Transcript level analyses of genes expressed during rice meiosis in wild type and *Msp*1 mutant

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Abstract: 【Objective】 This study aimed to find some candidate genes for modifying rice development process by biotechnology, and further understand the molecular mechanism of rice gametogenesis during meiosis. 【Method】 The 22 K Ailent cDNA microarray was used to study the gene expression difference between wild type and Msp1 mutant during meiosis stage. 【Result】 After comparing the genes expressed during meiosis between wild type rice and Msp1 mutant by microarray, 208 genes whose expressions changed significantly ($P \leqslant 0.01$ and lg Ratio $\geqslant 0.2$) when rice meiosis were explored. The potential functions of these genes were predicted by ontology analysis and clustered in 18 groups based on GO classification, ranging from GTP, DNA Binding, cell wall, kinase activity, chloroplast, meiosis, ovule, chromosome, anther, endomembrane, cell division, metabolism, ubiquitin, transport, proteolysis, Ca^{2+} binding, RNA binding, and unknown. It showed that 99 genes, 47.60% of 208 genes, were involved in chromosome activity; 39 genes, 18.75% of 208 genes, were involved in endomembrane and transport process respectively. Among these 208 genes, one gene was related to meiosis intimately; three of them were related to Ca^{2+} binding; the expression of three genes was significantly higher in *Msp*1 mutant compared with wild type. It implies they might control the development of tapetum. Moreover, microarray and RT-PCR results demonstrated that rice OSC6 (oxidosqualene cyclase) homolouges gene Os11g0582500 (AK064672) was tapetum specific and involved in tapetum development. The relationship between meiosis and ubiquitin, and the role of three Ca^{2+} binding genes in rice meiosis were discussed. 【Conclusion】 After comparing the gene expression between wild type and *Msp*1 mutant by 22 K chips, 208 genes were found involved in rice meiosis control at various aspects such as cell metabolism, tapetum development, ion transport, nucleic acid metabolism, kinase activity. This study provided an experimental support for the further understanding of the molecular mechanism of rice meiosis and gametogenesis.

Key words: rice (*Oryza sativa* L.); cDNA microarray; *Msp*1 mutant; wild type; meiosis; gametogenesis

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野生型和 *Msp*1 突变体水稻减数分裂期转录水平上的基因表达分析

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[摘 要] 【目的】深入探索水稻减数分裂期间配子体发育的分子机理,大规模发掘与水稻配子体发育相关的基因,以便更有把握地利用基因工程手段改造水稻配子体发育的进程。【方法】利用 22 K Ailent cDNA 芯片,在全基因组水平上对野生型水稻和 *Msp*1 突变体在减数分裂期间表达基因的表达情况进行研究和分析。【结果】有 208 个基因在野生型和 *Msp*1 突变体中的表达差异极显著($P\leqslant 0.01$,lg Ratio$\geqslant 0.2$);对这些基因的核酸序列进行分析、GO(Gene Ontology)注释以及功能预测后,将其划归为 18 个大类,分别涉及到 GTP、DNA Binding、细胞壁、激酶活性、叶绿体、减数分裂、胚珠、染色体、花粉囊、内膜、细胞分裂、泛素、转运、代谢、蛋白解、Ca^{2+} 结合、RNA 结合、以及未知功能等;统计分析表明,有 99 个基因与染色体有关,占总数的 47.60%;39 个基因与内膜系统有关,占总数的 18.75%;有 1 个基因与减数分裂紧密相关,3 个基因与 Ca^{2+} 结合过程有关;另外有 3 个基因在 *Msp*1 突变体中的表达极显著高于野生型,表明这 3 个基因很可能参与了绒粘层的发育调控。基因芯片和 RT-PCR 检测结果表明,水稻基因 AK070642 特异于绒粘层,说明其参与了绒粘层的发育调控。【结论】研究通过基因芯片技术,将野生型水稻与其 *Msp*1 突变体在减数分裂期间表达基因的表达情况进行大规模对比后发现,有 208 个基因分别从细胞代谢、绒毡层的发育、离子转运、核酸代谢、激酶活性等方面调控水稻的减数分裂过程,为深入理解水稻减数分裂和配子体发育的分子机理提供了实验支撑。

[关键词] 水稻;cDNA 基因芯片;*Msp*1 突变体;野生型;减数分裂;配子体发育

Meiosis is not only an extremely important stage for genetic research but also essential for plant breeding. Plants have always been at the forefront of genetic and cytogenetic studies, but it is only after the plant genomic revolution has used *Arabidopsis thaliana* as a model can the first genes involved in plant meiosis be cloned[1]. Since then, in less than 10 years, close to fifty plant meiotic genes have been functionally characterized mainly in *Arabidopsis*[2]. Further, understanding meiosis in the cereals could enable the manipulation and significantly enhance the efficiency and pace of breeding programs. The completion of the rice genome sequencing, and the rapid progress in genomics *i.e.*, microarray technology has opened a door for screening large number of genes involved in rice meiosis[3].

Although, many studies have identified and characterized genes involved in meiosis, only a small number are validated for specific involvement in this complex developmental process[2,4]. In mouse, CDK2, which recruits CDC45 to replication origins, is essential for prophase I of meiosis but not for mitotic cell divisions[5]. Azumi et al. (2002) have shown that a novel cyclin-like protein, SDS, in *Arabidopsis* is essential for normal homolog synapsis and bivalent formation and suggest that a CDK may play a role in meiotic prophase I[6]. Other plant-specific cases include an *Arabidopsis* SKP1 homolog that is essential for male meiosis and yeast and human SKP1 genes that regulate the mitotic cell cycle[7]. Premeiotic S-phase is of particular interest because of its link with recombination and synapsis events of meiosis[8-9]. Moreover, anther and tapetum specific genes also have been isolated from *Arabidopsis*[10], maize[11] and tobacco[12]. In rice, MSP1 (MULTIPLE SPOROCYTE 1) gene plays a similar role in EMS1/EXS1 in meiosis[13]. One of the ARGONAUTE family gene, MEL1 (MEIOSIS ARRESTED AT LEPTOTENE1), has been identified in the rice from the analysis of seed-sterile mutants. It shows that this germ cell-specific rice MEL1 gene regulates the cell division of premeiotic germ cells[14]. However, the functions of

most of these genes are not validated. Moreover, lots of uncertainties exist as to how these genes are related to meiosis.

Considering the complexity of meiosis, it is necessary to explore additional genes that are contributing to normal meiosis at the genome level. The rice *Msp*1 mutant provides us the ideal material for this study. According to Nonomura et al. (2003), the function of the rice gene MSP1 controls early sporogenic development. The formation of anther wall layers is disrupted and the tapetum layer is completely lost in the *Msp*1 mutation. The development of pollen mother cells is arrested at various stages of meiotic prophase I, which results in complete male sterility [13]. Comparing the gene expression between *Msp*1 mutant and wild type will tell us which genes are controling the development of rice gametogenesis during meiosis.

Our studies were performed to identify genes controling gametogenesis during meiosis on a larger scale, particularly with the application of microarray techniques. It will contribute towards understanding the molecular mechanism of meiosis and further characterize the genes involved in meiosis and gametogenesis.

1　Materials and Methods

1.1　Crop husbandry and sampling

Seeds of rice (*Oryza sativa* L. cv. Nipponbare, IRTP 06669) were obtained from the International Network for the Genetic Evaluation of Rice, International Rice Research Institute (IRRI), Philippines. Seeds of *Msp*1 mutant are kindly donated by Dr. Nonomura from National Institute of Genetics (Japan). Plants were grown individually in pots containing clay loam soil under glasshouse conditions with natural sun light and photoperiod. Spikelets of size 3 mm were collected from wild type and *Msp*1 mutant at the same time. 2.3－3.4 mm Nipponbare spikelet covered most of the meiosis stage[15]. The tissue samples were frozen immediately in liquid nitrogen and stored at －80 ℃ for future RNA extraction. The growing condition and sample collection of *Msp*1 mutant was the same with wild type.

1.2　RNA extraction and purafication

Total RNA was extracted by pooling 3 mm spikelets from wild type and *Msp*1 mutant by Trizol method, according to the instructions from the manufacturer (Invitrogen, Carlsbad, CA). RNA samples were then treated with RNase-free DNase (Promega, Madison, WI) to remove any contaminating genomic DNA. Total RNA at a ratio of $OD_{260}/OD_{280}>1.9$ was further used to prepare mRNA using an mRNA Extraction Kit (Qiagen, Valencia, CA, USA).

1.3　Microarray hybridization, data analysing and gene anotation

The 22 k cDNA chip from Ailent company was used in the microarray hybridization experiment. The hybridization was repeated four times, including two biological repeats and two dye repeats. RNA purification, hybridization and washing were performed according to the manufacturer's instructions. 0.5 μg of polyA$^+$ RNA was labled with the fluorescence dye Cy3 or Cy5 following instructions from CyScribe Post-Labeling Kit. The slide images were scanned with a DNA microarray scanner (Agilent Technologies, http://www.home.agilent.com/) using the manufacturer's Feature Extraction software. Only the spots whose signal intensity was at least four folds higher than the background were further analysed.

The analysis of microarray data was carried out by SSH software from IRRI (International Rice Research Institute). Only data with $|\lg \text{Ratio}| \geqslant 0.2$ and P-value $\leqslant 0.01$ in all four replicates were clustered with software Mev4.0 from TIGR (http//:www.tigr.org).

In order to evaluate the predicted biochemical functions of the genes involved in rice meiosis three major rice databases were used for both BLASTn, and tBLASTn analysis: NCBI (http//:www.ncbi.nlm.nih.gov), TIGR (http//:www.tigr.org) and Agilent (http//:www.agilent.com). The gene ontology analysis was processed on KOME (http://cdna01.dna.affrc.go.jp/cDNA) and http://www.geneontology.org to predict the pathways and

functions of these genes. The protein localization analysis was carried on PSORT (http://psort.ims.u-tokyo.ac.jp/). GO Slim terms for genes (probes) were obtained from the TIGR database site (http://www.tigr.org/tdb/e2k1/osa1/batch_downloads.html).

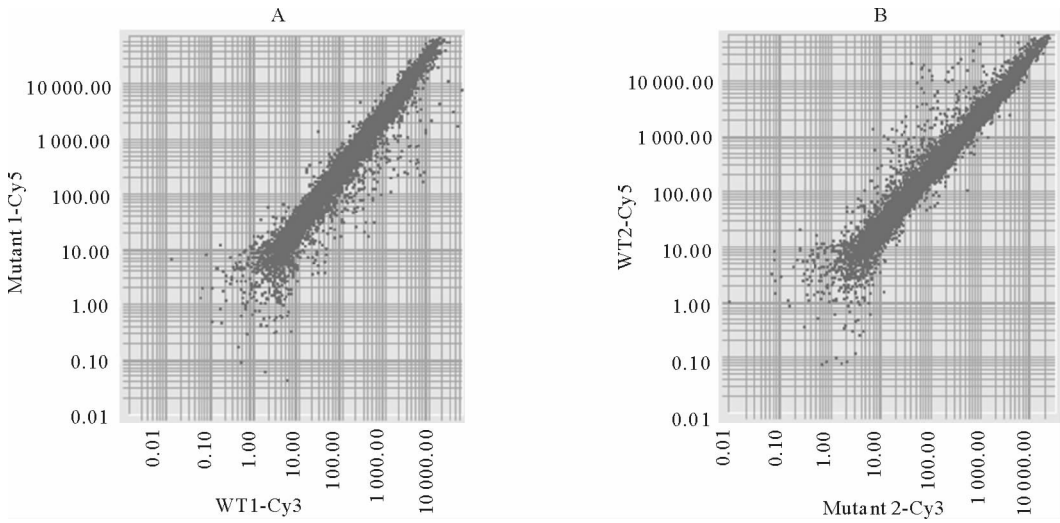
1.4 Reverse transcription polymerase chain reaction (RT-PCR)

To further study the gene expression patterns, reverse transcription-polymerase chain reaction (RT-PCR) was performed with SuperScript™ one-step RT-PCR with Platinum *Taq* (Invitrogen), according to the manufacturer's instructions. Similarly, total RNA for RT-PCR was extracted from 3 mm spikelet used for microarray. Gene specific primers were designed by Oligo5 software and synthesized by SBS company (Beijing, China). Cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as a control for verifying successful amplification and absence of genomic DNA. The gene specific primers were amplified with SuperScript™ one-step RT-PCR kit with Platinum *Taq* (Invitrogen). RT-PCR products were separated by electrophoresis using 1.5% agarose gels, post-stained using ethidium bromide and viewed using Gel Doc™ XR System (Bio-Rad, Sydney, Australia).

2 Results and discussion

2.1 The reliability of cDNA microarray in profiling gene expression during rice meiosis

We performed four replicates including two dye-exchange replicates with RNA extracted independently from different batches of samples to minimize the false-positive results in our microarray experiment. The results showed an overall balance of the two dyes in one hybridization and the expression level changes of genes detected from two replicates were superimposed (Fig. 1A and Fig. 1B). The correlation coefficients between two dye-exchange replicates ($R=0.989\ 2$) and between two biological repeats ($R=0.974\ 1$) were very high, suggesting that these microarray hybridization results are generally producible and reliable.



A. WT1-Cy3 vs. Mutant1-Cy5 (WT1 is wild type bio-replicate 1, Mutant1 is *Msp*1 mutant bioreplicate 1

B. WT2-Cy5 vs. Mutant2-Cy3 (WT2 is wild type bio-replicate 2; Mutant2 is *Msp*1 mutant bioreplicate 2

Fig. 1 Reproducibility analysis according to the ratio of the replicates with Cy3 and Cy5 (Distribution of about 22 000 genes in one hybridization)

2.2 SOTA clustering and Ontology analysis

After preliminary analysis of microarray data using SSH software from IRRI (International Rice Research Institute), only the genes, $|\lg \text{Ratio}| \geqslant 0.2$ and P-value$\leqslant 0.01$, in all four replicates, were clustered with software Mev 4.0 from TIGR (http://www.tigr.org). 212 genes fitting the above criteria showed significant changes in expression between wild type and *Msp*1 mutant in 3 mm spikelet, suggesting they may be possibly involved in rice meiosis. Among them, 208 genes had highly significant increase in expression during meiosis in

wild type；4 genes had a similar increase in expression in *Msp*1 mutant than in wild type.

So we further clustered the 208 highly expressed in wild type genes separately. SOTA Clustering result showed that all the 208 genes could be clustered in two major categories，namely，we could put these genes into two groups according to their contribution to rice gametogen esis during meiosis，and they could be further grouped into 11 specific sub-clusters according to their differences in expression intensity (Fig. 2).

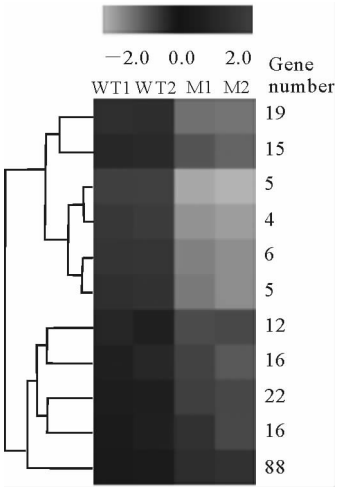


Fig. 2　SOTA Clustering of the 208 genes differentially expressed in meiosis between wild type and *Msp*1 mutant

WT1. Wild type vs. *Msp*1 mutant，repeat 1；WT2. Wild type vs. *Msp*1 mutant，repeat 2；M1. *Msp*1 mutant vs. wild type，repeat 1；M2. *Msp*1 mutant vs. wild type，repeat 2

According to the ontology analyses，the 208 genes，representing 0.95% of the 22 000 genes in the microarray chip，were divided into 18 groups based on their potential functions by GO slim term namely GTP，cell wall，chloroplast，ovule development，DNA binding，kinase activity，meiosis，chromosome activity，anther development，endomembrane，cell division，metabolism，ubiquitin，transport processes，proteolysis，calcium ion binding，RNA binding and unknown physiological processes.

The GO analysis results showed that 99 (47.60%) of 208 genes，were involved in chromosome activity；39 genes (18.75%) were involved in endomembrane and transport process respectively，7 in cell wall，32 in chloroplast development，24 in metabolism，37 in kinase activity，12 each in DNA binding and ubiquitin respectively，and 10 in proteolysis. 2 genes were involved in RNA binding while 2 other genes were found to be involved in ovule devel opment and 1 gene in meiosis and 3 genes in GTP and Ca^{2+} binding respectively. Further，4 genes were found involved in anther development and another 4 genes in cell division. However，there were 48 genes，nearly 23.1% of 208 genes，assigned to the unknown function group (Fig. 3).

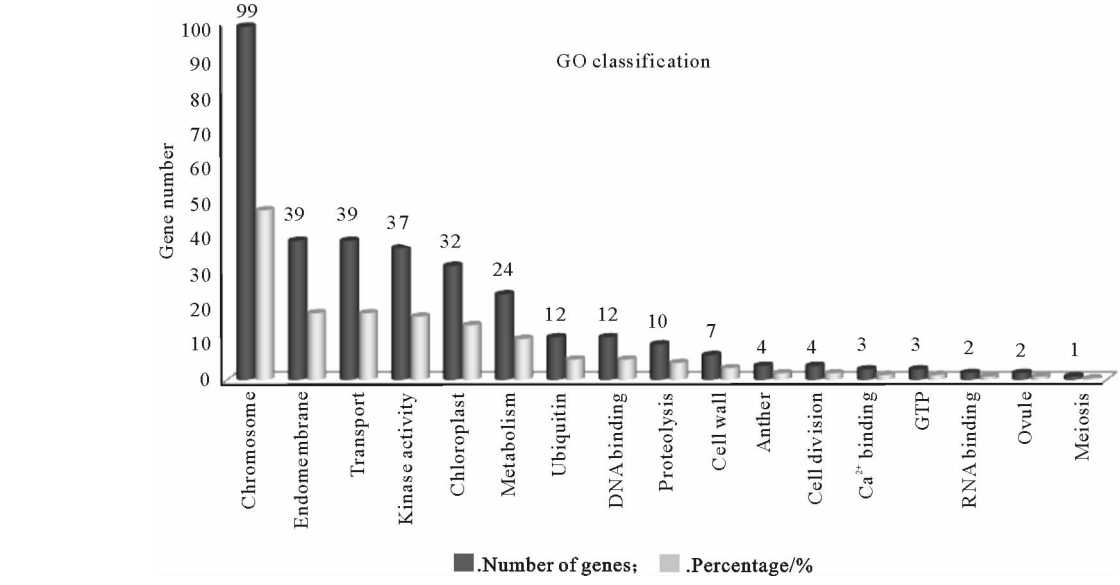


Fig. 3　Gene classification based on gene ontology (GO) in 17 clusters

Expression profiles of 208 significantly expressed genes between wild type and *Msp*1 mutant are classified according to GO analysis

Interestingly, although 208 genes were analyzed, the total gene number in all GO classification was 378. It showed that some genes had more than one function and simultaneously assigned to more than one functional group.

2.3 Potential genes related to rice meiosis and tapetum development

The research of Itoh et al. (2005) demonstrated that the meiosis of rice pollen mother cell is at Pachytene stage (homologous chromosome recombination) when the length of floret is around 2.3—3.4 mm, and the MMC (megaspore mother cell) is at zygotene to interkinesis stage when the length of floret is around 2.9—3.4 mm. Moreover, meiosis in rice anther is at diakinesis (breakdown of nuclear envelope); with meiosis in rice MMC from pachytene to interkinesis[15]. Due to the large complexity and diversity with the organism development, differences between morphological indicators and physiological process, determining the meiosis stage precisely by length of rice floret only is not completely reliable. However, for *Nipponbare*, it is confirmed by our sectioning results (data not shown) that floret length of 2.5—3.5 mm ranges from prophase I to telophase I. Moreover, Nonomura et al. (2003) showed that the development of pollen mother cells of *Msp*1 mutant is arrested at various stages of meiotic prophase I. This arrest in meiosis is accompanied by disrupted anther wall layers and com plete loss of the tapetum layer in *Msp*1 mutant[13]. It clearly shows that *Msp*1 mutant is the ideal material for reliably exploring genes involved in meiosis and tapetum development.

From the microarray analyses, in comparison with *Msp*1 mutant, the number of the genes with significant increase in expression at meiosis in wild type is much more than the number of genes with decreased expression. These results verify the arrest of the pollen mother cells development at meiotic prophase I and complete loss of tapetum layer in the *Msp*1 mutant. Moreover, the Ontology analyses showed that 35.7% of the genes have a role in general metabolism including metabolism, transport, chloroplast to endomembrane activities. Since the tapetum of *Msp*1 mutant is completely lost[13], which implies that these genes might be involved in tapetum development. Further, our annotation results also confirmed this prediction.

According to Nonomura et al. (2003), although the mutation never affects homologous chromosome pairing and chiasma maintenance, the development of pollen mother cells is arrested at various stages of meiotic prophase I, which results in complete male sterility[13]. Ontology analyses predict 99 genes have a role in chromosome activity, and that they should be involved in chromosome metabolic activities from prophase I to telophase I. Although it is unclear at which stage the expression increases, it clearly shows they are involved in chromosome activities through different pathways.

Among the 208 genes, Sinapis alba RNA—binding protein homologue (grp2a) gene Os05g0223200 (AK106308) and Plasmodium falciparum RNA-binding protein Puf1 gene Os03g0191700 (AK106885) could play a role in RNA binding based on the characteristics of their encoding proteins. It is reported that Mei2 gene controls the transportation of RNA involved in meiosis[16-19]. In order to accumulate convincing evidence about these two genes and their relation to meiosis, we further analyzed their sequences on PSORT (http://psort.ims.u-tokyo.ac.jp/). The protein localization analysis showed AK106308 expressed in the cytoplasm, and AK106885 in the mitochondrial matix space. The result of tBLASTn and BLASTn showed that these two genes as non-homologues of rice Mei2 further question their function in meiosis and RNA transportation.

The microarray results also showed rice (japonica cultivar-group) AP2-1 gene Os11g0129700 (AK112088) and another gene Os08g0442400 (AK106769) possibly involved in ovule development based on GO analysis. The result of BLASTn and tBLASTn showed both of them are homologues of arabidopsis BABY BOOM (AtBBM) gene. Ontology analyses also showed both these genes had a role in DNA binding. But AK106769

was identified with kinase activity, and AK112088 involved in metabolism. As the microspore is hypoplastic in *Msp*1 mutant, it is possible that these two genes have a role in microspore development.

2.4 Ubiquitin and rice meiosis

Selective proteolysis of regulatory proteins mediated by the ubiquitin pathway is an important mechanism for controlling many biological events[20-21]. Our microarray result showed that rice osk3 (Oryza sativa (japonica cultivar-group) skp1-like) gene Os09g0273800 (AK107960) was highly expressed in wild type during meiosis. The sequence analysis showed that AK107960 is a homologue of Arabidopsis SKP1/ASK1 (ARABIDOPSIS SKP1-LIKE1) gene Atlg75950 with high E value (5e-46). SKP1/ASK1is a key component of the SKP1-Cullin-F-box-protein (SCF) ubiquitin ligases that functions within the complex to link the substrate-recognition subunit to a cullin that in turn binds the ubiquitin-conjugating enzyme[22-23]. In *C. elegans*, SKP1-Related gene family performed critical functions in regulating cell proliferation, meiosis, and morphogenesis[23]. The Arabidopsis gene ASK1 is known to be essential for male meiosis, flower development, and auxin response[24]. Wang and Yang (2006) reported that the ARABIDOPSIS SKP1-LIKE1 (ASK1) protein acts predominately from leptotene to pachytene and represses homologous recombination in male meiosis[25]. It is also reported that ASK1 normally plays a repressive role in male recombination in Arabidopsis. The research in animal and other organism also indicted that SCF complex involved in meiosis, and the same with APC/C, SCF co-activate with CDK[26-28].

In rice, the function of SCF complex in meiosis has to be further verified, but the gene ontology classification predicts AK107960 is possibly involved in male meiosis with the protein localized in the cytoplasm. All these analyses imply that the encoding protein of AK107960 might negatively regulate recombination during meiosis similar to the Arabidopsis homologue. Further exploration of AK107960 and its involvement in meiosis would be an opening to understand the role of SCF complex in rice meiosis.

2.5 Ca^{2+} and rice meiosis

Elevated ion currents are crucial events in triggering the complex machinery involved in both gamete maturation and fertilization[29]. Calcium ions (Ca^{2+}) are the most common second messengers in animal cells (reference). Moreover, it is well established that calcium is involved in the physiology of the oocyte from oogenesis to maturation and fertilization in animals[30-33]. Particularly, it has been described that the transition from one meiotic phase to another is regulated by cell cycle control checkpoints which are in turn modulated by a transient increase of intracellular calcium in many animal species[34-35]. The research of Deguchi and Osanai (1994) showed that meiosis reinitiation from the first prophase is dependent on the levels of intracellular Ca^{2+}[36]. But it is not fully elucidated how the external calcium enters the cell through the plasma membrane ion channels.

Our microarray data showed that three rice genes, Conserved hypothetical protein gene Os04g0522800 (AK068328), Musa acuminata calmodulin-like protein gene Os03g0769500 (AK107501) and Hordeum vulgare calreticulin (CRH 1) gene Os03g0832200 (AK070712), expressed significantly higher in wild type than in *Msp*1 mutant. Gene ontology analysis indicated that all of these three genes have a role in Ca^{2+} binding. BLAST result implied that AK068328 encoded protein might be Oxygen evolving enhancer protein, and expressed in the cytoplasm. The encoding protein of AK107501 is putative to the regulator of gene silencing calmodulin-related protein and is predicted to be expressed in the mitochondrial matrix space. The encoding protein of AK070712 might be a calreticulin precursor and expressed in the endoplasmic reticulum. These results showed the involvement of the three genes in Ca^{2+} transportation in varying degrees during rice meiosis. The expression of these three genes are significantly higher in wild type than in *Msp*1 mutant implied that there might exist a meiotic arrest in rice, although it is not as clear as in the animal meiosis. Concentrating efforts on investigating of the role of

these three genes in calcium transportation during meiosis will help us to understand the role of Ca^{2+} in rice meiosis and answer the question of external calcium entering the cell.

Interestingly, it is reported that in the absence of intracellular calcium elevation spontaneous resumption of meiosis *in vitro* does not occur in animal[37]. It has been consistently shown that injection of calcium in mouse oocytes induces parthenogenetic activation and subsequent normal development[38]. It will be very useful for synthetic apomitic rice if Ca^{2+} has a similar function in rice meiosis as in mouse. The over-expression of these three genes might help to make process in developing apomictic rice.

2.6 Tapetum specific gene and its role in rice tapetum development

It is confirmed from the microarray data that the expression of Os11g0582500 (AK064672) is more than 100 folds higher in wild type than in *Msp*1 mutant. Sequence of AK064672 was homologues to Lotus japonicas rice OSC6 (oxidosqualene cyclase) protein NP_001068158.1. PSORT analysis predicted the protein of AK064672 can be expressed in the vacuole. RT-PCR results of AK064672 in 3 mm spikelet shows there is no amplification in *Msp*1 mutant spikelet but it is abundant in 3 mm wild type spikelet. Furthermore, RT-PCR with different sizes of wild type and *Msp*1 mutant spikelets showed no amplification in 1 mm to 7 mm *Msp*1 mutant spikelet (Fig. 4A). It implied that Os11g0582500 (AK064672) was only expressed in the tissue which was abundant in *Msp*1 mutant. Since the *Msp*1 mutant is characterized with no tapetum and with the development of pollen mother cells arrested at various stages of meiotic prophase I[13], AK064672 should be specific either to tapetum or to meiosis stages after prophase I. RT-PCR in different tissues showed that the mRNA expression in 3 mm, 5 mm and 7 mm wild type spikelet (Fig. 4B), with no product in 1 mm spikelet, 10 days root, 10 days shoot, flag leaf and second leaf. The amplification of AK064672 appearing in 7 mm wild type spikelet and lost in 1 mm wild type spikelet (Fig. 4A and Fig. 4B), indicates that AK064672 is tapetum specific.

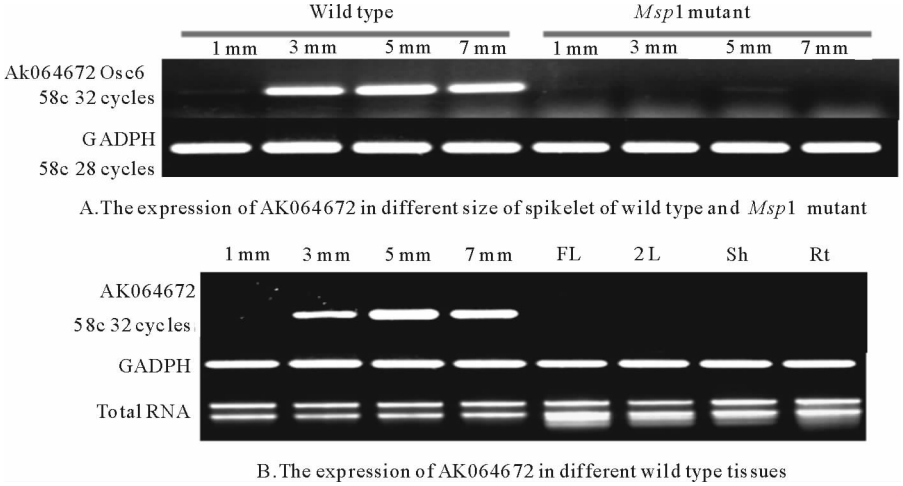


Fig. 4 Expression pattern of AK064672 in different tissues of wild type

1 mm is 1 mm spikelet (before meiosis); 3 mm is 3 mm spikelet (during meiosis); 5 mm is 5 mm spikelet (after meiosis); 7 mm is 7 mm spikelet (matured pollen). FL is flag leaf at heading stage; 2L is the second leaf at heading stage; Sh is 10 days shoot; Rt is 10 days root

It is reported that the OSC6 is abundantly expressed in anthers containing tetrads and uninucleate microspores, but are not expressed in leaves or roots[39]. The OSC6 mRNA was further showed to be localized only in the tapetal cells of the rice immature anthers, but not in the microspores or the mature pollen by in situ localization[40]. Our results are in confirmation with both these reports. The research of Tsuchiya et al. (1994) also showed that the 5′-upstream region of OSC6 was found to regu-

late beta-glucuronidase expression in the tapetum of transgenic tobacco[40].

3 Conclusion

212 genes significantly changing in expression during rice meiosis were identified and further explored by 22 K Ailent cDNA microarray. The expressions of 208 genes are significantly higher in wild type than in *Msp*1 mutant. 47.60% of these 208 genes are involved in chromosome activity, 18.75% of these 208 genes in endomembrane and transport process respectively.

OSC6 homologue gene Os11g0582500 (AK064672) is tapetum specific and is involved in tapetum development. It is important to rice gametogenesis.

The expression of three Ca^{2+} binding genes, Os04g0522800 (AK068328), Os03g0769500 (AK107501) and Os03g0832200 (AK070712), is significantly higher in wild type than in *Msp*1 mutant. The over-express of these genes might help to make process in developing apomictic rice.

We propose that the ubiquitin related genes such as osk3 gene Os09g0273800 (AK107960) also have an important role in gametogenesis during rice meiosis as their homologues in other organisms.

Further characterization of these candidate genes is required to the understanding of the molecular mechanism of rice meiosis and gametogenesis.

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