ed from northern, southern and particularly far eastern provenances. The separate position of the Chinese provenance can be confirmed on the basis of growth traits and insect damage.

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Morphological Changes in Transgenic *Populus* Carrying the *RolC* Gene from *Agrobacterium rhizogenes*

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Dedicated to WOLFGANG LANGNER on the occasion of his 90th birthday

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Summary

We have employed the reporter gene *rolC* gene from *Agrobacterium rhizogenes* as a morphologically detectable marker system for investigating growth alterations in *Populus*. A hybrid aspen (*P. tremula* L. x *P. tremuloides* MICHX.) clone, Esch5, was transformed using different chimeric gene constructs including the *rolC* gene to study its effect on morphological and physiologically-conditioned parameters. Mainly, transgenic aspen carrying the *rolC* gene under control of the cauliflower-mosaic-virus 35S-promoter and the light inducible *rbcS* promoter from potato were compared with controls. Other gene constructs, in which *rolC* expression is prevented by insertion of the transposable element *Ac* from maize were also included. Differences in growth parameters (e.g. plant height, stem diameter, number of leaves), and growth arrest and terminal bud formation were observed between the control and the *35S-rolC* transgenic aspens. Evaluation of onset of dormancy in the autumn and flushing in the next spring revealed differences between untransformed controls and, in particular, the *35S-rolC* transgenic plants. These tree-specific morphological and developmental characteristics are discussed in the light of the transferred foreign genes in aspen-*Populus*, a woody plant model system.

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Key words: Agrobacterium, aspen, bud release, dormancy, *rolC,* transgenic *Populus.*

FDC: 165.3; 165.72; 161.4; 172.3 *Agrobacterium;* 176.1 *Populus tremula* x *tremuloides.*

Introduction

Genetic engineering of plant species has successfully been introduced as a new tool in plant breeding programs. However, for forest trees many questions are still left, e.g. is it relevant to ask if foreign genes will be stably integrated and expressed (AHUJA, 1988a and b), and remain active during the long life cycle of trees. In genetically engineered crop species, a number of known reporter genes have been used. For long-term investigations in forest trees reporter genes having no phenotypic effect, for example, those coding for neomycin phosphotransferase (*npt*) and glucuronidase (GUS or *uidA*) can be used in transient or stable transformation experiments. However, reporter genes which affect the morphology of the plant are of special interest, as they can be used as visual markers throughout the life of a plant.

The *rolC* gene of *Agrobacterium rhizogenes* as a morphologically selectable marker gene has been tested earlier in annual plant species like tobacco (SPENA et al., 1987; SCHMÜLLING et al., 1988) and potato (FLADUNG, 1990; FLADUNG and BALLVORA, 1992; KAENDLER et al., 1996). Following transfer of the *rolC* gene to tobacco and potato, species-specific alterations in plant

height, coloration and shape of leaves, photosynthetic and transpiration rates, yield parameters and responses to pathogenes have been observed (SPENA et al., 1987; SCHMÜLLING et al., 1988; FLADUNG, 1990; FLADUNG and BALLVORA, 1992; FLADUNG and GIEFFERS, 1993; FLADUNG et al., 1993). The *rolC* gene product was suggested to be a cytokinin-β-glucosidase, which apparently releases free active cytokinins from inactive conjugates (ESTRUCH et al., 1991). Measurements of hormonal levels in different plant tissues, in fact, revealed dramatic changes in hormonal levels in both tobacco and potato (NILSSON et al., 1993; SCHMÜLLING et al., 1993).

Thus, it is possible that transgenic aspen (*Populus tremula*) carrying the same *rolC* gene would also reveal pleiotropic effects. However, before such transgenic aspen plants can be used for evaluation of transgene stability in greenhouse or field experiments, a detailed evaluation of the transgenics under study has to be performed. In this paper, results of the greenhouse experiment on phenological and morphological traits are presented. Based on the results of this study, a detailed phenological and morphological study of different transgenic aspen clones carrying the *rolC* gene under control of the *35S* and *rbcS* promotor grown under field conditions is underway, which will supplement the results of this greenhouse experiment.

Material and Methods

Constructs

Four different constructs were used for transformation experiments (*Table 1*). In all constructs the *rolC* gene from *Agrobacterium rhizogenes* was employed as common part. Two promoters were selected to control expression of *rolC*, the *35S*-promoter from the cauliflower mosaic virus (*35S-rolC*; SCHMÜLLING et al., 1988) and the light-inducible promoter of the large subunit of the ribulose-bisphosphate carboxylase from potato (*rbcS-rolC;* SCHMÜLLING et al., 1993). Additionally, the maize transposable element *Ac* has been inserted between the promoter and the *rolC* coding region. In this case, the expression of the gene is expunged (*35S-Ac-rolC*; SPENA et al., 1989; *rbcS-Ac-rolC*; JONES et al., 1992).

Table 1. – Number of integrated copies of the *rol*C gene in independent primary transgenic aspen clones included in this study carrying different gene constructs. For assessment of morphological and phenological traits only transgenics with one integrated copy were used.

Construct	No. of independent transgenics	Number of *rolC* integrated copies		
		1	2	≥3
35S-rolC	15	11	3	1
rbcS-rolC	15	12	2	1
35S-Ac-rolC	16	12	3	1
rbcS-Ac-rolC	13	10	3	0

Transformation, regeneration of aspen leaf discs and cloning of plants

Leaf discs of the hybrid aspen (*Populus tremula* L. X *P. tremuloides* MICHX) clone Esch5 were transformed with different gene constructs using the *Agrobacterium* vector system and regenerated at high frequencies with kanamycin as a selective marker as described by FLADUNG et al. (1996). Briefly, leaf pieces harvested from 3 to 4 weeks old axenic shoot cultures were treated with an overnight culture of *A. tumefaciens* carrying the desired binary vector, washed and incubated on a solidified WPM regeneration medium. Shoots appearing after 3 to 6 weeks were excised and rooted on a 'Woody Plant Medium' (WPM) rooting medium.

The number of independent transformants used in this study is shown in *table 1*. In order to obtain a large number of cloned transgenic plants from single independent transformants, 4 to 8 shoots were devided in 5 to 7 explants each which were rooted again on WPM rooting medium. Out of these, a varying number of 20 to 90 plants per construct were transferred into the greenhouse per month, while some 4 to 8 plants were subcultured again on WPM rooting medium for further multiplication. Using this propagation method, we were able to produce a total of 630 plants which were successfully rooted *in vitro* and transferred into the greenhouse. Out of these, 164 plants were transferred in December 1993, 245 plants in January 1994 and 221 plants in February/March 1994. From each different independent transformant clone, 3 to 15 plants were morphologically analyzed. Due to the low and uneven number of plants derived per transgenic, the observations are based on all plants available.

Plant cultivation and morphological evaluations

Plants grown *in vitro* were transferred and cultivated in a standard S1-greenhouse (this designation implies no risk) under natural daylight (photoperiod, light) conditions. Temperature was low (4 °C) during the winter months and increased to 20 °C to 25 °C in summer (on rare occasions up to 35 °C).

Plants were watered daily but supplemented with mineral fertilizer once during the growing season. Temperature and humidity in the greenhouse were recorded. Control and transgenic plants were grown side by side in the same greenhouse, so that daily fluctuations due to greenhouse cultivation would affect all plants simultaneously.

For the analysis of morphological traits, plants grown in greenhouse were examined and measured 6 months after transfer according the following traits: the formation of a terminal bud (growth stop), plant height, number of leaves of the main shoot, number of side shoots, stem diameter (2 cm above soil surface), and length to width ratios of leaves of the upper, middle and lower region of the plant.

For assessment of flushing, the plants were scored 6 months after transfer from *in vitro* into the greenhouse. A plant was recognized as flushed, when one bud of the plant reached the stage no. 3 of the classification scale ranging from 1 (fully undeveloped bud) to 6 (fully flushed bud including elongation of the stem) for bud development (modified after KLEINSCHMIT and SVOLBA, 1979). The stage no. 2 indicates a partly green bud starting to swell, and stage no. 3 means, that the bud is swollen, with unfolded leaves, only margins of the new developing leaves are visible. In the following year, recording of flushing date was repeated using the same plant material and the same score.

Molecular analysis

DNA extraction from leaves for PCR analysis and Southern hybridization for establishing the number of *rolC* integrated copies is described in FLADUNG and AHUJA (1995) and FLADUNG et al. (1996). PCR amplification for the *rolC* coding sequence was carried out using a specific primer pair as described in FLADUNG et al. (1996). Prehybridization and hybridization was done by a non-radioactive method using the DIG-system (NEUHAUS-URL and NEUHAUS, 1993; FLADUNG and AHUJA, 1995).

Results

Transformation, PCR and Southern analysis

On the basis of a well established method of tissue culture of aspen and hybrid aspen and regeneration of mature plants

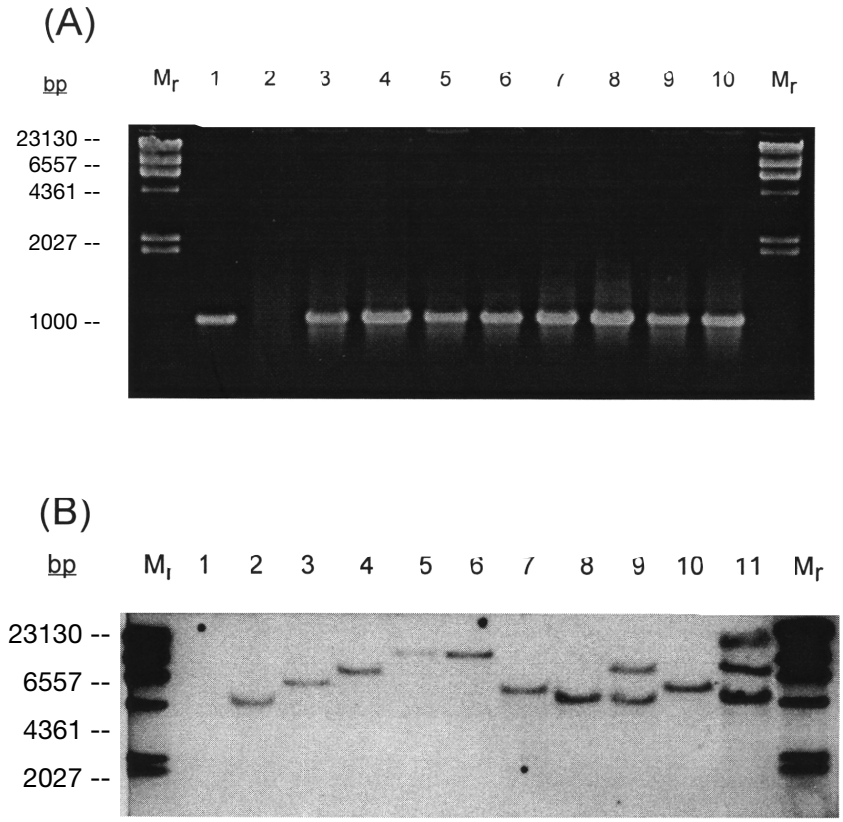

Figure 1. – PCR amplification and Southern analysis of transgenic aspen plants, carrying the *rol*C gene of *A. rhizogenes*. A. PCR amplification of the *rol*C coding sequence (lane 1), negative amplification in untransformed Esch5 clone (lane 2), and *rol*C positive, independent regenerated transgenic plants (lanes 3 to 10). Molecular weight markers are included on left and right. B. Non-radioactive Southern blot analysis of *35S-rol*C transgenic hybrid aspen plants showing 1 to 3 integrated copies of the transgene (lanes 2 to 11). Untransformed Esch5 (lane 1) and molecular markers are shown on the left and right.

(AHUJA, 1986, 1987), a transformation method was developed, by which a fairly large number of putative transformed plantlets were obtained (FLADUNG and AHUJA, 1996). Such clones morphologically characterized as *rolC* transgenics were tested positive in the PCR analysis (*Figure 1A*) as well as in Southern blot experiments (*Figure 1B*). Transgenics carrying the *Ac-rolC* gene constructs considered in this study were positive in PCR analysis as well as in Southern hybridization.

A total of 59 different transgenic clones have been investigated based on their number of *rolC* integrated copies (*Table 1*). The number of copies was established by restriction of genomic DNA with enzymes flanking either the right or the left site of the *rolC* containing cassette and non-radioactive Southern blots using a DIG-labeled *rolC*-specific probe. Whereas 45 (10 to 12 for each construct) of the independent primary transgenic clones used in this study for morphological investigations contained one *rolC* integrated copy, only 3 to 4 transgenics carried 2 or more integrated copies (*Table 1*). Morphological analysis and comparisons to controls were only made on transgenics, revealing one integrated copy of the gene construct.

Morphological analysis and formation of terminal buds

During the process of regeneration, transgenic plants were propagated *in vitro* continuously and transferred to the greenhouse at 4 different dates, starting December 1993 (short day

Table 2. – Formation of terminal buds in control and transgenic aspen plants in relation to transfer date from *in vitro* into the greenhouse, and flushing of buds after 6 months of growth.

Date of transfer (month/year)	Construct	Terminal bud	
		No. and (%) of plants forming terminal buds/plants total	No. and (%) of plants flushed
12/93	Control	18/18 (100%)	0 (0%)
	35S-rolC	19/33 (58%)	18 (95%)
	rbcS-rolC	37/37 (100%)	3 (8%)
	35S-Ac-rolC	59/59 (100%)	3 (5%)
	rbcS-Ac-rolC	17/17 (100%)	0 (0%)
1/94	Control	13/27 (48%)	13 (100%)
	35S-rolC	0/51 (0%)	-
	rbcS-rolC	16/20 (80%)	14 (88%)
	35S-Ac-rolC	50/90 (56%)	39 (78%)
	rbcS-Ac-rolC	30/57 (53%)	24 (80%)
2/ and 3/94	Control	0/17 (0%)	-
	35S-rolC	0/62 (0%)	-
	rbcS-rolC	8/67 (12%)	8 (100%)
	35S-Ac-rolC	3/57 (5%)	3 (100%)
	rbcS-Ac-rolC	3/18 (17%)	3 (100%)

conditions), January 1994, and February/March 1994. At each transfer date, controls and transgenic plants were transferred into the greenhouse and exposed to daylight conditions.

When untransformed control and transgenic plants transferred in December 1993, all control and transgenic aspen plants with exception of 14 out of 33 transformants of the *35S-rolC* plants stopped growth and formed a terminal bud (*Table 2*). However, when plants were transferred in January 1994 to the greenhouse, 48% of control plants and up to 80% of transgenic plants (*rbcS-rolC, 35S-Ac-rolC, rbcS-Ac-rolC*) revealed growth stop, but none of the *35S-rolC* transgenics. None of the controls and only a few transgenic plants responded with terminal bud formation, when transferred in February 1994 or later into the greenhouse.

After 6 months of growth in the greenhouse, some morphological parameters were determined in order to characterize the growth of the transgenic plants compared with the untransformed aspen transferred to greenhouse at the same time. First, an evaluation became necessary to classify the transgenics based on bud formation and release from dormancy (see also *Table 2*): (a) plants which formed terminal buds and were not flushed (*Figure 2A*, right plant); (b) plants which formed terminal buds and were flushed (*Figure 2B*, right plant; *Figure 2C*, plants in the foreground); and (c) plants which had not developed buds at the time of observation (*Figure 2A*, *B*, left plants; *Figure 2C*, plants in the background). Interestingly, only a small number of plants (control and *rbcS-rolC, 35S-Ac-rolC, rbcS-Ac-rolC* transgenics) which were transferred in December 1993 to the greenhouse, were flushed in June 1994 (values ranging from 0% to 3%; see *Table 2*), and revealed therefore small plant size, low number of side shoots and leaves (*Table 3*). However, nearly all *35S-rolC* transformants (95%) classified with terminal bud formation were flushed after half a year in the greenhouse. When the plants were transferred in January 1994 into the greenhouse, 78% to 100% of the controls and transformed plants were flushed after 6 months of growth in greenhouse (*Table 2*).

After 6 months of growth in greenhouse, the plant height of the *35S-rolC* with no growth stop (96.4 cm ± 3.8 cm) was lower as compared to untransformed controls with no bud (133.8 cm ± 7.8 cm). However, the number of leaves were 2- to 3fold higher (*Table 3*) in the *35S-rolC* transgenics (110 ± 4.0) as compared to controls (43 ± 2.0). A higher length to width ratio in the leaves of the *35S-rolC* transgenics (1.72 to 2.00) indicates a more lanceolate leaf form in this group of transformants compared to the controls (1.32 to 1.59). All other transgenics, including the *rbcS-rolC* plants, did not reveal any significant phenotypic differences as compared to untransformed aspen plants during growth period in the greenhouse.

In *35S-rolC* transgenic aspen coloration of leaves seems to be dependent on the age of the shoot on which the leaves are formed. During the first year of cultivation in the greenhouse, light-green leaves (including young and older ones) were observed on the one-year-old shoots of *35S-rolC* and *rbcS-rolC* transgenic aspen in comparison to the normal green leaves of control plants. However, on 2 or 3 years old *35S-rolC* plants, freshly formed young leaves following bud flush were yellow-green, while young leaves of control aspen were only slightly light-green. Older leaves of this 2- or 3-years old *35S-rolC* transgenics became as green as leaves from control plants.

A difference in flushing date between *35S-rolC* and control plants was also found in spring 1995 on the same greenhouse plants under greenhouse conditions (*Table 4*). Flushing of only one control, 5 *35S-rolC* and 1 *rbcS-rolC* transgenic was observed end of January 1995. Mid of February 1995, the majority of *35S-rolC* transgenics (87%) were flushed, whereas the *Ac* containing lines started to flush. Maximum flushing of control (80%), *35S-Ac-rolC* (71%) and *rbcS-Ac-rolC* transgenics (79%)

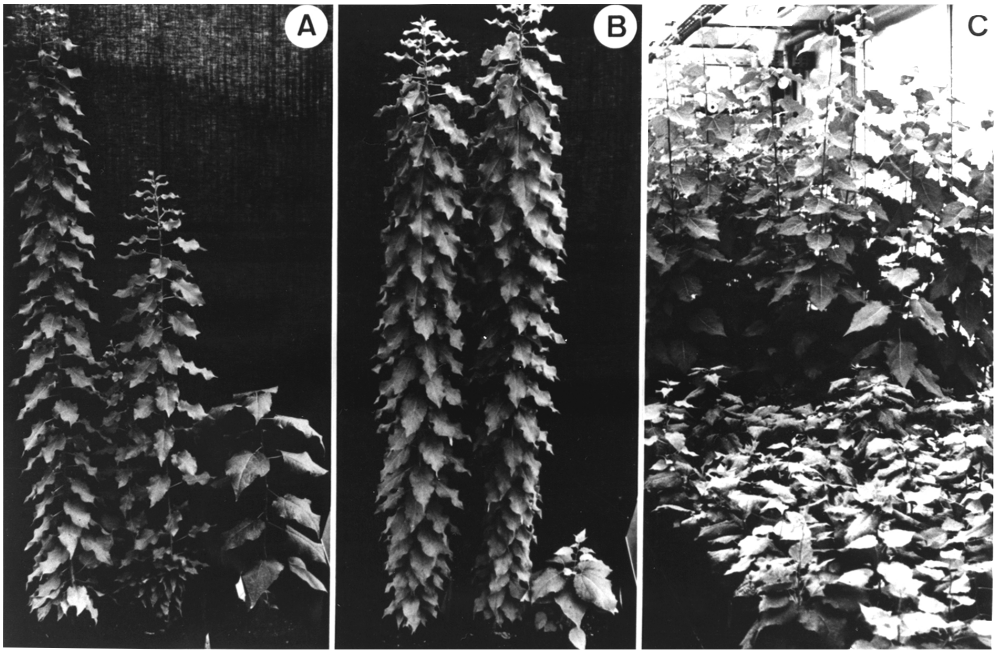

Figure 2. – Control and transgenic aspen plants which formed buds. A. Comparison of 2 *35S-rol*C transgenic clones (middle and left) with control plant (right) transferred in December 1993. The *35S-rol*C transgenics formed no terminal bud, or when they did, it was only during a short time, whereas controls revealed a prolonged time for dormancy. B. *35S-rol*C transgenic aspen clone (middle and left) which had not set buds at all compared with a recently flushed control plant. C. Transgenic *35S-Ac-rol*C and *rbcS-Ac-rol*C aspen plants in the greenhouse (June 1994). Plants transferred in December 1993 or January 1994 which were still in dormancy at time of observation (foreground), and plants transferred in February or March 1994 which had not developed buds (background).

Table 3. – Morphological traits of control and transgenic aspen plants after 6 months of growth in greenhouse (mean ± S.E.).

Construct	Formation of terminal bud	No. of plants*	Plant height (cm)	No. of sideshoots	Stem diameter (mm)	No. of leaves	Length to width ratio of leaves		
							upper	middle	lower
Control	yes, not flushed	18	23.5 ± 2.5	0.2 ± 0.1	4.8 ± 0.3	17 ± 1	1.59 ± 0.04	1.50 ± 0.01	1.55 ± 0.03
	yes, flushed	13	69.3 ± 8.6	2.3 ± 0.8	6.0 ± 0.3	33 ± 2	1.47 ± 0.03	1.32 ± 0.02	1.57 ± 0.05
	no	31	133.8 ± 7.8	2.1 ± 0.7	7.5 ± 0.4	43 ± 2	1.46 ± 0.02	1.41 ± 0.02	1.53 ± 0.03
35S-rolC	yes, not flushed	1	13	1	4.6	67	2.0	1.8	--
	yes, flushed	18	41.5 ± 3.7	2.1 ± 0.4	5.1 ± 0.3	87 ± 5	1.85 ± 0.03	1.86 ± 0.04	1.83 ± 0.04
	no	127	96.4 ± 3.8	1.1 ± 0.2	5.5 ± 0.2	110 ± 4	1.74 ± 0.02	1.72 ± 0.02	1.80 ± 0.02
rbcS-rolC	yes, not flushed	36	29.0 ± 1.7	0.3 ± 0.2	4.6 ± 0.2	23 ± 1	1.60 ± 0.03	1.52 ± 0.02	1.57 ± 0.02
	yes, flushed	25	63.6 ± 9.0	1.7 ± 0.5	4.9 ± 0.3	40 ± 3	1.42 ± 0.04	1.43 ± 0.04	1.55 ± 0.04
	no	63	137.6 ± 6.3	0.7 ± 0.3	6.6 ± 0.2	48 ± 2	1.47 ± 0.02	1.44 ± 0.02	1.56 ± 0.02
35S-Ac-rolC	yes, not flushed	67	17.4 ± 1.1	0.5 ± 0.1	4.1 ± 0.1	14 ± 1	1.59 ± 0.03	1.63 ± 0.02	1.61 ± 0.02
	yes, flushed	45	41.1 ± 2.8	2.2 ± 0.4	4.7 ± 0.2	31 ± 1	1.37 ± 0.02	1.39 ± 0.02	1.58 ± 0.02
	no	94	136.8 ± 5.3	0.4 ± 0.1	6.8 ± 0.2	44 ± 2	1.38 ± 0.02	1.40 ± 0.02	1.53 ± 0.02
rbcS-Ac-rolC	yes, not flushed	23	14.2 ± 0.9	0.4 ± 0.2	4.0 ± 0.2	14 ± 1	1.75 ± 0.04	1.59 ± 0.04	1.52 ± 0.03
	yes, flushed	27	48.3 ± 4.8	2.8 ± 0.4	5.2 ± 0.3	32 ± 2	1.39 ± 0.02	1.44 ± 0.05	1.57 ± 0.03
	no	42	139.2 ± 6.9	0.2 ± 0.1	7.3 ± 0.3	44 ± 2	1.43 ± 0.02	1.43 ± 0.02	1.49 ± 0.02

(*) see *Table 2*

was observed in mid of March 1995 (*Table 4*). In all transgenics, flushing was completed by the end of March 1995.

Discussion

Trees may require special considerations when genetically transformed due to their long life and extended vegetative growth phases. In our study, we have chosen the genus *Populus* as a model system to determine stability/instability of transgene expression in forest tree species. As a morphologically selectable marker system the *rolC* gene of *A. rhizogenes* under expressive control of 2 different promoters was employed for transformation, and the transposable element *Ac* of maize inhibiting *rolC* expression. However, *rolC* induces severe phenotypic and physiological changes in herbaceous transgenics like tobacco (SPENA et al., 1987; SCHMÜLLING et al., 1988) and potato (FLADUNG, 1990; FLADUNG and BALLVORA, 1992). Since *rolC* is expressed at the morphological level, it is important to analyze morphological and, in particular, developmental alterations more in detail in trees.

Expression of the *rolC* gene in transgenic aspen (*Populus tremula*) or hybrid aspen (*P. tremula* X *P. tremuloides*) also alters growth and development (OLSSON et al., 1992, 1995; FLADUNG et al., 1996). Transgenic aspen carrying the *rolC* gene under control of the constitutive *35S* promoter of the cauliflower mosaic virus are characterized as small plants due to reduced internodal length with small but light-green leaves. When the *rolC* gene is controlled by the light-inducible *rbcS*-promoter of potato the size of transgenic aspen seems unchanged.

Table 4. – Number and percent (%) of control and transgenic aspen plants grown in the greenhouse that flushed at different dates in spring 1995.

Date	Constructs				
	Control	*35S-rolC*	*rbcS-rolC*	*35S-Ac-rolC*	*rbcS-Ac-rolC*
January 15, 1995	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
January 31, 1995	1 (2%)	5 (7%)	1 (1%)	0 (0%)	0 (0%)
February 15, 1995	0 (0%)	63 (87%)	11 (9%)	5 (2%)	4 (4%)
February 28, 1995	9 (15%)	4 (6%)	48 (41%)	73 (27%)	15 (16%)
March 15, 1995	49 (80%)	0 (0%)	58 (49%)	191 (71%)	77 (79%)
March 31, 1995	2 (3%)	0 (0%)	0 (0%)	1 (0%)	1 (1%)
April 15, 1995	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total number of plants	61	72	118	270	97

Morphological measurements at half a year in the greenhouse clearly show that *35S-rolC* are reduced in height compared to controls, and forming small, lanceolate, light-green leaves. These results confirmed visual observations made on *35S-rolC* transgenic aspen compared to control aspen (OLSSON et al., 1992, 1995; FLADUNG et al. 1996). The *rbcS-rolC* transgenic plants, however, showed a similar plant height and leaf length to width ratio, as compared to controls, but still revealing slightly smaller, light-green leaves. The result of similar height is surprising because it can be assumed that photosynthetic rates are also reduced in *rbcS-rolC* transgenic aspen as shown for potato (FLADUNG et al., 1993). Therefore, unlike in potato *rbcS-rolC* transgenic aspen plants might be able to compensate reduced CO_2 assimilation to reach similar height as controls under the conditions of greenhouse growth. Possibly, measurements under natural light conditions in the field might reveal differences between *rbcS-rolC* aspen and controls.

The *rbcS-rolC* transgenics as well as those carrying the *rolC* construct in which expression is inhibited by insertion of a transposable element clearly demonstrate that random insertion of foreign sequences into the poplar genome (FLADUNG et al., 1996) do not induce other morphological changes. Therefore, the abnormalities observed in *35S-rolC* transgenic aspen are clearly induced by constitutive expression of the *rolC* gene under control of the *35S* promoter. This assumption is also confirmed by 15 different independent *35S-rolC* transgenics all revealing the similar morphological alterations which seem independent from the integration site of the *rolC* gene construct.

An indication of developmental differences between control and *35S-rolC* transgenic plants were obtained, when plants were transferred into the greenhouse during several months of the winter season. Transfer of plants in the middle of the winter season (e.g., December, January) is, of course, highly unphysiological, because plants do not achieve the normal gradual decrease in day-length and temperature. This can lead to a failure of flushing even after growth for several months in conditions that would normally release the plants from dormancy. However, even if transferred to the greenhouse

during the winter season, controls and transgenic plants can be compared when transferred at the same time and possible differences between them can be deduced either to one of the effects of the transferred gene in the transgenic plants or possibly to variations induced during the regeneration process.

In particular, when forming terminal buds, a strong difference between *35S-rolC* and control plants were observed. Moreover, all plants of that part of the *35S-rolC* transgenics transferred in December 1993, which formed a terminal bud, were flushed at half year evaluation, whereas most of controls and transgenic plants (*rbcS-rolC, 35-Ac-rolC, rbcS-Ac-rolC*) were still in dormancy or just releasing from it. This clearly indicates an alteration in the developmental process of bud formation and bud release in *35S-rolC* transgenic aspen plants, which was also shown after flushing evaluation in the following year. Therefore, it would appear that dormancy in trees may be influenced by either exogeneous environmental (temperature, light) or endogeneous factors (hormonal levels).

In this study, all transgenics investigated were produced using the same untransformed hybrid aspen clone Esch5. Therefore, the transgenic plants differ from the controls in having an alien gene. When cultivated under the same environmental (greenhouse) conditions, a significant difference in release from dormancy (altered bud break) found in *35S-rolC* transgenics might be related to physiological alterations induced by the *rolC* gene under expressive control of the *35S* promoter. This change might be correlated to hormone action, as the *rolC* gene product is believed to influence hormonal levels in transgenic plants (NIELSSON et al., 1993; SCHMÜLLING et al., 1993). Therefore, onset of dormancy and bud release could be regulated by hormone levels, as early suggested by KOZLOWSKI (1971). Further analysis on bud release as well as determination of hormonal levels in several tissues of control and *35S-rolC* transgenic plants (leaves, buds) might reveal some more insights in a possible hormonal regulation during bud development and bud release.

Due to the successful regeneration and transfer of plants into the greenhouse an unequal number of transformants per construct were considered in this study. Therefore, a preliminary investigation of phenological and morphological traits of *rolC* transgenic plants in comparison to controls was possible. However, a detailed phenological and morphological analysis of different transgenic aspen clones carrying the *rolC* gene under control of the *35S* and *rbcS* promoter has already been initiated in the field.

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