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Poster Abstract - D.54

MDR-LIKE ABC TRANSPORTER AtPGP4 IS INVOLVED IN AUXIN-MEDIATED LATERAL ROOT AND ROOT HAIR DEVELOPMENT

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AtPGP4 belongs to the MDR (multidrug-resistance protein) ABC transporter subfamily in *Arabidopsis thaliana*. MDRs were first identified in mammalian cells because their overexpression confers a multidrug resistance phenotype. The first plant ABC protein gene to be cloned was *AtPGP1*. Noh et al. (2001) and Geisler et al. (2003) showed that auxin transport activity was greatly impaired in *atmdr1* (*pgp19*) and *atmdr1-atpgp1* double mutant plants. Epinastic cotyledons and reduced apical dominance were phenotypes consistent with disrupted basipetal flow of auxin, suggesting that MDRs may be essential for normal auxin transport and the development of plant form.

In this study two independent T-DNA Salk lines *pgp4-1* and *pgp4-2* have been screened by PCR for the homozygous genotypes, and the number of T-DNA insertions checked by Southern Blot. *pgp4* mRNA was not detectable neither in root nor in shoot of *pgp4-1* seedlings, while still faintly visible in *pgp4-2* mutant tissues. In wild type plants *pgp4* was most strongly expressed in the root, especially in the lateral root and in the root elongation zone, early in the plant development. GC-MS analysis of wild type and *pgp4* mutants root extracts at 5 dag (day-after-germination) showed that the mutants accumulated IAA in the root at significantly higher level than wild type, consistent with a possible defect in transport and redistribution of auxin in this part of the plant. Lateral root formation at 5dag was increased in both mutant seedlings when compared to wild type, in agreement with the observation that auxin promotes lateral root formation. However, the relative differences in the number of lateral roots decreased with the age of the plant (7 dag and 9 dag), suggesting that PGP4 might be required for the initial control of lateral root formation, correlating with the fact that the gene is mainly expressed in the first developmental stages of the plant. When the plants germinated in the presence of synthetic auxin IAA, the relative differences in lateral root formation between wild type and the mutants were reduced. We also found that *pgp4* mutants were able to produce lateral roots even in the presence of the polar auxin transport inhibitor *N*-1-napthhylphalamic acid (NPA), indicating that the mutation directly altered auxin sensitivity and transport at the root level. Root hairs on the mutants were significantly longer than wild type root hairs and more variable in length. Auxin and ethylene are known to promote root hair elongation in *Arabidopsis*; indeed, IAA and NPA treatment led to the development of longer root hairs than untreated seedlings both in wt and mutants, but *pgp4* mutants were less responsive to the treatment compared to wild type plants, in agreement with the observed lateral root phenotype. When seedlings germinated in the presence of the ethylene biosynthesis inhibitor, aminovinylglycine (AVG), which abolishes root hairs, we observed that the inhibition ratio in *pgp4* mutants was significantly reduced compare to wt, meaning that higher intracellular level of auxin in the mutant seedlings might play an important role in promoting the root hair elongation in the absence of ethylene. Seedlings grown in hydroponic culture were loaded with ^{3}H-IAA in a physiological solution along a time course experiment and the relative amount of radioactivity in 1 cm of root apex was counted. *pgp4* seedlings showed less ^{3}H-IAA content in the root apex, suggesting that PGP4 might be involved in the import of auxin within the cell.

These observations indicate that AtPGP4 is a key regulator in auxin-mediated lateral root and root hair development in *Arabidopsis*.