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AN APPROACH TOWARD UNDERSTANDING SOME OF THE MORPHOGENETIC BASES OF PHYLOGENY OF STREPTOCARPUS (GESNERIACEAE)

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AN APPROACH TOWARD UNDERSTANDING SOME OF THE MORPHOGENETIC BASES OF PHYLOGENY OF *STREPTOCARPUS* (GESNERIACEAE)

by

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ABSTRACT

An Approach Toward Understanding Some of the Morphogenetic Bases of Phylogeny of *Streptocarpus* (Gesneriaceae)

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By utilizing an experimental approach, phenovariants of *Streptocarpus* (Gesneriaceae) species expressing ancestral morphological features have been induced. Since these phenovariants resulted from use of plant growth regulators, a beginning has been made toward understanding phylogeny (evolutionary diversification) of this taxon in chemical terms.

Two morphogenetic patterns have been critically involved in speciation within the Gesneriaceae. Subfamily Cyrtandroideae is characterized by accrescence of one of the two cotyledons as a result of continued activity of basal intercalary meristems, accompanied by suppression of growth of the second cotyledon.

In the second pattern, sustained growth of the accrescent cotyledon accompanied by suppression of the displaced shoot apical meristem gives rise to an acaulescent, dorsiventral vegetative plant body (phyllomorph) which characterizes species of subgenus *Streptocarpus* and also several other genera within Cyrtandroideae.

Exogenous plant growth regulators were applied to several species in subgenus *Streptocarpus*, especially *S. prolixus (gracilis)* at critical stages under three sets of experimental conditions. These were axenic plantlets regenerated from lamina discs, axenically grown seedlings, and pot grown seedlings. In all instances, the growth regulators were present at time of initiation of regenerants, or at time of or within two days of germination.

Gibberellin A_3 at levels of 10^{-5}M and higher caused suppression of cotyledonary accrescence and early activity of the shoot apical meristem, with production of a plumule. Sustained application of GA_3 resulted in caulescent plants with radial symmetry.

Similar isocotylous, caulescent seedlings were induced by application of the auxin antagonist 2,3,5-triiodobenzoic acid. Such seedlings also resulted from coating upper cotyledonary surfaces with a variety of pastes two days after germination; a possible role for ethylene was indicated.

A single exogenous application to germinating seedlings of the cytokinin benzyladenine at 10^{-6}M and higher caused desuppression of the second (micro-) cotyledon, resulting in accrescence of both cotyledons and growth of bi-phyllomorphic acaulescent plants.

Since these phenovariants were induced by exogenous application of growth regulators, there is strong indication that endogenous growth regulators control the unusual morphogenetic patterns found in subgenus *Streptocarpus* and elsewhere in subfamily Cyrtandroideae. Phylogenetic changes in balances among the regulators at critical stages in ontogeny probably account for evolutionary diversification within the Gesneriaceae.

A preliminary hypothesis regarding roles of phytohormones in controlling morphogenesis in subgenus *Streptocarpus* is offered. Further experimentation can expand understanding in this area and may lead to isolation of substances and processes at the molecular level which are mediated by the regulators.

ACKNOWLEDGEMENT

Knowing of my great interest in horticultural and morphological aspects of *Streptocarpus*, Professor Dominick Basile, who later became my adviser, showed me a number of years ago a reference to the Rossini and Nitsch paper on axenic regeneration of *S. nobilis*. From this beginning followed a short laboratory project along similar lines, which expanded in various directions, finally becoming a dissertation project. Dr. Basile's guidance, technical assistance, and constant encouragement throughout were indispensable in completing this work.

It was my good fortune that, shortly after the project began, Hilliard and Burtt's book, "Streptocarpus", was published which, in turn, led me to K. Jong's dissertation on seedling development in *Streptocarpus*. These references provided the descriptive base upon which experimental morphology could proceed.

I wish to thank Dr. Arthur Cronquist and Professors Peter Nelson and Jack Valdovinos for useful suggestions and advise relating to this dissertation.

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INTRODUCTION

Two main sources of evidence are traditionally utilized in deriving information regarding evolutionary processes and relationships among living organisms: the fossil record and studies in comparative morphology of extant organisms. In the case of angiosperms, the former is highly impoverished (Stebbins, 1974a), necessitating major reliance on the latter.

Observable differences in form can be traced to differences in morphogenetic processes. One little used approach to elucidating phylogenetic relationships among putatively related taxa is to improve our understanding of the factors and processes by which 'taxonomically' important characters develop. This is the premise from which this dissertation proceeds.

The Gesneriaceae are a highly evolved plant family containing members with varied distinctive and often exceedingly specialized vegetative morphology (Hilliard and Burtt, 1971). The genus *Streptocarpus* has been well studied in several areas: genetics, development, morphology and ecology. It exhibits in its two subgenera a wide range of features characteristic of the family, including some of the most extreme. Therefore, investigations which help improve our understanding of the morphogenetic basis of speciation in *Streptocarpus* should also contribute to a better understanding of phylogeny and direction of evolution within the Gesneriaceae.

The Family Gesneriaceae

The Gesneriaceae constitute a medium sized dicotyledonous family, ca 2500 species and 130 genera (Burtt, 1977) within the subclass. Asteridae, order Scrophulariales (Cronquist, 1968). Its major features, with few exceptions, are unilocular ovary with parietal placentation, zygomorphic flowers and simple leaves. It has been divided into subfamilies based on differences in cotyledonary development (Burtt, 1963). One subfamily, Gesnerioideae, is characterized by equal, non-accrescent cotyledons and possession of a plumular bud at germination; vegetative morphology is conventional for dicots.

Cyrtandroideae, the other subfamily, is characterized by cotyledons which are equal in size at germination, but in which accrescence of differing duration among the species occurs in one cotyledon shortly after germination (anisocotyly). It is this character which permits extremes in vegetative morphology, such as is found in those species in subgenus *Streptocarpus* in which the entire above-ground vegetative structure consists of an enormous accrescent cotyledon (up to 1 meter in length and about as broad) growing from the distal end of a very short petiole-like axis.

Anisocotyly is universal in all species of Cyrtandroideae in which seedling development has been studied (Burtt, 1977). At one extreme, it persists for a few days, with barely observable difference in cotyledon size, accompanied by short delay in appearance of the plumule. At the other extreme, cotyledonary accrescence persists for several years before flowering occurs, and no plumule appears. Such bizarre acaulescent morphology has so far been found in several genera, in ad-

dition to *Streptocarpus*, which belong to two tribes, Didymocarpeae and Klugieae (Burtt, 1970a; Jong and Burtt, 1975) of the four currently accepted (Burtt, 1977).

The Genus *Streptocarpus*

Because of widespread scientific and horticultural interest, the monograph "Streptocarpus", a survey of the genus, was published by Hilliard and Burtt in 1977, and is an invaluable source of information. The genus contains approximately 135 species, nearly all confined to Africa (3 or 4 species in Asia may be erroneously included). Its distinguishing character is a capsule which twists following fertilization.

Sub-genus *Streptocarpus*, with about 90 species, is found from southern Ethiopia southwest to Angola, with the most widespread species distribution in Natal Province of South Africa. Several species are also found on the island of Madagascar. Many growth forms occur, including unifoliates, with the only foliar organ being the accrescent cotyledon; plurifoliates, with one or more foliar organs produced each year; rosulates, with a rosette of foliar organs. Many species do not fall into these categories.

Although the overwhelming majority of the species are acaulescent, with no plumular bud ever appearing, there are exceptions. In some of the latter, caulescence is correlated with and appears to be secondary to inflorescences. Typically, inflorescences occur at the junction of the lamina and the petiole-like axis. Following the appearance of flower buds, intercalary growth below the buds produces long peduncles. In a few species, additional foliar organs develop on the inflores-

Fig. 1. Successive stages in the development of seedlings in subgenus *Streptocarpus*.

A. Unifoliate species.

1-3, *S. solenanthus*, 4-5, *S. grandis*

B. Rosulate species.

6-8, *S. primulifolius*, 9-10, *S. gardenii*

Co, macrocotyledon

co, microcotyledon

pd, petiolode

Cph, cotyledonary phyllomorph

ph2, first additional phyllomorph

Adapted from Hilliard and Burtt "Streptocarpus" (1971)

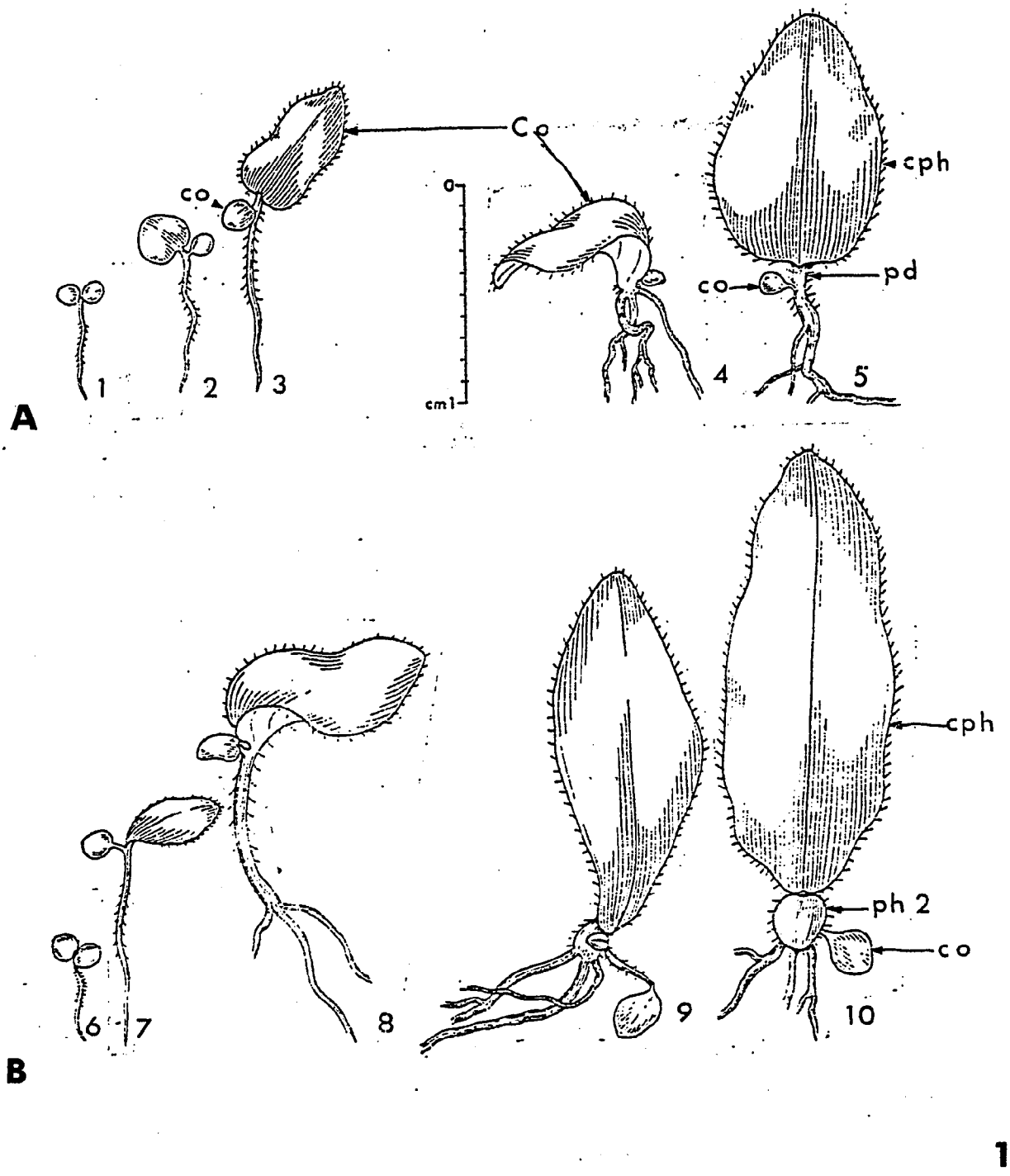

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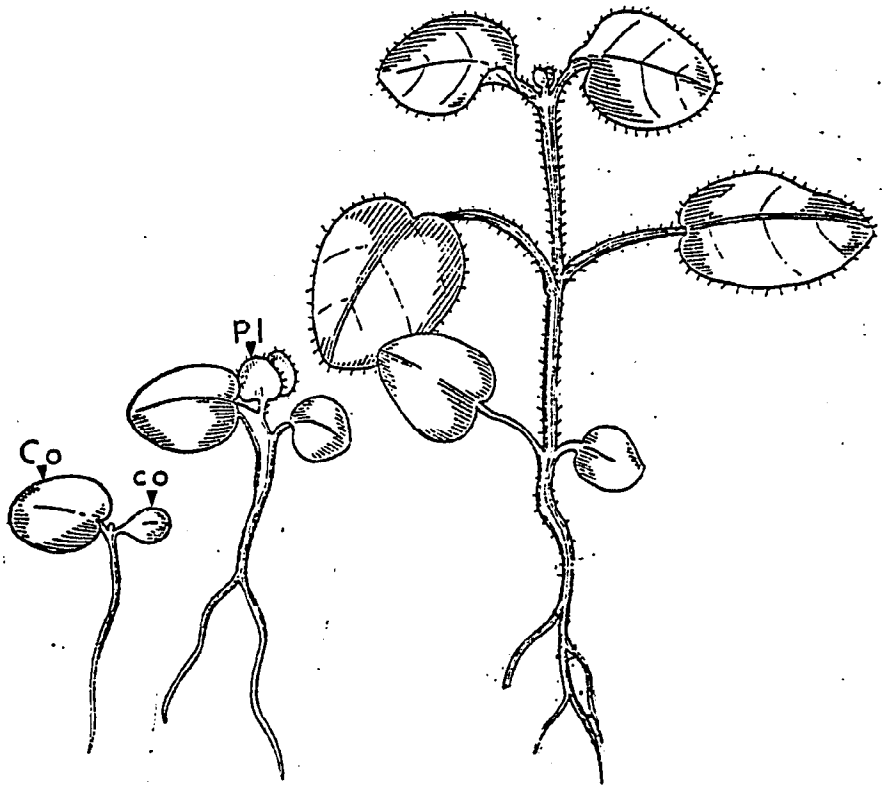
Fig. 2. Successive stages in the development of a seedling in subgenus *Streptocarpella*. *S. hilsenbergii*

Co, macrocotyledon

co, microcotyledon

pl, plumule

Adapted from Hilliard and Burtt "Streptocarpus" (1971)



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cences leading to secondary caulescence (e.g. *S. decipiens*). A most unusual example of delayed vegetative caulescence is found in *S. schliebenii*, whose seedling development has not yet been studied in detail.

Subgenus *Streptocarpella*, with about 45 species, occurs predominantly in an east-west distribution across tropical Africa. Although much less thoroughly investigated than subgenus *Streptocarpus*, its early seedling development is similar to the latter. The accrescent cotyledon may reach considerable size, even larger than foliar leaves, but with the same shape as the latter. After a delay of several days to many weeks, a shoot bearing true leaves appears. Adult caulescent morphology resembles that of typical dicots.

Both subgenera share similar flower and fruit characters. The basic differences are in vegetative morphology and in cytology. In subgenus *Streptocarpus*, diploid chromosome count is 2n=32 (two polyploid species are known) (Ratter, 1975). In subgenus *Streptocarpella*, all species examined to date are diploid with 2n-30 (Ibid).

Hypotheses Regarding Relationship Between Subgenera of *Streptocarpus*

Nearly all authors to the present time have regarded *Streptocarpus* as a single genus. Yet despite numerous attempts to create hybrids between the subgenera (Hilliard and Burtt, 1971; A. C. Zeven, 1972, personal communication; non-published reports by others, and my own efforts), none has succeeded. Within subgenus *Streptocarpus*, on the other hand, numerous natural and artificial hybrids exist. Interspeci-

fic hybrids among several species in subgenus *Streptocarpella* have also been created in recent years (W. Saylor and others, unpublished), and these are also highly fertile.

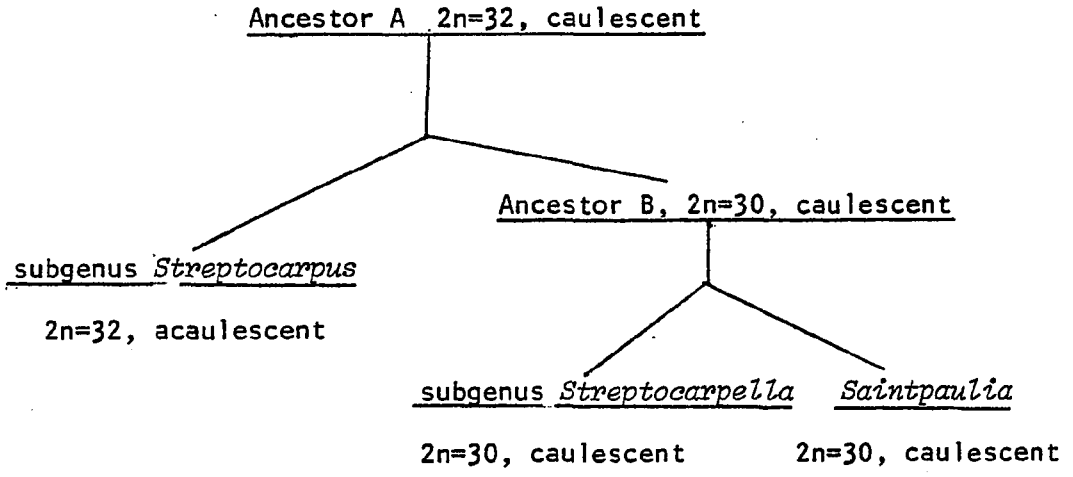
Numerous hypotheses have been published regarding the phylogenetic relationship between subgenus *Streptocarpus* and subgenus *Streptocarpella*. Hill (1938) suggested that within the genus acaulescence is the primitive condition and, therefore, subgenus *Streptocarpella* has evolved from subgenus *Streptocarpus*.

Most other authors disagreed with Hill. Since in general, pre-angiospermous land plants and dicotyledonous plants (including Gesneriaceae) are caulescent, they reason that ancestors of subgenus *Streptocarpus* must also be caulescent. Joshi (1938), Schenk (1942), Jong (1970) and Burtt (1970a) thus regarded subgenus *Streptocarpella* to be primitive within the genus. Hilliard and Burtt (1971), while agreeing with this viewpoint in general, considered the possibility that in some species within subgenus *Streptocarpella*, particularly *S. nobilis*, caulescence has re-evolved from acaulescent ancestry.

Ratter (1975) examined this question from the viewpoint of chromosome numbers as well as morphological similarities. Evidence accumulated from counts of about 200 of 1300 known species in sub-family Cyrtandroideae suggested to Ratter that the ancestral basic number of chromosomes was n=8 or 9, so that the numbers 2n=32 (as in the subgenus *Streptocarpus)* and 2n=30 (as in the subgenus *Streptocarpella* and another African genus, *Saintpaulia*), are of tetraploid origin. Further, he believed that n=15 probably evolved from n=16 by dysploid reduction. Ratter also pointed out that several recently discovered Madagascar

species in subgenus *Streptocarpella* bear strong morphological resemblance to *Saintpaulia* (Hilliard and Burtt, 1970 pp. 114-115). Ratter therefore concluded that *Saintpaulia* and subgenus *Streptocarpella* diverged from a common ancestor with 2n=30. Ratter also noted strong embryological evidence supporting his hypothesis for a close relationship between the genera *Streptocarpus* and *Saintpaulia* (Holmqvist-Gustavsson), 1964, cited in Hilliard and Burtt, p. 115).

My interpretation of the above, consistent with Ratter's ideas, is diagrammed below:



The implication of Ratter's hypothesis is that subgenera *Streptocarpus* and *Streptocarpella* are not in a direct linear ancestral relationship and may not necessarily be congeneric.

Studies of Development and Morphology in *Streptocarpus*

Cotyledon inequality was discovered independently by Caspary (1858) and Crocker (1860), both of whom observed this phenomenon in species of *Streptocarpus* (Crocker also observed anisocotyly in *Chirita*, a caulescent genus in Cyrtandroideae).

Dickson (1883) studied anisocotyly in *S. caulescens*, the first caulescent species in the genus to be brought under observation. He confirmed earlier observations of Dickie (1865) that in *Streptocarpus*, anisocotyly is accompanied by growth of an intervening segment that raises the accrescent cotyledon above the smaller one. Hielscher (1879) attributed increase in size of the enlarged cotyledon of *S. polyanthus* to persistently active meristematic tissue at the base of its lamina, which is absent in the smaller cotyledon.

Schenk (1942) published extensive and detailed studies of early seedling development in several growth forms in *Streptocarpus*.

Jong (1970) after careful observations of seedling ontogeny in *S. fanniniae* and other species in subgenus *Streptocarpus*, formulated a new concept of morphology in this taxon. The foliar organ together with a more or less short axial segment on which it arises, is a novel, unconventional plant unit; it is neither leaf nor shoot but an intermediate plant form incorporating properties of both. Jong called it a phyllomorph. This concept has been further elaborated by Jong (1973, 1978) and by Jong and Burtt (1975) and is today widely accepted.

According to this concept, the phyllomorph consists of two organs:

a lamina which grows indeterminately as a result of coordinated activity of a complex of meristems at the base of the lamina throughout the vegetative phase, and a petiole-like axial organ, the petiolode. The latter is composed of hypocotyl and tissue intercalated between the two cotyledons; it is dorsiventral in symmetry producing adventitious roots on the ventral surface, and inflorescences, accompanied in some species by additional phyllomorphs, on the dorsal surface. The phyllomorph is not limited to *Streptocarpus*.

Jong and Burtt (1975) pointed out that genera in Gesneriaceae with phyllomorphic growth patterns include in addition to *Streptocarpus*, several others in tribe Didymocarpeae, and *Epithema* and *Monophyllaea* in tribe Klugieae. Thus, it has apparently evolved independently more than once.

The morphogenetic events by which the seedling is transformed to this unusual form are as follows:

Several days after germination, one cotyledon begins to enlarge, accompanied by displacement of the small seedling apical meristem to its base. Within 2-3 weeks, a complex of 3 basal meristems is observable; these provide the growth pattern of the phyllomorph. Terminology for these meristems (Jong and Burtt, 1975) is:

1) The groove meristem (so named because it appears subepidermally in a visible groove just below the base of the lamina). It is derived from the laterally displaced apical meristem and is organogenic. In unifoliate species, it remains quiescent during vegetative growth, which may persist intermittently over several years. The groove meristem then enlarges, becoming dome-shaped. It splits, the proximal por-

Fig. 3. Seedling development in *S. fanniniae*, subgenus *Streptocarpus*. A-C, camera lucida drawings of 3 stages; all median longitudinal sections.

A. 1 week old, with embryonic apical meristem in fork between unequal cotyledons.

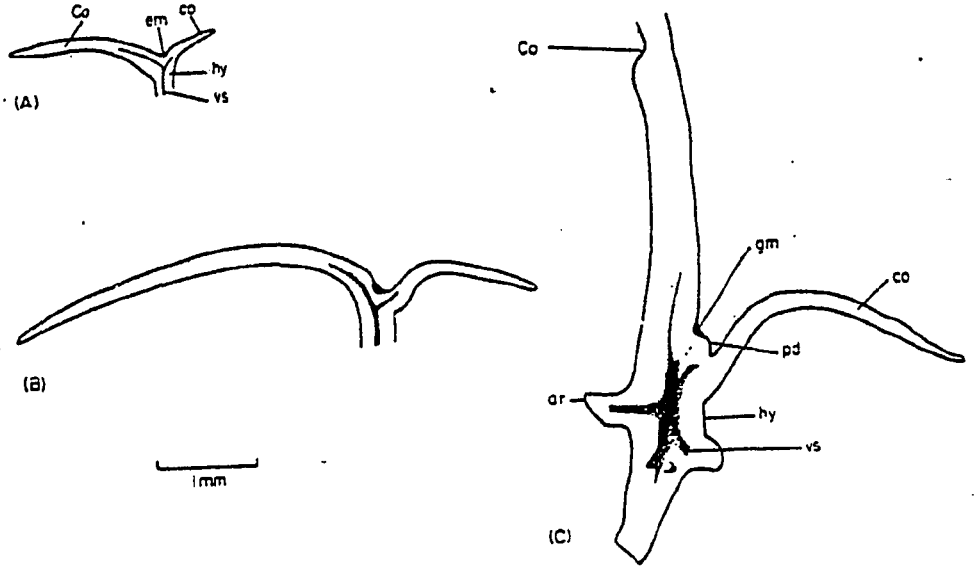
B. 4 weeks old, with apical meristem displaced to base of accrescent cotyledon.

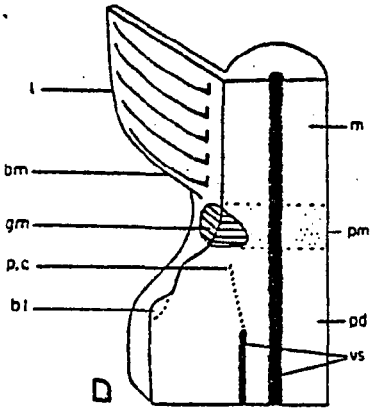
C. 6 weeks old, with petiolode and intercalary meristematic complex at base of macrocotyledon.

C. Diagrammatic representation of topological relations of meristems in longitudinal section.

Co - accrescent cotyledon
co - suppressed cotyledon
em - embryonic apical meristem
hy - hypocotyl
vs - vascular tissue
pd - petiolode
gm - groove (=displaced apical) meristem
ar - adventitious root
l - lamina
m - midvein
bm - basal meristem
pm - petiolode meristem
pc - procambial strand
bl - first detached meristem

Adapted from Jong and Burtt (1975)





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tion producing the first inflorescence primordium, while the distal portion generates subsequent inflorescences.

In many species, a portion of the groove meristem becomes detached and generates one or more additional phyllomorph primordia during vegetative growth (as in *S. fanniniae*) or at the time of flowering (as in *S. prolixus*).

Rosulate species develop in a somewhat different way. The displaced seedling apical meristem splits early, the major portion functioning as an apical meristem which generates phyllomorphs on a very short vertical or horizontal axis. The small detached axillary portion then functions as the groove meristem of the cotyledonary phyllomorph. It is evident that the diversified morphology found in *Streptocarpus* is traceable to the timing and mode of activity of the apical meristem.

2) The petiolode meristem, consisting of columns of vacuolated cambiform cells which divide anticlinally, extends through the petiolode at the level of the groove meristem, thus providing extension of the midrib of the lamina distally and of the petiolode proximally.

3) The basal meristems, immediately distal to and in contact with the groove meristem, generate lateral tissue in each half of the lamina.

This persistent complex of intercalary meristems is capable of producing in some species a lamina which may reach 1000 mm. in length and breadth. In almost all species so far studied, the phyllomorph is monocarpic. In those species in which the groove meristem generates additional phyllomorphs, the plant is perennial.

Experimental Approach to Speciation and Phylogency

The fundamental sources of morphogenetic patterns in plants remain among the most important unsolved problems in biology (Stebbins, 1974c). Changes in morphogenetic patterns lead to changes in morphology concomitant with evolution and speciation.

Such changes originate in mutations and/or genetic recombinations, but the pathways between alterations in DNA and their expression in new plant forms are understood in only general terms. Stebbins (1969) suggested that genes controlling morphological characters probably code for regulators or control systems affecting the action of other genes, or for structural proteins that can function properly only in conjunction with other proteins that are coded by different genes.

Studies in morphogenesis are needed to permit bridging by botanists of the gap between gene mutations and morphological trends; this is essential to evolutionists (Stebbins, 1974a).

A series of papers by Basile (1967, 1969, 1970, 1973, 1979, 1980) demonstrates the value of such studies. Evolution in leafy liverworts is generally assumed to have occurred in the direction from tri-radial to dorsi-ventral symmetry, i.e., from three to two rows of leaves. This evolution occurred independently in several families in more than one order.

Basile found that modifications of substrate in axenic cultures of dorsi-ventral species in five families of leafy liverworts produced radially symmetrical phenovariants. In a recent paper (1979) he was able to induce stable, uniform phenovariants in *Plagiochila arctica*

which permitted study of alterations in cell-division activity of derivatives of apical cells. Proteins containing high levels of hydroxyproline in cell walls are associated with suppression of cell division of ventral derivatives of apical cells in 'normal' dorsi-ventral species. Addition of substances antagonistic to normal metabolic incorporation of hydroxyproline in cell wall proteins (i.e., hydroxyproline and 2,2'-dipyridyl) produced phenovariants in which ventral derivatives of apical cells were not inhibited or suppressed, and the plants grew with radial symmetry.

The significance of Basile's studies to date lies in the following:

1) The genomes of highly evolved hepatics have not lost the capability to produce a third row of leaves as in the primitive ancestral condition.

2) The expression of the capability to form the third leaf row is directly related to the derepression of cell division (meristematic) activity of apical cell derivatives in one region. (Conversely, therefore, the evolved species possess only two rows of leaves because of phylogenetic suppression of cell division in derivatives of the apical cell in one plane.)

3) The change in morphogenetic patterns between normal and phenovariant plants is related to alteration in metabolism of cell wall hydroxyproline-containing protein(s).

Thus, Basile correlated evolutionary changes in form with morphogenetic changes depending on differential meristematic activity, and the latter with metabolic (biochemical) alterations.

Stebbins (1974b) cited Basile's studies as an example to be followed by botanists with other groups of plants. In the case of angio-

sperms, Stebbins (1974c) suggested two important characteristics of angiosperms in which they differ markedly from all other plant groups and that have made possible their great ability to adapt to altered environments by modifications in form. Those are, first, that angiosperm cells are much more responsive to phytohormones than, for example, gymnosperms; second, that angiosperm flexibility is often expressed via the activity of intercalary meristems.

In dicotyledonous plants apical meristems are responsible for continuing growth of stems, initiation and growth in length of leaves, root growth and flower initiation. Intercalary (non-apical) meristems produce stem internode and petiole elongation, leaf forms in all their diversity, and inflorescence growth. They also lead to increased complexity of flowers by such changes as adnation and connation of parts.

If, as is likely, Stebbins is correct in stressing the cardinal importance of intercalary meristems in flowering plant evolution, then studies focusing on those factors that initiate and subsequently regulate intercalary meristems are pivotal to understanding the morphogenetic bases of phylogeny. Until now, there have been few attempts to induce changes in morphogenetic patterns in flowering plants specifically aimed at obtaining information necessary to an improved understanding of morphological changes accompanying evolutionary processes.

In one example of such a study, meristem alterations have been produced in plants by use of 2,3,5-triiodobenzoic acid (TIBA). This substance acts by interfering with distribution of auxin in the plant (Heslop-Harrison, 1957). Thus, TIBA applied to the shoot apex of *Kalanchoe blossfeldiana* causes fusion of leaf primordia with transfor-

mation to a perfoliate leaf. The latter results from activity of intercalary meristems which lead to concrescence of parts (Harder and Opperman, 1952). Such a change to perfoliate leaves is taxonomically significant.

In another example, exogenously applied gibberellin induced alterations in shoot development and leaf shape in tobacco (Engelke, Hamzi and Skoog, 1973), again the result of changes in activity of intercalary meristems.

The experimental approach to phylogeny and speciation applied successfully in Hepaticae by Basile has motivated this research on *Streptocarpus*. Stebbins' suggestions regarding the importance of intercalary meristems and of the possible role of growth regulators in evolution of angiosperms, have provided the initial direction for my experiments.

Prior Reports on Effects of Plant Growth Regulators on *Streptocarpus*

There have been few attempts to study the effects of phytohormones and other plant growth regulators on morphogenesis, growth and morphology in *Streptocarpus*.

Rossini and Nitsch (1966) and Rossini (1967) regenerated assorted organs from sterilized leaf discs of *S. nobilis* in axenic cultures. The emphasis was on production of flower buds in this short day species belonging to subgenus *Streptocarpella*. They found that cytokinins and indoleacetic acid added to the agar medium increased flower bud production

whereas gibberellin, contrary to expectation (*S. nobilis* being a short day plant) completely inhibited flowering.

Appelgren and Heide (1972) studied regeneration from non-sterilized lamina discs cut from *S.* "Constant Nymph", a hybrid within subgenus *Streptocarpus*. The discs were floated on mineral-phytohormone solutions. They found that vegetative bud and root regeneration were enhanced by auxins. Cytokinins had only a small stimulatory effect on bud formation. Gibberellin A_3 inhibited both bud and root formation but was reversed by auxin. Abscisic acid in the presence of optimum levels of auxin enhanced bud formation, with little effect on root formation.

Effects of exogenous applications of gibberellins and 6-benzylaminopurine (benzyladenine) on morphology of species in sub-genus *Streptocarpus* have been reported in several publications by Michelle-A. Dubuc-Lebreux (Dubuc-Lebreux, 1976a, 1976b, 1978; Dubuc-Lebreux and Vieth, 1975, 1976). Seedlings of varying ages (6 1/2 to 14 months) of several species were treated with phytohormone solutions at weekly intervals and phenovariants resulted. In general, it was found that gibberellin application resulted in elongation of lamina and petiolodes. It also caused production of accessory phyllomorphs and thus conversion of unifoliates to plurifoliates with pseudaxial structure initiated on a few of the accessory phyllomorphs. Benzyladenine had little effect on vegetative form of plants in this age range.

It must be noted that none of the previous studies involving applications of phytohormones altered anisocotyly and acaulescence, patterns established shortly after germination of seedlings in subgenus

Streptocarpus. Nor did any investigators study the influences of phytohormones on location and activities of meristems, and their relationships to morphology of the adult plants.

In 1977 I reported achieving caulescence in subgenus *Streptocarpus* using gibberellin A_3; in 1980 I reported on caulescent phenovariants produced by GA_3, and on bi-phyllomorphic phenovariants produced by 6-BAP , along with meristem alterations. These reports were based on studies which are included in this dissertation.

The results I obtained are consistent with Basile's hypothesis that ancestral morphogenetic capacities may not be lost in derived taxa but are not normally expressed. They also confirm the correctness of Stebbins' suggestions on the importance of plant growth regulators and intercalary meristems in evolution of angiosperms.

MATERIALS AND METHODS

Species Employed in This Research

For investigating morphogenesis in subgenus *Streptocarpus* an early choice of species was made. *S. gracilis* B. L. Burtt was available, having been grown by me under fluorescent light with repeated vegetative propagation during several years. Seeds of this species were originally supplied by the Seed Fund of the American Gloxinia and Gesneriad Society.

This species was subsequently reduced to *S. prolixus* C. B. Clarke (Hilliard and Burtt). A plant of *S. prolixus*, somewhat different in appearance, was received in 1977 from O. H. Hilliard who had collected it in Natal Province. The two forms were grown side by side in the greenhouse for several years. Several character differences exist, and the plant formerly known as *S. gracilis* was found to possess advantages for research purposes and was thereafter used exclusively:

a) More rapid growth and flowering, with more perennation (additional phyllomorphs);

b) Greater seed production via artificial pollination;

c) Stouter peduncle and pedicel segments; thus, more suitability for axenic regeneration.

Following a nomenclatural suggestion for use of trinomials within a species made by Burtt (1970b) this form hereafter is referred to as *S. prolixus (gracilis)*.

S. prolixis (gracilis), although originally described by Burtt as unifoliate, under greenhouse conditions is a plurifoliate, flowering

Fig. 4. Greenhouse grown flowering plant of *S. prolixus (gracilis)*

from seed at about 10 months, and producing one or more additional phyllomorphs at about the same time. The cotyledonary phyllomorph is up to 23 x 15 cm; inflorescences may reach to 55 cm. long, with as many as 25 pairs of flowers. The inflorescence has the form of a sympodial cyme. At each node two flowers on 2 cm. pedicels appear, along with the subsequent peduncle segment. Normally, an inflorescence branches once. The small flowers contain two fertile stamens hidden near the center of the 1 cm. corolla tube. Artificial pollination requires dissection of the corolla.

Up to 6 inflorescences are produced basipetally by each monocarpic phyllomorph. After initial flowering 2 to 4 additional phyllomorphs are produced annually; these can be removed and rooted. By vegetative reproduction, artificial pollination and sowing of seeds, a constant supply of plants at all stages of growth was maintained in the greenhouse.

S. prolixus (gracilis) flourishes under a wide range of greenhouse conditions, flowering year round at the latitude of New York.

Many other species belonging to subgenus *Streptocarpus* were initially grown from seed supplied by the American Gloxinia and Gesneriad Society Seed Fund. Those maintained as stock plants or regrown periodically from seed and which were employed in experiments included:

S. haygarthii N.E. BR. ex C. B. Clarke (plurifoliate)

S. grandis N.E. Brown (unifoliate)

S. rexii (Hooker) Lindley (rosulate)

S. solenanthus Mansfield (Unifoliate)
(Source N.Y.B.G.)

S. schliebenii Mansfield listed in subgenus *Streptocarpella*

by Hilliard and Burtt, but changed to subgenus *Streptocarpus* by Milne (1975, addendum). Source was a cutting from Royal Botanic Garden, Edinburgh. This species is acaulescent for many months, becoming caulescent before flowering.

Of the several species in subgenus *Streptocarpella* that were grown, two were maintained by regrowth from seed, and were employed in the research:

S. nobilis C.B. Clarke (annual)

S. muscosus C.B. Clarke (perennial)

The source for these species was also Royal Botanic Garden, Edinburgh.

Potting mixes are listed in Appendix I.

Experimental Procedures

Axenic Culture

Several agar culture media, sterilizing techniques and inoculation methods were initially tried, from which evolved the most successful ones. Minerals are modified from the Murashige-Skoog (1962) Revised Tobacco Medium by reducing ammonium ion to 0.2 millimoles (Grunewaldt 1977). Vitamin additives are from Staba (1969). The composition of this medium is listed in Appendix II.

Sterilization of greenhouse grown plant parts to be inoculated was not difficult for seeds or inflorescences, for which the "washing machine" method of Basile (1973a) was adapted.

Streptocarpus lamina, however, are deeply grooved and bear a heavy indumentum, creating severe problems, especially regarding bacterial spores. After many trials, the method of Grunewaldt (1977), based on mercuric chloride, was successfully adopted for surface sterilization of phyllomorph lamina. (Appendix III).

Axenic cultures were grown under controlled environment: 12-14 hours light supplied by two 75 watt cool white fluorescent lamps providing 1720 lux intensity. Temperature was maintained at 18-20° C.

Exogenous Growth Regulators in Axenic Culture

Experiments were conducted on the morphogenetic effects induced by differing levels of various plant growth regulators added to plantlet regenerating medium (Appendix II). The growth regulators and concentrations used are given in the Results section of the text.

Lamina discs were cut aseptically from whole plants already in axenic culture or from sterilized lamina of greenhouse plants. Single discs were inoculated into 60 mm. petri dishes containing 5 ml. of medium.

Pot Culture

Seedlings were at first grown in pots containing various "soil" mixes in the greenhouse to study effects of plant growth regulators which were applied in solutions dropwise on individual seedlings. Later, when greater synchrony in germination had been achieved, solutions were applied as drenches at the expected time of germination.

A highly successful refinement used for most of the experiments employed a temperature controlled room (20°C) with fluorescent lighting. Seeds (30-60) were sown on the surface of 5.5 x 4.7 cm. plastic pots filled with Jiffy seed starting mix, then misted with deionized water to settle the seeds. The pots were placed on wetted capillary matting (Vattex, from U. S. Vattex Corporation, Center Moriches, N.Y.) in glass aquarium tanks 50 cm. long x 25 cm. wide x 30 cm. high. The tanks were covered by transparent acrylic covers which were kept open 10 mm. to retard evaporation while permitting gas exchange. A 12-13 hour day-length was provided by daylight type high output lamps (2400 lux). Moisture in the pots was maintained by watering the capillary matting as needed.

In order to promote early, uniform germination, fresh seeds, no older than 60 days after harvesting, were used wherever possible. Under these conditions, a high percentage of *S. prolixus (gracilis)* seeds germinated 8-10 days after sowing. Consequently, phytohormones to be tested for their influence on the initial stage of seedling development were applied as a 12-15 ml drench on day 7-9 after sowing.

When it was desired to sustain the effects of regulators, applications were repeated at 10 day intervals. For this purpose, solutions of the same concentration as the initial drench were applied to foliage using a pump type hand mist sprayer, 5-7 mls. sprayed on each pot.

In another set of experiments one or both cotyledons of 2-3 day old seedlings were coated on the upper surface (using a fine needle, under dissecting microscope observation), with a paste in which a solution of phytohormones or water alone was mixed. Initially, Aquaphore, a lanolized emulsifiable ointment, was used, mixed with solution or water in the ratio 9:1. Other ointments, as indicated in the appropriate por-

tion of the Results section, were used without phytohormones in subsequent experiments.

Histological Techniques

Seedlings were harvested at appropriate time intervals, cleansed of debris, then fixed in a solution of 3% glutaraldehyde in buffer solution of 0.1 M sodium cacodylate, pH 6.8, under a vacuum for 1 hour at 4°C.

Seedlings were then rinsed 3 times, 5 minutes each, in cold cacodylate buffer, followed by 3 similar rinses in distilled water. Vials could be stored up to 4 weeks at 4°C.

Photography was undertaken with either fresh or fixed material.

A simplified protocol for dehydration was employed based on the use of 2,2'dimethoxypropane (Postek and Tucker, 1976; Lin, Falk and Stocking, 1977) (Appendix IV).

Following sectioning, a simplified method for preparing slides was developed, based on staining with Toluidine Blue, a metachromatic stain (Appendix V).

In the earlier stages of this study, slides were prepared according to the method of Jong (1970), based on conventional fixation followed by dehydration in an alcohol series. Staining was by Delafield's hematoxylin, with Safranin O counterstain. The method described above was much simpler and faster.

Photography

All objects were photographed on Eastman-Kodak Ektachrome color slide film, using a Nikkomat FTN camera equipped with Nikon 55 mm micro-

lens. For small objects, a Nikon bellows attachment was used, with flood lamps. Smaller objects, e.g., very young seedlings, required fiberoptic lamps for illumination. Sections on glass slides were photographed with Nikon microscope and adapter.

For conversion to black and white, a Nikon copying attachment was used with the bellows, and negatives copied on Panatomic X film. These were then printed with cropping and further enlargement.

RESULTS

Experiments designed to investigate the possible relationship between growth regulators and morphogenetic patterns leading to radial vs. dorsiventral symmetry of the vegetative body of *Streptocarpus* species were of three kinds:

Tissue culture experiments from different plant parts; whole plant seedling culture experiments under axenic conditions and whole plant (seedling) culture under controlled but not axenic conditions. Each kind of experiment provided different insights and will be discussed in the order given.

The first experimental evidence that vegetative symmetry may be alterable came from tissue culture experiments in which inocula were derived from different plant parts. 5-8 mm. discs excised from lamina of *S. prolixus (gracilis)* phyllomorphs were inoculated on basal medium modified for plantlet regeneration (Appendix II). Plantlets began to appear at cut edges of discs in about 4 weeks; they followed developmental patterns similar to those in seedlings, as previously described (Jong, 1970; Hilliard and Burtt, 1971; Jong and Burtt, 1975) becoming anisocotylous and acaulescent.

Plantlets were then regenerated *in vitro* from inflorescences. Inflorescence segments of approximately 10 cm. length and containing terminal flowers and buds were cut from greenhouse plants of *S. prolixus (gracilis)*. These were sterilized and inoculated as nodes of approximately 8-10 mm. lengths into the plantlet regenerating medium. Callus formation was observed after 3-4 weeks at the cut ends, followed by

plantlets and roots in a ring at the periphery of the dome shaped callus at the proximal end. Two weeks later, additional plantlets were seen in flower axils. (Figure 5). Examination of the plantlets at 10 weeks revealed a diversity of form, unlike those regenerated from lamina discs. They consisted of 1-5 leaves and in some instances a flower or flower bud. The first two leaves were alternate or opposite, and equal, subequal or unequal; apical meristematic activity was apparent, and nearly all plantlets were caulescent. Some of the plantlets are illustrated in Figure 6.

Morphogenetic changes leading to caulescence in plantlets of subgenus *Streptocarpus* have not been reported previously. Obtaining them as a result of the first experiments suggested that differences between endogenous regulators present in peduncles and lamina might be responsible for caulescent regenerants from the former and acaulescent from the latter sources of inoculum.

To test this possibility, experiments were performed to find out if exogenously supplied growth regulators could induce regenerants from lamina discs to follow a caulescent pattern of morphogenesis. Auxin (indoleacetic acid) and cytokinin (benzyladenine) were components of the regeneration medium used in the first set of experiments. These regulators did not alter the regeneration pattern from lamina discs under the conditions employed. Since gibberellins had been reported to "incite stem formation" (Sachs, 1961), I decided to next study the influence of gibberellin A_3 on regeneration from lamina discs.

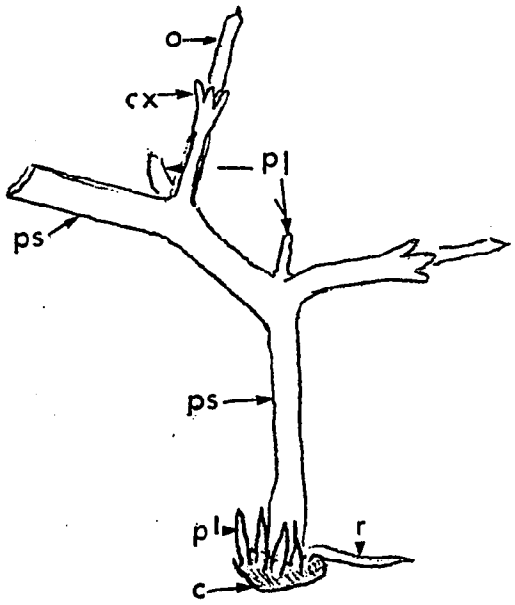
When GA_3 at 10^{-6}M to 10^{-5}M was incorporated into the regeneration culture medium, several distinct differences in regeneration and morphology were observed between plantlets developing on GA_3 supplemented

Fig. 5. Drawing showing regenerated plantlets from axenically inoculated inflorescence segment of *S. prolixus (gracilis)* grown on basal medium supplemented with indoleacetic acid and benzyladenine. 6 weeks.

ps - peduncle segment
p - pedicel
pl - plantlet
c - callus
r - root
cx - calyx of flower
o - unfertilized ovary

Fig. 6. Drawings of some examples of regenerated plantlets as in Fig. 5, but at 10 weeks.

l - leaf
b - bud
fb - flower bud
r - root



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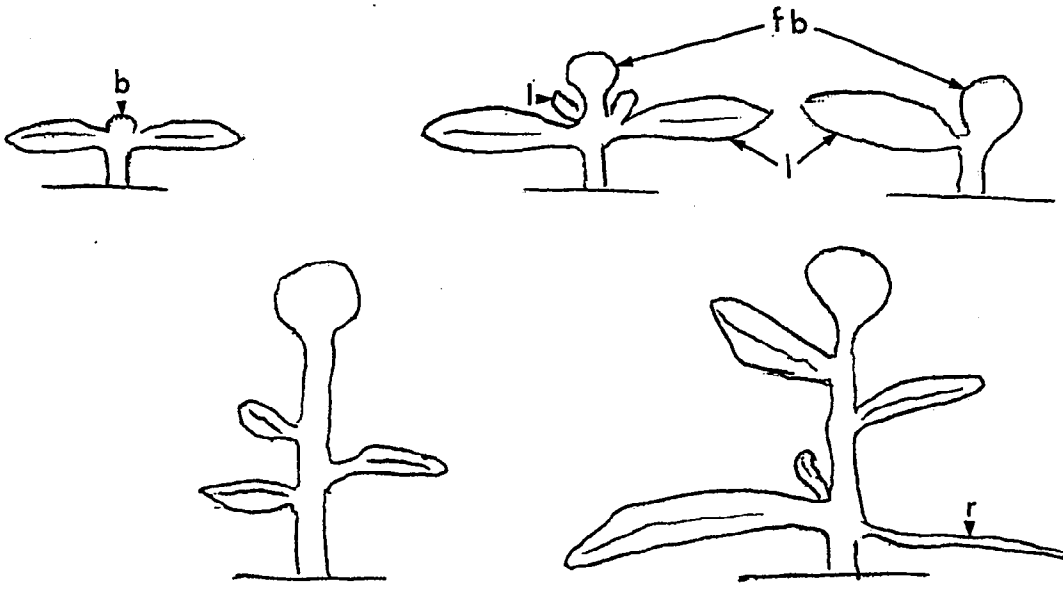

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medium and on non-supplemented medium. These differences are shown in Table 1. Of the features compared, the development of vegetative caulescent axes via a shoot apical meristem was the most profound. Besides providing additional evidence that an acaulescent species retained the capacity to manifest caulescence in its vegetative development, it provided evidence that differences in levels of gibberellins (or something regulated by gibberellins) might be involved.

Table 1. Comparison of morphogenetic features in plantlet regeneration from lamina discs of *S. prolixus (gracilis)* under axenic conditions in plantlet regenerating medium vs medium supplemented by GA_3 at $10^{-6}M$ or $10^{-5}M$.

	Non-supplemented	GA_3 supplemented
Regeneration rate	Plantlets appeared in 4 weeks	Plantlets appeared after 5 weeks and grew more slowly
Polarity	Plantlets evenly distributed around disc periphery	Plantlets clustered in one peripheral area
Root initiation at 10 weeks	Average 3 roots per disc	Average slightly less than 1 root per disc
Plantlet morphology at 10 weeks	Plantlets sessile on discs; small	Plantlets elongate (10-14 mm) and narrow (1 mm)
Orientation and tropism	Plantlets nearly horizontal	Plantlets vertical
Epinasty	None	Recurved 'hook' at nearly 135°, especially with GA$10^{-5}M$
Apical activity	None	Apical buds appear after 11 weeks; by 18 weeks 50% of regenerants had at least one extra leaf.

Having discovered that exogenously supplied GA_3 could induce a shift from acaulescence to caulescence in regenerants, I was interested in finding out whether this phytohormone could induce similar morphogenetic shifts in seedlings.

Effects of Gibberellin on Axenically Grown Seedlings

In this series of experiments, seedlings of *S. rexii*, a rosulate species, as well as of *S. prolixus (gracilis)* were used in pilot studies. Seeds were sterilized by the "washing machine" method of Basile (1973a), and were inoculated in 60 mm petri dishes containing basal medium supplemented with GA_3 at several concentrations.

Table 2. Effects of exogenous GA_3 on macrocotyledon, root and hypocotyl development under axenic conditions, measurements made at 5 weeks.

GA_3 Concentration	No. of plants	Average Macrocotyledon length, mm	Maximum root length mm	Average hypocotyl length, mm
0	6	4 (2-6)	6	<1
10^{-9}M	12	6 (2-10)	8	"
10^{-8}M	4	4	5	"
10^{-7}M	7	3 (1-5)	3	"
10^{-6}M	10	1-2	1	"
10^{-5}M	9	1	<1	1
10^{-4}M	n.a.	"	"	10
10^{-3}M	21	"	"	10

Seedlings which developed on medium with concentrations of GA_3 at 10^{-7}M and above showed obvious differences from the controls. The most important, with respect to this study, is the virtually complete suppresion of macrocotyledonary accrescence at concentrations of 10^{-6}M and above.

Results from the *S. rexii* experiment provided a basis for a similar but longer term experiment with seedlings of *S. prolixus (gracilis)*. Only two concentrations of GA_3 were used, 10^{-6}M and 10^{-5}M. Most of the cultures initiated in this experiment were lost to microbial contamination. Fortunately, 10 of the seedlings which developed on GA_3 supplemented media survived the 6 month incubation medium.

In all 10, not only was macrocotyledonary accrescence suppressed, but apical growth and caulescence were expressed. Seedlings bore as many as 14 leaves; the latter were small and elongate, ranging in length from 1 to 15 mm., but mostly near the lower size limit. Phyllotaxy was

mostly alternate but irregular; one plant had several pairs of decussate leaves and a terminal flower. Branching in cotyledonary axils was common.

Some seedlings in this experiment were germinated on basal medium, then transferred to GA_3 augmented medium at different intervals after germination. When transfers were made very soon after germination, cotyledons remained equal and the apical bud was centrally located. When transfers were delayed, partial accrescence of one cotyledon occurred. The apical bud in these was seen to be displaced laterally toward the base of the macrocotyledon. These latter exhibited developmental patterns analogous to the ones reported by Jong (1978) for rosulate species. It would seem then, depending on the "time"/stage of development at which the seedling was exposed to elevated levels of GA_3, this acaulescent plurifoliate species exhibited morphogenetic patterns reminiscent of either acaulescent rosulate species in subgenus *Streptocarpus* or of caulescent species in subgenus *Streptocarpella*.

In the next experiment, *S. prolixus (gracilis)* seeds were germinated in basal medium augmented by GA_3 at higher levels than before, 10^{-5} - 10^{-3}M. Transfers to fresh media were made about one month after germination, and observations continued for several months. In general, results were similar to those in the earlier experiments, the main difference being precocious flowering in some seedlings at these higher levels of GA_3. Flowers were generally single and terminal in position; in a few plants there was a second flower bud axillary to the first. Corollas were gamopetalous in most instances. Phyllotaxy was highly irregular and branching was seen in a few plants (Figures 7-10).

Figs. 7-10. Axenically grown seedlings of *S. prolixus (gracilis)* grown in basal medium supplemented by different levels of GA_3. 4 months.

7. Upper left - $GA_3 5 \times 10^{-5}$M. Extensive branching, no flowering. x7.5

8. Upper right - $GA_3 10^{-4}$M. Smaller plant with terminal flower bud. x3.6

9. Lower left - $GA_3 10^{-3}$M. Flower with unfused corolla lobes (not all seen in focus), pistil and stamens. x3.1

10. Lower right - $GA_3 10^{-3}$M. Flower bud, many leaves on short stem, and one branch. x3.4

c - cotyledon
b - branch
r - root
fb - flower bud
cx - calyx
col - corolla lobe
pi - pistil
st - stamen

B
C
D

Effects of Gibberellin on Pot-Grown Seedlings of *S. prolixus (gracilis)*

The experiments with axenically grown seedlings established two important points. First, gibberellin was capable of inducing morphogenetic changes leading to isocotyly and caulescence. Second, the time at which effective levels were present was critical. But due to the sensitivity of the minute (0.5-0.7 mm.) seeds to the surface sterlization solutions, it was difficult to rid seeds of contaminants without significantly decreasing their viability. Therefore, experiments with unsterilized germinating seeds similar to those performed *in vitro* were conducted using heat sterilized potting mixes as substrate. When it had been determined that germinating seeds responded to GA_3 in pot culture as they did *in vitro*, all subsequent experiments were conducted in this way. In the course of performing the first few experiments, refinements in procedure were made.

The results of one of the first pot culture experiments in which GA_3 at 10^{-4}M was applied as a single drench was given in Table 3.

Table 3. Quantitative effects of $GA_3 10^{-4}$M drench on pot grown seedlings of *S. prolixus (gracilis)*. 23 days

Treatment	No. of plants	Average dimensions, mm, macrocotyledon	microcotyledon	hypocotyl
Controls	40	5.5 x 3.5	2 x 1.5	3.5
$GA_3 10^{-4}$M	22	3.2 x 1.3	2.7 x 1.3	12

The results indicated that a single application of GA_3 was only sufficient to retard but not prevent anisocotyly. When, however, in a follow up experiment the initial drench application was supplemented by foliar sprays of the same concentration at 10 day intervals, the results given in Table 4 were obtained.

Table 4. Effects of GA_3 applied as drench supplemented by foliar sprays at 10 day intervals on pot grown seedlings of *S. prolixus (gracilis)*. 56 days

Treatment	No. of plants	Average Dimensions, mm. macro-cotyledon	hypocotyl	plant height	No. of extra leaves	Max. size of youngest leaf, mm.
Controls	24	16 x 5 (3 isocotylous=12%)	3	--	0 on 20 1 on 4 (=16%)	--
$GA_3 10^{-5}M$	22	1 x 1 (all isocotylous)	7	11-46 (highly variable)	1 on 13 2 on 8 3 on 1 (av.1.5)	8 x 2
$GA_3 3x10^{-5}M$	26	1 x 1 (all isocotylous)	9	22 (highly uniform)	1 on 6 2 on 14 3 on 4 4 on 2 (av. 2.1)	4 x 1

With sequential treatments, both concentrations of GA_3 suppressed cotyledonary accrescence and caused apical growth in 100% of seedlings. At the higher GA_3 level, plants were more uniform, shorter, and had more leaves. At both concentrations, the youngest leaves (those at greatest distance from the stem base) were escaping from GA induced growth suppression and were demonstrating accrescence. At $GA_3 10^{-5}M$, stem height was much more variable than at $GA_3 3x10^{-5}M$.

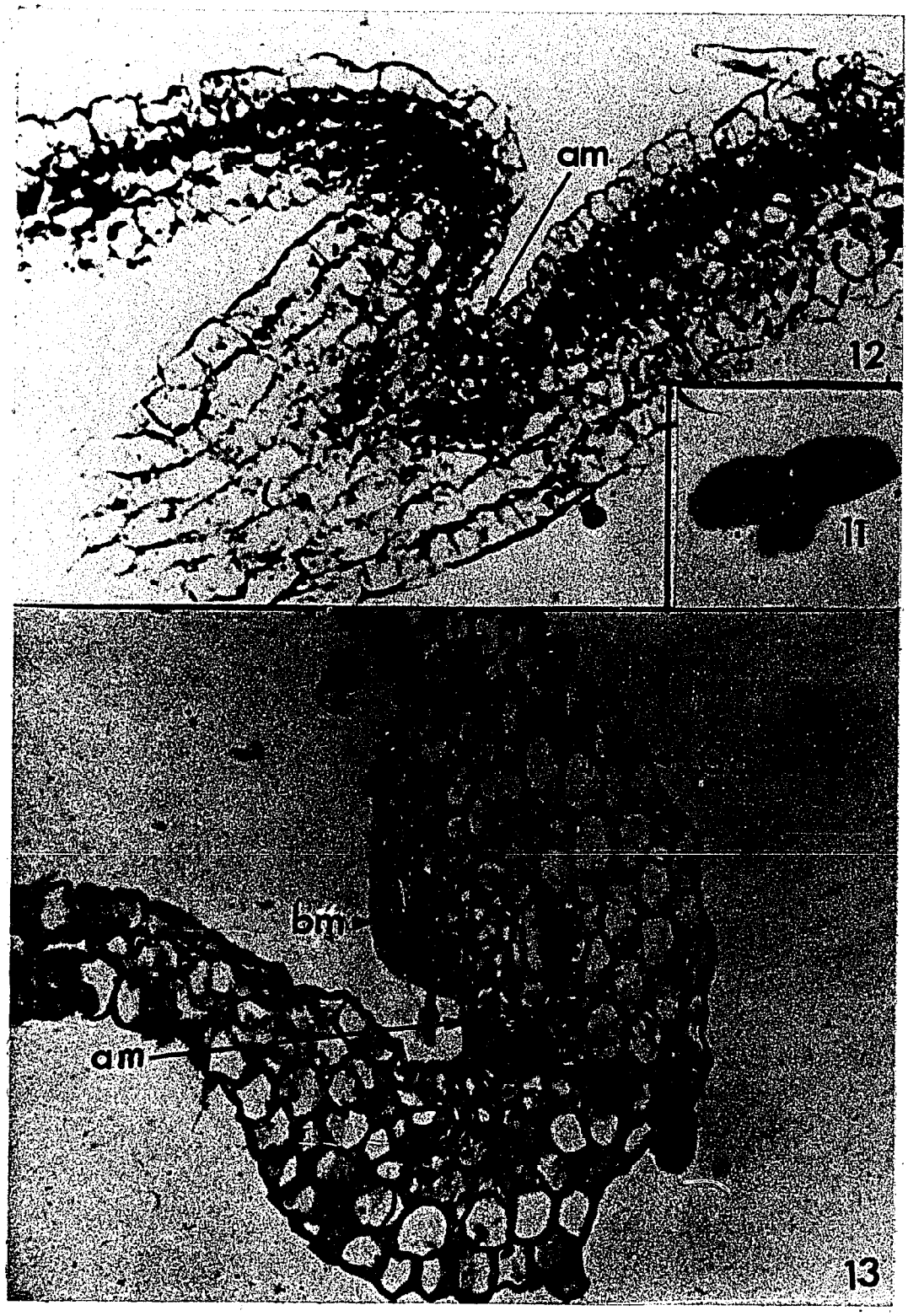
Figs. 11-13. Pot grown seedlings of *S. prolixis (gracilis)*, untreated.

11. Inset on upper figure. 3 days, cotyledons equal. x20

12. Upper. 3 days, median longitudinal section. Apical meristem consists of 2 cell layers of small densely staining cells. x1800

13. Lower. 13 days, median longitudinal section. Apical meristem has been displaced to base of accrescent cotyledon. Basal meristem is seen. x640

am - apical meristem
bm - basal meristem
Co - accrescent cotyledon


am
12
11
bm
am
13

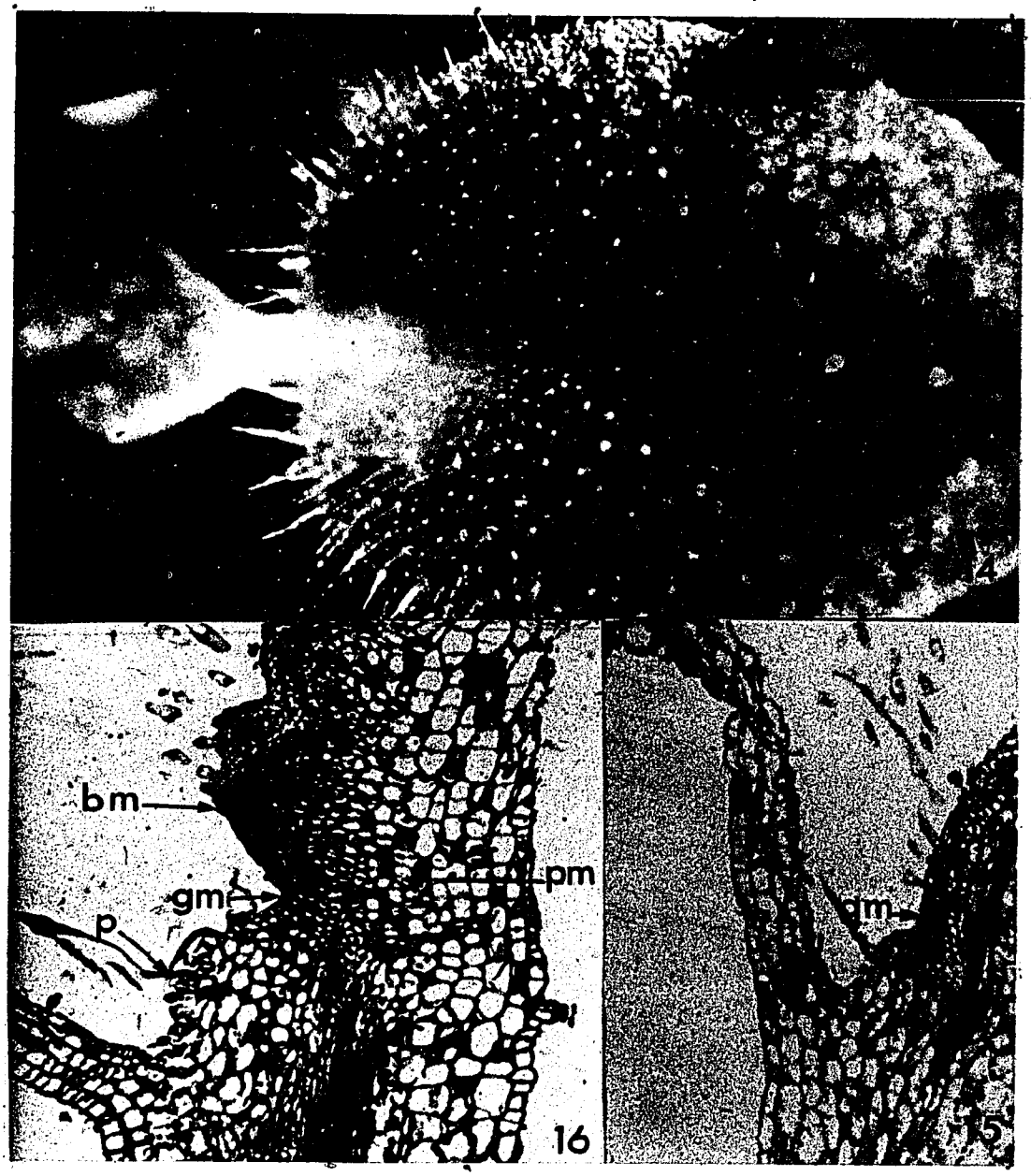
Figs. 14-16. Pot grown seedlings of *S. prolixus (gracilis)* untreated.

14. Upper. 29 days, top view.

15. Lower right, 29 days, MLS. Displaced apical meristem is now a groove meristem. x600

16. Lower left. 41 days, MLS. Petiolode and all 3 meristems seen. x600

p - petiolode
vs - vascular tissue
gm - groove meristem
pm - petiolode meristem
bm - basal meristem


bm
gm
p
pm
16
gm
15

Figs. 17-20. Pot grown seedlings of *S. prolixus (gracilis)* treated with single drench of Gibberellin A_3.

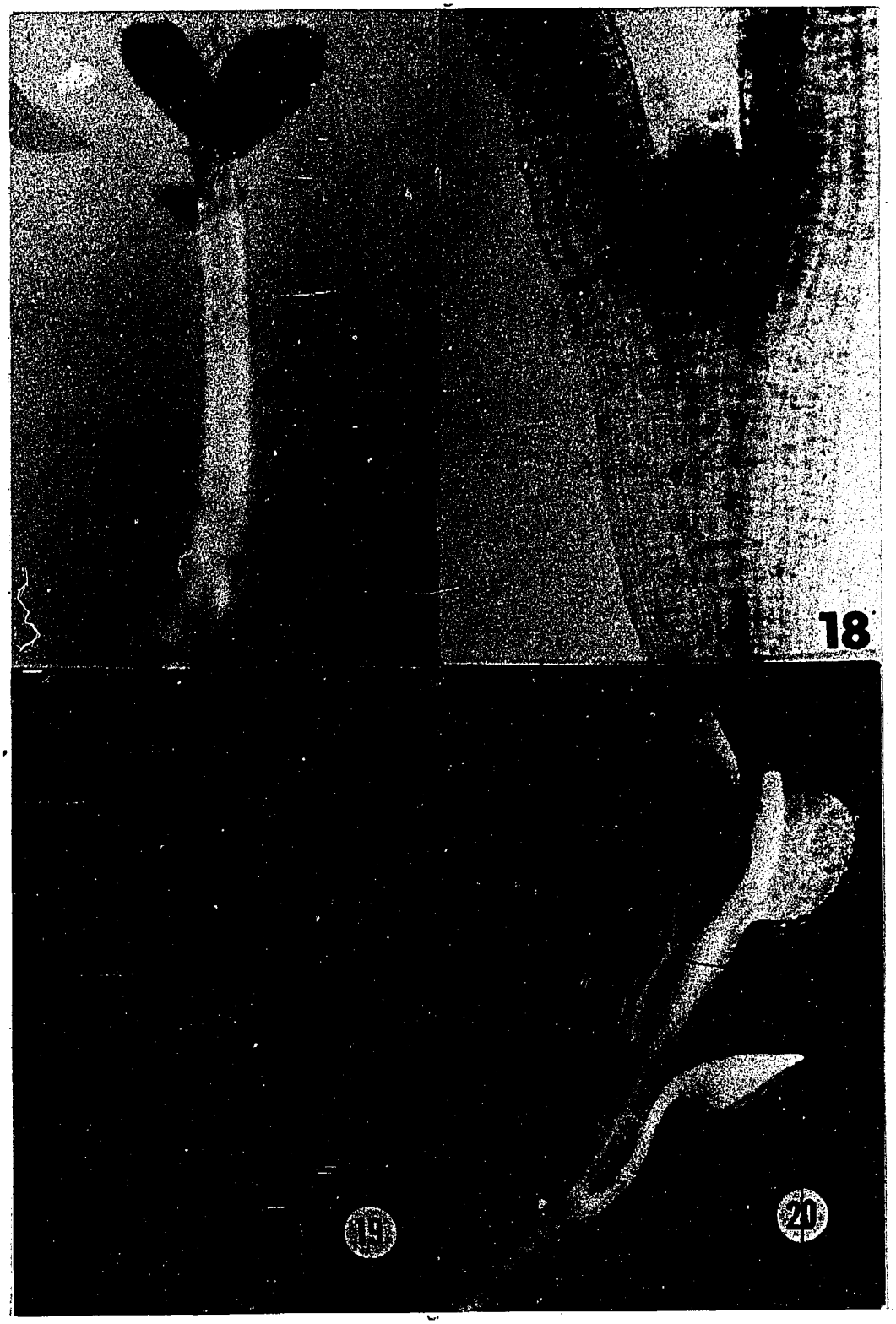
17-19 GA_3 at 5 x 10^{-4}M, 20 at 10^{-4}M.

17. Upper left. 3 days. x16

18. Upper right. 16 days. Cleared. Note apical bud. x50

19. Lower left. Isocotylous with central bud. 27 days. x14

20. Lower right. Slight cotyledonary accrescence; plumule has 2 leaves. 27 days. x14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19
20

Figs. 21-23. Pot grown seedlings of *S. prolixus (gracilis)*.

21. Upper right. Untreated, 7½ weeks. x4.6

22. Lower right. Single drench GA_3 at 10^{-4}M. 3 months. Cotyledons are equal and minute; one leaf of plumule has become accrescent. x2.8

23. Left. Treated at $GA_3 3 \times 10^{-5}$M by initial drench and foliar spraying at 10 day intervals. 7½ weeks. Several small leaves on each elongate seedling; upper branch seen on seedling at right. x10

Co - accrescent cotyledon
co - suppressed cotyledon
b - branch
L - accrescent leaf
l - minute leaf

23
22

Effects of Gibberellin on Seedlings of Other Unifoliate and Plurifoliate Species in Subgenus *Streptocarpus*

Early plumule appearance and suppression of cotyledonary accrescence in *S. prolixus (gracilis)* by gibberellin having been demonstrated, it was essential to test this phenomenon in other plurifoliate as well as unifoliate species in subgenus *Streptocarpus*.

In one experiment, sterilized seeds of 3 species were grown axenically on gibberellin augmented agar media for 6 weeks. *S. haygarthii* is a plurifoliate species, closely related to *S. prolixus* (Hilliard and Burtt); it typically produces one replacement phyllomorph annually. At GA_3 levels of 10^{-4}M and 10^{-3}M, plumules appeared in 65% of seedlings.

S. cooksonii, a large unifoliate species, exhibited plumular activity in 50% of treated seedlings. *S. eyelesii*, a slow growing unifoliate, produced a plumule in the only seedling that germinated; after 3 months, there were 7 leaves on 3 branches.

S. grandis, a large, fast-growing unifoliate species, was grown in the greenhouse in pots. With single drench GA_3 treatment, 2 of 8 seedlings possessed plumules at 5×10^{-5}M; 3 of 6 seedlings grew plumules at 10^{-3} M.

Seedlings of 3 species were grown in pots in a terrarium under fluorescent lights, treated by drench and 10 day sequential sprays of GA_3 at 10^{-4}M. *S. grandis* and *S. solenanthus* (also a fast-growing large unifoliate), as well as the plurifoliate *S. haygarthii*, responded with plumule production in nearly all seedlings. The response was similar to that of *S. prolixus (gracilis)*.

Fig. 24. Axenically grown seedling of *S. haygarthii*. Basal medium was supplemented by GA_3 at 10^{-5}M. 9½ months. Note many leaves and branches.

×2.8

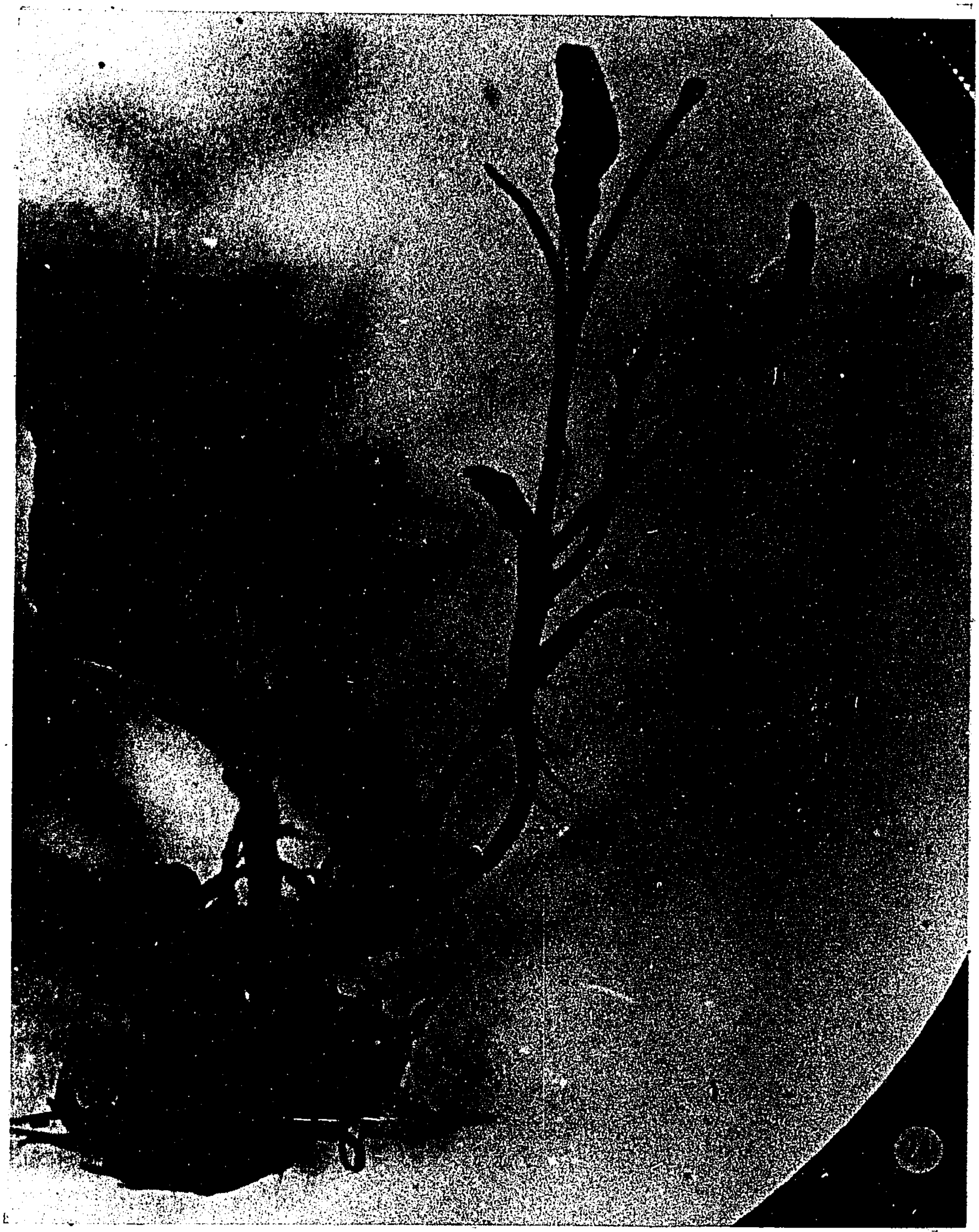


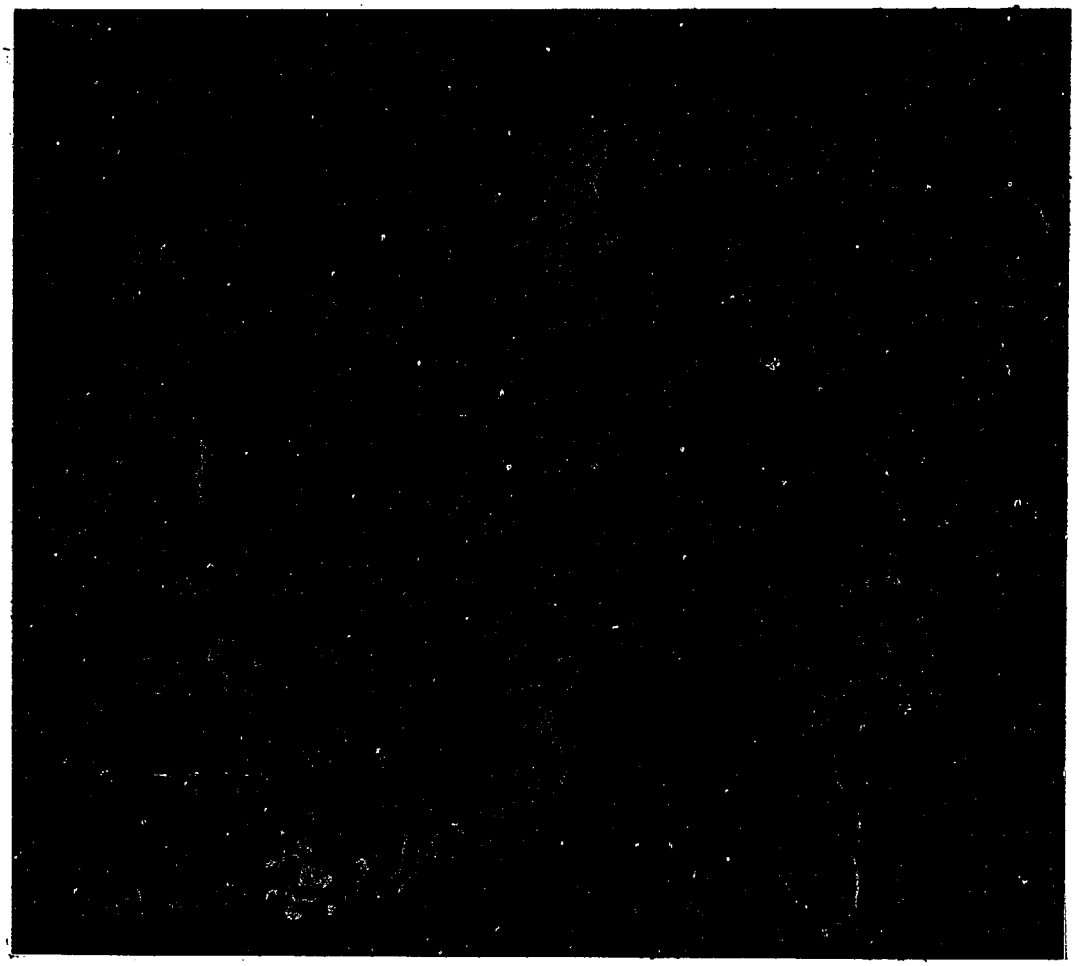
Fig. 25. Pot grown seedlings of *S. grandis*, a large, fast growing unifoliate. 7½ weeks.

× 3.4

right - untreated.

center - drench and 10 day sprays of GA_3 at 10^{-5}M. Macrocotyledon is long and narrow; note sharp 'hook' at junction of petiolode and lamina. No plumule present.

left - drench and 10 day sprays of GA_3 at 10^{-3}M. Stem and extra leaves of varying size are present. Note greatly reduced size of seedling.



It appeared in these experiments that higher levels of GA were required to suppress cotyledonary accrescence and produce early apical meristematic activity in fast growing large, unifoliate species than in smaller plurifoliate species.

These results closely parallel those obtained in several experiments with *S. prolixus (gracilis)*. The profound morphogenetic effects (isocotyly, caulescence) induced by gibberellin on seedlings were reproduced in all species of subgenus *Streptocarpus* that were tested.

Effects of Other Plant Growth Regulators on Species of Subgenus *Streptocarpus*

The successful induction of phenovariant regenerants and seedlings of gibberellin led to employment of other plant growth regulators, including those used in the original tissue culture experiments on regeneration from lamina discs.

Auxins

Drenches of three auxins were applied 8 days after sowing of freshly harvested seedlings of *S. prolixus (gracilis)* in pots of Jiffy mix. Treatments were:

Indoleacetic acid (IAA) at 10^{-5}M

Naphthaleneacetic acid (NAA) at 10^{-6}M

2,4-dichlorphenoxy acetic acid (2,4-D) at 10^{-6}M

These concentrations had been found to be the highest usable in agar media that did not result in obvious phytotoxicity.

No alterations in morphology or growth rate were observed.

Abscisic Acid

From prior experiments, maximum concentration without killing was found to be 10^{-3}M.

A drench at this concentration was applied 8 days after sowing to pots of *S. proxilus (gracilis)* in a terrarium. After 3 weeks, seedling growth was greatly inhibited, but morphology was unaltered.

2,3,5-Triiodo Benzoic Acid (TIBA)

This compound had been reported to induce phenovariants in plants by disrupting distribution of auxins leading to alteration of meristematic activity and resulting in fusion of leaf primordia and concrescence of flower parts. Several concentrations were used as soil drench in this experiment, with morphogenetic effects observed only at the two highest concentrations, 10^{-4}M and 10^{-3}M. At these levels, growth inhibition, macrocotyledon suppression and plumule organization were observed in some plantlets. At 10^{-4}M, inhibition was short-lived, and isocotyly and apical meristematic activity were observed in 15% of seedlings. Since this was approximately the same incidence observed in untreated seedlings, it was not considered significant.

At 10^{-3}M, however, isocotyly and apical meristematic activity with

production of one or a pair of true leaves was observed in nearly all plants. In all instances, such leaf pairs were seen to be fused in the form of a cup, forming one perfoliate leaf, the same effect as observed by Heslop-Harrison (1957) with *Kalanchoe blossfeldiana*. No further leaf production occurred within 9 months, although by then most seedlings were anisocotylous. Growth inhibition was still effective, no seedling being larger than 15 mm.

Auxin--Paste Experiments

Auxin, whether incorporated in media in *in vitro* experiments or supplied in drenches in pot culture experiments, did not alter the normal accrescent, acaulescent development of *S. prolixus (gracilis)* regenerants and seedlings. This was established by several experiments.

Yet the anti-auxin triiodobenzoic acid induced isocotylous, caulescent phenovariants, implying involvement of auxin. I decided to retest for exogenous auxin effects, but this time supplied in paste, on the possibility that a sustained targeted application to cotyledons might be required for this type of growth regulator.

For initial experiments, Aquaphore, a lanolized paste emulsion which becomes soft after incorporation of 10% water or aqueous solution, was used. Auxin (either indoleacetic acid or naphthaleneacetic acid) was dissolved with aid of sodium hydroxide in the 10% aqueous phase before blending with Aquaphore. Paste was applied by fine needle to coat the entire upper surface of one or both cotyledons, with the aid of a dissecting microscope, on the second day after germination. Results are

shown in Table 5.

Table 5. Effects of auxin pastes applied 2 days after germination on cotyledonary accrescence of *S. prolixus (gracilis)* after 10 days.

Paste Composition	One Cotyledon	Two Cotyledon
Aquaphore + water	Cotyledons mostly unequal*	Cotyledons mostly equal
Aquaphore + IAA 10^{-4}M	Same	Same
Aquaphore + NAA 10^{-5}M	Same	Same

*No correlation was observed between the cotyledon that was coated and the cotyledon that became accrescent.

Thus, coating both cotyledons, with or without auxin in the paste, tended to suppress cotyledonary accrescence, whereas coating only one cotyledon did not. The implication in these results was that the paste itself, by chemical or physical means, was acting in a morphoregulatory role.

In order to decide between these alternatives, a second experiment was then conducted with hormone free pastes of three differing chemical compositions applied to upper surfaces of both cotyledons 2 days after germination. Observations were made 25 days after treatment. All paste treatments resulted in much higher than expected frequency of plumule production. Results are shown in Table 6.

Table 6. Effects of topical application of hormone-free pastes to both cotyledons 2 days after germination of *S. prolixus (gracilis)*. After 25 days.

Paste	No. of Seedlings	Plumule Production		One Leaf Plumules		Two Leaf Plumules	
		No.	%	No.	%	No.	%
Aquaphore	96	58	60	53	55	5	5
Silicone Grease	55	41	75	29	53	12	22
Hydrocarbon Grease	81	43	53	37	46	6	7

The results given in Table 6 appeared to demonstrate that alteration of morphogenetic patterns with production of caulescent phenovariants was a consequence of the physical effect of coating the cotyledons, since the pastes were of differing chemical composition.

A confirmatory experiment was undertaken, this time including other pastes as well as water drench controls. Pastes were warmed to 35°C to decrease viscosity and improve spreading qualities. Other experimental conditions remained the same. Results are given in Table 7.

Table 7.

Paste	No. of Seedlings	Plants with Plumules No.	%	One Leaf Plumules No.	%	Two Leaf Plumules No.	%
Controls (Water)	66	15	22	10	15	5	7
Aquaphore	65	27	42	22	34	5	8
Lanolin	22	12	55	11	50	1	5
Petroleum Jelly	40	18	45	14	35	4	10
Silicone Grease	40	16	40	12	30	44	10

It is apparent that any of the several paste types, when coated on both cotyledonary upper surface, greatly increased plumule formation as compared with controls. The higher percentages with plumules in Table 6 might have resulted from a thicker coating layer formed by unwarmed paste. The ratio of paired to single leaves in the plumules was highly variable, but there were always more than twice as many plumules with one leaf than with two.

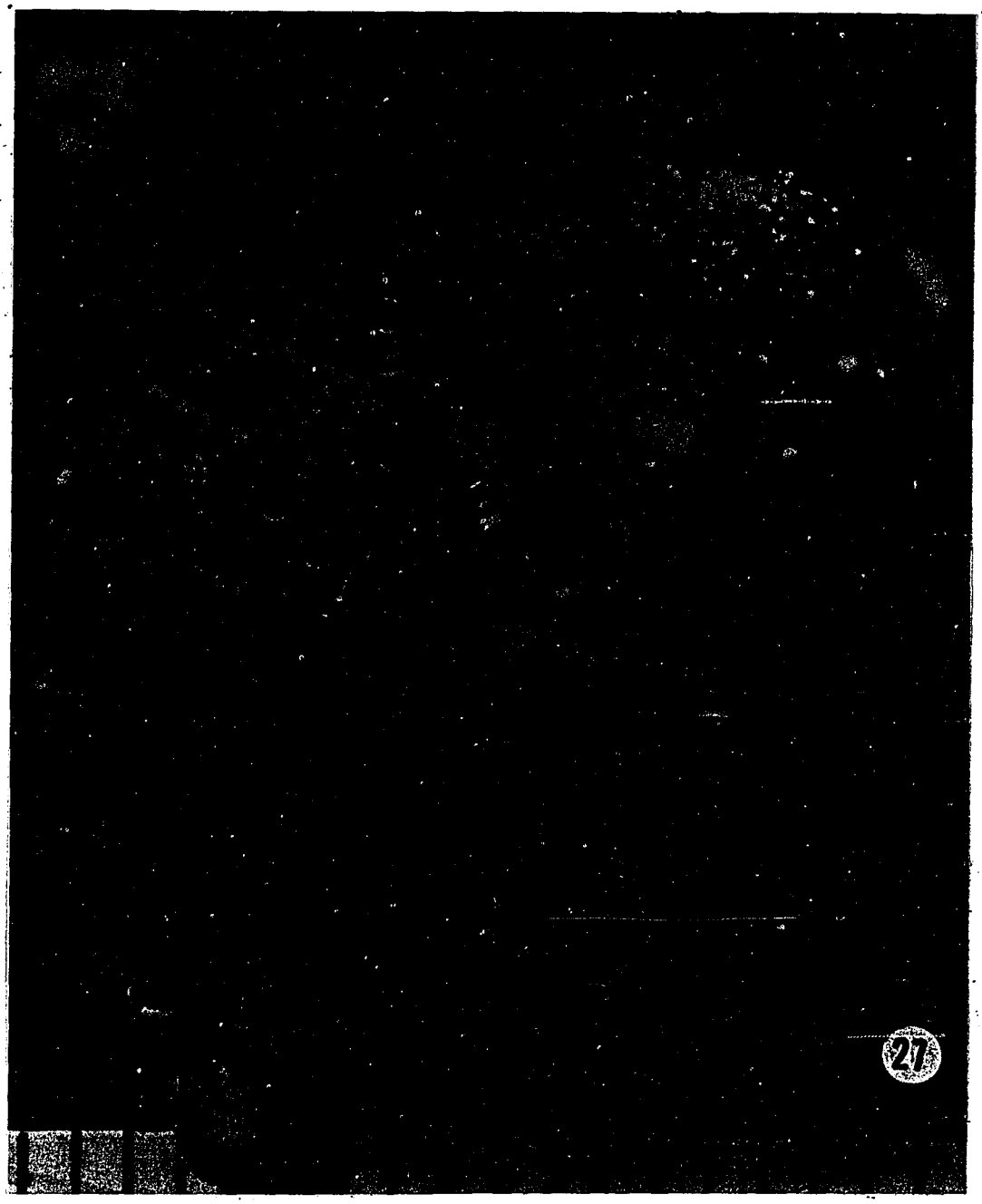
It is reasonable from these experiments to assume that the phenovariants with apical meristematic activity resulted from mechanical effects attributable to blocking of gas exchange through the upper cotyledonary surfaces. One possible explanation would be accumulation of the phytohormone ethylene within seedling tissues, resulting in an "anti-auxin effect" similar to that produced by TIBA.

The appearance of plumules in 22% of water drench controls confirmed what had already been observed in other experiments--that isocotylous, caulescent phenovariants will occur in 10% or more germinating seedlings

Figs. 26-27. Pot grown seedlings of *S. prolixus (gracilis)*

26. Top. Aquaphore paste was applied to upper surfaces of cotyledons on days 2 and 17 after germination. Isocotylous with plumule consisting of leaf pair. 26 days. x28

27. Bottom. Untreated spontaneous phenovariant. Isocotylous; plumule consisting of one leaf is accrescent; elongate shape due to overcrowding in pot. 4 months. Scale unit is 1 mm. x8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under normal conditions.

This rather high percentage of isocotyly easily escapes notice in the minute *Streptocarpus* seedling, since the first leaf or one of a pair of the plumule develops into a phyllomorph in the same manner as an accrescent cotyledon. In the process, the cotyledons are hidden and the seedling appears "normal". Foliar (not cotyledonary) accrescence was also greatly increased in single GA_3-drench treatment of germinating seeds.

Cytokinin Experiments

Pilot experiments to test the possible influence of various cytokinins on regeneration from lamina discs utilized kinetin, 6(γ,γ-dimethylallylamino) purine and 6-benzylamino purine (benzyladenine or BA). Of these, BA proved most effective and was, therefore, used in pot-culture experiments on developing seedlings.

Freshly harvested seeds of *S. prolixus (gracilis)* were sown in pots of Jiffy mix as before, then drenched 8 days later with aqueous solutions of benzyladenine at various concentrations. Seedlings treated at concentrations of 10^{-6}M and lower developed in the same way as controls.

At 10^{-5}M, however, BA caused unexpected morphogenetic alterations. All seedlings observed at 4 weeks had two equal accrescent cotyledons, each the size of the macrocotyledon in control plants. This phenomenon of desuppression of the microcotyledon was found to be irreversible after one treatment if given at the time of germination.

BA drenches at 3 x 10^{-5}M and 6 x 10^{-5}M also produced the same type of morphogenetic changes, but were increasingly inhibitory to cotyledonary growth.

Histological examination of BA treated plants revealed that the embryonic meristem in these remained central in location but was quiescent. Each cotyledon contained a complex of two intercalary meristems, comprising a petiolode meristem and a basal meristem at the base of each lamina lobe.

In subsequent experiments, comparable results were obtained with *S. grandis*, *S. solenanthus*, and *S. erubescens*, all unifoliates.

In every instance, the apical meristem remained inactive during vegetative growth. A few plants of each species were repotted and transferred to the greenhouse after 2-3 months. These flowered at the appropriate time, as twin 'phyllomorphic' plants, with separate series of inflorescences arising from the base of each phyllomorph. These are not phyllomorphs in the full sense, since groove meristems are lacking.

Treatment with BA resulted in a few instances in fusion of the twin phyllomorphs at their bases after 3-5 months.

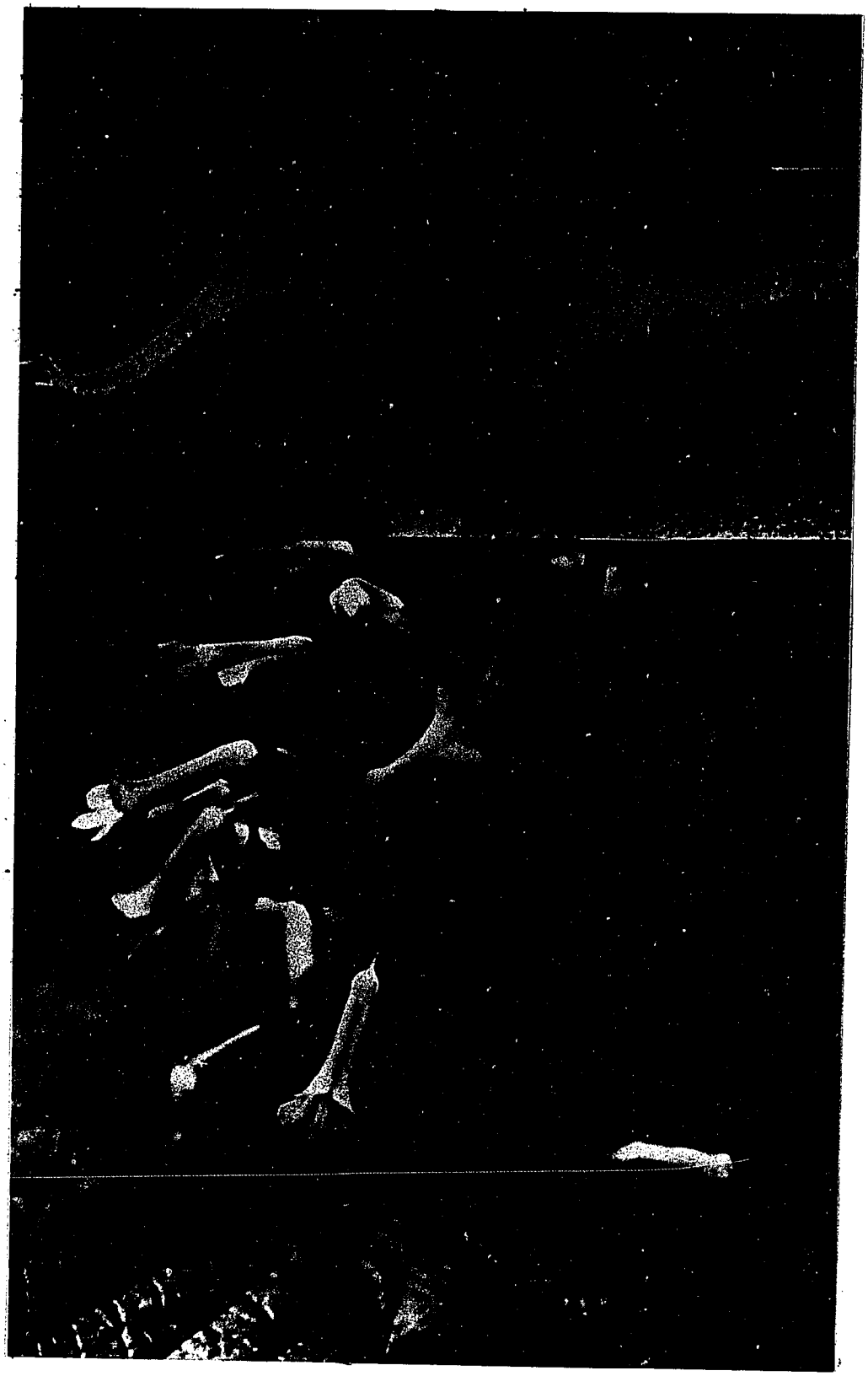
Effects of Anti-Gibberellins on Species in Subgenus *Streptocarpella*

Since it had been found that increased gibberellin induced caulescence phenovariants in subgenus *Streptocarpus*, the possibility was considered that in *Streptocarpella* endogenous levels of gibberellin were relatively high, thus accounting for earlier activity of the apical meristem and subsequent caulescence. Several experiments were undertaken

Figs. 28-29. Pot grown seedlings treated with drench of benzyladenine at time of germination.

28. top. *S. prolixis (gracilis)* treated with BA at 3×10^{-6}M. 7½ weeks. ×5

29. bottom. *S. grandis*. Bi-phyllomorphic plant in bloom at 15 months.



Figs. 30-31. Adjacent median longitudinal sections of pot grown seedling of *S. prolixus (gracilis)* treated with single drench of BA at 3×10^{-5}M. 41 days. x670

30. top - central apical meristem, dormant

31. bottom - petiolode and basal meristems

Sections are 10μthick.

am - apical meristem
bm - basal meristem
pm - petiolode meristem

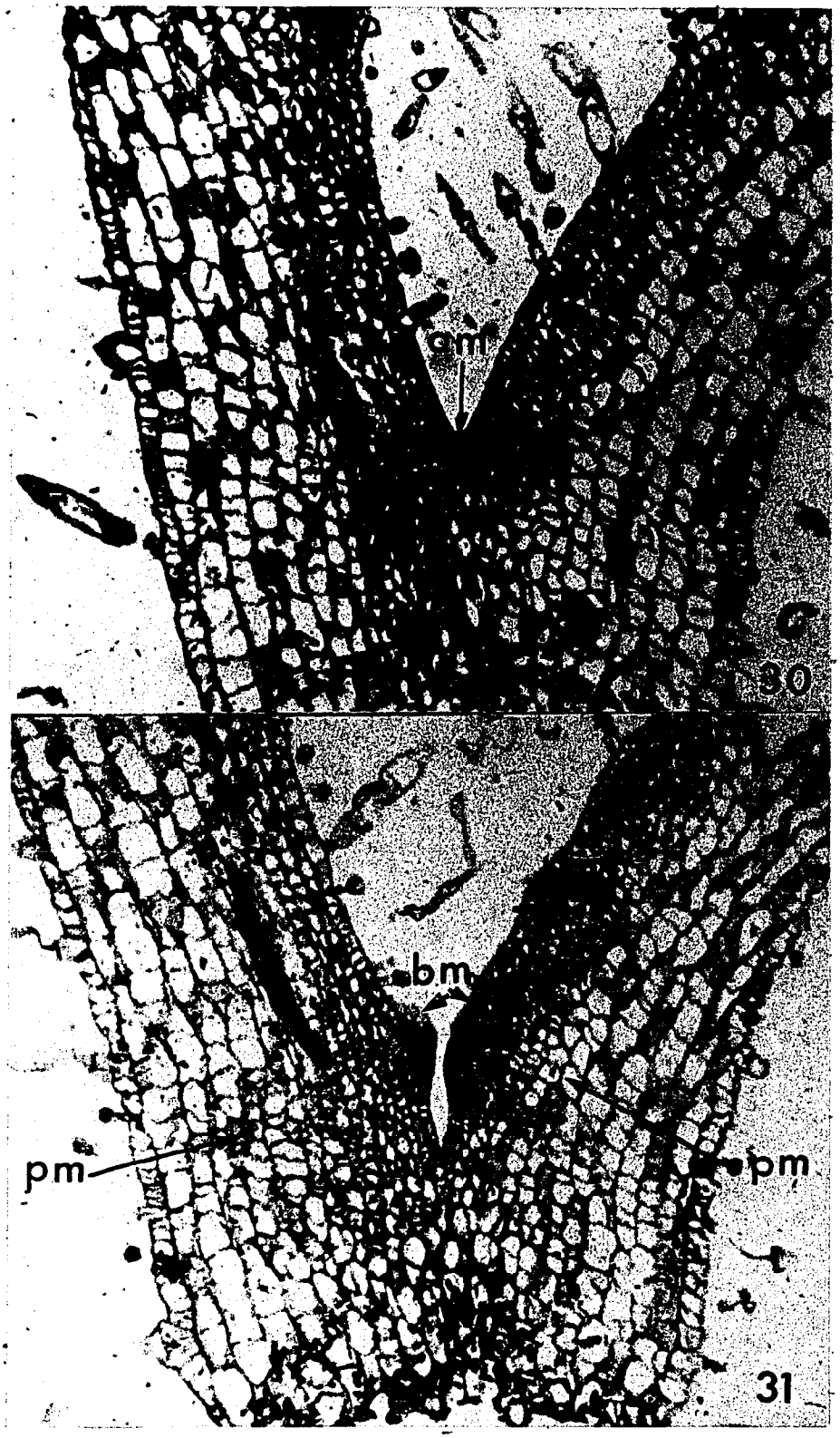

bm
30
bm
pm
pm
31

Fig. 32. Effects of single drench benzyladenine and gibberellin vs. controls on pot grown seedlings of *S. prolixus (gracilis)*. 31 days. x 3.5

left - controls

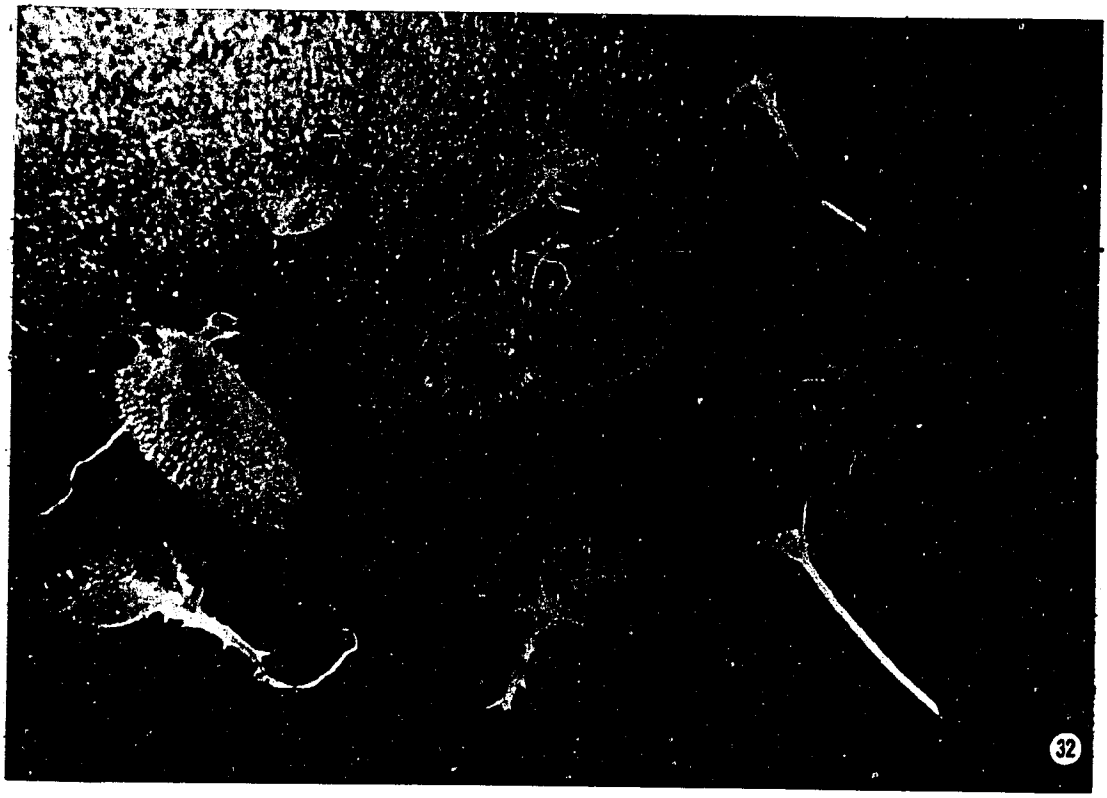
center - BA 3×10^{-5}M

right - GA_3 10^{-4}M

top row - *S. haygarthii*

middle row - *S. grandis*

bottom row - *S. prolixus (gracilis)*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to test this hypothesis. Exogenous anti-gibberellins were applied to germinating seeds of species in subgenus *Streptocarpella* in the expectation that the apical meristem might be suppressed. Many antagonists of gibberellin are known, ranging from abscisic acid to commercially utilized plant dwarfing agents (Audus, 1972; Green and Corcoran, 1975).

Abscisic acid was tested *in vitro* by adding it to Modified Tobacco Medium (Appendix II, Addendum) at levels ranging from 10^{-12}M to 10^{-6}M. Sterilized seeds of *S. nobilis* were then inoculated. Germination occurred at all levels. Five weeks after germination no effects caused by ABA at levels up to 10^{-10}M could be seen, compared with controls. At higher levels, however, phytotoxicity had occurred and most seedlings were dead or dying.

All other anti-gibberellins were tested in pot cultures. Seeds of *S. nobilis* were sown in pots of sand and soil drenched with Hoagland solution (Appendix VI) in the greenhouse. Once weekly Hoagland solution was poured over the pots; on the following day, anti-gibberellins were applied dropwise to seedlings after germination.

The dwarfing agents tested were tannic acid, Cycocel (2-chloroethyl trimethyl ammonium chloride, maleic hydrazide, and Amo-1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine carboxylate methyl chloride) at concentrations of 10^{-7}M, 10^{-5}M and 10^{-3}M. The experiment continued for 4 months after germination, with the following results:

Tannic acid treatments produced little or no dwarfing and no delay in plumule appearance at any level.

Cycocel at 10^{-3} resulted in reduction of height of seedlings from 125 to 75 mm compared with controls; plumules were present on all plants after 9 weeks.

Maleic hydrazide treatments resulted in considerable dwarfing at 10^{-3}M, height being reduced by 90% at 9 weeks, but plants were chlorotic and died one month later. At 10^{-5}M, variable dwarfing was seen at 9 weeks, but all seedlings had shoots.

Amo 1618 was apparently non-toxic at any level and was an effective dwarfing agent. After 4 months, seedlings treated at 10^{-3}M were about 10 mm tall (compared with 200 mm for controls) and only 1 of 9 had a plumule. Since this compound was the only one tested that showed signs of being capable of inducing acaulescence, this experiment was then repeated, but with Amo 1618 at five concentrations from 10^{-5}M to 10^{-3}M. Seedings of *S. nobilis* were grown in pots of vermiculite and fine gravel (growth was faster and more uniform than in the previous substrate). All seedlings had plumules after 4 months of treatment. At the highest concentration, 10^{-3}M, Amo 1618 had reduced plant height by 90%, but macrocotyledon length was reduced by only 25%. Plumules were present on all seedlings but were delayed several weeks compared with controls.

Similar results were obtained with Amo 1618 treatments of seedlings of *S. muscosus*, a small, slow growing species in subgenus *Streptocarpella*.

It is evident that the five anti-gibberellins failed to suppress apical meristematic activity in subgenus *Streptocarpella*. Even at concentrations highly effective in dwarfing seedlings, plumules appeared with relatively short delay.

Although experiments with anti-gibberellins did not result in causing caulescent species to manifest acaulescent morphogenetic patterns, they do not completely exclude the possibility that alternative developmental pathways leading to either acaulescence or caulescence may be de-

pendent to an important degree on critical levels of gibberellins at a critical stage of seedling ontogeny.

DISCUSSION

During the course of this study it was discovered that isocotylous and caulescent phenovariants could be produced at will by employment of growth regulators. The base has now been established for beginning to understand evolution of the morphogenetic processes responsible for unconventional morphology in subgenus *Streptocarpus* in terms of chemical regulation.

Alterations in Morphogenetic Processes by Growth Regulators

In typical dicotyledonous seedlings, such as species in subfamily Gesnerioideae (Gesneriaceae), a syndrome of processes occuring after germination can be stated as follows:

Equal but limited expansion of cotyledons, followed by early activity of the apical meristem which provides a plumule developing into stem, leaves and buds or branches.

In subgenus *Streptocarpus*, on the other hand, the apical meristem is dormant and becomes displaced laterally to the base of the accrescent cotyledon; a complex of active meristems at the base of the macrocotyledon causes continued growth of the latter.

Exogenous gibberellin transformed this syndrome to that of typical dicotyledons: accrescence of the macrocotyledon was suppressed and apical meristematic activity quickly produced a plumule.

This transformation in seedlings of subgenus *Streptocarpus* was achieved by other means as well:

1) by exogenous triiodobenzoic acid.

2) by coating upper cotyledonary surfaces with a variety of pastes.

In addition, it was observed in 10-22% of untreated seedings of *S. prolixus (gracilis)*.

In all these instances, in the absence of sustained treatments the syndrome of macrocotyledonary accrescence and apical meristematic dormancy was transferred from cotyledonary node to the first stem node. Sustained gibberellin treatment, however, led to continuation of the 'normal' dicot syndrome.

A different modification of the usual morphogenetic syndrome found in subgenus *Streptocarpus* resulted from treatment with exogenus cytokinin, viz., accrescence (desuppression) of the microcotyledon together with the expected dormancy of the apical meristem. These seedlings were acaulescent but bi-phyllomorphic.

Some obvious correlations can be seen:

A - persistent intercalary meristematic complex at macrocotyledonary base coincident with apical meristematic dormancy.

B - the inverse: absence of basal intercalary meristematic complex coincident with early apical meristematic activity.

Correlation B is found in subfamily Gesnerioideae.

Correlation A is found in subfamily Cyrtandioideae and may be unique to this taxon.

From the results obtained in this research, which was limited in scope, there is not yet sufficient evidence to explain fully morphogenesis either of normal plants of subgenus *Streptocarpus* or of the phenovariants induced by exogenously applied regulators.

It has long been known that phytohormones do not act independently; plant developmental processes are regulated by balances among several hormones and by their interactions (Audus, 1972). Disruption of these balances at a crucial stage of growth, in this instance at time of and shortly after germination, would have resulted from high level exogenous applications of one or more known plant growth regulators.

A tentative hypothesis to explain morphogenetic processes during early seedling development in subgenus *Streptocarpus* in terms of chemical regulation can now be offered, based on the results of my experiments.

The following statements of observed phenomena, relevant to such a hypothesis, are presented:

1) Seeds of species in Cyrtandroideae are minute, lack food reserves and contain no endosperm (Burtt, 1970, 1977).

2) In *Streptocarpus*, at time of germination, the radicle primordium is present as a slight protuberance; it increases in size slowly during the first 2-3 days. Development of the primary root is delayed, reaching only 1 mm after a few days (Jong, 1970, and my own observations).

3) Xylem differentiation is unequal in the cotyledons; the accrescent cotyledon is the one in which continuity of the vascular strand to the radicle is first accomplished (Jong, 1970).

Roots are a major source of cytokinins which are translocated in the xylem sap (ibid.). Cytokinins demonstrate a striking ability to stimulate cotyledon enlargement (Leopold and Kriedemann, 1975). It appears likely therefore, that cytokinins are in limited supply in *Streptocarpus* seedlings during initial development because of small seed size, absence of endosperm and delayed root development. Earlier vascular strand com-

pletion in one cotyledon would permit translocation to it of cytokinins as synthesis occurs in the primary root. Cotyledonary accrescence can then begin, including stimulation of meristematic activity in the basal region, where the growth occurs. Auxin synthesis is known to be stimulated in enlarging tissues and meristems (Audus, 1972). Under these circumstances, the cotyledonary meristems are capable of inhibiting the seedling apical meristem (lateral dominance), and also of sustaining the inhibition of growth of the microcotyledon.

Inhibitory effects of lateral organs on apical meristems are known in other angiosperms. Nozeran, Bancilhon and Neville (1971) cite several examples in which lateral organs such as young leaves of species of *Dipsacus* and *Gleditsia triacanthos*, and axillary buds *(Phyllanthus distichus)* suppress or limit meristematic activity of the main shoot axis. In these examples, regular removal of the lateral organs caused greatly increased activity of the apical meristem as measured by rate of formation of new leaves.

Evidence for this hypothesis is found in caulescent phenovariants that were induced by TIBA, a known antagonist of auxin transport (Audus, 1972). Similar effects followed paste coating of the cotyledons, which could have served to cause an increased concentration of ethylene in the cotyledons. Ethylene similarly has been shown to immobilize auxin (ibid). Both treatments led to early apical meristematic activity with suppression of the macrocotyledon (apical dominance).

The ability of exogenous cytokinin applied at time of germination to stimulate microcotyledon growth is consistent with the hypothesis presented above, i.e., paucity of cytokinins in the newly germinated seed-

ling. Once basal growth of the microcotyledon has been stimulated, an additional auxin source is present, reinforcing inhibition of the apical meristem.

Early plumule formation following exogenous application of gibberellin at time of germination may be due to the latter's ability to induce subapical mitotic activity (Sachs, 1961), thus 'inciting' stem formation. Gibberellin application is known to increase auxin levels (Kuraishi and Muir, 1964), in turn leading to inhibition of the basal meristems of the cotyledons, i.e., apical dominance.

In addition to providing some indication as to the growth regulators mediating the morphogenetic shifts to accrescence and acaulescence, the foregoing serve to focus attention on the roles of the meristems which they may be influencing. While the hypothesis presented may be too simple or erroneous, at least in part, it is readily testable.

The Cotyledonary Basal Meristematic Complex

Sustained growth of the accrescent cotyledon (which becomes the cotyledonary phyllomorph) is the product of the activity of two meristems. One of these, the petiolode meristem, composed of vacuolated cells dividing anticlinally, produces files of cells leading to elongation of the midvein and petiolode. It is equivalent to the rib meristem found in leaves. The second, the basal meristem, consists of plastid containing cells whose divisions produce the phyllomorph lamina, and is equivalent to the plate meristem of leaves. In morphogenetic terms, the only distinction between petiolode and rib meristems, and between basal

and plate meristems is the length of time during which the respective intercalary meristems are active.

In leaves of angiosperms, which are determinate in growth, intercalary meristematic activity in the plate meristem is somewhat prolonged but is not sustained. The accrescent cotyledon of non-phyllomorphic species in Cyrtandroideae is the product of intercalary meristematic activity which is still determinate but more sustained in one of the paired cotyledons. In *S. nobilis, Chirita micromusa* and some others, prolonged activity of these meristems produces accrescent cotyledons as large as or larger than subsequent leaves. Under normal environmental conditions, the displaced dormant apical meristem, after a short delay, ceases dormancy and generates a plumule which becomes the plant stem. Under unfavorable conditions, however, the dormant apical meristem after long delay generates inflorescences. *C. micromusa* seedlings, for example, grown under short day conditions, flowered at the base of the macrocotyledon. Some seedlings of the same sowing had stems with one pair of leaves at time of flowering.

The existence of the latter condition, intermediate between acaulescence and caulescence, demonstrates a continuity of growth forms in seedlings of Cyrtandroideae dependent on the reciprocal relationship between activity of the cotyledonary basal meristematic complex and activity of the displaced apical meristem.

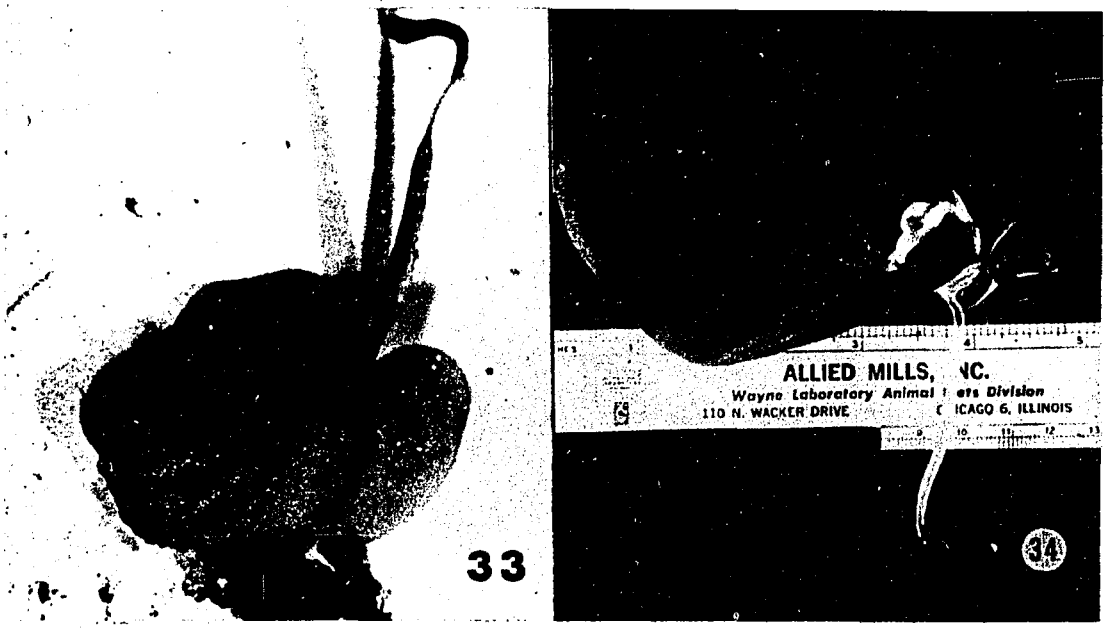
At one extreme, cotyledonary accrescence is short lived and the plumule appears after delay of several days to a few weeks. This is the situation in most genera and species of Cyrtandroideae. At the other extreme, cotyledonary accrescence continues intermittently for several years at the end of which the displaced apical meristem (=groove meris-

Figs. 33-34. *Chirita micromusa* seedlings in flower. Greenhouse grown during autumn. 4 months.

33. left. Acaulescent plant, 6 months, single fruit on microcotyledon. x3

34. right. 4 month seedling has 1 pair of leaves, flower on macrocotyledon. x1.5

Both were sown at the same time. Short days induce early flowering, with or without a stem.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tem) generates a series of inflorescences. This is the situation in large unifoliate species in subgenus *Streptocarpus*.

Between the extremes, many forms are possible, and indeed are found in nature. The distinctions between non-phyllomorph and phyllomorph, caulescence and acaulescence, are not necessarily clear cut, as shown by the short-day grown seedlings of *Chirita micromusa*.

Control Mechanisms in Subgenus *Streptocarpus*

From observations made by various observers, including Jong, Burtt, Schenk, and Hill, as well as myself, it is evident that the following mechanisms to regulate growth exist:

1. The macrocotyledon suppresses both the seedling apical meristem and the microcotyledon.

2. A complex of macrocotyledonary basal meristems produces phyllomorphic enlargement over a long period. When this growth ceases, suppression of the apical (groove) meristem ceases and the latter generates, via detached meristems, inflorescences and sometimes additional phyllomorphs. In many species, the groove meristem splits before phyllomorphic growth ceases, with the detached portion capable of generating an axis (in rosulates) or an extra phyllomorph (e.g., in *S. fanniniae*).

The capability of the accrescent cotyledon to suppress cell division elsewhere in the seedling may be due to one or more diffusible substances produced in it, as suggested by the following evidence: decapitation of the macrocotyledon (Hill, 1938) or exogenous cytokinin, as in this study, overcomes the suppression of the microcotyledon. On the other hand, exogenous gibberellin or 2,3,5-triiodobenzoic acid sup-

presses both cotyledons and simultaneously desuppresses the apical meristem.

An exquisite morphoregulatory system, different from those found in other dicots, has evolved in subgenus *Streptocarpus*.

Phylogenetic Implications

In Cyrtandroideae, a morphoregulatory pattern, probably unique in dicots, has evolved, in which one cotyledon is capable of continued growth at its base while simultaneously the seedling apical meristem remains dormant. In most genera and species of the subfamily, this reciprocal relationship is terminated relatively early, with cessation of basal growth in the macrocotyledon and initiation of apical growth followed by appearance of a stem.

In subgenus *Streptocarpus* cotyledonary basal meristems continue to produce lamina and petiolode tissue for long periods (many months or years) while the laterally displaced apical meristem remains dormant. When the cotyledon (phyllomorph) ceases growth, the desuppressed apical meristem generates inflorescences and/or phyllomorphs. Rarely, as in *S. schliebenii*, stems may be generated.

In this regard, it is important to note that mature leaf morphology in dicotyledonous angiosperms results from differential rates and durations of cell division and expansion, with basipetal maturation (Maksymowych, 1973). Thus, cell division and expansion are normally last to cease in the basal area of a leaf.

Growth of a phyllomorph represents an extreme variant in that cell division and expansion do not cease but continue indeterminately at the

base of the blade. In this respect phyllomorph development resembles that of some monocots, such as grasses, which exhibit prolonged basal intercalary growth. Its essential distinction from a leaf, then, is in the presence of an organogenic (groove) meristem at the base of the blade, derived from the embryonic shoot apical meristem.

Subgenus *Streptocarpus* has probably evolved through loss of a regulatory system present in an ancestral taxon within subfamily Cyrtandroideae which terminates macrocotyledonary growth early enough to permit formation of stem and leaves by the desuppressed apical meristem.

Another way of viewing these relationships is that in subgenus *Streptocarpus* apical dominance (suppression of axillary meristems) has been stood on its head: the cotyledonary basal meristems suppress the apex of the plant--lateral dominance.

Growth correlations in the nature of reciprocal influences of separate meristems are understandable in light of the knowledge that active meristems, wherever located, are principal sources of auxin (Audus, 1972), which plays a major role in "dominance" (ibid.). Early activity of apical meristems coupled with the predominantly polar transport of auxin favors apical dominance. Altered factors which modulate relative production and/or transport could result in a shift in relative dominance. Presumably, such alterations have occurred more than once in Cyrtandroideae, including *Streptocarpus*.

The general morphological distinction between (caulescent) subgenus *Streptocarpella* and (acaulescent) subgenus *Streptocarpus* can be viewed as a shift in dominance in a double sense: ontogenetically in the former and phylogenetically in the latter. In addition, speciation within sub-

genus *Streptocarpus* leading to secondary caulescence has apparently occurred.

Jong (1978) found that in rosulate species the groove meristem of the cotyledonary phyllomorph splits; the larger portion then quickly organizes a dome which functions as an apical meristem. The latter produces a rosette which consists of an axis generating phyllomorphs instead of leaves at nodes; the internodes are very short. This might be considered a case of quasi-caulescence.

A more complete return to caulescence seems to have occurred in *S. schliebenii*. Preliminary observations by me on development in this species indicate that a cotyledonary phyllomorph and a lateral axis as in those rosulates termed "excentric" by Jong (1978) are formed early. Several months later, a vertical stem with pairs of foliar organs is generated. The latter have the same gross aspect as the cotyledonary phyllomorph but behave as leaves, i.e., they are determinate and possess axillary buds which become branches or inflorescences. This species, and perhaps others not yet available for study, apparently demonstrate secondarily evolved caulescence.

Phyllomorphs have also evolved in other genera in a different tribe in Cyrtandroideae (*Monophyllaea* and *Epithema* in Klugieae). It is almost certain that these have evolved independently of *Streptocarpus* phyllomorphs because the two tribes, Didymocarpeae and Klugieae, are not closely related (Burtt, 1977). A possible parallel to secondary evolution of caulescence in subgenus *Streptocarpus* is found in *Monophyllaea* (Burtt, 1978) in *M. caulescens* and *M. ramosa*. Both species possess a cotyledonary phyllomorph and a straggling stem containing several

Fig. 35. *S. schliebenii*, a species in subgenus *Streptocarpus*. For many months it is acaulescent, resembling some rosulate species. It then becomes caulescent with leaves in pairs; buds in leaf axils become branches or inflorescences. Photographed before flowering for the second time.

blades bearing inflorescences.

Phylogenetic Relationship Between the Subgenera of *Streptocarpus*

Success in inducing caulescent phenovariants in subgenus *Streptocarpus* in this study does not bear directly on the question of phylogeny within the genus. Such phenovariants reinforce what was already assumed, namely, caulescent ancestry of subgenus *Streptocarpus*, but shed no new light on the identity of a particular caulescent ancestral taxon.

Further, inability to induce acaulescent phenovariants in subgenus *Streptocarpella* by an assortment of exogenous anti-gibberellins does not give unequivocal evidence regarding the phylogenetic relationship between the subgenera for the following reasons:

1. Exogenous gibberellin was not the only means by which caulescent phenovariants in subgenus *Streptocarpus* were induced. The underlying controls on morphogenesis in this taxon are still not known.

2. Facultative acaulescent phenovariants in subgenus *Streptocarpella* are already known in nature (in *S. nobilis*).

The morphogenetic evidence by itself does not yet permit choice among the three hypotheses previously stated, namely, that either subgenus is ancestral to the other or that they are not congeneric. Nevertheless, the results are pertinent to understanding some of the morphogenetic bases of phylogeny within the genus.

CONCLUSION

Two morphogenetic patterns have contributed to phylogenetic diversification (speciation) within the Gesneriaceae. The first, accrescence of one of the pair of cotyledons as a result of continued activity of basal intercalary meristems (anisocotyly), serves to differentiate subfamily Cyrtandroideae.

The second pattern, sustained growth of the accrescent cotyledon accompanied by dormancy of the displaced apical meristem, gives rise to an acaulescent, dorsiventral vegetative plant body (phyllomorph) which serves further to differentiate species of Cyrtandroideae in two tribes and several genera, including *Streptocarpus*.

Earlier descriptive studies (Jong, 1970; Jong and Burtt, 1975; Jong, 1978), analyzing the positions and relative contributions of apical and intercalary meristems in anisocotyly and acaulescence provide one level of explanation for the changes in form which correlate with phylogeny. They established a foundation as well as a point of departure for the studies reported here.

With these experimental results it begins to be possible to explain evolutionary diversification within this group at still another level, that of chemical regulation. As was suggested by Stebbins (1974c), the keys to this understanding were found in the effects of plant growth regulators on meristems, especially intercalary meristems.

The ability to induce phenovariants by exogenous growth regulators in these studies indicates that endogenous growth regulators control the unusual morphogenetic patterns found in subgenus *Streptocarpus* and in other genera and species in subfamily Cyrtandroideae of Gesneriaceae.

This knowledge opens the way to determine experimentally the existing balances among phytohormones and the roles of individual phytohormones which are responsible for these patterns. It also may lead to isolation of substances and processes at the molecular level which are mediated by the regulators.

APPENDIX I

Potting Mixtures Employed for Sowing Seeds and Growing *Streptocarpus* Plants in the Greenhouse

Seeds were sown in Jiffy mix, which contains sphagnum derived peat moss with an equal quantity of horticultural grade vermiculite and fertilizers (from Jiffy Products of America, West Chicago, IL 60185). Seedlings were pricked out at 2-4 months, then grown in the following mix:

1 part topsoil or compost or leaf mold

1 part sphagnum derived peat moss

1/2 part Silversand, a coarse silica with pH 8.9

1/2 part medium size Terragreen, a partially fired clay

0.4 oz/bu dried blood

2 oz/bu steamed bone meal

0.6 oz Greensand (for slow potassium release)

4 oz/bu powdered limestone

The mix was pasteurized by heating.

APPENDIX II

Composition of Basal Agar Medium Used for Starting Seedlings or Growing Axenic Stock Plants. Minerals are from Grunewaldt (1977) and represent a modification of Murashige-Skoog Revised Tobacco Medium (1962) with reduction of ammonium ion to 0.2 millimoles.

Vitamin composition is from Staba (1969).

Minerals	Conc. Mg/l	Vitamins	Conc. mg/l
NH_4NO_3	160	Cyanocabalamin	0.0015
KNO_3	1900	Folic Acid	0.5
$CaCl_2$	297	p-aminobenzoic acid	0.5
$MgSO_4 .7H_2O$	370	Riboflavin	0.5
KH_2PO_4	170	Biotin	1.0
$CoCl_2.6H_2O$	0.025	Choline chloride	1.0
$FeSO_4.7H_2O$	27.8	Calcium Pantothenate	1.0
H_3BO_3	6.2	Thiamine HCl	2.0
KI	0.83	Niacin	2.0
$MnSO_4.4H2O$	22.3	Pyridoxine HCl	2.0
$Na_2MoO_4.2H_2O$	0.25		
$Na_2EDTA.2H_2O$	37.3	Agar	8,000
$ZnSO_4.7H_2O$	8.6		
$CuSO_4.5H_2O$	0.025		

Organics	
Sucrose	30,000
Inositol	100

The basal medium was modified for plantlet regeneration from lamina discs or inflorescence nodes by addition of an auxin and a cytokinin before autoclaving. The additives used were:

indoleacetic acid	1.1×10^{-6}M
benzyladenine	5×10^{-6}M

Addendum

The Modified Tobacco Medium utilizes macronutrients at one half the concentrations given in the Murashige-Skoog Revised Tobacco Medium and are listed below:

Macronutrients	Conc,mg/
NH_4NO_3	825
KNO_3	950
$CaCl_2.2H_2O$	220
$MgSO_4.7H_2O$	185
KH_2PO_4	85

Micronutrients and organics remain as listed above.

APPENDIX III

Protocol for Sterilizing Lamina and Inflorescences

(Adapted from Grunewaldt, 1977)

In this technique, a solution was prepared containing 0.1% $HgCl_2$ and 0.1% Alconox, a laboratory detergent. Into a large glass petri dish, 100mm diameter x 85 mm high, was placed 300 ml of the sterilant solution and a magnetic stir bar. Tissue previously gently washed into soapsuds and then rinsed with deionized water was added, the dish covered and the contents stirred for 5 minutes. The dish was then placed in a transfer hood whose surfaces had been thoroughly sprayed with 2% Lysol (a mixture of cresols) along with 4 large covered petri dishes of the same size containing autoclaved demineralized water. The tissue was rinsed in 4 changes of sterile water, then discs were cut and inoculated aseptically. This method was employed for lamina discs and inflorescences of several species of *Streptocarpus* (for peduncle segments, ends were sealed in paraffin prior to sterilization).

APPENDIX IV

Protocol for Dehydrating, Embedding and Sectioning of Seedlings after Glutaraldehyde Fixation

Prepare acified 2,2' dimethoxypropane (DMP) by adding 3 drops 0.1 N HCl per 25 ml.

1) Decant water from fixed, washed seedlings at room temperature, replacing immediately with acidified DMP. Allow 5 minutes for dehydration.

2) Replace DMP twice, 5 minutes each.

3) Replace with 1:1 acetone: DMP, 5 minutes.

4) Replace twice with acetone, 5 minutes each.

5) Replace with 1:1 acetone: tertiary butyl alcohol (TBA), 15 minutes.

6) Replace 3 times with TBA, 15 minutes each (maintain at 20°C or higher). Add weakly colored safranin in TBA for last change, to enhance visibility of seedlings.

7) Melt paraffin in vial, cool to barely solid. Add seedlings in TBA to vial of paraffin (volume at least twice the volume of the TBA).

8) Place in oven to infiltrate. Allow 24 hours or more for TBA to evaporate.

9) Replace with melted paraffin twice. (Use fresh paraffin only in last change.)

10) Embed.

11) Section with rotary microtome at 8 to 15 microns. Mount on glass slide with Haupt's adhesive and formalin.

APPENDIX V

Protocol for Staining and Mounting Histological Slides

For staining, prepare stock aqueous solution of Toluidine Blue 0.5%. Keep tightly closed.

1) Using fresh 0.05% aqueous Toluidine Blue made from the stock solution, stain glass slides 5 minutes.

2) Rinse briefly in 3 changes of water to remove excess stain.

3) Remove excess water by blotting gently with bibulous paper. Dry on a slide warmer at least 24 hours.

4) Immerse in xylene 5 minutes to dissolve paraffin.

5) Mount coverslip with Permount or other resin. Dry on slide warmer.

APPENDIX VI

Hoagland Nutrient Solution

Macronutrients	g/l
$Ca(NO_3)_2.4H_2O$	0.94
$MgSO_4.7H_2O$	0.52
KNO_3	0.66
$NH_4H_2PO_4$	0.12

Micronutrients (stock solution, 100X)	g/l
H_3BO_3	0.28
$MnSO_4.H_2O$	0.34
$CuSO_4.5H_2O$	0.010
$2nSO_4.7H_2O$	0.022
$(NH_4)_6MO_7O_{24}.4H_2O$	0.010
H_2SO_4(conc.)	0.05 ml

Add 10 ml of micronutrients to 1 liter of macronutrients and adjust pH to 6.7.

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