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Flower Formation in Excised Tobacco Stem Segments

III. DEOXYRIBONUCLEIC ACID CONTENT IN STEM TISSUE OF VEGETATIVE AND FLOWERING TOBACCO PLANTS[1]

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ABSTRACT

A method has been developed that extracts DNA from stem tissue of flowering tobacco plants, *Nicotiana tabacum* cv. Wis. 38. The DNA content of stem tissue from a flowering tobacco plant is correlated with its capacity to flower *in vitro*. Stem segments known to form 100% floral buds contain 10 times more DNA per gram fresh weight than segments that form 5% floral buds and 95% vegetative buds, and in the uppermost 28 centimeters of flowering tobacco plant stems the DNA content decreases roughly in parallel with the floral gradient.

(^{3}H)-Thymidine incorporation into DNA in the internodes is inhibited by attached leaves in flowering tobacco plants but promoted in vegetative plants. Low indoleacetic acid concentrations (2.8 and 5.7 μM) inhibit DNA synthesis in internodes of defoliated stems of flowering tobacco plants to the same extent as attached leaves, whereas the same concentrations of indoleacetic acid promote DNA synthesis in internodes of defoliated stems of vegetative plants. The optimal concentration (11.4 μM) of indoleacetic acid supplied to defoliated stems of vegetative plants increased the rate of DNA synthesis to 4 times the rate in defoliated stems without indoleacetic acid treatment. This increase more than compensates for the promotion of DNA synthesis by the young leaves. Thus, the opposite effects of young leaves on DNA synthesis in internodes of flowering and vegetative tobacco plants may be related to their auxin content.

By *in vitro* cultivation of excised stem segments, flowering tobacco plants have been shown to have a pronounced apex to base gradient in the capacity to form floral buds (1, 2), which truly reflects a difference in the capacity to flower and not merely a decrease in the over-all capacity to form buds (32). Appropriate exogenous supplies of IAA effected a transition from floral to vegetative bud formation in such segments (31). It was suggested, therefore, that the gradient in floral expression may reflect an inverse gradient in endogenous IAA. This would be in agreement with Chouard and Aghion's (8) original proposal that the floral gradient may be due to gradients of (unspecified) endogenous growth substances in the stem and also in agreement with reports from Raghavan and Jacobs (23), Nitsch (22), and others (10, 13, 17, 18, 24) that IAA inhibits flowering in intact plants.

It was shown, furthermore, that in the cultured stem segments, RNA base analogues completely counteracted the inhibiting action of IAA on floral expression (32). This was true for all IAA concentrations which had been observed to decrease the number of floral buds and also held true for 5-fold higher IAA concentrations than required for complete inhibition. Nevertheless, when the auxin inhibition of flowering was completely counteracted by RNA base analogues, the floral gradient remained. This suggests that some factor, other than auxin, with a gradient distribution, also is involved in the regulation of floral expression. It is of interest that also in intact plants under certain conditions, treatments with base analogues promote floral expression (5, 14, 15, 28).

Other experiments with floral-induced tobacco stem tissue indicated that the capacity to flower *in vitro* persisted in successive subcultures up to the fourth transfer (30). A similar loss of capacity to produce flowers after three transfers has been observed in *Perilla*, cultured as described by Chailakhyan and Butenko (7, Chailakhyan, M., oral report, Xth Int. Bot. Congress, Seattle, Wash., 1969.) These results may be interpreted to mean that a flower-promoting material was being replenished through synthesis in the cultures. In view of all these results we have examined changes in nucleic acids associated with the capacity of tobacco stem segments to flower. This report deals with the DNA content in the stems of vegetative and flowering plants.

MATERIALS AND METHODS

Isolation of DNA. Flowering tobacco (*Nicotiana tabacum* cv. Wis. 38) plants were grown in 7-inch pots in the greenhouse until anthesis of the first flower; vegetative plants were grown in flats under cheesecloth and were harvested when 10 cm tall (30); young leaves about 17 cm long were cut from 30 cm tall vegetative plants grown in the greenhouse, and the midribs were removed. F[3] and V stem tissue and young V leaves were used for isolation of DNA.

Nuclear DNA was extracted from isolated nuclei according

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[3] Abbreviations: F: flowering tobacco plants; V: vegetative tobacco plants; SDS: sodium dodecyl sulfate; SSC: 0.15 M NaCl-0.015 M trisodium citrate, pH 7.0.

to the technique of Drlica and Knight (9) modified in the following manner. F and V stem and/or V leaf tissues were combined with isolation medium (1 ml/g stems, 3 ml/g leaves) and were chopped in a Sorvall Omnimixer (20 sec at 50% capacity followed by 20 sec at 100% capacity). The isolation medium, a modified Honda's medium (12), contained 25 mM tris-Cl (pH 7.8), 0.25 M sucrose, and 2.5% Ficoll. Following removal of large debris and chloroplasts from the crude pellet, the nuclei were sedimented through 1.8 M sucrose-10 mM $CaCl_2$ (Spinco SW 25.2 rotor, 23,000 rpm, 1 hr) to separate them from possible contaminating bacteria. The nuclei then were dissolved by stirring in 0.1 M NaCl-2.5% SDS for 3 hr at room temperature (1 ml/4 g tissue). The SDS was removed by adding solid sodium perchlorate (final concentration 1 M) to the solution and centrifuging the solution at 10,000g for 1 hr at 4 C. After the chloroform-octanol treatment, the solution was dialyzed overnight against 0.14 M NaCl-20 mM tris-Cl (pH 7.3) buffer solution at 4 C. Then it was incubated with pancreatic RNase (50 μg/ml, 45 min at 30 C). Pronase that had been self-digested for 2 hr at 37 C was added (100 μg/ml) to the solution, and the incubation was continued for one additional hour. Next the solution was mixed for 10 min at 4 C with an equal volume of neutralized phenol 20 mM potassium EDTA, which was removed by centrifugation at 12,000g for 10 min. The DNA was precipitated from the aqueous phase with 2.1 volumes of cold 95% ethanol and rinsed twice. The rinsing solution used here and elsewhere consisted of 1 volume of 0.1 M sodium acetate mixed with 4 volumes of absolute ethanol and had a pH of 7.0. Finally, the DNA was dissolved in buffer solution (0.5–1.5 mg/ml) and stored in a freezer. DNA concentrations were determined by absorbance at 260 nm; 1.0 A_{260} unit = 50 μg of DNA.

DNA was extracted from homogenates of V and F stem tissue by the following procedure. The tissue was macerated at room temperature in a mortar with an isolation medium containing 1.5% SDS, 75 mM NaCl, 15 mM tris-Cl (pH 8.0), 15 mM $MgCl_2$, and 4 mM 2-mercaptoethanol (1 ml/g tissue). The macerate was filtered through 10 layers of gauze and then through Miracloth, and the filtrate was centrifuged (all centrifugations were at 17,000g for 10 min at 4 C). The NaCl concentration of the supernatant was adjusted to 0.1 M (5.8 mg/ml); the nucleic acids were precipitated with 2.6 volumes of cold 95% ethanol; and the mixture was stored at −10 C. The following day the precipitate was collected by centrifugation and extracted twice with 3 M NaCl (1 ml/12 g tissue) to remove rRNA. The supernatants were combined and centrifuged. Cold 95% ethanol (2.1 volumes) were layered on top of the combined supernatants, and the phases were stored at 4 C. The next day the phases were mixed, and a spooly precipitate formed which was pulled out with tweezers, rinsed twice with rinsing solution, and dissolved in buffer solution (1 ml/6 g tissue). This solution is referred to as the spooly fraction. Solid sodium perchlorate (final concentration 1 M) was added to the spooly fraction, and the fraction was centrifuged. The supernatant was extracted with an equal volume of chloroform-octanol (10:1) and dialyzed overnight against buffer solution at 4 C. Then it was incubated with self-digested pronase (100 μg/ml, 45 min at 30 C). To remove pronase, 2.1 volumes of cold 95% ethanol were added to the solution, and the spooly precipitate which formed was pulled out immediately, rinsed twice with rinsing solution, and dissolved in buffer solution (1 ml/4 g tissue). Next RNase (20 μg/ml) was added to the solution, and the solution was incubated for 40 min at 30 C. To remove RNase, the solution was mixed with an equal volume of neutralized phenol 20 mM potassium EDTA for 10 min at 4 C. The phases were separated by centrifugation, and the aqueous phase was reextracted twice more with an equal volume of phenol 20 mM potassium EDTA. The DNA was precipitated from the aqueous phase with 2.1 volumes of cold 95% ethanol, rinsed twice, dissolved in buffer solution (0.5–1.5 mg/ml), and stored in a freezer.

Uptake of Radioactivity. For (^{3}H) or (^{14}C) incorporation into DNA, the top 10 cm of 4 tobacco plants, consisting of internodes and nodes with attached flowers or leaves, were placed with the stem ends in contact with 0.5 ml of labeling solution [(methyl-^{3}H)thymidine or (2-^{14}C)thymidine, 25 μg/ml chloramphenicol] contained in a 5-ml beaker. Next the tops were illuminated 20 cm from a 60-w Sylvania light bulb at room temperature. They absorbed the solution in about 30 min and were allowed to take up 1 ml of H_2O added to the beaker (an additional 1 hr). The tops were then placed with the stem ends in contact with H_2O in a 100-ml beaker and incubated at room temperature in the dark for 24 hr.

Measurement of Radioactivity. One hundred micrograms of bovine serum albumin and trichloroacetic acid to 5% were added to each sample. After 20 min at 4 C, each precipitate was collected on a glass fiber Millipore filter (Whatman GF/A, 2.4 cm), which was dried and placed in 5 ml of scintillation fluid (4 g Omnifluor/liter toluene). Counting was done in a Packard Tri-Carb liquid scintillation spectrometer.

Cesium Chloride Density Gradient Centrifugation. Isopycnic CsCl density gradient centrifugation was carried out as described by Flamm, Bond, and Burr (11) using a Spinco No. 50.1 rotor at 40,000 rpm for 65 hr at 20 C. The tubes were punctured, and 1-drop fractions were collected. The density gradients were calculated from refractive index measurements of every 10th fraction. The remaining fractions were diluted with 0.5 ml of H_2O, and the radioactivity was measured.

Thermal Denaturation. Simultaneous measurements of absorbance (260 nm) of four samples were made with a Model 2400 Gilford spectrophotometer equipped with a cell holder heated by circulating liquid. Temperature was regulated by a thermostat-controlled water bath, and an increase in temperature was effected by continually changing the setting of the thermostat by means of a small electronic motor. The temperature increase was less than 0.4 C/min. Fifteen micrograms per milliliter of extracted F stem DNA (from tissue homogenates), V stem DNA (9), or standard *Escherichia coli* strain K-12 DNA were dissolved in 1 × SSC. Three milliliters of each DNA solution and control SSC buffer were placed in separate stoppered quartz cuvettes. Denaturation was performed as described by Marmur and Doty (21). *E. coli* strain K-12 DNA, isolated and purified as described by Lovett and Young (19), was obtained from Dr. Paul Lovett, University of Maryland, Baltimore County.

Source of Chemicals. (Methyl-^{3}H)thymidine (>15 ci/mmole) and (2-^{14}C)thymidine (56 mc/mmole) were purchased from the New England Nuclear Corporation. Chloramphenicol was a product of Parke, Davis and Co. Crystallized RNase and DNase, electrophoretically pure, and pronase were from the Worthington Biochemical Company. Purified SDS was prepared from the Fisher Scientific Company product by crystallization from ethanol.

RESULTS

DNA from F and V: Methods of Isolation and Purity. In attempts to extract DNA from F, it was soon found that the method of Drlica and Knight (9), which reproducibly extracts DNA from stems or leaves of V, was inadequate for extracting DNA from F (Table I). Therefore, a new procedure was worked out, as described above, in which DNA was extracted from tissue homogenates instead of from isolated nuclei. It can be seen in Tables II and III that large quantities of DNA, as

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assayed by absorbance at 260 nm (A_{260}) and (^{3}H)thymidine incorporation, are obtained from F by this method. Although no DNA is obtained by this method from homogenates consisting solely of V stems, labeled V stems extracted together with DNA from F stems added as carrier give higher yields of labeled DNA than are extractable from isolated nuclei of the same material (see Tables I and II).

Table I. *Representative DNA Extractions from Isolated Nuclei of V and/or F Tissues*[1]

Labeled Tissue[2]	Nonlabeled Tissue	DNA	
		µg/g total tissue	*cpm/g labeled tissue*
...	Stems (V), 158 g	3	...
Leaves (V), 14.4 g	Leaves (V), 100 g	10	40,000
Stems (V), 7.3 g	Leaves (V), 100 g	16	52,000
Stems[3] (F), 10.4 g	Stems[3] (F), 90 g	0	0

[1] Analytical procedures according to Drlica and Knight (9).
[2] 0.5 mc (^{3}H) thymidine.
[3] Uppermost 10 cm of F.

Table II. *Representative DNA Extractions from Tissue Homogenates of F or V Stems*

Labeled Tissue[1]	Nonlabeled Tissue	DNA	
		µg/g total tissue	*cpm/g labeled tissue*
...	Stems[2] (F), 40 g	57	...
Stems[2] (F), 8.5 g	DNA[3] (F), 1.5 mg	...	1,220,000
Stems (V), 5.5 g	Stems (V), 100 g	0	0
Stems (V), 4.7 g	DNA[3] (F), 1.5 mg	...	210,000

[1] 0.5 mc (^{3}H) thymidine.
[2] Uppermost 10 cm of F.
[3] Added as carrier.

Table III. *Three Representative Extractions of DNA from Tissue Homogenates of F Stems*

Stem Tissue[1]	Total DNA Extracted	DNA
g	*mg*	*µg/g tissue*
300	10.4	35
300	9.6	32
300	10.1	35

[1] Uppermost 13 cm of F.

Table IV. *Interference of F Stem Tissue in the Extraction of DNA from Isolated Nuclei*[1]

Sample	Tissue	Fresh Weight	Estimated Extractable DNA[2]	DNA Actually Extracted	
		g	*mg*	*mg*	*cpm/g × 10⁻³*
1[1]	F[3]	0	0	...	...
	F	70	2.3	...	...
	V	70	0.7	...	...
	Total	140	3.0	0.5	...
2[1]	F[3]	8	0.5	...	...
	F	39	0.7	...	...
	V	70	0.7	...	...
	Total	117	1.9	0.9	51
3[4]	F[3]	9	0.5	...	...
	F	40	2.3	...	...
	V	0	0	...	...
	Total	49	2.8	2.3	109

[1] Drlica and Knight (9).
[2] Estimate for V leaves from Table I. Estimate for F stems from Fig. 3 (DNA): samples 2F[3], 3F[3] and 3F, uppermost 10 cm; sample 1F, uppermost 11 cm; and sample 2F, uppermost 14 cm of excised stem tops.
[3] 0.1 mc (^{3}H) thymidine.
[4] Tissue homogenates (see "Materials and Methods").

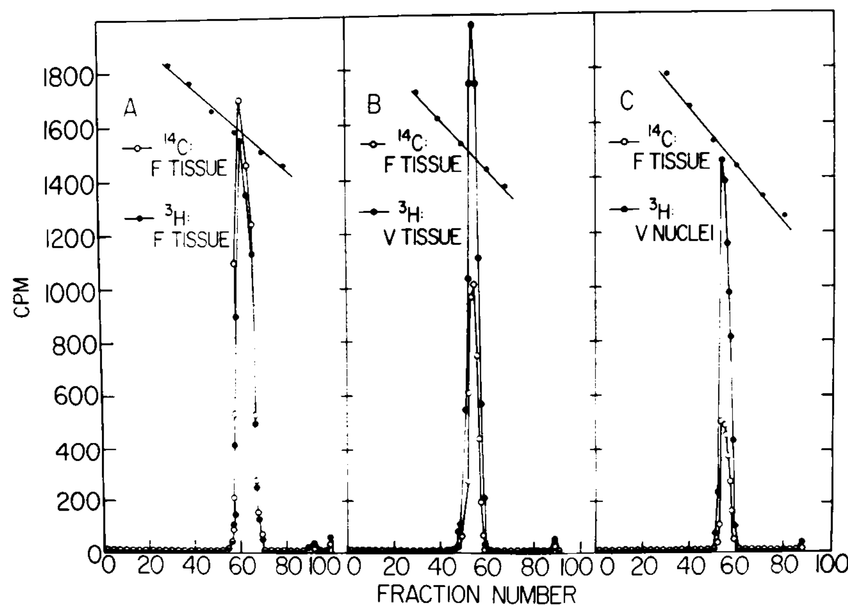

FIG. 1. Fractionation of DNA extracted from V and F stems in CsCl density gradients. Sixteen thousand cpm (20 µg) of (^{14}C)-DNA from F tissue plus 16,000 cpm (3 µg) of (^{3}H)-DNA from F tissue (gradient A), 10,000 cpm (13 µg) of (^{14}C)-DNA from F tissue plus 24,000 cpm (16 µg) of (^{3}H)-DNA from V tissue (gradient B), and 5,000 cpm (6 µg) of (^{14}C)-DNA from F tissue plus 13,000 cpm (30 µg) of (^{3}H)-DNA from V nuclei (gradient C) were centrifuged to equilibrium in CsCl. The buoyant density of all the DNA preparations is about 1.696 g/cm^3.

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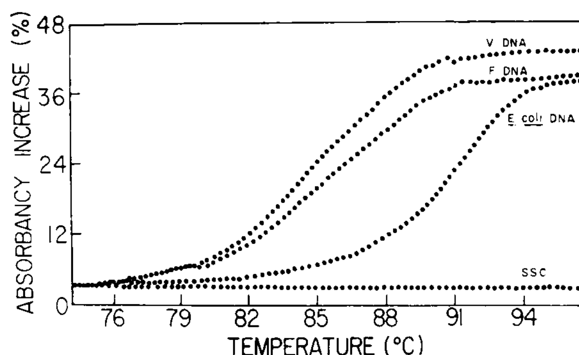

FIG. 2. Thermal denaturation profiles of V and F DNA extracted from stem tissue. Fifteen μg/ml of V DNA (nuclear), F DNA (tissue homogenates), or standard *E. coli* strain K-12 DNA were dissolved in 1 × SSC buffer. Three ml of each DNA solution and control SSC buffer were placed in separate stoppered quartz cuvettes, and the DNA solutions were denatured (see "Materials and Methods"). The T_m of DNA from V and F is 85.4 C as compared with a T_m of 90.4 C for standard *E. coli* DNA.

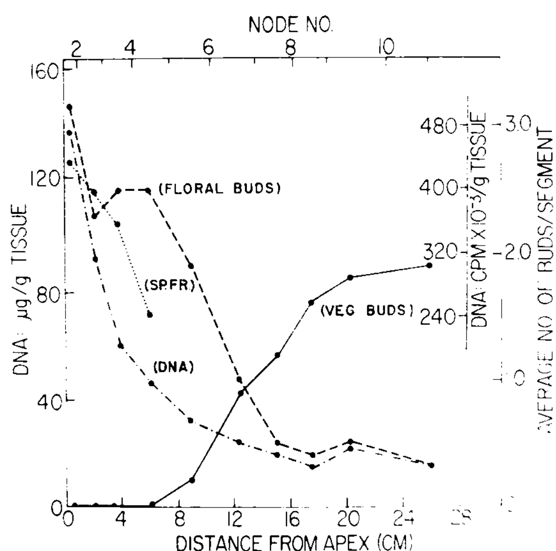

FIG. 3. DNA extracted from F: apex to base gradient in the region of the stem corresponding to the floral gradient. One hundred internodes excised from each of the uppermost 10 internodes of 100 F plants were pooled according to internode, and each of the 10 groups was weighed (10–100 g), and the DNA was extracted. Average total DNA/internode·g tissue is presented (DNA). The top 10 cm of three F plants, consisting of nodes and internodes 1 to 6 with attached flowers and leaves, were labeled with 0.5 mc (^{3}H) thymidine (see "Materials and Methods"). Then each set of three internodes was excised, weighed (about 6 g) and pooled with 12 g of nonlabeled F tissue. The spooly fractions (SP.FR.) were assayed for radioactivity. Average total cpm × 10^{-3}/internode·g tissue is presented (SP.FR.). The floral gradient, reflected by the average number of floral buds per segment and of vegetative buds per segment, obtained in an earlier study, is included for comparison.

Failure to obtain DNA from nuclei of F stems suggests that this tissue may have higher degradative enzyme activity than the V tissue. This is indicated by data presented in Table IV. It can be seen that when the F stem tissue is combined with an equal amount of V leaf tissue and the nuclei are isolated, the yield of DNA is only 17% of the quantity of total DNA extractable from tissue homogenates. When the F stem tissue is reduced to about half the amount of leaves, 47% of the total DNA in the sample is extracted. These results suggest that nucleases in F tissue interfere in the extraction of DNA by the Drlica and Knight procedure. The radioactivity extracted from isolated nuclei of F stem tissue in the presence of carrier indicates that the DNA in this tissue is mainly nuclear.

Analysis of DNA extracted by either method indicates a high degree of purity. All preparations give 260/280 ratios of 1.7 to 1.8; the DNA is resistant to alkali, and it is completely degraded to trichloroacetic acid-soluble products by DNase (50 μg/ml, 40 min at 30 C). Figure 1 shows the neutral CsCl density gradient analysis. In agreement with previous reports (27, 29, 34), the DNA prepared in the present study has a bouyant density of about 1.696, and guanine plus cytosine (G + C) calculated from the equation of Schildkraut *et al.* (25) is 35%. The chemical determined G + C is 40% (20, 29). The difference between these values is to be expected from the presence of 4 to 6% of 5-methyl substituted cytosine (20, 29), which decreases the density of DNA in CsCl (16). The sharpness of the DNA peaks in CsCl is evidence of homogeneity of the DNA.

The thermal denaturation profiles of tobacco and *E. coli* strain K-12 DNAs are shown in Figure 2. The T_m for tobacco DNA is 85.4 C as compared with 90.4 C for the *E. coli* standard DNA (21). A nucleotide composition of tobacco DNA, calculated from the T_m value using the equation of Marmur and Doty (21), of 39% of G + C is in agreement with the value of 40% reported from chemical determination. The hyperchromicity of tobacco DNA isolated by either method is 39% and the thermal transition curves are smooth.

Gradient Distribution of DNA in F. It is clear that there is an apex to base gradient in DNA content, which is roughly parallel to the floral gradient, suggesting that the capacity for floral expression of stem segments *in vitro* may be dependent

Table V. *Effects of Attached Leaves on DNA Synthesis in V and F Stem Sections*

For each of 4 treatments, 4 sections, consisting of internodes and nodes 1 to 4 of V or 3 to 5 of F with attached leaves, or internode and node 9 of F with attached leaf (numbering from the top of the plants) were preincubated with the stem ends in contact with H_2O in 100-ml beakers (sets of 2 explants/breaker) for 2 hr at room temperature. Then the sections were placed with the stem ends in contact with 0.5 ml of labeling solution [(^{3}H) thymidine or (^{3}H) uridine, 25 μg/ml chloramphenicol] contained in a 5-ml beaker. Following absorption of the labeling solution and an additional 1 ml of H_2O (see "Materials and Methods"), the sections were removed from the small beakers, and one set per treatment was defoliated before being placed with the stem ends in contact with H_2O in 100-ml beakers. The sections were incubated in the dark at room temperature for 24 hr and then were frozen at −10 C. The internodes from each set of sections were weighed (about 4 g) and pooled with 12 g of nonlabeled F tissue. The spooly fractions were extracted and assayed for radioactivity.

Experiment No. and Tissue	(^{3}H) Radioactivity[1]	Labeled Node(s) and Internode(s)	Radioactivity Incorporated		
			With leaves[2]	Defoliated[3]	Change
	mc		*cpm/g labeled stem tissue*		%
1 (V)	0.04 (T)	1–4	73,500	52,500	−28
2 (F)	0.04 (T)	3–5	46,000	70,000	+52
3 (F)	0.04 (T)	9	13,000	37,000	+185
4 (F)	0.04 (U)	9	22,000	18,000	−18

[1] T: thymidine; U: uridine.
[2] Leaves removed after isotope incorporation.
[3] Leaves removed before isotope incorporation.

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to some extent upon the quantity and synthesis of DNA in the tissue.

Effects of Young Leaves on DNA Synthesis in Excised F and V Stem Tissue. The effects of attached leaves on the incorporation of (^{3}H)thymidine into DNA in excised stem tissue of F and V stem sections were studied by procedures described in the legend to Table V. As shown in this table, leaves promote synthesis of DNA in V internodes, whereas they inhibit DNA

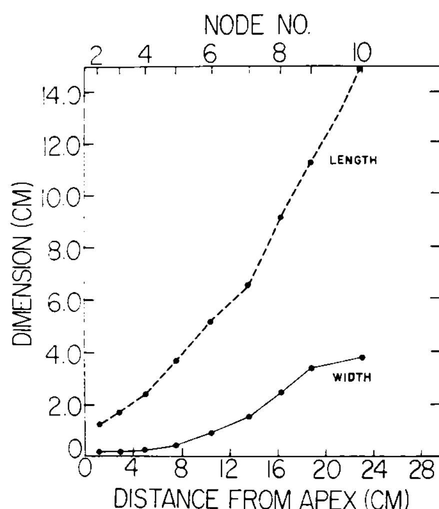

FIG. 4. Leaf dimensions in the region of the stem corresponding to the floral gradient. Leaves from each of the uppermost 10 nodes of 10 F plants were pooled according to node, and each leaf of the 10 groups was measured. The measurements are expressed as the average leaf length per node and the average leaf width per node.

Table VI. *Effect of IAA on DNA Synthesis in Defoliated F Stem Sections*

For each of 5 treatments, 2 sections, consisting of internode and node 9 with attached leaf, were preincubated with the stem ends in contact with H_2O or IAA solution in a 100-ml beaker for 2 hr at room temperature, and then they were placed with the stem ends in contact with 0.5 ml of labeling solution [(^{3}H) thymidine or (^{3}H) thymidine-IAA, 25 μg/ml chloramphenicol] contained in a 5-ml beaker. Following absorption of the labeling solution and an additional 1 ml of H_2O (see "Materials and Methods"), the sections were removed from the small beakers and sections in treatments 2 to 5 defoliated before being placed with the stem tops in contact with H_2O or appropriate IAA solution contained in 100-ml beakers. The foliated control sections of treatment 1 were placed with the stem ends in H_2O. The sections were incubated in the dark for 24 hr at room temperature and then were frozen at −10 C. The internodes from each set of sections were weighed (about 2.5 g) and pooled with 12 g of nonlabeled F tissue. The spooly fractions were assayed for radioactivity.

Treatment No. and Tissue	IAA	Radioactivity Incorporated		
		With leaves[1]	Defoliated[2]	Change
	μM	*cpm/g labeled stem tissue*		%
1-2 (F)	0	13,000	22,000	+70
3 (F)	0.6	...	22,000	+70
4 (F)	2.8	...	14,000	+8
5 (F)	5.7		13,000	0

[1] Leaves removed after isotope incorporation.
[2] Leaves removed before isotope incorporation.

Table VII. *Effect of IAA on DNA Synthesis in Defoliated V Stem Sections*

Following removal of the sections from the 5-ml beakers (see Table VI), sections in treatments 2 to 4, 6 to 9, and 11 to 15 were defoliated before being placed with the stem tops in contact with H_2O or the appropriate IAA solution contained in 100-ml beakers. The foliated control sections of treatments 1, 5, and 10 were placed with their stem ends in H_2O. The sections were incubated in the dark for 24 hr at room temperature and then were frozen at −10 C. The internodes from each set of sections were weighed (about 2.5 g) and pooled with 12 g of nonlabeled F tissue. The spooly fractions were assayed for radioactivity.

Experiment No. and Tissue	Treatment No.	Radioactivity Incorporated						
		With leaves[1]	Defoliated[2]					
			IAA (μM)					
			0	1.4	2.8	5.7	11.4	22.8
		cpm/g labeled stem tissue						
1 (V)	1–4	71,000	22,000	17,000	28,000	...	...	...
2 (V)	5–9	76,000	22,000	25,000	46,000	58,000	...	...
3 (V)	10–15	88,000	35,000	...	...	63,000	124,000	110,000

[1] Leaves removed after isotope incorporation.
[2] Leaves removed before isotope incorporation.

synthesis in F internodes. As expected from differences in size of the leaves (dimensions shown in Fig. 4), their inhibiting effect on DNA synthesis is much greater in internode 9 than in internodes 3 to 5. It should be noted that the large increase in (^{3}H)thymidine incorporation in F internodes is not accompanied by a parallel incorporation of (^{3}H)uridine into total RNA, indicating that the observed differences in (^{3}H)thymidine incorporation are not due to a translocation effect.

Effects of IAA on DNA Synthesis in Excised F and V Stem Tissue. The possibility that the opposite effects of young leaves on DNA synthesis in F and V stem tissue are related to auxin is suggested by the data presented in Tables VI and VII. We have found that 2.8 and 5.7 μM concentrations of IAA inhibit DNA synthesis in defoliated F internodes to the same extent as do attached leaves, whereas the same concentrations of IAA promote DNA synthesis in defoliated V internodes. The optimal concentration (11.4 μM) of IAA supplied to defoliated V internodes increases DNA synthesis to 4 times the rate in defoliated controls and thus more than compensates for the promotion of DNA synthesis by the young leaves.

DISCUSSION

This study extends the observation that one of the earliest indications of the transition of higher plants from vegetative to reproductive growth is an increased DNA content or synthesis in buds (3, 4, 6, 17, 26, 33, 35) to excised tobacco stem segments by showing that their capacity to flower *in vitro* is correlated with their DNA content at the time of excision. Experiments are in progress to test whether DNA extracted from F is specific for flowering.

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