Leaf movements and photoinhibition in relation to water stress in field-grown beans

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Abstract

Photoinhibition in plants depends on the extent of light energy being absorbed in excess of what can be used in photochemistry and is expected to increase as environmental constraints limit CO₂ assimilation. Water stress induces the closure of stomata, limiting carbon availability at the carboxylation sites in the chloroplasts and, therefore, resulting in an excessive excitation of the photosynthetic apparatus, particularly photosystem II (PSII). Mechanisms have evolved in plants in order to protect against photoinhibition, such as non-photochemical energy dissipation, chlorophyll concentration changes, chloroplast movements, increases in the capacity for scavenging the active oxygen species, and leaf movement or paraheliotropism, avoiding direct exposure to sun. In beans (*Phaseolus vulgaris* L.), paraheliotropism seems to be an important feature of the plant to avoid photoinhibition. The extent of the leaf movement is increased as the water potential drops, reducing light interception and maintaining a high proportion of open PSII reaction centres. Photoinhibition in water-stressed beans, measured as the capacity to recover \( F_v/F_m \), is not higher than in well-watered plants and leaf temperature is maintained below the ambient, despite the closure of stomata. Bean leaves restrained from moving, increase leaf temperature and reduce \( q_P \), the content of D1 protein and the capacity to recover \( F_v/F_m \) after dark adaptation, the extent of such changes being higher in water-stressed plants. Data are presented suggesting that even though protective under water stress, paraheliotropism, by reducing light interception, affects the capacity to maintain high CO₂ assimilation rates throughout the day in well-watered plants.

Key words: Beans, drought, paraheliotropism, photoinhibition, photosynthesis.

Introduction

Photoinhibition, resulting from down-regulation or damage, depends mainly on the proportion of light energy being absorbed in excess of the capacity of the photosynthetic organisms to use that energy in photochemistry. Therefore, not only high intensity light *per se*, but also any environmental constraint directly or indirectly limiting the capacity for CO₂ assimilation is likely to induce photoinhibition. One such constraint is water stress. It is commonly observed that water stress in the field is accompanied by high irradiance and high temperatures, exacerbating the water vapour deficit between air and leaf, leading to stomatal closure. Water stress limits photosynthesis by restricting CO₂ diffusion from air into carboxylation sites and through a more subtle effect on carbon metabolism (for a review see Lawlor and Cornic, 2002; Chaves et al., 2003). Under mild water stress, stomatal conductance is reduced by dehydration of guard cells and/or by chemical signals from the roots. Leaf mesophyll conductance of CO₂ has also been suggested to be of importance as water stress increases (Flexas and Medrano, 2002), interpreted as a limitation in the transfer of CO₂ from the intercellular air spaces to the chloroplast stroma in the chloroplasts (Bongi and Loreto, 1989). As water deficit increases, a biochemical limitation of the photosynthetic process is observed, in which the potential rate for CO₂ assimilation is not reached, despite CO₂ saturation (Lawlor and Cornic, 2002). Eventually, photosynthesis becomes limited from irreversible damage to the photosystems (Havaux et al., 1986). Such damage, particularly to PSII, is likely to result, not as a direct water-stress effect, but due to light-induced oxidative stress...
(Navari-Izzo and Rascio, 1999). In fact, absorbed energy, unable to be used in carbon reduction, could lead, on one hand, to the formation of singlet excited oxygen from energy transfer at the level of PSII complexes and also to superoxide at the electron acceptor side of PSI, both known to induce oxidative stress and damage.

Photosynthetic organisms, however, have evolved mechanisms to protect against photodamage. They consist of decreasing the excitation pressure on PSII reaction centres, repairing damaged complexes, and transforming or eliminating the toxic species produced by excess light. One of the most studied forms of protection is the non-photochemical energy dissipation resulting in a lower quantum yield of photosynthesis, similar to photoinduced damage, except that no maximal photosynthetic rate reduction is observed (Osmond, 1994; Demmig-Adams and Adams, 1996; Horton et al., 1996). Other mechanisms for protection consist of chlorophyll concentration changes in order to reduce the extent of the absorbed light (Giardi et al., 1996; Murchie and Horton, 1997); chloroplast movements, reducing the organelle and photosynthetic complexes exposure to light (Haupt, 1990); increases in the capacity for scavenging the active oxygen species (Bowler et al., 1992; Foyer et al., 1994); and leaf movement or paraheliotropism (light-avoiding), avoiding direct exposure to sun, thereby avoiding light and heat (Ludlow and Björkman, 1984).

Paraheliotropism is typical of leguminous species such as beans and has been described as a stress avoidance mechanism (Reed, 1987). Leaves move by means of turgor pressure changes at the pulvinus at the base of each lamina. In beans, the major driving stimulus for leaf movement is light, but it is also modulated by leaf water status and temperature. Apparently, light and heat driving paraheliotropism results from the environmental factors acting not only on the leaf blade but also on the pulvinus (Fu and Ehleringer, 1989; Donahue and Berg, 1990; Bielenberg et al., 2003). Light avoidance in plants capable of leaf movement, by means of reducing not just absorbed light but also heat and transpirational water loss, confers to the plant the capacity to protect against photoindihbition (Gamon and Pearcy, 1989). Even though the phenomenon has been mainly associated with water-stressed plants, it also occurs in well-watered plants but to a lesser extent, depending on the variety. The aim of the present study was to assess the importance of paraheliotropism in field-grown beans under water stress by means of restraining the leaf movement. Beans are a sensitive species to many environmental constraints, particularly water supply (Johansen et al., 1992; Slinkard et al., 1992; Monti et al., 1992), and as much as 60% of the crop is produced under conditions of significant drought stress in the developing world (Graham and Ranalli, 1997). Evidence is presented supporting the major protective role of paraheliotropism in beans under water stress, but suggesting that its occurrence in well-watered plants compromises the capacity for CO₂ assimilation.

**Materials and methods**

The experiments were conducted in 2000–2001 at the Agronomic Research and Experimental Station (Antunapo) at the Faculty of Agronomic Studies of the University of Chile in Santiago, central Chile. The experimental design was a randomized complete block with four replications with two plots per block. Each plot in a block corresponded to a water regime: well-watered and water-stressed, and was 10 × 6 m, with 10 rows, 0.6 m apart. Seeds were sown on October 2000. The two water regimes were set according to the water retention curve of the soil with a field capacity (FC) of 29% w/v, permanent wilting point of 11% w/v and available water capacity of 18% w/v. Well-watered plots were irrigated maintaining a 27% w/v close to FC. Water-stressed plots were sufficiently watered until plants were on their third trifoliate leaf, and then 50% of the available water capacity (20% w/v water in soil) was maintained until harvest. Irrigation was made by controlled drip by means of plastic tubes (NAAN, Israel) at 4.7 l m⁻¹ h⁻¹. Measurements were carried out 60 d after sowing. On each plot, at least 15 randomized plants were chosen for measurements on restrained leaves. The fourth trifoliate was restrained to horizontal position by means of sandwiching them between nylon strips in a metal frame. All measurements were carried out on the same day, after 48 h of leaves being restrained. All measurements consisted of at least four replicates.

**Chlorophyll fluorescence**

Photochemical (qP) and non-photochemical quenching (qN) of chlorophyll fluorescence were determined during the day, according to van Kooten and Snel (1990). Leaves were dark-adapted using leaf clips during the afternoon before measurements were taken. Early in the morning, in the dark-adapted leaves, the minimal (Fₘ) and maximal (Fₘ*), emitted fluorescence were determined with a modulated fluorimeter (Hansatech PEA, UK), and the leaf clips immediately removed after making its position in order to measure Fₘ, Fₘ*, and F₉ in exactly the same spot. Measurements were done with care so as not to disturb the leaf position and light exposure on the leaf. After Fₘ*, F₉ was recorded by means of a 3500 μmol photons m⁻² s⁻¹ PAR light flash and the leaf was darkened with a black cloth for 30 s after measuring Fₙ using far red light. All measurements were done with the same hardware configuration. Recovery of the maximum quantum yield, measured as F₉/Fₘ, was determined by means of a non-modulated fluorimeter (Hansatech PEA, UK), dark-adapted leaves for 30 min using leaf clips, on the same leaves during the day. Different leaves were used for the fluorescence quenching parameters and F₉/Fₘ.

**CO₂ assimilation**

CO₂ assimilation rates and stomatal conductance were determined using an IRGA. (ADC-pro, UK). Measurements were done on the same, previously labelled, leaves throughout the day, and maintaining the leaf angle. For the measurements, air was taken from 3.5 m above-ground. Measurements were recorded after an equilibration time of 60–90 s, as soon as steady-state assimilation was reached on every measurement.

**Leaf water potential**

Total water potential was assessed using a portable Scholander type pressure chamber (Scholander et al., 1965). Three leaves from different plants were measured per subplot, three times during the day: 08.30 h, 14.00 h, and 17.30 h. The leaf lamina was enclosed inside the chamber and the pressure (using a compressed nitrogen cylinder) was increased until free sap was visible at the petiole outside the chamber.

**Leaf temperature and incident light**

Leaf temperature was measured with an infrared thermometer (Extech, USA) on the same leaves used for determinations with the
modulated fluorimeter. Incident light was assessed using a PAR meter (LiCor, Li-182, Lincoln, USA) positioning the sensor above the leaf imitating the leaf angle.

**Leaf movements**

In order to assess the movement of leaves during the day, a visual scoring system was used, from 1 to 5, in which 1 represented leaves in a completely vertical position and 5, a completely horizontal position. Results correspond to observations in four random squares, 0.5 m² in every plot.

**Air humidity, temperature and light intensity**

Ambient PAR light was measured using a PAR light meter (LiCor, Li-182, Lincoln, USA) positioning the sensor perpendicular to the sunlight and parallel to the ground. Air temperature and relative humidity were obtained from a hygrothermometer (Extech 445900, USA), measured 1 m above the ground.

**Measurement of D1 protein content**

Leaf discs 28 mm diameter were collected from the field at 13.00 h and immediately frozen in liquid nitrogen, and kept at −80 °C until processed. D1 protein content was assessed in isolated thylakoids from the leaf discs. Leaf discs were homogenized at 0 °C in a grinding medium containing 50 mM HEPES-NaOH buffer, pH 7.5, 0.4 M sucrose, 15 mM NaCl, 5 mM MgCl₂, 2 mM Na₂EDTA, 0.1% (w/v) BSA, and 5 mM sodium ascorbate and protease inhibitors. The homogenized discs were centrifuged at 7000 g for 15 min at 4 °C. The pellet was resuspended in 0.3 ml buffer containing 50 mM HEPES-NaOH pH 7.5, 0.4 M sucrose, 15 mM NaCl, and 5 mM MgCl₂. The suspension was centrifuged at 350 g for 5 min at 4 °C. Supernatant was collected and centrifuged at 7000 g for 15 min. The thylakoids contained in the pellet were resuspended in 75 μl of the preparation buffer. D1 separation was performed by electrophoresis as in Laemmli (1970). D1 protein was assessed by western blot analysis. A specific polyclonal antibody raised against *Synechocystis* 6803, kindly supplied by Roberto Barbato was used. Gels were stained with Coomassie Brilliant Blue R-250, dried, and scanned on a scanner (ColorPage-Vivid 4 v1.0, Genius), and the images were analysed using the software package Kodak Digital Science 1D Image Analysis with the profile 1 gel densitometer application.

**Results**

As typically observed in the summer time in Mediterranean climates, the growing season was characterized by high light intensities and temperature. Already at 08.30 h in the morning, photosynthetic active radiation (PAR) perpendicular to the sun reaches 1400 μmol photons m⁻² s⁻¹ and 800 μmol photons m⁻² s⁻¹ measured horizontally, both nearly reaching 2000 μmol photons m⁻² s⁻¹ at midday (Fig. 1A). Air temperature increased from 22 °C early in the morning up to 32 °C at midday, slowly decreasing in the afternoon (Fig. 1B). As expected, relative humidity drops throughout the day as the air temperature increases (Fig. 1C). Watered soil resulted in a slightly higher relative humidity at the plant height, i.e. 40 cm at the time of the measurements, compared with the plots where water stress was imposed. Clearly, water shortages in the soil potentially affect the plant canopy by making the surrounding air more favourable for transpirational water losses. When measured 1 m

Fig. 1. Daily measurements of (A) PPFD horizontal to the ground and perpendicular to the sun; (B) temperature, and (C) relative humidity measured 1 m above ground and at the plant height (0.4 m above ground) in well-watered and water-stressed plots.
above ground, no differences in relative humidity were observed between plots.

As for the water status, stressed plants had a lower total water potential early in the morning, matching the value of watered plants at midday, and strongly decreasing in the afternoon. Watered plants, by contrast, were able to increase the leaf water potential during the afternoon to values close to the value observed early in the morning (Fig. 2) revealing a better water status.

Paraheliotropic movement of leaves was observed in the field-grown beans, therefore, avoiding the incident light. Both watered and stressed plants move their leaves from a horizontal position in the morning to a vertical angle during midday, slowly reversing again in the afternoon to the horizontal (Fig. 3). Leaves from water-stressed plants, however, resulted in a significantly more perpendicular position throughout the day compared with the control, except at midday. Incident PAR light varied according to the extent of leaf movement. As observed in Fig. 4A, leaves restrained from moving received from 1000 μmol photons m⁻² s⁻¹ early in the morning up to nearly 2000 μmol photons m⁻² s⁻¹ at midday, and decreasing again during the afternoon. Leaves from watered plants avoided direct sunlight during the day, intercepting a narrow range of light intensity from 1000 μmol photons m⁻² s⁻¹ in the early morning and late afternoon, to 1300 μmol photons m⁻² s⁻¹ in the mid-afternoon. Leaves from water-stressed plants, on the other hand, further escaped from direct light with a minimum light interception of 500 μmol photons m⁻² s⁻¹ at 15:30 h, the time of the day with the highest light intensity and temperature and lowest relative humidity (Fig. 1). As expected, leaf temperature was determined by the combination of water status of plants and the leaf angle (Fig. 4B). The highest leaf temperatures were reached by leaves from water-stressed plants, reaching in the restrained leaves values of 30 °C, and 25.5 °C on average, in the leaves free to move, both from 10:30 h to the afternoon. Leaves from watered plants, restrained from moving, reached 24 °C on average, a value higher than that observed for well-watered and free leaves, the latter being nearly constant throughout the day (Fig. 4B). Irrespective of the water status of the plant, leaves restrained from moving had a significant lower temperature than the free leaves early in the morning. This might be explained by the fact that beans move their leaves, very early in the morning, actually tracking the sun (data not shown) instead of escaping as in the rest of the day, therefore warming up faster than leaves forced to be horizontal.

The $F_v/F_m$ recovery after 30 min dark adaptation reached the lowest values at midday, particularly in leaves restrained from moving. This parameter which indicates the extent of photoinhibition, irrespective of down-regulation or damage, resulted in an even lower value in leaves from water-stressed plants compared with those from well-watered plants at mid-morning (Fig. 5), i.e. when temperature, light, and relative humidity were still favourable for gas exchange (Fig. 1). From midday to afternoon, no significant differences in $F_v/F_m$ were observed between restrained well-watered and water-stressed leaves, both having lower values than leaves free to move. During the

![Fig. 2. Total leaf water potential in well-watered and water-stressed plants during the day. Different letters indicates differences between water regime at same time of day at $P < 0.05$.](image1)

![Fig. 3. Leaf angle observed during the day in watered and stressed plants, according to a visual scoring system (see Materials and methods). Different letters indicates differences between water regime at same time of day at $P < 0.05$.](image2)
mid-afternoon, free leaves from water-stressed plants reached significantly higher $F_v/F_m$ values compared with well-watered plants (Fig. 5). As expected, the lowest $qP$ values were observed in leaves restrained from moving, particularly from midday throughout the rest of the day (Fig. 6A). The minimum value of 0.45, occurred at 15.30 h in restrained leaves from water-stressed plants followed by a 0.58 value in restrained well-watered leaves. Concurrent with the capacity to recover $F_v/F_m$ shown in Fig. 5, the highest $qP$ average value was observed in stressed, free leaves (Fig. 6A). On the other hand, $qN$ resulted in the highest values on the restrained stressed leaves as expected from the $qP$ data, particularly during the afternoon (Fig. 6B) and no significant differences were observed between free leaves from water-stressed and well-watered plants throughout the day. The latter two treatments decreased the values for $qN$ from mid-morning to the afternoon, suggesting a lesser need for non-radiative energy dissipation. The lowest average $qN$ value from mid-morning throughout the afternoon was observed in the restrained well-watered leaves (Fig. 6B).

The D1 protein, has long been recognized as the primary site of damage at the PSII reaction centres as a result of excess absorbed energy and the content of the intact polypeptide in leaf tissues is an indication of the relative activities of synthesis and damage (Andersson and Barber, 1996). As shown in Fig. 7, a strong effect of drought on the photosynthetic apparatus was observed in free leaves, in which the water-stressed plants reached a concentration of D1, 30% lower than in well-watered plants (Fig. 7). Restraining the leaves, on the other hand, resulted in a deleterious effect, with reductions in the D1 protein concentration in leaves from well-watered plants only, with no significant differences between free and restrained leaves from water-stressed plants.

Measurements of gas exchange confirms the expected effect of drought on CO$_2$ assimilation (Fig. 8A). Leaves from water-stressed plants reached the highest assimilation rates early in the morning, with only slightly lower values compared with well-watered plants at 08.30 h, but strongly decreasing to values below 5 µmol CO$_2$ m$^{-2}$ s$^{-1}$ in the afternoon (Fig. 8A), closely matching the stomatal conductance changes throughout the day (Fig. 8B). Leaves from well-watered plants maintained high assimilation rates...
during the day compared with stressed leaves (Fig. 8A). In the case of well-watered free leaves, assimilation slightly decreased from mid-morning to 15:30 h, with a small increase, on average, in the late afternoon. Restrained leaves from well-watered plants, on the other hand, clearly increased assimilation rates from early in the morning to midday, decreasing again throughout the afternoon, and being significantly similar to restrained leaves only in the extreme h of the day (Fig. 8A). The highest assimilation rate occurred in the restrained leaves from well-watered plants despite its lower stomatal conductance value compared with the free leaves (Fig. 8B).

**Discussion**

Beans are mainly produced in Mediterranean climates characterized by high intensity PAR light, high temperatures, and decreasing relative humidity in air throughout the day, similar to the conditions in the present study (Fig. 1). Such conditions are known to affect plant growth and yield because of the large water vapour concentration gradient between leaf and air, leading to reduced stomatal conductance and limiting photosynthesis by the reduced CO₂ concentration at the carboxylation sites. If water stress, a common feature of many bean production areas, is imposed under such environmental conditions, assimilation is further reduced leading to an overreduction of the PSII reaction centres and the formation of reactive oxygen species (Navari-Izzo and Rascio, 1999), harmful to the photosynthetic apparatus (Kaiser, 1979; Knox and Dodge, 1985; Asada, 1994).

As in previous studies (Pastenes et al., 2004), the water stress imposed on plants did not result in direct-light-induced deleterious effects on photosynthesis in free leaves compared with well-watered plants. Clearly, the capacity of bean plants to avoid light (Fig. 4A) and heat (Fig. 4B) acts as a protective mechanism, particularly for water-stressed plants as suggested from the great capacity for \(F_\text{v}/F_\text{m}\) recovery (Fig. 5) and high \(qP\) values (Fig. 6A). Paraheliotropism is induced by the incident light intensity, but is also modulated by the leaf water status and temperature (Donahue, 1990; Assmann, 1993; Yu and Berg, 1994). Consequently, water-stressed plants moved their leaves up
water-stressed plants, the temperature had already reached 30 °C at 10.30 h in the morning, much higher than the 25 °C air temperature at that time (Fig. 4B). In areas where air temperatures reach higher levels, drought becomes even more limiting to photosynthesis by increasing leaf temperature. Photosynthesis is the most susceptible process to heat in plants, PSII being the most sensitive component of the photosynthetic apparatus (Mamedov et al., 1993), particularly the oxygen-evolving machinery of PSII (Tanaka et al., 2000). Non-reversible damage to PSII occurs at temperatures higher than 40 °C (Pastenes and Horton, 1999), although temperatures close to the observed in restrained leaves from water-stressed plants have been reported to affect the carboxylation reaction in the Calvin cycle due to limitations in RubP regeneration and Rubisco activity (Crafts-Brandner et al., 2002; Wise et al., 2004).

The $F_v/F_m$ recovery after 30 min dark-adaptation has been widely used as an indication of photoinhibition, irrespective of down-regulation or damage, and by mid-morning restrained water-stressed leaves had already reached a significantly lower value compared with any other treatment (Fig. 5). It is known that reductions in $F_v/F_m$ recovery results from non-radiative dissipation of the absorbed energy by PSII (Horton and Ruban, 1994) and to direct damage to PSII (Aro et al., 1993; Andersson and Barber, 1996). The fact that the $F_v/F_m$ in the restrained leaves is similar to that of free leaves early in the morning, irrespective of water regime, suggests that down-regulation rather than damage is occurring. As for the leaves free to move, and contrary to what is expected in drought conditions (Souza et al., 2004), a significantly higher $F_v/F_m$ value was observed in water-stressed plants at 15.30 h (Fig. 5). Such higher capacity for $F_v/F_m$ recovery is clearly an indication of no overreduction of the photosynthetic electron system in free leaves from water-stressed plants, further demonstrated by the extremely high $q_P$ value during the afternoon (Fig. 6A). Such $q_P$ values results purely from the low incident light on the free leaves, rather than an increased activity for $q_N$ which, in the case of the stressed free leaves, is similar to that from free and restrained leaves from well-watered plants (Fig. 6B), both having a significant lower $q_P$ compared to the former. By contrast, restrained leaves from water-stressed plants reached the lowest $q_P$ values during the afternoon, close to 0.45, with concomitant high $q_N$. Even though no indication of damage can be inferred from the data from restrained-stressed leaves, it has been suggested that $q_P$ values close to 0.5 would be a threshold for long-term photoinhibition in many species and growth conditions (Oquist et al., 1992). Therefore, longer periods of restraining time for drought leaves could result in damage. In fact, the average D1 protein content in leaves from water-stressed plants, although not significant, is lower in restrained compared with free leaves (Fig. 7). Concentration of D1 results from simultaneous synthesis of the protein and degradation activity (Tyystajarvi and Aro, 1995).
1996) and in the case of well-watered plants a strong reduction of the protein is observed after restraining the leaves. The protein concentration in free stressed compared with free watered leaves is also strongly reduced but, contrary to leaf restraint, it results from a long-term effect induced by water stress in the field. The decreased D1 concentration in the stressed free leaves was paralleled by nearly 20% lower chlorophyll concentrations compared with well-watered free leaves (data not shown).

The protective effect of paraheliotropism in stressed leaves by maintaining lower temperatures, higher values for \( qP \), and a much higher capacity for \( F_{v}/F_{m} \) recovery in free compared with restrained leaves, did not result in a better performance on CO\(_2\) assimilation rates. Stomatatal conductance is highly responsive to water stress, thereby restricting CO\(_2\) entrance into the leaf (Cornic, 1994) and in this study, water-stressed plants were only capable of assimilation early in the morning under moderate PAR light and water-stressed plants were only capable of assimilation early in the morning under moderate PAR light and temperature as well as high relative humidity conditions (Fig. 8). A strikingly different situation occurred in well-watered plants. Even though the restrained leaves had increased leaf temperature, lowered \( qP \) values significantly reduced the capacity to recover \( F_{v}/F_{m} \) as well as consistently reduced the D1 protein concentration (higher degradation), CO\(_2\) assimilation was much higher compared with free leaves despite having a significantly lower stomatal conductance throughout the day (Fig. 8A, B). Apparently, bean leaves from well-watered plants are unable to express their potential capacity for CO\(_2\) assimilation as a result of light avoidance, even though for water-stressed plants paraheliotropism plays a central role in photoprotection.

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