Proc. Indian Acad. Sci. (Plant Sci.), Vol. 91, No. 1, February 1982, pp. 37–41.

Regeneration of plantlets from callus of *Elettaria cardamomum* Maton

N K SRINIVASA RAO, S NARAYANASWAMY*, E K CHACKO and R DORE SWAMY

Division of Plant Physiology and Biochemistry, Indian Institute of Horticultural Research (ICAR), Bangalore 560 080, India

*Emeritus Scientist, Council of Scientific and Industrial Research, New Delhi

MS received 3 November 1980 ; revised 1 October 1981

Abstract. Embryo callus and callus of rootstocks of *in vitro*-raised seedlings of *Elettaria cardamomum* were grown on MS medium supplemented with CW + 2, 4-D + BAP. Differentiation of shoot buds, roots and leaves leading to the development of plantlets could be induced in callus by withdrawing 2, 4-D or substituting it by IAA or NAA in low concentrations.

Keywords. Callus culture ; cardamom ; regeneration.

1. Introduction

Reports on induction of shoot buds and whole plants from tissue cultures of both monocotyledonous and dicotyledonous plants have been numerous in recent years as evident from the spate of publications on the subject (see reviews by Murashige 1974 ; Narayanaswamy 1977). Clonal propagation through tissue culture has been successful with many spice and condiment plants such as *Foeniculum vulgare* (Maheshwari and Gupta 1965), *Anethum graveolens* (Ratnamba and Chopra 1974), *Carum corvi* (Ammirato 1974) and *Capsicum annuum* (Gunay and Rao 1978). Spectacular rate of multiplication of turmeric (*Curcuma longa*) plants have been reported in cultures of young vegetative buds isolated from the root stock (Nadaguada *et al* 1978). This prompted us to investigate the potential for organogenesis in tissue cultures of the cardamom (*Elettaria cardamomum* Maton of Zingiberaceae) widely used as a condiment. This paper reports the successful regeneration of shootbuds and plantlets from seedling callus of the herbaceous perennial species.

2. Methodology and results

2·1. *Seed germination*

Dry seeds of cardamom were surface sterilised by 0·1% mercuric chloride solution to which a few drops of the detergent Teepol had been added. After washing

Contribution number 944 of Indian Institute of Horticultural Research.

thoroughly in sterilized water, the seeds were sown on White's (1963) nutrient agar. Slender seedlings were obtained in three weeks on incubation at 26° C. Whole seedlings bearing the first sheathing leaf and plumule were transferred to Murashige and Skoog's (1962) medium (MS) to which auxins, cytokinins and coconut water (CW) as specified (table 1) and sucrose (2%) had been added. Fe EDTA was used as the iron source. Each treatment comprised 12 replicates. Embryo callus was also obtained directly from seeds sown on the medium (figure 1) containing an auxin such as 2, 4-D (2,4-dichlorophyenoxy acetic acid).

2·2. *Callus induction*

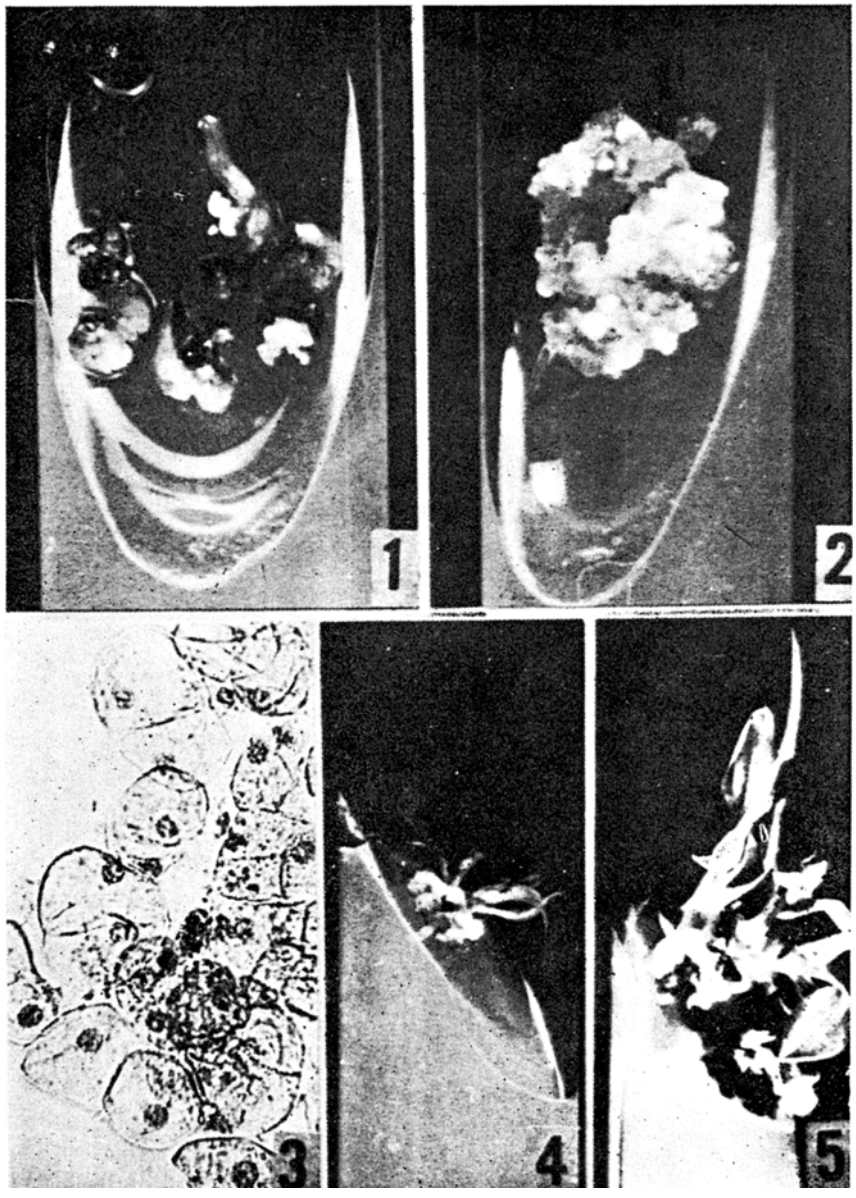
Five-week-old 3 cm long seedlings bearing the first sheathing leaf and plumule were transferred to MS medium to which CW (18% v/v) + 2, 4-D(2mg/l) or indole-3-butyric acid (IBA 2mg/l) or naphthalene acetic acid (NAA 2mg/l) and benzylaminopurine (BAP 2mg/l) had been added in combinations as needed. Proliferation of cells from the rootstock was observed in 75% of the cultures resulting in the formation of exuberant callus in 6 weeks after incubation (figure 2). MS supplemented with CW (18% v/v) + 2, 4-D (2mg/l) + BAP (0·5mg/l) was conducive for callus initiation and growth which could be augmented by the addition of casein hydrolysate (CH, 1gm/l) in the medium. Neither yeast extract 250mg/l nor malt extract (250mg/l) proved favourable for callus growth; 2, 4-D could, however, be replaced by IBA for callusing. Propionocarmine squashes of the proliferating callus showed cells of diverse sizes and shapes (figure 3). Tracheidal differentiation of cells was marked.

2·3. *Regeneration of shoot buds*

Callus growing on MS + CW + 2, 4-D was subcultured on medium devoid of 2, 4-D but containing IAA (2mg/l) or NAA (1mg/l). Three weeks after transfer green nodular structures developed in the callus indicating the initiation of organised growth centres. Callus grown on medium with higher concentrations of the auxin became friable and was not conducive for shoot bud induction. But induction

Table 1. Response of cardamom calli to growth regulators *in vitro* on sequentia transfer.

Sl. No.	Media composition (Hormone concentrations in mg/l)	Nature of response
1.	MS + CW (18% v/v) + 2,4-D (2)	Callusing good
2.	MS + CW (18% v/v) + 2,4-D (2) + CH (1g/l) + BAP (0·5)	Callusing exuberant
3.	MS + CW (18% v/v) + IAA or IBA (1) + NAA (2)	Callus grew as vascular nodules
4.	MS + CW (18% v/v) + IAA (1) + BAP (2)	Shoot bud initiation in callus (80%)
5.	MS + CW (10% v/v) + BAP (2-5) + IAA (1)	4-6 shoot buds per subculture



Figures 1–5. Callus induction and shoot bud regeneration in cardamom. **1.** Callusing of seedlings *in vitro* on MS + CW (18 %v/v) + 2,4-D (2 mg/l) +BAP (0·5 mg/l) 6 weeks after incubation. × 1·5. **2.** Nodular callus derived from root stock of seedling transferred to MS medium + IAA (1 mg/l) + NAA (2 mg/l) × 1·5. **3.** Propionocarmine squash preparation from root-stock callus piece showing free cells and cell aggregates × 800. **4.** Regeneration of a shoot bud in primary callus grown on MS + BAP (2 mg/l) + IAA (mg/l), × 1·5. **5.** Cluster of shoot buds regenerated from callus subcultures on MS + CW (10 %v/v) + BAP (2 mg/l) + IAA (1 mg/l) × 1·5.

occurred if MS medium was supplemented with CW (10% v/v) + BAP (2–5 mg/l) with or without the addition of IAA and incubated for 6 weeks under 500 lux (figures 4,5). Four to six shoot buds could be obtained from each callus subculture of uniform size. Rooting occurred at the base of individual shoot buds on prolonged incubation in the same medium (aged cultures) or when individual shootlets were isolated and grown on White's medium to which NAA (2mg/l) and sucrose (1%) had been added.

3. Discussion

Regeneration of plantlets in callus culture is an alternate means of propagation in cardamom. Callus subcultures could develop 4–6 regenerants, each of which was capable of rooting, when isolated and grown, forming a whole plant. Plantlets propagated thus might not conform to parent genotype, having been obtained from seedling calli. Nevertheless, tissue culture provides a method by which a large number of strains could be obtained for selection of desirable variants. Also, it offers a method of rapid multiplication of elite varieties through multiple shoot induction. Preliminary studies have shown that test tube plants could be successfully transferred to soil.

Acknowledgements

The authors thank Dr G S Randhawa, the then Director, Indian Institute of Horticultural Research, Bangalore, for his keen interest and for providing facilities for tissue culture work. Thanks are also due to Dr D Gundu Rao, Senior Plant Pathologist, for the supply of cardamom seeds.

References

Ammirato P V 1974 The effects of abscisic acid on the development of somatic embryos from cells of caraway (*Carum carvi* L.) ; *Bot. Gaz.* **135** 328–337

Gunay A L and Rao P S 1978 *In vitro* regeneration from hypocotyl and cotyledon explants of red pepper (*Capsicum annum*) ; *Plant Sci. Lett.* **11** 365–372

Maheshwari S C and Gupta R P G 1965 Production of adventitious embryoids *in vitro* from stem callus of *Foeniculum vulgare* ; *Planta* **67** 384–386

Murashige T 1974 Plant propagation through tissue culture ; *Ann. Rev. Plant Physiol.* **25** 135–166

Murashige T and Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures ; *Physiol. Plant.* **15** 473–497

Nadaguada R S, Mascarenhas A F, Hendre R R and Jaganathan V 1978 Rapid multiplication of turmeric (*Curcuma longa* Linn.), plants by tissue culture ; *Indian J. Exp. Biol.* **16** 120–122

Narayanaswamy S 1977 Regeneration of plants from tissue cultures; In *Fundamental and Applied Aspects of Plant Cell, Tissue and Organ Culture* (eds.) J Reinert and Y P S Bajaj (Berlin : Springer–Verlag) pp. 179–250

Ratnamba S P and Chopra R N 1974 *In vitro* induction of embryoids from hypocotyl and cotyledens of *Anethum graveolens* seedlings; *Z. Pflanzenphysiol.* **73** 452–455

White P R 1963 *The Cultivation of Animal and Plant Cells* (New York: Ronald Press)