Preliminary

Analysis of *Copia*-like Retrotransposons in Poplar (*Populus alba* L.)*[1]

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Retrotransposons are ubiquitous in the plant kingdom and the best-characterized class of mobile genetic elements, constituting an appreciable fraction of the repetitive DNA of plant genomes. *Copia*-like retrotransposons harboring long terminal repeat encode a reverse transcriptase and replicate through an RNA intermediate like retroviruses. It has been reported that they were activated under certain stress condition such as tissue culture, pathogen infection and wounding, though little is showed the activation in normal growth condition. Tissue culture of woody plants is an important means to propagate genetically identical individuals asexually and to produce transgenic plants. However, undesired genetic and cytogenetic modifications are frequently induced during tissue culture. The recent report indicated that the activation of rice retrotransposons is involved in tissue culture-induced mutations[1]. In this study, we tried to clone copia-like retrotransposons in poplar cultured cells utilized extensively as a well established experimental system in woody plants and to reveal their regulatory expression.

In order to search *copia*-like retrotransposons on poplar (*Populus alba* L.) genome, PCR method was used with degenerated primers based on conserved motifs (QMDVKT and YVDDM) corresponding to amino acid sequences in the reverse transcriptase domain[2]. The genome DNA was extracted from suspension cultured cells by CTAB method and purified by RNase A treatment[3]. The purified DNA was used as a template to amplify the domains of reverse transcriptase in poplar retrotransposons. Temperature cycling of PCR was performed with the following profile : initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 33°C for 1 min, 68°C for 1 min. Amplified products were separated on agarose gels. Target DNA fragments were isolated and subsequently cloned into the plasmid pGEM-T and then 237 clones were selected by blue/white screening and ampicillin selection. The selected clones were checked on the sizes of the inserted DNA by PCR with specific primers for the vector. Among them, 76 clones harboring the insert of expected size were visualized on agarose gels, and the nucleotide sequences were determined and reduced to amino acid sequences. While some of these clones showed homology to reverse transcriptase sequences, 17 clones were found to encode a single open reading frame uninterrupted by stop codons or translational framesifts.

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Clone No.                    *  *   ** *            *  *     *****
70          1 QMDVKTAFLH GDLEEEIYMS QPDGFKVTR- KENWACK-LK KSLYGLKQSP      50
7F          1 QMDVKTAFLH GDLDEEIYME QPEG-FEAKG KEQLVCK-LK KSLYGLKQAP      50
68          1 QMDVKTAFLH GDLDEEIYME QPEG-FEAKG KEQLVCK-LK KSLYGLKQAP      50
11          1 QMDVKTAFLH GELEEEIYML QPEGFAE-TG KENLVC-RLN KSLYGLKQAP      50
1           1 QMDVKTAFLH GELEEEIYML QPEGFAETC- KENLVC-RLN KSLYGLKQAP      50
5           1 QMDVKTAFLH GELEEEIYML QLEGFAE-TG KENLVC-RLN KSLYGLKQVP      50
3           1 QMDVKTAFLN GELNKEIYMD QPLG-FETKG QERKVCK-LK RSIYGLKQAS      50
16F         1 QMDVKTTFLN CELDEEVYMK Q-LKGFVLPG NEHKVFK-LI KSMYRLKQTP      50
4           1 QMDVKTTFLN CELDEEVYMK Q-LKGFVLPG NEHKVFK-LI KSMYRLKQTP      50

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70         51 RQWYKRFDKF MTEH-GYTRS QFDN--CVYF RRLL-DGSFI YLLLYVDDM     100
7F         51 RQWYKKFDSF MVDH-GYDRT --TSDHCVFM KRFP-DGNFI ILLLYVDDM     100
68         51 RQWYKKFDSF MVDH-GYDRT --TSDHCVFM KRFP-DGNFI ILLLYVDDM     100
11         51 RCWYKRFDSF I-ISLGYNRL --SSDHCTYY KRFEENDVFI ILLLYVDDM     100
1          51 RCWYKRFDSF I-ISLGYNRL --SSDHCTYY KRFKENDVFI ILLLYVDDM     100
5          51 RCWYMRFDSF I-ISFGYNKL --SSNYCTYY KRFEEDDDII ILLLYVDDM     100
3          51 RQWNIKFHQA ---ILKDGFT MMEEDHCVYL KRSNNSF--I ILSLYVDDM     100
16F        51 KYWH---DTF DYVILEYDFK YNSADRCIYS -RFTN-DFSV IIYFYVDDM     100
4          51 KYWH---DTF DYVILEYDFK YNSADRCIYS -RFTN-DFSV IIYFYVDDM     100
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Fig. 1. Alignment of deduced amino acid sequences in reverse transcriptase domeins for *copia*-like retrotransposons of poplar. Amino acids encompassed with boxes are utilized for PCR primers. Asteriks indicate amino acids conserved highly in plant *copia*-like retrotransposons. Hyphens showen in sequences depict gaps.

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These clones involved amino acids conserved in some previously determined *copia*-like reverse transcriptases (Fig.1). The sequences for this region of reverse transcriptase are distinct enough among other elements to clearly identify the clones as *copia*-like retrotransposon reverse transcriptase. The identity and similarity among 17 clones were ranged from 62% to 97% and from 41% to 96%, respectively. One of the isolated clones was used as a hybridization probe for Southern filter containing poplar genomic DNA. Smeared hybridizing signal was observed indicating that the clone was existed in poplar genome and as very high copy elements.

Phylogenetic analysis based on nucleotide sequences was conducted to assess relationships among *copia*-like retrotransposons possessed by poplar and other plants. The molecular phylogenetic tree had major three branches, and the sequences of clones isolated from poplar were found in each branch. Relationships of *copia*-like retrotransposons showed little congruence with the evolutionary relationships of their hosts.

The detection of RNA molecules corresponding to retrotransposons can be taken as an evidence that these elements are active, because transcription is the first step in transposition. The expressions for the random 4 clones isolated by this study were investigated by RT-PCR for total RNA isolated from poplar cultured cells. The specific primers for each clone were designed on the basis of the determined nucleotide sequences. However, there were no amplified bands and the expressions could not be confirmed under the ordinary culture condition. Moreover, reverse transcriptase domains were also amplified by PCR on the first strand cDNA synthesized from total RNA of poplar cells and were cloned. The attempt was not successful for the detection of prospective DNA fragments. These results suggested that *copia*-like retrotransposons are components in poplar genome but they are stable without activation under the normal culture condition.

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References

1) H. HIROCHIKA *et al.*: *Proc. Natl. Acad. Sci. USA*, **93**, 7783–7788 (1996)
2) D.F. VOYTAS *et al.*: *Proc. Natl. Acad. Sci. USA*, **89**, 7124–7128 (1992)
3) F.M. AUSBEL *et al.*: *"Current Protocols in Molecular Biology"*, John Wiley & Sons, New York, **Vol. 1**, 2.3.3–2.3.7 (1987)