***RolC*-Transgenic Carnation with Improved Horticultural Traits: Quantitative and Qualitative Analyses of Greenhouse-grown Plants**

Amir Zuker, Tzvi Tzfira, Gideon Scovel, Marianna Ovadis, Elena Shklarman, Hanan Itzhaki, and Alexander Vainstein¹

The Kennedy-Leigh Centre for Horticultural Research and The Otto Warburg Center for Biotechnology in Agriculture, The Hebrew University of Jerusalem, Rehovot 76-100, Israel

ADDITIONAL INDEX WORDS. genetic engineering, *rolC* gene, rooting, flower yield, *Dianthus caryophyllus*

ABSTRACT. Expression of the *rolC* gene under the constitutive CaMV 35S promoter led to several advantageous alterations in transgenic carnation (*Dianthus caryophyllus* L. 'White Sim'). The *rolC*-transgenic carnation plants exhibited increased axillary budbreak and development when grown under standard commercial greenhouse conditions. Carnation with *rolC* generated up to 48% more stem cuttings per mother plant than nontransformed plants. Stem cuttings from *rolC* plants exhibited better rooting ability, with up to five times higher root dry weight than controls. The improved rooting of *rolC*-transgenic stem cuttings was also apparent when the cuttings were treated with IBA. During the flowering season, *rolC*-transgenic plants produced up to three times more flowering stems than control plants. It should be noted that the latter alterations, namely increased flowering and rooting, are of major importance to the carnation industry. Chemical name used: indole-3-butyric acid (IBA).

As one of the major contributors to the cut-flower market and a commercial leader in terms of number of stems sold worldwide, carnation (*Dianthus caryophyllus*) is an important target for the breeding of new cultivars with novel characteristics (Jensen and Malter, 1995). Different carnation types have been developed as a result of hybridization (Holly and Baker, 1991) and they can be divided into two main groups, standard (midi and mignon carnation) and spray (micro and dianтини carnation); a minor group is formed by the pot carnations.

Carnation is propagated vegetatively and is affected detrimentally by inbreeding (Galbally and Galbally, 1997). Hence, controlled breeding is rather complicated and limited because selection of a desired trait in the siblings is performed on the genetic background of the two parents, and because of the high genetic variability among offspring. Furthermore, crosses within and between related species are limited by a rather small available gene pool for new traits (Holly and Baker, 1991).

Genetic engineering has already become an important and useful tool in basic plant research, as well as in the applied introduction of novel traits into many commercially important species (Potrykus et al., 1998). Genetic engineering of cut flowers, however, is rather limited, as their transformation is considered routine in only a few laboratories (Zuker et al., 1998).

New traits in cut flowers include not only yield improvement and resistance to insects or disease, they also consist of new

colors and novel plant morphology (Mol et al., 1995; Zuker et al., 1998). In fact, the latter are of great importance for the cut-flower market, where plant architecture and flower color are the main features determining consumer interest. Nevertheless, agronomic traits, such as yield, remain highly important for breeders and growers, as more vigorous and better performing plants can lower the time and cost required for growth and breeding.

Among the different genes affecting plant morphology, e.g., homeotic (Meyerowitz, 1997), *Agrobacterium* (Gaudin et al., 1994), phytochrome (Keller et al., 1989) and gibberellin (Coles et al., 1999) genes, the *rol* genes of *Agrobacterium rhizogenes* (Riker et al.) have been the most widely and successfully employed (Fladung et al., 1997; Nilsson et al., 1996; Schmulling et al., 1993; Scorza et al., 1994; van Altvorst et al., 1992). Although its precise mode of action is still unknown (Faiss et al., 1996; Nilsson and Olsson, 1997), *rolC* has attracted the most attention through its expression in transgenic plants, either under its own promoter or under the control of a cauliflower mosaic virus (CaMV) 35S promoter, leading to a series of morphological alterations. These include reduced apical dominance, altered leaf morphology, reduced seed production, reduced internode length, male sterility, small flowers and early flowering, bushy and compact phenotype, and even stem fasciation (Kurioka et al., 1992; Nilsson et al., 1993, 1996; Oono et al., 1993; Schmulling et al., 1988).

Nevertheless, studies on the *rolC* gene and its effects on plant development have been performed mostly in model herbaceous plants, e.g., tobacco (*Nicotiana tabacum* L.) (Schmulling et al., 1988), Irish potato (*Solanum tuberosum* L.) (Schmulling et al., 1993), and tomato (*Lycopersicon esculentum* Mill.) (van Altvorst et al., 1992), or tree fruits and timber species (Bell et al., 1999; Fladung et al., 1997; Nilsson et al., 1996). Studies on the use of the *rolC* gene in cut flowers are rather limited, the only report being in a woody ornamental, rose (*Rosa hybrida* L. 'Madame G. Delbard') (Souq et al., 1996). These transgenic rose plants, expressing the *rolC* gene under its native promoter, exhibited an array of phenotypic alterations, most of which were disadvantageous horticulturally. They included a dramatically reduced root

Received for publication 4 Jan. 2000. Accepted for publication 30 Aug. 2000. This research was supported by Grant IS-2610-95 from BARD, the United States-Israel Binational Agricultural Research and Development Fund, by the Ministry of Agriculture, and the Association of Israeli Flower Growers. We thank H. Voet and I. Zuker for assistance with statistical analysis and C. Vainstein for editing the manuscript. We wish to thank R. Shemi Ltd. (Shdema, Israel) for providing plant materials. The pUC18-CaMV35S-*rolC* plasmid was kindly provided by T. Schmulling, Tübingen, Germany; the pCGN1559 and pCGN7001 binary plasmids were kindly provided by Calgene Inc., Davis, Calif.; and *A. tumefaciens* strain AGLO was kindly provided by A. Ludwig, University of California, Santa Cruz. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

¹Corresponding author; e-mail vain@agri.huji.ac.il.

system, wrinkled leaves, high sensitivity to diseases, and desiccation of stems.

The purpose of this study was to determine the potential of the *rolC* gene for improving horticultural traits in carnation. The availability of an efficient and reproducible procedure for the genetic transformation of carnation plants (Zuker et al., 1999) enabled production of transgenic carnation carrying the *rolC* gene under the CaMV 35S promoter. These transgenic carnations exhibit enhanced yield of stem cuttings and flowering stems, as well as improved rooting ability. To the best of our knowledge, this is the first time that genetically engineered, improved agronomic traits have been generated and analyzed quantitatively in carnation plants.

Materials and Methods

GENE CONSTRUCT AND PLANT GENETIC TRANSFORMATION. A 1.46-kb *Xba*I fragment from plasmid pUC18-CaMV35S-*rolC* (Tzfira et al., 1997), carrying the *A. rhizogenes rolC* gene driven by a CaMV 35S RNA promoter, was inserted into the *Xba*I-digested pCGN1559 (McBride and Summerfelt, 1990) binary plasmid to create the binary plasmid pAM*rolC*. This plasmid contains the *nptII* selection gene (coding for neomycin phosphotransferase II (NPTII); Beck et al., 1982) also driven by a 35S promoter. The plasmids pAM*rolC* and pCGN7001 (Comai et al., 1990) were transferred to *A. tumefaciens* strain AGLO (Lazo et al., 1991) and used to transform 'White Sim' carnation as described previously (Zuker et al., 1999). The plasmid pCGN7001 is a control plasmid essentially identical to pCGN1559, but carrying the *uidA* gene coding for β -glucuronidase (GUS) driven by a mannopine synthetase (MAS) promoter and the *nptII* gene driven by a 35S promoter. Eighteen independent pAM*rolC* transformants were obtained, of which three randomly chosen *rolC*-transgenic lines (R-1, R-2, and R-3) were studied in more detail. The transgenic origin of the *uidA*-expressing control lines was confirmed previously. These plants were phenotypically and morphologically indistinguishable from nontransformed plants (Zuker et al., 1999).

DNA AND RNA BLOT ANALYSES. Total DNA and RNA were extracted from leaves of greenhouse-grown plants, as described previously (Zuker et al., 1999). DNA (10 mg) was digested with *Hind*III and electrophoresed through a 1% agarose gel using Tris-borate buffer (containing 1.3 M Tris, 0.7 M boric acid, and 24.5 mM EDTA, pH 8.4). *Hind*III is a unique restriction site within the T-DNA fragment, 5' to *rolC*. Gels were stained with ethidium bromide and photographed under ultraviolet light. Total RNA (10 mg) was electrophoresed through a 1.2% formaldehyde gel (Vishnevetsky et al., 1996). DNA and RNA were transferred to nylon membranes (Hybond N⁺; Amersham, Little Chalfont, United Kingdom) by capillary blotting as described by Maniatis et al. (1982). The 0.76-kb *Sal*I fragment from plasmid pUC18-CaMV35S-*rolC*, representing the 3' prime end of the *rolC* gene, served as a probe for that gene. Probe-labeling, prehybridization and hybridization of DNA and RNA blots were performed as described by Zuker et al. (1999) and by Vishnevetsky et al. (1996), respectively. The blots were visualized by exposure to Agfa Curix PR2 film (Agfa Co., Newark, Delaware) at -70°C for 5 to 20 h with an intensifying screen.

ACCLIMATIZATION OF TISSUE-CULTURED PLANTS AND ESTABLISHMENT OF PLANTS IN THE GREENHOUSE. About 3-cm tall, rooted plantlets were removed from the in vitro culture medium and their roots cleaned of agar. The plantlets were then transferred to

plastic planters filled with water-saturated no. 4 perlite (Agrical, Kibbutz Haborim, Israel) and kept for 2 weeks in a greenhouse (natural daylight and daylength) on a heated rooting table (20 °C) under intermittent mist (misting cycles of $\approx$ 3 min every 30 min, from 8 AM to 5 PM). The plantlets were then transferred to a no. 0.8 volcanic rock (Tof Marom Golan, Marom Golan, Israel) growing medium and grown for an additional 4 weeks in the same greenhouse. Hardened plants were then transferred to a regular greenhouse (natural light and temperature) and decapitated 4 weeks later to allow breaking and elongation of axillary shoots. These plants served as the source for standard stem cuttings (Zuker et al., 1995) used in all subsequent experiments.

rolC-transgenic and control plants were crossed with b.l. 12361 (pollen donor) to evaluate the effect of the *rolC* gene on the flower's female fertility. Open directed pollination and seed collection and germination were performed as described previously (Zuker et al., 1999).

ASSESSMENT OF DEVELOPMENTAL TRAITS IN THE GREENHOUSE. To evaluate the rooting performance of transgenic plants, stem cuttings were inserted, either directly (nontreated) or following treatment with a commercial rooting powder (Hormoril T-6, Asia Rize, Ramat Gan, Israel) containing 0.6% (w/w) IBA, in either no. 4 perlite or a rooting medium of 5 parts no. 4 perlite : 1 part no. 2 perlite : 2 parts no. 0.8 volcanic rock : 2 parts peat (v/v). The stem cuttings (50 cuttings per line) were maintained for 3 weeks on heated (20 °C) rooting tables. The cuttings which were inserted in perlite only were gently removed and washed of rooting medium. Root systems were dissected, dried at 65 °C for 48 h, and weighed. Cuttings with at least one root were counted as rooted cuttings in evaluating the percentage of cuttings developing roots out of total number of cuttings.

Stems cuttings treated with Hormoril and rooted in the peat medium were also transferred to no. 0.8 volcanic rock medium for growth under regular greenhouse conditions (natural light and temperature), to establish mother plants for further clonal propagation. Four weeks after transfer to the greenhouse, plants were decapitated to allow for axillary bud development. Stem cuttings with at least three internodes, originating from the breaking and elongation of the axillary buds, were then harvested from the mother plants.

For yield analyses (Table 1), stem cuttings were harvested from 40 mother plants (for each line), about every 4 weeks. Following five harvest cycles, mean number of stem cuttings per harvest per mother plant was determined. Side shoot analyses (Table 1) were performed with a random sample of 35 stem cuttings (per line), originating from the first harvest cycle of 40 mother plants. Type IV and V stem cuttings were dissected and side shoots, developed from axillary buds, were weighed and their developmental stage ranked according to the number of open leaves (Fig. 1). Plant height at flowering (10 plants per line) was measured from the soil line to the top of the apical flower bud on the flowering stem. The number of petals per flower bud, their length and dry weight (DW) were recorded from a random sample of 20 flowers (per line) at anthesis, during the flowering season (Nov. 1998 to Mar. 1999). The vase life of 25 flowers (per line) with 50-cm-long stems was measured in distilled water, at 20 °C, under a 12-h photoperiod provided by cool-white fluorescent lamps (60 μ mol·m⁻²·s⁻¹).

The data were subjected to analyses of variance using SPSS Base 10.0 (SPSS Inc., Chicago, Ill.). Means for *rolC*-transgenic and control lines were separated using Duncan's multiple range test, ($P \leq 0.05$).

Table 1. Yield and properties of stem cuttings of *rolC*-transgenic (R-1, R-2, and R-3) and control (C) carnation plants.

Line	Stem cutting yield ^z (no.)	Dry wt (mg) ^y for positions 4 and 5	Side shoots	
			Open pairs of leaves (no.) ^x	
			Position 4	Position 5
R-1	4.6 a ^w	47 a	1.6 a	2.0 a
R-2	4.2 ab	44 a	1.6 a	1.8 ab
R-3	4.0 b	36 b	1.1 a	1.4 b
C	3.1 c	29 c	0 b	0 c

^zStem cuttings were harvested from 40 mother plants a total of five times at 3- to 5-week intervals. Mean number of stem cuttings per harvest per mother plant is presented.

^yMean weight of side shoot at positions (nodes) 4 and 5, per stem cutting. A random sample of 35 stem cuttings was harvested from 40 mother plants.

^xMean number of open pairs of leaves per side shoot at positions 4 or 5.

^wMean separation within columns by Duncan's multiple range test ($P \leq 0.05$).

Results

CONFIRMING THE TRANSGENIC NATURE OF SELECTED LINES. Transgenic plants were generated following two regeneration and selection cycles under high kanamycin pressure as described previously (Zuker et al., 1999). All *rolC*- and control, *uidA*-transgenic plants exhibited high resistance to kanamycin, and the latter (Zuker et al., 1999) also exhibited strong GUS expression in all tissues tested. The *rolC*-transgenic plants (18 independent lines) were screened in vitro for phenotypic alterations, which included autonomous root growth in hormone-free media, axillary budbreak, increased growth rate, and altered internode length. Of 18 lines exhibiting altered morphology, three independent lines (R-1, R-2, and R-3) were hardened and transferred to the greenhouse for further study. Southern blot analysis of *Hind*III-digested DNA revealed integration of the *rolC* gene in all three selected lines (Fig. 2A). RNA blot analysis revealed expression of *rolC*, driven by the constitutive 35S promoter, in these three lines (Fig. 2B), thus confirming their transgenic nature.

YIELD AND MORPHOLOGY OF STEM CUTTINGS FROM *rolC*-TRANSGENIC VS. CONTROL PLANTS. About 50% of all stem cuttings from GUS-transgenic plants were type III (i.e., three internodes) (Fig. 1), and only about 6% were type V. In contrast, up to 97% of the *rolC*-transgenic cuttings were types IV and V (Fig. 3). The overall yield of cuttings per mother plant was up to 48% higher in *rolC*-transgenic plants than in control plants (Table 1).

Side shoots of *rolC*-transgenic stem cuttings exhibited up to 1.5-fold higher DWs than those originating from control plants (Table 1). Relative to control plants, the side shoots from *rolC*-transgenic plants were well developed (Table 1). In *rolC*-transgenic plants, the mean number of open leaf pairs on the side shoots at the 5th position (i.e., 5th node from apex) ranged from 1.4 to 2.0. In line R-1, all side shoots at the 5th position had two open pairs of leaves. Furthermore, side shoots at the 4th position in all *rolC*-transgenes averaged at least one pair of open leaves. In contrast, in control plants, none of the side shoots developing at the 4th or 5th positions had open leaves (Table 1).

ROOTING OF *rolC*-TRANSGENIC CARNATION PLANTS. When stem cuttings were not treated with IBA (Hormoril rooting powder), control (GUS-transgenic) cuttings yielded short and undeveloped adventitious roots, whereas *rolC*-transgenic cuttings developed a denser root system with greater DW than the control

cuttings (Figs. 4 and 5). Moreover, the percentage of cuttings developing roots of the total number of cuttings was about two times higher in R-1 and R-3 plants than in controls. In fact, nontreated stem cuttings of line R-1 exhibited a root system and DW similar to those of control plants treated with rooting powder

Fig. 1. Schematic illustration of a typical carnation stem cutting. Each cutting has three to five internodes (III to V). Each node carries two opposite leaves and two axillary buds which can develop into side shoots. The developmental stage of these side shoots was graded according to the number of open pairs of leaves on them (0, 1 or 2).

Fig. 2. Molecular analysis of three independent *rolC*-transgenic (R-1, R-2, and R-3) and control *uidA*-transgenic (C) carnation plants. (A) Southern blot analysis of *Hind*III-digested total DNA. (B) Northern blot analysis of total RNA. Both blots were hybridized with *rolC* probe.

Fig. 3. Distribution of stem-cutting types in *rolC*-transgenic (R-1, R-2, and R-3) and control (C) plants. Stem cuttings were classified as III, IV or V according to their number of internodes, as depicted in Fig. 1.

Fig. 4. Adventitious root dry weight and rooting percentages in stem cuttings of *rolC*-transgenic lines (R-1, R-2, and R-3) and control (C) carnation plants after 30 d on rooting tables, as described in Materials and Methods. Values represent means of 15 stem cuttings per line; vertical lines represent SE. Numbers above the bars represent the rooting percentages, i.e., percentage of cuttings developing roots out of total number of cuttings.

(Figs. 4 and 5). The enhanced rooting ability of *rolC*-transgenic plants vs. controls was also apparent following treatment of the cuttings with IBA. The *rolC*-transgenic plants developed up to twice the root DW of control cuttings. Moreover, while a period of approximately 3 weeks was essential for rooting of control carnation stem cuttings, a shorter period of only 2 weeks was required before *rolC*-transgenic carnation stem cuttings could be transferred for further development to standard greenhouse conditions.

PLANT GROWTH AND FLOWER DEVELOPMENT. To further assess the growth habits of *rolC*-transgenic and control (GUS-transgenic) plants, they were grown to flowering in the greenhouse. The *rolC*-transgenic clones developed a greater number of shoots

and a denser root system than control plants (Fig. 5). Furthermore, during the 5-month flowering season, the mean number of flowering stems per mother plant in R-1, R-2, and R-3 *rolC*-transgenic clones was significantly greater ($P \leq 0.05$) than in control plants: $(7.2 \pm 1.4, 8.1 \pm 1.7, 9.4 \pm 1.8$ and 3.2 ± 1.0 , respectively). The average number of flower buds on flowering stems was the same (4.5 ± 0.5 flowers/stem) in *rolC*-transgenic and control plants.

Height of the flowering stems of *rolC*-transgenic clones was not significantly different from that of control plants, being $85 \pm 5, 87 \pm 4$ and 95 ± 6 vs. 75 ± 5 cm in R-1, R-2, R-3 and control plants, respectively. Time to flowering was similar in *rolC*-transgenic clones and control plants, about 14 weeks after decapitation of mother plants. Vase life of flowers of control and *rolC*-transgenic clones was also the same (12 ± 2 d). An assessment of flower size during the flowering season revealed that R-1 and R-3 flowers are equal to controls, in all analyzed parameters, i.e., DW, petal number, and size. R-2 flowers (Fig. 5) were significantly smaller ($P \leq 0.05$): 410 ± 54 mg flower DW with 35 ± 3 petals (24 ± 3 mm in length) as compared to 696 ± 53 mg DW with 55 ± 2 petals (33 ± 3 mm in length) in control flowers. Crosses between R-1, R-2, R-3 and nontransformed male b.l. 12361 revealed that introduction of the *rolC* gene does not affect the flower's female fertility (data not presented). The effect of the *rolC* gene on male fertility could not be tested, because 'White Sim', the cultivar used in this study to generate transgenic clones, is male-sterile.

Discussion

To date, applicability of the *rolC* gene, alone or in combination with *rolA* and *rolB* genes, to cut-flower improvement has been tested only in roses (Souq et al., 1996; van der Salm et al., 1997). In that case, the *rolC* gene expressed under its native promoter led mainly to negative—from a horticultural point of view—alterations. In contrast, the use of the *rolABC* gene, similar to *rolB*, caused a significant improvement in the rooting of rose stem cuttings. To assess whether the *rolC* gene would exert a beneficial effect on carnation, 35S-*rolC*-transgenic plants were generated.

Although a slight reduction in apical dominance was observed in *rolC*-transgenic carnation plants, it did not result in a compact form or low growth rate as has been described previously for different 35S-*rolC*-transgenic plants (e.g., Bell et al., 1999; Fladung et al., 1997; Nilsson et al., 1996) and which would be considered undesirable in carnation. In fact, reduction in plant apical dominance resulted mainly in enhanced lateral shoot development, leading to a 1.48 times higher yield of stem cuttings per mother plant. Furthermore, the proportion of well-developed stem cuttings (types IV and V) of the total amount harvested was almost two times higher in *rolC*-transgenic plants than in control plants (Table 1, Fig. 3). Moreover, the yield of flowering stems per mother plant was approximately three times higher in *rolC*-transgenic plants (R-3) as compared to controls. A similar increase in the number of flower stalks has been observed in tobacco transgenic for *rolC* driven by its native promoter (Scorza et al., 1994).

Several studies have reported that introduction of *rol* genes, *rolC* in particular, leads to development of plants with wrinkled leaves and reduced flower size (Oono et al., 1993; Schmulling et al., 1988; Scorza et al., 1994; Souq et al., 1996). Wrinkled leaves were not observed in any of the 18 *rolC*-transgenic carnation lines generated in this study. With respect to flower size, line R-2 produced smaller flowers, while lines R-1 and R-3 showed true-

Fig. 5. Morphological alterations in *rolC*-transgenic carnation plants. (A) Rooted stem cuttings of control (left) and *rolC*-transgenic line R-1 (right). (B) Flowers of *rolC*-transgenic line R-2 (top) and control (bottom) plants. Bar = 12 mm. (C) Adventitious root formation in line R-1 (left) and control (right) plants. (D) Flowering stems of *rolC*-transgenic lines and control plants (from left to right: R-1, R-2, R-3, and control).

to-type flowers. In the remaining 15 *rolC*-transgenic lines, flower size was reduced in only one of them (data not presented).

The beneficial effect of the *rolC* gene in carnation was also reflected in the rooting capacity of stem cuttings. The root system of R-1 transgenic lines was actually quite similar to that of control plants treated with the commercial rooting powder Hormoril. The advantageous effect of *rolC* was apparent both with and without the addition of auxin, similar to the data reported on *rolABC*-transgenic rose (van der Salm et al., 1997). It should be noted that rooting of *rolB*-transgenic roses was negatively affected by auxin (van der Salm et al., 1997).

Based on the research reported to date, it is apparent that successful use of *rol* genes for plant molecular breeding depends on the plant system used. In carnation, the *rolC* gene improved rooting and increased the yield of stem cuttings and flowering stems. These are highly important traits to carnation propagators and growers. Improved rooting, for example, can reduce the labor

required to produce rooted stem cuttings. The importance of this trait is even more pronounced for commercial cultivars that exhibit very low rooting percentages despite application of auxin (Holly and Baker, 1991). With respect to the yield characteristics, their improvement will obviously lead to higher profitability for the carnation horticultural industry.

Literature Cited

- Beck, E., G. Ludwig, E.A. Auerswald, R. Reiss, and H. Schaller. 1982. Nucleotide sequence and exact location of the neomycin phosphotransferase gene from transposon Tn5. *Gene* 19:227–336.
- Bell, R.L., R. Scorza, S. Chinnathambi, and K. Webb. 1999. Transformation of 'Beurre Bosc' pear with the *rolC* gene. *J. Amer. Soc. Hort. Sci.* 124:570–574.
- Coles, J.P., A.L. Phillips, S.J. Croker, R. Garcia-Lepe, M.J. Lewis, and P. Hedden. 1999. Modification of gibberellin production and plant development in *Arabidopsis* by sense and antisense expression of gibberellin 20-oxidase genes. *Plant J.* 17:547–556.
- Comai, L., P. Moran, and D. Maslyar. 1990. Novel and useful properties of a chimeric plant promoter combining CaMV 35S and MAS elements. *Plant Mol. Biol.* 15:373–381.
- Faiss M., M. Strand, P. Redig, K. Dolezal, J. Hanus, H. Van Onckelen, and T. Schmulling. 1996. Chemically induced expression of the *rolC*-encoded β -glucosidase in transgenic tobacco plants and analysis of cytokinin metabolism: *rolC* does not hydrolyze endogenous cytokinin glucosides *in planta*. *Plant J.* 10:33–46.
- Fladung, M., K. Grossmann, and M.R. Ahuja. 1997. Alterations in hormonal and developmental characteristics in transgenic *Populus* conditioned by the *rolC* gene from *Agrobacterium rhizogenes*. *J. Plant Physiol.* 150:420–427.
- Galbally, J. and E. Galbally. 1997. Carnations and pinks. Timber Press, Portland, Ore.
- Gaudin, V., T. Vrain, and L. Jouanin. 1994. Bacterial genes modifying hormonal balances in plants. *Plant Physiol. Biochem.* 32:11–29.
- Holly, W.D. and R. Baker. 1991. Carnation production II. Kendall/Hunt Publishing Co., Iowa.
- Jensen, M.H. and A.J. Malter. 1995. Protected agriculture: A global review. World Bank Tech. Paper 253:144–146.
- Keller, J.M., J. Shanklin, R.D. Viestra, and H.P. Hershey. 1989. Expression of functional monocotyledonous phytochrome in transgenic tobacco. *EMBO J.* 8:1005–1012.
- Kurioka, Y., Y. Suzuki, H. Kamada, and H. Harada. 1992. Promotion of flowering and morphological alteration in *Atropa belladonna* transformed with CaMV 35S-*rolC* chimeric gene of the Ri plasmid. *Plant Cell Rpt.* 12:1–6.
- Lazo, G., P. Stein, and R. Ludwig. 1991. A DNA transformation-competent *Arabidopsis* genomic library in *Agrobacterium*. *Bio/Technology* 9:963–967.
- Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular cloning: A laboratory manual. Cold Spring Harbor Lab. Press, Cold Spring Harbor, N.Y.

- McBride, K.E. and K.R. Summerfelt. 1990. Improved binary vectors for *Agrobacterium*-mediated plant transformation. *Plant Mol. Biol.* 14:269–276.
- Meyerowitz, E.M. 1997. Genetic control of cell division patterns in developing plants. *Cell* 88:299–308.
- Mol, J.N.N., T.A. Holton, and R.E. Koes. 1995. Floriculture: Genetic engineering of commercial traits. *Trends Biotechnol.* 13:350–355.
- Nilsson, O., T. Moritz, N. Imbault, G. Sandberg, and O. Olsson. 1993. Hormonal characterization of transgenic tobacco plants expressing the *rolC* gene of *Agrobacterium rhizogenes* TL-DNA. *Plant Physiol.* 102:363–371.
- Nilsson, O., T. Moritz, B. Sundberg, G. Sandberg, and O. Olsson. 1996. Expression of the *Agrobacterium rhizogenes rolC* gene in a deciduous forest tree alters growth and development and leads to stem fasciation. *Plant Physiol.* 112:493–502.
- Nilsson, O. and O. Olsson. 1997. Getting to the root: The role of the *Agrobacterium rhizogenes rol* genes in the formation of hairy roots. *Physiol. Plant.* 100:463–473.
- Oono, Y., T. Suzuki, S. Toki, and H. Uchimiya. 1993. Effect of the overexpression of the *rolC* gene on leaf development in transgenic periclinal chimeric plants. *Plant Cell Physiol.* 34:745–752.
- Potrykus, I., R. Bilang, J. Futterer, C. Sautter, M. Schrott, and G. Spangenberg. 1998. Genetic engineering of crop plants, p. 119–159. In: A. Altman (ed.). *Agricultural biotechnology*. Marcel Dekker, New York.
- Schmulling, T., M. Fladung, K. Grossmann, and J. Schell. 1993. Hormonal content and sensitivity of transgenic tobacco and potato plants expressing single *rol* genes of *Agrobacterium rhizogenes* T-DNA. *Plant J.* 3:371–382.
- Schmulling, T., J. Schell, and A. Spena. 1988. Single genes from *Agrobacterium rhizogenes* influence plant development. *EMBO J.* 7:2621–2629.
- Scorza, R., T.W. Zimmerman, J.M. Cordts, K.J. Footen, and M. Ravelonandro. 1994. Horticultural characteristics of transgenic tobacco expressing *rolC* gene from *Agrobacterium rhizogenes*. *J. Amer. Soc. Hort. Sci.* 119:1091–1098.
- Souq, F., P. Coutos-Thevenot, H. Yean, G. Delbard, Y. Maziere, J.P. Barbe, and M. Boulay. 1996. Genetic transformation of roses, 2 examples: One on morphogenesis, the other on anthocyanin biosynthetic pathway. In: A. Morisot and P. Ricci (eds.). *Second Intl. Symp. on Roses*. Acta Hort. 424:381–388.
- Tzfira, T., C.S. Jensen, W. Wang, A. Zuker, B. Vinocur, A. Altman, and A. Vainstein. 1997. Transgenic *Populus tremula*: A step-by-step protocol for its *Agrobacterium*-mediated transformation. *Plant Mol. Biol. Rpt.* 15:219–235.
- van Altvorst, A.C., R.J. Bino, A.J. van Dijk, A.M.J. Lamers, W.H. Lindhout, F. van der Mark, and H.J.M. Dons. 1992. Effect of introduction of *Agrobacterium rhizogenes rol* genes on tomato plant and flower development. *Plant Science* 83:77–85.
- van der Salm, T.P.M., T.C.C. Hanisch, and H.J.M. Dons. 1996. Prospects for application of *rol* genes for crop improvement. *Plant Mol. Biol. Rpt.* 14:207–228.
- van der Salm, T.P.M., C.J.G. van der Toorn, R. Bouwer, T.C.C. Hanisch, and H.J.M. Dons. 1997. Production of *rol*-gene transformed plants of *Rosa hybrida* L. and characterization of their rooting ability. *Mol. Breeding* 3:39–47.
- Vishnevetsky, M., M. Ovadis, H. Izhaki, M. Levy, Y. Libal-Weksler, Z. Adam, and A. Vainstein. 1996. Molecular cloning of a carotenoid-associated protein from *Cucumis sativus* corollas: Homologous genes involved in carotenoid sequestration in chromoplasts. *Plant J.* 10:1111–1118.
- Zuker, A., A. Aharoni, T. Tzfira, H. Ben-Meir, and A. Vainstein. 1999. Wounding by bombardment yields highly efficient *Agrobacterium*-mediated transformation of carnation (*Dianthus caryophyllus* L.). *Mol. Breeding* 5:367–375.
- Zuker, A., P.-F.L. Chang, A. Ahroni, K. Cheah, R.W. Woodson, R.A. Bressan, A.A. Watad, P.M. Hasegawa, and A. Vainstein. 1995. Transformation of carnation by microprojectile bombardment. *Sci. Hort.* 64:177–185.
- Zuker, A., T. Tzfira, and A. Vainstein. 1998. Genetic engineering for cut-flower improvement. *Biotechnol. Adv.* 16:33–79.