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 $3 \times 10^{-4} M$ 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".

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.