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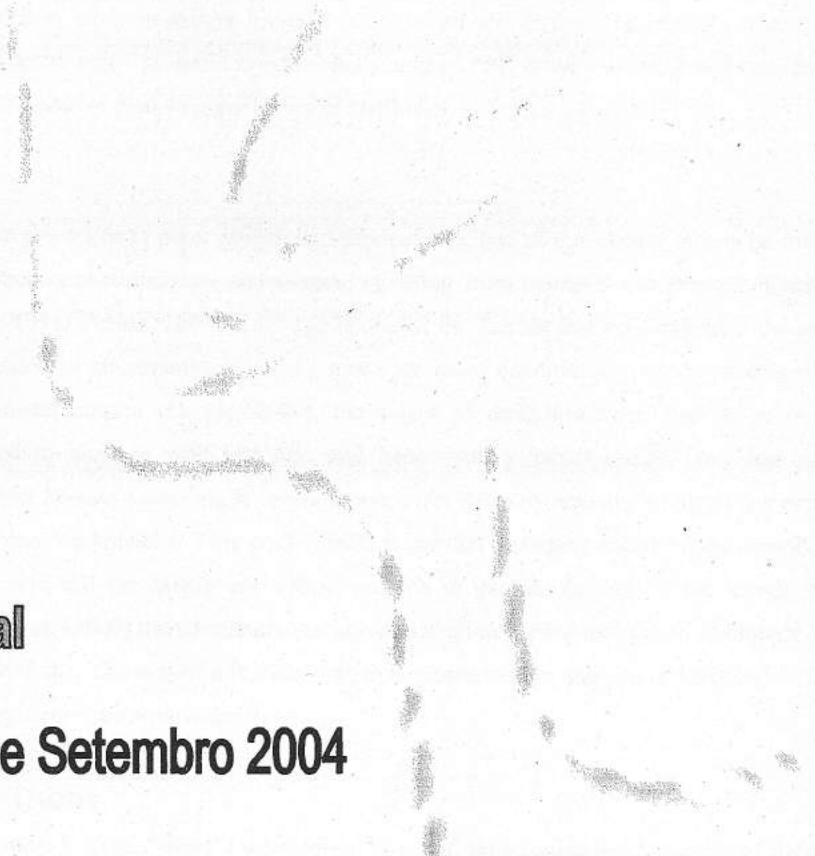

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THE EFFECTS OF DROUGHT ON NET CO_2 UPTAKE, CHLOROPHYLL FLUORESCENCE AND PHOTOSYNTHETIC PIGMENTS IN MATURE AND YOUNG LEAVES OF SUNFLOWER

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ABSTRACT

Photosynthesis, photosystem II activity and photosynthetic pigments were analysed in mature and young leaves of sunflower (*Helianthus annuus* L.) during dehydration and rehydration, in order to determine the influence of leaf age on the sensitivity to drought. Dehydration caused a more pronounced inhibition in photosynthetic rates in mature leaves compared with younger leaves, but 24 h after rewatering the recovery was complete in spite of leaf age. After exposure to drought, mature leaves showed a larger reduction in the actual PSII photochemical efficiency and a higher increase in nonradiative energy dissipation than young leaves. In well-watered plants the de-epoxidation level represented by the ratio A+Z/V+A+Z was higher in young leaves. However drought had opposite effects on both type of leaves: in young leaves A+Z/V+A+Z decreased with water deficit, while in mature leaves increased with soil drying. The results obtained suggest that leaf age influence the "strategy" to avoid irreversible damage of PSII activity under drought and that older leaves are more drought-sensitive than younger leaves of sunflower.

INTRODUCTION

Water deficit is a major constraint to plant growth and productivity, one of the earliest effects of soil drying being the decline in photosynthetic carbon assimilation, resulting from stomatal and mesophyll limitations (Kaiser, 1987; Chaves, 1991; Cornic, 2000). Leaf age is one of the factors that may influence the extent of drought-induced depression in photosynthesis. Under moderate stress conditions in which photosynthesis is mainly limited by stomatal closure (Cornic, 2000), the extent of drought-induced depression in carbon assimilation is expected to increase with leaf age, and hence young leaves should have less need for photoprotection than older leaves. According to recent reports, leaf age may actually influence the expression of different photoprotective mechanisms. Yang *et al.* (2003) found that leaf aging enhanced the contribution of both the xanthophyll cycle and the Mehler-peroxidase reaction to photoprotection in rice leaves, whereas according to Calatayud *et al.* (2000) the contribution of photorespiration to photoprotection of drought cassava leaves decreased with leaf age. The aim of this study was to compare mature and young leaves of *Helianthus annuus* as to their strategies to cope with water stress.

MATERIAL AND METHODS

Seeds of *Helianthus annuus* L. (var. "Giant") were sowed in 3 dm^3 pots containing a mixture of unfertilised peat and vermiculite (1:1, v:v). The plants (one plant per pot) were grown in a growth chamber (Fitoclima 16000 EHVP, Aralab, Portugal) under 12 h photoperiod, 70% relative humidity, 25/22 °C day/ night temperature and PPFD 500 $\mu mol\ m^{-2}\ s^{-1}$ at the plant level. Watering was done using a complete nutrient solution (Hoagland modified). From sowing until the beginning of soil drying cycles, the pots were regularly brought to field capacity (each 2 days). The onset of water stress imposition took place 18 days after sowing. A mild stress (MS) was reached after 9 days of soil drying, whereas a more severe (SS), was reached after prolonging the soil-drying period for 15 days. At the end of SS the pots were rewatered up to field capacity during the two following days (RW). Measurements and sampling of water-stressed plants took place in the last day of each soil-drying period, and 1 and 2 days after rewatering. Five plants were sampled per treatment and two leaves were sampled per plant: the most recently fully expanded leaf (young) and an older but non-senescent leaf (mature), which emerged before the beginning of water stress. Leaf water status was evaluated through the relative water content (RWC). Gas exchange measurements were conducted with an open gas exchange (HCM 1000 system, Walz, Effeltrich, Germany) under the conditions prevailing in the growth chamber. Chlorophyll fluorescence measurements were performed at room temperature, before the end of the dark period and 5 hours after lights were turn-on, using a portable pulse-modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany). Photosynthetic pigments were analyzed using a HPLC (Rivas et al. 1989).

RESULTS

The relative water content was affected by the water supply reduction: in SS plants the values of RWC were lesser than 70 %, being significantly lower in mature leaves than in young leaves. No significant differences were observed in net photosynthetic rate (NP) between leaves of different age of well watered and MS plants, in spite of stomatal conductance (g) being higher in control young leaves. However, by the end of the more prolonged soil-drying period, the value of g and NP decreased sharply and this drop was huge in mature leaves. Nonetheless, gas exchange rates recovered to the control levels on both types of leaves in the day after rewatering (Fig. 1). The rate of photosynthetic O_2 evolution at saturating CO_2 and PPFD (A_{max}) also decreased markedly at SS in both leaf types, but more drastically in young leaves (data not shown).

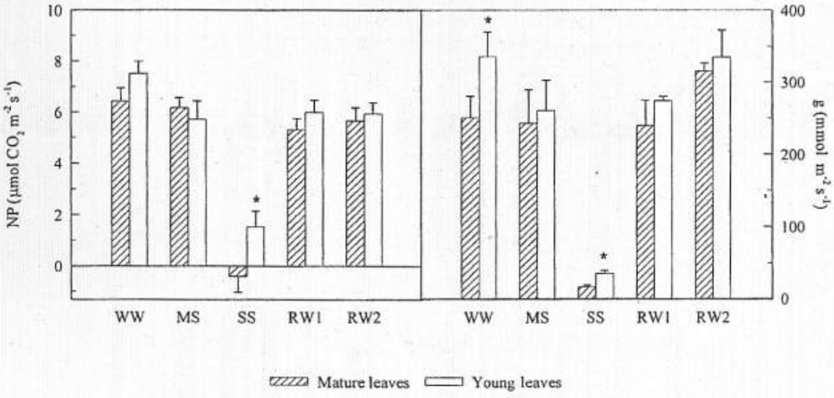

Fig. 1. Net photosynthesis (NP) and stomatal conductance (g) of mature and young leaves of sunflower plants well-watered (WW), moderate (MS) and severe (SS) water- stressed and after rewatering (24h-RW1 or 48h-RW2). Values shown are means ± s.e. of, 5 plants at least. * represent significant difference between mature and young leaves (t-test).

Pre-dawn values of the photochemical efficiency of PSII (F_v/F_m) were close to maximum (varying between 0.84 and 0.82) in the different treatments (data not shown). In mature leaves, the electron transport rate (ETR) decreased significantly in severe water-stressed plants in comparison with well-watered plants (Fig.2).

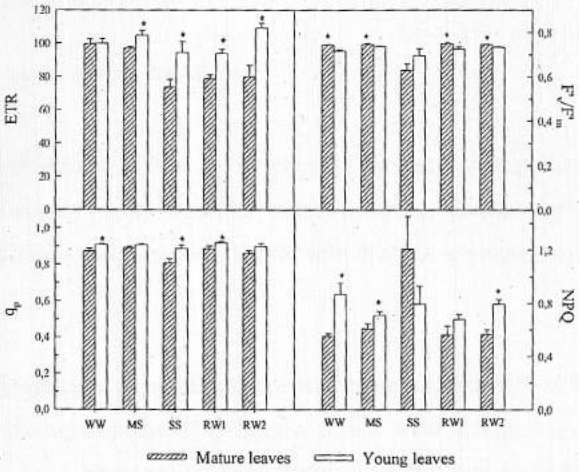

Fig. 2. Electron transport rate (ETR), efficiency of excitation energy capture by open PSII reaction centres (F'_v/F'_m), photochemical quenching (q_p) and nonphotochemical quenching (NPQ) of mature and young leaves of sunflower plants well-watered (WW), moderate (MS) and severe (SS) water-stressed and after rewatering (24h-RW1 or 48h-RW2). Values shown are means ± s.e. of, 5 plants at least. * represent significant difference between mature and young leaves (t-test).

The drought-induced restriction of electron transport in mature leaves was accompanied by a large increase in the reduction state of Q_A, as indicate by a significant decrease in photochemical quenching (q_p) and by a decline in the efficiency of excitation energy capture by open PSII reaction centres (F'_v/F'_m). Moreover, the portion of total energy dissipation by non-radiative processes (NPQ) increased considerably (Fig.2). All these values, excluding ETR, recovered within 24 h of rewatering to pre-stress levels. In young leaves drought did not significantly influence these fluorescence parameters. Therefore, in severe water stress, young leaves were able to maintain a higher proportion of oxidized to reduced Q_A (higher q_p values) and a higher quantum yield of PSII electron transport (ϕ_{PSII}) than mature leaves.

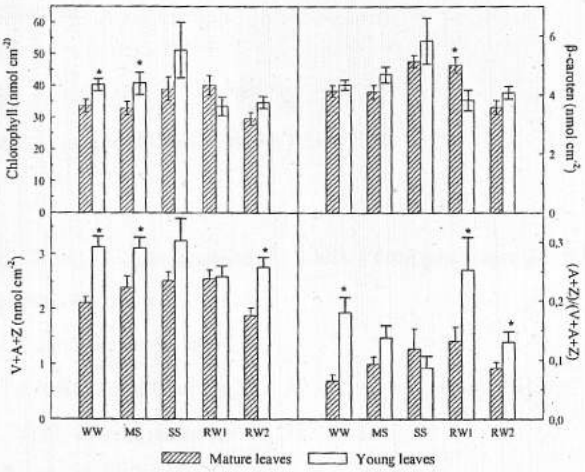

Fig. 3. Chlorophyll a+b, β-carotene and xanthophylls cycle pigments (V+A+Z) concentration and de-epoxidation level (A+Z)/V+A+Z) of mature and young leaves of sunflower plants well-watered (WW), moderate (MS) and severe (SS) water- stressed and after rewatering (24h-RW1 or 48h-RW2). Values shown are means ± s.e. of, 5 plants at least. * represent significant difference between mature and young leaves (t-test).

Independently of the water regime, pigment content analysis showed that the V+A+Z concentrations expressed on leaf area basis were present in young leaves in a higher amount compared to mature leaves. Water deficits and the subsequent rehydration did not significantly affect the pool of V+A+Z in leaves of both ages (Fig. 3). In well-watered plants the de-epoxidation level represented by the ratio A+Z/V+A+Z was higher in young leaves. However, drought had opposite effects on both type of leaves: in young leaves A+Z/V+A+Z decreased with water deficit, while in mature leaves increased with soil drying. The chlorophyll a+b concentration was statistically higher in young leaves and increased slightly with drought in young and mature leaves.

DISCUSSION

In the present work drought-induced stomatal closure was more accentuated in the older leaves, the same pattern occurring with net CO_2 uptake rate (PN). Similar results were obtained by Catalayud *et al.* (2000) in cassava leaves. In contrast, A_{max} decreased more severely in young than in mature leaves. To explain the strongest depression of A_{max} observed in young leaves, an increased accumulation of hexoses (Premanchandra *et al.*, 1992; David *et al.*, 1998) and/or a passive accumulation of inorganic ions (Kaiser, 1987) in young leaves cannot be discarded.

These results indicate that, albeit drought stress had affected negatively photosynthesis at the mesophyll level, stomatal limitation was still dominant in determining net CO_2 uptake rate. Despite the severe drought-induced depression in carbon assimilation, the maintenance of high values of F_v/F_m indicate that maximal PSII primary photochemistry was not affected by water stress or leaf age and that such decrease cannot be attributed to photoinhibition. On the other hand, results obtained in the present work point out that the importance of NPQ, and xanthophylls cycle, increases with leaf age. In a recent work with sweet potato (Haimerong and Kubota, 2003) also found that drought induced enhancement of NPQ was increased with leaf age, and this was attributed to the maintenance of higher stomatal conductance (and hence higher CO_2 assimilation rate, and less need for photoprotection) in young than in old leaves. In sunflower young leaves the absence of down-regulation of photochemistry, in spite of the decline in PN, indicate an other "strategy" to avoid photoinhibition of PSII activity under conditions of carbon starvation, such as increased photorespiration and/or Mehler-peroxidase reaction.

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