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CAMELLIA SINENSIS (TEA PLANT) – A REVIEW

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ABSTRACT

Tea, next to water is the beverage humans consume. It is increasingly appreciated that tea contains high amount of polyphenols and other components that may reduce the risk of chronic diseases such as cancer, diabetes etc. Tea has high composition of polyphenols. Catechins are polyphenols which is antioxidant found in tea may reduce the risk of various types of cancers. Several other components also serve as anti – inflammatory effects, enhances cognition in humans. The effect of Thidiazuron (TDZ) on the micro propagation of *Camellia sinensis* was compared with that of Benzyl Amino Purine (BAP) using nodal segments from in vitro raised seedlings. Extremely low concentrations of TDZ alone were effective in inducing shoot bud proliferation and maintaining high rates of shoot multiplication on hormone-free media. Callus tissues obtained from intact stem segments of tea seedlings. The callus from the epidermal layers form buds. Grafting or graftage is a horticulture technique whereby tissues of plants are joined so as to continue their growth together. The successes of this joining require that the vascular tissue grow together and such joining is called inoculation. The technique is most commonly used in asexual propagation of commercially grown plant for the horticulture and agriculture trades.

KEYWORDS: Catechins, Callus, Shoot formation, Micro propagation.

INTRODUCTION

A variety of bioactive phytochemicals in the human diet contribute functionality to a range of plant-based food-stuffs. Of particular interest are the antioxidants, which play an important part in reducing the risk of free radical- related oxidative damage associated with a number of clinical conditions and degenerative diseases. The main group of flavonoids in green tea are catechins. Black tea is subjected to more extensive 2 processing, during which a major part of the green tea catechins are converted into more complex condensation products, thioflavin and thiorubigin. Interpretation of epidemiological data and findings on biological effects of tea consumption in animal and human trials is currently hampered by the limited amount of information on the bioavailability and pharm kinetics of tea flavonoids.

Thidiazuron is an herbicide with intrinsic cytokinin-like activity and is known to stimulate high rates of regeneration and axillary shoot proliferation in many woody plant species. The impact of TDZ or BAP on the multiplication rates of responsive explants after their subculture to hormone-free medium has been compared and evaluated. The epidermal layers were separated from stem, and only epidermal layers were cultured and regenerated using callus induction. Micro grafting has been successfully reported in a range of horticultural plants, as a means to obtain clones free of viruses and virus-like diseases and also to detect graft in-compatibilities at an early stage. In the study presented here, we used a similar approach in tea in which the root stocks were juvenile seedlings or only 8- to 12-week-old plants with herbaceous stems. Prevailing constraints in the micro propagation of tea led us to assess the grafting of micro propagated shoots of selected stock Banuri-96 scions onto seedling root stock.

Catechins

Several epidemiological studies suggest that black tea consumption is associated with a reduced risk of degenerative diseases such as cardiovascular disease. There is increasing evidence from experimental studies that free radical-mediated damage may play a role in the aetiology of cardiovascular disease and that antioxidants may act in preventing this damage. Tea is a rich source of flavonoids and the beneficial health effects of tea consumption have been related to the antioxidant activity of these tea flavonoids. The main group of Flavonoids in green tea are catechins. Black tea is subjected to more extensive processing, during which a major part of the green tea catechins are converted into more complex condensation products, thioflavin and thiorubigin. Interpretation of epidemiological data and findings on biological effects of tea consumption in animal and human trials is currently hampered by the limited amount

of information on the bioavailability and pharm kinetics of tea flavonoids.

Catechins were determined as described previously. The colorimetric method comprises a solid phase extraction of blood with aluminium oxide followed by complexation of catechins with dimethyl amino cinnamaldehyde (DMACA). The 3 complexation is specific for flavones containing meta-oriented hydroxyl groups in the A-ring and a single bond in the 2, 3-position of the hetero- cyclic ring. The method shows, on a molar base, the same response factor for each of the catechins present in tea (namely, catechins, epicatechin, epigallocatechin, epigallocatechin gallate and epicatechin gallate). Thioflavin and thiorubigin are also detected, but their response factor is lower than that of catechins. The method is rapid, specific and sensitive but does not separate individual catechins as do other methods which require sophisticated equipment. For this, the total response of the spectrophotometric measurement is referred to as the concentration of total catechins was estimated (Table 4)

Table 4: Catechins content of green tea and black tea extracts.

Component	Green tea	Black tea
Total catechins (g/g)a	0.31	0.10
• Catechins (%)b	2	6
• Epicatechin (%)b	11	22
• Epigallocatechin (%)b	35	31
• Epicatechin gallate (%)b	12	25
• Epigallocatechin gallate (%)b	40	16

Venous blood samples were collected into Na2EDTA tubes, rapidly frozen and stored at -80 ° C. One ml of thawed blood was thoroughly mixed with 3.0ml methanol containing 1g/L butylated hydroxytoluene. After centrifugation, the supernatant was added to 100mg aluminium oxide. The mixture was vortexed, centrifuged and the supernatant was discarded. The residual aluminium oxide was washed with diethyl ether. Complexation of catechins was initiated by the addition of 0.5ml of DMACA, 6mmol/L in methanol/perchloric acid/water (8:1:1; v/v). After 6min, the absorption spectrum of the clear supernatant was measured from 500±750nm using DMACA reagent as reference.

Micropropagation

The effect of Thidiazuron (TDZ) on the micro propagation of Camellia sinensis was compared with that of Benzyl Amino Purine (BAP) using nodal segments from in vitro raised seedlings. Extremely low concentrations of TDZ (1pM–100nM) alone were effective in inducing shoot bud proliferation and maintaining high rates of shoot 4 multiplication on hormone-free media. On the other hand, higher concentrations of BAP (1–10µM) and its continued presence were required to initiate and sustain shoot proliferation. While wider ranges of BAP combined favourably with auxins like NAA or IBA, only specific combinations of TDZ and NAA were effective for shoot proliferation. TDZ treated explants yielded healthy shoots, with sturdy leaves, even during the initial stages of growth, whereas, the effect of BAP was cumulative over subcultures in attaining a high proliferative rate.

Seeds collected in the month of November from the Institute's Tea Experimental Farm were surface sterilized with 4% (w/v) sodium hypochlorite solution for 10 minutes and washed five times with sterile deionized water. The surface sterilized seeds were germinated on 1/2 strength MS medium supplemented with 30 gl−1 sucrose and 8 gl−1 agar. Plantlets which were obtained from the germinated seeds were then selected as the source material when they had attained a height of 3–4 cm after a period of 60 days. From such in vitro grown plantlets, nodal segments of about 1.0 cm were taken as explants and inoculated horizon- tally on two different basal media i.e. Woody Plant Medium or WPM or MS medium supplemented with 30 gl−1 sucrose and 0.8% agar.

The pH of the media was maintained at 5.8 prior to autoclaving. All cultures were maintained at 25± 2◦C under a photoperiod of 16 hours with cool fluorescent lights of 52 µmol m−2 s−1 each. Experiments were repeated four times. While the effect of either TDZ or BAP (0–100µM) alone was tested on both WPM and MS medium, the effect of different factorial combinations of auxins like 0, 5, 10 and 15µM 2, 4-D, NAA and IBA were tested with 0, 5 and 10µM of either TDZ or BAP for shoot and bud proliferation on MS medium only. After 4 weeks, the number of responsive explants were recorded and they were then transferred either to the same media or to hormone-free media and their multiplication rates evaluated.

Sub-culturing was done at regular intervals of 4 weeks up to 24 weeks. Observations on multiplication rates after each subculture were noted with respect to the number of shoots per explant, shoot length, and shoot diameter and the number of internodes and their lengths. For rooting, shoots (above 3.0 cm high) were treated with 500 mgl−1 IBA solution for 30 minutes and transferred directly to potting mix comprising of 9:1:1 :: garden soil : river bed sand : farm yard manure (pH 5.4) in Hikkotrays and the number of established rooted shoots recorded after 60 days.

Table 5: Effect of TDZ/BAP in MS/WPM media on the explant response (%).

Concentration	TDZ (MS)	BAP (MS)	TDZ (WPM)	BAP (WPM)
0	-	-	-	-
100µM	94.0 CF	-	94.7 CF	-
10µM	95.8 CF	33.8 SP	95.5 CF	1.5 SP
1µM	96.7 CF	12.8 SP	96.0 CF	1.3 SP
100 Nm	54.5 CF	-	43.5 SP	-
10 Nm	49.2 CF	-	38.5 SP	-
1 Nm	49.0 CF	-	39.0 SP	-

SP- shoot proliferation; CF-callus formation; - = no response

Callus Induction

The stem were taken from tea seedlings with three or four leaves growing in a greenhouse. The stems were sterilized with 7% chlorinated lime (calcium hypochlorite) for 20 minutes. Stem segments each 2mm long were sampled from first three nodes of seedlings. The epidermal layers were stripped of with a micro-scalpel after disinfection. Therefore three types of explants were inoculated: epidermal layer, intact stem segment (stem segment) and the segment without epidermal layer (stripped segment). The basal medium contained MS inorganic salts supplemented with 3% sucrose, 0.8% agar and 0.5 mg/l thiamine HCL, 0.5 mg/l pyridoxine, 2.0 mg/l glycine and 100 mg/l myoinositol. Plant growth regulators added to the basal medium were IBA and BA. The final pH was adjusted to 5.6-5.8 before autoclaving. The cultures were grown under a 16 hour photoperiod regime. The temperature was maintained under 26 ° C.

Callus induction from these plants were observed after 2 weeks of culture on the medium supplemented with 2 mg/l IBA, 4 mg/l BA and 4 mg/l IBA, 2 mg/l BA. Callus formation occurred four weeks after inoculated is illustrated in (Table 6 and Figure 1). Percentage of callus formation from the epidermal layer was lower than from other explants after 4 weeks of culture, but after 8 weeks of culture, most of the epidermal layers formed a callus and multiplication of the callus was identical in 3 types of explants.

Table 6: Percentage of callus formation from stem (4 weeks).

Growth regulators concentration (mg/l)	Epidermal layer	Stem segment	Stripped segment
IBA 2 mg/l, BA 4 mg/l	43.6	85.5	90.0
IBA 4 mg/l, BA 2 mg//l	53.1	100.0	95.5

Table 7: difference of bud formation in 3types of explants.

Types of explant	Number of inoculated cells	Percentage of bud formation
Epidermal layer	18	22.2
Stem segment	26	4.0
Stripped segment	20	0.0

(Callus tissues were cultured on MS medium supplemented with 0.5 mg/l IBA and 10 mg/l BA for 6 months)

After eight weeks the callus from the epidermal layer begins to form adventitious buds (Figure 2). These buds which were formed in about 20% of the callus were sub cultured monthly on the same medium until they grow sufficiently to be transferred to the rooting medium (Table 7, Figure 3). The callus obtained from the stem segment differentiated buds which were very small and failed to grow up in subsequent subcultures (Figure 4)

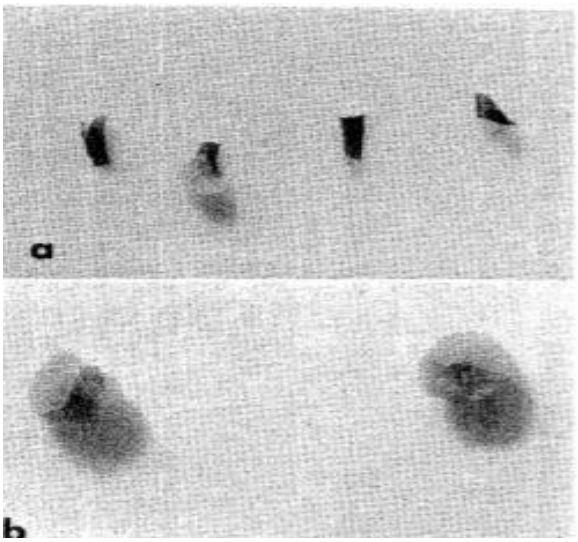

Figure 1 A) callus from the epidermal layer, B) the stripped segment, four weeks after inoculation on medium containing 2 mg/l IBA and 4 mg/l BA.

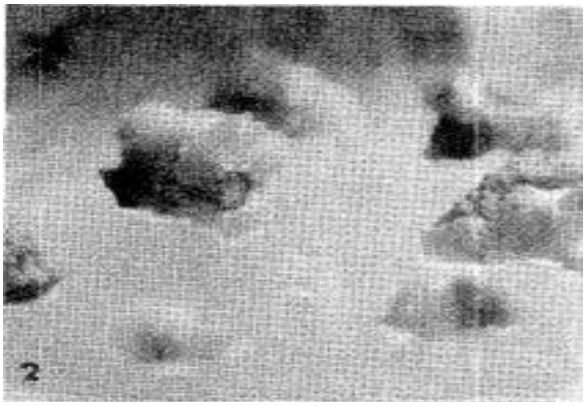

Figure 2. Bud differentiated from callus from the epidermal layer on medium containing 0.5 mg/l IBA and 10 mg/l BA.

Figure 3. Bud showing enough growth for enabling their transfer to the rooting medium with sub culture

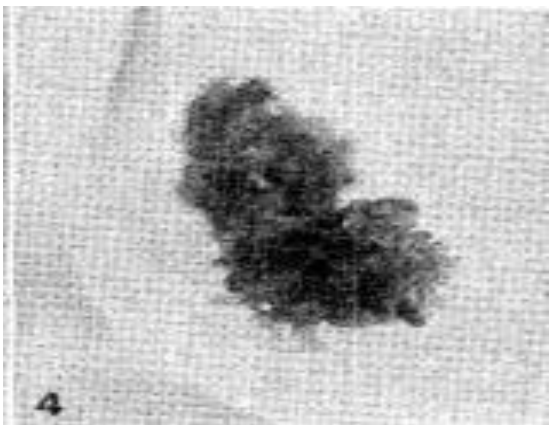

Figure 4. Buds from the callus from the stem segment.

Grafting

Tea micro shoots excised from well-established multiple shoot cultures grown in vitro and 8-week-old, three- to five-leaved seedlings from a local chinery stock (Banuri-96) and UPASI-9 (from southern India) were selected as scions and root stocks, respectively, for grafting. In addition, 4-month- and 12-month-old seedlings of Banuri-96 were also used as root stocks. Cut ends of root stocks and scions were pre-treated with varying concentrations of BAP and NAA for 10 min. A treatment of BAP (5 mg/l) and NAA (5 mg/l) to both scion and stocks in water renewed foliar development at a relatively early stage (40–60 days).

The grafted plants were kept in hardening chambers with CO2-enriched air. No significant difference was observed between autograft (scion and root stock of Banuri clone) and heterograft (scion of the Banuri clone and root stock of UPASI-9). Of the three types (in terms of age) of seedling-raised root stocks employed, grafts on young tea (4-month-old) performed the best (88.33%). Grafts made in early summer established relatively faster and at a high rate of success. The percentage survival of plants transferred to the field was 88.33%.

Figure 5 Grafting micro shoots of tea (*Camellia sinensis*).
A) Decapitation of seedling,
B) slanting cut applied to pre- treated (BAP+NAA; 0.5 mg l–1 each; 10 min) micro shoot,
C) wrapping moist cotton around graft,
D) securing graft union with parafilm

A significant difference was observed in auto- and hetero- grafts, i.e. grafts between scion and root stocks of the Banuri clone (autograft; 88.33%) scion of the Banuri clone and root stock of UPASI-9 (heterograft; 80%) while using 4-month-old young seedlings as root stocks (Table 8) when their establishment in soil was compared.

From our earlier experiments involving direct rooting of tea micro shoots, it was established that humid chambers enriched in CO2 (20/11×10–5 moles l–1 to 80/13×10–7 moles l–1) were suitable for the rooting and hardening of tea micro shoots. Tea is susceptible to misting and direct

watering during hardening. On the basis of results shown above the grafted plants were maintained in CO2-enriched humid chambers superimposed with lighting (15 µmol m–2 s–1) for 100 days. These conditions were suitable for sustaining the development of the union and inducing growth during the establishment of the graft, i.e. for a minimum of 60 days.

After this the plants could be maintained under polytunnels for 6–8 months. At the onset of spring the following year the plants became ready for field transfer (Figure. 6b). The success of plants transferred to the field, observed 100 days after field transfer, and was 88.33%. To date, about 400 plants have been transferred to the field. On comparing the rate of growth of seedling-raised tea (non-grafted) to that of grafted tea on young shoots and tea raised through direct rooting of micro shoots, we observed that the health of the 12-month-old tea seedling was better than that of the young tea graft, followed by rooted tea micro shoots (Figure. 6A). In the first, the new leaves that emerged after grafting were larger in size, and the plants looked relatively healthier than rooted micro shoots. However, when grafted tea shoots were compared with the conventional single-node cuttings, the former showed more vigorous growth than the latter at the 1-year stage. The major advantage was the time saved. Plants of tea with grafted micro shoots were transferred to the field within 10 months, whereas single node cutting-raised plants required 1.5 years before being transferred to the field.

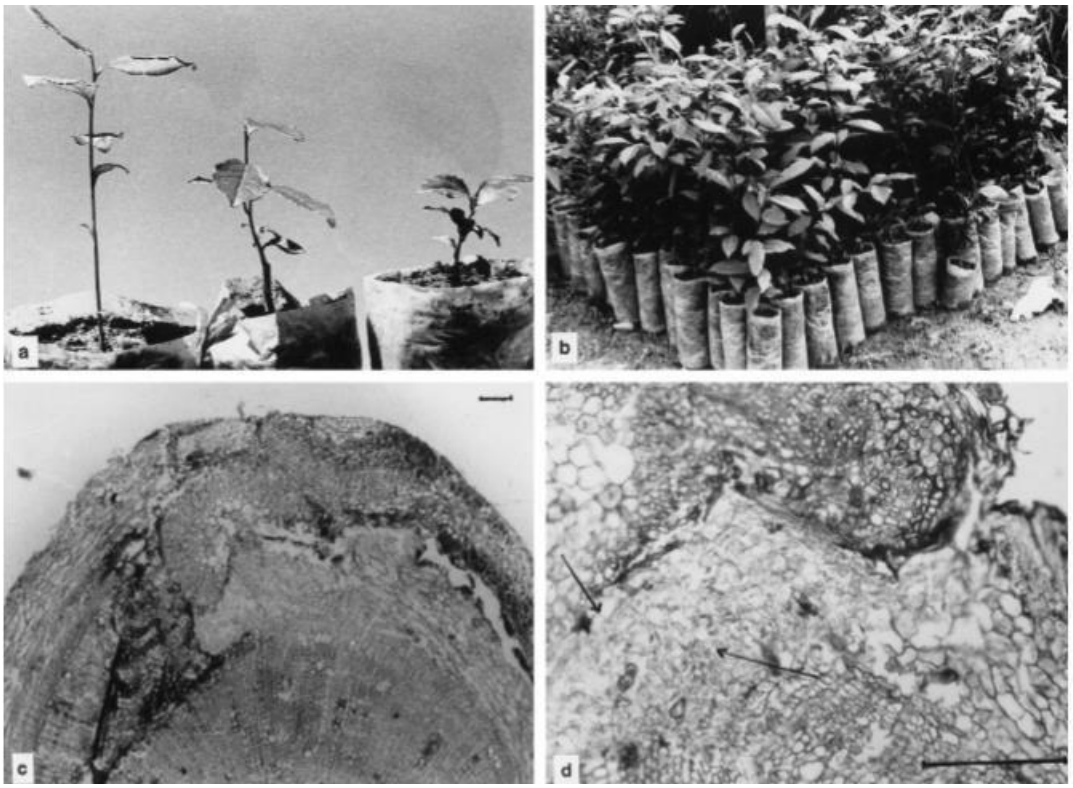

Figure 6. A) A comparison of growth of tea plants after 8 months, from left to right, seedling, grafted micro shoot and directly rooted micro- shoot.
B) Grafted tea plants growing in polysleeves before transplantation.
C) Cross-section at the point of union between root stock and scion.
D) Bridge between vascular bundles of stock and scion

Table 8: effect of age of the root stock on the graft union with micro shoots of tea.

Age of root stock grown from seed	Number of grafts	Number of successfully grafts	Percentage of establishment
2 months Banuri-96 UPASI-9	 500 250	 355 150	 71.00 60.00
4 months Banuri-96 UPASI-9	 180 200	 159 160	 88.33 80.00
12 months Banuri-96 UPASI-9	 55 120	 26 87	 47.00 72.50

CONCLUSION

Generally, the catechins epicatechin gallate, epigallocatechin, and epigallocatechin gallate are stronger antioxidants than the thioflavin and thearubigens, but both classes of tea polyphenols are much more potent as antioxidants than many of the more widely studied compounds, including glutathione, ascorbic acid, tocopherol, butylated hydroxy- toluene, butylated hydroxyanisole, and mannitol. The study shows that catechins from green and black tea are rapidly absorbed and that milk does not impair the bioavailability of the tea catechins. And the study accurately establishes a protocol for the method of use of TDZ in tea micro propagation. For high rates of shoot proliferation, it is necessary to subculture explants (initiated on medium containing TDZ) on to a hormone free medium.

During the initial stages of growth, the number of healthy shoots produced on medium containing TDZ are relatively more and this increases further with every subculture on to hormone free medium for up to over 24 subcultures. Since very low concentrations of TDZ are used only at the initial phase, the overall higher cost of TDZ is overcome. TDZ thus, appears to be a potent cytokinin for tea micro propagation with high proliferation rates. The callus from the epidermis layer as explants formed buds. But the stem segments containing vascular tissues without epidermis formed a callus and roots. These studies would be useful for successful development of tissue culture in materials considered to be difficult for regeneration as the tea plant.

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