Role of transporters in the mechanism of paraquat resistance of horseweed (*Conyza canadensis* (L.) Cronq.)

Vilmos Soós*, Balázs Jóri, Dóra Szegő, Zoltán Bratek, Ilona Rácz, Demeter Lásztity, Zoltán Szigeti

Department of Plant Physiology and Molecular Plant Biology, Eötvös Loránd University, Budapest, Hungary

ABSTRACT   In paraquat resistant biotype of *C. canadensis* inducible proteins are supposed to play a role in the mechanism of resistance. Here we studied the uptake and intracellular localization of Pq, the effect of selective inhibitors on the uptake and sequestration, and the Pq induced changes of gene expression in susceptible and resistant biotypes. In resistant plants Pq was found to reach the chloroplast in a short time, however, to the end of recovery period it was removed to vacuoles and stored for one month. Sequestration to vacuoles was inhibited by nitrate. Pq upregulated numerous genes in susceptible and resistant biotypes. Sequencing made the opportunity to identify 4 genes; the iron storage protein Ferritin2, a Myb transcription factor, CAT4 amino acid transporter and a xenobiotic transporter. Transporters expressed at higher levels in resistant plants as compared to susceptible ones. They are supposed to play important part in paraquat resistance.   **Acta Biol Szeged 49(1-2):191-193 (2005)**

KEY WORDS

Conyza canadensis
paraquat resistance
transport
Pq-induced genes

The bipyridylium herbicide paraquat (Pq) is effective as a non-selective herbicide upon application to plant leaves. Pq exerts its phytotoxic effect by diverting electrons from PSI to molecular oxygen generating reactive oxygen forms. Toxic effect of oxygen radicals is aggravated by the production of hydroxyl radicals by uncomplexed iron. Extensive use of Pq containing herbicides has led to the worldwide incidence of resistant weed biotypes. Despite efforts, the mechanism of Pq resistance is only partly understood. Inhibited translocation, sequestration to the vacuoles, or enhanced activity of oxygen radical detoxifying enzymes (Norman et al. 1993; Hart and DiTommaso 1994) were proposed as mechanisms of resistance.

In Hungary, Pq resistant (PqR) and Pq/atrazine coresistant (PqAR) biotypes of horseweed, Conyza canadensis (L.) Cronq. have been found. In these plants Pq can get to the chloroplast, as indicated by transitory inhibition of the functional activity (Lehoczki et al. 1992). Resistant 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 and the initial uptake of paraquat (Váradi et al. 2000). Pq inducible protein(s) presumably function by carrying Pq to metabolically inactive compartment (Szigeti et al. 2001).

The present work aims to study the uptake and intracellular localisation of Pq and the possible role of different transporters in the resistance mechanism of *C. canadensis* by studying the effect of selective transporter inhibitor on Pq resistance, as well as the changes in the expression level of the Pq-inducible genes in susceptible and resistant biotypes.

*Corresponding author. E-mail: vilcsok@hotmail.com

Materials and Methods

Paraquat resistant (PqR) and sensitive (S) biotypes of *C. canadensis* plants were grown hydroponically (illumination 130 mol m^{-2} s^{-1}, 16 h light period, 22-25°C). Plants at the rosette stage were used in the experiments.

Examination of Pq and transporter inhibitor on functional activity

For the kinetic experiments plants were sprayed with 5×10^{-4} mol L^{-1} paraquat. KNO$_3$ in 10^{-4} mol L^{-1} concentration solution was also sprayed on the leaves. Functional activity of leaves was characterized by variable fluorescence (Fv/Fm). Fluorescence parameters were determined by PAM fluorometer (Walz, Effeltrich, Germany).

Cell fractionation

Leaves were homogenized in three volumes of buffer (0.33 mol L^{-1} sucrose, 50 mmol L^{-1} Na$_2$HPO$_4$, 5 mmol L^{-1} MgCl$_2$, 0.1% (w/v) NaCl, 0.1% (w/v) serum albumin pH 6.5) 4h after Pq treatment. Nuclei were obtained by a centrifugation at 200 g for 2 min, chloroplasts at 3000 g for 5 min, mitochondria at 10 000 g for 5 min. Supernatant was regarded as cytosol+vacuolar fraction. Pq content was determined according to Kesari et al. (1997).

DDRT-PCR and DNA sequencing

mRNA content of three-week-old plants was isolated with a GenoPrep ™DirectmRNA Kit (GenoVision). DDRT-PCR was performed according to Tawe et al. 1998). Sequences which recurred at least four times were considered for further analysis. The nucleotide sequences were compared with se-

quences deposited in the databases (GenBank, EMBL, PDB). Translated DNA sequences were compared with protein sequences in databases (GenBank CDS translations, PDB, and SwissProt) using the BLAST 2.2.8. algorithm (Altschul et al. 1997). Similar sequences were aligned and the sequence data were analysed using the ClustalW built-in BioEdit (version 5.0.9) package.

Results and Discussion

Paraquat enters the cell with the aid of transporter molecules localized in the plasmalemma. In maize seedlings (Hart et al. 1994) putrescine inhibited the uptake. This implies that polyamine transporters can take part in the transport and sequestration of Pq. In the present work we examined the uptake and distribution of Pq in the intracellular compartments of S and PqR biotypes of horseweed and the possible role of transporters which can take part in Pq transport and sequestration. We found that Pq is taken up very quickly in both biotypes. In 1 hour after Pq spraying the maximum level (2-2,5 nM Pq/gr fresh weight) could be detected in the leaves. Nearly 10% was localised in chloroplasts, while mitochondria and nuclear fractions contained 4-5%. The amount of Pq in chloroplast and organellar fractions decreased during the recovery. In resistant plants no significant amount could be found in chloroplasts and organelles after 24 hour; the bulk of Pq was detected in the cytosol+vacuolar fraction. Although the vacuoles were not treated as a separate fraction, this probably means sequestration of Pq to the vacuoles, since resistant plants recovered their functional activity. In 1 month after treatment the Pq content in this fraction was similar.

According to literary data on Pq resistance, large family of transporters can transport and remove Pq and decrease its concentration near the target, resulting in recovery of functional activities. Transporter inhibitors can delay or prevent the recovery as it was detected in our earlier experiments. In PqR biotype DCCD (N^4N^1-dicyclohexylcarbodiimide), which blocks the membrane localised F_0 parts of channels, and the special antiporter inhibitor TPP (tetraphenylphosphonium-chloride), resulted in delay of recovery. Vanadate, which inhibits the ABC transporters in plants without influencing the function of vacuolar H^+ATPases (Martinoia et al. 2002), did not inhibit recovery. These results may refer to the participation of smaller, presumably not directly energized transporters in the sequestration process of paraquat. Here we proved that nitrate, which selectively inhibits tonoplast ATPases (Sze 1985) without affecting plasma membrane ATPases and ABC transporters, prevented recovery in PqR plants (Fig. 1). This refers to the energy requirement of vacuolar sequestration. Nitrate, however, had no detectable influence on Pq uptake into the cell, similarly to the ABC transporter inhibitor verapamil (data not shown).

Upregulation of numerous genes as a result of Pq treatment could be observed in both susceptible and resistant
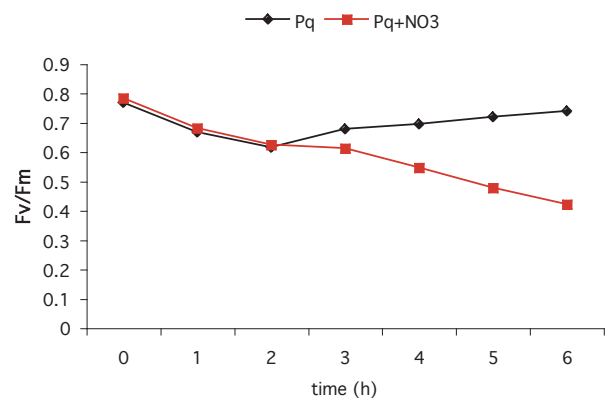

Figure 1. Changes of functional activity characterized by Fv/Fm in PqR biotype of *C. canadensis*. Nitrate was added 30 minutes after Pq treatment.

biotypes using DDRT-PCR gel chromatography. Sequencing of the ESTs made the opportunity to identify 4 genes; the iron storage protein Ferritin2, Myb transcription factor, an amino acid transporter and a xenobiotic transporter. Ferritine2 and Myb genes were equally upregulated in the S and PqR biotypes, while transporter genes exhibited much higher expression levels in resistant biotype. Ferritin2 and MYB proteins may play a role in general stress response reactions by decreasing the level of uncomplexed iron and regulating the transcription of Pq induced genes, respectively. (Sequence of Ferritin2 gene had been determined (GeneBank accession No. AJ786262) and submitted for publication.) One of the upregulated transporter genes is similar to the genes of *A. thaliana* CAT4 and *E. coli* PotE amino acid transporter (Fig. 2).

The CAT transporter family in Arabidopsis takes part in the amino acid and poliamine transport (Su et al. 2004). On the basis of sequence characteristics, CAT4 can be localized in tonoplast. The other transporter is similar to the gene of EmrE transporter of *E. coli*. This protein is responsible for paraquat and some other toxic compound resistance (Yerushalmi et al. 1995). Regarding their possible structure and function, CAT4 amino acid transporter and EmrE like protein can take part in transport and sequestration of Pq and play role in the mechanism of resistance. Determination of their complete sequence and examination of function is underway in our laboratory.
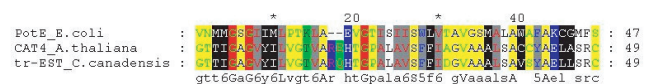

Figure 2. Alignement of the *Conyza canadensis* tr-EST sequence with the currently known amino acid transporter gene of *A. thaliana* and *E. coli*.

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