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Direct Organogenesis and Regeneration of Arabica Coffee Cultivars

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Abstract

Arabica coffee is a very important crop in the Cordillera region and one of the promising industrial crops in the highlands. To develop a tissue culture protocol for 'Typica', 'Mundo Novo' and 'Red Bourbon' cultivars, different growth hormone combinations of Benzyl Amino Purine (BAP), Kinetin, (Kin), Indole Acetic Acid, (IAA), and 2,4-Dichlorophenoxyacetic acid (2,4-D) with different concentrations of 1, 2, 3, 4, 6 ppm/L and light conditions were tested on the somatic embryo as an explant grown in MS medium. Result showed that using somatic embryo of 'Mundo Novo' inoculated in 2ppm BAP and 2ppm Kinetin at 16 hours light, shoot emerged after 18.2 days and shoot proliferated with an average of 4 shoots at 91.75 days after inoculated in 2ppm BAP+ 2ppm Kin + 1ppm IAA at 24 hours light. However, 'Typica' inoculated in 2ppm BAP, 2ppm Kin and 2ppm IAA at 24 hours light induced shoot emergence after 19 days and proliferated through direct organogenesis with an average of 5.43 shoots in 2ppm BAP +2ppm Kin + 2ppm IAA at 87.4 days after inoculation at 16 hours light condition. Subsequently, 'Red Bourbon' initiated shoot emergence after 35 days inoculated in 1ppm GA + 8ppm BA + 0.5ppm IAA in dark condition. Moreover, the regeneration of plantlets transferred to MS containing 2 ppm IBA, 2 ppm NAA and 2 ppm IAA were elongated and rooted 2-3 months after transplanting. Highest percentage of survival was recorded on 1 part of vermicompost: 1 part sand: 1 part of burnt rice hull on cultivar 'Red Bourbon' and 'Mundo Novo'. In addition, soil media with 1 part of BSU compost: 1 part sand: 1 part of burnt rice hull has a 100% survival rate on 'Typica' and 'Mundo Novo'.

Introduction

Coffee of the family Rubiaceae, genus Coffea consists of more than 124 species (Davis et al., 2011) of which two are known as economically important agronomic crops worldwide, namely, *C. canephora* and *C. arabica*. Within these cultivated species, even more so for *C. arabica*, there is a low genetic variability within populations which is linked to their origin, their adaptation to the environment and the manner in which they reproduce (International Coffee Genome Network [ICGN], 1999; Aga et al., 2003). It is recognized that the cultivated cultivars, *C. arabica* in particular, have a very narrow genetic base (Anthony et al., 2002). Cultivars of *C. arabica* are known to self-

fertilize and possess allotetraploid chromosomes (2n=4x=44) which make them genetically similar (Lashermes et al., 1997). The rest are diploids and have adaptated to prevent self-fertilization. This condition for *C. arabica* does not provide much option for breeders because of the narrow genetic base and a few traits to choose from, traits which are significant for determining the link to genetic factors important for growth and development. Having similar genetic composition also makes them vulnerable to natural selection and that they can be easily affected by any drastic change in the environment (Labouisse et al., 2008) or by emerging pests.

Coffee is one of the most important acceptable industrial crops not only in local markets but also in the global markets (Bolvenkel et al., 1993). In the Philippines, Arabica coffee (*Coffea arabica* L.) is a very important crop that existed for centuries in the Cordillera region and has been a part of the culture and lifestyle of the people. It is also regarded as one of the promising industrial crops in the Cordillera highlands. Arabica 'Typica' is the best cultivar planted in Benguet for the past 100 years (Killip, 2010).

In-vitro culture techniques have been developed for coffee germplasm ex-situ conservation techniques (Munoz-Sanchez & Hernandez-Stomayor, 2008; Ashebre, 2016). Different methods have been used for in-vitro multiplication of *C. arabica* including apical meristem, axillary bud culture, somatic embryogenesis, androgenesis, protoplast culture, and use of seeds (Carneiro & Ribeiro, 1989; Kumar et al., 2006; Ebrahim et. al., 2007). *C. arabica* is self-pollinated, thus characterized by homogeneity (Lashermes et al., 2000) resulting in uniform progenies from the seeds. This makes seeds as good starting material in the in-vitro establishment for Arabica coffee.

Despite the importance of Arabica coffee in the Cordillera, there is still lack of access to affordable and quality planting materials (Chaves, 2014). According to Ibrahim et al. (2013), conventional propagation is genetically stable, but very slow to produce a large number of coffee seedlings. Moreover, seed propagation of this species has slow rates of seed multiplication and short span of seed viability (Monaco et al., 1995). Thus, the need to find ways for rapid propagation through non-conventional means. This study aims to develop a protocol for direct organogenesis, multiplication, rooting and acclimatization of Arabica coffee cultivars from Benguet and Mountain Province.

Materials and Methods

This study conducted at the Horticulture Research and Training Institute (HORTI) Tissue Culture Laboratory. Figure 1 represents the overall process used in the study in producing tissue cultured Arabica coffee.

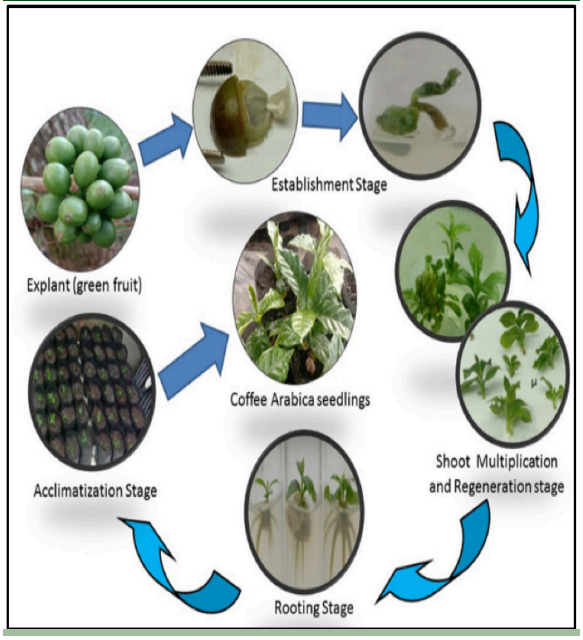
Establishment Stage/Culture Initiation

Explant Collection and Sterilization

Two hundred seventy (270) of young seeds of three cultivars ('Typica', 'Mundo Novo', and 'Red Bourbon') with pulp and hull were surface sterilized by washing with tap water and dishwashing soap. Then immersed again with distilled water and detergent soap with the addition of 2-3 drops of surfactant for 15 minutes and rinsed three times of sterilized distilled water. The young seeds was sterilized with 100% of sodium hypochlorite for 30 minutes with the addition of 2-3 drops of surfactant and rinsed with sterilized distilled water for three times.

Figure 1

Process in Producing Tissue-Cultured Arabica Coffee



Initiation and Inoculation of Explants

Under aseptic-condition, each young coffee seeds were excised to get the embryo and inoculated individually in a test tube containing 10ml of Murashige and Skooge (MS) medium with a different concentration of plant growth regulators of cytokinin (2,4,6,8 ppm BAP, 2,4,6 ppm Kinetin, 1,2,3,4,6 ppm TDZ), auxin (0.5,1,2,3,4,6 ppm IAA, and 1,2,3 ppm 2,4-D) and 1ppm Gibberellic acid GA3. Cultures were maintained in the culture room and subjected to three light conditions (dark, 16 hours light and 24 hours light).

Plantlets produced in the initiation stage were used in the shoot multiplication and inoculated with the same treatment used in the establishment stage.

Proliferation Stage (Adventitious Shoot Multiplication)

Micro shoots (10mm) were sub cultured in Erlenmeyer flasks (250ml) containing 50ml of solid MS media. The experiments used the study the best kind of cytokinin and concentration to maximize micro shoot production for each Arabica coffee cultivar. The cultured micro shoots were incubated under 26±2°C with different light condition which are 16 hours and 24 hours light (photosynthetic photon flux density; PPFD = (40-45) μmol/m²/sec) and dark condition. Treatments on the different concentration of growth regulators used were 2, 4, 6 ppm of BAP with a combination of 2, 4, 6 kinetin, 0.5,1,3 ppm TDZ, IAA, and 1ppm of Gibberellic acid used in the shoot multiplication.

Direct Organogenesis

Direct embryogenesis can be induced on certain explants. Direct somatic embryogenesis is the formation of somatic embryos from the explant without the formation of an intermediate callus phase (Raghavan & Sharma, 1995).

Regeneration of Micropropagated Plants

The regeneration of plants under aseptic and controlled environmental conditions is referred to as micropropagation, the use of very small pieces of plant tissue or organs as starting vegetative tissue (Davis & Becwar, 2007). It is dependent on the manipulation of the inorganic and organic constituents in the medium, as well as the type of explant and the species to be used. In most plants, successful regeneration from the callus or directly from the explants takes place after a series of sub-cultures in various media, in a sequence, which is often specific to the species, variety, or the newly introduced genotype (Christianson, 1987).

Elongation and Rooting Stage

Proliferated micro shoots of 'Typica', 'Mundo Novo', and 'Red Bourbon' were sub-cultured and transferred in fresh media containing half-strength of MS media having 30g/l sucrose as a basal medium. The basal media were supplemented with different combinations of 1,2 ppm of IAA, IBA and NAA. Murashige and Skooge was used as the control. An additional 100mg/l activated charcoal also enhanced root formation.

Acclimatization Stage/Hardening in the Greenhouse

Acclimatization was carried out by transferring rooted plantlets produced in the laboratory to greenhouse and left for one week. The well-rooted plantlets were removed from the medium freed of agar by washing in running tap water. Plantlets are soaked with commercial fungicide (2 ml/li) for five minutes to prevent disease infection. They are then transplanted into different treatments of different combination of growing media like 1 part Alnus compost: 1 part sand: 1 part of burnt rice hull. Another combination of growing soil media were 1 part of BSU compost: 1 part sand: 1part of burnt rice hull. And the last combination of growing soil media were 1 part of vermicompost: 1 part sand: 1part of burnt rice hull, which has been drench with the same fungicide solution. The approximated height of the plantlets when transferred to potting media has an average of 1.5 inches.

Data Gathered and Statistical Analysis

Data gathered in the study include number of days from inoculation to shoot emergence, to produce leaf, nodal produced, plant height after 90 days, in the establishment stage. Whereas on the proliferation stage, the number of days from shoots formation to sub division of multiple shoots, number of shoots produced, number of days from transfer to produced roots up to acclimatization were also recorded. Furthermore,

acclimatization include number of days to produce new shoots, leaves, length of roots, leaf diameter, leaves, petiole produced after 30 days, height of the plantlets and rate of survival. The experiment was laid out in a completely randomized design (CRD). Ten sample explants per replication served as the source of data for some specific parameters to be gathered. The data generated were analyzed through one-way ANOVA and the treatment means were compared for significance by LSD test at 0.05 P using GenStat.

Results and Discussion

Establishment Stage

Effect of Different Light Conditions and Different Rates of Growth Hormones on the Induction of Direct Organogenesis of Coffee arabica L. cultivar

Figure 2 presents the effect of light conditions in the three-tissue cultured Arabica coffee cultivars. Light condition significantly affected the shoot formation of 'Typica', 'Mundo Novo' and 'Red Bourbon'. Dark was the best condition in culturing 'Red Bourbon' and 'Typica'. However, 'Mundo Novo' was observed to produce shoots at 16 hrs light, which was comparable to embryo cultured in 24 hrs light. Miler et al. (2019), stated that, light quality directly affect the success of the tissue culture system and affects the growth of in vitro plants and light play an important role in the establishment of plantlets.

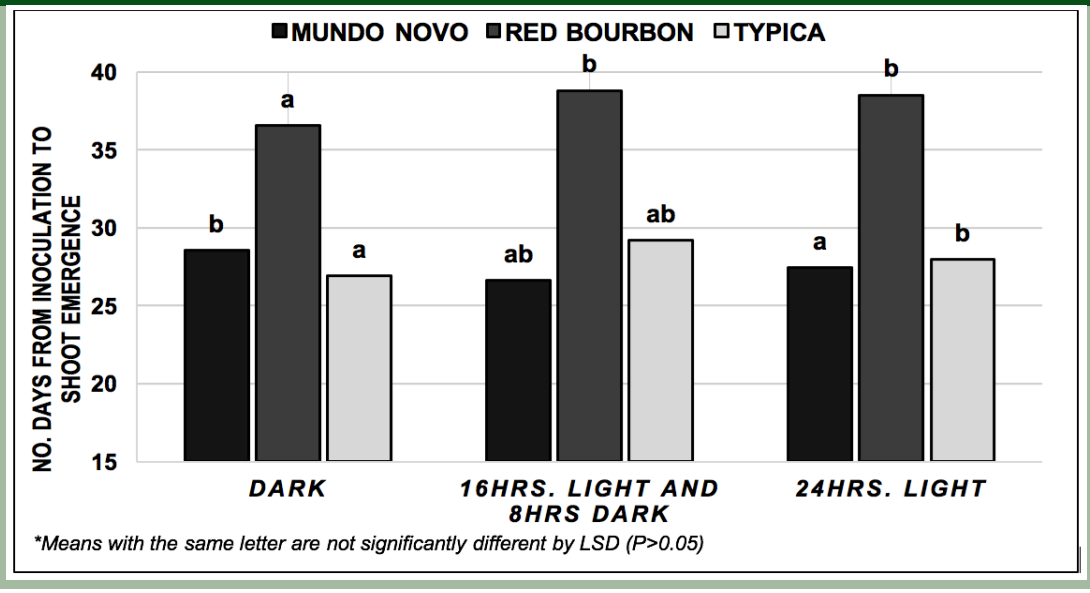
Among the three varieties evaluated, 'Typica' and 'Mundo Novo' were the earliest to produce shoots with an average of 27 days from inoculation while the latest was observed on Red Bourbon at 39 days.

Effect of Somatic Embryo Culture on the Establishment Stage in a Different Concentration of Plant Growth Regulators (PGR)

Somatic embryo culture of the three varieties responded to most of the growth regulator combinations. Cultured somatic embryo of the varieties showed different responses in terms of growth regulators, concentration and exposure to light. Explants in treatment 8 (MS medium + 6 ppm BAP + 6 ppm Kinetin + 3 ppm TDZ) at dark condition were the first to germinate while explants of 'Mundo Novo' germinated only after 7 days at treatment 3 (MS medium + 2 ppm Benzyl Amino Purine (BAP) + 2 ppm Kinetin) at 8 hours dark. This was followed by 'Red Bourbon' cultured in treatment 7 on continuous dark and eight hours dark condition, while 'Typica' first

Figure 2

Number of Days from Inoculation to Shoot Emergence of Three Arabica Coffee Cultured in Different Light Conditions



germinated at dark condition with a mean of 16 days on treatment 4 (MS medium + 4 ppm BAP + 4 ppm Kinetin + 4 ppm TDZ). This propagation challenges can be overcome by utilizing tissue culture techniques, which offer a viable alternative to traditional propagation methods in coffee (Kumar et al., 2006).

Effects of Kind and Rates of Hormones on the Induction of Somatic Embryo of Coffea arabica L. Cultivars

The different kinds and concentrations of the growth regulators significantly affected the time of shoot formation of the three Arabica coffee cultivars investigated. It was observed that each variety has specific combinations of growth regulators to shoot formation from inoculation. The 'Typica' cultivar started to produce shoot at 20.26 days after inoculation on MS supplemented with 2ppm BAP, 2ppm Kinetin and 2ppm IAA, followed by Mundo Novo on the same growth regulator but reduced in IAA at 21.73 days. On the other hand, Red Bourbon was first to produce shoot in treatment with growth regulators and concentration of 1ppm GA, 8ppm BA and .5ppm IAA. It was also the last to produce shoots among the three cultivars evaluated.

Proliferation Stage

The Influence of Light Conditions and Culture Medium Consistency on the Development of Direct Organogenesis and Regeneration of Coffea arabica L. Cultivars

Results showed that the different varieties inoculated in 24 hours light showed no differences in terms of days to shoot and shoot length. On the other hand, decapitated explants inoculated in dark condition did not produced shoots. However, the three cultivars of *C. Arabica* showed initiation of whitish to grayish callus from the cut edges of the explant. The callus observed to grow and change in color from grayish-brownish after 6 weeks of culture then start to deteriorate (Figure 5). This result was contrary to the study of Ismail et al. (2003) in which they stated that cultures in dark showed the best result than cultures incubated in light. This was further supported by Aga and Khillare (2017) whose result showed that cultures kept in dark condition survived for three weeks, then deteriorate and terminate. In addition, Reuveni and Elvenor (2007) stated in their study on P. hybrid that extended time period incubation in darkness abolish the ability to generate shoot. Moreover, that light play an important role in the establishment of plantlets, according to Montes (1982). In addition,

Figure 3

Embryo Cultures of Typica and Mundo Novo 16 Hours Light (A) Sterilized Seed Ready for Excision, (B) Excised Embryo for Inoculation; (C) Typica at 42 Days from Inoculation, (D) At 62 Days from Inoculation, (E) At 161 Days from Inoculation; (F) Mundo Novo at 28 Days from Inoculation, (G) Ready for Decapitation and (H) At 52 Days.

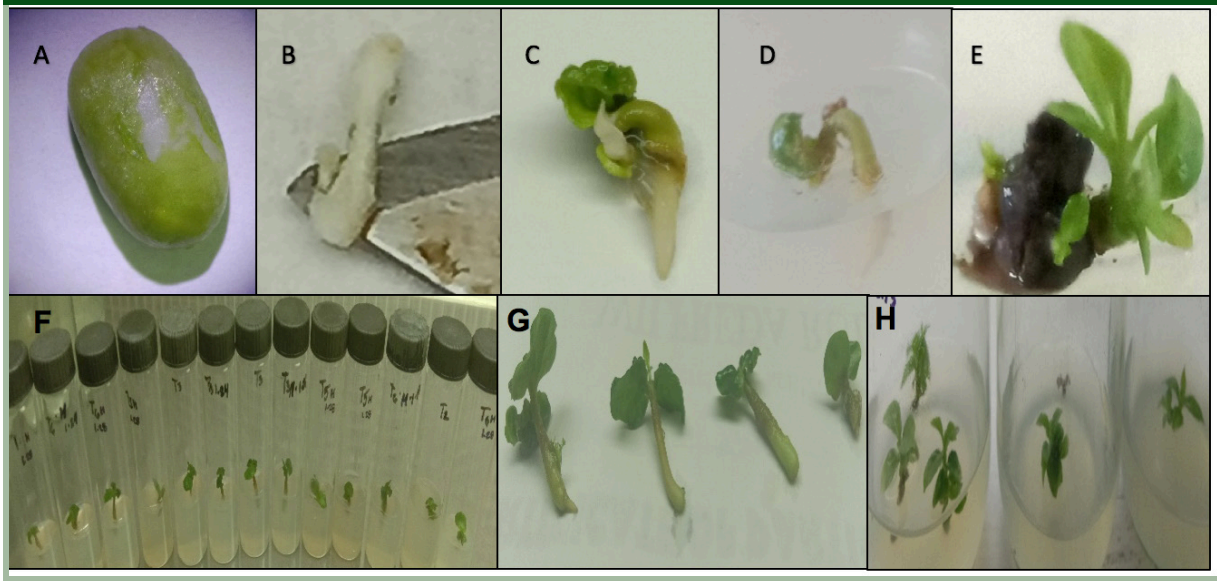
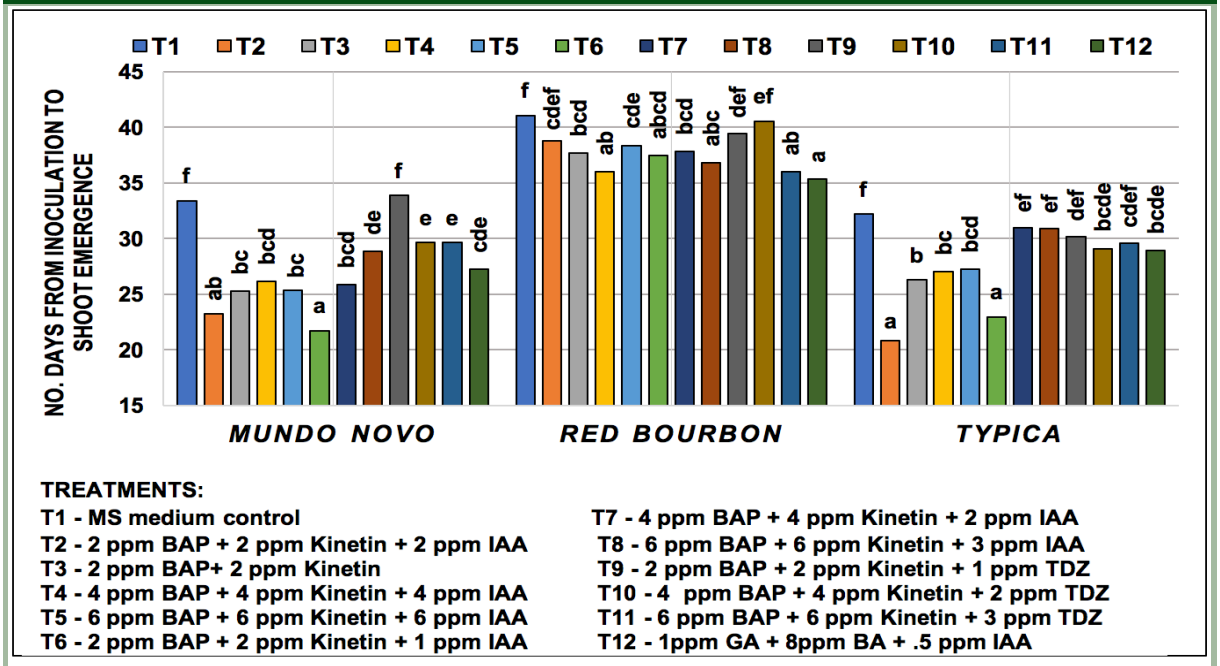


Figure 4

Number of Days from Inoculation to Shoot Emergence of Three Arabica Coffee in Different Kind and Concentration of Growth Regulators



that direct somatic embryogenesis is the formation of somatic embryos from the explant without the formation of an intermediate callus phase (Raghavan & Sharma, 1995).

Table 1 presents the effect of various concentration of plant growth regulators (PGRs) as affected by light exposure on regeneration of *C. arabica* ('Mundo Novo', 'Red Bourbon' and 'Typica') on days to shoot formation, number of shoot and shoot length. The excised explant of the three cultivars inoculated in different media started to produce shoots 31-55 days after culture in 16 hours light exposure while 32-47 days was observed on varieties cultured in twenty-four hours light.

On the number of shoot formation in 16 hours light exposure, 'Mundo Novo' (4.5 shoots) and 'Red Bourbon' showed the best results on MS medium + 1ppm GA + 8ppm BA + .5 ppm IAA, while 'Typica' on MS medium + 2 ppm BAP + 2 ppm Kinetin + 2 ppm IAA with the average of four shoots. On the other hand, varieties cultured in 24 hours light showed that 'Mundo Novo' recorded the highest number of shoots with the average of five shoots on MS medium + 4 ppm BAP + 4 ppm Kinetin + 2 ppm 2,4-D. However, this was comparable to 'Red bourbon' on MS medium + 2 ppm BAP + 2 ppm

Figure 5

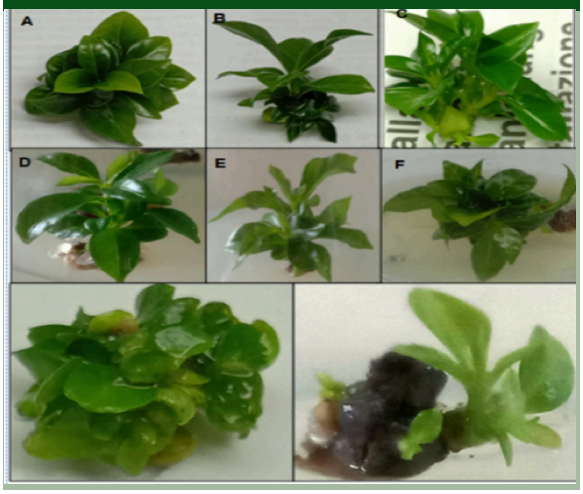
Callus Induction from Decapitated Explant Cultured in Dark Condition. (A and B) Mundo novo cultured in MS medium + 6 ppm BAP + 6 ppm Kinetin + 3 ppm IAA and MS medium + 4 ppm BAP + 4 ppm Kinetin + 2 ppm 2,4-D. (C and D) Red bourbon cultured on MS medium + 6 ppm BAP + 6 ppm Kinetin + 3 ppm IAA and - MS medium + 2 ppm BAP + 2 ppm Kinetin + 1 ppm IAA. (D and E) Typica Cultured in MS medium + 6 ppm BAP + 6 ppm Kinetin + 3 ppm 2,4-D and MS medium + 4 ppm BAP + 4 ppm Kinetin + 2 ppm 2,4-D

Kinetin + 2 ppm IAA and MS medium + 1ppm GA + 8ppm BA + .5 ppm IAA, 'Typica' on MS medium + 2 ppm BAP + 2 ppm Kinetin + 2 ppm IAA and 'Mundo Novo' on MS medium + 2 ppm BAP + 2 ppm Kinetin + 2 ppm IAA and MS medium + 2 ppm BAP + 2 ppm Kinetin + 1 ppm IAA.

In terms of shoot length of the plantlets at 45 days after shoot emergence, 'Red Bourbon' recorded the tallest shoot of 0.73cm in MS supplemented with 1ppm GA + 8ppm BA + 0.5 ppm IAA in 24 hours light exposure (Table 2). 'Mundo Novo' recorded 0.38cm in both 8 hours dark and 16 hours light condition and 24-hours light exposure on MS medium + 1ppm GA + 8ppm BA + 0.5 ppm IAA while Typica on MS medium + 2 ppm BAP + 2 ppm Kinetin + 1 ppm IAA was recorded with the average of 0.63cm (Figure 6).

Figure 6

Direct Organogenesis of Tissue Cultured Embryo (A and B) Mundo Novo at 207 days from Inoculation and at 68 days, (C) Typica at 141 days, (D) Mundo Novo at 34 days under 8 hours dark, (E) Mundo Novo at 69 days under 8 hours dark, (F) Mundo Novo at 125 days under 8 hours dark, (G) Red Bourbon at 55 days after inoculation, (H) Red Bourbon at 128 days and (I) Red Bourbon at 146 days under 24 hours light



Elongation and Rooting Stage

Effect of Auxins on Rooting of Arabica Coffee Cultivars 'Mundo Novo', 'Red Bourbon' and 'Typica'

Micro-shoots were rooted on half strength

Table 1

Effect of Culture Media to Different Concentrations of Cytokinin on Number of Days to Shoot Formation, Number of Shoots Produced and Shoot Length (cm) of Arabica Coffee

Culture Media	Days to Shoot			Number of Shoots			Shoot Length (cm)		
	Dark	16hrs light	24hrs light	Dark	16hrs light	24hrs light	Dark	16hrs light	24hrs light
MS medium + 2 ppm BAP + 2 ppm Kinetin + 2 ppm IAA	0	39.44^{bc}	39.97^{bc}	0	2.92^{ab}	3.75^{a}	0	0.33^{b}	0.46^{a}
MS medium + 2 ppm BAP + 2 ppm Kinetin + 1 ppm IAA	0	38.83^{b}	38.1^{1ab}	0	2.17^{bc}	2.67^{b}	0	0.34^{b}	0.40^{ab}
MS medium + 6 ppm BAP + 6 ppm Kinetin + 3 ppm IAA	0	41.06^{c}	40.44^{bc}	0	2.44^{b}	1.56^{c}	0	0.29^{b}	0.30^{bc}
MS medium + 4 ppm BAP + 4 ppm Kinetin + 2 ppm 2,4-D	0	40.11^{bc}	42.15^{cd}	0	2.17^{bc}	3.00^{ab}	0	0.25^{b}	0.24^{c}
MS medium + 6 ppm BAP + 6 ppm Kinetin + 3 ppm 2,4-D	0	44.75^{d}	43.89^{d}	0	1.36^{c}	2.44^{bc}	0	0.34^{b}	0.30^{bc}
MS medium + 1ppm GA + 8ppm BA + 0 .5 ppm IAA	0	33.75^{a}	36.28^{a}	0	3.67^{a}	3.33^{ab}	0	0.51^{a}	0.44^{ab}
% CV	0	6.3	9.6	0	49.1	41.0	0	50.5	53.2

Means with the same letters are not significantly difference at 5% LSD

Table 2

Number of Days to Proliferation, Number of Shoot and Shoot Length of Mundo Novo, Red Bourbon and Typica as Affected by the Different Kinds and Concentrations of Plant Growth Regulators and Light Conditions

Treatments	Dark			16hrs. Light			24hrs. Light		
	M	R	T	M	R	T	M	R	T
Numbers of Days to Shoot Formation									
T1	0	0	0	42.00^{de}	33.00^{ab}	43.33^{e}	3.50^{abcd}	1.00^{g}	4.25^{ab}
T2	0	0	0	42.75^{e}	30.50^{a}	43.25^{e}	2.25^{defg}	1.75^{efg}	2.50^{cdefg}
T3	0	0	0	52.50^{f}	38.67^{cd}	32.00^{a}	2.00^{defg}	2.00^{defg}	3.33^{abcde}
T4	0	0	0	52.00^{f}	36.33^{bc}	32.00^{a}	1.75^{efg}	2.00^{defg}	2.75^{bcdef}
T5	0	0	0	55.00^{f}	36.00^{bc}	43.25^{e}	1.50^{fg}	0.84^{g}	1.75^{efg}
T6	0	0	0	38.50^{cd}	31.00^{a}	31.75^{e}	4.50^{a}	4.00^{abc}	2.50^{cdefg}
CV%		0			6.3			9.6	
Numbers of Shoot Produced									
T1	0	0	0	3.50^{abcd}	1.00^{g}	4.25^{ab}	3.50^{abcd}	4.00^{ab}	3.75^{abc}
T2	0	0	0	2.25^{defg}	1.75^{efg}	2.50^{cdefg}	3.50^{abcd}	2.00^{def}	2.50^{bcdef}
T3	0	0	0	2.00^{defg}	2.00^{defg}	3.33^{abcde}	1.67^{ef}	1.33^{f}	1.67^{ef}
T4	0	0	0	1.75^{efg}	2.00^{defg}	2.75^{bcdef}	4.99^{a}	2.00^{def}	2.00^{def}
T5	0	0	0	1.50^{fg}	0.84^{g}	1.75^{efg}	3.00^{bcde}	2.33^{cdef}	2.00^{def}
T6	0	0	0	4.50^{a}	4.00^{abc}	2.50^{cdefg}	3.00^{bcde}	4.00^{ab}	3.00^{bcde}
CV%		0			49.1			41.0	
Shoot Length (cm)									
T1	0	0	0	0.33^{bc}	0.40^{abc}	0.25^{c}	0.33^{c}	0.63^{ab}	0.43^{bc}
T2	0	0	0	0.20^{c}	0.63^{a}	0.20^{c}	0.35^{c}	0.23^{c}	0.63^{ab}
T3	0	0	0	0.35^{bc}	0.23^{c}	0.30^{bc}	0.30^{c}	0.30^{c}	0.30^{c}
T4	0	0	0	0.23^{c}	0.23^{c}	0.30^{bc}	0.30^{c}	0.23^{c}	0.20^{c}
T5	0	0	0	0.33^{bc}	0.42^{abc}	0.28^{c}	0.30^{c}	0.37^{bc}	0.23^{c}
T6	0	0	0	0.38^{bc}	0.63^{a}	0.53^{ab}	0.38^{bc}	0.73^{a}	0.23^{c}
CV%		0			50.5			53.2	

Means with the same letters are not significantly difference at 5% LSD

*Legend: *M-Mundo Novo *R- Red Bourbon *T-Typica*
T1- MS medium + 2 ppm BAP + 2 ppm Kinetin + 2 ppm IAA
T2 - MS medium + 2 ppm BAP + 2 ppm Kinetin + 1 ppm IAA
T3 - MS medium + 6 ppm BAP + 6 ppm Kinetin + 3 ppm IAA
T4 - MS medium + 4 ppm BAP + 4 ppm Kinetin + 2 ppm 2,4-D
T5 - MS medium + 6 ppm BAP + 6 ppm Kinetin + 3 ppm 2,4-D
T6 - MS medium + 1ppm GA + 8ppm BA + .5 ppm IAA

MS media. When the nodular structure were fully develop as plantlets these were transferred to the different treatments of rooting media. Plantlets 3 to 4 cm in height are sub divide from the multiple shoot structure and plant in a rooting medium (Dumaslan, 2015). The concentration and combination of auxins had a significant effect on rooting of in-vitro coffee micro-shoots (Table 3). Increasing the auxins IBA, NAA and IAA to 2ppm per litter recorded the earliest to initiate roots. The combination of 1ppm IBA and 1ppm NAA was observed to produce the highest number of

roots followed by MS supplemented by 2ppm IBA, 1ppm NAA and 1ppm IAA with the mean number of roots of three, also recorded the longest roots among all the treatments tested. According to Harris and Stevenson (1979) stated that using ½ strength of MS salts was possible to increase the number of roots formed per shoot. With the addition of using Naphthalene Acetic Acid (NAA) significantly enhanced root formation.

MS medium supplemented with three different combinations of auxins either alone or in combination with each other were used on the three cultivars. 'Mundo Novo' was the earliest to produce root on 2ppm IBA, NAA and IAA with the average of 17 days after transplanting (Figure 7). 'Typica' followed on the same combination of auxin in lower concentrations of 1ppm of IBA, NAA and IAA. On the other hand, between all concentration and combinations tested, 'Red Bourbon' was observed only to initiate roots on medium with 1ppm of IBA and NAA.

Number of Roots. After a month of root initiation, 'Red Bourbon' recorded the highest number of roots with the average of six roots produced. An average of three roots produced were recorded on 'Mundo Novo' in the same treatment. Meanwhile, 'Typica' on 2ppm IBA, 1ppm NAA and 1ppm IAA produced the highest mean number of roots (Table 4).

Effect of the Combinations of Auxins on Root Length. Figure 7 showed that varieties have different reactions to combinations of auxin tested. 'Red Bourbon' has the longest root when cultured in 1ppm IBA and 1ppm IAA. This was followed by 'Typica' on 2ppm IBA, 1ppm NAA and 1ppm IAA and 'Mundo Novo' cultured in 1ppm IBA, NAA and IAA. Based on the rooting parameters observed, different cultivars of Arabica coffee has specific auxin combinations. It was observed that 'Red Bourbon' cultivar only responded on medium with 1ppm IBA and 1ppm NAA. 'Typica' was observed to have the highest number of roots and longest root on medium with 2ppm IBA, 1ppm NAA and 1ppm IAA however, last to initiate roots. On the other hand, 'Mundo Novo' cultivar was best on medium supplemented with 1ppm IBA, NAA and IAA in terms of growth and root length.

Table 3

Number of Days from Transplanting to Root Formation, Number of Roots Produced and Root Length of Arabica Coffee Cultivars

AUXINS	Number of days to root	Number of roots	Root length
MS control	19.69^{b}	1.09^{b}	1.51^{bc}
MS+1ppm IBA	0.00^{a}	0.00^{a}	0.00^{a}
MS+1ppm IBA and 1ppm NAA	18.37^{b}	3.82^{e}	1.76^{cd}
MS+1ppm IBA and 1ppm IAA	0.00^{a}	0.00^{a}	0.00^{a}
MS+1ppm IBA, 1ppm NAA and 1ppm IAA	19.50^{b}	2.03^{c}	1.82^{de}
MS+2ppm IBA, 1ppm NAA and 1ppm IAA	25.74^{c}	3.10^{d}	2.01^{e}
MS+2ppm IBA, 2ppm NAA and 2ppm IAA	17.92^{b}	1.86^{c}	1.32^{b}
CV%	16.0	44.2	25.1

Mean with the same letter are not significantly different at 5% level of LSD

Effect of Potting Media on Rooting of Arabica Coffee Cultivars 'Mundo Novo', 'Red Bourbon' and Typica

Interaction Effect

Effect of Different Kinds of Potting Media on the Shooting and Rooting During Acclimatization in the Greenhouse Plant Height, Leaf Diameter and Root Length. 'Mundo Novo' produced the tallest plants when grown in 1 part Alnus compost: 1 part sand: 1 part of burnt rice hull. The largest leaves were recorded in 'Typica' plantlets grown in 1 part of BSU compost: 1 part sand: 1part of burnt rice hull. Longest roots were obtained from 'Typica' grown in 1 part of vermicompost: 1 part sand: 1part of burnt rice hull (Figure 8). Aside from using different concentration of growing media, plantlets were covered with transparent plastic to enhance the relative humidity. Sherrington and George (1984) stated that tissue cultured plantlets grown in an artificial condition in a high

Table 4

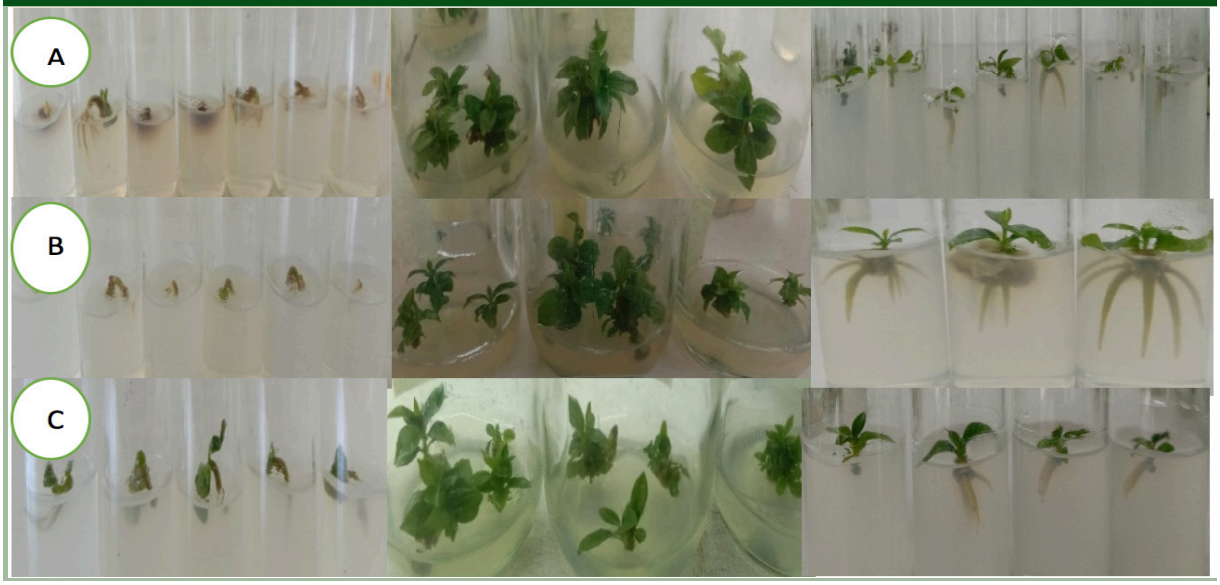
Number of Days to Root, Number of Roots and Root Length of Arabica Coffee Cultivars as Affected by Different Concentrations of Auxins

Treatments	Days to Root			Number of Roots			Root Length (cm)		
	Mundo Novo	Red Bourbon	Typica	Mundo Novo	Red Bourbon	Typica	Mundo Novo	Red Bourbon	Typica
MS control	20.09^{cd}	19.00^{bcd}	19.97^{cd}	0.88^{gh}	1.41^{efg}	1.00^{fgh}	1.40^{efg}	1.17^{f}	1.50^{defg}
MS+1ppm IBA	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{h}	0.00^{h}	0.00^{h}	0.00^{g}	0.00^{bcde}	0.00^{g}
MS+1ppm IBA and 1ppm NAA	20.00^{cd}	16.50^{b}	18.60^{bcd}	2.99^{bcd}	4.75^{a}	3.73^{ab}	1.40^{efg}	2.00^{g}	1.74^{abcde}
MS+1ppm IBA and 1ppm IAA	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{h}	0.00^{h}	0.00^{h}	0.0^{0g}	0'00^{a}	0.00^{g}
MS+1ppm IBA, 1ppm NAA and 1ppm IAA	21.00^{d}	18.84^{bcd}	18.67^{bcd}	2.00^{def}	2.34^{cde}	1.75^{efg}	1.80^{abcd}	1.94^{fg}	1.73^{abcde}
MS+2ppm IBA, 1ppm NAA and 1ppm IAA	26.15^{e}	25.06^{e}	26.00^{e}	2.88^{bcd}	3.40^{bc}	3.00^{bcd}	1.90^{abc}	1.94^{ab}	2.00^{ab}
MS+2ppm IBA,2ppm NAA and 2ppm IAA	17.00^{bc}	17.25^{bc}	19.50^{bcd}	1.67^{efg}	2.17^{de}	1.75^{efg}	1.17^{f}	2.13^{a}	1.35^{gf}
cv%		16			44.2			25.1	

Means with the same letters are not significantly difference at 5% LSD

Figure 7

Embryo Cultures of 'Mundo Novo', 'Red Bourbon', and 'Typica' column (A) Germinated Embryo at 30 days, column (B) Proliferated Embryos at 101 days ('Mundo Novo' and 'Typica') 122 days ('Red Bourbon'); (C) 'Mundo Novo' Rooted in 2 ppm IBA, 2 ppm NAA and 2 ppm IAA, 'Red Bourbon' on 1 ppm IBA and 1ppm IAA and 'Typica' in Treatments 1-7 at 48 Days



relative humidity were not photosynthetically self-sufficient and young leaves may not yet fully develop. Cuticular wax causing susceptible to water loss when plantlets transplanted to external environment. The cuticle is responsible for the glossy appearance of the leaves and gives additional protection by slowing down rate of water lost through transpiration. Therefore, plantlets grown under plastic-covered condition lessened gas exchange but increase relative humidity that decrease transpiration rate resulting to slower growth of the leaves.

Effect of the Different Potting Media on Height (cm), Leaf Diameter (cm²), Root Length (cm), Number of Leaves, Nodes and Roots. There was an average of 9 to 10 leaves from the three cultivars grown in all the potting media (Table 5). Likewise, all cultivars had an average of 4 to 5 leaves grown in all potting media. The most number of new roots produced was recorded in 'Mundo Novo' grown in 1 part of vermicompost: 1 part sand: 1 part of burnt rice hull. This was followed by 'Typica' grown in 1 part Alnus compost: 1 part sand: 1 part of burnt rice hull; 1 part of vermicompost: 1 part sand: 1 part of burnt rice hull; and 1 part of BSU compost: 1 part sand:1 part of burnt rice hull (Figure 8). Accordingly, potting medium with vermicompost had the best shooting and rooting performance. Dumaslan (2018) stated that, hardening of tissue cultured strawberries under greenhouse condition were transplanted into growing media combination of 1 part vermicompost + 1 part Mt. Sand + 1 part Burnt Rice hull is recommended to acclimatize the tissue cultured strawberry Hardening of tissue cultured strawberry plantlets should cover with plastic 14 days to promote growth and development.

Plant Survival. One hundred percent survival was observed in 'Mundo Novo' and 'Red Bourbon' grown in part of vermicompost: 1 part sand: 1 part of burnt rice hull and 'Typica' grown in 1 part of BSU compost: 1 part sand: 1part of burnt rice

Table 5

Effect of the Different Potting Media on Height (cm), Leaf Size (cm), and Root Length (cm) of Mundo Novo, Red Bourbon, and Typica Cultivars for Two-Months-Old Plantlets

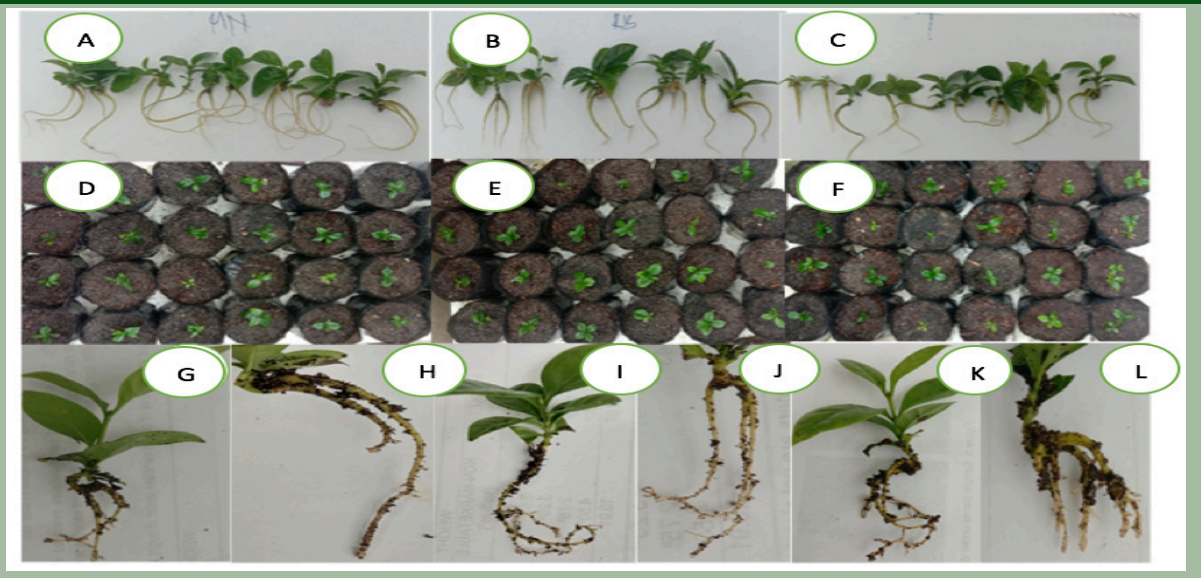
Treatments	Leaf size (cm)			Plant height (cm)			Root length (cm)		
	Mundo Novo	Red Bourbon	Typica	Mundo Novo	Red Bourbon	Typica	Mundo Novo	Red Bourbon	Typica
SM1	1.33^{b}	1.40^{ab}	1.45^{ab}	1.81^{b}	2.25^{ab}	2.90^{ab}	4.28^{cd}	6.30^{abc}	1.50^{e}
SM2	1.45^{ab}	1.65^{ab}	1.25^{b}	2.55^{ab}	2.25^{ab}	2.8^{ab}	3.50^{de}	6.20^{bc}	1.63^{e}
SM3	1.58^{ab}	1.90^{a}	1.20^{b}	2.77^{ab}	2.50^{ab}	3.00^{a}	4.90^{cd}	7.55^{ab}	1.18^{e}
SM4	1.25^{b}	1.70^{ab}	1.45^{ab}	2.30^{ab}	2.25^{ab}	2.90^{ab}	5.25^{bcd}	8.73^{a}	1.13^{e}
CV%		23.7			30.6			40.0	
	No. of nodes			No. of leaves			No. of roots		
	Mundo Novo	Red Bourbon	Typica	Mundo Novo	Red Bourbon	Typica	Mundo Novo	Red Bourbon	Typica
SM1	4.01^{cd}	9.996^{abcd}	8.013^{de}	7.50^{e}	9.996^{abcd}	8.013^{de}	6.03^{d}	20.97^{ab}	9.02^{cd}
SM2	5.00^{abc}	9.33^{bcde}	10.67^{ab}	10.00^{abc}	9.33^{bcde}	10.67^{ab}	9.75^{cd}	17.33^{abc}	14.67^{bcd}
SM3	5.75^{a}	8.5^{cde}	9.996^{abcd}	11.50^{a}	8.5^{cde}	9.996^{abcd}	7.75^{d}	17.00^{abc}	23.96^{a}
SM4	4.50^{bcd}	10.00^{abc}	10.00^{abc}	9^{bcde}	10.00^{abc}	10.00^{abc}	10.75^{cd}	17.00^{abc}	10.00^{cd}
cv%		16.6			14.2			44.6	

Means with the same letters are not significantly difference at 5% LSD

Legend: SM1-1part of animal manure: 1 part mountain sand: 1part of burnt rice hull
SM2-1 part alnus compost: 1 part mountain sand: 1part of burnt rice hull
SM3- 1 part of vermicompost: 1 part mountain sand: 1part of burnt rice hull
SM4- 1 part of BSU compost: 1 part mountain sand: 1 part of burnt rice hull

Figure 8

Plantlets of 'Mundo Novo' (A) 'Red Bourbon' (B) and 'Typica' column (C), (D) Acclimatized 'Mundo Novo' (E) 'Red Bourbon' and (F) 'Typica'. (G and H) 'Mundo Novo' on 1 part of alnus compost:1 part of mountain. sand:1part of burn rice hull and 1 part of vermicompost:1 part of mountain. sand:1part of burn rice hull, (I and J) 'Red Bourbon' in 1 part of BSU compost:1 part of mt. sand:1part of burn rice hull and 1 part of vermicompost:1 part of mountain sand



hull. Coffee plantlets grown in other potting media had 50-86 % survival (Table 6 & Figure 8).

Conclusions and Recommendations

In this study, the impacts of light condition and culture media on the micropropagation of *C. arabica*, specifically 'Mundo Novo', 'Red Bourbon', and 'Typica', were evaluated. The result indicates that there was an interplay between the cultivars and the culture media and environment of the culture media. It was observed that 'Mundo Novo' was best in medium with 8 ppm BAP, 0.5ppm IAA and 1 ppm GA cultured in 16 hours light condition in terms of number of shoots and shoot height of the shoots after 90 days of proliferation. On the other hand, 'Red Bourbon' on 16 hours light was observed to produced earlier shoot induction compared to decapitated explants on 24 hours light. However, in terms of number of shoot and shoot height medium with 8 ppm BAP, 0.5 ppm IAA and 1ppm GA in 24 hours light was best to obtain higher number of shoots and taller plantlets. 'Typica' cultured on 2 ppm BAP, 2 ppm Kinetin and 2 ppm IAA produced the highest average number of shoots either on 16 hours or 24 hours light.

Table 6

Number of Days from Transplanting to Root Formation, Number of Roots Produced and Root Length of Arabica Coffee Cultivars

Potting Medium	Survival (%)		
	Mundo Novo	Red Bourbon	Typica
1 part of animal manure: 1 part mountain sand: 1 part of burnt rice hull	57	71	50
1 part Alnus compost: 1 part mountain sand: 1 part of burnt rice hull	86	57	75
1 part of vermicompost: 1 part mountain sand: 1 part of burnt rice hull	100	100	50
1 part of BSU compost:1 part mountain sand:1 part of burnt rice hull	100	86	100

Elongation and rooting of plantlets can be accomplished by transferring to the culture to MS containing 2 ppm indole butyric acid (IBA), 2 ppm naphthalene acetic acid (NAA) and 2 ppm Indole acetic acid (IAA). Plantlets are maintained in the elongation and rooting medium for 2-3 months. When plantlets are elongated and produced roots, they are ready for the acclimatization stage wherein they are moved outside the laboratory.

A higher survival rate on acclimatized coffee cultivars was observed on 1 part of vermicompost: 1 part sand: 1 part of burnt rice hull on cultivar 'Red Bourbon' and 'Mundo Novo'. In addition, soil media with 1 part of BSU compost: 1 part sand: 1 part of burnt rice hull has 100% survival on 'Typica' and 'Mundo Novo'. The hardening of in-vitro raised plantlets is essential for better survival and successful establishment. Successful acclimatization provides optimal conditions for higher survival, subsequent growth and establishment of micropropagated plants.

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