4. Conclusions

1. Non-photosynthetic cytosolic isoform of NADP-malic enzyme (oxaloacetate decarboxylating) (NADP-ME; EC 1.1.1.40) was isolated from tobacco (*Nicotiana tabacum* L.) leaves. Specific activity of the obtained enzyme preparation was 0.95 $\mu mol.min^{-1}.mg^{-1}$, relative molecular mass of one subunit of tetramer approximately 67 000, pI 5.5 and pH optimum 7.1 - 7.4. Kinetic constants of this isoform were determined for L-malate and $NADP^+$ in the presence of various cofactors Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, similarly the dependencies of reaction rates on the concentration of divalent metal ions were observed. The dependence of reaction rate on Mn^{2+} concentration was sigmoidal with strong positive cooperativity and high value of Hill coefficient 7.5.

2. The kinetic mechanism of two-substrate reaction catalyzed by NADP-ME from tobacco leaves was determined as ordered sequential.

3. The regulation of the NADP-ME purified from tobacco leaves occurs predominantly in the presence of macroergic compounds (GTP, ATP and ADP), slightly by metabolites of glycolysis.

4. Biotic stress caused by *Potato virus Y* (PVY) (not only strain PVY^{NTN} but also PVY^{O}) increased NADP-ME activity in *N. tabacum* L. leaves, stems and roots during the infection. Milder PVY^{O} caused lower increase of NADP-ME activity than necrotic isolate of PVY^{NTN}. Significantly increased NADP-ME activity caused by PVY^{NTN} was accompanied by enhanced transcription and expression of cytosolic NADP-ME isoform.

5. Both transgenic plants *Nicotiana tabacum* L. carrying the gene for potyviral protein P3 from *Potato virus A* and transgenic plants *Nicotiana benthamiana* with the gene for potyviral multifunctional protein HC-pro from *Potato virus A* did not differ in NADP-ME activity during response to PVY^{NTN} infection from non-transgenic control.

6. An increase of NADP-ME activity in *Nicotiana benthamiana* plants as a result of PVY^{NTN} infection was not observed in leaves but in roots, similarly as in the case of *Nicotiana tabacum* L. roots. NADP-ME isoforms were found in various parts from both, the control and the infected *Nicotiana benthamiana* plants.

7. Abiotic stress caused by CO_2 deficiency or by drought conditions increased the NADP-ME activity in leaves of *Nicotiana tabacum* L. plants.