CATRINA (2009), 4 (3): 1 - 7

Activation tagging in aspen using a heat-shock inducible two component Ac/Ds-enhancer element system

Hamdy Atta-Alla[1], Mostafa Zaghloul[1], Abd El Kawee Waly[1], Matthias Fladung[2], and Fadia El-Sherif[1]*
[1]Department of Horticulture, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt
[2]Johann Heinrich von Thuenen-Institute (vTI), Federal Research Institute for Rural Areas, Forestry and Fisheries, Institute for Forest Genetics, Sieker Landstr. 2, D-22927 Grosshansdorf, Germany

ABSTRACT

From our earlier work on the genetic transfer of the maize transposable element *Ac* and its functional analysis in hybrid and pure aspen lines, it was shown that this element is active in transgenic aspen. However, a two element transposon tagging system where the *Ds* element and the transposase gene are put together in crosses is not feasible in trees due to the in part long vegetative phases. To overcome this barrier, an inducible two element HSP-*transposase* /ATDs element system is suggested to induce activation tagged variants following two independent transformation steps. The ATDs element is comprised by a 35S enhancer tetramer and outwards facing two CaMV 35S promoter located near both ends of the ATDs element. Following transposition of the ATDs element expression of genes can be elevated which are located adjacent to the new integration site of the element.

Keywords: Activation tagging - *rolC* gene - HSP-*transposase* - ATDs element.

INTRODUCTION

Activation tagging, as the result of random genomic insertion of either promoter or enhancer sequences, can produce novel, dominant mutations by over-expression of endogenous genes. This powerful genomics tool has been used extensively in annual plant species such as *Arabidopsis* (Springer, 2000).

In this study we describe the establishment of an activation tagging system in poplar based upon the maize *Ac*/Ds transposable element system. A modified Ds element (ATDs; Suzuki *et al.* 2001) containing two CaMV 35S promoters and four tandem repeats of enhancer fragments (En) of the 35S promoter, carrying *rolC* as phenotypic selectable marker was introduced into poplar. Second, the transposase gene under control of heat-shock inducible promoter (HSP) from soybean (Gmhsp17.5-E; Czarnecka *et al.* 1985) was tested for transcription following promoter induction.

Insertion of transposonse or T-DNAs in coding regions or promoters normally results in recessive loss-of-function mutants by disrupting the gene. In contrast, dominant gain-of-function mutants can be obtained with an insertion mutagenesis system called activation tagging (Kakimoto 1996, Weigel *et al*. 2000).

The preferential insertion of the maize *Ac* transposon near the coding regions of the aspen-*Populus* genome (Kumar and Fladung, 2003a) suggests that also transposon mutagenesis can be an important tool to discover gene functions by reverse-genetics strategies (Kumar, and Fladung, 2003b). Loss-of-function strategy using the maize *Ac* element was tested in haploid poplar (Deutsch *et al*. 2004, Fladung *et al*. 2004). However, besides the difficult handling of haploid tree material (slow growth, spontaneous diploidisation) screening of a large number of *Ac* excision events is very time consuming. So far, no report is available describing the use of a transposon system for activation tagging approach in a tree species. Using improved constructs carrying e.g. modified Ds elements together with the 35S promoter at its ends, and the transposase gene under control of an inducible promoter can be a starting-point for a systematic gain-of-function approach in the tree genus *Populus*.

MATERIALS AND METHODS

Gene constructs

Two constructs were designed carrying (a) the transposase gene under control of the heat–shock promoter (HSP Gmhsp17.5-E from soybean (Czarnecka *et al*., 1985), and (b) the ATDs element in combination with the phenotypically selectable marker rolC (Fig. 1). For (a) the plasmid P6-HSP-TP-OCS (12315 bps) derived from the ligation of P6-HSP-TP-OCS (2978 bps) into PLH6000 (9337 bps) plasmid (DNA Cloning Service, Hamburg, Germany). As plant selectable marker the construct carries the hygromycin resistance gene under control of the CaMV35S promoters. In the second construct either the *bar* or *npt-II* genes were used as plant selectable markers. Induction of transposase in HSP-*transposase* transgenic lines was achieved during a 24 h culture at 37 or 40 °C under continuous light. This treatment was sufficient to induce the transposase at high levels but without stressing the plants.

Plant material, culture and genetic transformation

In vitro culture of of hybrid aspen clones (*Populus tremula x tremuloides*, clone Esch5) was used for generation of the transgenic lines (Fladung *et al*. 1997). Plants were grown on solid McCown Woody plant medium (WPM, Duchefa M0220) (Lloyd and McCown, 1980) containing 2% Sacharose, 0.6% Agar (Agar Agar, Serva, 11396). Genetic transformations were carried out using the *Agrobacterium*-mediated approach (Fladung

* *Corresponding author: woroof@yahoo.com*

et al. 1997). WPM medium for regeneration of transgenic plants was supplemented with 0.01% Pluronic F-68 (Sigma P-7061), thidiazuron (0.01 µM/L) (601 medium) and antibiotic cefotaxime 500 mg/L for Agrobacterium elimination and kanamycin (50 mg/L) or hygromycin. (10 mg/L) for the selection of transgenic shoots. The cultures were kept under growth room conditions with 24 hour light and 22° C (light intensity: 5-8 x 103 lux, lamps: Phillips TLM 140W/33RS).

Extraction of DNA and RNA

The DNA extraction procedure was adapted from the method of Doyle and Doyle (1987) is described in Fladung *et al.* (1997). RNA was extracted according the procedure described by Logemann (1987).

DNA detection

Polymerase chain reaction (PCR) amplification analysis

PCR analysis was performed as described by Fladung *et al*. (1997). As a positive control in all PCR reactions primers amplifying an aspen–specific genomic region were added Kumar and Fladung (2002). Amplification was carried out under the following conditions in a thermal reaction. The initial denaturation for 2 min at 94 °C was followed by 40 cycles of denaturation (2 min at 94°C), annealing (2 min at 55 or 52 °C), extension (2 min at 72°C), and a final extension step of 5 min at 72 °C followed by 4°C .

PCR analysis to study Hsp/ *Ac* transposase

In the case of HSP-*transposase* gene construct PCR was performed by using primers (5'AAA GCC TGA ACT CAC CGC GA3') and (5'TGC GAG GAT CAC TTG TTT TAA3') to amplification a fragment from *hph* gene to *Ac Tpase* gene respectively.

PCR analysis to study the HSP-*transposase* / Ds-AT-*rolC* double transgenic aspen

400 µg total DNA from the double transformated plants were tested using PCR to check presence of the second gene construct ATDs using a primer pair amplifying a fragment of (5'GGC TGA AGA CGA CCT GTG TTC TCT3'), (5'ATC GTT GAA GAT GCC TCT GC3'), (5'ATC GTT GAA GAT GCC TCT GC3') and (5'ATG GAT TGC ACG CAG GTT CTC3') for amplification of *rolC* gene, *35S - rolC* gene, *hph* gene and *nptII* genes respectively.

PCR analysis in Activation tagging experiments using heat-shock

Ten PCR experiments were performed by using 400 µg DNA isolated from heat-shock induced and non-induced leaves and stems (ten different double transgenic aspen lines containing HSP-*transposase*/Ds-AT-*rolC* genes) to check presence of the second gene construct ATDs using a primer pair amplifying a fragment of (5'ATC GTT GAA GAT GCC TCT GC3') for amplification of 35S-*rolC* gene**,** and the primer pair amplifying a fragment of (5'ATG GAT TGC ACG CAG GTT CTC3' and 3'AAC TCA CCA GGT TCG AAC CTA5') to check for ATDs excision following the heat-shock treatment.

RT-PCR Method

RT-PCR reaction was carried out using One-Step Access RT-PCR system (Promega, USA). RT-PCR analysis to check for presence of the HSP-*transposase* and,/or HSP-*transposase* / Ds-AT-*rolC* double transgenic aspen system was performed by using RNA isolated from HSP-induced and non-induced leaves and stems of four different single transgenic aspen lines containing the HSP-*transposase* and/or HSP-*transposase* / Ds-AT-*rolC* double transgenic lines. The primer 5'TGC GAG GAT CAC TTG TTT TAA3' was used to amplification of the *Ac Tpase* gene.

Purification and sequencing of PCR products

The amplified fragments from the PCR product were purified using High Pure PCR Product Purification Kit (Roche Molecular Biochemicals Mannheim, Germany) according to manufacturers' recommendations. Sequencing of the purified fragments was done by (Star Seq Company Mainz Germany). The (http://blast.ncbi.nlm.nih.gov/Blast.cgi) sequencing data were analysed using BLASTN programs.

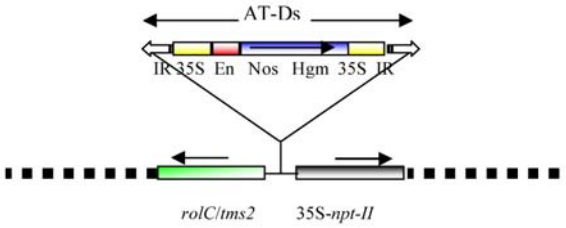

Figure (1): Structure of the activation construct (ATDs) (Suzuki *et al*. 2001). The ATDs also contains hygromycin phosphotransferase gene (Hgm) with a nopaline synthase promoter (Nos) as a transposition selection marker. The tms2 (A) or rolC gene (B) is outside of ATDs, respectively, and the CaMV 35S promoter on the left of the ATDs keeps these genes active under non-excised stage. As plant selectable marker the nptII gene is used.

RESULTS AND DISCUSSION

Production of Hsp/*Ac* transposase single transgenic aspen

To obtain transgenic aspen plants containing a single-copy of the transposase element, leaf discs and stem segments from hybrid aspen *(Populus tremula x Populus tremuloides*, clone Esch5) plants were transformed by *in vitro* co -cultivation with *Agrobacterium tumefaciens* strain carrying the binary vector P6-HSP-TP-OCS (gene construct), contains the *hph* gene as selectable marker gene. In mean, 36.5 independent transgenic plant lines were recovered via *Agrobacterium*-mediated transformation in six independent experiments under hygromycin selection. To confirm the presence of the T-DNA in the regenerated plants, some plants were subjected to PCR analysis (Table 1). PCR analysis results (Table 1) reveal that 35 independent transgenic lines carrying the HSP-*transposase* gene construct showed a clear band corresponding to the relevant sequence of the *Ac Tpase* and *hph* genes.

Several independent transgenic aspen lines carrying the *Ac Tpase* gene were obtained. Transgenic poplars have been obtained carrying the transposon *Ac* from maize (Fladung *et al.,* 1997, Fladung and Ahuja 1997, Fladung *et al*., 1999, Kumar and Fladung, 2003a).

Induction of transposase in HSP-*transposase* transgenic lines

Induction of transposase in HSP-*transposase* transgenic lines was achieved during a 24 h culture at 37 °C under continuous light. This treatment was sufficient to induce the transposase at high levels but without stressing the plants. To check for transposase transcription before and after heat shock treatment RNA was isolated from leaves. RNA quality and amount of the DNase digested RNA was sufficient for RT-PCR (Fig. 2a). RT-PCR of heat shock-induced and non-induced leaves of nine different single transgenic aspen lines containing the HSP-*transposase* gene construct was performed. RT-PCR results showed that out of eight HSP-*transpo-sase* transgenic lines seven lines showed transposase induction (Fig. 2a, b). For second transformation with the ATDs construct the lines N66-2 and N66-5 were chosen.

Establishment of double transgenic aspen

For second transformation with the ATDs construct the lines N66-2 and N66-5 were chosen. A number of double transgenic aspen carrying both the Ds element and the transposase gene have been produced. 12 and 39.5 independent transgenic poplar lines were recovered

Table (1): Transformation and PCR positive efficiency of single transgenic aspen lines obtained using the three different constructs established in this study

Construct	Means no. of transformed plants	Transformation Efficiency (%)	Transgenic lines tested PCR	Transgenic lines tested PCR positive	PCR efficiency (%)
HSP-*transposase*	36.5	10.6	41	35	85

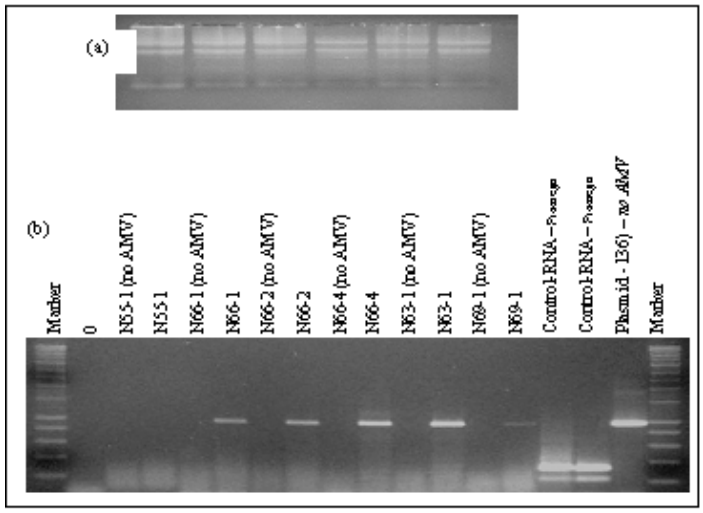

Figure (2): (a) RNA quality check. (b) RT-PCR of heat shock-induced leaves of five different single transgenic poplar lines containing the HSP-*transposase* gene construct. To check for DNA contamination, PCR was performed also without AMV.The line N66-2 was chosen for upper transformation with the ATDs construct. N55-1 is serves as control.

via *Agrobacterium*-mediated transformation under kanamysin selection (Table 2). Out of 15 and 10 putative independent transgenic lines which were tested in PCR experiments, 15 and 5 were positive, containing both HSP-*transposase* and Ds-AT-*rolC* (Fig 3a, b).

Activation tagging experiments using heat-shock

Using ten HSP-*transposase*/Ds-AT-*rolC* double transgenic lines an activation tagging experiment was established. The *in vitro* grown regenerating callus and plants (Fig. 4a) were used (eight Magenta vessels from each line containing nine pieces of calli and plants). Induction of transposase in HSP-*transposase*/Ds-AT-*rolC* transgenic lines was achieved during a 24 h culture at 42 °C C (instead of 37 °C) under continuous light. This treatment also induced the transposase at high levels but without stressing the plants. To check for transposase transcription before and after heat shock treatment RNA was isolated from the leaves of the plants before and after heat shock. The m-RNA amount of the DNase digested RNA was sufficient for RT-PCR (Fig. 5). RT-PCR results showed that out of ten HSP-*transposase*/Ds-AT-*rolC* double transgenic lines five lines showed transposase induction.

After heat-shock treatment the calli and plants were divided in about 39,309 regenerating microcallus pieces cut as small as possible and distributed on 1,766 petri dishes (Fig 4b, c, d). The number of regenerated shoots formed from the microcallis varied between the lines. 250 DNA samples were isolated from regenerated

Table (2): Mean number of independent transgenic aspen lines obtained using the different constructs established in this work and tested positive in PCR analyses.

Construct	Transgenic line	Transformation with 2nd construct	Means no. of double transgenic aspen lines	Transformation efficiency (%)	Double transgenic lines PCR tested	Double transgenic lines PCR positive	PCR efficiency (%)
HSP-*transposase*	N66-2	Ds-AT-*rolC*	39.5	12	15	12	80
HSP-*transposase*	N66-5	Ds-AT-*rolC*	12	3	10	5	50

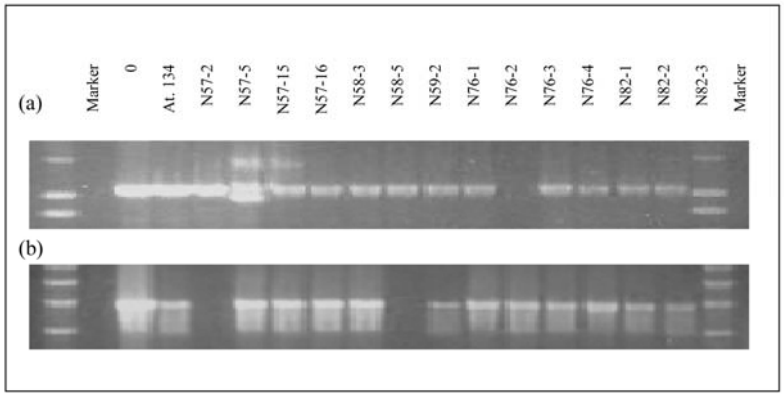

Figure (3): PCR analysis to check complete integration of the second gene construct ATDs at right (a) and left (b) border of T-DNA. (a) Primer pair 35S-promoter / *rolC* gene, (b) primer pair 35S-promoter / *nptII* gene. GIP-*transposase* / Ds-AT-*rolC*

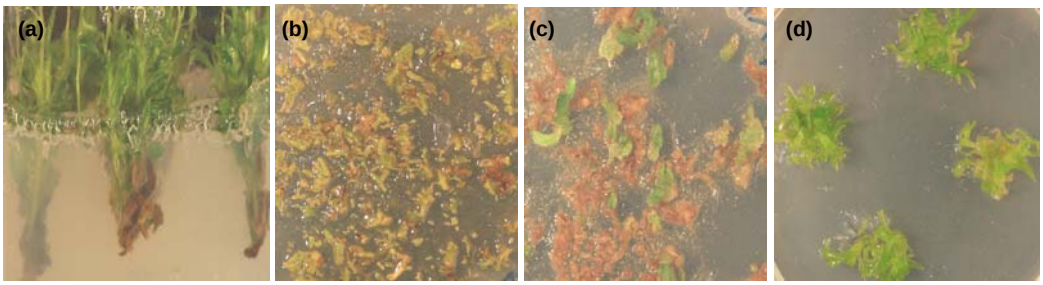

Figure (4): Activation tagging experiment 2: (a) Regenerating callus with shoots before starting the experiment, (b) Small callus pieces distributed on Petri dishes for regeneration of shoots after heat-shock treatment, (c) Callus with regenerated shoots after three weeks cultivation on 601 regeneration medium, and (d) Regenerated shoots after six weeks cultivation on 601 regeneration medium.

shoots after heat-shock treatment. Excision of the ATDs element following HSP treatment was confirmed in PCR experiments using primer located in the 5' *rolC* and 3' *nptII* genes (Fig. 6) or the primer located in the 35S-*rolC*. For the HSP-*transposase*/Ds-AT-*rolC* double transgenic lines a PCR fragment of about 1,700 bp is expected following ATDs excision using primer located in the *rolC* and *nptII* genes and no PCR fragment expected using primer located in 35S-*rolC* genes which located on the right border of ATDs because *rolC* gene located outside of the ATDs element, and following transposase induction the genes become promoterless upon excision of ATDs rendering them inactive.

Transposation of ATDs in aspen

DNA extracted from the HSP-*transposase*/Ds-AT-*rolC* double transgenic line was used for the PCR reaction to amplify the empty donor site of ATDs in aspen genom and/or 35S- *rolC* gene which is located on the right border of ATDs using primer located at the 5' *rolC* and 3' *nptII* and 35S-*rolC* genes, respectively. Up to 2% of the transformants showed a specific PCR amplification signal of 1700 bp using primer located in the 5' *rolC* and 3' *nptII* (Fig. 6). The occurrence of bands can be explained as ATDs excision giving first molecular evidence of primary ATDs excision in aspen. In total, 51% of the transformants showed a specific PCR amplification signal of 1,200 bp using primer located in the 35S-*rolC* gene due to ATDs-non-excised. Individual transformants displayed varying intensities of ATDs fragments suggesting that there was not enough transposase activity to produce excisions in the majority of transformants and also indicating variation among them.

Sequencing of empty donor sites

RolC and *nptII* genes located outside of the ATDs element, and following transposase induction the genes become promoterless upon excision of ATDs rendering

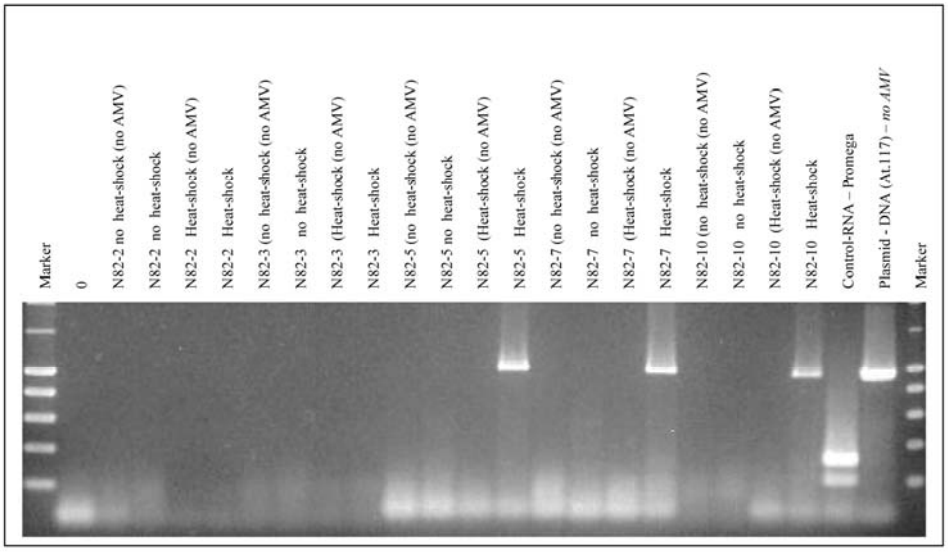

Figure (5): RT-PCR of heat shock-induced and non-induced leaves of five different double transgenic poplar lines containing the HSP-*transposase*/Ds-AT-*rolC* gene construct.

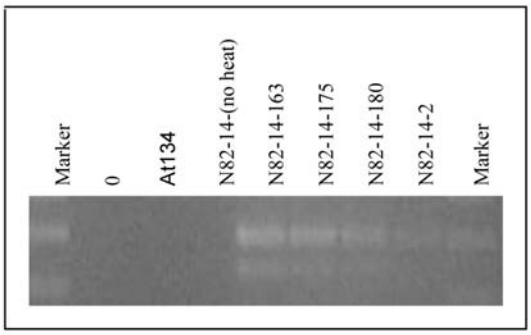

Figure (6): PCR analysis to check for ATDs excision following heat-shock and transposase induction using a pair amplifying a fragment of the 5' *rolC* and 3' *nptII* genes. The *Agrobacterium* strain 134 and the not-primer heaat shoched N82-14 line reveal a single amplification product of about 9 kb that only can be amplified using Long-Template PCR system (not shown here).

Table (3): EST of ATDs empty site of aspen genomic with similarity to sequences in public databases.

Transgenic line	Blastx similarity	Organism	Accession number positives (% amino acids) E Value		
HSP-*transposase*/Ds-AT-*rolC* #4	1-Panax ginseng RolC mutant rolC1-Pg (rolC) gene, complete cds	Panax ginseng	EU642406	442/448 (98%),	0.0
			AY818371	477/523 (91%),	0.0
	2- Kanamycin resistance plant expression vector pSAT4-nptII, complete sequence.	Kanamycin resistance plant expression vector pSAT4-nptII			

them inactive (Fig. 1). To confirm excision of the ATDs element and to detect putative footprints, the empty donor sites at the 5' and/or 3' ends of the ATDs element were sequenced. In total, eight genomic sequences were obtained which were subjected to BLASTN DNA homology searches using the NCBI Gen Bank (http://www.ncbi.nlm-.nih.gov/BLAST). The results of BLASTN DNA homology searches including sequences are summarized in Table (3). All sequences identified are significantly similar to *rolC* and *nptII* genes sequences represented in public databases (Table 3). From these results we concluded that ATDs excised in the lines tested.

Activation tagging in plants has been effectively applied using T-DNA inserts or using the maize (*Zea mays*) transposon system (Kardailsky *et al*., 1999; Ito and Meyerowitz, 2000; Lee *et al*., 2000; Van Der Graaff *et al*., 2003; Weigel *et al*., 2000; Huang *et al*., 2001: Zhao *et al*., 2001; Marsch-Martinez *et al*., 2002; Ayliffe *et al*., 2007; Shaohong *et al*., 2008).

Related activation cassettes have been incorporated into the transposable elements *Ac* and *En-I* (Suzuki *et al*., 2001; Marsch-Martinez *et al*., 2002). T-DNA insertions are often complex and characterized by multiple inverted or tandem copies or truncated T-DNA inserts, which often make a molecular analysis difficult (Nacry *et al*., 1998). One possibility to overcome this problem would be the use of single copy transposons insertions as the activation tags. In addition, transposons insertions can be remobilised germinally, thereby producing revertants that can confirm the phenotypic consequences of the insertion and activation tagging is more efficient with transposons than with T-DNA (Schneider *et al*., 2005).

The transposon system uses a method to mobilize the transposons containing the enhancer into different parts of the genome, and then segregates out the transposase responsible for the mobilization and thus generates transposons activation tags that are stable in their genomic positions (Marsch-Martinez *et al*., 2002). In this study, we tested an activation tagging approach by using the *Ac*/ATDs enhancer element system in aspen. It could be shown that individual transgenic lines displayed varying frequencies of ATDs transposition suggesting that transposase activity was not effective to induce excisions in the majority of the transformants and also indicating variation among them. An explanation could be that either heat-shock treatments were not sufficient or the transposase gene was silenced.

ACKNOWLEDGMENTS

We thank Olaf Nowitzki for technical assistance as well as other co-workers in the Institute of Forest Tree Breeding Grosshansdorf, Germany, for helpful discussion.

REFERENCES

AYLIFFE MA., M. PALLOTTA, P. LANGRIDGE AND A.J PRYOR. 2007. A barley activation tagging system. Plant Molecular Biology **64**(3):329-47.

CHARNG, Y. HUI-PING, L. HUNG-CHUN, CHANG, K. L. TZONG-HSIUNG, H. AND JENN, T. 2004. Fusion of the transposase with a classical nuclear localization signal to increase the transposition efficiency of *Ac* transposon . Bot Bull Acad Sin.45: 267-274.

DEUTSCH, K. KUMLEHN, J. ZIEGENHAGEN, B. AND FLADUNG, M. 2004. Stable haploid poplar callus lines from immature pollen culture. Physiol. Plant. 120:613–622.

DOYLE, J.J. AND J.L. DOYLE. 1987. Arapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11- 15.

FELDMAN, S. AND R. KUNZE. 1991. The ORFa protein, the putative transposase of maize transposable Ac ,has a basic DNA binding domain .EMBO Journal.10: 4003-4010.

FLADUNG, M. AND M.R. AHUJA. 1997. Excision of the maize transposable element *Ac* in periclinal chimeric leaves of 35S-*Ac*-*rolC* transgenic aspen-*Populus*. Plant Molecular Biology.33:1097–1103.

FLADUNG, M., F. DEUTSCH, H. HÖNICKA AND S. KUMAR. 2004. DNA and transposon tagging in aspen. Plant Molecular Biology.6 :5-11.

FLADUNG, M., S. KUMAR, AND M.R. AHUJA. 1997. Genetic transformation of *Populus* genotypes with different chimaeric gene constructs: transformation efficiency and molecular analysis. Transgenic Res. 6:111–121.

FLADUNG, M., S. KUMAR, AND M.R. AHUJA. 1999. Transposition of the maize transposable element *Ac* in transgenic aspen and its usability as a gene tagging system. In: Proc. of the Second International Wood Biotechnology Symposium in Canberra, Australien, März 1997, North Forest Products Limited, Australia, 125-135.

HUANG S., R.E. CERNY, D.S. BHAT AND S.M. BROWN. 2001. Cloning of an Arabidopsis patatin-like gene, *STURDY*, by activation T-DNA tagging. Plant Physiology 125: 573–584.

ITO T. AND E.M. MEYEROWITZ 2000. Overexpression of a gene encoding a cytochrome P450, *CYP78A9*, induces large and seedless fruit in *Arabidopsis*. Plant Cell 12, 1541– 1550.

Kakimoto, T. 1996. CKI1, a histidine kinase homolog implicated in cytokinin signal transduction. Science. 29: 982–985.

KARDAILSKY, I. SHUKLA, V. AHN, J. H. DAGENAIS, N. CHRISTENSEN, S. K. NGUYEN, J. T. CHORY, J. HARRISON, M. J. AND WEIGEL, D. 1999. Activation tagging of the floral inducer *FT*. Science 286: 1962-1965.

KUMAR, S. AND M. FLADUNG. 2003b. Forest Tree Transgenesis and Functional Genomics: From Fast Forward to Reverse Genetics. Silvae Genetica. 52 : 229-232.

KUMAR, S. AND M. FLADUNG. 2002. Transgene integration in aspen: structures of integration sites and mechanism of T-DNA integration .The Plant Journal **31**: (4) : 543–551.

KUMAR, S. AND M. FLADUNG 2003a. Somatic mobility of the maize element Ac and its usability for gene tagging in aspen. Plant Molecular Biology 51: 643-650.

LEE, H. SUH, SS. PARK, E. CHO, E. AHN, JH. KIM, SG. LEE, JS. KWON, YM. AND LEE, I. 2000. The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in Arabidopsis. Genes & Development. 14: 2366-2376.

LLOYD , G. AND B. MCCOWN. 1980. Combined proceedings of the international Plant Propagators Society 30: 421-427.

LOGEMANN, J. SCHELL., AND L. WILLMITZER 1987. Improved method for the isolation of RNA from plant tissues. Analytical Biochemistry. 163 : 16–20.

MARSCH-MARTINEZ, N., R. GRECO, G. VAN ARKEL, L. HERRERA-ESTRELLA, AND A. PEREIRA. 2002. ctivation tagging using the *EN-I* maize transposons system in *Arabidopsis*. Plant Physiol.129 :1544-1556.

NACRY, P., C. CHRISTINE, C. BÉATRICE, C. MICHEL, AND B. DAVID. 1998. Major Chromosomal Rearrangements Induced by T-DNA Transformation in *Arabidopsis*. Genetics 149: 641-650.

SCHNEIDER A., T. KIRCH, T. GIGOLASHVILI, HP. MOCK, U. SONNEWALD, R. SIMON, UI FLÜGGE. AND W. WERR. 2005. A transposon-based activation-tagging population in Arabidopsis thaliana (TAMARA) and its application in the identification of dominant developmental and metabolic mutations. FEBS Letters 29, 579(21):4622-4628..

SCOFIELD, B. JONES, D. JUNE, G. SWINBURNE., LLUIS, B. STEVEN, R. JONES, B. AND GEORGE, C. 1992. Elevated levels of activator transposase mRNA are associated with high frequencies of dissociation excision in Arabidopsis. American Society of Plant Physiologists 4: 583-595.

SHAOHONG Q., A. DESAI, R. WING AND V. SUNDARESAN 2008. A Versatile Transposon-Based Activation Tag Vector System for Functional Genomics in Cereals. Plant Physiology 146,189-199.

SPRINGER, P.S. 2000. Gene traps: tools for plant development and genomics. Plant Cell 12:1007-1020.

SUZUKI, Y., S. UEMURA, Y. SAITO, N. MUROFUSHI, K. SCHMITZ, AND H. YAMAGUCHI. 2001. A novel transposons tagging element for obtaining gain-of – function mutants based on a self-stabilizing Ac derivative. Plant Molecular Biology 54:123-131.

VAN DER GRAAFF, E., C.NUSSBAUMER AND B. KELLER. 2003. The *Arabidopsis thaliana rlp* mutations revert the ectopic leaf blade formation conferred by activation tagging of LEP gene. Molecular Genetics and Genomics. 270:243-252.

WEIGEL, D. AHN, J. H. BLÁZQUEZ, M. A. BOREVITZ, J. CHRISTENSEN, S. K. FANKHAUSER, C. FERRÁNDIZ, C. KARDAILSKY, I. MALANCHARUVIL, E. J. NEFF, M. M. NGUYEN, J. T. SATO, S. WANG, Z. XIA, Y. DIXON, R. A. HARRISON, M. J. LAMB, C. J. YANOFSKY, M. F. AND CHORY, J. 2000. Activation tagging in Arabidopsis. Plant Physiology 122:1003-1014.

ZHAO Y., S.K. CHRISTENSEN, C. FANKHAUSER, J.R. CASHMAN, J.D. COHEN, D. WEIGEL AND J. CHORY 2001. A role for flavin monooxygenase-like enzymes in auxin biosynthesis. Science 291: 306– 309.

Received July 20, 2008
Accepted May 10, 2009

الملخص العربـــى

الترانسبوزيم و الذى يعتمد تنشيط تخليقة على تعرض النباتات التى تحتوى على هذا الجين لدرجة حرارة 37 درجة مئوية لمدة 24 ساعة حيث تم تعريض اربع نباتات نباتات المحولة وراثيا النامية داخل معمل زراعة الانسجة لدرجة حرارة 37 درجة مئوية لمدة 24 ساعة تحت اضاءه مستمره ولكى يتم معرفة نشاط الجينى المسئول عن تكوين انزيم الـ*Ac* transposase تم الحصول على عينات ال دنا من الاوراق و السيقان النباتات المحولة وراثيا قبل وبعد المعاملة بدرجة الحرارة و عمل ال RT-PCR اظهرت النتائج نشاط الجينى للانزيم بعد المعاملة وتم اختيار نباتين لعمل النقل الوراثى للجين الاخر. حيث تم عمل نقل وراثى للجين Ds-AT-*rolC* بواسطة الأجروبكتريم *Agrobacterium tumefaciens* وتم بذلك الحصول على نباتات حور معدلة وراثيا تحتوى على كل من الجينات الاتية-HSP *transposase*/ATDs-*rolC* حيث تم تنشيط انزيم الترانسبوزم بتعريض النباتات المحولة وراثيا النامية داخل معمل زراعة الانسجة لدرجة حرارة 40 بدل من 37 درجة مئوية لمدة 24 ساعة تحت اضاءه مستمره وتم عزل ال رنا قبل و بعد التنشيط و عمل ال RT-PCR اوضحت نتائج ال PCRعلى انتقال ال (ATDs).