Manipulation of seed metabolism in transgenic pea plants to increase protein content

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Synthesis of storage protein is regulated at different levels during seed maturation. The availability and distribution of assimilates and nitrogen compounds are mostly important. During seed growth, sugar and nitrogen compounds confer regulatory control on storage activities. Thus, seed storage production could be regulated by the supply of nutrients.
We used transgenic approaches to manipulate different pathways of the seed metabolism: (1) The over-expression of the V. faba amino acid permease (VfAAP1) leads to an increase of the assimilate production in the seed. (2) Repression of an ADP-glucose pyrophosphorylase (AGP), due to RNAi construct, influences the starch and the protein content of the seed. Both genes were expressed under the control of the seed-specific LeB4 promotor.

(1) The amino acid transporter VfAAP1 was co-transformed with the selectable marker on a separate plasmid. This enabled to remove the marker gene by segregation. Northern analysis revealed over-expression of VfAAP1. Mature seeds of the homozygous marker gene-free line AAP14/10 showed higher protein concentration over 5 generations due to an increased globulin fraction. The effect of VfAAP1 was tested under field conditions in two growing periods and the data could be confirmed. (2) The plastidial enzyme AGP catalyzes the reaction of glucose-1-phosphate and ATP to pyrophosphate and ADP-glucose, which is the substrate for starch synthase. We show that AGP activity and transcript levels are strongly decreased in transgenic seeds. Repression of AGP results in a wrinkled seed phenotype obviously due to transient accumulation of free sugars during maturation. Mature seeds have reduced starch content whereas the protein concentration is higher due to increased fractions of albumins and globulins. In further analysis we test the influence of VfAAP1 and AGP on altered gene expression in developing cotyledons using cDNA-microarrays containing app. 6,000 genes from pea seeds.