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# Influence of Cutting Position, Medium, Hormone and Season on Rooting of Bush Tea (*Athrixia phylicoides* DC.) Stem Cuttings

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## ABSTRACT

The objective of this investigation was to study the effect of cutting position, rooting medium and hormone on the rooting of bush tea stem cuttings. The experiment was carried out in four consecutive seasons from 2002 to 2003 at the Hatfield Experimental Farm of the University of Pretoria, Pretoria, South Africa. The parameters recorded were cutting position (apical vs. basal), rooting medium (composted pine bark vs. sand), rooting hormone (with Seradix® No. 2 vs. no hormone), sampling days (15, 20, 25 and 30 days) and season (summer, autumn, winter and spring). Cutting position had a highly significant effect ($P<0.001$) on rooting and transplanting survival of bush tea with better rooting percentage, root length, root number and survival percentage from apical than from basal cuttings. Composted pine bark improved the number of roots developed but had no effect on rooting percentage, survival percentage nor on root length of the cuttings. The application of rooting hormone (Seradix® No. 2) during the propagation period increased root number and further increased shoot length after transplanting but not rooting percentage, survival percentage or root length. Season also showed highly significant differences ($P<0.001$) on rooting percentage, root number and root length. Rooting of cuttings was improved when propagated in autumn (longer roots) and spring (number of roots) but not in summer or winter. The results of this study suggest that vegetative propagation of bush tea could be achieved by apical cuttings propagated in composted pine bark with Seradix® No. 2 hormone in spring for 30 days since root number was the most important factor for the successful establishment of the cuttings.

**Keywords:** composted pine bark, days after planting, indole butyric acid, sand

## INTRODUCTION

*Athrixia phylicoides* DC. is one of the indigenous plants in South Africa commonly known as Bush-men's tea, Zulu tea or bush tea. It is an attractive shrub, about 50 cm to 1 m in height, much branched, with thin, white woolly stems and with pink to purple flowers (Fox and Young 1982; Roberts 1990). It is well known in open grassland and forest margins of the eastern parts of South Africa (Limpopo Province, Free State Province, KwaZulu-Natal and the eastern part of Eastern Cape Province) and Swaziland (Roberts 1990).

The people of South Africa have used bush tea for many years as a medicinal herbal tea. Throughout history people gathered this plant from the mountainous regions of their homeland and used it for cleansing or purifying the blood, treating boils, acne, infected wounds and cuts, for washing and as a lotion on boils or skin eruptions. It is also used for coughs and colds, and for loss of voice and for infected throats as a gargle (Roberts 1990). The stems of bush tea are also tied up in bundles for brooms and traded on a small scale in the Limpopo Province (van Wyk and Gericke 2000).

Bush tea leaves contain 5-hydroxy-6,7,8,3′,4′,5′-hexamethoxy flavon-3-ol considered by Mashimbye *et al*. (2006) to be a new flavonoid with no caffeine content or pyrrolizidine alkaloids (McGaw *et al*. 2007), thus justifying its medicinal potential. Other agronomic practices such as mineral nutrition have been reported to improve growth (Mudau *et al*. 2005), total polyphenols (Mudau *et al*. 2006, 2007b, 2007c), tannins (Mudau *et al*. 2007d) and total antioxidant contents (Mogotlane *et al*. 2007).

The potential of the species for domestication and development as a commercial health tea has been reported by van Wyk and Gericke (2000) and Mudau *et al*. (2007a). Data that describe vegetative propagation of bush tea by stem cuttings is lacking. It is known that the rooting success of cuttings is dependent on factors such as position of the cuttings on the shoots (Hasen 1986, 1988), rooting medium used (Jawanda *et al*. 1991; Hartmann *et al*. 1997), presence or absence of hormone and concentration (Al-Saqri and Alderson 1996), season when the cuttings were made (Leaky 1983, 1986; Klein *et al*. 2000) as well as physical and environmental factors (Wilson 1993).

The second step after the development of adventitious roots by cuttings is survival of rooted cuttings (Hartmann and Kester 1983). Cuttings of many plant species can develop roots but do not survive for a long time after rooting (Hartmann and Kester 1983), possibly due to attack by different microorganisms after rooting (Wassner and Ravetta 2000) or inability to recover after transplanting or due to their failure to adapt to the field environment (Berhe and Negash 1998). The survival ability of rooted bush tea stem cuttings is not known. In order to establish the most commercial viability and sustainability of the bush tea industry, the concept of plant domestication is very critical. Therefore, the present study was to establish how cutting position, rooting medium, hormone and season influenced rooting and transplanting survival of bush tea stem cuttings. The present results will facilitate the establishment of cheap, reliable and simple techniques of propagation which will be easy when establishing a bush tea industry in South Africa.

Received: 25 August, 2007. Accepted: 15 October, 2007.

Original Research Paper

## MATERIALS AND METHODS

### Experimental site and plant material collection

An experiment on vegetative propagation of bush tea was carried out on a mist bed in a greenhouse located at the University of Pretoria's Experimental Farm (25° 45′ S, 28° 16′ E). The propagation unit was supplemented with 24 hr a day misting and fogging systems (Environmist, Pretoria, South Africa) which worked automatically based on the humidity of the greenhouse. The used mist bed was 5 m long, 1.5 m wide and 1 m high supplied with an automatic misting system operating through misting nozzles. Throughout the experimental period, the temperature of the greenhouse was measured using a thermograph. The measured mean minimum and maximum temperatures during the study period were 17°C and 34.7°C in summer, 12.8°C and 29.6°C in autumn, 9°C and 27.8°C in winter and 13°C and 34.2°C in spring, respectively.

Six mature bush tea stock plants were selected from the mountain range of Venda in the Muhuyu village, Thohoyandou District during November 2002. As Hartmann *et al*. (1997) stated "stock plants that have been injured by frost or drought, stunted by excessive flowers or fruiting or by lack of soil moisture or proper nutrition should be avoided". Healthy, botanically authenticated true-to-name and type stock plants were dug out with intact soil around the root zone from the mountain range of Venda (Vhembe District, Limpopo Province, South Africa). The plants were potted in large black polyethylene bags (43 cm height; 32.5 cm width) containing composted pine bark as propagation medium, followed by taking them into a greenhouse with approximately 40% shading to be raised and left for about three months until full recovery from the shock of transplanting. The plants were then irrigated with approximately 2 L per day. In each season, the plants also received organic foliar fertilizer of NITROSOL® (8% N: 2% P: 5.8% K 4 ml per L of water; Bayer, Pretoria, South Africa) and a fungicide (Benomyl® (5 g per 10 L of water); Bayer, Pretoria, South Africa).

### Experimental design and treatment details

A randomised complete block design with four blocks and ten replications was used as a design to evaluate the effect of the source of the cuttings (position on the stem: apical or basal) with presence or absence of hormone using two different rooting media in four seasons (summer, autumn, winter and spring 2002 to 2003).

Two different propagation media, namely sand and composted pine bark were used in the experiment. The two media were randomly assigned to seedling trays with 5 × 3 × 4.5 cm (width, breadth and depth) cells. To get the medium moist before planting the filled trays were put under a mist system set to come on at 2 min intervals for 8 seconds. A mercury thermometer was inserted to a depth of 2 to 4 cm to measure the misting bed temperature. The measured temperature for a 48 hr period immediately prior to propagation varied between 17 to 29°C in the four seasons.

Shoots of 16 to 32 cm long were cut from the stock plants early in the morning (between 06:30 and 07:30), and wrapped with wet tissue paper followed by immediately placing them in plastic bags in order to keep them cool and turgid until taken to the working area. Working on the humid misting bed, shoots were further divided into a total of 320 semi-hardwood cuttings each with 8 cm length and 0.1 to 0.3 cm diameter range. Bottom leaves were clipped off, leaving only the top three followed by taking the fresh mass and initial circumference of each cutting. The bases of the cuttings were dipped in water and depending on the treatment type (with or without hormone) were then dipped into a rooting hormone powder (Seradix® No. 2) consisting of 0.3% IBA (4-indole-3-butyric acid) in a talc to a depth of approximately 1 cm. Excess rooting powder was tapped before planting. According to Hartmann and Kester (1983) in order to avoid brushing of the powder during planting, a trench was made in the rooting medium with a stick. The cuttings were directly planted after treatment into the pre-wetted rooting medium (composted pine bark and 0.8 mm sand) to a depth of 2 cm. Throughout the experimental period, cuttings were assessed for rooting, root number and length of the longest root and fresh and dry mass gained after 5, 10, 15 and 20 days in summer and 15, 20, 25 and 30 days in autumn, winter and spring from planting time.

Another experiment was conducted to evaluate the rooting success and transplanting survival of the cuttings. The setup was done based on the best rooting performance of the cuttings from the previous experiment. A total of 160 cuttings, two rooting media (sand and composted pine bark), two hormone treatments (with or without Seradix® No. 2) and two cutting positions (apical and basal) were used in a randomized complete design. The cuttings were left on the mist bed for 45 days. After 45 days from planting, all the rooted and non-rooted cuttings were transplanted to black polyethylene bags 17.5 cm high and 12.5 cm wide filled with composted pine bark. The transplanted cuttings were left in the propagation site for two weeks before moving them to a greenhouse in order to recover from the transplanting shock. After two weeks the plants were moved to the greenhouse where the stock plants were growing and were left to grow for two months. After two months from transplanting, the plants were assessed for rooting, root number, root length, root weight, leaf number and leaf area.

### Statistical analysis and data collection

Data collection was done by carefully separating the rooted cuttings from the seedling trays followed by washing the root zone with water. The collected data were subjected to ANOVA (analysis of variance) and the means were tested by confidence interval of 95% probability. Means were compared by Tukey test, with 5% level of significance. Data analysis was done using SAS program (Statistical Analysis System Institute Inc, 1999-2001).

## RESULTS

### Response of bush tea stem cuttings to rooting medium

Results in **Fig. 1** show that the propagation medium used did not affect the rooting percentage of bush tea stem cuttings regardless of season. However, rooting percentage was more favourable in autumn and spring.

The differences in root number were highly significant ($P<0.001$) during summer, autumn, winter and spring due to medium (**Fig. 2**). In summer (**Fig. 2A**), sand was better at 15 days after planting (DAP) but composted pine bark was better at 20 DAP. In autumn (**Fig. 2B**), high number of roots were produced in composted pine bark than sand with the highest root number at 20 DAP. In winter (**Fig. 2C**), at 15 DAP composted pine bark was better but the same as sand at 20 DAP. However at 25 and 30 DAP, high number of roots were produced in composted pine bark than sand. In spring (**Fig. 2D**), composted pine bark at 20 DAP was better

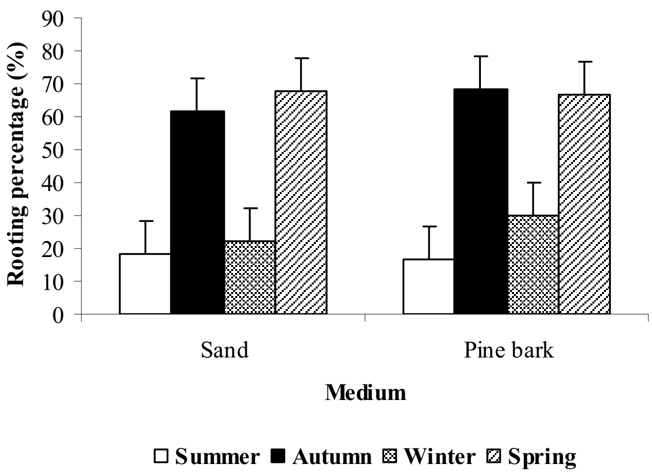


**Fig. 1** Effect of medium on rooting percentage (± standard error) of bush tea stem cuttings during summer, autumn, winter and spring. Values presented are means ($n$ = 40); means were compared by Tukey's comparison test at P<0.05.

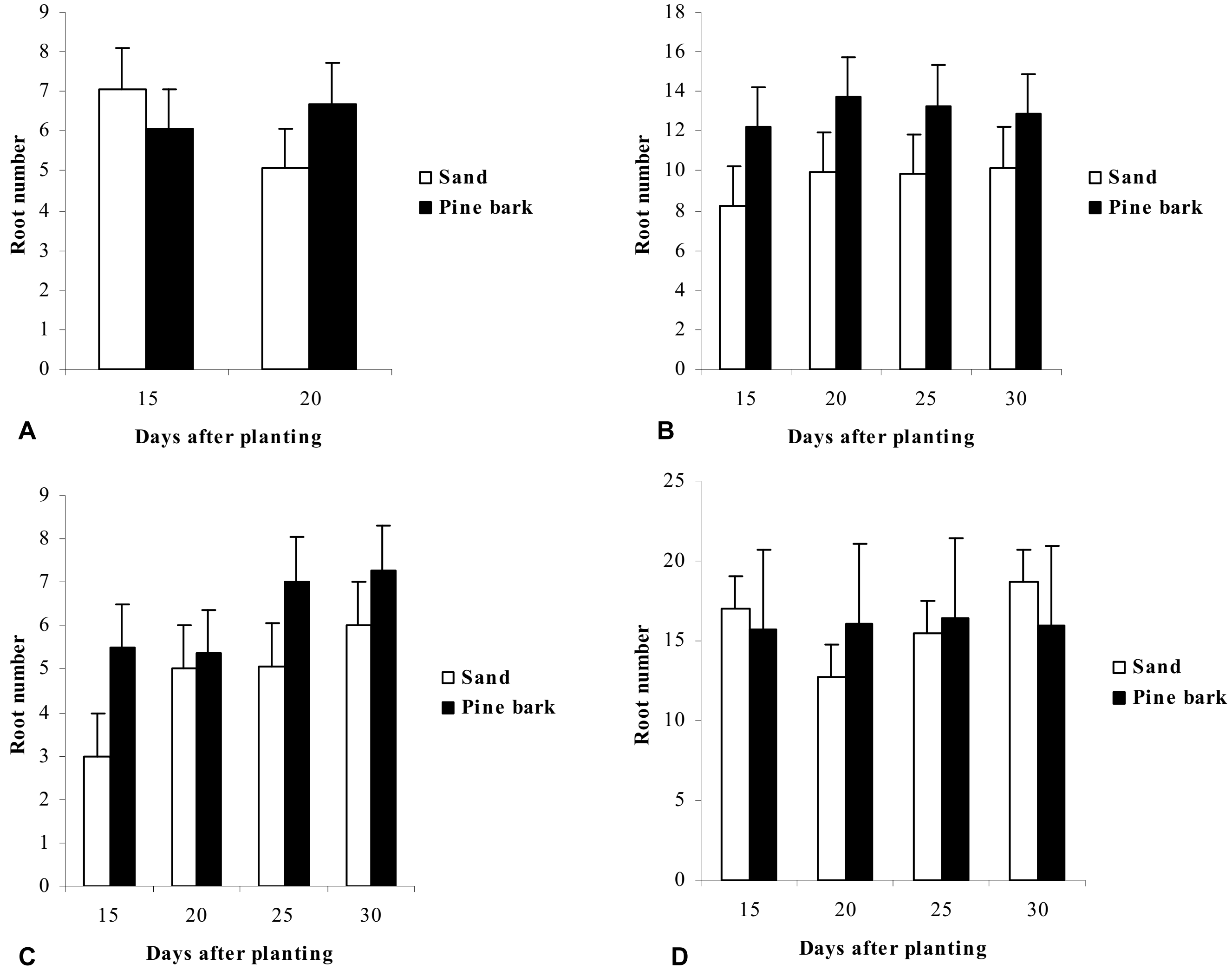


**Fig. 2** Effect of medium on root number (± standard error) per cutting during (**A**) summer, (**B**) autumn, (**C**) winter and (**D**) spring in bush tea stem cuttings. Values presented are means ($n$ = 40); means were compared by Tukey's comparison test at P<0.05.

but the same as sand at 15, 25 and 30 DAP.

Medium did not show significant differences on root length during summer (**Fig. 3A**) and winter (**Fig. 3C**). However, there were significant differences in autumn (P<0.05) and in spring (P<0.001). In autumn (**Fig. 3B**), longer roots were produced at 15 DAP in sand, but after 20 days and beyond length of the produced roots were about the same as composted pine bark. In spring (**Fig. 3D**), root length was about the same at 15, 20 and 25 DAP in sand and composted pine bark. However, at 30 DAP root length was better in sand than in composted pine bark.

## Response of bush tea stem cuttings to rooting hormone

Cuttings (apical and basal) did not show good response to the applied hormone (Seradix® No. 2) in terms of rooting percentage during summer, autumn and spring. However, there were highly significant differences (P<0.001) in winter (**Fig. 4**) due to hormone application. At 15 and 20 DAP rooting percentage was about the same with and without hormone application. However, at 25 DAP cuttings with hormone rooted to higher percentage while at 30 DAP cuttings without hormone rooted to higher percentage than cuttings with hormone.

There were significant differences in root number due to application of hormone regardless of season (**Fig. 5A-D**). With the exception of summer and 30 DAP in winter, in all other seasons cuttings with hormone produced more number of roots than cuttings without hormone. Similarly, uniformity and more number of roots after 15 days from planting were also observed when hormone was applied than without hormone application.

Furthermore, application of hormone (with or without) showed highly significant differences (P<0.001) on root length (**Fig. 6**) during autumn and spring. In autumn (**Fig. 6A**), longer roots were produced without hormone after 15 days from planting than cuttings with hormone. But after 20 days and beyond, longer roots were produced from cuttings with hormone than cuttings without hormone. Similarly, longer roots were produced from cuttings with hormone in spring (**Fig. 6B**) than cuttings without hormone. However, in summer and winter root length was not significantly influenced by hormone application.

## Response of bush tea stem cuttings to cutting position

Cuttings were found to be sensitive to cutting position from the stock plant in terms of adventitious root development. There were highly significant differences (P<0.001) between the apical and basal cuttings in summer, autumn, winter and spring (**Table 2**) in rooting percentage. Apical cuttings showed higher rooting percentage than basal cuttings in the four seasons. Fast and easy rooting ability of apical cuttings was also observed than basal cuttings.

Cutting position (apical or basal) had highly significant (P<0.001) effect on root number and root length (**Table 1**)

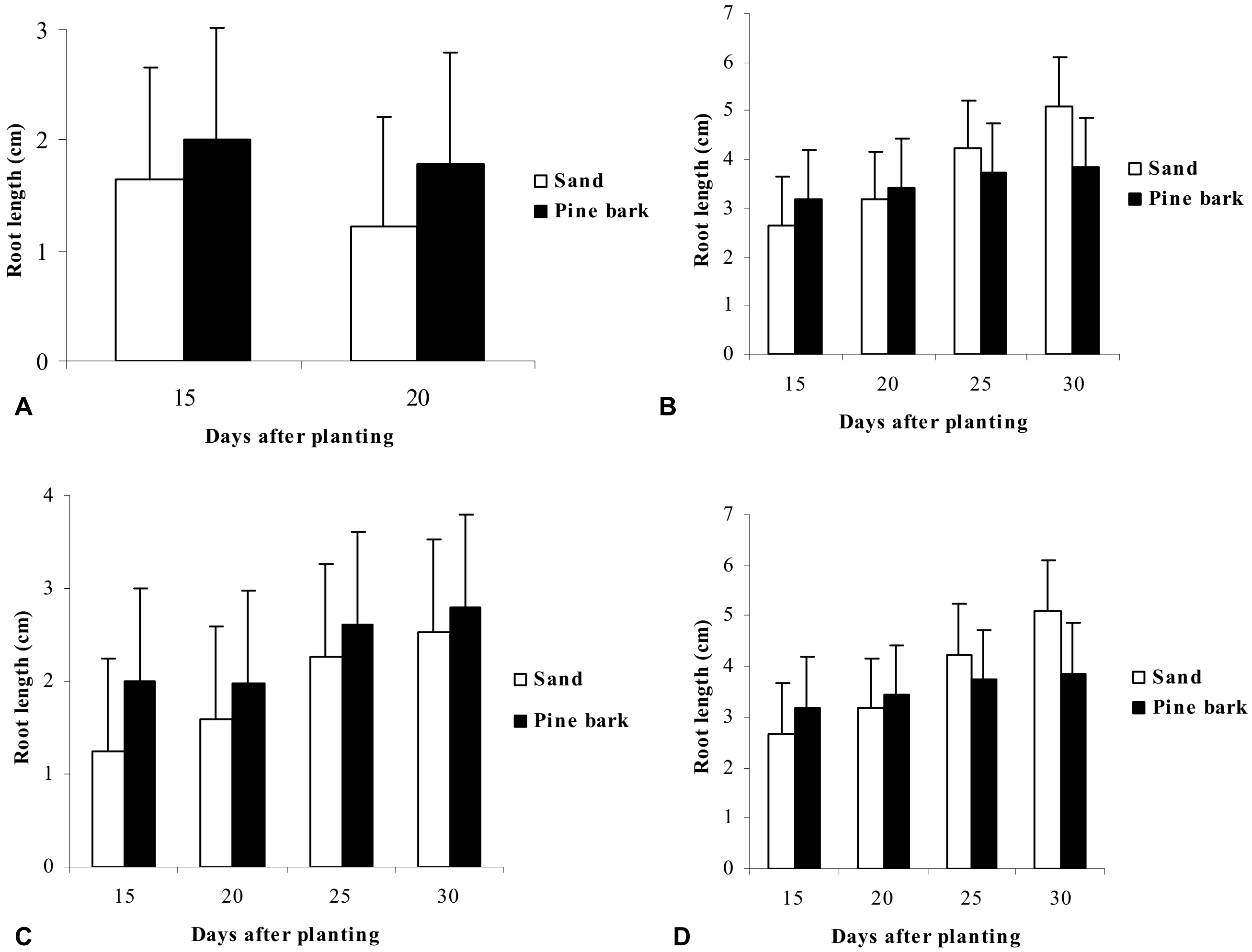


**Fig. 3** Effect of medium on root length (± standard error) (cm) per cutting during (**A**) summer), (**B**) autumn, (**C**) winter and (**D**) spring of bush tea stem cuttings. Values presented are means (*n* = 40); means were compared by Tukey's comparison test at P<0.05.

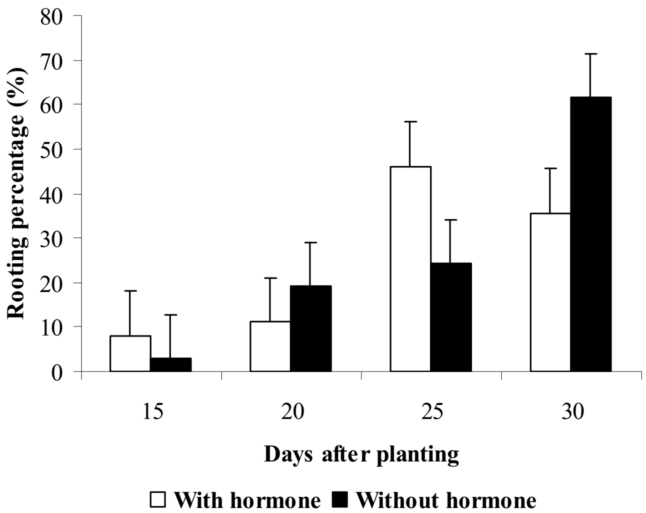


**Fig. 4** Effect of rooting hormone (with or without) on rooting percentage (± standard error) of bush tea stem cuttings during winter. Values presented are means (*n* = 40); means were compared by Tukey's comparison test at P<0.05.

regardless of season. Throughout the experimental period high number of roots and longer roots were produced from apical cuttings than basal cuttings. Apical cuttings also showed uniformity in the number of roots produced than basal cuttings with increasing DAP. In addition, length of the produced roots was observed to increase with increasing DAP from 15 to 30 days in both apical and basal cuttings.

## Response of bush tea stem cuttings to season

Rooting percentage of bush tea was affected by the season in which the cuttings were taken. High rooting percentage of bush tea was recorded in autumn and spring (**Table 2**). In both these two seasons 90 and 89.9% of the apical cuttings and 39.8 and 44.7% of the basal cuttings in autumn and spring, respectively, developed roots. In winter, rooting of apical (39.7) and basal (12.6) cuttings was low. Though the sampling day did not go beyond 20 days after planting in summer, low percentage (27.6%) of apical and basal (7.5%) cuttings was recorded as compared to autumn (apical 90% and basal 40%) and spring (apical 91.9% and basal 50%) after 20 days from planting.

Rooting percentage of cuttings was increased with increasing time after planting (**Table 2**). Percentage of rooted cuttings was low after 15 DAP but increases of 37.5%, 35% and 36.6% in autumn, winter and spring respectively, were recorded after 30 days from planting. This increase in rooting percentage with an increase in the number of days after planting (DAP) followed the same pattern throughout the experimental period except in spring for 25 DAP. The percentage of rooted cuttings after 25 days in spring was lower (67.6%) than those after 20 days (71.0%). However, the difference between them was not significant. This low percentage of rooted bush tea cuttings was due to 12.5% mortality of the cuttings before they started developing roots. On the other hand, in summer, the DAP did not go beyond 20 days since the experiment was set to see when bush tea cuttings started developing roots.

Season significantly (P<0.001) influenced the number of roots produced per rooted cutting (**Table 3**). More roots were developed during spring followed by autumn from

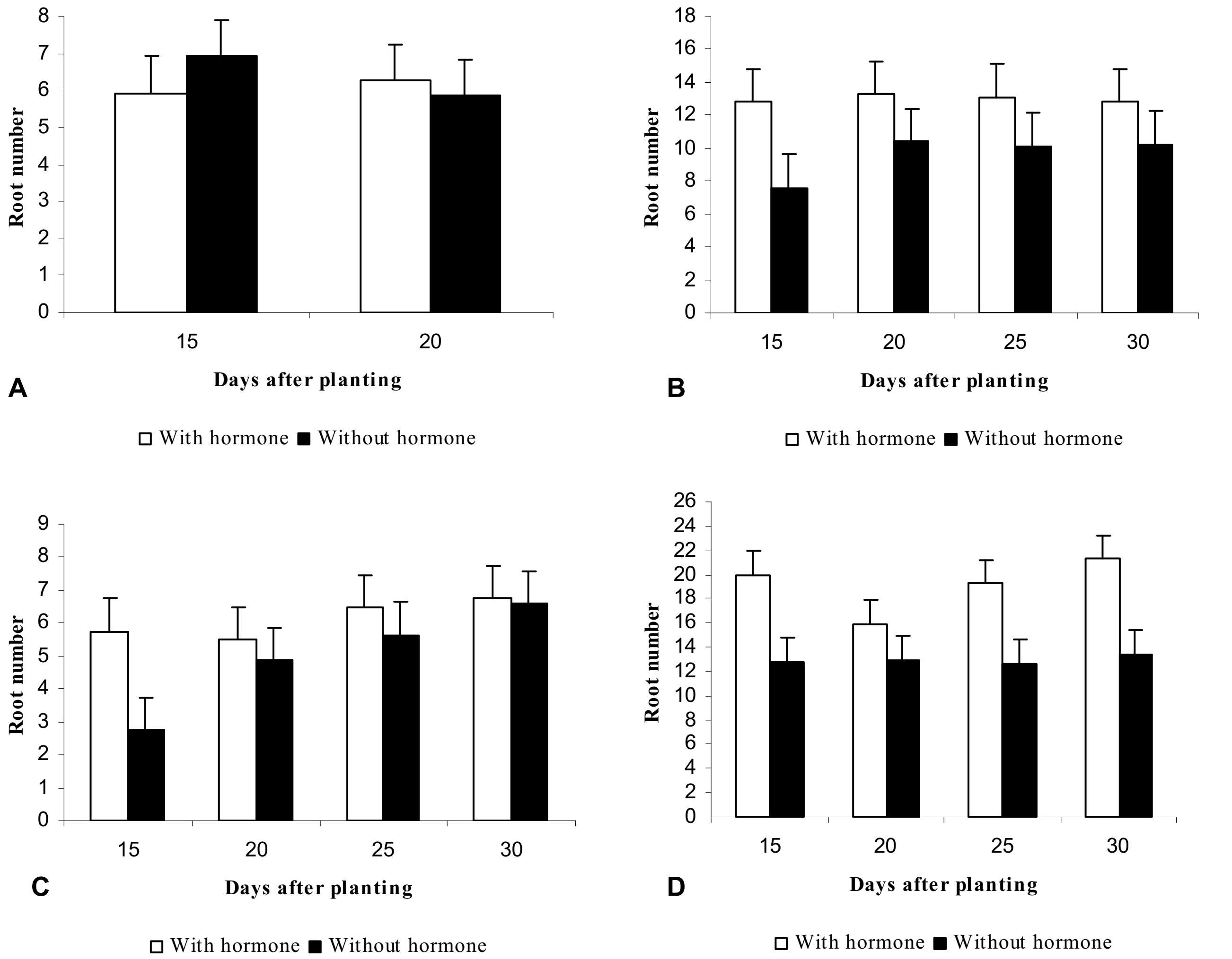


**Fig. 5** Effect of rooting hormone (with or without) on root number (± standard error) per cutting during (**A**) summer, (**B**) autumn, (**C**) winter and (**D**) spring for bush tea stem cuttings. Values presented are means ($n$ = 40); means were compared by Tukey's comparison test at P<0.05.

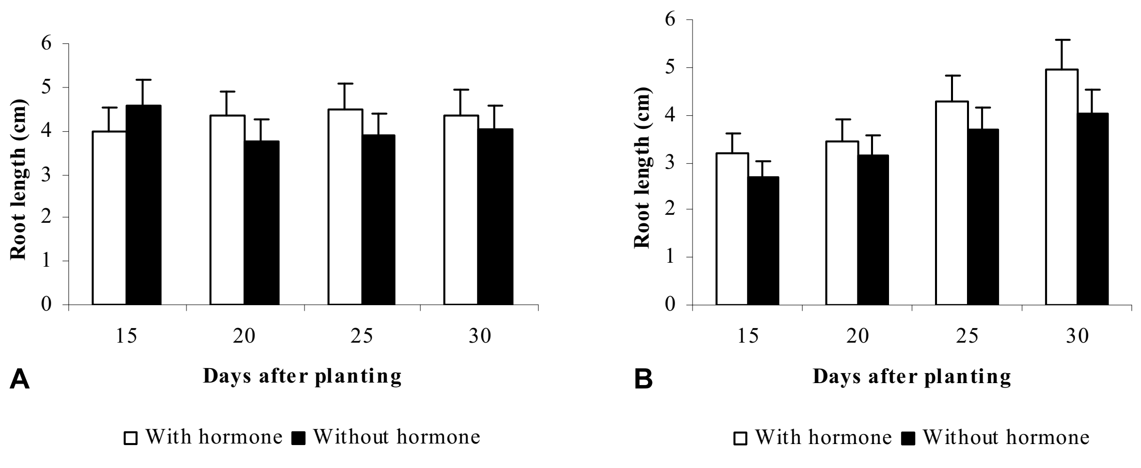


**Fig. 6** Effect of rooting hormone (with or without) on root length (± standard error) per cutting during (**A**) autumn and (**B**) spring for bush tea stem cuttings. Values presented are means ($n$ = 40); means were compared by Tukey's comparison test at P<0.05.

both apical and basal cuttings. Fewer numbers of roots were produced during winter than during autumn and spring. Though DAP did not go beyond 20 days in summer, number of roots produced were not much better than number of roots produced in winter. On the other hand, root number varied with increasing DAP (**Table 3**). During autumn, root number was uniform from 20 DAP and beyond. In spring, number of roots produced after 30 days was not much better than number of roots produced after 15 days from planting. However, during winter and summer more roots were produced with increasing DAP.

Root length was also affected by DAP (**Table 3**) with longer roots produced in winter and spring after 30 days than after 15 days from planting. Similarly, longer roots were produced after 20 days than after 15 days during summer. But in autumn, root length was the same at 15 and 30 DAP.

## Response of transplanted bush tea stem cuttings to position

Survival of rooted bush tea stem cuttings and rooting percentage of cuttings followed a similar pattern. Like rooting percentage, survival ability of rooted apical and basal cut-

**Table 1** Effect of cutting position on rooting percentage, root number and root length of bush tea in summer, autumn, winter and spring.

| Season | Cutting position | Rooting % ± SE | Root ± SE number | Root ± SE length (cm) |
|---|---|---|---|---|
| Summer | Apical | 27.6 ± 0.02 a | 9.02 ± 0.09 a | 2.04 ± 0.08 a |
| | Basal | 7.5 ± 0.02 b | 2.72 ± 0.17 b | 1.71 ± 0.09 b |
| Autumn | Apical | 90.0 ± 0.03 a | 14.76 ± 0.07 a | 5.18 ± 0.07 a |
| | Basal | 39.8 ± 0.03 b | 7.83 ± 0.13 b | 3.20 ± 0.12 b |
| Winter | Apical | 39.7 ± 0.03 a | 8.35 ± 0.12 a | 2.55 ± 0.07 a |
| | Basal | 12.6 ± 0.03 b | 3.44 ± 0.16 b | 1.29 ± 0.15 b |
| Spring | Apical | 89.9 ± 0.03 a | 19.47 ± 0.17 a | 4.14 ± 0.07 a |
| | Basal | 44.7 ± 0.03 b | 12.56 ± 0.29 b | 3.19 ± 0.12 b |

Means in a column followed by the same letter are not significantly different (P>0.05), using Tukey's comparison test.

**Table 2** Rooting percentages of bush tea cuttings after 15, 20, 25 and 30 days from planting in summer, autumn, winter and spring.

| DAP[z] | Rooting % Summer | Autumn | Winter | Spring |
|---|---|---|---|---|
| 15 | 25.0 a | 40.0 a | 5.0 a | 46.3 a |
| 20 | 45.3 b | 65.0 b | 21.7 b | 71.0 b |
| 25 | - | 75.0 bc | 28.8 bc | 67.6 b |
| 30 | - | 77.5 c | 40.0 c | 84.4 c |

DAP[z,] day after planting; Means in a column followed by the same letter are not significantly different (P>0.05), using Tukey's comparison test.

**Table 3** Mean root number and length (cm) of bush tea cuttings in summer, autumn, winter and spring after 15, 20, 25 and 30 days after planting.

| DAP[z] | Summer number | Summer length | Autumn number | Autumn length | Winter number | Winter length | Spring number | Spring length |
|---|---|---|---|---|---|---|---|---|
| 15 | 6.08 a | 1.43 a | 10.21 a | 4.28 a | 4.25 a | 1.63 a | 16.42 a | 2.93 a |
| 20 | 6.38 b | 1.90 b | 11.85 b | 4.05 a | 5.19 b | 1.78 a | 14.39 b | 3.30 a |
| 25 | - | - | 11.59 b | 4.20 a | 6.05 c | 2.44 b | 15.93 a | 3.98 b |
| 30 | - | - | 11.53 b | 4.20 a | 6.68 d | 2.67 b | 17.33 a | 4.48 c |

DAP[z,] day after planting; Means in a column followed by the same letter are not significantly different (P>0.05), using Tukey's comparison test.

**Table 4** Effect of cutting position on root number, root length (cm), leaf number, leaf area ($cm^2$) and branch number of bush tea stem cuttings.

| Cutting position | Root number | Root length | Leaf number | Leaf area | Branch number |
|---|---|---|---|---|---|
| Apical | 16.13 a | 22.97 a | 50.37 a | 103.58 a | 2.05 a |
| Basal | 14.83 b | 20.26 b | 72.75 b | 132.67 b | 3.68 b |
| LSD (α = 0.05) | 0.60 | 0.93 | 1.32 | 8.71 | 1.38 |

Means within a column followed by the same letter are not significantly different at (P<0.05), using Tukey's comparison test.

tings was also affected depending from where the cuttings were taken on the stock plants (P<0.05). A higher survival percentage was recorded from apical cuttings (84.4%) than from basal cuttings (62.5%) after two months from transplanting. However, differences in media and hormone application (with or without) used during the propagation period did not show significant differences in survival percentage of the cuttings. Interactions between medium and hormone application (with or without) and among cutting position, medium and hormone treatment also did not significantly affect the survival percentage of the cuttings.

Root number and root length of both cuttings (apical and basal) showed highly significant differences (P<0.001) (**Table 4**). Apical cuttings produced more and longer roots than basal cuttings. In addition, transplanted cuttings showed highly significant differences (P<0.001) (**Table 4**) in leaf number and area and number of branches produced. In these growth parameters, basal cuttings preformed better than apical cuttings.

### Response of transplanted bush tea stem cuttings to medium

Propagation medium also showed highly significant differences (P<0.001) in root number, but the difference in root length was not significant (**Table 5**). Cuttings propagated in sand had more roots than cuttings propagated in composted pine bark after transplanting. Furthermore, differences in propagation medium had a significant effect (P<0.05) (**Table 5**) on the number of leaves produced after transplanting. Cuttings propagated in composted pine bark produced more leaves than those propagated in sand.

### Response of transplanted bush tea stem cuttings to hormone treatment

Hormone treatment of cuttings during the propagation period showed a beneficial effect (P<0.001) by increasing the number and length of roots after transplanting (**Table 6**). Cuttings propagated with hormone (Seradix® No. 2) produced more roots than cuttings treated with no hormone. Similarly, longer roots developed from cuttings propagated with hormone treatment than those without hormone treatment after transplanting.

The interaction between cutting position, medium and hormone was highly significant (P<0.001) for root number (**Fig. 7A**). Hormone-treated apical cuttings developed more roots after transplanting when propagated in composted pine bark than untreated apical cuttings while hormone-treated basal cuttings produced more roots in sand than those without the hormone treatment. But this interaction did not show a significant difference in root length. On the other hand, root length after transplanting was affected by the interaction of cutting position and hormone treatment (P<0.001). More roots were produced by apical cuttings (**Fig. 7B**) with hormone but basal cuttings did not show any difference to applied hormone (Seradix® No. 2).

**Table 5** Effect of medium on root number, root length (cm) and leaf number of bush tea stem cuttings.

| Media | Root number | Root length | Leaf number |
|---|---|---|---|
| Sand | 16.41 a | 21.86 ns | 58.34 a |
| Composted pine bark | 14.67 b | 21.79 ns | 61.60 a |
| LSD (α = 0.05) | 0.60 | 0.93 | 1.31 |

Means within a column followed by the same letter are not significantly different at (P<0.05), using Tukey's comparison test; NS, non significant.

**Table 6** Effect of hormone treatment on root number and root length (cm) of bush tea stem cuttings.

| Hormone | Root number | Root length |
|---|---|---|
| With | 16.36 a | 22.38 a |
| Without | 14.79 b | 21.26 b |
| LSD (α = 0.05) | 0.60 | 0.93 |

Means within a column followed by the same letter are not significantly different at (P<0.05), using Tukey's comparison test.

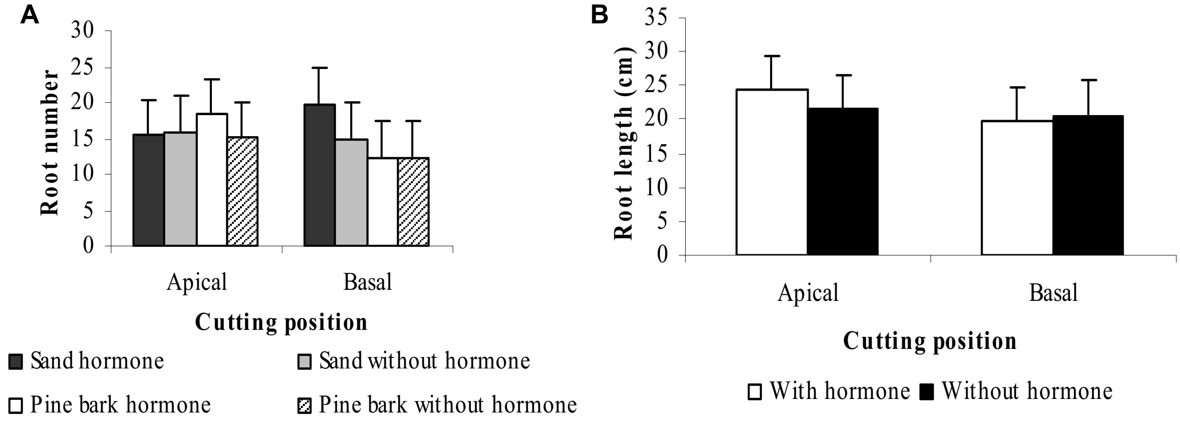


**Fig. 7** The interactive effect of cutting position, medium and hormone treatment (**A**) on root number (± standard error) (**B**) and on root length (+ standard error) of bush tea. Values presented are means ($n$ = 40; $n$ = 80, for **A** and **B**, respectively); means were compared by Tukey's comparison test at P<0.05.

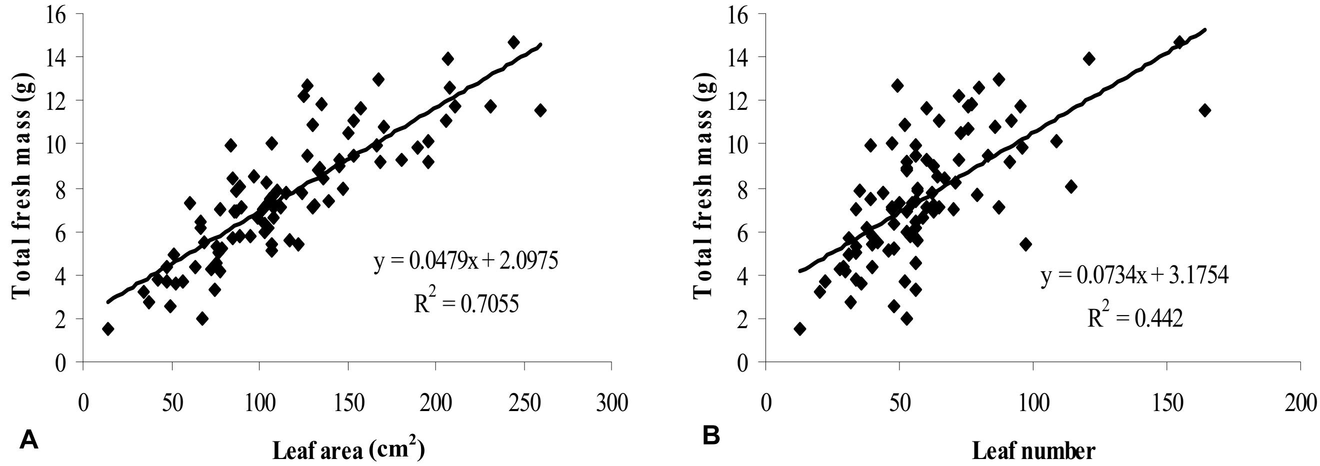


**Fig. 8** Effect of leaf area ($cm^2$) (**A**) and leaf number (**B**) on total fresh mass (shoot + root) of bush tea stem cuttings measured two months after transplanting.

## Effect of leaf area and leaf number on fresh mass of transplanted bush tea stem cuttings

Leaf area and leaf number of the cuttings had a positive linear relationship with the total fresh mass production (shoot + root) (**Fig. 8A**) and the there was also a weak relationship between leaf number and total fresh mass (**Fig. 8B**). The relationship was more prominent with leaf area ($R^2$= 0.71) rather than with leaf number ($R^2$= 0.44). This shows that, an increase in leaf area lead to a proportional increase in total fresh mass production of the cuttings than an increase in leaf number after two months from transplanting.

## Interactive effect of cutting position with hormone treatment and with medium on shoot length (cm) of transplanted bush tea stem cuttings

After transplanting, shoot length of bush tea cuttings was found to be affected by hormone treatment used on cuttings during the propagation period (P<0.001). Cuttings propagated with hormone showed longer shoots (50.10 cm) than those without hormone (47.60 cm) after transplanting. There were also highly significant differences (P<0.001) for the shoot length of cuttings, presumably due to the interaction of media and cutting position (**Table 7**). Apical cuttings that were propagated in composted pine bark produced longer shoots than those propagated in sand after transplanting. Basal cuttings that were propagated in sand produced longer shoots than cuttings propagated in composted pine bark, which was an opposite trend to apical cuttings.

**Table 7** Interactive effect of medium with cutting position and medium with hormone treatment on shoot length (cm) of bush tea cuttings.

| Media | Mean shoot length | | | |
|---|---|---|---|---|
| | Cutting position | | Hormone | |
| | apical | basal | with | without |
| Sand | 47.72 a | 50.77 a | 50.32 a | 48.18 a |
| Composted pine bark | 50.23 b | 46.62 b | 44.88 b | 51.96 b |

Means within a column followed by the same letter are not significantly different at (P<0.05), using Tukey's comparison test.

## Interactive effect of medium with hormone treatment on shoot length (cm) of transplanted bush tea stem cuttings

Interaction between medium and hormone treatment (with or without) during the propagation period affected shoot length (P<0.001) development after transplanting (**Table 7**). Hormone-treated cuttings propagated in sand developed longer shoots than untreated cuttings after transplanting. Longest shoots were developed from untreated cuttings propagated in composted pine bark than hormone-treated cuttings from composted pine bark. Cuttings transplanted with well-developed adventitious roots established more successfully compared to those that had fewer adventitious roots. Most of the cuttings transplanted with fewer adventitious roots failed to establish successfully.

## DISCUSSION

The results of these experiments indicated that bush tea stem cuttings on a mist bed could be propagated successfully. Optimum rooting percentage of cuttings was recorded, which implies that effective vegetative propagation of this species could be feasible. Mist beds have been used to propagate softwood, semi-hardwood, hardwood and herbaceous cuttings successfully. This system creates very small droplets of water (ideally 50-100 µm) on the leaf surface and the medium. Thus, it helps to cool down the leaves during hot periods and as a result reduces evapotranspiration or loses of water from the cuttings and the medium (Hartmann and Kester 1983; Hartmann *et al*. 1997). Even though this system has been used successfully to propagate many plant species, there are, however, certain factors that affect rooting of stem cuttings. These are position of the cuttings (apical or basal) on a plant shoot, type of the medium used, presence or absence of hormones and time of the year (season) when the cuttings are taken (Hartman *et al*. 1997).

In this study DAP (days after planting) showed significant effect on root development of bush tea cuttings. Apical and basal cuttings started to develop roots after 15 days from planting and the number of rooted cuttings was highest after 30 days. Maximum rooting of apical cuttings was recorded after 25 and 30 days from planting in autumn and spring. A positive correlation was also recorded on mean root number and root length with time from planting. Similar results were also reported by Ofori *et al*. (1996), where the rooting percentage of *Milicia excelsa* increased with time after planting.

Like many plant species the rooting ability of bush tea was sensitive to cutting position from the stock plant. In this experiment apical cuttings recorded higher percentage of roots, root number and root length per rooted cutting than the basal ones. Similar results were also reported by Palanisamy and Kumar (1997) in rooting of neem (*Azadirachta indica* A. Juss), where cuttings from the upper part of the branches rooted better than the lower ones. This was supported in the propagation of *Grindelia chiloensis* where none of the basal cuttings rooted (Wassner and Ravetta 2000). With the propagation of *Triplochiton scleroxylon*, a gradual reduction in rooting percentage was recorded with distance from the apex (Leakey 1983). This difference in rooting percentage of apical and basal 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 cells" which are capable of becoming meristematic (Hartmann and Kester 1983). The basal cuttings could be too mature and highly lignified to develop roots than the apical cuttings (Hartmann *et al*. 1990).

Rooting medium is one of the most important factors that affect the rooting success of cuttings (Berhe and Negash 1998; Leakey 1990). However, this experiment showed that bush tea can be propagated successfully using both sand and composted pine bark from the apical part of the shoots. Leaky (1990) also stated that cuttings of many species root successfully in a variety of rooting media. This result shows that there could be other factors interacting with medium to affect root development. These could be the difference in moisture holding capacity and aeration between the media. In this investigation, the two media showed differences in moisture holding capacity (48 and 39% for composted pine bark and sand, respectively).

Even though there were no significant differences in root development between the media, growing differences in the rooting systems were observed on the cuttings as a result of the chemical and physical property differences between the two media. Cuttings planted or propagated in sand tended to grow coarser and longer roots, whereas cuttings from the composted pine bark grew fine and highly branched roots. Hartmann and Kester (1983) also reported that when sand was used as rooting medium in some plant species, the root of the cuttings were long, non-branched, "coarse and brittle". However, when media such as a mixture of sand and peat moss, or perlite and peat moss were used, the cuttings had well branched, "slender" and flexible root types that were much more suitable for transplanting. This difference in rooting system or in the growth of the adventitious roots was related to the moisture holding capacity of the media.

The type of medium used also affected the mean number of roots and root length. Cuttings propagated in composted pine bark recorded a high root number and root length. Similarly, there was a positive response to the application of hormone on root number and length in composted pine bark than in sand. Composted pine bark had higher moisture holding capacity than sand. According to Grange and Loach (1983) and Loach (1992), the water uptake of cuttings is directly related to the water content of the medium and moisture is one of the most important factors in rooting success of cuttings. Al-Saqri and Alderson (1996) also reported that rooting response of cuttings could be influenced by the interactive effect of medium and rooting hormone, since the water and rooting hormone uptake are directly related. Composted pine bark was also reported to have phenolic compounds and this related with the theory reported by Hartmann and Kester (1983) where these compounds interact with auxins to promote the development of adventitious roots. The influence of medium on the number of roots produced and root length also varied with season, where high root number and root length were developed in spring and autumn, respectively.

Many studies stated that rooting hormones such as auxins have an important role in the development of adventitious roots, increasing rooting percentage, improving quality of roots and uniformity in rooting of cuttings. When rooting hormones are applied especially in difficult to root plants, clear differences in rooting were reported. But plants that root easily do not respond well to the application of rooting hormone (Hartmann and Kester 1983). Rooting percentage of bush tea was not found to respond well to the application of rooting hormone (Seradix® No. 2). Similar results were also reported by Shiembo *et al*. (1996) when no significance in rooting percentage occurred in vegetative propagation of *Genetum africanum* Welw when IBA from 0-250 µM was applied. Ofori *et al*. (1996) also reported similar results in *Milicia excelsa* with the application of IBA.

In this study the negative response of bush tea cuttings in terms of rooting percentage with rooting hormone application could be due to the high supplement of endogenous auxins in the shoots of the plant and these auxins might interact negatively with the application of exogenous rooting hormones. Similar suggestions were also reported by Ofori *et al*. (1996) in rooting of *Milicia excelsa*. The number of roots produced and root length per rooted cuttings of bush tea were found to respond well to the application of rooting hormone (Seradix® No. 2). Cuttings treated with the hormone produced more and longer roots than cuttings without hormone application. Similar results were reported by Ofori *et al*. (1996) where the mean number of roots per rooted cuttings was higher (80%) when the cuttings of *Milicia excelsa* were treated with IBA.

Time of the year or season when the cuttings are taken is one of the major factors for rooting success of cuttings and plant species respond differently. This might be very important to some plant species, whereas in others it might not make any difference in root development (Hartmann and Kester 1983; Klein *et al*. 2000). Similar to plant species, which are sensitive to the time of the year for rooting, the rooting percentage of bush tea was affected by season when the cuttings were taken. High rooting percentage was obtained in autumn and spring, 90 and 88% in apical and 39 and 47.13% in basal, respectively, but the difference between the two seasons was not significant. Similarly, Hartmann and Kester (1983) cited that red raspberry (*Rubus idaeus*) cuttings rooted best when the cuttings were taken from autumn to spring. They related this difference to the carbohydrate content of the stock plant since it was higher in autumn. Leafy olive cuttings also showed good rooting when taken during late spring (Hartmann and Kester 1983). In this experiment the stock plants were under active vegetative growth during spring and Hartmann and Kester (1983) stated that this is the right stage for excellent vegetative propagation of most plant species. A higher number as well as longer roots where also produced in autumn and spring by both apical and basal cuttings.

Rooting was low in winter season for apical (39.7%) and basal (12.6%) cuttings. According to Roberts (1990) bush tea starts to produce flowers from May to July. This was the time when the cuttings were taken for the winter season and the stock plants were at flowering stage. Hartmann and Kester (1983) reported that making cuttings when the plant is at flowering stage or when flowers are present has an opposite effect on vegetative propagation of the plant. This is related to the relationship between auxins and flowers since auxins inhibit the growth of flowers. Due to this, they recommended that for optimum rooting, cuttings should be made before or after flowering but not while the plants are at the flowering stage.

The other reason for low rooting during winter was the temperature of the microclimate (propagation mist bed). The minimum and maximum temperatures during this season were 3°C and 30°C, respectively, and this created 20% mortality of the cuttings before they developed roots. Most plant species root well when temperatures are about 21°C to 27°C with 15°C night temperature (Hartmann and Kester 1983). Similarly Puri and Vermat (1996) stated that cuttings of *Dalbergia sissoo* could be rooted in spring and monsoon seasons, while winter cuttings did not root at all. Hartmann *et al*. (1990) and Wilson (1993) also supported that softwood cuttings taken during spring and summer usually root more easily than cuttings taken in the winter. Generally, in most vegetatively propagated plants, growth activity is low during winter. However, as the temperature rises carbohydrates and growth promoters are mobilized and help root development (Puri and Vermat 1996).

Transplanting survival of bush tea was affected by cutting position. Apical cuttings recorded better survival percentage than basal cuttings. Also, they resulted in more and longer roots than basal ones. These differences between apical and basal cuttings could be due to a higher concentration of endogenous root promoting substances in the apical cuttings which arise from the terminal buds and also "more cells" which are capable of becoming meristematic (Hartmann and Kester 1983). Thus, apical cuttings produced more and longer roots than basal cuttings, which resulted in a higher survival percentage of the former. Basal cuttings had a higher leaf area, leaf number and number of branches than apical cuttings but these had no significant effect on survival percentage, root number and root length of the transplanted cuttings.

The difference in medium and hormone treatment during propagation did not have any direct effect on the survival and root length of transplanted cuttings. However, it had an effect on further development of roots. Cuttings rooted in sand produced further roots as compared to those propagated in composted pine bark after transplanting. The significant increase in rooting percentage of the sand-propagated cuttings could be related to the media used during transplanting (composted pine bark). Packer *et al*. (1999) stated that composted pine bark medium contain supplementary compounds (phenolic compounds), which increase root number. This effect appears to have been very limited with cuttings propagated in composted pine bark.

Application of hormone (Seradix® No. 2) during propagation had a further effect by increasing the number and length of roots produced after transplanting. An increase in root number and length of rooted cuttings after transplanting was also reported by Palakill and Feldman (1993) as a result of successful growth of the rooted cuttings. Semagn and Negash (1996) reported similar results in *Podocarpus falcatus* when plants propagated by cuttings survived well due to their greater root numbers. The same was evident for *Juniperus procera* where cuttings with more roots showed better growth and establishment than those with a low number of adventitious roots (Berhe and Negash 1998).

In conclusion, the results of these experiments demonstrated that stem cuttings on a mist bed could be used to propagate bush tea successfully. Optimum rooting percentage of cuttings was recorded, which implies that effective vegetative propagation of this species would be feasible. Cuttings started to develop roots after 15 DAP which increased with increasing the DAP to 30 days. This shows the importance of giving the cuttings more time on the mist bed for successful propagation. The major influencing factor for rooting success of bush tea cuttings was cutting position (apical or basal). It affected the root development, root number, root length and transplanting survival of the cuttings. Throughout the experimental period apical cuttings performed better in terms of rooting percentage, root number, root length and survival percentage than basal cuttings. Thus, apical cuttings are better planting materials for successful vegetative propagation of bush tea. Medium used and application of Seradix® No. 2 hormone had no significant effect on rooting percentage of the cuttings. However, it affected the number and length of roots produced. Compared with sand, cuttings propagated in composted pine bark produced a higher number of roots. Similarly, application of Seradix® No. 2 hormone in composted pine bark increased the number of roots produced. Since root numbers are most important in the establishment of a cutting, it is recommended to propagate bush tea cuttings in composted pine bark with the application of Seradix® No. 2 hormone. The other major factor in rooting of bush tea was time of the year or season when the cuttings were taken. High rooting could be obtained by taking cuttings during autumn and spring. Cuttings produced more roots in spring and longer roots in autumn. Therefore, for successful vegetative propagation of bush tea by stem cuttings, it is recommended to take apical cuttings in spring and propagate them in composted pine bark with the application of Seradix® No. 2 hormone.

## ACKNOWLEDGEMENTS

We would like to acknowledge Mr J. Marneweck and Mr L. van der Merwe at the Hatfield Experimental Farm, University of Pretoria, for their technical assistance. Eritrean Human Resource Development (EHRD) and the National Research Foundation (NRF) for funding the project.

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