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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 $\pm$ 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 $\times$ 3 growth regulators $\times$ 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