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Increased Plant Vigor by *in Vitro* Propagation of *Saintpaulia ionantha* Wendl. from Sub-epidermal Tissue[1]

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Abstract. **Differential regeneration capacities of epidermal and sub-epidermal tissues of *Saintpaulia ionantha* Wendl. 'Georgia' were dependent on the basal salts used in the tissue culture medium. Murashige and Skoog salts medium containing 0.1 mg/liter α-naphthaleneacetic acid (NAA) and 0.5 mg/liter benzyamino purine (BA) induced callus from petiole cross-sections and similar cross-sections with their epidermis removed. Shoot regeneration occurred only on the explants containing an epidermis. B5 salts medium, with the same growth hormone concentrations, induced shoot regeneration from both explant types. When grown to flowering, plants derived from sub-epidermal tissues were more vigorous, became 25% greater in fresh weight, 11% larger in diameter, and produced 28% more leaves, which were 14% wider with petioles 18% longer, than their epidermal derived counterparts.**

The *in vitro* propagation of *Chrysanthemum morifolium* Ram. from epidermal tissue produced plants with reduced vigor (3). Since the chrysanthemum propagation procedure of using stem tip cuttings does not change cell layer organization, this finding did not influence commercial production practices. However, numerous other plants propagated *in vitro* by commercial growers, especially members of the Gesneriaceae including *Achimenes, Streptocarpus, Sinningia* and *Saintpaulia*, naturally originate from the epidermal layer (6) and they possibly could be more vigorous if derived from sub-epidermal tissues.

This research compared the plant growth responses of one member of the Gesneriaceae, (African violet) when propagated *in vitro* from epidermal or sub-epidermal tissue.

Stock plants of African violet 'Georgia', of the German Optimara strain, were initially propagated commercially from leaf petiole cuttings and maintained in a greenhouse. Fully expanded young leaves including petioles were excised from stock plants and surface sterilized for 25 min in a commercial sodium hypochlorite solution (10% Domestos, Lever Brothers Ltd., U.K.). Following several sterile distilled water rinses, the petioles were sliced into 3 mm cross-sections. A stainless steel cork borer 3 mm in diameter was used to obtain uniform discs of sub-epidermal tissue from the petiole cross-sections. Petiole cross-sections and sub-epidermal explants were aseptically transferred to 5 cm plastic Petri dishes containing 10 ml of culture medium. Either MS (9) or B5 (4) macro- and micro-nutrients were used; both containing MS vitamins and amino acids (9), 2% sucrose, 0.6% agar (Sigma Chemical Co., U.K.), 0.1 mg/liter NAA, and 0.5 mg/liter BA. Cultures were maintained under Cool-White fluorescent light at 22 ± 2° C in an 18 hr photoperiod of 2 klx.

Twelve-week-old shoots regenerated adventitiously from petiole cross-sections on MS medium and sub-epidermal derived shoots from B5 salts medium were transplanted into plastic trays containing commercial African violet soil (Thomas Rochford and Sons Ltd., U.K.). The shoots were greenhouse grown for 4 weeks under decreasing humidity until acclimated and well rooted. Six plantlets per treatment were then transplanted into standard 9 cm plastic pots and grown for 19 weeks under greenhouse conditions suitable for *Saintpaulia*. Plants were watered and fertilized by a capillary mat to insure identical cultural conditions for the two treatments. Data assessing plant vigor were collected when all plants flowered.

The formation of shoots from the petiole cross-sections on MS medium was comparable to published results (2) but there was less stunting of shoots, probably due to cultivar differences. In contrast, shoot regeneration from sub-epidermal tissue did not occur on MS medium; only slight brown callus developed. Shoots regenerated, however, on B5 salts medium, from both petiole cross-sections and sub-epidermal tissue discs. Although shoot growth was initially rapid on B5 salts medium, the shoots from the petiole cross-sections gradually became slightly chlorotic. In general, both tissues responded more quickly on B5 salts medium than on MS medium producing both earlier callus growth (Table 1) and shoot regeneration. However, growth from the petiole cross-sections placed on MS medium gradually became more rapid and by transplantation time shoot size and number were comparable from both media (Fig. 1).

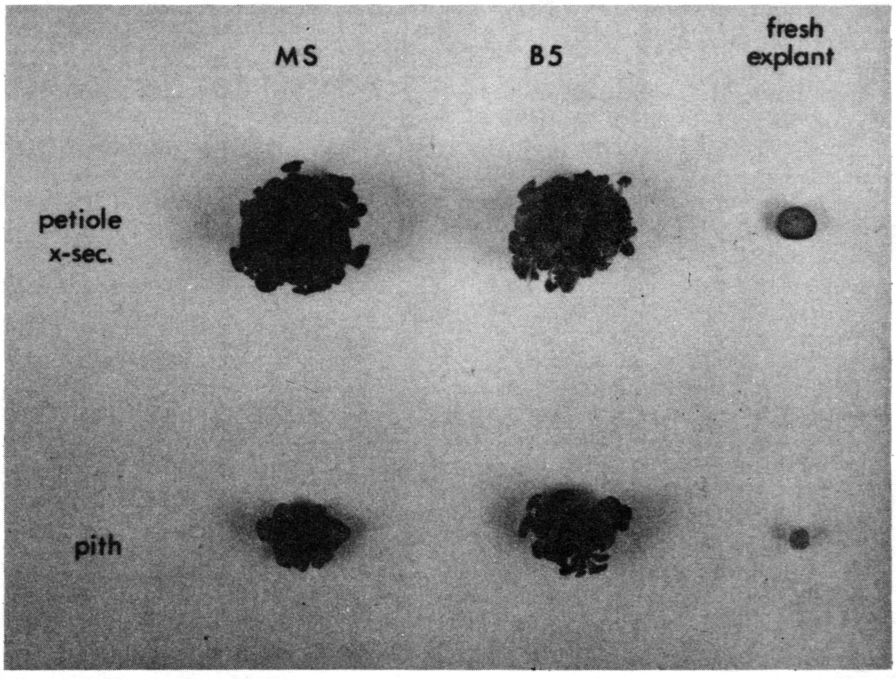

Fig. 1. Comparison between African violet 'Georgia' petiole cross-sections and sub-epidermal (pith) tissue grown 10 weeks *in vitro* on similar media containing either MS or B5 salts.

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Table 1. Explant growth of African violet 'Georgia' from petiole cross-sections and sub-epidermal (pith) tissue after 7 weeks on MS or B5 medium. Each treatment had 25 replicates.

Explant	Fresh wt (g)/explant	
	MS	B5
Petiole	0.20 a[z]	0.38 b
Pith	0.06 a	0.09 b

[z]Mean separation in rows by *t* test, 5% level.

Following transplantation into soil, plants regenerated from sub-epidermal tissue grew more vigorously than those derived from the epidermis. They were 11% larger in diameter, produced 28% more leaves, which were 14% wider with petioles 18% longer, than epidermal derived plants. Overall, the sub-epidermal derived plants were 25% larger, based on fresh weights, than their epidermal derived counterparts (Fig. 2). However, flower size and quantity did not differ between the plants from the two tissue sources (Table 2). The sub-epidermal derived plants, although possessing a faster growth rate, flowered on average 2 weeks later than the epidermal derived ones. Other than qualities attributed to vigor, plants from the two treatments appeared morphologically identical.

The different *in vitro* growth responses of sub-epidermal tissue caused only by variations in the macro- and micro-nutrient components in the media draw attention to their role with endogenous auxins and cytokinins in relation to shoot organogenesis. The B5 salts differ primarily from MS salts in the level of ammonium; MS containing 10× more than B5 (5). Possibly this high concentration of ammonium present in MS medium is responsible for the inhibition of organogenesis in the sub-epidermal tissue. The differential growth response of epidermal and sub-epidermal tissue to MS salts indicates considerable physiological variation between the two cell types. This variation aids research on the importance of tissue organization in the regulation of organogenesis (10). Further understanding of the growth response of sub-epidermal tissue cells *in vitro* is important for protoplast research as preliminary research at Nottingham has shown that protoplasts isolated from *Saintpaulia* leaves are primarily of sub-epidermal origin.

Bush et al. (3) attributed the difference in vigor between the epidermal and sub-epidermal derived chrysanthemum plants to factors such as rearrangement of the stock plant chimeral layers and/or mutations occurring in culture. These factors are unlikely causes for the differences in plant vigor from the tissue types of *Saintpaulia*. Chimeras in *Saintpaulia* are rare and not maintained through clonal generations since adventitious shoots arise from single cells (1), thus the *Saintpaulia* stock plants used were non-chimeral. Mutations specifically towards vigor caused by *in vitro* conditions are similarly unlikely as induced mutations in *Saintpaulia* occur randomly (7). The possibility of polyploidy causing the increased vigor of the sub-epidermal derived plants also appears unlikely as colchicine-induced tetraploid African violets are less vigorous than their diploid counterparts (1).

Most probably, the difference in plant vigor between the 2 tissue treatments is associated with the state of differentiation of the tissue's cells and their interaction with the basal media. Epidermal cells may be inherently less vigorous than sub-epidermal cells. Possibly, *Saintpaulia* epidermal cells undergo incomplete dedifferentiation during culture which later results in plants retaining the vigor character of the original explant cells. A plant which fails to undergo complete dedifferentiation *in vitro* is *Pterotheca falconeri* Hook (8). Mehra and Mehra (8) demonstrated that callus derived from roots of this species preferentially regenerated roots. Similarly, regeneration of isolated leaves and shoots was favored from leaf and shoot apex derived callus, respectively. A similar case of incomplete dedifferentiation appears to have occurred in a culture of 'Yellow Snowdon' chrysanthemum. A flower petal culture regenerated one plant whose leaves partially retained the petal character of the explant (Dr. B. Machin, personal communication). Clearly, a greater understanding of the control of plant vigor is necessary and *Saintpaulia* can provide appropriate tissue for such studies.

Plants with increased vigor are preferred by commercial growers as larger plants command higher prices. However, the commercial advantage of a larger sub-epidermal derived African violet will have to be weighed against the fact that it reaches maturity 2 weeks later than its conventional counterpart.

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Table 2. The growth response of epidermal- and sub-epidermal-derived-plants of African violet 'Georgia' at flowering. Each treatment had 6 replicates.

Plant origin	Plant Diam. (cm)	No. of leaves	Largest leaf width (cm)	Longest petiole length (cm)	Peduncle no.	Mean flower no. per peduncle	Flower diam. (cm)	Plant fresh wt. (g)
Epidermal	23.3a[z]	31.5a	6.3a	6.3a	7.3a	10.2a	3.1a	61.2a
Sub-epidermal	26.1b	40.4b	7.3b	7.4b	6.0a	10.1a	3.3a	76.5b

[z]Mean separation in columns by *t* test, 5% level.

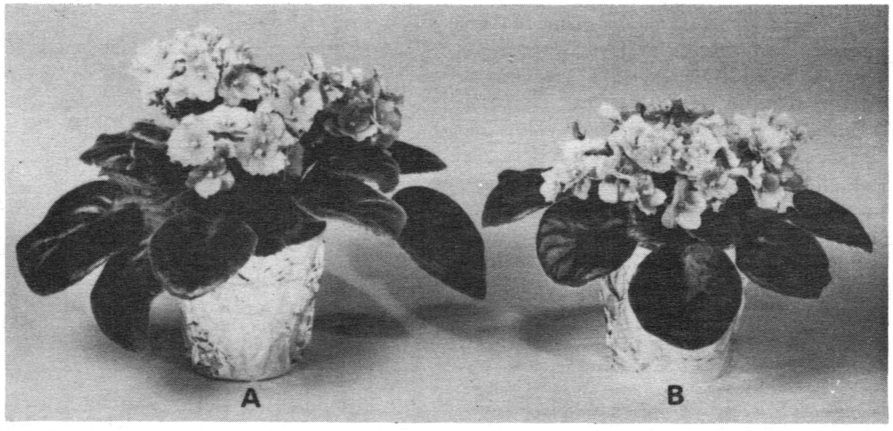

Fig. 2. Mature African violet 'Georgia' plants from sub-epidermal (A) and epidermal tissue (B), age 35 weeks.