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**ARTICLE**

# Recent advances in understanding of the mechanism of paraquat resistance

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**ABSTRACT** Cationic amino acid/polyamine transporter CAT4 is supposed to play essential part in paraquat resistance of horseweed. In order to get better understanding on its role in resistance mechanism here we examined and compared the expression level of this transporter in the susceptible rape *Brassica napus* and in the different (susceptible and resistant) biotypes of horseweed. We found that paraquat induced an increase in expression level of CAT4 in rape, similarly to its upregulation in horseweed. **Acta Biol Szeged 52(1):103-104 (2008)**

**KEY WORDS**

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Xenobiotic paraquat (Pq) is a non selective bipyridyl herbicide (1,1-dimethyl-4,4'-bipyridyl), widely used in the form of haloid salts for total weed control or defoliation and desiccation. Pq is effective upon application to plant leaves. Illumination of plants in the presence of Pq leads to the generation of reactive oxygen radicals and causes serious oxidative damages thus leading to plant mortality, since it diverts electrons on the reducing side of PSI to form highly reactive Pq cation radical. Application of Gramoxone in Hungarian vineyards resulted in appearance of resistant biotypes in populations of horseweed *Conyza canadensis* (L.) Cronq. Resistant plants have been extensively studied to reveal mechanism of resistance, however, Pq resistance is still only partly understood. In resistant biotype (PqR) of horseweed, Pq can penetrate into the chloroplast resulting in decrease of photosynthetic activity. This inhibition is transitional, within a few hours resistant plants start to return to normal state. (Lehoczki et al. 1992). Direct chemical determination of Pq indicated that its concentration declined in chloroplasts. Reduction in Pq content appears to be the result of the activation of a mechanism for moving Pq into a metabolically inactive compartment, the vacuole. According to our studies on uptake and intracellular localization of Pq and on changes of gene expression in susceptible (S) and PqR biotypes, resistance seemed to be induced by Pq itself and participation of not directly energized transporters are suggested in this process (Halász et al. 2002; Soós et al. 2006; Jóri et al. 2007). Here we aimed to examine and compare in horseweed as well as in the Pq sensitive rape (*Brassica napus*) the differential expression of one of these transporters, the cationic amino acid/polyamine transporter CAT4, to get better understanding on its role in mechanism of Pq resistance.

## Materials and Methods

Plants were grown hydroponically for 16-18 days in 1/4 strength Hoagland solution under phytotron conditions (illumination 130 μE $m^2$ $s^1$,16 h light period, 22–25ºC). For the examination of changes in gene expression, plants were sprayed with $2.5x10^{-3}$ mol/$dm^3$ paraquat (in the form of formulated Gramoxone A).

RNA was extracted from 0.2 g of adult leaves in 0, 60 and 90 minutes after Pq treatment. mRNA was prepared by Dynabeads® mRNA DIRECT™ Kit (Dynal Biotech) according to the instructions of manufacturer. cDNA synthesis was performed in the presence of RiboLock™ Ribonuclease Inhibitor (Fermentas) using $oligoT_{18}$ primer and ReverAid™ M-MuLV Reverse Transcriptase (Fermentas). Primers were tested by a two-step PCR using Dupla-Taq™ (Zenon Bio). Samples were separated by 1% agarose gel and then purified by Montage PCR Kit (Millipore). Sequencing was carried out by BigDyeTerm v3.1 CycleSeq Kit (Applied Biosystems) and the sequences were determined by BIOMI Ltd. (Gödöllő). Real-time PCR relative quantification test was carried out using iTaq SYBR Green Supermix With Rox (Bio-Rad) and Applied Biosystems 7500 Real Time PCR system. Final primer amount was 400 nM and template cDNA concentration was adjusted to 1ng/μl. Four replicates were performed to evaluate quantitative changes. The amplification of ACT2 was used as an internal control to normalize all data. Primers for ACT2: ACT2 forward: 5'-GTG GGA ATG GAA GCT GCT GG-3', ACT2 reverse: 5'-GAC CTG CCT CAT CAT ACT CGG-3'(Su et al. 2004). Specific primers for CAT4 EST of rape sequenced in our laboratory: BrCAT4 forward: 5'-GGA CCT GCT CTT GCT GTA TCA TT-3', BrCAT4 reverse: 5'-AGT GCC CAA CCA ACC AAC C-3'

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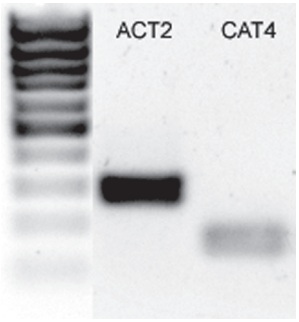


**Figure1.** Results of the primer testing with conventional two-step PCR (The length of ACT2 EST should be about 300 bp long and CAT4 EST 150 bp).

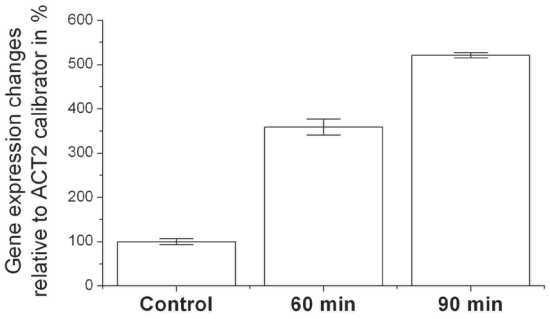


**Figure 2.** Relative quantification plot for CAT4 gene in Brassica napus. Control RNA was extracted before treatment, samples were taken in 60 and 90 minutes after Pq spraying.

## Results and Discussion

Previous examinations on Pq induced genes in S and PqR biotypes of horseweed revealed differential expression of four identified ESTs. Complete sequence of the Ferr2 gene has already been determined (Soós et al. 2006). Upregulated ESTs include two putative transporters. One of them is similar to a transporter responsible for Pq resistance in *E. coli*, and a membrane localised subunit of vacuolar $H^+$-ATPase from *A. thaliana* (Yerushalmi et al. 1995; Schumacher et al. 1999). The other one is similar to CAT4, a transporter of cationic amino acids and polyamines in vacuolar membrane of *A. thaliana* (Su et al. 2004). It is presumably able to transport Pq, which has similar intramolecular charge distribution. Earlier results on Pq induced upregulation of CAT4 in horseweed revealed, that S biotype - similarly to the susceptible *A. thaliana* – also responds by increasing expression of a number of genes, including a Myb factor (Camp et al. 2003). To reveal the role of CAT4 in Pq induced response reactions and get closer to its specific part in resistance mechanism, here we compared the time course of Pq induced changes in expression level of CAT4 in susceptible *B. napus* with those found in horseweed biotypes.

Using conventional PCR and sequence determination of the EST product from rape, we validated the specificity and aptness of primers for relative quantification by two-step PCR (Fig. 1). This was also confirmed by dissociation curve obtained during Real Time PCR reaction. According to our presumption, results of relative quantification test revealed, that Pq induces increase in the expression level of CAT4 gene in *B. napus*. As compared to the control plants, there is a considerable upregulation within 60 minutes and this increases in 90 minutes after Pq treatment (Fig. 2). Plants, however, die in 4 hours.

These results support the hypothesis, that Pq can induce upregulation of the specific amino acid transporter, CAT4, in S plants, either. In some respect, it is similar to the upregulation of a Myb factor in S biotype of *C. canadensis* and in *A. thaliana* (Camp et al. 2003; Jóri et al. 2007). Moreover, their interrelationship cannot be excluded, since Myb acts as a master switch in expression of number of genes regulated by the same signal. Recent results indicate, that one single transporter gene can be responsible for Pq resistance in various species and in transgenic constructions (Jo et al. 2004). In our case however, we cannot unambigously decide presently, whether an extensively increased expression of CAT4 (possibly due to a mutation in Myb factor), a mutation in CAT4 sequence itself, or both are responsible for the resistance due to higher effectivity of sequestration of Pq into vacuole. Sequence determination of differentially expressed genes seems to be unavoidable to give the answer. This may imply difficulties in case of a weed like *C. canadensis*, however it is currently underway in our laboratory.

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