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Lab Note

The cDNA cloning of a novel bacterial blight-resistance gene *ME137*

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Bacterial blight (BB) of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most serious diseases of rice worldwide [1]. Use of resistant varieties has become the most economic and effective method to control the disease. To date, more than 30 genes have been found and characterized. Five dominant R genes, *Xa1*, *Xa3*, *Xa21*, *Xa26*, *Xa27*, and two recessive R genes, *Xa5*, *Xa13*, have been cloned [2–8]. However, because of the differentiation of physiological race and the continually emerging of new toxic bacterial flora, cultivated varieties with narrower resistance spectrum will lose their resistance ability to BB in a short period [9]. Therefore, excavating and identification of new resistance genes are of significance to control BB.

Wild rice species have long been used as genetic resources in rice breeding. Some genes that confer resistance to blight disease have been identified in wild rice. *Xa21*, which was first reported by Khush *et al*. [10], was the first gene found in wild rice (*Oryza longistaminata*). After cloning and application in breeding resistant varieties, resistance genes were identified successively in *O. rufipogon*, *O. minuta*, *O. officinalis,* and *O. australiensis* [11–14]. A previous study revealed that some of *O. meyeriana* materials are immune to the BB disease [15]. So far the genome of *O. meyeriana* has not been demonstrated, thus it is difficult to investigate the resistance gene. Furthermore, because hybridization could not happen normally between *O. meyeriana* (GG genome $2n = 24$) and cultivated rice (AA genome), it is hard to use the resistance gene of *O. meyeriana* by conventional hybridization approaches for improving cultivated rice. All of these indicate that we should try some other approaches to find and identify novel disease-resistance genes. It is interesting why *O. meyeriana* can be immune to BB disease and what kind of genes is related to BB disease resistance in *O. meyeriana*. In this study, a novel BB resistance-related gene, named temporarily *ME137*, was cloned from *O. meyeriana*. This study would lay the foundation for finding, identification and usage of new resistance genes, and breeding resistant varieties.

The main materials used in this study were as follows: *X. oryzae* pv. *oryzae* C1, *O. meyeriana* planted in a vinyl house (Biotechnology & Genetic Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming, China) at natural temperature and light, RNAiso Plus (TaKaRa, Dalian, China), M-MLV-reverse transcriptase (TIANGEN, Beijing, China), 5′-Full RACE kit (TaKaRa), SanPrep DNA gel extraction kit (Sangon, Shanghai, China), and pMD18-T Vector (TaKaRa).

O. meyeriana was inoculated with C1 by the leaf-clipping method in the flowering stage. Fresh leaves inoculated were cut and collected for cloning cDNA. The fresh leaves at 1 d before *Xoo* induction and 1, 2, 3, 4, 5 d after *Xoo* induction were used for semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. All the materials were frozen in liquid nitrogen immediately after collection and stored at −80°C for further use. Total RNA was extracted from collected leaf tissues and amplified into first-strand cDNA. The Expressed Sequence Tag sequence that has low homology with previously cloned ones to obtain a novel resistance gene was chosen from suppression subtractive hybridization (SSH) library and confirmed by a pair of primers 137-1 (5′-GACCCTGCC CAACA-3′) and 137-2 (5′-ACCGCTGCCTGAT-3′). The SSH library was constructed by the conventional method, after *O. meyeriana* was inoculated with *Xoo* as tester, and non-inoculated materials as driver [16]. The primers used for amplification of *actin* gene were actin-F (5′-GTATGG TGAAGGCTGGATTTGC-3′) and actin-R (5′-ATTTCCCG CTCTGCTGTTGTGG-3′).

To obtain 3′ end of *ME137*, the primer AP-dT (5′-CG CGGATCCAAGCTTATCGATTTTTTTTTTTTTTTT-3′) instead of Oligo dT was used when amplified into first-strand cDNA. Then, a pair of primers AP (5′-CGCGGATCCAA GCTTATCGA-3′) and 137-1 (5′-GACCCTGCCCAACA-3′) were designed to get the 3′ end of cDNA sequence. 5′ RACE was performed as recommended by the manufactures. To obtain specific band, nested PCR was used. In the outer PCR, a pair of primers outer-F (5′-CATGGCTACATGCTG

ACAGCCTA) and outer-R (5′-TGATTTTCCCTGACCC TTGCCTC-3′) were used. In the inner PCR, another pair of primers inner-F (5′-CGCGGATCCACAGCCTACTGATGA TCAGTCGATG-3′) and inner-R (5′-TTCTTGGAGACGTG AACGACGAG-3′) were used. The PCR products were electrophoresed on 1% agarose gel together with 2000 bp DNA Ladder as a marker. The specific fragment from PCR reaction was purified and cloned into pMD18-T vector for sequencing. The samples were also sent to Beijing Genomics Institute for sequencing.

Sequence chromatograms were examined and edited using Bioedit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The full length of cDNA sequence was spliced by DNAMAN 6.0 program (Lynnon Biosoft, Quebec, Canada). The gene has 1802 bp in length and contains an open reading frame of 1308 bp. There is a 115-bp 5′ untranslated region and a 379-bp untranslated flank at 3′ end. The latter includes a polyadenylation signal AATAA located 52 bp to the poly A tail (**Fig. S1**). The gene was temporarily named *ME137*. Protein motif features were predicted using the Simple Modular Architecture Research Tool (SMART) (http://smart.embl-heidelberg.de/). Domain prediction of *ME137* protein showed that the protein belongs to AAA ATPases (**Fig. S2**). AAA ATPases belong to the AAA+ superfamily of ringshaped P-loop NTPases, which act *via* the energy-dependent unfolding of macromolecules [17,18]. AAA ATPases play a number of roles in cells including cell-cycle regulation, protein proteolysis and disaggregation, organelle biogenesis and intracellular transport. Some of them function as molecular chaperones, subunits of proteolytic complexes and independent proteases. They also act as DNA helicases and transcription factors [19]. Multiple sequence alignment of amino acid sequences of the *ME137* with 18 plant species including *O. sativa Japonica Group* (GenBank accession number ABG22614), *Brachypodium distachyon* (XP_003577195), *Deschampsia Antarctica* (AAP83928), *Hordeum vulgare* subsp. *Vulgare* (BAJ41041), *Triticum aestivum* (AAF71272), *Zea mays* (NP_001104921), *Sorghum bicolor* (XP_002451328), *Gossypium hirsutum* (AAG61120), *Cucumis melo* subsp. *Melo* (ADN34076), *Vitis vinifera* (XP_002270571), *Medicago truncatula* (XP_003616450), *Glycine max* (NP_001242531), *Ricinus communis* (XP_002524206), *Arabidopsis* (XP_002881673), *Populus trichocarpa* (XP_002312110), *Capsicum annuum* (ACB05667), *Populus trichocarpa* x *Populus deltoids* (ABK96359), *Acer rubrum* (ABI94078) was performed using ClustalW (http://www.ebi.ac.uk/clustalw) set in MEGA 5.05 program (http://www.megasoftware.net). Related sequences were found using the Blast program at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST). The phylogenetic tree was based on Neighbor-Joining Method in which the number of Bootstrap is 200 using MEGA 5.05 program. The phylogenetic tree showed that *Populus trichocarpa* appeared to be distinct from the *ME137* that was grouped closely with *O. sativa Japonica Group* and then with *Brachypodium distachyon* (**Fig. S3**).

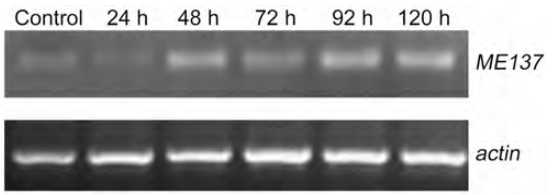

Figure 1 Expression levels of the *ME137* gene in different stages determined by semi-quantitative RT-PCR analysis The control was inoculated with sterile distilled water. The experimental groups were inoculated with *Xoo* at 24, 48, 72, 92, and 120 h. The *actin* gene is a housekeeping gene in rice.

To determine the detail expression patterns of the *ME137* in different stages, the cDNA levels were examined by semi-quantitative RT-PCR. The image of semi-quantitative RT-PCR was a representative of at least three independent experiments. Expression levels of *ME137* revealed that the *ME137* belongs to constitutive expression gene and the expression index is increasing in the 5 days after inoculating *X. oryzae* pv. *oryzae* (**Fig. 1**).

In conclusion, we have cloned the full length of cDNA about BB and observed its expression patterns in *O. meyeriana*. To our knowledge, we are the first to report the sequence of the AAA ATPase gene and its expression patterns in *O. meyeriana*. The results of this study suggest that *ME137* may play a critical role in the resistance of BB in *O. meyeriana*. This study would establish the foundation for excavating and cloning BB resistance-related genes from *O. meyeriana*. Although our work provides detailed information about *ME137* sequences; however, further studies will be required to explore the role of *ME137* in other gene regulation. Furthermore, the underlying molecular mechanisms associated with *Xoo* in *O. meyeriana* are needed to be clarified.

Supplementary Data

Supplementary data are available at *ABBS* online.

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