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ORIGINAL ARTICLES

Effect of 24-Epibrassinolide on Chlorophyll Fluorescence and Photosynthetic CO_2 Assimilation in *Vicia faba* Plants Treated with the Photosynthesis-Inhibiting Herbicide Terbutryn

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Abstract Simultaneous measurements of chlorophyll (Chl) fluorescence and CO_2 assimilation (A) in *Vicia faba* leaves were taken during the first weeks of growth to evaluate the protective effect of 24-epibrassinolide (EBR) against damage caused by the application of the herbicide terbutryn (Terb) at pre-emergence. *V. faba* seeds were incubated for 24 h in EBR solutions (2×10^{-6} or 2×10^{-5} mM) and immediately sown. Terb was applied at recommended doses (1.47 or 1.96 kg ha^{-1}) at pre-emergence. The highest dose of Terb strongly decreased CO_2 assimilation, the maximum quantum yield of PSII photochemistry in the dark-adapted state (F_V/F_M), the nonphotochemical quenching (NPQ), and the effective quantum yield ($\Delta F/F'_M$) during the first 3–4 weeks after plant emergence. Moreover, Terb increased the basal quantum yield of nonphotochemical processes (F_0/F_M), the degree of reaction center closure ($1 - q_p$), and the fraction of light absorbed in PSII antennae that was dissipated via thermal energy dissipation in the antennae ($1 - F'_V/F'_M$). The herbicide also significantly reduced plant growth at the end of the experiment as well as plant length, dry weight, and number of leaves. The application of EBR to *V. faba* seeds before sowing strongly diminished the effect of Terb on fluorescence parameters and CO_2 assimilation, which recovered 13 days after plant emergence and showed values similar to those of control plants. The protective effect of EBR on CO_2 assimilation was detected at a photosynthetic photon flux density (PFD) of 650 µmol m^{-2} s^{-1} and the effect on $\Delta F/F'_M$ and photosynthetic electron transport (J) was detected under actinic lightings up to 1750 µmol m^{-2} s^{-1}. The highest dose of EBR also counteracted the decrease in plant growth caused by Terb, and plants registered the same growth values as controls.

Keywords Brassinosteroids · Chlorophyll fluorescence · Epibrassinolide · Herbicide · Photosynthesis · s-Triazine · Terbutryn · *Vicia faba*

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Introduction

Brassinosteroids (BRs), now considered a sixth group of phytohormones, are natural growth-promoting compounds found at very low concentrations in pollen, seeds, and young plant tissues. These hormones promote elongation, bending, cell division, vascular differentiation, reproductive development, and modulation of stress processes in plants (Bishop and Yokota 2001). The molecular mechanisms and signal transduction pathways of BRs have been intensively studied in recent years (Vert and others 2005; Wang and others 2006). BRs have a broad spectrum of stimulatory and protective activities that cause a positive effect on the quantity and quality of crops (Khripach and others 2000). Under field conditions, 24-epibrassinolide (EBR) increases not only crop yield but also crop quality. In potato plants, it increases yield by about 20% and enhances starch and vitamin C content (Khripach and others 1996). BRs have a potential application in agriculture to increase yield and to stimulate crop growth under unfavorable conditions such as high salinity, low and high temperature, drought, or nutrient deficiency (Khripach and others 2000). Thus, the benefits of treatment with BRs have been described in plants subjected to chill stress, mild drought, and salt stress (Clouse and Sasse 1998; Krishna

2003; Kagale and others 2007). EBR enhances both cold and heat tolerance in bromegrass and tomato (Wilen and others 1995; Singh and Shono 2005) and also reduces the impact of salt stress on growth, restores pigment levels, and increases nitrate reductase activity in rice (Anuradha and Rao 2003). EBR protects barley and cucumber plants against fungi (Pshenichnaya and others 1997; Khripach and others 2000), and BRs also stimulate resistance to viral infection (Bobrik and others 1998).

BRs protect plants against pesticides and herbicides; however, only a few preliminary studies have addressed this topic (Cutler 1991; Krishna 2003). BRs diminish herbicidal injury to rice caused by simazine, symetrin, butachlor, and pretilachlor (Hamada 1986), perhaps by reducing transpiration and herbicidal absorption and by counteracting the herbicide-induced inhibition of photosynthesis (Mandava 1988). However, the mechanism of BR action has not been studied.

About half the herbicides known inhibit photosynthetic electron transport at the level of the PSII acceptor site. Herbicides compete with plastoquinone bound at the Q_B site and thus inhibit the electron transfer from Q_A to Q_B. Terbutryn (Terb) belongs to the s-triazine group. It specifically binds at the Q_B site of PSII, reduces the electron transfer to the plastoquinone pool (Xiong and others 1997), inhibits photosynthetic O_2 evolution, and affects flash-induced chlorophyll (Chl) fluorescence (Zimmermann and others 2006). The damage observed in intact plants after treatment with photosynthetic-inhibiting herbicides may be attributable to the radical chain reaction and lipid peroxidation initiated by the excited Chl molecule (Fedke 1982).

Chl fluorescence in vivo and its kinetics of relaxation are sensitive early indicators of damage to photosynthetic apparatus (Maxwell and Johnson 2000; Rohácek 2002) and are thus suitable parameters to study stress damage (Havaux and others 1988). Quenching phenomena are strongly influenced by several stress factors such as drought, high irradiance, and herbicides (Krause and Weis 1991). Thus, Chl fluorescence measurements, in combination with simultaneous analysis of leaf gas exchange, provide information about the partitioning of excitation energy between photochemical processes, which are responsible for CO_2 reduction, and nonphotochemical processes, of which radiationless dissipation is the most relevant (Sivak and Walker 1985).

Changes in the quantum yield of noncyclic electron transport in vivo can be evaluated from measurements of the fluorescence yield in the steady-state and maximal levels (Genty and others 1989). Thus, Chl fluorescence parameters mirror the effects of stress on the photosynthetic apparatus and other physiologic effects which feed back on photosynthesis (Bolhar-Nordenkampf and others 1989).

Vicia faba plants, grown in Mediterranean environmental conditions, are affected by Terb and other herbicides that inhibit photosynthesis (Caballero and others 1992; Vidal and others 1992). Furthermore, photosynthetic and Chl fluorescence responses to methabenzthiazuron, a photosynthesis-inhibiting herbicide, have been reported in this plant (Vidal and others 1995).

Here we studied the effect of EBR application to *V. faba* seeds on the photosynthetic response and growth of *V. faba* plants treated with Terb. For this purpose, we measured CO_2 assimilation (A) and fluorescence parameters to reveal the physiologic bases of the protective effect of BRs against this herbicide.

Materials and Methods

Plant Material, Growth, and Experimental Conditions

Seeds of *V. faba* cv. Reina Blanca (Semillas Fito S.A., Barcelona, Spain) were sown in 14-cm-diameter plastic pots with garden earth, perlite, and peat (2:7:1) and grown in a Conviron 5–15 controlled chamber under a long photoperiod (16 h light/8 h darkness) with a photosynthetic photon flux density (PFD) of 700 μmol m^{-2} s^{-1}. Day/night temperatures were 20/15°C and relative humidity was between 65 and 60%. Pots were watered regularly and fed weekly with Hoagland's nutrient solution (Hoagland and Arnold 1950).

EBR was applied to *V. faba* seeds before sowing. Thus, seeds were imbibed for 24 h in 2×10^{-6} or 2×10^{-5} mM EBR solutions (EBR_1 and EBR_2, respectively) and then immediately sown. Terb treatments were applied at pre-emergence at the recommended doses of 1.47 and 1.96 kg ha^{-1} ($Terb_1$ and $Terb_2$, respectively) to pots containing EBR_1- and EBR_2-treated seeds 4 days after sowing. There were five replicates per treatment. A set of plants was left untreated to be used as controls. Finally, other sets were treated only with one of the following: EBR_1, EBR_2, $Terb_1$, and $Terb_2$ as herbicide or EBR controls.

Sampling was initiated on the sixth day after plant emergence and repeated every 4–5 days for the next month.

Chl fluorescence was monitored using a pulse modulation fluorometer (PAM 101–103, Walz, Effeltrich, Germany), which provides a low-intensity, pulsed measuring beam (peak wavelength = 650 nm) from a light-emitting diode at frequencies of 1.6 or 100 kHz.

Detached leaves were dark-adapted for 30 min at room temperature before receiving a saturating pulse (4500 μmol m^{-2} s^{-1} photosynthetically active radiation, PFD) of 700-ms duration from a halogen lamp pulse source (FL 103, Walz), to determine the maximum level of Chl fluorescence (F_M) in dark-adapted state. The minimal fluorescence (F_0) was measured using a modulated red

radiation beam of 1 μmol m^{-2} s^{-1}. An actinic radiation source (Schott KL1500 halogen lamp, PFD = 650 μmol m^{-2} s^{-1}) was applied and then a train of saturating pulses of white light (700 ms, PFD = 4500 μmol m^{-2} s^{-1}) was applied repetitively at 20-s intervals. The nonphotochemical quenching coefficient (NPQ) was calculated as $(F_M - F'_M)/F'_M$. The degree of reaction center closure in the light-adapted state ($1 - q_P$) was calculated as $1 - [(F'_M - F'_0)/F'_M]$. The fraction of light absorbed in PSII antennae that is dissipated via thermal energy dissipation in the antennae, $1 - F'_V/F'_M$, was calculated as $1 - [(F'_M - F'_0)/F'_M]$. The maximal quantum yield of PSII (F_V/F_M) and the effective quantum yield ($\Delta F/F'_M$) were also calculated following the method of Schreiber and Bilger (1993). F'_0 was determined immediately after turning off the photosynthetic radiation following the method of Oxborough and Baker (1997).

Measurements of *A* were performed on intact plants using an infrared gas analyzer portable photosynthesis system (LICOR-6200, LI-COR Inc, Lincoln, NE, USA), working in a closed circuit. *A* was measured at 20°C, CO_2 concentration between 350 and 370 ppm, air flux of 500 μmol s^{-1}, and relative humidity of 40–50%. Conditions of measurements were similar to environmental conditions in the growth chamber. Chl fluorescence and *A* measurements were performed on the most recent fully expanded leaf.

Statistical Analysis

Mean values of fluorescence parameters, *A*, plant growth, and plant biomass in shoots and roots were compared by a one-way analysis of variance (ANOVA); differences between treatments were compared with the least significant difference (LSD) test.

Results

Photosynthetic CO_2 assimilation was affected by Terb treatments. Thus, *A* decreased by 56% in $Terb_2$ plants with respect to controls at the first sampling (Fig. 1a). The damage caused by $Terb_2$ to *A* decreased slowly during the growing period, but 18 days after emergence *A* values were still 29% lower than controls. Finally, at the last sampling, all plants showed the same *A* values. $Terb_1$-treated plants also showed a decrease of *A* with respect to the controls, but the effect was lower than the decrease observed in $Terb_2$-treated plants (Fig. 1a). Consequently, data corresponding to $Terb_1$ plants subjected to EBR application were omitted because the effects of herbicide and EBR treatments were not as apparent as in plants treated with the highest concentration of Terb. In the $Terb_2$ plus EBR_1 and $Terb_2$ plus EBR_2 treatments, *A* decreased by only 41 and 25%, respectively, compared to controls at the first sampling (Fig. 1b). Thus, EBR_1 and EBR_2 treatments maintained the rate of *A* 15 and 31%, respectively, higher than plants treated with $Terb_2$ only. Furthermore, when seeds had been treated with EBR, the damage caused by $Terb_2$ on *A* was not detected 13 days after plant emergence (Fig. 1b).

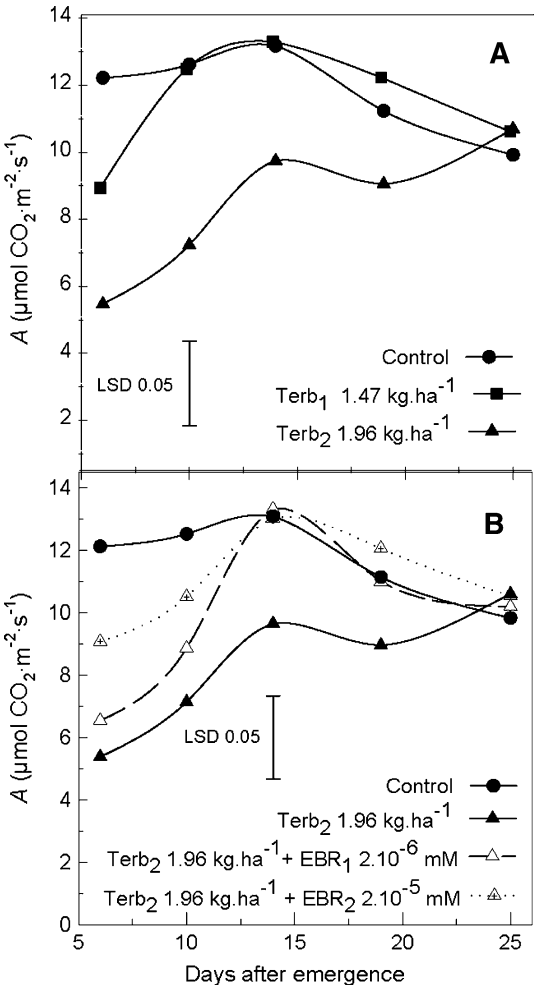

Fig. 1 Effect of terbutryn at doses of 1.47 kg ha^{-1} ($Terb_1$) and 1.96 kg ha^{-1} ($Terb_2$) (**a**) and $Terb_2$ and 24-epibrassinolide (EBR) (**b**) on photosynthetic CO_2 assimilation (*A*) of *Vicia faba* leaves during the first 25 days after plant emergence. EBR was applied at doses of 2×10^{-6} mM (EBR_1) or 2×10^{-5} mM (EBR_2). Each value represents the mean of five replicates. See Materials and Methods for more details

These initial effects of $Terb_2$ on photosynthetic parameters produced a reduction in plant growth, which was apparent at the end of the experiment (Table 1). Thus, $Terb_2$ treatment decreased significantly plant length, dry weight of roots and shoots, and the number of leaves compared to controls. With the $Terb_2$ plus EBR_2 treatment and even with the $Terb_2$ plus EBR_1 treatment, plant growth parameters did not decrease and they showed values not significantly different from those of controls.

Table 1 Effect of terbutryn and 24-epibrassinolide on growth parameters of *Vicia faba* plants at the end of the experiment

Treatment	Plant length (cm)	Dry weight of roots (g)	Dry weight of shoots (g)	Number of leaves
Control	26.17 ± 1.21^{a}	1.87 ± 0.10^{a}	2.94 ± 0.21^{a}	20.75 ± 1.08^{a}
$Terb_2$ 1.96 kg ha^{-1}	20.67 ± 1.52^{b}	0.98 ± 0.43^{b}	1.42 ± 0.60^{b}	13.67 ± 2.23^{c}
$Terb_2$ 1.96 kg ha^{-1} + EBR_1 2×10^{-6} mM	25.20 ± 2.37^{ab}	1.54 ± 0.21^{ab}	2.28 ± 0.38^{ab}	16.20 ± 1.73^{ac}
$Terb_2$ 1.96 kg ha^{-1} + EBR_2 2×10^{-5} mM	27.20 ± 0.66^{a}	1.75 ± 0.07^{a}	2.23 ± 0.16^{ab}	18.20 ± 0.72^{abc}

Data represent mean values ± standard errors of five experiments. Different letters in the same data column represent significant differences at the 5% level

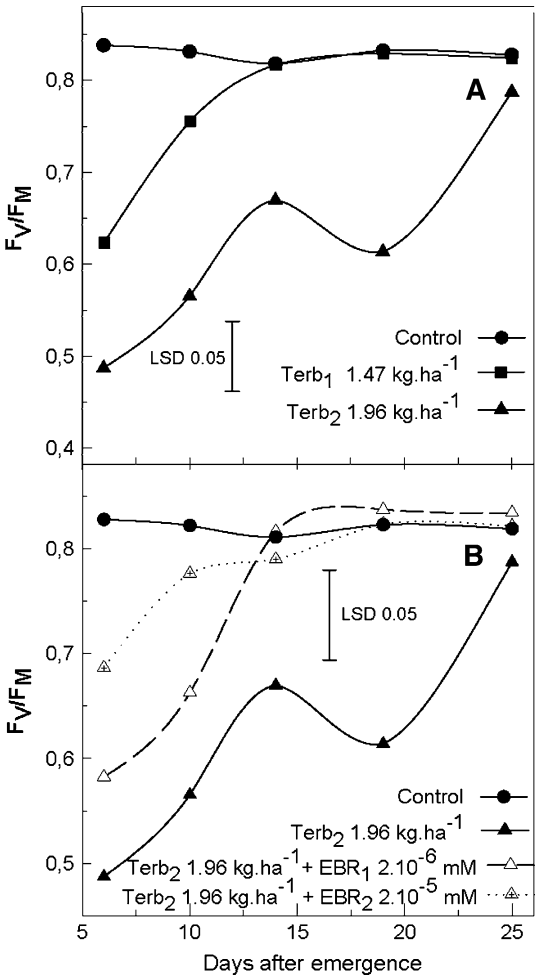

Fig. 2 Effect of (**a**) terbutryn (Terb) and (**b**) 24-epibrassinolide (EBR) and Terb on the variable fluorescence ratio F_V/F_M of *Vicia faba* leaves during the first 25 days after plant emergence. Terb was applied at doses of 1.47 kg ha^{-1} ($Terb_1$) and 1.96 kg ha^{-1} ($Terb_2$) and EBR was applied at doses of 2×10^{-6} mM (EBR_1) or 2×10^{-5} mM (EBR_2). Each value represents the mean of five replicates. See Materials and Methods for more details

Control plants maintained an almost constant F_V/F_M ratio throughout the study (Fig. 2a). The two Terb treatments caused a decrease in this parameter depending on the herbicide concentration applied. Thus, a decrease of 25 and 41% in F_V/F_M was observed in $Terb_1$ and $Terb_2$ plants, respectively. However, this initial decrease diminished progressively during plant growth; 25 days after plant emergence no differences were detected between control and $Terb_2$ plants. $Terb_1$ treatment caused less damage to plants and the initial decrease in F_V/F_M ceased 13 days after plant emergence (Fig. 2a).

The application of EBR_1 and EBR_2 to *V. faba* seeds before sowing strongly diminished the effect of Terb on fluorescence parameters. Thus, 6 days after plant emergence, plants treated with $Terb_2$ plus EBR_1 or EBR_2 showed a decrease in the F_V/F_M ratio of only 30 and 18%, respectively, compared to control plants (Fig. 2b). In addition, when the seeds had been treated with EBR_1 and EBR_2, the F_V/F_M ratio reached the control values more than 10 days before the plants treated with $Terb_2$ only. Data corresponding to $Terb_1$ plants subjected to EBR application were omitted because the effects of this herbicide were less relevant and EBR treatments were not as apparent as in plants treated with the higher concentration of $Terb_2$. Plants treated with only EBR_1 or only EBR_2 had the same F_V/F_M ratio as control plants (data not shown).

Basal quantum yield of nonphotochemical processes in the dark-adapted state, F_0/F_M, increased strongly in $Terb_1$- and $Terb_2$-treated plants (Fig. 3a) at the first sampling after plant emergence. However, 13 days after plant emergence, $Terb_1$-treated plants presented no significant differences with respect to the control plants, whereas $Terb_2$-treated plants showed important differences with respect to the control plants 18 days after plant emergence and recovered only at the last sampling. The application of EBR_1 and EBR_2 to *V. faba* seeds before sowing strongly diminished the effect of Terb on the basal quantum yield, as shown in Fig. 3b. $Terb_2$ plus EBR_2–treated plants had almost the same values as control plants 10 days after plant emergence. Thirteen days after plant emergence, the F_0/F_M ratios of all the EBR-treated plants had recovered totally, whereas $Terb_2$ plants had a mean F_0/F_M ratio that was 94% higher than control plants (Fig. 3b).

Fluorescence parameters related to the light-adapted state are shown in Figs. 4 and 5. As Fig. 4a illustrates, the degree of PSII reaction center closure, $1 - q_P$, remained almost constant throughout the study period in control plants but was drastically increased in $Terb_2$ plants (Fig. 4a). Thus, at the first sampling after herbicide treatment, $1 - q_P$ was

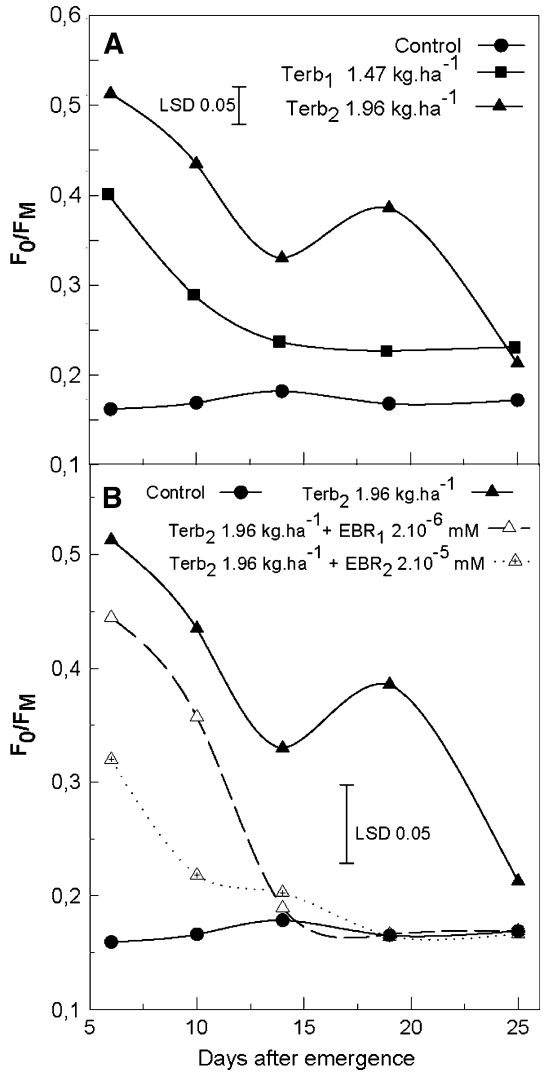

Fig. 3 Effect of (**a**) terbutryn (Terb) and (**b**) 24-epibrassinolide (EBR) and Terb on the basal quantum yield of nonphotochemical processes, F_0/F_M, of *Vicia faba* leaves during the first 25 days after plant emergence. Each value represents the mean of five replicates. See Materials and Methods for more details

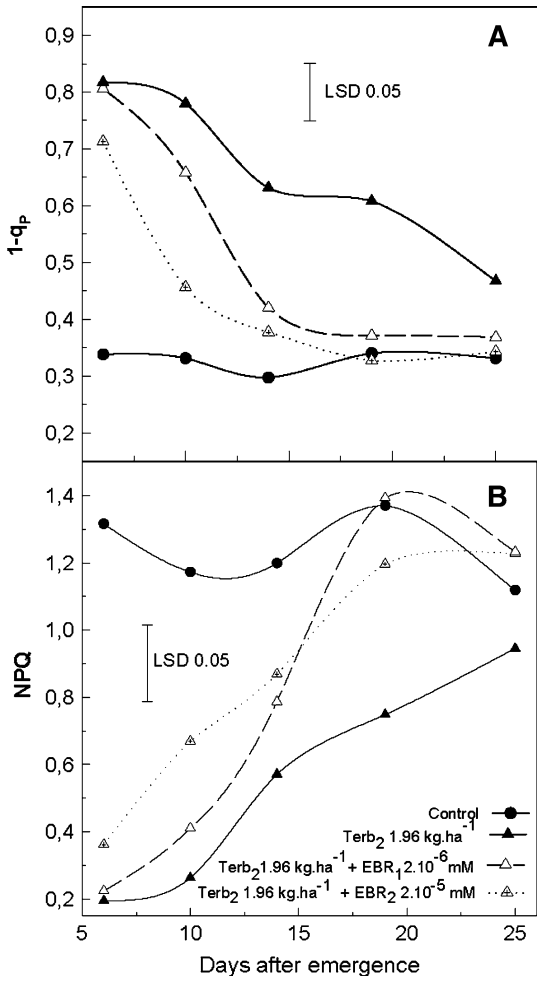

Fig. 4 Effect of terbutryn at 1.96 kg ha^{-1} ($Terb_2$) and of 24-epibrassinolide (EBR) on (**a**) $1 - q_P$ and (**b**) NPQ of *Vicia faba* leaves during the first 25 days after plant emergence. $1 - q_P$ and NPQ were determined in the steady-state after 10 min of fluorescence induction by saturating pulses (4500 μmol m^{-2} s^{-1} PFD) upon actinic irradiation (650 μmol m^{-2} s^{-1} PFD). EBR was applied at a dose of 2×10^{-6} mM (EBR_1) and 2×10^{-5} mM (EBR_2). Each value represents the mean of five replicates. See Materials and Methods for more details

141% higher than in the control plants. The effect of $Terb_2$ on $1 - q_P$ diminished progressively during the experiment but control values were almost reached at the last sampling (25 days after plant emergence). In the $Terb_2$ plus EBR_2–treated plants, the mean $1 - q_P$ values increased by only 111% at the first sampling and almost reached control values 10 days after plant emergence. The EBR_1 treatment had no effect at the first sampling but $1 - q_P$ values recovered rapidly and presented values similar to controls 13 days after plant emergence (Fig. 4a).

The nonphotochemical quenching, NPQ, of controls showed a slight decline throughout the growth period, falling by 17% from the first to the last sampling. The effect of $Terb_2$ was apparent at the first sampling when treated plants showed a mean NPQ 84% lower than controls, and 18 days after plant emergence the NPQ values of $Terb_2$ plants were still 46% lower than controls. Finally, NPQ showed no significant differences between control and $Terb_2$ plants at the last sampling (Fig. 4b). The NPQ of plants treated with $Terb_2$ plus EBR_2 showed values 73% lower than those of controls at the first sampling but reached the values of the controls 18 days after plant emergence. At the first sampling, the NPQ of $Terb_2$ plus EBR_1-treated plants was similar to that of plants treated with only $Terb_2$, but this parameter reached control values 18 days after plant emergence.

The $\Delta F/F'_M$ was also affected by $Terb_2$ treatment. This effect was maintained for a longer period because at the last sampling the $\Delta F/F'_M$ was still 20% lower in $Terb_2$ plants than in controls (Fig. 5a). The ameliorative effect of

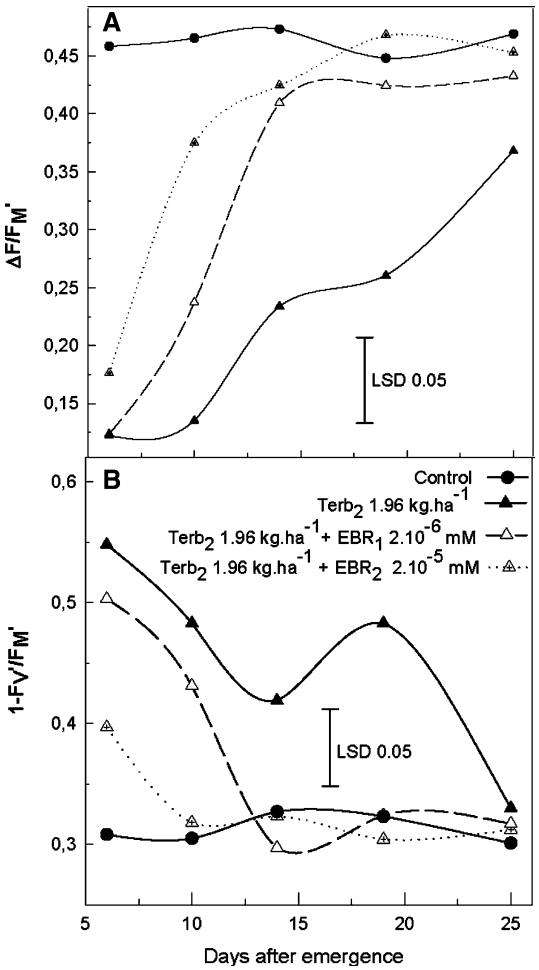

Fig. 5 Effect of terbutryn at 1.96 kg ha^{-1} (Terb$_2$) and 24-epibrassinolide (EBR) on (**a**) effective quantum yield, $\Delta F/F'_M$, and on (**b**) the fraction of light absorbed in PSII that is dissipated via thermal energy dissipation in the antennae, $1 - F'_v/F'_M$, of *Vicia faba* leaves during the first 25 days after plant emergence. EBR was applied at a dose of 2×10^{-6} mM (EBR$_1$) and 2×10^{-5} mM (EBR$_2$). Each value represents the mean of five replicates. See Materials and Methods for more details

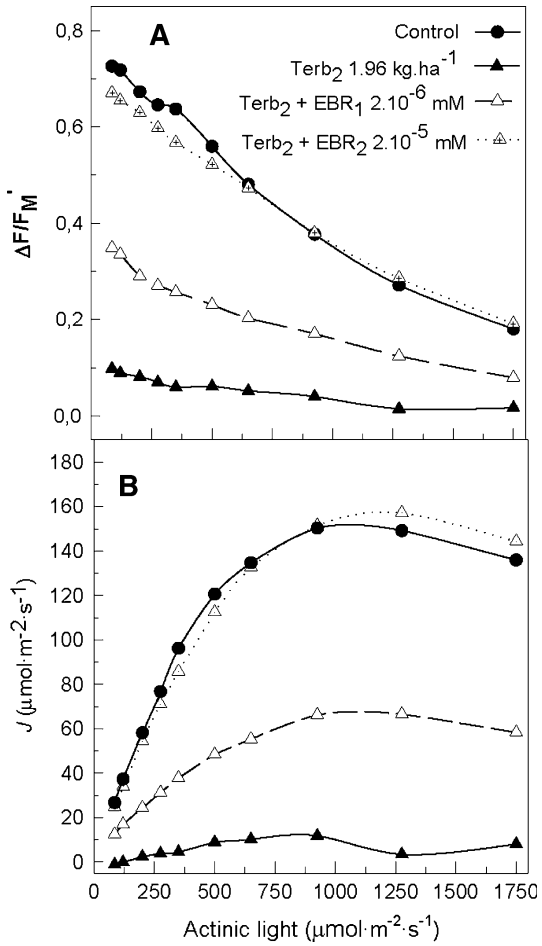

Fig. 6 Effect of terbutryn at 1.96 kg ha^{-1} (Terb$_2$) and 24-epibrassinolide (EBR) on (**a**) effective quantum yield, $\Delta F/F'_M$, and on (**b**) photosynthetic electron transport, *J*, of *Vicia faba* leaves 13 days after plant emergence. Measurements were made under actinic light up to 1750 μmol m^{-2} s^{-1}. EBR was applied at a dose of 2×10^{-6} mM (EBR$_1$) and 2×10^{-5} mM (EBR$_2$). Data correspond to one representative experiment. See Materials and Methods for more details

EBR treatments was also observed on the $\Delta F/F'_M$. Thus, EBR$_1$ and EBR$_2$ plants showed mean $\Delta F/F'_M$ values close to those of controls 13 days after emergence, whereas the plants treated with Terb$_2$ showed only a mean $\Delta F/F'_M$ value 51% lower than controls (Fig. 5a).

The fraction of light absorbed in PSII antennae that was dissipated via thermal energy dissipation in the antennae, $1 - F'_V/F'_M$, was strongly increased in Terb$_2$ plants that showed at the first sampling mean values 77% higher than control plants (Fig. 5b). This increase was progressively reduced and at the end of the experiment Terb$_2$ plants presented the same thermal dissipation values as controls. The ameliorative effect of EBR$_2$ was strong because at the first sampling the $1 - F'_V/F'_M$ of EBR$_2$ plants was only 25% higher than that of controls, and 10 days after plant emergence Terb$_2$ plus EBR$_2$-treated plants had the same thermal energy dissipation as control plants (Fig. 5b).

The protective effect of EBR was also reflected by measurements of $\Delta F/F'_M$ in plants subjected to an increasing range of actinic light (up to 1750 μmol m^{-2} s^{-1}) (Fig. 6a). Thus, the $\Delta F/F'_M$ of Terb$_2$ plants showed almost constant values in this range of actinic light. These values were 78-86% lower than controls, depending on whether the actinic light was low or high. However, plants treated with Terb$_2$ plus EBR$_2$ showed the same $\Delta F/F'_M$ as controls. The lower EBR concentration did not totally protect herbicide-treated plants but improved the response of $\Delta F/F'_M$.

The protective effect of EBR$_1$ and EBR$_2$ against damage induced by Terb$_2$ in *V. faba* plants was also indicated by measurements of the electron transport rate (*J*) in response to the increasing actinic lighting (Fig. 6b). Thus, EBR$_2$

abolished the inhibition of J caused by $Terb_2$, and EBR_2 plants showed the same J values as controls. The lower doses of EBR_1 maintained J rates 58–56% lower than controls, in contrast to the decrease of 98–96% caused by the application of $Terb_2$.

Discussion

The application of Terb at high recommended doses before plant emergence clearly reduced photosynthetic CO_2 assimilation and plant growth parameters (Fig. 1, Table 1) as well as affected fluorescence parameters in both the dark- and light-adapted state of *V. faba* leaves during the early weeks of growth (Figs. 2–5). The $\Delta F/F'_M$ was the most reduced fluorescent parameter, decreasing between 74 and 94% depending on the age of the plants and the photosynthetic actinic lighting applied (Figs. 5a and 6a). As $\Delta F/F'_M$ measures the proportion of light absorbed by Chl associated with PSII, which is used in photochemistry (Maxwell and Johnson 2000), our data indicate that Terb impaired the PSII photochemistry in a light-adapted state. This fluorescent parameter is probably the best measure of herbicide damage to *V. faba* plants because it has also been used to assess damage in *V. faba* plants treated with methabenzthiazuron (MBT) (Vidal and others 1995). The decrease in $\Delta F/F'_M$ was also associated with a drastic increase in $1 - F'_V/F'_M$, the fraction of light absorbed in PSII that is dissipated via thermal energy dissipation in the antennae (Fig. 5b), and in $1 - q_p$, which gives an indication of the proportion of PSII reaction centers that are closed (Fig. 4a). These changes indicate an inhibition of the electron transport rate. This inhibition is the predicted effect of Terb, which strongly binds at the Q_B site (Zimmermann and others 2006) and blocks PSII, thereby preventing the reoxidation of the primary acceptor Q_A and, consequently, electron transport to PSI. Thus, Terb inhibits PSII function by displacing Q_B from its binding site on the D_1 protein of this system (Xiong and others 1997). As a consequence of this inhibition, the NPQ was also strongly decreased (84% at the first sampling) and this effect was maintained until the end of the experiments (Fig. 4b).

Fluorescence parameters related to the dark-adapted state were also affected by Terb. Thus, F_V/F_M, which is one of the most frequently used fluorescence parameters and an indicator of the maximum quantum yield of PSII photochemistry in a dark-adapted state (Rohácek 2002), showed a considerable decrease in $Terb_2$-treated plants (41% at the first sampling) but a less important decrease than that presented by fluorescence parameters related to the light-adapted state. The basal quantum yield of non-photochemical processes in PSII, F_0/F_M, also increased strongly at the first sampling and remained high for 18 days (Fig. 3a). This observation is attributed mainly to an increase in F_0 (data not shown) because F_M values did not change significantly in these plants. A sustained decrease in dark-adapted F_V/F_M and an increase in F_0 indicated photoinhibitory damage in response to high or low temperature, excess PFD, and water stress (Maxwell and Johnson 2000). The increase in F_0 may be due to the initially damaged reaction centers of PSII (Lazár 1999).

It is also remarkable that the application of EBR to *V. faba* seeds did not cause a significant effect on fluorescence parameters, A, or growth (data not shown). However, $\Delta F/F'_M$, q_P-quenching, and A increased in *Cucumis sativus* when seedlings were sprayed with EBR (Yu and others 2004).

The palliative effects of EBR on $Terb_2$ damage in *V. faba* plants were seen in all fluorescence parameters, CO_2 assimilation, and growth (Figs. 1–5, Table 1). Thus, EBR induces the recovery of $Terb_2$ damage in *V. faba* plants detected by chlorophyll fluorescence and CO_2 assimilation. However, the strongest effect of EBR on $Terb_2$ damage corresponded to $1 - F'_V/F'_M$ (the fraction of light absorbed in PSII that is dissipated via thermal energy dissipation in the antennae), CO_2 assimilation, F_V/F_M, and F_0/F_M, the mean values of which were improved by 55–60% with respect to $Terb_2$-treated plant values at the first sampling (Figs. 1b, 2b, 3b, and 4b). The proportion of reaction center closure ($1 - q_p$) (Fig. 4a), the effective quantum yield ($\Delta F/F'_M$) (Fig. 5a), and NPQ (Fig. 4b) also showed the palliative effects of EBR_2 (21, 14, and 14%, respectively at the first sampling) on these parameters associated with the Terb-impaired effect on PSII photochemistry in the light-adapted state. As shown in Figs. 1–5, 13 days after plant emergence, EBR_2 provided total protection against the damage caused by $Terb_2$ on photosynthetic CO_2 (Fig. 1a) and fluorescence parameters F_V/F_M, F_0/F_M, $\Delta F/F'_M$, $1 - F'_V/F'_M$ (Figs. 2b, 3b, and 5, respectively). The effect on $\Delta F/F'_M$ was even more apparent in the response to a range of actinic lighting (Fig. 6a). It could be argued that during the experiment the differences between control and treated plants were reduced because plants were producing new, less affected leaves, whereas the older leaves were still severely affected by the herbicide. However, we believe that the comparisons are still valid because the measurements, recorded for the first fully expanded leaf, showed that the youngest leaves were healthier in controls and EBR-treated plants than in $Terb_2$ plants.

Our data do not reveal the mechanism whereby the EBR counteracts the herbicide treatment. However, we can suggest possible explanations. EBR could affect the $Terb_2$ inhibition of PSII by displacement of Q_B from its binding site on the D_1 protein of PSII. This protein is degraded when the photosynthetic system cannot process the energy of accumulated photons but little is known about the PSII

repair process, either at the level of protein synthesis, insertion, and concomitant assembly of the D_1 protein or at later functional post-translational assembly steps (Zang and others 2000). Thus, the D_1 protein of PSII must be degraded, resynthesized de novo, and reinserted into the PSII reaction center to repair the damage and reestablish PSII function (Asada 1999). It is known that BR could affect gene expression and protein synthesis (Lisso and others 2005; Vert and others 2005; Wang and others 2006). Thus, other authors (Deng and others 2007) describe two 29-kDa chloroplast ribonucleoproteins depending on BRs and several mutants of BR with proteomic changes that could directly or indirectly affect D_1 turnover in the thylakoid membranes (Ye and Sugiura 1992). Thus, ERB could be implicated in the control of D_1 damage and repair. However, other effects on the photosynthetic system cannot be ruled out. Thus, Chen and others (1995) reported a 29-kDa ribonucleioprotein involved in the mRNA stability of subunit IV of the cytochrome b_6/f complex. Besides, seed application of BR restored the Chl level and nucleic acids and soluble protein in rice plants grown in saline medium (Anuradha and Rao 2001, 2003). EBR may have caused the palliative effect on $Terb_2$-treated plants by enhancing the antioxidant enzyme system, as described by Ogweno and others (2008) in tomato plants subjected to high temperatures. The chlorophyll fluorescence parameters in the dark-adapted state indicate damage to the photosynthetic apparatus which could be alleviated by EBR protection from oxidative stress. It has also been described that BR protects against cadmium and aluminum toxicity or salt stress by stimulating the antioxidative enzymatic defense system (Nuñez and others 2003; Ali and others 2008; Hasan and others 2008). EBR application also reduced lipid peroxidation (expressed as malonaldehyde content) in tomato plants subjected to high-temperature stress (Ogweno and others 2008). This could explain the effect of BR on herbicides that inhibited elongation reactions in fatty acid synthesis, as described by Hamada (1986).

On the other hand, other BR effects cannot be ruled out. Thus, EBR could increase detoxification, thus affecting the reaction mechanisms of Terb degradation. Very little information is available on degradation processes, although at least three products have been identified; whether they are harmful is unknown (Eshel and others 1995; Kiss and others 2007). It is known that Terb induced detoxification enzymes in *Stodoptera frugiperda*, such as microsomal oxidases, glutathione S-transferases, and hydrolases, apparently by synthesis *de novo* (Yu and others 2004). BR-regulated proteins in *Arabidopsis* include at least seven glutathione S-transferases, two of them showing proteomic changes in BR mutants (Deng and others 2007).

Thus, more experimental work will be necessary to explain the mechanisms by which EBR palliates the effect of the Terb herbicide. However, our results are consistent with other BR-mediated stress responses reported, and they support the hypothesis that complex transcriptional and translational reprogramming occurs in response to BR and stress.

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