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Full Length Research Paper

High temperature induced fertility transition and anther carbohydrate metabolism modification in a canola recessive genic male sterile line

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High temperature stress is an important abiotic stress during plant growth and development that can damage canola male reproductive organ development. In this present study, we characterized a canola genic male sterile line that its fertility can be restored after high temperature induction with heterozygous but not homozygous condition. Anther length and weight was elongated and increased 2 and 5.7 times at 9 days after high temperature treatment in heterozygous line. Furthermore, viable pollen grain was produced in the anther. Anther carbohydrate content was assayed. The result showed that fructose and sucrose contents were much more stable under heat stress both in hetero- or homozygous lines. However, glucose and starch content was considerably increased after high temperature treatment in heterozygous line. In particular, anther glucose content was twice as much as that of starch content after 5 days high temperature treatment. Activity of neutral invertase, acid invertase, and sucrose synthase were increased in the anther of heterozygous line under heat stress. But activity of neutral invertase was the highest among these three sucrose cleavage enzymes. Both activity of sucrose phosphate synthase and starch phosphorylase were decreased under heat stress in the anther of heterozygous line. Activity of ADP glucose pyrophosphorylase was greatly increased in the heterozygous line, which was beneficial for starch accumulation. Transcription level of carbohydrate related genes was analyzed. 27 and 34 of the carbohydrate related genes were identified for down- and up-regulated in the anther of heterozygous line, respectively. Their function involved in the regulation of heat tolerance of heterozygous line was also discussed.

Key words: Canola, carbohydrate, gene.

INTRODUCTION

Appropriate temperature is important for canola (*Brassica napus* L.) growth and development. However, the temperature above (or below) its optimal range has a higher risk of high (or low) temperature stress. After flowering, as weather is getting warmer, canola plants always meet high temperature with irregular time, which affects its yield significantly (Angadi et al., 2000). Usually, the influence of heat stress on canola seed yield is adverse, which shows the reduction of seed yield. For example, Morrison (1993) illustrated that canola growth under 27/17°C light/dark condition throughout its life cycle showed almost totally sterile. Young et al. (2004) treated canola plants at 35°C for 1 or 2 weeks after the initiation of flowering and found that seed number and weight was

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Abbreviations: AGPase, ADP glucose pyrophophorylase; **AI,** acid invertase; **CMS,** cytoplasmic male sterile; **GMS**, genic male sterile; NI, neutral invertase; **SPS,** sucrose phosphate synthase; **SS,** sucrose synthase.

significantly declined due to the damage of micro- and mega-gametophyte fertility. Singh et al. (2008) reported that markedly heat tolerance among cultivars existed and the maximum temperature for canola tolerance was 33.7°C using pollen germination and pollen tube length as screening indices. All of previous examples indicated the negative effect of high temperature stress on canola reproductive organ development especially, the fertility change (from fertile to sterile). In addition to canola sterility, yield loss due to high temperature stress have also been reported in many crops such as cotton, rice, and groundnut (Prasad et al., 2000; Matrui and Omasa, 2002; Yasuor et al., 2006; Jagadish et al., 2007). Since its unfavorable influences on crop yield, identifying for high temperature stress tolerant germplasms and uncovering its mechanism should undoubtedly speed up canola heat stress resistance breeding progress.

In nature, thermo-sensitive materials that plant fertility can be changed from sterile to fertile phenomena exist. Youngner (1961) found that *Pennisetum clandestinum* was sterile under low temperature while fertile under high temperature condition. Li et al. (2009) characterized the fertility change in a wheat ecological male sterile line, BNS. They demonstrated that fertility of BNS can be changed from sterile to fertile as the temperature above 11.4°C. In canola, the famous cytoplasmic male sterile (CMS) line, pol CMS, also has high temperature fertile line according to the CMS types that are divided by Fu et al.(1990). Regardless of the fact that these materials were found, investigations on the mechanism for high temperature induced fertility transformation are very scarce. During heat stress, a lot of modifications on the physiological, biochemical, and molecular levels including carbohydrate metabolism correspondingly occur in plant male reproductive organs such as anther and pollen (Frank et al., 2009). Carbohydrate plays very important roles during plant growth and development (Rohde et al., 2004; Calenge et al., 2006; Nägele et al., 2010). During heat stress, Karni and Aloni (2002) demonstrated the reduction of the fructokinase and hexokinase activity in the bell pepper anther. In tomato, Pressman et al. (2002) documented that soluble sugars significantly reduced in the anther walls and pollen grains due to a decrease of starch concentration. Similar results were also reported from other studies (Jain et al., 2007; Frank et al., 2009; Snider et al., 2009). Thus, these results clearly revealed the close association between high temperature stress and carbohydrate metabolism.

In the present study, we characterized a type of recessive genic male sterile (GMS) canola line with high temperature fertile and low temperature sterile. The fertility transition can reduce the seed purity during hybrid seed production in GMS hybrid system because of the contamination of self-crossing. However, fertility restoration under heat stress provides the feasibility to breed high temperature tolerance variety in canola.

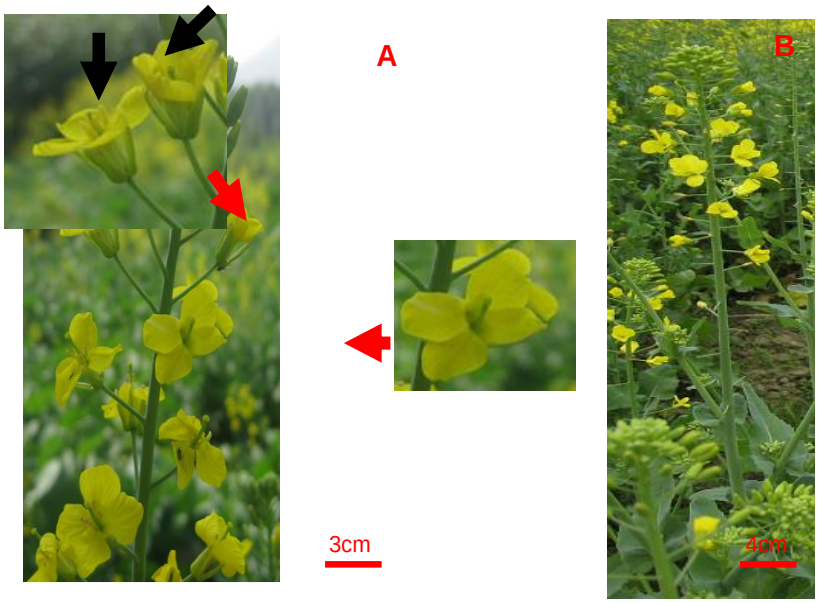
Therefore, interpreting the mechanism underlining GMS line fertility transformation is very meaningful. In our unpublished result from micro-array analysis during anther swelling, we identified many carbohydrate metabolism genes that were up or down-regulated. Combined with the importance of carbohydrate metabolism under heat stress as previously mentioned, we used a recessive GMS mutant that fertility can be changed under high temperature treatment as plant materials to assay anther carbohydrate metabolism including carbohydrate content determination, related enzymes activity measurement, and genes expression assay involved in the metabolism. The main purpose of this study was to understand the role of carbohydrate metabolism regulating anther fertility restoration with high temperature treatment.

MATERIALS AND METHODS

Plant material and growth conditions

A recessive genic male sterile *Brassica napus* line, ZA, was used throughout the experiments. ZA is a winter-type canola bred by our group and its homozygous and heterozygous genotype is *ms1ms1ms2ms2RfRf* and *ms1ms1ms2ms2Rfrf*, respectively. Fertility of the former is temperature stable while the latter is temperature dependent, which was based on the first observation in 2007 and in the consecutive two growing seasons in the field (Supplementary Figure 1A and B).

Two seeds were sown in a 4-L black pot filled with a mixture of natural soil from the field and peat moss. The pots were thinned to one seedling per pot during two-leaf stage. The pots were placed in a greenhouse with natural light and temperature before budding. Because ZA belongs to winter type, low temperature for vernalization was necessary in winter. In order to adapt to grow in the growth cabinet (GR48, Conviron, Winnipeg, MB, Canada), plants were transferred to growth chamber at budding stage with 16/8 h, 23/18°C, day/night cycles and 300 µmol m^{-2} s^{-1} using a combination of fluorescent and incandescent lights until anthesis. Two experiments were performed in this study. Pre-experiment (Experiment 1) was conducted for suitable high temperature and duration treatment screening during 2009 to 2010 in the growth chamber. The experiment was a three replicate randomly complete block design in a split-plot arrangement with different high temperature treatment as the main plot and stress time as the subplot. Each treatment contained 50 plants. High temperature was set from 25 to 31°C with 1°C interval. The procedure was: the temperature increased from 23 to 25°C (until 31°C), and maintained high temperature for 6 h, and was later ramped back to 23°C with daylight. Night temperature was kept at 18°C for 8 h. The duration for high temperature treatments was also recorded from 1 d to 5 days with 1 day interval until the anther was initially elongated. After heat treatment, plants were returned to their normal condition. Anther length and weight were recorded. In order to further test whether the pollen is really viable, pollen stain was also performed according to Peterson et al. (2010). Strict self-pollination was made after high temperature stress to ensure whether seeds can be harvested from the plants and we obtained seeds from self-crossing. Thus, additional seed germination was performed for the validation of its vigor (Supplementary Figure 2A and B). Experiment 2 was also a three replicate randomly complete block design for physiological and molecular studies with high temperature treatment. ZA with heterozygous and homozygous line was used as plant material and control, respectively. Plant treatment before anthesis was same as Experiment 1. After anthesis, canola plants were treated using the optima high temperature (30°C) and duration

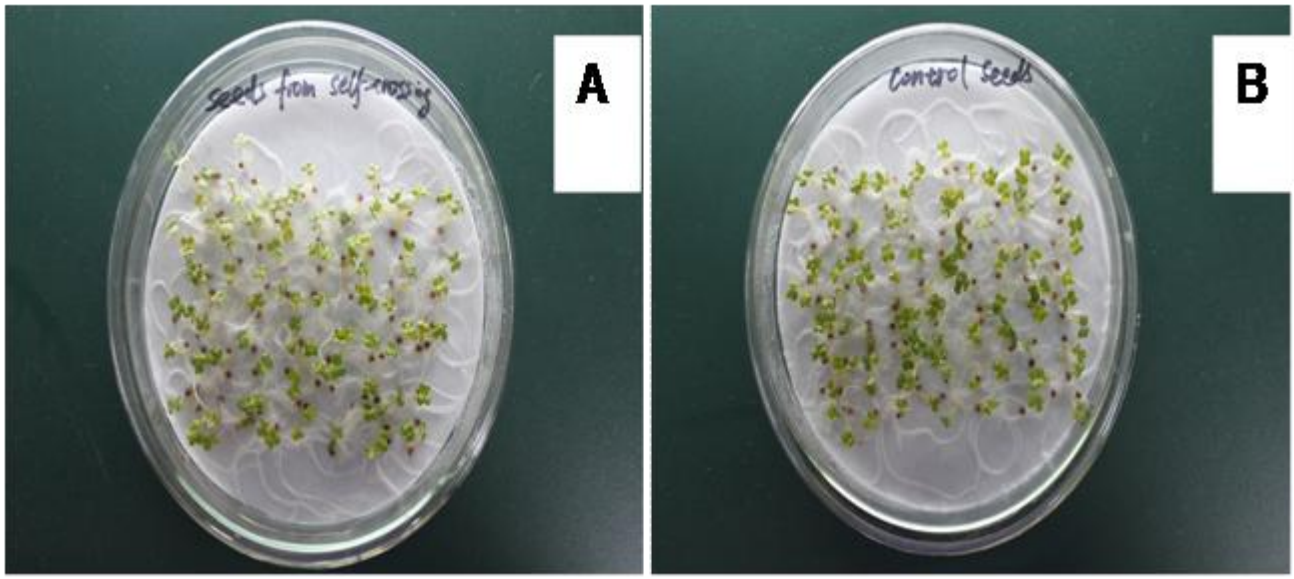


Supplementary Figure 1. Morphology of flower organ in the field condition of ZA heterozygous line (A) and homozygous line (B) when temperature above 30℃. Red arrow showed the magnification of fertile flower organ (left panel) and sterile flower organ (right panel). Black arrow showed the elongated and swell anther.

Table 1. Anther and filament length of ZA heterozygous and homozygous line after different high temperature treatment from 5 d to 9 d (mm).

Temperature	Genotype	5	6	7	8	9
25	Heterozygous	2.41	2.44	2.42	2.45	2.43
	Homozygous	2.42	2.43	2.42	2.44	2.43
26	Heterozygous	2.42	2.43	2.43	2.42	2.44
	Homozygous	2.41	2.43	2.44	2.43	2.44
27	Heterozygous	2.42	2.42	2.41	2.42	2.43
	Homozygous	2.44	2.43	2.23	2.44	2.44
28	Heterozygous	2.41	2.43	2.42	2.42	2.43
	Homozygous	2.43	2.42	2.44	2.43	2.43
29	Heterozygous	2.43	2.42	2.42	2.43	2.43
	Homozygous	2.44	2.42	2.43	2.43	2.44
30	Heterozygous	2.44	2.45	2.44	2.46	2.45
	Homozygous	2.60	2.93	4.50	5.11	5.16
31	Heterozygous	2.43	2.44	2.45	2.44	2.45
	Homozygous	2.63	2.95	4.47	5.12	5.13

(5 days) treatment (Table 1). The anther samples were collected from the 5th to the 9th day after heat stress treatment because the anthers without any elongation before the 5th day high temperature treatment. To minimize any possibility of drought stress during high temperature treatment in both experiments, plants were well watered. Carbohydrate content, related enzyme activities, and



Supplementary Figure 2. Seedlings were pictured from self-crossing of ZA heterozygous line after high temperature treatment (A) and control seeds (Zheshuang 72) (B) at 6 d after germination.

reverse transcript PCR analysis were performed.

Anther morphology image and paraffin section

Collected anthers were placed on the carrier under Lecica MZ 95 stereomicroscope and photographed. Anther length and weight was recorded by micro-ruler and balance. Anther paraffin sectionprocedure was according to Ye et al. (2010) as described using Zheshuang 72 (main cultivar in the downstream area of Yangzi River, China) as control, which was fertile.

Carbohydrate content analysis

Carbohydrate content from harvested samples with different stages after high temperature stress was conducted. Anther samples were ground in liquid nitrogen and boiled with 30 mL of 0.8 L L^{-1} ethonal twice for 30 min each time. Then the samples were centrifuged at3000 g and 200 mg of active charcoal was added into the supernatant to remove any possibility of chlorophyll. Sucrose, fructose, and glucose content in the anther were determined according to the method as described by Hendrix (1993). The pellets from soluble sugar extraction were used for starch extraction and measurement according to the procedure as described by Campbell et al. (1999) and Scofield et al. (2009).

Enzyme assay

Anther samples were homogenized with a 1: 5 tissue-to-buffer ratio. The extraction buffer contained 50 mmol L^{-1} MOPS-NaOH (pH7.5), 5 mmol L^{-1} $MgCl_2$, 1 mmol L^{-1} EDTA, 2.5 mmol L^{-1} DTT, 0.05% Triton X-100 (V: V), and 0.5 mg mL^{-1} BSA. Procedures for enzyme extraction of acid invertase (AI), neutral invertase (NI), sucrose synthase (SS), sucrose phosphate synthase (SPS), and measurement was according to Hubbard et al. (1989) as described. Protein content was determined by Lowry method (1951). Starch phosphorylase extraction was assayed according to Baun et al. (1970) as described. The activity was expressed as nmol Pi mmol · mg $protein^{-1}$ · min^{-1}. ADP glucose phosphorylase was measured following the study of Chen and Janes (1997).

RT-PCR

Total RNA was extracted from the anthers from 5 to 9 days after high temperature treatment. Total RNA extraction method was according to the instruction of TRIzoL® reagent (invitrogen™, Cat. no. 15596 to 026) and treated by DNase as described by Lee et al. (2002). cDNA synthesis was followed by the manufacturer (Takara, Cat. no. DRR019A). Choosing of cDNA fragments for amplification were based on the results of microarray including all of the up-regulated and down-regulated carbohydrate-related genes. Selected cDNA fragments were 2-fold greater or less to the relative expression of 5 days after high temperature treatment and their function annotations were listed in Table 2. Primer sequences were listed in Table 3. *Brassica napus ACTIN* (AF111812) was used as control. Because of the small size of gene fragment, we used polyacrylamide gel for transcripts detection due to its high resolution.

RESULTS

Change of anther morphology

The flower organ morphology of ZA with homozygous line was without any change (Figure 1B and D) while very significant filament elongation and anther swollen was found with heterozygous line under normal and high temperature treatment (Figure 1A and C). The anther and filament length of heterozygous ZA line at 9 days was twice as long as that before elongation after high temperature treatment (Figure 1K). Furthermore, the increment of anther weight reached 5.7 times as much as

Table 2. Annotation for the carbohydrate metabolic gene fragments studied in this experiment on the internet: http://campbio.dfci.harvard.edu/tgi/tgipage.html.

Fragment code	Annotation
TC161227	Rep: B component PTS system mannitol/fructose-specific enzyme II - *Bacillus clausii* (strain KSM-K16), partial (13%)
TC111191	*Brassica napus* galactinol synthase (GolS1) mRNA, complete cds
TC122369	Homologue to Cluster: NAC-domain protein 5-1; n=1; Brassica napus|Rep: NAC-domain protein 5-1 - *Brassica napus*, complete
TC138673	Homologue to Cluster: Fructose-1 6-bisphosphatase cytosolic; n=1; *Brassica napus*|Rep: Fructose-1, partial (15%)
TC111160	*Brassica napus* fructose 1,6-bisphosphatase mRNA, complete cds
TC159748	Rep: Fructose-bisphosphate aldolase - *Arabidopsis thaliana* (Mouse-ear cress), partial (8%)
TC152377	Rep: Fructose-bisphosphate aldolase - *Arabidopsis thaliana* (Mouse-ear cress), partial (13%)
TC146011	Rep: Fructose-bisphosphate aldolase - *Arabidopsis thaliana* (Mouse-ear cress), partial (45%)
TC136137	Rep: Glucose-1-phosphate adenylyltransferase large subunit 2, chloroplast precursor - *Arabidopsis thaliana* (Mouse-ear cress), partial (32%)
TC125843	Rep: Glucose-1-phosphate adenylyltransferase large subunit 3, chloroplast precursor - *Arabidopsis thaliana* (Mouse-ear cress), partial (14%)
TC138390	Rep: Glucose-1-phosphate adenylyltransferase large subunit 3, chloroplast precursor - *Arabidopsis thaliana* (Mouse-ear cress), partial (44%)
TC112033	Weakly similar to Cluster: Glucose-6-phosphatase 2; n=1; Homo sapiens|Rep: Glucose-6-phosphatase 2 - Homo sapiens, partial (6%)
TC125742	Homologue to Cluster: Glucose-6-phosphate 1-dehydrogenase 3 chloroplast precursor; n=1; *Arabidopsis thaliana*|, partial (38%)
TC114274	Rep: Glucose 6-Pi/Pi transporter - *Thellungiella halophila* (Salt cress), partial (98%)
TC122988	homologue to Cluster: Glucose and ribitol dehydrogenase homolog 2; n=1; *Arabidopsis thaliana*|Rep: Glucose and, complete
TC135360	Rep: Glucose and ribitol dehydrogenase homolog 2 - *Arabidopsis thaliana* (Mouse-ear cress), partial (57%)
TC120348	Rep: Plastidic glucose transporter 4 - *Arabidopsis thaliana* (Mouse-ear cress), partial (36%)
TC160913	Rep: Glutamine-fructose-6-phosphate transaminase 2 - *Arabidopsis thaliana* (Mouse-ear cress), partial (23%)
TC131147	Rep: Glutamine-fructose-6-phosphate transaminase 2 - *Arabidopsis thaliana* (Mouse-ear cress), partial (34%)
TC120035	Rep: Invertase - *Arabidopsis thaliana* (Mouse-ear cress), complete
TC154741	Homologue to Cluster: Putative neutral invertase; n=1; *Arabidopsis thaliana*|Rep: Putative neutral invertase -, partial (64%)
TC111032	Rep: Phosphoglucomutase, chloroplast precursor - *Brassica napus* (Rape), complete
TC120264	Rep: Probable carbohydrate esterase At4g34215 - *Arabidopsis thaliana* (Mouse-ear cress), partial (93%)
TC154333	Rep: Glucose-1-phosphate adenylyltransferase large subunit 3, chloroplast precursor - *Arabidopsis thaliana* (Mouse-ear cress), partial (6%)
TC139068	Homologue to Cluster: Putative UDP-glucose: glycoprotein glucosyltransferase; n=1; Arabidopsis thaliana|Rep:, partial (5%)
TC139098	*Brassica napus* sucrose transporter (SUT) mRNA, complete cds
TC113570	similar to Cluster: Pyrophosphate-fructose-6-phosphate 1-phosphotransferase-like protein; n=1; Arabidopsis, complete
TC139884	0194376 *Brassica napus* Apical meristem *Brassica napus* cDNA, mRNA sequence
TC111179	*Brassica napus* NAC-domain protein 1-1 (NAC1-1) mRNA, complete cds
TC133268	Rep: Sedoheptulose-bisphosphatase - *Arabidopsis thaliana* (Mouse-ear cress), complete
TC156381	Similar to Cluster: Soluble starch synthase 3 chloroplast precursor; n=1; *Solanum tuberosum*|Rep: Soluble, partial (1%)
TC157105	Rep: Soluble starch synthase, chloroplast precursor - *Arabidopsis thaliana* (Mouse-ear cress), partial (90%)
TC118694	Rep: Chromosome chr14 scaffold_27, whole genome shotgun sequence - *Vitis vinifera* (Grape), complete
TC123326	Rep: Starch branching enzyme class II - *Arabidopsis thaliana* (Mouse-ear cress), partial (33%)
TC114761	Homologue to Cluster: Sucrose-phosphatase; n=1; *Arabidopsis thaliana*|Rep: Sucrose-phosphatase - Arabidopsis, partial (51%)
TC128651	BN25.040D05F011026 BN25 *Brassica napus* cDNA clone BN25040D05, mRNA sequence

Table 2. Contd.

TC121549	0156016 *Brassica napus* Etiolated seedlings (pSPORT1) *Brassica napus* cDNA, mRNA sequence
TC119259	Homologue to Cluster: Sucrose synthase 2; n=1; *Arabidopsis thaliana*|Rep: Sucrose synthase 2 - Arabidopsis, partial (30%)
TC139098	*Brassica napus* sucrose transporter (SUT) mRNA, complete cds
TC144522	Rep: Sucrose transporter SUC2 - Brassica oleracea (Wild cabbage), partial (16%)
TC157011	Rep: Sucrose transporter SUC2 - Brassica oleracea (Wild cabbage), partial (11%)
TC119832	Rep: Sucrose-phosphate synthase 1 - Citrus unshiu (Satsuma orange), partial (23%)
TC154559	Rep: Sugar transport protein 2 - *Arabidopsis thaliana* (Mouse-ear cress), partial (22%)
TC130053	Rep: Sugar transport protein 2 - *Arabidopsis thaliana* (Mouse-ear cress), partial (35%)
TC131153	Rep: Sugar transport protein 4 - *Arabidopsis thaliana* (Mouse-ear cress), partial (46%)
TC130679	Similar to Cluster: Sugar transport protein 6; n=1; *Arabidopsis thaliana*|Rep: Sugar transport protein 6 -, partial (68%)
TC137135	Rep: Sugar transport protein 14 - *Arabidopsis thaliana* (Mouse-ear cress), partial (36%)
TC135620	Similar to Cluster: Sugar transporter ERD6-like 2; n=1; *Arabidopsis thaliana*|Rep: Sugar transporter ERD6-like, partial (20%)
TC157137	Sugar transporter EDR-like 6
TC136510	Rep: Sugar transporter ERD6-like 12 - *Arabidopsis thaliana* (Mouse-ear cress), partial (42%)
TC142439	Rep: Sugar transporter ERD6-like 12 - *Arabidopsis thaliana* (Mouse-ear cress), partial (36%)
TC123726	Homologue to Cluster: Sugar transport protein 9; n=1; *Arabidopsis thaliana*|Rep: Sugar transport protein 9 -, partial (60%)
TC141032	Rep: SUS2 (SUCROSE SYNTHASE 2); UDP-glycosyltransferase/ sucrose synthase/ transferase, transferring glycosyl groups - Arabidopsis thaliana, partial (25%)
TC121053	Homologue to Cluster: UDP-galactose 4-epimerase putative; 6572-4109; n=1; *Arabidopsis thaliana*|Rep:, complete
TC144075	Rep: UDP-glucose 4-epimerase - *Arabidopsis thaliana* (Mouse-ear cress), partial (56%)
TC126398	Rep: Probable UDP-glucose 6-dehydrogenase 2 - *Arabidopsis thaliana* (Mouse-ear cress), partial (46%)
TC112766	Rep: UDP-glucose glucosyltransferase - *Arabidopsis thaliana* (Mouse-ear cress), partial (48%)
TC141706	0173169 *Brassica napus* Leaf - drought *Brassica napus* cDNA, mRNA sequence
TC119263	BNSCS2CT_UP_024_G08_06JAN2005_052 *Brassica napus* seed coat BNSCS2CT *Brassica napus* cDNA 5', mRNA sequence
TC145133	Rep: UDP-glucose pyrophosphorylase - Oryza sativa subsp. indica (Rice), partial (14%)
TC158370	Rep: UTP-glucose glucosyltransferase - *Arabidopsis thaliana* (Mouse-ear cress), partial (30%)

that before swollen after high temperature treatment (Figure 1L). Though anther was elongated and swollen, further evidences were needed for its fertility change. Results from paraffin section of the elongated anther clearly showed that it produced lots of pollen grains compared to the Zheshuang 72 (Figures 1E, F, G and H). The pollen grain was stained and it showed its viability with red as with the control of Zheshuang 72 (Figures I and J). Results from the germination experiment of seeds from self-pollination indicated that heterozygous lines can produce normal seeds after fertility restoration is induced by high temperature (Supplementary Figure 2).

Taken together, all of the evidences supplied here and combined with supplementary Figures 1 and 2 indicated that the fertility changed from sterile to fertile in ZA heterozygous line while it did not in homozygous line under high temperature treatment.

Variation of carbohydrate content

Anther carbohydrate including fructose, glucose, sucrose, and starch content were measured in both of heterozygous and homozygous ZA line under high temperature treatment. Results showed that fructose content in heterozygous line was significantly higher on the 6^{th} day than after

Table 3. Sequence of forward and reverse primer pairs was used in the RT-PCR analysis with gene fragment in the experiment.

TC name	Forward primer 5' → 3'	Reverse primer 5' → 3'	Size (bps)
TC161227	ATGGGAAGATACTTGTGTG	CTTGTATGTCTTGAACCAG	82
TC111191	CCTGGTCTAGATACTTACGAG	CGCTAGGACGAGATTGTAAAC	141
TC122369	GAGAGGAGTGAAGACGAATTG	CCGACATAACACCCAATCATC	115
TC138673	CATTGATCGTCCCTCTTAGC	GAGATCGAGATCAGTGAGGA	139
TC111160	GACTGTGGTGTCTCCATTG	CCGTACATACAGTAACCCG	105
TC159748	GACAAGATTCGTCTCTTTGG	GATGGGTTATTTGGTAGTGC	113
TC152377	AGAGATGGTGGAGAAGTTC	CTTGAATTTGGTCAGGGTG	116
TC146011	GTACTGCTGCTAGCTTTGTG	GAACCCACAACTATTAGCGC	115
TC136137	CTAGTAGTAGTCCCTTTGGTC	CCTTGTCTGAATCTTCTGAGG	137
TC125843	GCATCGAAGACCTCTTCAAG	GCCCTGCAACAACATAATCC	85
TC138390	GGAACTAAACTCTTCCCTCTC	GCACGAAGATCTTGTTGATGC	127
TC112033	TTCGGTCGTGTTAGATGAAG	GTACCAAGATAATCTCCTCG	118
TC125742	GAACAGCAGTCAACGGTTAG	CAGGAAGACAGCCTTCATAG	109
TC114274	GTTCCTGATGAACAGTCAGG	GAAACGCGTTCAGAACCTTC	112
TC122988	GAGCGTGGCTTTCACTTACG	GTGGCGATCATGATGGGATC	114
TC135360	TGCGTTAGCTCTTCAGCTAG	GAGAAGGAAGCCGTTATCAG	93
TC120348	GGTGATGAGGAAGAAGCTG	CCCAAGGTGATAGCCAAAC	114
TC160913	GACAGCACATTCTCGAGGTC	GAGAGAGGGAATCGTCGATG	96
TC131147	GGATATAACTACGCAACAGC	GCGATAGGTAGATTCTCATC	134
TC120035	GGTAGTGAGCCATCTTCACAG	CACAATGTTACCCCAAACCGC	148
TC154741	GCTGAGAGCTTATGGAAAGC	GAACATGTCAAAGCCGTCAG	115
TC111032	GGCAGAAGACTGGAACAAGC	GTGGCGTCCTTATAGTCCTC	124
TC120264	ATAAGGTGAGAGAAGCACAG	CAAAGGATTGCAGCTAAAGC	78
TC154333	ATGTTCAAGAAGCGGATAGG	GTACCGTCTTGAATTGTAGC	91
TC139068	TGAAGACATGGAACTCAAC	CCAAGTCTTGTTCTGAATC	101
TC139098	GCTTGTTCAACCCATCGTC	CCATGCTGTGACCTAAGTC	146
TC113570	ATGATAAGAGCTGTACCTGC	CTCCTCTAAGGGGATATAAG	147
TC139884	GGAGAAGAGCATCAAGGACG	CTTGTCTCTAGCGTGGCTAG	121
TC111179	GGTTATTGGAAAGCGACTGG	GCAGATCGATCAACGTTAGC	158
TC133268	GTTAGGACAGCTTCTTGTGG	GTACTTCTTCAGAGCAAGCG	145
TC156381	GAAATTGTGTAACCGATGGG	GAGTATGGTGAGAGTACTAC	97
TC157105	CGTTCCTCCTGGTGATCAAG	GTAGCGTCTGAGGTCTCTTC	81
TC118694	GGTGAAGACTGTGTTCCTTC	CCTCTAACCTCAAGAGGAAG	90
TC123326	TGGGAAACGAATTTGGACACC	CTAAGATACTCTGCATCTCCC	145
TC114761	GAAACCAGAAAGTTCCCTGAG	GTGAAAGTTCCTTGGTCACTG	115
TC128651	CCTTCTCAGATTGCATCTCG	GCTCTAACGAGATGGGAAAC	115
TC121549	TCGAGGATGAGGAAGCAGAAG	GTAACGTATTGCTGCGTCGTG	116
TC119259	CTTGACTGGGCTAGTTGAGTG	CTCAGCCATTTCCTCTCTGTC	121
TC139098	CCGTTGCACCTCAAGATTCG	CCATGCTGTGACCTAAGTCG	113
TC144522	ATGGTGGTATCTGTGGGAGG	CAATATGCCACTCACCGCAG	105
TC157011	AGTGTTTCAGTGGCTATG	CCAAGGAGATGAATTTCC	90
TC119832	TCTACTCATGGCACCAAATC	CAAGATACAGCTTGTTTCCC	97
TC154559	GCACTTTCGTTATCGGACAG	CGTTCATGACCGCAAAGAAG	84
TC130053	ATGACCGCTACACAGATATC	CTTGTAATGGGTCCGATTAG	71
TC131153	ATGCCATCATGCGTGTTAC	CCACGATCAGATTAGCATC	106
TC130679	CACATCGTCTCTTTACCTAG	GACAGAAGCAAACTGCATAG	100
TC137135	GGTTTGGTTCTTGTGATCC	GATCTCACTCGGAACTAAC	90
TC135620	ATGGAATCTGCAAGTCTGC	CAACGGAAGTACTGAGAAG	91
TC157137	GAGAGAAGGGAAGATCACTTG	ACAAATGTGGGAGTAGCTGTG	86
TC136510	CGACCACTTCTTATGGCTTC	CTCGGGAACATCTTTAACTCC	87

Table 3. Contd.

TC142439	ATGACGGTGTTGTCTCTTATC	GCTTAAGCATAACCCAAATGC	114
TC123726	GACTGCTTACTGCAAATTCG	GTACTTCCTAGTCACAACTG	109
TC141032	CAGCCTGCTTTCTATGAAGC	GATGTGGAAGCCAGAAACTC	129
TC121053	CACTCTCTTGGAAGTCATGG	GTACAAGGAACCTCCTTTGG	96
TC144075	CTTATGGTCGCACTAAGCTG	GGATCTTCTCCAATCCTTCC	136
TC126398	GTCCACACATTGAAGTAGCAG	GCATTGCTTCACGATGTCTTC	113
TC112766	GAACTGTCTAGCTCCGTTC	GCTTCTCTGCAGCATCAAG	122
TC141706	CCACATTCCTTCTGCTCTTC	CTGAGATTGCATCTGCGTAG	92
TC119263	TGTACTTCGGCCTTATGGG	GGTCACACACCACTTTAGC	87
TC145133	CACATCTTGCTACTGATACC	CATGGTGGAAAAGACTTTGC	109
TC158370	CAGAAGGTGAATGCGTTCG	CCTGACGTCACTATCTTCC	162
BnACTIN	CCGAGAGAGGGTACATGTTC	CTCTTGCTCGTAGTCGAGAG	98

the 6th day under high temperature treatment, whereas it was very stable from the 7th day as shown in Figure 2A. There was no obvious variation of anther sucrose content under high temperature treatment both in homo- and heterozygous line (Figure 2C). As for glucose and starch content, a significant increment after high temperature treatment was observed (Figure 2B and D). However, in the heterozygous line, the glucose content was almost twice as much as the starch content on the 6th day. The extensive increase of anther glucose and starch content indicated the importance of these two types of carbohydrate in the regulation of fertility change under high temperature condition.

Alteration of enzymes activities

Invertase is an important type of enzyme for cleaving the sucrose in the plant cells. From Figure 3A, it was amazing that the neutral invertase activity was particularly high after high temperature treatment in heterozygous line. Although, the acid invertase was also markedly increased, its total activity was far less than that of neutral invertase under after temperature treatment (Figure 3B). The result exhibited the importance of neutral invertase involved in the sucrose molecular cleavage.

Besides invertase, sucrose synthase and sucrose phosphate synthase cleave sucrose as well. Figure 3C and D showed that the sucrose synthase and the sucrose phosphate synthase activity had the opposite trend: the former increased while the latter decreased after high temperature treatment. However, the maximum of sucrose synthase activity was below 12 nmol sucrose • g-1 protein • h-1, which was also far behind the neutral invertase activity. Both starch phosphorylase and ADP glucose pyrophosphorylase are important enzymes in the starch metabolism. Figure 3E showed that the anther starch phosphorylase activity dropped sharply on the 6th day after high temperature stress. However, the ADP glucose pyrophosphorylase was increased very quickly after high temperature treatment (Figure 3F).

From the aforementioned result, anther carbohydraterelated enzymes in heterozygous line was evidently changed as induced by high temperature while it was not so with the homozygous line.

Modification of transcripts

There were 27 of carbohydrate metabolically related genes down-regulated and 34 of genes up-regulated in heterozygous line in our unpublished microarray data. Of these 27 genes, we roughly classified them according to the carbohydrate type that was previously assayed. From Figure 4A, there were 9 of sucrose related genes that were down-regulated in the anther of heterozygous line after high temperature treatment, which ranked the first. Only one starch related gene was detected for down-regulation. As for the 34 of up-regulated genes, 13 of the glucose related genes (38%) were identified (Figure 4B). The number of fructose and sucrose related genes in up-regulation expression mode were less than that of down-regulation. A *BnGol1* was detected for its strong expression under high temperature treatment. A carbohydrate esterase gene and 6 of the sugar transporter genes were up-regulated after heat stress. Furthermore, there were some common properties for the down- and up-regulated gene expression mode. Firstly, the expression intensity was different at the same day after high temperature treatment. For example, expression of TC146011 and TC125843 were particularly strong while TC159748 and TC 152377 were very weak. Secondary, expression of some genes could be detected from the 5 to 9th day after high temperature treatment (that is to say, TC114274 and TC135360) while others could only be detected at the 5th day (that is to say,

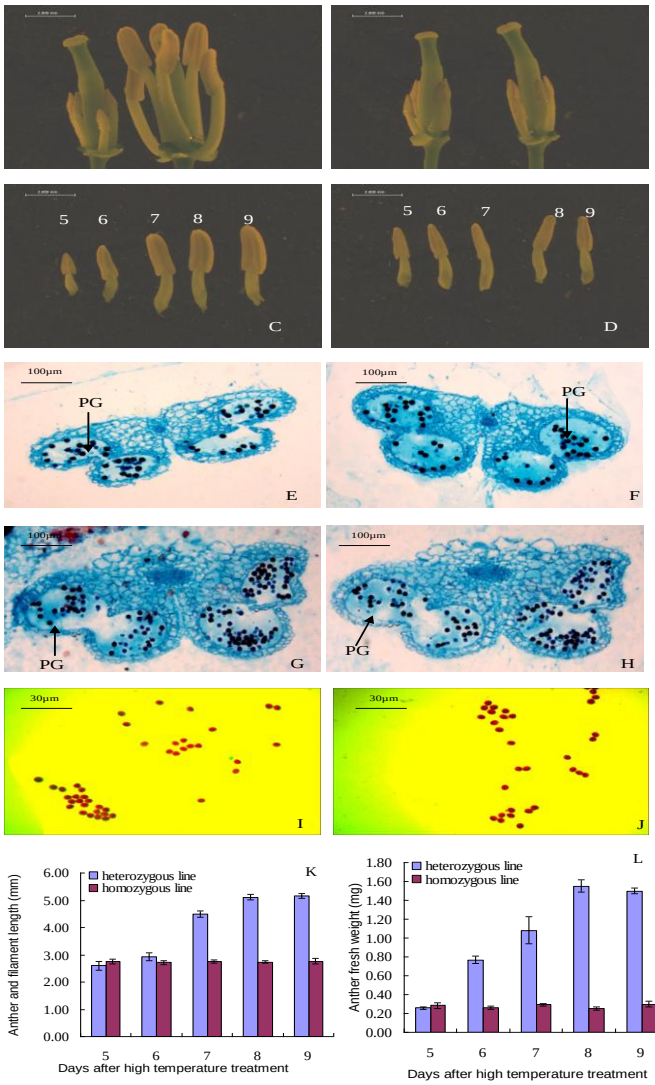


Figure 1. Morphology change of ZA male reproductive organ of recessive genic sterile line under high temperature treatment. A, anther and filament without elongation under control temperature (23°C, leaf panel) and with elongation after high temperature treatment (30°C, right panel) in heterogzygous line during flower opening. B, anther and filament without any elongation both under control temperature (23°C, leaf panel) and after high temperature treatment (30°C, right panel) in homozygous line during flower opening. C, anther and filament elongation dynamic from 5 d to 9 d after high temperature treatment in heterozygous line. D, anther and filament elongation dynamic from 5 d to 9 d after high temperature treatment in homozygous line. E and G, anther paraffin section at 6 d (about 3 mm length of bud) and 9 d after high temperature treatment in heterozygous line. F and H, anther paraffin section from control with similar stage (about 3 mm length and the day before opening bud) (Zheshuang 72, fertile plant, major open-pollinated variety in the Yangzi River of China). I, pollen grain stain from heterozygous line anther after high temperature treatment. J, pollen grain stain from control (Zheshuang 72). Figure 1k and L, anther and filament length from 5 d to 9 d after high temperature stress in heterozygous line and homozygous line. L, single anther weight from 5 d to 9 d after high temperature stress sin heterozygous line and homozygous line. Each value (K and L) was mean of three replicates. Bars on the histogram represent standard value. PG, pollen grain.

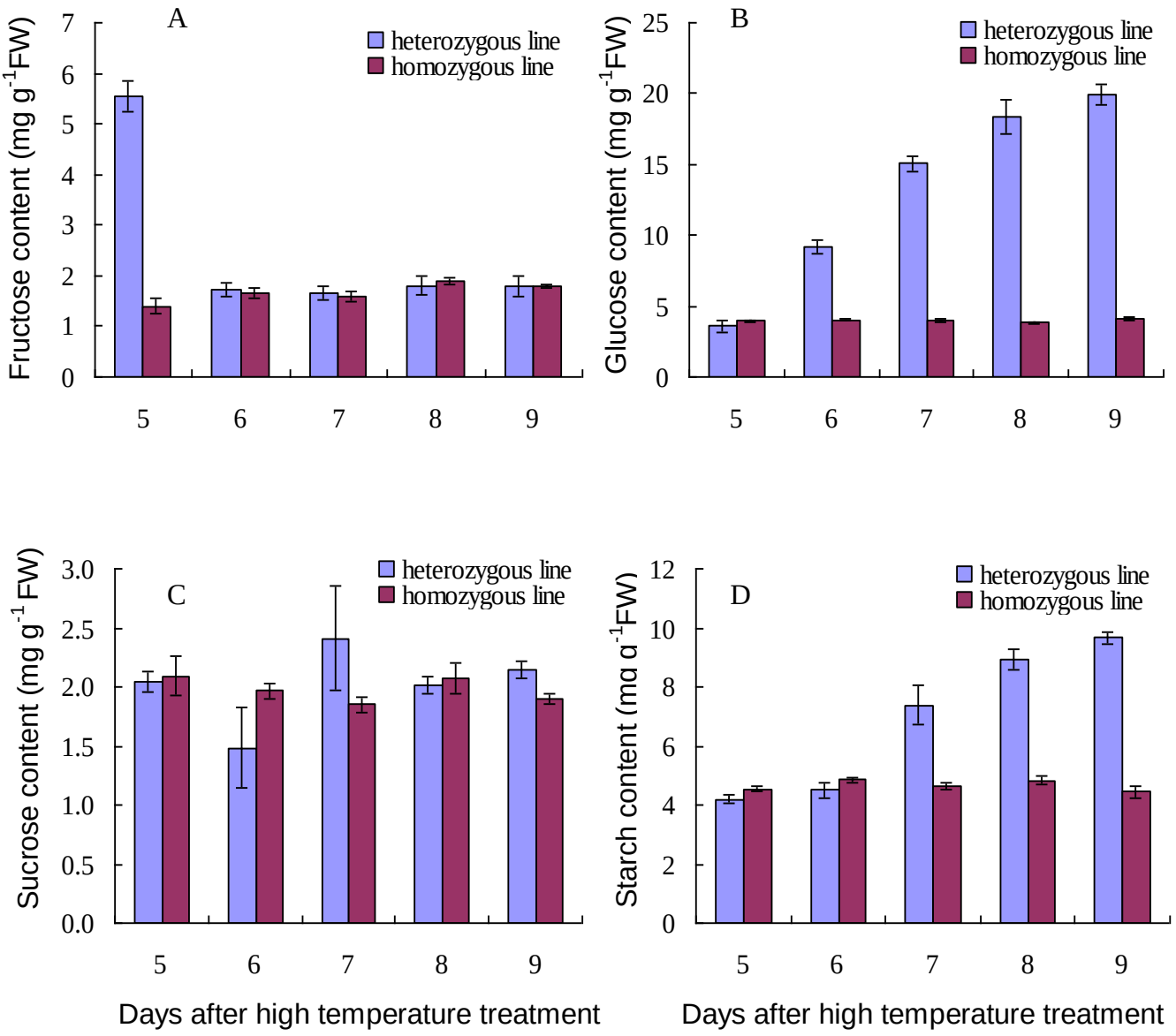


Figure 2. Carbohydrate content in anther of heterozygous and homozygous genic male sterile line after high temperature stress. A, Fructose content in anther of heterozygous and homozygous genic male sterile line from 5 to 9 d after high temperature treatment. B, Glucose content in anther of heterozygous and homozygous genic male sterile line from 5 to 9 d after high temperature treatment. C, Sucrose content in anther of heterozygous and homozygous genic male sterile line from 5 to 9 d after high temperature treatment. D, Starch content in anther of heterozygous and homozygous genic male sterile line from 5 to 9 d after high temperature treatment. Each value in A, B, C, and D was mean of three replications. Bars on each histogram indicated standard value.

TC162217 and TC138673) in heterozygous line of down-regulatedgenes.

Taken together, lots of transcripts involved in carbohydrate metabolism were modified including down-regulation and up-regulation in the anther of ZA heterozygous line after induction of high temperature.

DISCUSSION

Canola always suffers from various abiotic stress such as drought and heat stress. Different from animals, canola plants can not escape from these damages through self-moving. Therefore, they must struggle with these unfavorable factors to survive. Thus, some effective defense systems which show resistance to these forms of stress should be enhanced. As a result, those materials that are highly resistant or immune to adverseenvironment received great interest by researchers because of their potential value for canola breeding.

During reproductive growth stage, considerable studies

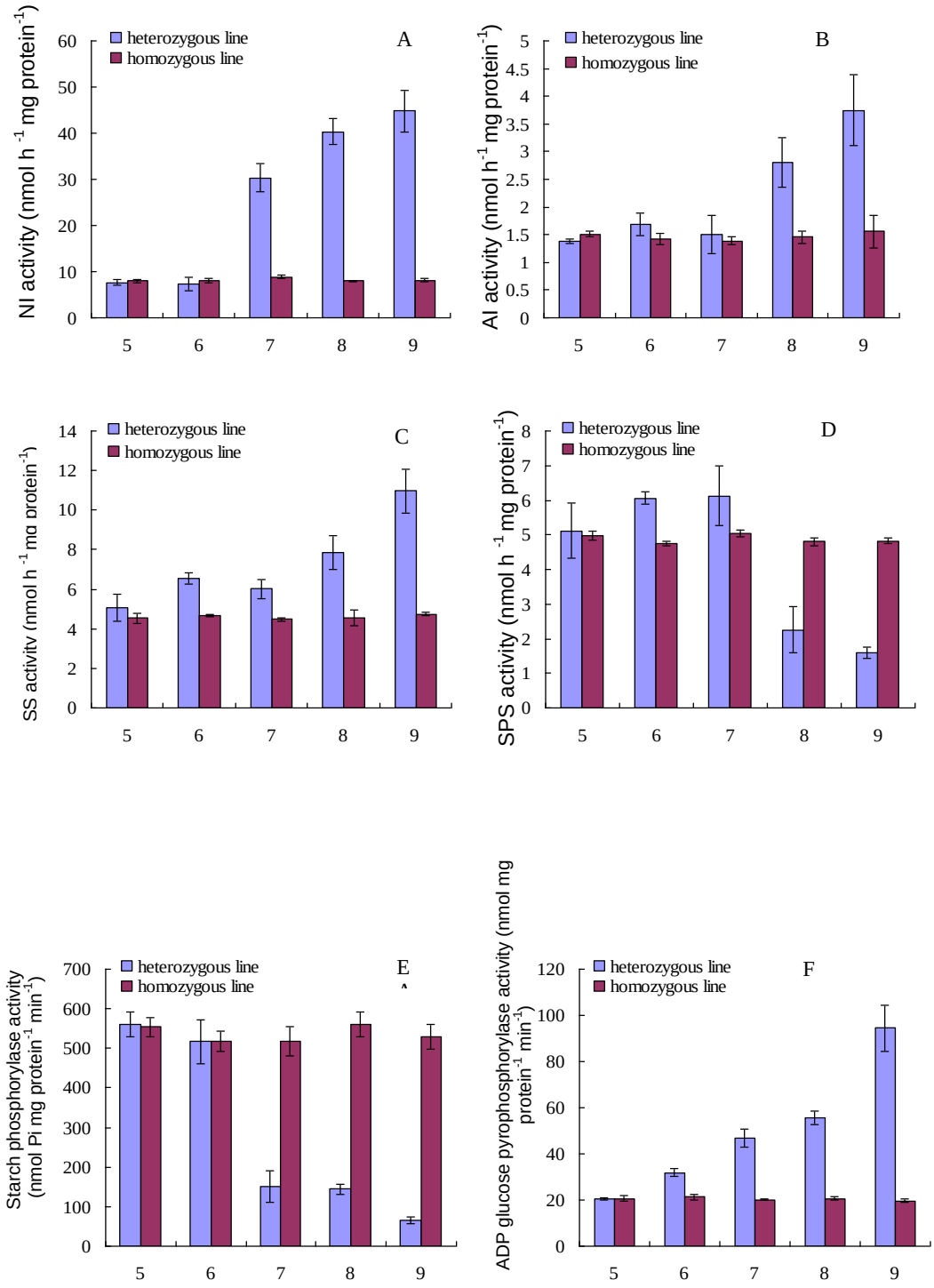


Figure 3. Carbohydrate metabolic enzymes in anther of heterozygous and homozygous genic male sterile line after high temperature stress. A, Neutral invertase activity in anther of heterozygous and homozygous genic male sterile line from 5 to 9 d after high temperature treatment. B, Acid invertase in anther of heterozygous and homozygous genic male sterile line from 5 to 9 d after high temperature treatment. C, Sucrose synthase activity in anther of heterozygous and homozygous genic male sterile line from 5 to 9 d after high temperature treatment. D, Sucrose phosphate synthase activity in anther of heterozygous and homozygous genic male sterile line from 5 to 9 d after high temperature treatment. E, Starch phosphorylase activity in anther of heterozygous and homozygous genic male sterile line from 5 to 9 d after high temperature treatment. F, ADP glucose pryphosphorylase activity in anther of heterozygous and homozygous genic male sterile line from 5 to 9 d after high temperature treatment. Each value in A, B, C, D, E, and F was mean of three replications. Bars on each histogram indicated standard value. NI, neutral invertase, AI, acid invertase, SS, sucrose synthase, SPS, sucrose phosphate synthase.

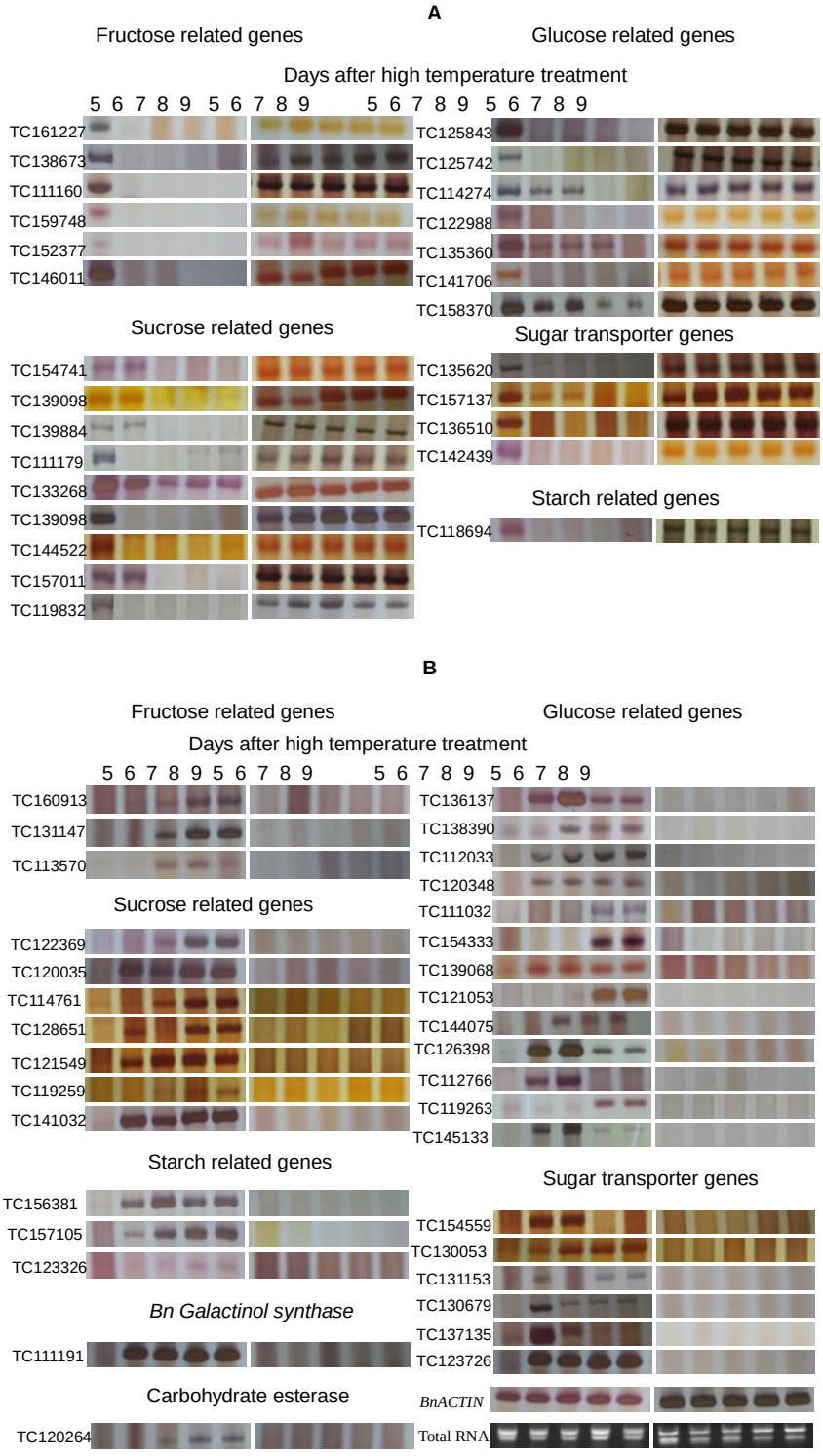


Figure 4. RT-PCR analysis of carbohydrate metabolic down-regulation and up-regulation genes in the heterozygous and homozygous anther of genic male sterile line after from 5 to 9 d after high temperature treatment. A, down-regulated genes. B, up-regulated genes. Annotation for the gene code was listed in supplementary table 2. Primer pairs for each individual gene were listed supplementary table 3. *BnACTIN* was used as the normalization control. Leaf panel was heterozygous genic male sterile line and right panel was homozygous genic male sterile line. Result was showed in polyacrylamide gel electrophoresis because of small fragment size and its high resolution. Total RNA was stained by ethidium bromide in agarose gel electrophoresis.

indicated that heat stress can damage canola reproductive organ development especially, pollen viability (Morrison, 1993; Young et al., 2004). Therein, screening for canola resistant to heat stress was carried out (Singh et al., 2008). In this present study, we obtained a mutant that was fertile under high temperature condition while sterile under low temperature in a recessive genic sterile line. It was interesting to notice that the fertility transition was only met in the heterozygous line but not homozygous line. After high temperature treatment, anther of the heterozygous line can elongate and produce viable pollen grain. Thus, its fertility restored after induction of high temperature.

Previous investigations in other plants found that carbohydrate metabolism was modified in anther afterheat stress. Because carbohydrate is a very important compound in cells and participates in many physiological metabolisms (Musgrave et al., 1998; Park et al., 2010). For example, carbohydrate such as starch deficiency in the pollen grain can result in sterile in rice (Jung et al., 2006; Zhang et al., 2010). It was found that the glucose and starch content in the anther of ZA heterozygous line was sharply increased after high temperature treatment. Maintaining carbohydrate content to a certain degree is necessary because anther swollen needs those carbohydrates for osmotic potential (Ackerson, 1981; Wang and Stutte, 1992). However, the anther fructose and sucrose content was not obviously increased. The result might suggest that 1) glucose was much more important than that of fructose; 2) lots of sucrose was converted into glucose. Indeed, the anther glucose content in heterozygous was the highest among these carbohydrates though it was not the only way to produce glucose. The result of very high activity of neutral invertase indicated that lots of sucrose might be cleaved and produced more glucose. The acid invertase could also have the contribution for sucrose cleavage to produce glucose. However, its activity was very low as compared with neutral invertase. SS was also a key enzyme for sucrose cleavage. Its activity was increased after high temperature treatment as well and was a medium between neutral and acid invertase. Therefore, neutral invertase, acid invertase, and sucrose synthase together have contributed in catalyzing sucrose into glucose. SPS was generally considered as sucrose synthesis direction (Baxter et al., 2003; Li et al., 2003) and was decreased after high temperature treatment indicating there were small amounts of sucrose synthesis in the anther. In plant, starch phosphorylase is an enzyme generally considered as starch degradation (Chang and Su, 1986). In our result, we detected its reduction activity under high temperature treatment, which was in accordance with the accumulation of starch in the anther of heterozygous line. We further determined the ADP glucose pyrophosphorylase (AGPase) activity and it increased very quickly after high temperature treatment. It is well known that AGPase is an important determinate of starch accumulation (Nakamura and Imamura, 1985; Bahaji et al., 2011). Therefore, increased AGPase activity is beneficial for anther starch accumulation after high temperature stress. In other words, carbohydrate and related enzymes in the anther of heterozygous line were greatly altered compared with homozygous line after high temperature induction.

In order to further explore the modification of carbohydrate metabolism, we generally analyzed the transcripts that were previously detected by microarray technology. Two types of fructose-2, 6-bisphosphatase (FBPases) were detected down-regulation. Because cytosolic FBPase plays an essential role in regulating photosynthetic carbon partitioning between sucrose and starch (Zrenner et al., 1996), reduction of the transcripts might hint on the accumulation of starch content in anther of ZA heterozygous line. The decrease of cytoFBPase transcript (TC138673 and TC111160) by down-regulation of SPS (TC119832) via the reduction of G6P Pi/Pi (TC114274) ratio brought about the reduction of the rate of sucrose synthesis (Huber and Huber, 1992; Stitt et al., 1987). It was striking that a neutral invertase transcript was found down-regulation (TC154741) though the neutral invertase activity was very high. However, another invertase transcript (TC120035) was strongly up-regulated. A possible interpretation for the down-regulation of TC154741 is that several isoforms for this enzyme exist in the cell (Karuppiah et al., 1989; Barrat et al., 2009). It was interesting that one glucose-1-phosphate adenlyltransferase (ADP glucose pyrophosphorylase; AGPase) gene (TC125843) was down-regulated and two of that (TC136137 and TC138390) were up-regulated. These three genes belonged to ADP glucose pyrophosphorylase large subunit. In other plants, several genes which encoded large subunits of AGPase were also identified and they had different transcript levels (Greene et al., 1996; Bejar et al., 2006). The fold of the up-regulated transcript was higher than that of the down-regulated transcript which may take advantage of the higher activity of AGPase, and thus, limit the enzyme meant for the biosynthesis of starch (Akihiro et al., 2005). Besides genes, a noticeable gene, *Brassca napus galactinol synthase* (*Bngols*) was abundantly expressed in the anther of the heterozygous line. Several evidences suggested that galactinol synthase was a heat shock gene induced by high temperature (Busch et al., 2005; Frank et al., 2009). Galactinol synthase utilized UDP galactose (TC121053) for the transfer of a galactosyl residue to myo-inositol and the production of galactinol, which belongs to the oligosaccharides of the raffinose family (Saravitz et al., 1987; Nishizawa et al., 2008). Another carbohydrate esterase gene, TC120264, was also identified in the up-regulation mode. The gene belonged to GDSL-motif family which played multiple roles in plant abiotic stress, disease defense and secondary metabolism. However, the function of GDSL motif in heat stress has not been

reported until now.

Taken together, fertility of a genic recessive male sterile with heterozygous condition changed after high temperature was induced and carbohydrate metabolism was greatly modified as compared with homozygous line. Analysis of carbohydrate related genes expression level should shed light on its molecular regulation mechanism.

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REFERENCES

Ackerson RC (1981). Osmoregulation in cotton response to water stress: Ⅱ. Leaf carbohydrate status in relation to osmotic adjustment. Plant Physiol., 67: 489-493.

Akihiro T, Mizuno K, Fujimura (2005). Gene expression of ADP-glucose pyrophosphorylase and starch content in rice cultured cells are cooperatively regulated by sucrose and ABA. Plant Cell Physiol., 46: 937-946.

Angadi SV, Cutforth HW, Miller PR, McConkey BG, Entz MH, Brandt SA, Volkmar KM (2000). Response of three Brassica species to high temperature stress during reproductive growth. Can. J. Plant. Sci., 80: 693-701.

Bahaji A, Li J, Ovecka M, Ezquer I, Muñoz FJ, Baroja-Fernández E, Romerno JM, Almagro M, Hidalgo M, Sesma MT, Pozueta-Romero J (2011). *Arabidopsis thaliana* mutants lacking ADP-glucose pyrophosphorylase accumulate starch and wild-type ADP-glucose content: further evidence for the occurrence of important sources, other than ADP-glucose pyrophosphorylase, of ADP-glucose linked to leaf starch biosynthesis. Plant Cell Physiol., 52: 1162-1176.

Barrat DHP, Derbyshire P, Findlay K, Pike M, Wellner N, Lunn J, Feil R, Simpson C, Maule AJ, Smith AM (2009). Normal growth of *Arabidopsis* requires cytosolic invertase but not sucrose synthase. Proc. Natl. Acad. Sci. USA 106: 13124-13129.

Baun LC, Palmiano EP, Perez CM, Juliano BO (1970). Enzymes of starch metabolism in the developing rice grain. Plant Physiol., 46: 429-434.

Baxter CJ, Foyer CH, Turner J, Rolfe RA, Quick WP (2003). Elevated sucrose-phosphate synthase activity in transgenic tobacco sustains photosynthesis in older leaves and alters development. J. Exp. Bot., 54: 1813-1820.

Bejar CM, Jin X, Ballicora MA, Preiss J (2006). Molecular architecture of the glucose 1-phosphate site in ADP-glucose pyrophosphorylases. J. Biol. Chem., 281: 40473-40484.

Busch W, Wunderlich M, Schoeffl F (2005). Identification of novel heat shock factor-dependent genes and biochemical pathways in *Arabidopsis thaliana*. Plant J., 41: 1-14.

Calenge F, Saliba-Colombani V, Mahieu S, Loudet O, Daniel-Vedele F, Krapp A (2006). Natural variation for carbohydrate content in Arabidopsis. Interaction with complex trait dissected by quantitative genetics. Plant Physiol., 141: 1630-1643.

Campbell JA, Hansen RW, Wilson JW (1999). Cost-effective colorimetric microtitre plate enzymatic assays for sucrose, glucose and fructose in sugarcane tissue extracts. J. Sci. Food Agri., 79: 232-236.

Cheng B, Janes HW (1997). Multiple forms of ADP-glucose pyrophosphorylase from tomato fruit. Plant Physiol., 113: 235-241.

Chuang T, Su J (1986). Starch phosphorylase inhibitor from sweet potato. Plant Physiol., 80: 534-538.

Frank G, Pressman E, Ophir R, Althan L, Shaked R, Freedman M, Shen S, Firon N (2009). Transcriptional profiling of maturing tomato (*Solanum lycopersicum* L.) microspores reveals the involvement of heat shock proteins, ROS scavengers, hormones, and sugars in the heat stress response. J. Exp. Bot., 60: 3891-3980.

Fu TD, Yang GS, Yang XN (1990). Studies on three line polima cytoplasmic male sterility developed in *Brassica napus* L. Plant Breeding, 104: 115-120.

Greene TW, Chantler SE, Kahn ML, Barry GF, Preiss J, Okita TW (1996). Mutagenesis of potato ADPglucose phyrophosphorylase and characterization of an allosteric mutant defective in 3-phosphoglycerate activation. Proc. Natl. Acad. Sci. USA, 93: 1509-1513.

Hendrix DL (1993). Rapid extraction and analysis of nonstructral carbohydrates in plant tissues. Crop Sci., 39: 1306-1311.

Horner HT, Palmer RG (1995). Mechanism of genic male sterility. Crop Sci., 35: 1527-1535.

Hubbard NL, Huber SC, Pharr DM (1989). Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing muskmelon (*Cucumis melo* L.) fruits. Plant Physiol., 91: 1527-1534.

Huber SC, Huber JLA (1992). Role of sucrose phosphate synthase in sucrose metabolism in leaves. Plant Physiol., 99: 1275-1278.

Jagadish SVK, Craufurd PQ, Wheeler TR (2007). High temperature stress and spikelet fertility in rice. J. Exp. Bot., 58: 1627-1635.

Jain M, Prasad PVV, Boote KJ, Hartwll JAL, Chourey PS (2007). Effects of season-long high temperature growth conditions on sugar-to-starch metabolism in developing microspores of grain sorghum (*Sorghum bicolor* L. Moench). Planta, 227: 67-79.

Jung K, Han M, Lee D, Lee Y, Schreiber L, Franke R, Faust A, Yephremov A, Kim Y, Hwang I, An G (2006). Wax-deficient anther 1 is involved in cuticle and wax production in rice anther walls and pollen development in rice (*Oryza sativa* L.). Plant Cell, 18: 3015-3032.

Karni L, Aloni B (2002). Fructokinase and hexokinase from pollen grains of bell pepper (*Capsium annuum* L.): Possible role in pollen germination under conditions of high temperature and CO2 enrichment. Ann. Bot., 90: 607-612.

Karuppiah N, Vadlamudi B, Kaufman PB (1989). Purification and characterization of solube (cytosolic) and bound (cell wall) isoforms of invertases in barley (*Hordeum vulgare*) elongating stem tissue. Plant Physiol., 91: 993-998.

Lee SC, Cheng H, King KE, Wang W, He Y, Hussain A, Lo J, Harberd NP, Peng JR (2002). Gibberellin regulates *Arabidopsis* seed germination via *RGL2*, a *GAI*/*RGA*-like gene whose expression is up-regulated following imbibition. Genes Dev. 16: 2175-2187.

Li CR, Zhang XB, Hew CS (2003). Cloning of a sucrose-phosphate synthase gene highly expressed in flowers from the tropical epiphytic orchid *Oncidium* Goldiana. J. Exp. Bot. 54: 2189-2191.

Li L, Ru Z, Gao Q, Jiang H, Guo F, Wu S, Sun Z (2009). Male sterility and thermo-photosensitivity characteristics of BNS in wheat. Sci. Agri. Sin., 42: 3019-3027.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Matsui T, Omasa K (2002). Rice (*Oryza sativa* L.) cultivars tolerant to high temperature at flowering: anther characteristics. Ann. Bot. 89: 683-687.

Morrison MJ (1993). Heat stress during reproduction in summer rape. Can. J. Bot., 71: 303-308.

Musgrave ME, Kuang A, Brown CR, Matthews S (1998). Changes in *Arabidopsis* leaf ultrastructure, chlorophyll and carbohydrate content during spaceflight depend on ventilation. Ann. Bot., 81: 503-512.

Nägele T, Henkel S, Hörmiller I, Sauter T, Sawodny O, Ederer M, Heyer AG (2010). Mathematical modeling of the central carbohydrate metabolism in reveals a substantial regulatory influence of vacuolar

invertase on whole plant carbon metabolism. Plant Physiol., 153: 260-272.
Nakamura Y, Imamura M (1985). Regulation of ADP-glucose pyrophosphorylase from *chlorella vulgaris*. Plant Physiol., 78: 601-605.
Nishizawa A, Yabuta Y, Shigeoka S (2008). Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. Plant Physiol., 147: 1251-1263.
Park J, Ishimizu T, Suwabe K, Sudo K, Masuko H, Hakozaki H, Nou I, Suzuki G, Watanabe M (2010). UDP-glucose pyrophosphorylase is rate limiting in vegetative and reproductive phases in *Arabidopsis thaliana*. Plant Cell Physiol., 51: 981-996.
Peterson R, Slovin JP, Chen CB (2010). A simplified method for differential staining of aborted and non-aborted polen grain. Int. J. Plant Biol., 1: e13-e16.
Prasad PVV, Craufurd PQ, Summerfield RJ, Wheeler TR (2000). Effects of short episodes of heat stress on flower production and fruit-set of groundnut (*Arachis hypgaea* L.). J. Exp. Bot., 51: 777-784.
Pressman E, Peet MM, Pharr DM (2002). The effect of heat stress on tomato pollen characteristics in associated with changes in carbohydrate concentration in developing anthers. Ann. Bot. 90: 631-636.
Rohde A, Morreel K, Ralph J, Goeminne G, Hostyn V, De Rycke R, Kushnir S, Van Doorsselaere J, Joseleau J, Vuylsteke M, Van Driessche G, Van Beeumen J, Messens E, Boerjan W (2004). Molecular phenotyping of the *pal1* and *pal2* mutants of *Arabidopsis thaliana* reveals far-reaching consequences on phenylpropanoid, amino acid, and carbohydrate metabolism. Plant Cell, 16: 2749-2771.
Saravitz DM, Pharr DM, Jr Carter TE (1987). Galactinol synthase activity and soluble sugars in developing seeds of four soybean genotypes. Plant Physiol., 83: 185-189.
Scofield GN, Ruuska SA, Aoki N, Lewis DC, Tabe LM, Jenkins LD (2009). Starch storage in the stems of wheat plants: Localization and temporal changes. Ann. Bot. 103: 859-868.
Singh SK, Kakani VG, Brand D, Baldwin B, Reddy KR (2008). Assessment of cold and heat tolerance of winter-grown canola (*Brassica napus* L.) cultivars by pollen-based parameters. J. Agron. Crop Sci., 194: 225-236.
Snider JL, Oosterhuis DM, Skulman BW, Kawakami EM (2009). Heat stress-induced limitations to reproductive success in *Gossypium hirsutum*. Physiol. Plant, 137: 125-138.
Stitt M, Gerhardt R, Wilker I, Heldt HW (1987). The contribution of fructose -2,6-bisphosphate to the regulation of sucrose synthesis during photosynthesis. Physiol. Plant, 69: 554-560.
Wang Z, Stutte GW (1992). The role of carbohydrates in acitive osmotic adjustment in apple under water stress. J. Amer. Soc. Hort. Sci., 117: 816-823.
Yasuor H, Abu-Abied M, Belausov E, Madmony A, Sadot E, Riov J, Rubin B (2006). Glyphosate-induced anther indehiscence in cotton is partially temperature dependent and involves cytoskeleton and secondary wall modifications and auxin accumulation. Plant Physiol., 141: 1306-1315.
Ye QQ, Zhu WJ, Li L, Zhang SS, Yin YH Ma H, Wang XL (2010). Brassinosteroids control male fertility by regulating the expression of key genes involved in Arabidopsis anther and pollen development. Proc. Natl. Acad. Sci. USA, 107: 6100-6105.
Young LW, Wilen RW, Bonham-Smith PC (2004). High temperature stress of *Brassica napus* during flowering reduces micro- and mega gametophyte fertility, induces fruit abortion, and disrupts seed J. Exp. Bot., 55: 485-495.
Younger VB (1961). Low temperature induced male sterility in male-fertile pennisetum clandetinum. Science, 133: 577-578.
Zhang H, Liang W, Yang X, Luo X, Jiang N, Ma H, Zhang D (2010). *carbon starved anther* encodes a MYB domain protein that regulates sugar partitioning required for rice pollen development. Plant Cell, 22: 672-689.
Zrenner R, Krause KP, Apel P, Sonnewald U (1996). Reduction of the cytosolic fructose-1,6-biphosphatase in transgenic potato plants limits photosynthetic sucrose biosynthesis with no impact on plant growth and tuber yield. Plant J., 9: 671-681.