Short-term responses of photosynthetic membrane lipids and photochemical efficiency in plants of *Phaseolus vulgaris* and *Vigna unguiculata* submitted to high irradiance

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Abstract

Primary leaves of young plants of common bean (*Phaseolus vulgaris* cv. Carioca and Negro Huasteco) and cowpea (*Vigna unguiculata* Walp cv. Epace 10) were exposed to high irradiance (HI) of 2 000 μmol m^{-2} s^{-1} for 10, 20, and 30 min. The initial fluorescence (F_0) was nearly constant in response to HI in each genotype except for Carioca. A distinct reduction of maximum fluorescence (F_m) was clearly observed in stressed genotypes of beans after 20 min followed by a slight recovery for the longer stress times. In common bean, the maximum quantum yield (F_v/F_m) was reduced slowly from 10 to 30 min of HI. In cowpea, only a slight reduction of F_v/F_m was observed at 20 min followed by recovery to normal values at 30 min. HI resulted in changes in the photochemical (q_P) and non-photochemical (q_N) quenching in both species, but to a different extent. In cowpea plants, more efficiency in the use of the absorbed energy under photoinhibitory conditions was related to increase in q_P and decrease in q_N. In addition, lipid peroxidation changed significantly in common bean genotypes with an evident increase after 20 min of HI. Hence the photosynthetic apparatus of cowpea was more tolerant to HI than that of common bean and the integrity of cowpea cell membranes was apparently maintained under HI.

Additional key words: chlorophyll *a* fluorescence; cultivar differences; French bean; lipid peroxidation; photoinhibition; photosynthesis; species differences.

Introduction

Photon energy is essential to the plants but can be harmful to the photosynthetic apparatus. According to Gilmore and Govindjee (1999), photoinhibition occurs whenever the absorbed photon energy exceeds the capacity of using the trapped energy by the photosynthetic electron transport. Under this condition, some changes occur in the physico-chemical properties of the thylakoids leading to an impairment of photosynthetic electron transport (Schansker and van Rensen 1999) and to oxidative damage to photosystem 2, PS2 (Long *et al*. 1994). As a result, a decrease in the quantum efficiency of the PS2 is observed (Aro *et al*. 1993).

Photoinhibition is a form of oxidative stress, because it is related to the production of reactive oxygen species (ROS). Although ROS may operate as a secondary messenger involved in signalling transduction in response to abiotic stress (Mittler 2002), high concentrations of ROS are highly harmful to the cell integrity and function (Wise and Naylor 1987a,b) causing lipid peroxidation at the membrane level (Choudhury and Behera 2001). Consequently, the ability of plants to maintain their membrane integrity under high irradiance (HI) stress determines their resistance capacity, and can be used as a criterion for stress resistance evaluation (Lauriano *et al.* 2000). As a defence, several photo-protective mechanisms are brought in action by plants in order to maintain the chloroplast function, such as the de-epoxidation of xanthophylls, the deviation of electron flux from photosystem 1 (PS1) to molecular oxygen, and the increase of pH dependent dissipation by ATP synthase (Hideg and Murata 1997, Gilmore and Govindjee 1999). In intact leaves, damaged PS2 is repaired by synthesis *de novo* of D1 protein which is responsible for maintaining the functionality of PS2 reaction centre (RC). Experiments have

Received 10 May 2006, *accepted* 12 June 2006.
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Abbreviations: Chl – chlorophyll; ETR – relative electron transport rate; F_0 and F_m – initial and maximum fluorescence; F_v/F_m – maximum quantum yield; HI – high irradiance; LI – low irradiance; MDA – malondialdehyde; PPF – photosynthetic photon flux; PS – photosystem; Q_A – quinone A; q_N – non-photochemical quenching; q_P – photochemical quenching; RC – reaction centre; ROS – reactive oxygen species.

indicated that the degree of the damage to PS2 is the result of an imbalance between light-induced inactivation and subsequent repair (Greer *et al*. 1986).

The effects of photoinhibition are usually monitored by the maximum quantum yield (F_v/F_m), which is associated to the maximum photochemical efficiency of the photosynthetic apparatus under photon saturation. For long-term responses, little is known about the consequences of HI on biomass productivity (Laing *et al*. 1995) especially in terms of chlorophyll (Chl) fluorescence analysis. Powles *et al.* (1983) demonstrated that the recovery from irradiance stress occurred between 4 and 8 h of low irradiance (LI) in *Phaseolus vulgaris*. In addition, plants of *P. vulgaris* grown in LI used more efficiently the protective systems, *i.e*. xanthophylls, with increased activity of de-epoxidation state, sufficient to scavenge ROS when irradiation was simulated by sunflecks at low temperature (Tsonev *et al.* 2003). However, little is known about the relation between the membrane integrity, resistance capacity, and photochemical efficiency, which can represent a selection criterion for abiotic stress.

In the present study, membrane integrity was used to assess the resistance to HI in *P. vulgaris* and *V. unguiculata*, which differ significantly in their responses to high temperature (Costa *et al*. 2002, Karim *et al*. 2003). The results are contrasted with analysis of fluorescence parameters of PS2 which were measured as the severity of the photoinhibitory treatment increased. These evaluations provide some clues to distinguish tolerance capacity to HI stress between the two species.

Material and methods

Plants and growth conditions: Seeds of two cultivars of *P. vulgaris* (Carioca and Negro Huasteco) and one cultivar (Epace 10) of *V. unguiculata* were sown in 300 cm^3 plastic pots containing organic substrate and grown in a *Biotronette IV* chamber (*Lab-Line Instruments*, USA) for 10 or 11 d. This time was considered optimum for the primary leaves to achieve the maximum size. The growth chamber allowed photosynthetic photon flux (PPF) of ~200 μmol m^{-2} s^{-1} with 12-h photoperiod, a temperature of 24–26±1 °C, and 48.0/72.5 % relative humidity (day/night), respectively. The PPF was measured by means of a photometer/quantometer/radiometer model *LI-159* (*Licor*, USA). The temperature and relative air humidity were registered by means of automatic sensors (model *250*, *Spectrum Technologies*, USA) coupled to a data logger (*WatchDog* Data Logger, *Spectrum Technologies*, USA).

HI treatment: Primary leaves at maximum size were utilised for the experiments. One leaf was exposed to HI (2 000 μmol m^{-2} s^{-1}) for 10, 20, and 30 min, and the second leaf was covered with aluminium paper in order to avoid irradiation. This leaf was considered as the control. The beam was passed across a water filter aiming the avoidance of heat over the photoinhibited leaf during irradiation. We used mean values ± S.E. of four replicates from two independent experiments.

Chl *a* fluorescence was measured using a *MINI-PAM* light-modulated fluorimeter (*Walz*, Germany) at room temperature (25±2 °C). After HI-treatment, the leaves utilised in the experiments were dark adapted for 30 min to guarantee the oxidised state of quinone A. The fluorescence parameters assessed were as follows: initial fluorescence (F_0) obtained with modulated low irradiance (<0.1 μmol m^{-2} s^{-1}), maximum fluorescence (F_m) determined with a pulse of saturating irradiance (~6 000 μmol m^{-2} s^{-1}) with duration of 0.3 s, and maximum quantum yield of PS2: $F_v/F_m = (F_m - F_0)/F_m$. Hence, these parameters were used for obtaining the photochemical quenching: $q_P = (F'_m - F)/(F'_m - F_0)$, and the non-photochemical quenching: $q_N = (F_m - F'_m)/(F_m - F_0)$. These quenching coefficients were automatically calculated by the *MINI-PAM*. Electron transport rate was estimated as $ETR = \Delta F/F'_m \times PAR \times 0.5 \times$ ETR factor, where $\Delta F/F'_m$ is the effective quantum yield of irradiated sample (variable fluorescence/effective maximum fluorescence), PAR is photosynthetically active radiation, and ETR factor corresponds to the fraction of incident radiation absorbed by green leaves (value of 0.84) (Schreiber *et al*. 1994). q_P, q_N, and ETR were measured in response to different PPF with constituting eight consecutive periods of "actinic light" (0, 190, 285, 432, 598, 894, 1 213, and 2 000 μmol m^{-2} s^{-1}). Each PPF period lasted about 10 s following a pulse of saturating radiation (6 000 μmol m^{-2} s^{-1}) of 0.3 s duration. Before the sequence of measurements with increasing actinic radiation, one measurement was performed in the dark.

Membrane integrity: The photoinhibited leaves were assayed for lipid peroxidation just after the Chl *a* fluorescence measurements. Firstly, leaf tissue was weighed and ground with a mortar and pestle with 5 cm^3 of 0.1 % trichloroacetic acid (TCA) in the presence of polyvinylpolypyrrolidone (PVPP). The use of PVPP is necessary for the protection against formation of phenolics that could interfere in the optical measurements. The homogenate was then centrifuged at 8 000×*g* for 5 min, and from the supernatant an aliquot of 1 cm^3 was added to 4 cm^3 of 20 % TCA containing 0.5 % of thiobarbituric acid. This mixture was maintained at 95 °C for 30 min followed by a rapid chilling in ice. After centrifugation at 8 000×*g* for 10 min, optical density was determined at 535 and 600 nm in a UV/VIS spectrophotometer (model *6405 UV/Vis*, *Jenway*, UK). The lipid peroxidation was estimated through malondialdehyde

(MDA) formation in the leaf extracts. MDA concentration was calculated utilising a coefficient of extinction of 155 mM cm^{-1}, according to Dhindsa *et al.* (1981).

Results and discussion

The genotypes tested in three periods (10, 20, and 30 min) exhibited distinct fluorescence responses to HI (2 000 μmol m^{-2} s^{-1}) as observed in Fig. 1. The most sensitive genotypes seemed to be N. Huasteco and Carioca, whose values for the photochemical efficiency of PS2 (F_v/F_m) decreased as the time of exposure to HI increased (Fig. 1*C*). Unlike the others, the values of F_v/F_m for Epace 10 demonstrated only small variations upon time of HI, decaying slightly at 20 min, and recovering at 30 min. This parameter, which characterises the maximal quantum yield of the initial photochemical reactions in dark adapted leaves, is the most suitable for indicating environmental stresses at irradiation excess (Maxwell and Johnson 2000) and this was confirmed in our investigation.

When the different genotypes were compared in relation to the F_0 level, an increase in N. Huasteco was observed initially (10 min) followed by an apparent maintenance (20 and 30 min) (Fig. 1*A*). The rise of F_0 under unfavourable conditions is usually due to the reduction of the plastoquinone pool and then Q_A, which in these conditions is prevented from being oxidised completely because of a limitation of electron flux across PS2 (Krause and Weis 1984, Krause 1988). Cv. Carioca exhibited a different behaviour with decreased values at 10 min followed by an apparent recovery at 20 and 30 min. This drop in F_0 has been associated with the destruction of the PS2 RC (Bolhàr-Nordenkampf *et al*. 1989), but this is not probably the situation in Carioca because of the fast recovery values within 20–30 min. In Epace 10, no difference was detected in F_0 values under photoinhibitory conditions.

In relation to F_m, all the genotypes showed decreasing values with increased exposure to HI but there was a tendency to recover in Epace 10 after 30 min (Fig. 1*B*). This drop in F_m is well characterised since it is due to a proteolytic degradation of the damaged D1 protein (Aro *et al*. 1993). The small recovery of F_m observed for Epace 10 at 30 min may suggest a repair of the PS2 RCs *via de novo* synthesis of D1 protein and reassembly of the functional PS2 RCs (Mishra and Ghanotakis 1994). This situation probably does not occur in the other genotypes, because PAR continues to reach the damaged PS2 complex and proteins other than D1, may be D2 (Schuster *et al.* 1988), CP43 and CP47 (Nedbal *et al*. 1990), and may ensue in irreversible loss of PS2 electron transport.

According to Ohad *et al.* (1984), LI is necessary for the recovery process from photoinhibition. Therefore, it is imperative to accept that situations of HI are not suitable for the recovery process, as occurred at transition from 20 to 30 min, especially in Carioca and N. Huasteco.

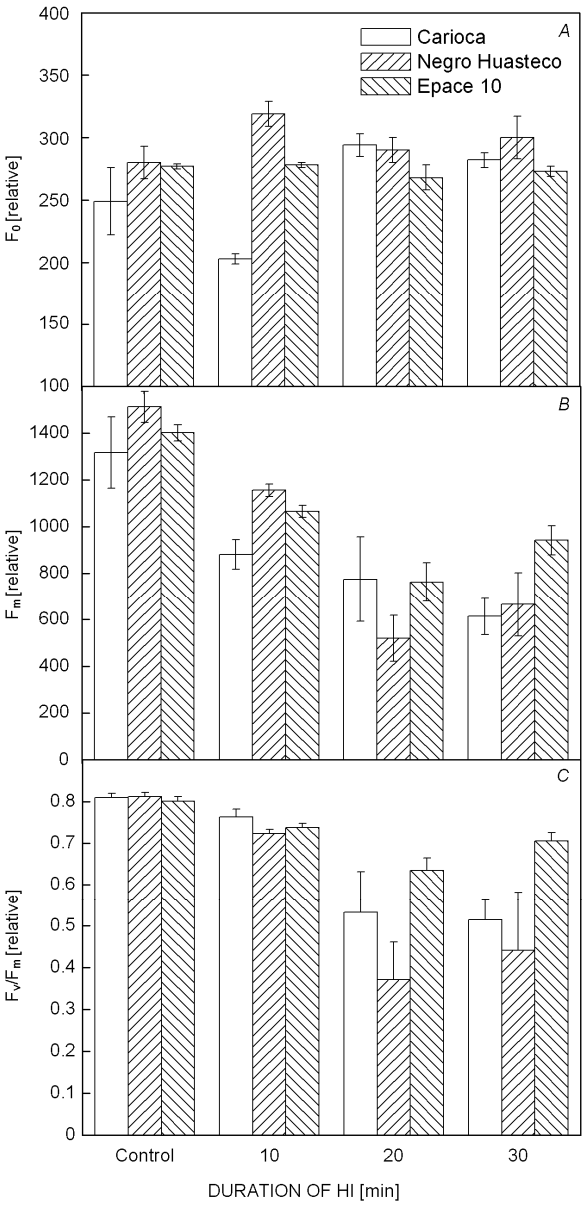

Fig. 1. (*A*) Initial fluorescence (F_0), (*B*) maximum fluorescence (F_m), and (*C*) maximum quantum yield (F_v/F_m) in leaves of *P. vulgaris* (Carioca and N. Huasteco) and *V. unguiculata* (Epace 10) exposed of 2 000 μmol m^{-2} s^{-1} for 10, 20, and 30 min. Measurements with *MINI-PAM* were done at 25 °C, 30 min after dark adaptation. Means (±SD) of five replications.

In these cultivars, an imbalance between PAR-induced inactivation and subsequent repair in the former may have lead to a strong loss of the quantum yield (Greer *et al*. 1986). This situation is related with the low values of F_v/F_m observed (Fig. 1*C*). Hence, a photo-induced

damage occurs in the PS2 complex in all the three genotypes, with an evidence of irreversible photoinhibition (Hideg and Murata 1997) in Carioca and N. Huasteco, and a fast repair process only in Epace 10.

We analysed the kinetics of fluorescence quenching in all genotypes in response to irradiance curve (Fig. 2) in order to assess the photosynthetic capacity of photoinhibited plants. In Carioca and N. Huasteco, the q_P measurements at 20 and 30 min could not be obtained by *MINI-PAM*, probably because of the high intensity of the imposed stress (Fig. 2*A,B*). The results for Epace 10 were interesting because major values were observed at 10 and 30 min and the treatment of 20 min was similar to the control (Fig. 2*C*). As compared with the other genotypes, Epace 10 exhibited a relative maintenance of q_P values under 2 000 μmol m^{-2} s^{-1} in the control and 20 min treatments, *i.e.* in 10- and 30-min treatments, distinct curves of q_P were observed with major values in response to HI. This seems to be particularly important for the regeneration capacity.

For q_N (Fig. 2*D–F*), an increase is expected as irradiance rises and this is clear for control plants of all genotypes. In general, there was a stimulus as the time of HI increased, except for N. Huasteco. The data suggest that this genotype might have the lowest capacity to support the imposed photoinhibitory conditions because of the major values achieved by q_N. As observed for q_P, smaller differences of q_N among the treatments were observed in Epace 10, showing a high capacity to support or to regenerate damaged photosynthetic apparatus under HI.

The ETR curves were stimulated in response to increasing irradiance in all genotypes (Fig. 3). The greatest difference among treatments was observed for Epace 10 (~90 % between control and 20 min). Markedly, the 20-min treatment was not saturated with 2 000 μmol m^{-2} s^{-1} in Epace 10 and N. Huasteco as occurred in Carioca. This situation can demonstrate a limited capacity of Carioca to respond to HI, even with stimulus of previous exposition to photoinhibitory conditions.

The variations of q_P and q_N under normal irradiance normally tend to decrease (q_P) and increase (q_N) as PAR is grown up and this is expected since decrease in q_P indicates the proportion of the open PS2 RCs (Maxwell and Johnson 2000). We found that the photoinhibitory treatment leads to a rise in q_P in all genotypes (Fig. 2) despite the lack of q_P curves for 20- and 30-min treatments for Carioca and N. Huasteco, as stated before. Instead of a drop, the increase of q_P indicates that as more PS2 RCs become inactivated, the pool on the electrons produced in the active centres by PS1 becomes stronger, resulting in more centres with Q_A oxidized and thus, increasing the number of open centres (Schansker and van Rensen 1999). In fact, Ivanov *et al.* (1998) pointed out that acclimation to HI increases PS1 mediated cyclic electron transport.

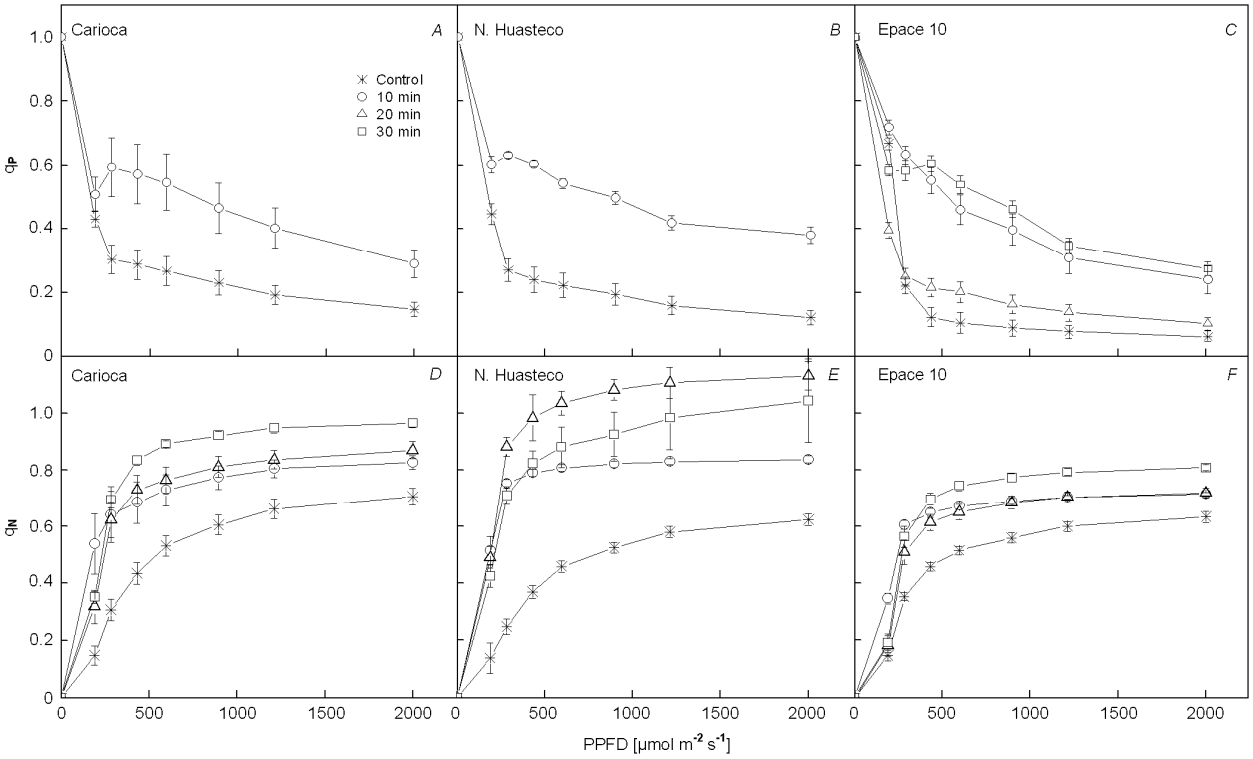

Fig. 2. Photochemical (q_P) and non-photochemical (q_N) quenching in plants of cvs. Carioca, Negro Huasteco (*P. vulgaris*), and Epace 10 (*V. unguiculata*) exposed to 2 000 μmol m^{-2} s^{-1} for 10, 20, and 30 min. Measurements with *MINI-PAM* were done at 25 °C, 30 min after dark adaptation. Means (±SD) of five replications.

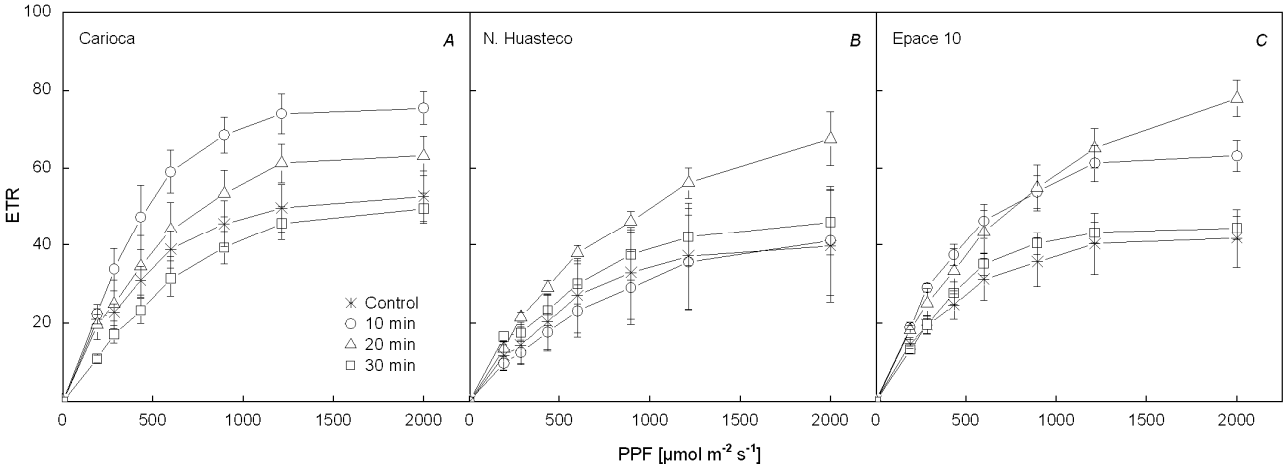

Fig. 3. Relative electron transport rate in response to photosynthetic photon flux (PPF) in plants of the cvs. Carioca, Negro Huasteco (*P. vulgaris*), and Epace 10 (*V. unguiculata*) exposed to 2 000 µmol m^{-2} s^{-1} for 10, 20, and 30 min. Measurements with *MINI-PAM* were done at 25 °C, 30 min after dark adaptation. Means (±SD) of five replications.

The changes in q_N measure the variation in the heat dissipation efficiency (Maxwell and Johnson 2000). The curve initiates with a rise in the photo-induced trans-membrane ΔpH followed by activation of ATP-consuming reactions of the Calvin cycle (Yordanov *et al.* 1997). This process requires the presence of a low pH in the thylakoid lumen and involves the HI-induced formation of zeaxanthin, which is a necessary condition for the photoprotective mechanism. According to Gilmore and Govindjee (1999), the first order of defence for a typical PS2 unit in short-term responses is the xanthophyll cycle-dependent energy dissipation. Because PS2 has a limited capacity to dissipate excess absorbed photon energy *via* the xanthophyll cycle-dependent mechanism and thus prevent molecular damage to the PS2, maybe there is the occurrence of a reversible phosphorylation of light-harvesting complex proteins, in a fast time scale, promoting an adequate distribution of energy between PS1 and PS2 (Horton *et al.* 1996), known as state transition. By this point of view, it is probable that Epace 10 used these two photoprotective mechanism more adequately than Carioca because that genotype demonstrated a fast recovery as shown by the F_v/F_m ratio at 30 min treatment.

We found important clues that the photosynthetic apparatus of Carioca and N. Huasteco demonstrates limitations imposed by time of exposure under HI. This situation is noticeable because photochemical efficiency was almost constant in Epace 10. Exposure of plants to normal irradiance usually leads to production of ROS due to limitations in ATP and NADP+ pools (Macpherson *et al.* 1993) and these deleterious molecules are minimised by an intricate defence system that involves several antioxidant enzymes (Scandalios 1993). With an inadequate availability of ATP and NADP+ pool, the excitation energy is subjected to transfer to O_2 molecules and this can promote the formation of harmful O_2^- or $^1O_2$ species.

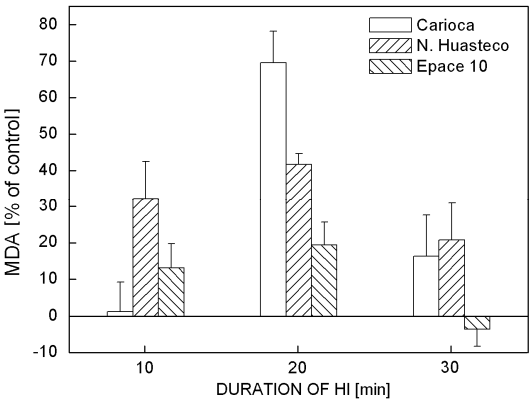

Fig. 4. Lipid peroxidation, measured by malondialdehyde (MDA) content in plants of the cvs. Carioca, Negro Huasteco (*P. vulgaris*), and Epace 10 (*V. unguiculata*) exposed to 2 000 µmol m^{-2} s^{-1} for 10, 20, and 30 min. Means (±SD) of five replications.

A thorough investigation was conducted to provide the levels of lipid peroxidation on leaf tissues exposed to HI (Fig. 4). This protocol was based on the fact that the inability to transport electrons *via* photochemical reactions results in an enhancement of MDA content (Buege and Aust 1978, Foyer *et al.* 1994). The evidence shows a higher MDA content in Carioca and N. Huasteco regardless the time of exposure to HI. In spite of MDA increases at 20 and 30 min for Epace 10, a substantial reduction back to control values was observed at 30 min, indicating a possible re-adaptation of the photosynthetic apparatus.

In this investigation, it has become clear that the photosynthetic apparatus of *V. unguiculata* (Epace 10) is distinguished from the other genotypes of *P. vulgaris* (Carioca and N. Huasteco) in terms of using photoprotective mechanisms against HI under short-term induction. Differently from *V. unguiculata*, genotypes are

exposed to mean irradiances of ~2 000 μmol m^{-2} s^{-1} or more in warm periods and this can result in loss of photosynthetic efficiency and consequently reduction of biomass production. In addition, HI is commonly associated with high temperature in the tropics, constituting optimised conditions for growing and productivity by *V. unguiculata* but deleterious for *P. vulgaris* (Costa *et al*. 2001, Silva 2001). We showed that Carioca and N. Huasteco are less tolerant and probably these cultivars suffer irreversible photoinhibition under daily HI in field conditions, as demonstrated for high temperature (Silva 2001). Despite fluorescence data can supply excellent approaches about efficiency of the photochemical activity under HI, new studies should be performed aiming to detect the biochemical mechanisms of tolerance to HI as D1 regeneration capacity, xanthophyll cycle dissipation mechanism, trans-thylakoid proton gradient, and Mehler reaction as well as the effect of the chronic photoinhibition on photosynthetic efficiency and productivity.

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