PLANT PROPAGATION BY MEANS OF ASEPTIC TECHNIQUES

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This discussion is to point out some of the commercial applications of aseptic methods of plant propagation. Many of you will have heard or read of the astounding rates of reproduction claimed for plants propagated using this technique, but it would be unrealistic of you not to express some scepticism of these theoretical figures of attainment. I aim to show that some of these high rates of vegetative reproduction are possible under commercial conditions. A whole new technology is arising from the application of these techniques but it is a long way from raising a dozen lilies or saintpaulias in test tubes to the successful commercial production of 5,000 or 100,000 plants of one clone in an aseptic environment.

According to the Oxford dictionary, the definition of the term "aseptic" is "not liable to putrefy" which, I am taking to imply, indicates a lack of all micro-organisms — except viruses. The conditions and techniques that I am going to outline, can almost always be so defined. Any micro-organisms that do manage to penetrate the aseptic cultures are easily detected and quickly eliminated. Production of virus-free plants, which involves an elaborate screening technique, may not be required and the necessary premium for such work is not always acceptable. It is almost inevitable and desirable that, in time, plants produced from tissue culture will be virus screened. The term "guaranteed virus-free" is unscientific and liable to misleading interpretation.

The greatest immediate benefits of aseptic propagation techniques are in the rapid multiplication of new hybrids from a wide variety of genera, but especially those producing bulbs or corms. The increased profits obtained by upgrading, and bulking selected stock more rapidly, should amply repay initial investment in tissue culture facilities. Costly research is necessary to identify the correct techniques for the production of particular species. It is, therefore, unlikely that plants from tissue culture will ever be particularly cheap. The price must be reviewed in the light of long-term savings in capital, labour, growing space, and the competitive introduction of new hybrids.

Some indication of the importance that is placed on the returns from this system may be gained from the fact that patents for at least three aseptic propagation techniques are now in operation. These have yet to be challenged however.

The third major application of this technique is the multiplication of rare or difficult-to-propagate plants. Due, perhaps, to the

changing whims of public demand, there are occasions when it is necessary to rapidly resurrect forgotten clones of plants of which only a few specimens remain in botanic gardens or private collections. Other groups of plants, such as the orchids, are difficult or slow to propagate by conventional means and produce heterogeneous seedlings; a tissue culture system of propagation can revolutionise the production of plants of this type. Banks of genetically pure material can be built up and maintained, although great care will need to be taken to prevent aneuploid and polyploid lines of cells from becoming dominant.

Finally, a tissue culture propagation system provides ideal material for genetic manipulation through the use of mutagens such as X-rays or colchicine. Under the optimum environmental conditions normally provided in the laboratory, a high proportion of mutants can be raised to maturity and screened for potential. Haploid plants can be raised from pollen or ovule cultures to aid the geneticist in his breeding programme, whilst the isolation and hydridisation of plant protoplasts provides exciting prospects for the future.

PROVISION OF DISEASE-FREE STOCK

I have termed this asexual method of reproduction "aseptic plant propagation", deliberately avoiding the terms "meristem" or "meristemming". An equally satisfactory but less embracing term would be "plant tissue culture". We, and especially those of us in the orchid trade, should avoid the use of all reference to the term 'meristem culture' except in the context of virus-free plants. The meristem — that apical dome of rapidly dividing undifferentiated cells — found at the tips of shoots and roots, is very rarely used alone in the propagation of plants by this technique. Its excision is a skillfull operation that provides only a relatively low success rate. The great majority of plants propagated aseptically arise from 1-2mm shoot or root tips, portions of the leaf lamina, inflorescences or stem sections, either directly from axillary or adventitious buds, or indirectly from callus tissue.

Procedures for the screening and elimination of virus diseases are familiar ones. Three screening methods are in current use — employing specific indicator plants, an electron microscope, or anti-sera production from animals. Before treatment the identity of the disease present is first established. Following this, the plant may be treated to a period of high temperature to induce rapid growth and internode elongation before it is attempted to excise and successfully culture the apex of the shoot, preferably in the form of the meristem itself. A number of plantlets are raised from this tissue and screened for the presence of virus, using the techniques already mentioned. Providing the results prove negative,

progress can be made in the rapid multiplication of the cultured tissue to produce the required number of indexed plants.

Having obtained his pathogen-free stock, the onus is on the grower to maintain meticulously clean conditions to prevent re-infection. If a grower wishes to obtain the maximum advantages that tissue culture has to offer, he should be prepared to fully commit himself to the system.

PREPARATION OF INOCULUM; CULTURAL CONDITIONS

In order to obtain our aseptic culture, it is first necessary to surface sterilize the tissue from which the explant is to be taken by means of immersion in a sodium hypochlorite solution, followed by copious rinsing in sterile water. The tissue usually excised at this point is the shoot tip in, for example, the *Orchidacae, Iridaceae* and *Geraniaceae,* but also employed are inflorescenses in the *Amaryllidaceae, Orchidaceae* and *Iridaceae,* and leaves in the *Gesneriaceae* and *Liliaceae.* Having so obtained this pathogen-free tissue, the morphogenetic expression can now be manipulated by means of alterations in the medium, environment, or cutting procedures.

Three types of multiplication can be recognised:

1. Encouraging the continuous development of axillary buds, as in the *Bromeliacae, Geraniaceae* and *Asparagus.*
2. The production of adventitious buds on the surface of stems and leaves, as in the *Gesneriaceae* and *Lilium.*
3. The development of plants from organized callus, as in *Pelargonium, Lilium* and the *Bromeliacae.*

Plants from axillary buds arise from predetermined sites whilst those from callus or adventitious buds are more likely to contain cells of different ploidy level that may give rise to mutants. However, all these systems have been successfully tested commercially.

The nutrient medium contains a carefully balanced ratio of organic and inorganic substances at a controlled pH level, in either a liquid or solid form. Of the organic substances, the carbohydrates and vitamins are fairly standard whilst the growth regulators are added in any number of combinations and concentrations to produce the desired growth form. The auxins IAA, NAA and 2,4-D are commonly employed to influence the production of callus, roots and internode elongation whilst the cytokinins, kinetin and 6-BAP influence the initiation and proliferation of shoots.

The environment in which the developing tissue is placed may have a profound effect on organogenetic processes, many of which may be photomorphogenic. Light quality can have impor-

tant effects on shoot induction and bulb and corm initiation; the light intensity is lower than that required by plants developing autotrophically. Photo- and thermo-periods must also be considered and care taken to produce plants in the correct physiological state for continued development in the field. Optimum temperatures somewhat lower than those often published are needed for the development of certain genera, especially those that require a dormant period in their growth cycle and also some orchids, such as those of the *Odontoglossum* tribe.

COMMERCIAL SYSTEMS OF PROPAGATION

1. **Orchids** (shoot tip culture). The first commercial applications of aseptic plant propagation came in the early 1960's following the discovery by the late Professor Georges Morel that excised *Cymbidium* shoot apices cultured on a sterile nutrient medium, gave rise to protocorms identical to those produced by germinating seeds (7). When sectioned, portions of the protocorm that included the epidermal tissue rapidly gave rise to similar structures on their surface thus providing a technique whereby up to 10,000 five centimetre plantlets could be produced within a year, contrasting with the conventional technique that gave only two plants from an initial one. Over thirty orchidaceous genera have been propagated from vegetative or floral primordia in this fashion. In the majority of cases it is the swollen bases of the leaf primordia and not the meristem itself that gives rise to the proliferation of propagules.

The initiation of propagules from leaves has been carried further in the genus *Phalaenopsis* with the routine production of protocorms from isolated leaves, and in *Cattleya* and *Epidendrum*, where the leaf tips have been induced to form propagules (3). Unorganised callus tissue of *Phalaenopsis* has been induced to form plantlets when placed on a deficient medium, but is one of the few examples of callus induction in the *Orchidaceae*.[1] The one outstanding genus that has so far failed to respond to the many techniques now available is *Paphiopedilum* which, ironically, develops seedling protocorms with the greatest of ease.

2. **Corms** (Shoot tip and inflorescence culture). *Freesia* and *Gladiolus* have been successfully propagated from both inflorescenses[2] and dormant shoot apices. Unopened flower buds and pedicel sections, respectively, were surface sterilized and placed on a nutrient medium for a period of several months, after which approximately 10% of the explants had given rise to callus from which plantlets freely developed. A more successful technique employing the use of dormant shoot apices from corms has

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[1] Unpublished data, this laboratory

[2] John Innes Institute, Annual Report 1971

been developed in this laboratory and has the advantage of providing plantlets via a more genetically stable technique. Cormlets of a controlled size can now be routinely produced under aseptic conditions and provide the ideal structure for enabling mechanization of culture systems to occur. The implication of such developments are only just being realised. Controlled production of cormlets of a particular size at any period of the year enables efficient use of growing rooms, storage of cormlets, and mechanization of planting. The period required for the production of 10,000 plants can be reduced from 6 years to 18 months.

Figure 1. *Freesia* — differentiation from callus induced on isolated flower buds.

3. **Bulbs** (Shoot tip and organ culture). Two aseptic techniques are available for the propagation of lilies — the induction of plantlets from callus (9) tissue, and the use of scales (8), the latter merely representing the traditional technique reduced in scale. Scaling aseptically enables the astounding number of 1 million plants to be produced within a year from 1 bulb. Given the correct cultural conditions each is capable of forming a bulblet that can be stored until required for planting. Other bulbous plants are not so obliging but commercial rates of increase have been achieved with *Amaryllis* and *Nerine*[3], whilst there is some progress with *Narcissus* and *Hyacinth*.

[3] Unpublished data, this laboratory

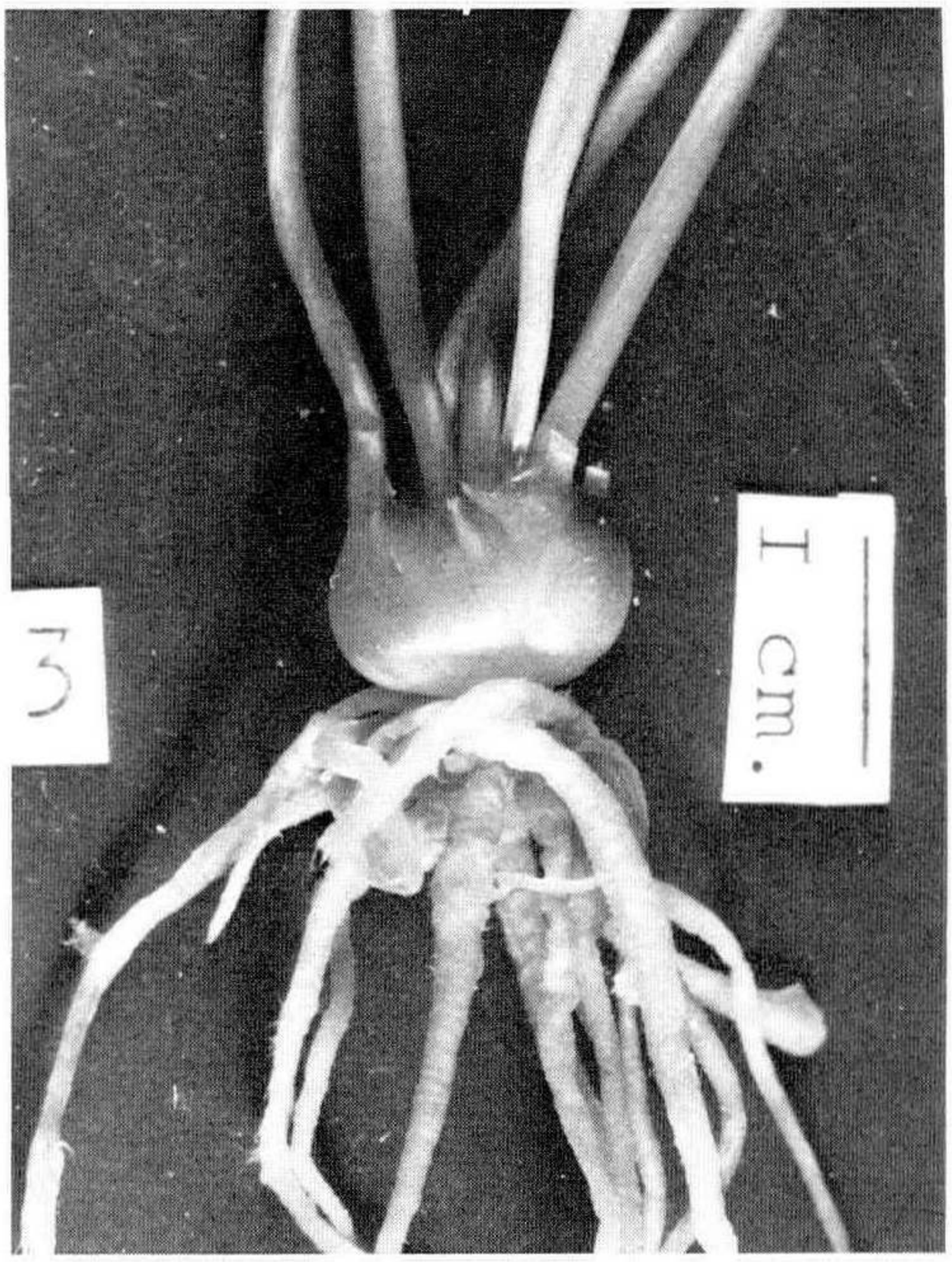

Figure 2. *Lilium* — single bulblet in an active state of growth 4 months after subculture to a basal medium.

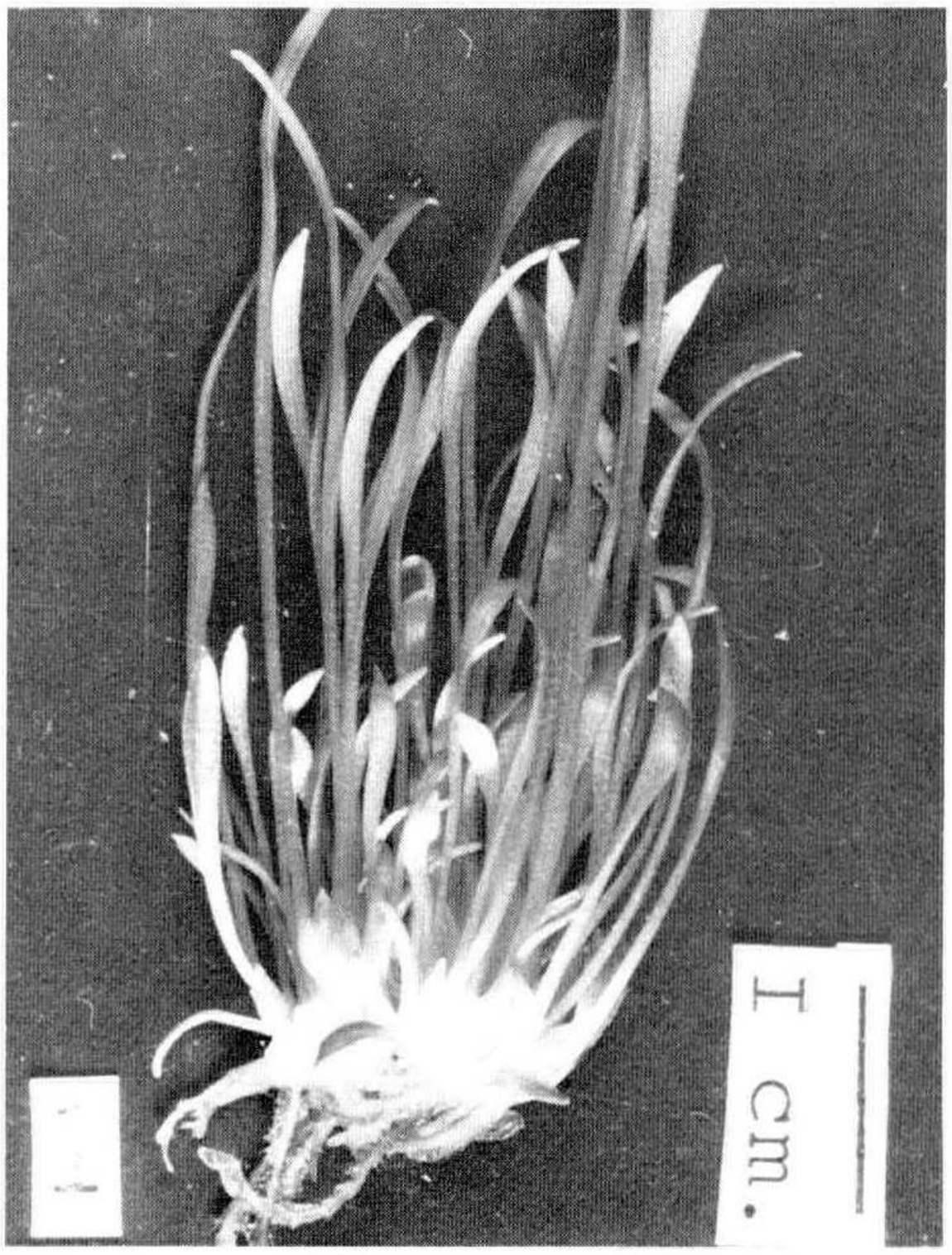

Figure 3. *Lilium* — massive plantlet regeneration from young bulb scales.

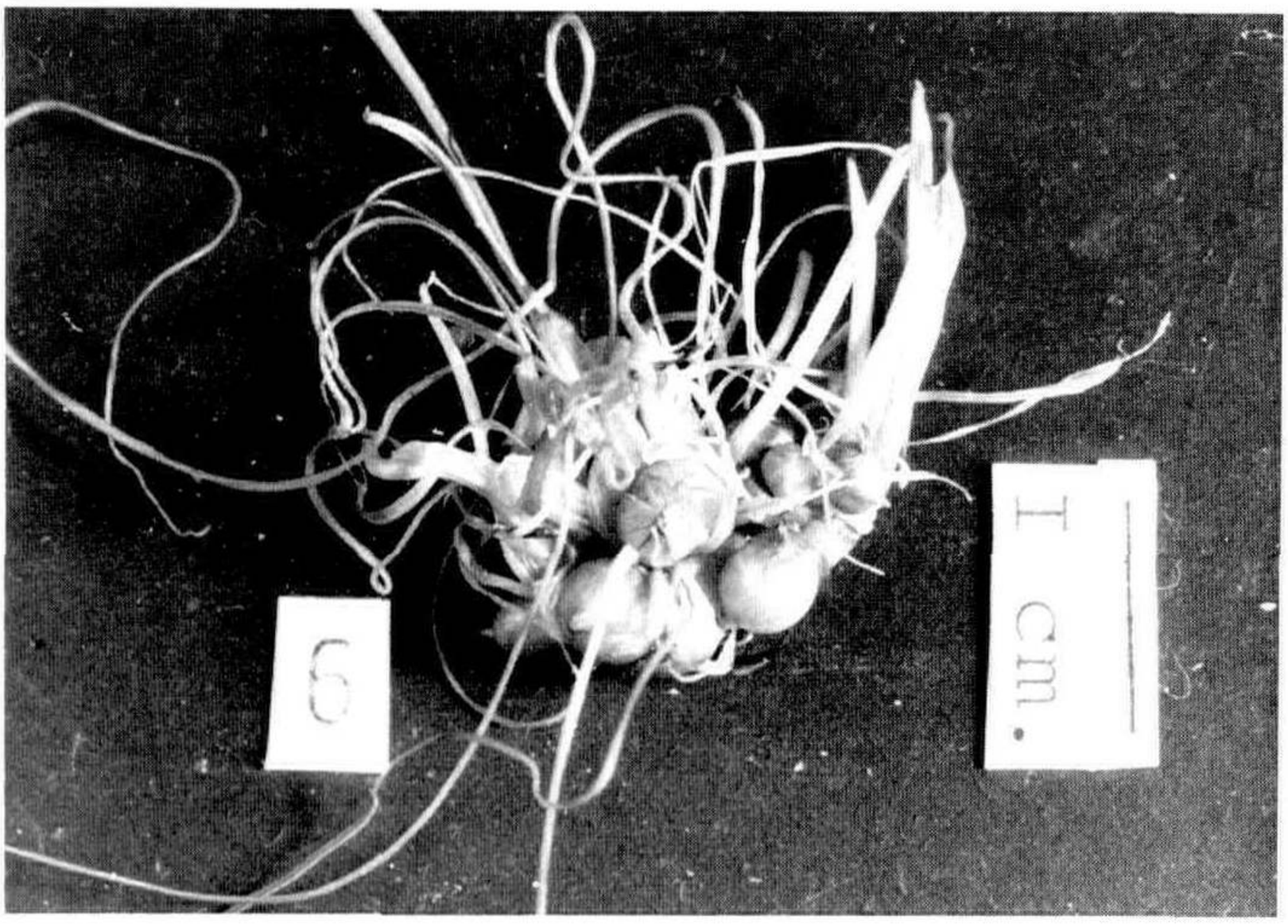

Figure 4. *Gladiolus* — Cormel formation in culture.

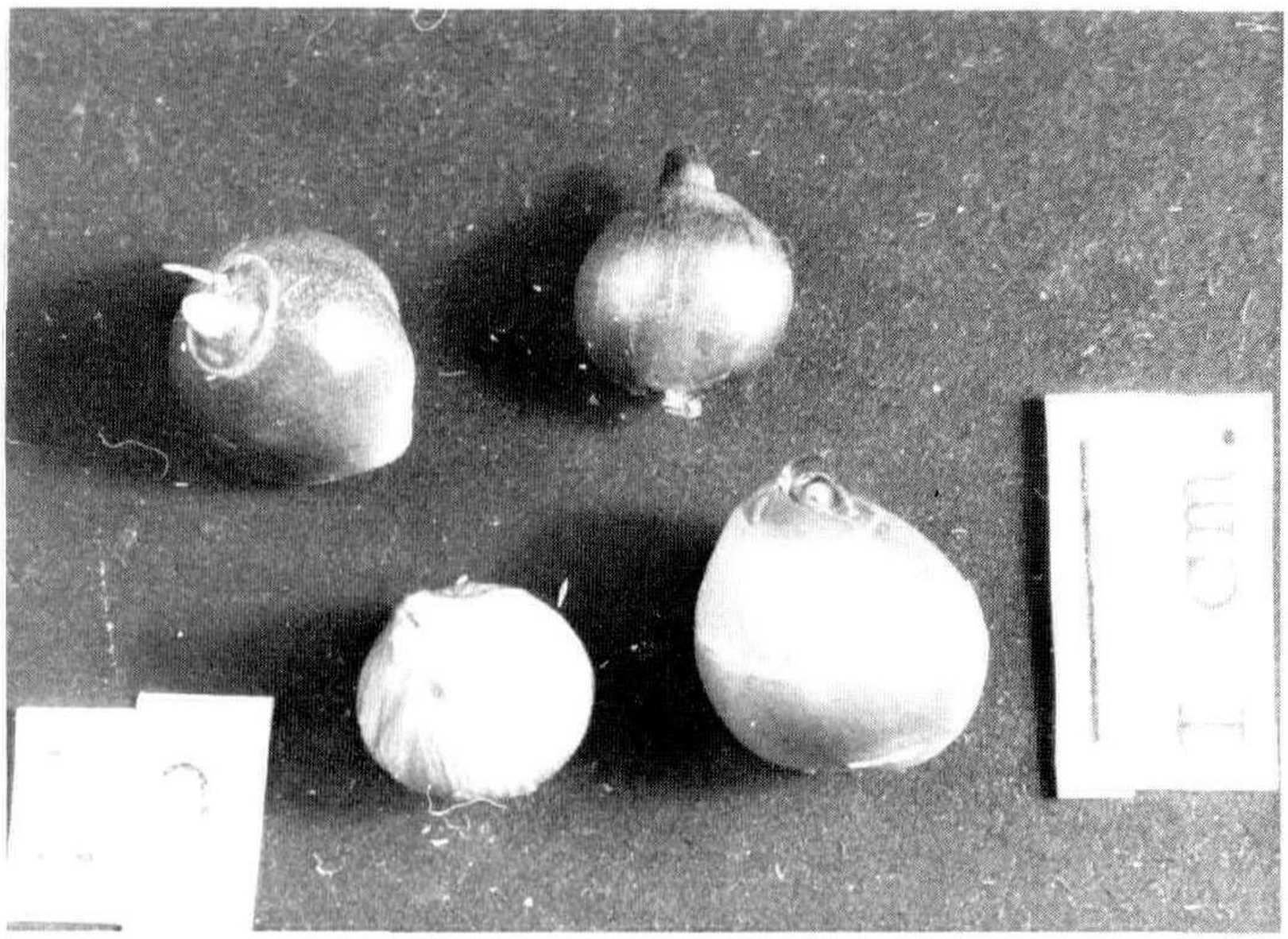

Figure 5. *Gladiolus* — Cormels, produced in culture, following prolonged storage at 3°C.

4. **Saintpaulia** (Tissue and organ culture). Scaled down conventional techniques have also been applied to the propagation of *Saintpaulia* cultivars. Portions of tissue from practically any part of the plant are inoculated onto a medium containing both auxins and cytokinins. Adventitious buds are rapidly formed, developing into plantlets from which petioles and leaves just 2mm in length, can be excised and induced to produce further propagules. The

numbers produced in a matter of months are probably at present greater than for any other plant of horticultural merit other than carrot plants produced from embryoids.

The proven rate of increase is one of x4 in 4 weeks, thus giving 10,000 plants from an original 10 leaves in 6 months. Many other members of the Gesneriaceae would probably respond similarly (4).

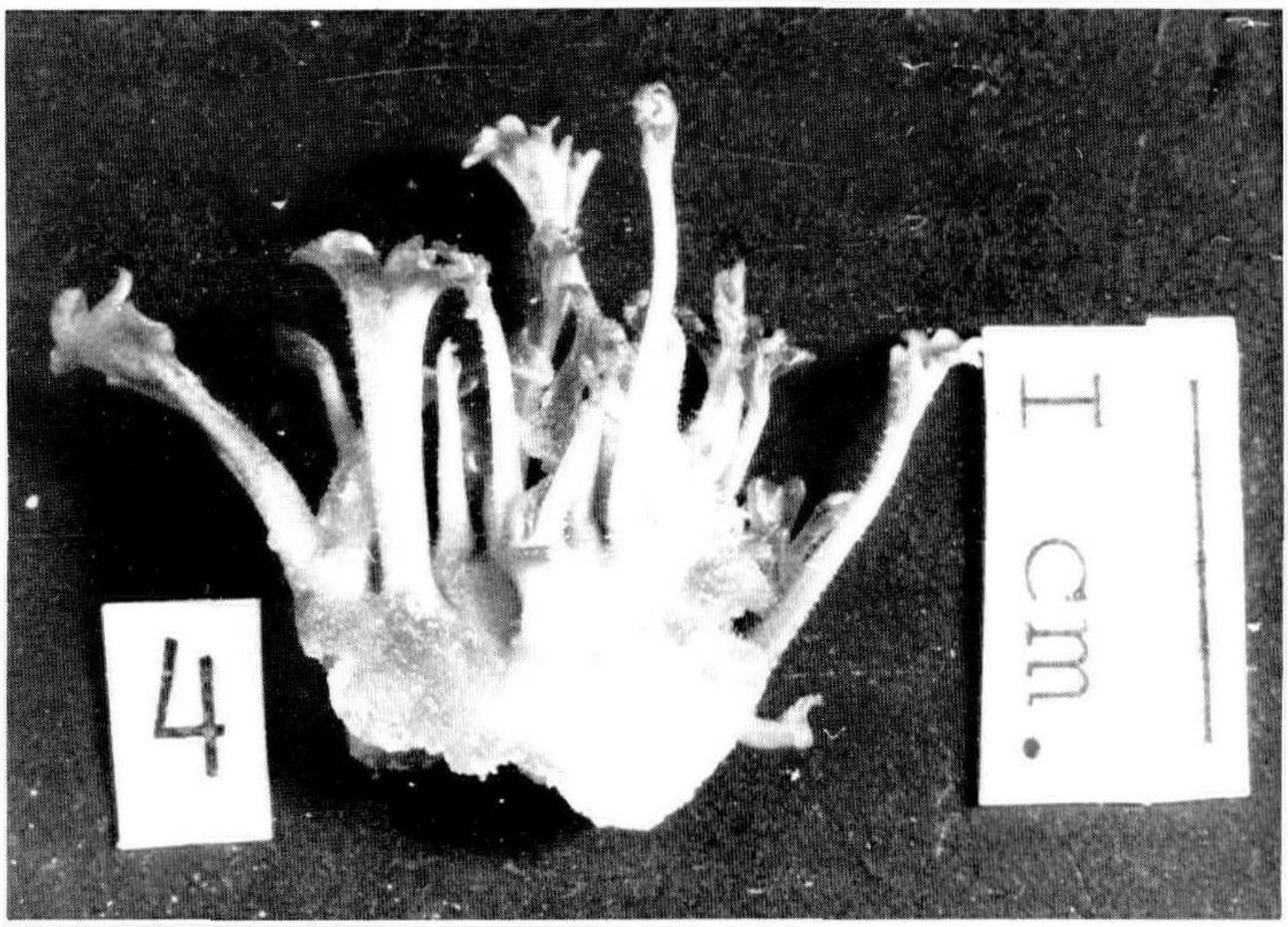

Figure 6. *Pelargonium* — Shoots initiated from callus and axillary buds.

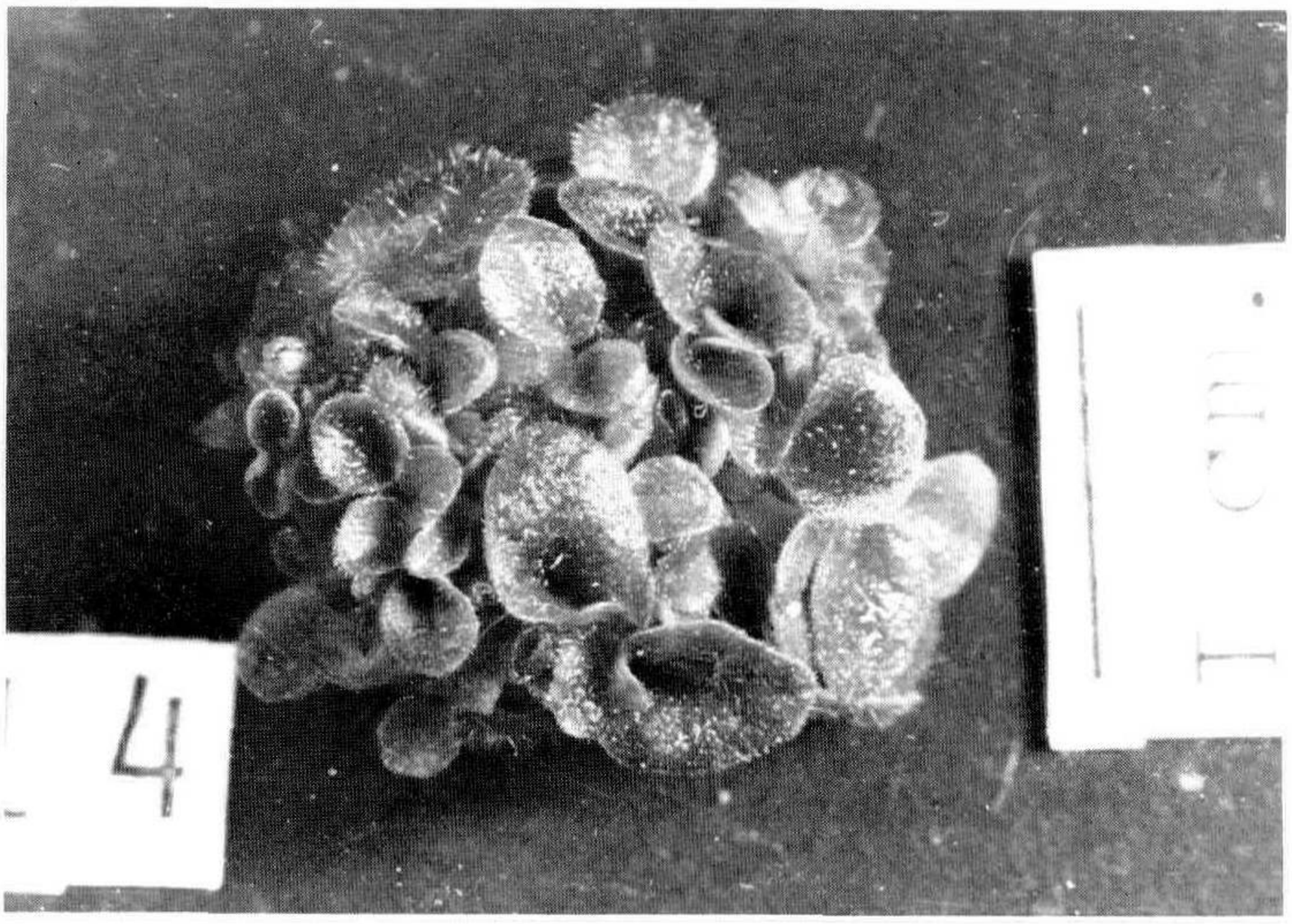

Figure 7. *Saintpaulia* — development of adventitious buds on isolated leaf fragments cultured at 27°C.

5. **Pelargonium and Chrysanthemum** (Shoot tip culture). Shoot apices are the material used for the initiation of cultures in *Pelargonium (1)* and *Chrysanthemum (5)*. Unorganised callus formation is easily induced by the use of auxins and from this tissue with the appropriate treatment, plantlets will develop. However, with repeated subculture the tissue gradually loses some of its morphogenetic potential and is less satisfactory than an axillary bud system of propagation. Even within this system a certain amount of selection is necessary to maintain and improve the productive ability of the tissue — an action that is necessary in certain other aseptically propagated genera. Thus, a secondary system of clone selection occurs, within the clone that is undergoing propagation. This selection does not appear to cause variation in the offspring although if carried to an extreme this might occur.

6. **Tropical Crops** (Shoot tip culture). The potential for the use of aseptic propagation techniques amongst tropical crops is enormous. The palms can only be propagated from seed, whilst pineapples and bananas are only vegetatively propagated — but stock is virus-infected and the provision of planting material on a large scale is a slow process. Proven systems are already available for *Ananas*[4] and research proceeds on *Elaeis* (10), *Coffea* (11), *Saccharum* (6), and *Musa* (2), amongst others. Independent research by workers in several countries has established that cultures of the edible pineapple can be aseptically propagated on a scale so as to give a million plants from a single crown in 1 year. The system involves the excision of axillary buds and multiplication in liquid culture of the resulting propagules. *Aechmea*, *Cryp-*

[4] Unpublished data, this laboratory

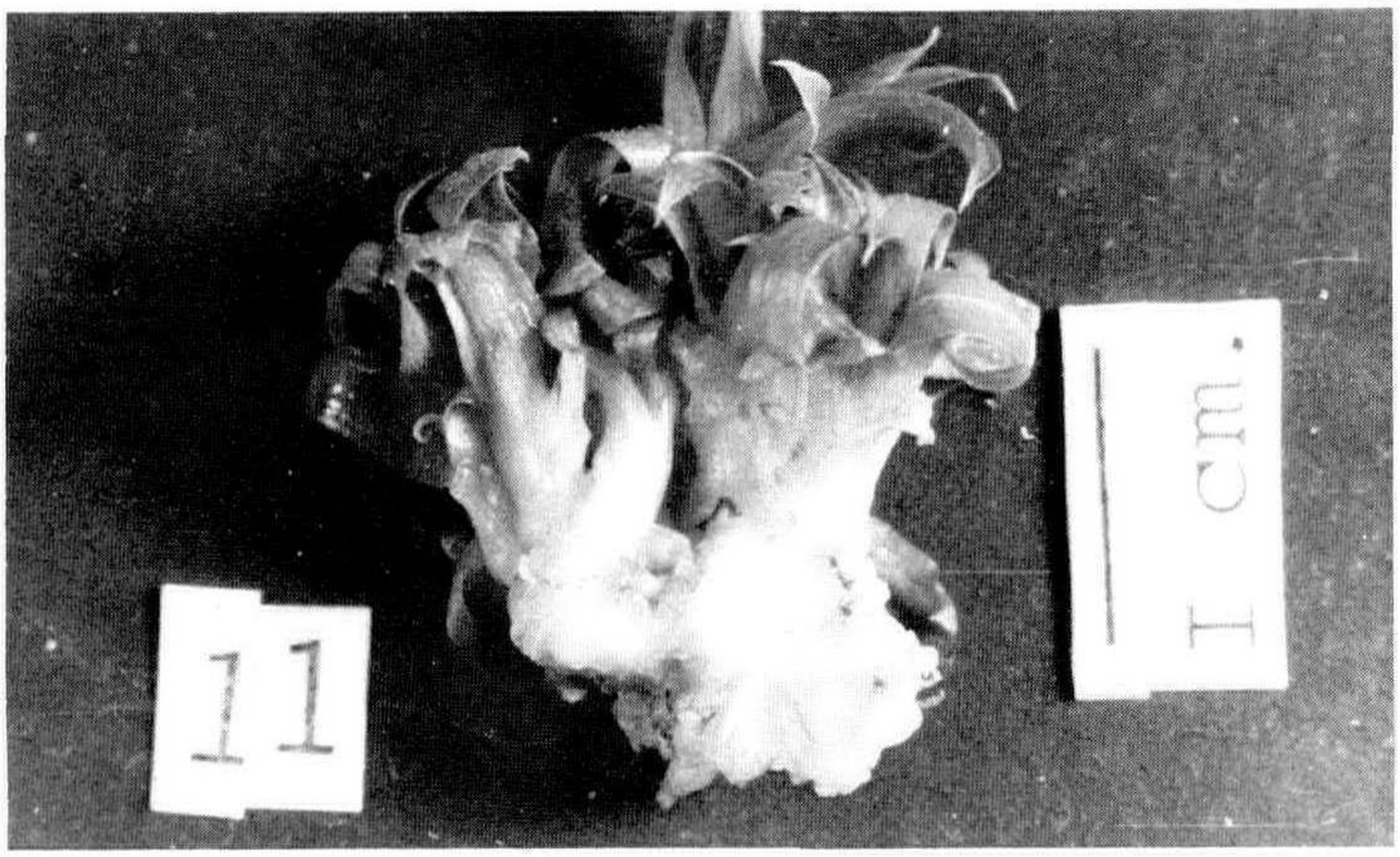

Figure 8. *Ananas* — plantlet production from callus grown in liquid medium on a klinostat.

tanthus, Dyckia, Bilbergia, Neoregelia and other members of the Bromeliaceae have been propagated by this means.

Figure 9. *Cryptanthus* — young plants from culture, ready for transfer to greenhouse environment.

PATENTS FOR PLANT PROPAGATION TECHNIQUES

A North American company has already patented a technique for the propagation of pineapples. The use of patents in this manner is a practice to be actively discouraged. Intolerable restrictions would be placed on scientists working in this field if the practice became widespread. The benefits from aseptic plant propagation should accrue to the horticultural industry as a whole and in particular to the long forgotten breeder who is now able to claim plant breeders' rights and avail himself of the advances in propagation techniques.

FUTURE EMPHASIS

The whole field of plant tissue culture is wide open for exploitation by both research workers and commerce. The two can only benefit from closer co-operation in this field with the former initiating research into topics that arise as spin-offs of horticultural applications and which, with a deeper understanding, could eventually prove commercially attractive. Considerable attention is now being focused on the propagation of a wide variety of woody plants, the successful mass production of which could revolutionize the forestry, fruit and hardy nursery stock industries. Aseptic conditions provide the ideal environment for seed germination and subsequent growth; apart from speeding up this process it provides a means by which certain phytosanitary regulations could be overcome. At present in the export trade we have the rather ludicrous situation where plant health inspectors are

required to examine plants growing under aseptic conditions which, by very definition, are free of diseases. Even if virus diseases were present, in itself unlikely, then symptoms would not be liable to show in such immature plant material; a system whereby plant tissue culture laboratories are licensed to export plants grown in sterile containers would surely be less time consuming. Finally, we should aim for closer co-operation with bio-engineers and plastics technologists to enable mechanization of labour intensive production lines and development of disposable containers for encapsulating the mini-plants. The next ten years should see some remarkable innovations in the propagation of many of our horticultural crops, particularly in the ornamental sector.

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