Influence of light on somatic embryogenesis in hypocotyls of *Albizzia lebbeck*

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Hypocotyls of *Albizzia lebbeck* when cultured on Gamborg's basal medium (devoid of any growth hormone) give rise to somatic embryos. The induction of somatic embryogenesis is influenced by light. Both short-duration red and far-red light, like white light, considerably enhanced the number of explants displaying embryogenesis over the dark controls. The number of embryoids per explant was also higher in red-light-irradiated explants but far-red light was not as effective and, in fact, reversed the effect of red light. The results suggest a possible involvement of phytochrome in light-mediated somatic embryo formation in this tree species.

LIGHT is a major physical factor influencing differentiation and morphogenesis in higher plants. Although photoperiod and light intensity have been known to influence morphogenesis in cultures, only limited information is available on the spectral quality of light regulating morphogenesis[1–7]. In a few studies, the role of phytochrome, a red/far-red reversible pigment system, has been investigated during organogenesis. In general, red (R) light was found to promote adventitious shoot formation in tobacco, lettuce, *Convolvulus*, *Pseudotsuga* and *Brassica oleracea*, and far-red (FR) reversed its effect[8–12].

Albizzia lebbeck hypocotyl is a novel system wherein both organogenesis and somatic embryogenesis can be induced, either way, from the same explant by controlling hormone levels in the nutrient medium[13,14]. A preliminary investigation indicated a marked influence of light on differentiation of somatic embryos from the hypocotyl explants of *A. lebbeck*[15]. In the present investigation, efforts have been made to study the effect of specific spectral regions of light to explore the identity of the photoreceptor involved in stimulating somatic embryogenesis in hypocotyl explants of *A. lebbeck*.

Seedlings of *A. lebbeck* were raised on B5 basal medium[16]. For induction of embryogenesis, hypocotyls from five-day-old seedlings were cut to 1 cm lengths and placed on B5 basal medium in Petri dishes, as described earlier[14], and subjected to various light treatments. Custom-built light chambers[17] were used for irradiating explants through broad-band filters. For R irradiation, light from eight cool white fluorescent tubes (Philips, TL 20 W, 6500 K) was filtered through one layer of no. 1 yellow Cinemoid and one layer of no. 14 ruby Cinemoid filter. FR light was obtained from eight 150 W tunsgten bulbs (Comptalux, Philips) and filtered through a 5 cm layer of running tap water, one layer of no. 5 deep orange and one layer of no. 20 deep blue pumary Cinemoid filters. Cinemoid sheets were obtained from Rank Strand Electric Co., UK. The output fluence rates were 7.5 $\mu mol\ m^{-2}\ s^{-1}$ for R light (between 600 and 700 nm) and 100 $\mu mol\ m^{-2}\ s^{-1}$ for FR light (between 700 and 800 nm), as recorded by a portable spectroradiometer (LI-COR, Lincoln, Nebraska).

The hypocotyl explants cultured on B5 basal medium, devoid of any growth regulator, split longitudinally along the entire length of the hypocotyl segments under con-

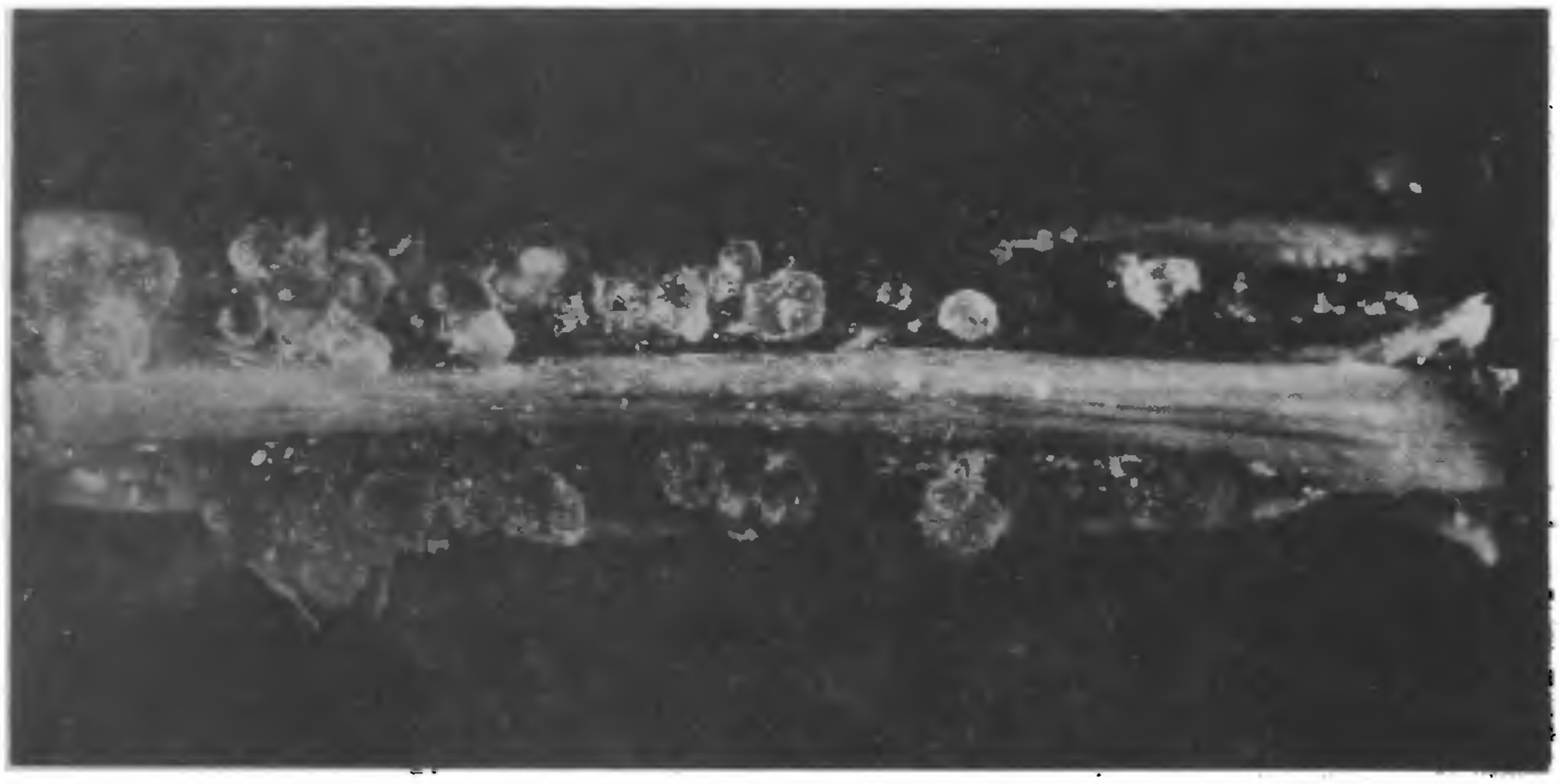

Figure 1. Hypocotyl explants of *Albizzia lebbeck* on B5 basal medium, displaying emergence of numerous globular embryoids after two weeks of culture.

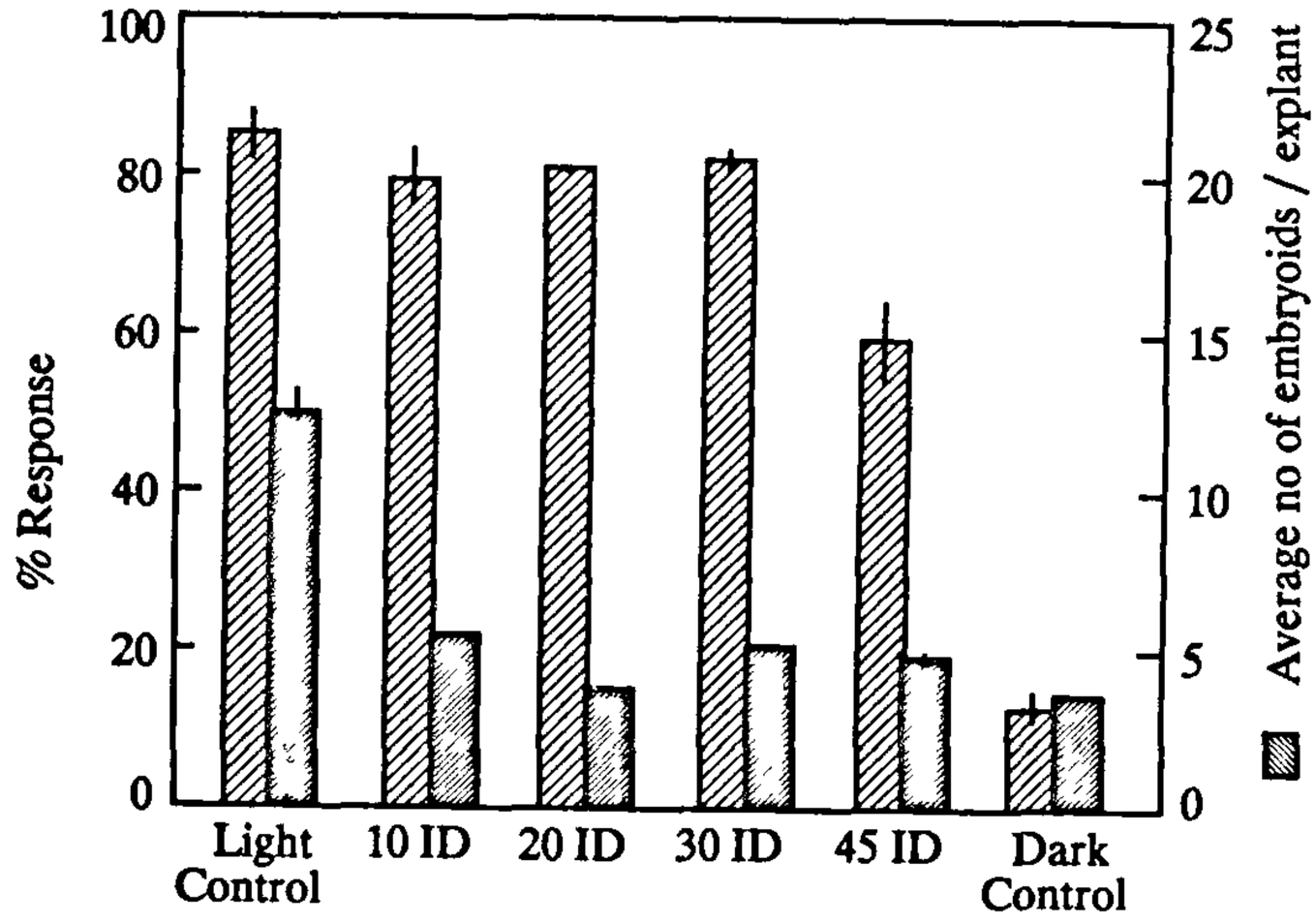

Figure 2. Quantitative determination of hypocotyl differentiation via somatic embryogenesis in cultures kept in total darkness (dark control), darkness interrupted (ID) by white light for 15 min every day for various durations (10, 20, 30 and 45 days), or a daily photoperiodic schedule of 16 h light and 8 h darkness (light control). All cultures were scored for embryogenesis employing a binocular after 45 days. The values plotted are means of four independent experiments (± S E).

ditions of 16 h daily light (Figure 1). This is followed by emergence of globular embryoids and various other stages of embryoid formation, which eventually gave rise to plantlets[13]. The response was fairly uniform, with about 90% of the explants differentiating 12–14 embryoids per explant (Figure 2).

When the hypocotyl explants inoculated on B5 basal medium were kept in darkness rather than in the regular photocycle of 16 h light and 8 h darkness, the number of explants displaying embryogenesis was reduced considerably and the average number of embryoids produced per explant was also reduced by 66% (Figure 2), and only rarely was any callus formed from the cut ends. Further experiments were conducted to verify whether the effect of light was of a catalytic nature or if it was obligatorily required for the entire culture duration to obtain the optimal response. Hypocotyl explants were exposed daily to 15 min white light for 10, 20, 30 or 45 d followed by incubation in darkness for the remaining duration of the experiment. All these cultures were analysed after 45 d. In these cultures, compared to results obtained with explants kept under 16 h photocycles (for 45 d), the percentage of explants showing embryogenesis was not affected much under any light schedule tried, except when kept in complete darkness, although the number of embryoids produced per explant decreased (Figure 2). It may thus appear that some other factor(s) besides light might also influence the intensity of embryogenic response in this system.

To check for the possible involvement of phytochrome in somatic embryogenesis, hypocotyl explants cultured on B5 basal medium and kept in darkness were irradiated with R, FR or R followed by FR light, for 15 min every day for 30 d. The average number of explants responding (exhibiting differentiation) to short-duration R irradiation was fairly comparable (about 75%) to controls kept under continuous light. Even with FR light

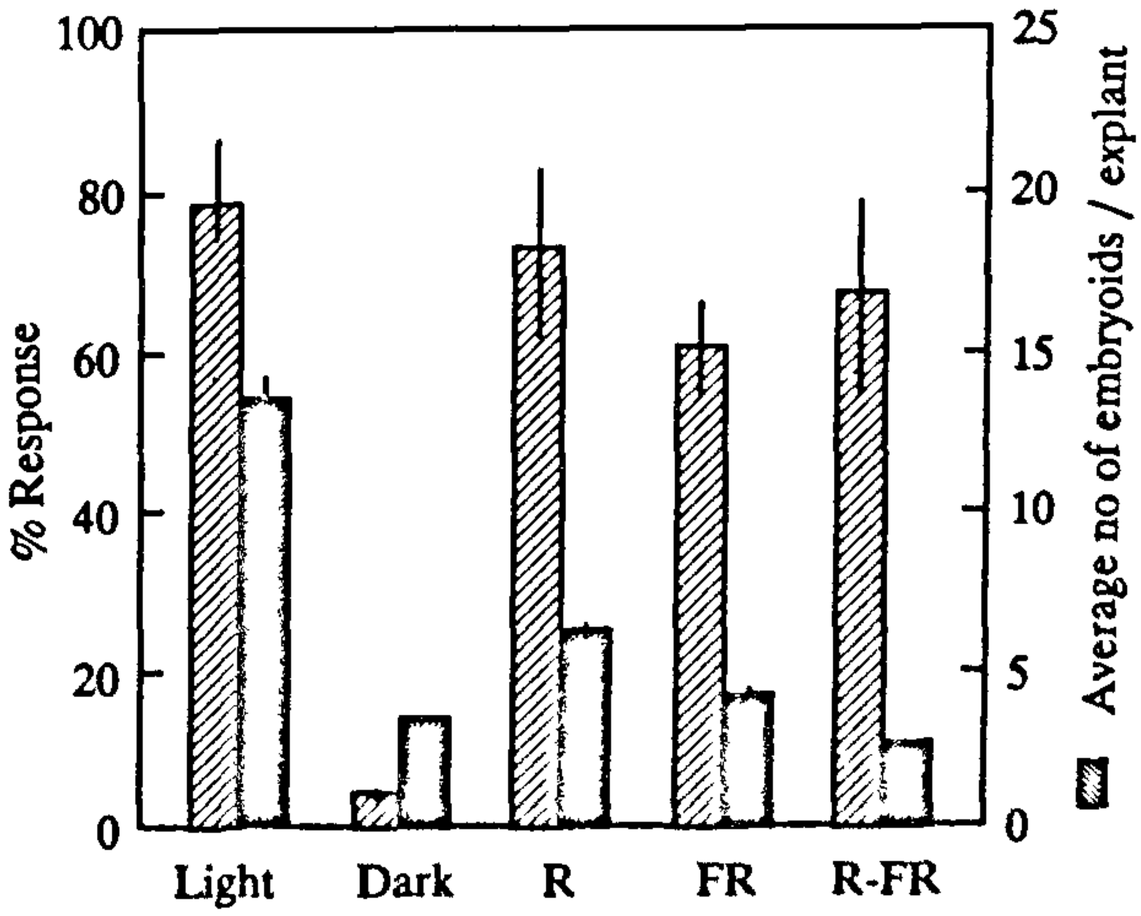

Figure 3. Effect of light quality on somatic embryogenesis. The cultures were kept in darkness and irradiated with red (R), far red (FR), or R followed by FR light for 15 min each day. Experiments were terminated after 30 days of culture period. The values plotted are means of four independent experiments (± S E).

given alone or following R irradiation, nearly 60% explants exhibited embryogenesis. Red light also affected the intensity of the response and, as is evident from Figure 3, the average number of embryoids per explant increased by nearly 75% over the dark control. FR light given immediately following R light reversed this effect, with the level of response being less than that obtained in explants irradiated with FR light alone (Figure 3). However, R light did not enhance the number of embryoids per explant as much as 16 h white light per day. The stimulatory effect of both R and FR light on embryogenic response (qualitatively) may appear intriguing but it is known that in certain very low fluence responses FR may mimic the effect of R light (i.e. very low levels of Pfr, which can be generated both with short-term R or FR irradiation, can induce the response[17,18]). Further analysis with regard to phytochrome photoequilibrium relationships[19] as established by R and FR irradiations, however, needs to be performed to provide a more definitive evidence that phytochrome does indeed regulate somatic embryogenesis in *A. lebbeck* hypocotyls.

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Sachet technique – an efficient method for the acclimatization of micropropagated grapes (*Vitis vinifera* L.)

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***In vitro* rooted plantlets of grape cvs. 'Arka Neelamani', 'Thompson Seedless' and 'Black Champa' were planted in sachets/polythene bags (200 gauge) of 12 × 24 cm size filled to 1/3 height with establishment mixture (sand : loamy soil : soilrite mix TC, 2 : 1 : 1), misted, closed and incubated under supplemented light (30–40 μmol m^{-2} s^{-1}), for 16 h under ambient conditions (25–32°C). Ninety to hundred per cent plantlet establishment was recorded and the plants could be shifted to full sunlight at 4–6 weeks. This method, involving a single-step acclimatization process, is very simple, labour- and cost-efficient and gives better results compared to protray or minipot method of acclimatization.**

ACCLIMATIZATION or hardening of tissue-culture-raised plants is a major step in micropropagation which is generally not given much emphasis in the literature. In grapes there are a number of reports on micropropagation[1–5], but often these do not give a proper account of the process of acclimatization of the tender plantlets. The survival of micropropagated grapes is relatively low compared to most other woody crops[6]. However, some success has been obtained in the recent years through the use of growth retardants like paclobutrazol in cultures, through a reduction in the relative humidity in culture vessels[7] and through CO_2 enrichment in controlled environmental chambers[6,8]. The other reports describe cumbersome procedures involving different steps and extended periods[9–11].

Conventional propagation of different horticultural plants by planting in rooting media within closed polythene sachets has been reported[12–14]. To our knowledge, this method has not been exploited for acclimatizing tissue-culture-raised plants. Therefore, the closed-sachet method was tried with a view to develop a single-step process of acclimatization in comparison with the other two common methods of hardening employed by different laboratories, *viz.* protray method and minipot method.

In-vitro-raised plantlets (4–5 weeks old) of cvs. 'Arka Neelamani', 'Thompson Seedless' and 'Black Champa', obtained from subculturing of shoot tip or nodal microcuttings on Rugini Olive medium[15] supplemented with 3.0% sucrose and 1.0 μM IAA, gelled with 0.2% gelrite (Schweizerhall Inc., N.J., USA) and incubated at