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Cloning and Expression Level Analysis of Two *BnaANT* Candidate Genes in *Brassica napus*

CHEN Bo[1*], WANG Tai-xia[2*], WANG Han-zhong[1], LI Yun-chang[1], YAN Xiao-hong[1], WANG Li-jun[1] and WEI Wen-hui[1]

[1] *Institute of Oil Crops, Chinese Academy of Agricultural Sciences/Key Laboratory for Biological Sciences of Oil Crops, Ministry of Agriculture, Wuhan 430062, P.R.China*
[2] *College of Life Sciences, Henan Normal University, Xinxiang 453007, P.R.China*

Abstract

AINTEGUMENTA (*ANT*) gene has been proved to have a novel function on controlling the organ size and the seed weight in *Arabidopsis thaliana*, the research on its orthologous gene in *Brassica napus* will be of potential interest to elucidate the molecular mechanism of rapeseed development and increase the rapeseed output. A SMART cDNA library of the flower bud from *B. napus* cultivar Zhongshuang 9 was constructed, and the full-length cDNAs of two homologous *BnaANT* candidate genes were cloned by PCR in *B. napus*, namely *BnaX.ANT.a* (GenBank accession no. DQ211969) and *BnaX.ANT.b* (accession no. DQ211970). They shared 87.1 and 83.4% amino acid identity with *ANT* of *A. thaliana*, respectively. There is 87.4% amino acid identity between *BnaX.ANT.a* and *BnaX.ANT.b*. By quantitative real-time PCR, the obviously differential expression levels of the two *BnaANT* candidate genes were detected in the 28 DAF seeds of the big and small grains *B. napus* lines, the expression abundance of *BnaANT* in the 28 DAF seed of 9311 was over three times compared to that of 9260, which indicates that these two *BnaANT* genes are also likely related to the floral organ size and the seed weight in *B. napus*.

Key words: *AINTEGUMENTA* gene, *Brassica napus*, *BnaANT* gene, yield-related gene, quantitative real-time PCR

INTRODUCTION

The *AP2*/*EREBP* (*APETALA2*, ethylene responsive element binding protein) multigene family includes developmentally and physiologically important transcription factors in plants (Krizek *et al*. 2000; Krizek 2003; Western *et al*. 2004). *AP2*/*EREBP* genes are divided into two subfamilies, including *AP2* genes with two AP2 domains and *EREBP* genes with a single AP2/ERF (ethylene responsive element binding factor) domain. Based on previous phylogenetic analyses, *AP2* genes can be divided into two clades, *AP2* and *ANT* groups (Shigyo *et al*. 2006).

AINTEGUMENTA (*ANT*) was firstly identified as a gene that regulated the flower development, and was required for the formation and development of organ anlagen by Elliott *et al*. (1996), Klucher *et al*. (1996), Krizek (1999), Mizukami and Fischer (2000), and Azhakanandam *et al*. (2008). It also plays an important role in the formation of pistil and petal (Liu *et al*. 2000; Krizek *et al*. 2000). In the flower morphogenesis ABC model, *ANT* is belonging to the C-type gene that controls the formation of stamen and petal, and it

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Correspondence WEI Wen-hui, Associate Professor, Tel/Fax: +86-27-86817881, E-mail: whwei@oilcrops.cn
* These authors contributed equally to this work.

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negatively controls *AGAMOUS* (*AG*) gene, its function controlling the development of flower has been fully understood (Bowman *et al*. 1993; Elliott *et al*. 1996). *ANT* has conserved YRG and RAYD sequences (Okamuro *et al*. 1997), protein sequence analysis indicated that RAYD sequence could form an amphoteric α-helix and involve in the interaction between protein and DNA (Okamuro *et al*. 1997; Nole-Wilson and Krizek 2000). The conserved amino acid sequences enabled it to bind the DNA *cis*-element (Liu *et al*. 2006). The distribution of this gene is very wide, existing in all the angiosperm and gymnosperm. All these make the study of *ANT* gene great significance in crop plant breeding.

Brassica napus is one of the most important oil crops in China, a main purpose for its breeding is to increase the seed yield. However, the molecular genetic basis controlling seed yield of *B*. *napus* is unknown. In *Arabidopsis thaliana*, the quantitative trait loci (QTLs) controlling the seed yield have been located at the end of the short arm of chromosome 1 and the ends of the long and short arms of chromosome 5 (Alonso-Blanco *et al*. 1999). Recently, a single gene in rice was found controlling grain weight (Song *et al*. 2007), or plant height and panicle size (Xue *et al*. 2008). Whereas people have not found out those genes controlling the seed yield in *B*. *napus* so far, and thereby limited understanding of its molecular mechanisms and the performing of its genetic improvement. At present, how to find the major factors controlling the seed yield has become one of the focus tasks in the molecular breeding of *B*. *napus*. Although a number of genes determining the seed size and seed yield of plants, such as *GhWBC1* (Zhu *et al*. 2003), *ABORTED MICROSPORES* (*AMS*) (Sorensen *et al*. 2003), *GIBBERELLINs* (*GAs*) (Swain *et al*. 2004), *FRUITFULL* (*FUL*) (Gu *et al*. 1998), *Cytochrome P450* (Ito and Meyerowitz 2000), and *PRETTY FEW SEEDS2* (*PFS2*) (Park *et al*. 2005), have been cloned, the mutants of these genes were usually abortive, and could not be used in crop breeding directly. It has been proved that the heterogeneous expression of *ANT* gene has important influence on the organ size, especially on the seed mass. When *ANT* was over expressed under 35S promoter, tissue hyperplasias appeared in the every organs of *A*. *thaliana*, and the seed mass also increased (Mizukami and Fischer 2000). The discovery of the novel function of *ANT* makes it as one of the most important candidates to increase seed yield of crops.

To clone the orthologous *ANT* gene from *B*. *napus*, a SMART cDNA library was constructed with the flower bud tissue of *B*. *napus* cultivar Zhongshuang 9, and two copies of the full-length cDNA of *BnaANT* were cloned according to the homologous sequence. Two *B*. *napus* lines, 9260 and 9311, have obvious differences in the seed size and the silique length, an obviously different expression level of *BnaANT* could be detected in their 28 DAF seeds by quantitative real-time PCR. These results will be a basis for further study on the functions of *BnaANT* gene in *B*. *napus*.

MATERIALS AND METHODS

Materials

B. *napus* cultivar Zhongshuang 9 was provided by the Institute of Oil Crops, Chinese Academy of Agricultural Sciences. The pedigree of *B*. *napus* small grain inbred line 9260 is: The pollen grains of the F_1 hybrid between maternal yellow seed rapeseed line C2H01 and paternal black seed rapeseed line 96P54 were cultured to obtain haploid seedling, then doubled with colchicine treatment, the DH inbred line is named 9260. *B*. *napus* big grain inbred line 9311 was obtained as the same method above, the maternal was Youza 2 and the paternal was large-seed line 204-1. For 9260, the 1 000-seed weight was (3.153±0.083) g, the average length of silique was (6.5±0.1) cm, and for 9 311, the 1 000-seed weight was (5.230±0.060) g, the average length of silique was (11.1±0.2) cm.

Reagents

T4 DNA ligase, pyrobest *Taq* polymerase, and pMD18-T were bought from TaKaRa Company (Japan); RNeasy Plant Mini Kit, Oligotex mRNA and QuantiTect SYBR Green PCR Kit from Qiagen Co. (Shanghai); MMLV and DNase I from Promega Co.(Beijing); SMART cDNA Library Construction Kit from BD Co.(USA); DNA Purification Kit from Fermentas Con.(Canada); *Taq* polymerase from BioStar Co.(Canada), other reagents from Chinese Wuhan Zhongxin Boyer Bioengineering