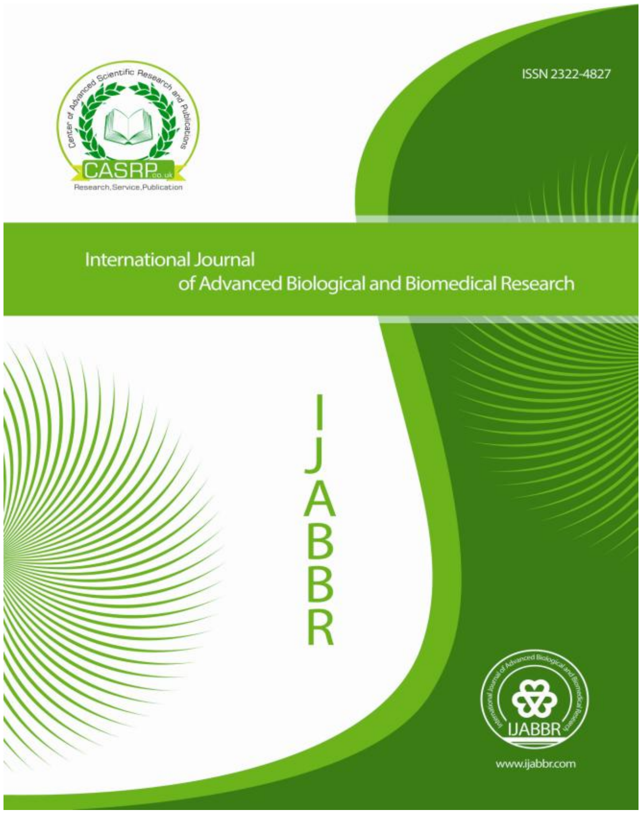
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Effect of cutting size and position on propagation ability of Sage (*Salvia officinalis* L.)

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Abstract

The investigation was conducted on wet and dry seasons of Ethiopia during the year 2012/2013 at Wondo Genet Agricultural Research Center Nursery site. Four levels of cutting size and three levels of cutting positions were arranged in randomized complete block design with three replications. Data on seedling height, branch number/seedling, root number/seedling, root weigh/seedling, root length/seedling and survival count were collected and analyzed except for root length/seedling in dry season. Cutting size exerted a significant influence (P<0.05) on all of the parameters considered in this study except on root length/seedling in dry season experiment. Cutting position also exerted a significant influence (P<0.05) on all of the parameters except on branch number/seedling, root length/seedling and root weight/seedling during dry season experiment. Interaction effect of cutting size and cutting positions didn't excreted a significant influence (P>0.05) on majority of the agronomic characters. But its effect was significant (P<0.05) on seedling height and root length/seedling during wet season experiment and on seedling height and branch number/seedling during dry season experimentation. Different perpetuation abilities of sage were observed in dry and wet season experiment. Top cuttings having a cutting length starting from 9 cm to 12cm can be recommended for sage propagation in wet season. However, bottom cutting position from 12cm to 15cm cutting size can be recommended for propagating sage in dry season using stem cuttings.

Keywords: Asexual propagation, Cutting position, Cutting size, Sage.

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1. Introduction

Sage (*Salvia officinalis*L.) is an evergreen, perennial and fast growing aromatic herb with grayish leaves and blue to purplish flowers (Hassanain, 2011). It belongs to the family Lamiaceae and is native to the Mediterranean region, though it has naturalized in many places throughout the world. The plant can grow up to 60 cm height on average. It has been grown for centuries for its many miraculous properties attributed for food and healing properties (Clebsch et al., 2003). It has a long history of medicinal and culinary use, and in modern times as an ornamental garden plant (Harrison, 2012). The leaves and essential oils are the economically important products of the sage plant.

Sage is a very common herb and its aromatic leaves are used as flavoring in cooked foods; its herbal tea is made from the fresh or dried leaves and it is said to improve digestion. So it is often used with heavy and oily foods (Phillips and Foy, 1990). The young leaves and flowers can be eaten raw, boiled, pickled or used in sandwiches; and the flowers can also be sprinkled on salads to add color and fragrance (Facciola, 1998). The leaves make excellent tooth cleaners simply by rubbing the top side of the leaf over the teeth and gums (Lust, 1983). The growing or dried plant is said to repel insects, it is especially useful when grown amongst cabbages and carrots (Haltom and Hylton, 1979; Riotte, 1978; Allardice, 1993). It was formerly used as a strewing herband has been burnt in rooms to fumigate insects (Phillips and Foy, 1990).

The processed products of sage leaves (essential oils) are known to possess carminative, antispasmodic, antiseptic and astringent properties (Lawrence, 2005). The biological properties of sage essential oil are attributed mainly to α-thujone and β-thujone, camphor and 1, 8-cineole (Raal et al., 2007). An essential oil obtained from the plant is used commercially to flavour ice cream, sweets, and baked goods (Usher, 1974; Facciola, 1998). It is also used in perfumery, hair shampoos (it is good for dark hair) and as a food flavouring (Hassanain, 2011). It is a very effective 'fixer' in perfumes and is also used to flavor toothpastes and is added to bio-activating cosmetics (Bown, 1995**).** Sage essential oil is reported to be a moderate skin irritant and is not recommended for use in aromatherapy (Barnes et al., 2007).

Productions of horticultural crops including sage are affected by a number of factors. From these, propagation is the one. Propagation success represents one of the most important gals for plant producers. During propagation, formation of adventitious roots and buds is dependent on plant cells to differentiate and develop into either root or shoot system (Araya, 2005). The process of differentiation is the capability of previously developed, differentiated cells to initiate cell divisions and form a new meristematic growing point (Hartmann et al., 1997). However, the development of adventitious roots in a variety of plant species can be influenced by different factors such as position of cutting, rooting hormone, rooting medium, environmental and physical factors (Wilson, 1993). The variation on propagation ability due to cuting posion, cutting size and season of propagation was observes for aromatic and medicinal herbs such as Lavender (Zewdinesh et al., 2013), Stevia (Beemnet et al., 2012) and Lemon verbena (Beemnet et al., 2012). Hartmann et al. (1997) also reprorted the effect of position seems to be species dependent and in some species basal or medial cuttings root best, whereas from other species apical cuttings root best. Sage was reported to be multiplied sexually by use of seeds or asexually by use of techniques such as cutting, divisions, air layering, shoot tip culture (Grzegorcazyk and Wysokinska, 2008; Nicola et al., 2005). As Sage doesn't produce fertile seed in Ethiopia, asexual propagation is the onley option to propagate sege plant in Ethiopia. Basing this, asexual propagation through stem cuttings has given attention for this particular study.

As it is stated above, sage plant has diverse uses and applications. Due to this fact, it has got an open and huge market potential for herbal preparation and extraction of essential oils in Ethiopia. Currently, Wondo Genet Agricultural Research Center is conducting different researches on this crop and there is a huge demand of investors to cultivate the plant in large scale. Despite sage is being very useful in food, pharmaceutical and cosmetics; and existence of interest from processors for commercial cultivation of the crop; there was no research conducted about propagation of this crops in Ethiopia. Therefore, this study was conducted to evaluate the effect of cutting size and cutting position on propagation ability of sage under wet and dry season of Ethiopia.

2. Materials and methods

A Sage plant maintained at Wondo Genet Agricultural Research Center botanical garden was used for this experiment.The experiments were conducted on dry and wet seasons at Wondo Genet Agricultural Research

Center nursery site during the year 2012/13. The site is located at 7°192 N latitude and 38° 382 E longitudes with an altitude of 1876m.a.s.l. The area receives mean annual rainfall of 1000 mm with maximum and minimum temperature of 12.02 and 26.72°C, respectively. Cuttings were taken from one year old healthy mother plants and planed in pots in November, 2012 for dry season experiment and in June, 2013 for wet season experiment. The experiment consisted of three levels of part used (top, middle and bottom) and four levels of cutting size (6cm, 9cm, 12cm and 15 cm) 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 twelve treatment combinations. Proper weeding,watering and carryings of the experimental pots were carried out uniformly whenever required. Throughout the experimental periods, incidence of disease, insect damage, frost and storm did not occur. The experiment was completed in February, 2013 for dry season and in September 2013 for wet season experiment.Data on seedling height, branch number/seedling, leaf number/seedling root number/seedling, root weigh/seedling, root length/seedling and survival count were collected. To statically analyze the differences in propagation ability of Sage caused by cutting position and cutting size, 20 samples were taken from each treatment. Experimental data was statistically analyzed using SAS PROC GLM, (2002) at $P < 0.05$. Differences between means were assessed using the least significance difference (LSD) test at $P < 0.05$.

3. Results and discussion

Mean square from the analysis of variance tested during dry and wet season at Wondo Genet Agricultural Research Center nursery site for evaluating the propagation ability of Sage are summarized in Tables 1 and 2. Result indicated that cutting position, cutting size, and their interaction contributed a significant influence on propagation ability of sage. Cutting size exerted a significant influence ($P<0.05$) on all of the parameters considered in this study except on root length/seedling in dry season. Cutting position also exerted a significant influence ($P<0.05$) on all parameters except on branch number/seedling, root length/seedling and root weight/seedling during dry season experiment. Interaction effect of cutting size and cutting positions did not excreted a significant influence ($P>0.05$) on majority of the agronomic characters. Interaction effect of cutting size and cutting position was significant ($P<0.05$) on seedling height and root length/seedling during wet season experiment and on seedling height and branch number/seedling during dry season experimentation. This indicates that cutting part, cutting size and season of propagation are the important factors to be considered during propagation of Sage. Similar results were also reported for lemon verbena (Beemnet et al., 2012), stevia (Beemnet and Solomon, 2012), Lavender (Zewdinesh et al., 2013), bush tea (Araya, 2005) and in many woody plant species (Hartman et al., 1997).

3.1. Effect of cutting size and position on propagation ability of Sage in wet season

The mean performance for agronomic characters of sage developed from different cutting sizes and positions tested under wet season are summarized in table 3. The interaction effects of cutting position and cutting size on seedling height and root length of sage are summarized in table 4.

3.1.1. Cutting size

The lowest values for branch number/seedling, leaf number/seedling, root number/seedling root weight/seedling and survival count were recorded for the shortest cutting size (6 cm). The values of these parameters were increased with increasing cutting size and the highest and statistically similar values were recorded at 9, 12 and 15cm of cutting size.This indicated that increasing cutting size have an impact on propagation ability of sage. In accordance with this result,propagation ability was found increased with increasing length of cutting in *Triplochiton scleroxylon* (Leakey and Mohammed, 1985) and in *Eucalyptus globules* (Wilson, 1993). However, in contrast to the present study, Howard and Ridout, (1992) reported non significant effect of cutting lenght on propagation ability of Madame Lemoine (*Syringa vulgaris*). The effect of cutting length was related to carbohydrate accumulation at the base of the cutting and that the carbohydrate amount was more optimal for root formation in cutting with long basal compared to short ones (Hartman et al., 1990). A correlation between diameter and cutting length was reported by Leaky and Mohammed, (1985) where thicker and longer cuttings rooted well than shorter and thinner ones, perhaps because larger cuttings contained more starch in the stem than thin cuttings. The better propagation ability of sage with longer cutting coud be due to the above reason.

Table 1
Mean squares of agronomic traits of sage as affected by Cutting size and Cutting position tested under wet season.

Source of variation	Df	Seedling height	Branch number	Leaf number	Root number	Root length	Root weight	Survival count
Replication(RP)	2	36ns	3.22*	119.47ns	97.63ns	7.56ns	0.24***	3.25ns
Cutting size (Cs)	2	216.33***	4.15**	618.82***	261.92**	61.01***	0.15***	70.77***
Cutting positio(Cp)	3	96.37**	3.18*	604.88***	484.12***	29.49*	0.3***	336.08***
Cs*Cp	6	59.51**	0.72ns	59.88ns	80.12ns	25.67*	0.03ns	16.71ns
Error	22	14.96	0.78	41.57	42.52	8.38	0.02	9.61
CV		21.4	43.24	25.09	36.09	23.04	41.22	29.77

***= Significant at *P*< 0.001; **= Significant at *P*< 0.01; *= Significant at *P*< 0.05; ns= Non significant at *P*< 0.05.

Table 2
Mean squares of agronomic traits of sage as affected by Cutting size and Cutting position tested under dry season.

Source of variation	Df	Seedling height	Branch number	Leaf number	Root number	Root length	Root weight	Survival count
Replication(RP)	2	8.36ns	0.16ns	29.44ns	634.46***	3.37ns	0.08ns	81.19***
Cutting size (Cs)	2	52.26***	15.67***	242.89***	697.57***	1.05ns	0.12*	71.81***
Cutting positio (Cp)	3	152.66***	0.3ns	251.07***	173.68*	1.85ns	0.01ns	28.78**
Cs*Cp	6	14.36*	2.38*	22.07ns	49.72ns	4.12ns	0.04ns	5.44ns
Error	22	5.59	0.54	16.73	46.69	3.52	0.034	4.59
CV		13.34	30.9	18.38	25.55	15.85	56.67	14.8

***= Significant at *P*< 0.001; **= Significant at *P*< 0.01; *= Significant at *P*< 0.05; ns= Non significant at *P*< 0.05**.**

3.1.2. Cutting position

The highest branch number/seedling (2.5), leaf number/seedling (30), root number/seedling (24), root weight/seedling (0.48 g) and survival count (16.17) were obtained for top cutting and the lowest values for all of these parameters were recorded for bottom cutting position. Middle cuttings were also demonstrated statistically similar values with top cutting positions for leaf number/seedling, root number/seedling and root weight/seedling.The values for branch number/seedling, leaf number/seedling root number/seedling, root weight/seedling and survival count were decreased with going from top to bottom cutting positions. This shows that top and middle cutting of sage have better propagation ability than bottom cutting during wet season. In agreement with this finding, Tirtoboma, (1988) reported that the advantage of top cutting on propagation ability and growth of stevia. Similarly, Gvasaliya et al., (1990) reported successful propagation of Stevia using top cuttings. More number of rooting using apical cuttings was also reported by Deen and Mohamoud, (1996) for rosemary (*Rosemarinusofficinalis*L.) and by Palanisamy and Kumar, (1997) for neem (*Azadirachtaindica* A. Juss). Wassner and Ravetta, (2000) also reported good propagation ability for top cuttings than none of the basal cuttings in *Grindelia chiloensis*. According to Araya et al., (1994), the difference in rooting due to cutting position can be related to the difference in the chemical composition of the shoots. As explained by Hartman and Kester, (1983) the difference in propagation ability of apical and bottom cuttings could be due to high concentration of endogenous root promoting substances in the apical cuttings which arise from the terminal buds and also more number of active cells which are capable of becoming meristematic.

3.1.3. Interaction effect of cutting position and cutting size

From the studied parameters, interaction effect of cutting position and cutting size demonstrated a highly significant ($P < 0.01$) influence on seedling height and a significant influence ($P < 0.05$) on root length/seedling only. Indicating seedling height and root length/seedlingare not consistent with the change in cutting size and position. For top cutting, the lowest values (19.47 and 20.1) of seedling height were obtained at 6cm and 15cm, respectively. Whereas the highest (23.33 & 21.2) values for this parameter were recorded at 9cm and 12cm of cutting size. For middle cutting, the lowest value (9.53cm) of seedling height was obtained at 6cm of cutting size and the values increased with increasing of cutting size and the highest and statistically similar values (20.8cm, 19cm and 21.83cm) were recorded at cutting size of 9, 12 and 15cm, respectively. Similarly, the lowest value of seedling height (3.8cm) for bottom cutting was recorded at 6cm of cutting size and increased with increasing cutting size and the highest seedling heights (19.34cm and 24.33cm, respectively) were obtained at 12 and 15cm of cutting size. Generally, for 6 and 9cm of cutting size the value of seedling height decreased when going from top to bottom; but for the longer cutting sizes (12 and 15cm), better seedling heights were observed when going from top to bottom. For root length, statistically similar and highest values were obtained at all level of cutting size for cuttings taken from top part. But for cuttings taken from middle and bottom part, the lowest value of root length/seedling obtained at 6 cm of cutting size and the highest and statistically similar values were obtained at 9, 12 and 15cm of cutting size**.** Similar to seedling height, root length/seedling decreased when going from top to bottom for the shorter cutting size.

Result obtained from wet season propagation study showed that, top and middle cuttings with cutting size from 9 to 15 cm demonstrated better values for most of the parameter considered than bottom cuttings with shortest cutting size. Thus, there is a variation in propagation ability of sage due to variation in cutting size and cutting position. The variation in propagation ability due to variation in cutting size and cutting position was also reported by Beemnet et al., (2012) in lemon verbena (*Alloysiatriphylla* L.), by Tirtoboma, (1988) in Stevia, by Araya, (2005) in Bush tea (*Aspalathuslinearis*(NL) Burm) and in many woody plant species by Hartman et al., (1997).

Table 3

Performance of agronomic traits of sage as affected by cutting size and position during wet season.

Treatments	Branch number	Leaf number	Root number	Root weight	Survival count
Cutting position					
Top	2.5^{a}	30.28^{a}	23.9^{a}	0.48^{a}	16.17^{a}
Mddle	$2.1a^{b}$	29.28^{a}	19^{a}	0.38^{a}	9.33^{b}
Bottom	1.48^{b}	17.52^{b}	11.3^{b}	0.17^{b}	5.75^{c}
$LSD_{0.05}$	0.75	5.46	5.52	0.12	2.63
Cutting size					
6 cm	1.04^{b}	13.6b	10.32^{b}	0.17^{b}	6.22^{b}
9 cm	2.4^{a}	30.13a	20.93^{a}	0.41^{a}	11.67^{a}
12cm	2.18^{a}	27.18a	22.4^{a}	0.46^{a}	12.1^{a}
15cm	2.53^{a}	31.87a	18.6^{a}	0.33^{a}	11.67^{a}
$LSD_{0.05}$	0.86	6.3	6.37	0.14	3.03

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

Table 4

Seddling height and Root length of sage as affected by cutting size and cutting position interaction during wet season experiment.

Cutting size	Cutting position					
	Seedling height (cm)			Root length (cm)		
	Top	Middle	Bottom	Top	Middle	Bottom
6 cm	19.47^{ab}	9.53^{cd}	3.8^{d}	14.29^{a}	7.73^{bc}	3.98^{c}
9 cm	23.33^{a}	20.8^{a}	14.08^{bc}	15.47^{a}	15.29^{a}	11.3^{ab}
12 cm	21.2^{a}	19^{ab}	19.34^{ab}	14.69^{a}	13.37^{a}	13.67^{a}
15 cm	20.1^{ab}	21.83^{a}	24.33^{a}	12.04^{ab}	13.97^{a}	15^{a}

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

3.2. Effect of cutting size and position on propagation ability of Sage in dry season

The mean performance for agronomic characters of sage seedling developed from different cutting sizes and positions tested under dry season are summarized in table 5**.** The interaction effects of cutting position and cutting size on seedling height and branch number/seedling are summarized in table 6**.**

3.2.1. Cutting size

The lowest values of leaf number/seedling (18), root number/seedling (18), root weight/seedling (0.2 g) and survival count (11.2) were recorded at the shortest cutting size (6cm). The values of these parameters increased with increasing cutting size and the highest values for leaf number/seedling (28) were recorded at 15cm,root number/seedling (32 and 36) at 12cm and 15cm, respectively, root weight/seedling (0.5g) and survival count (18) at 15 cm. As it is shown, most of the parameters increased with increasing cutting size which might be related to carbohydrate accumulation at the base of the cutting and that the carbohydrate amount was more optimal for root formation in cutting with long basal compared to short ones (Hartman et al., 1990). This indicated that increasing cutting size, increase propagation ability of sage. A similar propagation ability trend with increasing cutting size was recorded during the wet season experimentation. In agreement with this finding, increments of propagation ability with increasing cutting size were reported for *Triplochiton scleroxylon* by Leakey and Mohammed, (1985) and in *Eucalyptus globules* by Wilson, (1993).

3.2.2. Cutting position

The highest values of leaf number/seedling (25) and survival count (16) were recorded for cuttings taken from bottom part and the highest values of root number/seediling (27.5 and 30) were recordede for cuttings taken from top and middle part, respectively.The result obtained in dry season is on the reverse pattern of the wet seasons for these parameters (leaf number/seedling and survival count). In agreement to the present study, propagation ability difference byseason in bush tea was reported by Araya, (2005). Similarly, Hansen, (1986) and Hartman et al., (1997) reported higher rooting and survival percentage for many vegetatively propagated plants when cuttings were taken from the basal part of the shoot. Similar results were also reported by Al-Saqri and Alderson, (1996) for Rosa centifolia. Severno et al., (2011) also reported better shoot formation of basal portion in Jathropha (*Jathropha curcas* L.).

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). This result supported a higher value of root number/seedling recorded for top and middle cutting positions in the current study. Therefore, the existence of highly significant variation in propagation ability of Sage might be due to variability in thickness as well as availability of water soluble carbohydrates of the cutting materials used for propagation.

Table 5
Performance of agronomic traits of sage as affected by cutting size and position.

Treatments	Leaf number	Root number	Root length	Root weight	Survival count
Cutting position					
Top	16.98^{b}	27.53^{ab}	11.41	0.29	13.75^{b}
Mddle	24.62^{b}	30.08^{a}	12.18	0.34	13.42^{b}
Bottom	25.17^{a}	22.6^{b}	11.92	0.34	16.25^{a}
$LSD_{0.05}$	3.46	5.78	ns	ns	1.81
Cutting size					
6 cm	17.7^{b}	17.9^{b}	12.3	0.2^{b}	11.2^{c}
9 cm	18.1^{b}	20.8^{b}	11.5	0.2^{b}	13.7^{b}
12cm	24.9^{a}	31.8^{a}	11.9	0.4^{ab}	15^{b}
15cm	28.3^{a}	36.4^{a}	11.6	0.5^{a}	18^{a}
$LSD_{0.05}$	3.99	6.68	ns	0.18	2.09

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

3.2.3. Interaction effect of cutting position and size

As indicated in table 2, interaction effect of cutting size and cutting position had a significant influence only on seedling height and branch number/seedling. The lowest value of seedling height (10.4 and 11.2cm) was recorded at 6cm and 15cm, respectively of cutting size for cuttings taken from top part and the highest value (23.47cm) is recorded at the same cutting size for bottom cuttings. Concerning branch number/seedling, the lowest value were recorded for the lowest cutting size for all of cutting position used and increased with increasing cutting size. The overall highest value of this parameter was obtained at 15cm of cutting size for cuttings taken from top part.

Result obtained from dry season experiment indicated, most of the parameters recorded highest values at 12cm and 15cm of cutting size. Concerning cutting position, bottom part demonstrated highest leaf number/seedling, survival count and seedling height. Root number/seedling was highest at top and middle cutting; and branch number at top part cutting. For many years propagation ability has been known to vary between cuttings from different parts of the same plant 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). Hartman and Kester, (1983) reported that the difference in propagation ability is due to the difference in carbohydrate content of the stock, activeness of the vegetative growth and difference in micro climate of the propagation site.

Table 6
Seedling height and root length/seedling of sage as affected by cutting size and cutting position interaction.

Cutting size	Cutting positions					
	Seedling height			Branch number		
	Top	Middle	Bottom	Top	Middle	Bottom
6 cm	10.4^{e}	15.87^{d}	23.47^{a}	0.27^{g}	0.93^{fg}	1.6^{ef}
9 cm	18.07^{cd}	20.07^{abc}	19.13^{bcd}	0.93^{fg}	2.27^{cde}	1.73^{def}
12 cm	19.27^{bcd}	16.53^{cd}	20.33^{abc}	4.27^{ab}	2.93^{cd}	2.33^{cde}
15 cm	11.2^{e}	15.53^{d}	22.93^{ab}	4.67^{a}	3.47^{abc}	3.2^{bc}

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

4. Conclusion

Generally it was observed that propagation season, cutting position and cutting size had an effect on propagation ability of sage. Top cutting part with cutting length starting from 9-15 cm demonstrated better propagation ability of sage at wet season and bottom cutting with cutting length from 12-15 cm demonstrated better propagation ability during dry season. Therefore, top part with cutting length of 9 cm and bottom part with cutting length of 12 cm can be recommended at wet and dry season, respectively for the development of sage seedlings using stem cuttings. However, as this activity was the first to its kind on the crop, there are many questions are remaining unexplored under Ethiopian condions. Some of them are effect of hormones on its propagation, physiology of rooting, media of propagation, etc. Should emamided for the future.

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