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OsCOI1, a Putative *COI1* in Rice, Show MeJA and ABA Dependent Expression*

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Abstract A novel gene, which was a homologue of *Arabidopsis COI1* was isolated from rice (*Oryza sativa* L.) by RT-PCR and designated as *OsCOI1*. It encoded a protein of 595 amino acids. The similar F-box motif and 16 leucine-rich repeats were found in the deduced protein OsCOI1. OsCOI1 and COI1 showed high homology (74%) at amino acid level. Semi-quantitative RT-PCR and Northern blot analysis demonstrated that the expression of *OsCOI1* in rice varied obviously after treatment with MeJA and ABA but was not affected by SA and ET, suggesting that the specific function of OsCOI1 in JA signal pathway and related ABA pathway.

Key words rice, F-box motif, semi-quantitative RT-PCR, Northern blot, JA signal pathway, ABA pathway

Plant responses to many biotic and abiotic stresses are orchestrated locally and systemically by signaling molecules known as the Jasmonate (JA). Jasmonates (JAs) regulate *Arabidopsis thaliana* wound and defence responses, pollen development, and stress-related growth inhibition. Recent study indicated that JAs also probably played a role in the senescence program[1~7].

COI1 (Coronatine Insensitive 1) is a key factor in the jasmonate signal transduction pathway in *Arabidopsis* [8]. The *coi1* mutant is male sterile. It decreased resistance to insect attack and reduced response to wound damage[9,10]. The *COI1* gene encodes a 66-ku protein with an F-box motif and 16 leucin-rich repeats (LRRs) and is not induced by JA[11]. COI1 has been shown to form a functional E3 ubiquitin ligase, SCF^{COI1}, together with cullin and SKP1 in *Arabidopsis* [12~15]. Thus, SCF^{COI1} was thought to target key regulators of JA pathway for ubiquitination and subsequent degradation by ubiquitin-proteasome pathway, and trigger appropriate JA responses in either defence responses or stamen and pollen development. Recent report suggested that SCF^{COI1} associated with the COP9 signalosome *in vivo* and they mediated JA responses collaboratively[16].

Recent study of jasmonate responses was mostly focused on *Arabidopsis*, a model plant of dicotyledon, and less progress has been made in monocotyledonous plants. In order to investigate the mechanism of jasmonate signal transduction pathway in rice, we tried to isolate the rice homologue of *COI1*, the key factor of JA response pathway. A candidate cDNA, *OsCOI1* (*Oryza sativa COI1*), was isolated from rice, and its expression patterns responding to methyl jasmonate (MeJA), salicylic acid (SA), abscisic acid (ABA) and ethylene (ET) were investigated.

1 Materials and methods

1.1 Rice materials

Seeds of *Oryza sativa* L. ssp. *Japonica* var. Zhongshu No.9520 was provided by Shanghai Baoshan Orchard.

The seeds were sterilized and soaked in water at 30℃ for 3 days, then spread on gauze and germinated at 30℃ in greenhouse for 16 h light/8 h dark. After 5 days, leaf and root materials were collected from seedlings, the rest of the seedlings were then divided into five treatments: water (as control), 10 μmol/L

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MeJA, 500 μmol/L SA, 0.1% ET(commercial ethylene is 40% solution (ethylene/H_2O, *w*/*v*) named as ethephon) and 100 μmol/L ABA. The leaves and roots of seedlings were collected after 0 h, 2 h, 10 h, 24 h, 48 h, and 72 h treatment, respectively. Those materials were frozen by liquid nitrogen and then stored at −80℃.

1.2 Blast in GenBank database to get candidate sequence(s)

In GenBank database, using COI1 amino acid sequence to blast (tblastn) against the EST database and the nucleotide non-redundant database of *Oryza sativa*, we found four EST fragments (accession numbers is BF430818, AU075473, AU032235, CA765272), showing high homologue with *COI1*, and a genomic fragment (accession number is AP003279.2) in accordance with those ESTs. By analyzing and aligning those EST fragments, a partial cDNA including stop code sequence showed up, then the downstream primer OsCOI13'1 (GCCCCA-GGGATAGATAGG) was designed accordingly. There was a conservative sequence (cgatccgatgg) resided around translation start code of *Arabidopsis COI1* and the genomic fragment of rice mentioned above. So we presumed this area to be the possible translation initiation region of rice COI1 gene and designed the upstream primer OsCOI15'1 (ACCTGGTTCGGGC-TAGATC) according to the genomic sequence.

1.3 Isolation and analysis of *OsCOI1* cDNA

Total RNA was extracted with Trizol Reagent (GIBRCOL) from seedlings cultivated for five days, according to the manufacturer's instruction. Reverse transcription (RT) reaction was carried out with Ready-to-go kit (Shenergy Biocolor Biological Science & Technology Company), using OsCOI13'1 as 3' primer and 1 μg total RNA as template. The reverse transcription product was subjected to amplify rice *COI1*, using OsCOI15'1 and OsCOI13'1 as primers. PCR condition was: 94℃ initial denaturation for 2 min; then 94℃ denaturation for 30 s, 60℃ annealing for 50 s, 72℃ elongation for 180 s, 30 cycles in total; with final extension at 72℃ for 7 min. The 1 904 bp PCR product was cloned into pGEM-T Easy Vector (Promega) and sequenced.

The nucleotide sequence of the new *OsCOI1* cDNA and putative protein sequence were compared with *COI1* using DNAStar and GeneDoc.

1.4 Semi-quantitative RT-PCR

Based on the cDNA sequence, a set of primers were designed: OsCOI15'2 (ACTTTCGGCTTGTGC-TACTT) and OsCOI13'2 (GAGGTGTAAACTCGA-TGTTCCA), with which a 420 bp PCR product can be obtained. The housekeeping gene *ACTIN1*[17] was used as control.

Total RNA was isolated from the seedlings, which treated with water, JA, SA, ABA, ET respectively for different time. The gene fragments of *OsCOI1* and *ACTIN1* were amplified using the two sets of primers (OsCOI15'2-OsCOI13'2 and actin5'1 (TCCGTGACATCAAGGAAAAG)-actin3'1 (GATA-TCAACATCGCACTTCATG)) in one PCR reaction system. The PCR condition was: 94℃ denaturation for 2 min at first; then 94℃ denaturation for 30 s, 58℃ annealing for 50 s, 72℃ elongation 60 s, 30 cycles totally; and 72℃ elongation for 7 min at last. The equal amount of PCR products from each amplification reaction was separated by 1.5% (*w*/*v*) of agarose gel. The quantity of the fractions of the PCR products was determined by light density scanning using gel image analysis system. The relative expression level of *OsCOI1* in different conditions was calibrated against the expression level of internal control gene *ACTIN1*.

1.5 Northern-blot analysis

Total RNA was isolated from the seedlings, which treated with MeJA and ABA respectively for different time. Northern-blot analysis was performed using 20 μg of total RNA and was separated with electrophoresis in 1.2% denaturing agarose gel. It was then transferred to Hybond nylon membrane. Probes were generated from 5' up-stream specific sequence of *OsCOI1* gene and radiolabeled with [α-^{32}P]dCTP using the random primer system. Standard procedures were used for RNA blot analysis[18].

2 Results

2.1 Cloning and sequence analysis of *OsCOI1* gene in rice

Sequence analysis of the 1 904 bp RT-PCR product showed that it contained entire cDNA coding sequence of *OsCOI1* (The accession number of *OsCOI1* cDNA in GenBank is AY 168645, Wang *et al*, submitted in 2002). *OsCOI1* cDNA sequence and its putative protein sequence are shown in Figure 1. In comparison with *OsCOI1* cDNA and the relative genomic fragment (accession number is AP003279.2), *OsCOI1* gene is about 3.3 kb, consists of 3 exons and 2 introns.

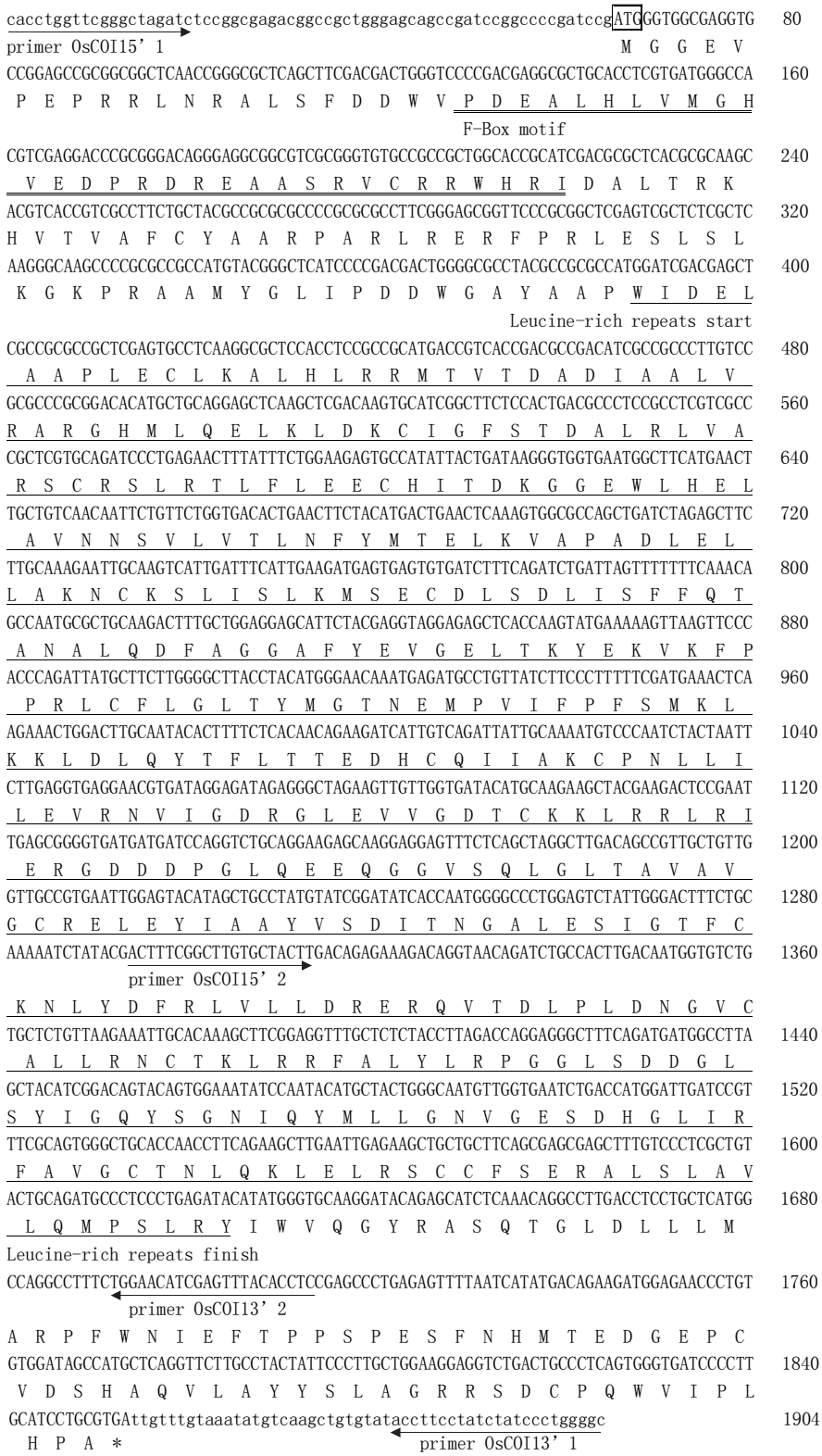

Fig. 1 Nucleotide sequence and deduced amino acid sequence of *OsCOI1* gene

Noncoding regions were shown in lowercase. F-box motif was indicated by double underline. Leucine rich repeats were marked by single underline. The *OsCOI1* cDNA primers (OsCOI15'1 and OsCOI13'1) and semi-quantitative RT-PCR primers (OsCOI13'2-OsCOI15'2) were also indicated below the nucleotide sequence.

The putative protein encoded by *OsCOI1* consists of 595 amino acids. OsCOI1 contains an F-box motif and 16 leucine-rich repeats (LRRs), which is the characteristics of COI1 in *Arabidopsis*. There is 74% similarity (56% identity) between these two proteins, suggesting that *OsCOI1* is probably the homologue of *COI1*.

2.2 Expression pattern of *OsCOI1* in different conditions

Semi-quantitative RT-PCR showed that the expression of *OsCOI1* induced obviously by MeJA in rice. *OsCOI1* transcript was upregulated after treatment with MeJA for 10 h, peaked around 48 h (Figure 2a, Figure 3). Whereas the transcript level of *OsCOI1* in materials treated by water has no distinct change (Figure 2e, Figure 3). Interestingly, we found that RNA transcript level was also upregulated by the induction of ABA (Figure 2b, Figure 3), and the expression pattern was consistent with that of MeJA. However, treatment with SA and ET didn't obviously affect the expression of *OsCOI1* (Figure 2c and 2d, Figure 3).

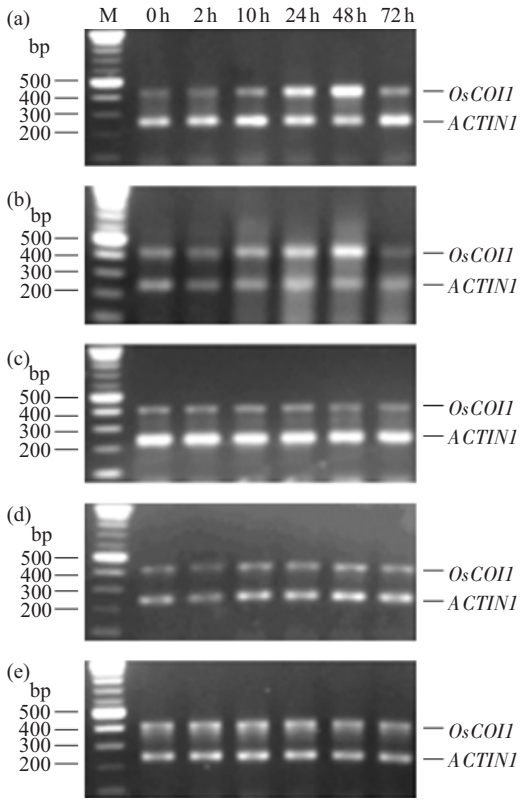

Fig. 2 The expression analysis of *OsCOI1* by semi-quantitative RT-PCR

(a), (b), (c), (d) and (e), the relative expression of *OsCOI1* in rice treated with MeJA, ABA, SA, ET and water (control), respectively.

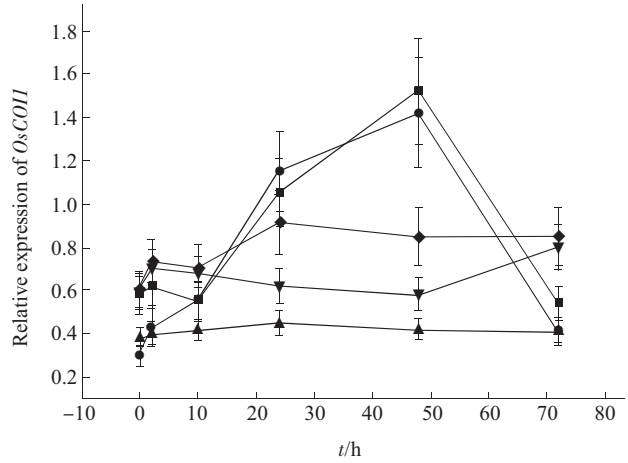

Fig. 3 Relative expression level of *OsCOI1*

The quantity of the fractions of the PCR products was determined by light density scanning gel in Figure 2, using gel image analysis system. The relative expression level of *OsCOI1* in different conditions was calibrated against the expression of *ACTIN1*. Results are the means of three independent experiments (error bars indicate *SD*). ■—■: MeJA; ●—●: ABA; ▲—▲: SA; ▼—▼: ET; ◆—◆: H_2O.

Northern-blot analysis also suggested that the expression of *OsCOI1* induced obviously by MeJA and ABA in rice (Figure 4)

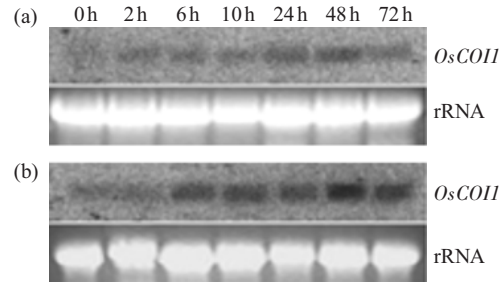

Fig. 4 Northern-blot analysis of *OsCOI1* gene expression

(a) and (b), rice seedling was treated with MeJA and ABA for different time, respectively.

3 Discussion

Recently from GenBank database, we found 2 sequences of rice, AK121543 and NM 190647, which were alternative splicing model. AK121543 is the same as *OsCOI1*. However, NM 190647 is a predicted mRNA of rice, its putative protein sequence contained 630 amino acids. Comparing these two splicing models, NM 190647 has one more exon before the first exon of AK121543/*OsCOI1* and its second exon was 49 bp longer than *OsCOI1* (Figure 5).

Based on the specific sequences of AK121543 and NM 190647, we designed two sets of new primers:

AKf (CCACCTGGTTCGGGCTAG) and NMf (ATGCCTCCGTATGAAACAGC), as the specific forward primer respectively, and reverse primer ANr (CCCTTGAGCGAGAGCGACT) was the same (Figure 5). If both of the two splicing models existed in rice, we would get 326 bp and 365 bp RT-PCR fragments using Akf-ANr primers and NMf-ANr primers respectively. However, we could not get the 365 bp fragment, which represented the predicted mRNA encoding 630 amino acids, from rice flower or seedling treated with ABA. Contrarily, the 326 bp fragment was there and still showed MeJA and ABA inducible character. This result suggested that NM 190647 was not a real splicing model or it only showed up in some special conditions that we did not get.

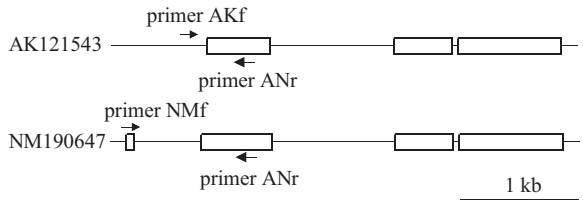

Fig. 5 A diagram of the exon/intron structures of AK121543/OsCOI1 and NM190647

Specific primers were synthesized for each form of mature mRNA.

It has been demonstrated that the site of action of JA was located downstream of ABA in certain wound response[19], which was consistent with our result. Early report suggested that ABA could promote the expression of some genes responding to wound signals *via* JA signal transduction pathway. It was possible that exogenous ABA promotes the endogenous JA, and in turn, the increase of endogenous JA enhanced the *OsCOI1* transcription in rice.

The characteristic F-box motif and LRRs existed in both COI1 and OsCOI1. And the two protein sequences showed high homology at amino acid level. However, semi-quantitative RT-PCR and Northern blot analysis demonstrated that expression of *OsCOI1* was induced by MeJA and ABA, while COI1 is not inducible by JA in *Arabidopsis* (data no shown), suggesting that *OsCOI1* probably worked in different way in the JA signal transduction pathway in rice.

Recent study has provided evidence that proper responses to JA were dependent on COI1 dosage. Most COI1-dependent JA-responsive genes require COI1 in dose-dependent manner and distinct JA responses have different sensitivities to COI1 abundance[16]. Although JA responses were also investigated in rice and other crops due to its important function in defence responses, the molecular mechanism remained unclear. Discovery of *OsCOI1* will provide valuable clue about the JA pathway in rice. Further studies on OsCOI1 are in progress.

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OsCOI1，水稻中一个受茉莉酸甲酯和脱落酸诱导表达的 F-box 家族基因 *

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摘要 利用 Blast 检索、EST 分析和 RT-PCR, 在水稻中分离到一个与拟南芥 *COI1* 同源的新基因，命名为 *OsCOI1*. *OsCOI1* 编码 595 个氨基酸. 推测的 *OsCOI1* 编码蛋白有一个 F-box motif 和 16 个富含亮氨酸的重复序列，这与拟南芥 *COI1* 相似. OsCOI1 在氨基酸水平上和 COI1 有很高的同源性(74%). 经半定量 RT-PCR 法和 RNA 印迹分析，表明水稻中 *OsCOI1* 表达水平在经茉莉酸甲酯和脱落酸处理后呈明显变化，但不受水杨酸和乙烯的影响，说明 OsCOI1 可能在茉莉酸信号途径和脱落酸途径中具有特定功能.

关键词 水稻，F-box motif，半定量 RT-PCR，RNA 印迹，茉莉酸信号途径，脱落酸途径
学科分类号 Q5，Q7

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