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PROTOPLASMA

Dark Requirement for Cell Regeneration and Colony Formation by Mesophyll Protoplasts of *Nicotiana plumbaginifolia* Viv.

Brief Report

RAVINDER GILL *, A. RASHID, and S. C. MAHESHWARI

Department of Botany, University of Delhi, Delhi

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Summary

The protoplasts of *Nicotiana plumbaginifolia* required darkness for cell regeneration and colony formation. Maximal plating efficiency of the protoplasts could be achieved by keeping the cultures in dark instead of light or dark/light sequence. Only two days of darkness prior to the illumination at 400 or 3,000 lux resulted in appreciable plating efficiency, than those of light from the beginning, but these values could not match the high plating efficiency in total darkness.

Keywords: Nicotiana; Dark requirement; Protoplast regeneration.

1. Introduction

The role of light in cell regeneration and colony formation by mesophyll protoplasts is not clear and at times controversial. NAGATA and TAKEBE (1971) found continuous light of 2,300 lux to be satisfactory for the division of *N. tabacum* mesophyll protoplasts. In a more detailed study of light effects by ENZMANN-BECKER (1973) on the same plant an initial incubation at 400 lux for 48 hours followed by 3,000 lux resulted in better plating efficiencies. In addition, different species of *Nicotiana* are reported to have different requirements of light (BANKS and EVANS 1976). In view of these reports it was in order to investigate the effect of light on the regeneration of protoplasts of *Nicotiana plumbaginifolia* which we had sometime ago cultured successfully (GILL *et al.* 1978).

* Correspondence and Reprints: Department of Botany, University of Delhi, Delhi-110007, India.

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2. Methods

The protoplasts were isolated from *in vitro* grown plants (GILL *et al.* 1978) and were cultured in a medium containing salts according to DURAND *et al.* (1973) with 2,4-D (1 mg/l) and BAP (1 mg/l). Sucrose (14%) served both as osmoticum as well as the carbon source. The method of plating has been described in our earlier report. Petriplates were incubated at a temperature of 25 ± 1.5 °C in the dark unless mentioned otherwise. Five replicates were raised for each experiment and the entire experiment has been done twice with identical results.

3. Results and Discussion

The protoplasts of *N. plumbaginifolia* divided in dark with a plating efficiency of about 61%. By contrast, illumination at low (400 lux) or high (3,000 lux) light intensity resulted in lower plating efficiencies—39% and

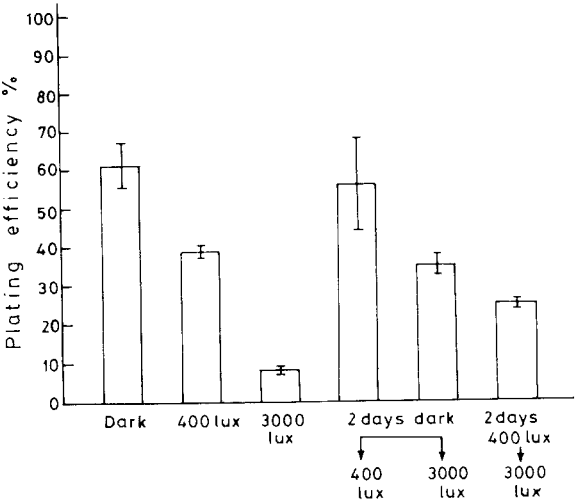

Fig. 1. Plating efficiencies as a function of various light conditions

8% respectively (Fig. 1). However, a pre-incubation in dark for 2 days resulted in marked increase in the division frequency and in colony formation at 400 as well as at 3,000 lux. On an average, the plating efficiencies were 56% at 400 lux and 35% at 3,000 lux. Also the plating efficiency was relatively lower (25%) when cultures were exposed to 400 lux light intensity for initial 2 days and then transferred at 3,000 lux. The further development of colonies was poor at 3,000 lux than at 400 lux, but better than under conditions when cultures were kept under continuous illumination from the very beginning. Whereas no difference in the pattern of growth was apparent in colonies developed at 400 lux or in darkness.

In the work on *N. tabacum* by NAGATA and TAKEBE (1971) the plating efficiency was shown to be intensity dependent. Better results were obtained at high light intensity (2,300 lux) as compared to low light intensity (700 lux). Contrarily, in another investigation (ENZMANN-BECKER 1973)—employing, however, another cultivar—a considerable increase in plating efficiency was obtained if the cultures were kept at lower light intensity, *i.e.*, 400 lux rather than at 3,000 lux. A further increase could be obtained by restricting the 400 lux light treatment to only 48 hours and then transferring at 3,000 lux. None of the above reports mentions results of experiments in dark.

In *N. plumbaginifolia* light did not favour the growth of protoplasts and the maximal plating efficiency was recorded when cultures were kept in dark. High light intensity has been found to be inhibitory in *Petunia hybrida* (FREARSON *et al.* 1973), and darkness enhances the plating efficiency in *Asparagus officinalis* (MACKENZIE *et al.* 1973), *Lycopersicon esculentum* (ZAPATA *et al.* 1977), *Solanum nigrum* (NEHLS 1978), and *Lilium speciosum* (SIMMONDS *et al.* 1979). However, these reports do not indicate of an obligate requirement for darkness. In *N. plumbaginifolia* only two days of initial darkness resulted in appreciably high plating efficiency. More detailed investigations are necessary to resolve the inhibitory role of light.

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