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TISSUE CULTURE INVESTIGATIONS OF BAMBOO

I. Callus Cultures of *Bambusa*, *Phyllostachys* and *Sasa*

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Abstract

A series of investigations was initiated to explore and develop tissue culture systems of bamboo. ***Bambusa oldhamii***, ***B. multiplex***, ***Sasa pygmaea***, and ***Phyllostachys aurea*** were used as test species. This report describes requirements and behavior of their callus cultures. Shoot tips, 3-6 mm tall, were used as explants. Callus of all species was initiated and maintained through unlimited subcultures in a medium containing Murashige and Skoog salts, 3% sucrose, White vitamins and glycine, 100 mg/l i-inositol, 3 mg/l 2, 4-D, and 0.8% TC agar. Vigorous growth of callus was attained in darkness and at constant 27°C. Callus of the two ***Bambusa*** species was moist, friable, smooth, and creamy-white. That of ***Sasa*** and ***Phyllostachys*** was creamy-yellow to tan colored and granular to nodular in texture. The ***Phyllostachys*** callus turned purplish-red when grown under light; that of the others retained their creamy appearance. The callus appeared to originate mainly in leaves of the shoot-apex explant. Intraclonal differences in callus growth rate and pigmentation were observed. Polyacrylamide gel electrophoresis of callus extracts disclosed distinct glutamate-oxaloacetate-transaminase isozymes among the four species.

Introduction

The bamboos are monocotyledonous evergreens, belonging to the Poaceae. They are set apart from the other grasses as a subfamily, Bambusoideae. More than 75 genera and 1,000 species of bamboo have been recorded, many of which are probably synonyms (Lawson, 1968; McClure, 1966; Munro, 1868; Soderstrom and Calderon, 1979). They constitute a major part of the natural vegetation of tropical, subtropical, and mild temperature regions, from sea level to snow line. Several species are cultivated extensively for their young

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shoots that are eaten as a vegetable and for their mature culms which are used in handicrafts and furniture making, as construction material, and in paper manufacturing (Austin and Ueda, 1970). Some species are also grown as landscape ornamentals.

Improvement of bamboo cultivars has not been possible by traditional breeding methods. Flowering occurs irregularly and at unpredictably long intervals. For some species, 60 years and longer must pass before seedlings reach flowering age. Once mature, flowering does not occur annually; the plant may die after one flowering cycle or may not rebloom for many years (Lawson, 1968; McClure, 1966). A series of investigations was therefore initiated to develop tissue culture methods as alternatives in bamboo hybridization and variety improvement. This report focuses on callus cultures; subsequent reports will deal with cells in liquid suspension, cell plating, protoplasts, plant regeneration, and other relevant aspects.

Although *in vitro* cultures have been used extensively with other grasses, especially the cereals, they have remained virtually untried with bamboos. The literature contains only two papers that deal even remotely with bamboo tissue culture. One is a note that describes aseptic development of embryos excised from mature seeds (Alexander and Rao, 1968), and the other simply included *Bambusa* among several plant genera that have been examined for protoplast release (Tseng, Liu, and Shaio, 1975).

Materials and Methods

Species investigated

Four species were compared: *Bambuas oldhamii* Munro (syn. *Sinocalamus oldhamii*), *B. multiplex* (Loureiro) Raeuschell (syn. *B. nana, B. argantea, Leleba multiplex*), *Sasa pygmaea* (Miquel) E. G. Camus (syn. *Arundinaria pygmaea, B, pygmaea*), and *Phpllostachys aurea* A. and C. Riviere (syn. *B. aurea, P. aurea, Sinarundinaria aurea*). *B. oldhamii* plants were available on the landscape of the University campus; the other three were established as potted plants in the department's greenhouse from specimens kindly furnished by ABC Nursery of Gardena, California.

B. oldhamii was the largest of the four species. Its culms were 4–5 cm in diameter and 7–10 m tall (Fig. 1). It was the primary species used in the investigations. *B. multiplex* was smaller but morphologically very similar to *B. oldhamii*. It had thin-walled, relatively smooth green culms, about 1 cm in diameter near the base and 250 cm in height; the culms drooped fairly readily (Fig. 2). *S. pygmaea* plants were the smallest. Potted plants had culms that barely reached 15 cm in height and 1.5 cm in diameter (Fig. 3).

Alike *B. multiplex*, *P. aurea* was a moderately sized bamboo (Fig. 4). However, in differing from the *Bambusa*, *P. aurea* had stiff, erect, and golden-colored culms, about 1.5 cm in diameter at the base and rising to 250 cm in height. *P. aurea* culms also showed prominently furrowed internodes, directly above each axillary bud. The internodes were also progressively shorter toward the culm base.

Explants and their preparation

Lateral shoots, 1–2 cm long and shortly after emergence from buds, were used as explant sources of *B. oldhamii*, *B. multiplex*, and *P. aurea*. Culms possessing desired shoots were severed at their bases and divided into single-node segments (Fig. 5). The shoots were separated from the culms and placed in a freshly prepared solution of 100 mg/l ascorbic and 150 mg/l citric acids. Several shoots were usually obtained from each node.

Lateral shoots of suitable size were not available in *S. pygmaea*, so explants were obtained from terminals of actively growing culms (Fig. 6). Culms were easily forced by clipping all older growths back to near soil level. The new shoots were 3–4 cm tall when used.

The outer leaves, sometimes composed mainly of sheaths, of lateral and terminal shoots were removed, and the shoots were washed with household detergent. They were washed further with phisohex, rinsed with distilled water, and immersed in 0.5% sodium hypochlorite solution for 10 minutes under vacuum. The hypochlorite solution contained a few drops of Tween 20 emulsifier to enhance spreading. The 0.5% solution was decanted and replaced with a 0.005% solution of sodium hypochlorite. The shoots remained in this solution until final excision of explants.

Aided by a dissecting microscope, the remaining green outer leaves were removed, leaving attached only the softer, pale-white inner leaves. The leaves were shortened to 2–5 mm, and the subjacent stem was reduced to 1 mm; together they comprised the shoot-tip explant (Fig. 7). The explants were transferred to nutrient medium at a rate of one per culture.

Stock cultures of callus

More uniform tissues for the experiments were assured by utilizing callus that had been maintained in stock culture. Approximately 300 mg quantities of callus were subcultured in fresh medium every 4 weeks.

Nutrient media and their preparation

The basal constituents were Murashige and Skoog salts (Murashige and Skoog, 1962) and, in mg/l: sucrose, 30,000; i-inositol, 100; thiamine · HCl, 1; nicotinic acid, 0.5; pyridoxine · HCl, 0.5; glycine, 2; and TC agar, 8,000. The

three vitamins and glycine together have sometimes been identified as the White vitamin and glycine supplements. The pH of all media was set at 5.7, using 1 N KOH or 1 N HCl, just prior to final dilution and addition of agar. The media were autoclaved at 1.05 kg/cm^2 for 3–10 minutes, the exact duration depending on their volumes, to dissolve the agar. They were then dispensed into 25 × 150 mm glass culture tubes at a rate of 25 ml per tube. The tubes were capped with polypropylene closures (Bellco kaputs) and autoclaved 15 minutes at 1.05 kg/cm^2. The nutrient tubes were cooled at 30° slants.

The main focus of callus culture experiments was the determination of nutrient requirements. Detailed studies were performed with auxins, cytokinins, vitamins, glycine, inositol, sugars, and levels of Murashige and Skoog macro-nutrient salts.

Incubation conditions

The bamboo callus cultures were initiated and maintained at constant 27°C and under continuous darkness.

Growth measurements

At least 10, usually 20, cultures comprised each treatment. Most experiments lasted one passage of 4 weeks' duration. The tests with BA (N^6-benzyladenine), vitamins, and glycine spanned four successive passages, or three subcultures; this procedure was intended to exclude masking effects by residual supplies of substances carried over from stock culture.

With cultures of freshly excised shoot-tips, the data recorded were limited to notes of callus initiation frequency and callus size and variability of behavior among explants. Experiments with established callus evaluated pigmentation, texture, intraclonal variations, and fresh-weight yields. The term growth value as used in this report refers to the ratio of final to initial fresh weights. Tissues were weighed aseptically when intended for further culture.

Standard errors of means were computed whenever appropriate according to Snedecor (1946). When data were expressed as percentages, the 95% confidence limits of binomially distributed attributes were obtained from tables published by Snedecor (1946) and Colquhoun (1971).

Histological and scanning electron microscopic observations

Callus development in shoot-tip explants was followed by examining cultures histologically. Samples were fixed in Craf V solution overnight under vacuum, dehydrated through the ethanol series, and embedded in paraplast (Berlyn and Miksche, 1976; Johansen, 1940; Sass, 1958). Dehydration

with tertiary butanol gave poor infiltration of bamboo tissues. Sections 12 μm thick were obtained, stained with saffranin-fast green, and mounted in balsam. At least five randomly selected cultures were fixed every 3 days. Microslides were prepared of all sections, obtained serially from each tissue.

Some samples of established callus were examined by SEM (scanning electron microscopy). The tissues were fixed in FAA (2:1:10:7, v:v:v:v, 37% formaldehyde: glacial acetic acid: 95% ethanol: distilled water), dehydrated through acetone solutions, further dried by critical drying point with liquid CO_2 (Tousimis Research Corp., 1975), attached onto aluminum mounts (E.F. Fullam, Inc.), sputter-coated approximately 200 angstroms thick with gold (Technics U.S.A.), and observed at 15 KV with the JEOL model JSM-35C scanning electron microscope.

Isozyme analysis

Callus samples of the four bamboo species were analyzed for GOT (glutamate-oxaloacetate transaminase) isozymes, using a procedure adapted from Torres, Soost, and Diedenhofen (1978) and Torres and Tisserat (1980). The enzyme extracts were prepared by homogenizing 5 g samples of callus in 2.5 ml volumes of extraction buffer. The callus was ground until uniformly fine, and the homogenates were transferred to plastic tubes and centrifuged at 15,000 rpm for 20 minutes (Sorvall RC-5 super speed refrigerated centrifuge). The supernatant fractions containing the proteins were removed to clean tubes and stored in an ice bath. The extracts were diluted as necessary with extraction buffer and subjected to polyacrylamide gel electrophoresis.

An LKB 2001 vertical slab unit was employed. The stacking gel acrylamide concentration was 5%; that of the resolving gel, 12%. One hundred μl of extracts, diluted and undiluted, were placed in sample wells. Using two slabs simultaneously, electrophoresis was carried out at constant 40 mA for 12 hours, the current being provided by an LKB 2197 power supply. The temperature of the running buffer was maintained at 15°C by circulating coolant through cooling tubes that came furnished with the apparatus.

Following electrophoresis, the gels were removed to separate pyrex baking dishes and stained. The stain solution was prepared and placed in the dishes just before introducing the gel slabs. The dishes were covered with aluminum foil to exclude light, placed on a gyratory shaker, and agitated for 10 hours at 50 rpm. The slabs were then rinsed by flooding the dishes with running tap water overnight. The positions of isozymes were recorded, and the gels were photographed. The gels were preserved by drying under vacuum onto Whatman 3 MM filter paper.

Results

Preliminary trials

Experiments performed during the summer of 1976 with *B. oldhamii* disclosed that the excised shoot tips generated callus within 2 weeks when cultured in a medium supplemented with 3 mg/l 2, 4-D (2, 4-dichlorophenoxy-acetic acid), and 0.1 mg/l BA. The callus showed sustained growth when subdivided and transferred to fresh medium of the same composition. The subculturing process was repeatable indefinitely.

Beginning in early 1978, the investigation undertook a detailed study of the nutrient requirements of bamboo callus cultures. Using the 1976 medium as base, each organic constituent was evaluated separately, and the macronutrient salts were examined collectively, with *B. oldhamii* serving as primary species. Those components that displayed significant effects were applied directly to or tested further with the other species.

Hormonal substances

Auxins. The initiation and sustained growth of callus of all four bamboo species required the provision of a potent auxin. Either 2, 4-D or tordon (Picloram, or 4-amino-2, 3, 5-trichloropicolinic acid) was satisfactory, but not IAA (Indole-3-acetic acid) or NAA (α-Naphthaleneacetic acid). The best callus yields with the latter two auxins were only 1/10 of that obtained with an optimum concentration of 2, 4-D or tordon. Furthermore, the callus obtained with IAA or NAA was brown and generally necrotic. Tordon was as effective as 2, 4-D and probably more effective for some species, e.g., *B. multiplex*; but it was not used routinely because of its scarcity.

Figure 8 shows the relationship between 2, 4-D concentration and initiation of callus in excised shoot tips of *B. oldhamii*. The basal medium of this experiment contained 0.1 mg/l BA. No callus developed in the absence of 2, 4-D. A concentration of about 3 mg/l was optimum, and concentrations 10 mg/l and higher appeared toxic. Callus initiation in shoot-tip explants of the other species showed essentially the same relationships, with highest incidences of callus formation occurring in the 1–3 mg/l range.

The same 2, 4-D optimum was encountered in subcultures of the callus. Figure 9 illustrates the typical response with data taken from an experiment with *B. multiplex*. The maximum callus growth was realized in a medium supplemented with about 3 mg/l 2, 4-D; repression resulted at higher levels.

Cytokinins. Exogenous cytokinin was unnecessary for initiation of callus in any of the bamboo species. The results of an experiment with *B. oldhamii* shoot tips are shown in Fig. 10. The basal medium contained 3 mg/l 2, 4-D. None of the BA supplements promoted callus formation.

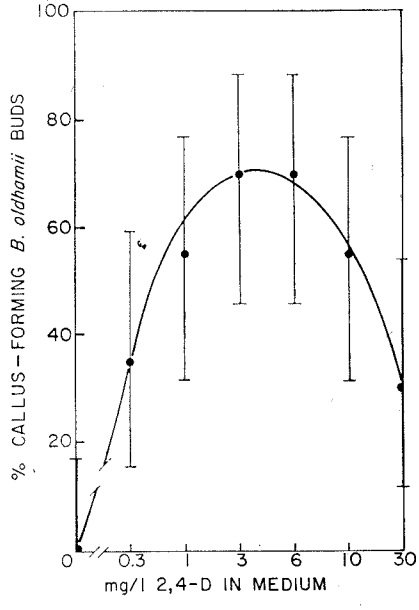

Fig. 8. Relationship between 2, 4-D concentration and frequency of callus-forming *B. oldhamii* shoot tips. Basal medium contained 0.1 mg/l BA.

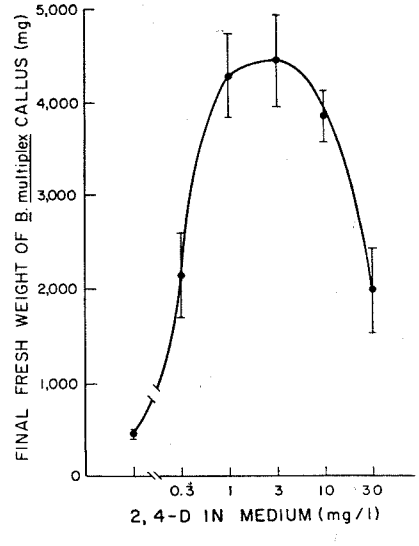

Fig. 9. Growth of subcultured *B. multiplex* callus as influenced by 2, 4-D concentration of medium. Basal medium contained 0.1 mg/l BA.

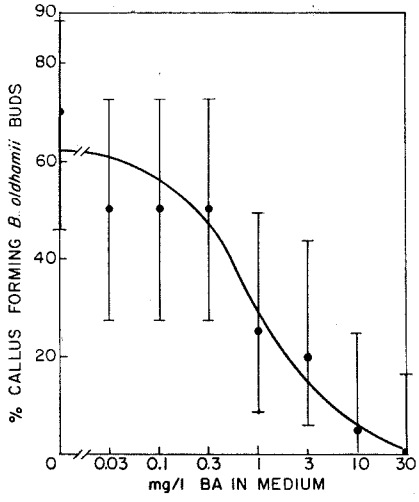

Fig. 10. Effects of BA addenda on callus initiation in *B. oldhamii* shoot tips. Basal medium contained 3 mg/l 2, 4-D.

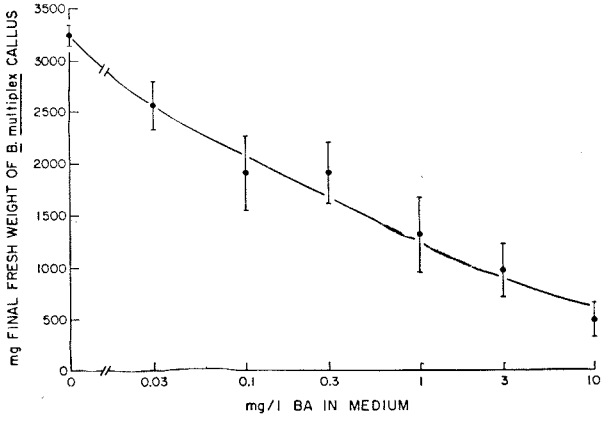

Fig. 11. Growth of subcultured *B. multiplex* callus as influenced BA addenda. Basal medium contained 3 mg/l 2, 4-D and 0.1 mg/l BA.

On the other hand, BA concentrations of 1 mg/l and higher reduced the frequency of callus-forming explants.

Subcultures of bamboo callus also showed no callus promotion by BA. Additions of BA usually resulted in reduced callus yields. The data from a representative experiment with *B. multiplex* can be seen in Fig. 11, where a concentration as low as 0.03 mg/l was already inhibitory. The absence of promotive effects by BA was unrelated to the kind of auxin used; the same trends were observed when the cytokinin was tested in media with tordon

or NAA as auxin.

The cytokinins kinetin, N^6-isopentenyladenine, and zeatin were also ineffective in concentrations ranging from 0.03 to 0.3 mg/l. All were distinctly inhibitory at the 1 mg/l level.

Tissues subcultured repeatedly in cytokinin-free media continued to respond negatively to cytokinin addenda.

White vitamins and glycine

The vitamins and other nonhormonal constituents were tested only with established callus stocks of *B. oldhamii*. Application of the findings to callus cultures of other species produced anticipated results. The experiments employed a basal medium that had been enriched with 3 mg/l of 2, 4-D.

Thiamine · HCl. This vitamin stimulated growth of *B. oldhamii* callus dramatically, but maximum effects from its addition were not evident unless the test callus had been grown previously for at least one passage in thiamine-free medium. Brown, necrotic areas developed in thiamine-deficient callus. As apparent in Fig. 12, the reductions in growth, expressed as ratios of final/initial fresh weights, became progressively more marked with successive subcultures in thiamine-less medium. The optimum thiamime · HCl concentration was determined in other experiments to be 1 mg/l, whether thiamine · HCl-depleted callus or callus obtained directly from stock culture was used. Higher concentrations gave no additional enhancement.

Pyridoxine · HCl. Inclusion of pyridoxine · HCl in concentrations of 0.3 to 100 mg/l neither promoted nor repressed *B. oldhamii* callus growth. Masking, residual effects were excluded by conducting the test through four consecutive passages, or three successive subcultures, in media lacking

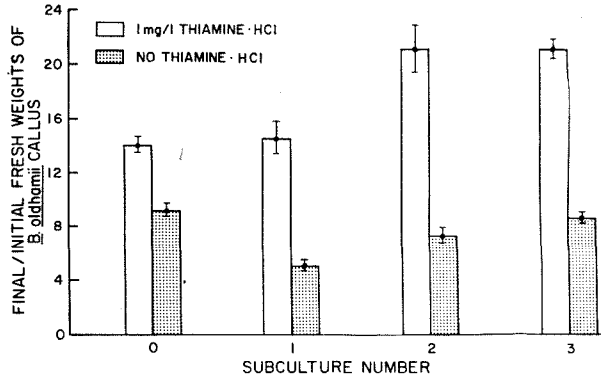

Fig. 12. Effects of repeated subculturing in thiamine-free medium on growth of *B. oldhamii* callus. Basal medium contained 3 mg/l 2, 4-D and 0.1 mg/l BA.

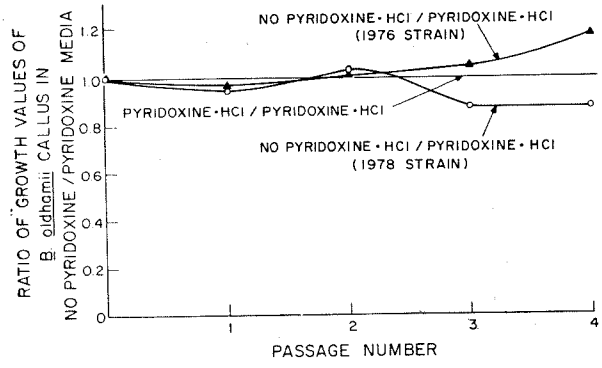

Fig. 13. Growth effects of subculturing *B. oldhamii* callus repeatedly in pyridoxine-free or pyridoxine-supplied medium. Basal medium contained 3 mg/l 2, 4-D. Pyridoxine HCl was used at a 0.5 mg/l rate.

pyridoxine. Two strains of *B. oldhamii* callus, one established in 1976 and another in 1978, were used in the tests. Both strains originated in the same plant. The data can be seen in Fig. 13. No significant lowering of growth values, or final/initial fresh-weight ratios, was observed among tissues that were repeatedly subsultured in pyridoxine-free medium. The slight decline of the 1978 strain and the small increase of the 1976 strain during the third and fourth passages in pyridoxine-free medium were probably not significant. There was also no visible deterioration of tissues in cultures deprived of pyridoxine · HCl. The uniformly creamy-white appearance of *B. oldhamii* tissue was retained.

Nicotinic acid. Quantitatively, bamboo callus cultures showed no depressions when nicotinic acid was withheld from the medium. This was evident even in tissues that had been subcultured repeatedly in media lacking the vitamin. Qualitative changes were readily observed, however. Brown areas developed, sometimes becoming necrotic, and the smooth texture of *B. oldhamii* callus was replaced by a more granular one (Fig. 14). The nicotinic acid-deficient tissues eventually recovered their more normal color and texture when returned to vitamin-supplemented medium, but not in the first passage. There were no differences in effectiveness among concentrations of nicotinic acid ranging from 0.03 to 100 mg/l; thus, the 0.5 mg/l level of the basal medium was kept as standard.

Glycine. Glycine failed to promote *B. oldhamii* callus when tested in concentrations of 0.3 to 100 mg/l. Residual effects by amino acid provided in the stock medium were probably not involved, since repeated subcultures in glycine-free medium showed no diminishment of growth. No toxic effects were observed, even at the 100 mg/l level.

Inositol

The hexitol, i-inositol, stimulated *B. oldhamii* callus noticeably, with maximum effect being obtained in a concentration as low as 30 mg/l. The 100 and 1,000 mg/l levels produced no further stimulation, but were not toxic.

Sugars

Sucrose gave highest callus yields when provided in concentrations of 1.5–3%. Additional increments, from 4.5–10.5%, progressively repressed growth (Fig. 15). Glucose was as effective as sucrose, with optima in the range, 1.5–4.5%. Levels of glucose, 6% and higher, were increasingly inhibitory.

Inorganic salts

Lowering the macro-nutrients of the Murashige and Skoog salt formula to 3/10 or 1/10 of their originally recommended levels only reduced bamboo callus growth. The 1/10 level produced callus yields that were less than half of those obtained at normal levels of salts. Raising the salt levels to three times their standard concentrations was neither stimulatory nor inhibitory.

Other requirements

B. oldhamii callus was clearly repressed by light. A 25% reduction in final fresh weights was observed in cultures maintained under 16-hour daily illumination with 1,000 lux GroLux light.

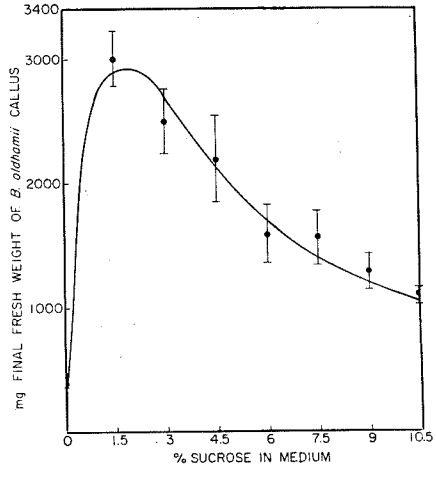

Fig. 15. Growth of *B. oldhamii* callus as influenced by sucrose provision. Basal medium included 3 mg/l 2, 4-D and 0.1 mg/l BA.

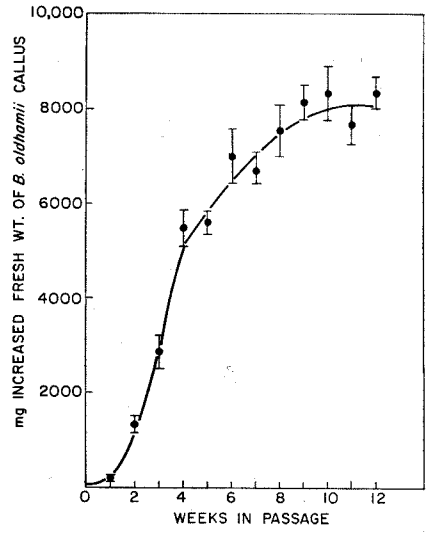

Fig. 16. Growth curve of *B. oldhamii* callus cultured in basal medium supplemented with 3 mg/l 2,4-D.

The final yield of callus was directly related to the quantity of tissue initially placed in culture, particularly for inocula of 50 to 300 mg dimensions. A 400 mg inoculum showed no further growth increase over the 300 mg quantity.

Characteristics of bamboo callus

The typical growth pattern obtained in the finally adopted callus culture medium is illustrated in Fig. 16, using data from an experiment with *B. oldhamii*. The exponential period of fresh-weight increase occurred during the first 2–4 weeks of the passage. The callus continued to grow up to about 8 weeks, but at a slower rate; there was no further growth thereafter. For subcultures, therefore, the transfers were performed every 4 weeks. Although growth occurred, the tissue quality appeared to deteriorate when left in the same medium for longer periods.

Sample cultures of callus of the four species can be observed in Fig. 17. The callus cultures of the two *Bambusa* were the most vigorous, with that of *B. oldhamii* usually growing slightly faster than that of *B. multiplex*. The two *Bambusa* species produced creamy-white, smooth, moist, and friable calls whereas those of *Sasa* and *Phyllostachys* were creamy-yellow to tan in color and granular to nodular in texture.

Intra-clonal variations were also observed among callus cultures. Strains differing in growth rates, even though originating in the same plant and explanted at the same time, were encountered among *B. oldhamii* callus.

Three strains of *B. multiplex* callus, distinguishable by their pigmentation, were also isolated. In addition to the typically creamy-white callus, gray and brown strains of *B. multiplex* were selected out and established as stocks. The three strains did not differ in growth rates.

Histological and SEM observations

Paraffin sections of *B. oldhamii* shoot tips, sampled during their development in callus-forming medium, revealed that much of the callus originated in leaves, although some callus was seen to arise from the basal cut surface of the stem. Proliferation began after 9 days among the outer, older leaves and spread slowly to the inner, younger leaves. The leaf bases, probably sheaths, initiated callus first. Callus development could be observed in the lamina region, extending to the leaf tips, only after 12 days. The newly generated callus was composed of tightly packed, small, relatively isodiametric cells. Older callus contained mainly loosely packed, large, highly vacuolated, and diversely shaped cells.

SEM observations revealed that the surface of a smooth bamboo callus, e.g., of *B. oldhamii*, featured a network of elongated, irregularly twisted, tube-like structures. There were large gaps and cavities within the network. A granular or nodular callus, e.g., of *P. aurea*, showed a more compact surface, covered with clusters of spherical bodies. The identity or significance of the surface structures has not been determined.

GOT isozymes

The four bamboo species were distinguishable by their GOT isozymes. Figure 18 shows zymograms produced from two concentrations of callus extracts. The *B. oldhamii* and *B. multiplex* callus displayed four identical GOT isozymes, but that of *B. oldhamii* possessed a fifth isozyme that was absent in *B. multiplex*. The *S. pygmaea* and *P. aurea* callus extracts showed four common GOT isozymes; however, that of *S. pygmaea* contained three proteins that were not found in *P. aurea*. When all four species were considered together, only one GOT isozyme was identifiable as being common to all.

Discussion

The ultimate goal has been to develop parasexual alternatives for genetic improvement of bamboo cultivars. To that end, a variety of tissue culture procedures has been explored. This report focused on establishment of callus cultures.

Shoot tips of actively growing laterals or terminals were used as explants, simply because they were abundant and other tissues were unavailable.

Callus cultures of the four species used, *Bambusa oldhamii B. multiplex, Sasa pygmaea,* and *Phyllostachys aurea,* disclosed an absolute requirement of a potent auxin, but not of cytokinin. Tordon seem as effective as, or more potent than, 2,4-D, but the latter was chosen for routine use because tordon has not been readily available. There were also indications that tordon might have cumulative toxic effects when used continuously; such effects were apparent in liquid suspension cultures. IAA and NAA were definitely ineffective in bamboo callus cultures.

The necessity to supply auxin, but not cytokinin, has been consistent with observations of callus cultures of other Poaceae members, e.g., *Avena* (Cure and Mott, 1975), *Hordeum* (Cheng and Smith, 1975), *Oryza* (Inoue, Maeda, Yoshida, and Oritani, 1979), *Pennisetum* (Vasil and Vasil, 1981), *Poa* (Zilkah and Gressel, 1977), *Saccharum* (Cheng, Liu, and Chao, 1979), *Secale* (Rybczynski, 1978), *Sorghum* (Dunstan, Short, and Thomas, 1978), *Triticum* (Chin and Scott, 1977), and *Zea* (Gengenbach and Green, 1975). In this investigation, the synthetics, BA and kinetin, as well as the naturally occurring cytokinins, zeatin and N^6-isopentenyladenine, showed no growth promotions, but were repressive in relatively low concontrations. The absence of a cytokinin requirement is interpreted as reflecting an adequate biosynthesis of the hormone by excised bamboo cells. Inoue *et al.* (1979) observed significant quantities of zeatin, zeatin riboside, and N^6-isopentenyladenine in the callus of rice, another Poaceae member.

In evaluating substances that are required in minute quantities, it may be necessary to first deplete or sufficiently dilute any residual supplies that may be carried over by the callus from stock culture or other sources. This has been accomplished in the investigation by subculturing test tissues one or more times in question. By following this procedure, two vitamins were found to be critical for bamboo callus cultures. Exclusion of thiamine resulted in reduced yield and necrosis. Whereas not affecting callus yield, deprivation of nicotinic acid caused deterioration of tissue quality; browning and necrosis resulted. Thus, excised bamboo cells are unable to synthesize adequate quantities of the two vitamins.

Pyridoxine · HCl was excludable without lowering callus yield or quality. Nevertheless, pyridoxine · HCl has been retained in the bamboo medium, since some untested species or cultivar will probably require the vitamin. Glycine has been retained for the same reason.

As generally observed among plant tissue cultures, inositol has been clearly beneficial to bamboo callus, and its retention in the basal medium seems justified.

Glucose promoted growth of bamboo callus as effectively as sucrose.

Favorable effects of glucose have been similarly reported with other grasses, e.g., *Sorghum bicolor* (Davis and Kidd, 1980). Nevertheless, sucrose has been retained as the standard carbon source of bamboo callus, mainly because it has been virtually universally effective in plant tissue cultures. Moreover, the glucose effect was not clearly superior to that of sucrose.

The inorganic salts were retained at levels as originally specified in the Murashige and Skoog medium. No advantage was gained by raising their levels, whereas considerable growth reductions were associated with lowering of the macro-nurtient concentrations.

The finally adopted nutrient formula for bamboo callus cultures contains the Murashige and Skoog salts and, in mg/l: sucrose, 30,000; thiamine · HCl, 1; nicotinic acid, 0.5; pyridoxine · HCl, 0.5; glycine, 2; i-inositol, 100; 2,4-D, 3; and TC agar, 8,000. More recently, the agar has been replaced by a better gelling agent, Kelco gelrite, in a 2,000 mg/l concentration. The only change, based on experimental findings, from the basal medium has been the addition of 2,4-D.

For best results, bamboo callus should be maintained in darkness. It should be subcultured every 4 weeks; whereas growth continues slowly, the tissue deteriorates if kept in the same medium for longer periods.

As a parasexual alternative in genetic improvement of bamboo cultivars, callus cultures have shown some potentially useful characterietics. Intraclonal variants have been isolable. Furthermore, distinct isozyme patterns have been observed among callus of different bamboo species. As subsequent reports will disclose, the callus has been used to initiate cell suspension cultures, which in turn has been employed in cell plating and as a source of protoplasts. It will also be shown that plants can be regenerated from some callus cultures.

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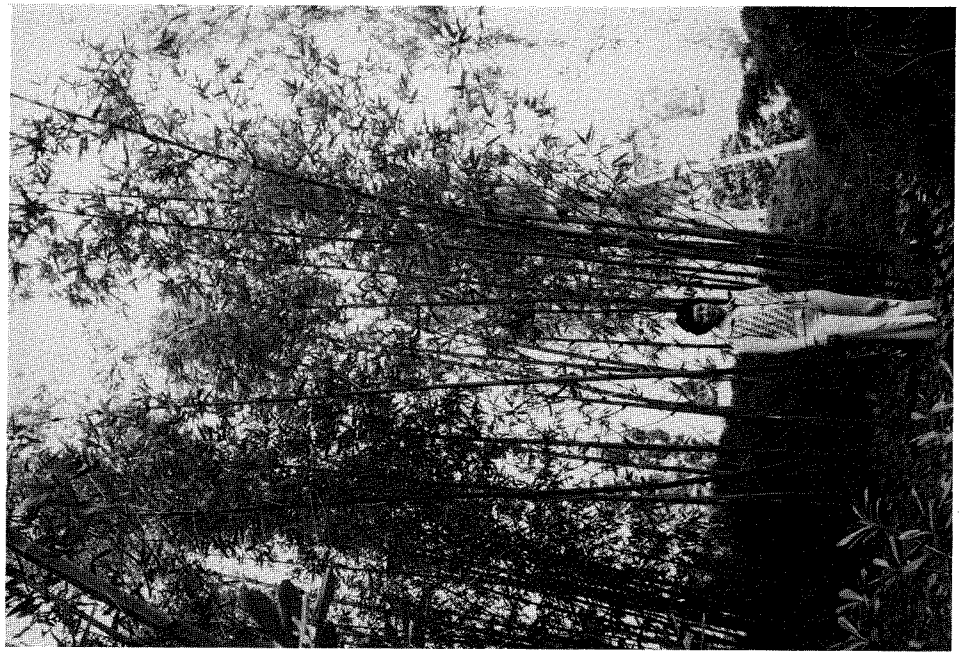

Fig. 1. Plants of *B. oldhamii*, the principal species used in the investigation.

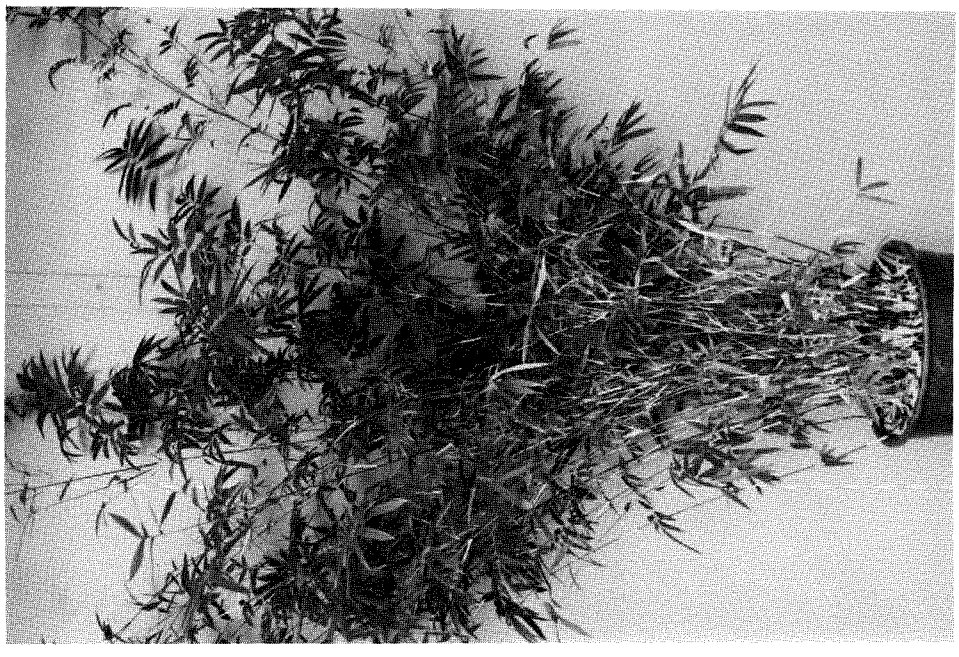

Fig. 2. Specimen plant of *B. multiplex*.

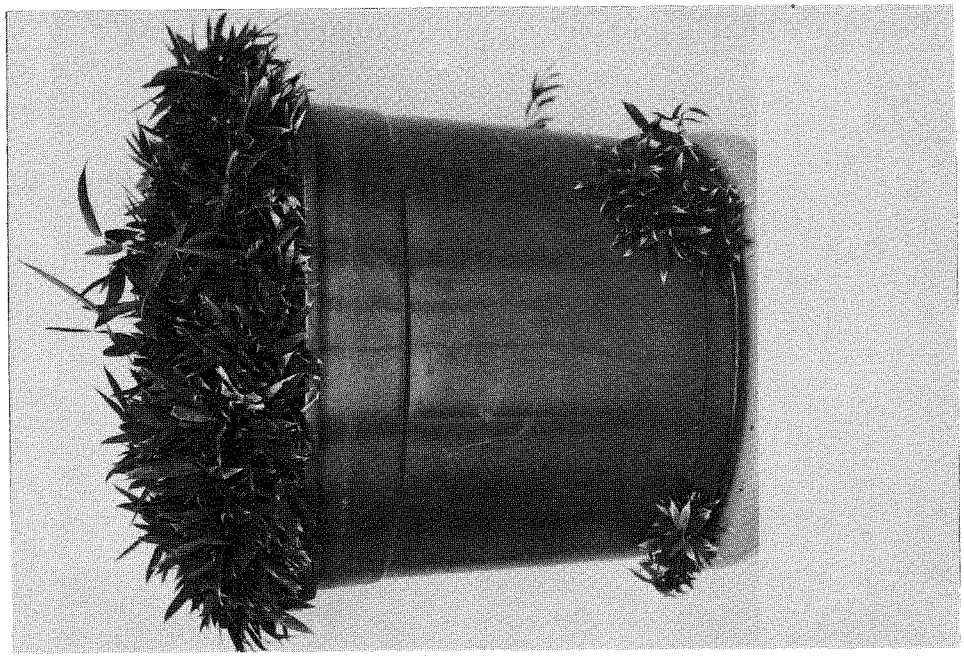

Fig. 3. *S. pygmaea* specimen plant.

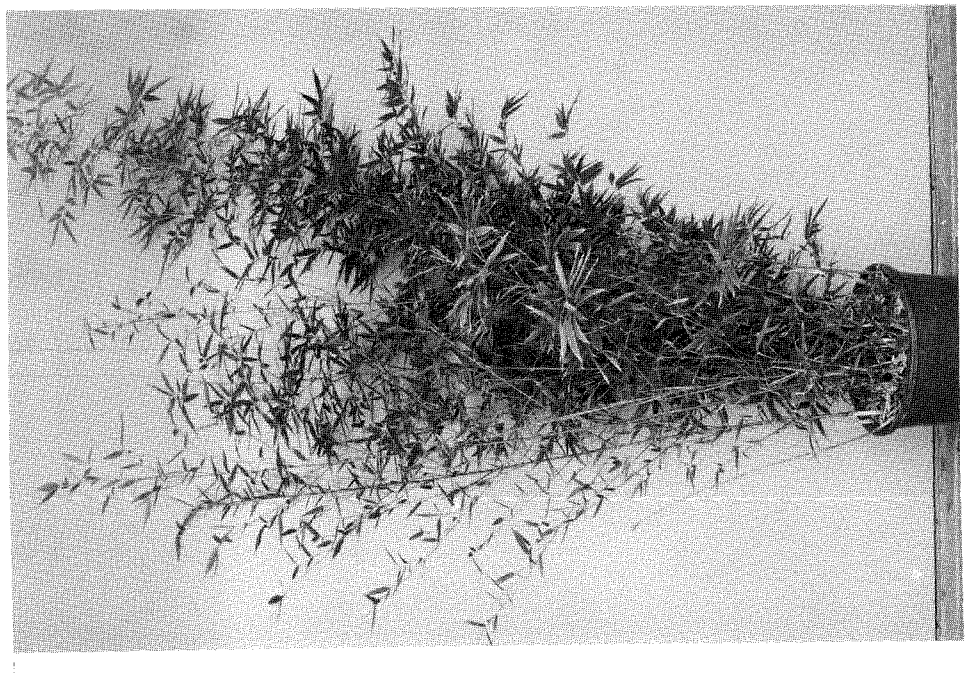

Fig. 4. Greenhouse-grown *P. aurea* used in investigation.

Fig. 5. Node section of ***B. multiplex***, showing cluster of shoots used as explant donors. At right is one of the shoots removed.

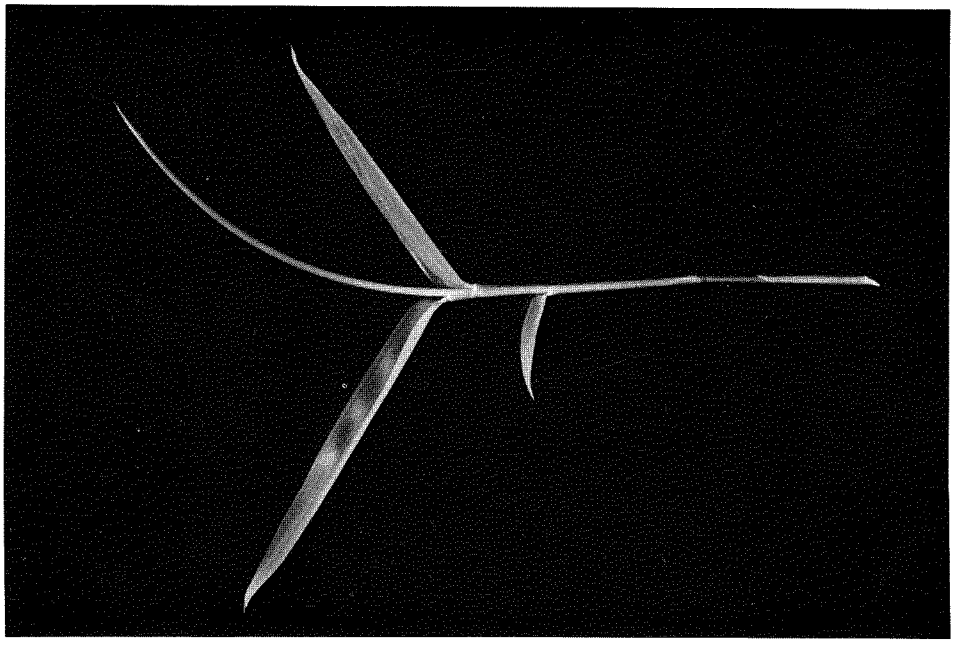

Fig. 6. Terminal shoot used as a source of ***S. pygmaea*** shoot tip.

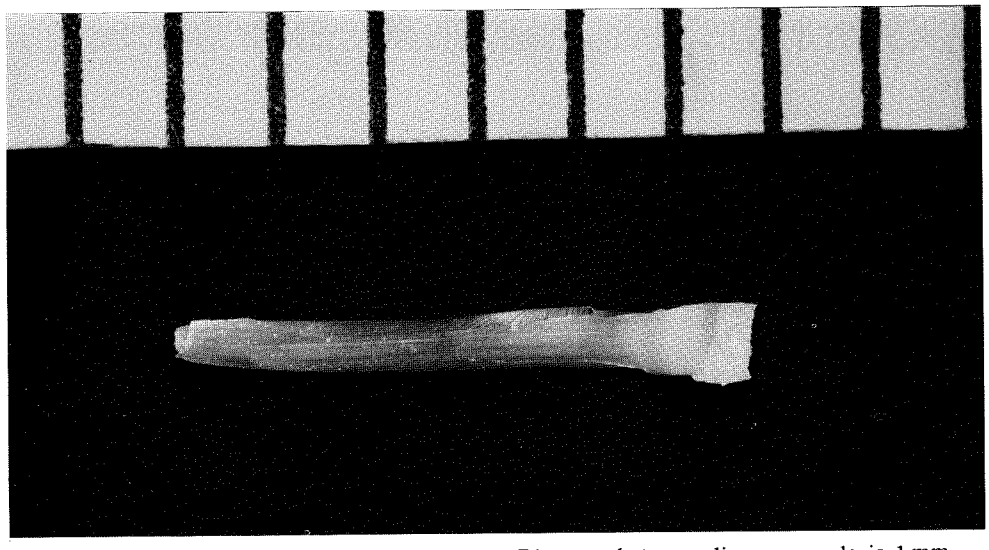

Fig. 7. Shoot-tip explant of ***B. multiplex***. Distance between lines on scale is 1 mm.

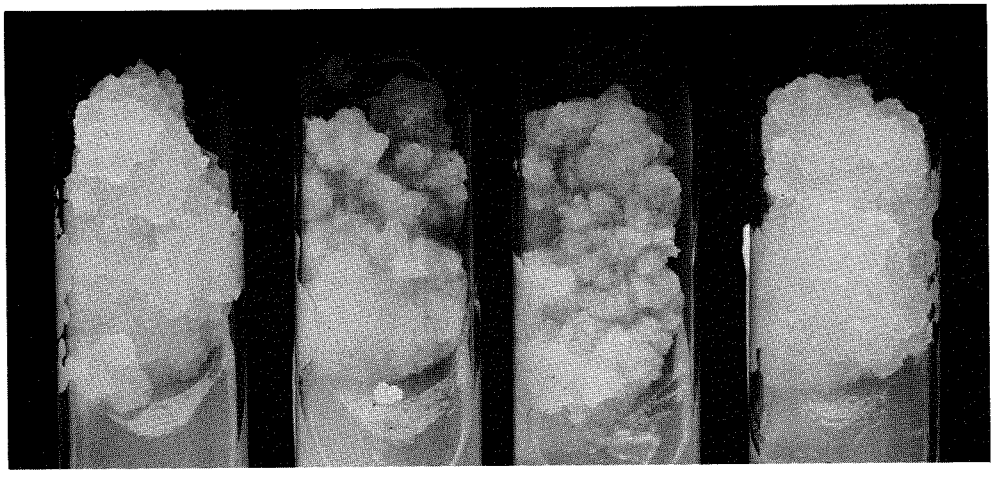

Fig. 14. Deterioration in quality of ***B. oldhamii*** callus when subcultured continuously in nicotinic acid-free medium. Left to right: callus grown continuously in 0.5 mg/l nicotinic acid-containing medium, callus subcultured repeatedly in nicotinic acid-free medium, callus in first passage following return from nicotinic acid-deprived to nicotinic acid-supplied medium, and callus in first transfer from stock culture to nicotinic acid-free medium. Basal medium contained 3 mg/l 2, 4-D.

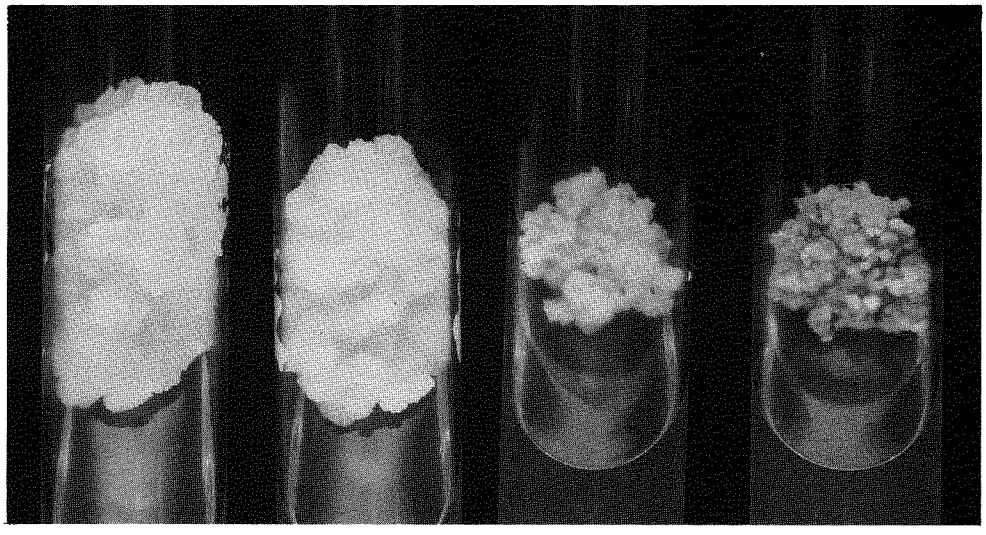

Fig. 17. Sample callus cultures of, left to right: ***B. oldhamii***, ***B. multiplex***, ***S. pygmaea***, and ***P. aurea***.

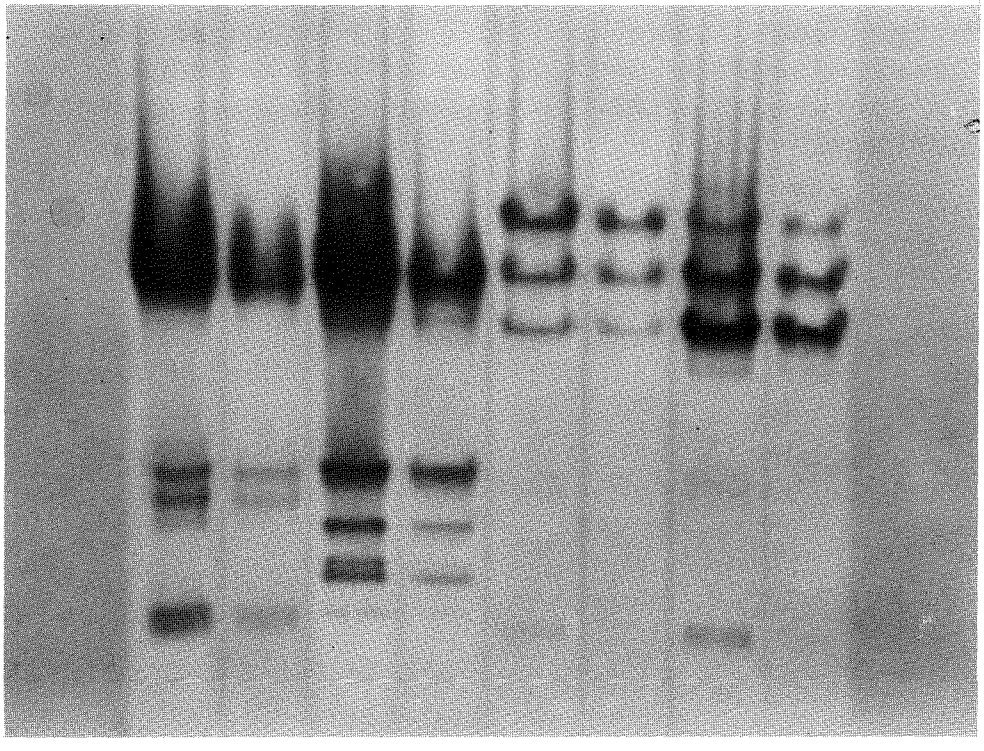

Fig. 18. Glutamate-oxaloacetate transaminase isozymes separated from callus extracts in polyacrylamide gel electrophoresis. Left to right, in pairs: ***P. aurea***, ***S. pygmaea***, ***B. multiplex***, and ***B. oldhamii***. Undiluted extract on leaf of each pair, 4X-diluted on right.

竹類植物組織培養之探討

I. *Bambusa*、*Phyllostachys* 和 *Sasa* 癒傷組織之培養

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以竹子爲組織培養的材料，從事一系統的研究是此實驗的目的之一。所用之四品種爲 *Bambusa oldhamii*, *B. multiplex*, *Sasa pygmaea* 和 *Phyllostachy aurea*。此篇報告敍述的部份爲癒傷組織的培養條件及其特徵。培養的材料選用 3 至 6 毫米高的側芽莖頂，置於下述培養基中，其成份爲 Murashige 和 Skoog 鹽，蔗糖 3%，White 綜合維生素和 glycine, inositol 100 毫克/升，及 TC agar 0.8%，在暗處及恆溫 27°C 下即可得繼代無限繁殖，生長迅速的癒傷組織，其色澤因品種不同而異，兩種 *Bambusa* 呈乳白色，細胞含水量高及鬆軟，*Sasa* 和 *Phyllostachy* 則呈乳黃色顆粒狀至根瘤狀的組織。*Phyllostachys* 的癒傷組織移於光下培養則轉變成紫紅色，其他三種不呈此種轉變仍保留原色。從石蠟切片可證明癒傷組織源自莖頂幼葉，癒傷組織生長的速度及色素都發生變異。四品種癒傷組織可用 polyacrylamide 之電泳分析法，分離 Glutamate-oxaloacetate-transaminase 之同位酵素而鑑定之。