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Tea tissue culture: *In vitro* shoot regeneration and callus induction in tea

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ABSTRACT: *Camellia sinensis*, or Tea, is one of the most important crop plants in the world. Biotechnological interventions in the propagation of tea have become necessary as conventional methods prove insufficient. From the present study, MS medium supplemented with 0.5 mg L^{-1} Kinetin was found to be effective in inducing *in vitro* shooting from apical and nodal explants derived from 3-month-old seedlings of *Camellia sinensis* cv. UPASI-9. For callus induction in *in vitro* grown leaves, MS medium supplemented with 0.5 mg L^{-1} NAA and 0.5 mg L^{-1} 2,4-D was found to be most effective.

KEYWORDS: *Camellia sinensis; In vitro; Propagation; Callus*

Introduction

Tea is the second most consumed beverage, surpassed only by water. The global requirement for tea has resulted in an increase in cultivation of *Camellia sinensis* (L.) Kuntze – the crop plant of tea. As with every crop plant, numerous varieties of *Camellia sinensis* exist. Many varieties have unique traits such as high caffeine content, resistance to specific diseases, drought resistance, etc.[1] Tea being slow-growing, conventional methods alone are insufficient for creating new varieties and cultivars. With advances in biotechnological methods, plant tissue culture has facilitated the creation of newer, better varieties. Hence, it is necessary to develop efficient protocols for *in vitro* propagation to aid the biotechnological interventions involved. In the present study, a protocol for the establishment of *in vitro* shoots using seedlings as explants has been described. The leaves from *in vitro* grown plants were further used for callus induction studies. The cultivar used for the present study was UPASI-9, a high yielding drought-tolerant variety.

Methodologies

Explant Source

Apical and nodal segments from 3-month-old seedlings of *Camellia sinensis* cultivar UPASI-9 were used to obtain *in vitro* shoots.

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Explant Treatment

The explants were surface disinfected by immersing them for 1 hr in a solution of 0.1% (*w/v*) bavistin and 1% (*v/v*) bacillocide in distilled water. After surface disinfection, the disinfecting solution was drained off, and the subsequent treatment was carried out under aseptic conditions inside a laminar air-flow unit. The material was rinsed 2–3 times with sterilized distilled water (SDW) to remove any traces of the disinfecting solution. The explants were then subjected to a two-step surface sterilization treatment. The material was first immersed in 0.1% (*w/v*) mercuric chloride with constant swirling for 60 sec. The mercuric chloride was then drained off, and the material was rinsed thoroughly 4–5 times with SDW to remove any traces of the treatment solution. Next, the material was immersed in 70% (*v/v*) ethanol with constant swirling for 30 sec. The treatment solution was then drained off, and all traces of the alcohol were removed with 4–5 rinses with SDW. This treated material was then used for *in vitro* culture establishment.

Shoot Induction

The apical and nodal segments of *C. sinensis* were inoculated on modified Murashige and Skoog medium (MS medium)[2] supplemented with various plant growth regulators (PGRs). The growth regulators used were thidiazuron (TDZ), benzyl aminopurine (BA) and 6-furfuryl aminopurine (Kinetin). The PGRs concentrations used were 0.1, 0.5 and 1.0 mg L^{-1}. The PGRs were used singly as well as in combination. The medium was gelled with 8% (*w/v*) agar. The pH of the medium as adjusted to pH 5.7±0.05 prior to autoclaving for 15 min at 121°C and 15 lb psi. Cultures were maintained under a photo-

Table 1: Comparison of plant growth regulator treatment for shoot induction in *C. sinensis*

PGR (mg L^{-1})	Mean No. of Leaves	Time (Week)
0.5 BA	$2.3^{b} \pm 0.943$	17
0.5 Kinetin	$7.3^{a} \pm 0.942$	16
0.5 TDZ	Callus induction	12

period regime of 16/8 hrs light/dark using 3,000 lux cool white fluorescent tubes. Temperature was maintained at 25±2°C (ref. Table 1).

Callus Induction

Leaves from *in vitro* cultured shoots of *C. sinensis* were used as explants for callus induction. The leaves were cut into small pieces of approximately 1 cm^2 such that each piece contained the mid-rib portion. These pieces were then inoculated on modified MS medium supplemented with various PGRs. The PGRs used were α-naphthalene acetic acid (NAA), indole 3-acetic acid (IAA) and 2,4-dicholophenoxyacetic acid (2,4-D). The PGR concentrations were varied from 0.1, 0.5 and 1.0 mg L^{-1}. The medium was gelled with 8% agar (*w/v*). The pH of the medium as adjusted to pH 5.7±0.05 prior to autoclaving for 15 mins at 121°C and 15 lb psi. Cultures were maintained under a photoperiod regime of 16/8 hours light/dark using 3000 lux cool white fluorescent tubes. Temperature was maintained at 25±2°C (ref. Table 2).

Callus Growth Rate Calculation

Relative growth rate may be calculated as follows:[3]

$$\text{Relative growth rate } (RGR) = [\text{Ln } WF - \text{Ln } WI] / T$$

where *WI* is the initial weight (g) of the callus, *WF* is the final weight (g) of the callus and *T* is the duration of the treatment.

Table 2: Comparison of plant growth regulator treatment for callus induction in *C. sinensis*

PGR (mg L^{-1})	Relative Growth Rate RGR (week 1) mean*± SD
0.5 2,4-D	$2.3^{b} \pm 0.943$
1.0 2,4-D	$7.3^{a} \pm 0.942$
0.5 2,4-D + 0.5 NAA	Callus induction

*Means with the same letters are not significantly different at $P<0.05$.

Results and Discussion

Shoot Induction

The induction was shoot was seen on medium supplement with 0.5 mg L^{-1} kinetin and 0.5 mg L^{-1} BA. Of these, 0.5 mg L^{-1} kinetin showed the fastest response, with the shoot induction being observed 4 weeks after inoculation. In case of 0.5 mg L^{-1} BA, shoot induction was observed 6 weeks after inoculation. Higher concentrations of the BA and kinetin (1.0 mg L^{-1}) showed stunted shoot growth; while lower concentrations (0.1 mg L^{-1}) failed to induce any response. All the explants produced only one shoot in case of nodal segments, while elongation of apical bud was seen in the apical segment explants. When TDZ was used for shoot induction, callus induction was observed in the explant inoculated on 0.5 mg L^{-1} TDZ. The initiation was callus, observed after 12 weeks of inoculation. Lower concentration of TDZ (0.1 mg L^{-1}) failed to induce any response, while explant blackening was seen in higher concentration (1.0 mg L^{-1}).

Since the number of shoots induced in all the treatments was the same (1 shoot/ explant), the optimum medium for shoot induction was decided based upon the response time and number of leaves formed per shoot. Based on these parameters, modified MS medium supplemented with 0.5 mg L^{-1} kinetin was the optimum medium for shoot induction. An average of 7 leaves/shoot was observed in this medium in 16 weeks. The second highest number of leaves was observed in medium supplemented with 0.5 mg L^{-1} BA, which gave 2.3 leaves/shoot in 17 weeks. It was reported that multiple shoot induction of tea in ½ strength MS medium supplemented with 3 mg L^{-1} BA in 16–24 weeks.[4] The explant used in their study was nodal segments from *in vitro* grown plantlets. Kinetin has also been reported to be effective in shoot induction in tea plants when shoot tips and nodal segments from field grown plants were cultured on MS medium supplemented with 2 mg L^{-1} IAA and 8 mg L^{-1} kinetin.[5] The response was seen in 8–12 weeks after inoculation.

Callus Induction

Explants cultured in all the media tested showed initiation of callus within 4–5 weeks after inoculation. When the PGRs were used singly, all concentration of 2,4-D showed callus induction; with 1.0 mg L^{-1} 2,4-D showing the best response. However, when NAA was used singly, only 0.5 mg L^{-1} NAA showed induction of callus. In the case of NAA + 2,4-D combinations, response for callus induction was seen at all the concentration combinations tried. The combination of 0.5 mg L^{-1} NAA +

0.5 mg L^{-1} 2,4-D proved to be most effective. The effect of 2,4-D and NAA on callus induction on tea is well reported.[1] While the callus grown on 1.0 mg L^{-1} 2,4-D showed more biomass accumulation with a higher relative growth rate (0.0884 ± 0.00091, week 1), spontaneous root induction occurred in the callus after 8 weeks of maintenance on the same medium. Hence, the combination of 0.5 mg L^{-1} NAA + 0.5 mg L^{-1} 2,4-D was chosen as the optimum medium for callus induction despite having a slightly lower relative growth rate (0.0868 ± 0.0042, week 1) than 1.0 mg L^{-1} 2,4-D.

From our findings, we can conclude that MS medium supplemented with 0.5 mg L^{-1} kinetin is the most effective medium to induce shoot growth from seedling derived apical and nodal segments. Callus induction in *in vitro* grown leaves was most effective in MS medium supplemented with 0.5 mg L^{-1} NAA in combination with 0.5 mg L^{-1} 2,4-D. The efficiency of the protocol will facilitate further studies on increasing the content of high value products of tea using callus cultures.

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