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Full Length Article

Sucrose Metabolism and Accumulation is Disturbed during another Development in the T-type Cytoplasmic Male Sterility of Wheat (*Triticum aestivum* L.)

Qingsong Ba[1,2], Guiping Li[1], Yulong Song[2*], Hongzhan Liu[3*], Gensheng Zhang[1], Chu Chen[1], Yunxian Song[1] and Zhaolin Fu[1]

[1]*School of Life Science, Huaibei Normal University, Huaibei, 235000, Anhui, P.R. China*
[2]*Key Laboratory of Crop Heterosis of Shaanxi Province, Northwest A&F University, Yangling, 712100, Shaanxi, P.R. China*
[3]*College of Life Science and Agronomy, Zhoukou Normal University, Zhoukou, 466001, Henan, P.R. China*
[*]For correspondence: baqs1234@163.com

Abstract

T-type cytoplasmic male sterility (T-CMS) plays a significant role in hybrid seed production of wheat. An obstruction of starch accumulation in the T-CMS pollen grain insinuated that an anomaly in sugar metabolism might be associated with pollen abortion. To elucidate the relationship between sugar metabolism and T-CMS, levels of sucrose levels and its decomposition products, activities of key enzymes and gene expression level involved in its sucrose metabolism at mononucleus stage were investigated. The content of sucrose in T-CMS anthers remained higher than that in the fertile line, but the contents of glucose and fructose in T-CMS anthers were dramaticly decreased in contrast with the fertile line. The activities of cell wall bound invertase (CWIN), vacuolar invertase (VIN) and neutral invertase (NIN) during sucrose accumulation in anthers of T-CMS were also decreased to 17.4%, 57.8% and 55.9%, respectively. The results of Real-time PCR also manifested that the expression levels of CWIN gene (*IVR1*), VIN gene (*IVR5*) and sucrose transporter gene (*TaSUT1*) were significantly down regulated in T-CMS anther. It showed that the accumulation of sucrose in T-CMS anthers might involve a decrease in activity and a reduction in content of invertase. It can be concluded that an inability to metabolize incoming sucrose to hexoses resulted in T-CMS pollen developmental lesion. © 2019 Friends Science Publishers

Keywords: Male sterility; Sucrose metabolism; Invertase; Wheat

Introduction

Breeding of hybrid wheat is a promising pathway for increasing wheat production. Utilization of hybrid wheat could achieve nearly 15% increase in yield (Singh *et al*., 2015). However, the nature of self-fertilization of wheat makes it difficult to hybrid seed production. Several pathways have been used to create male sterile systems which include engineered male sterility, male sterility induced by chemical killing agents, nuclear cytoplasmic male sterility, nuclear male sterility, and photo/thermos/photo-thermo/thermo-photosensitive male sterility system (Singh *et al*., 2015). The discovery of cytoplasmic male sterility in Aegilops-Triticum crosses by Kihara established the initial interest in hybrid wheat breeding (Kihara, 1951). In subsequent studies, to develop commercial hybrid wheat, dependable male sterility systems were identified in the genetic background of *Triticum timopheevi* Zhuk. cytoplasm with the substitution of the nuclear genome of bread wheat (*Tritcum aestivum* L) by Wilson and Ross (1961).

Wheat T-type cytoplasmic male sterility (the cytoplasm came from *T. timopheevi* cytoplasm, T-CMS) (Mukai and Tsunewaki, 1979), which lacks negative cytoplasmic effects and possess more restoration resources, has been used commercially to a large extent (Singh *et al*., 2010). Despite some research on mechanism of T-CMS, a lot of achievements have been scored (Hernould *et al*., 1993; Hedgcoth *et al*., 2002; Xu *et al*., 2008; Sinha *et al*., 2013). However, the underlying mechanism involved in abnormal carbohydrate metabolism in wheat T-CMS is not fully explored.

Sucrose could be unloaded from the phloem in anthers, which is capable of exporting to microspores (Lalonde *et al*., 2003). Transport of sucrose during pollen development is the most important step for starch biosynthesis, and the downstream process of sucrose metabolism and starch synthesis depends on sucrose supply (Kong *et al*., 2007). Anther-specific expression of sucrose transporter genes (SUTs) have been reported in some plant species such as tobacco (Lemoine *et al*., 1999), arabidopsis (Sivitz *et al*., 2008), potato (KÜHN *et al*., 2010) and rice

(Hirose, 2010; Eom *et al*., 2016). In wheat, the homologous SUT genes may have crucial functions in transport of sucrose molecules (Aoki *et al*., 2002; Deol *et al*., 2013). Sucrose transport is accompanied by sucrose hydrolysis into hexose sugars by sucrose synthases (SUS: EC 2.4.1.13) and invertases (INV: EC 3.2.1.26), and the resulting hexoses serve as the major substrate for starch synthesis (Emes *et al*., 2003). The *INV* catalyzed conversion of sucrose to glucose and fructose, and participate in sugar partitioning (Sturm, 1999). Invertases can be categorized as subcellular location, that is, neutral invertase (NIN), cell wall bound invertases (CWIN), and vacuolar invertase (VIN). In wheat, the cell wall bound invertases (IVR1) and the vacuolar invertase (IVR5) were cloned respectively, which were also abundantly present in the tapetum (Koonjul *et al*., 2005). Moreover, inhibition of the anther specific *Nin88* gene (a cell wall bound invertase gene) impeded pollen development (Goetz *et al*., 2001).

Carbohydrates have a variety of functions in the development of pollen (Goetz *et al*., 2001; Hirsche *et al*., 2009; Kunz *et al*., 2014; Hirsche *et al*., 2017). However, little or no starch was observed in the mature pollen in T-CMS (Joppa *et al*., 1966). To better understand the relationship between sucrose metabolism and T-CMS, sucrose metabolism in wheat T-CMS anther was drilled down. Experiments have shown that the pollen abortion of the T-CMS lines mainly occurred at the mononucleate stage (Yao *et al*., 2003), so the expression of *TaSUT1A*, *IVR1* and *IVR5* genes from mononucleus stage of anther were analyzed via quantitative real-time PCR (qRT-PCR). Moreover, invertase activity and sucrose content from the same stage of anthers were also determined in the T- CMS anthers.

Materials and Methods

Plant Materials

A wheat T-type CMS line (90-110, TA) and a maintainer line (90-110, TB) (Ba *et al*., 2014), were sown in experimental plot in the Binhu campus of Huaibei Normal University, China. Fertility appraisal of the CMS line and its maintainer line were carried out for microspore observations with KI-I_2 solution staining. To identify the developmental stages, we collected some spikes using conventional acetic red dying techniques and 4',6-diamidino-2-phenylindole staining method (Fig. 1A and B). We collected the anthers in the middle florets of spikes at mononucleus stage of pollen development from the main stems of TA and TB. The harvested anthers were placed immediately into liquid nitrogen and stored at -80℃.

Microscopic Observation

Scanning electron microscope (JSM-6360LV, Japan) was used to observe pollen morphology. Fluorescence microscope tested pollen fertility using 0.1% iodine-potassium iodide staining method.

Measurement of Sugar and Determination of Invertase Activity

The methods of testing sucrose and hexose were determined according to Oliver *et al*. (2005). The invertase activities were determined according to the optimization method (Koonjul *et al*., 2005).

Gene expression Analysis

The qRT-PCRs were performed for gene expression analyses according to the protocol. The primers of invertase (*IVR1* and *IVR5*) and one sucrose transporter gene (*TaSUT1*) were designed on the basis of Primer-Premier 5.0. Gene-specific primers (Zhu *et al*., 2015) were used for gene expression analyses (Table 1). Furthermore, *18S* rRNA gene was as control for normalization, the relative transcript amount of each sample was expressed according to the optimization method (Yuan *et al*., 2006). Data analysis was carried out through SPSS18.0 software.

Results

Pollen Grains in T-CMS are Depleted in Starch

Pollens from TB displayed starch staining (Fig. 1C), meanwhile, pollen is round (Fig. 1E). However, pollens from plants in TA did not exhibit any starch staining (Fig. 1D), and the absence of starch resulted in pollen wall depression (Fig. 1F). These results indicated that an obstruction of starch accumulation in TA.

Anthers in Wheat T-CMS Accumulate Sucrose

To deepen understanding of mechanism of starch deficiency in T-CMS, we detected the levels of sucrose, glucose and fructose (Fig. 2). Sucrose level in TA was increased upto 3.2-fold ($P < 0.01$; Fig. 2a), compared with TB. This suggests that sucrose accumulated in TA anthers is metabolized or relocated in the process of pollen development. Compared with TA, the levels of glucose and fructose in TB anthers fortified 1.2 times and 2.1 times, respectively. However, ratio of sucrose to hexoses (glucose and fructose) was more in TA anthers (1.18), than in TB anthers (0.19), which indicated that sucrose massively accumulated in wheat TA anthers. One possibility is that the process of converting sucrose into glucose is blocked.

Changes in Anther Invertase Activity in Wheat T-CMS

In T-CMS anther, CWIN activity was significantly reduced compared with TB (± 49.2%; $P < 0.01$; Fig. 3a). VIN activity in TA anther was nearly 5-fold lower than those in TB. Meanwhile the activity of NIN in TB was more than double those in TA. The significant reduction in invertase activity may result in disruption of sucrose conversion.

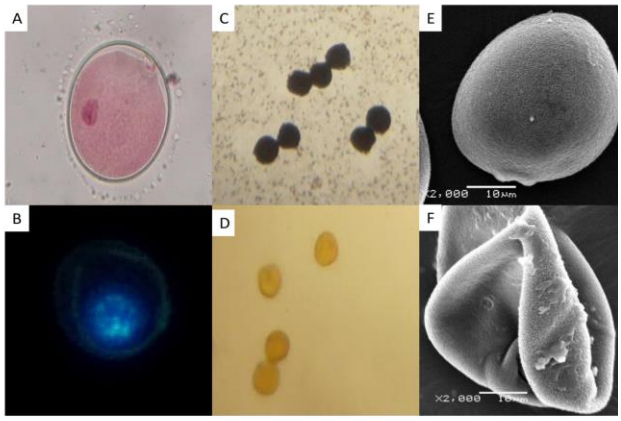

Fig. 1: Microspore development and pollen fertility in wheat
A, B were stained with acetocarmine and 4',6-diamidino-2-phenylindole at uninucleate stage. Pollen grains of C and D stained with 1% I_2–KI solution. Scanning electron microscopy analysis of the pollen surface from E and F. The pollens of C, E and D, F were from TB (maintainer line) and TA (T-type cytoplasmic male sterility line), respectively

Alteration of Expression of Anther Invertase Genes in Wheat T-CMS

To explore the relationship between gene expression in sucrose metabolism and the observed changes of the sucrose levels, we used the *IVR1* and *IVR5* genes and *TaSUT1*gene to analyze the expression level (Fig. 4).

Compared with the maintainer TB, the transcription of *IVR1* in T-CMS was severely suppressed (56.2%, $P < 0.01$), and *IVR5* transcription in T-CMS was nearly 4-fold decreased, simultaneously the TaSUT1 in T-CMS was prominent in anthers ($P < 0.01$).

Discussion

Male sterility is often related to abnormal accumulation of starch, brought about by abiotic stresses. There are some clews demonstrating carbohydrate metabolism participates in the formation of microspores in adverse environmental conditions. Low temperatures during pollen development lead to depletion of starch in pollens (Oliver *et al*., 2005). High temperature treatment reduced barley anther length, and impeded pollen starch formation (Sakata *et al*., 2010). Drought-induced pollen abortion in rice is related to inhibition of starch accumulation in pollen (Sheoran and Saini, 1996), and drought-induced wheat male sterility is related to a drop of invertase activity in pollen (Dorion *et al*., 1996).

Previous researches have demonstrated that the emergence of male sterile line is closely related to abnormal carbohydrate metabolism. Transcriptome analysis reveals starch metabolism had blocked male sterility in *rape*seed. (Li *et al*., 2015). Furthermore, sucrose metabolism was disturbed by chemical hybridizing agent SQ-1 with little starch in wheat pollen (Zhu *et al*., 2015). Compared with the maintainer line, generous genes in cotton (*Gossypium hirsutum* L.) genetic male sterility (GMS) lines were involved in starch and sucrose metabolism, and lack of starch accumulation was in male sterile pollen (Zhang *et al.*, 2015). Our observations also indicated that pollen grains of T-CMS fail to develop normally at maturity (Fig. 1F). Majority of pollens in T-CMS displayed little starch staining by I_2–KI staining (Fig. 1D). Thus, an impediment in starch accumulation was in the T-CMS pollen grains. Thus, a decrease of starch granules is a universal phenomenon of multiple male sterility types.

An important finding of present study was the amount of glucose in anthers which was approximately 2-4 times greater than that of fructose throughout anther development (Fig. 2). Fructose resulting from sucrose cleavage was probably phosphorylated very rapidly, since pollen grains have very high fructokinase activity (Nikolov and Nikolov, 2010). The process of converting sucrose into glucose was blocked, and sucrose accumulated in T-CMS anthers (Fig. 2). This interruption of converting sucrose into glucose in pollen is probably a key contributor to T-CMS, because the supply of glucose to starch synthesis in pollen plays an important role in pollen fertility (Lee *et al*., 2016).

Sucrose is generally converted to hexoses by invertase (INVs). In anthers, INVs are the crucial enzymes of sucrose conversion in pollen development (Saini and Westgate, 2000). As the experiment confirmed, CWINs play a dominant role in regulating the sucrose to hexose ratio (Kocal *et al*., 2008). CWINs have been also shown to participate in different aspects of physiological actions (Albacete *et al*., 2011; Albacete *et al*., 2014). Tissue-specific antisense repression of cwINVs resulted in an early arrest of pollen development (Zanor *et al*., 2009; Engelke *et al*., 2010). Thus, the two genes could be deemed to regulate sucrose transport from the tapetum to wheat pollen grains. An inhibition of any of the steps in sucrose metabolism could limit starch accumulation in T-CMS pollen. Our data also revealed that sucrose vastly accumulated in T-CMS anthers (Fig. 2). Simultaneously, expression of the wheat anther *IVR1* and *IVR5* in T-CMS were inhibited (Fig. 4). Furthermore, expression of *IVR1* and *IVR5* were suppressed in T-CMS anthers, and the tendency of this suppression was similar to the reduction in invertase activities (Fig. 3). The reduced invertase activity affects the generation of a metabolic signal as it is apparently the case during pollen development (Lee *et al*., 2016).

Sucrose transporter genes (SUTs) are prerequisite for conveying sucrose unloaded from the phloem to tissue sinks (Stadler *et al*., 2005; Milne *et al*., 2017). The homologous gene wheat SUT gene *TaSUT1* is expressed in large quantities in anthers (Sauer, 2007; Nguyen *et al*., 2010). A significant decline in *TaSUT1* gene transcription manifested that sucrose inversion of sucrose was smothered (Fig. 4A). The results supported the former hypothesis that sucrose conversion in T-CMS was suffocated in T-CMS pollen, and supply of glucose to the developing microspores was restricted.

Table 1: Target genes for analysis of expression profiles

Gene name	Genebank ID	Primer sequence (5'–3'□)	cDNA (bp)	annealing temperature
18S	AY049040	[F]AGTAAGCGCGAGTCATCAGCT [R]CATTCAATCGGTAGGAGCGAC	80	57
TaSUT1	AF408842	[F] GTGCTCTGATGGCTGATT [R] GAGGAACGGAAACCACTT	140	60
IVR1	AF030420	[F] TCGCCCTCAGGACATTG [R] CCAGACGCTTGTTCATCG	204	58
IVR5	AF069309	[F] TTCACTGTGCCTGTGCTCG [R] TCCGTCGGATACACCCTC	123	58

F, forward primer; R, reverse primer. Primer reference

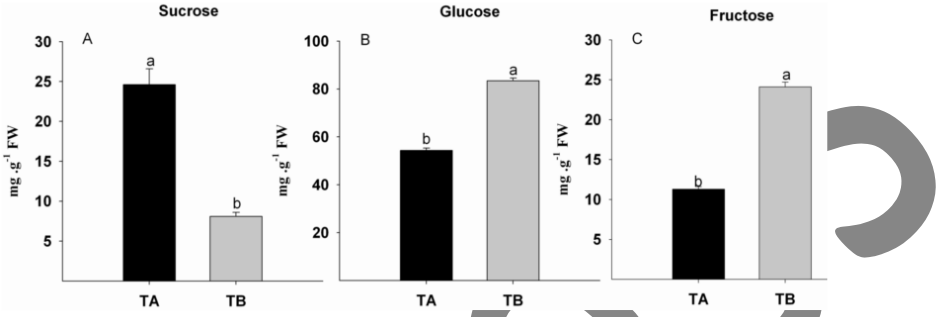

Fig. 2: Amount of sucrose (A), glucose (B) and fructose (C) of anthers from TA (T-type cytoplasmic male sterility line) and TB (maintainer line)
Different lowercase letters mean significant differences

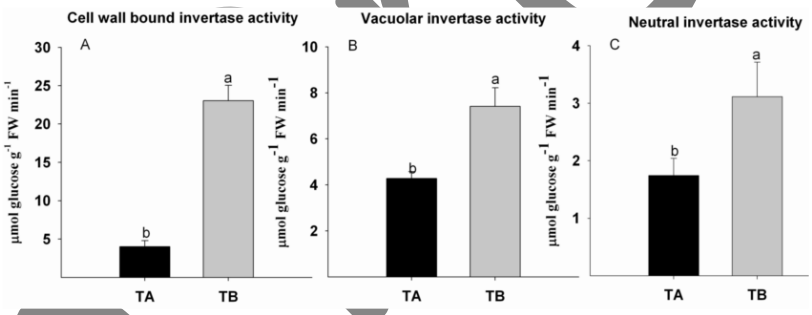

Fig. 3: Activities of cell wall invertase (A), vacuolar invertase (B) and neutral invertase (C) of anthers from TA (T-type cytoplasmic male sterility line) and TB (maintainer line). Different lowercase letters mean significant differences

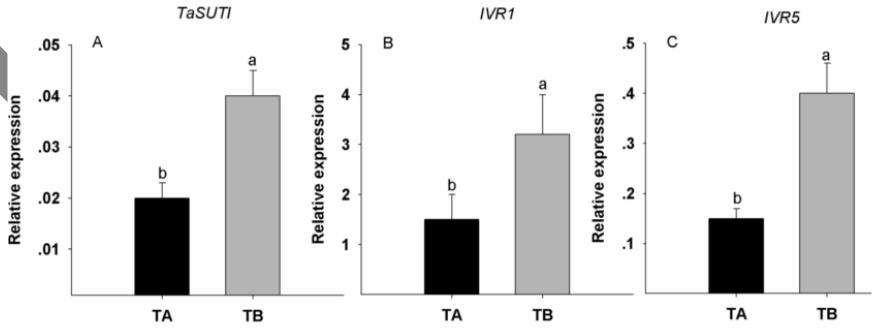

Fig. 4: Expression of *TaSUT1A* (A), *IVR1* (B), and *IVR5* (C) of anthers from TA (T-type cytoplasmic male sterility line) and TB (maintainer line). Different lowercase letters mean significant differences

Conclusion

Invertase activity and its gene expression are inhibited in T-CMS, as a consequence, inversion of sucrose is hindered in wheat anthers, accounting for absence of starch in T-CMS pollen.

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