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CALLUS FORMATION FROM EXPLANTED ORGANS OF TEA (*CAMELLIA SINENSIS* L.)

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Explant browning was noticed when different plant parts of tea (*Camellia sinensis* L) were cultured. Lowering the salt concentrations to 1/20th of the Murashige and Skoog medium was effective in reducing browning. Stem pieces produced better callus when high auxins, 2,4-D and NAA (2×10^{-5}M) and low cytokinins, BA and kinetin (10^{-5}M) were present in the medium. 2,4-D (4.5×10^{-5}M) and yeast extract (0.2%) together were found conducive for callus growth. Growth of buds occurred on BA (10^{-5}M) when the cultures were incubated in the dark for the first 10 days before maintaining under light.

INTRODUCTION

The propagation of plants by tissue culture has proved to be an alternate tool for rapid multiplication than traditional means (Murashige, 1974). In tissue culture one would expect to rear the seedlings on a large scale and to obtain variations for further selection via callus culture. All the plant parts of tea may not be suitable due to its complex chemical nature (Wickremasinghe, 1978). Shoot tips of tea were used mainly to study the biosynthesis of caffeine and polyphenols (Forrest, 1969; Koretskaya and Zaprometov, 1975 a, b; Ogutuga and Northcote, 1970; Shipilova, Koretskaya and Zaprometov, 1978), besides exploring its possibility as an alternative to propagation by tissue culture (Wu, 1976).

As there are few reports on the propagation of tea by tissue culture, several variations of the explant type and nutrient medium have been examined in the present studies and the possible solution of explant browning in culture is discussed.

MATERIALS AND METHODS

Eight tea bushes (*Camellia sinensis* L.) were maintained in the glasshouse of the School of Biological Sciences, Bath University, U. K. Different parts of these bushes excised for callus induction came from the branches which were up to one-year-old. Excised plant material was surface sterilized by 25% solution of sodium hypochlorite with two drops of decon as wetting agent for 15 minutes.

Explant browning

In explant browning experiments 5-10 mm long stem pieces were cut into halves. One half went directly to the culture medium which was treated as a control. The other half was immersed for two minutes in different chemical solutions (M) before culture: ascorbic acid (10^{-2}), catechol (10^{-2}, 3×10^{-3}), L-cysteine (10^{-2}), phloroglucinol (10^{-2}), phenylthiourea (10^{-2}), PVP 10 (10^{-3}), sodium diethyldithiocarbamate (SDDC) (10^{-2}), sodium fluoride (5×10^{-2}), and thiourea (10^{-2}). In another experiment (Table 1), stem pieces of 5-10 mm long were cut in halves. One half was cultured on Murashige and Skoog (1962) medium (MS) used for callus induction and was treated as a control. The other half went into different media with or without activated charcoal (1%), where the MS inorganic salts were reduced to 1/2, 1/5, 1/10, 1/20th times of the concentration. The extent of explant browning within one week of culture was graded as follows: explant remained green (0), 1/4th explant turned brown (1), 1/2 explant turned brown (2), 3/4th explant turned brown (3), and complete browning of the explant (4). Each experiment was replicated 9 times.

Callus induction

Callus induction was tried using different plant parts as explants which were flower stalks, leaf pieces, petioles, immature cotyledons, stem pieces, and shoot tips. When the youngest 3-4 leaves were used as explants they were cut into pieces 3-4 mm wide and 5-10 mm long. Two to three pieces were cultured together in 175 ml glass jars having metallic tops in 12 replications. Petioles were cut into halves and both halves were cultured together. Immature cotyledons were grouped into 4 categories, i.e. milky stage, just condensing stage, condensed stage and immature cotyledons. Stem pieces of 5-10 mm long were cut into halves to increase the cut surface area and each half was cultured separately in different jars. Cultured tips were 5-10 mm long. Initiation of callus was followed by grading after 42 days of culture. Amount of callus formed was given 5 grades: no callus formed (0), explant started swelling (1), little callus formed (2), callus formed but not enough to be separated (3) and profuse callus formed (4).

The medium used for callus induction (Table 2) was of 0.7% agar solidified MS which also contained 2,4-D+NAA together (2×10^{-5}M), BA+kinetin together (10^{-5}M) and 2% sucrose. The pH of the medium was maintained at 4.8 before autoclaving. Cultures were incubated in the dark at 30°±2°C in nine replications.

RESULTS

Explant browning

The different chemical solutions tried to control explant browning remained ineffective.

I. *Sterilization effect*

When the stem pieces were cut into halves after sterilization no immediate explant browning was noted. All the cut stem pieces turned to milky white in sterilizing solution. Later this white colour changed to brown and all the explants died in culture. Immature cotyledons did not brown at any stage, before or after culture.

II. *Effect of medium salt concentration*

When the inorganic components of the MS medium were reduced to 1/2, 1/5, 1/10, and 1/20th times the final concentrations, variations in explant browning were noted (Table 1). The reduction in the extent of explant browning was more when the concentration of inorganics were reduced to 1/20th of the final concentration.

TABLE 1 — *Extent of explant browning of the tea stem pieces in culture*

Treatment	Explant browning							
	Treatment				Control			
(Days)	1	2	3	7	1	2	3	7
1/20 MS inorganics	0.6	0.7	0.7	1.0	1.4	1.9	1.9	2.0
1/10 „ „	1.1	1.4	1.4	1.4	1.9	2.3	2.3	2.3
1/5 „ „	0.7	0.7	0.9	1.1	1.8	1.9	1.9	2.5
1/2 „ „	0.7	0.7	1.1	1.2	1.4	1.7	1.7	1.8
1/20 MS inorganics + activated charcoal	0.2	0.8	1.0	1.8	0.2	0.2	0.6	1.3
1/10 „ „	0.4	1.3	1.6	2.1	0.6	1.1	1.1	2.0
1/5 „ „	0.4	1.7	2.1	3.0	0.6	1.1	1.7	2.7
1/2 „ „	0.4	1.1	1.8	2.8	0.9	1.3	1.9	2.9

Each value is the mean of nine observations

Callus induction

The flower stalks could not escape browning (Table 2). Leaf pieces produced callus in 33% explants while the other types of explants turned brown and died. After 42 days of culture, these leaf pieces having produced the callus were transferred to the same fresh medium where little callus development was noticed. Only 88% of petiole explants produced little callus which was not enough to be separated. Immature cotyledons of milk and condensing stage turned black within 10 days of culture. All the explants of condensed stage and immature cotyledons survived browning and produced callus. The immature cotyledons produced the maximum amount of callus. All shoot tips turned brown and died within 10 days of culture. Ten buds each when cultured on 1/2 and 1/10th concentrations of MS medium, 0.5% activated charcoal, BA (10^{-5} and 2×10^{-5}M), kinetin (2×10^{-5}M), remained green when incubated in the dark. After 10 days, when transferred to 16 h photoperiod, 50% buds started swelling, grew in length and produced 2-3 leaves on BA (10^{-5}M) while only two buds showed swelling on 2×10^{-5}M BA medium. Kinetin was unable to support the growth of the buds.

Stem pieces produced varying amounts of callus on MS having two auxins together, 2,4-D and NAA (5×10^{-6}, 10^{-5}, 2×10^{-5}M) and two cytokinins together viz. BA and kinetin (10^{-5}, 2×10^{-5}, 4×10^{-5}M). Callus exhibited maximum growth where yeast extract (0.2%) and 2,4-D (4.5×10^{-5}M) together were part of the medium. After a few weeks, old callus started to dry and new callus was formed on the periphery.

TABLE 2 — *Callus induction on different types of explants*

Explant type	*No. of explants cultured*	*No. of explants died of browning*	*No. of explants produced callus*	*Callus formation (grade)*
Flower stalk	20	20	0	0
Leaf pieces	12	8	4	2
Leaf petiole	35	4	31	2
Cotyledon				
Milk stage	9	9	0	0
Just condensing stage	9	9	0	0
Condensed stage	9	0	9	2
Immature cotyledon	9	0	9	3
Shoot tip	9	9	0	0
Stem pieces	126	97	31	4

DISCUSSION

Tissue browning is a widespread phenomenon in tea cultures. The present study indicates that the explants turned brown before or after culture which retarded the callus induction. Such harmful effect of browning was reported before by Staritsky (1970) working with coffee.

The different chemicals used to control browning proved ineffective. Inorganic salts of MS at low concentrations reduced the explant browning (Table 1). Beneficial effect of low salt concentration was also noted in Azaleas (Anderson, 1975; Ma and Wang, 1977; Preil and Engelhardt, 1977), and in *Papaver somniferum* (Ilahi, 1983). In the present studies activated charcoal in a medium had little effect to control explant browning. It seems that the choice of explant is important in establishing tea cultures at the start of an experiment. It can be postulated that explant browning is related to the type of explant used since no browning was noticed when immature cotyledons were used as explants which was reported previously in tea (Wu, 1976). The method of sterilization has also some effect on browning as the explant browning had increased when the stem explants were sterilized after they were cut into halves. In the presence of browning and less number of replicates left after browning it is difficult to draw a clear conclusion but it shows that auxin and cytokinin together are necessary for good callus formation.

The effect of light on explant browning was not studied separately as reported before (Liu, 1981). It was found that low salt concentration, absence of light and the presence of activated charcoal, all together have exhibited favourable effect on tea shoot development since shoot buds could only survive browning where these three factors were present together.

The use of this culture procedure has helped to establish tea cultures which is a prerequisite for *in vitro* propagation. This work can be regarded as the first organized study to control tea explant browning in culture. Continued efforts and investigations need to be pursued in order to study the innumerous problems involved in this field.

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