Phosphoenolpyruvate carboxylase (EC 4.1.1.31; PEPC) plays many roles in plants; it connects the metabolism of saccharides and amino acids. PEPC is regulated at many levels including phosphorylation of serine residue near N-terminus. The aim was to found out, if the composition of cultivation medium affects the activity of PEPC and metabolically related enzymes such as NADP-malic enzyme (EC 1.1.1.40), pyruvate, phosphate dikinase (EC 2.7.9.1; PPDK) and enzymes participating in nitrogen assimilation, thus nitrate reductase (EC 1.7.1.1; NR), glutamine synthetase (EC 6.3.1.2; GS), glutamate synthase (EC 1.4.1.14; GOGAT), NAD-glutamate dehydrogenase (1.4.1.2; NAD-GDH) and NADP-glutamate dehydrogenase (1.4.1.4; NADP-GDH).

Tobacco plants (*Nicotiana tabacum* L., cv. Petit Havana SR1) were grown *in vitro* in Murashige-Skoog agar. The effect of the presence of 2% succrose, reduced content of nitrogen compounds or phosphate and limited supply of CO₂ was followed.

Sucrose added to Murashige-Skoog medium caused the increase of enzyme activity of PEPC, NADP-ME and most of enzymes related to nitrogen uptake and metabolism – NR, GS, GOGAT, NAD-GDH a NADP-GDH.

Reduced content of nitrogen compounds and phosphate in cultivation medium decreased activity of all monitored enzymes. Enzyme activities were also affected by the availability of CO₂. Lower activity of PEPC, NR, GS and GOGAT and higher activity of NADP-ME, NAD-GDH and NADP-GDH were found in plants with limited supply of CO₂ compared to unaffected plants.

This work showed that the availability of nitrogen compounds, phosphate and CO_2 /succrose significantly affected studied enzymes.