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Research Article

Influence of rooting media, number of nodes and seedling growing methods on rooting, seedling establishment and early growth of Chaya (*Cnidoscolus aconitifolius* McVaugh) stem cuttings at Dire Dawa, Eastern Ethiopia

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Abstract

Establishment of seedling growing method(s) is priority activity to the distribution of newly introduced vegetable crops. Chaya is proposed as candidate vegetable crop to distribute in eastern Ethiopia. However, recommendation is lacking for rooting media and type of cutting to produce seedlings. Therefore, this study was conducted to assess effect of rooting media and number of nodes on rooting, seedling establishment and early growth of chaya at Dire Dawa from February 2018 to July 2018. Three level of rooting media (top soil only, 3:2:1 top soil: farmyard manure: sand, 3:1 top soil: farmyard manure), three levels of node number (2, 3, 4) and two seedling growing methods (seedbed/bare root and polyethylene bag) in factorial combinations were evaluated in completely randomized and randomized complete block designs with three replications for nursery and field experiments, respectively. Seedling growing methods significantly influenced all traits, rooting media, number of nodes and interaction of the main factors had significant effect on percentage of root initiated cuttings. Fresh and dry weight of leaves and roots, dry weight of leaves and root significantly influenced by interaction of the three factors. The seedlings raised on seedbed filled with 3:1 top soil: farmyard manure (91.6%) and stem cuttings with four nodes (92.7%) showed high percentage survival. Generally, either one or more of the three main factors rooting media, number of nodes and seedling growing method or one or more of the possible two and three main factors interactions significantly influenced the seedlings growth and establishment of seedlings at the field.

In conclusion, chaya seedlings from cutting responded well to type of rooting media containing top soil: farmyard manure: sand, cutting length with three and four nodes and growing method (seedbed). Therefore, in the study area it could be advised to use combined mixtures of 3:2:1 top soil: farmyard manure: sand and stem cutting with four nodes grown on nursery bed and field establishment.

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Introduction

Chaya (*Cnidoscolus aconitifolius* McVaugh) belongs to Euphorbiaceae family [1,2]. Chaya is native to Mexico and its common name includes chaya, tree spinach, Mayan spinach. It is a bush sub-perennial and self-seeding, which grows to a height of 5 m when it is cultivated in subtropical climates [3]. Chaya is a fast-growing, drought-tolerant, perennial shrub. Its principal uses are as a vegetable, forage and medicine; that the leaves being the part used most. It is grown for its dark-green leaves, which is produced in abundance [1,4].

Its high nutritive value, productivity, tolerance of poor growth conditions, resistance to pests and disease makes it a valuable potential crop that could benefit people of many regions [5]. The nutrition values found in a 100 g serving of chaya is 32% to 53% of the recommended daily intakes of iron which is two times the iron as spinach, 27% of daily intakes of vitamin A and 275% to 342%, 20% to 30% and 12% to 15% of daily intakes of vitamin C, calcium and protein respectively [6].

Chaya has been given the name "famine plant" due to its importance under drought, low soil fertility conditions, tolerance to insect pest. The crop can grow in the tropics and subtropics throughout the world. It is tolerant to heavy rain and drought, grows in most soils but not acidic soils, cold sensitive but may recover, propagated from stem cuttings, slow until established then fast and become large shrub (3 m - 5 m) [7]. It grows best in areas where annual daytime temperatures are within the range of 20 °C - 32 °C, but can tolerate 12 °C – 38 °C. It prefers a mean annual rainfall in the range 1000 mm - 2000 mm, but tolerates 500 mm - 2500 mm (ECHO, 2006). Plants grown under partial shade will produce large and tender leaves. Annual dry leaf yields of up to 12 tons per hectare are possible on fertile soil with about 9000 plants per hectare [7].

Chaya makes a very good living fence to go around home gardens. Chaya uses to made compost from pruned chaya branches (high in nitrogen) and can be used as fertilizers. Dried or fresh chaya leaves and branches make good fodder for chickens and help to increase egg production, growing chaya at health centers could give good opportunity to teach people about the nutritive benefits of using chaya in family meals. Generally chaya out-performs most green leafy vegetables nutritionally. The spinach tree and or chaya has a great potential to alleviate deficits in population of developing countries as it is rich in protein, vitamin and mineral [8,9].

Vitamin A deficiency is a severe public health problem affecting young children and mothers and reaching alarming levels especially in rural areas. Anemia is also a public health problem in eastern Ethiopia that the prevalence is56.8% of them 1.2% was severely anemic, 26.7% were moderately anemic and 28.9% were mildly anemic [10]. About a quarter of school children suffer from anemia and their educational potential is likely to be affected especially for those with moderate and severe anemia. Realizing the problem, Haramaya University is conducting research to introduce chaya in the region which is rich in Vitamin A, Iron and Zinc. The chaya was introduced to Ethiopia by an individual before four decades but was not distributed to more than few individuals who used as fence plant and beautifying the garden (Bekelu Tola, Personal Communication). No higher education or research institutions conduct any sort of research in the country so far.

The chaya recently introduced is being studied for its adaptation at Haramaya University. However, many production practices are also needed for this nutritionally important vegetable. Some plant species are hard to propagate sexually and they also show complexities and undesirable characters [11]. Thus, vegetative propagation using stem cuttings is the most vital and sole method to reproduce these plants species unless needed for improvement purposes [12,13]. In vegetative propagation, the rooted plants have many advantages, such as faster growth rate, greater stock stand uniformity, better site matching and true-to-type planting material production. Cuttings can be categorized into three groups; easy to root, difficult to root and obstinate to root [12,14].

The chaya lacks recommended media for rooting and seedling growth and best part of the plant used for cuttings to propagate. Predominantly, chaya is propagated through vegetative means mainly from stem cuttings collected from healthy and vigorous mother plant. Experiences are lacking about the number of nodes to be retained per cutting and ideal rooting media for rooting and seedling growth of chaya. A good medium provides several essential functions: it holds the cuttings in place, it maintains high moisture content yet is well drained, it allows adequate air exchange around the base of the cutting and it must be free of disease and insects. The best choice for rooting is a media with low capacity of water retention [15].

The types of rooting media used have a major influence on the rooting capacity of cuttings [16]. Sand media is the best one for rooting percentage. There was a positive relationship between shoot lengths and rooting percentage so that by increase in rooting percentage, shoot length was also enhanced [15].

The length of cuttings used for vegetative propagation is commonly determined by the availability of mother plants [17]. Initial cutting size is a very important factor involved in rooting ability and growth performance [18-20]. Longer cuttings result in better survival and growth under normal conditions [20-22], due to larger cuttings store more carbohydrates [23]. Root initiation and length were lower in the short cuttings and associated with the lowest values of number of roots and rooting percentage [24]. Cutting length can affect both hedge productivity (number of cuttings per harvest) and rooting of a clone. In addition, cutting length is also an important causal factor of within- and between-shoot variation in rooting ability [25]. Determining optimal cutting length is essential as a very long cutting will be a waste of valuable coppice material, with limited or no benefit in rooting percentage, whereas a short cutting may not result in the development of sufficient roots (possibly due to a lack of sufficient storage reserves).

Most planting materials growth parameters such as number of leaves, length of shoot and root dry weight increase as the

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number of nodes per original stem cutting was increased in vanilla plants [26]. The use of short cuttings (30 cm long with 3 to 4 nodes) is widespread due to shortage and unavailability of the preferred planting materials [27]. Therefore, to generate information on appropriate media type and node number for successful propagation of chaya plant, this research was initiated with the following objectives:

- ✓ To assess effect of rooting media, number of nodes and seedling growing methods on rooting, seedling establishment and early growth of Chaya.

Materials and methods

Description of experimental site

The field experiment was conducted at the research farm of Haramaya University in Dire Dawa (Tony farm) during the period from February 2018 to July2018. The farm is located at an altitude of 1197 m above sea level and lies at 9º 6'N latitude and 41º8' E longitude in the eastern part of Ethiopia. The station lies in the semi-arid belt of the eastern rift valley escarpment with a long-term average rainfall of 612 mm. The mean maximum and minimum temperatures range from 28.1 ºC to 34.6 ºC and 14.5 ºC to 21.6 ºC respectively (Belay, 2002). The soil is classified as Eutric Regosol and the field has a gentle slope (3% - 8%) [28]. The texture and structure of the topsoil (0-30 cm) are sandy loam and sub angular blocky respectively. The soil has an average pH (H_2O 1:2.5) of 8.54 and organic matter content of 1.94% at (0 cm - 15 cm) and 1.84% at (15 cm - 30 cm) soil depth [29].

Description of experimental materials, treatments and design

In this study, experimental materials stem cuttings of chaya; farmyard manure, pure sand and soil were used. Well decomposed farmyard manure was collected from Haramaya University dairy farm and stem cuttings of chaya were collected from Adama private backyard. The woody cuttings were taken from the semi hardwood of a stem leaving extreme bottom and tip portions of the shoot. The cuttings were prepared to different nodes (two nodes, three nodes and four nodes) were prepared; leaves were removed from cuttings and hardened under shade for two days.

The cuttings were planted in black polythene bags(plastic pots) having 14 cm diameter *25 cm length size as suggested by [30] or with a pot volume of 3,846.5 cm³and lightly or gently firmed down till the surface just about 5 cm below the container's top edge. The pot volume was adequate in size to provide enough rooting space and nutrients for the plants over the whole study duration [31] and seedbed. The polythene bags were filled with well-prepared media of soil media (top soil only, 3:1 top soil: well decomposed FYM and 3:2:1, top soil: well decomposed FYM: sand) and seedbed directly without containers (top soil only, 3:1, top soil: well decomposed FYM and 3:2:1, top soil: well decomposed FYM: sand). The seedbeds were refilled at the depth of 25 cm (as equal height of the polythene bags) with the soil media compositions as per the treatments.

Therefore, this experiment consisted of three different node numbers, three soil media and two seedling growing methods (polyethylene bag vs. directly to seedbed) and a total of 18 treatments in factorial combination. The seedlings were planted in the field in a hole size of 60 x 60 cm spaced at 1 and 0.6 m between rows and plants respectively. The treatments were arranged as 3 x 3 x 2 using CRD factorial combination with a total of 18 treatments for nursery and RCBD factorial combination for field experiments (Table 1).

The treatments were assigned each in one plot as single row which one plot consisted five plants spaced at 1 m and 0.6 m between rows and plants, respectively. The spacing between plots and replications were 1 m and 2 m, respectively. There were total 12 plots in which 10 cuttings were planted per row and 60 seedlings were found in each plot. A total of 540cuttings were planted in 60 cm x 60 cm hole size in the field.

Experimental procedures

After field preparation, well decomposed farmyard manure and sand were applied to soil one month before planting to enable the decomposition of the organic matter so as to release plant nutrients slowly. The semi hard wood cuttings with different node number were prepared by slight cut below nodes to 45° angle and planted in polyethylene bag containers and directly to the seedbed on different soil media depending on the number of nodes in each cutting treatment. The cuttings in polythene bags and seedbeds were kept moist but not overwatered to protect rotting. Regular hand weeding was exercised to keep weed free throughout the experiment period. The seedbeds were covered with shade using plant leaves and the cover of the shade was reduced by half after all cuttings produced leaves to allow sun light to reach on seedbeds.

The planted seedlings received water at field capacity during planting and every three days until the seedlings established in the field (one month).

Soil and farmyard manure analysis

In order to determine the physical and chemical properties of the soil three representative soil samples were taken using auger from the depth of 0 cm to 30 cm for each block of the experimental site before planting and application of any type of fertilizer. The samples were mixed, after which about 1 kg of a single composite soil sample were prepared. The sample was taken to the Haramaya University soil testing laboratory and tested for total nitrogen, soil pH, organic carbon, available phosphorus, potassium, Cation Exchange Capacity (CEC) and texture analysis. Soil pH was measured in 1: 2: 5 soil water

Table 1: Description of three main factors and treatment combinations.

Soil media (Factor A)	Type of cutting (No. of nodes on cutting) (Factor B)			Seedlings growing method (Factor C)	
Top soil only	2	3	4	Seedbed	Polythene bag
3:1 top soil: FYM	2	3	4	Seedbed	Polythene bag
3:2:1, top soil: FYM: sand	2	3	4	Seedbed	Polythene bag

Seedbed and Polythene bag represented bare root and container seedlings growing methods, respectively.

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ratios using an electrodes pH meter. Organic carbon content of the soil was determined by Walkley and Black method [32]. Available P was estimated following the standard procedure of [33]. Total nitrogen was estimated by the Kjeldahl method [34].

Soil analysis for relevant parameters including pH, organic matter content, organic carbon, total nitrogen, exchangeable potassium and available phosphorus were done at the soil laboratory of Haramaya University.

Similarly, the farmyard manure (FYM) sample applied to the experimental field before planting was analyzed for some selected chemical properties like pH, available P, total N, organic C and Cation Exchange capacity (CEC). The data on these chemical properties were determined in a laboratory.

Data collected

Data collection at nursery: Number of days to initiate regrowth (first leaf bud formation): was recorded as the number of days from the day of planting of cuttings up to when 50% of the plants in a plot produce visible light green leaf as a sign of initiation of regrowth and the cuttings green leaf tips break and becomes visible but before unfolding of the longitudinal sections of the bud [35].

Days to first leaf appearance: was recorded as the number of days from the day of planting of cuttings up to when 50% of the cutting produce first leaf visible and become with fully expanded leaf or spread away from the shoot [36,37]. At this stage new leaves are clearly visible, new sprout continues to grow and longitudinal sections become unfolded.

Number of leaves: Numbers of leaves produced in five sample seedlings were counted at four months (120 days) after planting and the average was calculated for each treatment.

Stem height (cm): The heights of regrowth stem(s) from five sample seedlings were measured and the average was calculated for each treatment (at four months of cuttings planting).

Rooted cuttings (%): the proportion of cuttings producing seedlings in percent were calculated for each treatment after four months of planting as suggested by [38] using the formula:

$$\text{Rooting percentage}(\%) = \frac{\text{Number of cuttings rooted}}{\text{Total number of cuttings planted}} \times 100$$

Fresh weights of regrowth shoot (g): the fresh weight of stem(s) and leaves of five randomly selected seedlings were measured after detaching off stem(s) and leaves from five sample seedlings at four months of planting.

Fresh leaves weight (g): The fresh weights of leaves from five sample seedlings were measured and the average weight of leaves per seedling for each treatment was calculated after four months of planting.

Number of roots: The numbers of roots produced from five sample seedlings were counted and the average number of roots per seedling for each treatment was calculated after four months of planting.

Fresh weight of roots (g): The fresh weight of roots from five randomly selected samples from each treatment was measured and the average weight of root weight was calculated after four months of planting.

Dry weight of regrowth shoots (g): the regrowth stem(s) and leaves separately from five sample seedlings of each treatment was kept in oven at 78°C until constant weight was attained and the weight of dry leaves and stem(s) were measured separately.

Dry weight of leaves (g): The leaf from five sample seedlings of each treatment was kept in oven at 78°C and the weight of the dry leaves were measured.

Dry roots weight (g): the roots of five sample seedlings of each treatment was detached and kept in oven at 78°C and the weight of dry roots was measured.

Shoot to root dry weight ratio: was obtained after dividing the total above ground regrowth dry weight of (stem(s) and leaves) from sample seedlings by total dry weight of roots for each treatment.

Relative growth rate (RGR) ($g\ g^{-1} day^{-1}$): the relative growth rate expresses the dry weight increase in four months of cuttings planting in relation to initial weight as a modified compound interest equation [39] was employed as indicated below. The dry weight of the stem was taken before planting the cutting from each treatment and it was considered as initial weight.

$$RGR = (\log_e W_2 - \log_e W_1) / (t_1 - t_2)$$

Where: RGR= Relative Growth Rate, W_1 = dry weight of sample cuttings, W_2 = dry weight of sample cuttings and regrowth/shoots, t_1 = day of cuttings planting and t_2 = day of cuttings maintained after planting for regrowth (four months)

Data collection after transplanting to the field

The raised seedlings were transplanted to the field after four months with soil for both seedbed and polyethylene bag. The data for field experiment were collected from the five transplanted seedlings after two months (60 days) of transplanting as follows.

Number of stems: The numbers of stem(s) produced on each seedling of each treatment within two months of transplanting (if any) were counted and the average was calculated for each treatment and for each replication.

Stem height (cm): the height of longest stem was measured for each seedling and the average was calculated for each treatment at the end of two months of transplanting and the average was calculated for each treatment for each replication.

Number of leaves: Numbers of leaves in each seedling were counted at the end of two months of transplanting and the average was calculated for seedling in each plot of each replication.

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Survival/establishment (%): the proportion of seedlings established in the field in percent was calculated in each plot of each replication at the end of two months of transplanting.

Data analysis

The data collected were subjected to analysis of variance as per the design for the experiment [40]. Data recorded as percentage were subjected to appropriate data transformation before analysis of variance conducted. Treatment means that exhibited significance difference was separated using Duncan Multiple range test (DMRT) at 5% level of significance.

Results and discussion

Growth of Chaya seedlings at nursery

Parameters of regrowth initiation in cuttings: Number of nodes on cuttings and seedling growing methods had highly significant (p < 0.01) effect on percentage of root initiated on cuttings, number of days to initiate regrowth and numbers of days to first leaf appearance. Rooting media had significant effect on percentage of root initiated on the cuttings. The interaction of the three main factors (Seedling growing methods x Rooting media x Number of nodes) had significant influence on three regrowth parameters while the interaction of Rooting media x Number of nodes had significant effect on number of days to initiate regrowth and numbers of days to first leaf visible.

Days to initiate regrowth (first leaf bud formation): The cuttings required about 8 to 13 days to initiate regrowth (first leaf bud formation) with 10.5 average numbers of days to initiate regrowth. The early first leaf bud formation of about 8 days (7.83 and 8 days) was observed in stem cutting with two nodes grown in polyethylene bag filled with 3:2:1 top soil: FYM: sand and 3:1 top soil: FYM (Table 2).

Initiation of regrowth significantly delayed when stem cuttings with four nodes planted on seedbeds prepared by media filled with3:1 top soil: FYM and top soil only. Stem cuttings with four followed by three nodes grown on seedbeds regardless of rooting/soil media had delayed initiation of regrowth than stem cuttings with two nodes [41]. Argued that bud break and initial growth in cuttings are dependent on stored carbon which was differed depending on length of the cuttings.

The tendency of delayed initiation of regrowth in stem cuttings with four and three nodes grown in polyethylene bags was also observed though inconsistency was observed with the interaction of rooting/soil media. This may be due to better water holding capacity of applied FYM around root zone of cutting which enables initiation of roots and buds to sprout early.

The growing of chaya seedlings in polyethylene bag filled with soil media containing FYM with or without sand had early leaf bud formation indicated that FYM and polyethylene bag had significant role in modifying the growing conditions and speeds up budding of plants, which indicated that application

Table 2: Interaction effects of rooting media, number of nodes and seedling growing methods on three regrowth parameters of chaya cuttings grown at Dire Dawa in 2018

Treatment			Regrowth parameter		
Rooting media	Number of nodes	Seedling growing methods	Days to initiate regrowth	Days to first leaf appearance	Root initiated cuttings (%)
	N2	Seedbed	9.73^{ef}	19.50^{ef}	70.00^{b-f}
M1		Polyethylene bag	9.43^{f}	19.01^{f}	60.00^{eg}
	N3	Seedbed	11.07^{b}	20.75^{b}	80.00^{abc}
		Polyethylene bag	8.60^{g}	18.12^{g}	70.00^{def}
	N4	Seedbed	12.27^{a}	21.78^{a}	83.33^{a}
		Polyethylene bag	11.07^{b}	20.58^{bc}	83.33^{a}
	N2	Seedbed	9.73^{ef}	19.32^{f}	63.33^{d-g}
M2		Polyethylene bag	8.00^{h}	17.58^{g}	66.67^{d-g}
	N3	Seedbed	10.47^{cd}	19.98^{cde}	73.33^{bcd}
		Polyethylene bag	10.67^{bc}	20.19^{bcd}	70.00^{de}
	N4	Seedbed	12.73^{a}	22.25^{a}	83.33^{a}
		Polyethylene bag	9.60^{ef}	19.12^{f}	70.00^{de}
	N2	Seedbed	10.04^{de}	19.62^{def}	66.67d^{-g}
M3		Polyethylene bag	7.83^{h}	17.52^{g}	70.00^{de}
	N3	Seedbed	11.13^{b}	20.65^{b}	83.33^{a}
		Polyethylene bag	9.73^{ef}	19.25^{f}	70.00^{b-f}
	N4	Seedbed	11.20^{b}	20.72^{b}	86.67^{a}
		Polyethylene bag	10.93^{bc}	20.45^{bc}	80.00^{ab}
Overall mean			10.236	19.799	73.89
LSD (5%)			0.37	0.59	8.728

Means in a column followed by the same letter(s) had non-significant difference at 5% level of probability. LSD (5%) = least significant difference at P < 0.05, M1= top soil only, M2= 3:1 top soil: FYM, M3= 3:2:1 top soil: FYM: Sand, N2= two nodes, N3= three nodes, N4= four nodes.

of FYM is very important for root initiation and proliferation which speeds up budding of plants [42]. Observed that fastest sprouting in *Albizialebbeck* cuttings planted on potting mixture (cow dung + top soil + washed river sand) [43]. Suggested that the application of FYM increased water retention around root area and enhanced plant growth and development because of the presence of humic acids due to water retention around root area of chaya plants which helped the cuttings to initiate bud earlier [44]. Also reported that days taken to sprouting was observed minimum in cuttings of rose root stock in media containing sand and cattle manure [45]. Also observed that the application of FYM in sweet potato cuttings hasten bud sprouting. The decrease in time taken to root initiation might be due to the excellent drainage, good aeration and higher temperature in manure supplemented media.

The early and delayed leaf bud formation observed in stem cutting with two and four nodes, respectively, regardless of rooting/soil media and growing methods might be due to the shorter cuttings has less stress such as higher transpiration as compared to longer cutting and due to a faster mobilization of auxin in shorter cuttings and a short cutting may be weak in the development of sufficient roots (possibly due to a lack of sufficient storage reserves). In agreement with the current study findings [18], reported that initial cutting size for

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improving the growth performance of *Salix alba* L. and it is a very important factor involved in rooting ability and growth performance [46]. Also reported that carbohydrates enhance metabolic activities that occur at the base of the cuttings to aid cell division which brings about root initiation.

The high number of sprouts recorded from the longer stem cutting could also be attributed to the effect of buds on the cuttings. Buds on a cutting have a strong promoting effect on rooting [47].

This might be due to the increased peroxidase activity and fewer growth inhibitors in the cuttings thereby early bud opening. Similar result was reported by [37] that the increased peroxidase activity of own rooted cuttings boosts the time for bud opening. The available carbohydrates in stem cuttings and the nitrogen reserves within the cuttings increase the potential for callusing [48].

The main reason for earlier bud opening might be linked to the performance of the media to maintain high internal temperature. The solarisation effect of plastic bag also might have provided proper warmth to the media. These factors are in favor of early sprout and seedling growth as observed. The study of [49,50] agreed with the current result that high internal temperature of rooting media enhances the time of bud opening in cuttings.

Days to first leaf appearance: The stem cutting required about 18 to 22 days to produce visible leaf with 19.5 average numbers of days. The early visible leaf taken at about 18 days (17.52 and 17.58 days) which was observed in stem cutting with two nodes grown in polyethylene bag filled with top soil only and 3:1 top soil: FYM media respectively (Table 2).The increase in new leaf emergence might be due to high porosity and water holding capacity, good nutrient content and aeration of media containing sand and manure. The high aeration in the sand medium might have created a good environment for increased respiration at the base of the cuttings which encourages rooting and growth [51]. Polyethylene bag capture more solar energy which helps stem cutting to assimilate more reserved food to produce visible leaf early.

Stem cuttings with four nodes followed by three nodes grown on seedbeds regardless of rooting/soil media had delayed days to visible leaf than stem cuttings with two nodes. Concomitant with this finding [52], reported that longer cutting lengths significantly increased the number of days to first leaf emergence. Thus, the quicker emergence of new leaves on the shorter cuttings might be attributed to the earlier growth of the root system and wound healing of cuttings. The tendency of days to first leaf visible in stem cuttings with four and three nodes grown in polyethylene bags were also observed though inconsistency was observed with the interaction of rooting/soil media (Table 2).

Less number of days was required for cutting to produce visible leaf grown in polyethylene bag with two nodes as compared to three and four nodes. The four nodes cuttings grown on seedbed having 3:1 top soil: FYM media and top soil only produce visible leaf late. This might be due to the shorter cuttings has less stress such as higher transpiration as compared to longer cutting and due to a faster mobilization of auxin in shorter cuttings.

On the other hand, a delay of five days was recorded for the visible leaf development in 3:1 top soil: FYM and stem cutting having four nodes and cutting grown on seedbed (Table 2). Nutrition is the key factor affecting rooting because of its involvement in determining the morphogenetic response of the plants and the success rate of vegetative propagation programs depends on the optimal plant nutrient status, which is important for initial growth. In line with this finding [49] reported that the leaf growth in cuttings was hastened by the total nitrogen content of the rooting media.

Root initiated cuttings

The minimum (60%) cuttings that initiated roots was observed in stem cuttings with two nodes grown in polyethylene bag filled with top soil only, while the maximum (86.67%) cuttings that initiated roots was observed in stem cuttings with four nodes grown on seedbed regardless of rooting media (Table 2). This may be due to improved drainage of the sand and the availability of phosphorous nutrient available in FYM that increased rooting ability of stem cutting and high water retention capacity. In agreement with current findings [21], suggested that longer cuttings root at higher percentages than short cuttings in Ayous (*Triplochiton scleroxylon*). Similarly [24], suggested that rooting percentage values of Norway spruce [*Picea abies*(L.) Karst] cuttings increased with increasing cutting length. This might be due to the factor that the level of endogenous auxin and other rooting-inducing factors may be lower in smaller cuttings, which leads to reduced rooting percentage or the absence of rooting in short cuttings [53] and the larger cuttings store more carbohydrates [23].

Carbohydrates in longer cuttings contribute to the formation of roots by supplying energy and carbon necessary for cell divisions, establishment of the new root meristems and root formation itself [54].

[55], indicates that rooting capacity is positively correlated with carbohydrate availability. The importance of carbohydrate to nitrogen (C/N) ratio in plant growth and development has been hypothesized by [46]. High carbon to nitrogen ratio in cutting tissue promotes rooting. The ability of a stem to root has been shown to be due to an interaction of inherent factors present in the stem cells as well as transportable substances produced in leaves and buds, the major one being carbohydrate with others as nitrogenous compounds and vitamins [56].

[57] Reported that highest rooting percentage was recorded from rooting media having fine sand from cutting with three nodal cutting of Domat and Ayvalik Olive [15]. Also reported that the high rooting percentage was achieved by stem cuttings of guava in sand. This could be due to the fact that low bulk density of sand media invariably allows for greater root penetration leading to better root initiation [58]. Reported that high rooting percentage was recorded from the longest

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stem cutting of *Khaya senegalenss*. This might be due to better initial carbohydrates reserves stored of longer cuttings and polyethylene bag absorbs more solar energy which increase heat in the container and prohibit more rooting of cutting [23,54].

Growth of seedlings

Seedling growing methods (bare root and polyethylene bag) had highly significant ($p < 0.01$) effect on number of leaf, number of root and regrowth stem height. Rooting media significantly affected number of root and number of nodes had significant effect on number of leaves. The interaction of rooting media and seedling growing methods significantly affected number of leaves and regrowth stem height, while the interaction of number of nodes and seedling growing methods significantly affected number of leaves.

Regrowth stem height

The maximum regrowth stem height (42.78 cm) and (42.49 cm)was registered in stem cutting grown on seedbed filled with 3:1 top soil: FYM and 3:2:1 top soil: FYM: sand respectively, while the minimum regrowth stem height (4.96 cm) was registered from stem cuttings grown in polyethylene bag filled with 3:2:1 top soil: FYM: sand (Table 3). Osaigbovo *et al.* (2010) suggested that potting medium with top soil and cow dung in ratio of 2:1 produced the best seedling attributes in term of plant height. This finding is in concomitant with [60], who noted that maximum plant height observed in planting media having garden soil and FYM. Similarly [59], reported that maximum plant height of *Delonix Regia* was registered when stem cutting grown in soil mixture containing top soil and cow dung manure [61]. Suggested that maximum plant height of pineapple seedling was observed in media containing soil + manure (1:1) [62]. Suggested that maximum leaf number and plant height of mango (*Mangifera indica* L.) were recorded in soil + sand + FYM (2:1: 1).

This might be due to polyethylene bags often produce seedlings with deformed and spiraled root systems that lead to root girdling and weak performance after out planting as compared to seedbed [63,64]. Seedbeds have more available space for root spread and the plants capable of absorbing nutrients better than those in polyethylene bag. Also, seedbed allows better environment for seedling growth which facilitates circulation of warm air around the seedling and optimal relative humidity as compared to the polyethylene bag has little space between the seedlings.

Number of leaves

The maximum number of leaves(116.71, 116.78 and 119.16) were obtained from stem cuttings grown on seedbeds with all three rooting media while the lowest number leaves(25.38) was observed in stem cuttings grown in polyethylene bag filled with 3:2:1top soil: FYM: Sand (Table 3).

On the other hand, the highest number of leaves (119.51 and 129.36) were registered when stem cuttings with three and four nodes grown on seedbed, whereas the minimum number of leaves (33.64, 34.51 and 37.38) were registered when stem cuttings with three, four and two nodes grown in polyethylene bag (Table 4).

The superior performance of rooting media with FYM and longer cuttings could be attributed to the numerous nodes available for branching and leaves initiation and may be due to nitrogen from FYM might have contributed in producing new shoots and vigor in vegetative growth which is directly responsible in increasing the leaf number [61]. Suggested that maximum leaf number of pineapple was observed in media containing soil + manure (1:1).

[65] Suggested that increase in leave numbers of longer vines as a result of higher number of branches produced and also [66] suggested that the smallest cuttings had the least number of leaves for Eucalyptus hybrid clones [67]. Worked on response of olive hardwood cuttings to different growth media for propagation observed that the media with considerably higher organic matter content gave maximum number of leaves due to availability and release of essential nutrients which initiated early root development. This result is also in agreement with the work of [16] who reported that highest leaf number of vanilla stem cutting was recorded from forest soil: decomposed animal manure: fine sand rooting media and Smith and [26,68] also suggested that the maximum number

Table 3: Interaction effects of rooting media and seedling growing methods on growth of seedlings of chaya cuttings grown at Dire Dawa in 2018.

Treatment		Growth of Seedlings	
Rooting media	Seedling growing methods	Regrowth stem height (cm)	Number leaves per plant
	Seedbed	36.42^b	116.78^a
Top soil only	Polyethylene bag	6.90^c	44.36^b
	Seedbed	42.78^a	116.71^a
3:1 Top soil: FYM	Polyethylene bag	5.53^c	35.8^{bc}
	Seedbed	42.49^a	119.16^a
3:2:1 Top soil: FYM: Sand	Polyethylene bag	4.96^c	25.38^c
Overall mean 23.18			76.4
LSD (5%)		4.56	11.64

Means in a column followed by the same letter(s) had non-significant difference at 5% level of probability.

Table 4: Interaction effects of number of nodes and seedling growing methods on number of leaves grown at Dire Dawa in 2018.

Number of nodes	Seedling growing methods	Number of leaves
Stem cutting with two nodes	Seedbed	103.78^b
	Polyethylene bag	37.380^c
Stem cutting with three nodes	Seedbed	119.51^a
	Polyethylene bag	33.640^c
Stem cutting with four nodes	Seedbed	129.36^a
	Polyethylene bag	34.510^c
Overall mean		76.4
LSD (5%)		11.64

Means in a column followed by the same letter(s) had non-significant difference at 5% level of probability. LSD (5%) = least significant difference at $P < 0.05$.

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of leaves of vanilla was recorded in longer cutting with five nodes. This might be due to the longer cutting has more carbohydrates accumulation which helps in vegetative growth of the seedlings as compared to shorter cuttings [23].

Number of roots

The maximum number of root (10.43) were registered from chaya stem cuttings grown on rooting media filled with 3:2:1 top soil: FYM: sand, which was about 19% more than the lowest value recorded in media having 3:1 top soil: FYM (Table 5). On the other hand, the maximum number of root (10.6) was recorded from stem cutting with four nodal cutting, which was about 28.6% more than the lowest value recorded from stem cutting with two nodes. On the other hand, the maximum number of roots (10.96) was recorded in stem cutting grown on seedbed, which was about 41.96% more than the lowest value recorded from stem cutting grown in polyethylene bag (Table 5). This might be due to the polyethylene bag may hinder further root growth and leaf area expansion which helps the crop to absorb water, nutrition and to carry out photosynthesis for better growth of the plant respectively. The better performance of rooting media having more FYM to produce more root might be due to more nutrient, heat, porosity, aeration and higher water holding capacity of the rooting media in farmyard manure. Farmyard manure improves root growth and hence creates stable aggregate which reflect in improving water movement. FYM decreases soil pH as a result of decomposing process and microorganism's activity which affect directly on improving root growth [69]. Likewise [70,71] observed that media having FYM produce maximum number of root in Wax begonia.

Improved rooting characteristics in manure and sand based medium might be due to good drainage, aeration, better nutrition and water holding capacity of media [72]. Suggested

Table 5: Effects of rooting media, number of nodes and seedling growing methods treatment on number of root of chaya seedling grown at Dire Dawa in 2018.

Rooting Media	Number of root
Top soil	8.83[b]
3:1 Top soil: FYM	8.75[b]
3:2:1Top soil: FYM: Sand	10.43[a]
Node Number	
Stem cutting with two nodes	8.23[b]
Stem cutting with three nodes	9.19[b]
Stem cutting with four nodes	10.59[a]
LSD (5%)	1.33
Overall Mean	9.34
Treatment	Mean
Seedling growing methods	
Seedbed	10.96[a]
Polyethylene bag	7.72[b]
LSD (5%)	1.084
Overall Mean	9.34

Means in a column in each treatment followed by the same letter(s) had non-significant difference at 5% level of probability. LSD (5%) = least significant difference at $P < 0.05$.

that the maximum root number of stevia [*Stevia rebaudiana* (Bertoni) Hemsl.] was recorded in 1:1:1 soil: sand: FYM [73] Found more number of roots per cutting in geraniums by using a mixture of garden soil, compost and sand [74]. Suggested that materials added to the top soil to form a good rooting medium included animal dung and animal composting.

[68] Reported that the maximum number of roots of vanilla was recorded in longer cutting with five nodes [26]. Observed that maximum root number of vanilla was recorded in four nodal cuttings [24]. Also observed that maximum root number was recorded in longer cutting of Norway spruce [*Picea abies* (L.) Karst.]. This might be due to the fact that larger cuttings store more carbohydrates [23] and root growth is dependent on carbohydrates in the leaf and stem.

Fresh and dry weight of regrowth of seedlings

Fresh and dry weight of leaves and roots: Rooting media and seedling growing method highly significant influenced on fresh and dry weight of leaves and roots. Rooting media and seedling growing method interacted to influence fresh and dry weight of leaves and roots. In addition, number of nodes on cutting and seedling growing method interacted to influence fresh and dry weight of roots.

The highest fresh weight of leaves(2.68 g) and (2.96 g) were registered from stem cutting grown on seedbed filled with rooting media 3:1 top soil: FYM and 3:2:1 top soil: FYM: sand respectively, which was about 127.7% more than the lowest value recorded from stem cutting grown in polyethylene bag filled with top soil only (Table 6). [71], also suggested that potting medium with top soil and cow dung in ratio of 2:1 produced the best seedling attributes in term of dry weight of shoot and roots [62]. Also reported maximum fresh and dry weight in *Mangifera indica* L. seedlings grown in a mixture of soil + sand + FYM.

The maximum fresh root weight (46.67 g) was registered in chaya stem cutting grown on seedbed filled with rooting media 3:2:1 top soil: FYM: sand, which was about 322.7% more than the lowest value recorded from chaya stem cutting with top soil only grown in polyethylene bag (Table 6) [16], reported that the maximum fresh root weight of vanilla was recorded in cutting grown in 1:1:1 mixture of forest soil: decomposed animal manure: sand. Teshome *et al.* (2016) also suggested that the maximum fresh weight of root in tea (*Camellia sinensis* L.) was observed in media consisting top soil and FYM. Longer roots can have an ability of deeper penetration and therefore, greater capacity in water and nutrient absorption leading to heavier root fresh weight in this media (3:2:1 top soil: FYM: sand).

On the other hand, the maximum fresh root weight (41.41 g) was recorded from chaya stem cutting with four nodes grown on seedbed, which was about 270.39% more than the lowest value recorded from chaya stem cutting with two nodes grown in polyethylene bag (Table 7). [16] observed that the maximum fresh root weight of vanilla was recorded in five nodal cutting grown in 1:1:1 mixture of forest soil: decomposed animal

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manure: sand [26]. Also suggested that maximum fresh weight of roots was recorded in four nodal cuttings of Vanilla. This may reveal that the longer cutting denotes longer roots due to more carbohydrate accumulation. Similarly [66], suggested that the maximum root dry weight of Eucalyptus hybrid clones was recorded from longer cuttings.

The high NPK content present in the FYM and also release of soil nutrients contribute improved seedling growth. Soil and FYM mixture facilitates growth by modifying soil structure and permeability. FYM render optimum rooting environment due to its good water holding capacity and aeration. The increased biomass content may be attributed to the rapid absorption of these elements by the plant surface, especially the leaves and their translocation within the plant. Phosphorus present in the media could have led to better root growth and increased physiological activity of roots to absorb nutrients and moisture which in turn results in higher vegetative growth [75].

The maximum dry weight of root (9.33 g) was observed from seedlings grown on seedbed filled with media consisting of 3:2:1 top soil: FYM: sand, which was about 322.17% more than the minimum value registered from seedlings grown in polyethylene bag consisting of top soil only (Table 6) [76]. Suggested that maximum root dry weight of boxelder (*Acer negundo L.*) was observed in media containing soil, sand and FYM. This might be due to poor root development and low survival in cutting raised in polyethylene bag.

On the other hand, the maximum dry weight of root (8.28 g) was observed from stem cutting with four nodes grown on seedbed, which was about 269.64% more than the lowest value observed from stem cutting with two nodes grown in polyethylene bag (Table 7) [22]. Observed that maximum dry weight of root was registered in four nodal cuttings of vanilla. The higher value of dry weight in rooting media containing top soil, FYM and sand is due to the higher vegetative growth seen in the same medium. Growth is affected by factors like nutrient content, good aeration and moisture content. Rooting media containing top soil, FYM and sand, which provides all these factors enhance the physical and chemical properties of the soil and favors production of higher biomass. This may be due to the fact that as the nutrient absorption increased, it might have contributed to the increased photosynthesis and eventual assimilation of cellular material in the leaves. Increased leaf area index and duration for which it was operated and leaf production enhanced the size of photosynthetic tissue, which led to accelerated growth of main stem as well as branches that in turn increased the fresh and dry weight of plant.

Table 6: Interaction effects of rooting media and seedling growing methods on fresh and dry weight of leaves and roots of chaya seedlings grown at Dire Dawa in 2018.

Treatment					
Rooting media	Seedling growing method	Fresh weight of leaf (g)	Dry weight of leaf (g)	Fresh weight of root (g)	Dry weight of root (g)
	Seedbed	1.54bc	0.308bc	27.09c	5.42c
M1	Polyethylene	1.30c	0.260c	11.04d	2.21d
	Seedbed	2.68a	0.536a	33.88b	6.78b
M2	Polyethylene	1.59bc	0.318bc	12.47d	2.49d
	Seedbed	2.96a	0.592a	46.67a	9.33a
M3	Polyethylene	1.88b	0.376b	16.16d	3.23d
Overall mean 1.994			0.710	24.55	4.91
LSD (5%)		0.41	0.049	6.7	1.34

M1 = top soil only, M2 = 3:1 top soil: FYM, M3 = 3:2:1 top soil: FYM: Sand, Means in a column followed by the same letter are not significantly different at 5 % level of probability.

Table 7: Interaction effect of number of nodes and seedlings growing methods on fresh and dry weight of roots of chaya grown at Dire Dawa in 2018.

Treatment			
Node Number	Seedling growing methods	Fresh weight of root (g)	Dry weight of root (g)
	Seedbed	38.67a	7.73a
N2	Polyethylene bag	11.18c	2.24c
	Seedbed	27.56b	5.51b
N3	Polyethylene bag	17.14c	3.43c
	Seedbed	41.41a	8.28a
N4	Polyethylene bag	11.34c	2.27c
Overall mean 24.55			4.91
LSD (5%)		6.7	1.34

Means in a column followed by the same letter are not significantly different at 5 % level of probability. N2 = stem cutting with two nodes, N3 = stem cutting with three nodes, N4 = stem cutting with four nodes

On the other hand, the maximum leaf dry weight (0.536 g) and (0.592 g) was registered in rooting media filled with 3:2:1 top soil: FYM: sand and 3:1 top soil: FYM grown on seedbed respectively (Table 6) [16]. Reports that the highest leaf dry weight was recorded from stem cutting grown on 1:1:1 mixture of forest soil: decomposed animal manure: fine sand rooting media. This might be due to the nitrogen supply in experimental plot and FYM improves dry weight of leaf in chaya. Polyethylene bag absorb more solar energy which affect leaf area expansion.

Fresh and dry weight of regrowth shoot

Fresh and dry weight of regrowth shoot highly significant influenced by rooting media and seedling growing method and their interaction.

The maximum fresh weights of regrowth shoot (557.7 g) and (511.2 g) obtained in rooting media filled with 3:2:1 top soil: FYM: sand and 3:1 top soil: FYM grown on seedbed respectively (Table 8). However, the lowest fresh weights of regrowth shoot (119.7 g), (130.5 g) and (153.8 g) were recorded from stem cutting grown in polyethylene bag regardless of rooting media. On the other hand, the maximum dry weights of regrowth shoot (59.73 g) were recorded in media having 3:2:1 top soil: FYM: sand grown on seedbed which was about 372.55% more than the lowest value registered from top soil only grown in polyethylene bag (Table 8).

Likewise [16,71] suggested that the maximum shoot dry weight was registered from four and five node cutting grown on 1:1:1 mixture of forest soil: decomposed animal manure: fine

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sand [76]. Suggested that maximum shoot dry weight of boxelder (*Acer negundo* L.) was observed in media containing soil, sand and FYM [77]. Observed positive effects of organic fertilizer on vegetative growth parameters that could be attributed to their effects on supplying plants with the requirements of various nutrients for relatively long time, as well as their effect on lowering soil pH which could aid in facilitating the availability of soil nutrients and improving physical characters in favor of root development for higher water and nutrient uptake of the plant and dry matter accumulation.

This might be attributed to the fact that farmyard manure constitutes important mineral nutrients such as N, P, K, as well as micronutrients and provides decomposable organic matter thereby increasing soil aggregations which in turn improves physico-chemical conditions of the soil such as water holding capacity. In addition, the carbon content in the manure is utilized as food by soil microorganism, which increases microbial activity to convert unavailable plant nutrients to available forms through biological transformation (mineralization). This property of manure may have promoted chaya leaf and stem growth and expansion in this experiment.

Shoot to root dry weight ratio and relative growth rate

Shoot to root dry weight ratio significantly and relative growth rate was highly significant influenced by seedling growing method. Number of nodes highly influenced the relative growth rate. The interaction of number of nodes and seedling growing method significantly influenced shoot to root dry weight ratio and highly influenced relative growth rate.

The highest shoot to root dry weight ratio (9.88) was observed from stem cutting with three nodes grown on seedbed, which was about 87.83% more than the lowest value obtained from stem cutting with three nodes grown in polyethylene (Table 9). [20] observed that the shoot to root ratio of *Populus cl.* Max cuttings are higher in longer cutting [78], in line to this, concluded that a relatively high conservation of photo-assimilate in shoots increases the plant's photosynthetic leaf area while decreasing root biomass and the plant's capacity for water and nutrient uptake.

Shoot to root ratio (partitioning of the dry matter between shoot and root) can be affected by different factors like supply of all macronutrients (N, P, S, K, Mg and Ca), C: N ratio, carbohydrate accumulation in shoot [79]. The increase in shoot to root dry ratio might be associated with increased growth due to nitrogen supply from the rooting media and experimental plot and ratio may increase by supply of micronutrients [80]. The partitioning of photo assimilate between roots and shoots has frequently been analyzed as a balance between shoot and roots activity.

The highest relative growth rate (0.015 g $g^{-1}day^{-1}$ and 0.012 g $g^{-1}day^{-1}$) was observed from stem cutting with three and four nodal cutting grown on seedbed, which was about 82.9% more than the lowest value recorded in stem cutting with two nodal cutting grown in polyethylene bag.

Table 8: Interaction effects of rooting media and seedling growing methods on fresh and dry weight of regrowth shoot of chaya seedlings grown at Dire Dawa in 2018.

Treatment			
Rooting media	Seedling growing method	Fresh weight of regrowth shoot (g)	Dry weight of regrowth shoot (g)
Top soil only	Seedbed	399.3^b	42.61^c
	Polyethylene bag	119.7^c	12.64^d
3:1 top soil: FYM	Seedbed	511.2^a	54.74^b
	Polyethylene bag	130.5^c	13.79^d
3:2:1 top soil: FYM: Sand	Seedbed	557.7^a	59.73^a
	Polyethylene bag	153.8^c	16.16^d
Overall mean		312	33.28
LSD (5%)		54.68	4.82

Means in a column followed by the same letter(s) had non-significant difference at 5% level of probability. LSD (5%) = least significant difference at $P < 0.05$, SB = Seedbed and PT = Polyethylene bag

Table 9: Interaction effect of number of nodes and seedling growing method on shoot to root dry weight ratio and relative growth rate of chaya.

Treatment	Shoot to root dry weight ratio		Relative growth rate (g g^{-1} day $^{-1}$)	
Node Number	Seedbed	Polyethylene bag	Seedbed	Polyethylene bag
N2	6.82^b	6.76^b	0.0082^e	0.0082^e
N3	9.88^a	5.26^b	0.0121^b	0.0110^c
N4	7.46^b	7.46^b	0.0152^a	0.0101^d
Overall mean	7.27		0.01077	
LSD	2.16		0.000954	

Means in a column followed by the same letter(s) had non-significant difference at 5% level of probability. LSD (5%) at $p < 0.05$, N2 = stem cutting with two nodes, N3 = stem cutting with three nodes, N4 = stem cutting with four nodes

Establishment of Chaya in field

The seedlings raised on nursery were transplanted to the field after four months. Before transplanting, the experimental site was carefully prepared and watered to humid the soil. Among the 10 seedlings raised on nursery 5 seedlings were transplanted to see the survival percentage of the seedling under field condition. Field establishments of seedlings can be taken as an indicator of survival and potential growth after transplanted. During field establishments maximum leaf number, high survival percentage was observed in seedling raised on seedbed filled with 3:2:1 top soil: FYM: sand and 3:1 top soil: FYM and cutting with four nodes. The following parameters were observed from the seedlings transplanted to the field two months after transplanting.

Survival and/or establishment

Chaya seedlings planted in rooting media having top soil: FYM: sand at the ratio of 3:2:1 having four nodes grown on seedbed recorded better field establishment (90%) over other treatment combinations. The seedling raised on rooting media filled with 3:2:1 top soil: FYM: sand on nursery had the highest survival percentage (91.6%), which was about 15.37% more than the least survived seedling raised on top soil only (Table 10). The current result is in line with the finding of [72], who observed that highest field survival percentage of stevia [*Stevia rebaudiana* (Bertoni) Hemsl.] from seedling raised in media

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having 1:1:1 top soil: FYM: sand. In agreement with [81] who obtained maximum success of seedlings was observed in media filled with FYM + soil (1:1) at field.

Sonam *et al.* (2018) also suggests that the maximum field survival of rose was recorded in cuttings grown in manure and sand supplemented media. The increase in field survival percentage might be due to the profuse root production of cuttings in manure rich media having more nutrients, good aeration and water holding capacity. Sand is usually recognized good medium for rooting of cuttings. However, supplementation of sand with some organic products has given better results [82].

The result is in line with the finding of [83] who suggested that soil, sand and compost in the ratio of 1:1:2 is the best for growth and survival of Acacia catechu Willd [76]. Suggested that seedlings of boxelder (*Acer negundo* L.) survive more in media containing soil, sand and FYM.

Similarly, the seedling raised from the longer cutting (four nodes) survive by more percentage (92.66%), which was about 17.47% more than the least survived seedlings raised from shorter cuttings (two nodes) (Table 10).

Stem cuttings with a larger diameter and longer length result in better survival and growth under normal conditions [20-22]. This might be due to the longer cutting accumulates more carbohydrates which enables the seedlings to survive. In agreement with the current study [18] reported that initial cutting size for improving the growth performance of *Salix alba* L. and it is a very important factor involved in rooting ability and growth performance [46] has also reported that carbohydrates also enhance metabolic activities that occur at the base of the cuttings to aid cell division which brings about root initiation to survive.

Number of leaves

The seedlings raised on the rooting media filled with 3:1 top soil: FYM and 3:2:1 top soil: FYM: sand produced the maximum number of leaf (54.74) in the field, which was about 7.35% more than the minimum value recorded from seedling raised on top soil only. The seedlings raised from four nodes cutting produce the maximum number of leaf (54.48) at field, which was about 3.8% more than the lowest value recorded from seedlings raised from two nodes (Table 10).

Table 10: Survival, number of leaf and growth performance of chaya after 2 months of transplanting in field at Dire Dawa in 2018.

Rooting Media	Survival percentage (%)	Number of leaves per plant at field
Top soil only	3.967^b (79.40)	50.99^b
3:1 top soil: FYM	4.033^b (80.66)	54.74^a
3:2:1 top soil: FYM: Sand	4.583^a (91.60)	54.70^a
Node Number		
Stem cutting with two nodes	3.944^b (78.88)	52.48^c
Stem cutting with three nodes	4.006^b (80.12)	53.47^b
Stem cutting with four nodes	4.633^a (92.66)	54.48^a
LSD (5%)	0.34	0.71
Overall Mean	4.194	53.48

Means in a column followed by the same letter are not significantly different at 5% level of probability.

Conclusion

In conclusion, chaya seedlings from cutting responded well to type of rooting media containing top soil: FYM: sand, cutting length with three and four nodes and seedbed growing method. Therefore, in the study area it could be advised to use combined mixtures of3:2:1 top soil: FYM: sand and stem cutting with three or four nodes grown on nursery bed for raising planting materials and field establishment. However, this study was conducted only one season at one location and thus, similar studies have to be done under various agro-climatic and soil condition to make a conclusive recommendation.

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