was internal aminoacid replacement, indicating translational errors due to the presence of high concentrations of aminoacids[14].

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EFFECT OF CERTAIN PROANTHOCYANIDINS ON THE CHLOROPHYLL CONTENT OF *LEMNA PAUCICOSTATA* HEGELM.

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PLANT growth regulators like cytokinins, gibberelins auxins and abscisic acid play an important role in the maintenance of chlorophyll levels in plants and literature in this aspect has been reviewed by Thimann[1]. Proanthocyanidins (formerly referred to as leucoanthocyanins) are an important group of growth regulating phenolic compounds[2-7]. The present study aims at evaluating the effect of proanthocyanidins on the chlorophyll contents of *Lemna paucicostata* Hegelm plants. The extraction and isolation of various compounds used in this work have been reported earlier[6,8].

Clonal and axenic cultures of *L. paucicostata*, maintained on modified Bonner and Devirian medium[9] were used as inoculum. The basal medium (100 ml, without sucrose) poured into 250 ml Erlenmeyer flasks was autoclaved and supplemented with filter-sterilized proanthocyanidin solution at five concentrations (0.01, 0.05, 0.1, 0.5 and 1 ppm). Ten *Lemna* plants each with 3 fronds were introduced aseptically into each flask and the cultures were maintained under a light intensity of 5000 lux, at 25 ± 1 C. On the 10th day chlorophyll was extracted from 5 mg plant material in 5 ml 96% methanol and the chlorophyll content was estimated adopting the following formulae given by Holden[10]

$$\text{Chlorophyll a(mg/l)} = 16.5\, D_{665} - 8.3\, D_{650},$$
$$\text{Chlorophyll b(mg/l)} = 33.8\, D_{650} - 12.5\, D_{665},$$
$$\text{Total Chlorophyll (mg/l)} = 25.5\, D_{650} + 4.0\, D_{665}.$$

All the compounds enhanced the levels of chlorophylls in *Lemna* cultures (table 1). The earlier studies on the growth promoting activities of proanthocyanidins[6,7] and the results obtained in this study showed that there was a good correlation between the enhancement of growth and chlorophyll content in *Lemna* by proanthocyanidins. Leucocyanidin from *Tamarindus indica* (0.1 ppm), procyanidin from *Phoenix sylvestris* (0.1 ppm), procyanidin from *Anona squamosa* (0.5 ppm) enhanced the growth to a maximum extent[6] and these compounds also caused the

Table 1 *Effect of Proanthocyanidins on the chlorophyll content of L. paucicostata—Results expressed in terms of µg chlorophyll g fresh weight**

Compound	Chlorophyll a					Chlorophyll b					Total chlorophyll				
	Concentration					Concentration					Concentration				
	0.01 ppm	0.05 ppm	0.1 ppm	0.5 ppm	1.0 ppm	0.01 ppm	0.05 ppm	0.1 ppm	0.5 ppm	1.0 ppm	0.01 ppm	0.05 ppm	0.1 ppm	0.5 ppm	1.0 ppm
Leucocyanidin	296	296	445	395	246	106	106	158	141	144	402	402	603	536	396
Procyanidin from *P. sylvestris*	355	486	560	544	461	88	109	181	183	201	443	695	741	737	662
Procyanidin from *A. squamosa*	362	395	544	651	346	111	141	193	194	123	473	536	737	845	469
Propelargonidin from *C. javanica*	247	272	321	346	272	88	96	114	123	96	335	368	435	469	368
Propelargonidin from *P. pterocarpum*	279	329	395	438	223	119	136	141	116	78	398	465	536	544	301
Prorobinetindin	305	404	494	247	223	71	106	176	88	78	376	510	670	335	301
Proanthocyanidin from *D. cinera*	272	296	404	296	225	96	106	106	106	72	368	402	510	402	298
Proanthocyanidin from *X. dolarbriformis*	272	395	295	445	402	96	141	141	158	106	368	536	536	602	403
Control:			272					96					368		

* Each value represents the mean of 3 replicates

maximum production of chlorophylls at the respective concentrations. Propelargonidin from *Peltophorum pterocarpum*, prorobinetinidin and proanthocyanidin from *Dichrostachys cinera* which inhibited growth at 1.0 ppm concentration[6,7] caused a considerable decrease in chlorophyll content. Reduction in chlorophyll associated with growth inhibition by nalidixic acid was reported by Frick *et al*[11] in *L. minor*. The suggestion made by Steward and Shantz[4] that leucoanthocyanins and other phenolics exert their influence on growth through nucleic acid and protein metabolism was confirmed in the case of proanthocyanidins[12]. The results obtained in the present study and the earlier investigations[6,7,12] indicate that growth promotion by proanthocyanidins in *Lemna* is associated with increase in the amount of chlorophyll, RNA and protein.

Hillis[13] reported that during spring flush of growth in the eucalypts the youngest leaves were red except for the extreme tip (the area of most active cell division), which was pale green. Appreciable amounts of proanthocyanidins were found in the leaf (along with anthocyanins) but the greatest concentration of the former was noted in the extreme tip[13]. The gradual development of green colouration in the leaf was associated with a fall in the levels of proanthocyanidins (to a minimum)[13] indicating that proanthocyanidins have some role in chlorophyll development.

The rise in chlorophyll content (caused by proanthocyanidins) was also associated with increased dry weight of *Lemna* plants[7]. This suggests that higher levels of chlorophyll content might have facilitated more photosynthetic rate and dry matter yield. But high levels of chlorophyll may not be taken as a measure for high rates of photosynthesis, which is a complex process and controlled by diverse factors. However, Saunders and McClure[14], based on the occurrence of flavonoids in the chloroplasts of the leaves of 25 species of plants, suggested that phenolics have some role in the process of photosynthesis or at least they seem to protect chloroplasts from UV damage. Mohr and Drumm-Herrel[15] suggested that anthocyanins might protect the juvenile seedlings of mustard, milo, tomato and wheat from the injurious effects of UV radiation. Einhellig *et al*[16] reported that scopoletin inhibited the growth and also reduced photosynthetic rates in seedlings of tobacco, sunflower and pigweed. They attributed growth inhibition by

scopoletin to reduced photosynthesis. The distribution of proanthocyanidins in the leaves and their role in photosynthesis need a detailed investigation.

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A DOUBLE-EMBEDDING TECHNIQUE FOR SECTIONING EMBRYOIDS

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INVESTIGATIONS on plant tissue cultures, particularly those involving elucidation of the process of embryoidogenesis (a term for the development of embryoid *in vitro*) and the morphology of the embryoids, the histological characteristics of tissue organization, the mode of vascular differentiation, etc, would involve the sectioning of embryoids at different stages of growth. The minuteness of some of these materials, particularly the embryoids, renders them extremely difficult to manipulate if processed by the customary methods. The celloidin-spray method of Bhandari[1] is quite acceptable but for the high cost of celloidin and the stringent conditions for material preparation. The present communication describes a much simpler and less expensive procedure. In fact, this method, designated the double-embedding method gave extremely satisfactory results in the androgenetic investigations on *Nicotiana tabacum*[2,3]. This method involves the embedding of materials twice; first in agar sol and then the solidified agar blocks with the materials are re-embedded in paraffin by the customary procedure.

The procedure for double-embedding is given below:

1. Dissolve about 1 g of good quality agar-agar powder (Difco or other good make) in 100 ml of hot water.
2. Get the materials to be embedded ready: fixed as well as fresh materials can be chosen. Prior staining of material is optional for fixed materials.
3. Prepare a paper boat of convenient size and apply a thin coat of glycerine on its inner surface.
4. Pour a required quantity of agar sol into the paper boat. Transfer the materials into the boat and arrange them carefully leaving enough space between materials for easy partitioning of individual blocks later.
5. Place the boat in a cold chamber to facilitate rapid and uniform solidification.
6. Cut out each agar block containing the material, taking care to retain a uniform sheath of agar around the material.
7. Process the agar blocks for microtomy by customary methods[4]. If fresh materials are agar-embedded, proper fixation is essential before pro-