

**EFFECT OF MEDIA, EFFECTIVE MICROORGANISMS
INOCULATION AND CUTTING TYPES ON ROOTING AND GROWTH
OF ABA-BUNA HYBRID COFFEE (*Coffea arabica L.*) AT JIMMA**

M.Sc. Thesis

BY

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Jimma University

Jimma, Ethiopia

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Rooting and Growth of Aba-buna Hybrid Coffee (*Coffea arabica L.*) at Jimma

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JIMMA UNIVERSITY,
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**EFFECT OF MEDIA, EFFECTIVE MICROORGANISMS
INOCULATION AND CUTTING TYPES ON ROOTING AND GROWTH
OF ABA-BUNA HYBRID COFFEE (*Coffea arabica L.*) AT JIMMA**

M.Sc. Thesis

Submitted to the School of Graduate Studies

Jimma University College of Agriculture and Veterinary Medicine

In partial Fulfillment of the Requirements for the Degree of Masters in

Horticulture (Coffee, Tea and Spices)

BY

Tadesse Benti

June, 2013

Jimma University

DEDICATION

Dedicated to my family

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STATEMENT OF THE AUTHOR

I declare that this thesis is my genuine work and that all sources of materials used for this thesis have been duly acknowledged. This Thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree at Jimma University, College of Agriculture and Veterinary Medicine and is reserved at the University Library to be made available to borrowers under rules and regulations of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate. Brief citations from this thesis are allowable without special permission, provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the School of Graduate Studies when in his/her judgment the proposed use of the material is for scholarly interest. In all other instances, however, permission must be obtained from the author.

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BIOGRAPHICAL SKETCH

The author was born in May 1968 in Arbaminch. He attended his elementary and secondary school education at Kulfu elementary and Arbaminch comprehensive high school, respectively. In 1983 he joined the then Debre Zeit College of Agriculture and then transferred to the then Alemaya College of Agriculture now Haramaya University and graduated with Diploma in Crop Production and Protection Technology on July, 1985.

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LIST OF ABBREVIATIONS

AMF	Arbuscular Mycorrhizal Fungi
EIAR	Ethiopian Institute of Agricultural Research
EM	Effective Microorganisms
IAR	Institute of Agricultural Research
IBA	Indol-3-butryc Acid
JARC	Jimma Agricultural Research Center
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
PGPR	Plant Growth Promoting Rhizobacteria
PGR	Plant Growth Regulator

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HYBRID COFFEE (*Coffea arabica* L.) AT JIMMA

ABSTRACT

The study was conducted at Jima Agricultural Research Center (JARC) in 2013 (January to June) to investigate the effect of media, effective microorganisms (EM) inoculation and cutting types on rooting ability and growth of stem cuttings of hybrid coffee variety Ababuna to identify best alternatives for production of adequate planting material. Eight types of media, composed of top soil (Ts), sub soil (Ss), farm yard manure (M), decomposed coffee husk (Ch) and sand (S), were combined with two levels of EM (with or without) and cutting types (whole node or half node) factorial arrangement and laid down in a Randomized Complete Block Design (RCBD) with three replications. Significant ($p \leq 0.05$) variation among treatments was observed for all root and shoot parameters. The highest rooting percentage (88.89) was recorded for whole node cutting type when grown on rooting media composed of 6TS:3M:0Ch:2S(M6) with EM and 2Ts:1M:1S + top 10cm Ss(M7), with or without EM. Half node cuttings grown on rooting media M6 with or without EM, respectively produced 80.58 and 83.33 percent rooting, which is statistically at par with the highest values recorded for whole node cuttings and the same type of cutting propagated on 2Ts:1M:1S + top 10cm Ss(M7), with or without EM with the values 77.78 percent. Highest percent survival for whole node (97.22) and half node (86.66) was obtained from M6. Significantly higher or better performances of most root and shoot growth parameters for both cutting types were also observed from M6 with EM. Low percent rooting (11-22) and complete death of half node cuttings was observed when propagated on 1Ts:1M:1Ch:1S(M1), 3Ts:1M:2Ch:1S(M3) and 3Ts:2M:1Ch:1S(M4). From the present study, considering all parameters, M6 with EM could be recommended as a best potting media. Similarly, if there is financial limitation to purchase sand and EM, M6 and M7, both without EM, could also used as an alternative potting media and depending on propagules required for planting, both cutting types could be recommended for vegetative propagation of hybrid coffee (Ababuna) by stem cutting. Furthermore, Pot rooting under propagator constructed from inexpensive material could be employed to reduce cost of production. However, further investigation, including the remaining two hybrids was suggested to come up with conclusive recommendation.

1 INTRODUCTION

Coffee belong to the family *Rubiaceae* and the subgenus *Coffea* consists of approximately 100 taxa so far identified in African and Madagascar inter-tropical forests. *Coffea arabica* L. is both the most widely cultivated species of *Coffea*, contributing to 80% of the world coffee trade and the only tetraploid species ($2x = 44$) in the genus. Arabica coffee has its primary centre of origin and genetic diversity in the high lands of South West Ethiopia and the Boma Plateau of Sudan (Bridson and Verdcourt, 1988).

In Ethiopia, Arabica coffee is the backbone of the economy and takes the lion's share of the foreign exchange earning of the country. Considering its role in the national economy, much attention has been given to coffee research in Ethiopia to develop improved technologies for the farmers. Hence, due to the efforts made over the last four decades, 37 improved coffee varieties of which three are hybrids have been developed and released for production in the different coffee growing agroecologies. Although, the hybrid varieties produce high yield (2300-2600 kg clean coffee per hectare) as compared to the pure line varieties that give 700-1500 kg clean coffee per hectare, the distribution to the growers is found to be very low due to limitations in supply of adequate planting material for large scale production (Bayetta, 2001; JARC, 2010).

Inadequate labor, very short days available for hand emasculatation and pollination before flower opening and subsequently low rate of fruit set have been identified as major bottleneck to the production of sufficient hybrid seeds thus hindering the rapid distribution and adoption of the varieties for commercial production under our condition. As indicated by different authors, (Cliford and Wilson, 1965; Van der Vossen, 2001), vegetative propagation is found to be the best option for such problems. Vegetative propagation in coffee is a method of producing plantlets using plant vegetative parts instead of seed through either rooting of cuttings or tissue culture. Propagation by cutting is the cheapest (Hartmann *et al.*, 2002) and one of the extensively practiced methods of propagating improved clones of *C.canephora* and hybrid varieties of Arabica coffee though there is difficulties in achieving satisfactory efficiency of rooting in the latter case (Wamatu and King'oro,1992).

According to research results obtained elsewhere, rooting media, types of cutting, propagation environment and rooting hormones have been found among the main factors affecting the rooting success in Arabica coffee hybrid. Wamatu and King'oro (1992) reported that only 50% of rooting is achieved using intermittent mist propagation system from cuttings of Ruiru 11 Arabica coffee hybrid. In addition, the use of different plant growth regulating hormones and concentrations did not show significant improvement over the control treatments in success rate of rooting cuttings of Robusta clones and Arabica coffee hybrid (Wamatu and King'oro, 1992; Oloyede *et al.*, 2004).

On the other hand, results of experiments conducted under similar propagation condition in Ethiopia at Jima Agricultural Center (JARC) indicated that Arabica coffee hybrid (Ababuna) showed 89 % rooting success on media composed of top soil, manure and river sand in a ratio of 2:1:2 respectively, without using any plant growth regulators (Behailu *et al.*, 2004). However, it was reported that this percentage is reduced to 60 after rooted cuttings are transplanted to potted nursery media probably due to transplanting shock or during the hardening-off process in the preparation for field transplanting

In general, difficulties in achieving satisfactory multiplication rates of rooting of cutting, the high overhead cost of rooting of cuttings under mist system of propagation, the problems associated with seed production through hand pollination and also delay in tissue culture protocol optimization for mass propagation make the condition more difficult under our condition to implement supply of adequate planting material to the growers for commercial production of released hybrid varieties. At present, however, the demand for planting materials of the varieties is enormously increasing and becoming a great challenge that needs research focus to develop low cost vegetative propagation options that could be easily adopted by users.

As experiences elsewhere indicated, such problems were solved through applying other alternatives such as use of closed non-mist propagator which is constructed with simple and inexpensive locally available materials, planting cuttings directly in poly bags filled with suitable rooting media and using half-node cutting. With these methods, the production

efficiency of rooting cocoa cuttings had been raised from 45 % to 65 % and over 70 % rooting success of Robusta coffee has been achieved using half-node stem cuttings (Wood, 1985; Coste, 1992; Hartmann *et al.*, 2002; Adeyemi *et al.*, 2004).

Furthermore, recent studies on vegetative propagation of different species of plants also indicate the use of mixed cultures of beneficial and naturally occurring microorganisms (mainly photosynthetic bacteria, lactic acid bacteria and yeasts) for adventitious root initiation (Higa1991). Accordingly, Konoplya and Higa (2010) reported that rooting of lemon cuttings increased by 10 % with effective microorganisms (EM) application. Similarly, better quality of root and shoot parameters have been achieved with treatments receiving EM over the control in both potted Taiwan Cherry (*Prunus campanulata* Maxim), pigweed (*Amaranthus dubians*), and Robusta coffee seedlings (Chagas *et al.*, 1997; Chrispaul Muthaura *et al.*, 2010; Iou zen and cheng-yang, 2010).

However, there is no information on rooting cuttings by planting directly in poly bags, use of half-node cuttings and inoculation of EM for vegetative propagation of Arabica coffee hybrids. This experiment therefore, was initiated to evaluate the effectiveness of these alternatives on rooting of Arabica coffee hybrid for commercial production.

Objective

General objective: To identify feasible and low cost vegetative propagation options for accelerated multiplication of improved hybrid coffee varieties for large scale production.

Specific objectives:

- ❖ To identify optimum combination of type of potting media and effective microorganisms (EM) inoculation for propagation of Arabica coffee hybrid from different types of soft wood stem cuttings by evaluating rooting ability, root and shoot growth parameters.

2 LITERATURE REVIEW

2.1 Origin and Economic Importance of Coffee

Coffee-trees belong to the family *Rubiaceae* and the subgenus *Coffea* consists of approximately 100 taxa so far identified in African and Madagascar inter-tropical forests. *Coffea arabica* L. The global coffee production and its industry, however, depends on only two economic species, commonly known as Arabica coffee (*Coffea Arabica* L.) and Robusta coffee (*Coffea canephora* Pierre ex Froehn), which account for about 80% and 20% of the total world production, respectively. All species of the genus *Coffea* including the two commercial species are of tropical African origin. The equatorial lowland forests of West and Central Africa that stretches from Guinea to Uganda are the home of diverse forms of *C.canephora*, while the natural populations of *C.arabica* are restricted to the Montane rain forests of South Western Ethiopia (Berthaud and Charier, 1988).

After oil, coffee is the most valuable traded commodity worldwide, with global retail sales estimated to be US\$ 90 billion. Brazil is the largest world's coffee producer, followed by Vietnam and Colombia. Coffee is the major export product of some countries such as Uganda, Burundi, Rwanda and Ethiopia. In Ethiopia, Arabica coffee is the backbone of the economy and takes the lion's share of the foreign exchange earning of the country.

Arabica coffee breeding program in Ethiopia, has been in progress since 1968. Over the last forty years, several attempts have been made to boost production and productivity through developing and providing high yielding and disease resistant coffee varieties processing commercially acceptable quality accompanied by better crop management and post harvest technologies). In this regard, 34 improved pure line and three hybrid coffee varieties were released and distributed to the coffee producers. Although, these hybrid varieties produce 2300 to 2600 kg clean coffee per hectare the distribution for the growers is found to be very limited due to limitation in supply of adequate planting material for large scale production (Bayetta, 2001; JARC, 2010).

2.2 Propagation of Coffee

The production of healthy and vigorous seedlings is the first step for the formation of a productive coffee crop. In any Arabica coffee producing countries, propagation through seed is very popular as this method is simple and easy to generate large number of plants. In *C. arabica*, establishment of new plantations depends basically on seedlings originating from seeds. However, with the possibility of using hybrid vigor for productivity in that species, propagation of F1 hybrids either by seed through hand emasculation and pollination or vegetative/clonal means is very important for production in commercial scale (Bueno *et al.*, 2006).

However, there are certain limitations in hybrid coffee production through hand pollination among which inadequate labor, low fruit set, and unfavorable weather condition at the time of flowering are the major factors that result in shortage of adequate supply of planting material for commercial production. Inadequate labor, especially if flower is triggered by rainfall in the entire seed orchard leads to wastage since the four days available for emasculation and pollination before flower open is not adequate. Furthermore, unskilled small-scale farmers cannot easily adopt and/or practice such method (Cliford and Wilson, 1965; Wamatu, 1993; Van der Vossen 2001).

2.2.1 Vegetative propagation of coffee

Vegetative propagation is asexual reproduction of plants using different vegetative parts like shoot, leaf, root etc., of selected plants. A group of such vegetatively reproduced plantlets constitutes to what is known as a 'clone'. Its genetic makeup is identical to that of the mother plant from which it is created. Vegetative propagation offers the unique advantage of preserving all the characteristics of mother plants in their offspring. The clonal materials would also start producing yields earlier than seedling plants. Selection of elite mother plants having desirable characteristics like high yield potential, tolerance to pests, diseases and superior quality etc., is very important before initiating vegetative propagation. Recently, the importance of vegetative propagation has increased greatly due to the increasing demand for

hybrid varieties due to high agronomic performance over the pure line varieties and segregating improved clones of Robusta coffee when propagated by seed (Opile and Agwanda, 1993; Omandi, 2001; Oloyede *et al.*, 2004).

Unlike that of *C.canephora*, vegetative propagation methods are not efficient for large-scale propagation of *C.arabica* (a self fertilized species), due to the difficulty observed to achieve satisfactory multiplication rates. The sexual means is also insufficient for propagation of Arabica coffee hybrids due to the problem of segregation in F2 generation. Therefore, true-to-type propagation of Arabica hybrids is only possible with hand pollination followed by seed multiplication, or using vegetative propagation techniques, be it macro-propagation (cutting or grafting) or micro-propagation (tissue culture) (Wamatu and King'oro, 1992; Hartmann *et al.*, 2002).

Research is currently being undertaken to study the feasibility of micro propagation by tissue culture. Results obtained so far are promising and the method may be used to boost commercial production of planting materials. However, regardless of its advantage for mass propagation planting material, the current high cost for micro propagation in terms of the supply of culture containers, media, chemicals, equipment and instruments has caused major impediments to the direct use of micropropagation in many programs. Even the relative cost of labor and expertise is very high in micropropagation compared to macro propagation by stem cuttings which is generally more cost effective (Ezekiel, 2010). Although macro propagation by stem cuttings has low cost compared to micropropagation, in both methods there are potential factors to be considered in order to lower propagation costs.

2.2.2 Propagation by cutting

A cutting can be defined as any vegetative plant part, which, when detached from the parent, is capable of regenerating the missing organ or organs. It can be described as a method of propagating plants by the use of detached vegetative plant parts which when placed under conditions favorable for regeneration, will develop into a complete plant, similar in all characteristics to the parent plant. Based on the plant part taken, cuttings can be

classified as stem cuttings (hard wood, semi-hard wood, soft wood and herbaceous), leaf cuttings, leaf-bud cuttings (single-eye or single-node cuttings) and root cuttings (Hartmann and Kester, 1983).

In coffee two types of shoots *viz.*, horizontally growing 'plagiotropic' shoots and vertically growing 'orthotropic' shoots or 'suckers' are produced. The horizontal shoots are the main cropping wood in a coffee bush and are not suitable for propagation as they always grow laterally. On the other hand, vertically growing orthotropic shoots/suckers are ideally suitable for vegetative propagation because of their capacity to produce a plant structure similar to those obtained from seedlings. From orthotropic shoots/suckers, usually two types of cuttings *viz.*, hard wood and soft wood single node cuttings can be prepared (Haarar, 1962; Wintgens, 2004).

It is a well-established fact that adventitious root formation is a prerequisite to successful cutting propagation. Rooting of soft wood stem cuttings, which are obtained from six months old orthotropic suckers, is the most commonly used method of vegetative propagation to mass propagation of hybrid coffee and other economically important perennial crops (Wamatu and King'oro, 1992; Omandi, 2001; Hartmann *et al.*, 2002). This has been well tested at Jima Agricultural Research Center (JARC) and found promising (Behailu *et al.*, 2004).

Furthermore, whole node cuttings can be further split into two to increase the source of materials for cutting and reduce the unit cost of planting materials. Rene Coste (1992) reported in the trials in Madagascar where both whole and half-node stem cuttings from both species were used to enhance rapid multiplication of vegetative propagules. Similarly, exploring the possibility of half-node stem cutting as a means of propagating Robusta coffee in Nigeria in order to meet the high demand for improved planting materials that will breed true to has been reported (Harrar, 1962; Adeyemi *et al.*, 2004).

2.3 Factors Affecting Propagation by Cuttings

It is known that the capacity of the stem cutting to emit roots is a function of the interaction of endogenous factors and the environmental conditions offered to the rooting. Literatures indicate that the development of adventitious roots in a variety of plant species can be influenced by various factors such as physiological state of the stock plant, environmental conditions, the origin of cutting, type of propagating structure, propagation medium and growth regulators, etc (Wilson, 1993; Leakey *et al.*, 1994).

2.3.1 Effect of plant growth regulators

Many workers have observed the existence of variation in rooting and sprouting capacity of cuttings taken from different species. Different plant species are categorized based on their rooting potential as difficult to root and easy to root species. According to Harrar (1962) and Coste (1992), Robusta coffee is among the easy to root species and Arabica is categorized among the difficult to root groups and requires maximum care in considering external factors during the initiation for propagation by rooting cuttings.

Plant growth regulators are one of the most important endogenous factors contributing to the rooting process of cuttings. Different classes of plant growth regulators have been proven to influence root initiation, including auxins, cytokinins, gibberellins, ethylene, as well as inhibitory substances such as abscisic acid, growth retardants, and phenolics (Hartman *et al.*, 2002). Plant growth regulators are frequently used in vegetative propagation to promote rooting of cuttings (Fibijian *et al.*, 1981).

In addition to the endogenous ones, indole-3-butyric acid (IBA) and X-naphthalene acetic acid (NAA) are the two main synthetic hormones that are reliable to enhance rooting in cuttings (Hartmann *et al.*, 1997). According to Davis and Hassig (1990), the production of adventitious roots in plants through cell division, multiplication and specialization is also controlled by plant growth substances especially auxins. This implies that treating or exposure

of stem cuttings with plant growth substances can increase the percentage of rooting, root initiation and number of roots.

In coffee, however, experiments in Kenya showed that the use of different types and rate of concentration of hormones has no significant effect on number of rooted cuttings and on some other shoot and root growth parameters (except on few) compared to the control treatments (Wamatu and Kigaro, 1992). On the other hand, results of some experiments conducted under similar propagation condition in Ethiopia, indicated that Arabica coffee hybrid variety known as Ababuna showed 89% rooting success without using any plant growth regulators (Behailu *et al.*, 2004).

2.3.2 Environmental factors

Apart from physiological factors with in any one genotype, success in rooting depends on the environmental conditions that support the expression of the inherent rooting potential (Howard and Harrison-Murray, 1995). The major environmental factors determining full expression of the genotypic rooting potential of a species include: rooting media types and compositions, temperature, light, relative humidity, and type of the propagating structure. Presence of optimal temperature and moisture are one of the critical environmental factors that determine success during propagation by cutting. However, day temperatures of about 21⁰C to 27⁰C and night temperatures of about 15⁰C are satisfactory for the adequate rooting of most species (Hartmann *et al.*, 1997) though some species root better at lower temperatures. In both commercially important coffee species, a temperature of 25⁰C to 30⁰C inside the propagating structure is required during root induction (Haarar, 1961; Cost, 1992; Opil and Wamatu, 1993; Agwanda, 1993; Wintgens, 2004).

Light is also one of the major contributing factors for the formation of adventitious roots and buds on cuttings. Available reports reveal the presence of great variation in the response of plant species to different light conditions. In general, provision of adequate light conditions for the cuttings in the propagator is vital to succeed in rooting (Moe and Anderson, 1988). Similarly, relative humidity inside the propagator is one of the most critical environmental

factors that limits success rate in rooting of cuttings. In case of the two species of coffee, reports indicated that high relative humidity (90-100 %) inside the propagator is required during root induction. According to various research reports, the mist-system and enclosed non-mist type of propagator with over-head shade are being employed in a commercial way in different coffee growing countries. However, the former is found to be expensive in terms of installation cost and is not feasible to be implemented under farmer's condition (Haarar, 1961; Cost, 1992; Agwanda, 1993; Opil and Wamatu, 1993; Wintgens, 2004).

Although, the rooting of cuttings under mist propagation system is comparatively easy and rooted cuttings were well established in the field, it has certain limitations with regard to use for large scale propagation. The main problems include, death of the rooted cuttings after they have been transplanted to potted media probably because of transplanting shock or during the hardening-off process in the preparation for field transplanting which result in low (60%) production efficiency (Behailu *et al.*, 2004). Furthermore, this propagation system is found to be expensive in terms of cost of materials and equipments used for installation (Haarer, 1961; Wrigley, 1988; Coste, 1992) and not affordable by small-scale farmers' groups interested to produce planting material for their own use or supply seedlings to the other coffee growing farmers in the area.

2.4 Effect of Type of Cutting on Adventitious Root Initiation

Even though the physiological factors that determine the capacity of root initiation are poorly understood, rooting potential is highly influenced by the age of the cutting and/or the degree of lignifications of its tissues, by the age of the stock plant, being greatest in juvenile plants (Brown *et al.*, 1997). In most vegetatively propagated species, older, lignified wood cuttings are difficult to root than newly formed stems (Hartman *et al.*, 1990). In Arabica coffee, rooting of soft wood stem cuttings is the most commonly used method of vegetative propagation to mass propagation of hybrid coffee (Omandi, 2001; Behailu *et al.*, 2004). It was also observed that semi-hard wood cuttings gave significantly better rooting than terminal cuttings (Ofori-Gyamfi *et al.*, 1998).

Size of the stem cutting (diameter) may also contribute to variation in rooting ability of cuttings, since stem diameter varies along the shoot (Wilson, 1993). A positive relationship between cutting diameter and the number and length of roots has been reported in rooting of *Azadirachta indica* (Palanisamy and Kumar, 1997). Andres *et al.* (1999) had also obtained similar result in propagation of *Clutea arborescens* and explained the greater total carbohydrate content of the thick basal cuttings to be probably responsible for the greater rooting capacity and the production of well-developed plants. However, sometimes thinner-stemmed cuttings are also reported to root better (Howard and Ridout, 1992).

On the other hand, a positive relation between stem diameter and shoot growth was recorded in *Eucalyptus grandis* cuttings, but the relationship here was attributed primarily to root development; i.e. thicker cuttings rooted more prolifically, which in turn enhanced subsequent production of shoot mass (Wilson, 1994). Cameron *et al.* (2001) also reported similar result in propagation study with *Cotinus coggygia*.

The presence of leaves on cuttings also plays a significant role on root initiation of many plant species (Hartmann *et al.*, 1990). Research results in Nigeria showed that leafy half node cuttings had significant effect on sprouting of Robusta coffee with 61 percent success whereas cuttings with no leaf produce no sprouting at all (Adeyemi *et al.*, 2004). According to Leakey and Coutts (1989), apart from the actual presence of leaves, the leaf surface area on each cutting is vital for its photosynthetic efficiency, leaf water potential, transpiration and subsequent rooting percentage as well as the number of roots per cutting.

2.5 Effect of Rooting Media on Propagation by Cutting

The success of rooting stem cuttings depends mainly on the growing conditions of the cutting source mother trees in the field, composition of the rooting media (capacities for filtering excess water, retaining suitable moisture with good aeration, providing adequate nutrient) and the type of the propagator. According to Haarar (1962) and Cost (1992), the rooting media should have a good drainage capacity and provide nutrient for better root initiation healthy development of rooted cuttings. Various materials and mixtures of materials are used for

rooting cuttings; however, there is no universal or ideal rooting mix for cuttings of plant species (Hartman *et al.*, 1997).

An appropriate rooting medium generally has to have an optimal volume of gas filled pore space and oxygen diffusion rate adequate for the needs of respiration (Fonteno and Nelson, 1990). According to Caron *et al.* (2000), media physical properties should not be constrained to just measurements of air-filled porosity, water holding capacity and bulk density, but also gas exchange characteristics. According to Gordon (1992), an ideal rooting medium provides sufficient porosity to allow good aeration and has a high water holding capacity, yet is well-drained, and free from pathogens. Propagation media used in horticulture and forestry consists of a mixture of organic and inorganic components, which have different but complementary properties (Hartmann *et al.*, 1997).

Larson (1980) also indicated that the best planting media must have a pH conducive to plant growth, a structure that will permit gaseous exchange to provide aeration for the root and permit water infiltration and movement. Experiments in India indicate that soil mixture containing forest soil; well rotted farmyard manure and fine sand in 6:2:1 ratio was found to be desirable for coffee seed germination and early growth (Veerendra and Raju, 1988).

Different authors used various compositions of rooting media for vegetative propagation of the Arabica coffee hybrid. Van der Vossen *et al.* (1976) cited by Wamatu and King'oro (1992) used and recommended compost consisting of sub-soil, sand and swamp soil (1:1:1). This indicated that the best rooting media for rooting cuttings also depended on the conditions of the trial.

Numerous experiments are undertaken in different parts of the world to develop low-cost potting-mixes that could be readily available at the local level (Gordon, 1992). The materials commonly used as constituents of potting media in vegetative propagation of coffee include: top soil, coarse sand, manure, composts and coffee husk that are alone or mixed with different proportions. The coarse component in the potting mix is basically used to improve drainage and aeration by increasing the proportion of large, air-filled pores.

On top of the type of rooting medium used, compatibility of the medium-mix with the overall nature of the cuttings used and also the rooting capacity of particular cuttings under the given media composition are reported to be critical to the rooting process. Cuttings of many species root successfully in a variety of rooting media, but the performance of rooting both in number and percentage may be greatly influenced by the kind of rooting medium used (Hartmann *et al.*, 1990; Leakey *et al.*, 1990).

When rooting of soft wood cuttings of the Arabica coffee hybrid was tested, 89 percent of rooting was obtained in Ethiopia (JARC) from media composition of top soil, manure and sand in a ratio of 2:1:2, respectively. Wamatu *et al.* (1993) also found conventional nursery media topped with 10 cm sub soil with better performance in rooting of Arabica coffee hybrid Ruiru 11 in Kenya. This information indicates that possibilities of identifying suitable rooting media by blending top soil with various organic and inorganic sources for efficient multiplication of Arabica hybrid coffee variety Ababuna.

2.6 Influence of Effective Microorganisms (EM) on Adventitious Root Initiation

Beneficial soil microbes can help improve plant growth, nutrition and competitiveness and plant responses to external stress factors by an array of mechanisms They can also inhibit soil-borne plant pathogens and induce plant resistance to these (Vessey, 2003; Lucy *et al.*, 2004; Rodríguez *et al.*, 2006). Available literature indicated that soil dwelling fungi such as arbuscular mycorrhizal fungi (AMF) and plant growth promoting bacteria as a group, may have the single largest effect on plant performance functioning as an extension of the root system of the plant and increasing absorptive area (Leake *et al.*, 2004).

Growers have attempted to stimulate rooting by applying growth regulators and various chemical substances. Among the plant growth regulators, it is well known that auxin play a major role in the initiation and development of roots. However, the intensive use of exogenous plant growth regulators could result in environmental problems. Recently, environmental problems have raised interest in environmentally friendly sustainable

agricultural practices (Salantur *et al.*, 2005). Therefore, the use of growth promoting bacteria (PGPR) can overcome environmental problems.

Recent studies showed that bacteria in several genera (*Agrobacterium*, *Bacillus*, *Streptomyces*, *Pseudomonas* and *Alcaligenes*) induce root formation in stem cuttings (Ercisli *et al.*, 2004). Nelson (2004) also indicated that plant growth promoting rhizobacteria (PGPR) are able to exert a beneficial effect upon plant growth such as increases root growth and root weight probably different strains working through various multiple mechanisms. Similarly, experiments done to determine the effect of bacteria inoculation on root formation of vegetable cuttings such as mint performed by dipping the cuttings into *Agrobacterium rubi* (strain A 16), *Burkholderia gladii* (strain BA7), *Pseudomonas putidea* (strain BA8), *Bacillus subtilis* (strain OSU142) and *Bacillus megatorium* (strain M3) bacterial suspensions showed more consistent results from cuttings inoculated with bacteria genera in improving different root parameters (Kaymak *et al.*, 2008). Generally, microorganisms isolated from rhizosphere and rhizoplane of various plants are more active in producing auxins than those from root-free soil (Kampert *et al.*, 1975).

In addition, recent studies on vegetative propagation of different species of plants also indicate the use of mixed cultures of beneficial and naturally occurring microorganisms (Effective Microorganisms) for adventitious root initiation (Higa, 1991). Accordingly, (Konoplya and Higa, 1991) reported that rooting of lemon cuttings increased by 10 percent and length of roots was 12.2 ± 0.7 cm whereas it was 9.6 ± 0.4 cm without EM application. Similarly, better quality of root and shoot parameters have been observed over the control in both potted Taiwan Cherry (*Prunus campanulata* Maxim), pigweed (*Amaranthus dubians*), and Robusta coffee seedlings (Chagas *et al.*, 1997; Chrispaul Muthaura *et al.*, 2010; Iou-zen and Cheng-yang, 2010). Research has also shown that the inoculation of EM cultures to the soil plant ecosystem can improve soil quality, soil health and the growth, yield and quality of the crops (Kengo and Hui-lian, 2000). However, there is no information so far on the effects of inoculating effective microorganisms on vegetative propagation of Arabica coffee hybrid.

3 MATERIALS AND METHODS

3.1 Description of the Experimental Site

The experiment was conducted at Jima Agricultural Research Center (JARC) under the condition where the propagator was constructed from locally available materials in 2013 (January to June). The Center is located at 7° 46' N latitude and 36° 47'E longitudes coordinate with an altitude of 1753 meters above sea level, 365 km south west of Addis Ababa, and 12 km from Jimma town. The soil type of the area is nitosol. The area receives mean annual rainfall of 1572mm and the mean minimum and maximum air temperatures are 11.6°C and 26.3°C, respectively. The relative humidity of the area ranges from 35 to 95 percent (JARC, 2010).

3.2 Experimental Material

The experimental materials used in this study include, rooting media composed of top soil (Ts), sub soil (Ss), farm yard manure (M) decomposed coffee husk (Ch), river sand (S), mother culture solution of Effective microorganisms (EM) and stem cuttings of hybrid coffee variety Ababuna (741 x F59). In addition Eucalyptus tree, elephant grass and 30 micron thick white plastic sheet was used to construct the propagator.

3.2.1 Construction of propagating structure

Raised nursery bed with 1.2m width X 10.5m length were prepared to arrange the treatments. Then, simple and inexpensive non-mist propagator was made from wooden frame (eucalyptus tree). The frame was covered with 30 micron thick white translucent plastic sheet. Artificial shade supported with wooden poles were made at a height of 2 m above the ground and covered with elephant grass to provide 70 to 75 % shade (Behailu *et al.*, 2006), and both sides of the propagator was also protected with the same material to avoid direct sunlight.

3.2.2 Rooting media preparation

The basic media used for the preparation of the potting mixes were top soil (Ts), sub-soil (Ss), coarse river sand (S), farm yard manure (M) and coffee husk (Ch) were collected from available sources. The sand was purchased from supplier and sieved through 2.5 mm mesh wire to remove wood debris and other undesirable materials. Top soil was collected from the upper 25 cm layer of uncultivated land and the sub soil next to the layer of the top soil at about 30-35 cm depth was also collected from the same area. Well decomposed animal dung and coffee husk were collected from coffee hulling factories and dairy farming enterprise respectively, around Jimma town. These materials were sun dried, crushed and also sieved through mesh before mixing with other media categories. Finally, a total of eight rooting media types with the following proportions (V/V) were prepared and analyzed for various soil physico-chemical properties (Appendix Table 1).

$$\mathbf{M1} = 1\text{Ts} + 1\text{M} + 1\text{Ch} + 1\text{S}$$

$$\mathbf{M2} = 2\text{Ts} + 1\text{M} + 0\text{Ch} + 2\text{S} \text{ (bed-rooting medium)}$$

$$\mathbf{M3} = 2\text{Ts} + 0\text{M} + 1\text{Ch} + 2\text{S}$$

$$\mathbf{M4} = 3\text{Ts} + 1\text{M} + 2\text{Ch} + 1\text{S}$$

$$\mathbf{M5} = 3\text{Ts} + 2\text{M} + 1\text{Ch} + 1\text{S}$$

$$\mathbf{M6} = 6\text{Ts} + 3\text{M} + 0\text{Ch} + 2\text{S} \text{ (coffee nursery medium)}$$

$$\mathbf{M7} = 2\text{Ts} + 1\text{M} + 1\text{S}, \text{ topped with } 10 \text{ cm sub soil (tea medium)}$$

$$\mathbf{M8} = 2\text{Ts} + 1\text{M} + 1\text{S}, \text{ topped with } 10 \text{ cm } 4\text{Ss} + 1\text{M} + 1\text{Ch} + 1\text{S}$$

3.2.3 Inoculation of effective microorganisms (EM)

An activated effective microorganisms (dilution of one liter of effective microorganisms with 18 liters of water and 100 g of molasses) culture solution was purchased from the supplier. The culture solution was further diluted 1000X as to the recommendation to apply onto soil. Volume of each media composition required for each treatment groups receiving the EM was determined based on the volume of the poly bag and moistened with water and then, inoculation of EM at a rate of 100 ml per pot (Iou-Zen and Cheng-Yung, 2010) was

uniformly applied and thoroughly mixed and filled in to the poly bags three days before planting the cuttings. The inoculation was continued at monthly interval with similar rate until data collection was completed.

3.2.4 Preparation of cutting types

Ababuna coffee hybrid (741 X F-59) already established in the clonal garden of JARC and horizontally bent to initiate orthographic sucker growth was used as a source of stem cuttings. Procedures described by Wamatu and King'oro (1992) were strictly followed during the preparation of cutting types. Uniform and healthy suckers with 6 to 7 nodes were harvested early in the morning when the shoot and the leaves are turgid using sharp and clean pruning sheer. The suckers were placed immediately in the bucket containing clean tap water to prevent dehydration and, then transported to the actual propagation site where the whole operation is carried out under shaded condition to provide protection against sun light.

Single node cuttings were prepared by cutting the suckers just above each node and the woody and young parts from the lower and upper ends of the suckers, respectively were discarded. Half-node cuttings were prepared by dissecting the whole node cuttings in to two symmetrical halves using buddle knife. The leaves on both types of cutting were trimmed to half to reduce the rate of transpiration. Slash cut at the base of each cutting were made before setting them in the rooting media. To maintain internal turgidity, all the cuttings were kept in a plastic box containing clean water. Finally, they were inserted to a depth of 3-4cm into the potted media in January 2012 and watered up to field capacity. The polythene sheet was then buried along the edges of the bed to provide humidified environment for the cuttings.

3.2.5 After planting care

To maintain the required level of moisture, temperature and relative humidity, hand water application using 10 liter capacity plastic watering cane was done depending upon the prevailing weather conditions and fine water droplets retained on the covering plastic sheet.

Removal of weed, dead and diseased cuttings and other important management activities were carried out accordingly by opening and closing back the polyethylene sheet. A daily minimum and maximum temperature inside the propagator was recorded using thermometer and the range was 24-29⁰C. The relative humidity (RH) inside the propagator was also recorded daily and the average was 89 percent.

3.2.6 Hardening-off process

Four months after planting, hardening-off /acclimatization process was carried out for a subsequent two months by opening the polyethylene sheet suspended over the propagator from one side. This was done early in the morning for five to ten minutes for the first three days and was gradually increased according to the prevailing weather condition and the physical condition of the newly growing suckers until the complete removal of the poly sheet which was carried out after two months. In addition, the over-head shade was gradually reduced for further hardening-off process as usually done for seedlings raised from seed before field transplanting.

3.3 Experimental Design and Treatments

Eight media types, two level of EM inoculation (with and without) and two types of cutting (whole node and half-node) were combined with factorial arrangement (8x2x2) and laid down in a Randomized Complete Block Design (RCBD) with three replications (Table 1). Each treatment contained 16 cuttings and a total of 1536 cuttings were used for the experiment. The cuttings were inserted directly in the media filled in 16 cm wide and 25 cm long black polyethylene bags and randomly assigned in the propagator with two rows and 10 cm spacing between treatments.

Table 1. Treatment combinations

Media	EM	Cutting types
1Ts:1M:1Ch:1S (M1)	0	WN
1Ts:1M:1Ch:1S	0	HN
1Ts:1M:1Ch:1S	+	WN
1Ts:1M:1Ch:1S	+	HN
2Ts:1M:0Ch:2S (M2)	0	WN
2Ts:1M:0Ch:2S	0	HN
2Ts:1M:0Ch:2S	+	WN
2Ts:1M:0Ch:2S	+	HN
2Ts:0M:1Ch:2S (M3)	0	WN
2Ts:0M:1Ch:2S	0	HN
2Ts:0M:1Ch:2S	+	WN
2Ts:0M:1Ch:2S	+	HN
3Ts:1M:2Ch:1S (M4)	0	WN
3Ts:1M:2Ch:1S	0	HN
3Ts:1M:2Ch:1S	+	WN
3Ts:1M:2Ch:1S	+	HN
3Ts:2M:1Ch:1S (M5)	0	WN
3Ts:2M:1Ch:1S	0	HN
3Ts:2M:1Ch:1S	+	WN
3Ts:2M:1Ch:1S	+	HN
6TS:3M:0Ch:2S (M6)	0	WN
6TS:3M:0Ch:2S	0	HN
6TS:3M:0Ch:2S	+	WN
6TS:3M:0Ch:2S	+	HN
2Ts:1M:1S + top 10cm Ss (M7)	0	WN
2Ts:1M:1S + top 10cm Ss	0	HN
2Ts:1M:1S + top 10cm Ss	+	WN
2Ts:1M:1S + top 10cm Ss	+	HN
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S (M8)	0	WN
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	0	HN
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	+	WN
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	+	HN

EM; 0 = without, + = with EM. Cutting type; WN = whole node, HN = Half/dissected node

3.4 Data Collection

Destructive data were collected after six months (June, 2012) of planting. Rooting percent was determined based on all survived cuttings per plot and the average was taken. Five selected sample cuttings from each plot were considered and separated in to root and shoot parts and evaluated for the different parameters. The parameters measured and the methods used are presented as follows:

3.4.1 Data collected

3.4.1.1 Soil parameters

Soil sample from each rooting medium was prepared before planting the cuttings and analyzed for the following physical and chemical properties using standard laboratory procedures and methods (Sahlemedhin Sertsu and Taye Bekele, 2000). The analysis was carried out in JARC's soil laboratory by qualified technicians.

I. Physical properties (before planting)

- **Texture:** media texture was determined by the modified Bouyoucos hydrometer method.
- **Bulk density (g/ml):** mass of dried media (g)/volume of dried media (ml) was calculate and taken for analysis.
- **Water holding capacity (%):** it was calculated using the following formula.

$$WHC = \frac{\text{Weight of water in the saturated media(g)}}{\text{Weight of saturated media(g)}} \times 100$$

II. Chemical properties

- **pH:** The pH of the media was potentiometrically measured by water (pH H₂O) in the supernatant suspension of soil to water solution ratio of 1:2.5.
- **Organic carbon (%):** Organic carbon content of the media was determined by the wet combustion procedure of Walkley and Black.

- **Total nitrogen (%):** Total nitrogen content of the soil was determined by wet-oxidation procedure of the Kjeldahl method.
- **Available phosphorus (ppm):** The available phosphorus content of the media was determined by 0.5M sodium bicarbonate extraction solution (pH 8.5) method of Olsen (1954).
- **Available potassium (ppm):** The available potassium content of the media was determined by using atomic absorption or flame photometer.

3.4.1.2 Root parameters

- **Percentage of rooted cuttings (%):** poly bags containing seedling with shoot were cut vertically and the soil was removed by immersing it in to a bucket of water and cuttings with adventitious roots were counted per plot and the average was taken.
- **Root number:** the newly growing roots were counted for five selected sample roots per plot and the average was taken.
- **Root length (cm):** the length of the longest root for the sample cuttings was measured from the point of emergence to the tip by using a ruler and the average was taken
- **Root Girth (mm):** the diameter of the longest root per cuttings was measured by digital caliper (Model Fowler, USA) 1cm from the point of emergence and average was taken.
- **Root fresh weight (g):** newly growing sample roots were carefully detached from the point of attachment to the cuttings and thoroughly washed to remove the soil. Then, weight was measured using sensitive balance (Model CTG-6H+, USA).
- **Root volume (ml):** root volume was measured by water displacement method using graduated cylinder half filled with water. The volume of water displaced due to the immersion of each sample was calculated and the average was taken as root volume.
- **Root dry weight (g):** after drying the roots in an oven drier (at a temperature of 105⁰ C to constant weight) weight was measured using a sensitive balance and the average was calculated for each treatment.
- **Root to shoot dry weight ratio:** was determined by dividing dry weight of root to shoot of each sample cuttings and the average was calculated for each treatment.

- **Total dry matter yield (g):** the weight of oven dried whole plant parts (shoot and root) was summed up and the value was taken

3.4.1.3 Shoot parameters

- **Survival of cuttings (%):** the ratio of alive cuttings to the number of originally planted was calculated and the average was taken
- **Shoot sprouting:** total number of cuttings with newly growing shoots were counted and the average values were used for analysis
- **Shoot number:** the newly growing shoots (suckers) were counted for each sample cuttings and the average was taken for each treatment.
- **Shoot length (cm):** the length of each newly developed shoots of the sample was measured from the point of attachment on the cutting/stem to the tip of the shoot using a ruler and the average of plot was taken for each treatment.
- **Leaf number:** the newly growing leaves were counted for each sample cutting and the average was calculated for each treatment.
- **Leaf area (cm²):** Sample leaves from each cutting were taken and leaf area was calculated following the method described by Yacob *et al.* (1993). Then, the average value per cutting was taken.
- **Shoot fresh weight (g):** the weight of newly growing shoots detached from the sample cuttings was measured using sensitive balance and the average was taken for each treatment..
- **Shoot dry weight (g):** after drying the shoots in an oven drier (at a temperature of 105⁰C to constant weight) weight was measured using a sensitive balance and the average was calculated for each treatment.

3.4.2 Data analysis

Data collected for various root and shoot parameters were checked for meeting the assumptions for ANOVA. The percentage data collected for rooting ability and survival percent were transformed using the Angular transformation method and then, ANOVA was

carried out for the whole data set using the SAS (version 9.2) statistical software. For those significant treatment mean differences, least significance difference (LSD) method was utilized (Montgomery, 2005).

The model

Three factor analysis of variance model was used with general linear model (GLM) procedures of SAS Version 9.2.

$Y_{ijk} = \mu + (\beta) + A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk} + \Sigma_{ijk}$, where;

- Y_{ijk} is the observation of the i th, j th & k th treatment (response variables)= the response measurement for the ijk th observations
- μ is the mean
- β is the replication or block effect, and
- A_i, B_j & C_k is the treatment of the i th, j th, & k th treatment
- AB_{ij} is interaction effect of A_i and B_j treatment
- AC_{ik} is interaction effect of A_i and C_k treatment
- BC_{jk} is interaction effect of B_j and C_k treatment
- ABC_{ijk} is interaction effect of A_i, B_j and C_k treatment
- Σ_{ijk} is the experimental error

4 RESULTS AND DISCUSSION

4.1 Rooting Ability of Cuttings (%)

Rooting ability of stem cuttings was significantly influenced by rooting media, effective microorganisms (EM) inoculation and cutting types. The interactions between media and application of EM, media and cutting type, EM and cutting type and media by EM by cutting type were also significant. However, EM alone did not show significant differences for rooting of stem cuttings (Appendix Table 2).

Significant ($P \leq 0.05$) differences among treatments were observed for percent rooting ability of stem cuttings (Table 2). The highest percent rooting (88.89%) was recorded for whole node (WN) cutting grown in media composed of 6TS:3M:0Ch:2S with EM and 2Ts:1M:1S + top 10cm Ss with and without EM. However, this value was statistically similar with the rooting percent (83.33%) obtained for the same type of cutting grown in 6TS:3M:0Ch:2S without EM, 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S with EM and half node (HN) cutting type propagated on 6TS:3M:0Ch:2S with and without EM. Complete death was recorded for HN cutting propagated on rooting media compositions 1Ts:1M:1Ch:1S with EM, 3Ts:1M:2Ch:1S and 3Ts:2M:1Ch:1S with and without EM. The least rooting percent (11.11%), on the other hand, was observed for WN and HN cutting types planted in rooting media composed of 3Ts:2M:1Ch:1S and 1Ts:1M:1Ch:1S without EM.

In general, as compared to other rooting media, significantly higher rooting percent was recorded for both cutting types when propagated on 6TS:3M:0Ch:2S, 2Ts:1M:1S + top 10cm Ss and 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S, with or without EM. This indicates that both cutting types are more responsive to rooting in these media types than others. Furthermore, EM application did not show significant difference on percent rooting of survived cuttings when each rooting media is considered independently which indicated that its effect is media specific. However, the performance of half node cuttings was slightly better in 6TS:3M:0Ch:2S with or without EM than did on 2Ts:1M:1S + top 10cm Ss and 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S.

Thus, the reason for the higher rooting percentage of both cutting types obtained from 6TS:3M:0Ch:2S, 2Ts:1M:1S + top 10cm Ss and 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S with and without EM is reflected by positive and high interactions among the three factors, viz a viz, media, EM and cutting types. The relatively lower pH (4.55-5.89) and good physic-chemical properties of these media (Appendix Table 1) and thus, good aeration and water infiltration and movement in the rizosphere, as indicated by Larson (1980) might have contributed to the initiation of adventitious root. In addition, low pH of the media could played important role in loosening the cell wall of cuttings and presumably activated certain cell-wall-degrading enzymes that act in promoting active cell division and which are inactive at higher pH as described by Cleland (1987).

Generally, overall improvement in physical and chemical properties of the media which are associated with the balance between nutrient supply as well as optimal volume of pore spaces and adequate oxygen diffusion rate to satisfy the need of respiration (Fonteno and Nelson, 1990; Gordon, 1992; Caron *et al.*, 2000) could have attributed to higher percent rooting of both cutting types on the above mentioned rooting media. In addition, slightly better rooting recorded for both cutting types grown on EM treated media than not treated, could probably be due to the absorption of the amino acids, nucleic acids, bioactive substances and sugars which are produced by photosynthetic bacteria by cuttings, which act in promoting active cell division contributing to the better and early root initiation and development, as described by Higa (1996). The variations among the cutting types might possibly be due to variation in size and the accumulation of carbohydrates and growth regulating substances at the base of cutting types (Hartmann *et al.*, 1997).

In general, as far as rooting ability, the simplicity of media preparation and locally availability of the material are concerned, the result obtained from the present study was encouraging that indicates possible alternatives for propagation of hybrid coffee through rooting of stem cuttings. Furthermore, appropriate rooting media mixes, especially with organic source, such as manure which favors improvement of soil physic-chemical properties, provides suitable conditions for root initiation and subsequent growth and development are considerable factors in vegetative propagation of hybrid coffee by stem cutting.

The result also indicates that HN cutting could have equal rooting potential with that of WN cuttings provided that the rooting media is composed of appropriate organic and inorganic mixes. Despite the absence of synthetic rooting hormones, coupled with the fact that the cuttings were HN which made it more fragile than WN cutting type, a percent rooting of 70-83 was encouraging and thus, paved a way for utilizing such types of cuttings and media mixes to maximize the production of planting material for large scale plantation.

The results of the present study are in agreement with the findings of Wamatu and King'oro (1992), who reported rooting media composed of manure and other inorganic sources are suitable for rooting of Arabica coffee hybrid and observed significant difference between whole and half node cuttings. The result is also in agreement with findings of Wamatu *et al.*(1993) who have obtained best results from both cutting types when propagated on recommended potted mixture of top soil + sand + manure in a 3:2:1 ratio topped with 10 cm sand in a polythene bag placed in a propagator. This suggests that better results could be obtained if rooting is done directly in poly bags filled with appropriate media mixtures thus, avoiding losses due to expected death of rooted cuttings during transplanting and subsequent hardening of rooted cuttings which is a common problem with mist system of propagation.

These results were also in line with the findings of Wamatu and King'oro (1992), in other experiments done to study the effects of rooting hormone and cutting types on vegetative propagation of the same cultivar, which showed the superiority of HN cutting over whole single node cutting, indicating that potential of such kind of cuttings to develop adventitious roots as equal as or even better than whole node cuttings. The results of the present study are also in agreement with the findings of Mawardi and Purwadi (2004), who found highest rooting percentage, number of roots, root length, number of shoots and shoot length from different clones of Robusta coffee propagated on media composed of top soil, manure and sand at 1:1:2 ratios. Similarly, a mixture of forest soil, well decomposed farm yard manure and sand in 6:2:1 ratio was found to be desirable for coffee seed germination and early growth (Veerendra and Raju, 1988).

The results of the present study are also substantiated by the work of Muhabat *et al.* (2006), who found comparable results to the best and recommended media for propagation of ficus in media

composed of soil, silt and FYM at 1:1:1 ratio. Furthermore, the best performance of both cutting types observed in the present study revealed that a propagator constructed and shaded from inexpensive and locally available materials could be considered as the best environment for the propagation of Arabica coffee hybrid using rooting of soft wood stem cuttings. In general, the results obtained from the present study imply that both types of cuttings are equally good in their rooting ability provided that suitable rooting media and propagation conditions are used. It was also observed that top soil, manure and sand could be a good source of rooting media mixes though the proportions of each varies in different experiments.

Table 2. Interaction effects of media, effective microorganisms and cutting types on percent rooting ability of hybrid Arabica coffee Ababuna

Media	EM	Cutting types	Percent rooting
1Ts:1M:1Ch:1S (M1)	0	WN	13.89(21.66) ^{ij}
1Ts:1M:1Ch:1S	0	HN	11.11(19.22) ^j
1Ts:1M:1Ch:1S	+	WN	16.67(24.10) ^{ij}
1Ts:1M:1Ch:1S	+	HN	0.00(0.00) ^k
2Ts:1M:0Ch:2S (M2)	0	WN	63.89(53.09) ^{efg}
2Ts:1M:0Ch:2S	0	HN	55.56(48.25) ^{gh}
2Ts:1M:0Ch:2S	+	WN	69.45(56.49) ^{def}
2Ts:1M:0Ch:2S	+	HN	61.11(51.45) ^{fgh}
2Ts:0M:1Ch:2S (M3)	0	WN	55.55(48.20) ^{gh}
2Ts:0M:1Ch:2S	0	HN	50.00(45.00) ^h
2Ts:0M:1Ch:2S	+	WN	61.11(51.45) ^{fgh}
2Ts:0M:1Ch:2S	+	HN	55.55(48.2) ^{gh}
3Ts:1M:2Ch:1S (M4)	0	WN	16.67(23.63) ^{ij}
3Ts:1M:2Ch:1S	0	HN	0.00(0.00) ^k
3Ts:1M:2Ch:1S	+	WN	22.22(28.03) ⁱ
3Ts:1M:2Ch:1S	+	HN	0.00(0.00) ^k
3Ts:2M:1Ch:1S (M5)	0	WN	11.11(19.22) ^j
3Ts:2M:1Ch:1S	0	HN	0.00(0.00) ^k
3Ts:2M:1Ch:1S	+	WN	19.45(26.07) ⁱ
3Ts:2M:1Ch:1S	+	HN	0.00(0.00) ^k
6Ts:3M:0Ch:2S (M6)	0	WN	83.33(66.37) ^{ab}
6Ts:3M:0Ch:2S	0	HN	80.56(64.62) ^{abc}
6Ts:3M:0Ch:2S	+	WN	88.89(70.78) ^a
6Ts:3M:0Ch:2S	+	HN	83.33(65.90) ^{ab}
2Ts:1M:1S + top 10cm Ss (M7)	0	WN	88.89(70.78) ^a
2Ts:1M:1S + top 10cm Ss	0	HN	77.78(61.97) ^{bcd}
2Ts:1M:1S + top 10cm Ss	+	WN	88.89(70.78) ^a
2Ts:1M:1S + top 10cm Ss	+	HN	77.78(61.97) ^{bcd}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S (M8)	0	WN	72.22(58.25) ^{cde}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	0	HN	72.22(58.25) ^{cde}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	+	WN	83.33(66.37) ^{ab}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	+	HN	77.78(61.97) ^{bcd}
LSD (P<0.05)			6.54
CV (%)			(9.60)

Values in parenthesis are arcsine transformed values.

Values followed by the same letter are not significantly different.

EM0=without EM, EM+=with EM, Cutting type; WN= whole node, HN= half/dissected node).

Ts =top soil, M=farm yard manure, Ch=coffee husk, S=Sand.

4.2 Root Growth Parameters

The analysis of variance for most of the root growth parameters revealed the presence of highly significant differences for main factors, two and three-way interaction (Appendix Tables 3-12). The results therefore, are discussed in detail below.

4.2.1 Root fresh weight (g)

In this study, both the two and three way interactions were not significant for root fresh weight. The analysis of variance indicated that root fresh weight of coffee stem cuttings were highly significantly ($P<0.01$) affected by the rooting media (Appendix Table 3). Significant ($P<0.05$) differences were observed between media types on root fresh weight (g) of cutting types (Table 3). Maximum root fresh weight value (2.157g) was recorded for rooting media mix of 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S. However, this value is statistically similar with values 1.488, 1.385, and 1.351g recorded for cuttings grown on rooting media composed of 6TS:3M:0Ch:2S, 2Ts:1M:1S + top 10cm Ss and 2Ts:1M:0Ch:2S, respectively. The least root fresh weight value (0.678gm), on the other hand, was exhibited by cutting types grown on rooting media composed of 3Ts:1M:2Ch:1S.

Higher root fresh weight values for the aforementioned media types could be due to large number and length of roots produced by the cuttings as compared to others (Table 4). The present study is in line with the findings of Marwadi and Purwady (2004), who reported that percentage rooted cuttings and total root length are expected to be important characteristics on breeding program mainly dealing with the propagation cuttings. Farooqi *et al.* (1994) also investigated similar results from cuttings of *Rosa damascene*, where increases in number of roots and length of roots have direct influence on the roots fresh weight.

Table 3: Mean values of root fresh weight (g) of stem cuttings as affected by type of rooting media

Media	Root fresh weight (g)
1Ts:1M:1Ch:1S	0.808b
2Ts:1M:0Ch:2S	1.351ab
2Ts:0M:1Cm:2S	0.808b
3Ts:1M:2Ch:1S	0.678b
3Ts:2M:1Ch:1S	0.682b
6TS:3M:0Ch:2S	1.488ab
2Ts:1M:1S + top 10cm Ss	1.385ab
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	2.157a
LSD (0.05)	0.846
CV (%)	8.62

Means followed by the same letter are not significantly different

4.2.2 Root number

The analysis of variance has revealed a highly significant ($P < 0.001$) interaction among rooting media, EM inoculation and cutting type for average number of roots per cutting (Appendix Table 4). Significantly ($P < 0.05$) the largest number of roots per cutting (3.13) was produced by WN cuttings propagated in rooting media mixes of 2Ts:1M: 0Ch:2S treated with effective microorganisms (Table 4). It was, however, statistically at par with the range of values (2.67 to 3.07) obtained from the same type of cutting propagated on 6TS:3M: 0Ch:2S, 2Ts:1M: 1S + top 10cm Ss and 2Ts:1M: 1S + top 10cm 4Ts:1M: 1Ch:1S with and without EM inoculation. Whereas, the least root number (1.40) was observed for HN cuttings grown in 1Ts:1M: 1Ch:1S media blend without EM inoculation. In general, WN cuttings produced higher number of roots per cutting as compared to HN cuttings.

The observed variation with regard to the this response parameter, could be due to the combined effects of the three factors as well as the main effects of rooting media and the cutting type as the analysis of variance indicated. The variations among the cutting types may possibly be due to the difference in size of cuttings and, hence, accumulation of carbohydrates and growth regulating substances at the base of cuttings (Hartmann *et al.*, 1997). It could also be attributed to the characteristic differences in physico-chemical, properties of the rooting media mixes as well as their compatibility with the overall nature of the cutting types. Thus, the reason for the

maximum number of roots could be the availability of essential nutrients at surface layer of the medium so that effective absorption by the cuttings favored the production of more roots.

The result of the present study is also in line with earlier reports by Yeboah *et al.* (2010) who have found significant variation in number of roots per stem cuttings of Shea (*Vetilaria paradoxa gaernt*) where the highest value was recorded for those treated with selected herbicide and rooting hormones. Similarly, Hart and Gisler (1983) have reported that increasing air content of the rooting media improved rooting in poinsettia and hydrangea azalea stem cuttings, while at low air content (0 cm moisture tension) the formation and growth of roots was strongly inhibited. Berga (1990) also observed variation in root number per cutting grown on basic media such as soil, manure, sawdust and sand. Furthermore, Muhabat *et al.* (2006) have found significant variation in root number of *Ficus bennendijkii* 'Amstel queen' cuttings propagated on various rooting media where the maximum was recorded from leaf mold. In general, better or comparable root number was recorded from both cutting types when propagated on rooting media that showed sufficient rooting percentage.

4.2.3 Root length (cm)

From the analysis of variance root length was highly significantly ($P < 0.001$) influenced by the main, two-way and three-way interactions of media, EM inoculation and cutting types (Appendix Table 5). Significantly ($P \leq 0.05$) the longest average value (13.27) of root length per cutting was recorded for WN cuttings grown in rooting media composed of 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S with EM (Table 4). However, this value was statistically not different from values recorded for the same type of cutting grown in rooting medium mixes of 6TS:3M: 0Ch:2S (12.8 cm) with EM and 2Ts:1M:1S + top 10cm Ss with (13.07cm) and without (12.90Cm) EM, respectively. The shortest root length (5.73cm) per cutting was obtained from HN cuttings grown in 1Ts:1M:1Ch:1S medium mix with EM, but it was not statistically different for values (6.71cm) recorded for WH cuttings on the same media without EM. In general, root length of both cutting types propagated in EM treated media was significantly longer than those grown in rooting media without EM inoculation, except for 2Ts:1M:1S + top 10cm Ss where both types of cuttings produced similar values in both EM treated and un treated media and rooting media where complete death of cuttings were recorded for HN cuttings.

Likewise, differences in root length were non-significant for HN and WN cuttings in EM treated media or in un treated ones, except, for 6TS:3M:0Ch:2S with and without EM and 2Ts:1M:1S + top 10cm 4Ts:1M:1Ch:1S without EM inoculation, where WN cuttings produced significantly longer roots than did HN cuttings.

The highest root length obtained from both types of cuttings grown on EM inoculated media could be due to the absorption of the amino acids, nucleic acids, vitamins, enzymes and bioactive substances that are produced by effective microorganisms and favor in promoting active cell division which leads to subsequent growth and development of the root system as described by Higa (1996). In addition, gaseous exchange of the rooting media to provide aeration for the root and permit water infiltration and movement, according to Larson (1980), could have developed more positive synergistic effect in rooting media compositions where encouraging rooting was recorded and resulted in the observed variation.

Result of the present study was in agreement with the findings of Konoplya and Higa (2010) who have reported greater increment in root length of lemon cuttings under the action of EM than without EM. The result was also in line with investigation made by Rezende *et al.* (2010) who have also observed significantly larger root length for semi hard wood cuttings than woody and green cuttings of Arabica coffee, Catuai Amarelo. Similarly, in other works, root length was found to be greater when mint cuttings are inoculated with plant growth promoting rhizobacteria of some strains (Kymac *et al.*, 2008). Besides, results of the present study was in agreement with findings of Mawardi and Purwadi (2004), who have reported significant variation in root length among cuttings of Robusta coffee clones propagated in mixture of sand, top soil and cow dung manure in 2:1:1 ratio without using any plant growth regulators. Teferi (2012) has also reported similar result, indicating that hormone treated cuttings of Arabica coffee hybrids propagated in forest soil potted medium showed highest root length.

Table 4: Effects of media, EM and cutting type on various root growth parameters of Arabica coffee hybrid stem cuttings

Media	EM	CT	RN	RL (cm)	RG (mm)	RV (ml)
1Ts:1M:1Ch:1S (M1)	0	WN	1.93 ^{efgh}	6.71 ^m	1.410 ^j	1.250 ^{cd}
1Ts:1M:1Ch:1S	0	HN	1.40 ⁱ	5.73 ^m	1.377 ^j	0.800 ^{f-i}
1Ts:1M:1Ch:1S	+	WN	2.27 ^{cde}	7.27 ^{kl}	1.460 ^j	2.000 ^b
1Ts:1M:1Ch:1S	+	HN	0.00 ^j	0.00 ⁿ	0.000 ^k	0.000 ^j
2Ts:1M:0Ch:2S (M2)	0	WN	2.60 ^{bcd}	10.60 ^{fg}	2.653 ^{gh}	0.760 ^{ghi}
2Ts:1M:0Ch:2S	0	HN	1.87 ^{e-i}	9.93 ^{gh}	2.593 ^h	0.733 ^{ghi}
2Ts:1M:0Ch:2S	+	WN	3.13 ^a	12.33 ^{abc}	2.870 ^{def}	1.167 ^{cde}
2Ts:1M:0Ch:2S	+	HN	1.80 ^{e-i}	11.67 ^{cde}	2.730 ^{fgh}	0.860 ^{fgh}
2Ts:0M:1Ch:2S (M3)	0	WN	1.53 ^{hi}	9.27 ^{hi}	1.490 ^j	1.183 ^{cde}
2Ts:0M:1Ch:2S	0	HN	1.77 ^{e-i}	8.50 ^{ij}	1.363 ^j	0.867 ^{fgh}
2Ts:0M:1Ch:2S	+	WN	1.53 ^{hi}	10.47 ^{fg}	1.520 ^j	1.417 ^c
2Ts:0M:1Ch:2S	+	HN	1.53 ^{hi}	10.07 ^{fgh}	1.440 ^j	0.767 ^{ghi}
3Ts:1M:2Ch:1S (M4)	0	WN	1.63 ^{f-i}	6.61 ^m	1.923 ⁱ	1.333 ^{cd}
3Ts:1M:2Ch:1S	0	HN	0.00 ^j	0.00 ⁿ	0.00 ^k	0.000 ^j
3Ts:1M:2Ch:1S	+	WN	1.60 ^{ghi}	8.30 ^{ijk}	1.867 ⁱ	2.250 ^{ab}
3Ts:1M:2Ch:1S	+	HN	0.00 ^j	0.00 ⁿ	0.000 ^k	0.000 ^j
3Ts:2M:1Ch:1S (M5)	0	WN	1.87 ^{e-i}	6.20 ^m	1.817 ⁱ	1.417 ^c
3Ts:2M:1Ch:1S	0	HN	0.00 ^j	0.00 ⁿ	0.000 ^k	0.000 ^j
3Ts:2M:1Ch:1S	+	WN	1.60 ^{ghi}	8.10 ^{jk}	1.920 ⁱ	2.333 ^a
3Ts:2M:1Ch:1S	+	HN	0.00 ^j	0.00 ⁿ	0.000 ^k	0.000 ^j
6TS:3M:0Ch:2S (M6)	0	WN	2.67 ^{abc}	10.63 ^{efg}	2.767 ^{efg}	1.250 ^{cd}
6TS:3M:0Ch:2S	0	HN	1.87 ^{e-i}	9.80 ^{gh}	2.593 ^h	0.867 ^{fgh}
6TS:3M:0Ch:2S	+	WN	3.07 ^{ab}	12.80 ^{ab}	3.173 ^{ab}	2.250 ^{ab}
6TS:3M:0Ch:2S	+	HN	1.93 ^{e-h}	11.73 ^{cd}	2.933 ^{cd}	0.933 ^{efg}
2Ts:1M:1S + top 10cm Ss (M7)	0	WN	2.93 ^{ab}	13.07 ^{ab}	2.990 ^{cd}	0.867 ^{fgh}
2Ts:1M:1S + top 10cm Ss	0	HN	2.13 ^{def}	12.20 ^{bc}	2.743 ^{e-h}	0.633 ^{hi}
2Ts:1M:1S + top 10cm Ss	+	WN	2.81 ^{ab}	12.90 ^{ab}	3.320 ^a	1.083 ^{def}
2Ts:1M:1S + top 10cm Ss	+	HN	2.07 ^{efg}	12.20 ^{bc}	2.973 ^{cd}	0.800 ^{f-i}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S (M8)	0	WN	3.03 ^{ab}	11.10 ^{def}	2.908 ^{de}	0.933 ^{efg}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	0	HN	1.60 ^{ghi}	9.93 ^{gh}	2.763 ^{efg}	0.533 ⁱ
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	+	WN	2.93 ^{ab}	13.27 ^a	3.098 ^{bc}	1.250 ^{cd}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	+	HN	1.73 ^{f-i}	12.57 ^{abc}	2.963 ^{cd}	0.860 ^{fgh}
LSD 0.05			0.52	1.04	0.166	0.30
CV (%)			18.09	7.47	5.03	18.44

EM0 = without EM, + = with EM. CT; Cutting type WN= whole node, HN= half/splited node .

Values in parenthesis are arcsine transformed values. Figures followed by the same letter are not significantly different.

4.2.4 Root girth (mm)

Root girth was highly significantly ($P \leq 0.001$) affected by the main factors, and both two and three-way interaction (Appendix Table 6). The thickest average value (3.32mm) of root girth was recorded for WN cuttings grown on rooting media composed of 2Ts:1M:1S + top 10cm Ss, followed by 6TS:3M:0Ch:2S (3.17mm) and 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S (3.07mm) both with EM (Table 4). Similarly, HN cuttings showed the highest root girth (2.973mm) for rooting media composed of 2Ts:1M:1S + top 10cm Ss with EM treatment. This value was, however, statistically at par with the values observed by WN cuttings propagated in the same media (2.99mm), and in 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S (2.907mm) both without EM treatment. The thinnest root girth, on the other hand, was recorded for HN cuttings grown in 1Ts:1M:1Ch:1S medium without EM inoculation.

In general, both cutting types produced thick root girth when propagated on EM treated media, especially on those compositions where better performance of other shoot and root parameters were recorded. Furthermore, comparable performance of HN cuttings grown in EM treated media with WN cuttings grown in medium mixes without EM indicated that the potential of the former could be improved through application of effective microorganisms.

The variations among the cutting types may possibly be due to the differences in the accumulation of carbohydrates and growth regulating substances because of the nature/size of cuttings (Wilson, 1993; Hartmann *et al.*, 1997) besides, differences in physical and chemical properties of the rooting media mixes might have also contributed to the observed variations in root girth (Larson, 1980). On the other hand, activity of essential substances produced by the EM (Higa, 1991), may also hasten the adventitious root formation and absorption of nutrients, which, in turn, lead to better development of root and shoot systems.

The results obtained from the present study was in line with findings of Konoply and Higa (2010), who reported that EM-1 application in plant cultivation causes acceleration of development and increased growth in plants, which among others, is determined by improvement in root system formation. Similar results have also been reported by Chrispaul *et al.* (2010) based on a study conducted on effective microorganisms and their influence on growth and yield of pigweed

(*Amaranthus dubianca*). The result was also in line with result reported by Teferi (2012) have also reported that application of growth regulating hormones (auxin and NAA) improved production of adventitious roots and other root parameters in propagation of Arabica coffee hybrid by stem cutting.

4.2.5 Root volume (ml)

The analysis of variance indicated that root volume per cutting was very highly significantly ($P < 0.001$) influenced by the interaction of media, EM application and type of stem cutting (Appendix Table 7). The highest root volume (2.333 ml) was registered for WN cuttings grown in EM inoculated rooting media composed of 3Ts:2M: 1Ch:1S. This value was, however, statistically at par with the value (2.25 ml) recorded for the same type of cutting grown on 6TS:3M: 0Ch:2S and 3Ts:1M: 2Ch:1S both with EM inoculation (Table 4). The lowest value (0.533ml) was also exhibited for HN cuttings planted in rooting media composed of 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S without EM. On the other hand, the highest value (0.933 ml) was recorded for HN cuttings grown in rooting media composed of 6TS:3M:0Ch:2S with EM inoculation on which the highest root volume for WN cuttings was also recorded.

Furthermore, both cutting types exhibited better root volume when they are grown in EM treated than on un treated media, though WN cuttings had relatively higher values than did HN cuttings. This could be due to the positive effects of substances produced by the effective microorganisms and the suitability of the media that enhance root initiation and further growth and development of roots. On the other hand, significantly higher values were observed for WN than for HN cuttings which could be attributed to higher number of roots produced by the former due to larger surface area and higher internal carbohydrate concentration than in the later case (Wilson, 1993).

4.3 Shoot Growth Parameters

In this study different shoot growth parameters were considered and the analysis of variance (ANOVA) was performed, except for shoot sprouting, accordingly (Appendix Table 8-13). Results from the ANOVA therefore, are presented and discussed below.

4.3.1 Survival percent of cuttings

The analysis of variance for survival percent of cuttings indicated that the three way interaction and the main effect of EM were not significant ($P > 0.05$). However, noticeable significant interaction ($P \leq 0.05$) were observed for media and EM inoculation, media and cutting type and EM inoculation and cutting type (Appendix Table 8). The highest (93.06%) survival rate of cuttings was registered for rooting media composed of 6TS:3M: 0Ch:2S treated with EM, followed by the same media but without EM (90.28%) and those composed of 2Ts:1M: 1S + top 10cm Ss with and without EM and 2Ts:1M:1S + top 10cm 4Ts:1M:1Ch:1S, with EM (84.75-87.5%) (Table 5).

On the other hand, very low survival percent of cuttings were exhibited from rooting media mixes of 1Ts:1M: 1Ch:1S, 3Ts:1M: 2Ch:1S and 3Ts:2M: 1Ch:1S with average values ranging from 12.5 to 23.62%. When the same rooting media is concerned, survival percent of cuttings was not significantly affected by the application of EM. However, slight improvement in survival percent of cuttings was observed from EM inoculated media except in 1Ts:1M: 1Ch:1S, where the death rate of cuttings grown on EM treated media was high. In general, the effect of EM was found to be media specific and the observed differences among the treatments could be due to physico-chemical variation among the rooting media and the enhanced uptake of nutrients.

Table 5. Survival rate of stem cuttings of Arabica coffee hybrid as affected by rooting media and effective microorganisms inoculation

Media	EM	Survival percent
1Ts:1M:1Ch:1S	0	23.61(28.57) ^f
1Ts:1M:1Ch:1S	+	16.67(17.58) ^f
2Ts:1M:0Ch:2S	0	66.67(54.92) ^{de}
2Ts:1M:0Ch:2S	+	72.21(58.52) ^{cde}
2Ts:0M:1Cm:2S	0	58.33(49.85) ^e
2Ts:0M:1Cm:2S	+	65.28(53.92) ^e
3Ts:1M:2Ch:1S	0	13.89(15.88) ^f
3Ts:1M:2Ch:1S	+	15.28(16.75) ^f
3Ts:2M:1Ch:1S	0	12.5(14.89) ^f
3Ts:2M:1Ch:1S	+	15.28(16.7) ^f
6TS:3M:0Ch:2S	0	90.28(75.73) ^{ab}
6TS:3M:0Ch:2S	+	93.06(79.17) ^a
2Ts:1M:1S + top 10cm Ss	0	86.11(68.35) ^{a-d}
2Ts:1M:1S + top 10cm Ss	+	87.50(71.61) ^{abc}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	0	77.78(62.42) ^{b-e}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	+	84.72(68.94) ^{a-d}
	LSD 0.05	14.18
	CV (%)	13.23

EM1=without EM, EM2 with EM.

values in parenthesis are arcsine transformed values. Figures followed by the same letter are not significantly different.

Similarly, Percent survival was highly significantly affected by the interaction effect of media and cutting type (Appendix Table 8). The highest survival rate (97.22%) was registered for WN cuttings grown on rooting media composed of 6TS:3M: 0Ch:2S, followed by the same type of cutting propagated on 2Ts:1M:1S + top 10cm Ss (91.67%) which, however, was statistically similar with the values, 86.11 and 84.72 %, recorded for HN and WN cuttings grown on 6TS:3M:0Ch:2S and 2Ts:1M:1S + top 10cm 4Ts:1M:1Ch:1S, respectively (Table 6). On the other hand, complete death of HN cuttings was observed for rooting media composed of 3Ts:1M:2Ch:1S and 3Ts:2M:1Ch:1S, while low percent survival (8.33%) was obtained from the same type of cutting grown on 1Ts:1M:1Ch:1S medium. Similarly, WN cuttings also showed low percent survival mean values ranging from 27.78 to 29.17% when grown on rooting media where complete death of HN cuttings was exhibited. This indicates that the basic media types blended in such proportions are not suitable for rooting of stem cuttings of hybrid coffee Ababuna. Moreover, the variations between the two types of cuttings when grown on the same rooting media could be due to difference in their carbohydrate accumulation, as more accumulation in the WN cuttings supply more energy until they

develop new roots and shoots for the production of additional energy source through photosynthesis (Hartmann *et al.*, 1990; Al -Saqri and Alderson, 1996).

Table 6. Percent survival rate of stem cuttings of Arabica coffee hybrid as affected by interaction of different rooting media and cutting types

Media	Cutting types	Survival Rate (%)
1Ts:1M:1Ch:1S	WN	31.94(34.33)h
1Ts:1M:1Ch:1S	HN	8.33(11.81)i
2Ts:1M:0Ch:2S	WN	75(60.22)ef
2Ts:1M:0Ch:2S	HN	63.89(53.22)fg
2Ts:0M:1Cm:2S	WN	63.89(53.12)fg
2Ts:0M:1Cm:2S	HN	59.72(50.65)g
3Ts:1M:2Ch:1S	WN	29.17(32.63)h
3Ts:1M:2Ch:1S	HN	0.00(0.00)j
3Ts:2M:1Ch:1S	WN	27.78(31.59)h
3Ts:2M:1Ch:1S	HN	0.00(0.00)j
6TS:3M:0Ch:2S	WN	97.22(84.41)a
6TS:3M:0Ch:2S	HN	86.11(70.50)bc
2Ts:1M:1S + top 10cm Ss	WN	91.67(74.80)b
2Ts:1M:1S + top 10cm Ss	HN	81.94(65.16)cde
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	WN	84.72(69.28)bcd
2Ts:1M:1S + top 10cm Ss:1M:1Ch:1S	HN	77.78(62.08)ed
LSD (P<0.05)		8.07
CV (%)		13.23

Cutting type 1= whole node (WN), cutting type 2= half/splited node (HN),

Values in parenthesis are arcsine transformed values. Figures followed by the same letter are not significantly different

As far as the interaction effects of EM and cutting types is concerned, percent survival rate was highly significantly ($P \leq 0.05$) influenced by the combined effect of both factors (Appendix Table 8). The highest and significantly different ($P < 0.05$) percent survival rate (65.28%) was recorded for WN cuttings with EM inoculation (Table 7). This value was, however, statistically at par with the same type of cutting without EM treatment (60.07%). The lowest percent survival with similar values (47.22 %), on the other hand, was obtained from HN cuttings with and without EM inoculation. The significant variation observed between the two types of cuttings could probably be due to the difference in accumulation of carbohydrate. Increase in the respiration rate in creation of new “sink area” around the wounded site that stimulates cell division and production of root primordial require energy as described by Hartmann *et al.* (1997). Consequently, as compared to the WN cuttings, the low carbohydrate accumulation in the HN cuttings coupled with increased rate of respiration and

ethylene production in the wounded area could have led to early depletion of reserve energy and caused high death of cuttings prior to root and shoot initiation.

Table 7. Percent survival rate of stem cuttings of Arabica coffee hybrid as affected by interaction cutting types and effective microorganisms inoculation

Cutting types	EM	Survival Rate (%)
WN	0	60.07(52.38)ab
HN	0	47.22(40.28)b
WN	+	65.28(57.73)a
HN	+	47.22(38.08)b
LSD p<0.05		14.56
CV (%)		13.23

EM1= without EM, EM2 = with EM. *Cutting type 1= whole node (WN), cutting type 2= half/splited node (HN). values in parenthesis are arcsine transformed values. Figures followed by the same letter are not significantly different.*

4.3.2 Shoot sprouting

In this study, 100 % of shoot sprouting was recorded for both cutting types in all the rooting media both treated and not treated with EM except HN cuttings for which complete death of was recorded when propagated in rooting media composed of Ts:1M: 1Ch:1S with EM, 3Ts:1M: 2Ch:1S and 3Ts:2M: 1Ch:1S with and without EM (Table 8) . Therefore, analysis of variance for this parameter was not performed as it was observed no variation for shoot sprouting.

4.3.3 Shoot number

The effect of main factor and that of two and three way interactions was highly significant for number of shoot per cutting (Appendix Table 9). Different rooting media with and without EM inoculation had however, no effect on average number of shoots for all survived HN cuttings. Hence, HN cuttings were able to produce a single shoot per cutting since only on part of potential shoot developing buds were remained on this cutting type. Whereas, number of shoots per cutting, where whole node cutting is considered, was highly significantly ($P < 0.05$) affected by the interaction of the three factors considered in the study. Consequently, the highest average number of shoots per cutting (1.87) was recorded for WN cutting grown on 6TS:3M:0Ch:2S with and without EM (Table 8). This value,

however, was not statistically different from values recorded for the same type of cutting propagated in 1Ts:1M:1Ch:1S, 2Ts:1M:0Ch:2S, 2Ts:0M:1Ch:2S, 3Ts:1M:2Ch:1S, and 3Ts:2M:1Ch:1S both with and without EM and 2Ts:1M:1S + top 10cm Ss with EM inoculation. The lowest average number of shoots per WN cutting, on the other hand, was observed for rooting media composed of 3TS+1M+2CH+1S with value 1.58, followed by 1Ts:1M:1Ch:1S with EM and 3Ts:2M:1Ch:1S without EM. In general, EM application did not show considerable effect on shoot number of both cuttings in all treatments.

The most probable reason for the variation of shoot number per cutting is combined effect of cutting types, media blends and EM inoculation. The highest number of shoots was recorded for WN cuttings grown in rooting media composed of 6TS:3M: 0Ch:2S and other media mixes that showed statistically similar values with and without EM inoculation might be due to the physical and chemical properties of the media which is associated with the balance between nutrient supply as well as water holding capacity and adequate oxygen diffusion rate around the bases of cuttings (Gordon, 1992; Fonteno and Nelson, 1990; Caron *et al.*, 2000). The significant difference between the WN and HN cuttings could mainly be attributed to the variation in their nature, as the later contains only one bud from which shoot sprouting and subsequent growth could occur.

Table 8: Interaction effects of media, EM and cutting types on various shoot parameters of Arabica coffee hybrid

Media	EM	CT	ShSp (%)	ShN	ShL (cm)	ShG(mm)	LN	LA(cm ²)	ShFW(gm)
1Ts:1M:1Ch:1S (M1)	0	WN	100	1.78 ^{ab}	9.39 ^{bcd}	2.78 ⁱ⁻ⁿ	6.24 ^{tgh}	37.59 ^{d-g}	2.77 ^{efgh}
1Ts:1M:1Ch:1S	0	HN	100	1.00 ^e	9.06 ^{cde}	2.69 ^{k-n}	5.98 ^{gh}	23.83 ^{lm}	1.75 ^{ijkl}
1Ts:1M:1Ch:1S	+	WN	100	1.83 ^{ab}	9.56 ^{bcd}	2.77 ^{j-n}	7.12 ^{ef}	41.67 ^{a-e}	2.87 ^{e-h}
1Ts:1M:1Ch:1S	+	HN	0	0.00 ^f	0.00 ^l	0.00 ^p	0.00 ⁱ	0.00 ⁿ	0.00 ⁿ
2Ts:1M:0Ch:2S (M2)	0	WN	100	1.80 ^{ab}	9.27 ^{cde}	3.60 ^{d-g}	7.83 ^{de}	37.91 ^{c-g}	3.23 ^{cde}
2Ts:1M:0Ch:2S	0	HN	100	1.00 ^e	8.65 ^{d-g}	3.68 ^{c-f}	7.75 ^{de}	31.38 ^{hij}	1.90 ^{jk}
2Ts:1M:0Ch:2S	+	WN	100	1.80 ^{ab}	10.67 ^a	4.25 ^{ab}	10.56 ^{ab}	42.90 ^{ab}	3.60 ^{abc}
2Ts:1M:0Ch:2S	+	HN	100	1.00 ^e	9.15 ^{cde}	4.00 ^{a-d}	10.27 ^b	31.73 ^{hij}	2.40 ^{hi}
2Ts:0M:1Ch:2S (M3)	0	WN	100	1.73 ^{a-d}	8.94 ^{def}	2.65 ^{l-o}	5.92 ^h	39.09 ^{a-g}	3.07 ^{def}
2Ts:0M:1Ch:2S	0	HN	100	1.00 ^e	7.78 ^{g-j}	2.38 ^{no}	5.92 ^h	23.71 ^m	1.60 ^{kl}
2Ts:0M:1Ch:2S	+	WN	100	1.73 ^{a-d}	8.70 ^{d-g}	2.18 ^o	9.00 ^c	40.14 ^{a-f}	2.97 ^{d-g}
2Ts:0M:1Ch:2S	+	HN	100	1.00 ^e	8.21 ^{e-h}	2.47 ^{mno}	7.00 ^{efg}	30.36 ^{ijk}	1.80 ^{ijkl}
3Ts:1M:2Ch:1S (M4)	0	WN	100	1.70 ^{bcd}	9.46 ^{bcd}	2.93 ^{i-m}	8.37 ^{cd}	38.11 ^{b-g}	3.07 ^{def}
3Ts:1M:2Ch:1S	0	HN	0	0.00 ^f	0.00 ^l	0.00 ^p	0.00 ⁱ	0.00 ⁿ	0.00 ⁿ
3Ts:1M:2Ch:1S	+	WN	100	1.58 ^d	10.4 ^{ab}	2.78 ^{j-n}	8.49 ^{cd}	43.29 ^a	3.17 ^{c-f}
3Ts:1M:2Ch:1S	+	HN	0	0.00 ^f	0.00 ^l	0.00 ^p	0.00 ⁱ	0.00 ⁿ	0.00 ⁿ
3Ts:2M:1Ch:1S (M5)	0	WN	100	1.83 ^{ab}	9.34 ^{bcd}	2.87 ^{i-m}	6.52 ^{fgh}	40.27 ^{a-f}	3.17 ^{c-f}
3Ts:2M:1Ch:1S	0	HN	0	0.00 ^f	0.00 ^l	0.00 ^p	0.00 ⁱ	0.00 ⁿ	0.00 ⁿ
3Ts:2M:1Ch:1S	+	WN	100	1.76 ^{abc}	9.26 ^{cde}	3.01 ^{h-l}	7.99 ^{cde}	42.68 ^{abc}	3.57 ^{abc}
3Ts:2M:1Ch:1S	+	HN	0	0.00 ^f	0.00 ^l	0.00 ^p	0.00 ⁱ	0.00 ⁿ	0.00 ⁿ
6Ts:3M:0Ch:2S (M6)	0	WN	100	1.87 ^a	9.11 ^{cde}	3.46 ^{e-h}	7.63 ^{de}	37.05 ^{efg}	3.80 ^{ab}
6Ts:3M:0Ch:2S	0	HN	100	1.00 ^e	8.00 ^{d-g}	3.24 ^{f-j}	7.67 ^{de}	25.77 ^{klm}	2.07 ^{ijk}
6Ts:3M:0Ch:2S	+	WN	100	1.87 ^a	10.05 ^{abc}	4.39 ^a	11.43 ^a	42.37 ^{a-d}	4.03 ^a
6Ts:3M:0Ch:2S	+	HN	100	1.00 ^e	9.14 ^{cde}	4.12 ^{abc}	10.36 ^{ab}	35.7 ^{fgh}	2.70 ^{fgh}
2Ts:1M:1S + top 10cm Ss (M7)	0	WN	100	1.67 ^{bcd}	6.99 ^{ijk}	3.34 ^{f-i}	5.72 ^h	30.53 ^{ijk}	1.87 ^{jk}
2Ts:1M:1S + top 10cm Ss	0	HN	100	1.00 ^e	6.25 ^k	3.15 ^{g-k}	5.54 ^h	29.68 ^{ijk}	0.93 ^m
2Ts:1M:1S + top 10cm Ss	+	WN	100	1.80 ^{ab}	7.16 ^{h-k}	3.35 ^{f-i}	6.09 ^{fgh}	31.72 ^{hij}	2.10 ^{ij}
2Ts:1M:1S + top 10cm Ss	+	HN	100	1.00 ^e	6.72 ^{jk}	3.20 ^{f-j}	5.80 ^h	28.64 ^{ijkl}	1.33 ^{lm}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S (M8)	0	WN	100	1.60 ^{cd}	8.19 ^{e-h}	3.68 ^{c-f}	7.71 ^{de}	34.55 ^{hig}	3.43 ^{bcd}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	0	HN	100	1.00 ^e	7.71 ^{g-j}	3.90 ^{b-e}	7.16 ^{ef}	26.94 ^{j-m}	2.17 ^{ij}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	+	WN	100	1.67 ^{bcd}	9.30 ^{cd}	4.47 ^a	11.08 ^{ab}	40.14 ^{a-f}	3.87 ^{ab}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	+	HN	100	1.00 ^e	7.94 ^{f-i}	4.46 ^a	10.62 ^{ab}	29.33 ^{jk}	2.50 ^{ghi}
LSD 0.05				0.170	1.079	0.480	1.08	4.82	0.48
CV(%)				8.59	9.05	10.48	10.01	10.07	12.99

ShSp = shoot sprouting. ShN = shoot number. ShL = shoot length. ShG = shoot girth., LN= leaf numbers. LA=Leaf area, ShFW=shoot fresh weight. Values in the same column followed by the same letter are not significantly different.

4.3.4 Shoot length (cm)

Length of new shoots emerged from stem cuttings was very highly significantly ($P < 0.001$) influenced by the interaction of media, EM and cutting type (Appendix Table 10). Significant ($P \leq 0.05$) variations were observed among treatments, where the longest shoot length (10.67cm) was recorded for WN cuttings grown in EM treated rooting media composed of 2Ts:1M:0Ch:2S, followed by 3Ts:1M:2Ch:1S and 6TS:3M:0Ch:2S with average shoot length values of 10.4cm, and 10.05cm, respectively, for the same type of cutting (Table 8). Furthermore, survived HN cuttings grown on rooting media with EM showed statistically similar shoot length with that of WN cuttings grown on media with and without EM inoculation except for rooting media 2Ts:1M:0Ch:2S and 2Ts:1M:1S where average values recorded from the later was significantly higher for the parameter. The least value (6.25cm) was also recorded from half node cuttings grown on media composed of 2Ts:1M:1S + top 10cm Ss.

In the present study both cutting types grown on EM inoculated media showed better shoot length than those on rooting media without EM treatment. This could be due to the positive effect of EM application that causes accelerated development and increased growth in plants (Konoply and Higa, 2010). In addition, the physico-chemical nature of rooting media blends (Appendix Table 1) especially, those having low pH (2Ts:1M:0Ch:2S, and 6TS:3M:0Ch:2S) might have favored good rooting and shoot growth. On the other hand, the highest shoot growth recorded for 3Ts:1M:2Ch:1S combination could be due to relatively the high pH and percent N content that hastened the development of suckers at the expense of delayed root initiation and development.

This result is also in agreement with the findings of Hamid *et al.* (2006) who have observed in their study on effect of soil pH in rooting and growth of tea cuttings (*Camellia sinensis* L.) propagated in polythene sleeves filled with different level of acidic media and obtained the maximum shoot length, number of leaves and number of roots from the treatment combinations with relatively low pH reactions.

Similarly, Chagas *et al.* (1997) have compared the growth of rooted cuttings of Robusta coffee, cultivar conlin under two treatments (viz, sucks filled with usual soil mixes of the nursery with chemical fertilizer application and the other sucks filled with conventional soil + Bokashi and EM) and have found significant variation in number of pairs of leaves and plant height where the highest values were recorded from nursery media with Bokashi + EM treatment. Shah *et al.* (2001) have also reported maximum shoot length of maize recorded for crops treated with 25 t ha⁻¹ FYM + 30 t ha⁻¹ EM.

4.3.5 Shoot girth (mm)

Girth of newly emerged shoots from stem cuttings of Arabica coffee hybrid was very highly significantly ($P < 0.001$) influenced by the interaction of media, EM inoculation and cutting type (Appendix Table 11). There was significant ($P \leq 0.05$) variation for shoot girth among the treatments (Table 8). The highest average shoot girth with values of 4.47mm, and 4.46mm recorded for WN and HN cuttings grown in of 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S with EM, respectively. However, these values were statistically similar with the values recorded for WN cuttings propagated in rooting media mixture 6TS:3M:0Ch:2S (4.39mm), 2Ts:1M:0Ch:2S (4.25mm). Furthermore, HN cuttings also produced similar values to that of WN cuttings with values 4.12mm, 4.00mm when grown in rooting media 6TS:3M:0Ch:2S and 2Ts:1M:0Ch:2S with EM, respectively. The lowest shoot girth for both types of cuttings, with average values of 2.18mm to 2.65mm, were recorded for media mixture 2Ts:0M:1Ch:2S with and without EM.

Generally, shoot diameter recorded for both cutting types when grown on EM inoculated media was higher than the non treated ones, indicating that the effect of one factor resulted in positive synergy on the other factors. The difference for shoot girth however, was significantly high for cutting types grown on EM treated media mixes of 6TS:3M:0Ch:2S, 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S and 2Ts:1M:0Ch:2S. This may indicate that such media compositions are more suitable for EM activity in terms of physico-chemical properties. The increased shoot girth probably reflects balanced allocation of assimilates between root and shoot systems and availability of essential nutrients in the media and growth promoting substances produced by the EM which are suitable for the cuttings to develop vigorous shoots.

Chrispaul *et al.* (2010) obtained similar results from the study designed to evaluate the effect of inoculation of EM on growth and yield of pigweed where the thickest shoot was recorded for plants grown in a media blend of top soil, EM and organic manure. Teferi (2012) also obtained highest girth from hormone treated and cuttings with pair of leaves retained than leaves reduced to half. In their pot experiment to observe the effects of EM on the growth of Wisconsin Fast Plant (*Brassica rapa*) and on the microbial density in soil, Winget and Gold (2007) have also found the thickest diameter of stem from media treatment with EM bokashi plus EM solution than treatments with chemical fertilizer.

4.3.6 Leaf number

Total number of true leaves development on Arabica coffee stem cuttings was very highly significantly ($P < 0.001$) affected by the interaction of media, EM and cutting type (Appendix Table 12). The highest average number of true leaves with values ranging from 10.27 to 11.43 per stem cuttings was counted for the two types of cuttings grown on media composed of 6TS:3M: 0Ch:2S, 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S and 2Ts:1M:0Ch:2S with EM. On the other hand, the least values of 5.54 and 5.72 were recorded for HN and WN cuttings, respectively, grown on 2Ts:1M:1S + top 10cm Ss without EM (Table 8).

This could be due to the positive effect of EM application that causes accelerated development and increased growth in propagules as it was observe in other plants (Konoply and Higa, 2010) and the suitability of the media in supplying essential nutrients and micro climates around the root zone which might have favored good root and shoot growth. This is further reflected by longest shoot growth recorded for both types of cuttings when propagated on 6TS:3M: 0Ch:2S, 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S and 2Ts:1M:0Ch:2S with EM, where large number of leaves are expected along the shoot length.

Similarly, Chagas *et al.* (1997) have compared the growth of rooted cuttings of Robusta coffee, cultivar conlin, under two treatments (viz, sucks filled with usual soil mixes of the nursery with chemical fertilizer application and the other sucks filled with conventional soil + Bokashi and EM) and found significant variation in average number of leaves and plant height where the

highest values were recorded from nursery media with Bokashi + EM treatment. The result of the present study is also in agreement with research works reported by Crispaul *et al.* (2010) who have observed the highest number of leaves on pigweed grown on a media mixes of top soil and organic manure inoculated with EM.

4.3.7 Leaf area (cm²)

Total leaf area was very highly significantly ($P < 0.001$) influenced by interaction of media, EM inoculation and cutting types (Appendix Table 13). Significantly ($P \leq 0.05$) the largest average leaf area (43.29cm²) per stem cuttings was registered for WN cutting type when grown on rooting media composed of 3Ts:1M:2Ch:1S with EM. However, this value was statistically at par with the values recorded for the same type of cutting grown on most rooting media treated with EM, except for 2Ts:1M:1S + top 10cm Ss mixture (Table 8). Similarly, HN cuttings grown on EM inoculated rooting media 6Ts:3M:0Ch:2S produced significantly larger total leaf area than those grown on most rooting media with or without EM except on 2Ts:1M:0Ch:2S where no difference was observed. The lowest value of leaf area per stem cuttings (23.72 cm²) was also recorded for HN cuttings when grown in 2Ts:0M:1Ch:2S, without EM inoculation. .

Larger leaf area produced by survived cuttings when grown in EM inoculated media, except for 2Ts:1M:1S + top 10cm Ss mixture, could probably be to improved physical and chemical conditions of the rooting media, synthesis of vitamins glucose, growth regulators and amino acids by the EM and the enhanced rate of absorption of such substances by the cuttings promoting growth with increased cell division, chlorophyll formation and photosynthetic rate as compared to those untreated with EM.

This result is in line with the findings reported by Crispaul *et al.* (2010) who observed larger leaf area production from pigweed (*Amarantus dubians*) when grown on media blends of top soil, EM and organic manure. The difference revealed between the two types of cuttings could be attributed to the variation in their size and the number of leaves retained on them. The larger leaf area produced by WN cutting could be due to a higher total carbohydrate content and pair of leaves retained on it or it could be due to the accumulation of natural auxins in the buds, as

developing buds and leaves are considered primary sources for endogenous auxin synthesis (Blakestey *et al.*, 1991b; Liu and Reid, 1992)

4.3.8 Shoot fresh weight (g)

Shoot fresh weight was also very highly significantly ($P < 0.001$) affected by the interaction of the three factors (Appendix Table 14). There was a significant variation among the factor combinations for fresh weight of new shoots (Table 8). Accordingly, the highest mean shoot fresh weight (4.033gm) was recorded for WN cuttings propagated in rooting media mixes of 6TS:3M:0Ch:2S with EM inoculation. However, this value was statistically similar with the values (3.867 and 3.60) recorded for the same cutting type grown in 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S and 2Ts:1M:0Ch:2S media compositions with and 6TS:3M:0Ch:2S without EM. The lowest values (0.93 and 1.33), on the other hand, were recorded for HN cuttings grown in a 2Ts:1M:1S + top 10cm Ss without and with EM inoculation, respectively.

The performance of the two types of survived cuttings, in general, was better on EM treated media especially where good rooting percentage was observed. This could be explained by the enhanced uptake of nutrient, water and substances produced by the EM and balanced partitioning of total dry mater among newly growing root and shoot parts. This result is in agreement with findings of Crisppaul *et al.* (2010) who have reported that plants inoculated with EM manure recorded highest fresh weight than those without EM manure. The lower shoot fresh weight recorded for HN cuttings as compared to the WN cuttings was probably due to the variation in moisture content and number of shoots between the two types where the HN produced only single shoot as compared to the later that produced more than one mean number of shoots per cutting and consequently contained high water content inside the shoot system.

4.4 Dry Matter Production and Partitioning

4.4.1 Shoot dry weight (g)

Like in the case of other shoot parameters mentioned above, a very highly significant interaction ($P < 0.001$) was observed among the three factors for shoot dry weight (Appendix Table 15). There was highly significant ($P < 0.05$) differences in shoot dry weight per cutting among treatments (Table 9). The highest value (0.907g) was recorded for WN cuttings grown in 6TS:3M: 0Ch:2S media blends with EM. This value, however, was statistically at par with that (0.85g) recorded for the same type of cutting propagated in EM treated 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S mixes and in a 6TS:3M: 0Ch:2S without EM treatment (0.833g). Half-node cuttings grown in 2Ts:1M:1S + top 10cm Ss with and without showed the lowest shoot dry weight per stem cuttings, with average value of 0.19 and 0.29gm, respectively. In general, EM application did not show pronounced effect on shoot dry weight. However, both cutting types grown on most EM treated rooting media had exhibited better shoot dry weight than those grown without EM inoculation. This could be explained by the positive synergy of the factor combination, where inoculation of EM can increase the available nutrition for newly growing roots and improve photosynthesis and the improved physical and chemical properties of the rooting media favored better root growth and nutrient absorption and translocation it to the site of photosynthesis which increased the net carbon assimilation in the shoot system (Crisppaul *et al.*, 2010).

On the other hand, the lowest values registered for rooting media composed of 2Ts:1M:1S + top 10cm Ss could probably be due to early root initiation and grow that may cause delay in shoot initiation and development or accelerated translocation of assimilates from the upper part of the cuttings to their basal ends by increasing the activity of enzymes (Blazich, 1988). This condition was reflected by low values of majority of the shoot parameters measured for this rooting media.

4.4.2 Root dry weight (g)

In the present study, root dry weight was highly significantly ($P < 0.001$) influenced by the main factors and both two-way and three-way interactions (Appendix Table 16). Accordingly, the highest dry weight of roots (0.673g) was recorded for WN cuttings propagated in 6TS:3M:0Ch:2S rooting media with EM, followed by the same type of cutting grown in 2Ts:1M:0Ch:2S (0.62 g) treated with EM (Table 9). The lowest root dry weight (0.25 g), on the other hand, was recorded for HN cuttings type planted in media composed of 2Ts:0M: 1Ch:2S. Similarly, WN cutting type grown on the same media also produced the lowest root dry weight (0.293 g). Nevertheless, HN cuttings propagated in EM treated media produced better root dry weight than those grown on rooting media without EM application.

In general, alike shoot dry weight, EM application did not show pronounced effect on shoot dry weight. However, both cutting types grown on most EM treated rooting media had exhibited better shoot dry weight than those grown without EM inoculation. Thus, high values for root dry weight indicated that assimilated carbon in dry bases was better for cuttings grown on EM treated media than without (Burdett, 1990).

Results of the present study was quite in agreement with the work of Kymac *et al.* (2008), who reported that rooting performance, root length and dry matter contents of roots of cuttings of mint treated with bacterial solutions showed better performance than the control. The findings of Chrispaul *et al.* (2010) have also indicated that dry weight of pig weed (*Amaranthus dubias*) was highest in soil without organic manure but inoculated with effective microorganisms.

4.4.3 Root to shoot dry weight ratio

Root to shoot ratio was highly influenced by three-way interaction of both factors (Appendix Table 17). Significant ($P \leq 0.05$) difference was observed among treatments, where the highest value (2.47) was recorded for HN cuttings grown in 2Ts:1M:1S + top 10cm Ss media without EM followed by value (1.66), recorded for the same cutting type grown in the same media with EM (Table 9). Similarly, the WN cuttings propagated on this media, with or without EM,

exhibited better root to shoot ration with no significant difference. The lowest value (0.43), however, recorded for WN cutting type grown in rooting media composed of 2Ts:0M:1Ch:2S without EM. Farther more, both cuttings exhibited balanced and better root to shoot ratio when grown in rooting media composed of 6TS:3M:0Ch:2S and 2Ts:1M:1S + top 10cm 4Ts:1M:1Ch:1S with or without EM.

In addition, root to shoot ratio for HN cuttings was better than that of WN cuttings in all treatments except in treatments where complete death of cuttings observed. The probable reason for more root to shoot ratio observed in rooting media 2Ts:1M:1S + top 10cm Ss could be due to biomass partitioning is typically more directed to root organs and remained in dry form than shoot. This was manifested by low values recorded for majority of shoot variables from this rooting media with or without EM.

Balanced root to shoot ration observed for both types of cuttings grown in rooting media composed of 6TS:3M:0Ch:2S and 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S, with or without EM could be due to equal partitioning of biomass to root organs and shoot organs. Thus, the result indicated that the suitability of these rooting media to produce high quality plating material with the right proportion of root and shoot growth that can insure maximum field establishment.

Results of the present investigation was in agreement with the work of Teferi (2012), who reported that cuttings from Arabica coffee hybrids exhibited better root to shoot ratio when treated with rooting hormone (IBA) than the control treatment.

Table 9: Dry mater production and partitioning as affected by interaction effect of media, EM and cutting types

Media	EM	CT	Rdw (g)	Shdw (g)	R : Sh	Tdmy (g)
1Ts:1M:1Ch:1S (M1)	0	WN	0.403 ^{ij}	0.600 ^{hij}	0.68 ^{ghij}	1.003 ^h
1Ts:1M:1Ch:1S	0	HN	0.390 ^{ik}	0.330 ^{op}	1.18 ^{cd}	0.720 ^l
1Ts:1M:1Ch:1S	+	WN	0.437 ^{ghi}	0.62 ^{ghi}	0.70 ^{f-i}	1.063 ^{gh}
1Ts:1M:1Ch:1S	+	HN	0.000 ^o	0.000 ^r	0.00 ^k	0.000 ⁿ
2Ts:1M:0Ch:2S (M2)	0	WN	0.430 ^{hi}	0.703 ^{defg}	0.62 ^{hij}	1.133 ^{fg}
2Ts:1M:0Ch:2S	0	HN	0.427 ^{hij}	0.417 ^{mno}	1.03 ^{de}	0.843 ^{jk}
2Ts:1M:0Ch:2S	+	WN	0.620 ^b	0.790 ^{bcd}	0.79 ^{e-h}	1.410 ^b
2Ts:1M:0Ch:2S	+	HN	0.473 ^{efg}	0.527 ^{kl}	0.91 ^{efg}	1.000 ^h
2Ts:0M:1Ch:2S (M3)	0	WN	0.293 ^m	0.673 ^{e-h}	0.43 ^l	0.967 ^{hi}
2Ts:0M:1Ch:2S	0	HN	0.250 ⁿ	0.363 ^{nop}	0.71 ^{f-i}	0.613 ^m
2Ts:0M:1Ch:2S	+	WN	0.353 ^{kl}	0.653 ^{fgh}	0.54 ^{ij}	1.007 ^h
2Ts:0M:1Ch:2S	+	HN	0.337 ^l	0.390 ^{mno}	0.86 ^{efg}	0.727 ^l
3Ts:1M:2Ch:1S (M4)	0	WN	0.500 ^{ef}	0.670 ^{e-h}	0.74 ^{f-i}	1.177 ^{ef}
3Ts:1M:2Ch:1S	0	HN	0.000 ^o	0.000 ^r	0.00 ^k	0.000 ⁿ
3Ts:1M:2Ch:1S	+	WN	0.540 ^{cd}	0.707 ^{d-g}	0.77 ^{f-i}	1.247 ^{de}
3Ts:1M:2Ch:1S	+	HN	0.000 ^o	0.000 ^r	0.00 ^k	0.000 ⁿ
3Ts:2M:1Ch:1S (M5)	0	WN	0.503 ^{de}	0.687 ^{e-h}	0.74 ^{f-i}	1.190 ^{def}
3Ts:2M:1Ch:1S	0	HN	0.000 ^o	0.000 ^r	0.00 ^k	0.000 ⁿ
3Ts:2M:1Ch:1S	+	WN	0.540 ^{cd}	0.7433 ^{c-f}	0.73 ^{f-i}	1.283 ^{cd}
3Ts:2M:1Ch:1S	+	HN	0.000 ^o	0.000 ^r	0.00 ^k	0.000 ⁿ
6Ts:3M:0Ch:2S (M6)	0	WN	0.610 ^b	0.833 ^{abc}	0.73 ^{f-i}	1.443 ^b
6Ts:3M:0Ch:2S	0	HN	0.420 ^{ij}	0.453 ^{k-n}	0.94 ^{def}	0.873 ^{ij}
6Ts:3M:0Ch:2S	+	WN	0.673 ^a	0.907 ^a	0.74 ^{f-i}	1.580 ^a
6Ts:3M:0Ch:2S	+	HN	0.543 ^c	0.593 ^{hij}	0.92 ^{efg}	1.137 ^g
2Ts:1M:1S + top 10cm Ss (M7)	0	WN	0.583 ^b	0.437 ^{lmn}	1.34 ^c	1.020 ^h
2Ts:1M:1S + top 10cm Ss	0	HN	0.473 ^{efg}	0.193 ^q	2.47 ^a	0.667 ^{lm}
2Ts:1M:1S + top 10cm Ss	+	WN	0.603 ^b	0.460 ^{klm}	1.33 ^c	1.063 ^{gh}
2Ts:1M:1S + top 10cm Ss	+	HN	0.463 ^{fgh}	0.293 ^p	1.66 ^b	0.757 ^{lk}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S (M8)	0	WN	0.603 ^b	0.753 ^{cde}	0.82 ^{e-h}	1.357 ^{bc}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	0	HN	0.403 ^{ij}	0.483 ^{klm}	0.85 ^{e-h}	0.887 ^{ij}
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	+	WN	0.593 ^b	0.850 ^{ab}	0.70 ^{f-i}	1.443 ^b
2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S	+	HN	0.42 ^{hij}	0.543 ^{ijk}	0.82 ^{e-h}	0.970 ^{hi}
LSD 0.05			0.039	0.094	0.25	0.102
CV (%)			5.96	11.76	19.58	7.00

+, - = with and without EM respectively. CT = cutting type. Rdw = root dry weight Shdw = shoot dry weight R : Sh = root to shoot ratio., Tdmy = total dry matter yield. Values in the same column followed by the same letter are not significantly different.

4.4.4 Total dry matter yield (g)

Average total dry matter yield was highly influenced by the interaction of media, EM inoculation and cutting types (Appendix Table 18). Significant ($P \leq 0.05$) differences were observed among treatments, where the highest value (1.58g) was recorded for WN cuttings grown in 6TS:3M: 0Ch:2S media treated with EM followed by values (1.443 g) recorded for the same cutting type grown in EM treated media mixes of 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S, which, however, was statistically at par with values recorded for whole node cuttings propagated on 6TS:3M:0Ch:2S and 2Ts:1M:0Ch:2S without and with EM inoculation, respectively (Table 9). The lowest values 0.613gm and 0,667gm, on the other hand, were exhibited by HN cuttings propagated in media mixes of 2Ts:0M:1Ch:2S and 2Ts:1M:1S + top 10cm Ss without EM inoculation, respectively. However, the highest values (1,137gm and 0.97gm) of total biomass of HN cuttings were obtained from rooting media composed of 6TS:3M:0Ch:2S and 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S treated with EM, respectively.

Similar to other dry matter parameters, application of EM did not show pronounced effect on total dry matter yield production of cuttings when each rotting media is considered separately except on 6TS:3M:0Ch:2S. Both cutting types grown on EM inoculated media, however, was found to be better in dry matter content than without EM. Increased total dry matter observed for both types of cuttings when propagated on rooting media composed of 6TS:3M: 0Ch:2S, with or without EM, 2Ts:1M:1S+top 10cm 4Ss:1M:1Ch:1S, indicated their suitability for rooting of stem cuttings of hybrid coffee to produce high quality plating material with the right proportion of root and shoot growth that can insure maximum field establishment.

The highest values recorded for total dry matter yield could be due to production of high performance of cuttings with regard to both root and shoot parameters in rooting media mentioned above (Tables 4, 5 and 6). The highest values for these parameters, in turn, could be attributed to better absorption of nutrients and water from the rooting media, production of more carbohydrates due to increased photosynthetic rate and assimilation of the phtosynthets in to the newly growing organs, contributed to higher dry matter yield. This result is in line

the findings of Taye *et al.* (1997), who reported that blended nursery media and water application at moderate intervals favor desired root and shoot vegetative growth with higher total dry matter yield of coffee seedlings at nursery. Tesfaye and Berga (1997) have also reported similar findings with respect to production of high quality transplantable coffee seedlings during the nursery period.

4.5 Correlation Analysis among Root and Shoot Variables

Results from correlation analysis showed positive and highly significant ($P \leq 0.05$) association among all growth parameters except shoot fresh and dry weight with root to shoot ratio for which weak and non significant correlation (0.17) was observed (Appendix Table 19). Percent rooting was highly correlated with root length, root girth, root number and shoot girth with values 0.94, 0.91, 0.79 and 0.83, respectively. This indicates that more emphasis might be given to these characteristics when evaluating growth parameters for vegetative propagation of hybrid Arabica coffee varieties. Similar result was also reported by Mawardi and Purwadi (2004) who observed high heritability of percent rooting and total root length in their study on genetics of rooting and sprouting ability on cuttings propagation of Robusta coffee clones. Similarly, the remaining shoot and root growth parameters including dry matter production and partitioning had showed 0.55 – 0.75 degrees of association with rooting percent except for root volume and root fresh weight for which the lowest association, 0.32 and 0.43 respectively was recorded. On the other hand, root dry weight, shoot, girth, total dry matter yield, shoot sprouting, and shoot length had also showed higher degree of association with most root and shoot parameters. Therefore, it is more important to focus on the above mentioned parameters during evaluation of hybrid coffee varieties for vegetative propagation by stem cutting.

5 SUMMARY AND CONCLUSIONS

Ethiopia is the primary center of origin and genetic diversity for Arabic coffee (*Coffea arabica* L.) and it is the backbone of the economy and takes the lion's share of the foreign exchange earning of the country. Considering its vital role in the economy, improved coffee varieties with full package agronomic recommendations were released and disseminated to producers in different coffee growing agroecologies.

Among the released coffee varieties, the dissemination of the three hybrids viz, Aba-buna, Melko CH2 and Gawe, as compared to the pure line coffee varieties, was found to be very low mainly due to limitations in supplying adequate seed or seedlings for planting. Among others, method of propagation of the varieties have been identified as major bottleneck to the production of sufficient hybrid seeds thus hindering the rapid multiplication, distribution and adoption of the varieties for commercial production under our condition.

To fill the gap between the demand and supply for the planting material the present study was initiated with objectives to determine best potting media, suitable cutting type and evaluate effective microorganisms (EM) application that would help in establishing effective, feasible and low cost vegetative propagation options by evaluating rooting ability and other root and shoot growth parameters of stem cuttings of hybrid coffee variety aba-buna using 8x2x2 factorial combinations of the three factors (media, EM, and cutting type) laid in RCBD with three replications.

The result of the experiment showed that rooting ability and most root and shoot growth parameters of stem cuttings were significantly ($P \leq 0.05$) influenced by three-way interaction except survival rate and root fresh weight. It was observed that whole node (WN) cuttings grown on rooting media mixes of 6Ts:3M:0Ch:2S, with EM, and 2Ts:1M:1S + top 10cm Ss, with or without EM, had rooted (88.89%) better than most of the treatments. Half node (HN) cuttings propagated in rooting media composed of 6Ts:3M: 0Ch:2S with and without EM, had also exhibited 83.33 % rooting. In general, both cutting types grown in rooting media 6Ts:3M:0Ch:2S, 2Ts:1M:1S + top 10cm Ss and 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S, both

with or without EM inoculation had showed significantly higher rooting (72.22 to 88.89%) performance as compared to the others factor combinations. In addition, 100 % shoot sprouting was recorded for all survived cutting types grown in rooting media with or without EM inoculation.

Among the rooting media where better rooting was obtained, highest values of shoot girth, leaf number, leaf area, root length, root girth, root fresh weight, root & shoot dry weight, percent survival rate and TDM per cutting with balanced root to shoot ratio were observed for both cutting types propagated in rooting media composed of 6Ts:3M:0Ch:2S (conventional nursery media used to raise coffee seedlings from seed) with EM inoculation. Similarly, both cutting types propagated on rooting media composed of 6Ts:3M: 0Ch:2S without EM, 2Ts:1M: 1S + top 10cm Ss and 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S, with or without EM, had exhibited comparable performance with that of EM treated media mixes of 6Ts:3M:0Ch:2S for root number, root length and root girth. In addition, both cutting types propagated on rooting media composed of 2Ts:1M:1S + top 10cm Ss, with or without EM, exhibited high root to shoot ratio (in dry bases) than other treatments. On the other hand, complete death and very low percent survival was recorded for HN and WN cuttings respectively, propagated on rooting media compositions 1Ts:1M: 1Ch:1S 3Ts:1M: 2Ch:1S and 3Ts:2M: 1Ch:1S, with and without.

The results obtained from present study have therefore, indicated that vegetative production of hybrid coffee (Ababuna) could be successfully attained by use of both whole and half/splited soft wood stem cuttings directly inserting in the potted rooting media. Accordingly, 26% increase of field transplantable rooted propagules, as compared to the findings (63 %) of earlier works of JARC, was observed for WN cuttings from the present study. The results had also revealed that comparable rooting potential of HN with that of WN cuttings and options to get over 100 percent increase in supply of planting material as compared to the results (63 %) obtained for WN cuttings from previous findings in Ethiopia.

In general, the results obtained from the present study, imply that both cuttings are equally good in their rooting ability provided that suitable rooting media and propagation conditions

are used. Significantly higher or relatively better performance for most shoot and root growth parameters were recorded for both cutting types when propagated on rooting media composed of 6Ts:3M:0Ch:2S treated with EM. Similarly, significantly higher percent of rooting for both cutting types was recorded from rooting media mixes of 6Ts:3M:0Ch:2S, 2Ts:1M:1S + top 10cm 4Ss:1M:1Ch:1S and 2Ts:1M:1S + top 10cm Ss, with or without EM. This indicates possible options for vegetative propagation of hybrid coffee (Ababuna) by rooting either whole or half (splited) node soft wood stem cuttings. Furthermore, considering survived cuttings pronounced effect of EM treatment was observed for root and shoot growth parameters rather than percent rooting though its application had showed slight improvement in most treatments except in 2Ts:1M:1S + top 10cm Ss where similar values were recorded for both cutting types grown with or without EM treatment.

Therefore, depending up on number of propagules required for planting, both whole and half node cutting types could be recommended for vegetative propagation of hybrid coffee (Ababuna). Similarly, to obtain high number of rooted cuttings coupled with superior performance of both shoot and root growth & development, directly inserting the cuttings in the poly bags filled with conventional coffee nursery media (6Ts:3M:0Ch:2S) inoculated with EM could be recommended for efficient vegetative propagation of hybrid coffee (Ababuna). In addition, if there is shortage of resource to purchase sand and limitation in sustainable supply of EM, the conventional nursery media and 2Ts:1M:1S + top 10cm Ss, both without EM, could also be recommended as an alternative potting media for propagation of both cutting types. Moreover, non-mist propagation system that can be located anywhere and easily adopted by both large and small scale coffee producers could be used to reduce cost of production for multiplication of the hybrid coffee.

However, it was suggested that further investigations, focusing on different concentrations and methods of application of EM and evaluating hybrid coffee varieties (Melko CH2 and Gawe) be included in future study so as to come up with comprehensive conclusion and recommendation.

Future directions

The present work provided information on low cost option for both the propagation system, the types of stem cuttings to be used, effects of EM, rooting media options and the rooting ability of hybrid coffee (Ababuna) cuttings directly inserted in potted rooting media for production of sufficient and true-to-type planting materials for coffee farmers. However, the following gaps need to be taken in to consideration as future line of work.

1. Evaluation of different concentrations and methods of application of EM.
2. Investigation of rooting potential for the hybrid varieties not included in the present study.
3. Evaluation of rooting response of cuttings harvested during dry and wet seasons.
4. Compost of coffee husk fermented with effective microorganisms is also another area of focus to be evaluated for rooting of hybrid coffee cuttings.

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7 APPENDICES

Appendix Table 1: Physical and chemical properties of different rooting media used in the present study

Rooting media	Chemical properties					Physical properties			
	pH	% OC	% OM	P (ppm)	% N	K(meq/100gm)	B.D	Tex.	W.H.C
1Ts:1M:1Ch:1S (M1)	6.45	9.91	17.09	417.24	0.56	14.07	1.51	Sl	40.34
2Ts:1M:0Ch:2S (M2)	5.7	4.37	7.53	177.50	0.36	1.92	1.51	Sl	29.97
2Ts:0M:1Ch:2S (M3)	6.1	4.51	7.78	184.00	0.35	3.84	1.48	Sl	32.18
3Ts:1M:2Ch:1S (M4)	6.33	12.97	22.36	336.87	0.61	5.43	1.48	Sl	46.37
3Ts:2M:1Ch:1S (M5)	6.15	14.29	24.64	307.46	0.60	6.39	1.47	Scl	43.88
6TS:3M:0Ch:2S (M6)	5.87	6.29	10.85	209.65	0.53	1.28	1.43	Scl	40.38
Sub soil	4.55	1.80	3.10	2.39	0.12	0.38	1.31	Sc	32.24
2Ts:1M:1S (M7) (<i>bottom</i>)	5.82	5.97	10.29	200.33	0.48	0.97	1.48	Sl	36.99
4Ss:1M:1Ch:1S (M8) (<i>top</i>)	5.7	4.41	7.60	191.52	0.35	1.60	1.34	Sc	36.00

OC=organic carbon, OM=organic matter, P=phosphorus, N=nitrogen, K=potassium, B.D =bulk density, Tex=texture, W.H.C=water holding capacity

Appendix Table 2: Analysis of variance for rooting ability of stem cuttings of hybrid coffee variety Ababuna

Source	DF	SS	MS	F Value	Pr > F
Rep	2	24.71823	12.35912	0.76	0.4707
Media	7	50177.71	7168.244	442.35	<.0001
Em	1	57.84615	57.84615	3.57	0.0635
Cutting	1	2654.827	2654.827	163.83	<.0001
Media*Em	7	392.6459	56.09227	3.46	0.0034
Media*Cutting	7	1796.971	256.7102	15.84	<.0001
Em*Cutting	1	156.8771	156.8771	9.68	0.0028
Media*Em*Cutting	7	268.0833	38.29761	2.36	0.0331
Error	62	1004.711	16.20502		
Total	95	56534.39			

Appendix Table3: Analysis of variance for root fresh weight (g) per cutting of hybrid coffee variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	2.84489	1.422445	1.32	0.2732
Media	7	22.7722	3.253171	3.03	0.0083
Em	1	1.876004	1.876004	1.75	0.1911
Cutting	1	3.124817	3.124817	2.91	0.093
Media*Em	7	8.073996	1.153428	1.07	0.3905
Media*Cutting	7	13.98048	1.997212	1.86	0.0916
Em*Cutting	1	0.372504	0.372504	0.35	0.558
Media*Em*Cutting	7	9.390796	1.341542	1.25	0.2901
Error	62	66.56024	1.073552		
Total	95	128.9959			

Appendix Table 4: Analysis of variance for root number per cutting of hybrid coffee variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	0.208125	0.104063	1.01	0.3715
Media	7	41.5499	5.935699	57.4	<.0001
Em	1	0.055104	0.055104	0.53	0.4682
Cutting	1	28.71094	28.71094	277.62	<.0001
Media*Em	7	1.234063	0.176295	1.7	0.1244
Media*Cutting	7	7.191563	1.027366	9.93	<.0001
Em*Cutting	1	0.525104	0.525104	5.08	0.0278
Media*Em*Cutting	7	2.217396	0.316771	3.06	0.0078
Error	62	6.411875	0.103417		
Total	95	88.10406			

Appendix Table 5: Analysis of variance for root length (cm) per cutting of hybrid coffee variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	0.621458	0.310729	0.76	0.472
Media	7	1230.038	175.7197	429.74	<.0001
Em	1	16.83375	16.83375	41.17	<.0001
Cutting	1	192.1004	192.1004	469.8	<.0001
Media*Em	7	52.72458	7.532083	18.42	<.0001
Media*Cutting	7	188.1313	26.87589	65.73	<.0001
Em*Cutting	1	7.820417	7.820417	19.13	<.0001
Media*Em*Cutting	7	27.14792	3.878274	9.48	<.0001
Error	62	25.35188	0.408901		
Total	95	1740.77			

Appendix Table 6: Analysis of variance for root girth (mm) per cutting of hybrid coffee variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	0.043502	0.021751	2.18	0.122
Media	7	78.75197	11.25028	1125.7	<.0001
Em	1	0.072051	0.072051	7.21	0.0093
Cutting	1	10.75351	10.75351	1075.99	<.0001
Media*Em	7	2.127974	0.303996	30.42	<.0001
Media*Cutting	7	12.67572	1.810817	181.19	<.0001
Em*Cutting	1	0.259376	0.259376	25.95	<.0001
Media*Em*Cutting	7	1.294916	0.184988	18.51	<.0001
Error	62	0.619631	0.009994		
Total	95	106.5986			

Appendix Table 7: Analysis of variance for root volume (ml) per cutting of hybrid coffee
variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	0.090052	0.045026	1.37	0.2607
Media	7	2.034141	0.290592	8.87	<.0001
Em	1	1.940859	1.940859	59.23	<.0001
Cutting	1	18.5944	18.5944	567.46	<.0001
Media*Em	7	0.828516	0.118359	3.61	0.0025
Media*Cutting	7	9.696641	1.385234	42.27	<.0001
Em*Cutting	1	2.297109	2.297109	70.1	<.0001
Media*Em*Cutting	7	1.557266	0.222467	6.79	<.0001
Error	62	2.031615	0.032768		
Total	95	39.0706			

Appendix Table 8: Analysis of variance for percent survival of stem cuttings of hybrid coffee
variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	115.1534	57.5767	1.48	0.2351
Media	7	52925.22	7560.746	194.61	<.0001
Em	1	59.3776	59.3776	1.53	0.221
Cutting	1	6043.931	6043.931	155.56	<.0001
Media*Em	7	598.3795	85.48279	2.2	0.0462
Media*Cutting	7	2845.208	406.4583	10.46	<.0001
Em*Cutting	1	340.9588	340.9588	8.78	0.0043
Media*Em*Cutting	7	424.1408	60.59154	1.56	0.1644
Error	62	2408.805	38.85169		
Total	95	65761.17			

Appendix Table 9: Analysis of variance for shoot number per cuttings of hybrid coffee variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	0.01354	0.00677	0.62	0.5425
Media	7	4.784691	0.683527	62.36	<.0001
Em	1	0.082251	0.082251	7.5	0.008
Cutting	1	27.1469	27.1469	2476.64	<.0001
Media*Em	7	0.620591	0.088656	8.09	<.0001
Media*Cutting	7	4.313441	0.616206	56.22	<.0001
Em*Cutting	1	0.106001	0.106001	9.67	0.0028
Media*Em*Cutting	7	0.756841	0.10812	9.86	<.0001
Error	62	0.679594	0.010961		
Total	95	38.50385			

Appendix Table 10: Analysis of variance for shoot length (cm) per cuttings of hybrid coffee variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	1.232965	0.616482	1.4	0.2551
Media	7	279.4315	39.91878	90.43	<.0001
Em	1	0.573504	0.573504	1.3	0.2587
Cutting	1	300.1215	300.1215	679.9	<.0001
Media*Em	7	65.47175	9.353107	21.19	<.0001
Media*Cutting	7	338.9444	48.42063	109.69	<.0001
Em*Cutting	1	11.9992	11.9992	27.18	<.0001
Media*Em*Cutting	7	54.30378	7.757683	17.57	<.0001
Error	62	27.3681	0.441421		
Total	95	1079.447			

Appendix Table 11: Analysis of variance for shoot girth (mm) per cuttings of hybrid coffee variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	0.23664	0.11832	1.37	0.2621
Media	7	101.8883	14.55547	168.33	<.0001
Em	1	0.118301	0.118301	1.37	0.2466
Cutting	1	21.68851	21.68851	250.82	<.0001
Media*Em	7	10.04604	1.435149	16.6	<.0001
Media*Cutting	7	35.17307	5.024724	58.11	<.0001
Em*Cutting	1	0.671676	0.671676	7.77	0.0071
Media*Em*Cutting	7	5.099566	0.728509	8.42	<.0001
Error	62	5.361227	0.086471		
Total	95	180.2833			

Appendix Table 12: Analysis of variance for leaf number per cutting of hybrid coffee variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	0.383108	0.191554	0.44	0.6482
Media	7	459.6862	65.66946	149.67	<.0001
Em	1	36.96443	36.96443	84.25	<.0001
Cutting	1	178.5149	178.5149	406.85	<.0001
Media*Em	7	84.82152	12.11736	27.62	<.0001
Media*Cutting	7	238.423	34.06043	77.63	<.0001
Em*Cutting	1	13.0169	13.0169	29.67	<.0001
Media*Em*Cutting	7	27.86947	3.981353	9.07	<.0001
Error	62	27.20383	0.438771		
Total	95	1066.883			

Appendix Table 13: Analysis of variance for leaf area (cm²) per cutting of hybrid coffee variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	41.24851	20.62425	2.37	0.1015
Media	7	3292.194	470.3135	54.13	<.0001
Em	1	55.1915	55.1915	6.35	0.0143
Cutting	1	8603.117	8603.117	990.19	<.0001
Media*Em	7	550.0794	78.58278	9.04	<.0001
Media*Cutting	7	5048.105	721.1579	83	<.0001
Em*Cutting	1	117.2405	117.2405	13.49	0.0005
Media*Em*Cutting	7	558.4181	79.77401	9.18	<.0001
Error	62	538.6769	8.68834		
Total	95	18804.27			

Appendix Table 14: Analysis of variance for shoot fresh weight (g) per cutting of hybrid coffee variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	0.074115	0.037057	0.44	0.6478
Media	7	37.16268	5.308955	62.65	<.0001
Em	1	0.406901	0.406901	4.8	0.0322
Cutting	1	81.12565	81.12565	957.29	<.0001
Media*Em	7	3.638307	0.519758	6.13	<.0001
Media*Cutting	7	17.76456	2.537794	29.95	<.0001
Em*Cutting	1	0.197109	0.197109	2.33	0.1323
Media*Em*Cutting	7	2.726432	0.38949	4.6	0.0003
Error	62	5.254219	0.084746		
Total	95	148.35			

Appendix Table 15: Analysis of variance for shoot dry weight (g) per cutting of hybrid coffee variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	0.006215	0.003107	0.94	0.3979
Media	7	1.861913	0.265988	80.06	<.0001
Em	1	0.0216	0.0216	6.5	0.0133
Cutting	1	3.977204	3.977204	1197.11	<.0001
Media*Em	7	0.143483	0.020498	6.17	<.0001
Media*Cutting	7	0.804113	0.114873	34.58	<.0001
Em*Cutting	1	0.006667	0.006667	2.01	0.1616
Media*Em*Cutting	7	0.102617	0.01466	4.41	0.0005
Error	62	0.205985	0.003322		
Total	95	7.129796			

Appendix Table 16: Analysis of variance for shoot dry weight (g) per cutting of hybrid coffee variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	0.006215	0.003107	0.94	0.3979
Media	7	1.861913	0.265988	80.06	<.0001
Em	1	0.0216	0.0216	6.5	0.0133
Cutting	1	3.977204	3.977204	1197.11	<.0001
Media*Em	7	0.143483	0.020498	6.17	<.0001
Media*Cutting	7	0.804113	0.114873	34.58	<.0001
Em*Cutting	1	0.006667	0.006667	2.01	0.1616
Media*Em*Cutting	7	0.102617	0.01466	4.41	0.0005
Error	62	0.205985	0.003322		
Total	95	7.129796			

Appendix Table 17: Analysis of variance for root to shoot ratio (g) per cutting of hybrid coffee variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	0.040558	0.020279	0.88	0.4182
Media	7	14.72148	2.103068	91.69	<.0001
Em	1	0.299267	0.299267	13.05	0.0006
Cutting	1	0.000267	0.000267	0.01	0.9145
Media*Em	7	1.291417	0.184488	8.04	<.0001
Media*Cutting	7	5.554217	0.79346	34.59	<.0001
Em*Cutting	1	0.456504	0.456504	19.9	<.0001
Media*Em*Cutting	7	1.171246	0.167321	7.3	<.0001
Error	62	1.422042	0.022936		
Total	95	24.957			

Appendix Table 18: Analysis of variance for total dry matter yield (g) per cutting of hybrid coffee variety Ababuna

Source	DF	SS	MS	F value	Pr > F
Rep	2	0.008856	0.004428	1.13	0.3289
Media	7	5.392579	0.770368	196.98	<.0001
Em	1	0.059004	0.059004	15.09	0.0003
Cutting	1	9.741004	9.741004	2490.72	<.0001
Media*Em	7	0.591379	0.084483	21.6	<.0001
Media*Cutting	7	3.044146	0.434878	111.2	<.0001
Em*Cutting	1	0.063038	0.063038	16.12	0.0002
Media*Em*Cutting	7	0.431979	0.061711	15.78	<.0001
Error	62	0.242477	0.003911		
Total	95	19.57446			

Appendix Table: 19 : correlation analysis among different root and shoot parameters

	PR	RN	RL	RG	RV	Rfw	Rdw	ShS	ShL	ShG	LN	LA	Shfw	Sdw	TDM	RSr
RN	0.79 ***	1														
RL	0.94 ***	0.85 ***	1													
RG	0.91 ***	0.86 ***	0.94 ***	1												
RV	0.32 ***	0.55 ***	0.48 ***	0.43 ***	1											
Rfw	0.43 ***	0.42 ***	0.47 ***	0.47 ***	0.28 ***	1										
Rdw	0.75 ***	0.88 ***	0.87 ***	0.89* **	0.70 ***	0.47 ***	1									
ShS	0.55 ***	0.81* **	0.69* **	0.65* **	0.79 ***	0.34 ***	0.83 ***	1								
ShL	0.62 ***	0.76 ***	0.77 ***	0.72 ***	0.74 ***	0.40 ***	0.85 ***	0.86 ***	1							
ShG	0.83 ***	0.83 ***	0.91 ***	0.93 ***	0.53 ***	0.54 ***	0.89 ***	0.71 ***	0.85 ***	1						
LN	0.72 ***	0.75 ***	0.83 ***	0.81 ***	0.67 ***	0.48 ***	0.84 ***	0.73 ***	0.89 ***	0.89 ***	1					
LA	0.61 ***	0.78 ***	0.76 ***	0.72 ***	0.81 ***	0.39 ***	0.87* **	0.91 ***	0.92 ***	0.80 ***	0.85 ***	1				
Sdw	0.56 ***	0.76 ***	0.68 ***	0.67 ***	0.79 ***	0.40 ***	0.82 ***	0.89 ***	0.86 ***	0.76 ***	0.84 ***	0.89 ***	0.99 ***	1		
Tdm	0.67 ***	0.85 ***	0.79 ***	0.8 ***	0.79 ***	0.45 ***	0.94 ***	0.91 ***	0.89 ***	0.85 ***	0.88 ***	0.92 ***	0.96 ***	0.96 ***	1	
RSr	0.62 ***	0.54 ***	0.68 ***	0.68 ***	0.23 **	0.28 ***	0.62 ***	0.37 ***	0.46 ***	0.59 ***	0.42 ***	0.45 ***	0.17 ns	0.17 ns	0.37 ***	1

PR=% rooting, RN= root number, RL=root length, RG= root girth, RV=root volume, Rfw=root fresh weight, Rdw=root dry weight
 ShS=shoot sprouting, ShL-shoot length, ShG= shoot girth, LN=leaf number, LA=leaf area, Sdw=shoot dry weight, Tdm=total dry matter
 RSr=root to shoot ratio

Appendix 20. List of plates



Plate 1. Propagating structure/frame made of eucalyptus tree



Plate 2. Rooting media components ready to be mixed



Plate 3. Pots arranged for cutting insertion



Plate 4. Transporting six months old Suckers to the propagation site



Plate 5. Whole node cuttings ready for insertion



Plate 6. Cuttings dissected in to two symmetrical parts (half node cuttings)



Plate 7. Watering cuttings immediately after insertion



Plate 8. Propagating frame covered with white polyethylene sheet right after insertion



Plate 9. Partial view of sprouting cuttings in the propagation structure/frame



Plate 10. Partial view of cuttings with growing shoots on different rooting media



Plate 11. Whole node cuttings ready for distractive data collection



Plate 12. Half node cuttings ready for distractive data collection



Plate 13. Whole node cuttings grown on rooting media 6Ts:3M:0Ch:2S with EM



Plate 14. Whole node cuttings grown on rooting media 6Ts:3M:0Ch:2S without EM



Plate 15. Half-node cuttings grown on rooting media 6Ts:3M:0Ch:2S with EM



Plate 16. Half-node node cuttings grown on rooting media 6Ts:3M:0Ch:2S without EM



Plate 17. Whole node cuttings grown on rooting media 2Ts:1M:1S+top 10cmSs with EM



Plate 18. Whole node cuttings grown on rooting media 2Ts:1M:1S+top 10cmSs without EM