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BRIEF COMMUNICATION

Nutrient deficiency-dependent anthocyanin development in *Spirodela polyrhiza* L. Schleid.

V. KUMAR and S.S. SHARMA*

Department of Biosciences, Himachal Pradesh University, Shimla 171005, India

Abstract

Spirodela polyrhiza L. Schleid. plants developed anthocyanin (AC) on the abaxial frond surface when grown on Hoagland nutrient solution diluted 1:≥40; AC content was maximum at 1:80 dilution. Till 1:20 dilution there was no or very little AC formation. The formation of AC seems to rely on the availability of excess carbon skeleton in relation to nitrogen in plants. Thus, addition of saccharose to 1:20 diluted medium resulted in a concentration-dependent AC formation. Also fructose, glucose, mannitol, and sorbitol induced AC synthesis. Conversely, urea and ammonium nitrate, when added to the 1:80 diluted medium, suppressed the AC formation. Omission of micronutrients from the growth medium led to the formation of a little amount of AC whereas polyethylene glycol and Cd treatments were ineffective. AC produced under starvation (7 d on distilled water) did not get turned over upon the transfer of plants to nutrient sufficient (1:5 diluted medium) conditions indicating that AC formed does not serve as a reversible C-reserve.

Additional key words: ammonium nitrate, Cd, chlorophyll, C:N ratio, micronutrients, polyethylene glycol, saccharides, urea.

Anthocyanins are ubiquitous in angiosperms with the exception of order *Centrospermae* where they are replaced by a superficially similar group of pigments, the betacyanins. They render an array of colours to various plant parts, often in a species-specific manner. Besides being the normal plant chemical constituents, ACs respond to various stresses, *e.g.*, exposure to UV radiation (Tevini *et al.* 1991, Li *et al.* 1993, Ambasht and Agrawal 1997), drought (Do and Cormier 1990), heavy

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* Author for correspondence; fax: (+91 177) 230 775

metals and ozone exposure (Heller and Forkmann 1988) *etc.*, by a rise in concentration. ACs together with other phenolic compounds serve as epidermal UV screens and thus help plants avert the damage to macromolecules such as nucleic acids in the mesophyll cells. They also facilitate the pollination process by constituting visual signals to the pollinator insects and influence plant growth and development.

ACs, when present in relatively greater amounts, can conveniently delimit the plant species from the ones altogether lacking these pigments or possessing them in minute quantities. For example, purple pigmentation on the abaxial frond surface in *S. polyrhiza* is among the features distinguishing this from other duckweeds (Bamber 1916, Maheshwari 1963, Bhandari 1978). To be of use as reliable taxonomic markers, however, it is important that the concerned chemical constituent(s) exhibit(s) a reasonable qualitative as well as quantitative consistency. We here report that the AC contents in *S. polyrhiza* strongly depend on the nutrient status of the plants.

Table 1. Growth of *Spirodela polyrhiza* on nutrient solution of varying strengths. Values after 7 d. Initial frond number: 16 - 20 (fresh mass, 80.33 ± 1.49 mg). Values are arithmetic means of three replicates (± SE).

Fold dilution	Frond number	Fresh mass [mg]	Total chlorophyll [g kg^{-1}(f.m.)]	Relative anthocyanin
5	103.33 ± 7.20	435.00 ± 14.40	1.58 ± 0.040	0
10	109.67 ± 6.51	496.70 ± 30.40	1.50 ± 0.070	0
20	96.00 ± 8.64	451.30 ± 33.40	1.40 ± 0.060	0.01 ± 0.00
40	78.67 ± 4.75	355.00 ± 42.62	1.29 ± 0.200	0.16 ± 0.01
80	61.33 ± 2.88	304.70 ± 11.99	1.13 ± 0.031	0.44 ± 0.03

S. polyrhiza L. Schleid. plants were collected from a natural pond near Bilaspur, Himachal Pradesh (India). We cultivated them in *Corning* glass trays on nutrient solution (Hoagland and Arnon 1938) diluted 1:5 in an incubator at 25 ± 2 °C under continuous irradiation provided by white fluorescent tubes (PAR 40 μmol m^{-2} s^{-1}). The nutrient solution was aerated daily for about 30 min with an aquarium air pump. The composition of full strength nutrient medium was: 1 mM KH_2PO_4, 5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 4 mM $MgSO_4$, 4.6 μM H_3BO_3, 9.1 μM $MnCl_2$, 0.7 μM $ZnSO_4$, 0.3 μM $CuSO_4$, 0.1 μM H_2MoO_4, and 0.2 mM Fe-citrate. All dilutions of the nutrient solution were prepared with glass-distilled water. Healthy and uniform plants (frond number: 16 - 20) were used for experiments. Fresh mass of the plants was recorded after appropriately blotting them between the layers of filter paper. Chlorophyll was extracted in 80 % acetone and estimated spectrophotometrically using the equations given in Harborne (1973). Anthocyanins were extracted and determined as described by Lange *et al.* (1971). Briefly, the plant samples were submerged in 10 cm^3 isopropanol-HCl-H_2O (18:1:81, v/v/v) solution and the extraction tubes were immersed in boiling water bath for 1 min. Thereafter the samples were left in the extraction solution for 24 h in dark at 25 ± 1 °C to allow complete extraction. The

extractants were centrifuged at 5 000 *g* for 20 min, and the final volume of the supernatant was adjusted to 10 cm^3 with the extractant mixture. The absorbance was read at 535 nm (A_{535}) and 650 nm (A_{650}). The A_{535} values were corrected for scattering using the A_{650} and Rayleigh's formula: corrected $A_{535} = A_{535} - 2.2\ A_{650}$. The relative amount of the AC was expressed as A_{535} (corrected) per 100 mg(f.m.) of plants (extract volume: 10 cm^3).

S. polyrhiza plants maintained for 7 d on the nutrient medium diluted 1:5 or 1:10 were devoid of AC. At dilutions greater than 1:20, the AC production progressively increased with increasing dilution. Maximum AC content was observed in plants grown on 1:80 dilution beyond which no further increase was evident even when plants were maintained on distilled water. The induction of AC formation coincided with the onset of nutrient deficiency as reflected in substantial reduction of plant growth (frond number and fresh mass) and chlorophyll content from 1:40 dilution onwards (Table 1). The AC formation in the test plants grown on 1:80 diluted nutrient medium (the maximum dilution in our experiments) was inhibited in a dose-dependent manner when urea or ammonium nitrate (Fig. 1*A*,*B*) was added to the medium. Urea at 10 mM was toxic to the test plants.

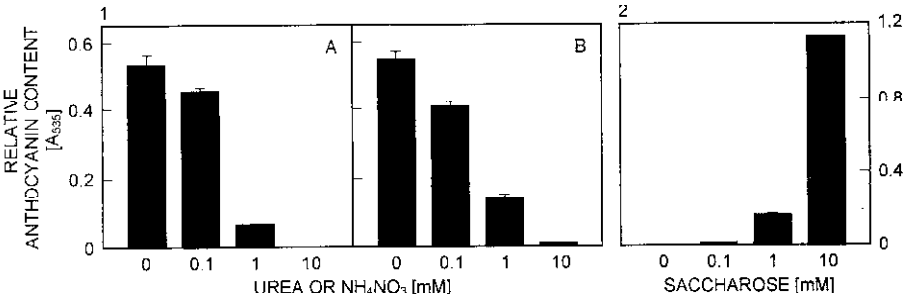

Fig. 1. Effects of urea (*A*), or ammonium nitrate (*B*) added to 1:80 diluted nutrient medium on anthocyanin development in *Spirodela polyrhiza*. Values after 7 d, arithmetic means of three replicates (± SE). Relative anthocyanin contents expressed as absorbance at 535 nm. For details see the text.

Fig. 2. Effects of saccharose added to 1:20 diluted nutrient solution on anthocyanin development in *Spirodela polyrhiza*. Values after 7 d, arithmetic means of three replicates (± SE). Relative anthocyanin contents expressed as absorbance at 535 nm.

In order to check whether a surplus of saccharides (C-skeleton) stimulates AC production, the effect of exogenous saccharose added to the highest-no-pigment induction dilution of nutrient medium (1:20) was monitored: the AC content increased in relation to saccharose concentration (Fig. 2). A stimulation of AC formation was induced also by the addition of fructose (12.2 ×), glucose (15.7 ×), mannitol (21.2 ×) and sorbitol (23.7 ×) supplied at 1 mM. Light was invariably essential for AC formation under all above conditions; no measurable AC was produced in dark even in the presence of 10 mM saccharose that was responsible for a high AC production in light (values not shown).

Water deficit (1 and 10 % PEG 6000) and heavy metal treatment (Cd in a range of 5 to 25 μM), though substantially inhibited the plant growth (values not shown), did not induce the development of ACs in plants grown on nutrient medium (1:5). The choice of PEG concentrations used was based on Frick and Golt (1995) who showed 1.15 and 11.5 % PEG (corresponding to osmotic potential -0.486 and -0.597 MPa) to inhibit growth of *Lemna minor*. A little pigmentation (A_{535} = 0.480 ± 0.003) was observed upon omission of micronutrients from the medium.

To check if the AC produced under nutrient starvation could serve as carbon reserve by getting turned over under nutrient sufficient conditions, 7 d-starved plants on distilled water (possessing high AC content: A_{535} = 0.681 ± 0.015) were shifted for another 7 d to the medium diluted 1:5. The plants multiplied normally and the newly formed fronds were all green; the number of pigmented fronds underwent no change. There was no evidence for a statistically significant turnover of AC during a 7 d period (A_{535} = 0.800 ± 0.124). Green fronds were devoid of AC.

The AC formation in *S. polyrhiza* is mainly a response to the perturbed C:N ratio in plants, an apparent consequence of N depletion in the nutrient medium. As soon as plant C-surplus (in relation to N) approaches a certain threshold, the AC synthesis gets triggered. This is evident from changes in AC production in response to saccharose, urea, or ammonium nitrate addition. Also some stress effects on the AC formation are attributable to a shift of C:N in favour of C. A slight promotion in AC formation by exclusion of micronutrients from the growth medium was in conformity with the findings of Thimann and Edmondson (1949).

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