SUMMARY

Humans and monogastric animals are unable to synthesise the nine essential amino acids. Therefore these have to be obtained from food or feed, which are essentially from plant origin. A major nutritional drawback of many crop plants is their low content of several essential amino acids, particularly lysine. Lysine belongs to the aspartate family of amino acids and its biosynthesis is regulated by several feedback loops in plants. Dihydrodipicolinate synthase (DHDPS) is the first enzyme of the lysine specific branch of the aspartate pathway. The enzyme is strongly feedback inhibited by lysine in most plant species and consequently has been found to play an important role in regulation of lysine biosynthesis. One approach for increasing the lysine content in plants is de-regulating the metabolic pathway through modification of the allosteric site of DHDPS. In this study, the mutant *dhdps-r1* gene from *Nicotiana sylvestris*, encoding a feedback insensitive DHDPS, was expressed in transgenic *Nicotiana plumbaginifolia* under control of the constitutive 35S promoter and expressed in transgenic *Brassica napus* under control of the seed specific *At2S2* promoter.

In the first part of this study, we wanted to find out whether the promoter of a plant *dhdps* gene would be suitable for overexpression of DHDPS coding sequences. As the promoter of the *dhdps-r1* gene has not been isolated, the *Arabidopsis thaliana dhdps1* (*Atdhdps1*) promoter was studied. The *Atdhdps1* promoter fused to the *Gus* reporter gene has been expressed in transgenic *N. plumbaginifolia* plants. Unexpectedly, GUS expression was restricted to anthers, suggesting that the *Atdhdps1* promoter is not properly recognised in *N. plumbaginifolia*.

In the second part of the thesis we studied the expression of the *dhdps-r1* gene in both *N. plumbaginifolia* and *B. napus* plants. Transformed lines were obtained based on kanamycin selection and verified by PCR, while Western blot analysis confirmed the presence of the DHDPS-R1 protein in transgenic *N. plumbaginifolia* plants. Transgenic *N. plumbaginifolia* plants with a high overproduction of free lysine (up to 28-fold in T0 leaves) developed abnormally, leading to branched and totally sterile plants. A moderate increase of free lysine levels (up to 3.6-fold and from 4.4 up to 5.3-fold in T1 and T2 leaves, respectively) correlated with a mixed

phenotype, determined by axillary branching with small, thick and rigid leaves and by reduced fertility. Plants with a lower increase in lysine overproduction (up to 3.1-fold and 3.6-fold in T1 and T2 leaves, respectively) showed a normal phenotype similar to wild type plants. DHDPS activity in T2 leaves of all transgenic lines was increased from 2 up to 4-fold and was only 18% inhibited in the presence of 100µM L-lysine, a concentration at which 98% inhibition of DHDPS activity occurs in wild type plants. In T2 mixed immature seeds, the free lysine increases from 2.3 up to 3.7-fold, whereas in seeds from transgenics with a normal phenotype the free lysine increases only up to 1.6-fold compared to wild type. The free lysine in T2 mixed mature seeds increased from 4.7 up to 7.5-fold, while in mature seeds of transgenic plants with a normal phenotype the free lysine increased to maximum 3.2-fold compared to wild type plants.

The transgenic *B. napus* lines showed no phenotypic differences with wild type plants. In *immature seeds*, the DHDPS activity increased up to 2-fold in transgenic plants compared to wild type. The DHDPS activity was inhibited to a maximum of 20% in the presence of 100 µM of L-lysine, compared to 80% inhibition in wild type. Free lysine content in *immature seeds* increased up to 2-fold compared to wild type control. In *mature seeds*, the DHDPS activities of transgenic plants are comparable with those of the wild type plants, while their lysine contents varied from less than 1 to 2-fold the level of wild type. Total lysine content in T2 *immature* seeds from some transgenic lines increased about 2-fold compared to the control, while in *mature seeds* total lysine was almost comparable to the value observed in wild type.

These results suggested that overexpression of a single enzyme (DHDPS) modified in its feedback properties can already exert a significant effect on the flux of carbon through the aspartate pathway resulting in the overproduction of lysine. Unfortunately, when this was applied to *B. napus* by using *At2S2* promoter, a strong increase in lysine levels in the mature seeds was not obtained. To reach that goal it might be necessary to combine overexpression of DHDPS with inhibition of lysine catabolism and/or with expression of a lysine-rich protein to trap the overproduced lysine.