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Rooting and survival of *Lobostemon fruticosus* (L) H. Buek stem cuttings as affected by season, media and cutting position

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ABSTRACT

The eight-day healing bush (*Lobostemon fruticosus*) belongs to the Boraginaceae family and is one of the oldest medicinal plants used in the Cape by Khoisan and early settlers. The natural populations of *L. fruticosus* are being destroyed by veld fires and overharvesting, leading to a need for propagation of plant material especially for commercial use. The objective of the study was to investigate the effect of season, plant growth regulator, growth media and cutting position on survival and rooting of *L. fruticosus*. A randomised complete block design was used with 2 block replications per season and 10 cuttings as an experimental unit. Treatments included four media, three plant growth regulator treatments and two cutting positions. Improved survival and better root quality was observed in cuttings planted in autumn (survival percentage 85.6% and root score 4.3) compared to spring (survival percentage 50.1% and root score 2.3). In autumn, cuttings planted in peat (91.25%) had the best survival percentage, however, only differed significantly from those planted in bark (77.5%), while in spring, cuttings planted in bark (68.33%) had significantly higher survival percentage compared to all other media {peat (52.92%); peatpol (51.67%) and sandpol (27.5%). Survival percentage was significantly higher in heel cutting planted in both bark (85.83%) and peat (78.33%) as compared to apical cuttings planted in the same growth media (bark 60% and peat 65.83%). While cuttings planted in peat consistently outperformed most other season-media treatment combinations in autumn an increase in rooting percentage, root length and root quality score was recorded in cuttings planted in bark during spring. Increased budding was recorded in cuttings planted in spring, except for cuttings planted in the sandpol media. Survival percentage, rooting percentage, root quality and budding leaves significantly increased in heel cuttings {survival percentage (61.46%); rooting percentage (41.88%); root score (3.25) and budding leaves (58.96%)} planted in spring as compared to apical cuttings {survival percentage (38.75%); rooting percentage (17.08%); root score (1.34) and budding leaves (38.54%)} of the same season. Rooting percentage (66.25%), root length (57.76 mm), root score (4.99) and budding leaf percentage were significantly higher in heel cuttings planted in bark as compared to apical cuttings. Application of PGR's produced a significantly better rooting percentage {Dip 'N Grow® (52.66%) and Seradix B® No. 2 (47.81%), significantly longer roots {Seradix B® No. 2 (36.23 mm) and Dip 'N Grow® (35.34 mm)} and a significantly better root quality score {Seradix B® No. 2 (3.64); Dip 'N Grow® (3.62)} compared to the control. Based on the current findings it is therefore recommended that, *L. fruticosus* stem cuttings can be propagated successfully using heel cutting type, Seradix B® No. 2 or Dip 'N Grow® as growth regulator application and, coco-peat as growth media if propagating during autumn or bark if propagating during spring.

1. Introduction

Lobostemon fruticosus (L.) H. Buek (Cowan and Anderson, 2014), also known as the eight-day healing bush, is one of the plants widely used in traditional medicine in the Cape provinces of South Africa (Van Wyk et al., 2013). Decoctions of the fresh or dried leaves and younger twigs are used to treat wounds, skin diseases and ringworm, while aqueous infusions are taken orally to treat internal problems, gynecological disorders and to act as a blood purifier (Van Wyk and Gericke, 2000; Van der Walt, 2005). Traditional healers also believe in the plant's anti-HIV properties (Lunat, 2011). *In vitro* studies conducted at The Nelson Mandela Metropolitan University on *Lobostemon* leaf extracts have shown that the extracts have a potent HIV-1 Reverse transcriptase inhibitory effect, suggesting that it could aid HIV-positive patients (Harnett et al., 2005). The anti-cancer properties of the plant have also been reported (Ndlovu, 2015).

The increase in demand for *L. fruticosus* as a medicinal plant has prompted a need to increase production in order to supply both the

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formal and informal market. Currently, the plant is harvested mostly from the wild and this could lead to depletion of natural populations. Although vegetative propagation of *L. fruticosus* has been reported to be difficult (Lodama et al., 2016), stem cuttings remain a method that can produce mature plants in a shorter time compared to seed (Rice and Rice, 2011) while at the same time maintaining certain desired characteristics.

Besides intrinsic genetic abilities of a plant, several physiological and environmental factors influence the success of vegetative propagation. The major pre-requisite in successful stem cutting propagation is the ability of the stem cutting to form a new adventitious root system as the probable shoot is already in existence (Hartmann et al., 1997). The rooting ability of cuttings is affected by several factors such as season, growth media, plant growth regulators (PGR's) and cutting position. Other elements include size, diameter and age of the cutting and the concentration of the PGR applied (Araya, 2005; Sharma and Verma, 2011).

Several studies have shown a significant variation in rooting ability of a number of species in relation to the time of the year at which stem cuttings are taken. Time of the year or season is also related to the physiological state of the plant and not merely a calendar date (Hartmann et al., 1997; Swamy et al., 2001). Functions of the growth media are to hold the cutting in place, provide moisture and permit exchange of air at the base of the cutting. A study conducted by Lodama et al. (2016) confirmed that growth media had a significant effect on rooting ability of *L. fruticosus* cuttings. Variations in rooting ability for cuttings taken from different positions from the shoot are often observed (Al-Salem and Karam, 2001; Agbo and Obi, 2007). Different cutting positions from the shoot can furthermore also affect the overall quality and subsequent growth habit of rooted stem cuttings (Tchoundjeu and Leakey, 1995).

Exogenous PGR's such as indolebutyric acid (IBA) play an important role in controlling the rooting ability of stem cuttings (Tchoundjeu et al., 2002; Husen and Pal, 2007). In many cases, IBA and indole-3-acetic acid (IAA) are highly recommended for the promotion of rooting in cutting propagation (Ofori et al., 1996; Guo et al., 2009). A delayed auxin treatment application has also been shown to positively affect rooting of *L. fruticosus* stem cuttings (Lodama et al., 2016).

If *L. fruticosus* is to be propagated on a commercial scale then detailed information is required on appropriate treatments which should be applied to stock plants and cuttings in order to obtain consistently high rooting success and survival. Vegetative propagation information for *L. fruticosus* is limited and successful commercialization of any product from this plant would require detailed propagation and cultivation information to ensure a sustainable supply of material.

The objectives of this study were thus to assess the effect of growth media, PGR's, cutting position, shading and season on adventitious root formation and survival of *L. fruticosus* cuttings.

2. Materials and methods

2.1. Plant material

Lobostemon fruticosus stem cutting material was obtained from mature *L. fruticosus* plants grown at the Agricultural Research Council Vegetable and Ornamental Plants in Roodeplaat (near Pretoria, Gauteng, South Africa). The stem cuttings were taken from established stock plants growing under 30% shade cloth. Stock plants were originally planted in 2010 and have been regularly cutback for use as cutting material. Cutting material was approximately 12-month-old stems. Cuttings were taken in spring (September 2013) and autumn (May 2014) from new shoots just after a flush of growth occurred and the wood was partially matured. Each cutting was collected randomly from a number of the mother plants. Desirable stock plants selected were disease free, moderately vigorous, true-to-type and uniform.

The diameter of the cuttings varied from 2.10 to 4.90 mm and the length of the apical cuttings were all approximately 80 mm. The average length of the heel cuttings varied from 61 to 155 mm. Leaves on the bottom half of the cuttings were removed and the remaining leaves on each cutting was cut back by 50%.

2.2. Treatments

In both spring (Sept) and autumn (May) trials, four different growth media were used namely: (1) sandpol 2:1:1:4 (*v*/v) washed river sand: sifted palm-peat: vermiculite: polystyrene; (2) peatpol 1:1 (v/v) coco-peat: polystyrene; (3) peat: coco-peat and (4) bark: composted pine bark. Three different PGR treatments were used namely: (1) Seradix® B No.2 (active ingredient 4-[indol-3-yl]-butyric acid 3 g/kg); (2) Dip 'N Grow® (active ingredients indole-3-butyric acid 1 g/kg and 1-naphthaleneacetic acid 0.5 g/kg), combined with Dip Gel™ diluting gel (polysaccharide carbohydrate polymer) and (3) control with no growth regulator treatment. The two different shade treatments used were: (1) fleece and (2) plastic. The two different cutting positions used were (1) apical and (2) heel. Many studies have found that the application of exogenous PGRs such as IBA is beneficial for the promotion of rooting in cuttings (Mesen et al., 1997; Araya et al., 2007; Guo et al., 2009; Kontoh, 2016).

For planting of cuttings, sanitised polystyrene trays (56 ml/7.168 ltray plugs) were filled with one of the four growth media. The trays were then kept under constructed hoop houses with either plastic or fleece sheeting inside of a polycarbonate greenhouse with fan and pad set-up. Before planting cuttings were completely immersed in a solution of Sporekill™ plant sanitiser (active ingredient didecyl dimethyl ammonium chloride 120 g/l) solution for about 5 s, to reduce the incidence of pathogens (Fourie and Halleen, 2006). The basal part of each cutting was then dipped into one of the selected growth regulator treatments and planted into prepared seedling trays. Each entire seedling tray with planted cuttings was then covered individually with fleece to maintain high relative humidity for the first 10 days after planting. The seedbeds (bottom heating) were heated to a temperature of (25 ± 2 °C) and misting in the greenhouse was scheduled to irrigate for 60 s every 60 min from 8 h00 to 16 h00, daily for the duration of the experiment. Water holding capacity of the four media was measured before the start of the trials using the equation:

Water holding capacity = Percentage porosity – percentage airspace (Gessert, 1976).

Water holding capacity of the four media calculated was: bark – 40%, peat – 90%, peatpol – 68% and sandpol 20%.

2.3. Experimental design

A split-plot set-up with randomised complete block design with two replications was used with stem cutting position as the main effects. The subplot treatments were arranged as a 4x3x2 factorial combinations (4 media × 3 growth regulators × 2 shade) randomised within each of the 2-main plot treatments. An experimental unit consisted of a tray with 10 cuttings. The complete design above was replicated with different randomisations in the two different seasons.

2.4. Data collection

Data were collected at weekly intervals starting at 7 days after planting. Parameters measured were: number of browning leaves per cutting, browning stems, rotting, budding flowers per cutting, budding leaves per cutting and survivability. Harvesting was done after 11 and 12 weeks in both the autumn and spring trials respectively. At harvest stage, the cuttings were assessed for: survivability, length of longest root and a root quality score. Due to the roots' brittle and herbaceous nature, it was impossible to remove all the media from the roots, therefore the fresh and dry weights of the roots could not be measured. The presence of media on the roots did not influence the measuring of the root length. The root quality was measured using the following scoring system: (1) no roots, (2) callus, (3) one to two roots, (4) roots branching, (5) roots fill one quarter of the cavity, (6) roots fill one half of the cavity, (7) roots fill three quarters of the cavity, (8) roots emerging from the bottom of the cavity (few), (9) roots fill entire cavity and (10) roots emerging from the bottom of the cavity (many) (Low and Hackett, 1981; Peer and Greenwood, 2001).

2.5. Statistical analysis

Data of the two seasons were first tested for comparable magnitude (equal variances) using Levene's test (Levene, 1960). An appropriate split–plot analysis of variance of the combined data was performed using the two seasons in combination with the main plot factors (John and Quenouille, 1977). The Shapiro–Wilk's test was performed on the standardised residuals to test for deviations from normality (Shapiro and Wilk, 1965). In cases where there was significant deviation from normality and it was due to skewness outliers were removed until the distribution of the standardised residuals was normal or symmetrical around zero (Glass et al., 1972). Student's t-LSD (Least Significant Difference) was calculated at a 5% significance level to compare means of significant source effects (Snedecor and Cochran, 1967). All the above data analyses were performed with SAS version 9.3 statistical software (SAS, 1999). The Pearson's coefficient of correlation was calculated between all the measurements.

3. Results and discussion

The shading did not affect any of the parameters measured and will not be discussed further. The PGR application had an effect on the rooting ability of *Lobostemon* stem cuttings and did not affect any of the other parameters measured. The PGR application produced a significantly better rooting percentage {SeradixB (47.81%); DipGrow (52.66%) and control (34.38%)}, significantly longer roots {SeradixB (36.23 mm); DipGrow (35.34 mm) and control (24.77%)} and a significantly better root quality score {SeradixB (3.64); DipGrow (3.62) and control (2.66)} than the control treatment (Table 1). Many studies have shown that exogenous PGR application such as auxins play an important role in the development of adventitious roots, thus improving root quality and uniformity of rooting in cuttings (Mesen et al., 1997; Araya, 2005; Guo et al., 2009; Yeboah et al., 2010). Lodama et al. (2016) reported that *L. fruticosus* stem cuttings with delayed treatment of PGR auxin had significantly higher rooting percentage than those without treatment.

3.1. Survival of cuttings

Significant interactions between growth media and season, cutting position and season and cutting position and growth media were observed. Season and growth media had a significant interactive effect on survival percentage (Fig. 1a). During autumn cuttings planted in the peat (91.25%) growth medium resulted in the best survival percentage, although the survival percentage only differed significantly from that of the cuttings planted in bark (77.5%). Although cuttings planted in bark had the lowest survival percentage in autumn compared to other media in the same season, cuttings in bark (68.33%) displayed the best survival during spring, with significantly higher values than the other three growth media (Fig. 1a). Lower ambient temperatures in autumn may have led to less transpirational loss from cuttings, resulting in waterlogging and a lack of oxygen to roots (anoxia) in bark media, causing death of cuttings before root initiation could occur (Hartmann et al., 1997). The bark media is likely to have reduced the amount of airspace relative to the other media used due to its higher water-holding capacity.

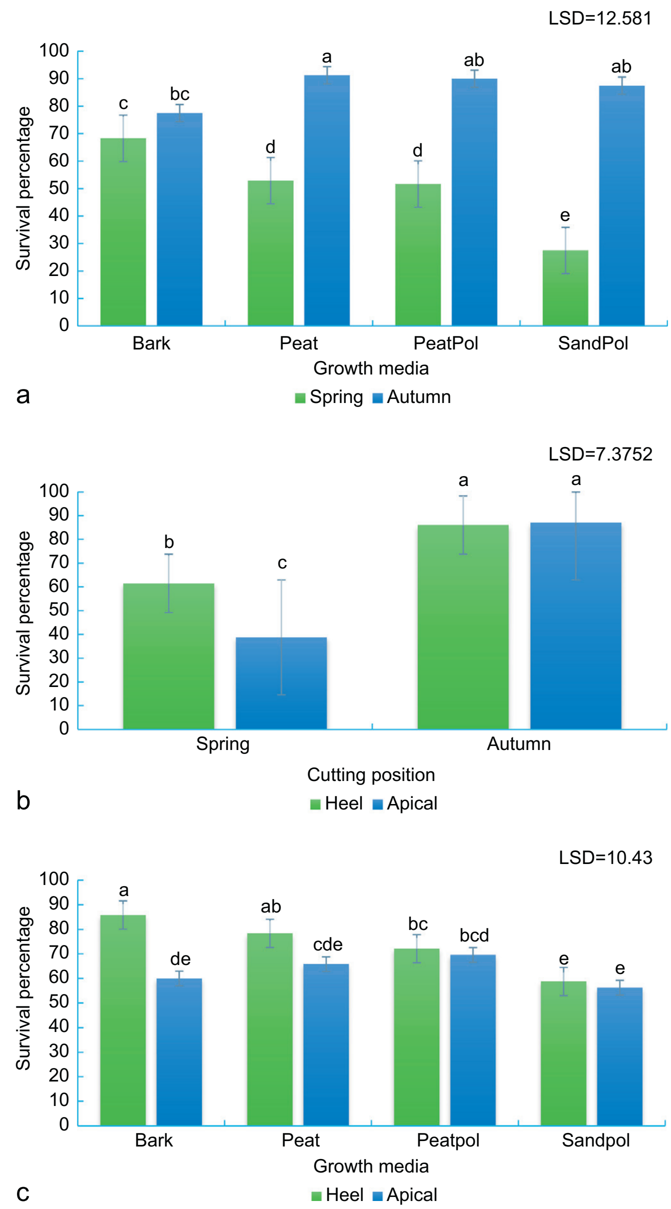

Fig. 1. a) The interactive effect of season and growth media on survival percentage of *L. fruticosus* stem cuttings. b) The interactive effect of season and cutting position on survival percentage of *L. fruticosus* stem cuttings. c) The interactive effect of cutting position and growth media on survival percentage of *L. fruticosus* stem cuttings.

Cutting position did not affect the survival percentage of cuttings during autumn, but heel cuttings resulted in a significantly higher survival percentage during spring (Fig. 1b). The cuttings planted in spring during higher temperatures may have increased transpirational loss and stress, compared to those planted in autumn. Heel cuttings may have performed better in spring due to the piece of wood attached, which increased the surface area for water uptake. The increase in volume created by the heel in the basal cuttings may have provided an

Table 1
Effects of PGR's on survival percentage, rooting percentage, root length and root quality score of *L. fruticosus* stem cuttings.*

PGR application	Survival %	Rooting %	Root length (mm)	Root quality score
Seradix® B	72.66	47.81a	36.23a	3.64a
Dip 'N Grow®	68.59	52.66a	35.34a	3.62a
Control	63.7	34.38b	24.77b	2.66b
$LSD_{(Pr = 0.05)}$	NS	7.9486	7.5468	0.6294

* Means with the same letter are not significantly different.

increase in storage for assimilates. Tchoundjeu and Leakey (1995) suggested that cuttings which have lower volumes do not provide storage capacity for assimilates coming from leaves. Similar results were reported by Saifuddin et al. (2013) basal cuttings of *Peltophorum pterocarpum* had a significantly higher survival percentage compared to apical cuttings, although cuttings were taken during the dry season (February–August) in Malaysia and temperatures ranged from (21–32 °C).

The combination of heel cuttings and bark (85.83%) growth media resulted in the best survival percentage although not significantly different from the heel cutting/peat combination (78.33%). Both the heel cuttings and apical cuttings reacted similarly in peatpol and sandpol with regard to survival, but apical cuttings had a significantly lower survival in bark (60%) and peat (65.83%) if compared to heel cuttings in the same media (Fig. 1c). Heel cuttings may contain more phytohormones compared to apical cuttings (Idun et al., 2011) resulting in higher survival percentages. The high water holding capacity of the bark media that probably resulted in waterlogged conditions in autumn, may have resulted in better survival results in spring when transpiration rates were higher.

3.2. Rooting percentage, root length and root quality of cuttings

Rooting parameters were significantly affected by the season–media interaction (Table 2). Cuttings planted in spring in general had lower values for all rooting parameters than those planted in autumn. Cuttings planted in peat consistently outperformed most other season–media treatment combinations during autumn, but during spring bark resulted in the highest rooting percentage, root length and root quality score (Table 2) and outperformed peat in terms of root length and root quality. Similar results were reported by Lodama et al. (2016), where *L. fruticosus* cuttings planted in peat had the highest rooting percentage compared to other medium combinations. Interestingly, season and growth media also had a significant interactive effect on percentage of budding leaves. In spring, cuttings generally had more budding, except for the cuttings planted in sandpol media. The increase in budding during spring may have occurred at the expense of rooting as most of the cuttings' assimilates may have been directed at producing buds, and not been produced as a result of rooting (Araya, 2005). These rooting ability results are similar to those found in the survival results and therefore could share the same explanation. Seasonal variation also had an effect on rooting and conflicting results were reported by Ling and Zhong (2012), where cuttings of *Robinia pseudoacacia* harvested and planted in spring had higher rooting percentages than those taken during autumn. The results of this study suggest that the differences in rooting may be linked to the level of endogenous carbohydrate and root-promoting substances at the time cuttings are harvested and planted (Hartmann et al., 1997).

Significant interactions were also observed between seasons and cutting position with regard to rooting percentage, root score and

Table 2
Interactive effect of season and growth media on rooting percentage, root length, root quality score and budding leaves.*

Season	Medium	Rooting (%)	Root length (mm)	Root quality score	Budding leaves %
Spring	Bark	45.83 cd	39.98bc	3.75bc	66.25a
Autumn	Bark	53.75bc	44.70b	3.81bc	18.33c
Spring	Peat	37.08d	20.23de	2.68de	52.50ab
Autumn	Peat	75.42a	59.84a	5.43a	41.667b
Spring	PeatPol	22.50e	11.63ef	1.69ef	50.00b
Autumn	PeatPol	65.83ab	41.89bc	4.60ab	43.33b
Spring	SandPol	12.50e	6.88f	1.05f	26.25c
Autumn	SandPol	46.67 cd	31.73 cd	3.42 cd	45.00b
$LSD_{(Pr = 0.05)}$		12.98	12.324	1.0279	13.997

* Means with the same letter are not significantly different.

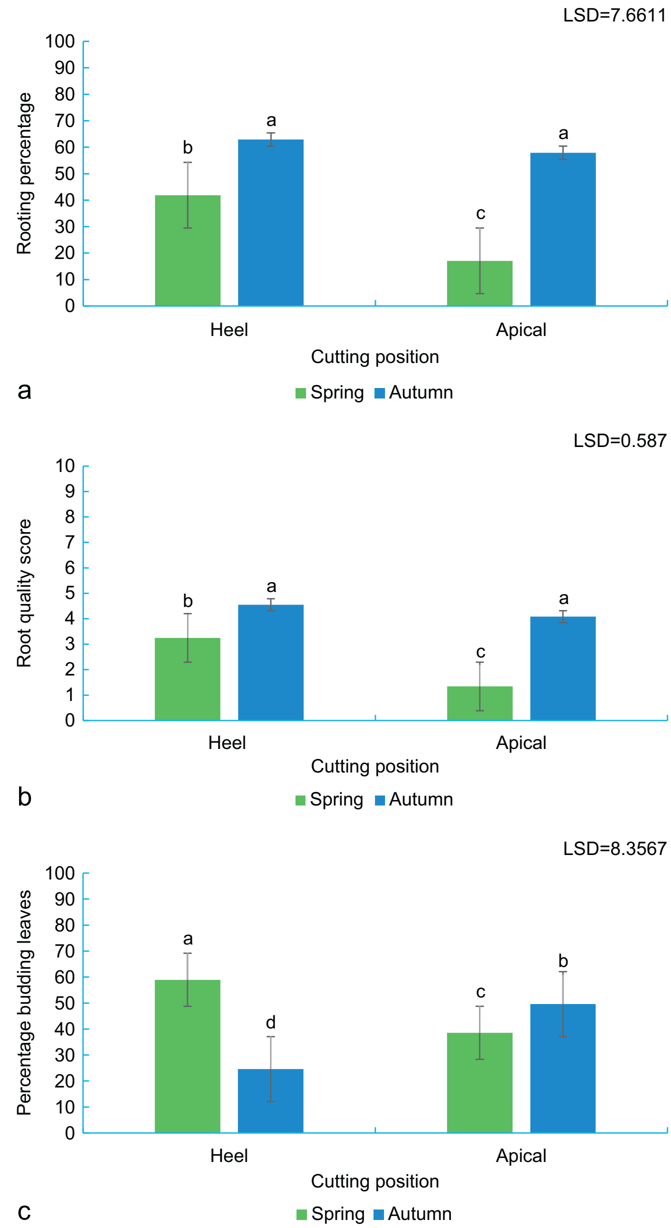

Fig. 2. a) The interactive effect of season and cutting position on rooting percentage of *L. fruticosus* stem cuttings. b) The interactive effect of season and cutting position on root quality score of *L. fruticosus* stem cuttings. c) The interactive effect of season and cutting position on percentage budding leaves of *L. fruticosus* stem cuttings.

budding (Fig. 2a–c). During autumn, the cutting position did not affect the rooting parameters, but heel cuttings did significantly better in terms of rooting percentage and root score in spring compared to apical cuttings. Interestingly, season and cutting position also had a significant interactive effect on budding leaves. In spring, the heel cuttings had a significantly higher percentage of budding leaves compared to apical cuttings. In autumn, apical cuttings had a significantly higher percentage of budding leaves compared to heel cuttings. Following root initiation and development, the budding of leaves is strongly dependant on the presence of roots in the cutting (Otiende et al., 2010), however, the physiological state of the mother plant at time cuttings are taken may have had an effect on the budding of leaves before roots were formed.

In spring, when *L. fruticosus* is naturally in flower (Pienaar and Smith, 2011), auxins and other rooting co-factors made by leaves and buds,

Table 3
The effect of growth media and cutting position on rooting percentage, root length, root quality score and budding.*

Media	Cutting position	Rooting %	Root length mm	Root quality score	Budding %
Bark	Apical	33.33de	26.92 cd	2.57 cd	34.58 cd
Bark	Heel	66.25a	57.76a	4.99a	50.00ab
Peat	Apical	48.33b	35.18bc	3.44b	46.25abc
Peat	Heel	64.17a	44.89b	4.67a	47.92ab
PeatPol	Apical	43.33bcd	25.69cde	2.98bc	52.92a
PeatPol	Heel	45.00bc	27.83 cd	3.32bc	40.42bcd
SandPol	Apical	25.00e	16.27e	1.85d	42.50abc
SandPol	Heel	34.17cde	22.34de	2.62bcd	28.78d
$LSD_{(Pr = 0.05)}$		10.834	10.269	0.8302	11.818

* Means with the same letter are not significantly different.

may be translocated to the base of the stem, therefore heel cuttings may contain more nutrient value, increasing bud formation while the plant is also flowering (Hartmann et al., 1997). Apical cuttings planted in spring may have limited reserves within the shoots to produce buds as well as flowers. According to Guo et al. (2009), endogenous hormone levels differed at various seasonal collection dates and cuttings of *Paeonia* “Yang Fei Chu Yu” performed better when the plant was in a vegetative state (lowered nitrogen and increased carbohydrate). Increased carbohydrate levels and lowered nitrogen levels have been linked to successful root formation (Hartmann et al., 1997).

Rooting percentage, root length, root score and budding was significantly affected by a media-cutting position interaction (Table 3). Heel cuttings in general performed better with regard to rooting parameters than apical cuttings in all media, but the differences were not significant in peatpol and sandpol. Heel cuttings had a significantly better root score in peat than apical cuttings, and heel cuttings planted in bark significantly outperformed apical cuttings in all rooting parameters measured. Heel cuttings that were planted in bark had a significantly higher percentage of budding leaves compared to sandpol and did not differ from peat and peatpol. Apical cuttings that were planted in bark, however had a significantly lower percentage of budding leaves compared to peatpol but did not differ from peat and sandpol. Bark in combination with heel cuttings gave the best budding leaf percentage out of the other heel–media combinations, but if bark was combined with apical cuttings, the percentage budding leaves were one of the lowest compared to other apical–media combinations.

In this study, rooting of *L. fruticosus* stem cuttings occurred at the very base of the cutting on the cut surface, and not at nodal position, this suggests that heel cuttings may have also provided a larger surface area from which to develop roots. The morphology of the heel cutting may have also ensured that the cutting did not have any vascular damage due to tissue softening and dessication.

It is theorised that hormone-like substances are manufactured in the developing buds and are translocated by the phloem to the basal end of the cutting, promoting root formation. This suggests that a factor besides auxins, presumably manufactured by the bud is necessary for root formation. This hypothetical rooting substance is known as ‘rhizocaline’ (Hartmann et al., 1997).

4. Conclusion

This study concludes that season, which can be linked to the physiological state of the stock plants and temperature fluctuations, had an effect on all parameters measured. Improved survival and better root score was observed in cuttings planted during autumn, compared to spring. The interaction between season and media has to be taken into consideration as certain types of media perform differently during the year due to changes in seasons. In autumn, peat performed the best, followed by peatpol and bark and lastly sandpol. However, in spring, bark performed the best and sandpol was unsuitable. Heel stem cuttings generally performed better than apical cuttings, especially in spring, with significantly higher survival percentage, significantly better root quality score compared to apical cuttings. The use of PGRs, Seradix B® and Dip ‘N Grow®, proved to be beneficial as it resulted in cuttings with better rooting ability compared to those left untreated.

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