Induction and growth of callus derived from rachilla explants of young inflorescences of coconut palm

YUKIO SUGIMURA[1] AND MYRNA J. SALVAÑA

Quality Control / Research and Development Department, Pilipinas Kao, Inc., P.O. Box 15, Cagayan de Oro City, Philippines

Received January 7, 1988

SUGIMURA, Y., and SALVAÑA, M. J. 1989. Induction and growth of callus derived from rachilla explants of young inflorescences of coconut palm. Can. J. Bot. **67**: 272–274.

When suitable stages of rachilla tissues derived from coconut palm were selected as an explant source, a higher frequency of callus induction was observed in medium containing activated carbon and 20–30 ppm 2,4-dichlorophenoxyacetic acid (2,4-D).

SUGIMURA, Y., et SALVAÑA, M. J. 1989. Induction and growth of callus derived from rachilla explants of young inflorescences of coconut palm. Can. J. Bot. **67** : 272–274.

Lorsque l'on choisit des tissus du rachille de la noix de coco comme source d'explants à des stades convenables, l'on observe une fréquence élevée d'induction de cals sur un milieu contenant du carbone activé et 20 à 30 ppm d'acide dichloro-2,4 phénoxyacétique.

[Traduit par la revue]

Clonal propagation of coconut palm by tissue culture has been attempted on numerous occasions (2, 3). When the rachilla tissues of young inflorescences were used as an explant source, they were capable of producing nodular structures, which later developed into leaf- and (or) root-like growths via embryogenesis (4, 8).

A prominent problem of browning has been noted in coconut tissue culture (1, 7, 10). This browning response might be minimized by selecting a suitable stage of explant cultured under defined conditions. In this study, using explants from different stages of immature inflorescences, the frequency of callus induction and browning response are quantified for several culture and slicing media.

A series of inflorescences was collected from approximately 30-year-old coconut palm (*Cocos nucifera* L. cv. Typica). Immature inflorescences ranging from 0.4 to 7.8 cm in length were selected. The corresponding rachillae (inflorescence branches) were isolated from the rachis (Fig. 1). The basal portion of each rachilla was discarded to exclude the female flower meristems. The remaining portion of rachilla with numerous male flower meristems was used as a source of explants.

For surface sterilization, inflorescences enclosed by outer and inner spathes were dipped in 3% H_2O_2 for 15 min and 80% ethanol for 5 s under aseptic conditions. After thorough washing with sterilized water, the outer and inner spathes were peeled off. The rachilla tissues were then sliced transversely (0.2–0.5 mm thick) in a medium composed of Y3 formulation (5) supplemented with 3% sucrose. Except for vitamins, which were added at one-half strength, the medium described previously (4) was used for callus induction. The cultures were kept in darkness inside an incubator controlled at 28 ± 2°C until well-developed calluses were obtained.

Coarse slices of nonuniform explant size exhibited browning regardless of the stage of the inflorescence. Reducing the thickness of the transverse slices minimized browning; for example, using a 4.6-cm length of inflorescence, 32% browning occurred in 1-mm slices and 11% in 0.5-mm slices. Blake (2) mentioned that smaller explants have a better survival rate than larger explants, which correlates with their reduced browning.

For slicing the rachilla tissues, different liquid slicing media were tried, such as sterile water, Y3 formulation, and basal medium for callus induction. Among the slicing media tested, the Y3 plain medium was most effective in preventing browning.

Initially, to determine a suitable stage of explant, tissue slices taken from a series of inflorescence lengths ranging from 0.4 to 7.8 cm were evaluated in terms of callus induction and browning. Observations showed (*i*) explants taken from inflorescences more than 3.8–4.7 cm long had slight callusing and a higher rate of browning; (*ii*) explants derived from inflorescences less than 0.4–0.8 cm long had minimal browning, but the expansion of tissues was very slow and induction of callus seldom occurred; and (*iii*) explants taken from inflorescences between 0.8 and 3.8 cm long were relatively higher callus induction and showed minimal browning.

On the basis of preliminary results, inflorescences of five stages comprising 0.98-, 1.4-, 1.6-, 2.5-, and 4.1-cm lengths were considered. One-hundred tissue slices for each stage were then inoculated into media containing a wide range of 2,4-dichlorophenoxyacetic acid (2,4-D) concentrations (5–100 ppm) in the presence of 0.25% activated carbon. Discoloration of tissues and initiation of callus were closely monitored during the initial culture period of 1–1.5 months. As shown in Table 1, stages 4 and 5 seemed to be satisfactory sources of explants, since a high rate of callus induction could be obtained with minimal browning. Stages 1, 2, and 3 showed

[1]Present address: Tochigi Research Laboratories, Kao Corporation, 2606 Akabane, Ichikaimachi, Haga, Tochigi, 321-34, Japan.

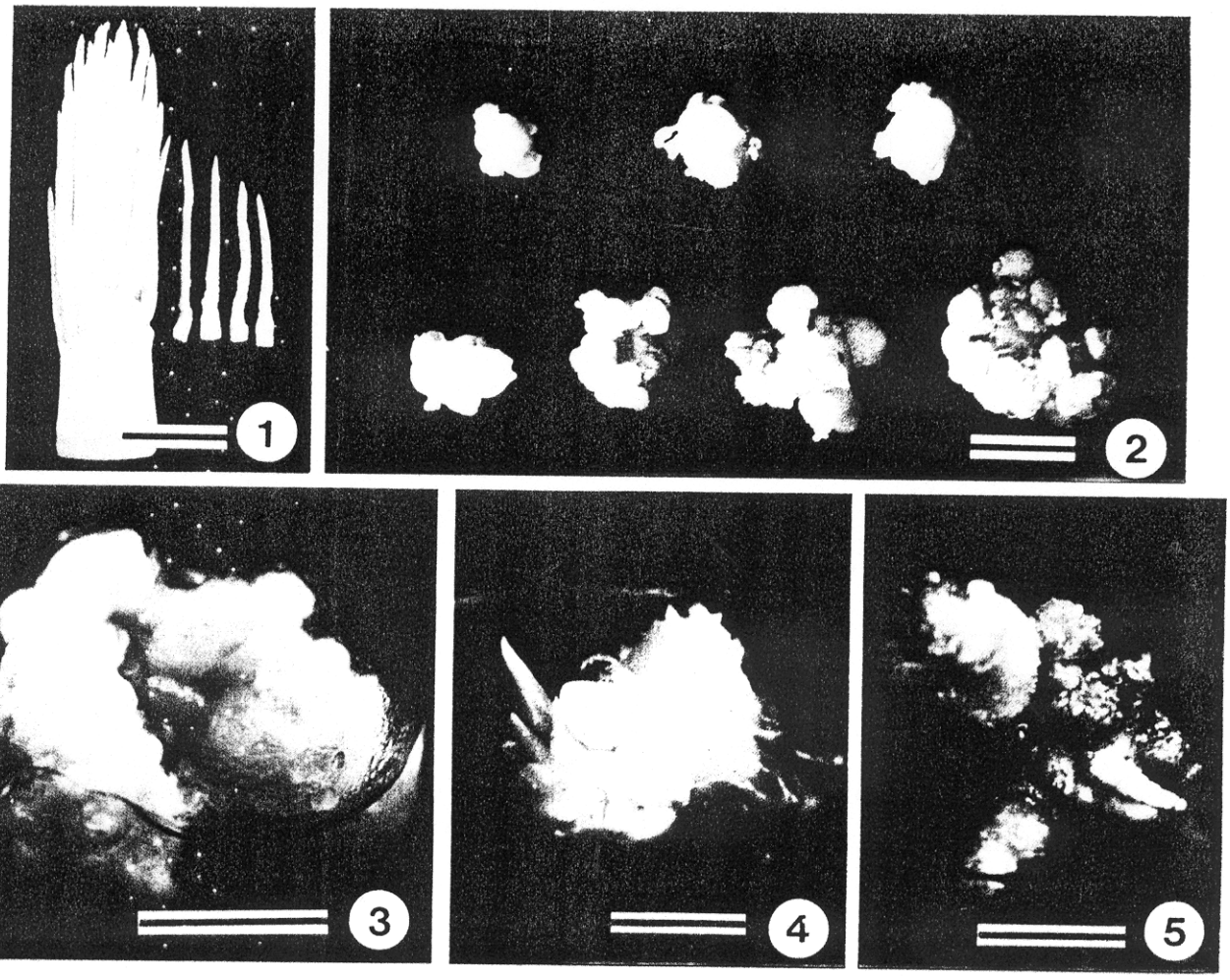

FIG. 1. Immature inflorescence and rachillae isolated from rachis for use as a source of explants. FIG. 2. A series of developments of finely sliced explants during culture in 20 ppm 2,4-D. Small nodular callus initiated at the regions of male flower meristems continued to grow into larger masses 2–3 months after the start of culture. FIG. 3. Callus proliferated during subcultures in 20 ppm 2,4-D at monthly intervals. FIG. 4. Shoot-like structure initiated from nodular mass. The concentration of 2,4-D was reduced stepwise from 20 to 1 ppm. FIG. 5. Abnormal projections developed from top part of rachilla cultured in 20 ppm 2,4-D for 6 months. Bars represent 1 cm.

a lower frequency of callus induction. Considering the various concentrations of 2,4-D tested, better induction and growth of callus could be demonstrated consistently at 10–30 ppm 2,4-D and particularly at 20–30 ppm. At 50 and 100 ppm 2,4-D, severe browning of tissues was observed regardless of the stage and size of cultured tissues. When explants were cultured with 10 ppm 2,4-D without activated carbon, browning was induced in all explants. The inclusion of activated carbon played a key role in minimizing browning. Although addition of activated carbon may reduce the availability of 2,4-D at higher concentrations, these high concentrations are still fatal to the rachilla tissues. In contrast, for date palm, callus could be initiated successfully at 100 ppm 2,4-D in the presence of activated carbon (9). In fact, in most palm explants the incorporation of activated carbon probably gives its beneficial effects by minimizing browning (10).

Using cultures with 20 ppm of 2,4-D, the development of rachilla callus was continuously observed. It was noticed during the initial culture period that explants expanded quickly. This was followed by initiation of small nodular calluses at the regions of male flower meristems (Fig. 2). Subculturing of the calluses was successful using the initial medium composition. They continued to develop into nodular masses when subcultured at monthly intervals (Fig. 3). When transferred to a reduced concentration of 2,4-D (5–10 ppm), more defined structures such as white nodular and translucent masses appeared after three or four subcultures. During further subculturing on low concentrations of 2,4-D (1–5 ppm), green shoot-like structures developed under fluorescent illumination (Fig. 4).

Other responses were also observed in explants derived from the top part of rachilla. After expansion of cultured tissues, instead of callus induction, abnormal projections were induced. These continued to elongate during subculture (Fig. 5). Similar projections have been observed previously (3, 6). In natural conditions, male flower blooming usually commences from the top portion of each inflorescence branch. Thus, asynchronous development may take place in floral meristems between the top and basal parts and the different responses may depend on the developmental stages of the undifferentiated male flowers.

In conclusion, to obtain a relatively uniform response and a high frequency of callus induction, it is essential to select a particular portion of rachilla, excluding the top and basal portions, and also to use the fourth and fifth stages of the immature inflorescence.

TABLE 1. Effect of 2,4-D concentration on callus induction in rachilla explants from different inflorescence stages

2,4-D concn. (ppm)	Stage of explant*	Callus induction (%)	Browning (%)
5	1	0	18
	2	58	3
	3	46	9
	4	52	10
	5	80	9
10	1	21	21
	2	59	0
	3	80	5
	4	88	8
	5	92	0
20	1	35	10
	2	64	0
	3	86	0
	4	92	2
	5	90	0
30	1	62	0
	2	68	0
	3	70	8
	4	98	0
	5	98	0
40	1	46	0
	2	55	19
	3	64	8
	4	92	2
	5	85	4

*Explant stages were classified by length of inflorescences used: stage 1, 0.98; stage 2, 1.4; stage 3, 1.6; stage 4, 2.5; and stage 5, 4.1 cm.

Acknowledgements

We thank Dr. K. Okamoto and Mr. W. Higashi for their valuable support and encouragement in this undertaking, and also Mr. S. Ueda for his technical assistance.

1. APAVATJRUT, P., and BLAKE, J. 1977. Tissue culture of stem explants of coconut (*Cocos nucifera* L.). Oleagineux, **32**: 267–271.
2. BLAKE, J. 1983. Tissue culture propagation of coconut, date and oil palm. *In* Tissue culture of tree. *Edited by* J. H. Dodds. Croom Helm, London. pp. 29–50.
3. BLAKE, J., and EEUWENS, C. J. 1981. Culture of coconut palm tissues with a view to vegetative propagation. *In* Proceedings of the Committee on Science and Technology in Developing Countries (COSTED) April 28–30, 1981, symposium on tissue culture of economically important plants. COSTED and ANBS (Asian Network for Biological Sciences), Singapore. *Edited by* A. N. Rao. Singapore. pp. 145–148.
4. BRANTON, R. L., and BLAKE, J. 1983. Development of organized structures in callus derived from explants of *Cocos nucifera* L. Ann. Bot. (London), **52**: 673–678.
5. EEUWENS, C. J. 1976. Mineral requirements for growth and callus initiation of tissue explants excised from mature coconut palm (*Cocos nucifera* L.) and cultured *in vitro*. Physiol. Plant. **36**: 23–28.
6. EEUWENS, C. J., and BLAKE, J. 1977. Culture of coconut and date palm tissues with a view to vegetative propagation. Acta Hortic. **78**: 277–286.
7. FISHER, J. B., and TSAI, J. H. 1978. In vitro growth of embryos and callus of coconut palm. In Vitro, **14**: 307–311.
8. GUPTA, P. K., KENDURKAR, S. V., KULKARNI, V. M., SHIRGURKAR, M. V., and MASCARENHAS, A. F. 1984. Somatic embryogenesis and plants from zygotic embryos of coconut (*Cocos nucifera* L.) *in vitro*. Plant Cell Rep. **3**: 222–225.
9. REYNOLDS, J. F., and MURASHIGE, T. 1979. Asexual embryogenesis in callus cultures of palms. In Vitro, **15**: 383–387.
10. TISSERAT, B. 1984. Date palm. *In* Handbook of plant cell culture. Vol. 2. *Edited by* W. R. Sharp et al. Macmillan Publishing Co., New York. pp. 505–545.

Seed bank populations in upland coniferous forests in central Alberta

JAMES W. FYLES[1]

Department of Botany, University of Alberta, Edmonton, Alta., Canada T6G 2E3

Received January 6, 1988

FYLES, J. W. 1989. Seed bank populations in upland coniferous forests in central Alberta. Can. J. Bot. **67**: 274–278.

The species composition and abundance of viable seed in organic forest floor and surface mineral soil from two high-latitude jack pine dominated and two white spruce dominated stands were determined by enumerating germinants from samples placed on moist peat-moss beds. Estimated seed density ranged from 500 to 2600 seeds/m^2, representing 13 species of trees, shrubs, and forbs. About half of the species in the seed bank were present as mature plants in each stand. The high seed densities recorded are inconsistent with the previously proposed poleward decline in the abundance of buried seed. The role of buried viable seed differed among species in relation to inherent seed dispersal capabilities and seed longevity. Several species were recorded that are considered to have a very short period of viability in the soil. It is suggested that even a short residence in the seed bank may be adaptive in areas where spring burning accounts for a large proportion of the total area burned annually.

[1]Present address: Department of Renewable Resources, Macdonald College of McGill University, Sainte-Anne-de-Bellevue, Que., Canada H9X 1C0.