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GABI-POP: Isolation of tree-specific genes and promoters
by a transposon tagging approach

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Final Report

of

the research project 0312291:

GABI-POP: Isolation of tree-specific genes and promoters by a transposon tagging approach

For the period

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Written by:

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(Results were obtained by Frank Deutsch and Sandeep Kumar)

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Introduction

The objective of the proposal was the identification, cloning and sequencing of genes and promoters from the tree species *Populus tremula*. The sequences obtained were submitted to the GABI databank, and new sequences with known or unknown functions can be subject for patenting.

We have tested a transposon tagging technique to identify tree-specific genes and/or promoters in a model tree system. Transposon tagging is a powerful tool for the identification of genes in many plant species but its use is limited by the low transformation efficiency and low regeneration capacity during tissue culture. The transposon tagging strategy used in poplar is based on our earlier experiments (Fladung 1999a) using constructs containing the *Ac* transposon from maize and the phenotypically visible marker genes *rolC* or *iaaL*. It was shown that *Ac* excises from its original position within the transferred gene construct and re-integrates somewhere in the genome. The excision of *Ac* during leaf development causes restoration of the marker gene, for example, *rolC* expression was indicated in the 35S-*Ac*-*rolC* transgenic aspen plants by light-green sectors in green leaf background.

To overcome difficulties in tree species such as long vegetative life cycles and dioecy, haploid poplar trees can be used for studying gene function following the induction of knock-out variants. Unfortunately, the haploid line provided by a different institution which was proposed for this project turned out to be diploid. Therefore, we initiate activities on the following topics:

1. Further analysis of variant phenotypes of already obtained transgenic lines
2. Transformation of a (*Populus tremula* x *P. tremuloides*) x *P. alba* hybrid with the 35S-*Ac*-*rolC* and rbcS-*Ac*-*rolC* constructs
3. Sequencing of genomic regions flanking the new *Ac*-insertion sites
4. Induction and establishment of stable haploid hybrid poplar lines
5. Transformation of the haploid line and molecular analysis of transformants
6. Submission of sequences to the GABI databank

Material and Methods

Plant material and gene constructs as well molecular and cytological methods used for the investigations have already been described in detail in the first annual report, and Fladung and Ahuja (1995), Fladung et al. (1997). For *Ac* insertion, thermal asymmetric interlaced (TAIL)-PCR (Liu et al., 1995) was performed with DNA extracted from pale-green leaf sectors. For Sequencing the T-DNA left and right genomic flanking regions, the Invers-PCR was used (Fladung 1999b). The Inverse- and TAIL-PCRs were performed using the Expand™ Long Template PCR system (Roche Molecular Biochemicals, Mannheim, Germany) according to manufacturer's recommendations.

Results and Discussion

1. Analysis of variant phenotypes of already obtained transgenics

Transgene flanking genomic regions of in total 30 transgenic lines including the lines showing variant phenotypes were analysed using Inverse-PCR strategy. In total, 27 right T-DNA/plant junctions, 20 left T-DNA/plant junctions, and ten target insertions from control plants were obtained. The right end of the T-DNA in 18 transgenic lines was conserved up to the cleavage site and the right border repeat was deleted in nine junctions. Nucleotides from the left border repeat were present in 19 transgenic lines out of 20 cases analysed (Kumar and Fladung 2002a).

Comparison of the genomic target sites prior to integration to the T-DNA revealed that T-DNA inserted into the plant genome without any notable deletion of genomic sequence in three transgenic lines. However, deletions of a few nucleotides to more than 500 bp were observed in other transgenic lines. Filler DNA of up to 235 bp were observed on left and/or right junctions of six transgenic lines which in most of the cases originated from the nearby host genomic sequence or from T-DNA. Short sequence similarities between recombining strands near break points without any bias to the left or the right T-DNA end were obtained in most of the lines analysed suggesting that both T-DNA ends may interact during the integration process. Small sequence similarity occurring at the T-DNA 3´ end could act as a primer for DNA fill-in synthesis, whereas at the 5´ end it could adjoin the recessed strand of the partner terminus and be fixed by ligation.

The integration model is reminiscent of the model for the repair of genomic DSB in somatic plant cells based on synthesis-dependent strand-annealing (SDSA). The sequences obtained were submitted to the GABI databank. The detailed analyses of the phenotypes, the length of the sequences obtained and their homologues databases were described in the last annual report.

2. Transformation of a (*Populus tremula* x *P. tremuloides*) x *P. alba* hybrid with the 35S-*Ac-rolC* and rbcS-*Ac-rolC* constructs

More than 20 different clones of the progeny *Populus tremula* x *P. tremuloides* (Esch5) and *P. alba* were used for transformations with the constructs 35S-*Ac-rolC* (#23) and rbcS-*Ac-rolC* (#28). These clones obtained from the parthenogenesis experiments mentioned in section 3 (for details please see last year report) exhibited haploid as well as diploid leaves. Overall, 75 different independent transgenic lines were obtained in the transformation experiments, 36 carrying the 35S-*Ac-rolC* and 39 containing the rbcS-*Ac-rolC* gene construct (Table 1). Out of these, light-green patches characteristic for *rolC* expression and, thus, indicating *Ac* transposition was found in 64 transgenic lines.

Regenerated plantlets were rooted and propagated *in vitro*. So far, 60 independent lines have been transferred into the greenhouse (date: December 2001). The remaining 15 lines will be transferred in

January 2002. Under *in vitro* culture 5 different variants were observed: „Necrotic spots“, „Dwarf“, „Mottled“, „Lanceolate leaf“, „Partial chlorophyllless“. No variant phenotypes were observed in control cultures.

The stability of the variant phenotype of the 5 mentioned lines will be investigated in the greenhouse also. Further, the transgenic lines will be screened for occurrence of additional variations.

Table 1: Transformation of haploid and diploid leaves harvested from different clones of the progeny *Populus tremula* x *P. tremuloides* (Esch5) and *P. alba*

Transformation number	Agro strain	Clone	Leaves	Independent transgenic lines	Efficiency
1	#23	EA (Esch5 x P.alba)	107	4	4%
2	#23	EA	80	9	11%
3	#23	EA13	100	0	-
4	#23	EA14	54	0	-
5	#23	EA15	53	0	-
6	#23	EA17	50	0	-
7	#23	EA18	41	0	-
8	#23	EA19	82	12	14%
9	#23	D2-2A	20	8	40%
10	#23	D2-2	19	3	15%
11	#28	EA	112	3	2%
12	#28	EA	80	0	-
13	#28	EA16	28	0	-
14	#28	EA	110	0	-
15	#28	EA + EA16	40	1	2%
16	#28	EA20	55	0	-
17	#28	EA	43	0	-
18	#28	D2-1-L1	110	35	31%
Total			**1184**	**75**	**6%**

More than 60 different independent transgenic lines were transferred into the greenhouse in December 2001. None of the *in vitro* observed variants showed the variant phenotype in the greenhouse.

3. Sequencing of genomic regions flanking new *Ac*-insertion sites

Three independent transgenic lines (35S-*Ac*-*rolC* #2, #3 and #10) containing a single copy of 35S-*Ac*-*rolC* transgene as shown by Southern analysis (Fladung et al. 1997) and showing light-green sectors on leaves indicating active excision of *Ac* were selected for the determinations of the *Ac* “Flanking Sequence Tags” (FSTs). The light-green sectors were harvested for further molecular analyses (Kumar and Fladung 2002b). DNA from several independent light green leaf sectors was used in Inverse-PCR or TAIL-PCR reactions to amplify aspen genomic DNA flanking the *Ac* insertions. The sequences obtained were compared to public databases for similarity to known proteins and genomic sequences.

The sequences analyzed in three independent transgenic lines and the BLASTx search results are summarized in Table 2. In total, 75 FSTs have been submitted to the GABI database so far.

The data show that out of 75 sequences analyzed 22 sequences gave significant BLASTx hits. This might be a minimal estimate keeping in view the small amount of information available on aspen genome in the public databases. However, we can expect DNA sequences disrupted by these insertions to become available as the poplar genome sequencing project progresses.

Table 2: Frequency of *Ac* insertions into predicted genes

Transgenic line	FSTs analyzed	Significant BLASTx hit
Esch5:35S-*Ac-rolC*#3	41	12 (29%)
Esch5:35S-*Ac-rolC*#10	24	6 (25%)
Esch5:35S-*Ac-rolC*#2	10	4 (40%)
Total	75	22 (29%)

Such a catalogue of *Ac* flanking DNA sequences could be used to make a FST database, suitable for aspen reverse genetics. The total frequency of FSTs in predicted genes, summarized in Table 1, was calculated to be 29%. Details of the *Ac*-tagged hits are given in Table 3. The frequency seems to be consistent among individual transgenic lines ranging from 25-33%. This suggests that about one third of all the *Ac* insertions are in genes that can be predicted on the basis of homology to the public databases. FSTs in predicted genes like DNA methyltransferase can provide information on the unique function of these genes in a long-lived tree system. Poplar is a model plant for the discovery of gene functions in trees. The preferential insertion of the *Ac* element in the coding regions of the aspen genome as indicated by our results suggests that transposon mutagenesis will be a very important tool to discover gene functions by reverse genetics or forward genetics strategies.

Table 3: *Ac* tagged aspen genomic sites with similarity to sequences in public databases

Transgenic line	Blast similarity	Organism	Accession number	Positives (% amino acids)	E value
35S-*Ac-rolC*#2	Probable anthranilate phosphoribosyltransferase	*A. thaliana*	T04208	60/62 (96%)	2e-44
	Putative GARI protein	*A. thaliana*	AC009540	38/42 (90%) 38/39 (96%)	3e-29
	Acetyl-CoA:benzylalcohol acetyltransferase	*Clarkia concinna*	AF121852	67/122 (54%)	6e-12
	Hypothetical protein F20P5.21	*A. thaliana*	C96723	45/74 (60%)	5e-10
35S-*Ac-rolC*#3	Receptor protein kinase-like protein	*A. thaliana*	AP000607	32/51 (62%)	1e-05
	Mutator-like transposase	*A. thaliana*	H84668	69/127 (54%)	7e-14
	Hypothetical protein F17J16.150	*A. thaliana*	T47792	27/28 (96%)	7e-07
	Genomic DNA	*A. thaliana*	AB026654	40/54 (73%)	4e-09
	Unknown	*T. aestivum*	AF325198	42/77 (54%)	1e-10
	Hypothetical protein T2L5.1	*A. thaliana*	T01952	39/76 (51%) 37/77 (47%)	1e-07
	Hypothetical protein AT4g03540	*A. thaliana*	H85044	45/78 (56%)	1e-05
	Genomic DNA	*A. thaliana*	AB005236	36/66 (53%)	7e-14
	Hypothetical protein F3I6.12	*A. thaliana*	T00649	44/74 (58%) 23/39 (58%)	6e-12
	Hypothetical protein T18N14.120	*A. thaliana*	T46070	95/135 (69%)	4e-34
	Hypothetical protein T2L5.1	*A. thaliana*	T01952	71/116 (60%)	2e-24
	Cytokinin receptor CRE1b	*A. thaliana*	AB049935	60/71 (83%)	2e-23
35S-*Ac-rolC*#10	Hypothetical protein F28P22.11	*A. thaliana*	G96751	25/30 (82%)	5e-05
	Late embryonic abundant protein EMB7	*Picea glauca*	T09288	34/44 (76%)	3e-10
	Hypothetical protein F316.13	*A. thaliana*	T00650	29/46 (62%)	7e-04
	Probable 24-sterol C-methyltransferase	*Glycine max*	T06780	30/30 (99%)	1e-10
	Hypothetical protein F26K9.80	*A. thaliana*	T48054	46/67 (67%)	4e-09

A similar analysis was also made for FSTs from the T-DNA tagged transgenic aspen lines (Table 4). Only 16% of the T-DNA insertions landed into or near known or predicted genes. This frequency is about one half of the frequency of *Ac* insertion hitting coding sequences in the same aspen system. Hence, the *Ac* system seems to be more effective for gene tagging and promoter trapping studies in aspen.

Table 4: T-DNA tagged aspen genomic sites (out of 32 transgenic lines analyzed) with similarity to sequences in public databases

Transgenic line	Blastx similarity	Organism	Accession number	Positives (% amino acids)	E Value
Brauna 11-*35S-rolC*#2	Probable methyltransferase	*A. thaliana*	E85112	41/46 (88%)	2e-21
Esch5-*35S-rolC*#5	Receptor-like kinase	*Oryza sativa*	AF238472	19/22(86%) 16/24 (65%)	3e-04
W52-*35S-rolC*#3	Polyprotein	*Sorghum bicolor*	AF061282	44/60 (72%) 9/10 (90%)	3e-11
Esch5-*35S-rolC*#11	Potassium channel 2	*P. tremula X P. tremuloides*	PTR271447	17/17 (100%)	0.003
Brauna 11-*35S-rolC*#5	Hypothetical protein F9E11.7	*A. thaliana*	H96769	128/269	7e-27

4. Aspen FSTs and GABI-KAT resources

The T-DNA/*Ac* FSTs obtained from aspen genome were searched for T-DNA tagged *Arabidopsis* lines in GABI-kat database. We obtained five *Arabidopsis* lines where T-DNA appears to be inserted into coding regions that are similar to aspen T-DNA/*Ac* FSTs (Table 5). Three of these five FSTs belong to T-DNA out of which two transgenic lines (Brauna11:35S-rolC#2, Esch5:35S-rolC#5) show variant phenotype. We have ordered seeds of these *Arabidopsis* lines for a comparison and in order to obtain quick results from *Arabidopsis*, which has a very small generation cycle compared to aspen.

Table 5: GABI-KAT search results of T-DNA/*Ac* flanking genomic sequences obtained from aspen

Aspen sequence (GABI-PD code)	Transgenic line	T-DNA/*Ac*	Gene annotation	Gene code	GK line ID
B2_2_RE_B	Brauna11:35S-rolC#2	T-DNA	Putative methyltransferase	At4g10760	353B10
E2_5RE_B	Esch5:35S-rolC#5	T-DNA	Receptor-like kinase	At5g38280	254G07
SK316CUP	Esch5:rbcS-rolC#11	T-DNA	potassium channel	At4g22200	152C05
SK467	Esch5:35S-*Ac*-rolC#2	*Ac*	putative phosphoribosylanthranilate transferase	At4g11610	004G08, 147G08, 234C05
SK510	Esch5:35S-*Ac*-rolC#3	*Ac*	Hypothetical protein	At4g03540	301G06

5. Induction and establishment of a haploid hybrid poplar line

As already described in the previous reports, the haploid line that was proposed for the current project turned out to be diploid. Following a literature survey and subsequent requests it was not possible to locate any haploid poplar line worldwide. Therefore, we decided to start our own experiments to induce haploidy in poplar based on the following activities:

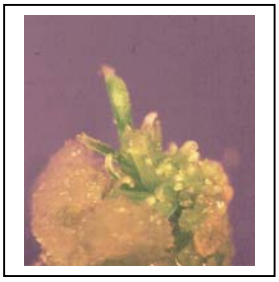
a. Gynogenesis: *In-vitro* culture of non-fertilised ovules
b. Androgenesis: *In-vitro* culture of immature anthers
c. Pseudogamy: Induction of parthenogenesis
d. Isolation and culture of microspores

Results of the experiments (a) to (c) can be summarized as following:

Ad a) Gynogenesis:
Populus tremula × *P. tremuloides* (Klon Esch5)
ca. 400 non-fertilized ovules 1 callus
Regeneration: not possible

Ad b) Androgenesis: anther culture in two species of *Populus*:
P. tremula (Clone „Parkplatzbaum“): ca. 4000 anthers 0 callus
(Clone W52): ca. 4000 anthers 1 callus
Regeneration: not possible
P. nigra (Clone „Auegrund“): ca. 3000 anthers appr. 3000 calli
Regeneration: 6 to 10 regenerations of shoots (Fig. 1)

Figure 1: Regeneration of shoots from anther callus of *P. nigra*



The obtained calli and shoots were not investigated in respect to their ploidy level.

Ad c) Pseudogamy: Induction of parthenogenesis

The method used is based on a publication by Illies (1975) in which successful production of haploid poplar is described. A female poplar hybrid (*Populus tremula* × *P. tremuloides* [Esch5]) was cross-pollinated with pollen of a male *P. alba* line. Following 15 hours a solution of 10ppm Toluidinblue was sprayed on the catkins to stop pollen tube growth. The advantages of this cross are that the hairy lower leaf surface of *P. alba* is dominant to the non-hairy leaf surface of Esch5. Haploid seedlings should reveal a non-hairy lower leaf surface. The results of the crosses are summarized in Tab. 6.

Table 6: Summary of the cross *Populus tremula* × *P. tremuloides* [Esch5] X *P. alba*.

Experiment No.	Catkin	Seeds	Seedling	Germination rate	No. of plants
	No.	No.	No.	Seedlings/ seeds	No.
1	42	51	44	0,86	32
2	25	931	847	0,91	735
3	17	1080	408	0,44	219
4	23	8	7	-	3
sum Toluidine-treated	107	2070	1306	0,63	989
Controls (not treated)	ca. 30	503	329	0,65	220
5 *In-vitro*-plants	-	128	125	0,98	120

No obvious phenotypes of the seedlings were detected. All seedlings showed a more or less hairy lower leaf surface. From 435 plants the ploidy level was determined using flow cytometry. Out of these 404 plants were diploid, 26 triploid, 15 aneuploid und 3 plants revealed haploid leaves. In one plant, different ploidy levels (haploid, diploid, triploid) were detected in one leaf.

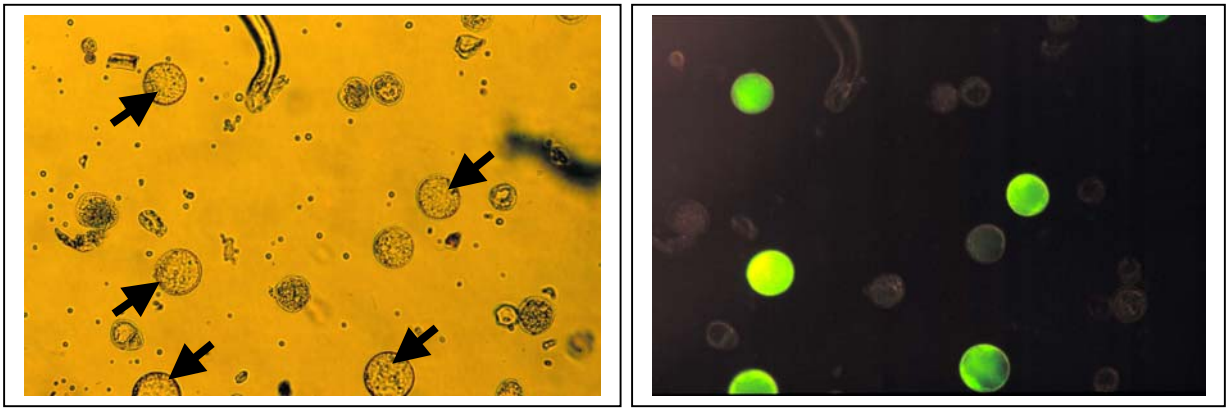
Taken all the results together, no haploid lines were obtained following gynogenesis and androgenesis. The progeny which were obtained after induction of parthenogenesis (c) contained plants with haploid

leaves. However, the haploid status was not stable haploid but switched to diploid as detected in flow cytometer measurements made later.

Ad d) Isolation and culture of microspores

In cooperation with Dr. J. Kumlehn (University of Hamburg) a method was developed to isolate microspores from male flowers of *P. tremula* and *P. nigra* (Fig. 2). Taken the results together, the microspore cultures of the year 2001 produced 113 calli larger than 1 mm, 19 calli per responsive bud. The cultures of the year 2002 produced 1375 calli larger than 1 mm, 81 calli per responsive bud, four times more than the cultures of 2001. Out of these, 100 calli showed further growth or formed embryoid like structures (embryoids). The yields of the microspore cultures are shown in Table 7.

Figure 2: Isolated microspores of *Populus nigra* hybrid. The microspores were stained with DAPI and photographed under normal light (left) and UV (right). Arrows in the left figure mark the high-lightening (thus living) microspores from the right figure.



The data of the regeneration from calli and embryos of the years 2001 and 2002 are summarized in Table 7. In the year 2001, calli with at least 1 mm in size (approximately after four to eight weeks) were transferred onto semi-solid regeneration media "Woody Plant Medium" (WPM) and Gamborg B5 without growth regulators. One third of the number of calli and embryos turned green in light and some embryos formed green cotyledons and hairy roots. Most of them died, but few were rescued using 0.02 mg/l NAA and 0.5 mg/l BAP. Two embryos and two calli formed adventitious leaves or shoots.

In the year 2002, 1375 calli were subcultured on hormone containing media. More than one third turned green and one sixth initiated organogenesis (Table 7). No significant increase in frequency of green calli formation was achieved with media supplemented with sucrose or maltose as carbohydrate source. Most of the transparent calli turned brownish but some formed green cores of solid regenerative cells and shoots. So far, twelve lines have rooted *in vitro*.

Table 7: Yield of microspore culture 2001 and 2002: Induction of callogenesis

Donor tree	Buds[(a)] processed	Responsive buds	Calli (> 1mm)	Calli / Responsive buds
Year 2001				
Aue 1	8	4	104	26
Aue 2	3	2	8	4
Total of 2001	11	6	112	19
Year 2002				
Aue 1	14	8	358	45
Aue 2	19	9	1017	113
Total of 2002	33	17	1375	81

[(a)] Only buds without infection were considered.

In 2001, analysis of the ploidy level of regenerants and calli using flow cytometry revealed haploid, diploid and tetraploid plants and calli. Haploid tissue was detected in two regenerants: M28 from the *Populus nigra* hybrid 'Aue2' and M22-1c5 from *Populus nigra* hybrid 'Aue1'. In M28 both haploid and diploid tissue was found indicating that the haploid status is not stable. In the second line M22-1c5, however, so far stable haploid level was detected after repeated measurements. The regenerative callus was sub-cultivated and multiplied (Figure 3, left, middle). Regenerated plants were rooted (Figure 3, right) and used for transformation (see next topic).

Figure 3: Regenerative callus of the haploid line M22-1c5 in 2001, generated from microspores of the *Populus nigra* hybrid 'Aue1' (left, middle). Rooted haploid plantlet (right).

In 2002, the M22-1c5 regenerative callus line still formed regeneration buds with small leaves (Figure 4). Occasionally evolving shoots from these buds could be rooted and transferred into soil one year after culture initiation (Figure 5). The regenerants are dwarfish and slow growing. Unfortunately, these regenerated plantlets are double-haploid. However, the regenerative callus line M22-1c5 is still haploid in *in vitro* culture for more than 24 months.

Figure 4: Regenerants from the embryo M22-1c5 in 2002.

Figure 5: Regenerants from the embryo M22-1c5 in soil in 2002. The large plant (left) is a control diploid plant regenerated *in vitro* from the "father" poplar.

Ploidy level measurements of regeneratants obtained in 2002

Flow cytometer measurements of 66 regenerative calli obtained in **2002** revealed that five calli were haploid, 58 diploid (including two calli showing a second peak at n and two calli at 4n), two tetraploid, and one callus was aneuploid (Table 8). All regenerative calli analysed in flow cytometer measurements were investigated for their haploid origin using five microsatellites- (SSR-) markers. The microsatellites were heterozygous in the "father" trees 'Aue 1' and 'Aue 2'. All haploid and most diploid and tetraploid calli were homozygous for either alleles, but some diploids and tetraploids revealed heterozygous genomes (Table 8).

Table 8: Ploidy level and 5 microsatellite loci of 66 regenerative calli obtained in 2002.

	Haploids	Diploids	Tetraploids
Homozygotic loci	5	52	2
Heterozygotic loci as the donor trees		2	1
Homo- and heterozygotic loci		4	
In total	5	58	3

One haploid regenerative callus line (FD25) regenerated also haploid plants. The line FD25 is well growing in *in-vitro* culture despite its haploid status. At present (a) measurements are repeated to confirm long-term haploidy in the regenerative callus line, and (b) regeneration experiments from leaves of regenerated haploid plants are underway. The haploid plantlets are currently multiplied for transformation experiments.

6. Transformation of the haploid lines and molecular analysis of transformants

Transformation experiments using callus and leaf tissue from the haploid lines with the 35S-*Ac*-*rolC* construct were performed. Three stable transgenic haploid *Populus* lines carrying *Ac* were obtained and being tested for *Ac*-excision at present (Table 9). Regeneration of *Ac*-transgenic plantlets is in progress. The haploid M22-1c5#23-1 transgenic line was investigated for *Ac* transposition. *Ac* insertion in one case has been found in the *npt-II* gene of the T-DNA, however, Southern analysis are underway to confirm this result.

Table 9: Transgenic lines carrying the 35S-*Ac*-*rolC* gene construct

Transgenic line	**Ploidy level**	**PCR check *npt-II***	**PCR check *rolC, Ac***
M22-1c5#23-1	1	+	+
M22-1c5#23-2	2	+/-	-
M22-1c5#23-3	2	+/-	-
M22-1c5#23-4	2	+/-	-
M22-1c5#23-5	2	+/-	-
FD4#23-1	2	n.a.	n.a.
FD4#23-2	2	n.a.	n.a.
FD4#23-3	2	n.a.	n.a.
FD25#23-1	1	+	+
FD25#23-2	1	+/-	+/-
FD25#23-3	1	+	+
FD25#23-4	n.a.	n.a.	n.a.
FD25#23-5	n.a.	n.a.	n.a.

n.a.=not analysed

7. Submission of sequences to the GABI databank

More than 100 sequences have been submitted to the GABI databank and are available to the GABI community.

8. Summary, conclusions and outlook

In this project, significant achievements have been made that are summarised below:

(a) T-DNA tagged *Arabidopsis* lines available from the GABI-KAT project with similar flanking genomic regions to T-DNA tagged aspen variants will be screened.

(b) T-DNA flanking sequences obtained from a number of T-DNA tagged variants showed homologies to known genes.

(c) At least two stable haploid poplar lines were obtained.

(d) Regeneration of plantlets from the haploid line in *in vitro* culture occurs via single cells, thus, no protoplast culture is necessary.

(e) It was shown that *Ac* excises and re-integrates in the aspen genome.

(f) *Ac* inserts frequently in or near coding regions.

(g) Three *Ac* transgenic haploid poplar line are available. In these lines, *Ac* transposition will be investigated.

(h) A large number of poplar sequences were obtained and submitted to GABI databank.

Outgoing from the results of this project the haploid line(s) can be used for the production of knock-out mutants. Using known recombination systems a targeted gene transfer using side-specific-recombination will be established in haploid poplar. In addition, due to the high frequency insertion of *Ac* element in coding regions in the aspen genome, a protocol for efficient activation tagging in diploid poplar is suggested.

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Dissemination

The results described here were published in part in scientific journals as well as presented in talks and posters on several congresses. Participation of the congresses were very important because of the very fruitful discussions generated with colleagues working in the same field.

Publications:

Deutsch, F., Kumlehn, J., Ziegenhagen, B., Fladung, M. (submitted) Haploid plant regeneration from poplar microspore culture.
Fladung, M., Kumar, S. (2002) Gene stability in transgenic aspen-Populus – III. T-DNA repeats influence transgene expression differentially among different transgenic lines. Plant Biol. 51, 329-338.
Kumar, S. Fladung, M. (2000) Transgene repeats in aspen: molecular characterisation suggests simultaneous integration of independent T-DNAs into receptive hotspots in the host genome. Mol. Gen. Genet. 264, 20-28.
Kumar, S. Fladung, M. (2001) Controlling transgene integration in plants. Trends in Plant Science, London, 155-159.
Kumar, S.; Fladung, M. (2001) Gene stability in transgenic aspen (Populus). II. Molecular characterization of variable expression of transgene in wild and hybrid aspen. Planta, Berlin, 213, S. 731-740.
Kumar, S., Fladung, M. (2002) Gene Targeting in plants. In: SM Jain, DS Brar, BS Ahloowalia (eds) Molecular Techniques in crop improvement. Kluwer Academic Press, 481-499.
Kumar, S., Fladung, M. (2002) Transgene integration in aspen: structures of integration sites and mechanism of T-DNA integration. Plant J. 31, 543-551.
Kumar, S. Fladung, M. (2002) Somatic mobility of the maize element *Ac* and its usability for gene tagging in aspen. Plant Mol. Biol. 51, 643-650.

A Meeting on “German Woody Plant Genomics” (1. GWPG) was organized by M. Fladung from 06.10.-09.10.2002 in Ahrensburg and Großhansdorf. On this meeting the “German Network on Woody Plants Genomics” (GeNetWG) was initiated.

Talks and Poster 2000-2002:

2000

DEUTSCH, F; FLADUNG, M.: Versuche zur Erzeugung haploider Pappeln. Tagung der deutschen Sektion der IAPTC, Bonn, 6.10.2000.
FLADUNG, M.: Isolation of tree-specific genes and promoters by a transposon-tagging approach. In: German-French Workshop GABI-GENOPLANTE, Abstracts and Project Lists Workshop in Bonn am 30. und 31.5.2000, S. 51.
KUMAR, S.; FLADUNG, M.: Molecular characterization of stable and unstable transgenic aspen

(*Populus*). Biotechnology 2000. The World Congress on Biotechnology, Berlin, 3. bis 8.9.2000.

2001

DEUTSCH, F.; FLADUNG, M.; KUMLEHN, J.: Embryogenese von isolierten Mikrosporen einer Schwarzpappelhybride. Forschungsseminar 2001, Fachbereich Biologie der Universität, in Hamburg, 4. und 5.7.2001.

DEUTSCH, F.; KUMLEHN, J.; FLADUNG, M.: Etablierung einer Mikrosporenkultur bei Pappeln: besteht die Möglichkeit einer Transformation vor der Diploidisierung? Meeting der Deutschen Sektion der IAPTC in Bonn, 13.7.2001.

FLADUNG, M.: GABI- POP: Isolation of tree specific genes and promoters in poplar. 1. Statusseminar zur Pflanzengenomforschung GABI, Bonn am 20. und 21.2.2001.

FLADUNG, M.: Gentechnik in der Forstwirtschaft: Verein der Deutschen Papierfabriken, Axel-Springer-Haus, in Berlin am 22.11.2001.

FLADUNG, M.; DEUTSCH, F.; KUMAR, S.: GABI-POP: Isolation of tree-specific genes and promoters by a transposon-tagging approach. 10th International Meeting of the IUFRO Working Party on Molecular Biology of Forest Trees ”Tree Biotechnology in the New Millenium”, in Stevenson, Washington/USA vom 22. bis 27.7.2001.

FLADUNG, M., KUMAR, S.: The *Ac*-transposon from maize transposes in trees. Plant and Animal Genome IX Meeting, San Diego, USA, 13.-17.01.01

FLADUNG, M.; KUMAR, S.: The *Ac*-transposon from maize transposes in trees. Plant and animal genome IX meeting, in San Diego/USA, vom 13. bis 17.1.2001.

KUMAR, S.; FLADUNG, M.: Transgene integration in aspen: mapping of the integrated T-DNAs and genomic target sites. Plant and animal genome IX meeting, in San Diego/USA, vom 13. bis 17.1.2001.

FLADUNG, M., KUMAR, S.: Transgenic trees: From tree improvement to functional genomics. 10th International Meeting of the IUFRO Working Party on Molecular Biology of Forest Trees ”Tree Biotechnology in the New Millenium”, in Stevenson, Washington/USA vom 22. bis 27.7.2001.

FLADUNG, M.; KUMAR, S.; KALDORF, M.; GRÜNWALD, C.; DEUTSCH, F.; MUHS, H.-J.: Integration und Expression von fremden Genen in Zitterpappeln. 14. Tagung "Molekularbiologie der Pflanzen", Dabringhausen vom 28.2. bis 3.3.2001.

FLADUNG, M.; NOWITZKI, O.; KUMAR, S.: Transgenic aspen: transgene integration, stable expression, and functional genomics. Internationale Konferenz ”Wood, Breeding, Biotechnology and industrial expectations. Bordeaux/Frankreich, 11. bis 14.6.2001.

KUMAR, S., FLADUNG, M. Transgene integration in aspen: mapping of the integrated T-DNAs and genomic target sites. Plant and Animal Genome IX Meeting, San Diego, USA, 13.-17.01.01

KUMAR, S.; FLADUNG M.: Transgene integration, stable expression and gene targeting in aspen. 10th International Meeting of the IUFRO Working Party on Molecular Biology of Forest Trees ”Tree Biotechnology in the New Millenium”, in Stevenson, Washington/USA vom 22. bis 27.7.2001.

2002

DEUTSCH, F.; KUMLEHN, J.; KUMAR, S.: FLADUNG, M.: Induction of haploid poplar through microspore culture. Stará Lesná/ Slowakai, 26-30.08.2002 (Poster).

DEUTSCH, F.; KUMLEHN, J.; KUMAR, S.; FLADUNG, M.: Induction of haploid poplar through microspore culture. Forschungstage Biologie, in Hamburg, 03. und 04.07.2002 (Poster).

DEUTSCH, F.; KUMLEHN, J.; KUMAR, S.; FLADUNG, M.: Haploid production in poplar. 1. GWPG-Meeting, in Ahrensburg/Großhansdorf, 06.-09.10.02 (Poster).

FLADUNG, M.: Kann die Gentechnik in der Forstwirtschaft Anwendung finden? Jahrestreffen des Südtiroler Forstvereins, Bozen/Italien, 21. bis 23.02.2002.FLADUNG, M.: Untersuchungen zur Expression und Stabilität von Transgenen in angiospermen Gehölzen am Beispiel der Zitterpappel (Aspe). Ahrensburg, 24.04.2002.

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FLADUNG, M.; DEUTSCH, F.; KUMAR, S.: Genomics in hybrid poplar. IUFRO Symposium 'Population and Evolutionary Genetics of Forest Trees', in Stará Lesná/ Slowakai, 25. bis 29.08.2002.

FLADUNG, M.; HÖNICKA, H.: Etablierung von männlicher und weiblicher Sterilität in transgenen Zitterpappeln zur Verhinderung eines vertikalen Gentransfers in forstliche Ökosysteme. Statusseminar ‚Biologische Sicherheit und Monitoring', in Hannover am 21. und 22.03.2002.

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FLADUNG, M.; KUMAR, S.: Establishment of a gene targeting strategy in aspen. DFG-Gutachtensitzung im Rahmen einer geplanten Forschergruppe ‚Poplar as a model to address tree-specific questions', in Göttingen, 21. und 22.04.2002.

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HÖNICKA, H.; FLADUNG, M.: Etablierung von männlicher und weiblicher Sterilität in transgenen Zitterpappeln zur Verhinderung eines vertikalen Gentransfers in forstliche Ökosysteme. Treffen des BMBF-Verbundprojekts "Spezifische Umweltwirkungen transgener Gehölze", in Freiburg, 20. und 21.06.2002.

KUMAR, S.; FLADUNG, M.: Somatic activity of the maize element *Ac* in aspen and its usability for gene isolation. International Poplar Symposium III, in Uppsala/Schweden, 25. bis 29.08.2002 (Poster).

KUMAR, S.; FLADUNG, M.: Somatic activity of the maize element *Ac* in aspen and its usability for gene isolation. *Populus* Functional Genomics Workshop, in Umeå/Schweden, 30.08.2002 (Poster).

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