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Floral Morphogenesis and Flowering in Aseptic Cultures of *Browallia demissa* L.

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Abstract. Floral buds of *Browallia demissa*, at three stages of development, were cultured on Nitsch and Nitsch basal medium. The supplements used include IAA; several cytokinins — benzyladenine, kinetin and 6-benzyl-9 tetrahydropyran-adenine (SD 8339); gibberellic acid (GA_3); 2, 3, 5-triiodobenzoic acid (TIBA); arginine and cysteine. All three stages of floral buds failed to complete development. In some treatments stages II and III produced callus and/or roots from the morphological basal end. Cytokinins promoted bud formation whereas both IAA and GA_3 depressed bud formation The shoots differentiated *in vitro* were capable of setting flowers, fruits and seeds in all the treatments. The seeds were viable. Comparative studies of development of flowers *in vivo* and *in vitro* were made. In some treatments the flowers exhibited abnormal corolla, androecium and gynoecium. Factors affecting normal bud initiation, organization and development are discussed.

Studies of *in vitro* culture of flowers and floral parts have proved useful in our understanding of floral morphogenesis (GALUN, JUNG and LANG 1963, TEPFER et al. 1963, NITSCH 1967). These studies have indicated that the failure to obtain complete development of excised floral buds in culture are due to lack of capacity to elaborate the necessary stimuli which may be present in parts other than the flower. Even in instances in which fairly complete development has been obtained, the nutritional requirements are complex (BLAKE 1966, PORATH and GALUN 1967).

Besides excised floral buds, flowers differentiated *in vitro* have also been studied in a few species (NITSCH 1967). But most of these studies have been confined to floral initiation and rarely to floral organization and development. The present study on *Browallia demissa* covers not only organization and development both in excised floral buds and in buds formed *in vitro*, but also initiation of floral buds *in vitro*. Efforts have been directed toward obtaining normal flowers and to study time relation in *in vitro* and *in vivo* flowers at different stages of development. Observations were also made on flowering of seedlings reared from test tube-formed seeds and *in vivo* seeds. Since in the course of investigation abnormal flowers were obtained, the usefulness of such flowers in our understanding of floral morphogenesis is discussed.

Material and Methods

Browallia demissa (Solanaceae) is a winter annual (about 30 cm tall) bearing blue-violet flowers. In our departmental garden it flowers from late August to March. Aseptic floral buds excised at the following three stages were cultured on K formula of NITSCH and NITSCH (1967 a) basal medium without glycine (NB).

Stage I: 0·1 to 0·2 cm long — sepals and stamens well formed; anthers contained microspore mother cells; petals still at primordial stage; stigma, style and ovules not initiated.

Stage II: 0·3 cm long — as in I but petals differentiated; microspores formed; stigma and style at young stage; ovular primordia just initiated.

Stage III: 0·5 cm long — calyx-mouth open; corolla not exserted; pollen grains formed and ovules just initiated.

Interaction of some growth substances was studied by supplementing the basal medium with IAA, some cytokinins — kinetin, benzyl-adenine and 6-benzylamino-9-tetrahydro-2-pyran-adenine (SD 8339), and gibberellic acid (GA_3) at 0·5 to 5 ppm. In addition, effects of 2, 3, 5-tri-iodobenzoic acid at 1 to 10 ppm and amino acids, l-arginine, and l-cysteine, at 10^{-5} M were also examined. In all treatments three per cent sucrose was used; pH was adjusted to 5·5; and 20 ml of the medium was dispensed into culture tubes (15 cm long and 2·5 cm in diameter).

The cultures were grown under 12 to 16-h day and 12 to 8-h dark conditions, respectively, at 24° day and 22° night temperatures and 50—60 per cent relative humidity. The combination of fluorescent and incandescent lamps gave an illumination of about 580 ft.-c. at the surface of culture tubes. The period of cultures varied from 6 to 11 weeks. Each treatment was replicated thrice and each time 12—24 cultures were raised. In studies on *in vitro* floral initiation, the number of days for visible appearance of floral buds, number of leaves and length of the differentiated shoots were recorded. For microscopic examination, the cultures were fixed in formalin-acetic-alcohol at different stages. Following customary methods of dehydration and paraffin-embedding, serial sections were cut 8—15 microns thick and stained with haematoxylin or safranin-fast green.

Results

The growth responses of floral buds, stages I to III, were evident within the first three weeks of culture. A general account of these responses is presented and the marked deviations from the general pattern are pointed out.

Responses of Stage I Floral Bud

The calyx that was already present at the time of culture did not show any visible change. The corolla remained at the primordial stage; consequently the stamens remained *in situ*. Microspore mother cells failed to undergo meiosis; ovule initiation was inhibited; and only partial development of the stigma and style took place. The explants did not callus or form roots.

Responses of Stage II Floral Bud

In some treatments the calyx elongated (as much as 0·7 cm in NB + IAA (1 ppm) + kinetin (0·5 ppm). The corolla differentiated. Nevertheless it failed to become exserted. In treatments with GA_3 alone and in GA_3 with IAA or cytokinins, the corolla tube became flexuose. The stamens showed only a slight elongation but the anthers were compressed. There was no pollen formation. The placenta bore presumptive ovule primordia. Rooting occurred sporadically on IAA-medium. In several treatments the cut-end of the pedicel callussed, particularly in those of cytokinins and IAA. The callus showed interesting morphogenesis.

Responses of Stage III Floral Bud

The calyx showed normal growth but tended to become swollen. In media supplemented with IAA or GA_3 alone or in combination, and with IAA and cytokinins, the corolla mouth became exserted although the corolla tube continued to be flexuose and inserted. The anthocyanin pigmentation of the corolla was not intense. With TIBA treatment, the region of the corolla lobes just above the point of insertion of the stamen elongated considerably.

The anthers were shrivelled and pollen grains mis-shapen. The differentiation of ovules progressed beyond the primordial stage, but only a few ovules developed. Nevertheless, the ovules failed to develop embryo sacs irrespective of the treatments but possessed endothelium (except in TIBA treatment). In TIBA treatment even the integument did not differentiate (Fig. 1). Both the outer epidermis of the single integument and the placental epidermis stained intensely with haematoxylin in cytokinins and IAA media (Fig. 2). The ovary wall also showed undulations. The inner epidermis, and the inner hypodermal layer of the ovary wall were sclerenchymatous (Fig. 3).

Both callussing and rooting occurred from the cut-end of the pedicel, although the incidence of rooting was as low as 20% and restricted to treatments of IAA, IAA and cytokinins, and arginine and cysteine. The callus showed morphogenesis similar to that of the callus formed in floral explants of stage II. However, the growth and differentiation of the callus was much rapid in stage III explants. All experimental observations were therefore made on callus stage III explants.

Morphogenetic Responses of Floral Callus

The callus developed nodules or "meristemoids" (TORREY 1966). The nodules differentiated either roots only, or both roots and shoots, except in basal medium and in medium supplemented with cytokinins in which only shoots were formed. The callus rarely produced flowers in the medium containing IAA (1 ppm), kinetin (1 ppm) and GA_3 (1 ppm) (Fig. 4). Root formation was optimal in IAA treatments in which not only the roots were initiated earlier but also the number of roots was greater than in others. Bud development was maximal in medium supplemented with cytokinins. The number of vegetative shoots that developed in a treatment was considered the bud number (Table 1).

Rapid differentiation of the vegetative shoots occurred in cytokinin treatments 2—5 ppm. Basal medium, GA_3, IAA, arginine and cysteine, treatments

retarded the rate of growth of vegetative shoots. The *in vitro* differentiated shoots ranged 0·5—20 cm in length. GA_3 promoted internodal elongation but suppressed leaf expansion. With increased cytokinins, although the shoots differentiated rapidly, the leaves were far from normal and the region of the shoot in contact with the agar medium became fasciated and developed shoulders. In TIBA treatment the fasciation of the shoot comprised flat-

Table 1

Number of shoots differentiated in floral callus formed in floral buds stage III*

Treatment**	5 weeks	9 weeks
Basal medium	1·8	3·4
GA_3 (1—2)	1·5	3·1
IAA (1) + GA_3 (1)	1·3	3·8
IAA (1)	2·1	4·6
l-cysteine (12)	2·1	4·1
l-arginine (17)	2·7	4·1
IAA (1) + kinetin (1) + GA_3 (1)	2·2	4·2
Kinetin (1—5)	3·2	7·8
Benzyladenine (2)	3·4	4·2
IAA (1) + kinetin (2)	5·0	9·2
IAA (1) + SD 8339 (2)	7·1	12·2
SD 8339 (2—5)	7·3	10·2

* Average of 24 cultures in 4 replicates.
** Concentrations in mg/l are those which elicited normal vegetative growth.

tened stem, involuted leaves and miniature enations. But, to TIBA medium, addition of kinetin (1 to 2 ppm) overcame the effects of TIBA in the later-formed shoots.

In nature flowering does not occur in Delhi until after the formation of nine leaves (seven vegetative and two cotyledonary). In contrast, the vegetative shoots differentiated from the callus flowered even when they bore as few as four leaves (Table 2) (Fig. 5). Cytokinin treatments not only induced early flowering but also increased the number of floral buds (maximum being 40 in SD 8339 and kinetin treatments). IAA and GA_3, in combination as well as given alone, reduced the number of floral buds per culture (Fig. 6).

The seedlings raised in treatment with cytokinins alone or in cytokinins together with auxin flowered earlier than in basal medium, in gibberellic acid and amino acid treatments (Table 3). Interestingly, responses of seedlings raised from *in vivo* seeds as well as seeds from *in vitro* differentiated flowers were similar.

Irrespective of the material from which the explants were derived, the morphology of the flower formed *in vitro* was characteristic of the species — bisexual and hypogynous. They were solitary and axillary below, and in a more or less one-sided raceme above. In nature the time between exsertion of the corolla tube and anthesis ranged from 6 to 8 days, while in cultures from 10

Table 2
Correlation between vegetative growth and flowering of *in vitro* shoots

Treatment*	Shoot length (in cm)	Average number of leaves before appearance of first flower	Number of floral buds per culture**
Basal medium	7·5 ± 0·3	7·2 ± 0·23	+
GA_3 (1)	7·7 ± 0·4	8·0 ± 0.29	+
IAA (1) + GA_3 (1)	8·0 ± 0·25	7·6 ± 0·2	+
IAA (1) + GA_3 (1) + kinetin (1)	8·2 ± 0·2	5·4 ± 0·18	+
TIBA (5)	Fasciation	Fasciation	+
l-arginine (17)	8·1 ± 0·02	5·4 ± 0·27	+
l-cysteine (12)	7·7 ± 0·03	7·2 ± 0·02	+
IAA (1)	4·9 ± 0·29	4·8 ± 0·27	++
SD 8339 (2)	3·3 ± 0·16	4·2 ± 0·2	+++
IAA (1) + kinetin (1)	6·0 ± 0·22	5·0 ± 0·2	++++
Kinetin (2)	4·8 ± 0·25	3·9 ± 0·2	++++
IAA (1) + SD 8339 (2)	5·6 ± 0·3	4·2 ± 0·2	++++

* Average of 24 cultures in 4 replicates. The concentrations in mg/l elicited normal vegetative growth (except TIBA treatment).
** 9 weeks after culture;
\+ indicates at least five flower buds.

Table 3
Flowering response of seedlings

Treatment*	Number of days to first flower bud appearance	Length of seedling (in cm)	Number of vegetative leaves at time of flowering
Basal medium	54·1 ± 1·5	7·2 ± 0·18	7·0 ± 0·37
GA_3 (1)	58·0 ± 0·5	6·3 ± 0·39	6·5 ± 0·2
l-cysteine (12)	58·3 ± 2·0	7·4 ± 0·29	7·0 ± 0·5
l-arginine (17)	57·5 ± 2·1	7·1 ± 0·32	7·0 ± 0·03
IAA (1) + GA_3 (1) + kinetin (1)	59·9 ± 1·6	9·2 ± 0·6	6·4 ± 0·03
SD 8339 (2–5)	57·5 ± 2·3	6·0 ± 0·28	6·4 ± 0·25
IAA (1) + SD 8339 (2–5)	51·0 ± 1·8	6·9 ± 0·4	6·4 ± 0·23
IAA (1–2)	57·2 ± 2·6	7·4 ± 0·2	5·8 ± 0·29
Kinetin (1)	43·4 ± 1·1	5·3 ± 0·2	5·9 ± 0·26
IAA (1) + kinetin (1)	46·9 ± 0·9	4·6 ± 0·2	4·5 ± 0·16

* Concentrations of substances are in mg/l which elicited normal growth. 4–6 seedlings were reared in each tube. Average of 24 seedlings in two replicates.

to 14 days. Not all the flowers developed in racemes as well as in the lower axils on the *in vitro* differentiated shoots arising from the calli did anthesize in the majority of the treatments. The number of flowers which showed anthesis ranged from 30 to 80 %, the percentage being lower for early-formed flowers.

Among the abnormalities observed in cultures were the development of duplex flowers (Fig. 7), inhibition of corolla development (Fig. 8, 9), re-

duction in number of corolla segments, receptacular origin of stamens (Fig. 10), *in situ* germination of pollen grains, and formation of open carpels (Fig. 10).

An unusual feature observed in high concentrations of cytokinins was the expansion of the corolla mouth without the elongation of the corolla tube. The number of corolla segments in the species is five. But, in cultures, flowers often showed only four, three or even two segments (an analysis of flowers gave the following data: out of 160 *in vitro* differentiated flowers 20% had 4-lobed corolla). Flowers bearing 4- and 5-segmented corolla developed on the same shoot. The number of veins was also fewer in 4-segmented corolla. The pigmentation of corolla was normal in all the treatments except in GA_3 and TIBA in which it was of low intensity.

The androecium comprised four epipetalous, didynamously inserted stamens. In high concentrations of cytokinins and TIBA they became exserted (Fig. 7). In treatments in which the corolla failed to develop, the stamens originated from the torus itself. There was generally a delay in the development of pollen grains and embryo sacs *in vitro* (*in vivo* flower buds 0·5 cm long contain pollen grains and megaspores while *in vitro* flower buds of the same size contained archesporium and megaspore mother cells). High concentrations of plant growth regulators caused an early degeneration of microspore mother cells, formation of microsporangia devoid of contents and dilation of the connective tissue.

Except in early formed flowers, pollen formation was normal in most treatments (Fig. 11). The pollen grains were binucleate and viable. They occasionally showed germination *in situ*. Observations on stigma and style excised from flowers of different ages showed that self pollination had occurred *in vitro* and there was normal growth of pollen tubes. Both fruit and seed set were common.

The development of gynoecium was normal in all treatments. The number of ovules in IAA and kinetin-medium was comparable to that *in vivo* (Fig. 11). In flowers formed early in SD 8339 (concentrations above 5 ppm) "naked" ovules developed on a "column" (Fig. 10). These ovules tended to acquire an anatropous condition. In TIBA treatment the ovules remained as a mass of tissue without differentiation into nucellus and integument. In general, TIBA treatment caused a reduction in size of the floral organs.

The development of embryo sac conformed to the description by MOHAN (1966). Fruits were formed in all the treatments. They matched in size with those *in vivo*. Fruit dehiscence was normal. However, seed number was low (as low as 2—30 in vitro against 20—120 seeds per capsule in nature). The seeds were viable and produced normal seedlings which set flowers and fruits.

Discussion

Various factors such as size of the explant, mineral nutrition, photoperiodism, carbohydrates and growth regulators are known to influence flower formation *in vitro* — both initiation and organization (CAPLIN and GRIESEL 1967, MARGARA, RANCILLAC and BOUNIOLS 1965, NITSCH and

NITSCH, 1967b, RAGHAVAN 1961). Throughout the present study three per cent sucrose and a photoperiod of 12 to 16-h light and 12 to 8-h dark, respectively, were used. Explants of stage I succumbed in all treatments. Those of II and III elicited only partial development. That the normal development of flowers could not be accomplished *in vitro* suggests that either the requirements were not adequate or a more complex medium was needed. The results with *Aquilegia* (TEPFER et al., 1963) and *Viscaria* (BLAKE 1966) have demonstrated that even on a complex medium normal pollen formation and ovule differentiation could not be achieved (*Aquilegia*) and an intervention of vegetative phase was necessary for normal flowering *in vitro* (*Viscaria*).

Whereas explants of stage I did not callus on any of the treatments, those of stage II and III did on media supplemented with IAA, GA_3 or cytokinins, alone or in combination. This may be attributed to the endogenous regulators in the different stages of explants. Both IAA and cytokinins promoted bud formations but GA_3 depressed it. Inhibition of shoot formation and root initiation has been reported in tobacco tissue cultures by GA_3 (MURASHIGE 1965). Promotion of bud development *in vitro* by adenine and cytokinins are well documented (SKOOG and MILLER 1957, TORREY J. G. 1966, NITSCH and NITSCH 1967a). In the present study adenine was present in all the treatments. Although IAA and cytokinins promoted bud initiation, only cytokinins evoked rapid differentiation of these buds. This indicates the need for recognition of the events — bud initiation and bud development as distinct from one another and probably controlled by different regulators. Similar have been the conclusions of NITSCH and NITSCH working with *Plumbago indica* (1967a).

The shoots differentiating from the callus of the floral explants set normal flowers, fruits and seeds. The number of leaves present before flowering varied in different treatments. In most treatments 4—6 leaves were present. This number is less than that obtained in seedlings (6—8 leaves in addition to 2 cotyledonary leaves).

Rarely, flowers have also been obtained directly from the callus. Such an observation has also been made in *Ranunculus sceleratus* (KONAR and NATARAJA 1964) and *Lunaria annua* (PIERIEK 1967). In *Lunaria annua*, coconut milk from immature nuts if present in the medium promoted flower development directly from calli. If flowers could be differentiated directly from the callus routinely, the study of floral morphogenesis would be greatly advanced.

As with vegetative buds, the number of floral buds also decreased in treatments with GA_3 alone or in combination with IAA. But in treatments with IAA alone and with cytokinins the floral bud number, like that of vegetative buds, also increased.

The role of amino acids in flowering is not clear. STEINBERG (1948) did not find any enhancement of flowering by amino acids in *Nicotiana*. Whereas NITSCH and NITSCH (1967b) found fewer flower buds in *Plumbago* with arginine treatment.

Flowers *in vitro* matched with those *in vivo* in size and in pigmentation. However, TIBA reduced the flower size but increased the length of the pedicel. Of the floral whorls, calyx was the least affected both in culture of explants of all three stages and in flowers differentiated *in vitro*. Corolla was

affected in explant cultures. Elongation and unfolding of corolla are known to be affected by chemical factors (GOLDSCHMIDT and MONSELISE 1966).

The time between the appearance of floral bud and anthesis was 10—14 days *in vitro* as against 6—8 days in nature. In media supplemented with increased concentrations of SD 8339, kinetin and benzyladenine, inhibition of tube elongation with or without opening of the corolla mouth was noticed. But it is significant that on these media vegetative growth was also often far from normal. On transfer to media with low concentrations or without these chemicals, not only vegetative growth improved but also newly-formed flowers became normal.

The characteristic number of corolla lobes for *Browallia demissa* is five. In cultures, however, less than five corolla lobes differentiated in some flowers in media with IAA, kinetin, SD 8339 and their combinations, or cysteine. This tendency towards a reduction in the number of corolla lobes suggests interesting studies on symmetry and aestivation of corolla.

Flowers both *in vivo* and *vitro* bore four stamens. In early formed flowers on cytokinins-supplemented media, degeneration of sporogenous tissue, resorption of tapetal cells, and intrusion into the anther locules were frequent. Similar effects reported elsewhere have been attributed to unfavorable photoperiods and phytogametocidal compounds (HESLOP-HARRISON and HESLOP-HARRISON 1958, RUSTAGI 1967).

In *Browallia demissa* stamens are not exserted. In contrast to this, in treatments with high concentrations of cytokinins and TIBA, the stamens protruded beyond the corolla or beyond the calyx in instances in which the corolla elongation was affected.

Carpel development occurred normally on a variety of cultures where vegetative growth was satisfactory. In flowers formed early, with increased SD 8339 in the medium, open carpels were formed. The formation of open carpels have also been observed with treatments of phytogametocidal compounds (see MOHAN RAM and RUSTAGI 1966).

The requirements for ovule development are still obscure. There was invariably a reduction in the ovule number in all the treatments. In addition, TIBA induced other abnormalities in ovule formation. Although there was reduction in the ovule number, the general structure of the ovule and the type of development of embryo sac was not affected in the majority of the treatments. Whether or not the embryo sac development proceeded normally, the endothelium formed invariably.

The fruits formed *in vitro* were comparable to those *in vivo* in size but in number only 15% of the flowers formed *in vitro* produced fruits. Like fruit number, seed number was also below average *in vitro*. This could be attributed to: (a) reduction in the ovule number itself; (b) decreased production of pollen; and (c) low incidence of self-pollination in cultures. MURASHIGE and NAKANO (1966) attributed the low seed set in *Nicotiana* species to the tetraploid nature of shoots differentiated *in vitro*. However, the ploidy could not be considered as a causal factor to low seed set because chromosomal analysis has shown that *in vitro* plants of *Browallia demissa* are diploid ($2n = 22$).

The usefulness of the technique of *in vitro* floral initiation, organization and development can hardly be minimized. One of the several possibilities is of

obtaining clonal populations from vegetative buds differentiated *in vitro*. However, caution due to a variability in chromosomal constitution *in vitro* should be exercised. The growth of floral buds without detectable anthocyanin synthesis and a later pigment development which can be correlated with bud length make *Browallia demissa* an ideal material for pigment studies.

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Květní pupeny *Browallia demissa* ve třech vývojových stádiích byly pěstovány na základním mediu Nitsch a Nitsch. Do media byla přidávána IAA, některé cytokininy — benzyladenin, kinetin a 6-benzyl-9-tetrahydropyran-adenin (SD 8339); kyselina giberelová (GA_3); kyselina 2,3,5-trijodbenzoová; arginin a cystein. V žádné variantě podle stádia pupenu nedošlo k úplnému vývoji. V některých variantách stádia II a III docházelo ke vzniku kalusu a nebo kořenů z báze pupenů. Cytokininy napomáhaly tvoření pupenů, zatímco jak IAA tak GA potlačily jejich vznik. Stonky diferencované *in vitro* byly schopné vytvořit květy, plody a semena ve všech variantách. Semena byla životaschopná. Byl srovnáván vývoj květů *in vitro* a *in vivo*. V některých variantách se v květech vytvářely abnormální květní obaly, androceum a gyneceum. Jsou diskutovány faktory ovlivňující normální vývoj a organizaci pupene.

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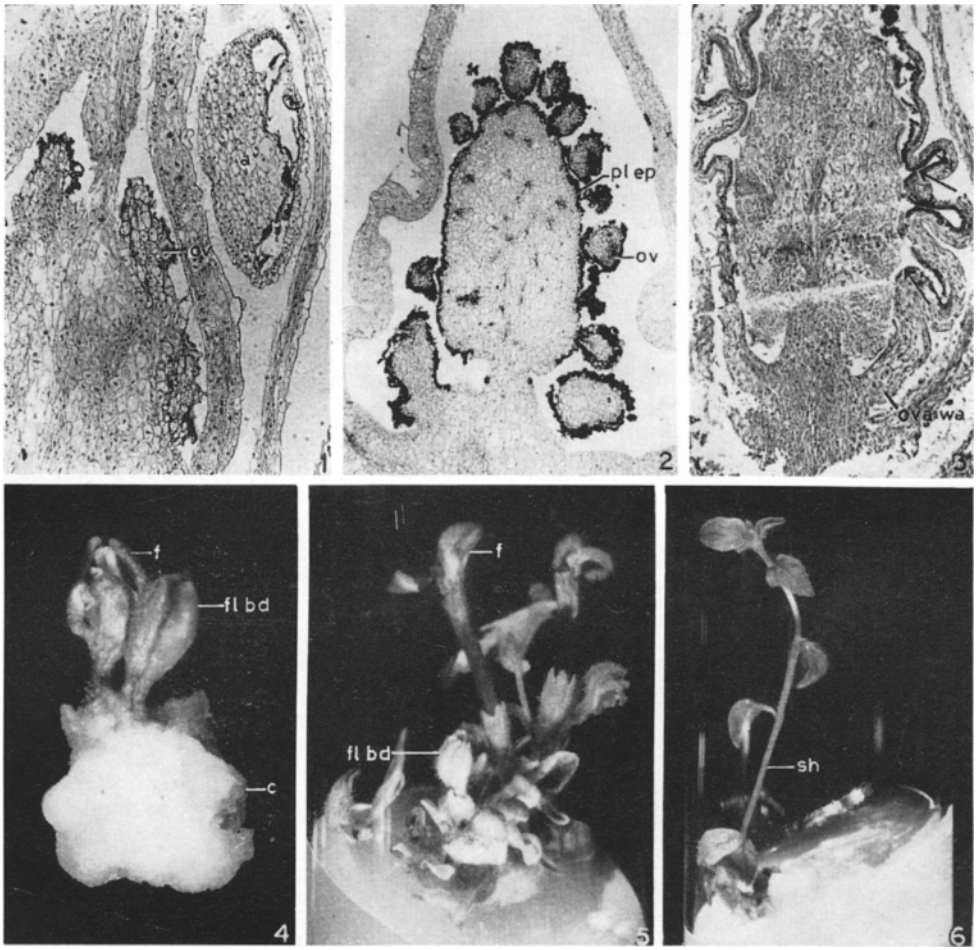
Выращивались цветочные почки *Browalia demissa* на трех стадиях развития на основной среде по Нитч и Нитч. Добавки к среде: ИУК, некоторые цитокинины — бензиладенин, кинетин и 6-бензил-9-тетрагидропиран-аденин (SD 8339); гиббереллловая кислота (GA_3); 2,3,5-трийодбензойная кислота; аргинин и цистеин. Полное развитие не происходило ни в одном из вариантов. В некоторых вариантах II и III стадии образовался каллус или корни из основания почек. Цитокинины способствовали образованию почек в то время как ИУК и ГК подавляли их возникновение. Стебли дифференцированные *in vitro* были способны образовать цветы, плоды и семена во всех вариантах. Семена были жизнеспособны. Сравнивали развитие цветов *in vivo* и *in vitro*. В некоторых вариантах в цветах образовывались аномальные корона, андроцей и гинецей. Обсуждаются факторы влияющие на нормальное развитие и организацию почки.

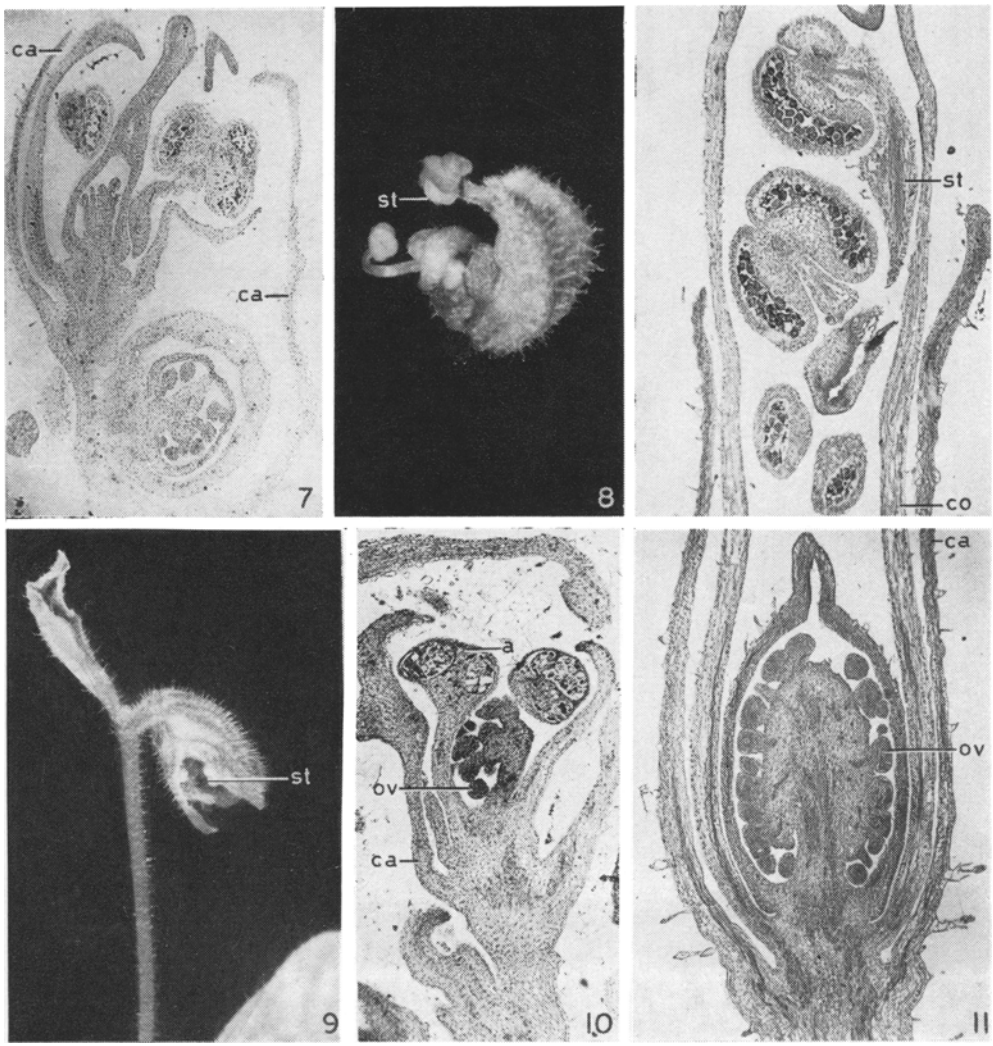
The plates will be found at the end of the issue.



Figs. 1–3. Behaviour of floral explants stage III on basal + TIBA, basal + IAA + kinetin and basal + IAA + kinetin + GA_3 (3 weeks after culture), respectively.

Fig. 1. Flower bud. Note the non-differentiation of the normal ovules and the empty anther locules. Magnification 114×. Fig. 2. Ovary showing the intense staining of the outermost layer of the integument of the ovules and the placental epidermis. Magnification 44×. Fig. 3. Ovary showing wall undulations, sclerenchymatous inner epidermis and inner hypodermal layer of the wall. Magnification 41×.

Figs. 4–6. *In vitro* flowers and shoots. Fig 4. Flowers developing directly from the callus on IAA, kinetin and GA_3 medium. Magnification 8×. Fig. 5. *In vitro* differentiated shoots on cytokinins supplemented medium Note the small stature of the shoots and the large number of buds and flowers. Magnification 3×. Fig. 6. *In vitro* differentiated shoot on IAA medium. Note the elongated internodes and sparse floral bud development. Magnification 2·2×. (Abbreviations: *a* — anther; *c* — callus; *f* — flower; *fl bd* — floral bud; *ov* — ovule; *ova wa* — ovary wall; *pl ep* — placental epidermis; *sh* — shoot).



Figs. 7—11 *In vitro* differentiated flowers. Fig. 7. Duplex flower differentiated on IAA and kinetin medium. Note the common calyx for both the flowers. Magnification 40·5×. Figs. 8 and 9. Flowers with inhibited corolla development; note the stamens visible outside the calyx cup. Magnification: Fig. 8 8×, Fig. 9 15×. Fig. 10. Flower differentiated on increased concentration of SD 8339; note the open carpel, absence of style and stigma and the origin of stamens directly from the torus. Magnification 55·5×. Fig. 11. Flower differentiated on IAA and kinetin medium; the flower has normal anther and ovary structure. Magnification 38·2×. (Abbreviations: *a* — anther; *ca* — calyx; *co* — corolla; *ov* — ovule; *st* — stamen.)