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EFFECTS OF DIFFERENT GROWTH FACTORS ON *GOMPHRENA GLOBOSA* CALLUS TISSUE

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The presence of pigments in plant tissue culture has been reported previously, in *Haplopappus gracilis* (1, 2), *Daucus carota* (3-5), *Lithospermum erythrorhyzon* (6, 7), *Sorghum vulgare* (8), *Chenopodium amaranticolor* (9), *Beta vulgaris* var. *crassa* (10), and *Euphorbia millii* (11). Betalains have been identified in callus cultures of different varieties of *Beta vulgaris* (10, 12) and in beet root tissue culture (13). The effect of blue light, sucrose concentration, and temperature on the biosynthesis of pigments such as anthocyanin has been reported by different authors (1, 2, 14). For example, blue light induced anthocyanin formation in cultures of *H. gracilis* (2). With *Coleus blumei*, increasing sucrose concentration in the medium from 2% to 7% stimulated both callus growth and synthesis of rosmarinic acid (14).

Preliminary studies with calli cultures of *Gomphrena globosa* L. (Amaranthaceae) demonstrated the presence of pigments in morphologically undifferentiated calli. This observation led us to believe that the system might be suitable for further studies on the control of cell differentiation. The pigment in *G. globosa* consists of a mixture of at least seven betacyanins (15).

Inasmuch as the aim of this work was to determine the optimum conditions for callus growth and to characterize the factors involved in pigmented formation by this tissue, based on the reports mentioned above, we studied the effect of different factors (hormone concentration, wavelength of light, and concentration of carbon source) upon the growth and pigment production in calli cultures of *G. globosa*.

RESULTS AND DISCUSSION

Callus formation was obtained with all three media employed. However, their quality was different depending on the hormone combinations, sucrose content, and the illumination conditions employed. The concentrations of the hormone combinations used here were obtained from our laboratory experience of growing dicotyledon cultures (16).

We used the following hormone combinations: 10^{-5}M naphthalene acetic acid (NAA) with 10^{-9}M benzyladenine (BA), 10^{-5}M NAA with 10^{-8}M BA, and 10^{-6}M 2,4-dichlorophenyoxyacetic acid (2,4-D) with 10^{-8}M BA. Calli were induced in all media containing 3%,5% or 7% of sucrose concentrations when incubated in blue, white, or red light, but propagation was not obtained in all cases. The media containing 10^{-6}M 2,4-D with 10^{-8}M BA produced the best calli growth; these calli were friable and showed no morphological differentiation such as shoots or roots (Table 1). In the case of the 10^{-5}M NAA with 10^{-9}M BA medium containing 3% of sucrose concentration, the calli were poorly propagated, but in the media containing 5% or 7% of sucrose, differentiated parts such as shoots or roots appeared, with the roots being the most abundant; differentiation also occurred using a 10^{-5}M NAA with 10^{-8}M BA medium. However, in this case, shoots predominated with little or no differentiation into roots.

Callus growth rate was determined with material that had been subcultured ten times. Increases in fresh weight (6.9

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TABLE 1. Effect of the Sucrose Concentrations and the Different Illuminations on the Callus Growth of *Gomphrena globosa*

Media	sucrose (%)	Blue light			White light			Red light		
		weight[a]		appearance[b]	weight[a]		appearance[b]	weight[a]		appearance[b]
		fresh (g)	dry (g)		fresh (g)	dry (g)		fresh (g)	dry (g)	
2,4-D 10^{-6}M	3	18.7±1.04	0.74±0.017	good	14.6±1.07	0.75±0.06	good	10.8±1.01	0.79±0.02	good
and	5	12.1±1.02	0.78±0.017	good	8.9±1.00	0.84±0.06	medium	8.0±1.004	0.87±0.01	medium
BA 10^{-6}M	7	19.6±1.04	0.96±0.004	good	16.3±1.01	1.14±0.06	medium	14.3±1.02	1.19±0.05	medium

[a]Fresh and dry weights are given in g/30 ml of solid medium. The calli were grown for 24 days, incubated at 28°.
[b]Good=homogeneous, friable, white; medium=homogeneous, friable, yellowish, wih some growth.

times) and in dry weight (1.87 times) were observed after 24 days of incubation (Figure 1).

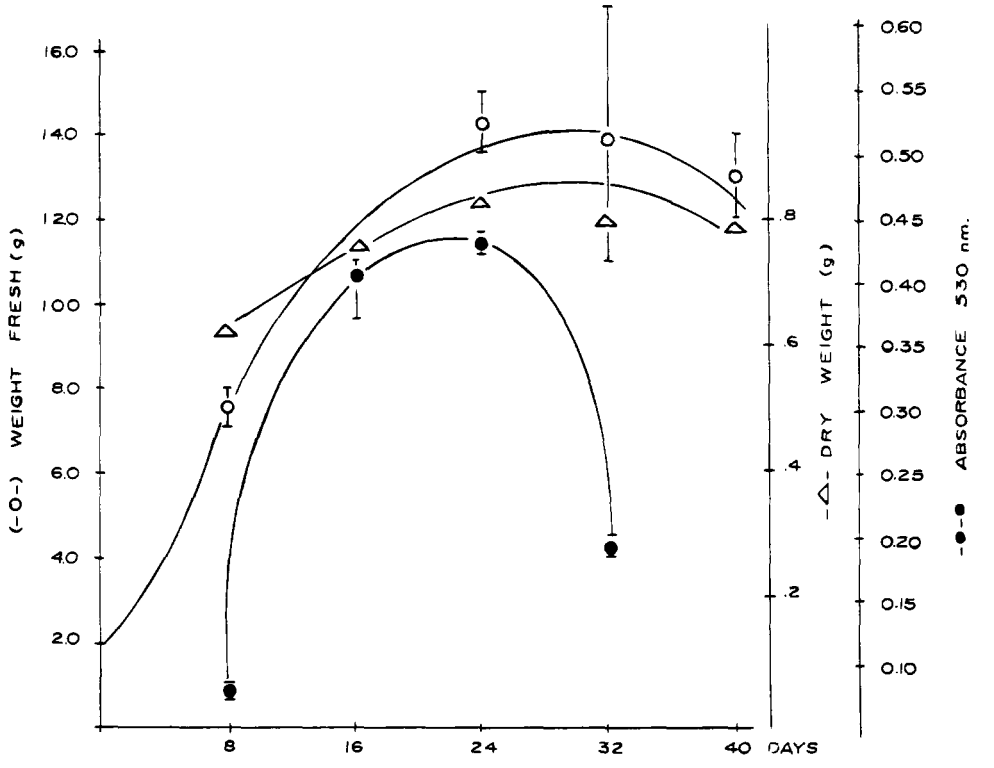

FIGURE 1. The callus culture cycle as a function of the incubation time in blue light at 28°, in a 10^{-6}M 2,4-D and 10^{-8}M BA medium, with 3% sucrose and the pigment content (—●—) in the cultured callus at different stages of the cell growth cycle: (—O—) fresh and (—△—) dry weight. The initial inoculations was of 2 g of tissue. Each point in the growth curve is the average of three different experiments. In a recent experiment, each determination (pigment and weight) represents the mean of five flasks. Fresh and dry weights are in g/30 ml medium.

By increasing sucrose concentrations, we observed increases in dry weight of the tissue under the three light regimens employed. Yield in fresh weight was greatest with 3% and 7% sucrose. (Table 1).

These increases in the fresh and dry weight observed in the media employed, containing high levels of sucrose and their incubation in different conditions of light, do not necessarily reflect an optimum growth of the callus culture.

Based on the results obtained, the best culture was that grown in 10^{-6}M of 2,4-D and 10^{-8}M of BA, 3% of sucrose concentration under blue light. This was therefore considered to be the optimal sucrose concentration for the induction and propagation of *G. globosa* callus. Blue light induced a larger production of pigment than white or red light.

The biosynthesis of betalains in *G. globosa* is affected by the temperature, as in the case of anthocyanins (17,18). Thus, when callus cultures are incubated at 24°, a greater pigmentation is observed than in those incubated at 28°, although callus culture growth decreased at lower temperatures.

Quantification of betalains was obtained from absorbency measurement at 530 nm, during different times of the growth cycle, with cultures incubated with blue light at 28°.

From Figure 1, it is clear that maximum pigment contents are present around the 24th day of incubation, after

which a decrease of the pigments was observed.

In the present work, we obtained *G. globosa* callus culture under different conditions of incubation. The best growth of callus was obtained using Murashige and Skoog medium containing 10^{-6}M 2,4-D, and 10^{-8}M BA and 3% of sucrose. Higher sucrose concentration increased dry weight. This, however, was not necessarily due to an increase in cell number. Staining the tissue with iodine revealed an increase in starch content with increasing sucrose concentrations. Therefore, the increase in dry weight was most likely due to starch accumulation by the cells.

The wavelength of the light showed an effect on callus growth; white and red light induced higher growth as measured by dry weight, compared with those tissue cultures grown under blue light. Stickland and Sunderland (2) found similar results in *H. gracilis* tissue cultures. These authors suggested that under red light, the auxin content of the tissues is raised, and therefore, the rate of growth is greater. As shown in Table 1, the effect of the light on fresh weight seems to be different than on dry weight. Fresh weight accumulation was the highest under blue light. However, this effect was due to water content as shown in the results. In medium with 5% and 7% of sucrose concentration, the callus culture did not grow well, even though the dry weight appeared higher. In addition, a considerable amount of differentiated parts appeared.

Maximum betalain content was observed when callus was grown in Murashige and Skoog medium containing 3% of sucrose and 10^{-6}M 2,4-D and 10^{-8}M of BA. Both growth and pigmentation were poor when the hormone combination used were 10^{-5}M of NAA and 10^{-8}M of BA or 10^{-5}M of NAA and 10^{-9}M BA.

Constabel *et al.* (12) reported the absence of betalains in *B. vulgaris* tissue cultures in a medium containing 2,4-D, but production occurred in the presence of gibberellic acid as soon as the auxin level in the media was reduced. Similarly, betalains in beet root tissue cultures were obtained in a medium containing NAA and kinetin (13).

Pigments in *G. globosa* callus cultures were present in those cultured callus grown under blue light, and a smaller amount of the pigments was present in cultures grown under red light. When the cultures were grown under white light, only a few red colonies were present in the callus tissue.

In *H. gracilis* callus cultures (1,2), the red pigmentation was due to anthocyanins, which accumulated at the highest rate under blue light, and in a medium containing auxins (NAA or 2,4-D) (2).

In some plants, light seems to be an absolute requirement for pigment biosynthesis, while in others, betalain accumulation takes place also in the darkness (19,20). The results presented here show that in *G. globosa* callus cultures, blue light and 2,4-D are conditions for obtaining good growth and pigmented callus cultures.

EXPERIMENTAL

CALLUS INDUCTION.—Seeds of *G. globosa* were sterilized by soaking them in 10% calcium hypochlorite solution for 10 min, followed by three successive rinses with sterile distilled H_2O. Sterilized seeds were germinated in three media as described below. The same media were also used for callus propagation.

CULTURE MEDIA.—The basal medium of Murashige and Skoog (21) was used throughout; the hormone concentrations employed were: 10^{-5}M NAA and 10^{-9}M BA; 10^{-5}M NAA and 10^{-8}M BA; and 10^{-6}M 2,4-D and 10^{-8}M BA.

CALLUS GROWTH.—Samples of callus from hypocotyl (2 g) were incubated at 28° in 30 ml of various media during different time intervals using the following conditions of light: white, 1200 lux; blue, 80 lux; red, 75 lux. Fresh weight increases were determined during different periods after removing excess moisture from the tissue by filtration with a mild vacuum. Dry weight was determined after drying the tissue at 60° for 48 h. (See footnote to Figure 1.)

PIGMENT EXTRACTION.—After the callus culture was grown in the medium containing 10^{-6}M 2,4-D and 10^{-8}M of BA, incubated at 28° and exposed to continuous illumination with blue light (80 lux), the pigments were extracted according to Stafford (22) in the following way: 1 g of the fresh tissue was homogenized in 6 ml of 0.1% HCl in MeOH, and the homogenate was centrifuged at 300×*g* to remove debris. The supernatant was extracted with Et_2O. The final aqueous phase containing crude pigments was adjusted to 10 ml and pigment concentration measured at 530 nm in a Unicam SP 1800 spectrophotometer. The pigments were authenticated by their characteristic absorption at 475 and 530 nm, respectively, by their characteristic color reactions in 2 N HCl and lead acetate, as well as their migration on paper chromatograms (BuOH-HOAc-H_2O, 20:5:11).

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