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Improving rooting of *Lobostemon fruticosus* L. cuttings with delayed auxin treatment

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ABSTRACT

Lobostemon fruticosus is a semi-woody shrub used medicinally for treating wounds, blood poisoning, ringworms, skin diseases and syphilis. The material used is mostly wild harvested, leading to decline in natural populations. Propagation and cultivation methods to establish commercial production can assist in conservation of the species. The objective of this study was to determine the effect of delaying rooting hormone application on the success of vegetative propagation of *L. fruticosus* using basal stem cutting, cut at an angle of 30° at the base. It was done in a mist bed at the Agricultural Research Council-Vegetable and Ornamental Plant Institute (ARC-VOPI) (Pretoria, South Africa) in a factorial randomized complete block design with two growth media (cocopeat and cocopeat + potting soil1:1; *v*/*v*), rooting hormone (Seradix No. 1 and control) and six application times, with five replications. The parameters assessed were callus development, rooting percentage, shoot and root length, survival rate and stem cutting anatomical analysis. Rooting percentage significantly increased with delaying the application of rooting hormone for one to two weeks from planting, as compared to the control (without hormone) and hormone application at the time of planting (week zero). Cocopeat medium gave the highest rooting percentage (67.1%) and lowest mortality rate (18%), whereas potting soil + cocopeat medium gave the lowest root development (31.5%). Anatomical observations in this study showed that with delayed auxin application up to two weeks after planting, the callus tissue started to develop from the vascular cambium close to the cut end of the cutting. Parenchyma gaps in the phloem fiber ring close to the cut end of the cuttings was also observed. This study concludes that delaying hormone application for two weeks after planting improved rooting of *L. fruticosus* cuttings.

1. Introduction

Lobostemon fruticosus (Boraginaceae) is among the most important perennial shrubs that are medicinal species in the Western Cape Province of South Africa (Buys, 2011). It is one of the species that has been looked at more closely with regard to its medicinal properties. It is also known as "agtdaegeneesbos" (Afrikaans; meaning eight day healing bush) due to its apparent ability to heal a condition in eight days. Decoctions are used to treat wounds, skin disease, ringworm and ulcers while infusions are used for general internal problems and purifying the blood (Van Wyk et al., 1997; VanWyk and Gericke, 2000). Traditional health practitioners also believe that this plant has anti-HIV properties. The plant is gaining more attention and popularity due to the high demand for medicinal extracts from the leaves used by traditional healers (Van Wyk et al., 1997).

Lobostemon plants are currently harvested from the wild and this can lead to a decline in natural populations or even extinction from its natural habitats. Unfortunately, due to difficulty of vegetative propagation, *L. fruticosus* is rarely grown in the nursery trade. The development of successful vegetative propagation methodology, followed by cultivation can therefore help to minimize the wild harvest pressure on the species.

Poor rooting ability in some plant species has been attributed to the presence of growth inhibitors (Barlow et al., 1961), a lack of or imbalance of hormones or rooting cofactors (Raviv et al., 1986). The most common technique to promote rooting in difficult to root plant species is to use exogenous rooting hormones (Muhammad et al., 2006; Owuor et al., 2009; Jordan et al., 2010). Rooting hormones such as indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) are more effective than naturally occurring phytohormones like indole-3-acetic acid (Henrique et al., 2006) to optimize rooting of cuttings. In most of the cases best results were obtained when rooting hormones were applied directly before planting, especially with easy-to-root species such as *Rosa damascenae* Mill. (Muhammad et al., 2006); *Lippia javanica* (Soundy et al., 2008) and *Guindilia trinervis* Gillies (Jordan et al., 2010). However, Luckman and Menary (2002) observed that delaying the application of indole-3-butyric acid (IBA) for six weeks after

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planting cuttings, many roots formed on *Eucalyptus nitens*. According to Hartmann et al. (1997) and Ofori-Gyamfi (1998), rooting performance further depends on the type of medium used in the propagating program as well as the type of rooting hormone used to improve root initiation. Auxins often hasten root initiation, increase the number of cuttings rooted, as well as improve the quality and uniformity of rooting cuttings (Newton et al., 1992; Al-Saqri and Alderson, 1996).

Several anatomical studies have suggested a correlation between difficulty in rooting and the presence of a pericyclic sclerenchyma layer (Goodin, 1965; Beakbane, 1969; Edwards and Thomas, 1980). Amissah et al. (2008) also suggested that the presence of a continuous sclerenchyma layer might act as a physiological barrier to adventitious root initiation or as a mechanical barrier to root emergence. Roots arising from the cambium and phloem regions in *Vaccinium corymbosum* hardwood cuttings were reportedly impeded by a continuous layer of lignified pericyclic fibers and by the epidermis (Mahlstede and Watson, 1952).

There is hardly any information on rooting of cuttings of *L. fruticosus* and an urgent need therefore exists to develop appropriate techniques, including the best rooting substrate to ensure good root initiation and shoot formation from stem cuttings that can easily be adopted by start-up nursery enterprises as well as rural communities.

The objectives of this study were (1) to determine the effect of delaying rooting hormone application on the success of vegetative propagation of stem cuttings of *L. fruticosus*; (2) identifying the site(s) of primordial initiation of adventitious roots and recording the progress of root formation with the view of (3) establishing whether the sclerenchyma ring can be interpreted as a barrier to the initiation and emergence of adventitious roots that can be broken by delayed hormone application.

2. Materials and methods

2.1. Study area and plant material collection

The experiment was carried out at the Agricultural Research Council Vegetable and Ornamental Plant Institute (ARC-VOPI) (latitude 25°9′S, longitude 28°35′E and altitude 1200 m.a.s.l), Pretoria, Republic of South Africa, during the 2013 winter (May to July) and spring (September to November) seasons. The experimental unit was a glasshouse supplemented with 24 h a day misting which worked automatically based on the humidity of the greenhouse. Throughout the experimental period, the temperature of the greenhouse was recorded using a data logger (Tinytag View 2, TV-1500, UK). Mean maximum and minimum temperatures during the study period were 25 and 13 °C, respectively.

Shoots containing secondary xylem were obtained from three year old stock plants of *L. fruticosus* at the ARC-VOPI medicinal plant nursery. The shoots were collected early in the morning and were immediately placed in a bucket filled with water in order to keep them cool and turgid until taken to the working area, following the protocol given by Soundy et al. (2008). The immature top parts of the shoots were removed before cutting them into 8 cm cuttings, using sterile pruning shears. Bottom leaves were removed from the base of the cuttings, leaving only one pair of leaves at the top node with the basal end cut at an angle of 30°.

2.2. Rooting medium preparation

Seradix No. 1 hormone rooting powder was used for this experiment. It contains 4-(indol-3-yl)-butyric acid and is an Auxin class plant growth regulator (PGR) which is used to promote and accelerate root formation of plant clippings and to reduce transplant shock of non-food ornamental nursery stock (Al-Saqri and Alderson, 1996). Two commercial rooting substrates were tested in this study. The first was a commercial rooting substrate (M1) made up of a 1:1(*v*/*v*) mixture of potting soil (90% composted bark and 10% sand) and cocopeat. The second rooting substrate (M2) was cocopeat obtained from coconut husk fibers which is a by-product of the coconut, also known as coir. The moist rooting substrates were used to fill seedling trays with 98 cavities each, measuring 4 × 4 × 9 cm (width, breadth and depth). After planting the cuttings were kept at 80% RH in a mist bed system which was set to spray for 15 min at 1 h intervals.

2.3. Experimental design and treatment details

The layout of the experiment was a factorial randomized complete block design with 16 treatment combinations and five replications. The design consisted of 2 rooting substrates × 8 treatments of delayed Seradix No. 1 rooting hormone containing 4-(indol-3-yl)-butyric acid); 0.1% IBA) applications (2 × 8). For each treatment 14 cuttings were used.

The eight hormone (Seradix No. 1) treatments consisted of a control (no Seradix applied at all), or Seradix applied immediately before planting (week zero), one week after planting (week 1), two weeks after planting (week 2), three weeks after planting (week 3) four weeks after plating (week 4), five weeks after planting (week 5) and six weeks after planting (week 6). The latter six treatments were applied once only on the specific week by removing the cuttings from the medium, rinsing the base of the cuttings with water, followed by quick dipping (3–4 s.) in Seradix No. 1 solution at a concentration of 1 mg/10 ml of distilled water. After hormone treatments, the cuttings were re-planted in the same cavity in the tray. Cuttings which had already started root initiation after week five were also treated carefully. The idea behind treating cuttings which had already started root development was to increase root initiation, number of roots, and uniformity of rooting and callus formation. Callusing, root initiation, shoot number, shoot length and survival were observed at the end of the experiment (week 9). The amount of callus formation at the bases of the cuttings was assessed using a four point rating scale where 0 = no visible callus and 4 = callus mass formation at the whole cut end of the cutting or greater (Luckman, 1996).

2.4. Stem anatomy

Three cuttings of each of the eight treatments were investigated, the basal 1 cm of the cuttings (including the slanted cut) were removed and fixed for two days in formalin:alcohol:glacial acetic acid (FAA) solution (1:9:1; *v*/*v*/*v*) using 70% (*v*/*v*) ethanol in distilled water, after which they were dehydrated using a series of 30%, 50%, 70% and 100% (2×) ethanol. The ethanol was substituted by means of a series of 30%, 50%, 70% and 100% (2×) xylol, followed by infiltration of wax (O'Brien and McCully, 1981). Transverse sections of about 10 μm thick were made from the bottom part of the cuttings, using a rotary microtome (Reichert-Jung). Sections were mounted on slides, stained with safranin and counter stained with fast green and photographed using a digital camera (DXC900; Sony Corp., Tokyo) mounted on an optical microscope (BX 60; Olympus Optical Co., Tokyo).

2.5. Data collection and statistical analysis

Cuttings were finally assessed for root number, root length, shoot length and cutting survival nine weeks from planting. The survival percentage was calculated as the total number of cuttings with shoot growth divided by the total number of cuttings planted per treatment. Rooting parameters were measured by gently separating rooted cuttings from the seedling trays, followed by washing the root zone with water. The data collected were transformed using log transformation, which is normally used for measurable data such as length, whereafter S.A.S. (Statistical Analysis System version 9.1) was used to perform analysis of variance (ANOVA).