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Plant regeneration from petiole segments of some species in tissue culture

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Abstract

The regeneration ability of 21 plant species belonging to 14 families was tested. The method of tissue culture in vitro was applied, on basic MS medium with an addition of growth regulators from the auxin and cytokinin groups. From among the investigated plant groups *Peperomia scandens* and *Caladium* × *hortulanum* were capable of plant regeneration, *Passiflora coerulea* regenerated shoots, *Hedera helix, Begonia glabra, Coleus blumei, Fuchsia hybrida, Passiflora suberosa* and *Peperomia eburnea* formed callus and roots, *Kalanchoe blossfeldiana, Pelargonium grandiflorum, P. peltatum, P. radula, Coleus shirensis* and *Magnolia soulangeana* produced callus, *Philodendron scandens, Rhododendron smirnovii, Hibiscus rosa-sinensis, Coprosma baueri, Cestrum purpureum* and *Solanum rantonnetii* did not exhibit any regeneration reactions.

INTRODUCTION

The method of in vitro culture of isolated plant organs and tissues is more and more frequently applied in studies of various processes occurring in plant organisms. In vitro cultures found particularly wide application in vegetative multiplication of valuable ornamental plants and in investigations on plant morphogenesis. In most of the plant species tested the processes of organogenesis are conditioned by growth regulators of the auxin and cytokinin groups added to the medium (Harris and Hart, 1964; Gautheret, 1966). Skoog and Miller (1957) proved that essential in this case is also the auxin: cytokinin ratio in the medium. The studies of Grushvickii et al. (1970), Zenkteler (1972), Rao et al. (1973), Prabhudesai and Narayanaswamy (1974) on the possibility of breeding whole plants from fragments of various organs demonstrated that the regeneration potential of plants varies within a family, genus and even species. It depends, among other things, on the kind of organ isolated. Thus, the ability

of organogenesis of various organs of the same plant or of the same organ from various plants can be studied.

Much attention has been devoted to the regeneration ability of leaf blades (Schraudolf and Reinert, 1959; Writh, 1959; Heide, 1964; 1965; Geeta et al., 1967; Bigot and Chlyah, 1970; Bigot, 1972, Nag and Johri, 1970; Zenkteler, 1971; Bajaj, 1972; Bajaj and Mader, 1974; Kato and Kawahara, 1972; Kukułczanka and Suszyńska, 1972; Havranek and Novak, 1973; Pierik, 1976), whereas not many data are available concerning the regeneration potential of isolated petioles completely deprived of leaf blade (Pierik, 1972; Pierik et al., 1974; Fonnesbach, 1974a, b; Langhe et al., 1974; Devos et al., 1976).

The present investigations were undertaken in order to: (1) test the regeneration potential of petioles of various plants under in vitro culture conditions, (2) study the influence of growth regulators of the auxin and cytokinin groups and of external factors on organ differentiation in cultures in vitro.

The finding of the ability of plant regeneration from petioles might eventually be useful in gardening practice for vegetative multiplication of these plants. This method would be particularly useful for reproduction of species represented by very few specimens (e.g. in garden collections) since it does not require the destruction of the mother plant.

PLANT MATERIAL AND METHODS

The experiments were performed in the period 1974-1976 by in vitro culture. The object of study were petioles of 21 tropical or subtropical plant species representing 14 families. The material for experiments was obtained from the collection of the Botanical Garden of the Wrocław University. It was collected in two vegetation seasons: spring (March–April–May — experiments A) and in summer (June–July–August — experiments B). The petioles were detached from the leaves at the stage of fully developed leaf blade from young one-year shoots. Only the petioles of *Caladium* × *hortulanum* Birdsey were taken from sterile plants obtained in in vitro culture from fragments of tubers by Kukułczanka and Prędota (1974). The culture was kept in a thermostated room at 24°-26°C under constant illumination of 2700 lux, in darkness and under photoperiodic conditions (17 h of light). The MS medium (Murashige and Skoog, 1962) was used without edamin and with sucrose content increased to 4 per cent. The medium was solidified with agar (Difco-Bacto). After sterilization in an autoclave the pH of the medium was 6.0. The MS was differentiated by the addition of growth regulators of the group of auxins and cytokinins in various

concentrations and combinations. From among auxins naphthyl-1-acetic acid (NAA — 2 mg/l), 2,4-dichlorophenoxyacetic acid (2,4-D — 1 mg/l) and indolyl-3-acetic acid (IAA — 0.5 and 1 mg/l) were used. The cytokinins applied were: 6-furfuryl-aminopurine (kinetin — 1, 2, 4 mg/l); 6-benzylaminopurine (BA — 1, 2, 4, 8 mg/l) and 6(-4-hydroxy-3-methyl-trans-2-butenyl-aminopurine (zeatin — 1, 2, 4 mg/l). For the explants of some plants the medium was enriched with yeast extract (YE — 2 g/l).

The petioles were disinfected with 0.5 per cent mercuric chloride for 30 to 60 s in dependence on the species. The disinfected material was washed repeatedly with sterile distilled water and divided into parts 0.5 cm long. Each explants was placed horizontally in a test tube on the surface of the medium. Explants with callus and differentiated roots or shoot buds were transferred at 8-week intervals to fresh medium of identical composition. Only explants of *Peperomia scandens* Ruiz. remained on the same medium for 16 weeks, then the regenerated plants were transferred to larger test tubes or pots. Observations were recorded at 2- or 4-week intervals. Each medium variant was used for 20 replications (explants).

In order to establish the contribution of the particular tissues to the process of organogenesis anatomical preparations were made. The material was fixed with Cr-A-F (Filutowicz and Kużdowicz, 1951). The paraffin method was applied. The sections were stained with safranin and fast green and then embedded in Canada balsam.

RESULTS

Plant forming explants

Peperomia scandens Ruiz. — Fragments of petioles of *P. scandens* at first markedly increased in size and after 4 weeks shoot buds or roots

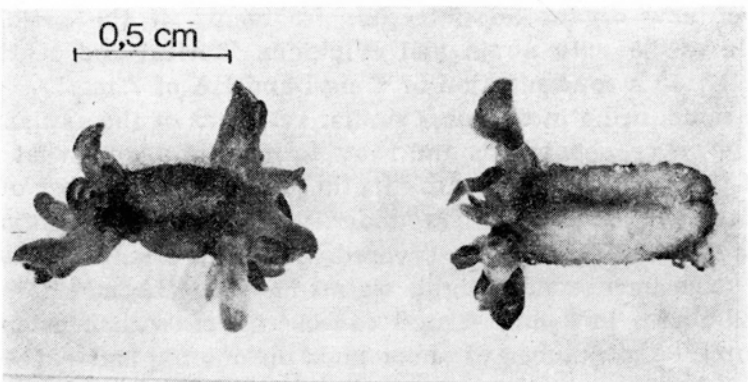


Fig. 1. Bud formation on petiole segments of *Peperomia scandens* Ruiz. after 4 weeks on MS medium with NAA (2 mg/l) and BA (2 mg/l)

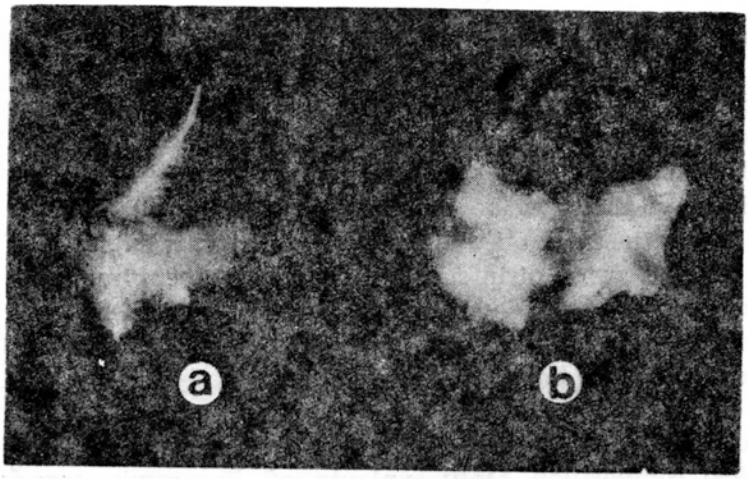


Fig. 2. Root formation on petiole segments of *Peperomia scandens* Ruiz. on MS medium with NAA (2 mg/l): a) in light, b) in darkness

formed on them, mainly close to the wound surface (Figs. 1, 2). No regularity was noted in the successive formation of organs — roots and shoot buds. Shoots developed after 12 weeks. The young plants were transferred after 16 weeks to fresh medium (Fig. 3) or planted in pots. The number of regenerating explants and the growth and development of the organs formed depended above all on the growth regulators added to the medium. The light conditions were not without effect on the number of explants forming young plants.

Spring experiments (A)

On medium with NAA shoot buds and roots formed only under light. In darkness the explants only increased in size (Table 1). Appearance of new organs on numerous fragments of the petioles was observed in media with auxin and cytokinin. Kinetin and zeatin were most effective in a concentration of 4 mg/l and BA of 2 mg/l.

On the same media in darkness similar reactions of the explants were observed, however, shoot buds and roots formed on a somewhat smaller number of them. On medium with kinetin (4 mg/l) the number of regenerating petioles was higher than under light. Roots differentiating in darkness were short and densely covered with hairs (Fig. 2b).

In the experiment under light, on media with BA added in 1 and 2 mg/l and zeatin in 2 and 4 mg/l concentrations with constant NAA level of 2 mg/l the number of shoot buds on one explant exceeded 10. In darkness, however, on the same media this number was always lower. On these media growth of the forming organs was always most intensive.

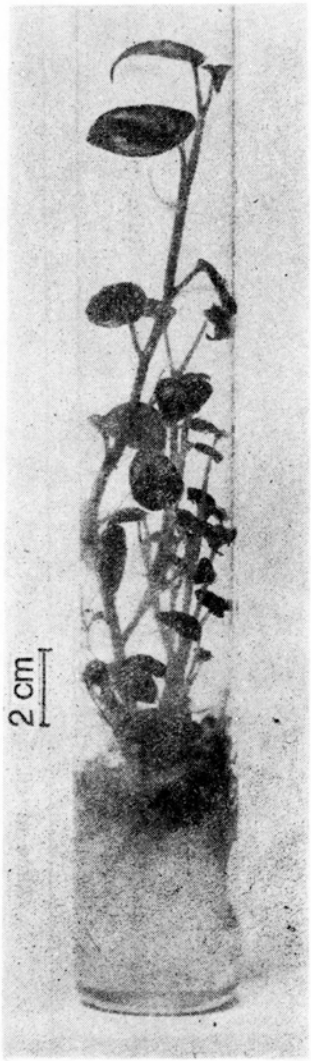


Fig. 3. *Peperomia scandens* Ruiz. plantlets after 24 weeks of culture on MS medium with NAA (2 mg/l) and BA (2 mg/l)

Summer experiments (B)

On medium with NAA or 2,4-D shoot bud initiation occurred on a small number of explants (Table 2). On medium containing 2,4-D the shoot buds did not develop to shoots and roots did not form. In media with auxin and cytokinin simultaneously added interaction of these substances was observed in the process of organogenesis. Kinetin, BA and zeatin with constant NAA level induced young plant formation from all explants in the particular combinations of the experiment (Fig. 4a,

Table 1

Influence of light conditions and growth regulators on organ formation on *Peperomia scandens* Ruiz. petiole segments cultured on MS medium (in per cent)
Experiments A

Growth regulators mg/l	Time of culture																							
	4 weeks						8 weeks						12 weeks						16 weeks					
	light			darkness			light			darkness			light			darkness			light			darkness		
	sb	sbr	r	sb	sbr	r	sb	sbr	r	sb	sbr	r	s	sr	r	s	sr	r	s	sr	r	s	sr	r
—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2 NAA	0	0	0	0	0	0	0	0	0	0	0	0	15	5	0	0	0	0	0	20	0	0	0	0
2 NAA; 1 kinetin	0	0	0	0	0	0	0	0	5	0	0	5	5	15	10	0	6	15	0	40	0	0	12	20
2 NAA; 2 kinetin	0	0	0	0	0	0	0	0	10	0	7	7	0	5	5	0	7	20	0	10	30	0	7	20
2 NAA; 4 kinetin	5	0	5	0	0	0	0	18	22	0	45	0	0	40	10	0	80	0	0	60	18	0	87	0
2 NAA; 1 BA	0	0	0	5	0	5	18	6	18	6	18	5	0	50	0	0	50	0	0	78	0	0	77	0
2 NAA; 2 BA	40	0	20	25	0	15	58	30	0	24	35	5	0	88	12	0	70	0	0	100	0	0	80	0
2 NAA; 4 BA	0	0	0	0	0	0	15	0	7	18	0	5	18	28	10	35	12	0	0	82	0	0	50	0
2 NAA; 1 zeatin	6	0	10	5	0	5	0	40	20	0	23	15	0	70	7	0	77	0	0	90	0	0	77	20
2 NAA; 2 zeatin	0	0	0	7	0	0	7	53	20	0	15	0	0	73	13	0	25	15	0	86	0	0	52	12
2 NAA; 4 zeatin	10	0	10	10	0	5	30	0	30	0	43	7	12	88	0	0	85	0	0	100	0	0	92	0

sb — only shhot buds, sbr — shoot buds and roots, r — only roots, s — only shoot, sr — shoot and roots.

Table 2

Influence of growth regulators on organ formation on *Peperomia scandens* Ruiz. petiole segments cultured on MS medium (in per cent)
Experiments B

Growth regulators mg/l	Time of culture													
	4 weeks			8 weeks			12 weeks				16 weeks			
	sb	sbr	r	sb	sbr	r	sb	s	sr	r	sb	s	sr	r
—	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2 NAA	0	0	0	10	0	10	10	0	20	0	0	0	40	0
2 NAA; 4 kinetin	0	10	10	20	30	10	0	15	70	0	0	0	100	0
2 NAA; 2 BA	12	30	5	25	60	5	0	25	75	0	0	0	100	0
2 NAA; 4 BA	15	10	0	20	30	10	0	35	50	0	0	0	100	0
2 NAA; 2 zeatin	0	12	5	10	60	5	0	10	90	0	0	0	100	0
2 NAA; 4 zeatin	5	12	5	30	40	0	0	0	80	0	0	0	100	0
1 2,4-D	0	0	0	0	0	0	5	0	0	0	10	0	0	0
1 2,4-D; 4 kinetin	0	0	0	0	0	0	0	0	0	0	10	0	0	0
1 2,4-D; 2 BA	0	0	0	60	0	0	90	0	0	0	0	100	0	0
1 2,4-D; 4 BA	0	0	0	50	0	0	80	0	0	0	0	100	0	0
1 2,4-D; 2 zeatin	0	0	0	0	0	0	0	0	0	0	90	0	0	0
1 2,4-D; 4 zeatin	0	0	0	10	0	0	30	0	0	0	100	0	0	0

sb — only shoot buds, sbr — shoot buds and roots, r — only roots, s — ontly shoot, sr — shoot and roots.

c, e, g, i). Shoot buds retarded, with inhibited development (Fig. 4b, d, f, h, j) formed on media containing cytokinin and a constant 2,4-D concentration. Shoots developed exclusively on medium with BA added. Roots did not differentiate on medium with 2,4-D and cytokinins.

An intensive growth of differentiated organs was noted similarly as in experiment A on media with NAA and BA (2 mg/l) added as well as with NAA and zeatin (2 mg/l).

Inspection of the anatomical preparations demonstrated that the increase of the petiole fragment in size occurred owing to the division of subepidermal and parenchymal cells (Fig. 5). Then, close to the surface along which the explant was cut, on the whole periphery of the wound nodules appeared (Fig. 6). At the tip of the protuberances shoot apical meristem was found in the outer cell layers (Fig. 7). The roots formed in close connection with the vascular bundles of the petiole (Fig. 8).

Caladium × *hortulanum* Birdsey. — On the surface of the wound on the petiole fragment of *Caladium* × *hortulanum* callus formed with compact tuberculous structure. After 8 or 12 weeks shoot buds and roots grew out of the callus (Fig. 9). The explants which first formed roots did not later produce buds. On the other hand, explants which first formed shoot buds could form roots. After 16 weeks leaf rosettes developed which spread when transferred to larger test tubes. The per cent of explants forming shoot buds and roots depended on the growth regulators added to the medium and the light conditions.

Table 3

Influence of growth regulators on organ formation on *Caladium* × *hortulanum* Birdsey petiole segments cultured on MS medium (in per cent)
Experiment I

Growth regulators mg/l	Time of culture							
	4 weeks		8 weeks		12 weeks		16 weeks	
	sb	callus	sb	callus	sb	callus	sb	callus
—	0	0	0	0	0	0	0	0
2 NAA	0	0	0	0	0	0	0	0
2 NAA; 4 kinetin	0	0	0	10	0	18	0	25
2 NAA; 1 BA	0	20	0	40	25	40	40	50
2 NAA; 2 BA	0	18	0	35	20	55	34	66
2 NAA; 4 BA	0	10	0	40	18	80	20	80
2 NAA; 2 zeatin	0	5	0	27	10	40	16	40
2 NAA; 4 zeatin	0	3	0	23	0	38	0	50

sb — shoot buds,

Table 4

Influence of growth regulators on organ formation on *Caladium* × *hortulanum* Birdsey petiole segments cultured on MS medium (in per cent)
Experiment II

Growth regulators mg/l	Time of culture															
	4 weeks				8 weeks				12 weeks				16 weeks			
	sb	sbr	r	callus	sb	sbr	r	callus	sb	sbr	r	callus	sb	sbr	r	callus
—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1 2,4-D	0	0	0	0	0	0	0	13	3	0	3	23	3	0	3	23
1 2,4-D; 1 kinetin	0	0	0	2	3	0	5	19	4	0	5	20	5	8	5	29
1 2,4-D; 2 kinetin	0	0	0	5	5	0	3	16	5	0	3	20	7	0	3	24
1 2,4-D; 4 kinetin	0	0	0	8	1	0	0	24	6	0	0	32	9	0	0	44
1 2,4-D; 1 BA	0	0	0	0	0	0	0	10	10	0	0	25	15	10	0	35
1 2,4-D; 2 BA	0	0	0	2	0	0	0	12	0	0	0	17	0	5	0	35
1 2,4-D; 4 BA	0	0	0	8	1	0	0	18	5	0	0	29	6	0	0	38
1 2,4-D; 1 zeatin	0	0	0	0	0	0	0	5	0	0	0	12	0	0	0	20
1 2,4-D; 2 zeatin	0	0	0	0	5	0	0	17	10	5	0	35	8	15	0	55
1 2,4-D; 4 zeatin	0	0	0	0	0	0	0	20	0	0	0	42	40	0	0	60

sb — only shoot buds, sbr — shoot buds and roots, r — only roots.

In experiment I on control medium and the same with 2 mg/l NAA added, the explants, underwent necrosis both under light and in darkness. The callus and shoot buds formed only under light on media containing NAA and cytokinin (Table 3). The growth intensity of callus tissue on the tested MS medium variants was very low. The shoot buds differentiated in the callus on medium containing BA and zeatin in suitable concentrations with constant 2 mg/l NAA dose. Explants forming roots exclusively were never observed. The newly formed leaf rosettes produced roots as late as after 20 weeks of in vitro culture.

In experiment II the callus tissue arose on explants cultures both on medium with 2,4-D and that with auxin and cytokinin (Table 4). Intensive callus growth was observed on medium with in 1, 2, 4 mg/l kinetin or with BA in 2 and 4 mg/l concentrations or zeatin in 4 mg/l concentration with constant dose of 2,4-D (1 mg/l). On 2,4-D the shoot buds and roots differentiated sporadically. More numerous explants showed regeneration on media containing 2,4-D and cytokinins. Shoot buds did not form at all only on medium with 1 mg/l zeatin and 1 mg/l 2,4-D added. Cytokinins added in high concentration retarded root initiation.

On medium with IAA (0.5 and 1 mg/l) in experiment III callus tissue appeared late and on a small number of explants (Table 5). When kinetin was also added to the medium, particularly in a 2 mg/l concentration, callus formed on a greater number of petioles. This effect was slightly enhanced when the medium was additonally enriched with 2 g/l YE. Shoot buds and roots differentiated on media with auxin and cytokinin, with the exception of the medium containing 0.5 mg/l IAA and 1 mg/l kinetin on which these organs were not found. IAA (0.5 and 1 mg/l) stimulated only root formation.

In experiment IV explants placed under continuous illumination formed callus tissue and shoot buds when cultured on the variants of MS medium tested, the medium with kinetin or zeatin in 2 mg/l concentration excepted (Table 6). Under 17th illumination callus and shoot buds formed only on medium with kinetin or zeatin in 2 mg/l concentration. All cytokinins inhibited root differentiation.

Observation of anatomical preparations showed that callus tissue forms on the injured surface of the explants owing to division of parenchymal and epidermis cells (Fig. 10, 10a). In the outer callus layers groups of cells were observed forming the growth apexes of shoots and roots (Fig. 11).

Callus and shoot forming explant

Passiflora coerulea Linn. — After a dozen or so days of culture the *Passiflora coerulea* petioles produced callus tissue on the surface of the

Table 5

Influence of growth regulators on organ formation on *Caladium* × *hortulanum* Birdsey petiole segments cultured on MS medium (in per cent)
Experiment III

Growth regulators and YE mg/l	Time of culture															
	4 weeks				8 weeks				12 weeks				16 weeks			
	sb	sbr	r	callus	sb	sbr	r	callus	sb	sbr	r	callus	sb	sbr	r	callus
0.5 IAA	0	0	0	0	0	0	0	0	0	0	0	5	0	0	8	11
0.5 IAA; 1 kinetin	0	0	0	0	0	0	0	10	0	0	0	10	0	0	0	25
0.5 IAA; 2 kinetin	10	0	0	10	10	0	10	50	25	5	10	55	0	40	10	67
0.5 IAA; 2 kinetin 2000 YE	0	0	0	20	25	0	0	50	38	0	0	60	50	10	0	72
1 IAA	0	0	0	0	0	0	0	7	0	0	7	13	0	0	15	20
1 IAA; 1 kinetin	0	0	0	0	0	0	0	30	0	28	0	30	0	30	0	45
1 IAA; 2 kinetin	0	0	0	25	30	0	0	50	52	0	10	62	60	0	10	74
1 IAA; 2 kinetin 2000 YE	23	0	0	40	37	20	5	67	10	40	7	75	10	53	7	80

sb — only shoot buds, sbr — shoot buds and roots, r — only roots, yeast extracts.

Table 6

Influence of light conditions and growth regulators on organ formation on *Caladium* × *hortulanum* Birdsey petiole segments cultured on MS medium (in per cent)
Experiment IV

Cytokinins	Time of culture															
	4 weeks				8 weeks				12 weeks				16 weeks			
mg/l	photoperiod		continuos light		photoperiod		continuos light		photoperiod		continuos light		photoperiod		continuos light	
	sb	callus	sb	callus	sb	callus	sb	callus	sb	callus	sb	callus	sb	callus	sb	callus
—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2 kinetin	0	15	0	0	20	20	0	0	20	30	0	0	26	40	0	0
4 kinetin	0	0	0	10	0	0	8	10	0	0	10	18	0	0	10	25
2 BA	0	0	0	20	0	0	20	20	0	0	25	28	0	0	30	35
4 BA	0	0	0	13	0	0	17	20	0	0	20	27	0	0	26	39
2 zeatin	0	10	0	0	12	15	0	0	15	22	0	0	18	25	0	0
4 zeatin	0	0	0	8	0	0	5	10	0	0	10	12	0	0	15	15

sb — shoot buds. Photoperiod — 17h illumination.

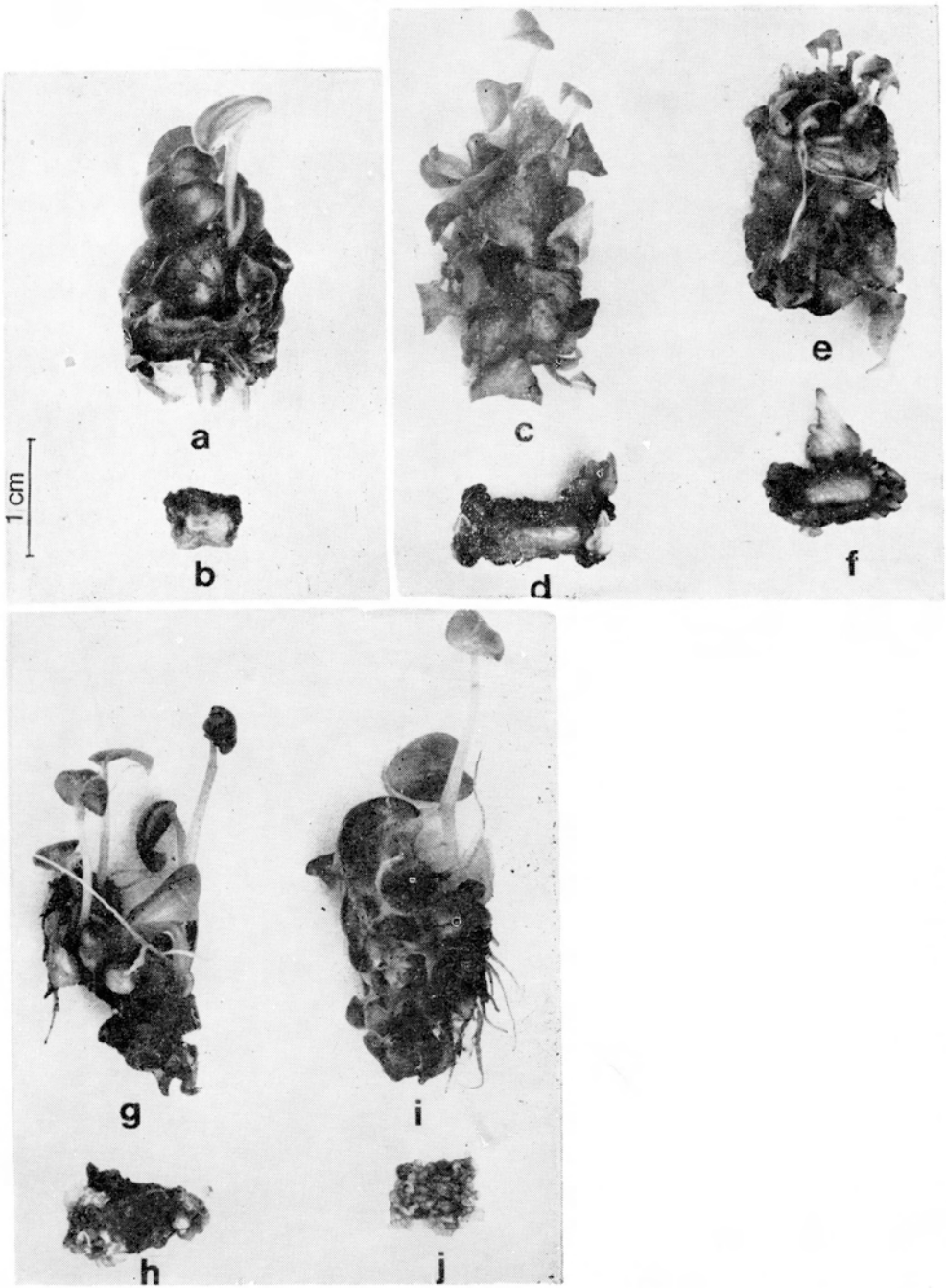
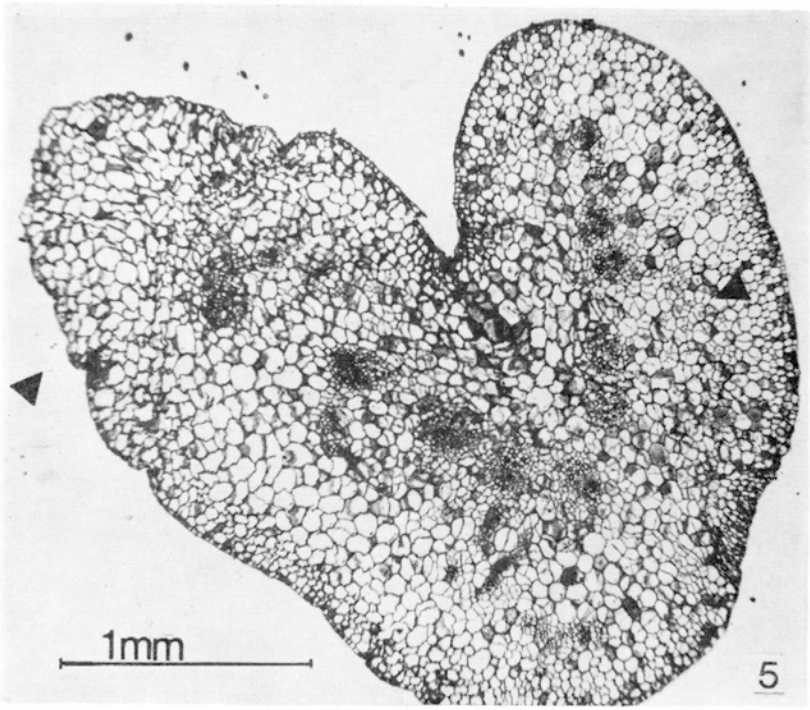


Fig. 4. Influence of growth regulators on plantlet formation on *Peperomia scandens* Ruiz. petiole segments after 12 weeks of culture on MS medium with: a) NAA (2 mg/l) and kinetin (4 mg/l), b) 2,4-D (1 mg/l) and kinetin (4 mg/l), c) NAA (2 mg/l) and BA (2 mg/l), d) 2,4-D (1 mg/l) and BA (2 mg/l), e) NAA (2 mg/l) and BA (4 mg/l), f) 2,4-D (1 mg/l) and BA (4 mg/l), g) NAA (2 mg/l) and zeatin (2 mg/l), h) 2,4-D (1 mg/l) and zeatin (2 mg/l), i) NAA (2 mg/l) and zeatin (4 mg/l), j) 2,4-D (1 mg/l) and zeatin (4 mg/l)



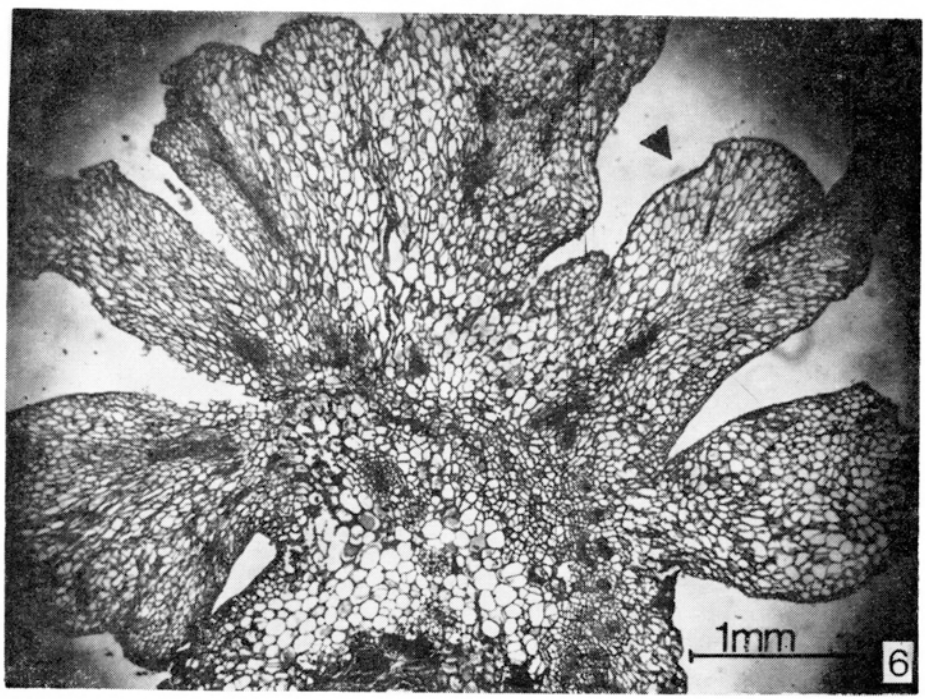
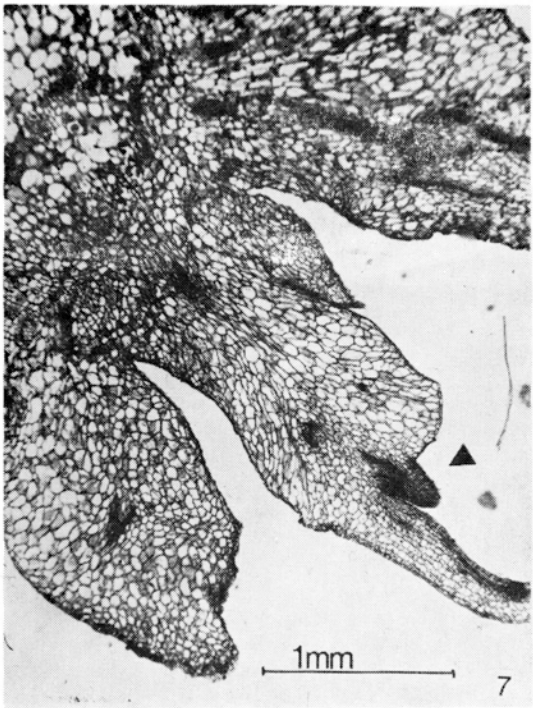


Fig. 5. Cross section through petiole of *Peperomia scandens* Ruiz. showing proliferation of subepidermal and parenchymal cells (arrows)

Fig. 6. Cross section through petiole of *Peperomia scandens* Ruiz. near the wounded surface showing nodules without callus formation arrow



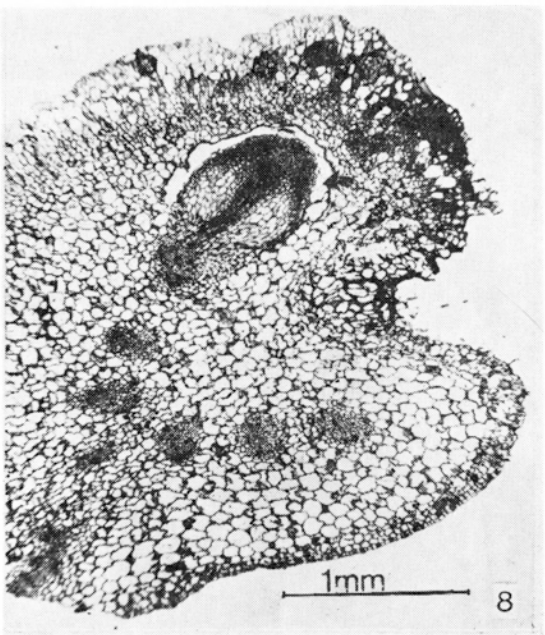


Fig. 7. Cross section through petiole shown in Fig. 6. Arrow indicates a shoot apical meristem

Fig. 8. Cross section through petiole of *Peperomia scandens* Ruiz. showing the formation of root

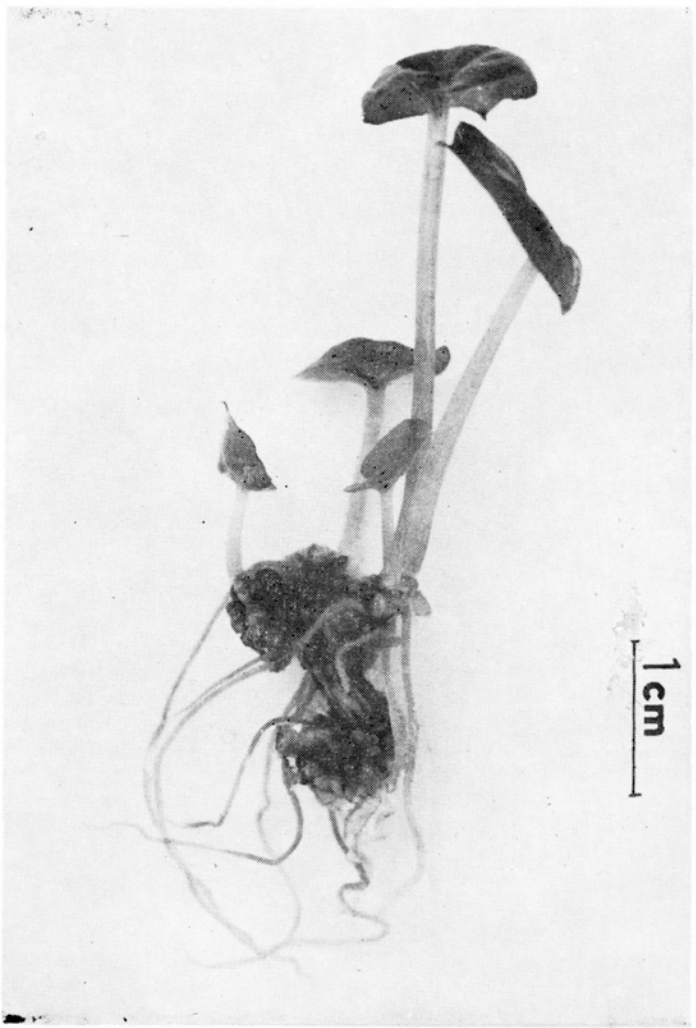
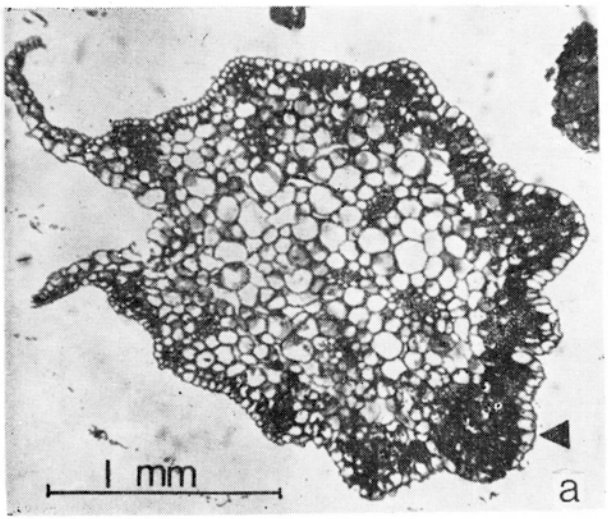


Fig. 9. *Caladium* × *hortulanum* Birdsey plantlets after 24 weeks of culture on MS medium with 2,4-D (1 mg/l) and kinetin (1 mg/l)



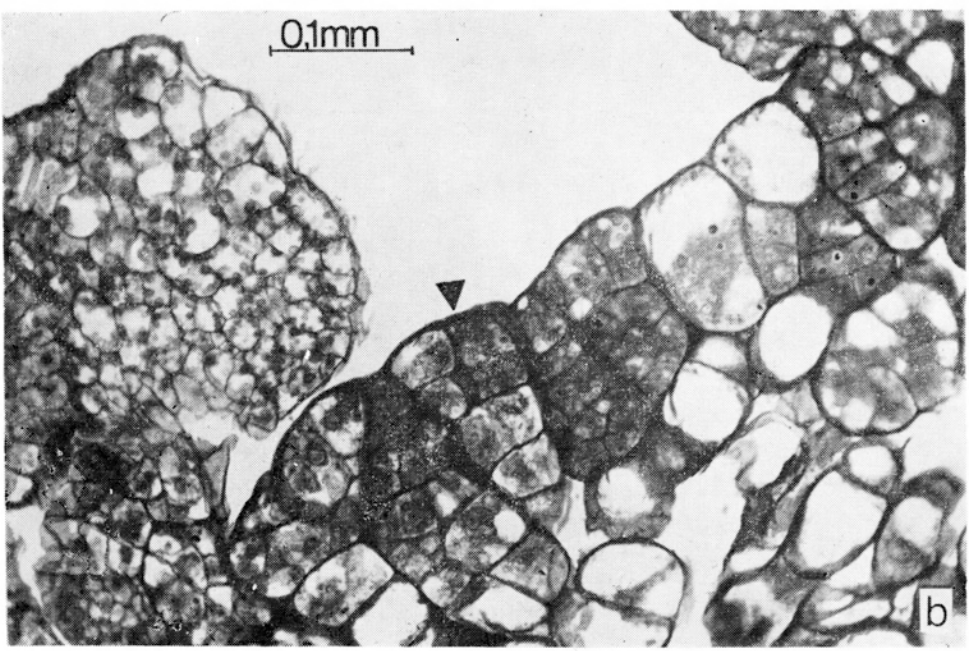
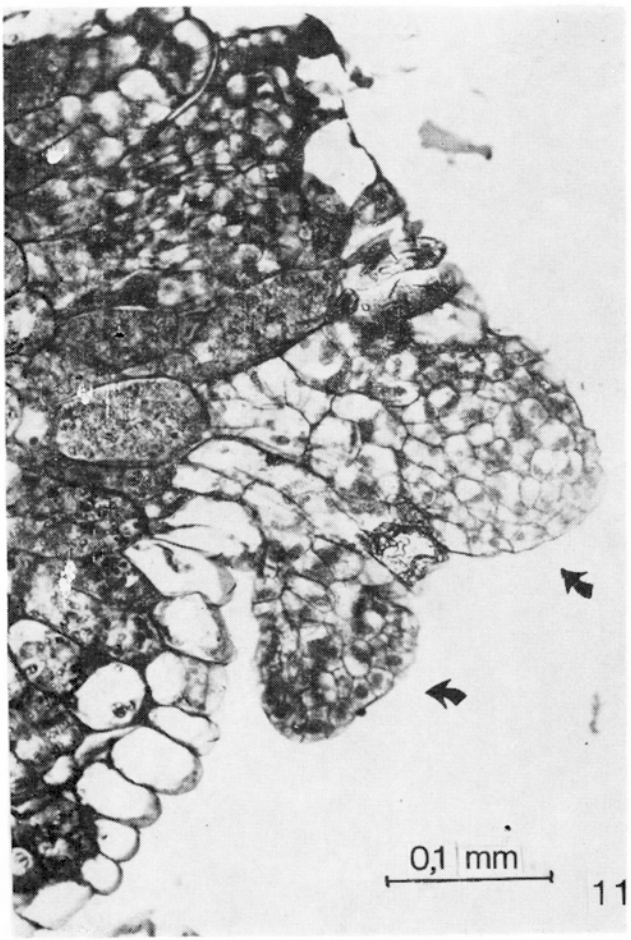


Fig. 10. Cross section through petiole of *Caladium* × *hortulanum* Birdsey. a — (near base) showing proliferating parenchymal cells; b — showing proliferating epidermal cells



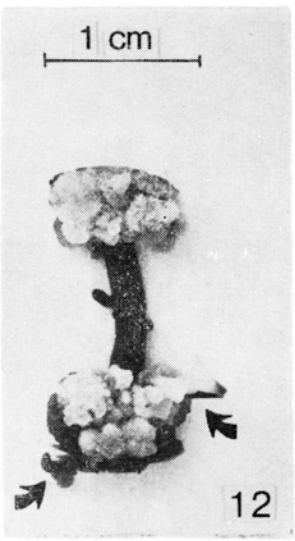
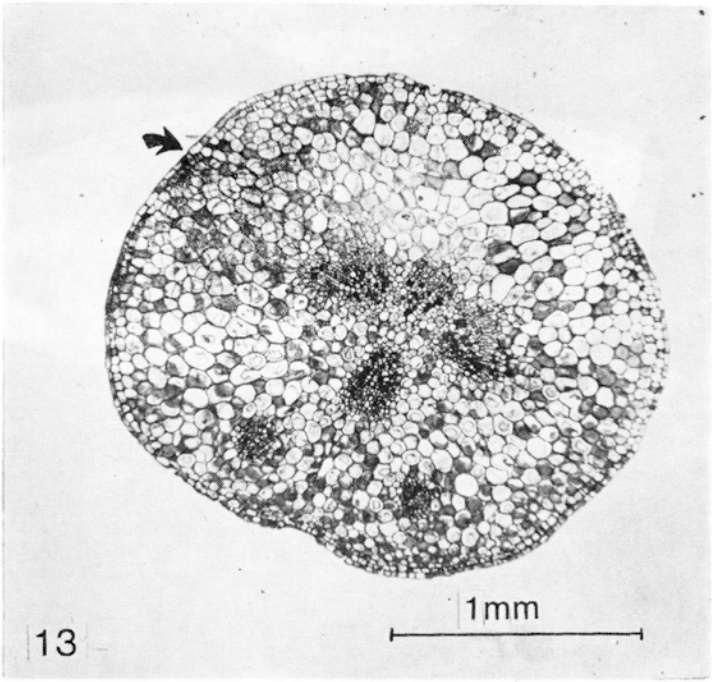


Fig. 11. Cross section through petiole of *Caladium* × *hortulanum* Birdsey with callus. External groups of callus cells form shoot apical meristems

Fig. 12. Bud formation on petiole segments of *Passiflora coerulea* Linn. after 8 weeks of culture on MS medium with 2,4-D (1 mg/l) and zeatin (4 mg/l)



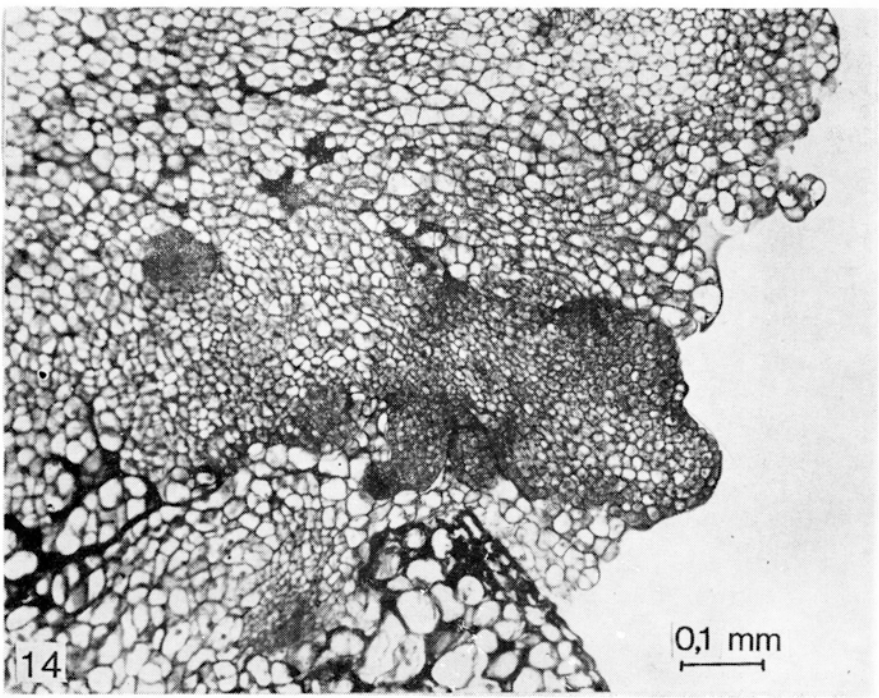


Fig. 13. Cross section through petiole of *Passiflora coerulea* Linn. showing proliferating parenchymal cells

Fig. 14. Apical meristem of *Passiflora coerulea* Linn. shoot, differentiating from callus cells

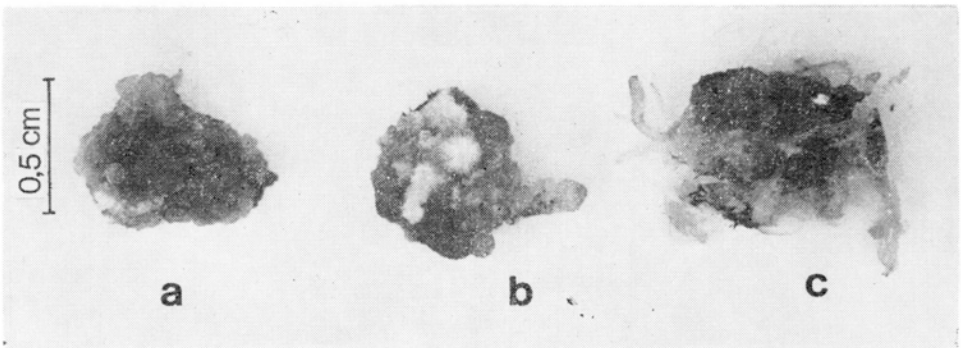


Fig. 15. Callus and root formation on *Coleus blumei* Benth. petiole segments after a) 4 weeks, b) 8 weeks of culture on MS medium with 2,4-D (1 mg/l)

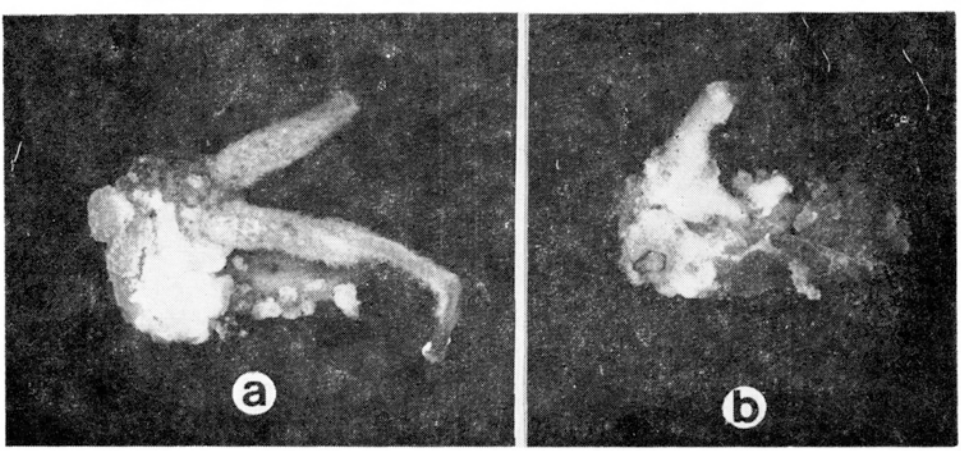


Fig. 16. Callus and root formation on *Hedera helix* Linn. petiole segments after 8 weeks of culture on MS medium with: a) NAA (2 mg/l) and BA (2 mg/l), b) NAA (2 mg/l) and BA (4 mg/l)

Table 7

Influence of light conditions and growth regulators on organ formation on *Passiflora coerulea* Linn. petiole segments cultured on MS medium (in per cent)

Experiments A

Growth regulators mg/l	Time of culture: 4 weeks light callus	4 weeks light sb	4 weeks darkness callus	4 weeks darkness sb	8 weeks light callus	8 weeks light sb	8 weeks darkness callus	8 weeks darkness sb	12 weeks light callus	12 weeks light sb	12 weeks darkness callus	12 weeks darkness sb	16 weeks light callus	16 weeks light sb	16 weeks darkness callus	16 weeks darkness sb
—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2 NAA	30	0	35	0	35	0	40	0	35	0	45	0	35	0	45	0
2 NAA; 1 kinetin	30	0	48	0	45	0	60	0	50	5	65	0	50	5	70	0
2 NAA; 2 kinetin	32	0	50	0	40	25	80	10	60	30	82	10	62	30	85	10
2 NAA; 4 kinetin	30	0	40	0	35	0	60	0	45	0	60	0	45	0	62	0
2 NAA; 1 BA	15	0	25	0	20	20	50	8	35	25	50	10	40	28	50	10
2 NAA; 2 BA	40	0	62	0	60	30	80	20	72	40	80	20	75	40	85	20
2 NAA; 4 BA	38	0	42	0	45	0	70	0	50	0	72	0	50	0	75	0
0,5 IAA	0	0	0	0	12	0	30	0	20	0	35	0	20	0	37	0
0,5 IAA; 1 kinetin	20	0	20	0	35	0	90	0	40	0	95	0	55	0	95	0
0,5 IAA; 2 kinetin	40	0	90	0	45	0	90	0	60	10	90	0	70	20	90	0

sb — shoot buds.

lesions. This callus was compact and friable, green under light conditions and yellow-white in darkness. After 8 weeks on some variants of MS medium shoot buds differentiated (Fig. 12), from which some few developed into shoots with tiny leaves. The young shoots died after several weeks. *P. coerulea* explants did not form roots. Initiation of callus and shoot buds depended on growth regulators and light conditions.

Spring experiments (A)

The explants formed callus tissue (Table 7) on medium with an addition of auxin (NAA or IAA) as well as auxin and cytokinin (kinetin or BA). Both cytokinins were most effective in 2 mg/l concentrations. Analogous reactions of explants were observed in experiments carried out in darkness. The number of petiole fragments, however, with callus tissue was higher than under light. This tissue grew intensively on media with 2 mg/l NAA and 4 mg/l kinetin or 2 mg/l NAA and 1 mg/l BA added. Callus grew more intensively in darkness than under light. Shoot buds differentiated on media with cytokinin and auxin, however, the former in 4 mg/l concentration inhibited shoot bud initiation. In darkness the number of petiole fragments producing shoot buds was lower than under light.

Summer experiments (B)

On medium with NAA a large number of explants formed callus both in light and darkness conditions (Table 8). On medium with 2,4-D callus appeared only in darkness on few explants. Similarly as in the spring experiments (A) callus formed in a large number of explants on media with auxin and cytokinin. Only on medium with 2,4-D and kinetin in 4 mg/l concentration callus tissue did not form. In darkness the number of petioles with callus was the same or higher than under light. Callus grew intensively both under light and in darkness on media containing 2 mg/l NAA and 2 or 4 mg/l BA and 2 mg/l NAA and 2 or 4 mg/l zeatin as well as 1 mg/l 2,4-D and 2 or 4 mg/l zeatin or 2 mg/l BA. Shoot buds were initiated on media only with 2,4-D and BA (2 mg/l) or with zeatin (2 and 4 mg/l). Shoot bud formation was not, however, observed on media with NAA and cytokinin as was the case in experiments A. In darkness the shoot buds were initiated on media with addition of the same growth regulators, but the number of explants with shoot buds was lower than under light conditions.

Analysis of the anatomical preparations demonstrated that mainly

Table 8

Influence of light conditions and growth regulators on organ formation on *Passiflora coerulea* Linn. petiole segments cultured on MS medium (in per cent)
Experiments B

Growth regulators mg/l	Time of culture: 4 weeks light callus	4 weeks light sb	4 weeks darkness callus	4 weeks darkness sb	8 weeks light callus	8 weeks light sb	8 weeks darkness callus	8 weeks darkness sb	12 weeks light callus	12 weeks light sb	12 weeks darkness callus	12 weeks darkness sb	16 weeks light callus	16 weeks light sb	16 weeks darkness callus	16 weeks darkness sb
—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2 NAA	0	0	10	0	40	0	40	0	55	0	60	0	60	0	70	0
2 NAA; 1 kinetin	30	0	25	0	50	0	40	0	58	0	45	0	75	0	80	0
2 NAA; 2 kinetin	30	0	30	0	40	0	45	0	60	0	60	0	85	0	80	0
2 NAA; 4 kinetin	32	0	30	0	50	0	58	0	88	0	80	0	92	0	90	0
2 NAA; 1 BA	15	0	20	0	30	0	30	0	45	0	50	0	72	0	70	0
2 NAA; 2 BA	55	0	50	0	85	0	70	0	95	0	90	0	100	0	100	0
2 NAA; 4 BA	50	0	60	0	60	0	65	0	80	0	78	0	92	0	100	0
2 NAA; 2 zeatin	70	0	50	0	80	0	65	0	85	0	90	0	94	0	95	0
2 NAA; 4 zeatin	60	0	60	0	80	0	80	0	90	0	85	0	100	0	100	0
1 2,4-D	0	0	0	0	0	0	5	0	0	0	7	0	0	0	7	0
1 2,4-D; 4 kinetin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1 2,4-D; 2 BA	10	0	20	0	10	0	30	2	32	5	50	2	32	5	55	4
1 2,4-D; 4 BA	5	0	10	0	5	0	25	0	22	0	40	0	30	0	40	0
1 2,4-D; 2 zeatin	40	0	40	0	50	7	55	7	50	12	60	7	50	13	60	7
1 2,4-D; 4 zeatin	40	0	40	0	70	6	60	0	68	21	60	5	70	21	80	5

sb — shoot buds.

the cells on the surface of the injury in the explants or near it underwent divisions. Subepidermal and parenchymal cells divided (Fig. 13), thus leading to the formation of callus tissue. The well visible groups of smaller callus cells dividing on various planes give rise to the growth apices of shoots (Fig. 14).

Callus and root forming explants

Hedera helix Linn., *Begonia glabra* Aublet., *Coleus blumei* Benth., *Fuchsia hybrida* Voss., *Passiflora suberosa* Linn., *Peperomia eburnea* hort. formed callus tissue and roots on petiole fragments cultured on appropriate media in vitro (Table 9). Explants of these species did not produce shoot buds in spite of application of various auxin and cytokinin levels. Callus tissue formed on both the injured surfaces or on one of them, it then extended covering the entire explants. After 4 or 8 weeks roots grew out of the callus tissue (Figs. 15, 16).

Explants forming callus exclusively

Fragments of petioles of *Kalanchoe blossfeldiana* v Poelln., *Pelargonium grandiflorum* Willd., *P. peltatum* Ait., *P. radula* L'Her. *Coleus shirensis* Baker., *Magnolia soulangeana* Soul. formed exclusively callus tissue in in vitro culture (Table 9). Auxins and cytokinins applied in various concentrations did not induce organ differentiation, but increased the number of explants forming callus and the intensity of growth of the latter. Neither did organs differentiate in callus transferred to agar and liquid medium.

Explants showing no regenerative reactions

Petioles of *Philodendron scandens*, C. Koch, H. Stello., *Rhododendron smirnovii*, Trautv., *Hibiscus rosa-sinensis* Linn., *Coprosma baueri* Endl., *Cestrum purpureum* (Lind.) Standl., *Solanum rantonnetii* Carr. were cultured on media with various auxin (NAA, 2,4-D, IAA) concentrations and cytokinins (kinetin, BA) added. On none of the tested MS medium variants did the explants of these species show regenerative reactions and after two weeks or so they wilted.

DISCUSSION

Of the 21 plant species examined, petioles of only 3 species formed shoot buds (*Caladium* × *hortulanum*, *Peperomia scandens*, *Passiflora*

coerulea), and of these three only two species also produced roots (*Caladium* × *hortulanum*, *Peperomia scandens*). The remaining species formed callus and roots or only callus or else showed no noticeable changes. The differences in the reaction of the explants from various species within one genus, and even in different varieties within one species (Miszke, 1972) indicate that the regeneration potential is genetically conditioned. The unequal regeneration ability of various species of plants was reported among others by Grushvickii et al. (1970) in the family *Araliaceae*, Zenkteler (1972) in the family *Solanaceae*, and Prabhudesai and Narayanaswamy (1974) in the family *Asclepiadaceae*. In the cases described callus formation and organ differentiation were induced by exogenous growth regulators (auxins and cytokinins). It depends, however, on the properties of the given species what the reaction of the explants will be to the same medium variants, and with what intensity and abundance callus tissue will form.

Caladium × *hortulanum* — On medium containing NAA and increasing cytokinin doses (BA, zeatin) callus tissue formation is stimulated and the ability of organogenesis is distinctly depressed. A similar reaction was noted by Prabhudesai and Narayanaswamy (1973) in petioles of *Nicotiana tabacum*. According to these authors, BA in 4 mg/l concentration together with IAA and adenin reduce the ability of organogenesis. This is probably connected with an unsuitable quantitative proportion of auxin to cytokinin, which according to Skoog and Miller (1975), Heide (1965) and Kartha et al. (1974) has an important influence on organ differentiation processes in in vitro cultures. If 2,4-D is present in the medium, the increasing kinetin and BA doses evoke similar effects in callus and shoot bud formation as those observed in the presence of NAA. All the cytokinins applied in 4 mg/l concentration together with 2,4-D inhibit root formation. Schraudolf and Reinert (1959) when investigating leaf segments of *Begonia* sp. found that cytokinins not only inhibit root differentiation, but abolish the stimulating action of 2,4-D. Probably cytokinins in the concentration used evoke the same effect in petiole explants of *Caladium* × *hortulanum*. Kinetin in 1 and 2 mg/l concentrations together with IAA stimulates callus, shoot bud and root formation.

Peperomia scandens — Explants of this species produce plants on media with NAA and cytokinins, but on media with 2,4-D and cytokinins inhibition of shoot buds and roots occurs. According to Schraudolf and Reinert (1959), 2,4-D stimulates root initiation on begonia leaves and retards or inhibits the development of shoots. It would seem, therefore, that after appearance of shoot buds on petiole explants of

Table
Morphogenetic responses of petiole segments

Growth regulators mg/l	*Caladium* × *hortulanum*	*Passiflora coerulea*	*Passiflora suberosa*	*Peperomia scandens*	*Peperomia eburnea*	*Hedera helix*
Control	—	—	—	—	—	—
2 NAA	—	callus, r	callus, r	sb, r	callus, r	callus, r
2 NAA; 1 kinetin		callus, sb	callus	sb, r	callus	callus, r
2 NAA; 2 kinetin		callus, sb	callus	sb, r	callus	callus, r
2 NAA; 4 kinetin	callus	callus		sb, r		callus, r
2 NAA; 1 BA	callus, sb	callus	callus	sb, r	callus, r	callus
2 NAA; 2 BA	callus, sb	callus	callus	sb, r	callus, r	callus, r
2 NAA; 4 BA	callus, sb	callus, sb		sb, r		callus, r
2 NAA; 8 BA						callus
2 NAA; 1 zeatin				sb, r		
2 NAA; 2 zeatin	callus, sb	callus		sb, r		
2 NAA; 4 zeatin	callus	callus		sb, r		
1 2,4-D	callus, sb, r	callus	—	sb		callus
1 2,4-D; 1 kinetin	callus sb, r		—			
1 2,4-D; 2 kinetin	callus, sb, r		—			
1 2,4-D; 4 kinetin	callus, sb	—		sb		callus
1 2,4 D; 1 BA	callus, sb, r		—			callus
1 2,4-D; 2 BA	callus, sb, r	callus, sb	—	sb		callus
1 2,4-D; 4 BA	callus, sb	callus		sb		callus
1 2,4-D; 8 BA						callus
1 2,4-D; 1 zeatin	—					
1 2,4-D; 2 zeatin	callus, sb, r	callus, sb		sb		
1 2,4-D; 4 zeatin	callus, sb	callus, sb	callus	sb		
						callus, r
1 IAA	callus, r		callus			callus
1 IAA; 1 kinetin	callus, sb, r					
1 IAA; 2 kinetin	callus, sb, r		callus			callus
1 IAA; 1 BA	callus, sb, r					
0,5 IAA	callus, r	callus				
0,5 IAA; 1 kinetin	callus	callus				
0,5 IAA; 2 kinetin	callus, sb, r	callus, sb				
1 kinetin						
2 kinetin	callus, sb					
4 kinetin	callus, sb					
1 BA						
2 BA	callus, sb					
4 BA	callus, sb					
2 zeatin	callus, sb					
4 zeatin	callus, sb					

sb — shoot buds, r — roots.

9
to various combinations of MS medium

Coleus blumei	*Coleus shirensis*	*Fuchsia hybrida*	*Magnolia soulangeana*	*Kalanchoe blossfeldiana*	*Begonia glabra*	*Pelargonium grandiflorum*	*Pelargonium peltatum*	*Pelargonium radula*
—	—	—	—	—	—	—	—	—
callus, r	callus	callus, r	callus	callus	callus, r	callus	callus	—
callus, r	callus	—	callus	callus	callus	callus	callus	callus
callus, r		—	callus	callus	callus	callus	callus	callus
callus		—	callus	callus	—	callus	callus	callus
	callus	—	callus	—	—	callus	callus	callus
callus		—						
callus								
callus		callus						
callus, r								
callus, r								
callus, r	callus	callus	—	—	—	callus	callus	—
	callus	—	callus	callus	—	callus	callus	callus
callus								
	callus	—	callus	callus		callus	callus	callus
callus								
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callus								
callus								
	callus		callus	—	—	callus	callus	callus
	callus		callus	callus	—	callus	—	callus
	callus		callus	callus		callus	callus	callus
	—							
—								
—	—							
—								

Peperomia scandens, inhibition of their growth and development is the consequence of the inhibitory action of 2,4-D. Analogously, the absence of roots may be attributed to the inhibitory action of cytokinins. Only on medium with BA and 2,4-D did the shoot buds develop into shoots. This may be explained as do Heide (1965) and Rao et al. (1973) by a higher BA activity as compared with that of kinetin and zeatin. A similar morphogenetic reaction of tobacco petioles to BA in the medium was found by Prabhudesai and Narayanswamy (1973). Moreover, Rao et al. (1973) reported that 2,4-D together with BA (0.2 mg/l) inhibits shoot bud development on explants of leaves and shoots of two species of the genus *Petunia*. The different effect produced by these substances in *Peperomia scandens* explants, that is development of buds to shoots may be ascribed to the higher concentration of BA in the medium (2 and 4 mg/l). Havranek and Novak (1973) observed, when investigating *Alium sativum* leaves, that on medium with 2,4-D added callus formed in which only vascular elements differentiated. On the other hand, when this auxin was absent, they observed shoot bud initiation. A similar reaction of isolated *Torenia fournieri* leaves to auxin 2,4-D was observed by Bajaj (1972). This author noted thickening of the leaves and callus formation with simultaneous inhibition of shoot bud differentiation.

Passiflora coerulea — Fragments of petioles of *Passiflora coerulea* on some MS medium variants exhibit an ability to organogenesis. Increasing cytokinin doses in the presence of NAA stimulate callus tissue formation. Bud initiation occurs at cytokinin concentrations of the order of 1 and 2 mg/l kinetin and BA. Higher cytokinin concentrations inhibit organogenesis like in the case of *Caladium* × *hortulanum*. 2,4-D, particularly together with BA and zeatin, stimulates callus formation. Combination of this auxin with kinetin is unfavourable as in the case of *Peperomia scandens* and does not cause any noticeable changes in the explants.

The effect of light and darkness on the behaviour of the explants of the studied plant species is similar to that observed by other investigators (Vasil and Hildebrandt, 1966; Gautheret, 1966; Pierik, 1972; Caponetti et al., 1971). The petioles of *Peperomia scandens* form better developed roots in darkness, but the number of initiated shoot buds per explant is higher under light. The differentiating influence of light was also noted in petioles of *Passiflora coerulea*. The per cent of explants forming shoot buds under continuous illumination was higher, but callus growth was less intensive. Darkness favours the growth of callus, it depresses, however, the number of explants differentiating shoot buds. Under conditions of darkness only the explants of *Caladium* × *hortulanum* die.

CONCLUSIONS

I. Morphogenetic reactions of petiole fragments of the studied plants

1. The morphogenetic reactions of isolated petiole fragments of various plant species under the in vitro culture conditions differ according to species:

(a) petioles of *Peperomia scandens* and *Caladium* × *hortulanum* regenerate roots and shoots,

(b) petioles of *Passiflora coerulea* from callus tissue and shoot buds which develop to shoots but then die,

(c) petioles of *Hedera helix, Begonia glabra, Coleus blumei, Fuchsia hybrida, Passiflora suberosa, Peperomia eburnea* form callus tissue und roots,

(d) petioles of *Kalanchoe blossfeldiana, Pelargonium grandiflorum, P. peltatum, P. radula, Coleus shirensis* and *Magnolia soulangeana* only form callus,

(e) petioles of *Philodendron scandens, Rhododendron smirnovii, Hibiscus rosa-sinensis, Coprosma baueri, Cestrum purpureum* and *Solanum rantonnetti* did not exhibit any regenerative reaction.

2. Different morphogenetic reactions of isolated petiole fragments from the studied species indicate their different regenerative potential:

(a) it is high in *Peperomia scandens* and *Caladium* × *hortulanum,*

(b) moderate in *Passiflora coerulea, P. suberosa, Hedera helix, Begonia glabra, Coleus blumei, Fuchsia hybrida* and *Peperomia eburnea.*

(c) and weak in *Kalanchoe blossfeldiana, Pelargonium grandiflorum, P. peltatum, P. radula, Coleus shirensis, Magnolia soulangeana.*

3. Growth regulators

(a) auxins stimulate mainly callus formation and root differentiation and in *Peperomia scandens* and *Caladium* × *hortulanum* also shoot bud production,

(b) cytokinins stimulate shoot bud differentiation and inhibit root initiation in the callus of *Caladium* × *hortulanum,* whereas they evoke no regeneration reaction in the petioles of *Coleus blumei* and *C. shirensis.*

4. Darkness stimulates root development in *Peperomia scandens* and profuse callus production in *Passiflora coerulea,* whereas light stimulates in the same plant shoot bud initiation.

5. In vitro cultures of petiole fragments from *Peperomia scandens* and *Caladium* × *hortulanum* may be utilised in gardening practice for vegetative reproduction of these plants.

II. Anatomical aspect of organ formation on fragments of petioles of the investigated plants

1. In *Peperomia scandens* the shoot buds and roots arise without the participation of the exuberant callus:

(a) shoot buds arise from differentiated subepidermal and parenchymal cells, mainly on the wounded surface,

(b) roots form from parenchymal cells adjacent to the vascular bundle of the explant.

2. In *Caladium* × *hortulanum* shoot buds and roots, and in *Passiflora coerulea* shoot buds differentiate from the exuberant callus tissue:

(a) callus tissue in *Caladium* × *hortulanum* forms from the epidermal and parenchymal cells situated on the petiole ribs,

(b) callus in *Passiflora coerulea* forms from subepidermal and parenchymal cells on the whole surface where the petiole was cut.

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Regeneracja roślin wybranych gatunków z fragmentów ogonków liściowych w kulturach in vitro

Streszczenie

Przebadano 21 gatunków roślin, należących do 14 rodzin, pod względem zdolności do regeneracji. W badaniach zastosowano metodę kultur tkankowych in vitro na pożywce podstawowej MS z dodatkiem regulatorów wzrostu z grupy auksyn i cytokinin. Spośród badanych gatunków roślin *Peperomia scandens* i *Caladium* × *hortulanum* regenerowały rośliny, *Passiflora coerulea* regenerowała pędy, *Hedera helix*, *Begonia glabra*, *Coleus blumei*, *Fuchsia hybrida*, *Passiflora suberosa* i *Peperomia eubernea* tworzyły kalus i korzenie, *Kalanchoe blossfeldiana*, *Pelargonium grandiflorum*, *P. radula*, *Coleus shirensis* i *Magnolia soulangeana* formowały kalus, *Philodendron scandens*, *Rhododendron smirnovii*, *Hibiscus rosa-sinensis*, *Coprosma baueri*, *Cestrum purpureum* i *Solanum rantonnetii* nie wykazywały żadnych reakcji regeneracyjnych.