CONCLUSIONS

I. The *ipt* expression under the senescence-specific *SAG12* promoter resulted in delay of leaf senescence, increase of content of bioactive cytokinins and nitrate reductase activity in leaves as well as to enhancement of nitrate influx in plants grown under limited N supply. However, the *SAG12::ipt* plants did not differ from WT plants in grain yield components including the number of grains and grain weight. Results suggest that delay of leaf senesce of wheat plants also delays translocation of nutrients and metabolites from leaves to developing grains after anthesis and in this way interferes with the reproductive strategy wheat as strictly monocarpic plant which is based on a programmed fast translocation of metabolites and nutrients from senescing leaves to the reproductive sinks shortly after anthesis.

II. Responding to the mostly vague statements that the physiological effects of cytokinins are dependent on plant genotype and affected by various internal factors we tested the efficiency of and aromatic (3OHBAR) and an isoprenoid (t-ZR) cytokinin on retention of chlorophyll in detached oat and wheat leaf apices. The efficiency of the two cytokinins in preservation of chlorophyll was different in the oat and wheat depending on local or whole adaxial leaf surface cytokinin application. The oat and wheat leaf pieces differed in the rates of uptake and the dynamics of [^{3}H]t-ZR and [^{3}H]3OHBAR translocation which was very fast in wheat and slower but steadily increasing with time in oat. The oat and wheat leaf apices also differed in the metabolism of applied t-ZR namely in its conversion to the storage dihydrozeatin-*O*-glucoside prevailing in oat and to the inactive t-Z *N9*-glucoside prevailing in wheat. The t-Z was more efficient in stimulation of cytokinin oxidase/dehydrogenase (CKX) than 3OHBAR and the activity of the enzyme was more increased by the two cytokinins in oat than in wheat leaf apices. The difference in activities of both cytokinins in retention of chlorophyll in wheat and oat can be minimized by their pulse application followed by incubation of leaf apices floating on water.

III. Root pressure xylem sap from de-topped oat plants contains CKX activity that is associated with a glycosylated protein. The pH optimum of the enzyme (8.5) is much higher than pH of root xylem sap (6.1) indicating suppression of cytokinin degradation by the enzyme during its transport via the xylem flow. Reported alkalization of the xylem sap in leaf apoplast and its enhancement in response to NO_3^- and water availability may create favorable conditions for metabolic degradation of co-transported cytokinins and thus decrease of cytokinin/ABA ratio at sites of high transpiration.

IV. Simultaneous determination of CKX and ZRED activities in pea leaves allowed comparisons of the actual roles of the two enzymes in control of cytokinin levels in plants, which represents a novel approach toward the investigation of the mechanisms maintaining hormonal homeostasis. Characterization of CKX activity revealed the existence of a non-glycosylated CKX isoform with relatively high pH optimum (pH 8.5) in pea leaves. The presence of non-glycosylated CKX and/or CKX with a very low degree of glycosylation is generally rather uncommon in plants, and the finding in pea suggests a more abundant occurrence as well as possible relevance and function in some legume genotypes. Also, the detection of ZRED activity in leaves (that is, vegetative organs) of pea is novel, because so far this enzyme activity had been isolated only from Phaseolus embryos (that is, generative organs; Martin et al. 1989; Mok et al. 1990). Although the proportion of ZRED to CKX activities was found to vary in a relatively broad range in pea leaves, a close relationship between conversions of Z-type cytokinins catalyzed by ZRED and their degradation by CKX is obvious. The fact that

ZRED activity converts cytokinins to forms protected from breakdown by CKX underlines a potential role of ZRED in cytokinin homeostasis.

V. The modified Bieleski's, as compared to two other tested extraction solvents, sufficiently suppressed dephosphorylation of cytokinin riboside monophosphates and reduced the extraction of compounds decreasing the RISRs of tested deuterated cytokinins. This solvent, lacking $CHCl_3$, is easier and safer to handle and appeared to be the most suitable for extraction of cytokinins. Purification of cytokinins using mixed-mode-SPE, as compared to DEAE Sephadex RP-C18 method, was powerful in the removal UV absorbing contaminants providing preparations exhibiting high RISRs of deuterated counterparts of natural cytokinins. This method was found simpler, faster and more operational. It also allows more complex plant hormone analysis by providing a partially purified fraction containing auxin and abscisic acid.