

MULTICELLED POLLEN PRO-EMBRYOIDS AND CALLUS FORMATION IN TEA ANTHHER CULTURES

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INTRODUCTION

Tea is an exclusively cross-pollinated crop and is also self-incompatible. Owing to this limitation, genetic studies, and more particularly breeding programmes have been considerably handicapped in this important cash crop.

In self-incompatible plants, particularly the economically important ones like tea, the production of a large number of haploids and subsequently homozygous lines through chromosome doubling, would not only be of great advantage to the further understanding of their genetics, but also, in hastening of and adding a new dimension to the breeding programmes. It was with this background that among about 25 species of crop plants studied in this laboratory for response to anther culture technique for the production of haploids (Iyer and Raina, 1972; Raina, 1977), tea was one of the species tried rather intensively.

MATERIALS AND METHODS

Anther culturing was carried out at the Tea Experimental Research Stations

at Jorhat (Assam), Coonoor (Tamil Nadu) and Palampur (Himachal Pradesh) using mostly Assam clones at the first two stations and China clones at the last. Culture media were transported to these places and after anther inoculations, the cultures were brought back to Delhi for incubation and further observations. Flower buds having anthers at mid-to late-uninucleate pollen stage were surface-sterilized with 0.2% mercuric chloride for 10 minutes, washed with sterile water, and/or at the time of anther excision, they were dipped in absolute alcohol for 3-5 seconds, and flamed lightly. During transportation, which generally took place one or two days after inoculation and involved an overnight or upto 36 hours journey, care was taken to keep the cultures at 20-30° C. Otherwise, the cultures were maintained in a culture room at 25°±1°C under a low light intensity of 100-150 lux. The following culture media** were tried, some of which have been used successfully in this laboratory or elsewhere, for eliciting anther

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** Auxin & Kinin concentrations in mg/l, N-Nitsch's (1969), LS-Linsmaier & Skoog's (1965) and B-Blaydes (1966) based media; CW-Coconut water from tender nuts, added prior to pH adjustment; 2,4-D-2,4-Dichloro-phenoxyacetic acid; IAA-Indole-3-acetic acid; K-Kinetin; NAA-Naphthalene acetic acid; BA-Benzyladenine. Unless specified, sucrose was used at 3%. All media were adjusted to pH 5.8, and gelled with agar 1%.

response and/or for organ regeneration.
(in other plant species):

1. N + IAA (0.1)
2. N + CW (15%)
3. LS + CW (15%)
4. B + CW (15%)
5. N + 2,4-D (2) + IAA(1.5) +
K(1.5) + CW(15%)
6. LS + 2,4-D (2) + IAA(1.5) +
K(1.5) + CW(15%)
7. N + NAA(2) + CW(15%)
8. N (Sucrose 6%) + NAA(2) +
CW(15%)
9. LS (Sucrose 6%) + NAA(2) +
CW(15%)
10. N + NAA(2) + BA(1) + CW
(15%)
11. N + IAA(1) + K(1)
12. N + IAA(2) + K(2)
13. N + NAA(2) + BAA(1)

RESULTS AND DISCUSSION

Within a week after inoculation, callus formation occurred from the cut end of the filament and from the rather massive connective (Fig. 1 a) which occasionally forced its way through the another lobe (Fig. 1 b), in all the cultivars and media tried (Table I). The initial rapid growth of the diploid callus from connective region almost engulfed the another quite often and obscured the detection of any pollen-callus formation that might have occurred. This was particularly marked in all the 2,4-D supplemented media where somatic callus was initiated earlier and had proliferated faster.

Cytological examination of anthers withdrawn from the cultures periodically, revealed rapid pollen degeneration in all cases except in a China hybrid

Table I. *Type of response in anther cultures of different tea clones*

Cultivars	Anthers inoculated (No.)	Media tried (Sl. No.)	Type of response
B/4/51	1250	2,3,4,5	CC, FC
B/4/42	960	2,3,4	" "
B/4/61	2260	1,2,3,4,5,6,7,8,9,10	" "
B/5/63	2300	2,3,4,5,6,7,8,9,10,11	" "
B/6/61	2670	2,3,4,5,6,7,8,9,10,11	" "
B/6/62	1680	2,3,4,5,6	" "
Badamtam	1150	2,3,4,5	" "
China hybrid-3	2230	2,3,4,5,6,7,8,9,10	PC (0.3% in medium 2), CC, FC
Dheodham	2580	2,3,4,5,6,7,8,9,10	CC, FC
Lingia	1600	2,3,4,5,6	" "
TV-9	1340	2,3,4,5	" "
Tumsong	730	2,3,4	" "

CC - Connective callus

FC - Filament callus

PC - Pollen callus

clone (China hybrid-3) where, about 2% of the anthers (from medium-2) showed 0.01% multinucleate or multi-celled pollen pro-embryoids (Fig. 1 c). After about 40 days in the same medium, a few anthers (0.3%) of this clone also showed pollen callus formation. These pollen-calli emerged from inside the anther lobes and could be distinguished from the somatic callus by their globular and fragile appearance (Fig. 1 d) with a tendency to readily fall off the anther lobes, indicating the absence of any physical connection with somatic tissues. The pollen-calli developed chlorophyll when such cultures were shifted to an alternating regime of 12 hr light (4000 lux) and 12 hr darkness. Initially, they were all transferred to fresh parent medium, and later the proliferated calli were subcultured on media 1, 2, 7, 10, 11, 12 and 13 for organ regeneration. However, the calli continued to proliferate in all the media without showing any obvious signs of organogenesis. Fresh inoculations of another 5,000 anthers of China hybrid-3 were carried out on medium-2. The pollen-calli were directly transferred to four different media (1, 2, 7 and 10) for inducing organ regeneration. As observed earlier, calli continued to proliferate slowly but no regeneration of any organs was observed.

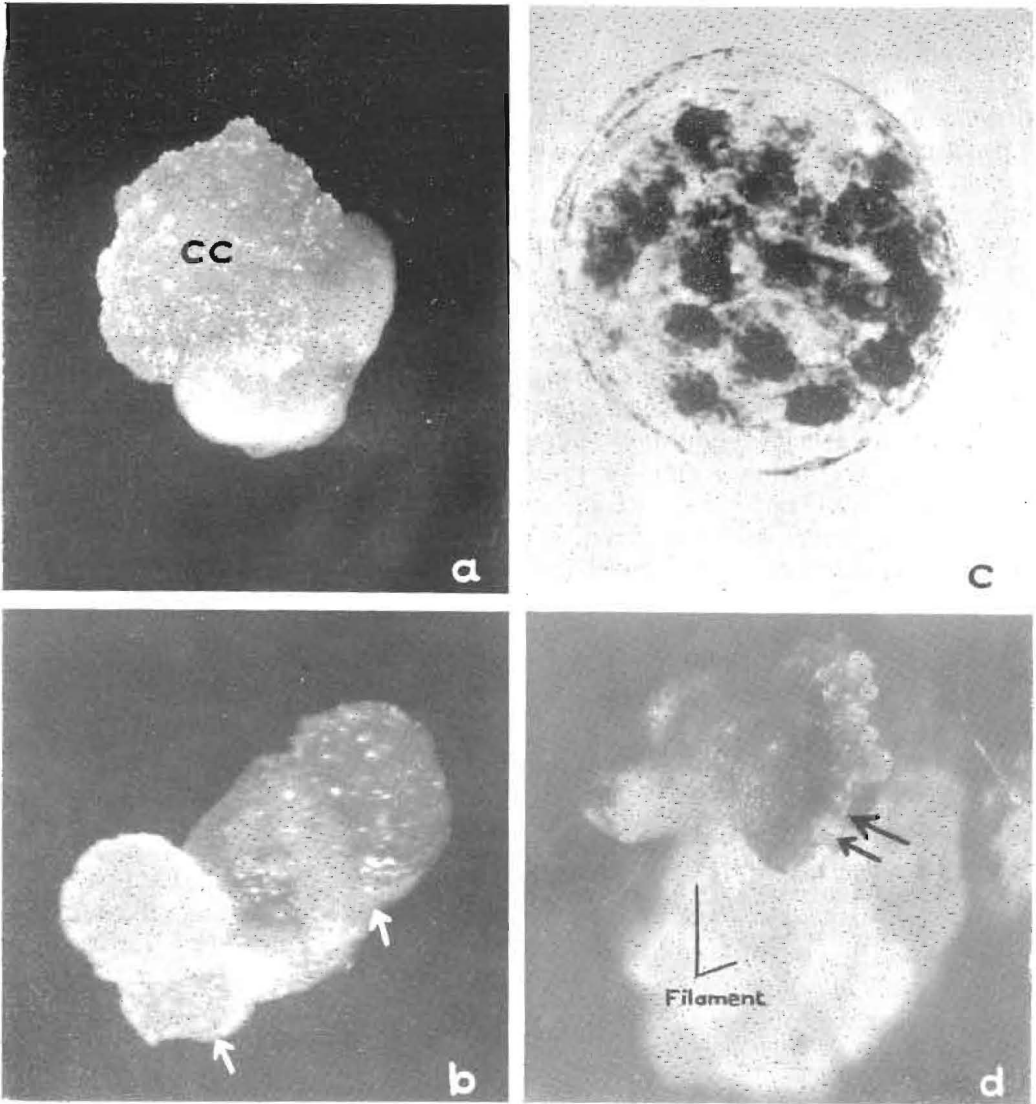
In a recent review of tea tissue and anther culture, Wu et al., (1981) have reported differentiation of plantlets from cotyledon derived callus cultures which were established earlier (Wu, 1976).

They have found that addition of 2,4-D (2 to 8 ppm) to the basal medium was indispensable for callus initiation, but for organogenesis, the interaction of IAA (2-4 ppm) and kinetin (2-4 ppm) was essential, 2, 4-D being inhibitory. However, no organogenesis occurred in anther and shoot-tip derived calli.

In spite of the large number of anthers cultured and a variety of media tried, the response was very low and limited to only one clone. Induction of organogenesis in pollen-calli still remains a challenge. Keeping in view the immense potential that this technique holds in augmenting the basic genetic information through haploids and particularly for achieving a rapid improvement of this crop, concerted efforts are called for not only for enhancing the frequency of anther-response but also regeneration of plants from pollen-calli.

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FIG. 1. *IN VITRO* CULTURE RESPONSE OF *CAMELLIA SINENSIS* ANTHERS

(a) Callus formation from the connective region (cc) in a 18-day old anther (cv Lingia), (b) Connective callus emerging through the anther lobes (at arrows), after 16 days in culture (cv China hybrid-3), (c) Multi-celled pollen pro-embryoid from a 35-day old anther (cv China hybrid-3), (d) Globular pollen-calli (arrows) along with somatic calli from anther wall, in a 45-day old culture (cv China hybrid-3).

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