In vitro proliferation of nutmeg aril (mace) by tissue culture

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ABSTRACT

Mace from *Myristica fragrans* Houtt. is one of the most expensive of spices. Mace tissue could be successfully multiplied on Mc Cown's Woody Plant Medium (WPM) supplemented with 0.5 mg l^{-1} of IBA. The multiplied tissue retained both the colour and flavour components even after 2 months of culture indicating that their biosynthesis is continuing in culture. Gas chromatographic analysis of the mace oil extracted from the cultured tissue was similar to that of original mace in its qualitative profile.

Key words : Nutmeg, *Myristica fragrans*, mace, *in vitro* multiplication

Abbreviations

BA : N^6-Benzyladenine
IBA : Indole-3-butryric acid
NAA : alpha-Naphthelene acetic acid
WPM : Woody Plant Medium (Mc Cown & Amos 1979)

Introduction

Nutmeg and mace are two important spices obtained from the tropical evergreen tree *Myristica fragrans* Houtt. belonging to family Myristicaceae. Nutmeg is the seed kernel whereas mace is the aril that surrounds it. Mace is more expensive of the two. It has stimulative, carminative and astringent properties and hence is used in flavouring food products, perfumery etc. The volatile oils present in it contain small amounts of myristicin and elemicin (Gopalakrishnan 1992) which have narcotic and hallucinogenic properties. Myristicin is also a potential cancer chemopreventive agent (Zheng, Kinney & Lam 1992). *In vitro* techniques were used by various workers for multiplying various plant tissues and for production of flavours and essential oils in tissue culture (Mulder-Krieger *et al.* 1988). In spice crops, techniques for

direct multiplication of saffron stigmas and synthesis of crocin, crocetin, picrocrocin and safranal by tissue culture were reported earlier (Sano & Himeno 1987; Sujata Viswanathan, Ravishanker & Venkata-raman 1990; Sarma *et al.* 1990 & 1991). In the present study, an attempt was made to standardize similar technologies for direct multiplication of mace with the objective of developing protocols for industrial production of the spice as well as its flavour components.

Materials and methods

Source of explant and establishment of cultures

Fully mature but unopened fruits were collected from high yielding nutmeg trees and were surface sterilized by swabbing them with 80% alcohol and then flaming. The outer pericarp was removed under aspetic conditions. The mace tissue was separated from the seed and cut into 1 cm^2 bits. These bits were inoculated on culture medium.

Table 1. Effect of growth regulators on *in vitro* proliferation of mace explants*

Concentration of growth regulators (mg l^{-1})			Morphogenetic response	% cultures showing response**
BA	NAA	IBA		
0	0.5	0	No response	—
0	1.0	0	No response	—
0	0	0.5	Proliferation of explants into mass of tissues or small mace-like structures which retain the reddish colour of the original explant	90
0	0	1.0	No response	—
1	0	0	No response	—
1	0.5	0	Slow callus growth from cut ends	20
1	1.0	0	Callus white	
2	0	0	Proliferation of explants into mass of tissues or small mace-like structures which retain the reddish colour of the original explant	70
2	0.5	0	No response	—
2	1.0	0	No response	—

* WPM (McCown & Amos 1979) was used as basal medium and cultures were incubated in dark

** Mean of 20 replications

Culture medium and culture conditions

The basal medium used was Woody Plant Medium (Mc Cown & Amos 1979) supplemented with BA (0, 10 and 2.0 mg l^{-1}), NAA (0, 0.5 and 1.0 mg l^{-1}) and IBA (0.5 and 1.0 mg l^{-1}) in various combinations. The pH of the medium was adjusted to 5.8 prior to autoclaving at 1 kg/cm^2 (121°C) for 20 min. The medium was solidified with 0.7% agar. The cultures were maintained both in dark as well as in 14 h photoperiod provided by cool white fluorescent light (35 μ mol$^{e\cdot1m\cdot2}$). The temperature was maintained at 25± 2°C.

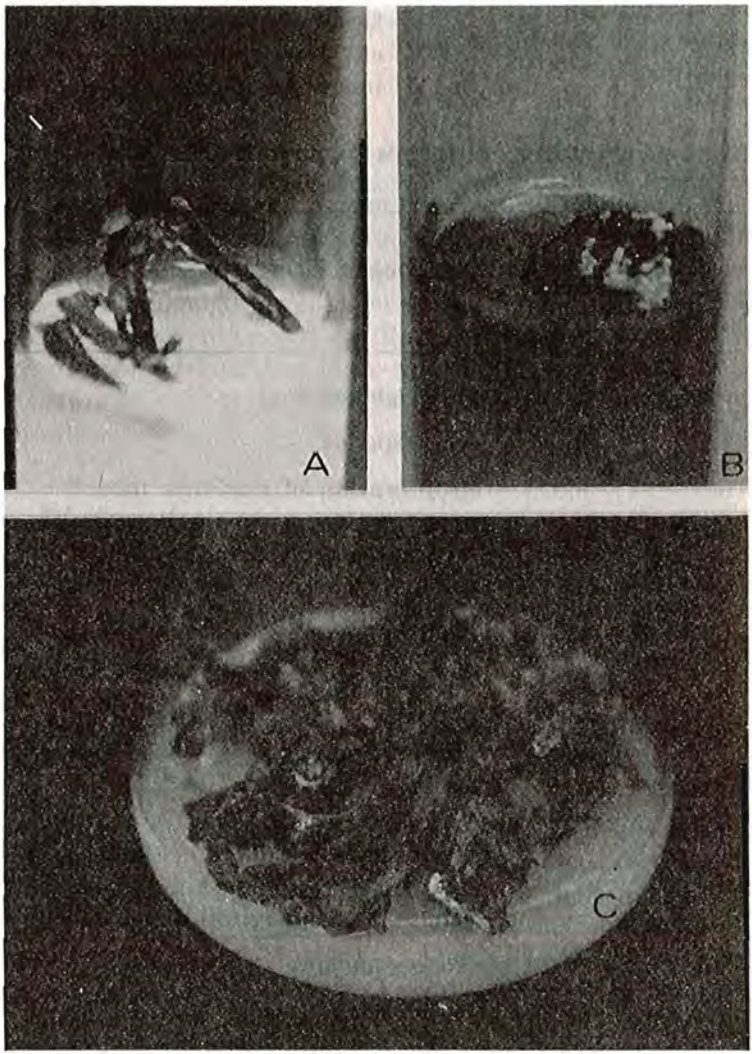

Fig. 1. *In vitro* proliferation of nutmeg aril (mace)

A. Bits of fresh mace in culture B. White callus C. Proliferation of mace tissue

Chemical analysis

The essential oils from cultured mace samples and normal mace were extracted by hydrodistillation using Clevenger's trap. The percentage volume of oil was computed on fresh weight basis. Gas chromatographic analysis of oils was done using Hewlett-Packard 5730A equipped with HP 3390-A integrater using Carbowax (2μ) column. The temperature was programmed from 70-200°C @ 4°C/min with nitrogen as carrier gas (20 ml/min), injector temperature at 200°C and FID (Flame Ionization Detector) temperature at 250°C. Majority of the peaks were identified by comparison of their retention times with those of authentic samples and their quantities were calculated from the integrated area percentages.

Results and discussion

The cultures incubated in light showed no response and slowly dried up after 30 days. However, the cultures incubated in dark showed growth indicating that darkness is required for growth of mace tissue. Twenty per cent of the explants incubated on WPM with 0.5-1.0 mg l^{-1} NAA and 1 mg l^{-1} BA showed callus development from the cultures (Table 1). The callus was white and rate of growth was very slow (Fig. 1B) But on WPM supplemented with either 0.5 mg l^{-1} IBA or 2 mg l^{-1} BA the explants showed good growth in 90% and 70% of the cultures, respectively. In these cultures explants started proliferating and produced either small aril-like growths or a mass of tissues (Fig. 1C). The WPM + 0.5 mg l^{-1} IBA was the best among the media tested for proliferation of mace tissue. The newly produced tissue retained the reddish colour of the natural mace indicating synthesis of the colouring compounds. The tissue doubled its volume in about 10 days. There was ten-fold increase in fresh weight of the tissue from the initial 0.148 g to 1.460 g within 2 weeks of culture. Tissue culture techniques were reported earlier for *in vitro* multiplication of saffron by various authors (Sano & Himeno 1987; Himeno & Sano 1987; Hori, Enomato & Nakaya 1988; Sarma *et al.* 1990). This is the first report of multiplication of mace tissue using tissue culture.

Sensory and chemical evaluation

Sensory evaluation indicated that the cultured tissue retained both flavour and pungency characters of the original spice. Mace oil could be extracted from the cultured tissues and the percentage recovery of the oil was lesser by 10 times when compared to that of normal mace. This may be due to high moisture content in the tissue cultured sample. Such reduction in tissue culture samples were reported earlier in saffron tissue cultures (Sujatha Viswanathan *et al.* 1990; Sarma *et al.* 1991). Gas chromatographic profile of cultured mace tissue, compared to that of normal mace, indicated that qualitatively they were almost similar escept for certain quantitative differences. There was reduction in percentage of beta-Pinine + Sabinine, gamma-Terpinine and Nerolidol. The content of alpha-Pinine, 1-8 cineole etc. increased in cultured samples. The percentage of myristicin was reduced in cultured samples from 7.35 to 5.43 while that of elimicin was almost the same (Table 2).

Thus, the results show that mace could be successfully multiplied by tissue culture and the cultured tissue could prove a possible substitute for commercial

Table 2. Chromatographic analysis of tissue cultured and normal mace oils

Compound	Composition		
	Rt	Mace oil	Tissue cultured mace oil
alpha-Pinine	1.15	6.700	11.500
beta-Pinine + Sabinene	2.08	46.280	43.460
alpha-Phellandrene	2.27	3.027	3.840
UI	2.61	1.660	1.580
1:8-Cineole + Limonene	2.82	3.850	6.650
UI	3.32	2.800	2.500
gamma-terpinene	3.98	2.600	1.290
UI	4.42	3.400	2.280
UI	6.74	0.094	0.071
UI	7.79	0.147	0.318
UI	8.77	0.231	0.340
UI	9.29	0.162	2.295
UI	11.00	0.525	0.391
UI	11.36	0.521	0.454
Myristicin	12.52	7.350	5.430
UI	13.20	0.413	0.343
UI	14.40	0.265	0.274
Elemicin	14.98	1.080	1.080
UI	16.25	0.479	0.413
UI	19.34	2.370	4.130
Garaniol	21.31	0.179	0.202
UI	23.16	1.126	1.037
UI	23.86	0.521	0.312
Nerlidol	24.76	0.869	0.458
UI	25.60	0.381	0.387
UI	27.16	1.240	0.791
UI	28.30	2.120	1.000
UI	28.78	0.916	—
UI	30.98	1.440	1.490

Rt = Retention time; UI = Unidentified

mace. Since flavour and other major components of mace are being synthesised in tissue cultures, this technique could form a base for possible *in vitro* production of myristicin which has anticarcinogenic properties.

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