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Participatory Development of Quality Seedlings in Lemon Verbena (*Alloysia triphylla* L.) Using Stem Cuttings

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ABSTRACT

This experiment fills in existing information and knowledge gaps on asexual propagation of lemon verbena by involving two farmer's research groups (FRGs) at Sembero Rogicha and Dawile Kebeles during 2011 starting from the beginning of August to the end of October. Each FRG has 10 member farmers. The experiment consisted of three levels of the part used (top, middle and bottom) and four levels of node number (three, five, seven and nine) in a factorial combination. The nursery experiments were laid out in a randomized complete block design with three replications. Data on survival count, survival percentage, number of branches/seedling, number of leaves/branch and number of leaves/seedling were recorded in a participatory approach. Mean squares from analysis of variance revealed the existence of a very highly significant influence ($P < 0.001$) of cutting position, node number and the interaction effect of cutting position with node number on all parameters considered in the study. Bottom cuttings with 3 and 5 nodes demonstrated significantly higher respective values of survival rate (81.67 and 78.33%), number of branches/seedling (6), number of leaves/branch (25) and number of leaves/seedling (104 and 137) and lowest values of these parameters were recorded for top cuttings. Similar results were obtained with the FRG member farmers' evaluation criteria. Therefore, bottom cutting position with 3 and 5 nodes could be recommended for the development of quality lemon verbena seedlings using stem cuttings under good nursery management.

Keywords: asexual propagation, lemon verbena, FRG, node number, part used

INTRODUCTION

Lemon verbena (*Aloysia triphylla* L.) is a perennial shrub that belongs to the Verbenaceae family (Gomes *et al*. 2006), named so due to its whorls consisting of three (tri) leaves (phylla) at each node (Beemnet *et al*. 2010). Lemon verbena is locally known as Lominat. It is native to Argentina, Paraguay, Brazil, Uruguay, Chile, Bolivia, and Peru (Armada and Barra 1992; Carnat *et al*. 1999; Rotman and Mulgura de Romero 1999; Vogel *et al*. 1999; Gomes *et al*. 2006; Rebekah 2009).

The leaves of lemon verbena are the most economical part of the plant that can be used anywhere to add a lemony taste in salads, tea, milk, ice creams and jellies (Beemnet *et al*. 2010; Hanna *et al*. 2011). Likewise, the fragrant flowers are also used in tea and culinary concoctions and the essential oil obtained through distillation of the leaves is used in fragrance industries, food flavoring industries, soft drink industries, and folk medicine (Duarte *et al*. 2005). In addition, essential oil of lemon verbena has also antibacterial and antifungal properties (Hanna *et al.* 2011). Antibacterial and antioxidant activity of *A. citriodora* have been demonstrated for the essential oils, tea, and tinctures (Cowan 1999; Valentão *et al*. 2002; Ohno *et al*. 2003; Sartoratto *et al*. 2004; Pereira and Meireles 2007; Bilia *et al*. 2008). Traditionally, lemon verbena is used as folk remedy in treatments of spasms, cold and fever (Carnat *et al*. 2004), asthma, flatulence, colic, diarrhoea, indigestion, insomnia and anxiety (Van Hellemon 1986; Newal *et al*. 1996) and as source of analgesic, anti-inflammatory and/or anti-pyretic remedies (Pascual *et al*. 2001). The sedative and anxiolytic activities of lemon verbena infusions were not confirmed in clinic trials (Pascual *et al*. 2001). Due to its diverse uses and applications, it has an open and huge market potential for herbal preparation and extraction of essential oils. Currently one kilo of fresh lemon verbena leaf is sold with 15 birr (= 0.852 US$) at Wondo Genet and thus farmers are interested in growing the crop (EIAR 2009).

Despite huge market demand for the leaves locally and internationally for herbal preparations, production of essential oils and existence of great interest of farmers to grow the crop, there exists a limited information on proper cultivation and exploitation of the crop. From the different interweaving production factors, propagation is the one. As the crop does not produce seed, asexual propagation through stem cuttings is the only option for its perpetuation.

In asexually propagated plants, the development of quality seedlings through stem cuttings are influenced by different factors such as position of cutting (Beemnet and Solomon 2012), rooting hormone, rooting medium, environmental and physical factors (Wilson 1993). Even if development of quality seedlings affected by numerous factors and asexual propagation is the only option for lemon verbena perpetuation, information about its propagation using cuttings is scanty in Ethiopia.

Hence, identification of proper cutting size and cutting positions for the development of quality seedlings is one critical area that needs to be explored for lemon verbena. Therefore, this activity was designed with the objective of developing quality seedlings of lemon verbena using stem cuttings by involving farmer's research groups (FRGs).

MATERIALS AND METHODS

One year old lemon verbena mother plant maintained at Wondo Genet Agricultural Research Center was used for this experimentation. The experiment was conducted through the involvement of two FRGs at Sembero Rogicha and Dawile Kebeles on farmer's

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Table 1 Mean squares from the combined analysis of variance for tested over two FRG trial nursery sites.

Source of variation	Df	Survival count	Survival percentage	Number of branches/seedling	Number of leaves/branch	Number of leaves/seedling
Replication	2	1.62	40.62	2.24	29.13	313.92
Locations	1	86.68***	2167.01***	15.93***	199.84*	617.37 ns
Treatments	11	178.76***	4469.03***	22.36***	334.05***	10217.83***
LOC*TRT	11	4.98***	16:20***	9.34***	201.46***	2834.00***
Error	46	1.10	27.58	0.96	30.63	298.26
CV (%)		20.17	20.17	24.22	38.73	27.18

* = significant at $P < 0.05$; ** = highly significant at $P < 0.01$; *** = very highly significant at $P < 0.001$ and ns = non significant at $P < 0.5$; Treatments = cutting position and node number

nursery site during 2011. Each FRG has 10 member farmers and a total of 20 farmers were involved in the activity.

The experiment consisted of three levels of part used (top, middle and bottom) and four levels of node number (three, five, seven and nine) in factorial combination. The experiment was laid out in randomized complete block design with three replications according to Gomez and Gomez (1984). Each replication contained 12 treatment combinations.

Cuttings were taken from one year old healthy mother plants and planed in pots on August, 2011. In both farmers' nursery sites, 20 pots per treatment were considered in each replication. Proper weeding and watering of the experimental pots were carried out uniformly whenever required in a participatory approach. Throughout the experimental periods, incidence of disease, insect damage, frost and storm did not occur. The experiments were completed on October, 2011.

Data on survival count, survival percentage, number of branches/seedlings, number of leaves/branches and number of leaves/seedlings were recorded for all the treatments considered in a participatory approach. To statically analyze the differences in propagation ability of lemon verbena caused by cutting position and number of nodes tested at two farmer's nursery sites, the experimental data were analyzed statistically by analysis of variance (ANOVA) using SAS PROC GLM (2002) at $P < 0.05$. Differences between means were assessed using the least significance difference (LSD) test at $P < 0.05$.

RESULTS AND DISCUSSION

Analysis of variance

Mean square from combined analysis of variance tested at two farmer's nursery sites for evaluating the propagation ability of lemon verbena is summarized in **Table 1**. The result indicated that treatments of cutting position and node number, nursery site management and interaction effect of treatments with nursery site management exerted a highly significant effect ($P < 0.001$) on propagation ability of lemon verbena. This indicates that cutting part, node number and nursery site management are the important factors to be considered during the propagation of lemon verbena. Similar results were also reported for lemon verbena (Beemnet *et al*. 2011), stevia (Beemnet and Solomon 2012), bush tea (Araya 2005) and in many woody plant species (Hartman *et al*. 1997).

Effect of cutting position and node number on propagation ability of lemon verbena

The effect of cutting position on propagation ability for the development of quality seedlings of lemon verbena is summarized in **Table 2**. The FRG member evaluation results were also summarized in **Table 3**. Variability was observed on survival count, survival percentage, number of branches/seedlings, number of leaves/branches and number of leaves/seedlings with the variation in using different cutting positions and number of nodes. The overall highest values on survival count, survival percentage, number of branches/seedlings, number of leaves/branches and number of leaves/seedlings were recorded for bottom cutting positions while the lowers values were recorded for top cutting position. This statistical analysis was consistent with the result of the FRG member farmer's observation results (**Table 3**). Survival count varied from 1 for top cuttings with three nodes to 16 for bottom cutting with three nodes. Survival rates varied from 4.17% for top cuttings with three nodes to 81.67% for bottom cuttings with 3 nodes. Higher number of branches/seedlings (6), number of leaves/branch (25) and number of leaves/seedlings (136) were recorded for bottom cuttings with five nodes and the lowest values were recorded for cuttings taken from top cutting positions with 3, 5 and 7 nodes, respectively.

In agreement with this study, Beemnet *et al*. (2011) reported a similar trend for the nursery activity conducted at Wondo Genet Agricultural Research Center for the same plant. For many years propagation ability has been known to vary between cuttings from different parts of the same plant, especially in woody species (Leaky and Mohammed 1985) and this was correlated with structure of the stem (Hartman *et al*. 1997) or difference in chemical composition of the plant along the stem (Hansen 1986; Hartman *et al*. 1997). Similarly, higher rooting percentage was obtained for many vegetatively propagated plants when cuttings were taken from the basal part of the shoot (Hansen 1986; Hartman *et al*. 1997). Likewise, basal cuttings produced significantly higher rooting percentage than the other types of cuttings (apical and medial) in three cultivars of high bush blueberry (*Vaccinium corymbosum*) (Hartman *et al*. 1990). Similar results were also reported by Al-Saqri and Alderson (1996) for *Rosa centifolia*. Basal portion of plum also tend to root more readily as compared to sub-apical portion (Jawanda *et al*. 1991). Severno *et al*. (2011) also reported, plants originated from stem cuttings obtained from the base of the branch grew more shoot structures (buds, stems, and leaves) than stem cutting from middle and apex of the branch in jatropha (*Jatropha curcas* L.). They also reported that short cuttings favor early sprouting, but long and thick cuttings promote more shoot and root growth in jatropha. The good rooting of basal cuttings of *Schfflera arboricola* (Leaky 1983) and *Strephanotis floribuna* (Hansen 1988) are in agreement with the general statement by Hartmann and Kester (1983) that the best rooting of the cutting is usually found from the basal portions of shoots and there is a gradient in rooting response from top to base. It was also reported that the ability to form roots increased with distance from the apex (Hansen 1986), since cuttings taken from the basal portion are known to have greater accumulation of photosynthetic products, mostly carbohydrates or it could be due to juvenility factors (Jawanda *et al*. 1991).

A correlation between diameter and cutting length was reported by Leaky and Mohammed (1985) where thicker cuttings rooted well than thinner ones, perhaps because thicker cuttings contained more starch in the stem than thin cuttings. They may lead mostly to mortality of thin cuttings before getting a chance to root (Hartman *et al*. 2002). But sometimes thinner-stemmed cuttings also root better (Howard and Ridout 1992). Therefore, the existence of highly significant variation in propagation ability lemon verbena might be due to variability in thickness as well as availability of water soluble carbohydrates of the cutting materials used for propagation.

Table 2 Mean comparison of different parameters as affected by number of nodes and cutting position.

Cutting position	Node number	Survival count	Survival percentage	Number of branches/seedling	Number of leaves/branch	Number of leaves/seedling
Top	3	0.83 g	4.17 g	1.00 e	9.50 cd	19.00f
Top	5	1.00 gf	5.00 gf	1.25 e	5.69 d	13.17 f
Top	7	2.17 def	10.83 def	1.49 e	8.64 cd	12.74 f
Top	9	1.17 gef	5.83 gef	2.86 d	5.65 d	31.47 fe
Middle	3	3.00 dc	15.00 dc	4.60 bc	26.95 a	107.54 b
Middle	5	3.00 dc	15.00 dc	4.40 c	13.54 cb	55.14 dc
Middle	7	4.17 c	20.83 cde	5.94 a	13.37 cb	65.97 dc
Middle	9	2.33 de	11.67 de	6.08 a	11.10 cbd	70.50 c
Bottom	3	16.33 a	81.67 a	4.32 c	24.70 a	104.07 b
Bottom	5	15.67 a	78.33 a	5.62 ba	25.01 a	136.73 a
Bottom	7	8.67 b	43.33 b	6.01 a	16.21 b	99.09 b
Bottom	9	4.17 c	20.83 c	5.20 bac	11.10 cbd	46.90 de
$LSD_{0.05}$		1.22	6.10	1.14	6.43	20.07
CV (%)		20.17	20.17	24.22	38.73	27.18

Mean followed by the same letter with in the same column are statistically non significant at $P < 0.05$ according to the least significant difference (LSD) test.

Table 3 Pair-wise ranking for lemon verbena.

Treatments	Top-3	Top-5	Top-7	Top-9	Middle-3	Middle-5	Middle-7	Middle-9	Bottom-3	Bottom-5	Bottom-7	Bottom-9	Score	Rank
Top-3	x	Top-5	Top-7	Top-9	Middle-3	Middle-5	Middle-7	Middle-9	Bottom-3	Botom-5	Bottom-7	Bottom-9	0	12
Top-5		x	Top-7	Top-9	Middle-3	Middle-5	Middle-7	Middle-9	Bottom-3	Botom-5	Bottom-7	Bottom-9	1	9
Top-7			x	Top-9	Middle-3	Middle-5	Middle-7	Middle-9	Bottom-3	Botom-5	Bottom-7	Bottom-9	2	8
Top-9				x	Middle-3	Middle-5	Middle-7	Middle-9	Bottom-3	Botom-5	Bottom-7	Bottom-9	3	7
Middle-3					x	Middle-5	Middle-7	Middle-9	Bottom-3	Botom-5	Bottom-7	Bottom-9	4	6
Middle-5						x	Middle-7	Middle-5	Bottom-3	Botom-5	Bottom-7	Middle-5	8	3
Middle-7							x	Middle-7	Bottom-3	Botom-5	Bottom-7	Bottom-9	6	4
Middle-9								x	Bottom-3	Botom-5	Bottom-7	Bottom-9	5	5
Bottom-3									x	Bottom-3	Bottom-3	Bottom-3	11	1
Bottom-5										x	Botom-5	Bottom-9	9	2
Bottom-7											x	Bottom-7	9	2
Bottom-9												x	8	3

Effect of nursery management on the propagation ability of lemon verbena

Analysis from the combined analysis of variance revealed that nursery management exerted a very highly significant ($P < 0.001$) influence on propagation ability of lemon verbena (**Table 1**). The survival counts, survival percentage, number of branches per seedlings and number of leaves per branches were higher in the Sembero Rogicha nursery site than in Dawile (**Table 4**). According to the farmers, the good performance in Sembero Rogicha nursery site was due to the proper follow up of the FRG member farmers in the site as compared to the less proper follow up and nursery management of the FRG member farmers at the Dawile nursery site. This indicated that nursery management is one of the important factors for the propagation of lemon verbena. In agreement to the present study, Araya (2005) also reported the influence of nursery management on the propagation ability of bush tea. Likewise, Hartman and Kester (1983) also reported the contribution of nursery site environment in the propagation ability of cuttings through its effect in the mobilization of carbohydrates and growth promoters. Hence it can be said that the propagation conditions under Sembero Rogicha nursery site are suitable for the mobilization of carbohydrates and growth promoters to help the root development and there by propagation ability of lemon verbena.

Generally it was observed that nursery management, cutting position and number of nodes had an effect on propagation ability of lemon verbena and propagation of the plant in good nursery management using bottom cutting position with 3 and 5 nodes was identified in a participatory approach and this finding can be advised to be used for the development of quality lemon verbena seedlings using stem cuttings. As the activity was done in farmer's participatory approach, farmers have properly understood on how to select and validate best technologies based on their own criterion. Likewise, the farmer's knowledge capacitated and tradition of joint experimentation was promoted both in FRG and non-FRG member farmers. However, as this activity was the first to its kind on the crop, there are many questions are remaining unexplored. Some of them are effect of hormones on its propagation, physiology of rooting, media of propagation, etc.

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Table 4 Mean performance comparison of different parameters as affected by FRG trial nursery sites.

Locations	Survival count	Survival percentage	Number of branches/Seedling	Number of leaves/branch	Number of leaves/seedling
Sembero Rogicha	6.31 a	31.53 a	4.54 a	15.96 a	66.46 a
Dawille	4.11 b	20.56 b	3.59 b	12.62 b	60.60 a
$LSD_{0.05}$	0.50	2.49	0.47	2.63	8.19
CV (%)	20.17	20.17	24.22	38.73	27.18

Mean followed by the same letter with in the same column are statistically non significant at $P < 0.05$ according to the least significant difference (LSD) test.

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