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RESEARCH ARTICLE

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

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 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.

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 (Tudzynski and Sharon, 2002). The production yields reported of *B. cinerea* ABA are 0 ~ 1.6 g/L in axenic culture (Wu and Zheng, 1997; Tan and Li, 1998; Wu and Shi, 1998; Tudzynski and Sharon, 2002; Liang et al. 2004). Now overproducing strains of *B. cinerea* are used for biotechnological production of ABA (Tan and Li, 1998). Recently, an ABA gene cluster that contains gene *bcaba1*, *bcaba2*, *bcaba3*, and *bcaba4* in *B. cinerea* SAS56 was identified (Siewers et al. 2004; Siewers et al. 2006).

The Whole Genome Shotgun (WGS) sequences of *B. cinerea* B05.10 are public available at http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi?organism=fungi. In this work, the putative ABA gene cluster of *B. cinerea* B05.10 was 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/GtRNAdb/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

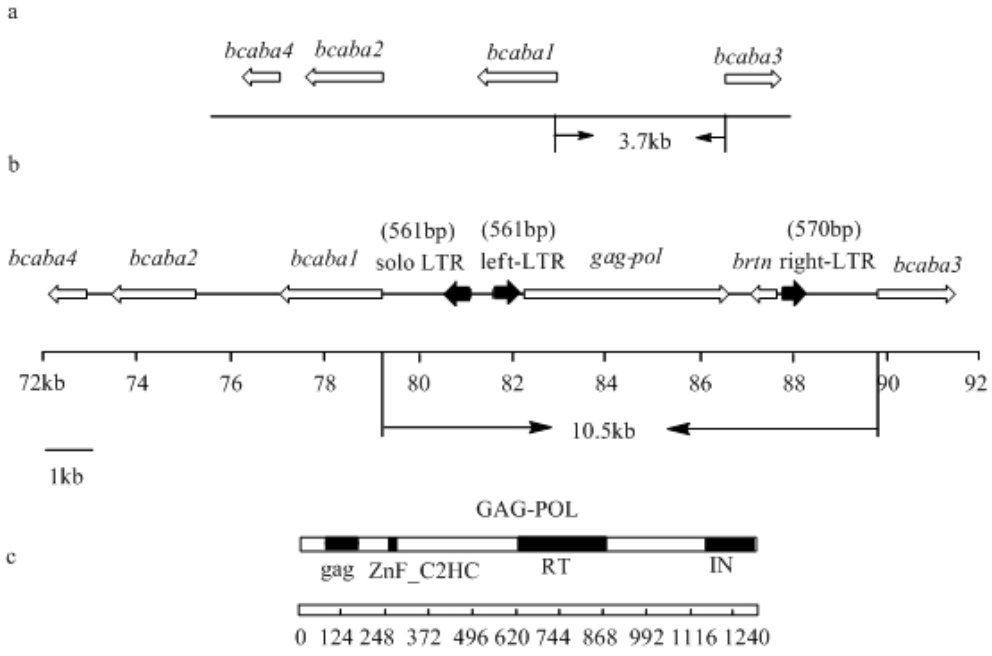

Figure 1. Representations of the structural features of :
(a) The ABA gene cluster in B. cinerea strain SAS56 (Siewers et al. 2006).
(b) The ABA gene cluster in B. cinerea strain B05.10.
(c) Protein GAG-POL of Boty-aba.

DNA depicted with ___, Genes were depicted with the ⇨, LTRs is indicated with ➡, and the putative conserved domains is indicated with ▬

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* were 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 demonstrates that both left and right LTR show significant similarity to the LTR of *Boty* (X81790), with 80.2% and 82.0% identity, respectively. Structure factures 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'-TTTGAGCAC-3') immediately downstream of the left LTR. They both use self-priming mechanism to initiate synthesis of reverse transcripts (Lin and Levin, 1997). The polypurine-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'-AGGCTAAGAAGGGGATAG-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.

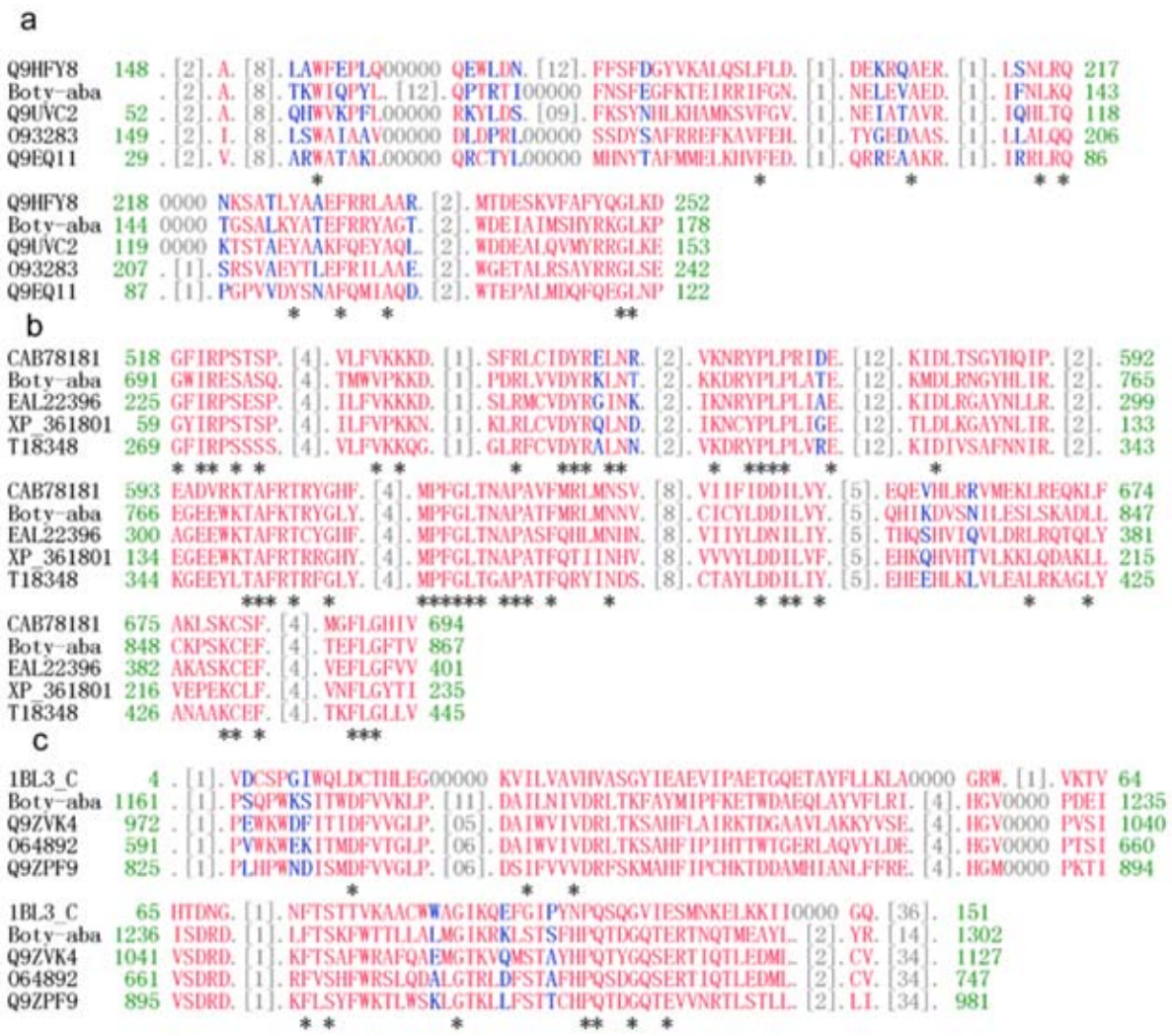

Figure 2. Analysis of the:
(a) gag domain.
(b) RT domain.
(c) IN domain of the *Boty-aba* GAG-POL protein.

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 (*CfT-I*) in *Cladosporium fulvum*; O93283 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 *Arabidopsis thaliana*; EAL22396 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 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 putative retroelement pol 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 divergence 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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