Two LTR retrotransposon elements within the abscisic acid gene cluster in *Botrytis cinerea* B05.10, but not in SAS56

Ming Zhao  
Chengdu Institute of Biology  
Chinese Academy of Sciences  
Chengdu 610041, PR China  
Graduate University of Chinese Academy of Sciences  
Beijing 100039, China  
Tel: 86 028 85219314  
Fax: 86 028 85219314  
E-mail: zhaoming02292002@yahoo.com.cn  

Jin yan Zhou  
Chengdu Institute of Biology  
Chinese Academy of Sciences  
Chengdu 610041, PR China  
Tel: 86 028 85219314  
Fax: 86 028 85219314  
E-mail: abath@cib.ac.cn  

You jiu Tan  
Chengdu Institute of Biology  
Chinese Academy of Sciences  
Chengdu 610041, PR China  
Graduate University of Chinese Academy of Sciences  
Beijing 100039, China  
Tel: 86 028 85219314  
Fax: 86 028 85219314  
E-mail: tanyou9@126.com  

Wei wei Song  
Chengdu Institute of Biology  
Chinese Academy of Sciences  
Chengdu 610041, PR China  
Graduate University of Chinese Academy of Sciences  
Beijing 100039, China  
Tel: 86 028 85219314  
Fax: 86 028 85219314  
E-mail: songweiwei426@sohu.com  

Zhi dong Li  
Chengdu Institute of Biology  
Chinese Academy of Sciences  
Chengdu 610041, PR China  
Tel: 86 028 85219314  
Fax: 86 028 85219314  
E-mail: abath@cib.ac.cn  

Hong Tan*  
Chengdu Institute of Biology  
Chinese Academy of Sciences  
Chengdu 610041, PR China  
Tel: 86 028 85219314  
Fax: 86 028 85219314  
E-mail: abath@cib.ac.cn  

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*Corresponding author
The plant hormone abscisic acid has huge economic potential and can be applied in agriculture and forestry for it is considered to be involved in plant resistance to stresses such as cold, heat, salinity, drought, pathogens and wounding. Now overproducing strains of Botrytis cinerea are used for biotechnological production of abscisic acid. An LTR retrotransposon, Boty-aba, and a solo LTR were identified by in silico genomic sequence analysis, and both were detected within the abscisic acid gene cluster in B. cinerea B05.10, but not in B. cinerea SAS56. Boty-aba contains a pair of LTRs and two internal genes. The LTRs and the first gene have features characteristic of Ty3/gypsy LTR retrotransposons. The second gene is a novel gene, named brtn, which encodes for a protein (named BRTN) without putative conserved domains. The impressive divergence in structure of the abscisic acid gene clusters putatively gives new clues to investigate the divergence in the abscisic acid production yields of different B. cinerea strains.

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Abbreviations: ABA: abscisic acid
IN: integrase domain
LTR: long terminal repeat
PBS: primer binding site
PPT: polypurine tract
RT: reverse transcriptase domain
TSD: target-site duplication
WGS: Whole Genome Shotgun

The phytopathogenic ascomycete Botrytis cinerea, is known to produce the plant hormone abscisic acid (ABA), which plays a major role in several steps of plant growth and development, such as stomatal closure, embryo and seed dormancy, seed germination and the adaptation to environmental stress (Tudzynski and Sharon, 2002). ABA has huge economic potential and can be applied in agriculture and forestry for it is considered to be involved in plant resistance to stresses such as cold, heat, salinity, drought, pathogens and wounding. Now overproducing strains of Botrytis cinerea are used for biotechnological production of abscisic acid. An LTR retrotransposon, Boty-aba, and a solo LTR were identified by an in silico genomic sequence analysis. To our surprise an impressive divergence in structure of the two ABA gene clusters was identified. An LTR retrotransposon and a solo LTR were detected within the ABA gene cluster in B. cinerea B05.10, but not in B. cinerea SAS56. For it has been shown that retrotransposons may contribute to the expression pattern of many host genes (Kashkush et al. 2003), this divergence in structure of the two ABA gene clusters putatively gives new clues to survey the divergence in the ABA production yields of different B. cinerea strains.

MATERIALS AND METHODS

Nucleotide sequences of bcaba1 (AJ609392), bcaba2 (AJ851088), bcaba3 (AM237449) and bcaba4 (AM237450) were obtained from GenBank and used to query the whole genome sequence (WGS) of B. cinerea B05.10 by BLASTN search provided by the Broad Institute (Altschul et al. 1997). The annotated features of the genome sequences from the bcaba4 gene to the bcaba3 gene were viewed using Browse Region provided by the Broad Institute. The scheme of the putative ABA gene cluster of B. cinerea B05.10 was drawn by the computer software Chem Draw ultra 8.0.

The DNA sequence of B. cinerea B05.10 from the bcaba4 gene to the bcaba3 gene was extracted using Browse Region provided by the Broad Institute. The repetitive elements were analyzed in RepeatMasker. The termini of the LTRs were confirmed by manual inspection. Protein domains were identified using RPSBLAST (Marchler-Bauer and Bryant, 2004).

Sequences of Saccharomyces cerevisiae tRNAs used for identification of PBSs were obtained from the Genomic tRNA Database (http://lowelab.ucsc.edu/GrtRnaDb/Scere) (Lowe and Eddy, 1997). The polypurine tract (PPT) and the target-site duplications (TSD) of LTR retrotransposon were investigated by manual inspection.

RESULTS AND DISCUSSION

The putative ABA gene cluster of B. cinerea B05.10 is located in the supercontig 41 from 72143 to 91420 bp (in the cont1.1706, NZ_AAID01001706). Sequence identity analysis reveals that each ABA gene of strain B05.10 shows a high degree of similarity to the ABA gene of strain SAS56 (gene bcaba1, bcaba2 and bcaba4 with 99% identity, respectively; gene bcaba3 with 98% identity). It is noteworthy that an astonishing divergence in structures of the two ABA gene clusters was identified. In B. cinerea SAS56, gene bcaba3 is located 3.7 kb upstream of gene bcaba1 (Figure 1a), while in B. cinerea B05.10, gene
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bcaba3 is located about 10.5 kb upstream of gene bcaba1 and two putative genes were detected within this region. A sequence similarity search by BLASTP and BLASTN revealed that the first gene is an LTR retrotransposon gag-pol gene, and the second is a novel gene (Figure 1b).

To investigate the structure features of ABA gene cluster of B. cinerea B05.10, about 20 kb of WGS sequences of B. cinerea B05.10 from gene bcaba4 to gene bcaba3 was extracted. The extract was analyzed with RepeatMasker. A solo LTR and a LTR retrotransposon that was designated as Boty-aba was identified. Boty-aba is flanked by 5 bp direct repeats (CATTC) representing target-site duplications (TSD). It contains 6603bp with a pair of LTRs flanking and two internal deduced genes. The LTRs contain 561bp (left) and 570bp (right), respectively. And they share 93.8% identity. A sequence similarity search by BLASTN revealed that the LTR of Boty (X81790), with 80.2% and 82.0% identity, respectively. Structure features analysis reveals that the three LTRs contain the 5'-terminal and 3'-terminal sequences (5’TG...CA3’) and perfect short inverted terminal repeats of 7 bp (TGTTACG...CGTAACA) (Diolez et al. 1995). The presumed TATA boxes are found in the three LTRs. Boty-aba and Boty contain the identical primer binding sites (PBSs) for first-strand reverse transcription with 9 nt (5’-TTTGAGGCAC-3’) immediately downstream of the left LTR. They both use self-priming mechanism to initiate synthesis of reverse transcripts (Lin and Levin, 1997). The polyuridine-rich sequence that corresponds to the primer binding site for plus-strand DNA (PPT) synthesis is located immediately upstream of the right LTR, and the sequence of PPT in Boty-aba is 5’-AGGCTAAGAGGGGATAG-3’. The solo LTR is located 448 bp upstream of Boty-aba and flanked by 5 bp direct repeats (CTCAT) representing TSD; it is inverted and is identical with the left-LTR of Boty-aba.

The first internal gene of LTR retrotransposon is a gag-pol gene. This gene has the same transcription direction as gene bcaba3 and contains 4255 nucleotides with 3 exons coding for 1302 amino acids. Conserved domain search analysis by RPSBLAST demonstrates that this gene encodes a polyprotein with a retrotrans_gag (gag) domain, a C2HC zinc fingers, a reverse transcriptase (RT) domain, and an integrase core (IN) domain (Figure 1c). This polyprotein does not contain protease and RNase H domains. The analysis of the conserved domains by RPSBLAST is shown in Figure 2. The fact that the domains of Boty-aba within the pol gene are arranged in the order RT and IN reveals that Boty-aba belongs to the Ty3/gypsy group of retrotransposons. The fact that the LTRs of Boty-aba are not identical and there are no protease and RNase H domains suggests that Boty-aba is an ancient element and it possibly cannot retrotranspose.
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Figure 2. Analysis of the:
(a) gag domain.
(b) RT domain.
(c) IN domain of the Boty-aba GAG-POL protein.

Absolutely conserved amino acids are indicated with an asterisk (*), identical residues are coloured red, similar residues are coloured blue, masked out regions are coloured gray. Q9HFY8 is the gag protein of an LTR-retrotransposon (Cgret) from Glomerella cingulata; Q9UVC2 is the gag polyprotein of an LTR-retrotransposon (CIT-) in Cladosporium fulvum; Q93283 is the gag polyprotein of the LTR retrotransposon from Takifugu rubripes; Q9EQ11 is the gag domain of Myelin expression factor-3-like protein from Mus musculus; CAB78181 is the putative reverse-transcriptase-like protein from Glomerella cingulata; T18348 is the RT domain of the hypothetical protein CNBB2750 in Cryptococcus neoformans var. neoformans B-3501A; XP_361801 is the RT domain of the hypothetical protein MG04275.4 in Magnaporthe grisea 70-15; T18348 is the RT domain of the gypsy retrotransposon in Magnaporthe grisea; 1BL3_C is the catalytic domain of HIV-1 integrase; Q9ZVK4 is the integrase core domain of the polyprotein from Arabidopsis thaliana; O64892 is the integrase core domain of the polyprotein from Ananas comosus; Q9ZPF9 is the IN domain of the F5K24.1 protein (putative polyprotein) from Arabidopsis thaliana.

Retrotransposons are a widespread and important class of eukaryotic mobile genetic elements that have a central role in the structure, evolution, and function of eukaryotic genomes (Bennetzen, 2000; Kidwell and Lisch, 2001). Recent reports have shown that retrotransposons contribute to the formation of genome structure and to the expression pattern of many host genes (Kashkush et al. 2003). For example, when Wis 2-1A retrotransposons are activated in wheat, the expression of several adjacent genes is activated or silenced by producing sense or antisense transcripts of those genes (Kashkush et al. 2003). Matsubara concluded that at least 3 transposable elements in Hf1 gene that plays a key role in the expression of floral colour in petunias govern anthocyanin biosynthesis of commercial petunias (Matsubara et al. 2005). It is not known whether Boty-aba has an affect on the expression of ABA genes. This diversity in structure of the ABA gene clusters gives new clues to survey the divergence in the ABA production yields of different B. cinerea strains.
The second internal gene of LTR retrotransposon is 597 bp long and encodes 199 amino acids. It is transcribed divergently. No putative conserved domain has been detected using RPSBLAST. On the basis of these results, we believe that this is a novel gene, which we designate brtn (B. cinerea retrotransposon novel) and the deduced protein is named BTRN. The structural features of Boty-aba are depicted in Figure 1b. We also identified an EST (W0AA017ZF01C1) shows high sequence similarity to gene brtn (100% identities). We presume that the internal promoter of the flanking LTRs drive the transcription of gene brtn.

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