

Cloning and functional characterization of genes from the tocopherol biosynthesis pathway in rapeseed (*Brassica napus* L.) and candidate gene based association studies of tocopherol content and composition

MSc Steffi Fritsche

1. Berichterstatter: Prof. Dr. Christian Jung

Rapeseed (*Brassica napus* L.) is the most relevant oil crop of temperate climates. Its oil is an important source for vitamin E, also known as tocopherol, in human nutrition. There are four naturally occurring tocopherol forms (α , β , γ , and δ) with different vitamin E activity. The α -tocopherol form displays the highest vitamin E activity whereas for oil-stability high amounts of γ - and δ -tocopherol are required. Thus, enhancing the content and composition of tocopherol is one important step to further improve the oil quality of rapeseed. All major genes of the tocopherol biosynthesis pathway have been cloned in the closely related model species *A. thaliana*. The aim of the present study was to functionally characterize homologous tocopherol genes from *B. napus* and to investigate the impact of sequence variations within major genes for tocopherol synthesis on the seed tocopherol content and composition in rapeseed.

In the first part, homologous genes of *A. thaliana* (*PDS1*, *VTE1*, *VTE2*) encoding key enzymes of tocopherol biosynthesis were investigated in rapeseed. Consensus primers derived from conserved regions of the *A. thaliana* genes and *Brassica* EST sequences were used to develop probes in order to screen a rapeseed BAC library. This strategy led to the isolation of rapeseed gene sequences with high sequence similarities to the respective *A. thaliana* genes. Furthermore, the predicted gene structure and high amino acid sequence similarity of the rapeseed proteins in comparison to the *A. thaliana* proteins indicated similar functions. The function of four rapeseed *PDS1* cDNA variants was evaluated by transformation into *E. coli* using a protein expression vector. As a result, the cDNA sequence variant *BnPDS1-A* was identified to encode a functional *PDS1* protein. The function of *VTE1* and *VTE2* was verified by transformation into *A. thaliana*. A rapeseed cDNA with high similarity to *A. thaliana VTE1* was transformed into *vte1* mutant plants. In seeds of transgenic plants (T_2 -generation) on average 185.84 mg kg⁻¹ γ -tocopherol were detected, indicating an active rapeseed enzyme. The expression of rapeseed *VTE1* in *A. thaliana* ecotype Col-0 (T_2 -generation) resulted in a 50 % increase of seed α -tocopherol content. Moreover, *B. napus* cDNA with high similarity to *A. thaliana VTE2* was transformed into *vte2* mutant plants and γ -tocopherol contents ranging between 1.56 and 5.12 mg kg⁻¹ were found. In transgenic *A. thaliana* ecotype Col-0 plants, 35S::*BnVTE2* averagely enhances the α -tocopherol content 1.45-fold compared to non-transgenic Col-0 plants.

In the second part of the work, a candidate gene-based mapping association approach was accomplished. Field trials were carried out with 229 accessions and tocopherol content and composition were analyzed. Sequence polymorphisms were identified by sequencing selected regions of 13 tocopherol candidate genes (*BnaX.VTE1.a*, *BnaX.VTE1.b*, *BnaA.VTE2.a*, *BnaX.VTE2.b*, *BnaX.VTE3.a*, *BnaX.VTE3.b*, *BnaA.VTE4.a*, *BnaX.VTE4.b*, *BnaX.VTE4.c*, *BnaC.VTE5*, *BnaX.PDS1.a*, *BnaX.PDS1.b*, and *BnaA.PDS1.c*) from 96 accessions. Population structure (Q) and relative kinship (K) were analyzed by SSR marker genotyping. Association studies were performed using the models GLM+Q and PK-mixed. Between 12 and 26 polymorphisms within two tocopherol candidate genes (*BnaX.VTE3.a*, *BnaA.PDS1.c*) were significantly associated with tocopherol traits. The SNPs explained up to 16.93 % of the genetic variance for tocopherol composition and up to 10.48 % for total tocopherol content. CAPS markers were designed for genotyping the remaining 133 accessions and significant associations with various tocopherol traits confirmed the results of the first experiment.

This work presents functional genes from the tocopherol biosynthesis pathway of rapeseed. High sequence similarities and functional conservation between *A. thaliana* and *B. napus* were demonstrated. For the first time, associations between tocopherol traits and allelic variations at various candidate gene loci in rapeseed were demonstrated. These polymorphisms are most promising candidates for the development of molecular markers which will have considerable potential for the use in marker-assisted breeding for rapeseed varieties with improved tocopherol content and composition.