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Effects of Some Growth Regulators on in vitro Flowering of *Streptocarpus nobilis*

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ABSTRACT

Leaf discs from vegetative *Streptocarpus nobilis* plants were cultured *in vitro* in media with cytokinin (BAP or K at 0.35 mg.l^{-1}) and auxin (IAA, NAA or 2,4-D at 0.1 mg.l^{-1}). Under short days (8-h photoperiod) in medium with IAA and BAP, floral buds developed in 100% of the cultures; under long days (16-h photoperiod) only shoots were formed. In medium with IAA and K, flowering was reduced. Flowers rarely formed in medium containing NAA and K, but roots developed profusely. NAA + BAP promoted leafy shoots which rarely flowered later. The effect of 2,4-D was to inhibit flowering completely and to induce callusing and formation of teratomous structures. *Abreviations:* BAP = 6-benzylaminopurine; K = kinetin; IAA = indoleacetic acid: NAA = naphthaleneacetic acid; 2,4-D = 2,4-dichlorophenoxyacetic acid.

INTRODUCTION

The use of *in vitro* techniques to study the flowering process is very convenient especially in species in which this process is controlled by photoperiodism and/or vernalization. In fact, by using such an experimental system, floral induction and all further events after the inductive stimulus can be made to occur in a single plant fragment such as a root, stem segment, or leaf piece (Nitsch *et al.* 1970).

Among the more suitable species to study *in vitro* flowering is *Streptocarpus nobilis*, an African gesneriad. In this short-day species, aspects of flower induction *in vivo* were studied by Nitsch (1967), Handro (1976), Handro and Monteiro-Scanavacca (1978), and Simmonds (1982). The *in vitro* neoformation of flower buds in leaf discs of this species were firstly reported by Rossini and Nitsch (1966). A more extensive paper was published in 1970 by Rossini, where some characteristics of *in vitro* flowering were studied as well as the effects of some growth regulators. Handro (1977a) described some histological aspects of *in vitro* bud neoformation and also the responses of different kinds of explants submitted to photoperiodic induction (1977b). Recently, Simmonds (1982) reported the morphogenetic responses of leaf explants cultured on media differing in mineral composition, sucrose and cytokinin content.

Despite all the available information, several points related to the control of *in vitro* flowering in *S. nobilis* remain unknown. This paper provides additional information on the *in vitro* flower induction in leaf discs of this species, mainly on the effects of different auxins on the organogenesis.

MATERIAL AND METHODS

Plants of *S. nobilis* were produced from seeds and maintained in a vegetative state in a greenhouse under 16-h photoperiod. Plants with about 10 leaf pairs were used as the source of the experimental material. Leaf explants were taken from between the 3rd and 7th node from the shoot apex. Leaves were sterilized in 5% calcium hypochlorite for 5 min, then rinsed in sterile distilled water. Leaf discs 1 cm in diameter and including the midrib were excised near the petiole with the aid of a corkborer and placed on the medium with the adaxial surface upwards. The basal medium employed in the experiments was described by Handro (1977a). Auxins such as IAA, NAA and 2,4-D when used were at 0.1 mg.l^{-1}, and BAP and K at 0.35 mg.l^{-1}. Before gelling with 0.8% Difco Bacto-Agar the pH was adjusted to 5.5. The cultures were kept under either short days (8-h photoperiod) or long days (16-h photoperiod), at 28°C during the light period, and 22°C in the dark period. Light was supplied by Philips Special Day Light fluorescent tubes complemented by incandescent lamps, giving about 5000 lux of intensity. Treatments were performed with 12 cultures each and repeated at least once.

RESULTS

Number of short days for flowering – Leaf discs from vegetative plants were cultured in the basal medium supplemented with BAP and IAA, under 8-h photoperiod. Every two days up to the 24th day, one set of 12 cultures was transferred to long days (16-h light). After 8 weeks of culture the results were recorded (number of cultures that produced flowers).

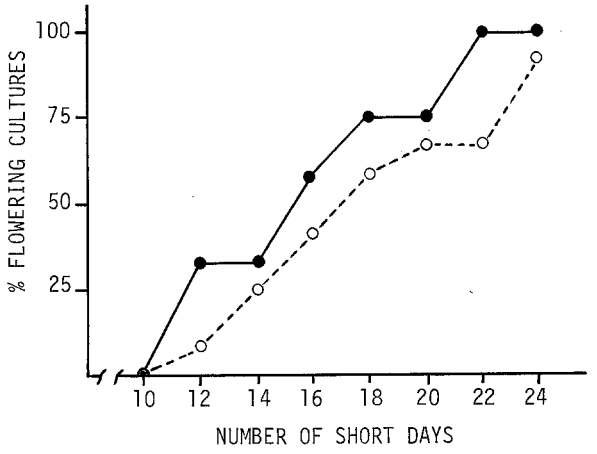

Fig. 1 - Effect of the number of inductive short days on flower initiation in leaf discs of *S. nobilis*. Results of two experiments.

Fig. 1 includes the results of two different experiments, showing that 10 short days were unable to induce flowering but with 12 short days some cultures flowered. With 24 short days almost 100% of cultures produced flowers or flower buds. Typical cultures after long or short-day treatment are shown in Fig. 2 and 3.

Effects of auxins on organogenesis - Three different auxins, IAA, NAA and 2,4-D at 0.1 $mg.l^{-1}$ were added to the basal medium containing either K or BAP (0.35 $mg.l^{-1}$). A control without auxin was also kept in culture. All cultures were maintained for 4 weeks under short days then transferred to long days. The results were recorded after 6 weeks of culture. The responses for the three auxins used were markedly different: when no auxin was incorporated to the medium, flower buds were rarely formed, these that did were small in size, or developed late. In medium with IAA + BAP, flower buds developed in the proximal region of the midrib, producing several flowers, generally without production of vegetative buds (Fig. 3). NAA + BAP promoted regeneration of small vegetative shoots all around the explant; most of them flowered some weeks later. When IAA was combined with K, the number of flower buds was reduced as compared with the effect of BAP. On the other hand, NAA combined with K induced rooting all around the explant, and rarely flowers were produced in this situation (Fig. 4).

The effects of 2,4-D were similar either when combined with BAP or K, and under short or long days. With this treatment leaf discs formed a yellowish callus on their surface and furthermore developed several teratomous structures. These structures are of two types: shoot-like with abnormal or reduced leaves up to one centimeter in length, or smaller and translucent bud-like structures, grouped or isolated, into different forms (cylinders, bottles, bunches, clubs etc), and rarely exceeding 3 mm in length (Fig. 5-7). Such structures did not develop into flowers or vegetative shoots and remained in their original form and size until the senescence of the cultures. Even after sectioning and preliminary examination of their structure, the vegetative or floral nature of the teratomata could not be determined.

DISCUSSION

Almost all previous reports on *in vitro* flowering of *S. nobilis* showed that the latter is easily achieved by using well defined conditions of culture, established by Rossini (1970). These conditions included low macronutrient medium (Knop) and the presence of a cytokinin and IAA. Our results also showed that IAA is necessary for a high production of floral buds. Recently Simmonds (1982) using leaf explants from induced plants, reported that IAA was inhibitory for *in vitro* flowering. Reports found in the literature (see Simmonds 1982) have shown that the effects of IAA on *in vitro* flowering are either inhibitory or stimulatory. It is difficult to explain these contradictory results. Perhaps they are due to the differences between the experimental systems employed, i.e. root or stem segments, leaf pieces, thin cell layers, etc. which originated in some cases from vegetative, and in others from flowering plants.

To understand such contradictory results, particularly the *in vitro* flowering of *S. nobilis*, we must consider the possibility that there may be inherent differences in explants taken from either induced or vegetative plants. If tissues from vegetative plants are placed into a culture, in the overall process of *in vitro* flowering three steps must occur: a) a wound reaction at the cut surfaces of the explant varying according to the auxin in the medium (see Aitchison *et al.* 1977, Yeoman and Forche 1979); b) the photoperiodic stimulus received by phytochrome, thus leading to alterations in cell metabolism, and conditioning a peculiar kind of morphogenesis (see Smith 1975); c) the regeneration of a bud from newly meristematic or dedifferentiated tissues (see Reinert *et al.* 1977, Handro 1977a). These steps must occur in part concomitantly, and exogenously added growth substances could be either stimulatory or inhibitory depending on the phase of the culture. As demonstrated in this paper a clear relationship exists between the number of inductive short days and the flowering response of leaf discs. Thus, the physiological and biochemical conditions of a leaf disc could be quite different on the first day of culture compared to the fifth or the thirteenth day. Since step *b* occurred previously in the induced plants and since the conditions of the explant at the onset of culture might be different, different responses are conditioned.

Thus, a better approach to the interpretation of the process of *in vitro* flowering might be the sequential isolation of the different steps during the time of culture, especially for the purpose of studying the effects of growth regulators.

Regarding the effect of other auxins such as NAA and 2,4-D which have never been used in *S. nobilis* cultures, they produced characteristic morphogenetic responses but inhibited flowering. The root-inducing capacity of NAA is well known and the occurrence of teratomous organ-like structures such as

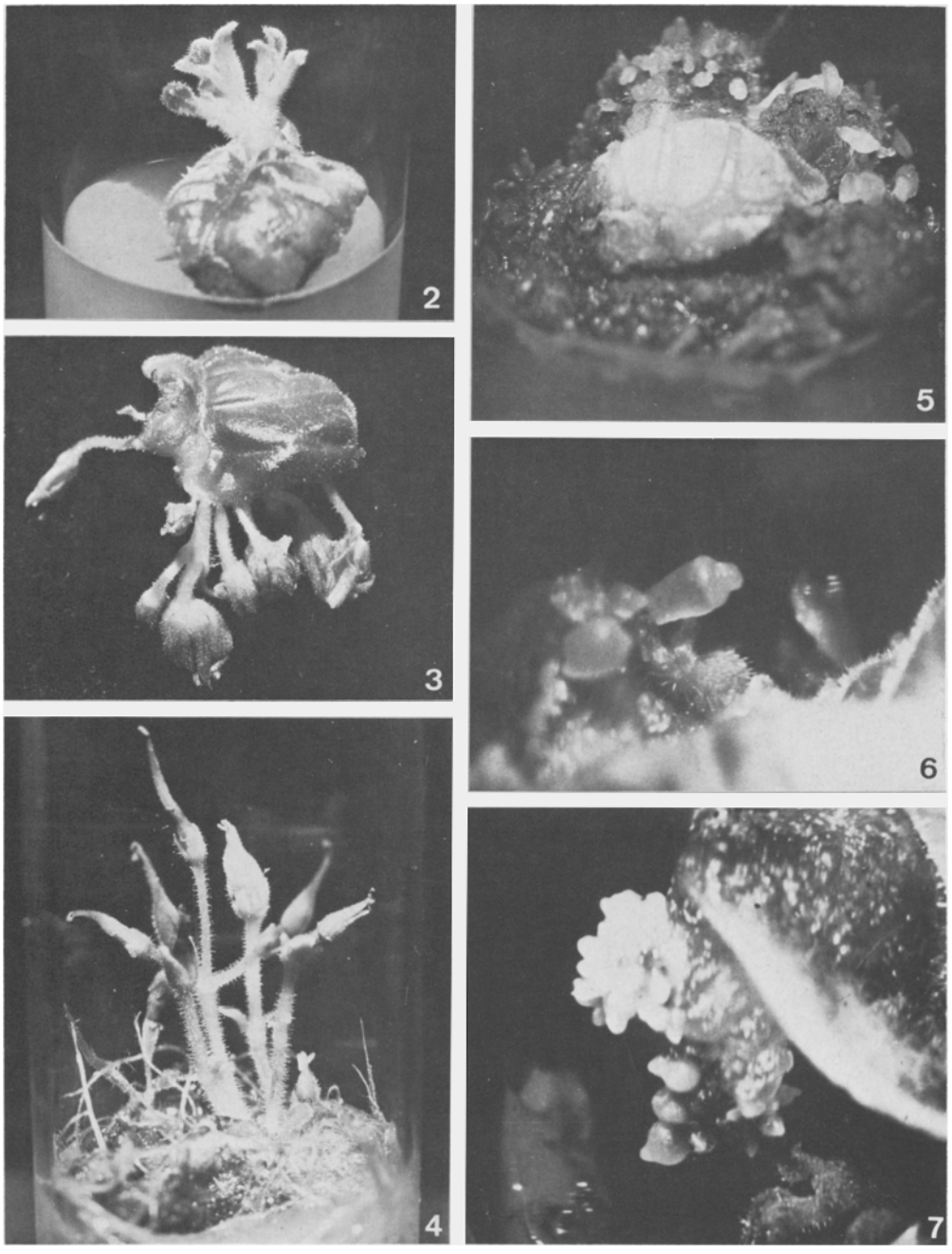

Fig. 2 - IAA-treated culture, under long days. Fig. 3 - IAA-treated culture, under short days. Fig. 4 - A rare NAA-treated culture that produced flowers. Fig. 5-7 - 2,4-D-treated cultures showing teratomous structures.

those produced in 2,4-D-treated cultures are occasionally reported in the literature (e. g. Braun 1959; Bowes 1971, 1975). However in these cases, they were not induced by 2,4-D. Similar responses were not found in leaf discs of *Streptocarpus x hybridus* treated with 2,4-D; this auxin poorly stimulated root and bud formation, when compared with other auxins (Apelgrenn and Heide 1972). Although 2,4-D has a high capacity of inducing embryoids or embryo-like structures (see Evans *et al.* 1981) such structures were never observed in our cultures.

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