Molecular analysis of *Arabidopsis thaliana transparent testa* (*tt*) genes in *Brassica napus*

Tina Lotz[1], Rod Snowdon[1], Renate Horn[2], Gieta Dewal[3], Bernd Weisshaar[3], Wolfgang Friedt[1], Harry Belcram[4], Michel Caboche[4] and Boulos Chalhoub[4]

[1] Institute of Crop Science and Plant Breeding I,Justus-Liebig-University, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany, e-mail: tina.lotz@agrar.uni-giessen.de

[2] Department of Genetics & Biochemistry, University of Clemson, 100 Long Hall, Clemson, SC 29634-0324, USA

[3] Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, D-50829 Köln, Germany

[4] Unité de Recherches en Génomique Végétale - Institut National de Recherche Agronomique, 2, rue Gaston Crémieux, CP 5708, 91057 Évry Cedex, France

ABSTRACT

The yellow seed colour trait, which is controlled by flavonoid biosynthesis, is of particular interest for rapeseed breeding because of the associated improvements in feed grain quality after oil extraction. Numerous genes involved in flavonoid biosynthesis have been identified in *Arabidopsis thaliana* that cause when mutated the *transparent testa* (*tt*) phenotype. We are applying the accumulated knowledge from *A. thaliana* to oilseed rape (*Brassica napus*), the most important European oilseed crop. Our goal is to identify and characterise homologous genes implicated in yellow seed colour of oilseed rape. Oligonucleotide primers derived from *A. thaliana* sequence data for different *tt* loci were used to identify corresponding sequences in oilseed rape by PCR screening of a *Brassica napus* BAC library. Positive BAC clones are being analysed in detail to determine gene structure in a polyploid context.

Key words: seed colour – transparent testa – *tt* loci – Arabidopsis – candidate genes

INTRODUCTION

The flavonoid biosynthetic pathway has been studied in detail at the biochemical and genetic level in a number of plant species, particularly in *Zea mays*, *Petunia hybrida*, *Antirrhinum majus* and the model flowering plant *Arabidopsis thaliana* (Winkel-Shirley 2001). With regard to the latter, numerous loci that play a role in the synthesis of flavonoids have been studied in detail (Shirley *et al*. 1995; Graham 1998) and insertional mutagenesis experiments indicate that several additional loci are also involved (Wisman *et al*. 1998). Gene loci that affect the synthesis of brown pigments (condensed tannins) in the *A. thaliana* seed coat are collectively named *transparent testa* (*tt*) loci. The locus *TT1* is involved in development of the seed endothelium, in which brown tannin pigments accumulate (Sagasser et al. 2002). Several further loci (*TT3*, *TT4*, *TT5, TT6* and *TTG*) are also required for the accumulation of purple anthocyanins in leaves and stems, and *TTG* also plays additional roles in trichome and root hair development (Koornneef 1994). Numerous single-copy *A. thaliana* flavonoid biosynthetic genes have been identified and correlated with specific loci: chalcone synthase (CHS) with *TT4* (Feinbaum and Ausubel 1988), chalcone isomerase (CHI/CFI) with *TT5* (Shirley *et al*. 1992), dihydroflavonol 4-reductase (DFR) with *TT3* (Shirley et al. 1992), flavanone 3-hydroxylase (F3H) with *TT6* (Wisman *et al.* 1998) and flavanone 3'-hydroxylase (F3'H) with *TT7* (Schoenbohm *et al*. 2000), respectively.

The different *tt* homologs identified in *B. napus* will be sequenced and compared both to each other and to orthologues identified in other species. Genetic distances between the different gene loci will be established and it is hoped that the homoeologous loci can ultimately be mapped in *B. napus* and compared to seed colour QTL identified in a project funded by the German genome programme "GABI" (see Badani et al. 2003), giving important information about the genome organisation of yellow seed colour genes in *Brassica napus.*

MATERIALS AND METHODS

A BAC library was constructed from the French oilseed rape cultivar 'Darmor-Bzh in the context of a Genoplante program. We adopted the strategy of identifying *B. napus* homologues from *tt*-genes of *Arabidopsis* at the nucleotide level. Primers for the comparative analysis of the flavonoid genes were designed by comparing *tt*-locus DNA sequences from *A. thaliana* to corresponding sequences from *B. napus* and other plant species when available.

The developed strategy, that is creating physical functional markers (PFM) from *A. thaliana* syntenic regions (Chalhoub *et al*. 2002, 2003 and in preparation), was used for contiguing BAC clones (Genoplante project).

RESULTS AND DISCUSSION

Homoeologues for six *TT* loci (*TT*1, *TT*3, *TT*4, *TT*5, *TT*6 and *TT*7) were identified in *B. napus* cultivar 'Darmor Bzh'. As expected, we detected multiple copies of these genes in rapeseed (Table 1), although these genes are single-copy genes in *A. thaliana* (Shirley et al.1995).

Table 1: Positive clones detected after screening of a *B. napus* BAC library with PCR primers designed from *A. thaliana transparent testa* loci.

Flavonoid Gene	*A. thaliana TT* locus	Positive BAC clones in *B. napus*	Expected alleles in *B. napus*
WIP zinc-finger family	*TT1*	47	> 4
DFR	*TT3*	11	> 2
CHS	*TT4*	115	> 10
CHI/ CFI	*TT5*	32	> 4
F3H	*TT6*	6	2
F3'H	*TT7*	8	> 1

To accurately determine the number of *TT* gene copies in *B. napus*, the respective gene loci were isolated for sequencing and to allow further comparative studies. Some of the duplicated regions identified with PFM primers (surrounding the *BnTT* genes) detect BAC clones that do not contain *TT* gene homologues (but surround PFM). We are continuing our microsynteny analyses of the respective genome regions in *A. thaliana* and *B. napus*.

The systematic search for *A. thaliana TT* gene sequences in rapeseed and investigation of the surrounding genome regions involved has allowed us to identify putative homologues of these genes in the amphidiploid *B. napus* genome and to study microsynteny in the genome regions containing the respective loci. In almost all cases multiple copies of the *A. thaliana* loci could be observed in *B. napus*, especially for CHS (chalcone synthase) where at least 15 copies of the gene appear to be present. Using locus-specific primers for the individual homoeologous loci in *B. napus* we hope to identify polymorphisms that will enable us to map these closely related genes. This would open the possibility to detect associations between gene loci involved in the flavonoid biosynthesis pathway in *B. napus* and seed colour QTL, potentially allowing the identification of candidate genes and ultimately genetic markers for the valuable yellow seed colour trait in oilseed rape.

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