Indian Journal of Exprimental Biology
Vol. 51, December 2013, pp. 1130-1136

Molecular cloning and mRNA expression profile of Sucrose Transporter Gene *BnSUT1C* from *Brassica napus* L.

FuPeng Li[1,2], Lin Yan[2], Jianxiong Lai[2], ChaoZhi Ma[1*], Mayank Gautam[1] & TingDong Fu[1]

[1]National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, P.R. China
[2]Spice and Beverage Research Institute, Chinese Academy of Tropical Agricultural Science, Wanning 571533, P.R. China

Received 8 November 2012; revised 10 September 2013

The genomic and cDNA sequences of *BnSUT1C* were isolated from *B. napus*. Combination of cDNA and genomic DNA sequences revealed that the *BnSUT1C* gene contained three exons and two introns. The cDNA encodes a protein of 513 amino acids with a calculated molecular mass of 54.7 kDa and an isoelectric point of 9.12. It exhibits typical features of sucrose transporter with 12 trans-membranes spanning domains. BnSUT1C showed highly homologous with AtSUC1 and AtSUC5. A histidine residue, which is conserved across all functional sucrose transporter proteins in higher plants, is located at position 66 of the BnSUT1C. Two putative pollen-specific *cis*-elements, AGAAA and GTGA motifs, are located in 5′-upstream of *BnSUT1C*. The spatial and temporal expression patterns carried out by semi-quantitative RT-PCR and Real-Time PCR, which indicated that *BnSUT1C* predominantly expressed in later developmental stages of anther, as tapetal cells began to shrink and collapse. BnSUT1C could mediate the uptake of sucrose in the pollen and retrieval of tapetal degenerated products during pollen maturation.

Keywords: Anther, *Brassica napus*, Expression pattern, Sucrose transporter

In higher plants, sucrose is major photosynthetic product in source (autotrophic) organs, and is the most common transported form in sink (heterotrophic) organs mediated through the phloem sieve element-companion cell complex (SE-CCC). Sucrose transporters play an irreplaceable role during sucrose trans-membrane process. Since, the first plant sucrose carrier was isolated from spinach (*Spinacia oleracea* L.)[1], termed as *SUT* (*SUC*), and sores of *SUT*s have been observed in various plant species[2-9]. SUTs were located in vein or SE-CCC and play a central role of sucrose loading and unloading[10,11]. In addition to phloem loading, SUTs have been shown to mediate sucrose uptake into storage tissues and developing filial tissues/organs. In developing seeds, all nutrients are translocated from maternal tissues to filial tissues, symplastically[12]. *VfSUT1* gene express in the epidermis of embryo in fava bean (*Vicia faba* L.)[13], imports high concentrations of sugars during storage phase. Overexpression of SUTs, regulated by seed-specific promoter, enhanced both sucrose influx rates into cotyledons and seed growth rates at the linear phase[14,15]. *LeSUT1* behaves an essential role in phloem loading; while *LeSUT2* controls fruit and seed development in tomato[16]. Three *TaSUT1* are highly expressed in filling grain in wheat[17].

Furthermore, it is reported that the expression and location of SUTs in anther are involved in pollen development. *AtSUC1* shows strong expression in pollen and involved in pollen development[18-20]. As expected, the germination rate of *AtSUC1* mutant pollen decreases[18]. *PmSUC1* and *LeSUT2* are expressed in pollen grains and pollen tubes in plantago and tomato, and involved in the import of sucrose to the developing pollen and growing pollen tube[16,21]. Moreover, *NtSUT3* is only detected in pollen and restricted to late pollen development, pollen germination and pollen tube growth[22]. *OsSUT1* mutant can result in pollen dysfunction in rice[23].

B. napus (AACC, 2*n*=38) is an amphidiploid plant arising from interspecies hybridization between *B. rapa* (AA, 2*n*=20) and *B. oleracea* (CC, 2*n*=18)[24]. *B. napus* is an oil crop and used for both direct human consumption and bio-fuel, all over the world. There is an increasing demand for high seed yield per unit in *B. napus*. In oilseed, source and sink organs are superfluous and assimilate translocation, which is the most critical factor limiting factor for seed yield[25,26],

*Correspondent author
Telephone: +86-27-87281676
Fax: +86-27-87280009
E-mail: yuanbeauty@mail.hzau.edu.cn

resulting in many abortive pods and shrunken seeds at maturity[27]. *BnA7.SUT1* alleles, located on A genome, show distinguished expression pattern, resulting in yield-related traits phenotype variation[28]. In the present investigation, the *BnSUT1C* gene, which show high identity to the *BoSUC1* of *B. oleracea*, was isolated and characterized from C genome, in order to understand the role of sucrose transporter in *B. napus*. *BnSUT1C* was showing expression only in flower buds and anther. Expression pattern of different developmental stages of flower buds showed that BnSUT1C may function in anther development during tapetum collapse stages.

Materials and Methods

Plant materials—A self-incompatible 'S-1300' *B. napus* line was used to isolate *BnSUT1C*. Two other semi-winter *B. napus* lines, '3706' and '3715', were planted for quantificational analyses. Oilseed plants were grown under field conditions during 2008-2010 at Huazhong Agricultural University, Wuhan, Hubei province, China. Leaves, shoots, roots, flower buds, pods and pericarp of pods, sepal, petal, anther and pistil were frozen in liquid nitrogen to perform expression analysis.

DNA extraction and RNA extraction—Genomic DNA was extracted from fresh leaves by CTAB methodology[29]. DNA concentration and purity was measured by a GeneQuantII spectrophotometer (Fisher Scientific, CA, USA) at a wavelength of 260 nm versus 280 nm, then adjusted to 50 ng/μL in TE buffer (10 m*M* Tris, 1 m*M*, EDTA *p*H 8.0).

Total RNAs were extracted from respective tissues using Tripure reagent (Bioteke, http://www.bioteke.com/chn/). Final concentration was adjusted to 1 μg/μL in double-distilled water treated with 0.1% DEPC. The total RNAs (1.0 μg) were reverse-transcripted (RT) using M-MLV Reverse transcriptase (Fermentas, Ontario, Canada) according to the manufacturer's instructions. The resultant first-strand cDNA mixtures were diluted 50-fold with double-distilled water and used as a template for performing further PCR.

Isolation of BnSUT1C and its promoter—Three *Brassica* fragments (GenBank accession numbers AY190281, AY065839, AC189334), which showed high sequence homology with *AtSUC1* (*Arabidopsis*) sequence[30], were obtained from The *Arabidopsis* Information Resource (TAIR) database. Conservative sections were used to design primers and obtain core *BnSUT1C*. Remnant fragments and the promoter region were isolated by thermal asymmetric interlaced (TAIL) PCR[31,32]. Based on the contig, gene specific primers were developed. The full-length coding domain sequence (CDS) and genomic DNA sequence were amplified with PCR reaction volumes of 20 μL, 50 ng genomic DNA, 1 unit *Taq* polymerase (MBI Fermentas, Lithuania), 2 μL 10×*Taq* buffer with $(NH_4)_2SO_4$, 2 m*M* $MgCl_2$, 0.2 m*M* dNTP mixes (Sangon, Shanghai, China), 0.5 μ*M* of each primer. PCR amplifications were carried out: initial denaturation for 4 min at 94 °C, 30 cycles of 45 sec at 94 °C, annealing at 60 °C for 45 sec and extension for 60 sec at 72 °C, followed by an extension of 10 min at 72 °C. PCR products were size-separated by 1.2% agrose gel electrophoresis and purified using the Gel Purification Kit (Sangon, Shanghai, China). The specific PCR products were ligated into the pMD18-T vector (TaKaRa, Dalian, China), and then the recombinants were transferred into *Escherichia coli* Top10. Positive transformed clones were screened for sequencing.

Sequence analysis—Sequence homology were carried out using GenBank BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequence homology analysis was carried out using the DNAMAN program (version 5.0, Lynnon Biosoft). Characterization and secondary structure of the protein were analyzed by Expert Protein Analysis System (http://expasy.org/tools/). *Cis*-elements of *BnSUT1C* were predicted by PLACE Web Signal Scan (http://www.dna.affrc.go.jp/PLACE/).

Expression analysis—For semi-quantitative RT-PCR, product of the first-strand cDNA synthesis reaction was used as a template for amplification in a PCR reaction with primers: EX-P1 (5'-GAG ACG CGA GGT TGA AGC-3') and EX-P2 (5'-GCA TAT GGA CCG GCG ACT-3') for cDNA amplification to detect the *BnSUT1C* transcript or with primers RAC1-P3 (5'-CTA TCC TCC GTC TCG ATC TCG C-3') and RAC1-P4 (5'-CTT AGC CGT CTC CAG CTC TTG C-3') specific for *B. napus Actin* (GeneBank accession number: AF111812) as an endogenous control. The PCR reactions and conditions were performed following operation of *BnSUT1C* isolation. The amplified DNA fragments were detected on 1.2% agarose gel.

Moreover, Real-Time quantitative PCR was performed using SYBR Green Realtime PCR Master Mix (TOYOBO, Osaka, Japan). The PCR reactions contained 400 n*M* of both forward and reverse gene

specific primers and 8.4 µL of the 50-fold diluted RT reaction in a final volume of 20 µL. The thermal cycling protocol was followed by DNA polymerase activation at 95 °C for 3 min. The PCR amplification was carried out by 45 cycles of denaturation at 95 °C for 10 sec, primer annealing at 60 °C for 15 sec, and extension at 72 °C for 30 sec. Optical data were acquired following the extension step, and the PCR reactions were subject to melting curve analysis beginning at 65 °C through 95 °C, at 0.1 °Csec^{-1}. The data are presented as mean ± SD of three independently produced RT preparations used for PCR run, each having four replicates.

Results

Cloning and sequence analysis of BnSUT1C—The sequenced fragments were primarily analyzed and spliced the SEQMAN application of the DNASTAR software suite (Windows version 5.0.2; DNASTAR, Madison, Wis.). The complete open reading frame (ORF) was identified with program FGENESH (http://www.softberry.com/berry.phtml). Primers were designed in the predicted 5' and 3' untranslated regions of joint sucrose transporter-like gene : CDS-3-UP (5'-AAG ATG CGG CGG TTG TAG AG-3') CDS-3-LOW (5'-GGT GGT GAA GGT AAA ACG GT-3'). These primers were used to amplify cDNA sequences of *BnSUT1*. Forty-four cDNA sequences were isolated various organs/tissues, classifying into four clusters. Three clusters expressed in all test tissue/organs, two of which were located in A genome (A7) of *B. napus* and associated with yield-related traits[28]. The remaining cluster shared 100% CDS identity with *B. oleacea* sucrose transporter gene *BoSUC1* (GenBank accession number: AY065839); this cluster was located on C genome of *B. napus* and designated as *BnSUT1C*. Hence, Gene-specific primers were designed: SUT-3-UP (5'-CAA TGG GAG CTT TTG AAA CAG-3') and SUT-3-LOW (5'-GAA ACC TAA TGT AAA ACT AAT GG-3'). These primers were used to amplify the full length cDNA and genomic sequences of *BnSUT1C*, and 1.5 kb of the promoter was isolated by TAIL-PCR.

Combination of the cDNA and genomic DNA sequences revealed that *BnSUT1C* gene is 3,289 bp in length, containing three exons and two introns. The open reading frame (ORF) of *BnSUT1C* contains 1,542 bp and encodes a protein of 513 amino acids (Fig. 1) with a calculated molecular mass of 54.7 kDa and an isoelectric point of 9.12. Hydrophobicity profile analysis of BnSUT1C, revealed the presence of 12 trans-membrane spanning domains, with both N- and C-terminal domains located on the intracellular side of plasma membrane. This model is consistent with the structure of SUTs proposed in previous studies. Further, a histidine residue located at position 66 of the BnSUT1C, similar to previously shown site-directed mutagenesis in Arabidopsis AtSUC1, involved in sucrose binding during the

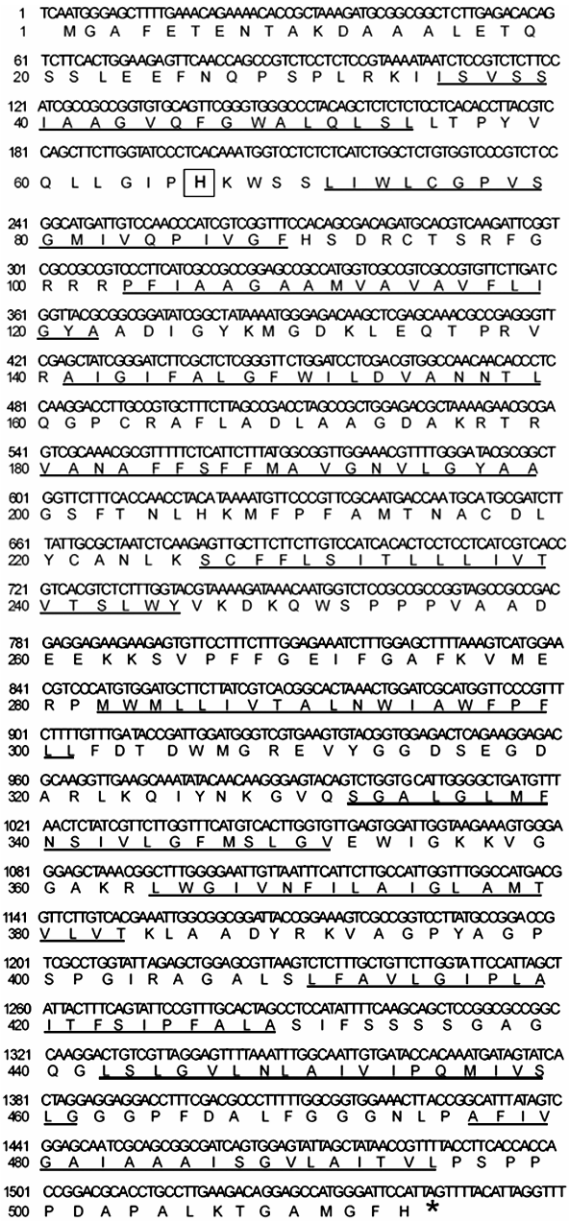

Fig. 1—cDNA sequence and deduced amino acid sequence of *B. napus BnSUT1C* sucrose transporter. The 66th conserve histidine of BnSUT1C is bold and boxed up. Predicted transmembrane regions (HMMTOP) are underlined and the stop condon is marked by an asterisk

transport process[33]. The *BnSUT1C* (GenBank no. JN020959) showed more than 85% identity with *AtSUC1* (AT1G71880). BnSUT1C is showing high homology to AtSUC1 and AtSUC5, and is classified to SUT1 subfamily[34].

Cis-elements of BnSUT1C promoter—A search for *cis*-regulatory elements in the 5'-upstream region of *BnSUT1C* (-1,366 to +171) was performed using PLACE program, in order to predict the function of *BnSUT1C*. Firstly, the putative TATA box was confirmed, a closed CCAAT box is present at -232 position, relative to the transcription start site of *BnSUT1C*. The promoter contains three copies of AGAAA motifs (POLLEN1LETAT52)[35,36] (-210, -243 and -305) and eight copies GTGA motifs (GTGANTG10)[37] (-170, -685, -692, -850, -963, -1005, -1112 and -1262) (Fig. 2), later shown to be which are associated with later anther and pollen development. Hence, it is speculated that *BnSUT1C* probably express in anther and involved in pollen development.

Expression pattern of BnSUT1C—Semi-quantitative RT-PCR analysis was performed with different tissues, to investigate the expression pattern of *BnSUT1C* in various organs/tissues.The *BnSUT1C* transcript was mainly detected in buds; diffused signals were detected in leaves, stems, roots, pods and pericarp (Fig. 3a). Interestingly, a higher transcript amount was initially detected in flower bud 5-6 mm of *B. napus*, corresponding to the pollen maturation stage, and weaker signal was obtained from flower bud 6-7 mm before flowering. However, no transcripts were detected from flower bud 1-2 mm, flower bud 3-4 mm and open flower (Fig. 3b).

In order to ascertain expression pattern of *BnSUT1C* in bud, flower bud (5-6 mm) were divided into sepal, petal, anther and pistil, and RT-PCR was performed. *BnSUT1C* transcript was showing high expression in anther, with diffused signal in petal tissues (Fig. 3c). These results were also validated by Real-Time PCR (Fig. 3) which further indicate that *BnSUT1C* was strongly expressed in anthers.

Discussion

B. napus is an allotetraploid derived from interspecific hybridization of *B. rapa* and *B. oleracea*[38]. *BnA7.SUT1* alleles, located on A genome, were significantly associated with yield-related traits[28]. *BnSUT1C* showed non-variation sequences in two *B. napus* lines 'S-1300' and 'Eagle' distinguished in vegetative and reproductive traits[39]. Therefore, in the present work, we focused on the other possible function of *BnSUT1C*.

The predicted BnSUT1C contains almost all conserved amino acids and motifs on sucrose transporter protein, and show the conversed secondary structure of plant SUTs[34]. Histidine plays a primary role in sucrose binding. Amino acid sequences showed that, histidine residue was conserved across all functional sucrose transporter proteins present in higher plants. Moreover, a histidine residue was located at position 66 in BnSUT1C. Higher similarity of BnSUT1C with other functional SUTs indicated that BnSUT1C proteins may play an important role in sucrose transport.

Interestingly, *BnSUT1C* expressed in flower bud exclusively, showed its potential function in anther development. The predominant expression of *BnSUT1C* in flower buds and anther seemingly indicated its active involvement in assimilates unloading process. Further, in the present study, *BnSUT1C* mRNA was restricted to express in the very late stages of flower development (flower bud

Fig. 2—Localization of *cis*-regulatory elements in *BnSUT1C* promoter nucleotide sequence. The transcription start site is in bold. The putative TATA box and CAAT box are indicated by a thin solid underline with names. The putative pollen-specific motifs AGAAA and GTGA are indicated by solid underline with rhombus and pentacle respectively.

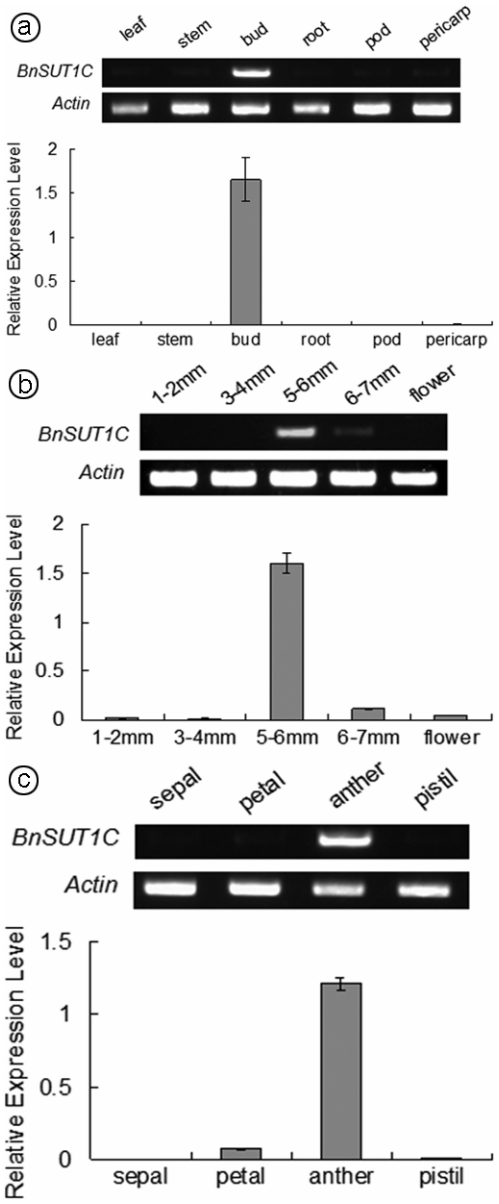

Fig. 3—RT-PCR and Real-Time PCR analysis of *BnSUT1C* expression. (a) Expression in various organs/tissues of plant during flowering, including leaves, stems, buds, roots, pods and pericarp. (b) Expression in flower bud 1-2, 3-4, 5-6, 6-7 mm and open flower. (c) Expression in respective tissues of bud, including sepal, petal, anther and pistil. Amplification of *Actin* cDNA was used as a control.

5-6 mm and 6-7 mm, Fig. 3), corresponding to the pollen maturation stage which are in consistency with previous study on the stamen developmental stages 11-13 in Arabidopsis[40,41]. Therefore, it could be suggested that sucrose is taken up by BnSUT1C for pollen development. However, the temporal phase of *BnSUT1C* appeared to be inconsistent with the presumed roles of *BnSUT1C*, as it was expressed more specifically during tapetum collapse of pollen maturation stage. Possibly, BnSUT1C could medicate the uptake of sucrose in the pollen and retrieval of tapetal degenerated products during pollen maturation. The tapetum plays a crucial role in biosynthesis and substrates supply. The majority of exine constituents are derived from tapetal cells during early pollen development stages; the tapetum secretes mainly lipidic components of pollen coats at later pollen developmental stages[42,43]. After tri-nucleate stage, tapetal cells begin to degrade and release abundant degenerated products. So, BnSUT1C might play a crucial in retrieval of sucrose and anther development.

Arabidopsis, a model plant and a member of *Brassicaceae* is a natural choice of the investigator for studying the gene function and their role in plant functionality[41,44,45]. However, it is difficult for anther investigation, as *Arabidopsis* buds are small to separate specific tissue. *Brassica napus* is one of the closest relatives of *Arabidopsis*, and sequence similarity show processes of anther development are highly similar. The buds of *B. napus* are large enough to separate different tissues of flower bud.

In conclusion, *BnSUT1C* was isolated from *B. napus* and showed higher homology with *BoSUC1*. Promoter and expression analyses, both indicated that *BnSUT1C* was expressed in anther of *B. napus* and might play a crucial role in pollen maturation, which in turn influences yield traits.

Acknowledgment

This work was financially supported by the State Key Basic Research and Development Plan of China (2007CB109001) and Hi-Tech Research and Development Programs of China (2011AA10A104) and the National Science Foundation of China (30971802).

References

1 Riesmeier J W, Willmitzer L & Frommer W B, Isolation and characterization of a sucrose carrier cDNA from spinach by functional expression in yeast, *EMBO J*, 11 (1992) 4705.

2 Gahrtz M, Stolz J & Sauer N, A phloem-specific sucrose-H^+ symporter from *Plantago major* L. supports the model of apoplastic phloem loading, *Plant J*, 6 (1994) 697.

3 Hirose T, Imaizumi N, Scofield G N, Furbank R T & Ohsugi R, cDNA Cloning and tissue specific expression of a gene for sucrose transporter from rice (*Oryza sativa* L.), *Plant Cell Physiol*, 38 (1997) 1389.

4 Weise A, Barker L, Kühn C, Lalonde S, Buschmann H, Frommer W B & Ward J M, A new subfamily of sucrose transporters, SUT4, with low affinity/high capacity localized in enucleate sieve elements of plants, *Plant Cell*, 12 (2000) 1345.

5 Aldape M J, Elmer A M, Chao W S & Grimes H D, Identification and characterization of a sucrose transporter isolated from the developing cotyledons of soybean, *Arch Biochem Biophys*, 409 (2003) 243.

6 Burkle L, Hibberd J M, Quick W P, Kuhn C, Hirner B & Frommer W B, The H^+-sucrose cotransporter NtSUT1 is essential for sugar export from tobacco leaves, *Plant Physiol*, 118 (1998) 59.

7 Barker L, Kühn C, Weise A, Schulz A, Gebhardt C, Hirner B, Hellmann H, Schulze W, Ward J M & Frommer W B, SUT2, a putative sucrose sensor in sieve elements, *Plant Cell*, 12 (2000) 1153.

8 Wahl R, Wippel K, Goos S, Kamper J & Sauer N, A novel high-affinity sucrose transporter is required for virulence of the plant pathogen *Ustilago maydis*, *Plos Biol*, 8 (2010) e1000303.

9 Decourteix M, Alves G, Brunel N, Améglio T, Guillio A, Lemoine R, Pétel G & Sakr S, *JrSUT1*, a putative xylem sucrose transporter, could mediate sucrose influx into xylem parenchyma cells and be up-regulated by freeze-thaw cycles over the autumn-winter period in walnut tree (*Juglans regia* L.), *Plant Cell Environ*, 29 (2006) 36.

10 Kühn C, Franceschi V R, Schulz A, Lemoine R & Frommer W B, Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements, *Science*, 275 (1997) 1298.

11 Schmitt B, Stadler R & Sauer N, Immunolocalization of solanaceous SUT1 proteins in companion cells and xylem parenchyma: new perspectives for phloem loading and transport, *Plant Physiol*, 148 (2008) 187.

12 Patrick J W & Offler C E, Compartmentation of transport and transfer events in developing seeds, *J Exp Bot*, 52 (2001) 551.

13 Weber H, Borisjuk L, Heim U, Sauer N & Wobus U, A role for sugar transporters during seed development: Molecular characterization of a hexose and a sucrose carrier in fava bean seeds, *Plant Cell*, 9 (1997) 895.

14 Rosche E, Blackmore D, Tegeder M, Richardson T, Schroeder H, Higgins T J, Frommer W B, Offler C E & Patrick J W, Seed-specific overexpression of a potato sucrose transporter increases sucrose uptake and growth rates of developing pea cotyledons, *Plant J*, 30 (2002) 165.

15 Zhou Y, Chan K, Wang T L, Hedley C L, Offler C E & Patrick J W, Intracellular sucrose communicates metabolic demand to sucrose transporters in developing pea cotyledons, *J Exp Bot*, 60 (2009) 71.

16 Hackel A, Schauer N, Carrari F, Fernie A R, Grimm B & Kühn C, Sucrose transporter LeSUT1 and LeSUT2 inhibition affects tomato fruit development in different ways, *Plant J*, 45 (2006) 180.

17 Aoki N, Whitfeld P, Hoeren F, Scofield G, Newell K, Patrick J, Offler C E, Clarke B, Rahman S & Furbank R T, Three sucrose transporter genes are expressed in the developing grain of hexaploid wheat, *Plant Mol Biol*, 50 (2002) 453.

18 Sivitz A B, Reinders A & Ward J M, *Arabidopsis* sucrose transporter AtSUC1 is important for pollen germination and sucrose-induced anthocyanin accumulation, *Plant Physiol*, 147 (2008) 92.

19 Stadler R, Truernit E, Gahrtz M & Sauer N, The AtSUC1 sucrose carrier may represent the osmotic driving force for anther dehiscence and pollen tube growth in *Arabidopsis*, *Plant J*, 19 (1999) 269.

20 Sivitz A B, Reinders A, Johnson M E, Krentz A D, Grof C P L, Perroux J M & Ward J M, *Arabidopsis* sucrose transporter *AtSUC9*: High-affinity transport activity, intragenic control of expression, and early flowering mutant phenotype, *Plant Physiol*, 143 (2007) 188.

21 Lauterbach C, Niedermeier M, Besenbeck R, Stadler R & Sauer N, Immunolocalization of the PmSUC1 sucrose transporter in *Plantago major* flowers and reporter-gene analyses of the *PmSUC1* promoter suggest a role in sucrose release from the inner integument, *Plant Biol*, 9 (2007) 357.

22 Lemoine R, Burkle L, Barker L, Sakr S, Kuhn C, Regnacq M, Gaillard C, Delrot S & Frommer W B, Identification of a pollen-specific sucrose transporter-like protein NtSUT3 from tobacco, *FEBS Lett*, 454 (1999) 325.

23 Hirose T, Zhang Z J, Miyao A, Hirochika H, Ohsugi R & Terao T, Disruption of a gene for rice sucrose transporter, *OsSUT1*, impairs pollen function but pollen maturation is unaffected, *J Exp Bot*, 61 (2010) 3639.

24 UN, Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization, *Jpn J Bot*, 7 (1935) 389.

25 Chhabra M L, Translocation pattern of assimilates in India mustard, Proc 7th Int Rapeseed Cong, Poznan, Poland, 11-14 May, 1987.

26 Shen J X, Fu T D, Yang G S, Ma C Z & Tu JX, Genetic analysis of rapeseed self-incompatibility lines reveals significant heterosis of different patterns for yield and oil content traits, *Plant Breeding*, 124 (2005) 111.

27 Jullien A, Mathieu A, Allirand J M, Pinet A, de Reffye P, Cournède P H & Ney B, Characterization of the interactions between architecture and source-sink relationships in winter oilseed rape (*Brassica napus*) using the GreenLab model, *Ann Bot*, 107 (2011) 765.

28 Li F P, Ma C Z, Wang X, Gao C B, Zhang J F, Wang Y Y, Cong N, Li X H, Wen J, Yi B, Shen J X, Tu J X & Fu T D, Characterization of Sucrose transporter alleles and their association with seed yield-related traits in *Brassica napus* L., *BMC Plant Biol*, 11 (2011) 168.

29 Doyle J J & Doyle J L, Isolation of plant DNA from fresh tissue, *Focus*, 12 (1990) 13.

30 Sauer N & Stolz J, SUC1 and SUC2: Two sucrose transporters from *Arabidopsis thaliana*; expression and characterization in baker's yeast and identification of the histidine-tagged protein, *Plant J*, 6 (1994) 67.

31 Liu Y G & Whittier R F, Thermal asymmetric interlaced PCR: Automatable amplification and sequencing of insert end fragments from P1 and YAC clones for chromosome walking, *Genomics*, 25 (1995) 674.

32 Liu Y G, Mitsukawa N, Oosumi T & Whittier R F, Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR, *Plant J*, 8 (1995) 457.

33 Lu J M & Bush D R, His-65 in the proton-sucrose symporter is an essential amino acid whose modification with site-directed mutagenesis increases transport activity, *Proc Natl Acad Sci USA*, 95 (1998) 9025.

34 Lalonde S, Wipf D & Frommer W B, Transport mechanisms for organic forms of carbon and nitrogen between source and sink, *Annu Rev Plant Biol*, 55 (2004) 341.

35 Bate N & Twell D, Functional architecture of a late pollen promoter: pollen-specific transcription is developmentally

regulated by multiple stage-specific and co-dependent activator elements, *Plant Mol Biol*, 37 (1998) 859.

36 Filichkin S A, Leonard J M, Monteros A, Liu P P & Nonogaki H, A novel endo-beta-mannanase associated with anther and gene in tomato *LeMAN5* is pollen development, *Plant Physiol*, 134 (2004) 1080.

37 Rogers H J, Bate N, Combe J, Sullivan J, Sweetman J, Swan C, Lonsdale D M & Twell D, Functional analysis of *cis*-regulatory elements within the promoter of the tobacco late pollen gene *g10*, *Plant Mol Biol*, 45 (2001) 577.

38 Parkin I A P, Sharpe A G, Keith D J & Lydiate D J, Identification of the A and C genomes of amphidiploid *Brassica napus* (oilseed rape), *Genome*, 38 (1995) 1122.

39 Li Y Y, Shen J X, Wang T H, Chen Q F, Zhang X G, Fu T D, Meng J L, Tu J X & Ma C Z, QTL analysis of yield-related traits and their association with functional markers in *Brassica napus* L., *Aust J Agr Res*, 58 (2007) 759.

40 Scott R, Dagless E, Hodge R, Paul W, Soufleri I & Draper J, Patterns of gene expression in developing anthers of *Brassica napus*, *Plant Mol Biol*, 17(2) (1991) 195.

41 Sanders P M, Bui A Q, Weterings K, McIntire K N, Hsu Y C, Lee P Y, Truong M T, Beals T P & Goldberg R B, Anther developmental defects in *Arabidopsis thaliana* male-sterile mutants, *Sex Plant Reprod*, 11 (1999) 297.

42 Piffanelli P, Ross J H E & Murphy D J, Biogenesis and function of the lipidic structures of pollen grains, *Sex Plant Reprod*, 11 (1998) 65.

43 Yi B, Zeng F Q, Lei S L, Chen Y N, Yao X Q, Zhu Y, Wen J, Shen J X, Ma C Z, Tu J X & Fu T D, Two duplicate *CYP704B1*-homologous genes *BnMs1* and *BnMs2* are required for pollen exine formation and tapetal development in *Brassica napus*, *Plant J*, 63 (2010) 925.

44 Stirnberg P, van De Sande K & Leyser H M, *MAX1* and *MAX2* control shoot lateral branching in *Arabidopsis*, *Development*, 129 (2002) 1131.

45 Sorefan K, Booker J, Haurogne K, Goussot M, Bainbridge K, Foo E, Chatfield S, Ward S, Beveridge C, Rameau C & Leyser O, *MAX4* and *RMS1* are orthologous dioxygenase-like genes that regulate shoot branching in *Arabidopsis* and pea, *Gene Dev*, 17 (2003) 1469.