

# In vitro shoot growth, direct organogenesis and somatic embryogenesis promoted by silver nitrate in *Coffea dewevrei*

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**Abstract** Direct differentiation of shoot buds in *Coffea dewevrei* was evident from the seedling shoots with collar region and also from collar region end of hypocotyl segments in presence of 40  $\mu\text{M}$   $\text{AgNO}_3$ , 8.88  $\mu\text{M}$  of BA and 2.85  $\mu\text{M}$  of IAA. Apart from this, shoot end of hypocotyl explants mainly supported yellow friable callus or somatic embryos. Subsequent transfer to the same medium induced secondary somatic embryogenesis. The collar region of the hypocotyl explants not only showed direct organogenesis by producing 1–3 shoots per explant and also able to produce globular somatic embryos and embryogenic yellow friable callus. Similarly direct somatic embryogenesis along with yellow friable embryogenic callus formation on 1/2 strength MS medium comprising 1.47  $\mu\text{M}$  IAA, 2.22  $\mu\text{M}$  BA and 40  $\mu\text{M}$   $\text{AgNO}_3$  was noticed from cut portion of in vitro leaf and stalk of regenerated plants. The microshoots rooted well upon subculturing onto the same medium in 6 weeks and showed 60 % survival in green house and resumed growth upon hardening.

**Keywords** Collar region · *Coffea dewevrei* · Organogenesis · Silver nitrate · Somatic embryos

## Abbreviations

$\text{AgNO}_3$	Silver nitrate
BA	Benzyladenine
IAA	Indole-3-acetic acid
MS	Murashige and Skoog
RH	Relative humidity

## Introduction

The genus *Coffea* (Rubiaceae) includes more than 60 species, out of this *Coffea arabica* and *C. canephora* are prominent in the trade of coffee beans and the cultivation of the other two minor species *C. liberica* and *C. dewevrei* is limited. *C. dewevrei* ( $2n=22$ ) belongs to section *Eucoffea* and subsection *Pachycoffea* (Carvalho and Monaco 1967). *C. dewevrei* is highly resistant to rust infections with good yield and also require temperature and good light for cultivation (Chevalier 1947; Lopes and Monaco 1979). The levels of caffeine in *C. dewevrei* is ~1.2 % (Ashihara and Crozier 2001) which is more than that of *C. arabica* (1.0 %) but less than that of *C. canephora* cv. Robusta (1.7–2.0 %). Earlier studies showed that in matured fruits of *C. dewevrei* the alkaloid caffeine metabolizes rapidly when compared to the other species (Mazzafera et al. 1991; Mazzafera 1993). When grown undisturbed *C. dewevrei* attain a greater height compared to that of *C. canephora* and *C. arabica* and it may require 4–5 years to bear fruiting. Moreover, it finds use in *C. canephora* breeding programmes. Though in vitro propagation and somatic embryogenesis protocols are well established for both *C. arabica* and *C. canephora* varieties (Giridhar et al. 2004; Vinod et al. 2006a) including the genetic transformation methods for caffeine regulation (Vinod et al. 2006b) no such reports are available with *C. dewevrei*. While pursuing the metabolic engineering of caffeine in *Coffea*, *C. dewevrei* could be used as a model for desired gene silencing. As a tool for such studies somatic embryos are highly useful for efficient gene transfer through *Agrobacterium* mediated transformation. Apart from this, in vitro propagation methods for *C. dewevrei* could be suitable for mass multiplication for its commercial cultivation in view of its superior features such as clustered fruit

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maturation, high seed weight and low caffeine content. In view of this a study has been taken up to standardize an efficient method of in vitro shoot multiplication and somatic embryogenesis for *Coffea dewevrei* from in vitro germinated seedling explants.

## Materials and methods

*Coffea dewevrei* De Wild. & T. Durand ripened and matured fruits were collected from Central Coffee Research Institute, Balehonnur, Karnataka State, India. The pulp is removed and the seeds were used for in vitro germination. Surface sterilization was performed as reported earlier for *Coffea* sp. (Sridevi et al., 2010) by using 1 % Bavistin (active compound Carbendazim 50%WP (wet product), BASF India Limited, Thane, India) for 20 min, then 1 min in 70 % alcohol followed by three times sterile distilled water. Subsequently soaked in 0.10 % (w/v) mercuric chloride ( $\text{HgCl}_2$ ) solution for 10 min and washed five times with sterile distilled water. Later the seeds were soaked in sterile distilled water for 48 h then blot dried and inoculated (2–4 seeds/bottle) into MS basal medium (Murashige and Skoog 1962) comprising with 2 % sucrose (Hi-media, Mumbai, India) and  $40 \text{ mgL}^{-1}$  cysteine HCl (Hi-media, Mumbai, India). The cultures were maintained at  $25 \pm 2^\circ \text{C}$  in the dark. The seedlings emerged were 6–7 cm long by 8–10 weeks and the fully opened cotyledonary leaves and hypocotyls were used as explants for experiment.

Seedling shoots with two cotyledons and hypocotyl base including collar region were used for in vitro shoot elongation under silver nitrate ( $\text{AgNO}_3$ ) treatment. Apart from this hypocotyl explants with collar region were also prepared. The main root system from the seedling was removed by cutting with scalpel just below the collar region. Then explants of ~1 cm in length were trimmed that included hypocotyl with collar region. The in vitro regenerated plants obtained from the above explants are further used for initiation of somatic embryogenesis. The leaves and stalk of these plants were used as explants. Fifty mm square explants were cut from the leaf blade with a scalpel, excluding the basal and apical portions, mid vein and margins. Stalk explants were cut into ten mm segments and collected in petri dishes containing 0.025 % cysteine HCl. The explants were cultured in  $100 \times 20$  mm disposable petri dishes containing approximately 25 ml of the medium. Leaf explants were cultured with their adaxial side in contact with the medium. All the growth regulators used for the study were procured from Sigma, St. Louis, MO, USA. The pH of the respective media was adjusted to  $5.7 \pm 0.2$  and gelled with 0.8 % Agar (Hi-media, Mumbai), followed by autoclaving at  $120^\circ \text{C}$  and pressure of  $1.06 \text{ kg/cm}^2$  for 15 min. The seedling shoots of length ~4.0 cms with cotyledonary leaves and

shoot tip were inoculated directly onto 40 ml growth medium comprising MS components supplemented with  $2 \text{ mgL}^{-1}$  6-benzyladenine (BA)  $2.85 \text{ }\mu\text{M}$  indole-3-acetic acid (IAA), 5–40  $\mu\text{M}$   $\text{AgNO}_3$  and 3 % sucrose (w/v). Some of the explants were as the controls and cultured continuously for 45 days. The other explants were cultured for 3 days on  $\text{AgNO}_3$  devoid medium and then inoculated after 3 days onto the same growth medium as fortified with 5–40  $\mu\text{M}$   $\text{AgNO}_3$ . The cultures were incubated at  $25^\circ \text{C}$  with 16:8 h photoperiod at a light intensity of 2500 lx for 45 days. The shoot length, number of nodes, number of leaves, leaf area were recorded (Table 1). In order to find out the optimal levels of IAA and BA for initiation of callusing/embryogenesis or for induction of direct organogenesis, hypocotyl explants with collar region were inoculated on MS medium (Murashige and Skoog 1962) supplemented with IAA 1.42–5.71  $\mu\text{M}$ , BA 6.66–8.87  $\mu\text{M}$ , sucrose 3 % (w/v) and with or without 40  $\mu\text{M}$   $\text{AgNO}_3$  (Table 2) as testified earlier for *C. canephora* (Sridevi et al. 2010). The explants of hypocotyl with the collar region were placed horizontally on 50 ml medium in 200 ml capacity tissue culture bottles and incubated at  $25 \pm 2^\circ \text{C}$  under 16 h photoperiod with fluorescent illumination of  $45 \text{ }\mu\text{mol/m}^2/\text{s}$  for 8 weeks. The micro-shoots of 2–3 cm length that obtained from the cut edges of explants were excised aseptically and transferred to fresh bottles containing the same medium i.e. MS medium supplemented with 2.85  $\mu\text{M}$  IAA and 8.87  $\mu\text{M}$  BA and 40  $\mu\text{M}$   $\text{AgNO}_3$  for obtaining elongated plantlets and also for in vitro rooting. The well rooted plantlets were removed from the medium freed of agar by washing in running tap water and planted in micro pots containing sand :compost mixture (1:2) for hardening for 30 days at 80 % relative humidity (RH) under polyethylene hoods in the green house.

To get direct somatic embryogenesis or callus from explants of in vitro leaf and surface of hypocotyl (prepared from silver nitrate treatment derived regenerated plant), the same were initially cultured on 1/2 strength MS medium comprising 1.47  $\mu\text{M}$  IAA, 2.22  $\mu\text{M}$  BA, 2 % sucrose and with or without 40  $\mu\text{M}$   $\text{AgNO}_3$  for 8 weeks. Embryogenic callus (weighing 250 mg) or small globular primary embryos (a clump of four embryos of approximately 50–60 mg) were used for proliferation to produce somatic embryos. For induction of secondary embryogenesis the same medium was used i.e. MS medium supplemented with 2.85  $\mu\text{M}$  IAA, 8.87  $\mu\text{M}$  BA and 40  $\mu\text{M}$   $\text{AgNO}_3$ . Mature embryos were cultured on half-strength MS medium containing 2 % sucrose, inositol ( $100 \text{ mgL}^{-1}$ ), thiamine HCl ( $8 \text{ mgL}^{-1}$ ), pyridoxine HCl ( $3.2 \text{ mgL}^{-1}$ ), IAA ( $2.85 \text{ }\mu\text{M}$ ), and BA ( $8.87 \text{ }\mu\text{M}$ ). After 6–8 week of growth, plantlets developed the first pair of leaves. At this time, cotyledons and roots were cut off and the shoots were transferred to MS basal medium for further development. To study the in vitro propagation of seedling shoots ten explants were used in each treatment and control. To get direct organogenesis from

**Table 1** Effect of silver nitrate on *C. deweyrei* shoot growth after 3 months

AgNO <sub>3</sub> (μM)	Shoot elongation [cm.]	Number of leaves [explants <sup>-1</sup> ]	Number of nodes [explants <sup>-1</sup> ]	Leaf area [explants <sup>-1</sup> cm <sup>2</sup> ]
control	7.02±0.08	4.4±0.20	2.2±0.20	2.5±0.09
10	9.0±0.04	6.7±0.26	2.8±0.19	4.42±0.08
20	10.5±0.09	7.3±0.26	3.3±0.15	3.54±0.09
30	11.2±0.21	10±0.29	4.7±0.15	6.71±0.12
40	18±0.72	11.3±0.26	5.3±0.21	13.07±0.16
50	17.5±0.85	11.1±0.31	5.2±0.29	12.03±0.05

Values are means of 10 explants ± SE

hypocotyl explants with collar region, the experiments were repeated twice with 50 explants each. Similarly, 100 explants each (leaf and hypocotyls) were used for callus induction and somatic embryogenesis. The experiments repeated twice and the mean ± S.E. values are given in respective tables and tested using the one-way ANOVA test and the results were analyzed using Tukey's Multiple Comparison.

## Results

In the present study, AgNO<sub>3</sub> showed good response for both organogenesis and somatic embryogenesis in *C. deweyrei*. Silver nitrate enhanced growth of *C. deweyrei* seedling shoot tips during the 3 months of culturing and at 40 μM it nearly tripled the growth of *C. deweyrei* shoots (18±0.72 cm). Even the number of nodes (5.3±0.23), leaves (11.3±0.26) and leaf area (13.07±0.16) were more (Table 1). The seedling based shoot tips were not exposed to AgNO<sub>3</sub> for the first 72 h, because it seemed likely that ethylene may be required during the first 72 h of culture for growth on the basis of a hypothesis advanced by Cho and

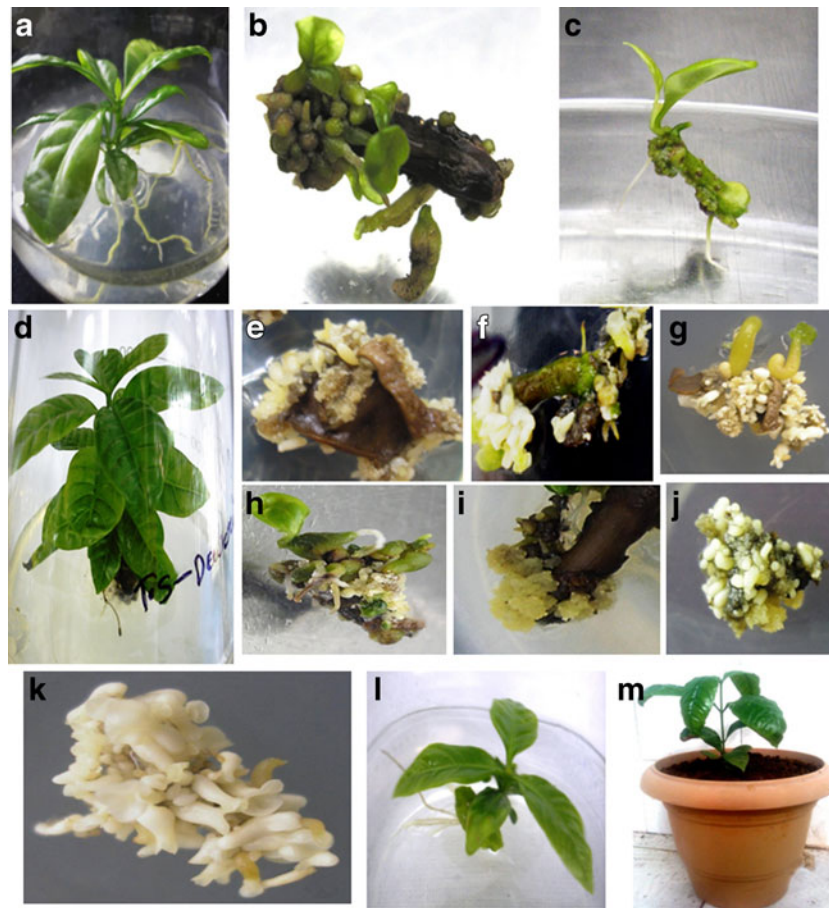
Kasha (1989,1992) with barley. During the prolonged incubation of 3 months culturing period efficient in vitro rooting was established and plants measuring 10–13 cm were obtained (Fig. 1a), Upon hardening and transplantation to pots showed 60 % survival in a green house. Embryogenic callusing mass (yellow friable), along with few torpedo shaped embryos was also noticed from the bottom of the shoots during 3–4 months culturing.

In the present study, direct organogenesis of shoot from the cut edges of the explants from the collar region of hypocotyl explants was significantly evident on MS medium containing 2.85 μM IAA and 8.87 μM BA along with 40 μM of AgNO<sub>3</sub> (Table 2, Fig. 1b). Similar organogenetic response was found in few other explants at shoot end of hypocotyl segments. Medium without growth regulators did neither support callusing nor embryogenesis. There was no shoot formation from the explants on medium devoid of AgNO<sub>3</sub>, but 30 % explants showed response for callusing along with somatic embryos (data not shown). The response for direct shoot regeneration from the collar region was 70 % in presence of 2.85 μM IAA, 8.87 μM BA and 40 μM of AgNO<sub>3</sub> (Fig. 1b) compared to the ones at other

**Table 2** Effect of different concentrations of IAA and BA in MS medium supplemented with 40 μM AgNO<sub>3</sub> on regeneration of shoots from hypocotyls explants with collar region of *C. deweyrei* after 8 weeks of culture

IAA [μM]	BA [μM]	Regenerating shoots [%]	Callus with somatic embryos [%]	Number of explants producing shoot buds	Number of shoots [explants <sup>-1</sup> ]	Length of shoot [mm explants <sup>-1</sup> ]	Number of somatic embryos [explant <sup>-1</sup> ]
-	-	-	-	-	-	-	-
1.42	6.66	-	-	-	-	-	-
1.42	6.66	-	20	-	-	-	-
1.42	8.87	-	30	-	-	-	-
1.42	11.09	-	40	-	-	-	-
2.85	6.66	30	30	-	1.0	4.33±0.12	-
2.85	8.87	70	50	50	2.6±0.17	9.98±0.38	16.3±0.77
2.85	11.09	40	40	10	1.0	4.25±0.14	4.9±0.37
5.71	6.66	-	30	-	-	-	-
5.71	8.87	-	20	-	-	-	-
5.71	11.09	-	20	-	-	-	-

Medium without growth regulators did not support callusing and embryogenesis. In absence of silver nitrate, only the combination of 0.5 mg IAA and 2.0 mg BA able to show (30 % explants) response for callusing with somatic embryos. Values are means of 20 explants ± SE



**Fig. 1** Organogenesis and somatic embryogenesis in *Coffea dewevrei*. **a** Elongated in vitro seedling shoot with established rooting upon prolonged culturing on MS medium containing 2.85  $\mu\text{M}$  of IAA and 8.87  $\mu\text{M}$  of BA and 40  $\mu\text{M}$  of  $\text{AgNO}_3$  established rooting (bar 30 mm). **b** Direct shoot formation from collar region end of hypocotyls explants (bar 10 mm). **c** Single shoot formation from shoot end of hypocotyl explants (bar 10 mm). **d** Three months old regenerated plant (~11 cm) with roots initiation from the base (bar 30 mm). **e** Direct somatic embryos formation from in vitro leaf explants in presence of 2.85  $\mu\text{M}$  of IAA and 8.87  $\mu\text{M}$  of BA (bar 10 mm). **f** Direct somatic embryos formation from in vitro stalk explants in presence of 2.85  $\mu\text{M}$

of IAA and 8.87  $\mu\text{M}$  of BA (bar 10 mm). **g** Augmented somatic embryos formation from in vitro leaf explants in presence of 2.85  $\mu\text{M}$  of IAA and 8.87  $\mu\text{M}$  of BA and 40  $\mu\text{M}$  of  $\text{AgNO}_3$  (bar 10 mm). **h** Augmented somatic embryos formation from in vitro stalk explants in presence of 2.85  $\mu\text{M}$  of IAA and 8.87  $\mu\text{M}$  of BA and 40  $\mu\text{M}$  of  $\text{AgNO}_3$  (bar 10 mm). **i** Proliferation of yellow friable callus and embryos formation from in vitro stalk explants (bar 10 mm). **j** Somatic embryogenesis from yellow friable callus of stalk explants. **k** Secondary somatic embryogenesis from primary globular embryo cultures (bar 10 mm). **l** Regenerated plant with roots (bar 20 mm). **m** 6 months old Potted plant (bar 50 cm)

end (shoot end) of the explants (Fig. 1c). In 50 % of explants 2–3 shoots were noticed. The appearance of ~1 cm long shoots with two leaves is evident by 7 weeks of culturing. Only single shoot formation was observed on medium containing 40  $\mu\text{M}$   $\text{AgNO}_3$ , 2.85  $\mu\text{M}$  IAA and 6.66–11.09  $\mu\text{M}$  BA. The initiation of direct organogenesis was noticed after 2 weeks of culturing with small protrusion at the cut edge of the explants. Simultaneously, some granular greenish-yellow callus mass at both the ends of the explants was observed and sporadically over the entire surface of the hypocotyls explants.

Yellow friable callus (yfc) production at cut regions of 50 % of hypocotyl explants with collar region was noticed on medium comprising 2.85  $\mu\text{M}$  IAA and 8.87  $\mu\text{M}$  BA and 40  $\mu\text{M}$  of  $\text{AgNO}_3$  (Fig. 1b), which also supported direct

organogenesis. Prolonged culturing of the explants on the same medium induced somatic embryos formation ( $16.3 \pm 0.77$  per culture) from the callus (Fig. 1b). Subculturing the primary embryogenic clumps on to the fresh medium leads to secondary somatic embryogenesis (data not shown). The elongation of microshoots was also achieved on MS medium containing 2.85  $\mu\text{M}$  IAA and 8.87  $\mu\text{M}$  BA and 40  $\mu\text{M}$   $\text{AgNO}_3$ , wherein, 4 cm long shoots with 3 internodes (6 leaves) obtained in 7 weeks with one subculturing. In vitro rooting was established in these elongated shoots upon subculturing onto the fresh medium that was used for elongation. After 11–14 weeks of culture, plants measuring 12–15 cms were obtained (Fig. 1d) which upon hardening and transplantation to pots showed 60 % survival.



**Table 3** Induction of somatic embryogenesis from in vitro leaf and stalk of in vitro regenerated plant of *Coffea dewevrei*

*Plant Growth regulator $\mu\text{M}$		Explants responded for embryogenesis [%]		Number of embryos [explants <sup>-1</sup> ]		Explants producing embryogenic callus [%]	
IAA	BAP	Leaf	stalk	Leaf	stalk	Leaf	stalk
1.42	2.22	-	-	-	-	-	-
1.42	2.22	40	-	10.15 $\pm$ 4.47	-	20	40
1.42	4.44	50	30	43.6 $\pm$ 1.22	16.36 $\pm$ 0.65	20	30
1.42	2.22	80	50	59.35 $\pm$ 2.29	29.8 $\pm$ 1.02	60	60
2.85	2.22	60	30	52 $\pm$ 3.15	19.6 $\pm$ 0.64	50	30
2.85	4.44	40	20	28 $\pm$ 2.45	9.65 $\pm$ 0.57	50	-
5.71	1.11	50	-	18 $\pm$ 0.98	-	60	20
5.71	2.22	50	-	14.2 $\pm$ 1.18	-	40	-
5.71	4.44	30	-	10.2 $\pm$ 0.92	-	20	-

\*Medium used: 1/2 strength MS medium with 2 % sucrose

MS medium containing 1.42  $\mu\text{M}$  IAA and 2.22  $\mu\text{M}$  BA along with 40  $\mu\text{M}$  of  $\text{AgNO}_3$  found good for direct somatic embryogenesis from leaf and stalk of in vitro regenerated plants. The somatic embryos appeared as small white globular masses, which germinated and passed through successive developmental stages. The effects of BA and IAA at optimal levels that used in this study showed influence on the frequency of embryo and callus formation per explant of *C. dewevrei* is described in Table 3. Direct somatic embryogenesis achieved from 90 % of in vitro leaf explants with 59.35 $\pm$ 2.29 somatic embryos (Fig. 1e) and 50 % of stalk explants produced 29.8  $\pm$ 1.02 somatic embryos (Table 3, Fig. 1f). Incorporation of 40  $\mu\text{M}$  of  $\text{AgNO}_3$  to the medium containing 1.42  $\mu\text{M}$  IAA and 2.22  $\mu\text{M}$  BA induced high frequency somatic embryogenesis (90 % explants) wherein, 190–230 globular embryos were produced from the cut surface of in vitro leaf explants (Fig. 1g). Similarly on the same medium in vitro regenerated plant stalk explants able to produce 50–60 somatic embryos (Fig. 1h).

Yellow friable callus was noticed in both leaf and stalk explants (60 %) wherein marginal callusing was noticed (Table 3) in presence of 1.42  $\mu\text{M}$  IAA and 2.22  $\mu\text{M}$  BA. But in presence of 40  $\mu\text{M}$  of  $\text{AgNO}_3$  the response was higher with different stages of somatic embryos appearance along with yellow friable callus (Fig. 1i). The higher concentration of  $\text{AgNO}_3$  in medium (>40  $\mu\text{M}$ ) reduced somatic embryogenesis in *C. dewevrei* from both cotyledonary and hypocotyls explants. Subculturing of the callus into same medium induced somatic embryos of globular stage in 6–8 weeks (Fig. 1j) and even secondary somatic embryogenesis was noticed (Fig. 1k). Matured embryos (greenish globular and tubular stage) physically removed from the explants and placed on developing medium to form individual plantlets (60–75 %) (Fig. 1l). They produced shoots after 4–6 weeks. All the regenerated plantlets

appeared to be morphologically normal. The regenerated plantlets were rooted on half strength MS basal medium within 6 weeks (Fig. 1f).

## Discussion

Silver nitrate incorporation to the medium containing optimal levels of IAA and BA promoted efficient growth of seedling shoots with collar base. Long shoots with number of nodes and big leaves were evident compared to controls (without  $\text{AgNO}_3$ ), which shows the efficiency of  $\text{AgNO}_3$  as supported by similar studies in *Vanilla planifolia* (Giridhar et al. 2001). According to earlier reports, very high levels of  $\text{AgNO}_3$  (20  $\text{mg l}^{-1}$ ) is required to get *C. arabica* shoots of 3 cm long in 70–80 days culturing on a medium containing 4.44  $\mu\text{M}$  BA (Ganesh and Sreenath 1996). In the present study lower levels of  $\text{AgNO}_3$ , with 2.85  $\mu\text{M}$  IAA and 8.87  $\mu\text{M}$  BAP containing medium produced long shoots with more nodes and leaves in only 45 days period as in case of other *Coffea* species i.e. *C. arabica* and *C. canephora* (Giridhar et al. 2003). Similarly, hypocotyl explants with collar region naturally showed good response for direct induction of shoots in the initial 3–4 weeks of culturing on MS medium supplemented with optimal levels of BA and IAA along with  $\text{AgNO}_3$ , and also showed embryogenic yellow friable callus and somatic embryos in significant number. The response for direct shoot regeneration from the collar region end of hypocotyl explants is high (70 %) wherein, mostly single microshoot was obtained. In medium devoid of  $\text{AgNO}_3$  there was no shoot formation from the explants. Alteration in IAA and BA to high or low in presence of  $\text{AgNO}_3$ , did not demonstrate any response for direct shoot formation except 2.85  $\mu\text{M}$  IAA and 6.66–11.09  $\mu\text{M}$  BA wherein, 30–40 % explants responded. Compare to other cytokinins, BA was reported to be good

for inducing direct organogenesis of shoots (Sonia et al. 1998). Upon subculturing onto MS medium containing 2.85  $\mu\text{M}$  IAA, 8.88  $\mu\text{M}$  BA and 40  $\mu\text{M}$   $\text{AgNO}_3$  the leafy microshoots elongated into 4 cm long shoots with 3 internodes (6 leaves) in next 6 weeks culturing. In most of these shoots single root (~1 cm long) was also present. However, one more subculturing onto same medium leads to efficient in vitro rooting wherein, plants with 4–5 roots (~2–3 cm long) were obtained which upon hardening showed 70 % survival. Overall, in the controls (MS medium) neither rooting nor effective shoot growth occurred. This is the first report on direct organogenesis in *C. dewevrei* variety from collar region end of hypocotyl explants of in vitro raised seedlings. Similarly the effect of  $\text{AgNO}_3$  on in vitro shoot regeneration of *C. dewevrei* has not been reported previously. A large number of reports are adding on the utility of  $\text{AgNO}_3$  in plant tissue culture and other applications, (Vinod et al. 2009). Silver nitrate being inhibitor of ethylene action (Beyer 1976) greatly improved the in vitro regeneration of many dicot and monocot cultures (Duncan et al. 1985). Promotive effect of optimal levels of  $\text{AgNO}_3$  during clonal propagation (Pestana et al. 1999; Giridhar et al. 2001), somatic embryogenesis and also in vitro rooting (Kong and Yeung 1994; Reddy et al. 2001; Giridhar et al. 2004) was well documented. The response what we got from hypocotyl based explants for organogenesis in the present study is similar to that of *Tamarindus indica* (Sonia et al. 1998) and *Psidium guajava* (Singh et al. 2002) but in both the cases the response was specific to different growth regulators wherein, silver nitrate was not used. It could be possible to get either direct shoots or somatic embryos along with yellow friable callus by using  $\text{AgNO}_3$ , which will produce somatic embryos upon in subsequent subculturing, hence this method is advantageous. Though the number of such shoots or somatic embryos produced in this method is not so high compared to earlier reports (Giridhar et al. 2004) direct or indirect somatic embryogenesis based on either leaf or hypocotyls, for obtaining even small number of transgenic cultures (plants or embryos), this method would be helpful as the percentage of transformation efficiency was good in our study. Silver nitrate incorporation to the medium at higher levels (>40  $\mu\text{M}$ ) did not show good response for shoot growth in our study which was in concurrence with a similar response in other plants (Giridhar et al. 2004; Sridevi et al. 2010) and the reasons for the same were attributed to silver ion mediated responses involvement in ethylene, polyamines and calcium-mediated pathways (reviewed by Vinod et al. 2009) that in turn influence the morphogenesis. From our study it is clear that  $\text{AgNO}_3$  supported efficient growth of seedling shoots of *C. dewevrei*, and also direct organogenesis with simultaneous embryogenesis from collar region of hypocotyl explants, in vitro leaf and stalk explants of regenerated plants. As seedling explants with collar region have shown better response the same could be used for mass multiplication through in vitro propagation.

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