The Effect of Light Intensity and Relative Exposure Under Light on the Expression of Direct or Indirect Somatic Embryogenesis from Common Mallow (*Malva sylvestris* L.)

J. Konstas, S. Kintzios, and J.Drossopoulos
Lab of Plant Physiology
Department of Agricultural Biotechnology
Agricultural University of Athens
Iera Odos 75, 11855 Athens, Greece

G. Sarlis
Inst. of Systematic Botany
Department of Agricultural Biotechnology
Agricultural University of Athens
Iera Odos 75, 11855 Athens, Greece

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Abstract

We investigated the effect of light intensity and the relative exposure under light and dark conditions on the expression of direct or indirect somatic embryogenesis from common mallow. Petiole explants were inoculated on Murashige and Skoog medium supplemented with 200 mg/L casein hydrolysate and incubated either continuously under a photosynthetic proton flux density (PPDF) of 150 µmol m^{-2} sec^{-1} or in increasing darkness (1, 2, 3, 4, 5, 6 or 7 days) for a total period of one week. The direct or indirect (with callus formation) induction of globular somatic embryos, as well as the number of induced globular embryos were assessed every day after inoculation. The quantitative effect of light on somatic embryogenesis was evaluated by incubating cultures under different light intensities (50, 150 or 250 µmol m^{-2} s^{-1}) for one week. The observed results indicated that increased light intensity was significantly associated with increased proliferation of mallow somatic embryos. However, a minimum initial incubation induction in darkness (at least for one day) was required for somatic embryogenesis to occur, while induction of somatic embryogenesis directly on the cut surface of the explants was observed if cultures were incubated in darkness for 2-6 days. Incubation for longer periods resulted in indirect induction of globular embryos. The relative duration of incubation in darkness also affected globular embryo proliferation, with maximum number of embryos observed after 4 days in the dark. The direct induction of embryogenesis was verified histologically by microscopical observation of explant sections. The results of the present study allow for further exploiting mallow petiole culture as a reliable, fast-responding model system for investigating the effect of various factors on the induction of somatic embryogenesis from dicotyledonous plant species.

INTRODUCTION

Malva sylvestris L. (Malvaceae), the common mallow, is an annual to short-lived perennial plant, abundantly distributed in temperate and tropical regions and virtually world-wide (Podlech, 1996; Kintzios, 2001). It is used for various folk medicinal purposes, such as the treatment of coughs and throat infections and other bronchial problems, as well as stomach and intestinal irritations. We have previously demonstrated (Kintzios et al., 1998a) that numerous spherical somatic embryos could be directly induced on mallow petioles within only three days of culture on a MS medium supplemented with 1.8-18 µM 1-naphthalenacetic acid (NAA) and 6-benzyladenine (BA), along with 0.5 g l^{-1} casein hydrolysate culture, and heart-shaped embryos (1-2 mm long) were observed two weeks after culture initiation. Therefore, we were able to provide a reliable, fast-responding model system for investigating the effect of various factors on the induction of somatic embryogenesis from dicotyledonous plant species. Furthermore, we have studied (Kintzios et al., 2001a) the promotive effect of casein on induction of direct somatic embryogenesis from mallow stem and petiole explants. This was associated with an increase in total chlorophyll and potassium accumulation, and in particular, somatic embryo induction was highly positively correlated with K^+

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accumulation.

In the present study we investigated the effect of light intensity and the relative exposure to light or darkness on the expression of direct or indirect somatic embryogenesis from common mallow.

MATERIALS AND METHODS

Petiole explants were received from young mallow plants (bearing 6-8 leaves), collected in the area of Attiki, Greece. Explants were surface sterilised for 10 min in 1% (w/v) sodium hypochlorite solution, containing 1-2% Tween-80, then rinsed 3 times in sterilised distilled water. One cm long explant pieces were excised and inoculated onto Murashige and Skoog (MS) (Murashige and Skoog, 1962) basal medium solidified with 0.8% agar and supplemented with 3% sucrose and 200 mg l^{-1} casein hydrolysate. Media were adjusted to pH 5.8 using 1N NaOH or 1N HCl, autoclaved at 121°C for 20 min and poured into polystyrene 100 x 20 mm Petri dishes (30 ml of medium/dish, 4 explants/dish). Inoculated dishes were sealed with Parafilm™ and incubated initially (for one day) in darkness and then under a PPFD of either 50, 150 or 250 μmol m^{-2} s^{-1} (16/8, from cool white fluorescent lamps) for six days. Numbers of globular embryos cm^{-2}, as well as culture growth (fresh weight) were recorded at the end of the culture period. In a separate experiment, cultures were incubated either continuously under a photosynthetic proton flux density of 150 μmol m^{-2} sec^{-1} or in increasing darkness (1, 2, 3, 4, 5, 6 or 7 days) for a total period of one week. The direct or indirect (with callus formation) induction of globular somatic embryos, as well as the number of induced globular embryos were assessed every day after inoculation. Histological examination of explants from all treatment was conducted according to Jensen (1962). Results were assessed by a standard analysis of variance for a completely randomised design, using MS-STATISTICA software.

RESULTS AND DISCUSSION

Increasing light intensity significantly promoted somatic embryo proliferation, but not culture growth (Table1, Fig.1). However, somatic embryogenesis did not occur if cultures were exposed to light immediately after inoculation. In other words, a minimum initial incubation induction in the dark (at least for one day) was required. In addition, induction of somatic embryogenesis directly on the cut surface of the explants, without prior induction of a callus tissue, was observed if cultures were incubated in darkness for 2-6 days (with maximum induction observed after 3, 4 or 6 days in the dark). Incubation for longer periods resulted in indirect induction of globular embryos. The relative duration of incubation in the dark also affected globular embryo proliferation, with maximum number of embryos observed after 4 days in the dark. The results of these effects are summarised in Fig. 2 and Table 2. The direct induction of embryogenesis was verified histologically by microscopical observation of explant sections (analytical data not presented), whereas abundant globular embryos were formed in cortex parenchyma.

Several investigators have reported that incubation of cell cultures under a low PPFD or in dark condition may be preferable for shoot induction and somatic embryogenesis from some species, such as cucumber (Colijn-Hooymans et al., 1988), melon (Gray et al., 1993; Kintzios et al., 1998b, Kintzios and Taravira, 1997) and rose (Kintzios et al., 1998c). As far as medicinal species are concerned, we have previously shown that lavender (*Lavandula angustifolia* Miller subsp. *angustifolia*) callus growth was improved under increased incubation in darkness while somatic embryogenesis was remarkably reduced under the same conditions. Contrary effects were observed with another Lamiaceae species, wall germander (*Teucrium chamaedrys*): incubation in darkness did not affect the growth of wall germander cultures, but improved somatic embryogenesis (Kintzios et al., 2001b). In conclusion, the results of the present study allow for further exploiting mallow petiole culture as a reliable, fast-responding model system for investigating the effect of various chemical factors on the induction of somatic embryogenesis from dicotyledonous plant species. Currently, this system is being used

for evaluating the growth regulatory potential of oligosaccharide fractions derived from various Malvaceae species (Project PENED 99).

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Tables

Table 1. Analysis of variance of culture growth and globular embryo proliferation from common mallow petioles in response to different light intensities.

		Tissue growth	Globular embryo proliferation
Source of variation	df	Mean square	
Light intensity	2	0.203596*	7262.8*
Error	12	0.0754	2504.41

* = p<0.05

Table 2. Analysis of variance of induction of callus, somatic embryogenesis (direct or indirect) and globular embryo proliferation from common mallow petioles in response to the duration of the culture incubation in darkness.

		Callus induction	Direct somatic embryogenesis	Indirect somatic embryogenesis	Globular embryo proliferation
Source of variation	df	Mean square			
Duration of incubation	7	0.285714**	0.714286***	0.1258*	226246*
Error	32	0.07518	0.10416	0.04166	66565.625

* = p<0.05,** = p<0.01,*** = p<0.001

Figures

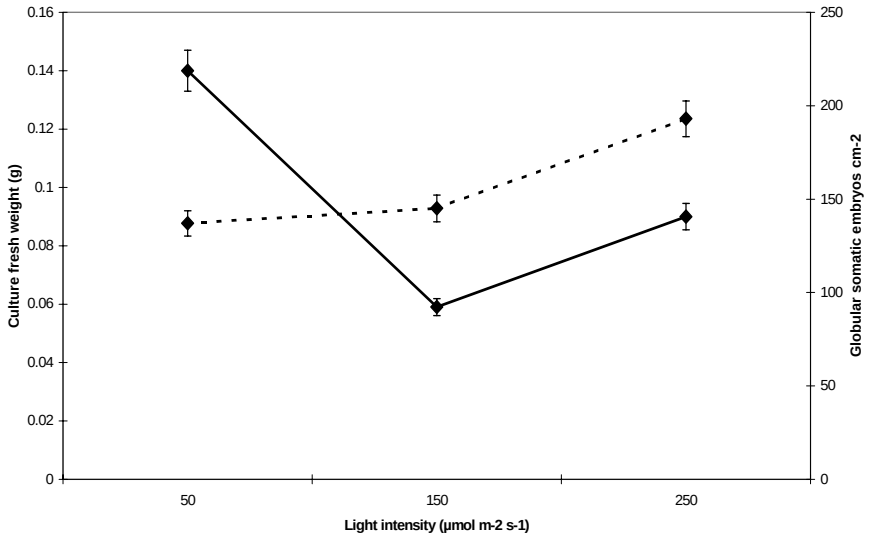

Fig. 1. Effect of light intensity on culture growth (solid line) and the proliferation of globular somatic embryos (dashed line) from mallow (*Malva sylvestris* L.) petiole cultures.

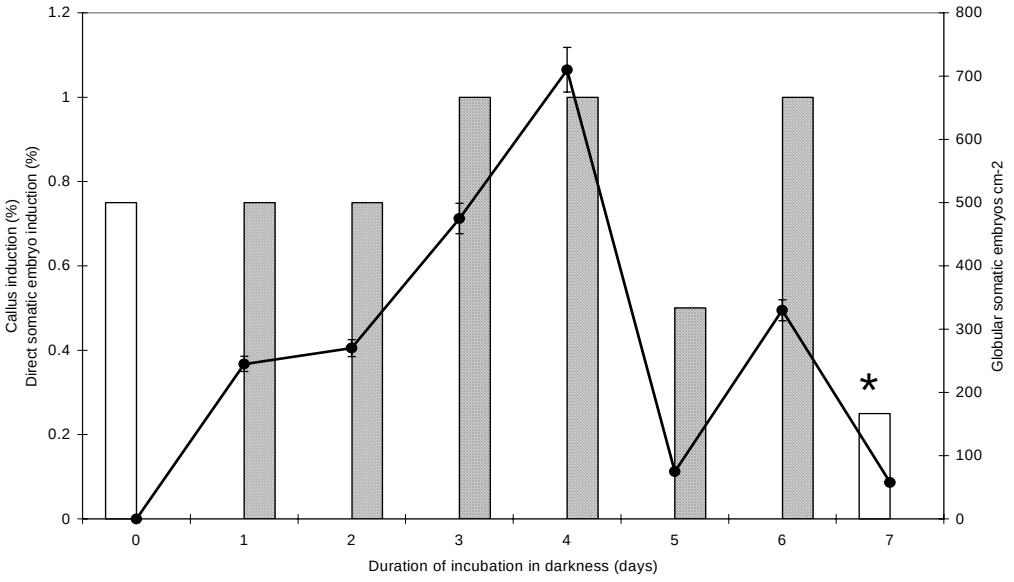

Fig. 2. Effect of the duration of incubation in darkness on the induction of callus (open bars), direct somatic embryogenesis (shaded bars) and the proliferation of globular somatic embryos (line) from mallow (*Malva sylvestris* L.) petiole cultures (asterisk indicates indirect induction of embryogenesis).