7. Abstract

Transgenic tobacco plants overexpressing the *M. crystallinum PPDK* gene were studied. Four different construct were generated, using two different promoters (CaMV-35S and B33) and two different cDNA sequences (one directed to plastids, the other to the cytosol) (Sheriff, 1994; Stenzel, 1997).

The effect of nitrogen on the behaviour of transgenic and wild type tobacco plants was tested during the first experiments. Treatments included a combination of nitrate and ammonium (20% of the total nitrogen as ammonium); nitrate (as only N-form supplied) and ammonium (as the only N-form supplied). Control plants received a solution low in N (3 mM NO_3^-). Different variables including growth (plant height, fresh weight, foliar area); concentration of different metabolites (chlorophyll, soluble protein, and free amino acids); and of CO_2-fixing proteins (RUBISCO and PEPC); and parameters of productivity (production of capsules and seed yield) were determined.

The mutants showed a significant higher growth when grown in nitrogen, whereas at low N supply their growth was reduced in comparison to the wild-type. Transgenic plants showed stronger tolerance to ammonium in comparison to the wild-type. Transgenic lines growth was directly correlated with the protein and amino acid concentration. Ammonium as the only form of N supply reduced the growth parameters only in wild-type plants. Under stress conditions (ammonium as the only N form and deficiency of nitrogen) the PEPC-concentration was increased. Seed yield in transgenic plants was higher in comparison to the wild-type. This suggests that the increase in growth and seed production in transgenic plants may be the result of a better supply of C-chains for the formation of amino acids. In the plastids the reversible PPDK-reaction is shifted in direction to phosphoenolpyruvate (PEP) because of the high pyrophosphatase-activity. PEP serves as substrate for the formation of oxalacetate (OAA) by the PEP-carboxylase. OAA can be incorporated into the citric acid cycle or directly reduced to malate. The results observed on transgenic lines showed that the PPDK-reaction in the cytosol leads to PEP-synthesis.

Transgenic tobacco plants and the wild-type where further analyzed to elucidate their growth response in the presence of Aluminium (Al). Protein concentration in roots, inhibition of root growth, exudation of organic acids from the roots and accumulation of Al in root tissues were measured. In the presence of Al, protein concentration was increased in transgenic plants, while in wild-type plants it was reduced. No significant reduction of root growth was observed in transgenic plants in the presence of Al. Al-accumulation in root tissues was specific detected in wild-type plants. Exudation of organic acids, specific malate, was higher in transgenic plants grown in the presence of Al.

These data indicates that Al tolerance observed in transgenic plants was increased via exudation of organic acids in the roots. Organic acids complex the Al, avoiding it uptake by roots. In wild-type plants, at least under Al stress conditions, supply of PEP may be more difficult. As overexpression of PPDK causes no visible phenotypical changes, but improves productivity and growth under a good nitrogen supply, PPDK may represent a possibility to increase tolerance to Al stress in plants.