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In Vitro Propagation of Coffea arabica cv. MCH2 Using Nodal Explants

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Abstract
A protocol for rapid in vitro propagation of Coffea arabica L hybrid (MCH2) through shoot induction from nodal explants was established. Proliferation of shoots from nodal segments was achieved on Murashige and Skoog medium supplemented with different concentrations of N^6-benzyladenin (BA). The highest number of shoots per explant (3.5±0.8) was obtained on MS medium supplemented with 44.4 µM (10 mg/l) BA. Moreover, 135.7 µM (25 mg/l) adenine hemisulfate could not enhance shoot proliferation. In vitro regenerated shoots rooted best on half-strength MS medium containing 7.35 µM (1.5 mg/l) IBA and 20 g/l sucrose. There was no significant difference between 20 or 30 g/l sucrose as well as no significant interaction effect were observed between sucrose and IBA on rooting. Rooted shoots, acclimatized in the green house with 87 % survival and were successfully transferred to field conditions.
Keywords: Coffee, nodal explants, shoot proliferation, N^6-benzyladenin, 3-indolbutric acid

1. Introduction

Coffee is one of the major source of export in Ethiopian economy as it contributes 25-30% of the Ethiopia's total export earnings (Abu Tefera, 2015). Beyond this, based on molecular evidence, South-western Ethiopia is the natural habitat and primary center of diversity of *Coffea arabica* (Lashermes et al., 1996). This immense genetic recourse provides huge opportunity for coffee improvement through pure line selection and hybridization for yield, disease resistance and quality.

Despite the existence of genetic variation for improvement, the national average yield is very low (6 qt/ha) (Behailu et al., 2008). The presence of old and poorly managed farm trees, lack of improved cultivars, occurrence of disease such as coffee berry disease, leaf rust and coffee wilt are among the major factors for this low productivity (Dubale and Tektay, 2000).

In order to address this production constraints, Jimma Agricultural Research Center (JARC) developed three hybrid coffee cultivars Aba buna, MCH2 and Gawe with average yield of 23.8,24, and 26 qt/ha clean coffee, respectively (Bellachew and Labouisse, 2007). In addition, Amaha and Bellachew (1983) reported higher hybrid performance for yield (18%-60%) among the cross of elite breeding materials in Ethiopia. Therefore, the demand for hybrid seed and seedling from coffee farmers as well as from coffee plantation owners is high. However, those high yielding coffee varieties were not distributed to coffee growers, due to lack of efficient seedling production techniques. Currently, JARC producing hybrid seed through conventional approach that is inefficient. Therefore, an economically feasible, clonal propagation method is needed to satisfy the growing demand of hybrid seed in order to make effective use of observed yield advantage.

Hybrid coffee is propagated from seed or by orthotropic steam cuttings. Seed propagation, which is associated with hand pollination to get F1 seed, is the major method used in Ethiopia. This method is time consuming and need large number of skilled labor. Consequently, using F2 leads to lack of uniformity as a result of segregation. Propagation of coffee by vegetative cuttings guarantees uniformity. Cuttings generate relatively low multiplication rates as they can only be obtained from orthotropic branches. Multiplication by tissue culture techniques could provide a best 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 those under the conventional methods (Kumar et al., 2006). Several studies have been carried out with the aim of micropropagation of superior coffee genotypes by using apical or axillary meristem and nodal cultures (Custers, 1980; Carneiro, 1993; Berthouly et al., 1994). Ribeiro and Carneiro (1989) reported genotype dependent response of different coffee cultivars on shoot proliferation potential. This necessitate optimization of multiplication protocol for mass propagation of elite hybrid coffee varities invitro. Therefore, the objective of this work was to optimize an efficient protocol for rapid clonal multiplication of *Coffea arabica* L hybrid (MCH2) through shoot induction from nodal explants.

2. Materials and Methods

2.1 Shoot establishment and multiplication

Single node segments were obtained from invitro plantlets for this experiment. To determine the effect of benzyladenin (BA) concentration on shoot multiplication, single node were cultured on MS (Murashige and Skoog, 1962) medium containing BA 5 mg/l (22.2 µM) and 10 mg/l (44.4 µM). The PH was adjusted to 5.6 before autoclaving. For each BA concentration, 40 single node were placed in culture. The culture were

maintained at 25±2 ^{o}C under cool white fluorescent lamps with ~ 68µmol.s^{-2}.m^{-2} light and 16 hr photo period. Observations were done on the number of shoots per node and comparison were done using student's t-test.
In order to see the effect of adenine sulfate (ADS) on shoot proliferation, 25 mg/l adenine sulfate + 22.2 µM BA were also compared with BA (22.2 µM) alone.

2.2 Rooting and acclimatization.
Shoots obtained from cultures multiplied on 10 mg/l (44.4 µM) BA were used for rooting studies. Shoots were first cultured on hormone free media containing 0.5 % (w/v) activated charcoal (Takayama and Misawa, 1980) in order to avoid carry over effect of BA. Regenerated shoots were excised when they reached about 4- 5 cm in length and transferred on to half-strength MS media supplemented with 2.45,4.90,7.35 and 9.8 µM 3-indolbutric acid (IBA) in combination with 20 or 30 g/l sucrose. To avoid heat degradation, IBA was added after filter sterilization to the autoclaved media using Millex-GP Syringe Filter (0.22 µm). The experiment was set up as completely randomized design (CRD) with 4 x 2 factorial design with three replication each 16 explants. After 10 weeks, observations were made on rooting percentage and rooting length. Then, plantlets were removed from the root induction medium and washed with tap water to remove the remnants of culture medium transplanted into seedling tray containing sand, vermi compost and top soil and covered with transparent polyethylene to ensure high humidity. Polythene cover were removed after two weeks to acclimatize plants to field condition. The surviving plants were transplanted to the field condition after eight months.

3. Results and discussion
3.1 In vitro shoot multiplication
The present investigation was carried out to optimize shoot proliferation potential of nodal explants of *C. arabica* hybrid MCH2. Shoot regeneration from *in vitro* cultured nodal segments was tested on MS medium supplemented with two BA concentration (22.2 and 44.4 µM). Multiple shoots sprouting appeared within 4 weeks of culture initiation. Significant differences in the number of shoots per explant were detected between two concentration of BA (P<0.05). Out of the two concentration tested, 44.4 µM resulted in the highest number of shoots (3.72 shoots per node) whereas, 2.74 shoot per node obtained using 22.2 µM BA. Shoot bud proliferation was not induced from nodal explants cultured on MS medium without plant growth regulators. This is consistent with the study of Custer, (1980) in which 2.8 shoots per node on average was obtained using 44.4 µM BA on *C. arabica*. Likewise, Ribeiro and Carneiro (1989) also obtained 6.8,2.5 and 2.7 shoots per node on average from three *C. arabica* cultivars, Caturra, Geisha and Catimor, respectively.

In order to enhance shoot proliferation, the effect of BA and adenine hemisulfate combination was also evaluated on multiple shoot proliferation from nodal segments. Nodal explants cultured on MS medium supplemented with 25 mg/l adenin hemisulfate with BA and BA alone was evaluated. Significant differences in number of shoots per explant were detected between BA with ADS and BA alone (P<0.05). Medium supplemented with 22.2 µM BA only induced a mean of 2.82±0.12 shoots per explants (Fig.3), while medium supplemented with 22.2 µM BA and 25 mg/l adenin hemisulfate resulted in a reduced number of shoots although with longer internodes (Fig. 4). BA together with ADS suppressed the shoot proliferation in nodal explants. Reduction in the number of shoots generated when ADS used was also reported for other plants. Jarret et al. (2008) found that ADS in the concentration range 30-300 mg/l was inhibitory for the formation of shoots from cultured tuber discs of potato. In contrary, the enhancing role of ADS for shoot multiplication has also been reported in various woody species like *Ficus religiosa* (Deshpande et al 1998; Siwach and Gill 2011), *Bauhinia vahlii* (Dhar and Upreti 1999), *Petrocarpus marsupium* (Husain et al 2008).

3.2 In vitro rooting
The regenerated shoots from nodal explants were excised and transferred to half-strength MS medium supplemented with 2.45,4.9,7.35 and 9.8 µM IBA in combination with 20 or 30 g sucrose. The analysis of variance for this experiment indicated that the sucrose effect and the sucrose x IBA interaction were not significant for root length; however, the main effects of IBA were significant (Table 1.). The maximum percentage of root formation (66%) (Table 2.) and length (2.4±0.4) of roots was achieved in half-strength MS medium containing 7.35 µM (1.5 mg/l) IBA with 20 g sucrose (Fig.5). Callus formation at root base was also observed on cultures treated with 9.8 µM (2 mg/l) (Fig. 2e). The effectiveness of IBA in rooting has been also reported in *C. arabica.* For instance, Kartha et al., (1981) induced root in *C. arabica* shoots using half-MS medium supplemented with 1µM IBA without sucrose. In contrast, Lubabali et al., (2014) obtained well developed root from hybrid coffee using half-MS with 9.8 µM IBA and 20 g sucrose. Our result also confirmed the benefit of sucrose for in vitro rooting of arabica coffee.

Rooted plantlets were transferred to 35-cell tray containing soil mix (2 top soil:1 sand:1 coffee husk) after washing extra agar on root surface. They were kept in box to maintain humidity for one week, then opened

gradually for the rest of acclimatization period with 87% survival. Plants then were transferred to plastic pots containing the same soil mix medium in lat house and grown for 7 months with 100% survival rate.

In conclusion, procedure for shoot regeneration from nodal segments of *C. arabica* hybrid MCH2 has been demonstrated. In vitro shoot multiplication were successful in MS medium supplemented with 44.4 µM (10 mg/l) BA and subsequently transferring to hormone free medium with activated charcoal. Then after, rooting were found effective on half-MS medium supplemented with 7.35 µM (1.5 mg/l) IBA and 20 g sucrose. The present study opens the way to scale-up studies to enhance mass propagation in liquid media with nodal segment cultures.

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Table 1. ANOVA of the effect of IBA and sucrose concentration on *C. arabica* cv. MCH2 root formation

Source of variation	df	Mean squares	F-value
IBA	3	8.166	11.266^{*}
sucrose	1	0.175	0.242^{NS}
IBA*sucrose	3	0.949	1.31^{NS}
Error	56	0.725	

*Significant at 5% significance level.

Table 2. Rooting percentage of *C. arabica* cv. MCH2 as affected by IBA and sucrose concentration level

	Sucrose concentration (g)	
	20	30
IBA concentration (µM)	Rooting (%)	
2.45	34	28
4.90	53	59
7.35	66	56
9.80	44	34

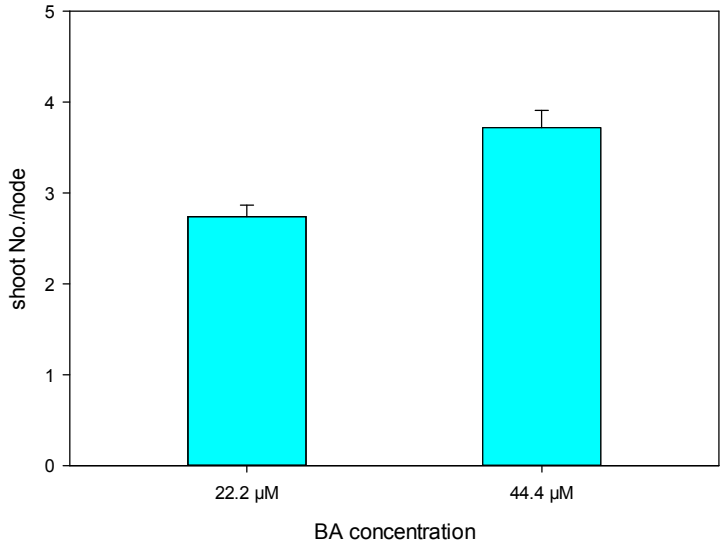

Figure 1. Effect of BA on shoot formation of *C.arabica* cv. MCH2. Data represent the mean ± s.e.m and $P < 0.05$,Student's t-test.

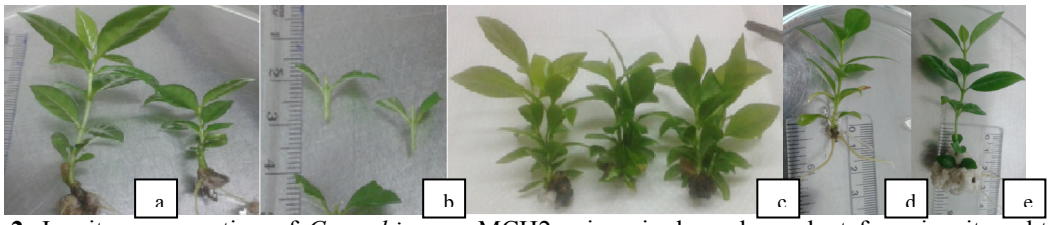

Figure 2. In vitro propagation of *C. arabica* cv. MCH2 using single node explant from in vitro obtained plantlets.(a) In vitro plantlets used as explant source,(b) single node explants (c) shoot multiplication on MS+ BA (44.4µM),(d) In vitro rooted plants on 1/2 MS + IBA (7.35µM) + 20 g sucrose, (e) In vitro rooted plants on 1/2 MS + IBA (9.8 µM) + 20 g sucrose.

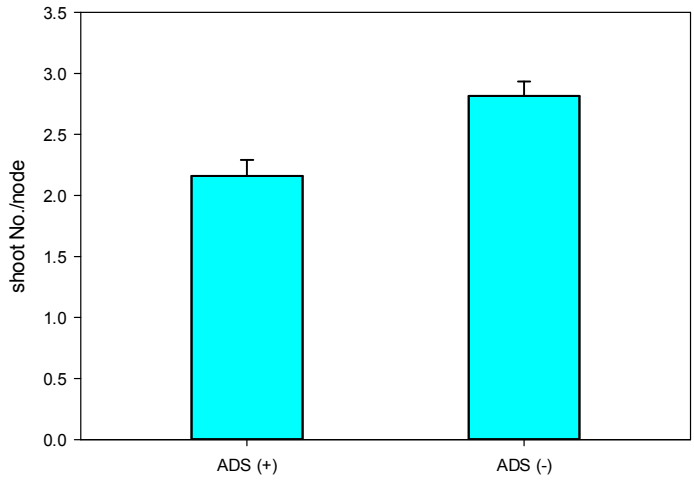

Figure 3. Effect of adenine sulfate on shoot proliferation of *C. arabica* cv. MCH2. Data represent the mean ± s.e.m and $P < 0.05$,Student's t-test. ADS (+) =BA (22.2 µM) + ADS (135.75 µM); ADS (-) = BA (22.2 µM).

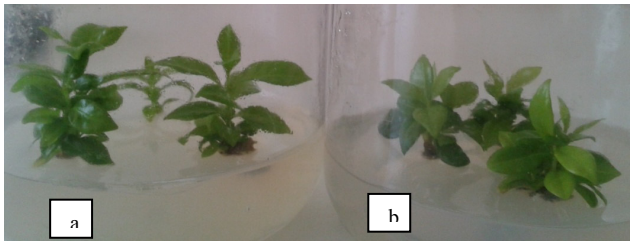

Figure 4. Comparison of shoot proliferation (a) with and (b) without adenine sulfate.

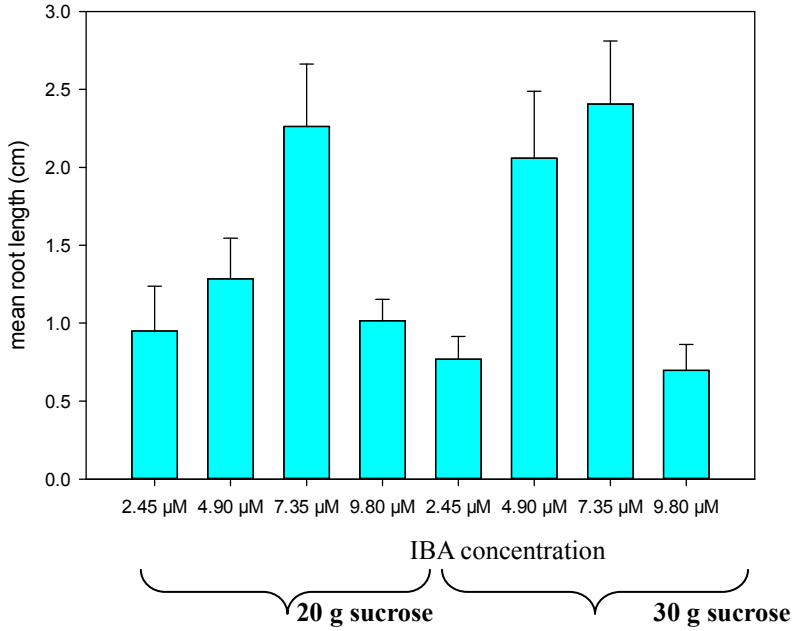

Figure 5. Effect of IBA and sucrose concentration on rooting of in vitro *C. arabica* cv. MCH2 shoots. Bars represent means ± s.e.m of 40 explants.