TISSUE CULTURE OF SOME MONOCOTYLEDONOUS PLANTS*†

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Of the approximately 100 species in Gautheret’s (1959) list of tissue cultures 10 only are monocotyledonous, and even though additions can now be made the list of monocotyledonous species is still short. Partanen (1963, pp. 218–19) stated: “Notably refractory to being cultured satisfactorily are the tissues of monocotyledonous plants, among which are to be found, unfortunately, some of the best materials for chromosomal studies.”

This communication reports the culturing of a number of monocotyledonous plants, several of which may be useful for cytological studies.

Methods

Difco Bacto yeast extract (YE), Oxoid acid-hydrolysed vitamin-free casein, and Oxoid dehydrated malt extract were used in preparation of media. Coconut milk (CM) was obtained from batches of at least 20 mature nuts, filtered through Whatman No. 1 filter paper, sterilized in the autoclave at 15 lb/in² for 15 min, and then stored in a refrigerator. 15 ml was generally added to 100 ml (final volume) media. Solid media were gelled with 0·6 g/100 ml Difco Noble agar and pH of all media adjusted to within the range 5·5–6·0.

White’s (1963) medium with Fe-EDTA prepared according to Jacobson (1951) replacing ferric sulphate was used as a basal medium (BM).

Cultures were grown in 15 by 2·5 cm Pyrex test tubes closed with Oxoid aluminium caps and containing 20 ml of medium. Incubation was in the dark at 27°C.

Results

Notes on species that were cultured successfully are now given.

(a) Onion (Allium cepa L.)

Gautheret (1959, p. 278) refers to unpublished work of Hurel-Py on apparently unsuccessful attempts to culture fragments of onion bulbs. Using a CM–2,4-dichlorophenoxyacetic acid (2,4-D) medium the author has obtained clones from three sources, viz. excised roots of cv. Hunter River Brown, seedling roots of cv. Early Flat Barletta, and bulb scales of an unknown cultivar.

(i) cv. Hunter River Brown.—Tissue was derived from excised roots grown in an attempt to establish a continuous culture using a technique resembling that of Bausa-Alcalde (1961) for Androcymbium gramineum whereby roots are alternated between a medium containing indolebutyric acid (IBA) and one containing adenine. The roots were excised from seedlings in January 1963 and grown initially in media containing glucose which was later replaced with sucrose.

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In August 1965, roots which had been 5 months in the adenine medium without appreciable growth were placed on an agar medium consisting of BM with CM and 6 mg/l 2,4-D. Most of the roots produced slow-growing callus which was subcultured 3 months later on the same medium. Over the following 6 months the tissue was taken through five passages, again on the same medium, and made more rapid growth (Fig. 1). For the sixth and subsequent passages the medium was modified by increasing the sucrose concentration to 4 g/100 ml and reduction of 2,4-D to 0·6 mg/l. The tissue is now in its 20th passage.

(ii) cv. Early Flat Barletta.—In May 1966, sterile seedlings were placed on an agar medium incorporating sucrose (60 g/l), indoleacetic acid (2 mg/l), and the minerals and vitamins used by Torrey and Shigemura (1957) for pea tissue. After 17 days the thickened roots were excised and transferred to the CM–2,4-D medium used for later passages of Hunter River Brown tissue. Callusing occurred and tissue has now survived into its 13th passage.

(iii) Unknown Cultivar.—Of 12 explants of bulb scale tissue in the form of approximately 6-mm disks placed on a BM–CM–6 mg/l 2,4-D agar medium, one only produced callus (after a delay of 2 months). The tissue has survived 10 passages over a period of more than 2½ years, in later passages with sucrose increased to 4 g/100 ml and 2,4-D reduced to 0·6 mg/l.

(b) Asparagus (Asparagus officinalis L.)

Attempts to grow excised asparagus roots using the technique of Bausa-Alcalde (1961) were unsuccessful and the roots showed only limited growth through a few subcultures. However, several roots in the last passage produced outgrowths of callus and this was transferred in January 1965 to an agar medium based on White’s medium but with sucrose replaced with glucose and the addition of 40 mg/l adenine and 0·1 mg/l naphthaleneacetic acid (NAA). The tissue grew slowly and 7 weeks later was subcultured to a YE medium used by Venketeswaran (1962) for broad bean. On this medium growth was much more vigorous and the tissue survived 15 passages, with occasional production of shoots (Fig. 2), over a period of more than 3 years before it was lost due to an accidental infection. However, tissue derived from a seedling of the cv. Mary Washington has been established on Venketeswaran’s medium since loss of the original tissue and is now in its fourth passage. This tissue has also grown into its third passage on a synthetic agar medium consisting of BM with the addition of 1 mg/l NAA.

Wilmar and Hellendoorn (1968) have reported the growth of asparagus in light on a synthetic medium.

(c) Rye (Secale cereale L.)

Carew and Schwarting (1958) have reported in vitro growth of callus derived from embryos of the cultivar Canadian Spring on a sucrose–YE–2,4-D medium. The author has obtained vigorously growing callus from a clone of excised roots of the cv. Black Winter. The roots were originally excised in May 1964 and first grown in light in the YE medium of Roberts and Street (1955) and then from March 1966 in the dark in a modification of the synthetic medium of Gonzalez-Alonso and Bausa-
Alcalde (1965). The minerals of their medium were replaced by those of White’s medium, the glucose content reduced to 2%, sodium pantothenate replaced with calcium pantothenate, and IBA omitted.

![Fig. 1](image1)
![Fig. 2](image2)
![Fig. 3](image3)
![Fig. 4](image4)
![Fig. 5](image5)

Fig. 1.—Onion root tissue, cv. Hunter River Brown, ninth passage, 29-day-old culture. ×1·0. Fig. 2.—Asparagus root tissue, tenth passage, 3-month-old culture (YE medium), with shoot developing on top. ×1·0. Fig. 3.—Rye root tissue, fourth passage, 28-day-old cultures. ×1·0. Fig. 4.—Spanish bluebell tuber tissue, seventh passage, 4-month-old culture (CM medium), with development of tuberized shoots. ×1·0. Fig. 5.—Beech orchid seedling, fourth passage, 3·5-month-old cultures. ×0·8.

In September 1966, roots in their tenth passage in synthetic medium were transferred to an agar medium containing BM with 2% glucose in place of sucrose, 100 mg/l myoinositol, 3 g/l YE, and 6 mg/l 2,4-D. The roots callused vigorously during the following 4 weeks and callus was subcultured to the same medium. In
this first passage growth declined in vigour and in the second passage it almost stopped. For the third passage 2,4-D was reduced to 2 mg/l and growth in a few tubes improved. Subculturing from these tubes yielded tissue of vigorous growth in subsequent passages (Fig. 3). Beginning with the ninth passage, 2,4-D was reduced to 1 mg/l. In its 13th passage the tissue died, which appears to be due to its being left for too long (4 months) without subculturing in the previous passage.

(d) Spanish Bluebell [Endymion hispanicus (Mill.) Chouard]

In August 1965, approximately 6-mm cubes of tissue were excised from surface-sterilized Spanish bluebell tubers and placed on a BM–CM–6 mg/l 2,4-D agar medium. Tissue in only 1 tube out of 10 proliferated and only after a delay of 6 weeks. For the first passage, 2,4-D was replaced with 0.1 mg/l NAA and very little growth was made. For the second passage, the tissue was subcultured back to the 2,4-D medium and growth improved. Subsequent passages were on the same medium with 2,4-D reduced to 0.6 mg/l and sucrose increased to 4%. Cultures now show moderately rapid growth and the tissue has survived 17 passages.

Some of the first passage tissue on the NAA medium was also transferred to Venketeswaran’s (1962) broad bean medium, on which it showed little activity for over 4 months. However, in subsequent passages on the same medium its vigour increased markedly, becoming comparable with that of tissue on the CM–2,4-D medium, and the tissue is now in its 16th passage.

On both media the tissue displays a marked capacity to retain organization and mostly proliferates into a mass of tuberized shoots (Fig. 4) plus some callus tissue. Attempts to subculture the callus have failed on both media.

This species has a remarkable power to retain organization in culture. On media which more or less completely suppress organization in other species, little callus is produced and the cultures are organ cultures rather than true tissue cultures.

(e) Daylily (a species of Hemerocallis L.)

Approximately 6-mm cubes of surface-sterilized tuberized roots of daylily were excised in August 1965 and placed on a BM–CM–6 mg/l 2,4-D agar medium. After a delay of 3 months, 1 explant out of 12 proliferated, yielding tissue which in subsequent passages grew slowly, usually with a somewhat brown appearance, and which sometimes produced a few short roots. Reduction of 2,4-D to 0.6 mg/l caused slackening of growth and the initial concentration was therefore maintained through all passages. Sugar concentration was increased to 4% for the fourth and subsequent passages. The tissue is now in its 14th passage.

(f) A Species of Liriope Lour.

Explants from rhizomes were taken in August 1965 and subjected to the same cultural conditions as for daylily tissue. They yielded slow-growing cultures with some tendency to produce short roots. The tissue is now in its 14th passage.

(g) A Species of Crinum L.

Of nine explants of bulb scale tissue placed on a BM–CM–6 mg/l 2,4-D agar medium in August 1965, two only produced new growth, which was quite limited
and appeared only after a delay of almost 2 months. For the first and second passages the 2,4-D was replaced with NAA at 0.1 mg/l and 1.0 mg/l respectively but growth was still limited and blackening of the older tissue occurred. Subsequent passages were on the complex opium poppy medium of Ranganathan et al. (1963), on which the tissue was at first precariously maintained. In later passages growth was more vigorous, though darkening of the older parts still occurred. The tissue is now in its 17th passage.

(h) Beech Orchid (Dendrobium falcrostrum Fitzg.)

Aseptically germinated beech orchid seedlings were placed in May 1966 on the opium poppy medium of Ranganathan et al. (1963) where they proliferated vigorously in the dark to produce a mass of non-green shoots. For the first passage, a single mass of shoots originating from one seedling was subdivided to form a clone which has maintained its organization (Fig. 5) and continued to produce shoots on the same medium through 15 passages.

Like Spanish bluebell, this species also markedly retains organization in culture.

References

Gautheret, R. J. (1959).—“La Culture des Tissus Vegetaux.” (Masson et Cie: Paris.)