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BRIEF COMMUNICATION

Effect of photoperiod and chlorogenic acid on morphogenesis in leaf discs of *Streptocarpus nobilis*

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Abstract

Leaf discs from vegetative plants greatly increase their phenolic content when cultivated *in vitro*. Under long days the values remained constant, and were higher when compared with short days cultures. Under short days total phenolics decreased after 10 d, corresponding to the induction and expression of *in vitro* flowering. The effect of photoperiod and chlorogenic acid (0.01 mM) on leaf discs cultured from induced and non-induced plants, were analyzed regarding the neo-formation of roots, as well as vegetative and flower buds. Chlorogenic acid enhances the regeneration of roots in all treatments tested, with the highest stimulation on induced leaf discs cultivated in short days. The flowering was not affected by chlorogenic acid, but an inhibitory effect was observed on the neo-formation of vegetative buds in non-induced explants maintained in short days. Vegetative buds were reduced by 50 % in flower-induced leaf discs cultivated under short days.

Additional key words: flowering, phenols, rooting.

Several studies have demonstrated that phenolic compounds play a relevant role in the control of morphogenetic processes in plants. Effects noted from adding phenols to culture media are mainly on enhancement of callus growth, more effective adventitious shoot formation, the improved rooting of shoots, and a higher rate of shoot proliferation in certain bud cultures (George 1993).

Chlorogenic acid, a natural constituent of plants, is a strong reducing agent and a negative cofactor of IAA-oxidase (Gaspar 1965), which has been added regularly to media containing auxin to promote *in vitro* shoot rooting in *Beta vulgaris* (Margara 1977), *Brassica napus* (Margara and Leydecker 1978), *Prunus cerasifera* (Hammerschlag 1982), and tuberization in *Solanum tuberosum* (George and Sherrington 1984). Chlorogenic acid can replace the photoperiodic effect for *in vitro* flowering of *Lens culinaris* (Gaspar *et al.* 1968) and enhances floral bud neo-formation in *Cichorium intybus* and *Plumbago indica* (Paulet and Nitsch 1964, Paulet 1965, Nitsch 1967).

A close relationship between the positive or negative action of phenolic effectors and peroxidases was proposed to be correlated with different aspects of growth, such as auxin metabolism, ethylene biosynthesis and cell wall lignification (Gaspar *et al.* 1991). As the endogenous levels of IAA are under the influence of peroxidase, a relationship between these compounds and IAA in the control of morphogenetic processes, such as flowering and rooting, could be envisaged.

This paper provides additional information on the induction of morphogenesis in leaf discs of *S. nobilis*, mainly regarding the effects of photoperiod and chlorogenic acid.

Plants of *Streptocarpus nobilis* C.B. Clarke were obtained from seeds and kept vegetative since germination under long-day conditions (16-h photoperiod) or induced to flower under short-days ones (8-h photoperiod). Leaf discs (10 mm in diameter) from plants with eight leaf pairs were prepared from leaves previously sterilized with 5 % sodium hypochlorite (5 min) and cultured on a medium containing Knop's

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macroelements (Gautheret 1959), microelements and vitamins (Nitsch and Nitsch 1965), 0.35 mg dm^{-3} 6-benzylaminopurine (BAP), 0.1 mg dm^{-3} indole-3-acetic acid (IAA), 1.5 mg dm^{-3} adenine, and 20 g dm^{-3} sucrose. The pH was adjusted to 5.5 and then the medium was gelled with 0.8 g dm^{-3} agar. Chlorogenic acid (0.01 mM) was added after filtration through *Millipore* membranes after autoclaving. The cultures were kept either under 16- or 8-h photoperiod, photon flux density of 150 µmol m^{-2} s^{-1} (day-light fluorescent lamps), and temperature of 26 ± 1 °C. After 12 weeks, the number of vegetative and floral buds were counted. Each treatment consisted of 20 culture tubes (25 × 125 mm) with one explant in each, and was repeated once again, with similar results.

Phenol extractions were performed according to Wescott and Henshaw (1976) and their dosage done according to Folin and Denis (1912) using chlorogenic acid (10 - 20 µg) as standard.

Leaf discs from vegetative plants greatly increase their phenolic content when cultivated *in vitro*. Under long days the values remained constant, and were higher when compared with short-day cultures. Under short days total phenolics decreased after 10 d (Fig. 1), corresponding to the phases of induction and expression of *in vitro* flowering.

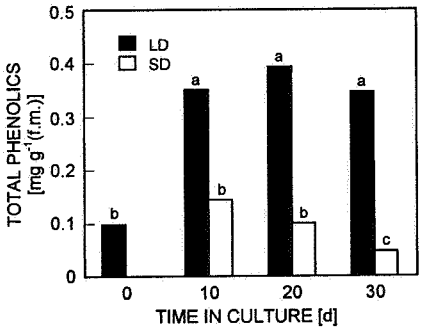

Fig. 1. Variation of phenolic contents in leaf discs from vegetative plants cultivated *in vitro*, under long days (LD) and short days (SD). Different letters indicate significant difference among treatments.

The natural rate of formation of some phenolic compounds has been observed to be dependent on the rate of growth of cultured tissues (Barz and Hoesel 1979) and on the auxin and cytokinin concentrations in the medium (Sargent and Skoog 1960, Skoog and Montaldi 1961). The occurrence of phenolics has frequently been correlated with the morphogenetic capacity of tissues (Tryon 1956, Hackett 1970, Kefeli and Kadyrov 1971). Rawal and Mehta (1982) found that shoot formation from haploid tobacco callus occurred when the content of natural phenolic substances declined, while cellular differentiation occurred with increasing phenolic accumulation.

Explants from vegetative plants cultured under long days, produced only vegetative leaf buds, whereas under short days they were induced to flower *in vitro*, producing also floral buds (Fig. 2). Explants from induced plants expressed the pre-existing floral state in the mother plant, and produced vegetative and floral buds, proving that the induction was stable in the leaf discs, as previously described (Handro 1976, Simmonds 1985). The highest flowering was observed when the cultures were kept under short days, independently of the previously photoperiodic conditions *in vivo*. Explants from induced plants cultured in short days showed the lowest neo-formation of roots, and the highest flower and vegetative bud neo-formation (Fig. 2).

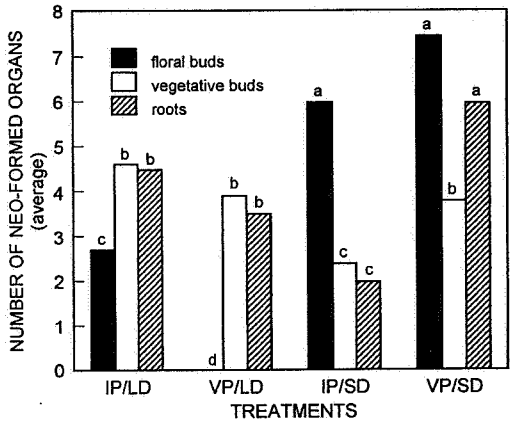

Fig. 2. Average number of neo-formed organs (floral buds, vegetative buds and roots) *in vitro*, in leaf discs originated from vegetative (VP) and induced plants (IP), and cultured under long days (LD) and short days (SD). Treatments: donor plant condition/photoperiodic condition of *in vitro* culture. Different letters indicate significant difference among treatments.

The neo-formation of vegetative and flower buds was not affected by the presence of chlorogenic acid (Fig. 3). On the other hand, chlorogenic acid incorporated to the medium, clearly enhances the neo-formation of roots in all the treatments tested. The highest stimulation was observed on leaf discs from flower induced plants cultured in short days, where rooting was five times higher than in untreated explants (Fig. 3*C*). Similarly, Hammerschlag (1982) was able to root 100 % of *Prunus cerasifera* shoots in light when chlorogenic and IAA were present in the medium, but only 30 % formed roots in response to IAA alone. Shoots kept in darkness all respond to IAA alone. In apple (Druart *et al.* 1982) and *Sequoiadendron giganteum* (Monteuuis *et al.* 1987) peroxidase activity in the shoots was the inverse of phenol content. Low phenol contents occur at the root induction phase, but higher contents occur when roots were initiated (Berthon *et al.* 1990).

Leaf discs from induced and non-induced plants will reflect their inherent differences at the onset of culture leading probably to different responses when exposed to different photoperiodic conditions, growth regulators and phenolic compounds. Our results suggest that the

understanding of the effects of photoperiod and phenolics on rooting and flowering in explanted leaf discs of *S. nobilis* must take into account the relationship between light and phenolic biosynthesis, and their effects on peroxidase activity and endogenous IAA content.

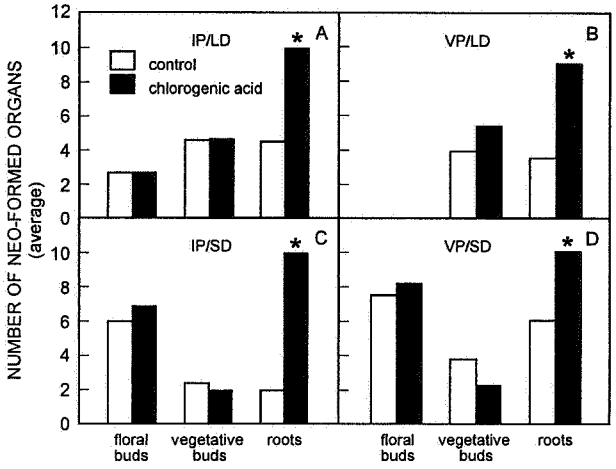

Fig. 3. Effect of chlorogenic acid (0.01 mM) on the percentage of neo-formed organs in leaf discs explanted from vegetative (VP) and induced plants (IP), cultured under long days (LD) and short days (SD). * - significant difference in relation to control at $P < 0.05$.

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