AN EVALUATION OF TISSUE CULTURE TECHNIQUES IN COCONUT AND TURMERIC

MS KURUVINASHETTI AND RD IYER

Central Plantation Crops Research Institute
Kasaragod-670 124, Kerala

ABSTRACT

In vitro studies in coconut and turmeric were undertaken with the main objectives of achieving rapid multiplication of elite genotypes.

In coconut a stage in the development of flower primordia was standardised for their conversion into vegetative structures on Y3 medium supplemented with 2mg/*l* BAP 0.5 mg/*l* Kinetin. Shoot-like structures were produced on the rachilla explants excised from the spadices which were extracted from the axils of first (mainly) to third open leaves on the crown. Rooting of these structures was not regular. Calli were induced from stem and petiole explants of one year old WCT seedlings on Y3 medium supplemented with 0,5 to 1.0 mg/*l*. 2,4-D. Culture of isolated embryos was done on MS medium supplemented with 200 mg/*l*. KC*l*. Embryos took four months to attain the first open leaf stage. Rooting of the seedlings was not regular. Seedlings when grown on liquid medium supplemented with 1 mg/*l* IBA produced roots.

In turmeric, a tissue culture method for rapid multiplication of clone 15 B was developed. On MS medium supplemented with 0.2 mg/*l* kinetin and 0. 4mg/*l* BAP, the rate of multiplication was of the order of eight plantlets from every bud cultured for two months, which works out to over two lakh plantlets per bud per year. For initiating callus cultures, buds were grown on the same medium supplemented with 2 mg/*l* IAA or 0.5 mg/*l* 2, 4-D in dark. These calli were soft and friable. When exposed to light, IAA induced calli underwent differentiation to produce several plantlets. These plantlets could be separated and grown further before they were transplanted to polybags. These plants are growing very vigorously in the glasshouse for the last 8 months.

INTRODUCTION

Success in tissue culture of coconut palm is at present limited to culture of immature and mature embryos, which is being regularly used for the multiplication of 'makapuno' mutant palms in

Philippines (Guzman and Rosario, 1974). Although initially the conversion of floral primordia into vegetative shoots appeared to be possible (Schwabe, 1973; 1976) this is yet to be established as a regular technique for vegetative propagation of elite coconut palms. Several groups of workers have reported the induction of callus growth on embryo, root, endosperm and seedling tissue explants (Guzman *et al.*, 1978, Fulford *et al.*, 1976, Fisher and Tsai, 1978; Blake; 1981). D'Souza (1980) reported the formation of bud-like outgrowths from hypocotyl region of normal coconut embryos and their morphogenesis into root and shoot-like structures.

In turmeric, high rates of bud multiplication have been achieved in clones, Duggirala and Tekurpeta (Nadgouda *et al.*, 1978) and in clone 15 B along with induction of callus and further morphogenesis of viable plantlets (Kuruvinashetti and Iyer, 1980).

Materials and Methods

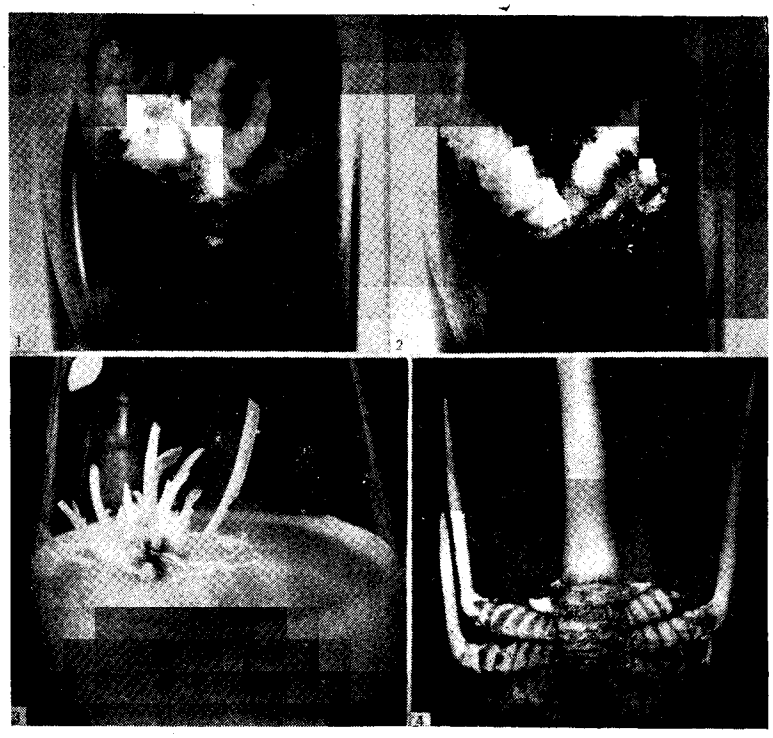
In all studies on coconut, the West Coast Tall variety was used. Rachilla explants were taken from spadices borne at the axil of the youngest, fully open leaf on the crown. Embryos were scooped out of mature coconuts after breaking them open. Experiments on multiple shoot induction in turmeric were carried out on fresh buds collected from sprouting rhizomes of clone 15 B of *Curcuma domestica*. Surface sterilization of coconut embryos and turmeric buds was done with freshly prepared chlorine water and of spadices with 70 per cent ethanol. Explants were cultured in tubes containing 10-20 ml of Eeuwen's (1976, Y-3) or Murashige and Skoog's (1962, MS) medium.

Results and Discussion

Coconut

Experiments conducted for the conversion of floral primordia into vegetative shoots, resulted in variable response. The stage of development of the floral primordium is a major factor in deciding what types of shoots would develop. The best response was obtained on Y3 medium supplemented with low auxin (NAA 0.02 mg/l) and high cytokinin (BAP 5.0 mg/l). Shoot-like structures produced from floral primordia had all the floral parts trans-

formed into scale leaves. Older flower buds produced well differentiated male and female flowers in culture. It appears that the central pistillode of the male flower have not been already differentiated if shoot-like structures with indeterminate apex are to arise. This transformation was a rare event. When the explants were at the appropriate stage of development, about 50 per cent of the primordia produced shoot-like structures (Fig. 1). Rooting



Figs. 1-2. Coconut. Shoot-like structures from rachilla explants
1. Six weeks old 2. Ten weeks old

3-4. Turmeric.
3. Multiple shoots 4. Rhizome from tissue cultured plant

occurred very rarely, but together with shoots they did not survive. Non-survival of such shoot-like structures in spite of well differentiated roots has also been reported by Eeuwens (1978).

Callus induction : In order to find out alternative methods for clonal propagation, studies on induction of callus from adult and seedling palm tissues were taken up. It is relatively easy to induce callus growth on shoot tips, young leaf bases of seedling and adult palms on MS medium supplemented with 2, 4-D alone or in combination with NAA (Table 1). Callus appears as a white mass from the cut surfaces of the explant (Fig. 2). Browning of the tissues on further transfer and subculture has become a major hurdle in establishing callus cultures. Attempts to maintain and multiply calli for inducing organogenesis are in progress. Use of activated charcoal (2-10 g/l) in the agar medium prevented browning of tissues to a large extent.

Table 1. Effect of NAA and 2, 4-D on callus induction in seedling tissues

BM	+2, 4-D	+NAA	+Charcoal	No. of cultures	No. of calli induced	Remarks
BM	0.5	0	0	10	0	Severe browning of tissues
BM	1.0	0	0	10	0	,,
BM	1.5	0	0	10	0	,,
BM	0.5	0	0.2	10	3	White callus
BM	1.0	0	,,	10	4	,,
BM	1.5	0	,,	10	2	,,
BM	1.0	5.0	,,	10	3	,,
BM	1.0	10.0	,,	10	6	,,
BM	5.0	0	,,	10	1	Calli not well formed
BM	0	5.0	,,	10	1	,,
BM	0	10.0	,,	10	1	,,

BM : MS+200 mg/1 KCl Coconut water 20% Sucrose 40 g/1.
Kinetin 0.5 mg/1, 2, 4-D and NAA are in mg/1.

Embryo culture : Mature coconut embryos of WCT variety were cultured on MS medium containing additional KCl (200 mg/l) and 40 g/l glucose. Sprouting of embryos was better on a medium without added growth regulators like Kinetin, IAA, NAA and BAP. First fully expanded leaf appeared in about four months of culture. Sprouted embryos did not produce well developed roots. Seedlings grown on liquid medium added with IBA (1 mg/l) induced healthy roots.

Turmeric

Multiple shoots : Buds excised from sprouting rhizomes of clone 15 B developed multiple shoots (Fig. 3) on MS medium supplemented with BAP, Kinetin and sucrose (40 g/1). Of the several combinations tried, BAP (2 mg/1) and Kinetin (1 mg/1) induced the formation of maximum number of shoots. Individual shoots separated and cultured on the same medium multiplied further at the rate of about 8 plantlets each during a culture period of 60 days. At this rate of multiplication, over two lakh plants could be produced from a single bud established in culture during an year.

Root induction and establishment of plantlets : Plantlets separated from a bunch of shoots hardly had one or two small roots which often got damaged during the process of separation. When subcultured on half the concentration of MS (liquid) minerals with sucrose (20 g/1), several healthy roots were produced in 1-2 weeks. Rooted plantlets established very well (90%) in polybags containing a mixture of top soil and farm yard manure (3 : 1). The plants grew vigorously in the glass-house till maturity.

In turmeric it is well known that the yield of rhizomes is proportional to the weight of planting material. Further, Nambiar (1979) has shown that turmeric plants raised from seeds produced only a small mother rhizome and a few root tubers during the first year. The situation with tissue culture raised plants is similar to the seedlings at least in one respect that both started with neither enough material reserves to support initial growth nor additional buds to produce sucker shoots. However, plants raised through tissue culture produced well developed rhizomes (Fig. 4). Ten plants harvested 8 months after transplantation had produced a sizeable quantity of rhizomes (av. 97 g/pl. fresh wt.).

Though it is not advisable to grow these plantlets in field commercially, this method can generate a large volume of planting material from single plant selections for use in plant improvement programmes. This would reduce the time lag between identification of a single promising clone and its further testing in large scale yield trials before its release as a commercial variety.

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