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BRIEF COMMUNICATION

CO_2 assimilation and water relations of almond tree (*Prunus amygdalus* Batsch) cultivars grown under field conditions

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Abstract

Gas exchanges and leaf water potential (ψ_w) of six-years-old trees of fourteen *Prunus amygdalus* cultivars, grafted on GF-677, were studied in May, when fruits were in active growing period, and in October, after harvesting. The trees were grown in the field under rain fed conditions. Predawn ψ_w showed lower water availability in October compared with May. The lowest ψ_w values at midday in May increased gradually afterwards, while in October they decreased progressively until night, suggesting a higher difficulty to compensate the water lost by transpiration. However, relative water content (RWC) measured in the morning was similar in both periods, most likely due to some rainfall that occurred in September and first days of October that could be enough to re-hydrate canopy without significantly increasing soil water availability. The highest net photosynthetic rate (P_N) was found in both periods early in the morning (08:00-11:00). Reductions in P_N from May to October occurred in most cultivars except in José Dias and Ferrastar. In all cultivars a decrease in stomatal conductance (g_s) was observed. Photosynthetic capacity (P_{max}) did not significantly change from spring to autumn in nine cultivars, revealing a high resistance of photosynthetic machinery of this species to environmental stresses, namely high temperature and drought. Osmotic adjustment was observed in some cultivars, which showed reductions of *ca.* 23 % (Duro d' Estrada, José Dias) and 15 % (Tuono) in leaf osmotic potential (ψ_π). Such decreases were accompanied by soluble sugars accumulation. The Portuguese cultivar José Dias had a higher photosynthetic performance than the remaining genotypes.

Additional key words: chlorophyll fluorescence induction; drought; leaf water potential; Mediterranean climate; osmotic adjustment; proline; soluble sugars; water relations.

Almond tree (*Prunus* a*mygdalus* Batsch) is well adapted to Mediterranean conditions. In Portugal most of yield is produced by rain-fed traditional almond orchards in the Northeast (Trás-os-Montes) and South (Algarve). The production of *ca.* 30 000 ton/year is assured by several genotypes which grow side by side. Although growing together and requiring 6 to 8 months from full bloom to nut maturation, and surviving in shallow soils and arid conditions, they are different in productivity. Such differences may be associated with changes in physiological mechanisms and biochemical characteristics related with gas exchanges and water relations (Roberts *et al.* 1979), and their study may be useful for breeding purposes. In Mediterranean climates plants are exposed to water stress during summer and usually withstand very low water potentials to maintain leaf turgor (Lambers *et al.* 1998). Accumulation of compatible solutes is a common metabolic response of higher plants to several stresses, namely water deficit (Morgan 1984). Among these solutes, soluble sugars (Leopold 1990) and proline (Samaras *et al.* 1995) strongly contribute to osmotic adjustment, which is an important mechanism for drought avoidance and

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Abbreviations: Chl = chlorophyll; F_v/F_m = photochemical efficiency of photosystem 2 under dark conditions; g_s = stomatal conductance; P_{max} = photosynthetic capacity; P_N = net photosynthetic rate; RWC = relative water content; ψ_w = leaf water potential; ψ_π = leaf osmotic potential.
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membrane protection against functional or structural damages caused by stress (Blum 1992).

In previous papers we studied diurnal and seasonal gas exchanges of young plants of foreign almond tree cultivars grown in the field (Matos *et al.* 1997, 1998). In the present work we compared foreign and local cultivars as regards several physiological and biochemical indicators that may predict which genotypes can best endure periods of little or no rain, and still be productive.

Six-years-old almond trees (*Prunus amygdalus* Batsch) grafted on GF-677 were grown in an orchard in a schistic soil at Quinta do Valongo/DRATM, Mirandela, Portugal (250 m altitude, 41°31'N latitude and 7°12'W longitude). Fourteen cultivars were studied, from which five were Portuguese (José Dias, Duro d'Estrada, Duro Italiano, Casanova, and Verdeal) and the remaining were foreign (Moncaio, Tuono, Guara, Ferraduel, Ferrastar, Ferragnés, Marcona, Non Pareil, and Garrigues). Irrigation was applied once at the beginning of summer and the field was fertilized according to soil analysis. Trees were then left under rain-fed conditions.

Diurnally, net photosynthetic rate (P_N), stomatal conductance (g_s), and leaf water potential (ψ_w) were measured in May and October, periods of high physiological activity (fruit filling) and post harvest, respectively. P_N and g_s were measured using a portable CO_2/H_2O gas exchange system *LI-6200* (*LI-COR*, Lincoln, USA) on individual attached sun exposed and fully expanded leaves from the middle of the canopy, in a well stirred cuvette (1 000 cm^3) to minimize boundary layer resistance. Diurnal courses of photosynthetically active radiation, air temperature, and relative humidity were followed. Photosynthetic capacity (P_{max}) was determined using a leaf disc oxygen electrode *LD2/2* (*Hansatech*, Kings Lynn, U.K.) under saturating irradiance (*ca.* 1 500 µmol m^{-2} s^{-1}) and CO_2 (*ca.* 7 %, obtained through a carbonate/bicarbonate buffer), to completely overcome the stomatal resistance, according to preliminary experiments. Photochemical efficiency of photosystem 2 (F_v/F_m) was measured using a portable *Plant Stress Meter Mark II* system (*BioMonitor Sci. AB*, Sweden), before predawn ψ_w determinations, and in the morning.

Three mature leaves per tree were harvested in the morning for determination of water relations and chemical analyses. Diurnal changes in ψ_w with a pressure chamber on three detached leaves per tree were determined according to Scholander *et al.* (1965) from predawn to sunset, always before the gas exchange determinations on adjacent leaves. Relative water content (RWC) was calculated according to Čatský (1960) from samples of 10 leaf discs of 0.5 cm^2, as RWC = (FM – DM/TM – DM)×100, where FM is fresh mass, TM is the turgid mass after overnight re-hydration of the discs in a humid chamber at room temperature, and DM is the dry mass after drying at 80 °C for 24 h. The discs for osmotic potential and chemical analyses were cut from the centre of the leaves and frozen in liquid nitrogen. Leaf osmotic potential (ψ_π) of cell sap was measured using a thermocouple psychrometer (*Wescor HR-33T-R* dew point micro voltmeter and *C-52* sample chamber, *Wescor*, Logan, Utah, USA). Psychrometers were calibrated with sodium chloride solutions on filter paper lining the bottom of the leaf chamber. Three foliar discs (0.5 cm^2 each), were homogenised in Eppendorf tubes to extract cell sap and immediately placed in the chambers. After 20 min for sample equilibration and cooling, readings were made. The discs for chemical analyses were cut from the centre of the leaves and frozen in liquid nitrogen. Proline and soluble sugars were extracted from 3 leaf discs of 0.5 cm^2 and quantified spectrophotometrically according to Bates (1973) and Ashwell (1957), respectively. Chlorophylls and carotenoids were quantified according to Lichtenthaler (1987).

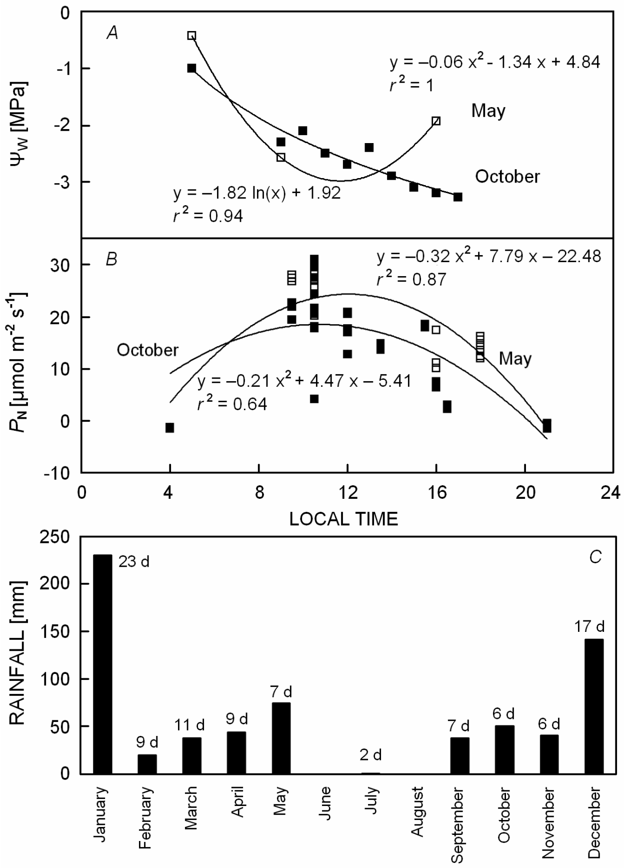

Fig. 1. Changes in mean daily courses of (*A*) leaf water potential (ψ_w; *top*) and (*B*) net photosynthetic rate (P_N; *bottom*) measured in May (□) and October (■) in fourteen *Prunus amygdalus* cultivars, grown in the field under rain-fed conditions. (*C*) Rainfall distribution for the year of 1996 in Mirandela (Trás-os-Montes, Portugal). Total number of days in which precipitation occurred are indicated per month.

Results were statistically analyzed by the *Statistix* program version *7*, 1998. For mean comparison, an LSD test was applied, considering a 95 % confidence level.

The days of measurements were very similar in irradiance, air temperature, and relative humidity. ψ_w showed different daily patterns in May and October (Fig. 1*A*), which can be expressed (between 04:00 and 20:00) by the included equations, where y = ψ_w and x = solar time. Predawn ψ_w reflected lower water availability in October than in May (Fig. 1*A*). The lowest ψ_w value occurred at 08:00 in May and was followed by an increase, while in October it decreased progressively until night, suggesting a higher difficulty to compensate the water lost by transpiration. However, RWC measured in the morning was similar in both periods, most likely due to solutes' accumulation along the growing season. Soluble sugar content was mostly higher in October (Fig. 2*A*) which can be associated with osmotic adjustment of plant tissues (Morgan 1984). The greater amount of sugars can explain the decrease of ψ_π, which occurred in one half of the cultivars; the highest reduction was observed in Duro d'Estrada and José Dias (*ca.* 23 %), followed by Tuono (*ca.* 15 %). Other solutes may be involved in the reduction of ψ_π (Lambers *et al.* 1998). Besides contributing to the maintenance of pressure turgor and, consequently, to sustain photosynthesis, soluble sugars may have a protective function, stabilizing proteins and membranes during dehydration (Paleg *et al.* 1985).

Proline content increased from May to October significantly only in Duro d'Estrada and Non Pareil (Fig. 2*B*), but it was probably not important for osmotic adjustment except for Duro d'Estrada. However, as October measurements were performed after a period of rainfall (Fig. 1*C*), proline might have presumably increased in most cultivars in the previous dry months, being afterwards metabolized. The proline role as an osmotic solute is controversial, it may accumulate as a result of metabolic disorders under stress (Larher *et al.* 2002).

Maintenance of volume or RWC by osmotic adjustment preserves photosynthetic activity. Daily P_N was measured in May and October: the highest activity occurred in both periods in the morning (*ca.* 09:00–11:00). José Dias (Fig. 1*B*), for example, followed polynomial patterns, respectively $y = -0.32\ x^2 + 7.79\ x - 22.48$ (r^2 = 0.87), for May and $y = -0.21\ x^2 + 4.47\ x - 5.41$ (r^2 =0.64) for October, where y = P_N and x = local time. Reductions in P_N from May to October occurred in most cultivars except in José Dias and Ferrastar (Fig. 2*C*). In all cultivars g_s decreased (Fig. 2*D*).

P_{max} was maintained or increased in Casa Nova, Ferraduel, Ferragnes, Guara, José Dias, Marcona, Tuono, Garrigues, and Moncaio (Fig. 2*E*). José Dias showed very high rates in May, which were maintained in October. Whole mean values of chlorophyll (Chl) content were 357±10 and 257±10 mg m^{-2} in May and October, respectively, although the maintenance of Chl contents was observed in some cultivars. The ratio Chl *a/b* was also maintained (4.2±0.1), indicating that the relationship between photosystems 1 and 2 was similar in May and October. The maintenance of carotenoid contents, observed in May and October in some cultivars, can help preserve P_{max}.

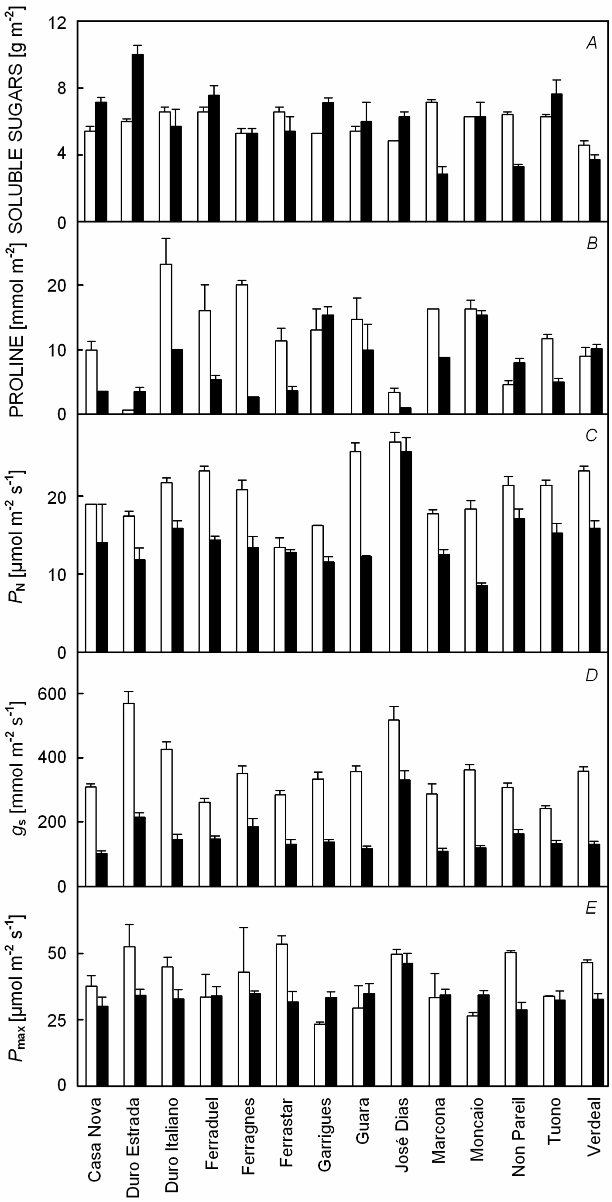

Fig. 2. Changes in leaf (*A*) soluble sugar and (*B*) proline contents, (*C*) mean diurnal net photosynthetic rate (P_N), (*D*) stomatal conductance (g_s), and (*E*) photosynthetic capacity (P_{max}) measured in May (□) and October (■) in fourteen *Prunus amygdalus* cultivars, grown in the field under rain-fed conditions. *Bars* are means of three measurements ± SE.

Little or no senescence symptoms occurred in leaves at the level of photosynthetic machinery in October in the majority of cultivars, as observed in our study. This was also supported by fluorescence measurements. Mean F_v/F_m values obtained in predawn in May and October changed from *ca.* 0.80 to *ca.* 0.76, respectively.

To summarize, osmotic adjustment was observed in some cultivars which showed reductions in leaf osmotic potential. Such decreases were accompanied by soluble sugars' accumulation. P_{max} did not significantly change from spring to autumn in nine cultivars, revealing a high resistance of photosynthetic machinery of this species to environmental stresses, namely high temperature and drought. Considering that the Portuguese cultivar José Dias did not show relevant seasonal changes in P_N and $P_{max,}$ presented the lowest g_s decrease and also showed osmotic adjustment, we suggest it has a higher photosynthetic performance than the remaining genotypes.

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