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Quantitative Proteomic Analysis of CMS-Related Changes in Honglian CMS Rice Anther

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Abstract Honglian (HL) cytoplasmic male sterility (CMS) is one of the rice CMS types and has been widely used in hybrid rice production in China. The CMS line (Yuetai A, YTA) has a Yuetai B (maintainer line, YTB) nuclear genome, but has a rearranged mitochondrial (mt) genome consisting of Yuetai B. The fertility of hybrid (HL-6) was restored by restorer gene in nuclear genome of restorer line (9311). We used isotope-code affinity tag (ICAT) technology to perform the protein profiling of uninucleate stage rice anther and identify the CMS-HL related proteins. Two separate ICAT analyses were performed in this study: (1) anthers from YTA versus anthers from YTB, and (2) anthers from YTA versus anthers from HL-6. Based on the two analyses, a total of 97 unique proteins were identified and quantified in uninucleate stage rice anther under the error rate of less than 10%, of which eight proteins showed abundance changes of at least twofold between YTA and YTB. Triosephosphate isomerase, fructokinase II, DNA-binding protein GBP16 and ribosomal protein L3B were over-expressed in YTB, while oligopeptide transporter, floral organ regulator 1, kinase and *S*-adenosyl-L-methionine synthetase were over-expressed in YTA. Reduction of the proteins associated with energy production and lesser ATP equivalents detected in CMS anther indicated that the low level of energy production played an important role in inducing CMS-HL.

Keywords Anther · Cytoplasmic male sterility · Isotope-code affinity tag · *Oryza sativa* · Proteome

Abbreviations

HL	Honglian
ICAT	Isotope-code affinity tag
CMS	Cytoplasmic male sterility
Rf	Restorer fertility
YTA	Yuetai A
YTB	Yuetai B

1 Introduction

Plant cytoplasmic male sterility (CMS) is a widespread phenomenon observed in flowering plant species [16]. Pollen in sterile plants is inviable while pistil remains functional to accept fertile pollens from normal individuals. In agricultural production, cytoplasmic male sterility is greatly used in many crops in producing hybrid seeds because the inactive pollen eliminated the costly detasseling procedure. Since the utilization of heterosis takes such an important role in agricultural production, especially in China, CMS was extensively studied in many crops [3, 33]. On the other hand, because the CMS related genes lie in cytoplasmic genome, while the restorer gene(s) of fertility (Rf) in nuclear can restore the fertility of pollen, the CMS-Rf is a good model for nuclear-cytoplasm interaction research.

After the CMS related gene was first identified in CMS-T maize [7], many CMS related genes were also found in many crop species. Until now, almost all the CMS related genes are identified to be chimeric ORF in mitochondrial genome [14]. In rice (*Oryza sativa*), several CMS/Rf

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systems defined by the different CMS cytoplasm with distinct genetic features have been identified. These include CMS-BT (Boro II), CMS-WA (Wild abortive), and CMS-HL (Honglian) [19, 25, 27]. Although the CMS-HL is the latest founded in the three CMS types, it has been widely used in rice hybrid seed production in China. Microscopical study showed that the morphology of pollen of YTA, comparing with that of YTB, was clearly changed mostly in early binucleate stage with some exceptions in uninucleate stage [42]. But changes of bio-macromolecules have been detected in uninucleate stage [32]. In CMS-HL rice, a novel sequence is inserted into the end of the gene *atp6*, and can be co-transcribed with *atp6* [38]. It codes for a peptide ORFH79 which is thought to be the key protein related to CMS in CMS-HL rice. Although many CMS related genes have been identified in many plant species, the mechanism of how CMS genes led to a specific abortion in male gamete is far from clear. In CMS-HL rice, few studies were focused on the CMS mechanism. Wan's [32] report presented that the more oxidative stress on mitochondria during microspores development lied in sterile lines than in fertile lines. And in Li's report, a program cell death procedure was detected in CMS-HL rice anther cells. [18].

Because it is not easy to find the direct way of how CMS genes affect pollen development, methods in globally detecting different expression between CMS line and maintainer line are useful in studying CMS pathways. Recently, a global scale of the nuclear gene expression profile was performed by cDNA microarrays in cytoplasmic male sterile *Brassica napus* flowers [2]. A large number of genes differed in expression between the CMS line and maintainer line and some of them were involved in energy production pathway. Global scale of detecting changes in transcriptome and proteome were also studied in WA-CMS rice [9]. Energy production related genes/proteins were also found changed in data of microarray and 2-D electrophoresis. These studies give some new clues of the CMS mechanism.

Isotope-code affinity tag is a powerful tool for proteomics analysis developed in recent years [10, 28]. Two protein samples are labeled with light isotope or heavy isotope at the specific amino acid, usually cysteine. After digested by a protein enzyme, the proteins complexes are cut into small peptides, the peptides labeled with isotope are selected and sequenced by LC–MS/MS, and then the quantitative message is detected based on the signal came from the labeled isotope. ICAT is very useful in identifying the protein profile of a tissue and in comparing the protein different expression in different tissues or cells. Compared with 2-D electrophoresis based proteomics analysis methods, ICAT can quantitatively, globally, and automatically measure gene expression with a relative low amount of cells and tissues [23].

In this study, we performed two separate ICAT analyses: (1) anthers from sterile line (YTA) versus anthers from maintainer line (YTB), and (2) anthers from sterile line versus anthers from HL-6 [the hybrid of sterile line and restore line (9311)]. Combining the two analyses, we constructed a primary protein profile of uninuclear stage rice anther. And the analysis between YTA and YTB was performed to identify and quantify the proteins that are up- or down-regulated in uninucleate stage rice anther. This was the first study of CMS related changes in protein expression by ICAT technology.

2 Materials and Methods

2.1 Plant Materials

A typical Honglian (HL) CMS line, Yuetai A (YTA), its corresponding maintainer line, Yuetai B (YTB) and the restored hybrid line HL-6 (YTA/9311, F) were planted in an experimental field at Linshui, Hainan province, China in the summer of 2005. The plants were tended under the routine management regime. Anthers at particular developmental stage were collected by hand for experiments after plants being grown for about 3 months. The particular stage anthers was primarily classified by the auricle distance (AD, uninucleate stage is 0–50 mm, binucleate stage is 50–100 mm) between the auricle of the flag-leaf (last leaf) and that of the penultimate leaf, and confirmed by microscope observing [13, 32]. Anthers were picked out from the spikelets of the top three branches of immature panicles with precision forceps and scalpels at 4 °C and collected for experiments. Three biological replicates were performed in anther collection for protein and RNA isolation and 40–50 individual plants were included in each biological replicates.

2.2 Protein Preparation

Uninucleate stage anthers were collected on ice, then, 500 mg anther sample was grounded in a mortar with prechilled pestle and powder for about 10 min until they had become a very fine powder. The resultant powder was suspended in 5 mL of 10% TCA in acetone containing 0.07% DTT, and sonicated with the tube on dry ice using a probe sonicator in 5 × 10 s busts at 7 microns with 60 s intervals between bursts. After standing for 1 h at −20 °C, samples were centrifuged at 35,000*g* for 15 min. The precipitates were washed with prechilled acetone containing 0.07% DTT, standing overnight at −20 °C. Then samples were centrifuged at 12,000*g* for 15 min. Remove the acetone and wash the precipitates again. Then dry the precipitates by lyophilization for 2 min for ICAT analysis.

2.3 ICAT Labeling and LC–MS/MS Analysis

As shown in Fig. 1, the extracted proteins from YTA and YTB anthers were re-suspended in 400 μL of 8 M urea, 100 mM Tris (pH 8.3), 0.05% SDS w/v and 5 mM TBP. ICAT^{TM} reagent labeling with the light (C_{12}) and the heavy (C_{13}) was performed as per the protocol provided (Applied Biosystems, Foster City, CA, USA). Essentially, equal amounts of the two labeled samples (100 μg) were combined and digested into peptides by trypsin (Sigma, USA). ICAT-labeled peptides were subsequently purified by cation-exchange chromatography and avidin-affinity chromatography. Peptide mixtures were analyzed by microcapillary HPLC-electrospray ionization (ESI)-MS/MS using a Q-TOF2 mass spectrometer (Waters, Milford, MA).

2.4 Database Searches

The acquired MS/MS spectra were automatically compared against the National Center for Biotechnology Information (NCBI) Oryza sativa sub-database using a Mascot 2.1 program (Matrixscience, UK, [24] allowing 1 missed cleavage (trypsin) and 1.2 and 0.6 Dalton mass accuracy for MS and MS/MS, respectively. Modifications allowed were typical: ICAT on Cys, Met oxidation. ICAT ratios were calculated manually based on the Mascot search results. The ICAT experiment by design produced fewer peptides, but the requirement of containing Cys increases the confidence of the one peptide hits when manually curated (as these were). The probability score of each peptide and protein were calculated by Mascot and we extracted tables of high-confidence (90%) matched peptides with their ratios and used them for further calculations. The quality of peptide matches of interest was checked manually.

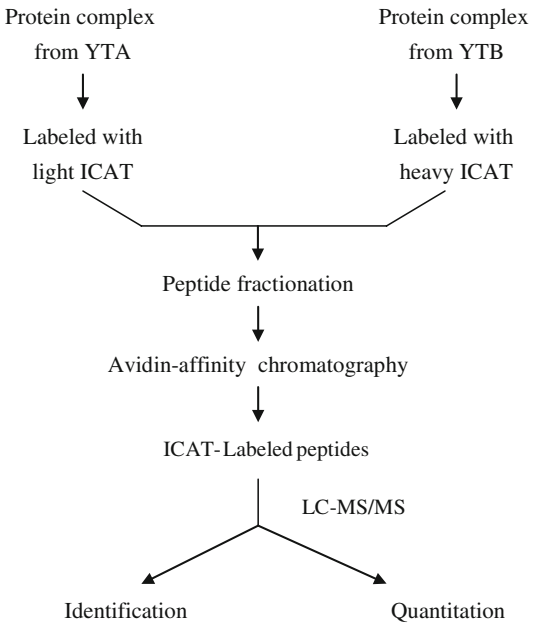

Fig. 1 ICAT approach for quantitative proteomic analysis. Protein sample from YTA and YTB were labeled with the isotopically light or heavy ICAT reagents, respectively. The labeled protein mixtures were then combined and proteolyzed; the combined peptide mixtures were further fractionated by cation exchange chromatography, and ICAT-labeled peptides were purified by avidin affinity chromatography. Next, these peptides were separated by microcapillary HPLC and subjected to MS/MS analysis. The proteins were identified by a database search of the MS/MS spectrum for matching peptides. The relative abundance was determined by the ratio of signal intensities of the tagged peptide pairs

2.5 RNA Isolation and Reverse Transcription-Polymerase Chain Reaction

Uninucleate stage anthers were frozen in liquid nitrogen and ground in a mortar and pestle. Total RNA was isolated using TRIzol reagent (Gibco BRL) according to the manufacturer's protocol. Total RNA was treated with DNase to remove residual genomic DNA. Aliquots of RNA (1–2 mg) were reverse-transcribed by using Superscript reverse transcriptase (Gibco BRL) at 42 °C. Reactions were primed with oligo(dT) and stopped by heating at 95 °C for 5 min. The first-strand cDNA pool then was diluted fivefold in dH_2O, and one-tenth of this was used in polymerase chain reaction (PCR) amplification. Primers used for PCR were listed in Supplemental Table 1. PCR amplifications were performed in 1.5 mM $MgCl_2$ and 2% DMSO for the following cycle conditions: 94 °C for 4 min, followed by 22 cycles at 94 °C for 45 s, 55 °C (according to primers, listed in Supplemental Table 1) for 1 min, and 72 °C for 1 min, and a final extension for 10 min at 72 °C. Reverse transcription (RT)-PCRs were repeated at least third times to verify the results.

2.6 ATP Measurements

The amount of ATP was measured by the luciferin-luciferase method [30] following the protocol of ATP detection kit (Beyotime, China). The rice anthers were collected on ice and immediately grinded with 200 μL lysis buffer from ATP detection kit. After centrifuged at 12,000*g* for 5 min at 4 °C, the supernatant was transferred to a new 1.5 mL tube for ATP test. The luminescence from a 100 μL sample was assayed in a luminometer (Perkin Elmer) together with 100 μL of ATP detection buffer from the ATP detection kit. The standard curve of ATP concentration was prepared from a know amount (1 nM–1 μM). All the experiments were carried out with triplicates; values reported are means of three replicates ±SD.

3 Results

3.1 Proteome Profile of Uninucleate Stage Rice Anther

Although clearly morphological changes of pollen of YTA were detected mostly in early binucleate stage, transcriptome and content of some enzyme were already changed in uninucleate stage anthers [32, 42]. In this study, uninucleate stage anthers were used for ICAT analysis. After the anther protein samples were extracted, we labeled the protein complex of YTA with light ICAT reagents, while YTB and HL-6 were labeled with heavy ICAT reagents. The labeled proteins were then combined, digested, fractionated, and quantification was accomplished using a suite of software tools [24, 39, 41]. In this study, we performed two separate ICAT analyses: (1) anthers from YTA versus anthers from YTB, and (2) anthers from YTA versus anthers from HL-6. The two analyses were used to construct a primary protein profile of uninucleate stage rice anther. Because YTA (sterile) and YTB (fertile) shared a same nuclear genome but had a different cytoplasmic genome, the ICAT analysis between YTA and YTB was used to find different expressing proteins which might relate to CMS-HL.

Under the error rate of 10%, we identified 69 proteins in the YTA/YTB experiment and 64 proteins in the YTA/HL-6 experiment. Totally, 97 unique proteins were identified in the two experiments (Supplemental Tables 2 and 3), of which 36 proteins were common between the two experiments, and 33 proteins were only identified in YTA/YTB experiment, while 28 proteins were only identified in YTA/HL-6 experiment (Fig. 2). Forty proteins (Data not shown) were previously identified in anther or pollen by 2-D based method [5, 6, 36].

3.2 Proteins Identified in Uninucleate Stage Rice Anther

The 97 identified proteins were classified to nine groups according to their function based on the GO consortium.

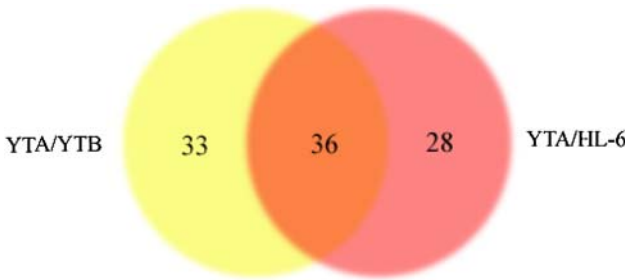

Fig. 2 Protein number identified in uninucleate stage rice anther. In both of YTA/YTB and YTA/HL-6 experiments, a total of 97 unique proteins were identified by the protein possibility threshold of 0.9, of which 36 proteins were common between the two experiments, and 33 proteins were only identified in YTA/YTB, while 28 proteins were only identified in YTA/HL-6

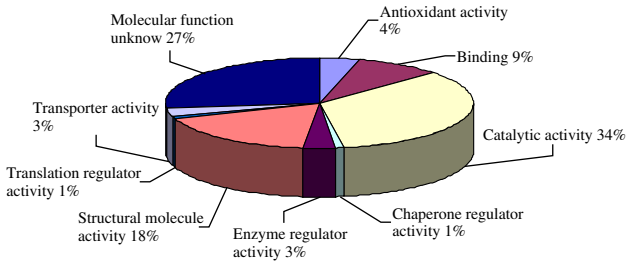

Fig. 3 Function classification of the identified proteins. The 97 proteins identified in uninucleate rice anther were classified into categories based on molecular function

The most of the proteins identified in uninucleate stage rice anther were enzymes (catalytic activity, 34%), and this was consistent with other studies [5, 6, 37]. The other groups are antioxidant activity 4%, binding 9%, chaperone regulator activity 1%, enzyme regulator activity 3%, structural molecule activity 18%, translation regulator activity 1%, and transporter activity 3%. Molecular functions for 27% of the proteins are still unknown (Fig. 3).

3.3 Proteins with at Least Twofold Change in Abundance Between YTA and YTB

The use of ICAT technology allows us to perform protein identification and quantification studies in the same experiment. The quantification of each protein is presented as a protein ratio between two samples tested. In this study, eight proteins showed different expression in uninucleate stage anther by at least twofold between YTA and YTB (Table 1). Of these proteins, oligopeptide transporter, floral organ regulator 1, kinase, *S*-adenosyl-L-methionine synthetase were over-expressed in YTA and triosephosphate isomerase, fructokinase II, DNA-binding protein GBP16, ribosomal protein L3B were over-expressed in YTB. The YTA/YTB ratio of the four proteins that over-expressed in YTA were from 2.3 to 4.8, while the YTB/YTA ratio of the four proteins that over-expressed in YTB were all around 2.0.

In this study, RT-PCR was used to further investigate the expression of the proteins listed in Table 1 in mRNA level. The primers used for RT-PCR were listed in Supplemental Table 1. *Actin* was used as a control to normalize the six templates. Furthermore, it can be used to show that there was no DNA contamination in cDNA templates because the primer of *actin* was designed to include both an ORF (720 base pairs) and an intron (249 base pairs), a big band (969 base pairs) could be detected if any DNA contaminated the cDNA template (Fig. 4).

In the RT-PCR experiment, we analyzed the eight genes expression pattern among sterile line (YTA), maintainer line (YTB) and the hybrid (HL-6) in both uninucleate stage and binucleate stage anthers. Expression patterns of gene

Table 1 List of proteins with at least twofold abundance change between YTA and YTB

Protein ID	Protein name	Ratio (YTA/YTB)	Molecular function
Over-expressed in YTB			
gi|169821	Triosephosphate isomerase	1/2.1	Catalytic activity
gi|2511541	DNA-binding protein GBP16	1/2.0	DNA binding
gi|37909690	Ribosomal protein L3B	1/2.3	Ribosomal protein
gi|16566704	Fructokinase II	1/2.4	Catalytic activity
Over-expressed in YTA			
gi|38347209	Oligopeptide transporter family	3.2	Other (oligopeptide transporter)
gi|27497120	Floral organ regulator 1	4.8	Floral development
gi|24899458	Kinase	2.3	Enzyme regulator
gi|1778821	*S*-adenosyl-L-methionine synthetase	4.2	Catalytic activity

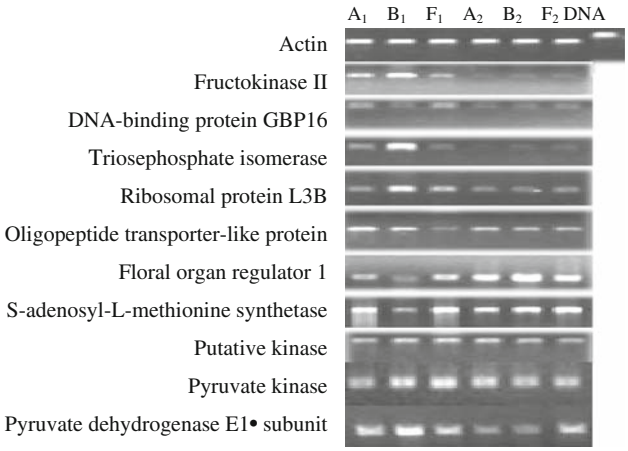

Fig. 4 Expression analysis of the gene listed in Supplemental Table 2 by RT-PCR. The genes were showed in *left*. The *Actin* gene was used as a control. A_1 means uninucleate stage anthers of YTA, B_1 means uninucleate stage anthers of YTB, F_1 means uninucleate stage anthers of HL-6. A_2 means binucleate stage anthers of YTA, B_2 means binucleate stage anthers of YTB, F_2 means binucleate stage anthers of HL-6. DNA means that in this cell, the template used in the PCR reaction is rice genomic DNA. Because the primer of *actin* is designed to include both an ORF and an intron, the big band only detected in the genomic DNA template cell can be used to show that there was no DNA contamination in the other cDNA templates

fructokinase II, *GBP16* and *triosephosphate isomerase* showed great difference between uninucleate stage and binucleate stage. These three genes might relate to anther development. On the other hand the difference of expression pattern between uninucleate stage and binucleate stage show that we have collected the right stage anthers.

In both ICAT and RT-PCR experiment, triosephosphate isomerase and fructokinase II, which is involved in energy metabolism, were down-regulated in YTA. Because some studies have showed that sufficient ATP supply is very important for anther cells development [35], we went through the ICAT data in YTA/YTB and found another two energy metabolism related proteins (pyruvate kinase and pyruvate dehydrogenase E1 α subunit) were also down-regulated in YTA. Although the YTB/YTA ratio of pyruvate kinase and pyruvate dehydrogenase E1α subunit didn't reach 2.0 (1.5 and 1.7, respectively), by further RT-PCR analysis the two proteins also showed distinct down-regulated in YTA. In total, four energy metabolism related genes were down-regulated in YTA. This indicated a relative low ATP level in YTA anther cells.

3.4 ATP Measurements

In order to compare the energy equivalents between YTA and YTB, we measured the amount of ATP by the luciferin-luciferase method [30]. Because tapetum was very important for pollen development, we measured the amount of ATP in rice anther but not only in pollen. The results were shown in Fig. 5. The amount of ATP in YTA anther was significantly lower than that in YTB both in uninucleate stage and in binucleate stage. This indicated that the energy production in YTA anther was restrained.

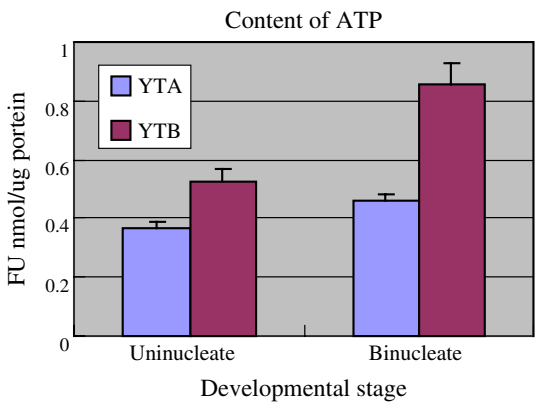

Fig. 5 Content of ATP in anthers. Content of ATP in uninucleate and binucleate developmental stage anthers. Data are the mean of three replicates ±SD

Moreover, the ATP level in binucleate stage was significantly increased than that in uninucleate stage in YTB anther. But the ATP level in binucleate stage was just slightly increased than that in uninucleate stage in YTA anther. This assumed that some of the energy production related pathways were further restrained between uninucleate stage and binucleate stage in CMS-HL anther.

4 Discussion

Although Cytoplasmic male sterility (CMS) is a widespread phenomenon in high plant and CMS has been widely used in producing hybrid seeds in many crops, the mechanism by which it specifically interrupts normal pollen development is not well understood. Previous studies showed that almost all the CMS in different plant species are caused by a specific ORF containing chimeric gene in mitochondrial genome [14]. To date, rarely similarity in different chimeric genes was found in different plant species. However, they share a same male gamete abortion phenomenon [11, 38]. While, the more interesting thing is how these different chimeric genes specifically interrupt pollen development but have no visible affection on pistils or other tissues. An increase in mitochondria per cell both in the tapetal cell layer (40-fold increase) and in the sporogenous cells (20-fold increase) were previously observed in normal maize anther [35]. These mitochondrial amplification events suggest an increased demand for energy during pollen formation [17, 34, 35]. However, a lowered ATP production was observed in some CMS flowers [1, 8, 31]. Therefore, one hypothesis has been put forward to explain the floral phenotypes of CMS lines is based on the idea that an increased demand for respiratory function and energy equivalents during flower development cannot be provided for by the abnormal mitochondria of a CMS line [12, 20, 26].

In vivo, ATP is mainly produced by three pathways: glycolysis, tricarboxylic acid (TCA) cycle and oxidative phosphorylation. Pyruvate dehydrogenase (PDH) is one of key regulators in the TCA cycle, a study in 2003 reported that depression of mitochondrial pyruvate dehydrogenase E1α subunit in anther tapetum can caused male sterility in sugar beet [40]. This data suggest that the affection on TCA cycle was enough to cause male sterility. In our study, two key enzymes (triosephosphate isomerase, fructokinase II) in glycolysis showed at least twofold changes between YTA and YTB. Besides this, pyruvate kinase and pyruvate dehydrogenase E1α subunit also showed different changes between YTA and YTB (Fig. 4). Totally, four key enzymes in glycolysis and tricarboxylic acid cycle decreased in YTA were identified in this study, and this implicated that a low level of energy production is present in YTA. Further measurement of ATP content also confirmed that the amount of ATP equivalents in YTA anther was significantly lower than that of YTB. It was clear that the energy production in CMS-HL rice anther was restrained. And this is in agreement with the hypothesis mentioned above. In addition, two similar results were also observed in CMS *Brassica napus* and WA-CMS rice by microarray analysis [2, 9]. Thirteen genes involved in glycolysis, in the citric acid cycle or are components of electron transport chains were significantly down-regulated in CMS *Brassica napus* flowers. And two genes involved in glycolysis and citric acid cycle were also down-regulated in WA-CMS rice. The further research on the effect of low energy production level on CMS-HL rice is ongoing in our laboratory.

In the ICAT data, four proteins were significantly overexpressed in YTA, they are oligopeptide transporter, *S*-adenosyl-L-methionine synthetase, floral organ regulator 1 and kinase. Because YTA and YTB share a same nuclear genome, the four proteins overexpressed in YTA should be induced by the CMS related gene (ORFH79) in mitochondria.

S-adenosyl-L-methionine synthetase is the enzyme responsible for the synthesis of endogenous SAMe from methionine and adenosine triphosphate. SAMe acts as a methyl group donor in many transmethylation reactions, including the methylation of phospholipids within the cell membranes whereby plasma membrane fluidity is restored. It is proposed that SAMe can contribute to the prevention of disruption of both cellular and mitochondrial membranes. In addition, oxidative damage on cell can also induce a corresponding increase of SAMe synthetase expression [21]. Moreover the oxidative damage on mitochondrial membranes can lead to a loss of mitochondrial potential and a consequent disruption of ATP formation. A significant elevation of SAMe synthetase in CMS line in our data indicated an oxidative damage on mitochondrial membranes in CMS-HL anther cells. Actually, an oxidative damage on mitochondrial has been observed in CMS-HL anther cells [32].

Oligopeptide transporter-like protein, floral organ regulator 1 and putative kinase also over-expressed in YTA. This indicated that they involved in inducing abortive pollen in CMS-HL rice. Although we had no idea about the possible function of these genes in CMS, some studies showed that they, at least, have some roles in flower development. Floral organ regulator 1 (FOR) was first discovered in Arabidopsis. It expressed specifically in flower organs and was supposed to contribute to flower development [15]. The oligopeptide transporter (OPT protein) is characterized by the ability of transporting peptides, which are usually tetra- and pentapeptides, across membranes in a carrier-mediated and energy dependent manner [22]. And a study [29] showed that a OPT protein is indispensable in

Arabidopsis flower development. Further functional studies of these genes are ongoing in our lab.

In this study, we performed three biological replicates for RNA isolation and three to five technical replicates for RT-PCR. The expression of protein GBP16 and Kinase was identified over-expressed in YTB and YTA, respectively, but GBP16 was increased in YTA and Kinase showed no difference in RT-PCR data. In fact, inconsistency between transcriptome and proteome was always found [4, 9]. The reason for that may attribute to the complicated post-transcript modification. In addition, almost no significant difference of these eight genes was found among YTA, YTB and HL-6 in binucleate stage anthers in mRNA expression, but differences can be found in uninucleate stage and ATP content both in uninucleate stage and binucleate stage anthers. This may because that (1) the significant enlargement of anther size from uninucleate to binucleate stage. This diluted the content of different expressed mRNA of microspore in the whole anther. (2) Carbohydrate and energy metabolism are very complicate regulated and connected, the different need of anther cells in different developmental stage can induce or reduce the mRNA expression of enzymes in glycolysis and TCA cycle. Anyhow, alterations in the transcriptome are not always or fully reflected in a similar alteration in the proteome, and predicts or conclusions based on transcriptome and/or proteome data should be verified by other methods.

Till now, lots of CMS related genes have been cloned in many important crops [20]. Although these CMS related genes showed rare similarity to each other, they all lead to a similar CMS phenomenon [20]. This implicates that there must be some common pathways in all kinds of CMS types and this common pathways should be more important than each individual CMS gene is. A quantitative proteomics overview is very helpful in finding the similar protein expression changes between different CMS plants. ICAT is a powerful tool for proteomics study [10, 28]. By this technology, we can easily and fast get a quantitative understanding of the information of protein different expression between two related samples. Use of this method and strict identification criteria we identified 97 non-redundant proteins in uninucleate stage rice anther, and 8 CMS related different expressing proteins. The ICAT data coupled with RT-PCR data indicates that energy metabolism may played an important role in CMS-HL rice. Overall, the data may help us to understand the molecular mechanism or the biochemical pathways leading to pollen abortive in CMS-HL rice. Although the use of ICAT labeling and LC–MS/MS for quantitative proteomics is becoming more widespread [37], this is the first sample of study CMS-induced changes. With the more quantitative proteomics data we get in different plant species or different CMS types, the common pathways of CMS will be clearer.

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