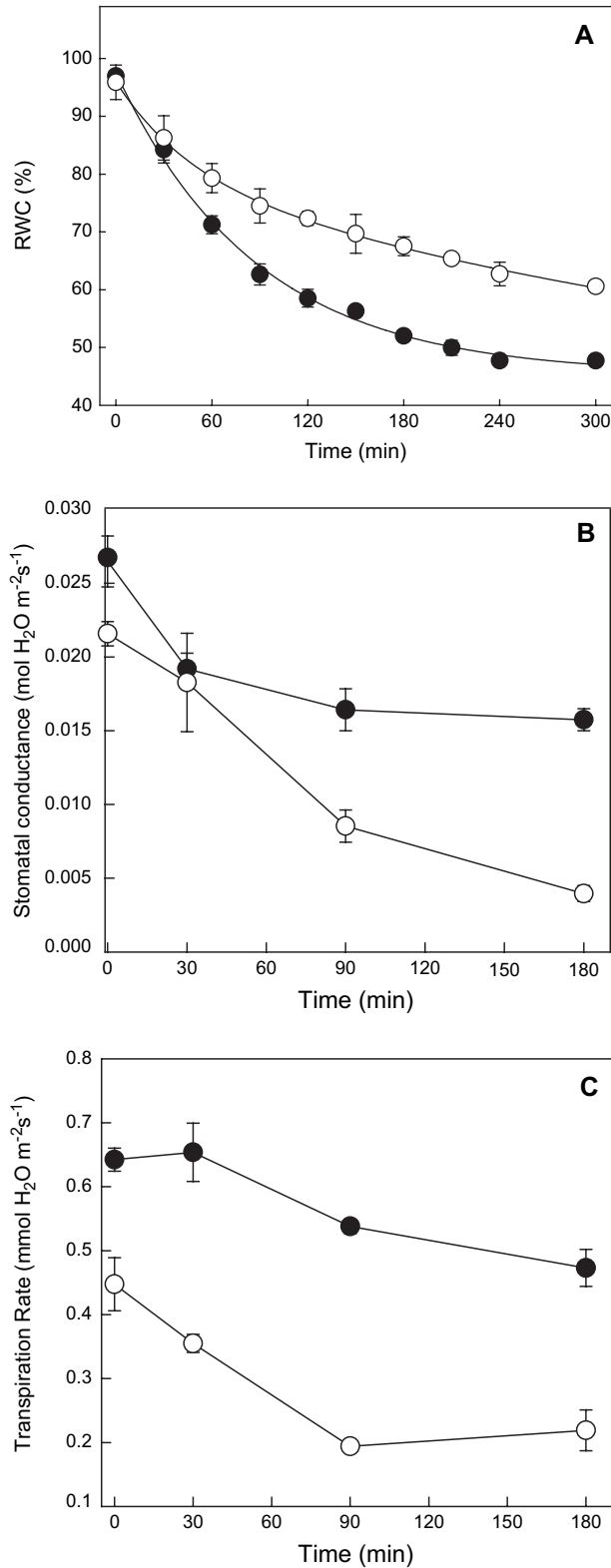
Abscisic acid is known to be a major factor controlling stomatal conductance. The response of stomata of Arroz and Orfeo to the application of ABA was therefore investigated. With well-watered plants, increasing concentrations of ABA caused a reduction in stomatal conductance in both Orfeo and Arroz (Fig. 8A). However, Orfeo was more responsive. Starting with a higher conductance,  $10 \mu\text{M}$  ABA induced a 4-fold decrease in conductance in Orfeo but only a 2-fold decrease in Arroz. Therefore, after this

application they had equal conductance. With  $100 \mu\text{M}$  ABA, the conductance of Orfeo was less than Arroz.

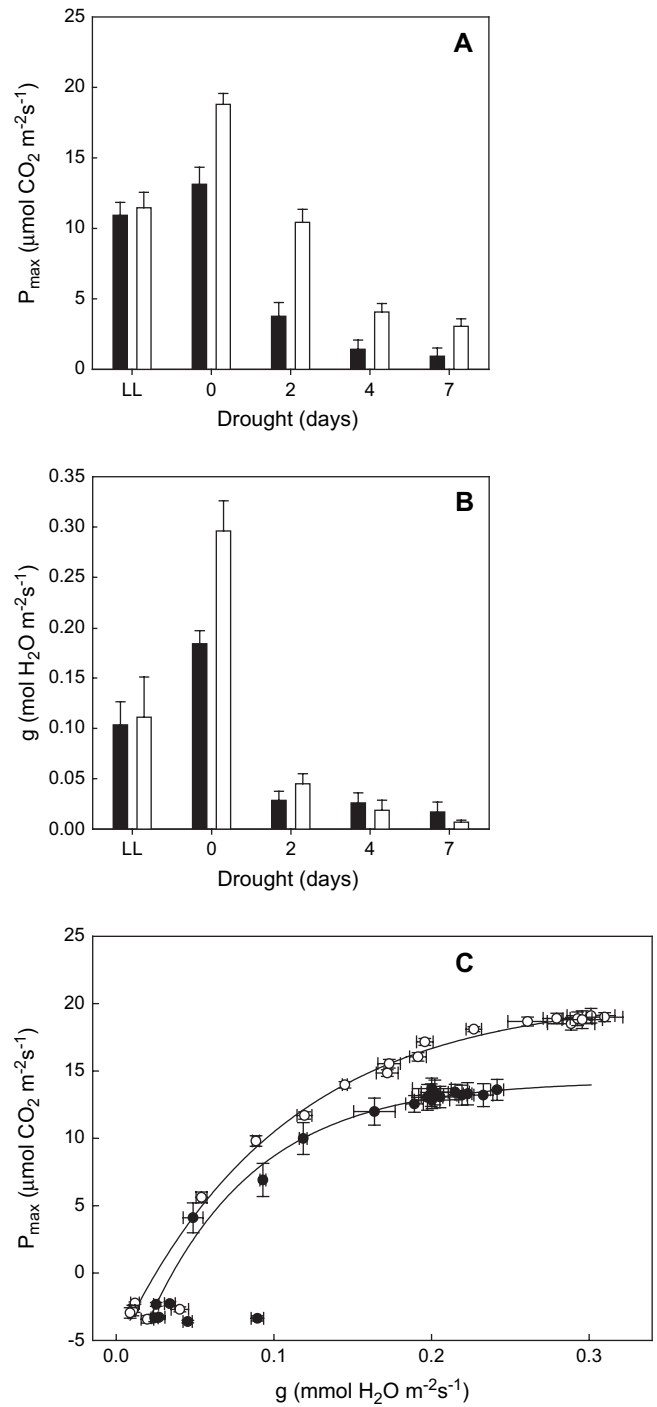
The synthesis of ABA was also investigated. Osmotic stress was used to induce ABA synthesis in leaf discs of both varieties. It was found that Arroz was less responsive than Orfeo. After 2 h and 4 h of incubation in mannitol, leaves of Arroz had only half the ABA content that was observed in Orfeo (Fig. 8B).

#### Leaf movement

Paraheliotropic leaf movement is a well-characterized response of bean leaves to drought (Pastenes *et al.*, 2005). Leaf movements in Arroz and Orfeo were therefore compared. Figure 9A shows the type of rotation of bean leaves observed when the plants were subjected to drought. Figure 9B shows the evolution of the movement of leaves after increasing periods of drought. Plants of Arroz proved



**Fig. 6.** The effect leaf removal and illumination at  $1000 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$  at  $35 \text{ }^\circ\text{C}$ . (A) Relative water content (*RWC*) of leaves; (B) stomatal conductance; and (C) transpiration rate. Arroz, closed symbols; Orfeo, open symbols. Time follows the removal of leaves from the plant. Results represent the means  $\pm$ SE for at least 12 leaves from six plants taken from at least two batches.



**Fig. 7.** Effect of drought treatment on photosynthetic capacity (A, *P*<sub>max</sub>) and stomatal conductance (B, *g*). Also shown in (A) is the *P*<sub>max</sub> of plants grown under low light ( $300 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$  at  $25 \text{ }^\circ\text{C}$ ). (C) The relationship between *P*<sub>max</sub> and *g*. Arroz, closed bars/symbols; Orfeo, open bars/symbols. Results represent the means  $\pm$ SE for at least 12 leaves from six plants taken from at least two batches.

to be more sensitive and started to move their leaves 24 h earlier than Orfeo. After 60 h of drought its leaves showed a rotation of  $60^\circ$  and 10 h later they reached a maximum of  $90^\circ$  of rotation with respect to their original position.



**Table 2.** Stomatal conductance characteristics of the bean varieties Arroz and Orfeo

Shown are data obtained for stomatal conductances during the experiments described in Fig. 7.  $g_{\max}$ , maximum steady-state stomatal conductance;  $g_{\min}$  dark, minimum steady-state stomatal conductance reached after 30 min in the dark;  $g_{\min}$  drought, minimum level of stomatal conductance in plants following 7 d of water deficit; Dynamic range, the range of stomatal conductances,  $R = g_{\max}/g_{\min}$  drought;  $t_{1/2}$  open and  $t_{\text{full}}$  open, times taken to reach 50% and 100%, respectively, of maximum stomatal conductance following illumination;  $t_{1/2}$  closed and  $t_{\text{full}}$  closed, the time taken to reach 50% and 100%, respectively, of minimum stomatal conductance following darkening.

Stomatal parameter	Arroz	Orfeo
$g_{\max}$ (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	0.220±0.007	0.322±0.006
$g_{\min}$ dark (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	0.025±0.005	0.0078±0.0016
$g_{\min}$ drought (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	0.016±0.001	0.005±0.002
Dynamic range (R)	13.51	64.50
$t_{1/2}$ open (min)	14.1	26.9
$t_{\text{full}}$ open (min)	25.7	46.3
$t_{1/2}$ closed (min)	9.3	5.3
$t_{\text{full}}$ closed (min)	16.9	9.3

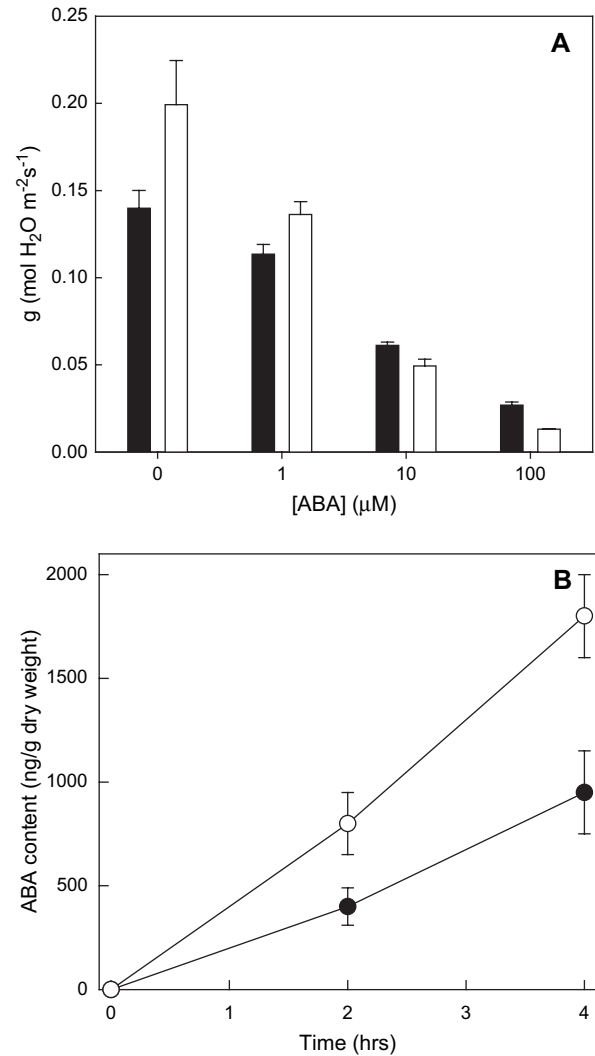
Leaf rotation in Orfeo only started after 48 h of drought and reached the maximum (90°) at 96 h, 24 h later than Arroz. The rate of leaf movement was approximately the same in both varieties, indicating no principal difference in the process itself.

#### Photoinhibition

The response of Arroz and Orfeo to photoinhibitory treatments was next investigated using plants grown in the growth room. The first mature leaves of well-watered plants, grown under HL conditions were illuminated with 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and the photochemical efficiency (measured as  $F_v/F_m$ ) was determined. Under this treatment Arroz showed an initially a fast decline (25%) in  $F_v/F_m$  after 30 min of illumination, followed by a further steady decrease to 50% after 180 min (Fig. 10A) After a similar illumination time, the photochemical efficiency of Orfeo was reduced by only 12% with respect to its original value. It is concluded that Arroz is more sensitive to photoinhibition than Orfeo.

In the next experiment, mature leaves were illuminated with the half of the intensity (1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) used in the previous experiment, but maintaining the plants for over a week under drought. Withholding of water initially induced a rapid decline of the photochemical efficiency of Arroz, which showed an almost 21% reduction in  $F_v/F_m$  after 2 d. In Orfeo for the same period this reduction was only 3% (Fig. 10B). After 1 week, the difference between the varieties was reduced, but Arroz still remained significantly more photoinhibited.

The higher sensitivity of Arroz to photoinhibition was corroborated in another experiment which examined the droughted detached leaves used previously (Fig. 6). After 3 h of drought, a strong decline in  $F_v/F_m$  was observed

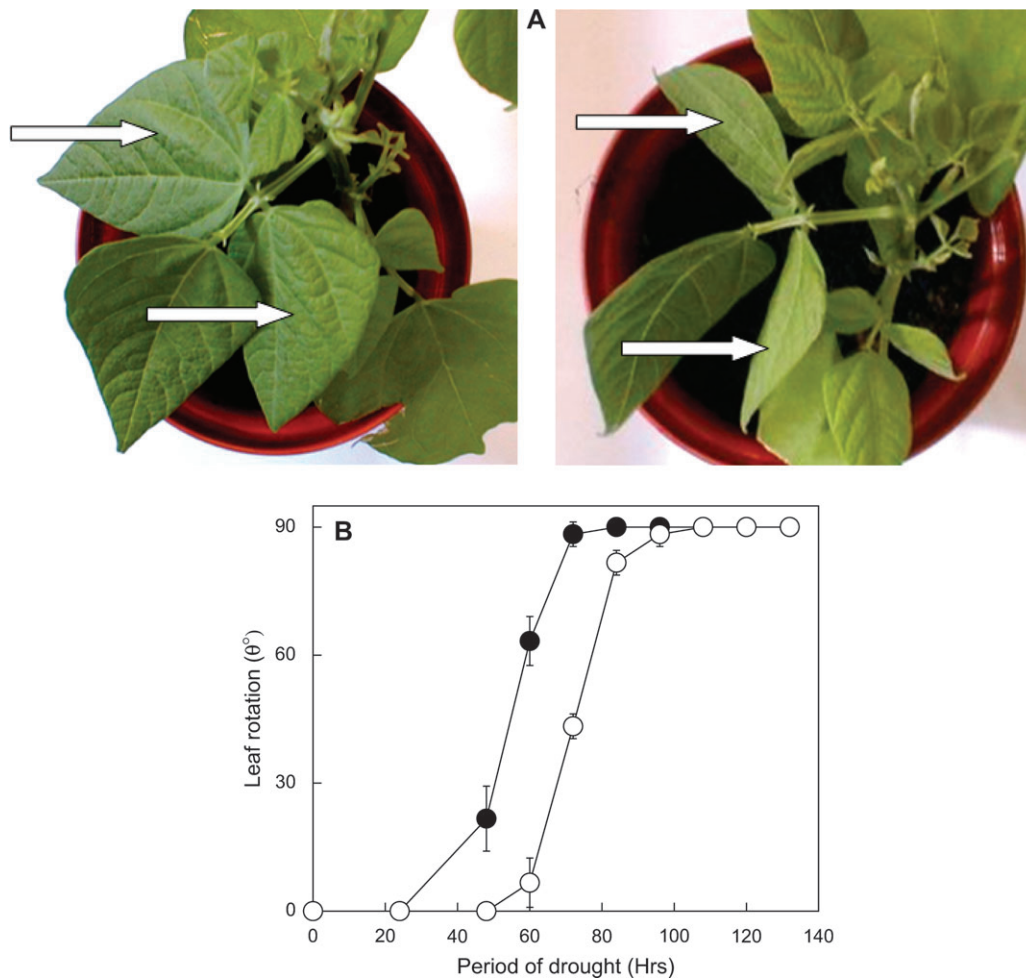


**Fig. 8.** (A) Effect of different concentrations of abscisic acid (ABA) on stomatal closure. Stomatal conductances of the middle trifoliolate leaves was recorded 2 h after ABA application to well-watered plants at 1000  $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$  at 35 °C. (B) Rate of ABA production from leaf discs incubated at 35 °C floated on mannitol solution with an osmotic potential of  $-1.6$  MPa. Arroz, closed bars/symbols; Orfeo, open bars/symbols.

in Arroz, but much less change was observed in Orfeo (Fig. 10C).

#### Anthocyanin and MDA levels

Accumulation of anthocyanin is commonly associated with exposure to abiotic stress. Figure 11A shows the levels of anthocyanin found in Arroz and Orfeo. Under low light conditions both varieties had very low levels of anthocyanin. When grown under high light conditions, there was little change in level in Arroz. However, in Orfeo, the anthocyanin increased by almost four times. Lipid peroxidation is an indicator of membrane damage by abiotic stress. Under low light, the MDA levels were low in both varieties, and almost undetectable in Orfeo (Fig. 11B). Growth under high light was associated with increases



**Fig. 9.** Leaf movement following increasing periods of drought. (A) Plants before (left) and after (right) drought-induced leaf movement. Leaf rotation was measured on flanking leaves (arrows) of the first mature trifoliate leaves. (B) Relationship between period of drought and leaf rotation angle ( $\theta$ ) in Arroz (closed symbols) and Orfeo (open symbols). Data are means  $\pm$ SE (n=18).

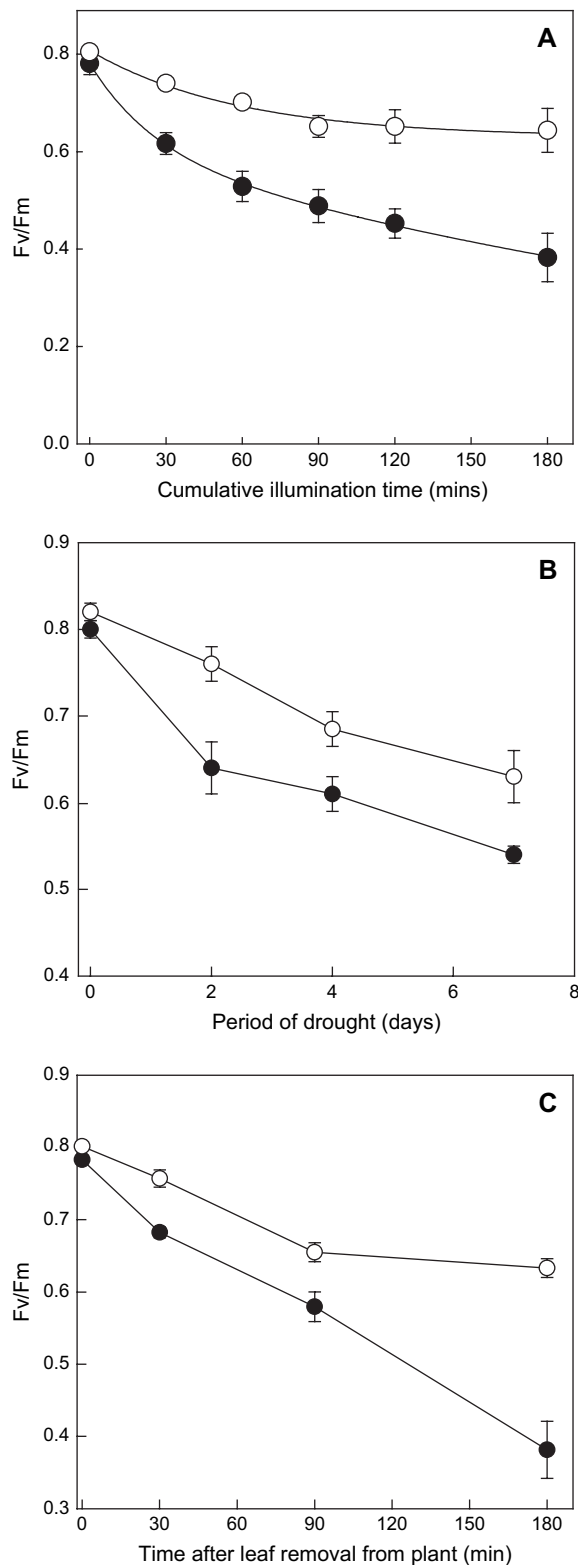
in MDA in both varieties. However, the level of lipid peroxidation was found to be higher in Arroz than in Orfeo.

## Discussion

In this work, two varieties of bean with very different yield stability have been studied. According to the method used to determine the yield stability, varieties with linear regression coefficients of the order of 1.0 have average stability over all environments. The variety Orfeo had a regression coefficient close to 0.9 which is not statistically different to 1.0, indicating that it has average yield stability. It produced very similar yields to the site average yields in all the conditions and can therefore be considered to have general adaptability. On the other hand, Arroz is typical of varieties which are sensitive to changes in the environment. Its yield stability is below the average with a regression coefficient of 1.84, significantly greater than 1.0. Under favourable conditions, Arroz was a high-yield variety and

can be described as specifically adapted to high-yield environments. However, small changes in the environment produced large changes in its yield. It produced much less grain in a low-yielding environment than the average, but as the environment improved, thus favouring higher yields, its yield increased.

Specific experiments using these two varieties revealed physiological characteristics that could explain their yield differences. Comparing well-watered and water-stressed conditions, Arroz showed much greater decreases in yield, mostly explained by pod and flower abscission. There was also a significant decrease in growth rate in Arroz under drought, whereas Orfeo was not much affected. Thus, whereas *RGR* was higher in Arroz under well-watered conditions, Orfeo grew faster when water was withdrawn. These differences in sensitivity to drought were, in turn, related to their responses to desiccation treatments, when Arroz lost water more quickly than Orfeo. Under these conditions, it was found that Arroz showed leaf movement much quicker than Orfeo. Since paraheliotropism is

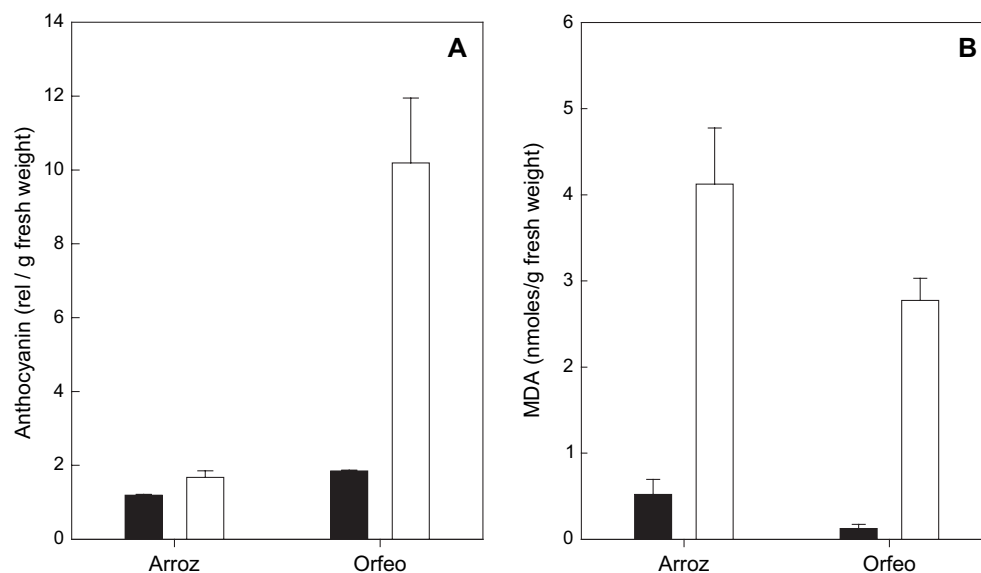


**Fig. 10.** Effect of photoinhibitory treatment of plants on the dark-adapted  $F_v/F_m$ . (A) Well-watered plants illuminated with high light ( $2000 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$ ); (B) droughted plants illuminated at  $1000 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$  as described in Fig. 7; (C) detached leaves illuminated at  $1000 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$  as in Fig. 6. Arroz, closed symbols; Orfeo, open symbols. Results represent the means  $\pm$  SE for at least 12 leaves from six plants taken from at least two batches.

well-documented as a response of leaves of bean and other species to drought (Pastenes *et al.*, 2005), this indicates an earlier onset of water stress in Arroz.

Differences in the rate of onset of water stress observed between plant varieties can be the result of differences in many mechanisms of adaptation located in different organs. For instance, differences in tolerance to drought stress between two eucalyptus varieties were recently largely attributed by Costa e Silva *et al.* (2004) to differences in the growth and the hydraulic characteristics of the root system. Thus similar differences in the root system could contribute to the observed differences between Arroz and Orfeo. However, it is clear that the higher stomatal conductance and transpiration rate observed in Arroz compared to Orfeo could give rise to the differences in susceptibility to drought. Water-use-efficiency, as defined by photosynthetic rate relative to the stomatal conductance was higher in Orfeo. In fact, under drought, there was a higher photosynthetic rate with a lower conductance in Orfeo compared to Arroz. Thus under stress, diffusional limitations (stomatal and mesophyll) to photosynthesis (Bota *et al.*, 2004) seem to be more important in Orfeo compared to Arroz, which, in turn, suggests that the higher water-use-efficiency observed in this variety, can be a consequence of a better functioning of its carboxylation machinery under those conditions (Parry *et al.*, 2002; Tezara *et al.*, 2002; Thimmaniak *et al.*, 2002). However, more detailed studies are necessary to confirm whether differences in carboxylation efficiency exist between these bean varieties. On the other hand, the contrasting dynamics of stomatal opening between Orfeo and Arroz were clearly consistent with their differing response to drought. Thus the faster and more complete stomatal closure showed by Orfeo could clearly be of significant advantage in coping with a sudden water shortage under field conditions. In part, this enhanced stomatal response may be explained by ABA, not only does Orfeo exhibit a higher rate of ABA synthesis upon exposure to osmotic stress but the sensitivity of the stomata to ABA appears greater.

The ability of Orfeo to retain water under drought appeared to result in less photoinhibition in this variety compared with Arroz. Furthermore, there appears to be an inherent difference between the susceptibility of these varieties to photoinhibition, even in well-watered conditions. Thus, the leaves of Orfeo seem generally to be more resistant to stress, not only exhibiting an increased ability to retain water but a higher photosynthetic capacity under high light conditions and an increased resistance to photoinhibition. This was confirmed by contrasting levels of anthocyanin accumulation, indicative of acclimation to stress, and MDA, indicative of membrane damage by stress, in the two varieties. These attributes are fully consistent with the high yield stability of Orfeo compared with Arroz. In the subsequent paper, further aspects of the adaptation of Orfeo to stress conditions are described.



**Fig. 11.** Leaf anthocyanin (A) and malondialdehyde (MDA) (B) content of two bean varieties (open bars). Also shown are data from low-light-grown plants (closed bars, 300  $\mu\text{mol m}^{-2}\text{s}^{-1}$  at 25 °C). Results represent the means  $\pm$ SE for at least 12 leaves from six plants taken from at least two batches.

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