Pak. J. Bot., 35(5): 829-834, 2003.

IN VITRO MULTIPLICATION OF *COFFEA ARABICA*

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Abstract

This study was conducted to develop a micropropagation protocol for *Coffea arabica*, one of the most economically important plant for coffee production. Apical and axillary buds and leaf explants were cultured in modified MS medium and incubated at 28°C ± 1°C temperature in both light and dark conditions. The apical bud incubated in the medium supplemented with coconut water and L-Cysteine HCl (10 mg/L) gave the best results. Coconut water promotes growth whereas L-Cysteine HCl inhibits oxidation of phenolic compounds in the medium.

Introduction

In the international trade market, coffee is one of the most wide spread permanent crop of the world and is of decisive importance in the economics of developing countries. The word coffee is used to characterize not only the plant but also the stimulatory drink obtained from the roasted and ground seeds of certain species of the genus *Coffea*, family Rubiaceae. The genus originally had a broad distribution in the tropical and sub tropical parts of Africa and Asia (Monaco *et al.*, 1995). *Coffea arabica* accounts for about 70 % of the total world coffee production and consumed in the world (George, 1996).

Conventionally, coffee is propagated from seed or by vegetative cuttings. Both these methods of propagation have disadvantages and limitations in production of clones in large numbers. Seed propagation is associated with inherent uncontrolled genetic variation in heterozygous cultivars, slow rates of multiplication of seed, and short span of seed viability (Monaco *et al.*, 1995). Propagation of coffee by vegetative cuttings guarantees uniformity. There is more uniformity within plants of the same clone than within plants of different clones.

Multiplication by tissue culture techniques could provide a viable alternative to these traditional methods of coffee propagation. Tissue culture methods permit the production of relatively uniform plants on a massive scale in a shorter period and with a narrower genetic base than is possible under the conventional methods. Coffee plant produced through tissue culture flowered 2-3 years earlier than the plant produced through conventional method (George, 1993).

Coffea arabica has two restrictions: reduction of productivity and susceptibility of the most productive cultivars to main fungal diseases (coffee berry disease by *Colletotrichum coffeanum* and coffee leaf rust by *Hemileia vastatrix*). Fungicides can control these diseases but this is not always economically feasible (Monaco *et al.*, 1995). Hence, exploitation of tissue culture techniques also gains importance in this context. Since the rate of contamination tends to increase with the age of the nodal segment, young plants grown in greenhouses are preferred as source of micro-cuttings (Staritsky, 1970). Furthermore, coffee contains high levels of phenols, making phenolic oxidation a serious problem in primary cultures.

Therefore, in the present study attempts were made to develop and standardize *in vitro* multiplication of *Coffea arabica*. The aims of our study were to identify the most suitable part of the plant as an explant source and the best medium for the growth of *C. arabica*.

Materials and Methods

Explant from mature trees were collected from the experimental field of PCSIR – laboratories, Karachi. Careful selection of undamaged and healthy explant has been made. Apical buds, axillary buds and leaf explants were taken to select the most suitable plant material for coffee in-vitro multiplication. Disinfection of coffee explants from green house was accomplished by washing in few drops of tween 20 and 10 % Na-hypochlorite solution for 25-30 minutes followed by a washing in a solution of L-cysteine HCL 10-100 mg/L. This was then followed by three rinses in sterile double distilled water.

Surface sterile explant were cultured in four different combinations (A, B, C, D, Table 1, modified after Murashige & Skoog, 1962, MS) of salts for shoot initiation and multiplication. The medium is supplemented with 5 % coconut water, 10 mg/L L-Cysteine HCl, 10 g/L Agar, 30 g/L sucrose. The growth regulators used were kinetin 0.1 mg/L and NAA 1 mg/L. The four combinations of these ingredients are: Medium A, only growth regulators were used and considered as control, Medium B, L-Cysteine HCl was used to control the oxidation of phenolic compounds, Medium C, coconut water with growth regulators was used to promote growth, Medium D, to find out the combined effect of L-Cysteine, HCl and coconut water.

The pH value of the medium was adjusted at 5.7 and autoclaved at 121° C for 20 minutes. Approximately, 30 mL of medium was dispensed in jars for tissue culture experiments with five replicates. Growth was recorded every third day after the inoculation and incubation. Cultures were maintained in a growth room at 28 ° C ± 1 ° C under a photoperiod of 16 hours supplied by cool white fluorescent light at 50 μ Mol. m^{-2} s^{-1}. Similarly, cultures were also set in cool incubator for complete darkness to observe the effect of darkness on the oxidation of phenolic compounds.

Table 1. Four different combinations of MS medium.

		A	B	C	D
1	NAA	1 mg./L	1 mg./L	1 mg./L	1 mg./L
2	Kinetin	0.1 mg/L	0.1 mg/L	0.1 mg/L	0.1 mg/L
3	Coconut water			5 %	5 %
4	L-Cysteine HCL		10 mg/L		10mg/L

Table 2. Growth response in dark and light conditions

	Apical buds		Axillary bud		Leaf explant	
Media	Dark	Light	Dark	Light	Dark	Light
A	+	+	-	-	-	-
B	++	+	+	-	-	-
C	++	+	+	-	-	-
D	+++	++	++	+	+	-

(+) = poor growth, (++) = average growth, (+++) = no growth

Figure 1

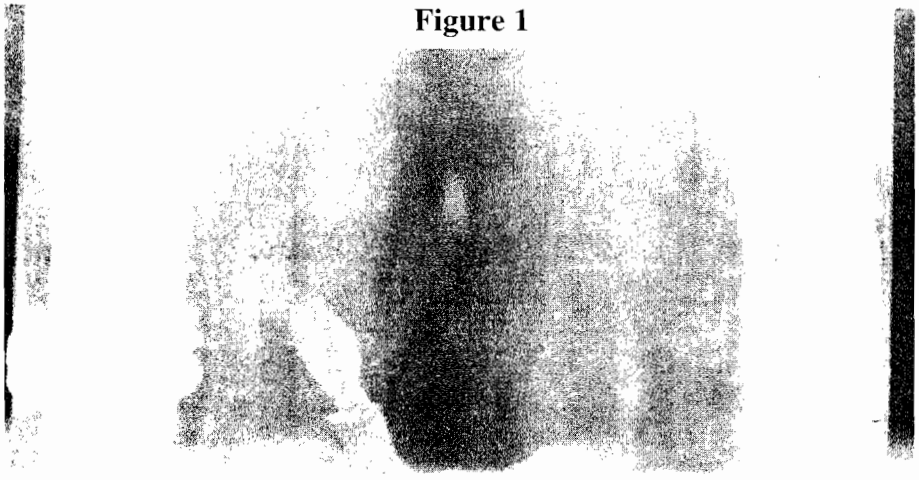


Fig. 1. Shoot initiation in apical bud explant.

Figure 2

Fig. 2. First pair of leave formation from apical bud.

Figure 3

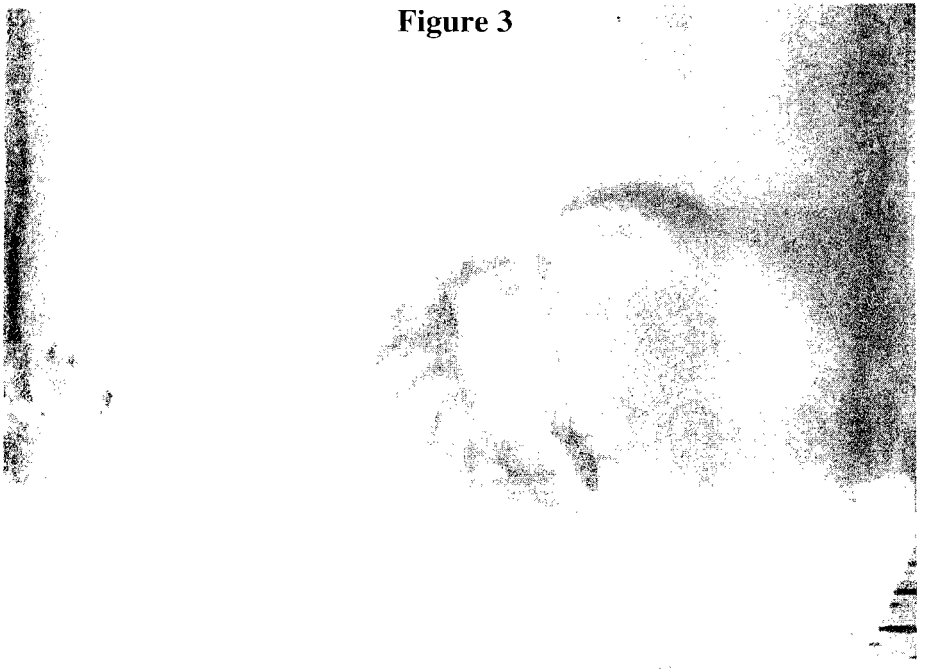
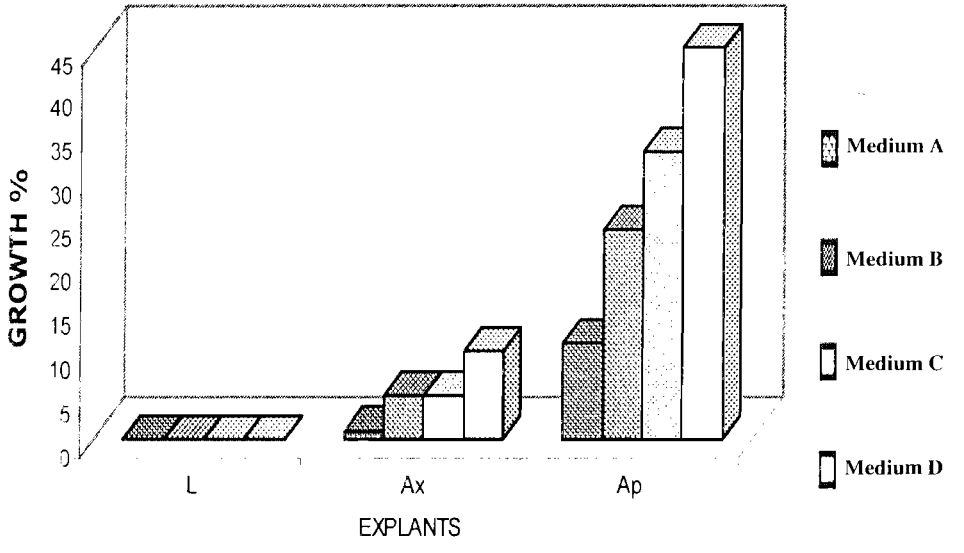


Fig. 3. Second pair of leave formation from apical bud.



L= Leaf expalnt, Ax = Axillary bud, Ap = Apical bud

Fig. 4. Effect of different combinations of medium on selected explants.

Results and Discussion

This study was performed to determine the best explant source like apical and nodal buds and leaves for *in vitro* multiplication of *Coffea arabica*. Monaco *et al.* (1995) reported that different parts as an explant showed difference in potentialities in respect of their origin. Number of scientists have used leaf as an explant source and obtained good results (Sharp *et al.*, 1973; Etienne *et al.*, 2002; Venegas *et al.*, 2002), whereas Sondahl *et al.* (1985) found that the rate of growth of axillary shoot improves and increases by tissue culture in coffee whereas, present study show some deviation *i.e.* apical bud are more vigourous in growth than axillary buds (Figs. 1-3).

According to our results, apical buds showed good results than the axillary buds, whereas leaf explant showed no growth at all (Table 2; Figs. 1-4). Growth was measured in terms of number and length of leaves. Apical bud showed growth in all of the four media, but maximum number and increase in size of leaves were attained in medium D (Fig. 4). Dodds & Roberts (1986) suggested that the apical bud showed best growth than other explants because it receives minimum wound and injury, thus secretes minimum phenolic compounds. Polyphenolases stimulated by tissue injury oxidizes these phenolic substances to growth-inhibitory dark-colour compounds. He further explained that during collection of explants, wounds and cuts initiate the oxidation process in coffee plant therefore, special precautions should be taken for preventing the accumulation of toxic products. Tissues containing relatively high concentrations of phenolic compounds like coffee plants are difficult to culture. The brown colour that frequently develops in cultures of coffee is due to the formation of quinones, which are well known to be toxic to microorganisms and inhibitory to plant's cellular growth (Monaco *et al.*, 1995). The data indicate that incorporation of L-Cysteine HCl in medium 'B' minimizes the formation of phenolic compounds and growth of apical and axillary buds increased at 24 % and 5 % respectively, as compared to control medium 'A' that showed very low growth (Fig. 4, Table 2) whereas, immersion of tissues in an isotonic solution supplemented with the same anti-oxidant also curtailed the oxidation of phenolics (Hu & Wang, 1983; Dodds & Robert, 1986; George, 1993, 1996; Monaco *et al.*, 1995).

It was noteworthy that combined effect of L-Cysteine, HCl and coconut water (medium D) enhance the growth comparatively and maximum length and number of leaves were attained (Fig. 4, Table 2). The MS media (C&D) supplemented with coconut water gave the good result *i.e.* 33 % and 5 % increase in growth of apical and axillary buds respectively, as compared to control medium 'A', because coconut water can induce plant cell to divide and grow rapidly (George, 1993). Gautheret (1942) found that coconut water could be used to initiate and maintain growth and tissue culture of several plants. Coconut water contains natural cytokinin, adding it to the medium often has the same effect as adding the compound cytokinin itself (George, 1993). Part of the growth promoting property of coconut water is due to its myoinositol content (Pollard *et al.*, 1961).

It was also notable that cultures incubated in the dark showed best results than the culture incubated in light (Table 2). Monaco *et al.* (1995) explained that maintenance of culture in the dark promotes growth, because illumination is stimulatory to the production of phenolics. Flick (1983) has also described that phenolic compounds are produced when grown in the light but not in the dark. Another advantage of incubating cultures in the dark is elimination of any possible photo-degradation in the tissues.

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