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Effect of transporter inhibitors on paraquat resistance of horseweed (*Conyza canadensis* /L./ Cronq.)

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ABSTRACT A paraquat inducible protein is supposed to play a role in the resistance of horseweed *Conyza canadensis* (L.) Cronq., which presumably functions by carrying paraquat to a metabolically inactive compartment. Here we studied the effect of transporter inhibitors (CCCP, DCCD, TPP and vanadate) on the decrease of functional activity caused by paraquat in order to reveal the possible roles of different types of transporters in the resistance mechanism. Results presented here imply the participation of not directly energized antiporters in paraquat sequestration.
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KEY WORDS

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Paraquat (Pq) is a foliage-active bipyridyl herbicide, which excerts its phytotoxic effect by diverting electrons from PSI to molecular oxygen and generating toxic oxygen forms. Extensive use of Pq containing herbicides has led to the worldwide incidence of resistant biotypes including weeds as well as cultivated plants. Numerous hypotheses have been evolved on possible reasons for the Pq resistance. Inhibited translocation, binding of Pq to the cell wall, its sequestration to the vacuoles, or enhanced activity of oxygen radical detoxifying enzymes (Norman et al. 1993; Hart and DiTomaso 1994) were proposed as mechanisms of resistance. Despite the efforts, Pq resistance is only partly understood.

In Hungary, Pq resistant (PqR) and Pq/atrazine coresistant (PqAR) biotypes of horseweed, *Conyza canadensis* (L.) Cronq., were observed. In these plants Pq can reach the chloroplast, as indicated by transitory inhibition followed by a gradual recovery of the functional activity (Lehoczki et al. 1992). These plants do not have enhanced activities of oxygen radical detoxifying enzymes (Turcsányi et al. 1998). An inducible mechanism can be proposed in Pq resistant biotypes of *C. canadensis* and light plays an important role in inducing both the resistance (Lehoczki et al. 1992) and the initial uptake of Pq (Váradi et al. 2000). Since cycloheximide inhibits the recovery (Darkó et al. 1994), Pq inducible protein(s) are supposed to play role in the resistance, which presumably function by carrying Pq to metabolically inactive compartment (Szigeti et al. 2001). According to the literature on Pq resistance and transport, large family of transporters can remove Pq - and wide variety of toxic molecules - in an energy dependent process and decrease their concentration near their target (Yerushalmi et al. 1995).

The present work aims to study the possible role of different transporters in the resistance mechanism of *C. canadensis* by studying the effect of various transporter inhibitors on the Pq resistance.

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Materials and Methods

Paraquat resistant (PqR) and susceptible (S) biotypes of *C. canadensis* plants were grown under laboratory conditions (illumination 130 μE $m^{-2}s^{-1}$, 16h light period, 22-25 ºC) for 3-4 months in soil containers and then transferred to field conditions. Five to six-month-old plants at the rosette stage were used in all experiments. Plants were sprayed with 1kg a.i./ha Pq as a 1 % (v/v) solution ($5x10^{-4}$ $molL^{-1}$) of Gramoxone. Carbonyl cyanide m-chlorophenylhydrazone (CCCP) in $1.5x10^{-4}$ $molL^{-1}$, N^4N^1-dicyclohexylcarbodiimide (DCCD), in 1.5 $molL^{-1}$, tetraphenylphosphonium-chloride (TPP) in $1.7 x10^{-4}$ $molL^{-1}$ and Na-orthovanadate in 10^{-4} $molL^{-1}$ concentration solution were also sprayed on the leaves of plants. Functional activity of leaves was characterized by variable fluorescence (Fv/Fm). Fluorescence parameters were determined by PAM fluorometer (Walz, Effeltrich, Germany).

Results and Discussion

Resistance of weeds is often based on their ability to remove herbicides from their site of action. Multidrug transporters can actively remove toxic molecules from their targets. These transporters can be divided into different families (Doige and Ames 1993). ABC transporters, which can directly utilize ATP, are the best known of them (Martinoia et al. 2002). The other family includes antiporters, which utilize proton electrochemical gradient to actively transport drugs. A third family comprises the smallest putative ion-coupled transporter proteins. One member of this group, the membrane protein EmrE, is responsible for paraquat and some other toxic compound resistance in *E. coli* (Yerushalmi et al. 1995).

The participation of similar types of transporters can also be supposed in the inducible resistance mechanism of *C. canadensis,* which removes Pq from its site of action and sequestrates it into the vacuole or the cell wall. In the present work we examined the effect of transporter inhibitors on the Pq resistance (by measuring the changes in Fv/Fm values) in order to reveal the possible role of these proteins in the resistance mechanism. Inhibitors were used either before or

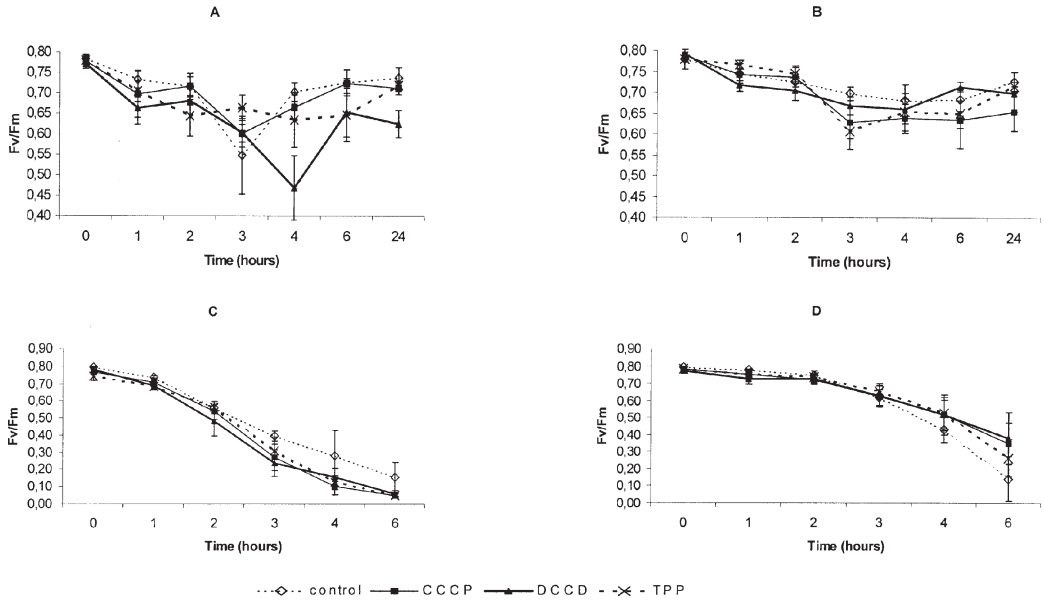

Figure 1. The effect of inhibitors carbonyl cyanide m-chlorophenylhydrazone (CCCP), N^4N^1-dicyclohexylcarbodiimide (DCCD) and tetraphenylphosphonium-chloride (TPP) on functional activity (characterized by variable fluorescence values Fv/Fm) of Pq treated *C. canadensis* resistant (PqR) and susceptible (S) biotypes. A,B: resistant (PqR), C,D: susceptible (S), A,C: treated with inhibitors by 2 hours after Pq treatment, B,D: treated with inhibitors by 2 hours before Pq treatment

after the Pq treatment to identify whether the inhibited transporter was involved in the Pq uptake, or in the sequestration (Fig. 1).

In the PqR biotype DCCD, which blocks the membrane localised F_0 parts of channels, and the special ion-coupled-type antiporter inhibitor TPP, both resulted in delay of recovery, when they were given by 2 hours after the Pq treatment. This indicates, that these types of membrane proteins may take part in intracellular transport of Pq during the process of recovery. When PqR plants were pre-treated with the inhibitors, CCCP exhibited the most remarkable effect resulting in some delay in the decrease of Fv/Fm values caused by Pq. Since CCCP results in collapse of pH gradients between the two side of membranes, this effect may refer to the strong requirement for energy during the uptake of Pq into the cells. The light dependence of both Pq uptake and recovery (Váradi et al. 2000) also support this idea. In the susceptible biotype the inhibitors slightly enhanced the effect of Pq when they were given after the Pq treatment. Applying before the Pq treatment, CCCP and DCCD had some protecting effect, also referring to the energy dependence of Pq uptake into the cells.

Vanadate, which inhibits the ABC transporters in plants without influencing the function of vacuolar H^+ATPases (Martinoia et al. 2002), did not inhibit recovery in resistant PqR biotype when it was used after the Pq treatment (data not shown). This may also refer to the participation of smaller, presumably not directly energized transporters in the sequestration process of paraquat.

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