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Original Article

Inhibition of cell proliferation, cell expansion and differentiation by the *Arabidopsis SUPERMAN* gene in transgenic tobacco plants

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Abstract. Plant development depends upon the control of growth, organization and differentiation of cells derived from shoot and root meristems. Among the genes involved in flower organ determination, the *cadaster* gene *SUPERMAN* controls the boundary between whorls 3 and 4 and the growth of the adaxial outer ovule integument by down-regulating cell divisions. To determine the precise function of this gene we overexpressed ectopically the *Arabidopsis thaliana* (L.) Heynh. *SUPERMAN* gene in tobacco (*Nicotiana tabacum* L.). The transgenic plants exhibited a dwarf phenotype. Histologically and cytologically detailed analyses showed that dwarfism is correlated with a reduction in cell number, which is in agreement with the *SUPERMAN* function in *Arabidopsis*. Furthermore, a reduction in cell expansion and an impairment of cell differentiation were observed in tobacco organs. These traits were observed in differentiated vegetative and floral organs but not in meristem structures. A potential effect of the *SUPERMAN* transcription factor in the control of gibberellin biosynthesis is discussed.

Keywords. Cell proliferation (expansion and differentiation) - Development - Gibberellin - *Nicotiana* - *SUPERMAN*

Abbreviations. GA: gibberellin GA₃: gibberellic acid RT-PCR: reverse transcription-polymerase chain reaction SAM: shoot apical meristem *SUP*: *SUPERMAN*

Introduction

Organogenesis in flowering plants results from the patterned control of the number, place and plane of cell divisions, coupled with a regulated and coordinated cellular expansion (for review, see Meyerowitz 1997). The characterization of mutants impaired in plant growth showed that cell number, size and shape of organ primordia are genetically determined. Cell division is the main activity in the shoot apical meristem (SAM) and root meristem. For example, *WUSCHEL* (*WUS*) is involved in the formation of the initial meristem in embryos as well as in the maintenance of cell divisions during flower development (Laux et al. 1996). Among the set of genes negatively regulating cell divisions, genes such as *CLAVATA* (*CLV1*, *CLV3*) control the number of cells in the SAM (Leyser and Furrer 1992; Clark et al. 1993, 1995; Alvarez and Smyth 1994). Cell expansion and differentiation occur afterwards, thus contributing to tissue and organ formation. Cell expansion depends upon cell elongation along the longitudinal axis and cell enlargement along the transverse axis. Phytohormones such as auxins, gibberellic acid (GA₃) and cytokinins are known to be involved in the control of this process. Mutants affecting gibberellin (GA) biosynthesis (Hooley 1994; Sun and Kamiya 1994; Yamaguchi et al. 1996; Heliwell et al. 1998), GA perception (Koomneef et al. 1985) or signal transduction (Ross 1994; Swain and Olszewski 1996) have a detrimental effect on the cell elongation process. Although some mutants and genes have already been identified, such as *FRUITFULL* (Gu et al. 1998), *PASTICCINO* (Faure et al. 1998), and more recently *SCHIZOID* (Parsons et al. 2000), the understanding of the cell expansion and differentiation process requires more investigations. In a recent report, Kater et al. (2000) have shown that the *Arabidopsis SUPERMAN* gene (*SUP*) represents a new candidate involved in the control of cell expansion.

In *Arabidopsis* flowers, *SUP* controls the boundary between whorls 3 and 4 and the growth of the adaxial outer integument (Bowman et al. 1992; Sakai et al. 1995) through regulation of cell division. *SUP* encodes a transcription factor that possesses a zinc-finger motif (Sakai et al. 1995). However, the analysis of the *SUP* potential to regulate cell proliferation is difficult since the organs where the gene is normally expressed are very small and hardly accessible. One useful means to investigate a gene function relies on the analysis of the dominant gain-of-function resulting from overexpression of the gene (Sablowski and Meyerowitz 1998; Mizukami and Fisher 2000). Thus, we aimed to overexpress constitutively the *Arabidopsis SUP* gene in tobacco and analyze the transgene effects in organs where *SUP* is not naturally expressed. Transgenic plants displayed a dwarf phenotype, which is due to a reduction in the cell number and an impairment of cell expansion. Cell length reduction is possibly caused by a GA-biosynthesis default. Furthermore, plant organs were impaired in the process of cell differentiation.

Materials and methods

Construction of the binary vector used for tobacco transformation

The clone pHS-supL1 encoding the *Arabidopsis thaliana* (L.) Heynh. *SUP* gene was introduced into the *Bam* HI site of pDH51 (Pietrzack et al. 1986) to produce p35S::*SUP* and then into the *Eco* RI site of the binary vector pPZP212 (Hajdukiewicz et al. 1994), giving rise to the plasmid pSUP. This plasmid without the *SUP* insert was called pH51 and used as a control. pSUP and pH51 were introduced by electroporation into *Agrobacterium tumefaciens* strain GV 3101. Transformation of *Nicotiana tabacum* L. SR1 cv. Petit Havana (Maliga et al. 1973) leaf discs was performed as described by Horsch et al. (1986).

Screening of transgenic plants

Transgenic plants were selected on hormone-free MS medium (Murashige and Skoog 1962) containing 200 mg l⁻¹ of kanamycin. The presence of the transgene expression was detected by reverse transcription-polymerase chain reaction (RT-PCR). Total RNA from in vitro plantlets was obtained according to Hernould et al. (1993). Five micrograms of RNA was used for RT-PCR reactions with the specific primers termVI (5'-TATGCTCAACACATGAGCG-3') and SUP5⁺ (5'-GATTATGATAATGCCAACAGG-3').

Morphometric analyses

Transgenic plants were transferred into the greenhouse at 25 °C under a photoperiod of 16 h light/8 h darkness. The epidermes from the 10th internode, and from the corolla tube were dissected and analyzed under a microscope. Cell width and length were measured on full-grown T₀ and T₁ plants. An average of 20 cells for each organ was analyzed. The determination of cell number per unit organ length was calculated by dividing the absolute length of the organ by the cell size. Data were compared by using the Student's *t*-test.

Hormone treatment

To test the hypocotyl elongation response, seeds were germinated on MS medium without hormone or supplemented with 0.1, 0.5 or 1 mg l⁻¹ of GA₃ (Sigma). Hypocotyl cells were measured as above, after 13 days of treatment.

Estimation of GA 20-oxidase transcript levels by semi-quantitative RT-PCR

In order to determine the relative transcript level for GA 20-oxidase from *N. tabacum* (GenBank #AB012856), semi-quantitative RT-PCR assays were performed as described by Joubès et al. (1999). Three micrograms of total RNA, extracted from mature leaves, was used in the RT reaction, and then a 10-fold dilution of the generated cDNAs was used in the PCR reaction with the specific primers NtGA20ox5⁺ (5'-CGCGGCCCAACAAGCATC-3') and NtGA20ox3⁺ (5'-GCAAGTGATTCTCTAGGAG-3'). The cDNA amplification products were electrophoresed, blotted and hybridized with the corresponding ³²P-specific labelled probes.

Histological analysis

To analyze plant tissue organization, samples were taken at the level of the 3rd midvein branching for leaves and of the 10th internode for stems. The material was cleared with sodium hypochlorite (5%) in order to eliminate the cell protoplasm, fixed for 4 h in paraformaldehyde-glutaraldehyde (4% and 0.25% (w/v), respectively) dissolved in a 100 mM phosphate buffer, and then treated as described by Gabe (1968). Sections (12 μm thick) were stained in carmine-green iodide (4 and 0.2% (w/v), respectively) and counterstained with toluidine blue (0.1%, w/v). Vegetative and floral meristems were directly fixed and treated as for leaves and stems. Longitudinal sections were 8 μm thick.

Results

Ectopic expression of *SUPERMAN* in *N. tabacum* leads to a dwarf phenotype

The gain-of-function strategy using the ectopic expression of transcription factors has been already used (Fridborg et al. 1999; Fukazawa et al. 2000; Garcia-Maroto et al. 2000; Hewelt et al. 2000; Nandi et al. 2000). Here, we used the same strategy to gain more insight into SUP function.

Ten plants transformed with pSUP and growing on kanamycin selective medium were obtained; they displayed an altered phenotype as early as the in vitro regeneration stage (compare Fig. 1A and B). Leaves were greenish, crinkled and swollen; roots were swollen, more numerous and branched compared to wild-type plants. Among these plants, two were exceptional in that they flowered, in vitro, as early as the formation of the first leaves (Fig. 1C), similar to *Arabidopsis emf* mutants (Sung et al. 1992). These plants died soon after their in vitro blooming, and consequently we were not able to investigate them in more detail.

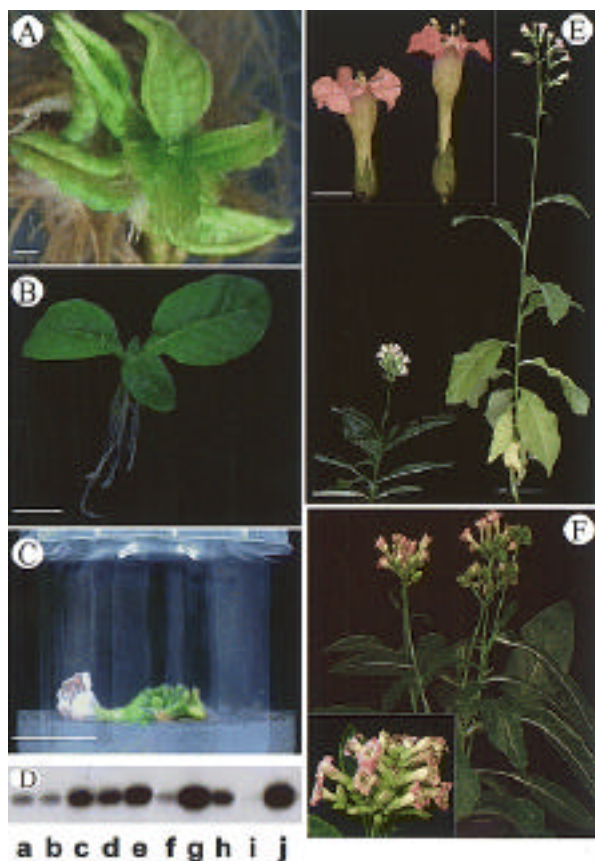


Fig. 1A-F. Phenotypic and molecular characterization of tobacco (*Nicotiana tabacum*) plants overexpressing the *Arabidopsis thaliana* *SUPERMAN* gene. **A** In-vitro-grown 35S::*SUP* plant. **B** In-vitro-grown wild-type tobacco seedling. **C** In-vitro premature blooming of a 35S::*SUP* plant. **D** Detection of 35S::*SUP* transcripts by RT-PCR. Amplification products were detected by DNA gel blot hybridization. Lanes a-h Transformants 35S::*SUP* 1, 2, 3, 4, 5, 6, 7 and 8, respectively; lane i wild-type tobacco (negative control); lane j pSUP plasmid (positive control). **E** Morphology of wild-type (right) and 35S::*SUP* 3 (left) plants. **Insert** For comparison, a higher-magnification image of wild-type (right) and transgenic (left) flowers is shown. **F** 35S::*SUP* 4 bushy inflorescence. Lack of apical dominance in T₁ progeny is expressed by the simultaneous development of several inflorescences (magnification $\times 2$ that in **E**). **Insert** Higher magnification (same as insert in **E**) of a transgenic inflorescence. Bars = 2 cm (**A-C**), 10 cm (**E**), 1 cm (**E**, insert)

The plants less affected in their phenotype were checked for transgene expression (Fig. 1D) and transferred into the greenhouse. All the plants were similar and displayed a dwarf phenotype. The data relating to organ size and number were comparable among the different plants (Table 1). At the flowering time, transgenic plants were twice as small as wild-type plants but internode number was comparable to that of wild-type plants (Fig. 1E, Table 1). Leaves were swollen with curled fringes and a fleshy aspect, dark-green and smaller than those of wild-type plants (Fig. 1E). Interestingly, very small leaves alternated regularly with larger leaves (Fig. 1E). However, and despite the important decrease in the surface, the length/width ratio was similar to that of wild-type leaves (Table 1). Control plants transformed with pH51, were similar to wild-type plants.

Table 1. Morphometric analyses of full-grown wild-type and transgenic *Nicotiana tabacum* plants

	Wild type	35S:: <i>SUP</i> ^c
Plant size (cm)	100.0±9.8 ^a	43.36±2.75 ^d
Internode number	16.0±0.0 ^a	14.7±0.5
Size of the 10th internode (cm)	6.25±0.32 ^a	2.93±0.27 ^d
Leaf length/width ratio	2.05±0.20 ^a	2.37±0.27
Flower number	32.0±3.0 ^a	40.0±0.8 ^d
Sepal size (cm) ^b	1.60±0.17	1.15±0.05 ^d
Petal size (cm) ^b	4.50±0.18	3.34±0.43 ^d
Stamen size (cm) ^b	4.20±0.25	2.95±0.85 ^d
Pistil size (cm) ^b	3.90±0.37	2.61±0.55 ^d

^aMean ± SD calculated from three plants

^bMean ± SD calculated from 10 flowers

^cMean ± SD calculated from eight T₀ plants

^dSignificantly different compared to wild type ($P < 0.05$)

Transgenic plants flowered 5-6 weeks after transplanting in the greenhouse. The inflorescences of T_0 plants (Fig. 1E) produced 20% more flowers than wild-type plants (Table 1). However, flowers were stacked due to a reduced size of the peduncle and significantly smaller than in wild-type plants (Fig. 1E, inset; Table 1). All flower organs were smaller (Table 1) but the global architecture was comparable to that of wild-type flowers. These modifications are similar to those observed in transgenic *Petunia* overexpressing *SUP* in petals and stamens (Kater et al. 2000). The functionality of reproductive organs was analyzed on three plants named 35S::*SUP 1*, 3 and 4. They produced functional pollen but their germination ability, ranging from 30 to 45%, was lower than in wild-type plants (70%). After self-pollination they produced seeds. Seedlings derived from 35S::*SUP 1*, 3 and 4 T_0 plants, segregated kanamycin resistance versus sensitivity as a mendelian trait. The abnormal morphology of T_0 plants was transmitted as a dominant trait to kanamycin-resistant T_1 plants while the kanamycin-sensitive T_1 plants recovered the wild-type phenotype. Furthermore, a loss of apical dominance and an increase in flower number (approx. 200 flowers/inflorescence) were observed in T_1 plants (Fig. 1F).

SUPERMAN controls cell number and size

According to the function of *SUP* in regulating cell proliferation (Sakai et al. 1995), a reduction in cell number was expected in transgenic plants. Thus, we counted and measured the epidermal cells from internodes and corolla tubes of full-grown plants.

Internodes of T_0 plants showed a significant epidermal cell-length reduction (15-45%) compared to wild-type plants (Table 2). This reduction was maintained or even increased in T_1 progenies. Cell width varied in the sense of a decrease in T_0 plants, but generally increased in T_1 progenies. The cell number per unit organ length showed a significant decrease (25-40%) in T_0 plants. This decrease in cell number was even worse in T_1 plants, confirming *SUP* function in cell proliferation. However, the global cell-length decrease and cell-width increase suggests that *SUP* is involved in the cell-expansion process.

Table 2. Analysis of cell length, width and number in the 10th internode and corolla of wild-type and 35S::*SUP tobacco* plants. For each T_1 progeny, 2 plants, taken at random from among the 15 plants grown in the greenhouse, were analyzed

Plants	Internode				Corolla			
	Organ length ^c (cm)	Cell length ^b (μ m)	Cell width ^b (μ m)	Cell number/organ length	Organ length ^c (cm)	Cell length ^d (μ m)	Cell width ^d (μ m)	Cell number/organ length
Wild type	6.2 \pm 0.32	251.5 \pm 41.5	43.7 \pm 8.0	254.9 \pm 43.5	4.4 \pm 0.1	166.2 \pm 29.0	13.0 \pm 2.4	272.9 \pm 47.9
35S:: <i>SUP 1</i>								
T_0	3.1 ^a	215.8 \pm 47.8	30.7 \pm 3.8 ^a	151.5 \pm 40.4 ^a	3.6 \pm 0.2 ^a	136.9 \pm 24.1 ^a	17.8 \pm 2.8 ^a	271.4 \pm 54.5
$T_1 1$	1.28 ^a	206.2 \pm 29.8 ^a	24.4 \pm 3.2 ^a	63.0 \pm 7.0 ^a	3.6 \pm 0.3 ^a	148.3 \pm 28.7	22.8 \pm 2.9 ^a	249.8 \pm 41.6
$T_1 10$	1.95 ^a	161.9 \pm 24.2 ^a	51.7 \pm 9.2	123.3 \pm 22.1 ^a	3.4 \pm 0.3 ^a	153.3 \pm 19.1	18.3 \pm 3.3 ^a	224.7 \pm 26.7 ^a
35S:: <i>SUP 3</i>								
T_0	2.9 ^a	175.0 \pm 24.1 ^a	49.2 \pm 7.6	169.7 \pm 23.0 ^a	3.7 \pm 0.2 ^a	141.8 \pm 19.5 ^a	14.6 \pm 1.9	214.4 \pm 29.4 ^a
$T_1 3$	3.0 ^a	181.8 \pm 46.2 ^a	63.6 \pm 9.9 ^a	179.6 \pm 55.8 ^a	3.1 \pm 0.1 ^a	163.5 \pm 28.0	19.7 \pm 4.0 ^a	194.5 \pm 32.2 ^a
$T_1 7$	1.0 ^a	100.6 \pm 16.0 ^a	51.9 \pm 7.4 ^a	97.8 \pm 16.4 ^a	3.1 \pm 0.2 ^a	81.7 \pm 11.0 ^a	24.7 \pm 4.4 ^a	386.0 \pm 52.6 ^a
35S:: <i>SUP 4</i>								
T_0	2.8 ^a	153.7 \pm 32.5 ^a	33.3 \pm 7.0 ^a	192.5 \pm 41.8 ^a	2.7 \pm 0.4 ^a	115.9 \pm 22.5 ^a	18.1 \pm 4.1 ^a	244.3 \pm 51.3
$T_1 2$	1.6 ^a	181.7 \pm 38.4 ^a	49.6 \pm 5.3	89.4 \pm 16.1 ^a	2.0 \pm 0.2 ^a	125.0 \pm 25.9 ^a	19.4 \pm 3.5 ^a	166.7 \pm 36.4 ^a
$T_1 12$	2.1 ^a	169.3 \pm 38.1 ^a	59.6 \pm 7.2 ^a	128.7 \pm 26.5 ^a	3.0 \pm 0.2 ^a	167.5 \pm 37.4	15.6 \pm 2.5 ^a	186.2 \pm 36.1 ^a

^aSignificantly different compared to wild type ($P < 0.05$)

^bAverage calculated from 20 measurements on the 10th internode

^cAverage calculated from 10 flowers

^dAverage calculated from 20 measurements per flower, over 3 flowers per plant

A similar analysis was performed for corolla tubes (Table 2). In T_0 plants, the epidermal cell length was significantly shorter than in the wild type, while cell width increased. In T_1 plants, cell lengths and cell widths were generally comparable to those of T_0 plants. The decrease in cell length and increase in cell width makes the cells dumpy, resulting in stunted flowers. A reduction in cell number per unit organ length was general in T_1 progeny of each transgenic plant. Investigations performed on stamen filaments as well as on the style of the pistil (the most elongated part of each organ) confirmed the reduction in both cell size and number (data not shown). Thus, the effect of *SUP* on cell proliferation and expansion already observed for the stem was confirmed in the corolla. Semi-quantitative RT-PCR was performed to estimate the expression of *SUP* in T_0 and T_1 plants. A strong correlation between severity of dwarfism (variation of cell length and cell width) and *SUP* expression was not observed (data not shown). We were therefore only able to correlate the dwarf phenotype with the ectopic expression of *SUP*.

In order to check whether the discrepancy in cell number between transgenic and wild-type plants was due to differences in meristem structure, sections of SAM were analyzed. Using a gauge of $10^3 \mu\text{m}^2$, cell number and size (assimilated to a square) were evaluated in the peripheral zone of meristems. Cell counting gave 16.2 ± 1.6 and 14.9 ± 1.5 (mean \pm SD) cells/gauge unit for transgenic and wild-type plants, respectively. Cell diameter was $8 \mu\text{m}$ in cross-sections in both cases. In floral meristems, cell number and size were similar in both plant types. In situ-hybridization performed on vegetative and reproductive meristems of the transgenic plants showed that the transgene was expressed throughout the meristems (data not shown). The effect of *SUP* expression, however, was visible only at later stages when these organs had elongated by cell expansion rather than cell division. Thus, we could not correlate the cell number and size discrepancy observed in flowers and internodes between the transgenic and wild-type plants with any structural differences in the meristems.

The cell elongation default is restored by GA_3 and correlates with the inhibition of *GA 20-oxidase* gene expression

Dwarf transgenic tobacco plants showed similarity to *Arabidopsis* *GA*-deficient mutants (Hooley 1994; Sun and Kamiya 1994; Yamaguchi et al. 1996; Helliwell et al. 1998; Fridborg et al. 1999). In these mutants, reduced stem cell elongation is responsible for the dwarf phenotype. We hypothesized that the dwarfism of tobacco plants could be related to *GA* deficiency. As hypocotyl growth is mainly due to cell elongation (Gendreau et al. 1997), seedlings of transgenic and wild-type plants were subjected to hormone treatment and hypocotyl cell length was measured after 13 days (Fig. 2A). In untreated material, cells were significantly shorter in transgenic seedlings than in wild-type seedlings. After GA_3 treatment a 4-fold increase in the cell size of 35S::*SUP 3* and 4 seedlings was observed.

This increase was only 1.5-fold in wild-type plants. The optimal enlargement was obtained with $0.5 \text{ mg l}^{-1} GA_3$. At this concentration, cells of transgenic seedlings recovered the average wild-type size. It is noteworthy that epidermal cell width and number were similar in treated and untreated material. Furthermore, seedling leaves retained the symptoms described for T_0 plants.

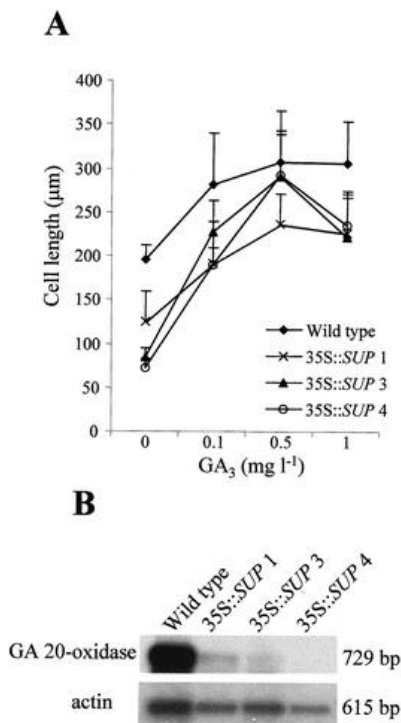


Fig. 2. A Effect of GA₃ on hypocotyl cell elongation of 13-day-old seedlings from transgenic and wild-type tobacco plants. For simplicity, SD bars are shown in only one direction. **B** Quantification of *GA 20-oxidase* gene expression by semi-quantitative RT-PCR in transgenic and wild-type plants. Actin was used as a control

These results indicate that the reduction in size of hypocotyl cells may be due to a GA deficiency. As a consequence, size reduction of stem cells of T₀ and T₁ plants may be attributable to a failure in GA biosynthesis.

To verify whether the size reduction is correlated with a failure in GA biosynthesis, the relative transcript level corresponding to the *GA 20-oxidase* gene was analyzed by semi-quantitative RT-PCR. Primers designed from the coding sequence of *GA 20-oxidase* were used. As an internal control, a cDNA for actin was also amplified. In mature leaves of transgenic plants only a very faint signal was observed compared to wild-type plants (Fig. 2B). The hybridization signal with the *actin* cDNA was comparable among the different plants. Thus it can be deduced that the transcript level for *GA 20-oxidase* was impaired in transgenic plants. Furthermore, the possible involvement of brassinosteroids in the dwarf phenotype was checked. The effect of 2,4 epibrassinolide (1–3 µM) on seedling growth was similar in wild-type and transgenic plants (data not shown). Thus, a default in brassinosteroid biosynthesis can not account for dwarfism.

Overexpression of *SUPERMAN* impairs tissue differentiation

With regard to the modifications of cell size and number in vegetative organs of transgenic plants, we analyzed leaves and stem sections to determine how cell shape and position could be affected by constitutive expression of *SUP*.

Wild-type leaves showed a classical organization. The midvein (Fig. 3A) consisted of a crescent shape vascular bundle surrounded by the bundle sheath (Fig. 3C) and the parenchyma tissue in which cells are organized according to an increasing gradient of size from the epidermis to the center. A single layer of palisade parenchyma cells and four layers of spongy cells classically constituted the leaf mesophyll delimited by the lower and upper epidermis (Fig. 3E). In transgenic plants, the global organization of leaf tissues was preserved. However, in the midvein (Fig. 3B), the vascular bundle no longer possessed the characteristic organization and delimitation observed in wild-type leaves. The vascular bundle sheath was indistinguishable from the surrounding parenchyma cells (Fig. 3D). Sections stained with green iodide, specific for lignin, showed that xylem vessels were disorganized and less abundant than those of wild-type plants. These defects were indicative of a failure in the cell differentiation process and to a lesser extent in the organization of vascular bundles. Furthermore, the perivascular parenchyma did not show the characteristic gradient of differentiation from the epidermis to the center of the leaf. Cells generally had an increased diameter and the number of cell layers was reduced to 5–6 rows while there were 9–10 rows in wild-type plants. Concerning the mesophyll (Fig. 3F), the lower and upper epidermes as well as the parenchyma consisted of highly vacuolated cells, and the distinction between the spongy and palisade parenchyma cells was not clear. Here, also, a defect in organization and especially in differentiation of leaf tissues could be observed.

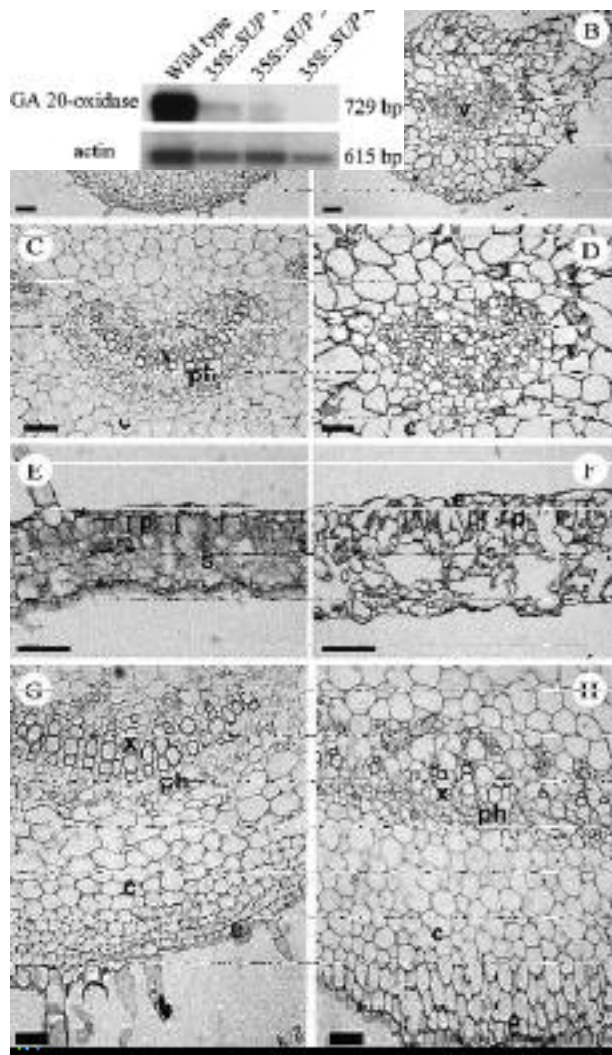


Fig. 3A-H. Anatomy of leaves and stems of full-grown transgenic and wild-type tobacco plants. **A, C, E, G** Wild-type plant. **B, D, F, H** Transgenic plant. **A, B** Transverse section of a leaf midvein. **C, D** enlargement of parts of **A** and **B**, respectively. **E, F** Transverse section of a leaf-blade. **G, H** Transverse section of the stem of fully grown plants. *c* Cortex, *e* epidermis, *p* palisade parenchyma, *ph* phloem, *pp* pith parenchyma, *s* spongy parenchyma, *v* vascular bundle, *x* xylem. Bars = 100 μ m

Transverse sections of wild-type tobacco stems (Fig. 3G) consisted of a single cell layer forming the epidermis, the cortical parenchyma made up of an average of 12 cell layers with size increasing gradually toward the central cylinder, the vascular system containing cellular rays of phloem and xylem, and finally the pith parenchyma. In transgenic plants, tissue organization appeared to be globally preserved (Fig. 3H). However, the cortical parenchyma contained only eight to nine cell layers and the vascular system showed defects in organization and lignification.

In summary, leaves and stem tissues of the transgenic tobacco showed a significant reduction in cell number, and a defect in differentiation of palisade and spongy parenchyma cells as well as vessels (xylem mainly). However, it should be stressed that the general organization, i.e. the positions of the respective tissues, was not modified.

Discussion

The putative function of *SUP* has been deduced from the mutant phenotype but it was not supported by a direct analysis at the cellular level. In order to clarify the function of the transcription factor *SUP*, we aimed to overexpress the *Arabidopsis SUP* gene in a heterologous plant, tobacco, and characterize phenotypically and cytologically the resulting transgenic plants. Our results provide evidence that cell size, cell number and cell differentiation are disturbed by the ectopic *SUP* expression. Furthermore, these modifications correlate with a decrease in *GA 20-oxidase* gene expression and probably with a failure in GA biosynthesis.

Constitutive expression of *SUPERMAN* affects severely vegetative and floral organ growth of tobacco plants

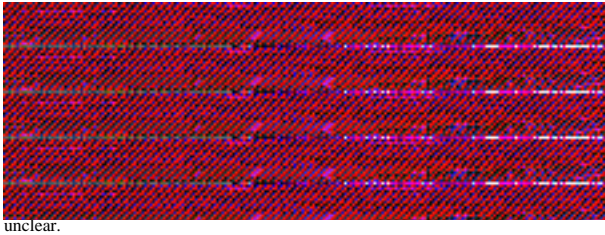
The transgenic lines obtained are easily distinguishable from wild-type plants, as they show a dwarf phenotype with severe modifications of vegetative organs. Flowers are also modified, but floral organ number and fertility in transgenic lines, were preserved. These data contrast with those obtained for rice (Nandi et al. 2000).

Interestingly, the phenotypic traits of 35S::*SUP* plants resemble those of *Arabidopsis shi* mutants (Fridborg et al. 1999). Indeed *shi* mutants are GA defective-like mutants, displaying short internodes, small leaves, swollen roots, reduced apical dominance, a delay in flowering period and an increased flower number. However, the application of GA does not correct the dwarfism of *shi* plants, indicating that they are defective in the perception or in the response to GA. In contrast, seedlings of dwarf 35S::*SUP* plants recover the wild-type growth habit when treated with GA as do 35S::*RSGbZIP*-transformed tobacco plants (Fukazawa et al. 2000). This suggests a defect in GA production as elongation of hypocotyls and cells is restored after the treatment in both types of transgenic plant. According to the results of the semi-quantitative RT-PCR, the overexpression of *SUP* induces a decrease in *GA 20-oxidase* transcripts. *GA 20-oxidase* is one of the enzymes involved in GA₁ biosynthesis and is known to be regulated by active GA content (Rebers et al. 1999). Similar results have been obtained in tobacco plants overexpressing the rice *OSH1* and the tobacco *NTH15* transcription factors (Kusaba et al. 1998; Tanaka-Ueguchi et al. 1998). In these reports it is demonstrated that the inhibition of *GA 20-oxidase* gene expression leads to a drastic reduction in GA₁ content. Further work is needed to elucidate the relationship between *SUP* function and the mechanism controlling the expression of *GA 20-oxidase*.

SUPERMAN prevents cell proliferation

In plants, organ cell number is determined primarily by cell proliferation (Mizukami and Fischer 2000). According to *SUP* function, it was expected that its constitutive expression affects cell proliferation in transgenic plants. Effectively, 35S::*SUP* vegetative organs in T₀ and T₁ plants contain fewer cells than wild-type plants. In flowers the situation is not as clear as in internodes, though T₁ flowers show a significant reduction in cell number. On the whole, the data confirm the function of *SUP* in the control of cell proliferation (Sakai et al. 1995) but contrast with those reported for transgenic petunia overexpressing *SUP* driven by an organ-specific promoter, since no any reduction in cell number was reported (Kater et al. 2000). The strength of the promoter used, the 35S promoter in our experiments and the *FBP1* promoter in transgenic petunia, could account for the difference.

However, at the level of SAMs (both vegetative and floral), no effect on cell proliferation and expansion, was observed. These observations are similar to those reported for transgenic petunia (Kater et al.



or expansion in organ primordia. The absence of modifications at the level of the SAM can be explained in the light of the results presented here (Fig. 2). The authors showed that even though the organ-identity genes are expressed at the inception of organ primordia, the effect of *SUP* on cell proliferation would not be observed in meristems but would be visible only during organ outgrowth (Kater et al. 2000) and herein.

differentiation. Cell length is 30% shorter on average than in wild-type plants while cell width is 2-fold that of wild-type plants compared with wild-type plants, resulting in distorted cell shape and indicating a failure in the differentiation process. The results presented here, which have already been reported for petals and anthers of transgenic petunia overexpressing *SUP* (Kater et al. 2000) in transgenic petunia, which concerns the cell differentiation process. The default in cell elongation can be restored to obtain the traits of transgenic plants. However, the relationship between cell expansion and cell differentiation remains unclear.

In conclusion, by using the strategy of gain-of-function we showed that *SUP* regulates both cell division and cell expansion (elongation and enlargement) and prevents cells from differentiating normally without disturbing the organization process. To understand how such a transcription factor affects cell proliferation, expansion and differentiation, its target genes must be identified.

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