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PHOTOPERIODIC INDUCTION OF FLOWERING ON DIFFERENT EXPLANTED TISSUES FROM *STREPTOCARPUS NOBILIS* CULTURED *IN VITRO*

INDUÇÃO FOTOPERIÓDICA DA FLORAÇÃO EM DIFERENTES TECIDOS ISOLADOS DE *STREPTOCARPUS NOBILIS* CULTIVADOS *IN VITRO*

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SUMMARY - Different kinds of explanted tissues from vegetative plants of *Streptocarpus nobilis* were cultivated *in vitro* under flower inducing photoperiods, and their ability to flowering compared. Isolated shoot apices and seedlings were able to produce flowers about 10 weeks after planting. Stem, petiole and leaf midrib segments rarely showed direct flower neo-formation as leaf discs do. In these cases, leafy buds were formed firstly, and the flowers developed 10 - 12 weeks later, in the neo-formed shoot.

RESUMO - Differentes explantes de plantas vegetativas de *Streptocarpus nobilis* foram cultivados *in vitro* em condições fotoperiódicas indutoras de floração, e comparada sua capacidade para florescer. Ápices caulinares e plântulas são capazes de florescer após 10 semanas em cultura. Segmentos de caule, pecíolo ou nervura mediana da folha raramente mostram neoformação de gemas florais diretamente do explante, como acontece nos discos foliares. Geralmente gemas com folhas diferenciam-se primeiro, e então estes meristemas tornam-se florais, ocorrendo a floração 10 - 12 semanas após o início da cultura.

INTRODUCTION

The *in vitro* flower induction on leaf discs of *Streptocarpus nobilis* C.B. Clarke (a short day plant) was reported by Rossini and Nitsch (1966) and Rossini (1970). Some histological features of the *in vitro* neo-formation of flower buds in this species were also studied (Handro, 1977). Flower induction on different explanted plant tissues from several species has been studied also: Paulet and Nitsch (1964) showed that root fragments from vegetative plants of *Cichorium intybus* were able to be induced to flowering; Pierik (1966) obtained flower induction by cold treatment on petiole segments of *Lunaria annua;* Harada (1967) reported flower induction on excised shoot apices of *Chrysanthemum* and *Pharbitis;* Nitsch and Nitsch (1967), working with stem segments of *Plumbago indica* succedeed in obtaining flower neo-formation induced by short photoperiods, and Culafic (1973) showed that isolated buds of *Spinacea oleracea* could be induced to flower *in vitro*.

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This paper reports the results obtained when different explanted tissues from vegetative plants of *Streptocarpus nobilis* were submitted to photoperiodic induction to flowering *in vitro*.

MATERIAL AND METHODS

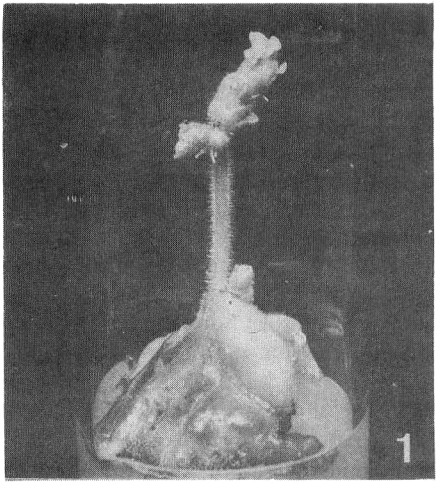
The experiments were carried out using explants excised from whole plants raised in a greenhouse, or from plants cultured aseptically from seeds, maintained vegetative under long day condition (16 hours of light). In the case of plants cultured in the greenhouse, leaves and apical parts were sterilized by soaking for 5 min in 5 per cent Calcium hypochlorite, and then washed in sterile water. Discs 1 cm in diameter or midrib sections 1 cm long were excised from leaves and planted in culture tubes. Apical parts were prepared for a final size of about 2 mm long, and then planted. Plants growing aseptically in large Erlenmayer's flasks, obtained from previously sterilized seeds sown on the culture medium, were used for flower induction or maintained vegetative to be used as a source of stem explants. The medium used in our experiments contained Knop's macroelements (Gautheret, 1959), microelements and vitamins (Nitsch and Nitsch, 1965), sucrose (20 g/l), benzyladenine (400 μg/l), indoleacetic acid 100 μg/l) and adenine (1.5 mg/l). The pH was adjusted to 5.5 and the medium was then gelled with 0.8 per cent Difco Bacto-agar. Auxins and cytokinins were omitted in the cultures of whole plants or apices. Murashige and Skoog's macroelements (1962) where employed with the same results.

RESULTS AND DISCUSSION

When leaf discs were cultured on adequate medium under short days (8 - 10 hours of light) flower buds were neo-formed, the flowers reaching complete development in about 6 - 8 weeks (Figure 1). Under long day condition (16 hours of light) only leafy buds were produced (Figure 2). To be compared with the leaf discs response, other kinds of explants were tried. Shoot apices 2 - 3 mm long (Figure 3) from vegetative plants were cultured on medium without growth regulators, under short days. The shoots developed producing leafy lateral buds and apical mature flowers, 8 - 10 weeks after planting (Figure 4). Stem and petiole segments were also explanted from aseptically cultivated plants. Generally vegetative buds appeared firstly and flowering occurred only on the neo-formed shoot, at about 8 - 10 weeks after planting. The neo-formation of floral buds without prior vegetative bud differentiation was rarely observed in this case, and the flowers never reached a complete development. When midrib segments from mature leaves were cultured *in vitro*, in some cases flower buds were neo-

Fig. 1 – Disco foliar 7 semanas em dias curtos, mostrando uma flor e botões. Fig. 2 – Disco foliar cultivado em dias longos, com diversos eixos vegetativos. Fig. 3 – Ápice isolado de planta vegetativa. Fig. 4 – A mesma cultura da figura 3, 10 semanas mais tarde. Fig. 5 – Segmento de caule após 10 semanas sob dias curtos, mostrando um botão floral. Fig. 6 – Plantinha com cotilédones e botoões florais, 12 semanas após a germinação da semente.

Fig. 1 – Leaf disc cultured 7 weeks under short days, showing one flower and buttons. Fig. 2 – Leaf disc cultured under long days, with vegetative shoot. Fig. 3 – Excised apex from a vegetative plant. Fig. 4 – The same culture shown in fig. 3, 10 weeks later. Fig. 5 – Stem segment after 10 weeks in culture under short days, showing a mature floral button. Fig. 6 – Plantlet with cotyledons and floral buttons, 12 weeks after seed germination.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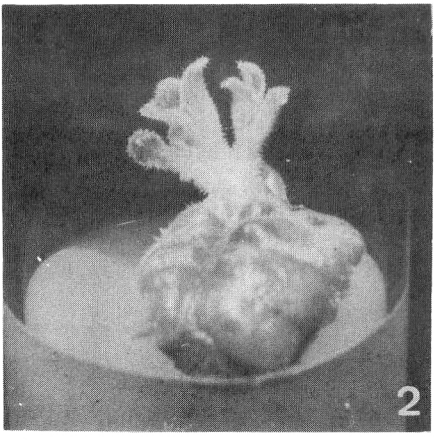
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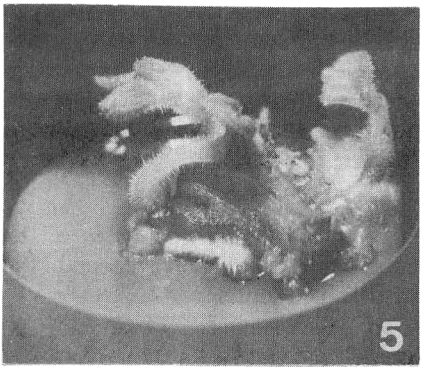
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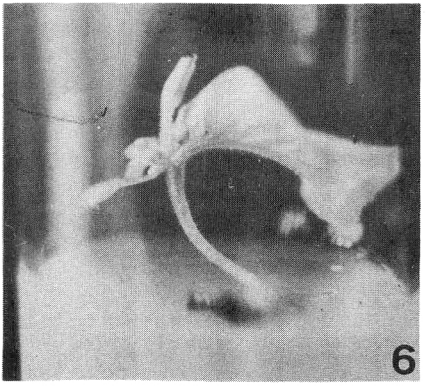
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-formed directly on the explanted tissue and succeeded in forming mature buttons (Figure 5) and flowers. From seeds sown on medium without growth regulators, flowering plants were obtained after 10 weeks in culture under short days (Figure 6). These plants presented only the characteristic distinct cotyledons and several flowers.

Our results showed that leaf discs, in this species, seem to be the most convenient system to study flowering *in vitro* for its fast response and complete development of the neo-formed organs. It should be remarked that in explanted tissues where buds are absent (leaf discs, stem and petiole segments, or leaf midrib) two distinct processes occur: photoperiodic induction with production of a stimulus to flowering, and a process of bud neo-formation, whereas in the shoot apex segments, or in seedlings, the only process is a photoperiodic induced change in the developmental pattern of a pre-existing vegetative meristem leading to a floral bud. Although shoot apices and seedlings have apical meristems, flowering is slower as compared with leaf discs. These differences may be ascribed to a distinctive sensitivity of the receptor for the photoperiodic stimulation: leaf discs were explanted from mature leaves and could have higher capacity to be stimulated than imature leaves as those in the shoot apex or seedlings. Handro (1976) showed that seedlings could be induced to flowering but the development of complete flowers is slower than in mature plants. Meanwhile, in other species as *Pharbitis*, the response of excised shoot apices is fast (Harada, 1967). This fact could be related to the ability of some species to be induced to flowering having only a small quantity of receptor tissue as *Pharbitis*, *Chenopodium* etc. (Lang, 1965). On the other hand, stem and petiole segments from *Streptocarpus* were unable to flower as easily as leaf discs. Since leafy buds are nornally produced by stem and petiole segments, it is suggested that their inability to form flower buds is mainly related to the photoperiodic stimulus.

The observed responses in other systems than leaf discs suggest further studies to explain how the sequential steps of flower initiation and its expression occur, and how variations in the inductive conditions or in the culture medium may affect the flower initiation in different tissues.

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