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DEVELOPMENT OF CLONAL PLANTLETS FROM IMMATURE PANICLES OF CARDAMOM*

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ABSTRACT

Cultured immature panicles of cardamom formed plantlets directly without the intervention of callus or embryoids, on MS medium supplemented with 0.5 mg/l NAA, 0.5mg/l kinetin, 1.0 mg/l BAP, 0.1mg/l calcium pantothenate, 0.1 mg/l folic acid and 10% coconut water. The stage of development of the explant and the growth regulators in the medium are the two major factors influencing the frequency of shoot formation in the immature panicle cultures. The transformation occurs due to the reversal of morphogenesis in the floral primordia into vegetative shoots.

INTRODUCTION

There are some reports of plantlet formation from inflorescence cultures through callus and embryoids in rice, wheat, sorghum, pearl millet and sugarbeet (Brettell, Wernicke and Thomas 1980; Coumas-Gilles et al., 1981; Vasil and Vasil, 1982; Ozias-Akins and Vasil 1982; Ling et al., 1983). Reports of plantlet formation direct from panicle cultures without a callus phase or embryoid formation are rare. Ling et al., (1983) has reported plantlet formation from immature floral buds in rice. Nadgauda, Mascarenhas and Madhusoodhanan (1982) have reported clonal propagation of cardamom using vegetative buds from rhizome, achieving a 1:3 rate of multiplication. In this paper, we report the development of plantlets directly from immature panicles of cardamom in culture media.

MATERIALS AND METHODS

The 'Malabar' cultivar of cardamom [*Elettaria cardamomum* (L) Maton] obtained from CPCRI Research Centre, Appangala (Coorg) was raised especially for the tissue culture work, at the Institute farm at Kasaragod under shade of coconut trees. The material used consisted of immature panicle buds at various stages of development.

The stage of the immature panicle : The immature panicles measuring 0.5 to 5 cms after initiation from the base of the pseudostem, were used as explant. The buds of appropriate size were separated and washed in running tap water. The outermost bracts were

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peeled off and after cleaning with 70% alcohol, the buds were soaked in 0.12 per cent (w/v) solution of mercuric chloride ($HgCl_2$) for 10 to 15 minutes and washed 6 times in sterilised water. Later, the buds were dissected out aseptically in petridishes, and cultured.

Medium: MS (Murashige and Skoog, 1962), White's (White, 1963) and B_5 (Gamborg, Miller and Ojima, 1968) were used as basic media supplemented with NAA, Kinetin, BAP, calcium pantothenate, Folic acid and coconut water (Table I). The pH was adjusted to 5.8–6.0 prior to the addition of agar (0.8%).

The cultures were incubated at 25±1°C under illumination of 1500 lux from fluorescent tubes. Humidity was maintained at 75% RH, in a BOD incubator.

After initial incubation of cultures for two weeks, they were subcultured on to fresh media of same composition as well as to media containing varying levels of growth regulators, for eliciting enhanced vegetative growth and rooting response to get normal plantlets.

RESULTS AND DISCUSSION

Of the several sizes of the inflorescence explants tried in culture, those measuring 1.5 to 2.5 cm proved most suitable for getting them converted into vegetative shoots. When the size of explant increased beyond 2.5 cm, we observed only development of normal flowers rather than vegetative shoots. Buds smaller than 1 cm retained their green colour for a long period although no elongation was observed. In initial experiments, sprouting buds were inoculated on the basal media without supplements. Of the three basal media tried, MS medium gave the best response. On MS basic alone, the explants retained their green colour without any further growth. This basic medium was used for further studies. The influence of auxins, cytokinins, vitamins and CW on growth of cardamom buds was studied separately as well as in combinations (Table I).

On MS medium supplemented with NAA alone (0.1 to 5.0 mg/l) even after incubation for 6 weeks no response was noticed. In a medium supplemented

Table I. *Effect of different media on shoot bud formation*

Media	Response
MS basic medium	+
MS + NAA (0.1 to 5.0 mg/l)	+
MS + Kn (0.05 to 2.00 mg/l)	+
MS + BAP (0.05 to 2.0 mg/l)	+
MS + BAP and Kn (0.5 and 0.5 mg/l)	++
MS + NAA (1.0 mg/l) + Kn (0.5 mg/ml) + BAP (0.5 mg/ml + CW (5%)	+++
MS + NAA (0.5 mg/l) + Kn (0.5 mg/l) + BAP (1.0 mg/l) + Calcium pantothenate (0.1 mg/l) + folic acid (0.1 mg/l) + CW, (10%)	++++

\+ buds green no elongation
++ buds green elongated (0.1–0.5 cm)
+++ buds green further elongation (1–2 cm)
++++ buds green forming bulbil shoots

with kinetin or BAP (0.01 to 2.0 mg/l), the buds retained their green colours but no elongation was noticed even after six weeks of incubation. The green buds elongated considerably after two weeks on MS medium supplemented with 1.0 mg/l NAA, 0.5 mg/l Kn, 0.5 mg/l BAP and 5% CW. However, the bracts remained unopened even after prolonged incubation. These buds when transferred to fresh medium with reduced auxin (0.5 mg/l) and increased cytokinin levels (0.5 mg/l Kn and 1.0 mg/l BAP), developed considerable swelling at the base. On further transfer to media supplemented with vitamins calcium pantothenate (0.1 mg/l) folic acid (0.1 mg/l and CW (10%), the lower bracts opened out and from their axils new shoot bud primordia started developing. On repeated subculturing, a large number of well-developed shoot buds were developed (Fig. 1).

In cases where the initial green bud was allowed to remain for more than 6 weeks on MS medium supplemented with 0.1 mg/l NAA, 0.5 mg/l Kn, 1.0 mg/l BAP and 10 per cent CW the panicle buds elongated considerably and from the axil of the upper bracts new shoot buds started sprouting. These buds grew further and formed a crown of vegetative shoots at the top of the panicle explant on subsequent subtransfer to fresh media of the same composition (Fig. 2).

Rooting : For the induction of roots, these shoot buds were transferred to a fresh medium containing higher auxin (2 mg/l NAA) and lower cytokinin (0.05 mg/l Kn) levels and incubated in dark for 5 days. The shoots also grew rapidly during this period (Fig. 3). In cases where the development of shoot buds was from the top of the panicle explant, enhanced vegetative growth of the sprouting bud was observed.

These buds have evidently developed by the reversal of the original developmental pathway from the floral to vegetative morphogenesis as a result of the stimulus provided by the medium. It can be due to one or more of the elements or a combination of auxin and cytokinins (NAA & Kn/BAP), although we cannot yet pinpoint the exact phenomenon responsible for this reversal of floral primordia into vegetative buds. This method could profitably be used for the rapid clonal multiplication of elite cardamom using floral primordia also, in addition to the production of multiple shoots from vegetative buds.

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Fig. 1. Bulbil shoots sprouting from immature floral buds cultured on MS medium containing Ca pantothenate (0.1mg/l), folic acid (0.1mg/l) and coconut water (CW) (10%). Fig. 2. Crown of vegetative shoots emerging from the top of immature spike cultured on MS medium supplemented with NAA (0.5 mg/l), Kinetin (0.5 mg/l), BAP (1 mg/l) and CW (10%). Fig. 3. Induction of roots on the bulbil shoots cultured on MS+NAA (2 mg/l) + Kinetin (0.05 mg/l) and incubated in dark for 5 days.

REFERENCES

BRETTELL, R. I. S., WERNICKE, W. and THOMAS, E. 1980. Embryogenesis from cultured immature inflorescences of *Sorghum bicolor*. *Protoplasma*. **104**: 141–148.

COUMAS-GILLES, M. F., KEVERS C. L., CENTEMANS, E. and GASPER, Th. 1981. Vegetative multiplication of sugarbeet through *in vitro* culture of inflorescence pieces. *Plant Cell Tissue and Organ culture* **1**: 93–101.

GAMBORG, O. L., MILLER, R. H. and OJIMA, K. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell. Res.* **50**: 151–158.

KURUVINASHETTI, M. S., and IYER, R. D. 1982. An evaluation of tissue culture techniques in coconut and turmeric. In S. Vishweswara, *et al.* (eds.); *Proc. PLACROSYM IV–1981*, Mysore. pp. 101–106.

LING, D. H., CHEIN, W. Y., CHEN M. F. and MA,Z.R. 1983. Direct development of plantlets from immature panicles of rice *in vitro*. *Plant Cell Reports*. **2**: 172–174.

MURASHIGE, T. and SKOOG, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant*. **15**: 473–493.

NADGAUDA, R. S., MASCARENHAS, A. F. and MADHUSOODHANAN, K. J. 1983. Clonal multiplication of cardamom *(Elettaria cardamomum* Maton) by tissue culture *J. Plant. Crops*. **11**: 60–64.

OZIAS-AKINS, P. and VASIL, I. K. 1982. Plant regeneration from cultured immature embryos and inflorescences of *Triticum aestivm* L. (Wheat) evidence for somatic embryogenesis. *Protoplasma*. **110**: 95–105.

VASIL, V. and VASIL, I. K. 1982. Characterisation of an embryogenetic cell suspension culture derived from cultured inflorescence of *Pennisetum americanum* (Pearl Millet: Gramineae). *Amer. Jour. Bot.* **69**: 1441–1449.

WHITE, P. R. 1963. *The Cultivation of Animal and Plant Cells*. Ronald Press, New York. pp. 59.