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THE INDUCTION OF FLOWERING *IN VITRO* IN STEM SEGMENTS OF *PLUMBAGO INDICA* L.

II. THE PRODUCTION OF REPRODUCTIVE BUDS

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Summary. Internode segments excised from vegetative *Plumbago indica* plants are responsive to photoperiodic treatments *in vitro*. Under long days, they produce vegetative buds; under short days, they develop inflorescences. These inflorescences can remain devoid of flowers ("vegetative inflorescences"), or produce normal flowers which open in the test tubes. The minimum duration of the short-day treatment capable of inducing flowering is of the order of 4 weeks.

The production of inflorescences under short days is affected by various factors. An adequate level of sucrose is necessary. Sucrose can be replaced by maltose and, to a small extent, by cellobiose, but not by mannitol or lactose. Auxins and gibberellins inhibit the production of flower buds, whereas cytokinins and adenine do not. Guanine, thymine, cytosine or uracil alone are ineffective, but thymine or its precursor, orotic acid, enhance the production of floral buds when adenine and kinetin are also present in the medium. Several amino acids, as well as glutamine and asparagine, tend to reduce inflorescence formation at 3×10^{-4} *M* or above; urea increases it slightly at the same concentrations. Both the *cis*- and the *trans*-isomer of abscisin II enhance inflorescence formation under short days, but have no such effect under long days.

High concentrations of adenine re-established the red coloration of the petals which is typical of the clone used. Otherwise, the color of the flowers grown *in vitro* was pink, presumably because of the depressing effect of kinetin on anthocyanin synthesis.

Introduction, Material and Methods

The search for a suitable system with which one could study the biochemistry of the flowering process, has led us to use excised stem segments of an absolute short-day species, *Plumbago indica* L., clone "Angkor". The first article of this series (NITSCH and NITSCH, 1967) has described the general techniques for aseptic culture of such segments, and the conditions under which they can be stimulated to produce buds. The present article will present the results of investigations aiming at determining the conditions under which these buds can be brought into the flowering rather than the vegetative state.

The techniques used have been described in the preceding article (NITSCH and NITSCH, 1966). All segments were excised from internodes of stock plants kept in completely vegetative conditions in a greenhouse by means of 16-hour photoperiods.

Experimental Results

a) Types of Inflorescences

Depending on the experimental conditions, the flower buds which were formed *in vitro* produced either flowers of normal size (Fig. 1) or inflorescences with bracts, but devoid of flowers. These inflorescences have been termed "vegetative inflorescences" (Fig. 2). They may bear some similarities to the "sterile cones" obtained by RAGHAVAN and JACOBS (1961) while culturing apices of *Perilla*. These inflorescences frequently did not reach the stage represented in Fig. 2, but turned brown and died; such inflorescences have been called "aborted inflorescences".

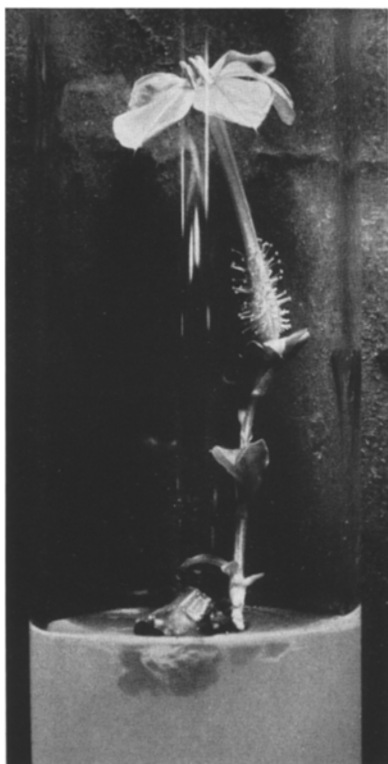

Fig. 1. Flower of *Plumbago indica* induced and developed *in vitro* on a 5 mm-long section of internode excised from a vegetative plant. (Magnification × 2)

b) Photoperiodic Induction in vitro

After being planted in test tubes, the internode segments were subjected to either long (18 hours of light) or short (9—10 hours) photoperiods. No inflorescences developed when the cultures were maintained under long days (Table 1). As already shown previously (NITSCH and NITSCH, 1967), the percentage of cultures forming buds at all was also much reduced under these conditions.

Determinations of the Critical Induction Period. In the case of whole plants, depending on the vigour and the temperature, one week of 9-hr. days or less is sufficient for irreversible flower induction (NITSCH, 1965b). When planted in test tubes, internode sections have first to recover from the shock of being severed from the plant. Then, they have to start producing buds. It is not surprising, therefore, that floral induction takes longer in the case of excised segments. By shifting cultures from short-day to long-day conditions at various time intervals, it was possible to show that a minimum of 4 weeks of short-days is necessary for the irreversible induction *in vitro* (Table 1). This is about the time at which buds start to become visible on the cultures.

c) Effect of Carbohydrates

As shown previously (NITSCH and NITSCH, 1965), sucrose has a determining influence upon the formation of flower buds in *Plumbago*. This phenomenon has been investigated further by testing disaccharides other than sucrose, and by studying the effect of their concentration.

Apart from sucrose, the formation of vegetative buds was also promoted by maltose, cellobiose and lactose. In the latter case, the buds

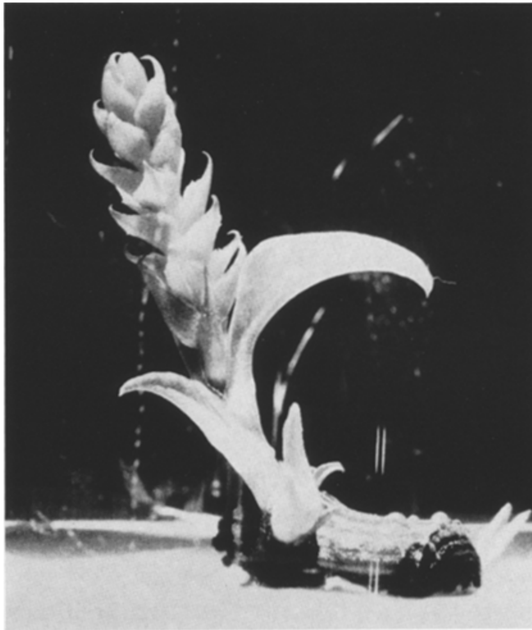

Fig. 2. "Vegetative inflorescence" developed on an internode section under short days on a medium containing 1% sucrose. (Magnification × 8)

remained very small. The carbohydrates differed, however, in their ability to support the formation of reproductive buds (Table 2). In general, sucrose was best, followed closely by maltose; cellobiose (at 6×10^{-2} M) was also able to allow the formation of some inflorescences, whereas lactose was totally ineffective.

As shown by the data of Table 2, sucrose, at a concentration of 10^{-2} *M*, promoted the formation of buds on 100% of the cultures, but these were all vegetative. At higher sucrose concentrations, a certain proportion of the cultures developed flower buds.

In order to determine whether this concentration effect was an osmotic one, various concentrations of mannitol were added to a relatively low sucrose concentration. Vegetative buds could be produced with

Table 1. *Effect of photoperiod on the induction of flowering in vitro*
Medium containing kinetin (1 mg/l), adenine (3×10^{-4} M) and 2.5% sucrose. Segments 10 mm in length.

Photoperiod	Number of cultures	After 8 weeks		After 14 weeks		
		Cultures (%) with		Cultures (%) with		
		Veg. buds	Inflores-cences	Veg. buds	Inflo-res-cences*	Flowers
9-hr days throughout	12	42	50	8	83	33
18-hr. days throughout	12	17	0	33	0	0
2 weeks of 9-hr. days, then 18-hr. days	12	42	0	42	8	0
3 weeks of 9-hr. days, then 18-hr. days	12	50	0	58	0	0
4 weeks of 9-hr. days, then 18-hr. days	12	42	25	42	42	8
5 weeks of 9-hr. days, then 18-hr. days	12	75	8	58	25	25
6 weeks of 9-hr. days, then 18-hr. days	10	50	30	20	60	17
7 weeks of 9-hr. days, then 18-hr. days	12	50	42	8	92	25
2 weeks of 18-hr. days, then 9-hr. days	12	30	8	8	42	25
3 weeks of 18-hr. days, then 9-hr. days	11	18	0	9	27	9
4 weeks of 18-hr. days, then 9-hr. days	12	8	0	17	25	0
5 weeks of 18-hr. days, then 9-hr. days	12	25	0	17	25	8
6 weeks of 18-hr. days, then 9-hr. days	11	25	0	18	27	0
7 weeks of 18-hr. days, then 9-hr. days	12	25	0	17	33	0

* Including those which produced open flowers (which are listed in the last column).

mannitol alone, but they remained very small (Table 3). When added to media containing 3×10^{-2} M sucrose, mannitol not only did not increase the percentage of cultures forming inflorescences, but actually decreased it (Table 3). Thus, the effect of high concentrations of sucrose in stimulating inflorescence formation cannot be duplicated by replacing part of the sucrose with mannitol.

d) Effect of Auxins, Gibberellins and Abscisins

Auxins and Related Compounds. As mentioned previously (NITSCH and NITSCH, 1967), IAA at relatively low concentrations tended to increase the percentage of cultures forming buds. This effect can be seen again in the results of Table 4. However, the addition of auxin decreased the percentage of cultures developing inflorescences.

Similar results, with variations depending on the compounds used and their concentrations, were obtained with L-tryptophane, isatin (purified as described by BUI DANG HA and NITSCH, 1966), phenylacetic acid, 1-naphthylacetamide, and 2,3-dichlorophenylacetic acid, a strong synthetic auxin (see GANDAR and NITSCH, 1959).

Table 2. *Effect of various carbohydrates on the formation of reproductive buds under short days*

Media containing kinetin (1 mg/l) and adenine (10^{-4} *M*). All carbohydrates were sterilized by filtration.

Compound	Concentration (*M*)	Number of cultures	Cultures (%) with	
			Vegetative buds	Inflorescences
None	0	12	0	0
Sucrose	3×10^{-3}	12	83*	0
	10^{-2}	12	100	0
	3×10^{-2}	12	75	25
	6×10^{-2}	12	67	8
	9×10^{-2}	12	17	8
	12×10^{-2}	11	0	9
Maltose	3×10^{-2}	12	100	0
	6×10^{-2}	12	75	8
	9×10^{-2}	11	36	36
Lactose	3×10^{-2}	12	100*	0
	9×10^{-2}	11	82*	0
None	0	10	20*	0
Sucrose	3×10^{-2}	8	25	25
	6×10^{-2}	12	17	33
	9×10^{-2}	11	9	36
Cellobiose	3×10^{-2}	12	83	0
	6×10^{-2}	12	58	8

* Very small buds.

Table 3. *Effect of mannitol as a total or partial substitute for sucrose on the formation of reproductive buds under short days*

Media containing kinetin (1 mg/l) and adenine (10^{-4} *M*). Mannitol sterilized by filtration.

Compounds and individual concentrations (*M*)		Total concentration ($M \times 10^{-2}$)	Number of cultures	Cultures (%) with	
				Vegetative buds	Inflorescences
None		0	10	20*	0
Sucrose	3×10^{-2}	3	8	25	25
	6×10^{-2}	6	12	17	33
	9×10^{-2}	9	11	9	36
Mannitol	3×10^{-2}	3	11	55*	0
	6×10^{-2}	6	11	18*	0
	9×10^{-2}	9	12	0	0
Sucrose	3×10^{-2} + mannitol 3×10^{-2}	6	11	18	18
Sucrose	3×10^{-2} + mannitol 6×10^{-2}	9	11	27	9

* Very small buds.

Table 4. *Effect of auxins and related compounds on the production of flower buds*
All media contained benzyladenine (10^{-6} *M*), adenine (10^{-4} *M*) and 2% sucrose. Cultures maintained under short days.

Compound	Concentration (*M*)	Number of cultures	Cultures (%) with			
			Veg. buds (total)	Reproductive buds producing		
				Flowers	Veg. inflor.	Total
None	0	11	45	9	36	45
IAA	10^{-8}	12	83	8	9	17
	10^{-7}	12	75	0	17	17
	10^{-6}	12	42	8	25	33
	10^{-6}*	12	83	0	17	17
	10^{-5}	10	40	0	20	20
L-tryptophane	10^{-6}	12	100	0	0	0
	10^{-5}	12	75	8	0	8
	10^{-4}	10	20	0	8	8
	10^{-3}	10	0	0	0	0
Isatin	10^{-4}	12	67	8	17	25
	10^{-3}	9	0	0	0	0
Phenylacetic acid	10^{-6}	12	42	8	33	41
	10^{-5}	12	50	0	0	0
	10^{-4}	10	0	0	0	0
1-Naphthylacetamide	10^{-6}	12	75	0	17	17
	10^{-5}	12	83	0	8	8
	10^{-4}	10	0	0	0	0
2,3-Dichlorophenylacetic acid*	10^{-8}	12	25	0	0	0
	10^{-7}	10	0	0	0	0
	10^{-6}	12	0	0	0	0

* Autoclaved (others filtered).

Table 5. *Effect of three gibberellins on the production of flower buds*
All media contained kinetin (1 mg/l), adenine (10^{-4} *M*) and 2% sucrose. All the gibberellins were sterilized by filtration. Cultures subjected to short days.

Gibberellin	Concentration (*M*)	Number of cultures	Cultures (%) with	
			Vegetative buds	Inflorescences
None	0	10	20	40
GA_1	10^{-7}	12	0	50
	10^{-6}	12	0	0
	10^{-5}	12	8	0
GA_3	10^{-7}	10	20	10
	10^{-6}	12	17	0
	10^{-5}	12	8	0
GA_7	10^{-7}	11	9	0
	10^{-6}	12	25	8
	10^{-5}	12	8	0

Table 6. *Effect of cis- and trans-abscisin II on the production of flower buds under short- and long-day regimes*

All media contained kinetin (1 mg/l), adenine (10^{-4} *M*) and 1% sucrose. The abscisins were sterilized by filtration.

Compound	Concentration (mg/l)	Photoperiod	Number of cultures	Cultures (%) with	
				Vegetative buds	Inflorescences
None	0	9 h.	12	42	33
Cis-abscisin	1	9 h.	12	75	0
	3	9 h.	12	17	67
	10	9 h.	12	0	50
None	0	9 h.	9	33	22
Trans-abscisin *	1	9 h.	12	42	42
	3	9 h.	12	0	42
	10	9 h.	12	0	0
None	0	18 h.	12	17	0
Cis-abscisin	1	18 h.	12	33	0
	3	18 h.	12	8	0
	10	18 h.	12	8	0
None	0	18 h.	10	40	0
Trans-abscisin *	1	18 h.	12	25	0
	3	18 h.	12	17	0
	10	18 h.	11	0	0

* Methyl ester.

Table 7. *Effect of purines on the production of flower buds*

All media contained kinetin (1 mg/l) and sucrose (2%). Sterilization by filtration. Cultures grown under short days.

Compound	Concentration (*M*)	Number of cultures	Cultures (%) with	
			Vegetative buds	Inflorescences
None	0	12	0	0
Adenine	10^{-6}	12	8	8
	10^{-5}	12	0	8
	10^{-4}	12	17	17
	3×10^{-4}	12	58	42
Guanine	10^{-6}	12	0	8
	10^{-5}	11	0	0
	10^{-4}	11	0	0
	3×10^{-4}	12	8	0

Gibberellins. Three gibberellins, GA_1, GA_3 and GA_7 were tested, each at three different concentrations. As shown in Table 5, except for the lowest concentration of GA_1, all three gibberellins reduced the percentage of cultures forming inflorescences.

Abscisins. Abscisin II, which has been found in ageing flowers such as the yellow lupin (van Steveninck, 1959), and is produced under short days by trees such as *Acer pseudoplatanus* (Cornforth *et al.*, 1965), was also tested on *Plumbago* internodes. Both the *cis-* and the *trans-*isomers were used. It was found that, under short days of 9 hours, neither one

Fig. 3. Effect of adenine concentration on the coloration of the petals. Left: 10^{-4} M; right: 3×10^{-4} M

inhibited flower bud production; in fact, they stimulated it (Table 6). However, under long days (18 hours), both isomers were unable to promote the formation of inflorescences.

e) Effect of Cytokinins, Nucleic Acid Constituents, and Other Nitrogenous Compounds

In contrast to auxins, cytokinins allowed the formation of floral buds, at least at concentrations of 10^{-6} M, as in the case of benzyladenine,

Table 8. *Effect of nucleic acid constituents and orotic acid in the presence of adenine on the production of flower buds*

All media contained kinetin (1 mg/l), adenine (3×10^{-4} *M*) and 2% sucrose. The added substances were all sterilized by filtration. Results after 3 months of culture under short days.

Compounds and concentrations (*M*)		Number of cultures	Cultures (%) with				
			Embryonic buds*	Vegetative buds	Reproductive buds producing		
					Flowers	Veg. inflor.	Total
None	0	11	45	0	18	27	45
Guanine	3×10^{-6}	12	25	8	33	17	50
	3×10^{-5}	12	17	0	42	25	67
	3×10^{-4}	10	20	20	30	20	50
Uracil	3×10^{-6}	11	9	18	9	64	73
	3×10^{-5}	12	8	8	25	24	50
	3×10^{-4}	12	25	0	8	50	58
Cytosine	3×10^{-6}	11	18	0	36	27	63
	3×10^{-5}	11	18	0	36	36	72
	3×10^{-4}	12	25	0	25	42	67
Ribose	3×10^{-5}	12	17	0	8	58	66
	3×10^{-4}	10	0	10	20	60	80
None	0	12	17	0	25	33	58
Thymine	3×10^{-7}	12	42	17	17	25	42
	3×10^{-6}	12	17	17	0	67	67
	3×10^{-5}	12	17	0	42	42	84
	3×10^{-4}	12	8	0	67	25	92
G + U + C** +							
Thymine	3×10^{-6}	12	50	8	8	33	41
	3×10^{-5}	12	25	0	25	42	67
	3×10^{-6}***	12	0	0	42	50	92
Orotic acid	3×10^{-5}	12	42	8	17	17	34
	3×10^{-4}	12	42	0	33	25	58
	3×10^{-3}	11	36	9	27	18	45
Ribose + orotic acid	3×10^{-4} + 3×10^{-4}	12	42	17	17	25	42

* Buds smaller than 2—3 mm from which it is not possible to tell whether they are vegetative or reproductive.

** 3×10^{-5} *M* each.

*** Plus ribose (3×10^{-5} *M*).

6-benzyl-9-tetrahydropyrane-adenine (SD-8339) and zeatin. In most of the reported experiments, kinetin at 1 mg/l was used, with good results with respect to the formation of flower buds.

Nucleic Acid Constituents. Constituents of nucleic acids, such as purines, pyrimidines and ribose, were added at various concentrations

Table 9. *Effect of amino-acids, amides and urea on the production of flower buds*
All media contained kinetin (1 mg/l), adenine (10^{-4} M) and 1.5% sucrose. All the nitrogenous compounds were sterilized by filtration. Cultures subjected to short days.

Compound	Concentration (M)	Number of cultures	Cultures (%) with		
			Embryonic buds*	Vegetative buds	Inflorescences
None	0	12	25	8	42
L-alanine	3×10^{-4}	12	17	25	0
	10^{-3}	12	17	8	17
	3×10^{-3}	12	8	8	8
L-arginine	3×10^{-4}	12	8	8	8
	10^{-3}	12	42	25	8
	3×10^{-3}	11	45	9	9
L-serine	10^{-3}	11	36	9	9
	3×10^{-3}	12	8	0	0
Urea	3×10^{-4}	12	0	17	58
	10^{-3}	12	8	17	50
	3×10^{-3}	12	0	25	33
None	0	12	8	0	42
L-aspartic acid	3×10^{-4}	11	27	0	36
	10^{-3}	12	8	8	8
	3×10^{-3}	11	9	0	9
L-glutamic acid	3×10^{-4}	9	44	0	22
	10^{-3}	10	30	0	10
	3×10^{-3}	12	33	0	0
L-asparagine	3×10^{-4}	11	45	18	9
	10^{-3}	11	36	9	27
	3×10^{-3}	12	8	0	0
L-glutamine	10^{-3}	11	18	9	18
	3×10^{-3}	11	45	9	0

* Buds which did not develop over a size of 1—2 mm.

to the basal medium in the presence of kinetin (1 mg/l) and 2% sucrose. As described in the previous article (Nitsch and Nitsch, 1967), formation of buds in general was greatly enhanced by adenine. The production of flowers was promoted in approximately the same proportion (Table 7).

At the highest concentration tested (3×10^{-4} M), adenine increased the intensity of the color of the petals, resulting in the formation of deep red flowers as they are found in this *Plumbago* variety in nature. Otherwise, the color of flowers produced in test tubes was usually pink (Fig. 3).

Guanine was completely ineffective when used alone (Table 7), and so were uracil and cytosine at 10^{-7} to 10^{-4} M, thymine (10^{-7} to 10^{-5} M) and ribose (10^{-5} to 10^{-3} M).

When the same compounds were tested in the presence of 3×10^{-4} *M* of adenine, they enhanced slightly the adenine effect (Table 8). Thymine was especially effective in this respect, notably more than its precursor, orotic acid.

Effect of Certain Amino Acids, Amides and Urea. In addition to the nitrate ions present in the K medium, various nitrogenous compounds were incorporated in the culture media. At 3×10^{-4} *M* and above, however, all the amino acids and the two amides tested reduced the percentage of cultures forming inflorescences (Table 9). Urea, in contrast, had a promotive, although slight effect.

Discussion

The Induction of Flowering in Vitro. Flowering can be induced *in vitro* in excised internode sections of *Plumbago indica* devoid of any preformed bud (NITSCH and NITSCH, 1965). Since the cultured sections did not bear any leaves either, it seems that the photoperiodic effect can be exercised directly on the stem segments. Presumably, these contain enough phytochrome to be sensitive to daylength. These results are in accordance with those of BALDEV (1962) who showed that stem tips of another short-day plant, *Cuscuta reflexa,* could be induced *in vitro* by the proper photoperiod to produce flower buds. In the case of this parasitic plant, however, buds were initially present, and BALDEV surmised that they were the photoreceptors. The presence of roots is not required for floral induction, since most *Plumbago* cultures, even though they produced vegetative shoots or inflorescences, never developed roots.

The Role of Carbohydrates. The nature and, especially, the concentration of carbohydrates in the medium has a determining influence on the formation of vegetative *versus* reproductive buds under short days. An effect of these factors has been found also in the case of "floral expression" in segments excised from flowering stalks of tobacco (AGHION-PRAT, 1965) or of *Cichorium intybus* (NITSCH, 1966a, b).

The experiments in which part of the sucrose concentration had been replaced with mannitol (Table 3) indicate that the concentration effect is probably not osmotic in nature. Rather it seems to be a true metabolic one. NITSCH (1965a, 1966a) has advanced the hypothesis that flower formation may be associated with an enhanced activity of the pentose pathway. An excess of soluble sugars may increase the metabolic flow through this pathway.

Relatively high sucrose concentrations (8—10%) stimulate flower formation in intact seedlings of *Nicotiana rustica* (STEINBERG, 1950) and *Pharbitis nil* (TAKIMOTO, 1960; KIMURA, 1963) cultured *in vitro*. It is even possible to promote flower formation in some short-day plants by

removing the leaves and feeding the plants sugar, as shown by LONA with *Chenopodium amaranticolor* (1948) and *Perilla* (1950). All these results agree with the ideas of KLEBS (1913) that a relatively high level of carbohydrates is necessary for flowering. They also are in accordance with the results of LIVERMAN and BONNER (1953) who demonstrated that the "high-intensity-light reaction" necessary for floral induction in *Xanthium* can be partly replaced by supplying sucrose to the leaves.

If the C/N ratio is important for flowering, an increase in the level of nitrogen should reduce the production of flower buds. Preliminary experiments in which the level of inorganic nitrogen has been varied have not yielded conclusive results. The data obtained with individual amino acids and amides (Table 9) have shown a general reduction of the formation of inflorescences, but the percentage of cultures forming vegetative buds was reduced also. One exception stands out, however, that of urea, a compound rich in nitrogen which actually increased flowering (Table 9).

Effect of Purines and Pyrimidines. Adenine, which has been found to be essential for bud formation in *Plumbago* (NITSCH and NITSCH, 1966), supported also the development of flower buds. It seemed also to enhance anthocyanin formation in the flowers, since petals were of a more intense color when the media contained $3 \times 10^{-4} M$ adenine than when the adenine level was lower (Fig. 3). This result is reminiscent of that of THIMANN and RADNER (1962) who showed in *Spirodela oligorrhiza* that purines and pyrimidines were involved in anthocyanin formation. These authors also observed that kinetin decreased anthocyanin formation. It is possible, therefore, that *Plumbago* flowers grown *in vitro* were usually pink instead of red, as those of the clone "Angkor" are in nature, because kinetin was routinely added to the medium. A relatively high concentration of adenine was necessary to restore the full color of the petals.

A beneficial effect of purines and pyrimidines on flower induction has been reported by CHAILAKHYAN *et al.* (1961) in the case of *Perilla* apices cultured *in vitro*. These authors were able to obtain flower buds even under long days when the cultures were supplied with adenine, adenosine or kinetin. With guanosine and cytosine also some floral buds were formed, but they did not develop into flowers. Although we never obtained any inflorescence under long days, the results reported for excised *Perilla* apices agree with ours in showing that a suitable level of adenine and kinetin is beneficial to flower formation.

Effect of Auxins and Gibberellins. Auxins and gibberellins have been found to reduce, or totally inhibit, the production of inflorescences in *Plumbago* (Tables 4 and 5). This effect is not surprising since auxins are notorious for inhibiting flower induction in short-day plants (see NITSCH,

1965a). The only apparent exception, that of the pineapple (*Ananas comosus*), has disappeared since BURG and BURG (1966) have demonstrated that the effect of applied auxin in this case was to trigger off the production of ethylene, which acts as the actual flower-inducing agent. Auxins have also been found to inhibit the induction of flowering *in vitro* of a long-day species, *Cichorium intybus* (PAULET and NITSCH, 1964). In the case of isolated *Perilla* apices, auxins and gibberellic acid were reported to be likewise inhibitory to the induction of flowering (CHAILAKHYAN *et al.*, 1961; RAGHAVAN, 1961). IAA even suppressed the expression of the floral state in cultures derived from tobacco inflorescences (AGHION-PRAT, 1965).

Conclusion. The results obtained with excised, internodal segments of *Plumbago* fit with what is generally known about the factors which influence the flowering process, such as an adequate level of sugars, the presence of nucleic acid constituents, or the inhibitory effect of auxins and gibberellins. This system can thus be used as a tool to investigate further the biochemistry of the flowering process.

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Literature

AGHION-PRAT, D.: Néoformation de fleurs *in vitro* chez *Nicotiana tabacum* L. Physiol. végét. **3**, 229—303 (1965).

BALDEV, D.: *In vitro* studies of floral induction on stem apices of *Cuscuta reflexa* Roxb. — a short-day plant. Ann. Bot. **26**, 173—180 (1962).

BUI DANG HA, D., et J. P. NITSCH: Stimulation par l'isatine de la prolifération cellulaire chez le Topinambour. C.R. Acad. Sci. (Paris) **262**, 1235—1238 (1966).

BURG, S. P., and E. A. BURG: Auxin-induced ethylene formation: its relation to flowering in the pineapple. Science **152**, 1269 (1966).

CHAILAKHYAN, M. KH., R. B. BUTENKO, and I. I. LYUBARSKAYA: Effect of derivatives of nuclei acid metabolism on the growth and flowering of *Perilla nankinensis*. Fiziol. Rast. 8, 101—113 (1961).

CORNFORTH, J. W., B. V. MILBORROW, G. RYBACK, and P. F. WAREING: Chemistry and physiology of "dormins" in sycamore. Identy of sycamore "dormin" with abscisin II. Nature (Lond.) **205**, 1269—1270 (1965).

GANDAR, J. C., et J. P. NITSCH: Activités comparées de quelques auxines dérivées de l'acide phénylacétique. Bull. Soc. bot. France **106**, 441—445 (1959).

KIMURA, K.: Floral initiation in *Pharbitis nil* subjected to continuous illumination at relatively low temperatures. II. Effect of some factors in culture medium on floral initiation. Bot. Mag. (Tokyo) **76**, 351—358 (1963).

KLEBS, G.: Über das Verhältnis der Außenwelt zur Entwicklung der Pflanze. S.-B. Akad. Wiss. Heidelberg, Ser. B, Nr 5 (1913).

LIVERMAN, J. L., and J. BONNER: Biochemistry of the photoperiodic response. The high-intensity-light reaction. Bot. Gaz. **115**, 121—128 (1953).

LONA, F.: La fioritura della brevidiurna *Chenopodium amaranticolor* COSTE et REYN. coltivata in soluzione nutritiza con saccarosio, in assenza di stimulo fotoperiodico euflorigeno. Nuovo giorn. bot. ital., N.S. **56**, 559—562 (1948).
— Il significato dei glucidi e del fattore antiflorigeno nel meccanismo di fioritura della pianta erbacee ed in particolore delle brevidiurne. R.C. Ist. lombardo Sci. **83**, 1—22 (1950).
NITSCH, C., and J. P. NITSCH: The induction of flowering *in vitro* in stem segments of *Plumbago indica* L. I. The production of vegetative buds. Planta (Berl.) **72**, 355—370 (1967).
NITSCH, J. P.: Phytohormones et genèse des bourgeons végétatifs et floraux. *In*: Les Phytohormones et l'Organogenèse (R. BOUILLENNE, éd.), p. 265—299. Liège: 1965a.
— Deux espèces photopériodiques de jours courts: *Plumbago indica* L. et *P. zeylanica* L. Bull. Soc. bot. France **112**, 517—522 (1965b).
— L'induction de la floraison *in vitro:* résultats et perspectives. Mém. Soc. bot. France **113**, (in press) (1966a).
— Towards a biochemistry of flowering and fruiting: contributions of the "*in vitro*" technique. Proc. XIIth Int. Hort. Congr. **2**, (in press) (1966b).
—, et C. NITSCH: Néoformation de fleurs *in vitro* chez une espèce de jours courts: *Plumbago indica* L. Ann. Physiol. végét. **7**, 251—256 (1965).
PAULET, P., et J. P. NITSCH: La néoformation de fleurs sur cultures *in vitro* de racines *Cichorium intybus* L.: étude physiologique. Ann. Physiol. végét. **6**, 333—345 (1964).
RAGHAVAN, V.: Studies on the floral histogenesis and physiology of *Perilla*. III. Effects of indoleacetic acid on the flowering of apical buds and explants in culture. Amer. J. Bot. **48**, 870—876 (1961).
—, and W. P. JACOBS: Studies on the floral histogenesis and physiology of *Perilla*. II. Floral induction in cultured apical buds of *P. frutescens*. Amer. J. Bot. **48**, 751—760 (1961).
STEINBERG, R. A.: Flowering response of a variety of *Nicotiana rustica* to organic compounds in aseptic culture. Amer. J. Bot. **37**, 547—551 (1950).
STEVENINCK, R. F. M. VAN: Abscission-accelerators in lupins (*Lupinus luteus* L.). Nature (Lond.) **183**, 1246—1248 (1959).
TAKIMOTO, A.: Effect of sucrose on flower initiation of *Pharbitis*. Plant and Cell Physiol. **1**, 241—246 (1960).
THIMANN, K. V., and B. S. RADNER: The biogenesis of anthocyanins. VII. The requirement for both purines and pyrimidines. Arch. Biochem. **92**, 270—279 (1962).

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