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The effect of photoperiod and growth regulators on organogenesis in thin-layer tissues

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Abstract

Thin layer tissue cultures are good model in studies of direct induction of various developmental programmes, particularly in floral determination. Despite an increasing number of related investigations, the factors that regulate flower formation *in vitro* are not yet clear. To evaluate the role of growth regulators in flower and stem meristem genesis, we determined the ratio of regenerating vegetative shoots and flowers as well as the dependence of the growth of regenerated organs on cultivation conditions. Genesis of vegetative organs ocurred after two to three days of cultivation in media with growth regulators. Flower buds appeared after three to four days of cultivation. The number of vegetative shoots continued to increase with the extension of growth regulator exposure from two to six days. However, the number of flowers did not change. The elimination of the stimulus after the initiation of the process showed an increase in the number of organs.

Key words: organogenesis, growth regulators, photoperiod, tobacco, thin-layer tissues.

Introduction

Plant growth and morphogenesis are controlled by meristems, organized tissues containing pluripotent stem cells whose identities and activities are regulated by intrinsic and environmental signals. Molecular studies have shown that the identity of the apical meristem and genesis of floral organs are governed by a network of gene expression, which depends on the interrelation between organs and tissues (Adams et al. 2001; Sharma, Fletcher 2002). However, these ordinary correlation links are disrupted in experiments performed with tissue explants cultivated *in vitro*. Thin-layer tissues are cell groups of the same differentiation level, e.g. subepidermal cells which *in vitro* can form roots, flower- or vegetative buds as well as irregular meristems without any intermediate callus, or can proliferate into callus without any subsequent organogenesis (Tran Thanh Van 1973). The aim of the present study was comparison of the effect of photoperiod and growth regulator exposure on flower and stem meristem genesis.

Materials and methods

The experiments were carried out with tobacco tissues having different photoperiodism

traits: long-day flowering garden tobacco – *Nicotiana alata* Link et Otto and photoperiodic neutral common tobacco – *Nicotiana tabacum* L. cv. 'Samsun'. Intact plants were cultivated under greenhouse conditions in a 16-h light photoperiod. Flower stalk thin-layer tissue explants of tobacco at stage IX of organogenesis (Kuperman 1984) were used. Thin-layer tissues composed of epidermis and three to six subepidermal cell layers were excised from flower stalks of *N. alata* and *N. tabacum*. The initial mass of the explant was 14 ± 3 mg. The tissues were cultivated for 30 days under two types of photoperiod: 16 h long-day and 8 h short-day light of 40 mM m^{-2} s^{-1} under cool white fluorescent tubes at 25 °C.

The basal cultivation medium contained Murashige and Skoog mineral salts (MS; 1962), 0.1 mg l^{-1} thiamine HCl, 100 mg l^{-1} myoinositol and 30 g l^{-1} glucose and 8 g l^{-1} agar (Altamura et al. 1998). A pH was adjusted to 5.7. The medium was supplemented with 1 μM indole-3-acetic acid (IAA) and 1 μM 6-benzylaminopurine (BA) for flower and vegetative bud induction. After 1, 2, 3, 4, 5, 6, 14 days of stimulation media with growth regulators, thin-layer explants were transferred to MS without growth regulators and cultivated up to 30 days. One of the samples of the performed experiments was cultivated in media supplemented with IAA and BA up to 30 days. The control consisted of tissues cultivated in media without growth regulators. Each experiment was carried out twice and at least 10 explants were used per test. The number of newly formed buds in an explant was estimated in relation to vegetative and to floral buds. The cultures were tested under the microscope to count the number of flower and vegetative buds formed and to determine the developmental stage of the regenerated organs. The average number of structures with differences statistically significant at $p \leq 0.05$ was estimated.

Results

The cultivation of thin-layer flower stem tissues with growth regulators in a long and short photoperiod indicated the dependence of flower regeneration on both photoperiodic reaction and photoperiod. In isolated tissue cultures the formation of flowers, similar

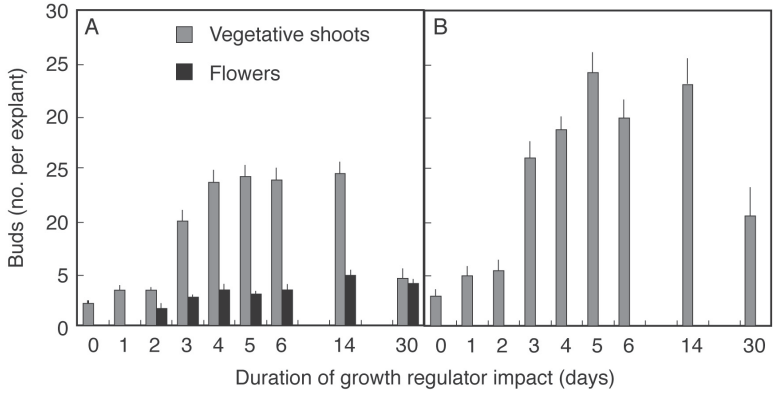

Fig. 1. Organogenesis in long day plant *Nicotiana alata* flower stalk thin-layer tissues in relation to the photoperiod and growth regulators. A, long day conditions; B, short day conditions. The number of days of thin-layer tissues cultivation on stimulation media with growth regulators is shown. After cultivation, the explants were transferred to MS medium without growth regulators and incubated up to 30 days. 0, control, explants 30 days cultivated in MS. Each experiment was carried out twice and at least 10 explants were used per test. The bars represent SE.

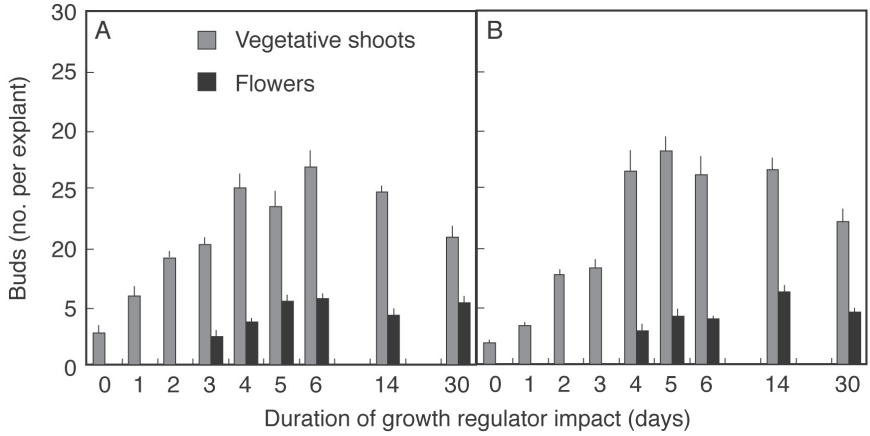

Fig. 2. Organogenesis in photoperiodic neutral *Nicotiana tabacum* 'Samsun' flower stalk thin-layer tissues in relation to the photoperiod and growth regulators. A, long day conditions; B, short day conditions. The number of days of thin-layer tissues cultivation on stimulation media with growth regulators is shown. After cultivation, the explants were transferred to MS without growth regulators and incubated up to 30 days. 0, control, explants 30 days cultivated in MS. Each experiment was carried out twice and at least 10 explants were used per test. The bars represent SE.

to vegetative shoots, needed an additional growth regulators stimulus. Initial (two to tree days) cultivation in media with IAA and BA was sufficient to evoke generation of vegetative organs, as compared with the control (Fig. 1, 2). Explants cultivated for three days in media with growth regulators produced five times more vegetative buds in long day and four times more in short day. Flower genesis was modified by a longer two-to-four day growth regulator exposure. In thin-layer flower-stem cultures the number of vegetative shoots continued to increase with the extension of growth regulator exposure from two to six days. However, the number of flowers did not change. A larger number of vegetative buds was observed after four days of cultivation in growth regulator media. Flower regeneration was less dependent on the media composition. Continuous cultivation of flower-stem tissues with growth regulators reduced regeneration of vegetative shoots.

Tissues of the long-day plant *N. alata* formed flowers (maximum was 4.58 ± 0.56 flowers per explant) only in a long day regime while only vegetative shoots were induced in a short day. Tissue of the *N. tabacum* 'Samsun', witch has a photoperiodic neutral reaction, formed flowers both under long- and short-day lighting, with maximum numbers 5.54 ± 0.33 and 4.36 ± 0.31 flowers per explant, respectively.

Discussion

Regions of dividing cells form meristematic centres and meristems, the morphogenesis of which depends on factors that modify the realisation of genetic information (Sharma, Fletcher 2003). Flower and vegetative shoot formation in thin-layer flower stem tissues requires a shorter exposure to growth regulators as compared with leaf tissues (Šauliene, Raklevičiene 2002). The observed increase in the number of vegetative organs as a response to removing growth regulators from the medium (Fig. 1, 2) suggests that growth regulator excess caused by *de novo* synthesis suppresses the morphogenesis. It is also possible that

growth regulator decomposition in the medium and the appearance of phenol compounds in the course of long-term cultivation could suppress the formation of new meristems (Scaramagli et al. 1999; Mizukami, Fischer 2000; Weyers, Paterson 2001; Torrigiani et al. 2003). Ways to enhance organogenesis may be to shorten the phytohormone effect of the medium as one of the possible reasons for the genetic instability of the regenerated organs and, after stimulation, to cultivate the tissues in hormone-free media.

Our experiments showed the importance of the photoperiodic reaction of a plant and photoperiod in flower genesis. The hypothesis that tobacco flower-stem tissues, in contrast to stems, possess a predetermined number of cells that can form flower meristems (Rajeevan, Lang 1993), is consistent with our results: as these cells are inactive under unfavourable photoperiod conditions and when growth regulators are lacking. These findings corroborate the hypothesis that the degree of tissue differentiation is one of decisive factors of flower regeneration in tissue cultures (Rajeevan, Lang 1993). It is reasonable to suggest, that at the beginning of flowering (gamete initiation), the complex modifying the photoperiodic reaction is labile; therefore, its manifestation does not depend on the photoperiod. The performed experiments showed that in tissues isolated from tobacco plants at a later stage of organogenesis the photoperiodic memory is a characteristic feature of the explant donor plant and is preserved in the first *in vitro* subculture.

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