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ARTICLE

Counteracting the effects of paraquat on photosynthesis by chlorogenic acid

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ABSTRACT The effects of chlorogenic acid on the actions of paraquat on photosynthesis were studied in green pepper (*Capsicum annuum*) leaves under medium- and low-irradiance conditions by following various characteristic parameters of chlorophyll fluorescence (e.g. fast fluorescence induction kinetics and light intensity-dependence of non-photochemical quenching (NPQ)). Simultaneous treatment with 1 mM chlorogenic acid for 30 minutes eliminated the light intensity-dependent elevation of the non-photochemical quenching (NPQ) induced by 1 mM paraquat, and it also counteracted the paraquat-induced decrease in variable fluorescence (F_v/F_m). These protective actions were seen both under medium- and low-irradiance conditions. Importantly, there was no need for pretreatment of the leaves with chlorogenic acid. These results indicate that chlorogenic acid is capable of protecting green plant cells from the actions of paraquat.
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KEY WORDS

chlorophyll fluorescence
NPQ
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Paraquat (1,1'-dimethyl-4,4'-bipyridinium) is a widely-used broad-spectrum contact foliar herbicide causing rapid death of plant cells upon application to green parts of plants in the presence of light. In green plant parts it is supposed to act as a redox-cycling electron acceptor in the chloroplasts where it mediates the transfer of electrons from ferredoxin to molecular O_2, thereby blocking the photosynthetic electron transport and the formation of NADPH (Dodge 1971; Farrington et al. 1973; Rabinowitch and Fridovich 1983). Reduction of O_2 leads to the generation of superoxide anion radical and other reactive O_2 species (ROS, Vaughn and Duke, 1983; Halliwell, 1991) that can attack the unsaturated fatty acids of membrane lipids causing lipid peroxidation and the deterioration of cell membranes (Bielski et al. 1983).

Chlorogenic acid (1L-1(OH),3,4/5-tetrahydroxycyclohexanecarboxylic acid; also known as 3-(3,4-dihydroxycinnamoyl)quinic acid) is a naturally occurring hydroxycinnamic acid, one of the most important polyphenols of plants, being especially abundant in coffee and bamboo. It was often found to counteract the effects of oxidative stressors and is therefore regarded as an antioxidant (Niggeweg et al. 2004; Lan 2007). In rats, it was found to protect the animals from the oxidative stress-inducing actions of paraquat (Tsuchiya et al. 1996).

Materials and Methods

Plant material and treatment

Green pepper (*Capsicum annuum,* cv. Senator) plants were grown from seeds using a light/dark regime of 12/12 hours, illuminated with white light of 700 µmol photons m^{-2} s^{-1} intensity at room temperature. Leaves from 12-16 week-old plants were removed and discs of 1.5 cm diameter were cut out of them. The leaf discs were floated on nutrient solution (1 mM KCl, 0.1 mM NaCl, 0,05 mM $CaSO_4$, 1 mM HEPES, pH 6.5) with their abaxial side facing the solution. Two light intensities were used: dim light (30 µmol photons m^{-2} s^{-1}) and medium light (600 µmol photons m^{-2} s^{-1}). 1 mM paraquat, 1 mM chlorogenic acid, or both were given to the solution and the leaves were kept in the respective solutions for 30 minutes in the light. After that the leaf discs were transferred to the sample holder of the PAM200 chlorophyll fluorometer and were dark-adapted for 30 minutes before starting the measurement.

Chlorophyll fluorescence measurements

The actinic light (AL)-response of the modulated chlorophyll fluorescence parameters was measured on leaf discs using a PAM-200 chlorophyll fluorometer (TEACHING-PAM) and Data Aquistion Software (Walz GmbH, Effeltrich, Germany). The standard setting of Run 8 was used according to the PAM 200 handbook (Operating Manual, 1st Edition, 1997). Before the start of Run 8, the initial level of fluorescence (F_o) and the maximal fluorescence (F_m) were determined, by using 1 s red (λ~650 nm) pulses of 3,000 µmol photons m^{-2} s^{-1} PPFD (sufficient to close all of the PSII reaction centres), followed by a 5-min pre-illumination at 210 µmol photons m^{-2} s^{-1} AL intensity applied in order to allow the light-adaptation of the sample and activation of the Calvin cycle enzymes, before Run 8. During Run 8, the red AL was changed to 60 µmol photons m^{-2} s^{-1} PPFD and then it was increased stepwise (10 steps) to 1,250 µmol photons m^{-2} s^{-1} with 5 min illumination

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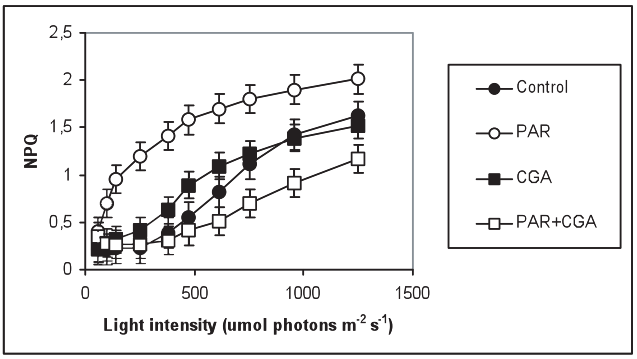

Figure 1. Light-intensity dependence of NPQ in green pepper leaves after 30-minute treatment with 1mM paraquat (PAR), 1 mM chlorogenic acid (CGA), and 1 mM paraquat + 1 mM chlorogenic acid (PAR+CGA) under medium light (600 µmol photons m^{-2} s^{-1}). Each point represents the mean ± S.D. from 9 independent experiments.

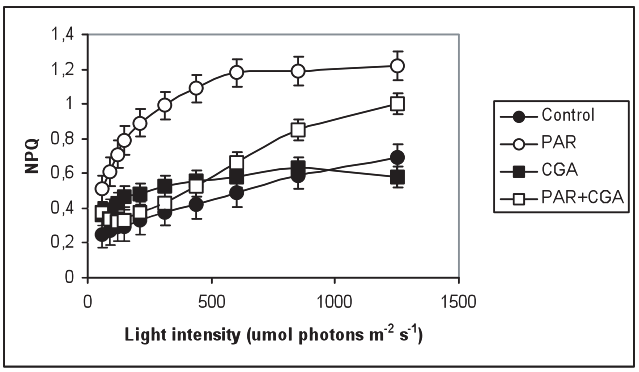

Figure 3. Light-inetnsity dependence of NPQ in green pepper leaves after 30-minute treatment with 1mM paraquat (PAR), 1 mM chlorogenic acid (CGA), and 1 mM paraquat + 1 mM chlorogenic acid (PAR+CGA) under dim light (30 µmol photons m^{-2} s^{-1}). Each point represents the mean ± S.D. from 9 independent experiments.

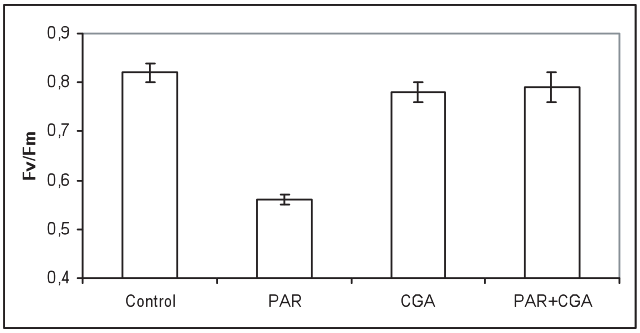

Figure 2. Effect of 30-minute treatment with 1mM paraquat (PAR), 1 mM chlorogenic acid (CGA), and 1 mM paraquat + 1 mM chlorogenic acid (PAR+CGA) on the value of F_v/F_m in green pepper leaves under medium light (600 µmol photons m^{-2} s^{-1}). Each value represents the mean ± S.D. from 9 independent experiments.

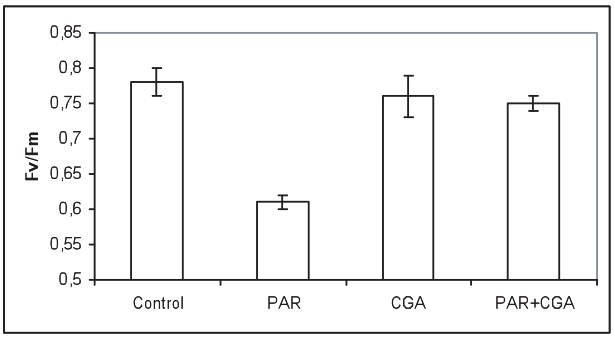

Figure 4. Effect of 30-minute treatment with 1mM paraquat (PAR), 1 mM chlorogenic acid (CGA), and 1 mM paraquat + 1 mM chlorogenic acid (PAR+CGA) on the value of F_v/F_m in green pepper leaves under dim light (30 µmol photons m^{-2} s^{-1}). Each value represents the mean ± S.D. from 9 independent experiments.

before sampling, when the quenched levels of maximum fluorescence (F'_m) and steady-state levels of fluorescence (F) were determined at each AL level. All experiments were carried out at room temperature.

Data analysis and statistical treatments

All experiments were repeated 9 times (3 parallel experiments on 3 different days) and the values are expressed as the mean ± SD. Statistically significant differences ($p<0.005$) were assessed by the use of Student's t-test.

Results and discussion

The effects of 30-minute treatment with 1 mM paraquat, 1 mM chlorogenic acid and 1 mM paraquat + 1 mM chlorogenic acid under medium light (600 µmol photons m^{-2} s^{-1}) on the light-intensity dependence of NPQ in leaves are shown in Figure 1. It is seen that paraquat increased the NPQ even at low light intensities. Chlorogenic acid in itself increased the NPQ only slightly, but it completely eliminated the paraquat-induced increase when the leaves were simultaneously treated with both compounds. The effects of the treatments on the F_v/F_m can be seen in Figure 2. Paraquat decreased F_v/F_m significantly after 30 minutes treatment. Chlorogenic acid in itself did not change the value of this parameter, but it eliminated the paraquat-induced decrease when applied together. These experiments were carried out under medium light (600 µmol photons m^{-2} s^{-1}) in order to study the action of chlorogenic acid on the paraquat-induced changes when the light intensity was high enough to activate the photosynthetic electron transport and therefore to set the stage for the action of paraquat as a mediator of electrons on the acceptor side of the PS1. On the other hand, the duration of the treatment (30 minutes) was not long enough to induce oxidative stress, so

the secondary effects of paraquat (inducing lipid peroxidation and membrane damage) are not yet present.

We also studied the action of chlorogenic acid on the effects of paraquat on photosynthesis when the light intensity was so low (30 μmol photons m^{-2} s^{-1}) that it practically did not activate photosynthetic electron transport. The effects on NPQ are shown in Figure 3, while those on F_v/F_m can be seen in Figure 4. Paraquat treatment again led to a significant increase in the NPQ (Fig. 3) as well as a significant decrease in the F_v/F_m (Fig. 4). Chlorogenic acid itself did not alter either NPQ, or F_v/F_m significantly, but it eliminated both the paraquat-induced elevation of NPQ (Fig. 3) and the paraquat-induced decrease in F_v/F_m (Fig. 4).

These results indicate that chlorogenic acid effectively counteracts the actions of paraquat both under low- and medium-light intensities. These findings extend earlier results on the protective action of chlorogenic acid on the paraquat-induced oxidative stress in rats (Tsuchiya et al. 1996) and offer the possibility that chlorogenic acid is capable of protecting plants from the effects of paraquat well before the symptoms of oxidative stress develop.

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