

Mechanism of paraquat resistance – from the antioxidant enzymes to the transporters

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ABSTRACT In this paper a review of the most important results on the paraquat resistance mechanism of weeds is given, with special respect on horseweed *Conyza canadensis* (L.) Cronq. There is no difference between susceptible and resistant plants in the activity of antioxidant enzymes and in the penetration of paraquat into the chloroplasts. The paraquat resistance is primarily based on higher expression of a putative amino acid/polyamine transporter which is responsible for the exclusion of paraquat into the vacuole in resistant horseweed.

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KEY WORDS

Conyza canadensis (L.) Cronq.
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The first paraquat resistant weed biotypes were detected 12–15 years after herbicides containing paraquat (Pq) were introduced in the sixties. These resistant plants also exhibited a lower extent of resistance to diquat.

It is well known, that bipyridyls exert their phytotoxic effect by diverting electrons on the reducing side of PS I from their normal physiological pathway, thus forming cation radicals and also preventing the formation of NADPH. Plants are known to possess a detoxification system, which eliminate the active oxygen forms arising in the chloroplasts under physiological conditions. The superoxide anion radical is transformed by the superoxide dismutase (SOD) enzyme into hydrogen peroxide and molecular oxygen, and subsequently the hydrogen peroxide is eliminated from the chloroplasts by the ascorbate-glutathione cycle, or by other enzymatic processes. The superoxide anion and Pq cation radicals generate hydroxyl radicals in presence of iron. It is well known that reactive oxygen forms exert strong membrane damaging effects. What is the reason of Pq resistance? For the answer on this question a series of hypotheses have been developed.

Hypotheses for the explanation of the Pq resistance

I. The idea that the redox potential relations at the entry site of Pq in the photosynthetic electron transport chain changed so that the Pq was incapable of accepting electrons was rejected at an early stage. It was found in several resistant (R) plants, that the active site was just as accessible to Pq as in susceptible ones.

II. The hypothesis that Pq becomes metabolised in the plant was also rejected, since no Pq metabolites had been detected in plants up till now.

III. A further hypothesis is that resistance is associated with the enhanced activity of antioxidative enzymes functioning in cooperation as a cycle (Shaaltiel and Gressel 1986). Dur-

ing oxidative stress the activity of these enzymes is generally enhanced. However, according to other observations, the enhanced activity of the enzymes in this cycle could not be detected in most of the paraquat-resistant (PqR) plants (Powles and Cornic 1987; Carroll et al. 1988). The protecting enzyme cycle, which utilises the NADPH pool of the chloroplasts is not capable to cope with the continuous generation of superoxide radicals indefinitely. The opinion prevails that the antioxidant enzyme cycle only provides a temporary protection, until some other type of mechanism(s) ensuring long-term survival starts to operate.

IV. According to another possible explanation, Pq is prevented from reaching its site of action in the chloroplasts of R plants. Pq enters the cell with the aid of a transporter molecule localized in the plasmalemma. Putrescine competitively inhibited uptake, which could indicate that a polyamine transporter is responsible for the uptake of Pq. Since polyamines can be transported into the vacuoles, it is probable that a polyamine transporter assists the Pq to enter the vacuoles (Pistocchi et al. 1988). From these results we can conclude, that the most probable mode of the resistance is the exclusion of Pq from its site of action.

Paraquat resistance of horseweed *Conyza canadensis* (L.) Cronq.

The first PqR *Conyza canadensis* plants found in Hungary developed in an atrazine-resistant population (Pölös et al. 1987). Later, horseweed populations resistant only to Pq were also found. In the rosette stage the resistance factor (RF) value of plants resistant only to Pq was 240 expressed as the change in F_v/F_m . In paraquat-atrazine coresistant (PqAR) plants, RF value was about 560. In the flowering stage RF values as high as 1000 were reported (Turcsányi et al. 1998).

After spraying with Pq the PqR plants exhibited transitory inhibition of their photosynthetic functions. Within two hours of treatment the inhibition no longer increased and thereafter

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gradually decreased. This clearly indicates that even in R plants Pq is rapidly able to enter the chloroplasts and exert the phytotoxic effect, which is then gradually eliminated by a mechanism induced and/or activated after the treatment. The gradual recovery indicates that resistance is not due to the ab ovo exclusion of the Pq from its site of action, but to the functioning of an inducible eliminating mechanism. Light plays an important role in inducing the resistance mechanism.

Experiments carried out on plants belonging to five different resistant biotypes of *Conyza* growing at various sites in Hungary convincingly proved that despite the very different – and relatively high – resistance factors there was neither a difference in SOD activity (Turcsányi et al. 1994) nor in ascorbate peroxidase, glutathione reductase and catalase activity after spraying with Pq (Turcsányi et al. 1998).

The activity of the SOD, APX and CAT enzymes in R plants was not higher in untreated plants or in those treated with Pq than in susceptible (S) plants. The possibility of an increase in SOD activity as part of the resistance mechanism was excluded by the simultaneous application of the Cu-chelator N,N-diethyldithiocarbamate (DDC) and Pq. DDC is the in vivo inhibitor of Cu/ZnSOD (Heikkilä et al. 1976). However, even after combined spraying with these two compounds, the R plants functionally recovered, which was not expected in the presence of the chelator if Cu/ZnSOD played a decisive role in the resistance mechanism. In the presence of 0.5 mM menadione, which compound is an active oxygen generator, the functional activity of PqR plants decreased to the same extent as that of S plants. These results indicate that in these horseweed biotype the antioxidant enzyme system is not involved in the resistance mechanism.

It was found that even R horseweed plants were destroyed if they were simultaneously treated with cycloheximide and Pq (Darkó et al. 1994). In the concentration applied (1 mg/ml), cycloheximide alone was not lethal to the plants. In vivo cycloheximide inhibits protein synthesis of the eukaryotic type. It can thus be concluded that the recovery of the functions in R plants might be caused by a nuclear-coded protein.

Possible role of transporters in paraquat resistance

Pq enters the cell with the aid of transporter molecules localised in the plasmalemma. In maize seedlings putrescine competitively inhibited the uptake, which could indicate, that a polyamine transporter was responsible for the uptake of Pq. According to literary data on Pq resistance and transport in prokaryotes and eukaryotes, large family of transporters can actively transport and remove Pq and wide variety of toxic molecules in an energy dependent process and decrease their concentration near their target (Yerushalmi et al. 1995; Shimizu et al. 2001). Similar observations were made on PqR horseweed plant using different transporter inhibitors.

We studied the effect of Pq when the uncoupler CCCP (carbonyl cyanide-m-chlorophenyl-hydrazone), the channel blocker DCCD (N4N1-dicyclohexyl-carbodiimide), the special inhibitor of ion-coupled-type transporter EmrE TTP (tetraphenylphosphonium chloride), or the ABC transporter inhibitor Na-orthovanadate was concomitantly used. In order to select between inhibition of uptake or exclusion, inhibitors were applied either before or after the Pq treatment.

From these experiment we can conclude that membrane localised Fo parts of channels and the special ion-coupled-type antiporter may take part in intracellular transport of Pq during the recovery. The Pq uptake has a strong energy requirement, and a smaller, presumably not directly energized transporters participate in the sequestration process of Pq.

In order to reveal the characteristics of the putative transporters and other proteins which can take part in Pq sequestration and transport or involved in the mechanism of resistance we also used in silico and molecular biological approaches.

Since Pq can be transported by putrescine and cationic amino acid transporters and the polyamine transporter PotE proved to be responsible for Pq resistance in lower organisms (*E. coli* and yeast), we tried to find homologues of this transporter in plant genomes either. Using in silico data bases searches we could identify the gene of a similar transporter in the Arabidopsis genome (with the EMBOSS program). This gene exhibited high level of similarity not only at the level of nucleotides but at the level of translated amino acids as well.

According to our gene expression studies using DDRT-PCR, this gene proved to be a Pq inducible one and exhibited much higher expression level in PqR biotype than in S biotype. Its differential expression was also confirmed by semi-quantitative RT-PCR and Real-Time PCR. On the basis of partial sequence data, it is a putative amino acid transporter, similar to CAT4 amino acid/polyamine transporter in *A. thaliana* (Su et al. 2004), and of PotE in *E. coli* and it is probably localised in tonoplast.

Upregulation of another putative carrier, similar to *E. coli* EmrE transporter and a homologue of the membrane localised subunits of H⁺-ATPases, was also detected in PqR plants. Regarding their possible structure and function, and the results of inhibition of recovery by selective transporter inhibitors, 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.

Among the differentially expressed cDNA fragments we could identify the genes of ferritin 2 and a Myb transcription factor. These genes were equally upregulated in the S and PqR biotypes. The increased level of the plastid-localised iron binding protein ferritin can result in decreased iron concentration and availability for the Fenton and Winterbourn reactions. We suppose its protective role in the first period after Pq treatment by decreasing the generation of reactive

oxygen radicals. MYB trans-acting factor can probably take part in the expression of Pq responsive genes and this way in the general stress response reactions after Pq treatment.

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