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FACTORS INVOLVED DURING *IN VITRO* CULTURE OF *CALAMUS ROTANG*

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ROY, A. & SAHA, P.K. 1997. Factors involved during *in vitro* culture of *Calamus rotang*. *Calamus rotang is* a subterranean woody monocot where *in vitro* multiplication technique may be used as an alternative process for rapid plant generation. Since subterrestrial young plantlets face a problem in establishment due to a high degree of contamination in the medium, to reduce contamination, chemicals were tested for sterilisation of the explant. Sodium hypochlorite (5.0 % v/v) in combination with mercuric chloride (0.5 % w/v) resulted in the highest response. Explant establishment was dependent on their lengths as well as the plantlets from where the explants were derived. Explants of length 1.5 - 2.0 cm derived from plantlets having a length of 10 - 20 cm showed a higher rate of growth under culture conditions. Bud proliferation in explant was achieved in the establishment medium supplemented with BAP (6-benzylaminopurine) (5-10 mg l^{-1}).

Keywords: *Calamus rotang* - establishment - monocot - propagation - sterilisation

ROY, A. & SAHA, P.K. 1997. Faktor yang terlibat semasa kultur *in vitro Calamus rotang*. *Calamus rotang* ialah tumbuhan monokot subterranean yang menggunakan teknik pendaraban *in vitro* sebagai proses alternatif bagi generasi tumbuhan yang pantas. Oleh kerana anak pokok muda subdarat menghadapi masalah penubuhannya akibat daripada kontaminasi yang tinggi dalam medianya, bahan kimia diuji bagi pensterilan tumbuhan luar untuk mengurangkan kontaminasi. Sodium hipoklorit (5.0 % v/v) dengan kombinasi klorida merkurik (0.5% w/v) menyebabkan tindak balas tertinggi. Penubuhan eksplant bergantung kepada panjangnya sama seperti anak pokok dari mana eksplant diperoleh. Eksplant yang panjangnya 1.5 - 2.0 cm diperoleh daripada anak pokok yang panjangnya 10 - 20 cm menunjukkan kadar pertumbuhan yang tertinggi di bawah keadaan kultur. Percambahan tunas dalam eksplant dicapai dalam media penubuhan disokong dengan BAP (6 - benzilaminopurin) (5 - 10 mg l^{-1}).

Introduction

Calamus rotang, commonly known as cane, is a shrub of the tropical rain forest. The plant is designated as one of the important minor forest products (MFP) and used in multitier silviculture practices as a lower tier member (Hore 1994). It is also used in making furniture, wicker work and basketry. The plant reproduces

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through vegetative propagation by proliferation of adventitious buds which are present in the rhizome and require a minimum age of three years (Johari & Che Aziz 1981). Due to the lower rate of plantlet production by vegetative means it is difficult to bridge the gap between requirement and production. The fact remains that the natural reproduction is season dependent and propagation through seeds is meagre (Manokaran 1982).

Application of tissue culture technique is becoming increasingly popular as an alternative to vegetative reproduction (Choo 1990). Large numbers of plantlets can be produced within a short period of time through *in vitro* micropropagation (Debergh 1987, Gunawan 1991). The micropropagation technique can also be used for germplasm conservation of several types of cane available (CSIR 1986, Renuka 1990). Direct plant regeneration from axillary and adventitious buds has been reported from a few monocotyledonous plants (Hussev 1976). Leaf sheath, petiole and stem have been successfully used as explant for development of plantlets of date-palm (Tisserat 1982).

Although *in vitro* propagation of several other genera of the family Arecaceae, vis. *Elaeis, Phoenix, Cocos,* etc. has been reported (Eeuwens 1978, Tisserat & Mason 1980), the same for *C. rotang* is not much in evidence.

Investigations were carried out on the propagation of *C. rotang* and the factors responsible for *in vitro* explant establishment and multiplication are reported.

Materials and methods

Material

Calamus rotang plants aged between four and five years were selected from the Higuli forest at Ranaghat, Krishnanagar Forest Division, West Bengal, India. Plantlets developing from the rhizome of each of the selected mother plants were carefully excised alone with a portion of the rhizome and the roots of the mother plant. The lengths of the collected plantlets were recorded.

Processing of the collected plantlets

The plantlets were washed under running tap water for 30 min, treated with mild surfactant (teepol 0.1 % v/v) on a magnetic stirrer for 15-20 min and washed twice in distilled water. The leaf bases were removed acropetally one after the other and the lengths of the plantlets were reduced to 6 - 7 cm. After reducing each length, the white coloured material (with 4-5 leaf bases) was treated with antioxidant solution. This was done by dipping the material in PVP (polyvinyl polypyrollidone) (0.5 % w/v) solution for 20 min.

Sterilisation

The material was subjected to sterilisation with several chemicals,viz. potassium permaganate (0.01 % w/v), ethanol (70 % v/v), sodium hypochlorite (1.0 % v/v) and mercuric chloride (0.1 % w/v) for preliminary screening of the appropriate sterilising agent(s) which were then selected for testing at different concentrations, either singly or in combination, to reduce contamination. When using a combination of two sterilising agents, the material was first treated with sodium hypochlorite (5% v/v) for 5 -7 min and washed in sterile water (thrice) again. The material was then dipped in mercuric chloride (0.5 % w/v) solution containing two drops of teepol (0.1% v/v) and washed (thrice) again. It was then dissected into different lengths having the apical shoot meristem and a portion of the rhizome prior to inoculation. These operations were performed under a laminar flow hood.

Media and cultural conditions

Eeuwens ' (1976) Y3 basal medium supplemented with 2, 4 - D (2.4 - dichlorophenoxyacetic acid) (5 mg l^{-1}) and BAP (6-benzylaminopurine) (1.2 mg l^{-1}) with sucrose (5 % w/v) was used for explant establishment medium. Activated charcoal (0.3 % w/v) was also used to prevent browning of the medium. The medium was gelled with agar (0.8 % w/v) and autoclaved at 1 kg cm^{-2} for 15 min after adjusting the pH to 5.8. The cultures were maintained at 25 ± 2°C for 16 h photoperiod under white fluorescent tube light (9 watts cm^{-2}) and 78 % relative humidity. The explants were subcultured after each culture cycle of 28 - 30 days.

Selection of explant and plantlet length

Experiments were performed with different explant lengths containing two or more leaf bases and a portion of the rhizome. Explants of different lengths 0 - 0.5, 0.5 - 1.5, 1.5 - 2.5 and 2.5 - 3.5 cm were inoculated in the established medium to select the optimum length of the explant for growth. A separate experiment was conducted to select plantlet lengths from where the explants were chosen. Plantlets of different lengths, i.e. 0 - 10, 10 - 20, 20 - 30 and 30 - 40 cm were taken and reduced to 1.5- 2.5 cm explant length and cultured.

Multiplication of explant

BAP at different concentrations (5 - 10 mg l^{-1}) were tested for proliferation of axillary and adventitious buds of the explants.

Experimental design

For all the experiments, three replicates per treatment were carried and mean standard errors were calculated.

Results and discussion

Sodium hypochlorite and mercuric chloride proved effective among the different chemicals tested for explant sterilisation. Treatment with sodium hypochlorite (5.0 % v/v) and mercuric chloride (0.5 % w/v) separately showed, respectively, 45 % and 15 % contamination-free responding explant after 14 days in culture (Figure 1). A combination of sodium hypochlorite followed by mercuric chloride resulted in 55 % contamination-free responding explants. Use of two successive sterilising agents (mercuric chloride and ethanol) in oil palm has been reported by Choo (1990). After 10-15 days in culture, a positive response of explant was evidenced by the proliferation of the inner green leaf base without contamination (Figure 4a).

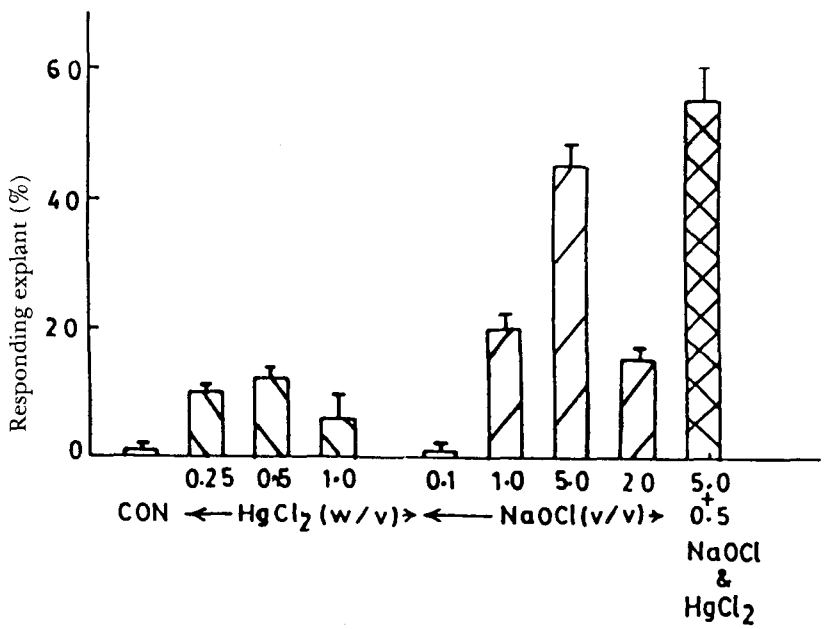


Figure 1. Effect of sodium hypochlorite (NaOCl) and mercuric chloride ($HgCl_2$) at different concentrations, as sterilising agents, either singly or in combination during explant establishment of *Calamus rotang* after 14 days in culture [CON - control]

Browning in both the outer surface as well as cut ends of the explant occurred within two days of culture. Accumulation of the exudate from the explants into the medium retarded the growth and subsequent degeneration of explants. Activated charcoal in the medium was capable of reducing the toxic effect of the exudate, showing a higher response (45 %) than that without charcoal (Figure 2). Activated charcoal probably acted as an adsorbent in the medium of the toxic exudate secreted from the cut ends. Surface oxidation was reduced by dipping the explants in antioxidant solution (PVP 0.5 % w/v) resulting in negligible surface browning in comparison with the untreated explants. In the date-palm, rapid browning of the explant was similarly prevented by dipping the explant in antioxidant solution prior to surface sterilisation (Tisserat 1982, Shaheen 1990). The *Calamus* explants of lengths 2-3 cm and with two or three white coloured leaf bases attached to the rhizome showed slight browning of the upper leaf sheath in the medium within the first week of culture. After 4 - 5 weeks, inner leaf bases proliferated with the emergence of leaflets on the sheath surface (Figure 4b). Subculture of these developing explants led to growth of the rachis and leaf in 8-9 weeks (Figure 4c). In culture, the leaves proliferated and spines developed in 12 weeks (Figure 4d). Entire leaf with leaflets developed in 16 weeks (Figure 4e).

Investigation with explant length (0-0.5 cm) showed that, within 2 - 4 days, 98 % turned brown and failed to respond (Table 1). Explants (2.5 - 3.5 cm) with more than three leaf bases showed growth but were prone to systemic contamination (80 %). Larger explants are not suitable due to their proneness to contamination as reported in *Nicotiana* by Murashige (1974). Explants of length between 1.5 and 2.5 cm showed maximum increase in length in the first cultural passage (Table l) in comparision with explants of other lengths.

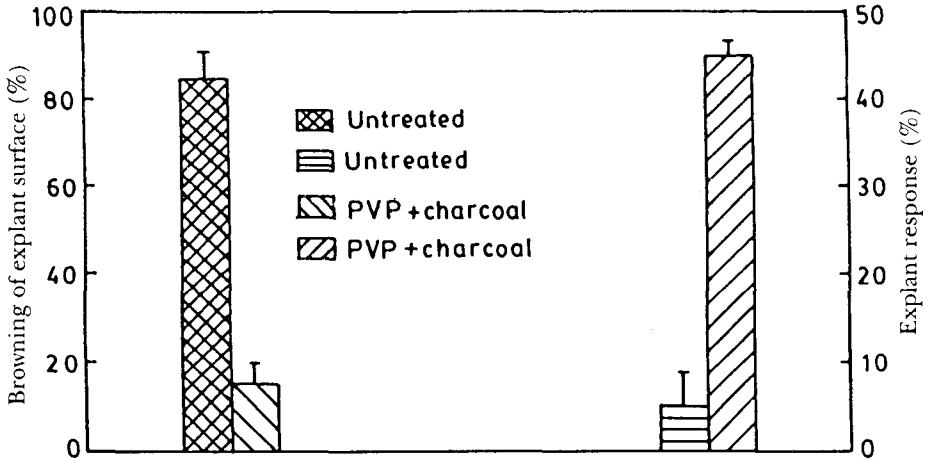


Figure 2. Influence of PVP on surface browning and response of explants of *Calamus rotang* in the activated charcoal containing medium

Table 1. Different explant lengths of *Calamus rotang* with respect to systemic contamination and response in culture conditions

Explant length inoculated (cm)	Presence of systemic contamination (%)	Increase in explant length after first cultural passage of 30 days (cm)
0.0 - 0.5	0	nil
0.5 - 1.5	10	1.5 ± 0.2
1.5 - 2.5	55	1.0 ± 0.2
2.5 - 3.5	80	0.5 ± 0.1

In the culture, inoculation of explants of length 1.5 - 2.5 cm showed variation in their growth response. This was possibly due to the different ages of the plantlets from which the explants were derived. The explants derived from plantlets of 30 - 40 cm length showed a higher rate of growth but were contaminated after 10 to 12 days (Figure 3). The explants derived from plantlets of length 20 - 30 cm showed less contamination but the growth rate was slow. Explants derived from smaller plantlets (0 -10 cm) showed negligible growth. However, the highest rate of establishment and growth (1.0 cm within 30 days) was recorded in explants derived from plantlets whose lengths ranged between 10 and 20 cm. The explants taken from plantlets vigorously growing in soil exhibited similar rapid growth in culture. In the oil-palm *(Elaeis guineensis)*, contamination was reduced by selecting rapidly growing plants (Staritsky 1970).

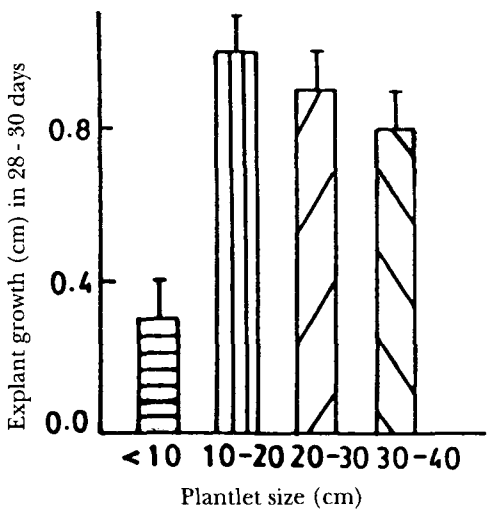
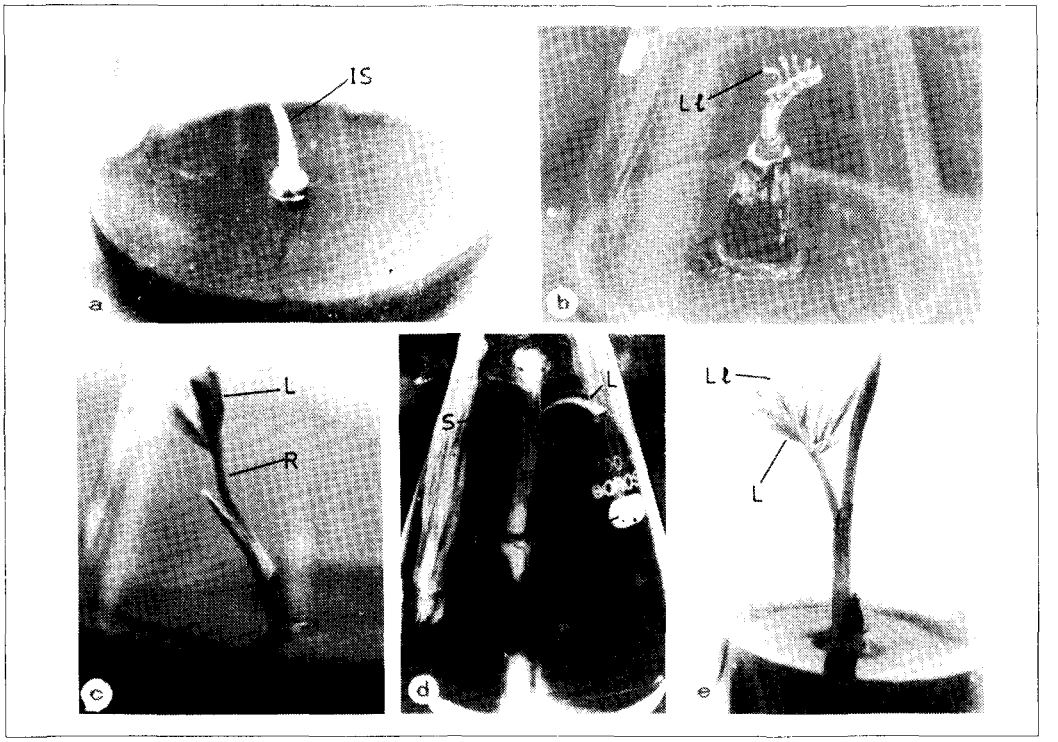


Figure 3. *In vitro* growth rate of explants derived from various plantlet lengths of *Calamus rotang* in the first cultural passage of 28-30 days

Figure 4



a. Explant showing proliferated inner leaf bases (IS) after 10-15 days in culture
b. Leaflets (Ll) emerging from sheath surface of the explant after 4-5 weeks (x3)
c. Rachis (R) and leaf (L) developing after 8-9 weeks (x2)
d. Twelve-week-old explant showing developed leaf (L) and spine (S) (x2)
e. Explant showing entire leaf (L) with leaflet (Ll) after 16 weeks (x2)

Figure 4. Photographs showing explants of *Calamus rotang* at different growth stages in the establishment medium; Y3 basal medium + sucrose (5 % w/v) + 2,4-D (5 mg l^{-1}) + BAP (1.2 mg l^{-1})

Initiation of axillary bud took place in 5.5 % of the explants in the medium with BAP (5.0 and 10.0 mg l^{-1}). Subculturing of these buds in the same medium, however, showed poor growth. Both adventitious and axillary meristem in the form of buds (0.5-2.0 cm) developed in close proximity to the apical meristem in *C. rotang* and, therefore, their easy access to the medium resulted in growth and proliferation. As cane is a monocot, multiplication of the established explant is difficult because of the absence of lateral cambial layer or axillary branch (CSIR 1986, Sharma *et al.*1980). In *Elaeis guineensis* (oil palm), after establishment the explant with adventitous buds treated with BAP showed bud proliferation (Deffossard 1976).

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