UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Vegetative propagation of *Pelargonium sidoides* D.C. (Rabassam) using leaf-bud cuttings

By

Mafamo Samuel Pholo

Submitted in partial fulfilment of the requirements for the degree MSc. (Agric) Horticultural Science

In the Faculty of Natural and Agricultural Sciences

University of Pretoria

Pretoria

Supervisor: Prof ES du Toit

Co-supervisor: Prof P Soundy

February 2019

© University of Pretoria

Abstract

Pelagonium sidoides (Rabassam) is an herbaceous plant of the Geraniaceae family used throughout history as a medicine for the people of South Africa which has and recently also been exported to other parts of the world. A number of studies have shown that the plant extracts have anti-microbial, anti-viral and immune boosting properties. Consequently, it has been harvested in the wild for these properties, exposing the species to possible extinction. This scenario can be addressed by artificially mass propagating and producing the species to satisfy demand. One method of propagating the plant is to use leaf-bud cuttings. Unfortunately, vegetative propagation of the species using leaf-bud cuttings is not documented. This study was therefore conducted to investigate the influence of rooting medium (sand and sand + coir), rooting hormone (0.1% indole-butyric acid (IBA), planting depth (1.5 and 3 cm) and season (summer, autumn, winter and spring) on rooting success of leaf-bud cuttings as well as impacts of these treatments on the survival and morphological characteristics of subsequent transplants.

The experiments were conducted on a mist bed in a greenhouse located at the Experimental Farm of the University of Pretoria (25°45'S, 28°16'E). A 2^3 factorial experiment laid out in a randomized complete block design (RCBD) with 3 replicates was carried out. Root length, root number, planting depth, growth media and hormone application, were assessed by destructive sampling at 5 to 25 days after planting (DAP). Results of the study demonstrated that sand medium, compared to a mixture of sand + coir medium, resulted in higher rooting percentage and more roots. However, sand + coir medium produced longer roots on the cuttings. Planting depth of 3 cm resulted in higher rooting percentage and better establishment of cuttings as compared to a depth of 1.5 cm. Application of the rooting hormone 0.1% IBA improved the number of roots but not rooting percentage nor root length. Rooting of cuttings was improved when propagated in autumn (longer roots) and spring (higher number of roots) than in summer or winter.

After one month transplanting of cuttings, significant differences ($P < 0.05$) were noted in survival percentage due to the media initially used. Cuttings initially propagated on sand showed a higher survival percentage (78.6%) and had a higher number of roots,

© University of Pretoria

which were longer, compared to cuttings initially propagated in a medium of sand + coir (61.2%). Cuttings initially propagated in sand and treated with hormone had significantly more roots after transplanting. Application of hormone also improved root and shoot length of transplants.

Based on observations, cuttings taken from younger mother stock plants had a higher chance of survival than cuttings taken from mature stock plants. In general, leaf-bud cuttings of *P. sidoides* can be artificially propagated without rooting hormone but the success rate will be low. The ideal regime is to propagate cuttings treated with IBA in a sand medium.

Key words: *Pelargonium sidoides*, medium, leaf-bud cuttings, IBA

© University of Pretoria

Declaration

I declare that this research project is my own work and I have acknowledged all the ideas that are not directly mine. This dissertation is submitted in partial fulfilment of the requirements for the degree MSc. Agric. (Horticultural Science) In the Faculty of Natural and Agricultural Sciences at the University of Pretoria. It has not been submitted before for any degree or examination in any other University. I further declare that I have obtained the necessary authorisation and consent to carry out this research.

Samuel Mafamo Pholo

……

February 2019

© University of Pretoria

Acknowledgements

Had it not been of an encouragement and a contribution of many individuals and organizations, this dissertation would not have been a success. Hence, I would like to express my sincere thanks and gratitude to the following:

First and foremost, I thank God for his wisdom and guidance throughout my life and study.

I would like to express my deepest gratitude to my supervisor Prof. E.S. du Toit and Co-Supervisor Prof. Puffy Soundy for their patient guidance, encouragement and excellent advice throughout this study, mainly during the preparation of this manuscript.

I greatly acknowledge Prof. P.J. Robbertse for helping me with the microscopic studies on *Pelargonium sidoides.*

I would also like to express my appreciation to Mrs J. Sommerville and Mr A. Swanepoel from the Department of Statistics, University of Pretoria, for their guidance in experimental layouts and statistical analysis.

I am thankful to Mr J. Marneweck and Mr L. van der Merwe at the Hatfield Experimental Farm, University of Pretoria, for their technical assistance.

My grateful acknowledgement also goes to the National Research Foundation (NRF) for funding my studies and the Pico-Gro Nursery in Midrand (Gauteng) for providing me with the stock plants and advice.

Last, but certainly not least, I would like to extend my gratitude to my wife, brothers, sister and my adorable daughters for their love and support.

© University of Pretoria

Table of Contents

Contents

© University of Pretoria

© University of Pretoria

© University of Pretoria

© University of Pretoria

© University of Pretoria

List of Figures

© University of Pretoria

© University of Pretoria

© University of Pretoria

List of Tables

© University of Pretoria

CHAPTER 1

1.1 INTRODUCTION TO THE RESEARCH PROBLEM

Herbal medicines have been and are still relevant in the livelihoods of the poor citizens of the world, especially in Africa and some parts of Asia. However, an interesting shift appears to have taken place with regards to the use and acceptance of traditional or herbal medicines in all societies. Botanicals, once synonymous with poor or African traditional practices, now have a prominent position even in pharmacies of the most affluent of societies. Africa, maybe due to its favourable climate and vast tracts of undeveloped land, is a notable supplier of such herbs (Kuete and Efferth, 2015).

South Africa has remarkable plant biodiversity and this has made it a supplier of useful botanicals to the world. Some of the most potent and useful medicinal herbs endemic to South Africa belong to the genus *Pelargonium* (Geraniaceae). Thirty five indigenous medicinal plants were accurately recorded to occur in the Cape region in the period 1650-1800 and more than six of the species on that list belonged to the genus *Pelargonium* (Scott and Hewett, 2008). Many scented and unscented *Pelargonium* species are used as traditional remedies by the Sotho, Xhosa, Khoi-San and Zulu tribes of South Africa (Watt and Breyer-Brandwijk, 1962; Viljoen *et al*., 2008) for wounds, abscesses, fever, colic, nephritis and suppression of urine, colds and sore throats, haemorrhoids and gonorrhoea. They are even used as an insecticide and for treating livestock (Lis-Balchin, 1996). The diversity in use is probably a function of the diversity of the species in general.

Two hundred and eighty species belonging to the *Pelargonium* genus are known to science (Singab *et al*, 2015). Approximately 80% of these species occur in South Africa where they are predominantly found in the south-western Cape region, a region which receives most of its rainfall in winter (Van der Walt and Vorster, 1988; Lis-Balchin, 1996). The remaining 20% of the species are found in other parts of southern Africa, New Zealand, Australia and the Far East (Mativandlela and Meyer, 2005). The concentration of most members of a species within a limited geographical range can be a threat to its long term survival (Rehm *et al*, 2015).

© University of Pretoria

Some species of the *Pelargonium* genus are dwindling in abundance due to over exploitation (Moyo *et al.*, 2015). This is because, as noted earlier, *Pelargonium* species play a key role in traditional medicine around the world. This species provides the world's pharmaceutical industry with crucial ingredients for herbal medicines (Fowler, 1983; Abraham *et al*., 2010). In addition to medicinal uses, they are also harvested for their essential oils for use in perfumes and body fragrances (Ali *et al*., 2015). Some of the species targeted for this use include *P*. *capitatum, P. graveolens and P. radens.* Such use have resulted in the species becoming an integral part of even the urban dwellers, not just the poor in the rural areas.

Dauskardt (1990) reported the emergence of urban herbalism in South Africa. This was a mirror of what was happening elsewhere in the world. More and more people were complimenting modern medicines with traditional herbs. Peltzer (2009) reported that the sole reliance on traditional healers by Africans declined from a range of between 3.6% and 12.7% to 0.1% between 1996 and 2009. The implications of the findings of these two authors point to the usage of botanicals even within people with access to modern medicines. This has resulted in the coining of the term complimentary/alternative medicines signifying the ability of herbal medicines in making positive contributions in people's lives (World Health Organisation, 2005). This global increase in the use of herbal medications can be attributed to the fear, justified or not, within the general population that chemicals used in the drug and food industries can cause cancers (Akbulut *et al*, 2013).

Increasing use of traditional herbs by consumers ignited a wave of research within the academic sector. Research in the efficacy of many botanicals in treating diseases has produced many interesting results. Scientific literature is awash with results of such studies. For instance, Tahan and Yaman (2013) reported that *Pelargonium sidoides* can be used to manage asthma; Barnes *et al*. (2002) reported that at least 24% of American civilians used natural products for health reasons in 2001 and Anekonda and Reddy (2005) working on mice reported that herbs were promising as treatment for Alzheimer's disease and that they had less side effects than synthetic drugs. These are just a few of the positive results that show the potential that medicinal herbs have in treating various ailments.

© University of Pretoria

Nature plays a role in the wellbeing of humans in two ways. Firstly it inspires design of new drugs and can provide useful leads for new drugs (Boris, 1996, Newman and Cragg, 2012). About 11% of drugs defined as basic are derived from angiosperms (Veeresham, 2012). These include quinine used for treating malaria which was first isolated from the bark of the tree *Cinchona pubescens* (Muraleedharan, 2000) and aspirin, which was first isolated from *Salix alba* (Veeresham, 2012).

The second way nature aids in the wellbeing of mankind is when natural herbs are consumed after minimal processing. More people, if compared to previous decades, are now opting for medications that relate more to nature than the laboratory. The corporate world, especially drug manufacturers, have not been blind to these changes in preferences. These changes in preferences have led large companies to acquire and package botanical values for onward selling to consumers, creating an industry worth $US 62 billion in 2007 (Jamil *et al*., 2007). Medications like Ginkgo oil from the tree *Ginkgo biloba* (Chen *et al*., 2013), Aloe gels and syrups from *Aloe vera* (George *et al*, 2009) and Moringa powder from the tree *Moringa oleifera* (Sale *et al*, 2015) are some of the natural product based medications that are packaged by multi-million dollar organisations.

The commercialization of medicinal herbs can expose some “on demand” herbs to the risk of extinction due to over-exploitation (Cunningham, 1991; Rai *et al*, 2000; Dubey *et al*, 2004; Tomlinson and Akerele, 2015). The over-exploitation of medicinal herbs can be partly minimised by intensive cultivation of species of interest in natural environments. However, growing most of the herbs from seeds is slow, unpredictable and generally difficult (Nadeem *et al*., 2000). Furthermore, seeds can also lead to genetic variation between individual plants. A lot of effort has been justifiably put by plant scientists in trying to find ways of efficiently growing some of the species in artificial environments.

Fortunately, scientific advances of the past century came handy in addressing some of the challenges emanating from over-exploitation of some species. Vegetative propagation is one of the techniques that can be used to quicken the propagation of plants. Therefore, this technique effectively goes some distance in helping to satisfy the growing appetite for herbal medicines. Consequently, vegetative propagation

© University of Pretoria

lessens the exploitation of wild species (Dixena and Patel, 2018). Vegetative propagation has been around for ages, but scientific research keeps on improving the science. Advances have been made in understanding how synthetic plant hormones affect growth, what the ideal cuttings to use are and also what is the ideal season to collect plant cuttings for propagation (Caplan *et al.*, 2018. However, research findings from studying one plant cannot be extrapolated to be true for all plants. There is thus need for targeted research in order to come up with the most efficient way to propagate a specific species (Danu *et al*., 2015).A typical species that falls under this category is *Pelargonium sidoides,* an important medicinal plant to South Africa. *P. sidoides* has velvety, heart- shaped leaves which are slightly scented (Figure 1.1A). The flowers are small and range from maroon to nearly black in colour (van der Walt and Vorster, 1988). Underground, the plant has tuberous rhizomes (van Wyk and Brendler, 2008), that are uprooted by harvesters to use for medicine, as a result killing the whole plant. At first glance, the plant is hard to distinguish from a closely related species, *Pelargonium reinforme* (White *et al.*, 2008). However, when in flower the two species can be easily differentiated, since *P. reniforme* has bright pink flowers in contrast to the darker petals of *P. sidoides* (van der Walt and Vorster, 1988).

Figure 1.1: Leaves (A) and rhizomes (B) of *P. sidoides*

In cross-section, the rhizomes are bright red in colour, typical of a number of *Pelargonium* species which are used in traditional medicine (van der Walt and Vorster, 1988). According to Newton (2009) young tubers are translucent or white but gradually

© University of Pretoria

change to pink and finally deep red over a number of years (Figure 1.1B). . *P. sidoides* has a wide distribution. It occurs throughout the Eastern Cape, Lesotho, Free State and southern and south-western Gauteng in the Republic of South Africa (Figure 1.2). It usually grows in short grasslands and sometimes with occasional shrubs and trees on stony soil, varying from sand to clay-loam, shale or basalt. *P. sidoides* is found at altitudes ranging from near sea level to 2300 m in Lesotho. It is found in areas which receive rainfall in summer (November to March) varying from 200 – 800 mm per annum (Lewu *et al*., 2007)

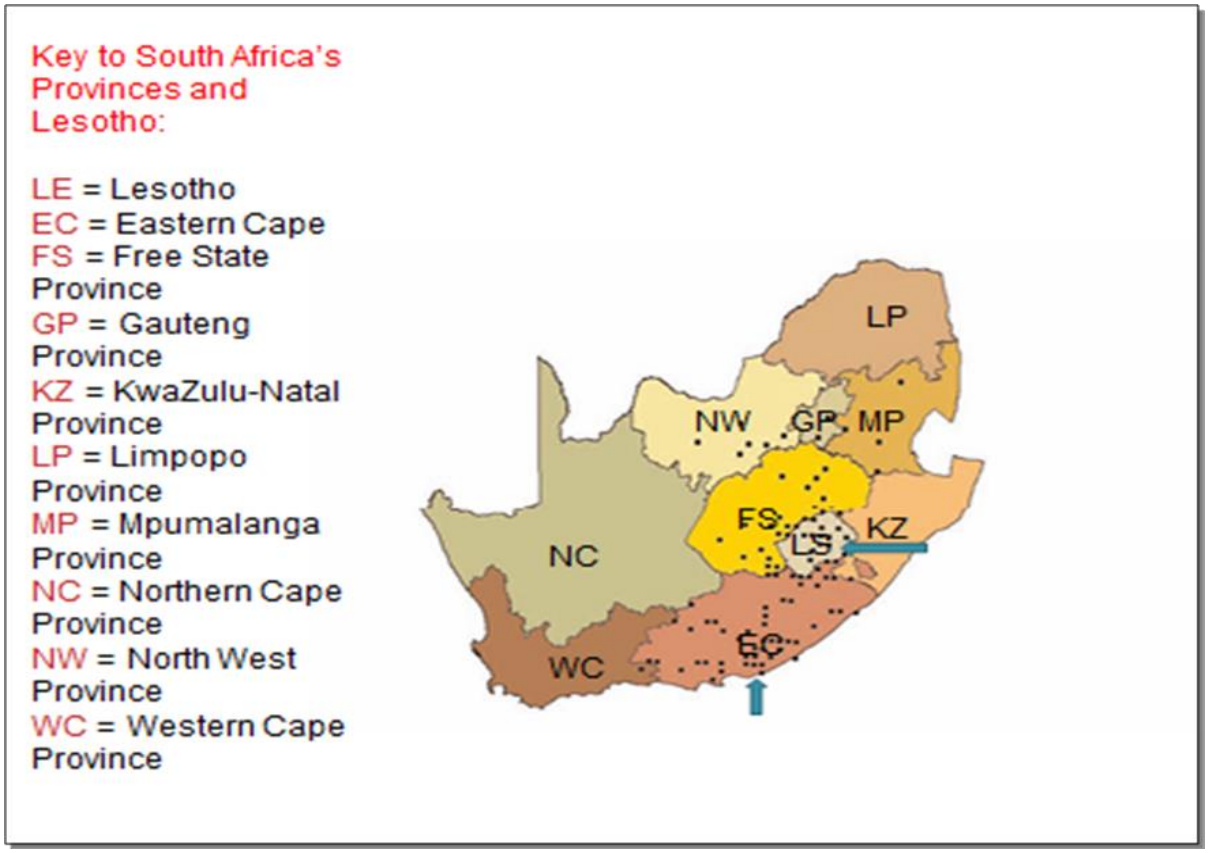

Figure 1.3: Distribution map for *P. sidoides* in South Africa and Lesotho (De Castro *et al*, 2010)

As seen on Figure 1.2, the species has a limited distribution and difference in climatic conditions limit the species from being grown naturally in other regions of South Africa (Munganyi *et al.,* 2018). Population growing in the areas shown on the map can be augmented by artificially propagated elsewhere, effectively lessening exploitation pressures of the wild species.

© University of Pretoria

Though it is possible to propagate *P. sidoides* from seed, this is not the most efficient way. Generally, the use of seed to propagate wild plants is slow and germination rates are poor (Abbo *et al*., 2014). Due to its importance as a medicinal herb, a number of researchers have worked on finding more efficient ways of multiplying or propagating the plant. Progress has been made in this regard over the past decades. Kumar *et al*., 2015 reported that they were able to propagate *P. sidoides* in-vitro using the process of somatic embryogenesis. Though commendable, this process is expensive and very technical to be efficiently used in some countries.

Using leaf bud cuttings can be a cheaper and more easily adaptable way of propagating *P. sidoides.* In addition to being faster than using seeds, vegetative propagated cultivars of *Pelargonium* are also better than seed generated plants in some traits. They have beautiful semi-double florets and stems are less likely to shatter during handling (Harney, 1982; Saxena *et al*., 2001). It however takes prudent scientific enquiry to come up with the most efficient way of artificially propagating *P. sidoides*, or any other plant.

This study therefore attempts to decipher the factors that determine the success of using leaf bud cuttings to multiply the medicinal plant, *P. sidoides*.

1.2 Problem Statement

Medicinal herbs have always played an important role in the livelihoods of African communities. A number of herbs prescribed by African traditional herbalists for centuries have been proven by modern scientific analysis to have medicinal impacts they are claimed to have. This does not only vindicate the herbalists, whose methods lack rigorous scientific enquiry and often attract ridicule and met with scepticism in some sectors, but also attracts pharmaceutical companies and middlemen seeking profits. This consequently has led to increased exploitation of herbal plants and some populations of natural plants prized for their medicinal properties have been depleted, some even got locally extinct.

There is also a shift in the type of medicines preferred by people. This shift is motivated by the general sentiment that medicinal herbs are safer than conventional drugs. This, in addition to challenges posed by global warming and habitat loss, has put additional

© University of Pretoria

pressure on the wild populations of medicinal plants. Its long term survival is threatened by unsustainable harvesting to satisfy both the local and international herbal medicine markets. It is therefore wise, in terms of both economics and conservation, to supplement the wild plant with artificially propagated plants.

Propagating wild plants from seeds is often fraught with difficulties and slow. Some of the difficulties inherent in the use of seeds for propagating new plants can be circumvented by using cuttings. Cuttings, though advantageous for propagating some plants, may need special treatment in some species and the exact species' requirements differ. Success in propagating plants from cuttings hinges on many factors like, presence of absence of rooting hormone, season the cuttings are collected and planted, as well as the rooting media used.

This study was therefore designed to determine the best conditions to artificially propagate *P. sidoides* from leaf bud cuttings. The ability of mankind to rapidly artificially grow medicinal plants like *P. sidoides* is important for the conservation of species affected by over-exploitation.

1.3 Aims and Objectives

1.3.1 Overall Objective

The overall aim of this research was to determine the optimal conditions needed to propagate the medicinal plant, *Pelargonium sidoides* artificially from leaf bud cuttings. The researcher hoped to generate and document the ideal protocol for the vegetative propagation of *P. sidoides.* Compared to other *Pelargonium* species which are mostly propagated using stem cuttings, *P. sidoides* has a distinctly different morphology. It has a crowded stem with heart-shaped long-stalked leaves and as such it may have different requirements to other species of the genus.

© University of Pretoria

1.3.2 Specific Objectives

- To determine the effects of the rooting hormone, Dynaroot™ No. 1 (0.1% indole-butyric acid) in powdered form, on rooting of *P. sidoides* leaf bud cuttings.
- To determine which rooting medium produces better results between sand only and a mixture of sand and coir.
- To determine the best time of the year or season for rooting of leaf bud cuttings for *P. sidoides*.
- To determine the ideal planting/sticking depth of cuttings.
- To evaluate the survival of the propagated plants and their development into complete plantlets.

1.4 Hypothesis Statements

H_0: The application of Dynaroot™ No. 1 has no effect on the rooting of leaf bud cuttings of *P. sidoides.*

H_1: The application of Dynaroot™ No. 1 improves rooting of leaf bud cuttings of *P. sidoides.*

H_0: Root development of leaf bud cuttings planted in a medium of sand alone is identical to root development of leaf bud cuttings planted in a medium of sand and coir.

H_1: Root development of leaf bud cuttings planted in a medium of sand alone is higher in number than root development of leaf bud cuttings planted in a medium of sand and coir.

H_0: The number and length of roots produced by leaf bud cuttings of *P. sidoides* is independent of the season the cuttings are planted.

H_1: Planting season for cuttings significantly affects the number and length of roots on *P. sidoides* cuttings due to varying temperatures which affects the growth of the plant.

© University of Pretoria

H_0: Rooting of leaf bud cuttings planted at a depth of 1.5 cm is the same as the rooting of leaf bud cuttings planted at a depth of 3.0 cm.

H_1: Root length and number of leaf bud cuttings planted at a depth of 1.5 cm with be less as compared to leaf bud cuttings planted at a depth of 3.0 cm due low water and nutrients availability.

H_0: The proportion of plantlets that grow into adult plants is independent to treatment given to the respective plants at the cutting stage.

H_1: The proportion of plantlets that grow into adult plants is dependent on the treatment given to the respective plants at the cutting stage.

© University of Pretoria

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The use of medicinal plants is an age old practice and humans owe part of their success as a species to the availability of botanicals with healing properties. Plants have been used in all cultures, societies and regions of the world for the treatment and prevention of various ailments and physiological abnormalities like fatigue. Medicinal plants have been playing a crucial role in the management and treatment of diseases for millennia and still have a place in modern society (Van Wyk and Wink, 2004).

In this modern age, medicinal plants are particularly useful in poor or developing countries where life threatening, infectious diseases are endemic and health facilities and services are inadequate (Samie *et al*., 2005). Botanicals therefore fill the gap in health care delivery since they are largely free and also are found more in less disturbed, poorly developed rural areas which lack adequate health facilities. It is therefore not surprising that medicinal plants are a resource which a majority of people in Africa depend on for physical, social, cultural and economic well-being (Marshall, 1998; WHO, 2002; Annika *et al*., 2009).

Medicinal plants can be described as nature's gift to mankind, containing active compounds used to alleviate illness. Plants can be an effective source of drugs or drug leads for the pharmaceutical industry. The World Health Organisation (World Health Organisation, 2014) now acknowledges their role in the well-being of human populations (David *et al.,* 2014). Increased population, rising poverty levels, land use changes (Gafna *et al*., 2017) and renewed interest in natural products by large pharmaceutical conglomerates (David *et al.,* 2014) expose medicinal plants to the threat of extinction through overexploitation. The role of wild plants as sources of raw materials makes them vulnerable to unmitigated and severe exploitation for both commercial and non-commercial purposes (Gupta *et al*., 1998; Ved *et al*., 1998).

A medicinal plant can be defined as a plant, naturally occurring or cultivated, that contains valuable chemical compounds known to confer well-being to people and cure

© University of Pretoria

illness. Seventy percent of the South Africa's population uses traditional remedies. They have always been considered important and effective for the treatment of many different diseases in Africa and other parts of the world (Adimoeja, 2000; Mpati *et al*., 2006). According to Gragg *et al*. (2005), twenty-five percent of modern medicines are derived from plants that have been used by traditional practitioners. It is a fact that traditional systems of medicine have become a topic of global importance. Although modern medicines are now easily accessible in many developed countries, people are still turning to alternative or complementary therapies, including medicinal herbs (Ameenah and Ossy, 2010).

Some of the medicinally valuable plants in South Africa belong to the genus *Pelargonium* and extracts from these plants have been an integral part of traditional healing practices in the country for centuries (Moyo and Van Staden, 2014). South Africa is considered to be a biodiversity hotspot for this genus as it accommodates approximately 90% of the species of this genus. The species is predominantly found in South Africa's Eastern Cape Province and the highlands of Lesotho (Moyo and Van Standed, 2015).

Pelargonium sidoides DC and *P. reniforme* Curt (Geraniaceae) are two of the most important species under the new section Reniformia due to their international commercial value (Viljoen *et al*., 2014). Additionally, these two species are close taxonomic allies and are only differentiated by subtle morphological features. Currently, herbal pharmacopeias or drug synthesis makes provision for the use of both species as acceptable ingredients to produce phytomedicines. Their prized healing properties, as indicated earlier, may result in overexploitation and local extinctions. Already, there are indications pointing to overexploitation as the species is currently classified as declining in South Africa (Kumar *et al*., 2015).

2.2 Methods of plant propagation

Continued survival of any organism is only guaranteed if the species has an effective way of propagating itself. Plant propagation is simply the multiplication of plants by both sexual and asexual means of reproduction. Artificial propagation of useful plants in huge quantities may not be the panacea for conservation challenges faced by mankind, but it goes a long way in mitigating the negative impacts of overharvesting.

© University of Pretoria

The ability to propagate useful plants enabled ancient man, 10 000 years ago, to fore gore his nomadic tendencies and establish cities and villages. The ability to propagate plants meant that man had more control over his and his animals' source of food. Successful propagation of plants require knowledge of ideal plant growth conditions, how the plant develops, morphology or form the plants can take and possible methods by which respective plants can be propagated (Caponetti *et al*., 2018). Beginning with ancient civilization, man has since discovered that plant establishment through vegetative propagation is an important technique (Hartmann and Kester, 1983; Mpati *et al*., 2006).This gave man more power to control his food source as cuttings are easier to grow for species like cane, sweet potatoes and cassava.

The journey of mankind is not over yet. New challenges emerge with each passing century and man is constantly called upon to address these challenges. Novel plant propagation techniques for useful plants are now needed more than ever before to alleviate the overexploitation of natural resources (Dakah e*t al*., 2013). Due to the increase in popularity of natural or herbal remedies, wild collections of plants of interest should be complimented by effective methods of plant propagation (Duchow *et al*., 2016). Debate has been raging with regards to feasibility of mass production of herbal plants (Moyo *et al.,* 2013). This has stimulated more research on artificial plant propagation techniques that may enable the mass production of medicinal plants (Majumder and Rahman, 2016) to minimise depletion of wild resources and to satisfy the seemingly insatiable demand for natural plant-derived pharmaceutical products (Moyo *et al.,* 2013).

The use of vegetative plant generation techniques are evident in literature of renowned individuals like Aristotle (384–322 B.C.), Pliny the Elder (23–79 A.D.) and Theophrastus (371–287 B.C.) among others (Haissig and Davis, 1994:275). Vegetative propagation has a number of advantages over other forms of propagation. It quickens the process of multiplying a plant population and also maintains the genotype of a plant, a feat impossible with sexual propagation (Hughes and Smith, 2014; Bonga, 1982). Seed germination is more unpredictable as the genotype and phenotype of the new plants cannot be fully determined by looking at the parent plant or seed. Seeds are also affected more by environmental factors such as availability of

© University of Pretoria

moisture, light, temperature and these factors vary with plant species (Hartmann and Kester, 1983; Copeland and McDonald, 1985; Hartmann *et al*., 1997; Mpati *et al*., 2006).

In addition to food crops, mankind has always relied on plants for useful fibres, paper, medicines and beautiful ornaments. Vegetative propagation is reported to be an extensively practiced and economical means of propagating a wide range of plants (Leaky, 1990; Mudge *et al*., 1995; Araya *et al*., 2005). Cuttings have played a huge role in various reforestation projects around the world and enabled the constant production of quality fruits in various orchards by circumventing the weaknesses that result from relying on seeds for new plants (Lelu-Walter *et al*., 2015). This traditional method of propagation is advantageous in that a lot of new plants can be quickly generated from a few stock plants (Buta *et al*., 2014). In other words it is usually less expensive, rapid and simple (Bonga, 1982). Though it often does not require special techniques to propagate new plants from cuttings, scientists are constantly on the lookout to improve the efficacy and success of propagation using cuttings.

Plant cuttings used for propagation come in different forms and the two major groups are leafy softwood and leafless hardwood cuttings from older and shoots which typically have already shed their leaves at the advent of dry or winter seasons (Leakey, 2004). The propagation environment is one of the important aspects that govern the success of a vegetative propagation endeavour. It is no surprise that a lot of research has gone into determining the most ideal environment for different plant species. Ofori *et al.* (1997), working on *Melicia excels* discovered that moisture content of growth medium was positively correlated to abundance of roots which in turn reduced mortality and leaf abscission. Buta *et al*. (2014) working on a *Mentha* x *piperita* hybrid discovered that the number of roots was higher in cuttings grown in peat and perlite than in sand. Vegetative reproduction has evolved greatly between the days of Aristotle and today. Modern methods of vegetative propagation employ complex chemicals like the auxin indole-3-butyric acid (IBA), benzyladenine and kinetin (Dakah *et al*., 2014). Also, the growth medium is not just soil but can be complex solutions like the Murashige and Skoog solution which is composed of a number of different chemicals like thiamine, niacin and glycin (Zenaro *et al*., 2015).

© University of Pretoria

Tissue culture is another vegetative plant propagation technique that has been employed by plant biologists. It is more expensive than all the other methods but in a world littered with conservation and food provision challenges, it has its place and uses (Janick, 2003). Plant tissue culture is a collection of different methods used to grow tissues, organs or whole plants in a sterile laboratory environment. Anis and Ahmad (2016) noted that the over exploitation of many medicinal plants has resulted in extinctions and tissue culture holds great promise in minimising the loss of plant biodiversity. Plant tissue culture can therefore positively impact on human welfare. Tissue culture has shown great promise in the propagation of the medicinal and ornamental plant *Laburnum anagyroides*. This small tree/shrub is known to be difficult to propagate using traditional or conventional propagation methods (Timofeeva *et al*., 2016).

The success of tissue culture depends on the medium used and a number of substances have been tried with deferent levels of success. Some of the substances that have been used include 1, 3, 5-trihydroxybenzene (phloroglucinol), a compound now prized for its superior growth enhancing properties. It enhances shoot formation and somatic embryogenesis in some horticultural and grain crops (da Silva, Dobránszki and Ross, 2013:1). Plant tissue culture has been used to improve commercial competitiveness for fruit plantations which include banana (Khalid and Tan, 2016:101), grow medicinal plants on a commercial level (Kumar and Rao, 2016:119) as well as for the conservation and management of *Podophyllum hexandrum* and *Picrorhiza kurroa, e*ndangered medicinal herbs (Dhiman, Patial and Bhattacharya, 2016:45). It is also the key method used to propagate transgenic cereals to avoid the loss of inserted genes (Stroud *et al*., 2013:1).

2.3 Pelargonium Species (Geraniaceae)

Phylogenetic studies of the genus *Pelargonium* have proved to be a challenge for botanists (Roschenbleck *et al*., 2014). This medicinally and horticulturally important genus belongs to the family Geraniaceae. The Geraniaceae family is made up of more than 800 species found across the world, mostly in temperate and subtropical regions. *Pelargonium* makes up about 280 taxa of Geraniaceae and is distinguished from the rest of the family by having a hypanthium which consists of an adnate nectar spur.

© University of Pretoria

Pelargonium, unlike other family members, also has a zygomorphic floral symmetry (Albers and van der Walt, 2007). The genus *Pelargonium* is more popular than other members of the family and this has resulted in the name *geranium* being erroneously used to refer to plants that should correctly be referred to as *pelargonium* (van der Walt, 1977).

Members of the *Pelargonium* genus occur in a number of different growth forms which include stem succulents, shrubs and herbaceous annuals (Roschenbleck *et al*., 2014). These different growth forms prompted De Candolle in 1824 to arrange members of the *Pelargonium* genus into sections (Dreyer *et al.,* 1992). De Candolle's characterisation has been refined further and the *Pelargonium* species are phylogenetically arranged into 16 sections (Linder and Hardy, 2004).

Plants in the *Pelargonium* genus play a key role in traditional medicine around the world and they also provide useful raw materials for the pharmaceutical industry (Fowler, 1983; Abraham *et al*., 2010). Most of the *Pelargonium* species are aromatic and in particular, *P. capitatum, P. graveolens and P. radens* are artificially cultivated for the ennoblement of `*geranium*' oil. In South Africa, many scented and unscented *Pelargonium* species are used as traditional remedies by the Sotho, Xhosa, Khoi-San and Zulus (Watt and Breyer-Brandwijk, 1962; Viljoen *et al*., 2008). They are used as remedies for wounds, abscesses, fever, colic, nephritis and suppression of urine, colds and sore throats, haemorrhoids, gonorrhoea and are also used for stimulating milk-production, for anti-helminthic infections and even as an insecticide (Lis-Balchin, 1996).

2.4 Medicinal Plants: Past, Present and Threats

The use of plants to manage ailments appears in 5000 year old Sumerian records. Archaeological findings suggest that plants or plant materials have been used as medicines or phytotherapy as far back as sixty thousand years ago in Iraq and eight thousand years ago in China (Pan *et al.,* 2014). In third world countries like Nepal, 80-85% of the population depends solely on herbal medicine (Dzotam *et al.,* 2016). It can therefore be said that natural plant products play an important role in the wellbeing of a lot of people in the world. A staggering 75% of the world's population depends on herbs for basic medical needs (Pan *et al.,* 2014).

© University of Pretoria

Plants have been used for millennia to manage medical conditions like epilepsy, diarrhoea and headaches amongst others (Elsas, 2015). Botanicals also offer a potentially valuable, and more tolerated mode of managing some diseases (Elsas, 2015). Botanicals can help people in the developing world get access to some medications to manage their infirmities. It is estimated that, for example, 90% of people with epilepsy in the developing world have no access to treatment. This has prompted researchers to actively look for botanicals in those countries that can be used to manage the disease (Sucher and Carles, 2015). This endeavour appears to be paying off as more than a dozen plants found in the developing countries have been proven to have anticonvulsant properties comparable to those of Anti-Epileptic Drugs (AED) used in the first world.

In addition to being a source of medication for the poor, there is a general paradigm shift in the use of herbal medicines, even within the rich communities of the world. Modern doctors are now more open to the idea of prescribing herbal medication to their patients (Pan *et al.,* 2014). There is renewed interest in the pharmaceutical industry also as more people are now getting more inclined to using natural herbal medicines. The increase in extraction of natural resources has resulted in some medical herb species becoming threatened with extinction (Pradhan and Badola, 2015). This has been exacerbated by the fact that some of the herbs or medicinal plants occur in limited isolated habitats. This is exemplified by the recently discovered herb *Pelargonium saxatile* which is found almost exclusively on the shallow soils of the Elandskloofberge (South Africa) sandstone rock pavements (Manning and Goldblatt, 2012). Antoniadis *et al*. (2017) reported that the abundance of the highly medicinal herb *Geranium wallichianum,* which is restricted to the Himalaya region of Pakistan has been reduced by more than 75% by anthropogenic activities.

In South Africa, medicinal plants are endangered by large scale harvesting for local use and also for increased export (Lange, 1997). One of the species that have been proven to be effective against a number of ailments in South Africa is *Pelargonium sidoides* (*Geraniaceae*). *Pelargonium sidoides* is a popular herb in traditional medication in South Africa. Its roots contribute to near extinction when harvested are even though the leaves have been proven to be equally effective against some

© University of Pretoria

disease causing agents (Lewu, Grierson and Afolayan, 2005). The herb is believed to treat diseases like cough, tuberculosis, bronchitis, sore throat, fatigue and general weakness of the body. In addition to the local exploitation, the herb is highly sought after in developed countries like Germany where it is used in syrup to treat bronchitis in children (Lewu *et al*., 2005).

Literature is littered with examples of how plants can address some of the health challenges faced by mankind. Prinyanka *et al*., (2013) working on the potency of botanicals to treat malaria, discovered that the plants, *Holarrhena antidysentrica and* Azadirachta *indica* had antiplasmodial properties against the malarial *Plasmodium berghei*. Kumari and Dutta (2013), working on mice suggested that the herb *Elettaria cardamomum* can significantly ameliorate the impacts of smoking on lungs. Rao *et al*., (2013) discovered that aerial parts of the plant *Melochia corchorifolia* have anti-oxidant properties that have the potential of lessening the risk of cancer.

The efficacy of the plants as agents to manage diseases makes them a blessing for mankind but at the same time makes the plants vulnerable to over exploitation, which can lead to extinction. Dold and Cocks (2002) reported that informal and unregulated trade in medicinal plants is a threat to the long term survival of some species in South Africa since demand appears to be more that what can be supplied naturally. Lewu et al. (2006) reported that uncontrolled harvesting of the medicinal plant *Pelargonium sidoides* in the Eastern Cape Province of South Africa, though this brings some income to the unemployed, poses a risk to the long term perpetuation of the species in some localities. The demand of the plant is driven not only by the large contingent of traditional healers in the country but also by international demand (Dold and Cocks, 2002).

Over-exploitation of medicinal herbs is a worldwide challenge and governments constantly have to protect more and more plants by some form of legal statutory instrument. In 2002, the Kingdom of Nepal had to control the exploitation of 51 herbal species (Bhattarai *et al*., 2002). In India, 90% of the 700 plants with medicinal value are harvested from the wild, many of which are on the verge of extinction. The 2012 edition of India's *Red Data Book* stated that 3,947 species are critically endangered, most of which are plants (Pan *et al*., 2014). Zheng *et al.* (2015) reported that some

© University of Pretoria

medicinally important herbs like *Curcuma wenyujin* have been harvested to the brink of extinction from the wild or their natural habitat in India.

The general acceptance of herbal medicine has increased the world over and the sale of herbals is expected to grow at a rate of 6.4% per year. In the USA, the number of people using herbal medication increased tenfold between 1991 and 2004. It is not surprising that maintaining a consistent supply of botanicals to satisfy an ever increasing market is proving to be a tall order. This has resulted in scientists the world over attempting to find new ways of growing plants of interest (Dakah *et al*., 2013). Scientific breakthroughs of the past decades offer promising avenues to address some of the challenges hindering the widespread and consistent availability of medicinal botanicals. *Pelargonium reniforme* and the closely related *P. sidoides* are intensively used as ingredients in a German remedy, marketed as “Umckaloabo”, which is used to treat bronchitis in children (Msomi, 2015).

2.5 Medicinal value of *Pelargonium sidoides*

Pelargonium sidoides has a long history as an effective herbal medicine. It first appeared in literature as a medicinal herb as far back as 1685 (Brendler and van Wyk, 2008). It stands above all the other members of the Geraniaceae family as the only herb that is widely used in Africa by traditional herbalists and also recommended by some first world doctors to their patients (Moyo and van Staden, 2014). This has resulted in a considerable amount of work being carried out to understand how *P. sidoides* work and to identify as many uses as possible. Results from around the world have indicated that *P. sidoides* is effective against a surprisingly wide spectrum of disease causing agents as well as other physiological disorders like fatigue.

Charles Henry Stevens is credited for introducing the herb to the English after it was used to cure him of tuberculosis by an African healer. *Pelargonium sidoides* is marketed as a herbal medicine under the name Umckaloabo (ISO Arzneimittel, Ettlingen, Germany). The active ingredient in the medicine is referred to as EPs 7630. EPs 7630 is made up of polyphenols and coumarins among other complex compounds (Witte *et al*., 2015). This herbal medicine is effective against a number of pathogens and one of its major advantages is that it does not promote microbial resistance. In

© University of Pretoria

Germany, EPs 7630 is approved for use on people with acute bronchitis (Careddu and Pettenazzo, 2018).

P. sidoides has a special place in folk medicine. It is very probable that its use predates earliest recorded accounts. Records indicate that it was already being used as an effective medication in 1895 and was used to cure dysentery (Smith 1895 cited in Brendler and van Wyk, 2008), treat colic (Phillips 1917 cited in Brendler and van Wyk, 2008) and to manage tuberculosis (Bladt, 1974). In addition to its use in treating diseases and managing wounds in humans, it is also used to prevent purging in horses and to treat diarrhoea in calves. It is therefore not an overstatement to conclude that this plant is integral to the history and culture of the people of South Africa, Namibia and Lesotho.

The plant is also playing a role in the health issues on man in the 21st century. Extracts of the plant have been shown to have potent anti-viral activity. This is especially important given the fact that new viral diseases have caused widespread panic recently and some of the most difficult diseases to cure are viral (World Health Organisation, 2009). In response to the new disease caused by the H1N1 virus (swine influenza) Theisen and Muller (2012) showed that extracts of *P. sidoides* can inhibit the virus from attaching to host cell receptors. Though these tests were done in vitro, they still hold great promise and hope that the plant species can be effective against new and emerging viruses. Helfer *et al.* (2014) showed that root extracts of the plant protects macrophages and peripheral blood mononuclear cells from being infected by some HIV-1 strains. This is extremely important given the realisation that HIV/AIDS is a huge challenge for health authorities and the plant is endemic to the region with some of the highest HIV/AIDS cases.

Pelargonium extracts are particularly effective against infections of the upper respiratory tract. Research has demonstrated that *Pelargonium* extracts may help relieve the symptoms associated with tonsillitis, bronchitis, sinusitis and other upper respiratory tract infections (Lewu *et al*., 2010). *Pelargonium* extract prevents the adhesion of bacteria and viruses to the respiratory tract epithelial cells and reduces the mucus where bacteria multiply and prevents them from penetrating cell walls and spreading infection. It is therefore not surprising that herb extracts are also effective

© University of Pretoria

against flu. They reduce the frequency of coughing and intensity of nasal congestion (Tahan and Yaman, 2013). The same researchers also discovered that it is effective against in preventing asthma attacks in children.

The plant extracts are also effective against bacteria, both gram positive and gram negative (Kayser and Kolodziej, 1997). This is particularly valuable in this era of antibiotic resistant strains of bacteria. *Pelargonium* extracts also stimulate the immune system and, in particular, the activation of natural killer cells and stimulation of phagocytosis enabling the body's own defense mechanism to fight more effectively against infections, effectively shortening the lengths of disease bouts.

In summary, *Pelargonium* extracts have been shown to have anti-microbial, anti-viral and immune boosting properties. This is probably the reason why there is an impressive array of medicine brands that claim to contain *P. sidoides* root extracts. These brands include Pelargonium, Medicherb UK; Pelargonium Syrup, Bioharmony Africa and Umckaloabo® and Spitzner. These brands are available internationally in countries including Germany, Mexico, Turkey, Brazil (e.g. Matthys *et al*., 2003; Chuchalin *et al*., 2005) and South Africa (White, 2007; White *et al*., 2008). According to White *et al*. (2008), the bioactive constituents isolated from *P. sidoides* root extracts include: the coumarins umckalin and 6, 8-dihydroxy-5, 7-dimethoxycoumarin (Kayser and Kolodziej, 1997), garlic acid and its methyl ester (+)-catechin (Kayser and Kolodziej, 1997; Kolodziej *et al*., 2003), and certain fatty acids (Seidel and Tayler, 2004) and tannins (Kolodziej *et al*., 2005).

2.6 Vegetative Propagation: An attempt to satisfy demand

The utilization of plant products in the medical and cosmetic industry is increasing and plants of the genus *Pelargonium*, especially *P. sidoides* and *P. reniforme*, are some of the most sought after herbs. Research on *Pelargonium* sidoides has intensively focused on its potency as a medicinal herb and the chemical composition of its essential oils (Williams and Harborne, 2002). Unfortunately much less work has been done on improving or understanding propagation to enable effective multiplication. Being able to rapidly grow a medicinal plant species is important because it guarantees or enhances supply to meet the ever increasing demand. Vegetative propagation is simply the process of establishing new plants without the use of seeds

© University of Pretoria

or by asexual means. A number of different methods are used to achieve this, ranging from the use of cuttings to more advanced methods like tissue culture.

2.6.1 Vegetative propagation using leaf-bud cuttings

A cutting can be defined as any vegetative plant part which, when detached from the parent, is capable of regenerating the missing organ or organs. It has long been known that, if exposed to the right conditions, certain plant parts are capable of developing into a complete plant similar in most characteristics to the parent plant (Hartmann and Kester, 1983).

Though cuttings were used by man for thousands of years, in-depth and more scientific understanding of the process can be traced back to around the late 1800s (Preece, 2003). Propagators back then understood that rooting of cuttings was associated with the presence of nodes, also called joints back then. It was also understood that rooting was easier when using young plants than older individuals (Preece, 2003). Hartmann *et al*. (1997) stated that plant regeneration can be achieved from any of the vegetative portions of a plant. These include stems, modified stems (rhizomes, tubers, corms and bulbs), leaves or roots. Cuttings can be classified using the part of the plant used. There are stem cuttings which can be hardwood, semi-hardwood, softwood or herbaceous, leaf-bud cuttings which can be single-eye or single-node cuttings and root cuttings.

Propagation of plants using leaf bud cuttings involves promoting the cuttings to produce adventitious roots so that the new plant can be transplanted and function independently like any other plant (Davies and Joiner, 1980). It is a rapid way of coming up with plants having a uniform genotype. Lauer and Lauer (1976) reported that using leaf bud cuttings is a quick, simple and effective way of increasing desired plant stocks. When propagating plants using leaf-bud cutting, the initial cuttings should consist of a current season leaf blade segment, petiole, and a short piece of stem with the attached axillary bud. They differ from leaf cuttings in that only adventitious roots need to form. The axillary bud at the nodal area of the stem provides the new shoot (Fatemeh and Zaynab, 2014).

© University of Pretoria

Several plant species such as the black raspberry (*Rubus cidentalis*), blackberry, boysenberry, lemon, camellia, maple, and rhododendron are equally started by leaf-bud cuttings, as well as many tropical shrubs and most herbaceous greenhouse plants (Hartmann *et al*., 2002). Investigations have shown that plant tissues in the region of buds are likely to be rich in food materials and hormones. Therefore, these tissues make good propagation material, even though the amount of wood used may be extremely small as compared with standard cutting with long internodes. The time required for the leaf-bud cutting to form a heavy root system depends upon the season, the variety or type of plant, and the medium into which the cutting is introduced (Watkins, 2015).

Leaf-bud cuttings are particularly useful when propagation material is scarce, because they will produce at least twice as many new plants from the same amount of stock material as can be started by stem cuttings (Hartmann *et al*., 1997). However, the rooting success of the cuttings is dependent on factors such as position of the cuttings on the shoots, rooting medium used, presence or absence of hormone and concentration, season when cuttings were made as well as physical and environmental factors (Wilson, 1993; Araya *et al*., 2005) and age of the cuttings (Davis and Joiner, 1980).

2.6.2 Position of cutting

It has long been known that the position of a cutting determines the success of rooting and the subsequent creation of a new plant (Preece, 2003). Rooting ability has been known to vary between cuttings from different parts of the same plant, especially in woody species (Leakey and Mohammed, 1985). Cuttings can be categorised in terms of their position on the parent plant. They can be apical, medial or basal cuttings. The impact of the position of cuttings is also species dependent. In some species, basal or medial cuttings produce the best root quantity and quality, whereas in others, apical cuttings root best (Hartmann *et al*., 1997).

Apical cuttings are mostly softwood or semi-hardwood cuttings with developing apex and associated young leaves, which may produce rooting promoters that could be inhibitors if present in excess (Hansen, 1986; Leakey and Coutts, 1989). Many studies comparing rooting in apical stems and in basal cuttings have reported inferior rooting

© University of Pretoria

in apical cuttings. Saifudin *et al*., (2013) compared rooting in *Peltophorum pterocarpum* stem cuttings and discovered that basal cuttings produced more roots than apical cuttings. Another study undertaken by Lebrum *et al*. (1998) on the influence of position on cuttings of *Syzygium paniculatum* gave comparable results to the study discussed above. They reported that the development of roots was higher among those cuttings taken from the median and lower parts of the parent plant than from the apical parts.

Poor rooting using apical cuttings was suggested to be related to the softness of the leaves and stem, and the difference in degree of maturity along the shoot or their rate of growth (Hansen, 1986; Leakey and Coutts, 1989). Smalley and colleagues (1991) suggested that there is a minimal threshold quantity of carbohydrates needed for rooting to take place. This was supported by Saifudin *et al*. (2013) who reported that the efficacy of various cutting positions in inducing rooting originates from differences in nutritional statuses of cuttings from different parts. Apical regions of parent plants are more likely to have less concentration of carbohydrates because of the actions of IBA which promotes the transfer of sugars to basal areas of plants (Hongmei *et al*., 2011).

However, for some species, vegetative propagation of older and more lignified woody cuttings is difficult. In these species, the use of apical stems produces better results (Hartmann *et al*., 1990). This was observed to be true for apical cuttings of rosemary (*Rosmarinus officinalis*) which produced more roots than the basal cuttings (Deen and Mohamoud, 1996; Araya *et al*., 2005).

Montarone *et al.* (1997), working on *Protea eximis,* reported that median cuttings from flower bud stems produced the best roots. In a number of species, basal and medial cuttings rooted significantly better than apical cuttings. Cuttings taken from the basal portion are known to have greater accumulation of photosynthetic products, mostly carbohydrates, thus it is reasonable to expect greater numbers of roots and root weight (Bal *et al*., 1991). In addition to more roots, basal and medial cuttings root faster when compared to apical cuttings (Lebrun *et al*., 1998). This is in tandem with what Palanisamy and Kumar (1997) found. These authors reported that basal cuttings of *Azadirachta indica* rooted better than apical cuttings and they postulated that this could

© University of Pretoria

be due to higher levels of endogenous auxin. Hansen (1998) and Mpati *et al*. (2006) reported that apical and basal cuttings of *Strephanotis floribunda* and *Lippia javanica* respectively produced fewer roots than medial cuttings.

Araya *et al*. (2005), working on bush tea, reported different findings than those of the authors discussed above. This researcher reported that apical cuttings produced more roots than basal cuttings (Table 2.1). Additionally, apical cuttings showed uniformity in the number of roots produced than basal cuttings and the quantity of roots was directly proportional to an increase in number of days after planting. Working on bush tea (*Athrixia phylicoides*), Soundy *et al*. (2008) reported that apical stems rooted earlier than basal cuttings but after 20 days, cuttings from both regions had similar roots. It was therefore concluded that cuttings from both regions can be used effectively to establish new plants.

Table 2. 1: Effect of season and cutting position on mean root number and root length of bush tea (Araya *et al*., 2005)

Cutting position	Season	Number of roots (Mean ± S.E)	Root length in cm (Mean ± S.E)
Apical	Summer	8.86 ± 1.72	2.10 ± 0.62
	Autumn	14. 80 ±2.66	5.16 ± 0.82
	Winter	8.85 ± 2.04	2.73 ± 0.57
	Spring	19.74 ± 5.84	4.23 ± 1.24
Basal	Summer	4.50 ± 1.83	1.79 ± 0.89
	Autumn	9.03 ±3.08	3.39 ±1.37
	Winter	4.93 ± 1.82	2.72 ± 1.06
	Spring	12.33 ± 3.05	3.28 ± 1.04
Significance		P < 0.0001	P < 0.0001

© University of Pretoria

2.6.3 Physical factors

Leakey (1983) stated that physical factors such as cutting length, stem diameter, cutting type, presence or absence of hormones and their concentrations play an important role in rooting ability of cuttings.

Plant cutting size (length and diameter)

The length of the cuttings can impact the quality and quantity of roots produced by the cutting and the optimal length varies with species. Palanisamy and Kumar (1998) reported that in *Azadirachta indica,* 100% of basal cuttings measuring 25 cm in length and 0.5 cm in diameter successfully rooted whilst those measuring 5 cm or less did not produce any roots. This could be attributed to the quantity of resources available to cuttings of different sizes. This study is very comparable to the findings of OuYang *et al.* (2015) working on Norway spruce (*Picea abies*), who reported that thin and short cuttings produced fewer roots than larger cuttings. They reported an optimum length of 9-12 cm and diameter of 0.3-0.4 cm. They also stated that rooting was positively correlated to both length and diameter of cuttings.

The impacts of cutting length on rooting are not always straight forward. Leakey (1983) reported that cutting length was significantly correlated with number of roots per rooted cutting and not with rooting percentage. The relationship between cutting length and percentage of rooting was stronger when cuttings increased in length acropetally than basipetally. Furthermore, thicker and longer cuttings rooted better than shorter and thinner ones. They suggested that it could be because that larger cuttings store more starch in the stem than smaller ones (Leakey, 1983; Leakey and Mohammed, 1985). Aminah *et al*. (2015) reported that cuttings of *Tinospora crispa* measuring 22.5 cm produced more roots than shorter cuttings. All the cuttings in that experiment were treated with IBA and this suggests that both length and hormones play important roles in root development. The abundance of starch in a cutting could also be linked to the rate of mortality of cuttings. Thin short cuttings are more prone to die before rooting because they lack enough starch to sustain them before photosynthesis commences (Hartmann *et al*., 2002). The relationship between rooting success and size is not always as reported above; sometimes thinner-stemmed cuttings also root better,

© University of Pretoria

because of other favourable growth parameters such as water and season (Howard and Ridout, 1992).

Effect of Leaves on rooting

The presence of leaves on cuttings plays an important role on root initiation of many plant species (Hartmann *et al*., 1990). Leaky and Mohammed (1985) reported that leafless cuttings of *Triplochiton scleroxylon* were unable to root whilst Ofori *et al*. (1990) reported that only 30% of leafless *Milicia excelsa* cuttings rooted. Similarly, Tchoundjeu *et al*. (2001) reported that leafless cuttings of *Prunus africana* did not root and rooting ability was positively correlated with leaf area up to 20 cm^2. Cuttings with a surface area of 25 cm^2 rooted at the same level as those with a surface area of 20 cm^2. It is possible that the increase in rooting success with increasing leaf area in *P. africanus* is related to the rate of photosynthesis. Photosynthesis provides the growing roots with energy needed for the complex physiological process of root formation. Hoad and Leakey (1996) measured photosynthesis in rooting cuttings of *Eucalyptus grandis* and discovered that rooting success was strongly correlated to the rate of photosynthesis.

The relationship between the presence of leaves and success of rooting in cuttings is not always clear. Leaves on the mother plants are important because they ensure that the harvested cuttings have nutrients. However, large leaves on cuttings has been found to result in increased water loss and consequently, a reduction in photosynthesis (Newton *et al*., 1992). Many leaves on a cutting can be harmful because they result in excessive transpiration which may lead to a water deficit. Vigl and Rewald (2015) warned against deploying cuttings with large exposed surface area as this will result in more transpiration and failure of the planted cuttings. This is especially true in moisture stress prone areas. This can impair rooting or cause mortality before rooting (Hartmann *et al*., 1990; Loach, 1992).This negative impact of leaves can be addressed by minimising the total surface area of available leaves. Excessive trimming may reduce photosynthesis and limit rooting such as in *Eucalyptus globulus* (Wilson, 1993). Hence, it is essential to have a balance between photosynthesis and transpiration for optimum rooting to occur (Leakey and Coutts, 1989; Newton *et al*., 1992). A ± 50 cm^2

© University of Pretoria

of leaf area on a single cutting was recommended by Wiessman-Ben and Tchoundjeu (2000) for best rooting of *Shorea leprosula* cuttings.

Planting depth and size of plant cuttings

Planting depth (the extent of underground stem) can influence establishment and different plants have different planting depth requirements. Edelfeldt (2015) reported that willows planted deeper were more susceptible to mortality than those planted shallower. The mortality rate was even greater when the cuttings planted deep were also small in size or length. Depth has been observed to have great impacts on stem growth. Holwerda and Ekanayake (1991) did extensive work on sweet potato. They reported that sweet potato cuttings planted at a depth of 5 cm had significantly less stem growth than those planted at 10 or 15 cm. Furthermore, the more nodes planted beneath the soil, the greater the reduction was in stem growth. The number of roots per internode increased significantly when the number of nodes per cutting decreased.

2.6.4 Season

The season of the year when cuttings are collected or planted is an important factor in determining the extent of rooting success of cuttings (Klein *et al*., 2000). Seasonal variation in rooting efficiency is very common in woody plants and there is optimal time for root establishment for each species (Howard, 1996). 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. A number of studies suggested that rooting of cuttings is facilitated when carbohydrates and growth promoters are in abundance (Puri and Vermat, 1996; Mpati *et al*., 2006). Hartmann *et al*. (1990) and Wilson (1993) also reported that simple rooting of softwood cuttings could be achieved when taken during spring and summer than in winter. Also, Peri 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. In contrast, root number and rooting percentage of Eastern red cedar cuttings was higher throughout winter (Henry *et al*., 1992). On the other hand, rooting of Myrtaceae family (*Chamelaucium* sp.*)* is unaffected by season (Curir *et al*., 1993).

© University of Pretoria

Efficiency of auxin was found to vary with season when rooting percentage and root number (87% and 5.4 respectively) of treated *Eastern redcedar* cuttings were maximized in January with 0.5% IBA (Henry *et al*., 1992). Similar results on season were reported on rooting of *Cephalotaxus harringtonia* where cuttings taken in December to February and treated with K-IBA had significantly higher rooting percentage, mean number of roots and mean root length than non-treated cuttings (Southworth and Dirr, 1996).

Hare (1978) reported that peak rooting success for cuttings of *Pinuselliottii* var. *elliollii* in Canada occurs during spring as temperature begins to warm. It is probable that during this time, it is neither too cold nor too dry. Danthu *et al*., (2008), in the first successful attempt to propagate the species *Ravevsara aromatica,* and *Aphloia theiformis* as well as the endangered *Prunus africana* from Madagascar forests, reported that rooting success could only be realised if cuttings are collected in the hot season. The authors suggested that this observed pattern could be attributed to the physiological condition of the parent tree. During the hot season, the conditions are ideal for photosynthesis and as such the concentration of carbohydrates in the cuttings are high.

2.6.5 Rooting hormone and media

A number of studies have shown the importance of rooting hormones in enhancing the formation of roots on cuttings (Topacoglu *et al*., 2016). Auxins are a group of tryptophan-derived hormones, which play a critical role in many aspects of plant development, including root development and have been extensively used to promote rooting in cuttings (Woodward and Bartel 2005). Studies on the efficacy of auxins as root stimulators are often paired with studies to determine which media gives the best results. Auxins promote the development of adventitious roots of cuttings through their ability to promote the initiation of lateral primordia and to enhance transport of carbohydrates to the cutting base (Leakey, 1983; Hartmann *et al*., 1990; Fogaca and Fett-Neto, 2005). This enhancement could be through their direct effect on the cuttings or through their interactions (Leakey, 1990; Ofori *et al*., 1996).

© University of Pretoria

2.6.5.1 Rooting hormone

The development of adventitious roots is a complex physiological process which is regulated by a number of phytohormones (Sun *et al*., 2016). The growth processes in plants are controlled by a class of hormones called auxins (Bertoni, 2011). Indole-3-acetic acid (IAA) is the most common in plants, occurring at different concentrations in different parts (Hao *et al.,* 2016). Another auxin, indole-3-butyric acid (IBA) is derived from IAA and is one the most commonly used compounds in plant propagation for stimulating adventitious roots in stem cuttings (Gehlot *et al.*, 2014). A number of auxins have since been discovered since the discovery of IAA by F.W. Went in 1928. Some of the auxins used include 1-naphthaleneacetic acid (NAA).

Adventitious root formation is regulated by complex interactions between endogenous and exogenous factors which affect the various developmental stages of root formation. In general terms, adventitious root follows three developmental phases: (1) differentiation, in which predetermined cells switch from their normal morphogenetic path to act as mother cells for the root primordia; (2) initiation, in which these cells start to divide and form the distinct structure of a root primordium; (3) elongation, during which the newly formed primordia form vascular connections and later protrude through the surrounding tissues to form roots (Hartmann *et al*., 2002, Fardin *et al*., 2015). Many physiological studies have shown that auxin plays a central role in the development process of root initiation (Jarvis, 1986). The promoting effect of IBA on rooting is mainly the result of its conversion to IAA in plant tissue. However, IAA, which is needed for the rooting process, is oxidised readily in the plant by peroxidases, whereas IAA released from IBA is not oxidised by peroxidases and remains at the base of the cutting (Abu-Zahra *et al*., 2012).

The impact of auxins varies with species, rooting media as well as concentration. Topacoglu *et al*. (2016) studied the effectiveness of IAA, NAA and IBA on cuttings of *Ficus benjamina.* They discovered that 100 ppm of IBA and 10 ppm of NAA resulted in the highest rooting percentages of 93.9% and 94.43% respectively. Ouyang *et al*. (2014) compared the effects of IBA, NAA and ABT rooting powder on cuttings of *Picea abies*. They determined that all hormones significantly increased rooting and IBA was more effective than the rest.

© University of Pretoria

According to Al-Barazi and Schwabe (1982), the purpose of treating cuttings with auxin is to increase the percentage of rooting, root initiation, number of roots and uniformity of rooting. Cooper (1936) showed that the application of IAA increased both the rate of rooting and number of roots in lantana. In Cooper's study, as evident on the graph (Figure 2.1), plants treated with IAA developed more roots quicker than plants without IAA. This means it is quicker to produce new plants from cuttings if one employs auxins.

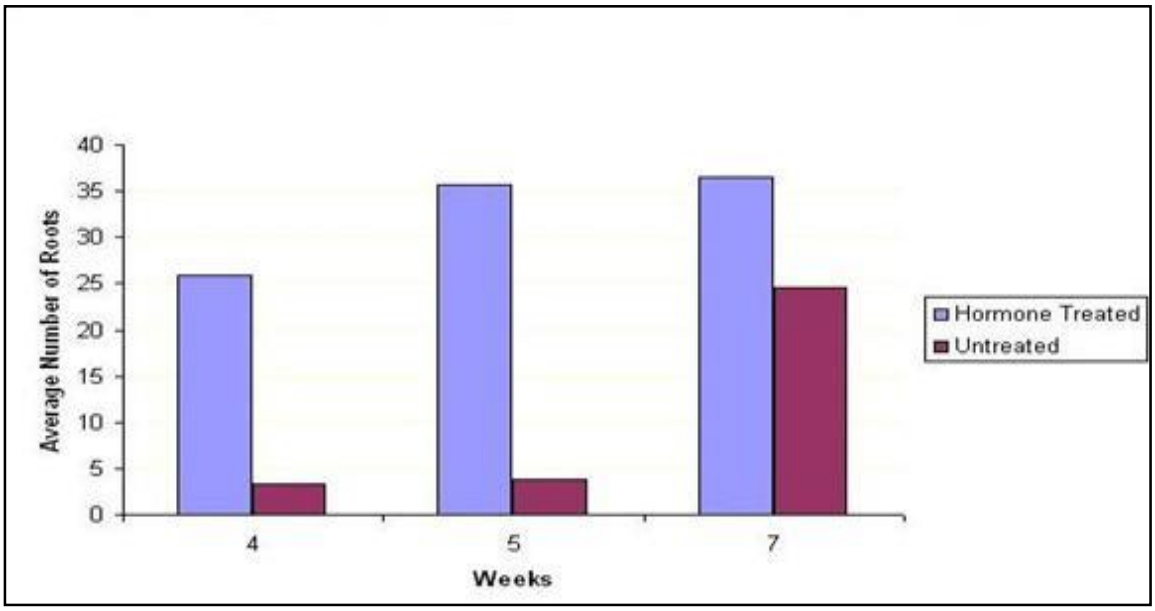

Figure 2.2: Effects of hormone treatment on number of roots on *Lantana camara* cuttings (Koike *et al,* 2017)

The exact way auxins work is still not fully understood up to this point, but they have been shown to accelerate the translocation of nutrients from upper parts of the cuttings to their basal ends where roots are formed, providing enough energy for cells involved in the rooting process (Arya *et al*., 1994). A report by Al-Barazi and Schwabe (1982) stated that IBA treatment seems to stimulate cell division in the ray cells between the primary bundles to improve root initiation. According to Jawanda *et al*. (1991), plum cuttings treated with 0.01% IBA recorded a higher rooting percentage followed by 0.005% IBA than the control. The final rooting percentage of *Milicia excela* was also increased by 9% above the control when treated with 0.002% IBA. Even though auxins

© University of Pretoria

promote adventitious root development of cuttings, the use of auxins at the appropriate concentration is essential because the wrong concentration can inhibit rooting or it can act as a growth retardant when applied in higher concentrations (Hartmann *et al*., 1990; Wiessman-Ben and Tchoundjeu, 2000). The rooting response of cuttings was also found to be dependent on concentration of auxins, and this was supported by Hartmann *et al*. (1990). According to Lebrum *et al*. (1990), high concentration added to the endogenous auxin of the plant, which self-increases as a consequence of taking cuttings, might have led to a level which disturbed the hormonal metabolism and inhibited rhizogenesis.

The rooting process in plant propagation is a process governed by environmental and endogenous factors (Gehlot *et al.,* 2014). The auxin IBA is effective in stimulating the production of adventitious roots but its effectiveness can be further enhanced by external factors. Amri (2015), working on *Dalbergia melanoxylon,* showed that inoculating cuttings treated with IBA with arbuscular mycorrhizal fungi (AMF) significantly increased the quality and health of the roots produced. This shows that there is a lot of work needed in our endeavour to effectively propagate useful plants.

It is reasonably evident that cuttings of different plant species require different optimum concentrations of auxins for the best rooting. Mesen *et al*. (1997) found that *Cordia alliodora* required a concentration of 1.6% IBA for optimum rooting percentage but it failed to root when no auxin was applied. Leakey and Mohammed (1985) also reported the same results for rooting of *Triplochiton scleroxylon*. In contrast, tropical trees species, such as *Nauclea dierrichii* and *Vochsia hondurensis* (Leakey, 1990), *Shorea macrophylla* (Lo, 1985), and *Milicia excelsa* (Ofori *et al*., 1996) successfully rooted without application of auxin. The efficiency or rooting hormone was also reported to vary when used in combination with each other (Smalley *et al*., 1991; Puri and Vermat, 1996). Combinations of NAA+IBA+thiamine auxins and IAA+IBA+2,4-D auxins proved to be the best auxin treatments, stimulating rooting in 50-60% of *P. cineraria* cuttings than when used singly (Arya *et al*., 1994). The effect of five different concentrations of IBA (0, 0.2, 0.4, 0.8 and 1.6%) on the rooting percentage and the number of roots per rooted cuttings of single-node, leafy stem cuttings of *Cordia alliodora* were investigated. Nine weeks after insertion, highly significant differences in rooting

© University of Pretoria

percentage were found between IBA concentrations with a value of 70% recorded in cuttings treated with 1.6% IBA, compared with only 10% for the control cuttings. The effect of IBA concentration on number of roots per rooted cutting was also significant. It was also confirmed by other that rooting was highly dependent on IBA concentration (Messen *et al*., 1997; Mpati *et al*., 2006).

The effect of auxins also varies with time of application (Hartmann *et al*., 1997; Boeing *et al*., 1999). According to Kromwijk and van Mourik (1992) dipping the base of *Ficus benjamina* cuttings in a solution containing 0.025% of IBA for 4 hours was found to be more effective than using higher IBA concentrations or a rooting powder.

2.6.5.2 Rooting medium

According to Watkins (2015) a congenial medium to hold the cutting during their period of rooting is of utmost importance. Additionally, there is no ideal or universal rooting medium for cuttings; no propagation method is going to work if the right media for growth is not used (Berhe and Negash, 1998). A medium is said to be suitable for propagation based on species, cutting type, season, propagation system used, its cost and availability (Hartmann *et al*., 1990). A suitable propagation medium has to provide the cuttings with good aeration, moisture, drainage, support, nutrients and must be free of disease causing pathogens (Hartmann *et al*., 1990). Most frequently used media contain combinations of sand, peat, sphagnum moss, vermiculite, perlite, compost, and shredded bark (Hartmann *et al*., 1997).

Even though the water uptake of cuttings is directly related to the water content of the medium, it is important to have a balance between the water and air content of the medium (Grange and Loach, 1983; Loach, 1992). Poor rooting of *Syzygium paniculatum* cuttings were reported when peat: sand was used as a medium. This was related to the compaction of the peat by water and as a result prevented proper aeration of the mixture and lead to rotting of the cuttings (Lebrum *et al*., 1998).

Temperature of the rooting medium was also found to affect the rooting of the cuttings (Hartmann *et al*., 1997). Additionally, Lebrum *et al*. (1998) also reported that when cuttings of *Syzygium paniculatum* were grown on a non-heated substrate (average temperature 22 ± 0.5^{o}C), the mean rooting rate exceeded 75% of the best substrates.

© University of Pretoria

Studies also show that root length and rooting percentage have some dependence on the type of medium used. Cuttings of *Rosa centifolia* planted in peat perlite resulted in a significantly higher rooting percentage and length than in vermiculite, although the media did not affect mean root number per rooted cutting (Al-Saqri and Alderson, 1996).

In general, the type of media used depends on growing region and species propagated, most frequently used material contains combinations of different mediums (Araya *et al*., 2005).

2.7 Chapter conclusion

This chapter highlighted some of the important aspects of phytomedicines. In general, plants offer an attractive alternative to conventional medicines. Research in identifying plants with beneficial medicinal uses is ongoing and building on the foundation laid by proven plant based medications like *P. sidoides* extracts. Threats posed by overexploitation were also highlighted as well as ways that can potentially mitigate the overexploitation of wild species. The chapter also highlighted the role that science can play in reducing the harvesting of wild plants and availing the healing herb to more people. It was shown that vegetative propagation could be useful in this regard. The efficiency of artificial propagation techniques can be improved by using phytohormones and appropriate growing media.

© University of Pretoria

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

This chapter attempts to explain in full the methods that were used in conducting this study. Deliberate effort was put in explaining each step so that the study can be replicated. This is also important to those who might want to take up the recommendations proffered in chapters that follow. Most of the work that went into the setting up was carried out by the author.

3.2 Preparation of cuttings

Parent or stock plants were acquired from *Pico-Gro* Nursery (Midrand, Gauteng Province, South Africa). A total of 960 leaf-bud cuttings measuring 15–20 cm were cut from the stock plants (using sterile blades). This was done early in the morning (between 06:00 am and 08:00 am) and the cuttings were immediately wrapped with wet tissue paper followed by placing them in plastic bags in order to keep them moist and turgid waiting for further treatment.

Three hundred and twenty cuttings were prepared for each replicate, but this applied for the summer season trial only because for the other seasons the number of cuttings differed due to changes in treatment combinations. The 15–20 cm long leaf-bud cuttings taken from the mother plants early in the morning were taken into the mist-beds for planting.

3.3 Propagation environment

The leaf bud cuttings of *P. sidoides* were propagated on a mist bed in a greenhouse located at the Experimental Farm of the University of Pretoria (25^{o} 45’ S, 28^{o} 16’ E and 1372 m above sea level). The propagation unit was supplemented with a 24 hour misting and fogging system (Environment, Pretoria, South Africa). The system was programmed to automatically release a mist of moisture depending on the humidity of the greenhouse. The mist bed was 5 m long, 1.5 m wide and 1 m high. Throughout the study period, the temperature and relative humidity of the greenhouse were measured and recorded using a thermo hygrograph (HOBOLD Temp/RH (H14-00). In

© University of Pretoria

summer, autumn, winter and spring the minimum and maximum temperatures ranged between 18.7ºC - 35.5ºC, 3.8ºC - 30.2ºC, 10.2ºC - 28.6ºC and 14ºC - 35.3ºC respectively.

3.4 Preparation of rooting media

Two different propagation media were used in the experiment (1 mm sand only and 1 mm sand + coir). The ratio of sand + coir mixture was 2:1.The two media types were randomly assigned to seedling trays with dimensions 5 x 3 x 4.5 cm (width, breadth and depth). Prior to planting, the filled trays were put under a mist system set to come on at 5 min intervals for 10 seconds and left over night to get moist.

3.5 Experimental design

The trial was a 2^3 factorial experiment laid out in a randomized complete block design (RCBD) with 3 replicates. Cuttings were replicated 8 times for each treatment. The treatments consisted of medium, hormone, planting depth and season. The exact number of cuttings varied with season. In the summer season, trial treatment combinations were: medium: sand or sand + coir (2:1 ratio), hormone (Dynaroot™ No. 2; 0.3% IBA and control) and planting depth (1.5 and 3 cm).

In autumn season, trial treatment combinations were: medium: sand or sand + coir (2:1 ratio), hormone (Dynaroot™ No. 2; 0.3% IBA, Dynaroot™ No.1; 0.1% IBA and control) planting depth (1.5 and 3 cm). A less concentrated version of the hormone (0.1 % IBA) was introduced into the treatments as a result of the summer trial observations.

In winter season, trial treatment combinations were: medium: sand or sand + coir (2:1 ratio), hormone (Dynaroot™ No.1; 0.1% IBA and control) planting depth (3 cm). In this season, a 0.3% IBA was dropped from the treatments and planting depth of 1.5 cm was also removed. In all seasons, samples for analysis were taken on 5, 10, 15, 20 and 25 days after planting (DAP).In spring season, the treatment combination were the same as in winter.

© University of Pretoria

3.6 Planting of *P. sidoides* cuttings

The initial fresh mass and circumference of each cutting was taken, followed by dipping the base of each cutting in water depending on the treatment type (with or without hormone). The cuttings to be treated with a hormone were then dipped into a rooting hormone powder (Dynaroot No.1 (0.1% IBA) or No.2 (0.3% IBA) to a depth of 1 cm and excess rooting powder was tapped off before planting. In order to avoid brushing off of the rooting powder, a hole was made on the rooting media with a stick (Hartmann and Kester, 1983).

The cuttings, after respective treatments, were directly planted into the pre-wetted rooting medium, sand or sand + coir to a depth of either 1.5 or 3 cm (Figure 3.1).

Figure 3.3: Experimental layout showing cuttings planted in both media in the mist bed (summer season)

3.7 Rooting Evaluation

Cuttings were assessed for rooting at 5 day intervals; that is after 5, 10, 15, 20 and 25 days after planting (DAP). This rooting assessment was done by carefully separating the rooted cuttings from the seedling trays so that no roots would break off, followed

© University of Pretoria

by washing the root zone with water. The number of cuttings that developed roots was noted, the length of the roots on cuttings and number of roots on cuttings were recorded and then re-plant.

3.8 Morphological Analysis of *P. sidoides* transplants

The morphological analysis of *P. sidoides* was carried out in the University of Pretoria's micro-analysis laboratory. Forty healthy rooted transplants or rooted cuttings were selected for destructive analysis and collected 20 DAP during spring season; this was done after other data was collected as described in section 3.7 above. Twenty of these cuttings were initially planted in sand and ten of these were initially treated with hormone (Dynaroot No.1) while the other ten were not. The other twenty cuttings were initially planted on sand + coir and ten of these were initially treated with hormone (Dynaroot No. 1) while the other ten were not.

The transplants were rinsed with tap water to remove all the growth medium, wrapped in a moist towel paper to prevent desiccation and taken to the microscope laboratory for analysis. At the microscope laboratory, the stems and part of the cuttings were dissected with a scalpel blade to enable clear observation. A camera mounted electron microscope was then used to observe the morphological structure of the rooted cuttings. The transplants were assessed for callus and radicle formation.

3.9 Survival rate of transplanted *Pelargonium sidoides* leaf-bud cuttings

An experiment was conducted to evaluate the success rate when transplants from cuttings were replanted into standard potting soil with 25% coco peat. To investigate this, a factorial experiment arranged in a randomized complete block design with four replications and five blocks was used. For this part of the study, a total of 100 transplants that were still healthy after the measurement of root attributes were used. These cuttings were initially rooted/grown in either sand or sand + coir with or without 0.1 % IBA treatment.

The media (standard potting soil with 25% coco peat), packed in black polythene bags measuring 17.5 cm in height and 12.5 cm in diameter, was pre-treated the same way as described previously for rooting media. The 100 transplants were then planted in

© University of Pretoria

these polythene bags (Figure 3.2) and kept at the propagation site for a week before moving them to a greenhouse in order to recover from transplanting shock. After recovery the plants were moved to the greenhouse in which the stock plants were growing.

Figure 3.4: Transplanted *Pelargonium sidoides* leaf-bud cuttings

The experiment on transplants' survival, with respect to time when the cuttings were transplanted showed that the successful growth of the *P. sidoides* transplants only started after two weeks from the date they were transplanted and as such it was decided to leave the transplants for 30 days in the glasshouse. The potted plants were fertilized with commercial foliar fertilizer (4 ml of NITROSOL®; 8% N: 2% P: 5.8% K in 1L of water). During this period "Benomyl" fungicide was applied as foliar spray to prevent transplants from getting fungal infection and were irrigated every two days. After 30 days (month) from transplanting, the transplants were assessed for survival rate, root number and length of roots in respect to their previous treatments during propagation.

First data collection was done after two weeks. Cuttings to be analysed were washed at the root zone and tangled roots were gently separated. The plants were assessed for root number and root length.

© University of Pretoria

3.10 Statistical analysis

Cuttings were analysed by destructive sampling after 5 to 25 DAP, with 64 cuttings harvested depending on the season from each replicate after every 5 days. The fresh mass of each cutting was determined, the stem diameter was measured using a vernier calliper, the number of leaves was counted and the root length was measured using a ruler. The data was analysed using the SAS programme and Krustal-Wallis test was done to determine the similarity or differences within the measurements taken. All statistics were done at a significance level of 5%.

© University of Pretoria

CHAPTER 4

RESULTS AND DISCUSSIONS ON PROPAGATION OF *P.SIDOIDES*

4.1 Introduction

This chapter summarizes the results obtained from the study and the results put into two sections. The first and longer section describes the effects of propagation media, season and hormone treatment (Dynaroot^{TM}) on the propagation of *P. sidoides* cuttings. The extent of the success of propagation, in this study, is judged by the length of roots on propagated cuttings as well as the number of roots on respective cuttings. The general appearance of the roots was also noted by visual inspection, an approach that can be described as subjective but none the less an important part of the study.

The second section assessed the effects of initial treatment of cuttings on the survival and performance of transplanted transplants that would have grown from the cuttings. This is also an important part of the study since the overall objective was to have healthy and productive plants from the cuttings.

Bar graphs were predominantly used to report on the findings because they are easy to understand and effective in conveying differences in the variables measured. Pictures were also used to reduce the subjectivity of visual inspection of the cuttings. Each graph or picture is always accompanied by an in-depth explanation.

4.2 Effects of rooting media on roots of *Pelargonium sidoides*

Media, in this context, refers to the substrate the cuttings were propagated on. Physical and chemical properties of the media can have profound impacts on its effectiveness or ability to support the rooting process of cuttings (Abdi, Hedayat and Modarresi, 2013).

Generally, cuttings grown in sand had longer, thicker and healthier looking roots (Figure 4.1). Through visual inspection, these cuttings showed well-developed adventitious roots which established more successfully and grew vigorously compared to those that had fewer adventitious roots.

© University of Pretoria

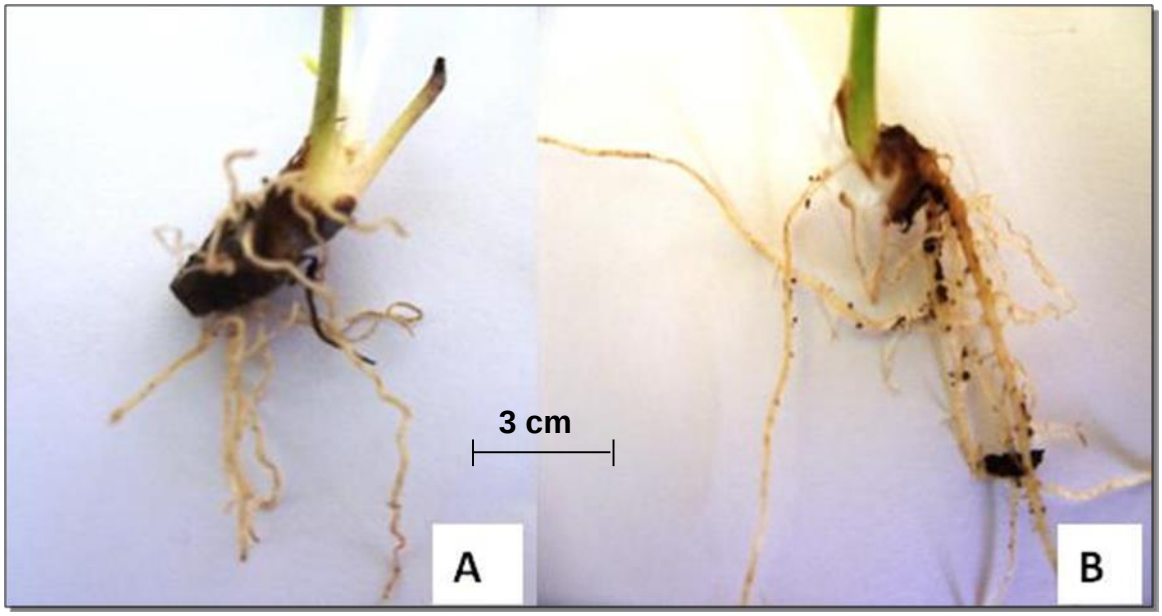

Figure 4.1: Root development and establishment on leaf-bud cuttings of *P. sidoides,* propagated in sand + coir (A) and in sand (B).

The differences in the appearance of roots grown in the two different media were evident as shown in Figure 4.1. Sand produced more roots which were much longer and looked healthier. These differences are probably a result of the water holding capacities of the respective media (Leenaars *et al*., 2018). As sand would hold water for a shorter time and quickly infiltrate, while sand and coir would hold water much longer and that resulting in waterlogging.

4.2.1 Effects of rooting media on length of roots of *Pelargonium sidoides* in summer

The quality of roots in plants is measured by a number of variables and the easiest to use is the size and number of roots on respective cuttings. Generally speaking, long roots function better than short roots and the same can be said for cuttings with more numerous roots (Hartmann *et al*., 2007). The quality of roots is affected by a number of variables. In this study, the effects of season, media, hormone and planting depth were assessed. Figure 4.2 shows the effect of media on root length during the summer season.

© University of Pretoria

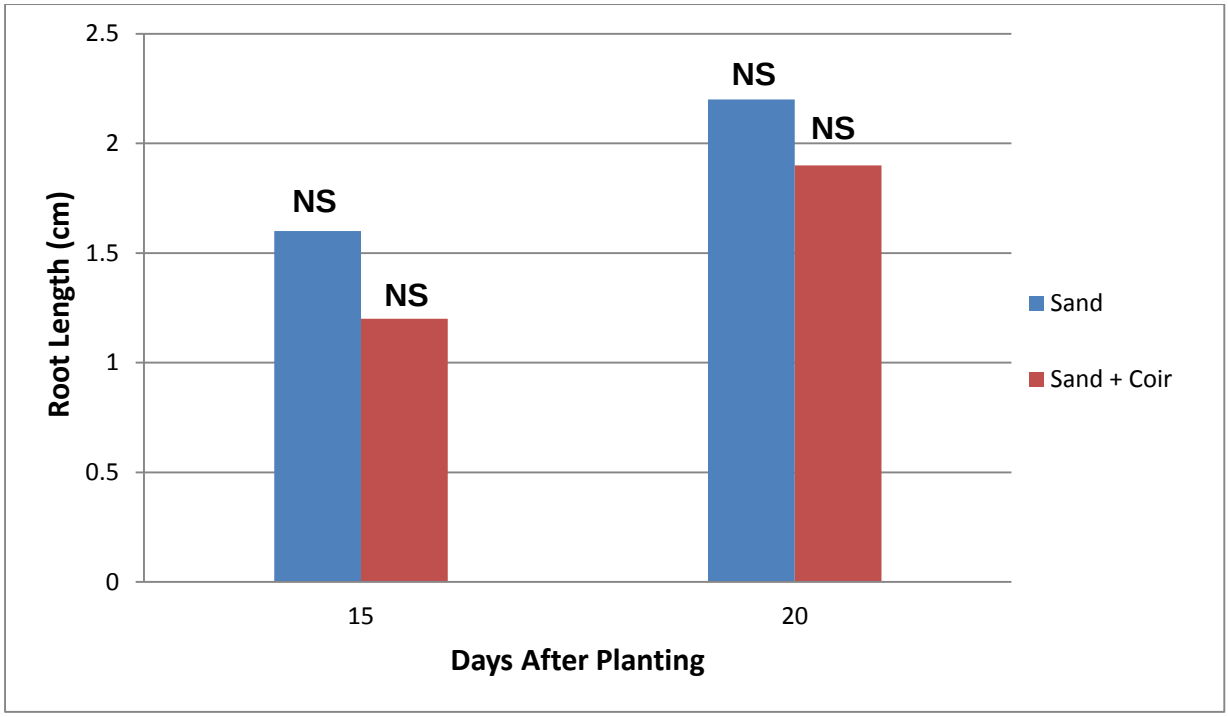

Figure 4. 2: Length of roots of *P. sidoides* grown in different media at 15 and 20 days after planting (DAP) in summer. No significant (NS) differences attributable to media were observed in root length (Kruskal-Wallis, P > 0.05).

In summer, the type of media used, sand or sand + coir, did not result in significant differences in root length. Sand tended to have longer roots at both 15 and 20 days after planting (DAP) but the differences in length were not statistically significant. No result was available for root length at 25 DAP because roots on most cuttings decayed.

4.2.2 Effects of rooting media on length of roots of *Pelargonium sidoides* in autumn

In autumn, the results were different than what was observed in summer. The differences could be due to differences in ambient temperatures between the two seasons. In this season, root decay was minimal and as such the researcher was able to get results for 25 DAP. The results, as presented in Figure. 4.3 show that the average root length was greater during this season if compared to summer.

© University of Pretoria

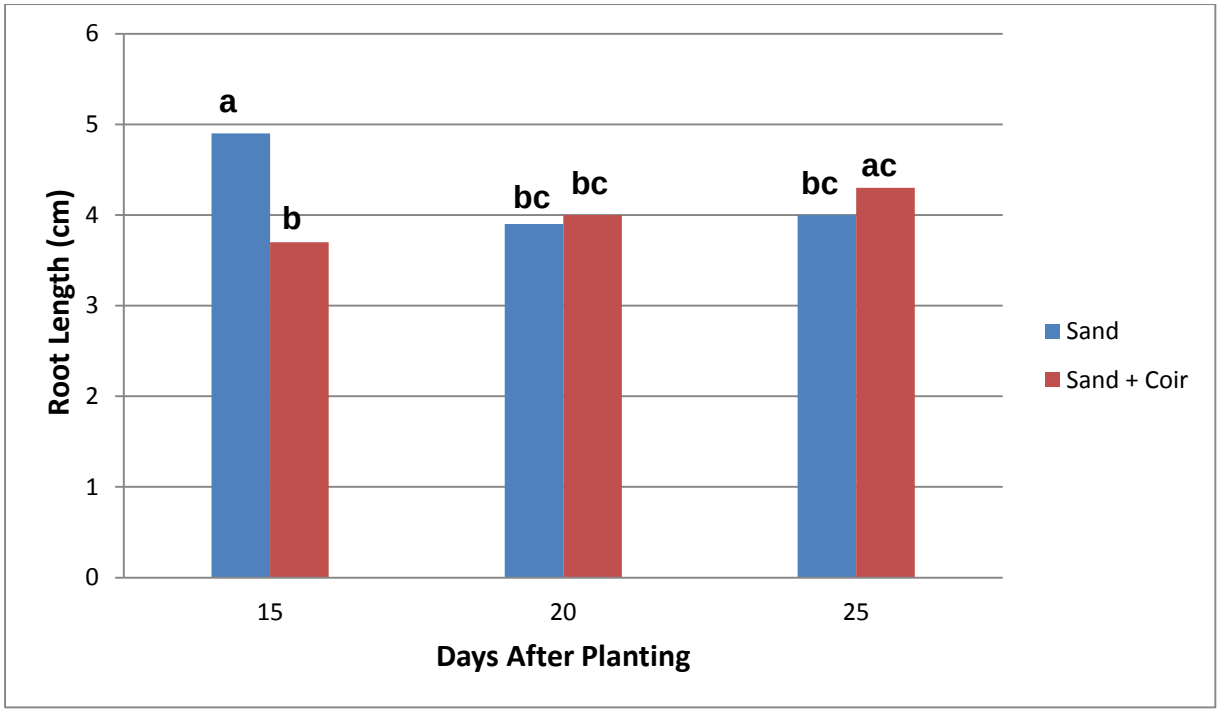

Figure 4. 3: Length of roots of *P. sidoides* grown in different media at 15, 20 and 25 days after planting (DAP) in autumn. Bars bearing different letters are significantly different (Kruskal-Wallis Test, P < 0.05)

In autumn, longest mean root length was observed at 15 DAP on roots of cuttings propagated on sand and their mean length was significantly greater than the length of cuttings grown on sand + coir at the same age. At 20 DAP, roots propagated in sand + coir were longer than those grown on sand but the difference in length was not significant. As the number of days increased from 15 to 20 growth was retarded and cuttings started to show signs of rotting due to waterlogging, so the maximum rot length was obtained at 15 DAP.

4.2.3 Effects of rooting media on length of roots of *Pelargonium sidoides* in winter

In general, when conditions are not optimal like in winter, plants grow slower and store resources for summer when growing conditions are more suitable. Due to low temperatures and lack of water in the wild during winter, the plants are evolutionary programmed to grow minimally and this may not be changed even if water is availed

© University of Pretoria

artificially (Korner, 2016). This could be because physiological processes in some plants are controlled by day length also and not just the availability of water or artificially high temperatures provided for by the greenhouse environment.

In general, cuttings grown in winter had shorter roots if compared to those grown in autumn. In addition to that, as shown on figure 4.4 below, there were no significant differences in root lengths at different days after planting for the cuttings grown in winter.

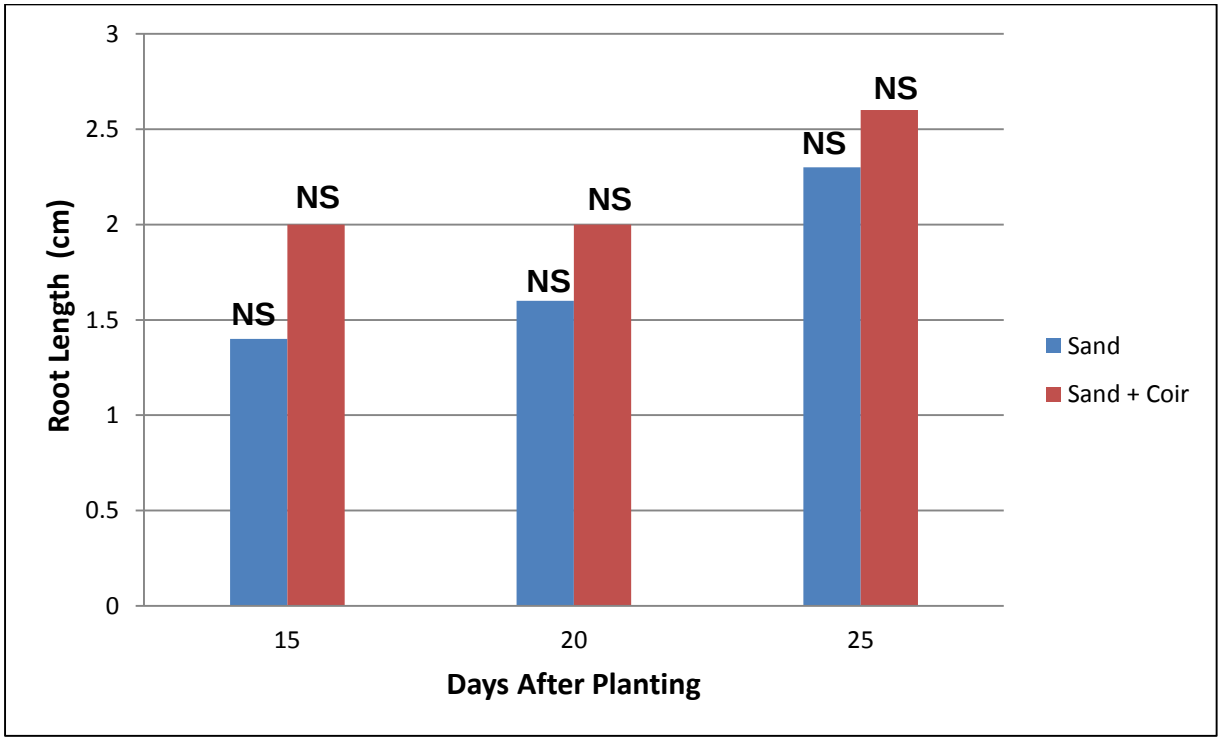

Figure 4. 4: The length of roots of *P. sidoides* grown in different media at 15, 20 and 25 days after planting (DAP) in winter. No significant (NS) differences in length were observed which could be attributed to media (Kruskal-Wallis, P > 0.05)

In winter, cuttings propagated in sand + coir generally had longer roots but the differences in length at respective days after planting were not statistically significant (Kruskal-Wallis test, P > 0.05). The lengths of the roots generally increased with increasing days after planting. Surprisingly, the roots were longer than what was observed in summer but shorter than what was observed in autumn.

© University of Pretoria

4.2.4 Effects of rooting media on length of roots of *Pelargonium sidoides* cuttings propagated in spring

In spring, the lengths of roots were measured at 15, 20 and 25 days after planting. Unlike in summer, fewer roots decayed and as such more samples were measured even at day 25. The results, as shown in Figure 4.5, show that the length of roots increased with increase in days after planting.

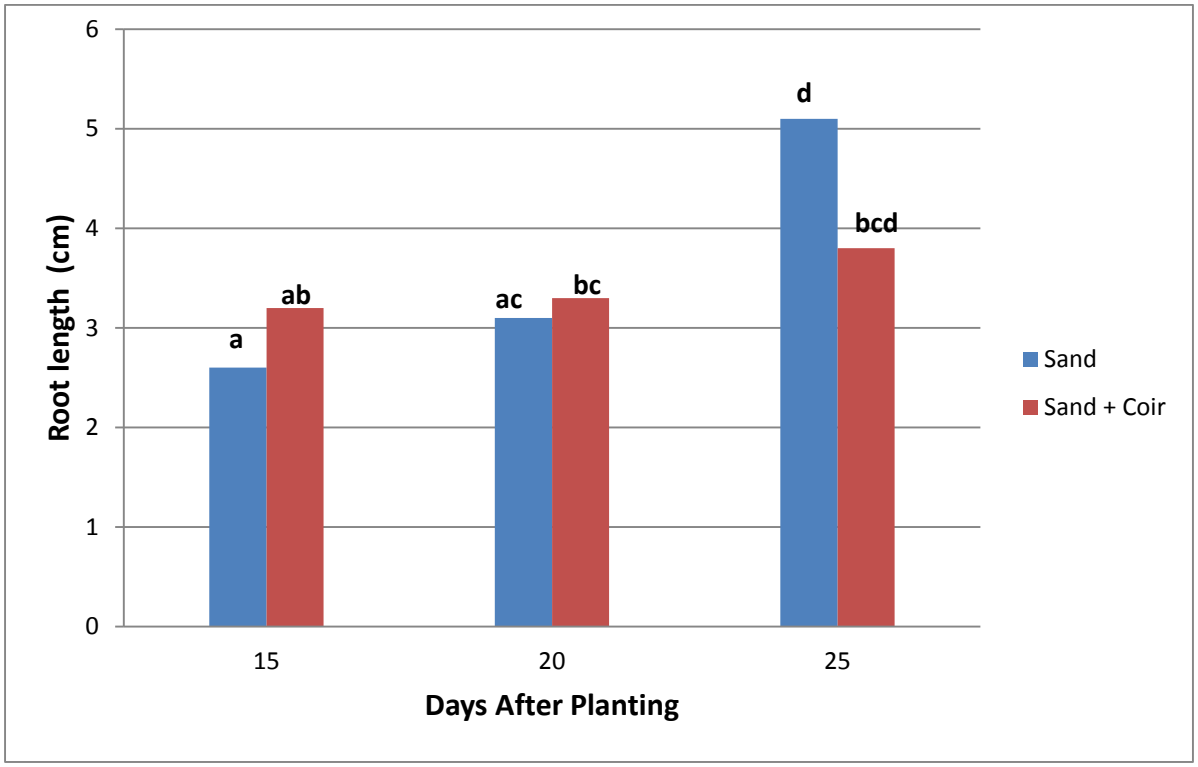

Figure 4. 5: Length of roots of *P. sidoides* grown in different media at 15, 20 and 25 days after planting (DAP) in spring. Bars bearing different letters are significantly different (Kruskal-Wallis Test, P < 0.05)

In spring, significant differences were observed in length of roots grown in different media at all sampling dates. At 15 and 20 DAP, cuttings grown in sand + coir had significantly longer roots than those grown in sand. Cuttings at 25 DAP grown in sand had the longest roots observed for the season, significantly longer than roots of

© University of Pretoria

cuttings grown in sand + coir at the same period. In summer, more of the developing roots on respective cuttings rotted if compared to spring. It can be concluded that in spring, it will be beneficial to grow cuttings for longer in the rooting media so that they develop longer roots without the risk of the roots rotting.

4.3 Effects of growing media on number of roots per cutting

The number of roots on plants is important. In general, for a particular species, more roots are superior than fewer roots. This is because more roots enable greater access to resources and in plants like *P. sidoides,* roots develop into rhizomes and more roots results in greater total mass of the medicinally important rhizomes.

The results in Figure 4.6 illustrates the impacts of growing media on the number of roots on respective cuttings during the autumn season.

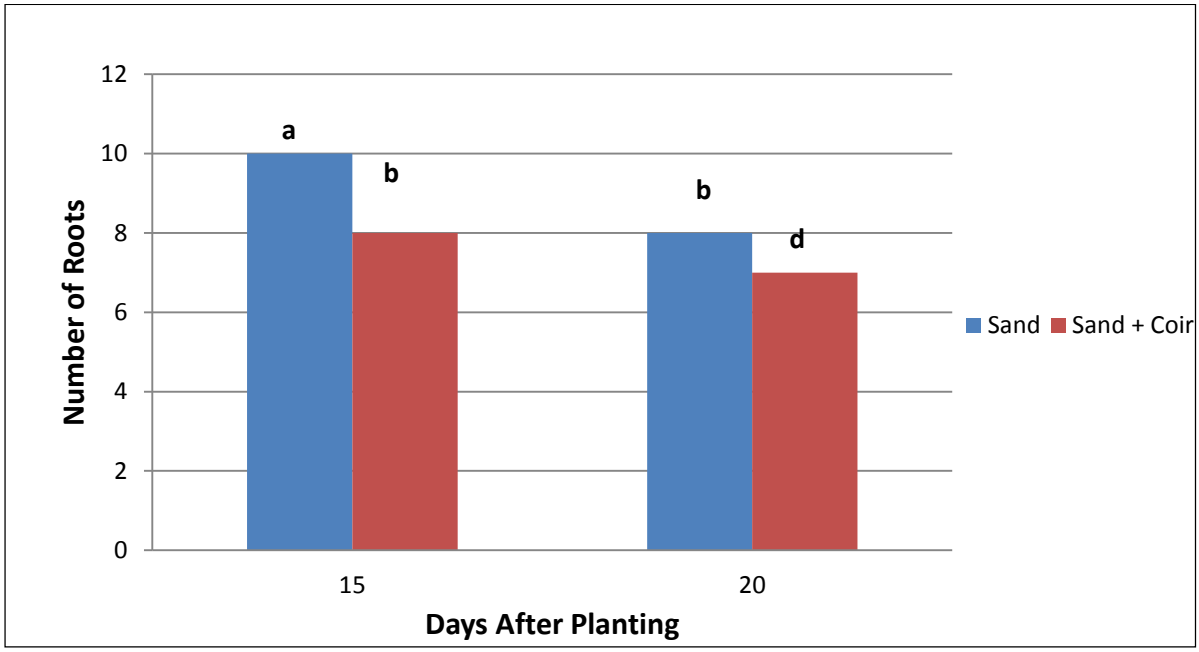

Figure 4. 6: Number of roots of *P. sidoides* grown in different media at 15 and 20 days after planting (DAP) in autumn. Bars bearing different letters are significantly different (Kruskal-Wallis Test, P < 0.05)

It was observed that cuttings planted in sand had more roots as compared to cuttings grown in sand + coir. At 15 DAP cuttings grown in sand had significantly more roots than cuttings grown on sand + coir ($P < 0.05$). It was noted that at 20 DAP the number

© University of Pretoria

of roots for all cuttings both grown on sand + coir and sand started rotting probably as a result of the organic content in coir and higher water holding capacity which lead to water logging and poor cuttings survival.

4.4 Effects of rooting media on rooting percentage

Rooting percentage refers to the proportion of cuttings that have at least one root in relation to the total number of cuttings planted. It may not be a useful indicator in terms of quality of rooting but it gives indications on the overall efficiency of the entire propagation process. Figure 4.7 illustrates the differences in rooting percentages for cuttings grown in sand and sand + coir respectively.

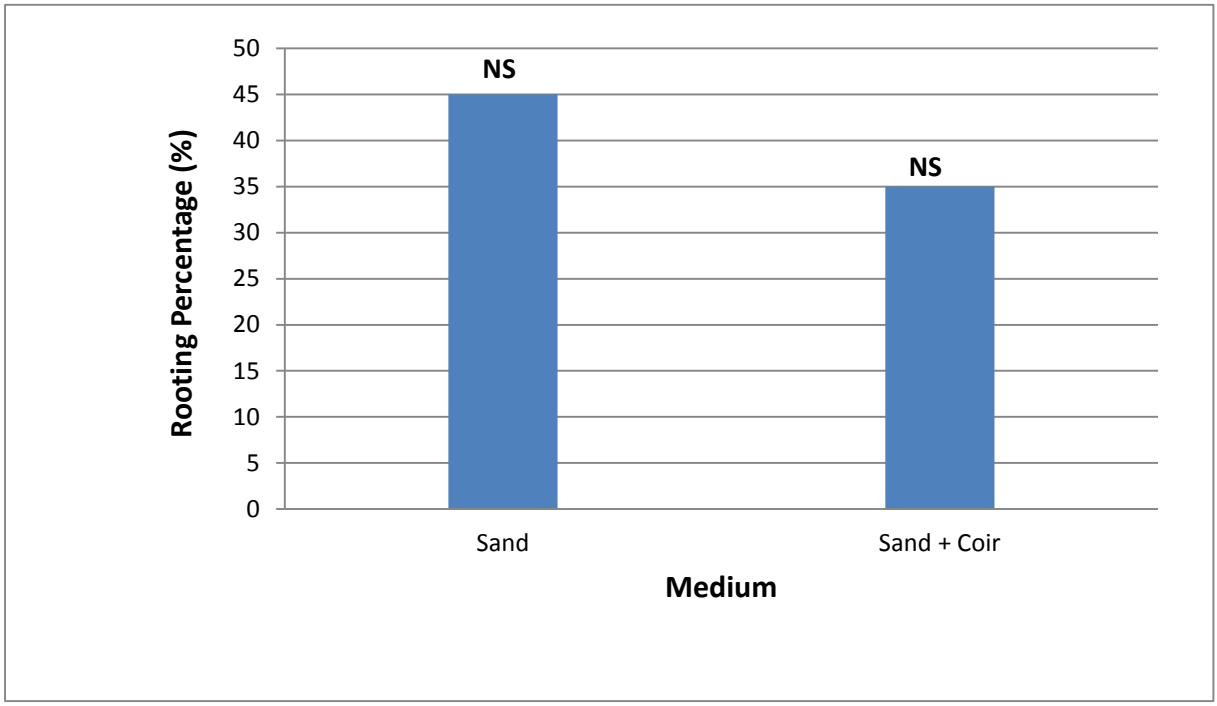

Figure 4. 7: Effects of rooting media on rooting percentage of *P. sidoides* leaf bud cuttings during summer. No statistically significant (NS) differences were observed (Kruskal-Wallis test, P < 0.05)

The data collected indicated that there were no significant differences in rooting percentages in cuttings grown in the two media types. Even though there were no significant differences in rooting percentages for the cuttings grown in the two different media, cuttings planted in sand looked much healthier than cuttings planted in sand +

© University of Pretoria

coir. Cuttings planted in sand tended to have a higher rooting percentage as compared to those planted in sand + coir, but it was not statistically significant.

4.5 Effect of season on rooting percentage

In the wild, plants are synchronised with various environmental attributes like temperature, length of daytime and activities of pollinators like bees (Jia *et al.,* 2016). These conditions will need to be replicated in the laboratory or greenhouse in order to achieve the best results. Some conditions like temperature and water availability can be easily manipulated but some are more difficult to manipulate. It is therefore necessary to determine how cuttings harvested during different periods of the year respond when propagated artificially. For instance, some plants are genetically pre-programmed to remobilize nutrients like nitrogen from senescing leaves to the trunks in winter and as such cuttings harvested in different seasons can have different amounts of stored nutrients hence the cuttings collected in different seasons are bound to perform differently once propagated (Masclaux-Daubresse, 2010).

The results indicated that rooting percentage of *P. sidoides* was affected by the season when the respective cuttings were collected. There were highly significant differences ($P < 0.001$) in rooting percentages depending on when the leaf-bud cuttings were taken (Figure 4.8). High rooting percentage of *P. sidoides* was recorded in autumn and spring. Rooting of cuttings was at 89% and 80% for autumn and spring respectively. There was low rooting of cuttings in summer and winter seasons at 29.9% and 39.8% respectively. There were no significant differences between autumn and spring as well as between summer and winter in terms of percentage of rooted cuttings.

© University of Pretoria

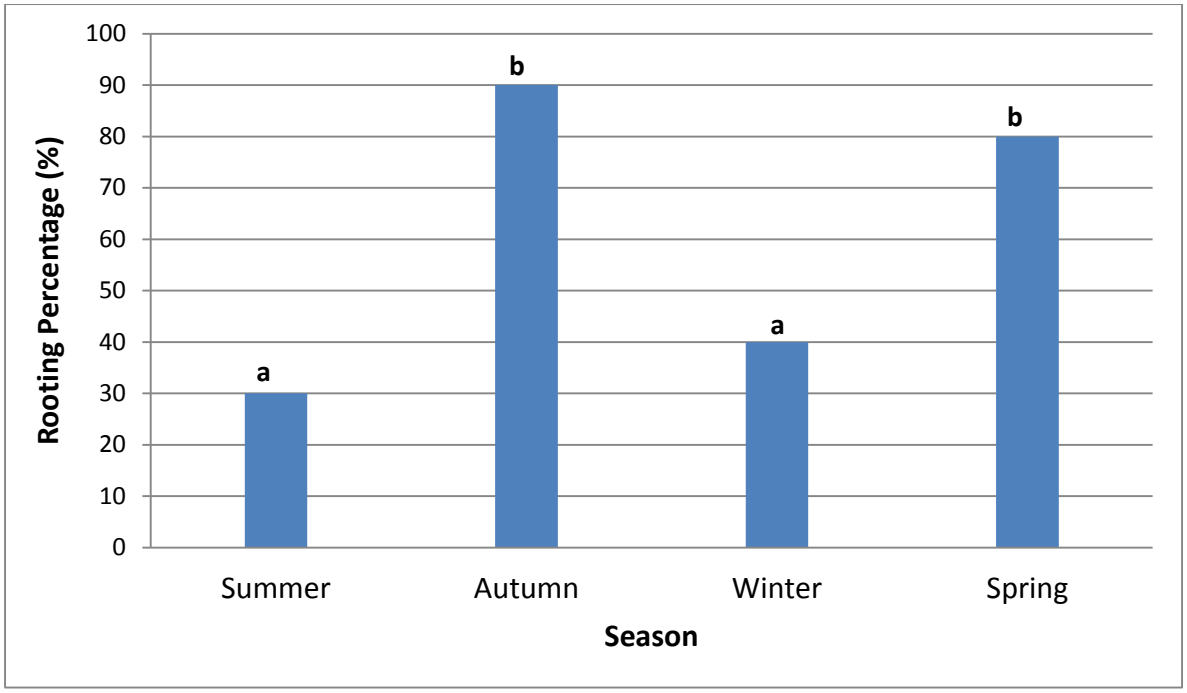

Figure 4. 8: Effect of season (summer, autumn, winter and spring) on rooting percentage of cuttings of *P. sidoides*. Bars bearing different letters are significantly different (Kruskal-Wallis Test, P < 0.05)

4.5.1 Changes in rooting percentage with time [Days after Planting (DAP)]

The development of roots on cuttings is not uniform. Plants of the same species can develop roots at different rates (Birlanga *et al*., 2015). It is therefore important to note the changes in rooting percentages of propagated cuttings over time. Determining how the rooting percentages change with time is important because it allows the plant breeder or grower to know the best time to transplant cuttings. It is tempting to assume that the more time the cuttings have after planting the more the rooting percentage will be. This may not always be true because some cuttings will simply never have roots and some will rot. Figure 4.8 is a graph detailing how rooting percentage changed with DAP in different seasons.

© University of Pretoria

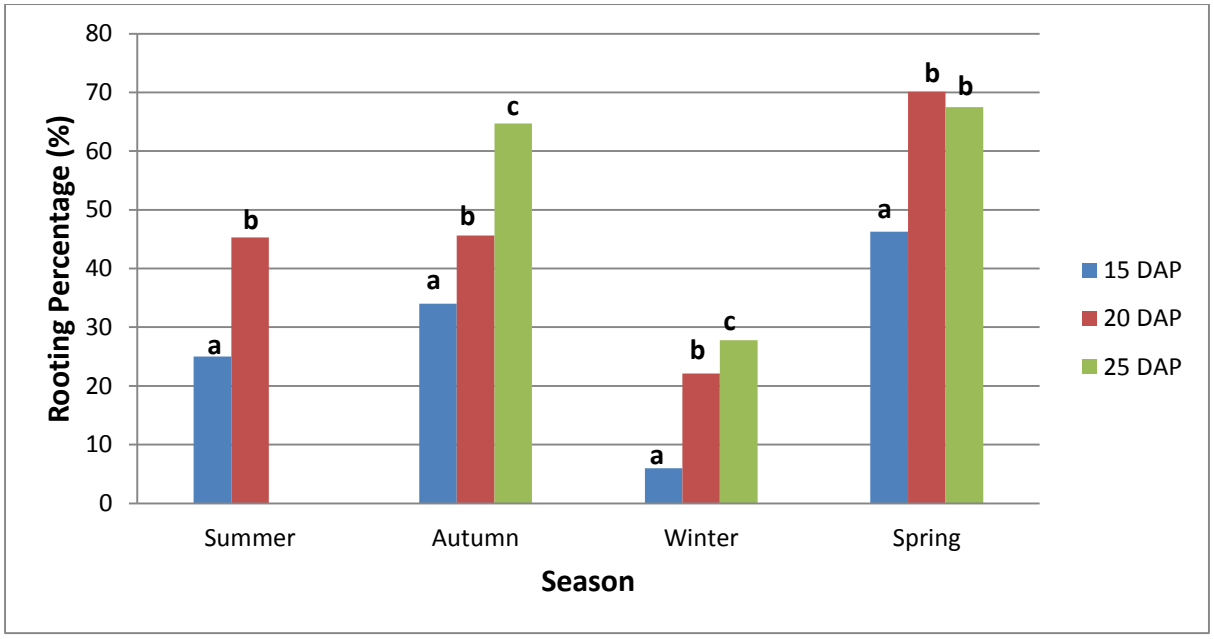

Figure 4. 9: Rooting percentage of *P. sidoides* cuttings at 15, 20 and 25 days after planting in summer, autumn, winter and spring. Bars bearing different letters within the same season are significantly different (Kruskal-Wallis Test, P < 0.05)

It was also observed that rooting percentage of *P. sidoides* cuttings was positively correlated to age of the cutting or days after planting (DAP). In autumn and winter seasons, rooting percentage increased significantly with increasing DAP (Figure 4.8). In spring, rooting percentage remained the same after 20 DAP. In summer, beyond 20 DAP, the cuttings started rotting.

4.6 Effects of IBA rooting hormone on cuttings of *P. sidoides*

Dynaroot™ is the trade name for a rooting powder which has 4-indole-3-butyric acid (IBA) as its active agent. It is widely used in South Africa and its efficacy in stimulating rooting is not in question. Roots naturally produce a number of different phytohormones and these include cytokinins and auxins. Auxins usually are produced in all parts of a plant whilst cytokinins are produced in roots and distributed to other parts of the plant (Lane *et al*., 2001). Plant biologists have discovered how to harness the power of these compounds to increase yields and improve plant production. Figure 4.10 shows findings on how Dynaroot affects the rooting percentage of *P. sidiodes* cuttings.

© University of Pretoria

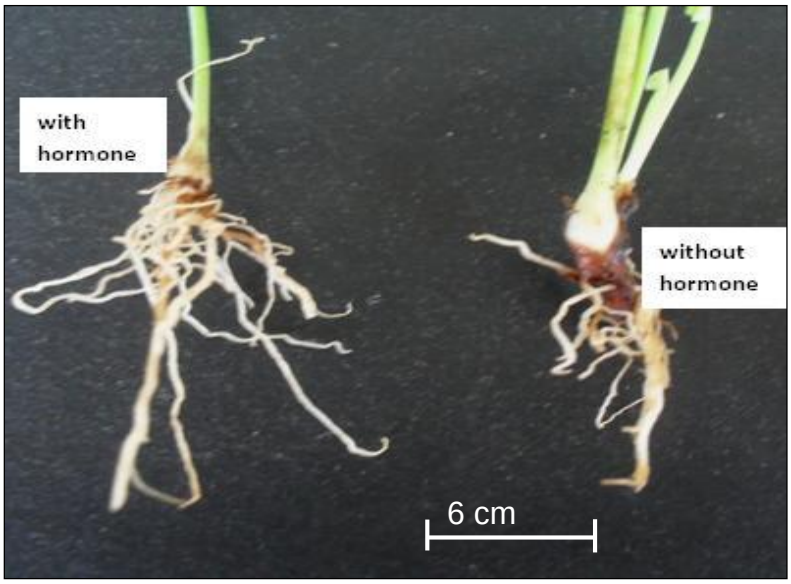

Figure 4. 10: Roots on a cutting treated with Dynaroot and roots on a cutting without any treatment

It was observed that the number of roots per rooted cutting was positively affected by the application of rooting hormone ($P < 0.001$) during all four seasons. The photograph (Figure 4.10) clearly shows the differences in rooting on cuttings as a result of the application of Dynaroot. Roots on cuttings that received hormone treatment were longer and thicker. The magnitude of the impacts of the rooting hormone differed with season, media and planting depth. This means that although rooting hormones positively affected root quality, their impacts are enhanced or hindered by other variables in the environment.

4.6.1 Effects of rooting hormone on rooting percentage

Hormones stimulate the rooting process in plants. One of the key organs that benefit from the action of hormones is the root system of plants. Roots need to consistently grow to satisfy the demand of water and other resources crucial as plants grow. In the artificial propagation of plants, the number of cuttings that develop roots is crucial for the success of the process. Plant biologists use phytohormones to stimulate rooting of some species that may not adequately root naturally and this is particularly true for wild species. The proportion of cuttings that develop roots is the rooting percentage.

© University of Pretoria

It was observed that the application of rooting hormone significantly increased the number of cuttings that developed roots at all sampling dates of the planted cuttings (Figure 4.11). Results showed that the best rooting percentage was on cuttings at 25 DAP. As mentioned earlier, the impacts of the rooting hormone is dependant also on other factors. It was therefore necessary to assess how rooting hormones interacted with season and media and the results are reported on sections that follow.

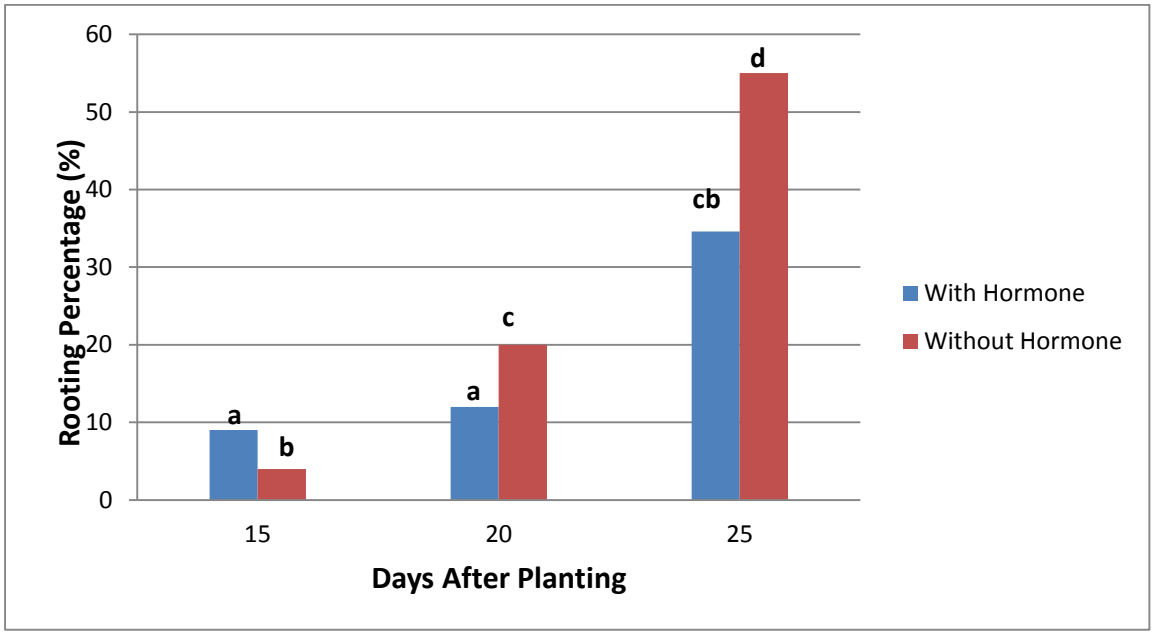

Figure 4. 11: Rooting percentage of *P. sidoides* cuttings after 15, 20 and 25 days after planting in winter. Bars bearing different letters within the same DAP are significantly different (Kruskal-Wallis Test, P < 0.05)

4.6.2 Effect of rooting hormone on number of roots per cutting in summer

As noted earlier, hormones are used by plant breeders and growers to improve performance or growth of plants. They increase the chances of cuttings developing roots before they dry or rot.

It was found out that the application of rooting hormone had no significant impacts on the rooting percentage of *P. sidoides* cuttings during summer at 15 DAP but significantly impacted rooting percentage 20 DAP (Fig 4.12). It was also observed that most cuttings in summer rotted and it is possible that other conditions like ambient temperatures negatively affected the functioning of the rooting hormone.

© University of Pretoria

Uncharacteristically high temperatures and water content of soil can result in stunted growth in plants (Leenaars *et al.,* 2018).

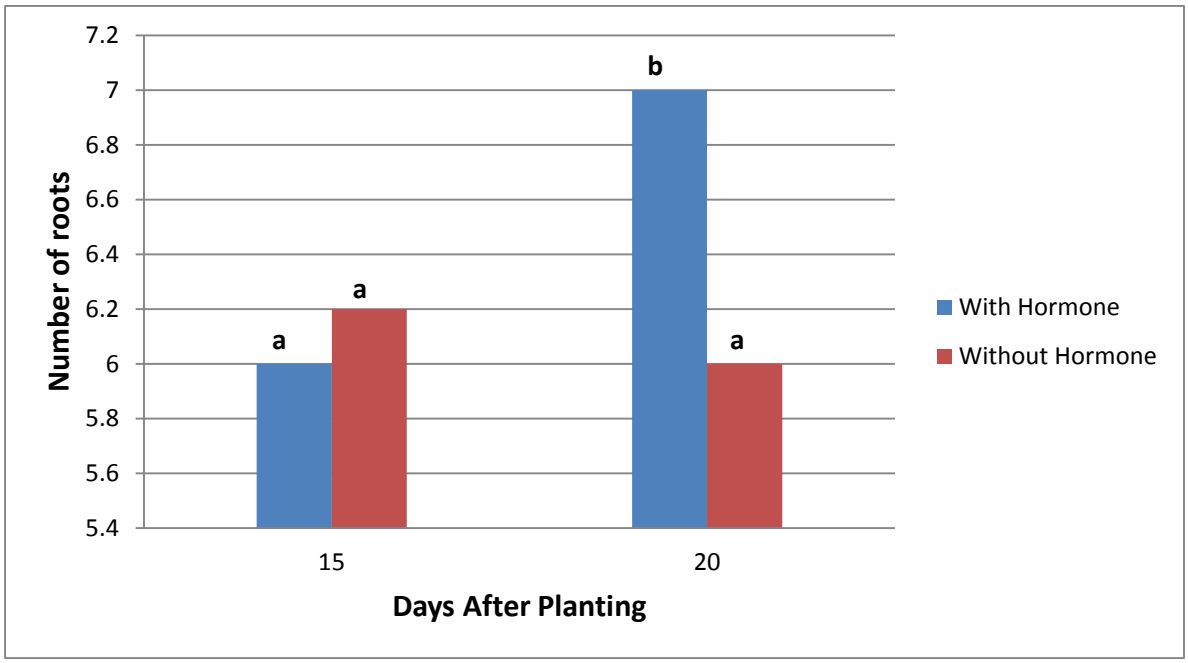

Figure 4. 12: Effects of rooting hormone on the number of roots per cutting during summer season. Bars bearing different letters are significantly different (Kruskal-Wallis Test, P < 0.05)

4.6.3 Effect of rooting hormone on number of roots per cutting in autumn

In autumn, cuttings treated with rooting hormone had significantly more roots than those not treated at 15, 20 and 25 DAP (Figure 4.13). Treated cuttings at 15 and 20 DAP had the same (statistically) number of roots and surprisingly, treated cuttings at 25 DAP had significantly fewer roots than treated cuttings at 15 and 20 DAP. Number of roots for cuttings that were not treated with hormone increased significantly between 15 and 20 DAP and like the treated cuttings, the number of roots per cutting dropped significantly between 20 and 25 DAP.

© University of Pretoria

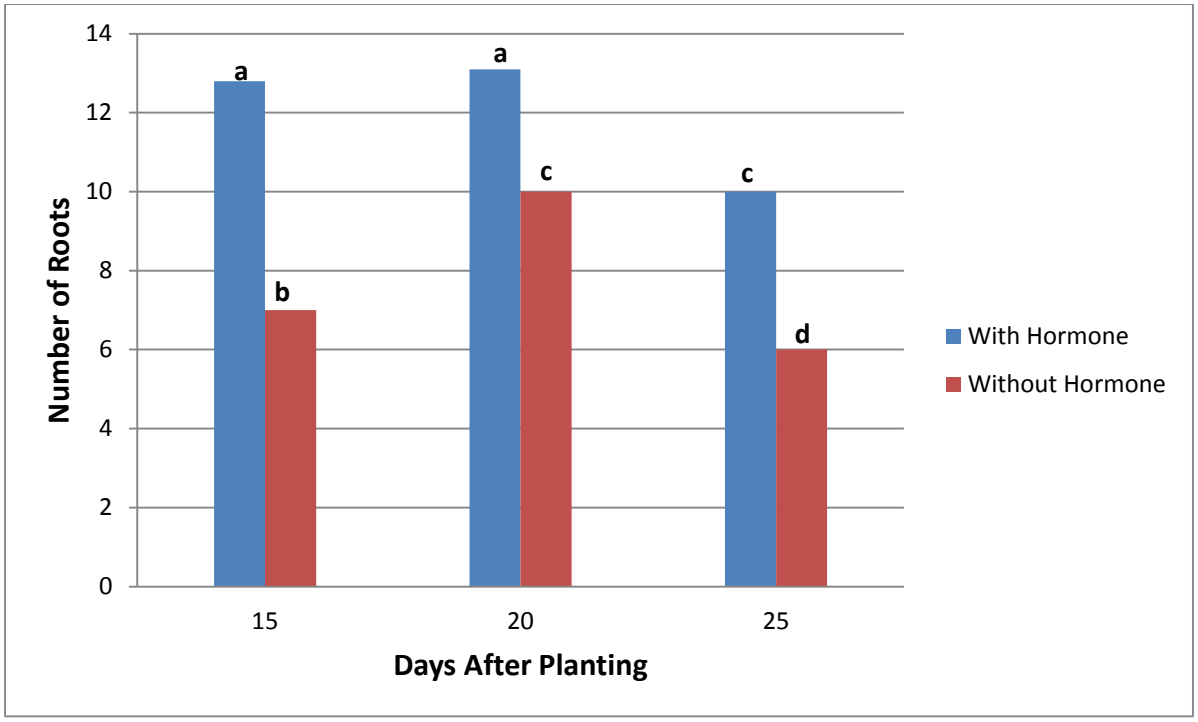

Figure 4. 13: Effects of rooting hormone on the number of roots per cutting during autumn. Bars bearing different letters are significantly different (Kruskal-Wallis Test, P < 0.05)

4.6.4 Effect of rooting hormone on number of roots per cutting in winter

In winter, treated cuttings (at specific DAP) also had more roots per cutting than untreated cuttings. Untreated cuttings had statistically similar number of roots at 15, 20 and 25 DAP (Figure 4.14). The number of roots per cutting for hormone treated cuttings significantly declined between 15 and 20 DAP. For treated cuttings, number of roots per cutting at 15 DAP were the highest.

© University of Pretoria

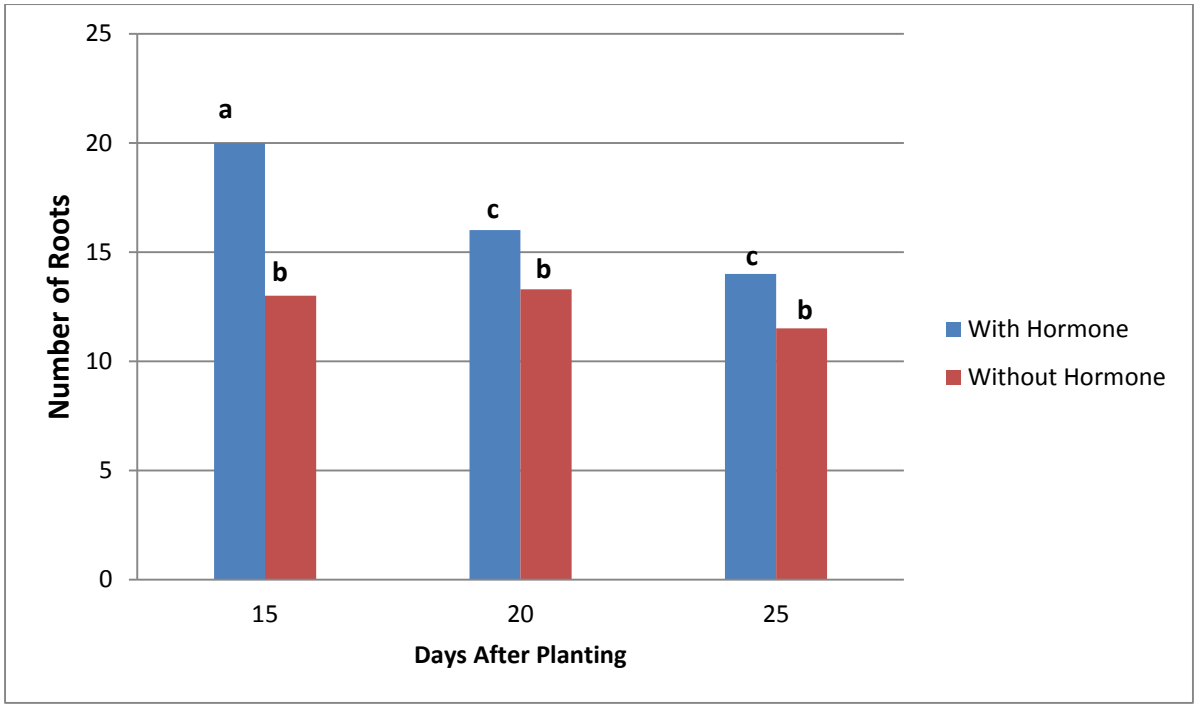

Figure 4.14: Effects of rooting hormone on the number of roots on each cutting during winter. Bars bearing different letters are significantly different (Kruskal-Wallis Test, P < 0.05)

4.6.5 Effect of rooting hormone on number roots in spring

In spring, the same trend as observed in other seasons was apparent. Treated cuttings had significantly more roots than untreated cuttings. The number of roots per cutting on treated cuttings declined significantly between 15 and 20 DAP and also between 20 DAP and 25 DAP (Figure 4.15). In untreated cuttings, the number of roots increased between 15 and 20 DAP and then declined at 25 DAP to numbers statistically similar to number of roots at 15 DAP. As observed the effect of hormone on cuttings planted in mist bed is highly effective to 15-20 DAP, and beyond this time there is no effect as rooting stays the same or even declined, this might be associated with high infiltration rate in which the media has been subjected to within the period of 0-15 days, resulting in loss of nutrients in media and hormone.

© University of Pretoria

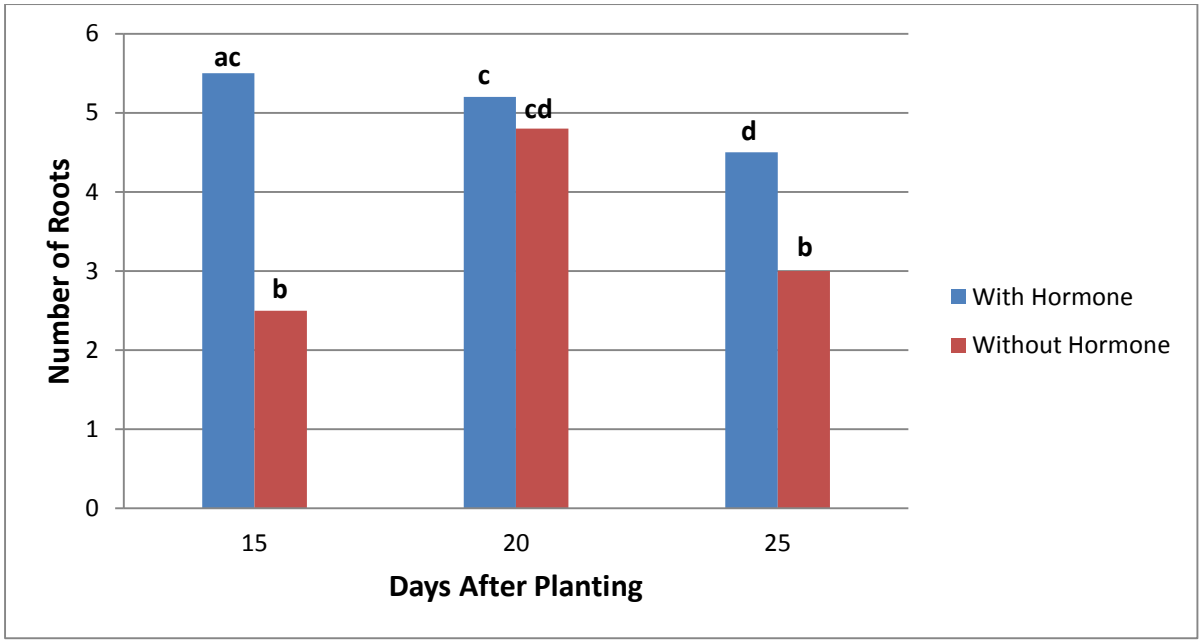

Figure 4.15: Effects of rooting hormone on the number of roots per cutting during spring. Bars bearing different letters are significantly different (Kruskal-Wallis Test, P < 0.05)

4.7 Effects of rooting hormone on root length

A number of studies have shown the effects of rooting hormones on root length. Sevik *et al.,* (2015) reported that phyto-hormones increased rooting percentage and root length in rooting percentage and some root characters in the Dwarf Umbrella tree, *Schefflera arboricola*. Root length is an important quality in plant breeding because it is directly linked to the success of establishment for transplants and make some transplant more resilient to water logging (Sundgren *et al.,* 2018).

4.7.1 Impacts of rooting hormone on root length in autumn and spring

Significant differences (P<0.001) in root length due to the effects of rooting hormone were observed only in cuttings propagated in autumn and spring. In autumn (Figure 4.16), the longest root lengths was recorded at 15 DAP and the shortest at 25 DAP, for both treatments (with or without rooting hormone). As observed *P.sidoides* leaf bud cuttings are very fragile and squeaky, so a longer period beyond 15 DAP in the mist bed does have not effect of root length, but in contrary the cuttings start to rot and wilt of. In spring (Figure 4. 16), the longest roots were observed in cuttings at 20 DAP and

© University of Pretoria

the shortest in plants at 25 DAP. In autumn, the longest roots were recorded at 15 DAP on cuttings that were treated with hormone. At this stage, 15 DAP, the root length on treated cuttings was significantly longer than for untreated cuttings. At 20 DAP; the differences in length of roots on treated and untreated were not significantly different. The shortest roots were observed on untreated cuttings at 25 DAP. Also, at 25 DAP, the length of roots on treated cuttings was observed to be statistically similar to length of roots at 20 DAP (with or without hormone).

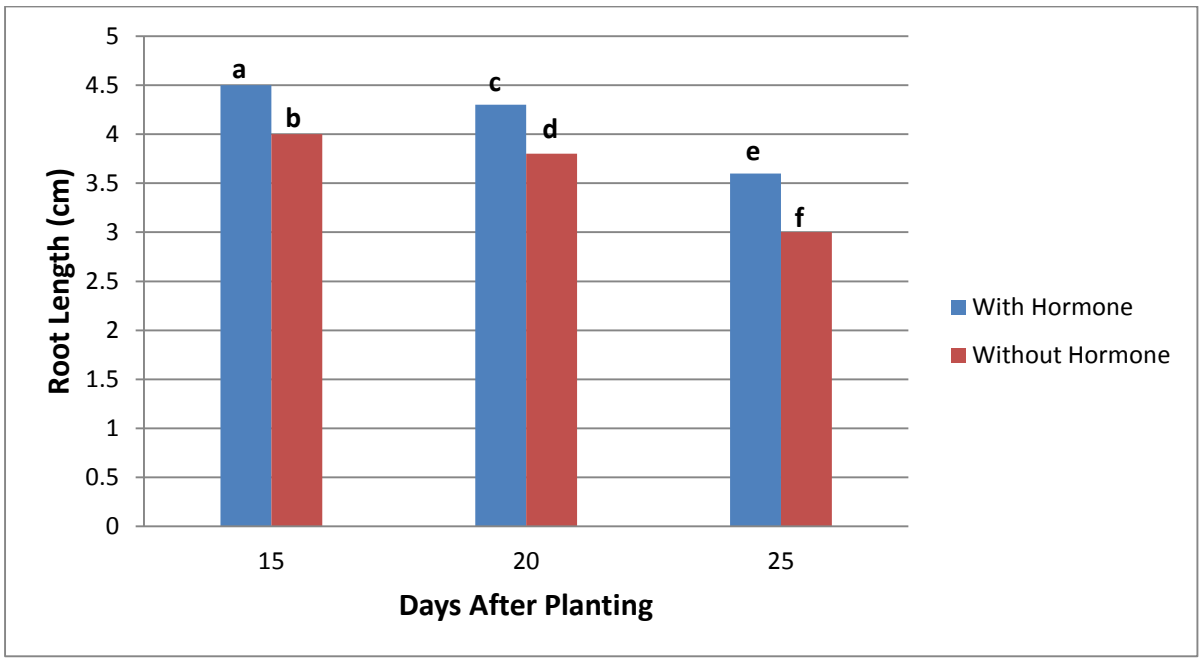

Figure 4.16: Effect of rooting hormone on root length during autumn. Bars bearing different letters within DAPs are significantly different (Kruskal-Wallis Test, P < 0.05)

In spring, significant differences in root length were observed between treated and untreated roots at 15 DAP (Figure 4.17). Treated cuttings at 20 DAP had the longest roots and the roots at this age from the two treatments were statistically similar in length.

© University of Pretoria

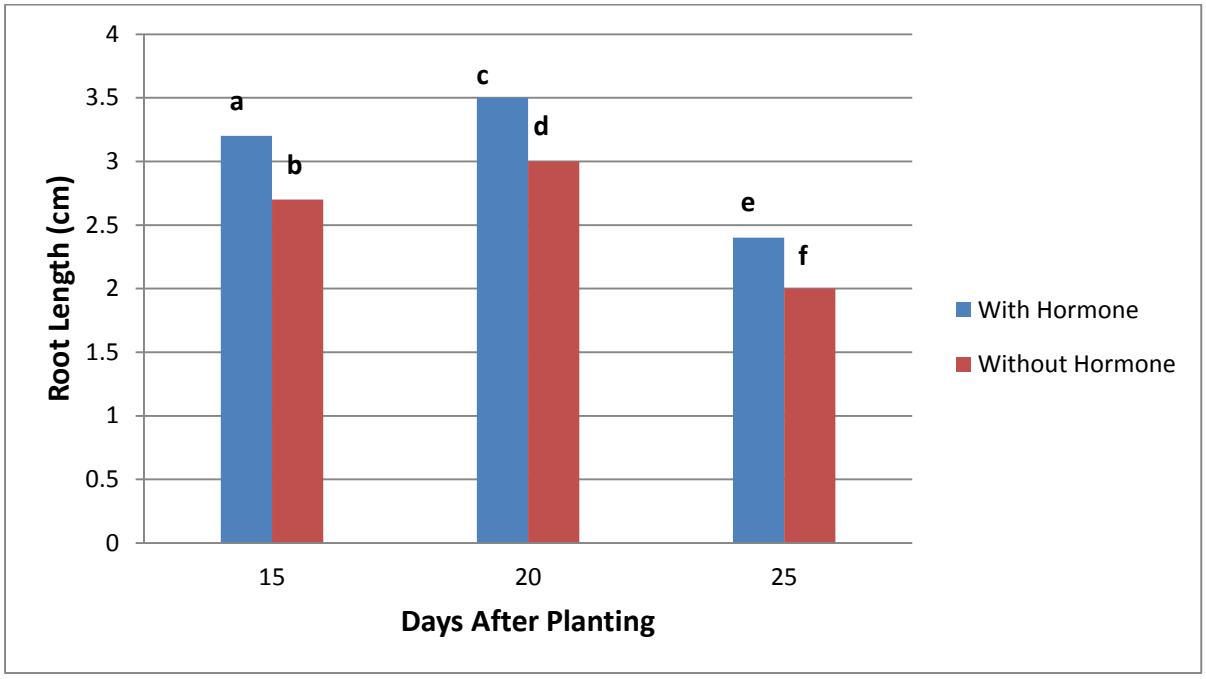

Figure 4.17: Effect of rooting hormone on root length during spring. Bars bearing different letters per DAP are significantly different (Kruskal-Wallis Test, P < 0.05)

4.8 Impacts of pH and Water Holding Capacity of media on rooting of *P. sidoides*

Different rooting media have different physico-chemical properties and consequently their impacts on cuttings may differ significantly. Two of the main properties of soil or artificial media are pH and water holding capacity. Media pH may affect aspects like functioning of enzymes whilst water holding capacity affects the amount of moisture available to the roots after watering as well as the aeration, as poor aeration can cause roots to rot Figure 4.18 is a summary comparison of these two factors.

© University of Pretoria

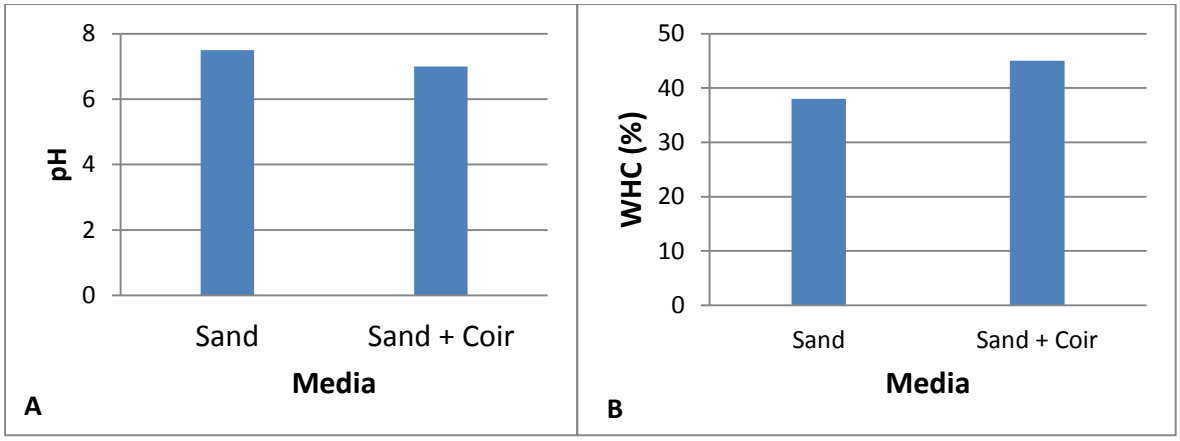

Figure 4.18: Comparison of physico-chemical properties of the media used: pH (A) and water holding capacity (B)

The combined sand + coir medium had a higher water holding capacity than sand only (Fig 4.18B).The pH was around neutral for both sand + coir at 6.9 and sand at 7.5 respectively as illustrated in Fig 4.18A. The pH for the potting soil in which the parent stock grew on ranged between 5.4 and 6.3.

4.9 Effects of different treatments on performance of resultant transplants

4.9.1 Effect of media on root length and number of roots

The type of medium used in growing transplanted cutting is just as important as media used for initial propagation. Different media have different properties and as such their impacts on plant growth differ. Some of the important properties include pH and water holding capacity. It is important to assess the magnitude of the differences in plant growth offered by different media. This allows for the most efficient use of resources.

© University of Pretoria

The results for the analysis on effectiveness of different media in growing transplanted cuttings are summarized in Figure 4.19.

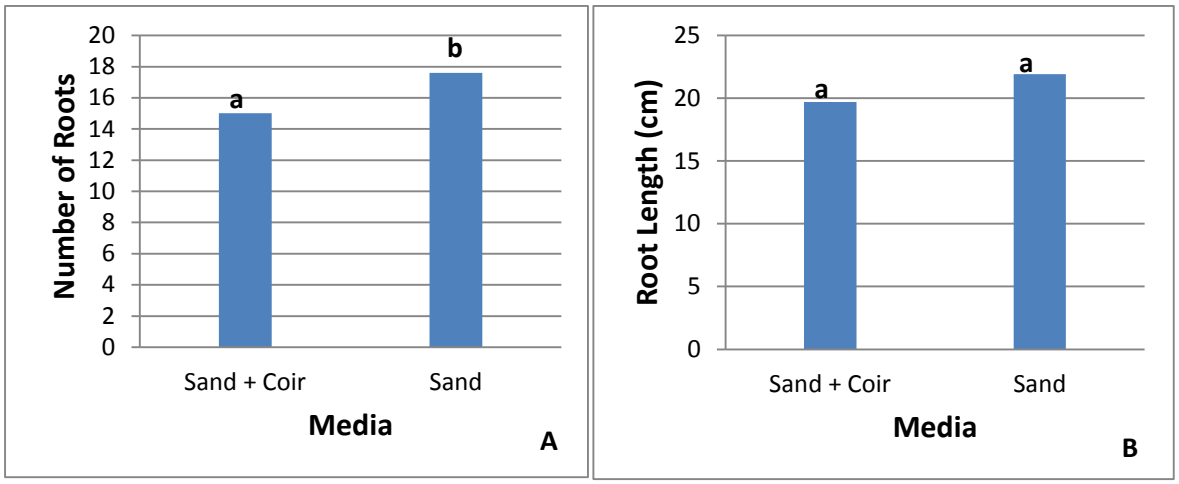

Figure 4.19: Impact of medium on number (A) and length (B) of roots on transplanted cuttings of *P. sidoides.* Bars bearing different letters are significantly different (Kruskal-Wallis Test, P < 0.05)

Survival of transplanted cuttings of *P. sidoides* followed similar pattern as that of rooting percentage of cuttings. Like rooting percentage, survival rate of transplanted leaf-bud cuttings was found to be highest in transplants from cuttings that were firstly propagated on sand medium and also treated with hormone. A closer examination of the roots showed that transplants planted in sand had significantly more roots than those planted in sand and coir mixture (Figure 4.19A). In terms of root length, transplants planted in sand also had longer roots but the differences in length were not statistically significant (Figure 4.19B).

4.9.2 Effect of rooting hormone on number of roots on transplants

Plant propagation has come a long way and plant biologists have found new and better ways of growing plants of interest. Phytohormones have been employed in improving yields of some crops (Hayat *et al*., 2001; Sakamoto, 2006). Their effectiveness is not uniform across all species and as such it is important to carry out species specific studies.

© University of Pretoria

Figure 4.20 presents the results from the study on the impacts of Dynaroot on performance of transplants.

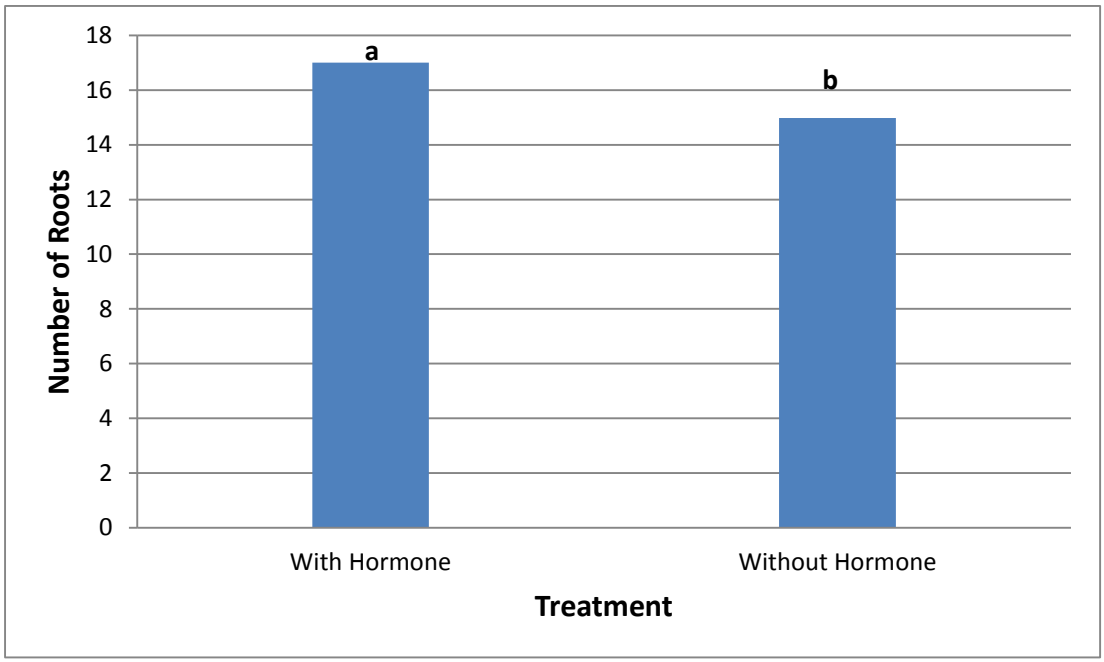

Figure 4.20: Impact of rooting hormone on number of roots on transplanted transplants of *P. sidoides.* Bars bearing different letters are significantly different (Kruskal-Wallis Test, P < 0.05)

In addition to the impacts of media, as seen in the section above, the rooting hormones also positively impacted on performance of cuttings. The average number of roots on transplants where higher on cuttings that were previously treated with rooting hormone. The average number of roots was 17.01 roots per plant for transplants previously treated with the hormone and 14.98 roots for the transplants from cuttings that were never treated with rooting hormone.

4.9.3 Effect of rooting hormone on length of roots on transplants

Roots are important for any plant species. They allow the plant to interact with the medium it is grown in and effectively allowing for the uptake of nutrients needed by the plant. It can therefore be assumed that healthier plants have more roots. Studies on impacts of hormone on plants will not be complete if the impacts of the hormones on roots is not analysed and in Figure 4.21 are the results of this analysis.

© University of Pretoria

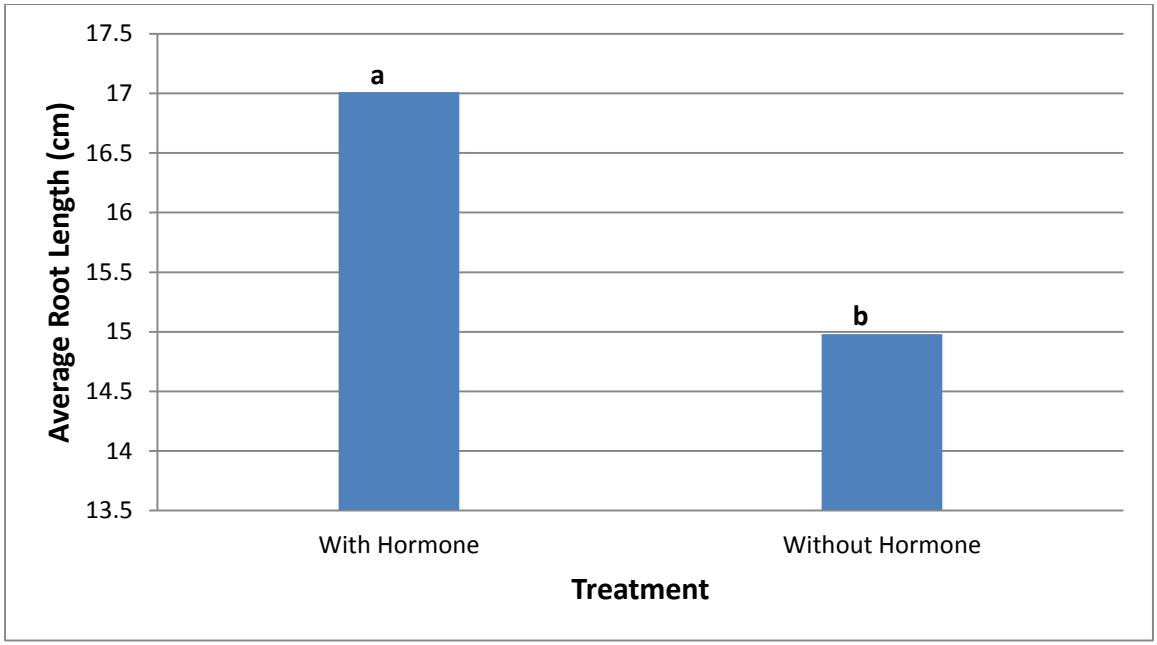

Figure 4.21: Impact of rooting hormone on length of roots of transplanted transplants of *P. sidoides.* Bars bearing different letters are significantly different (Kruskal-Wallis Test, P < 0.05)

The pattern for root length mirrors that was observed for number of roots, transplants from cuttings that were previously treated with hormone had significantly longer roots (17 cm) than those that were never treated with the rooting hormone (15 cm).

4.9.4 Interaction between hormone and media

This study focused on the impacts of two factors on plant propagation using cuttings. These two factors were media and Dynaroot hormone. It therefore makes sense to assess how these two factors interact. This is because interactions are always complex and may result in unexpected outcomes (Borchert *et al*., 1989).

The result, as illustrated in Figure 4.22, indicate that a combination of sand media and Dynaroot (IBA) produces the better plants if the criteria is number of roots per plant. Sand, in general, was a superior and ideal medium for growing transplants of *P. sidoides*. This is because untreated cuttings on sand tended to produce more roots than treated cuttings on sand+coir.

© University of Pretoria

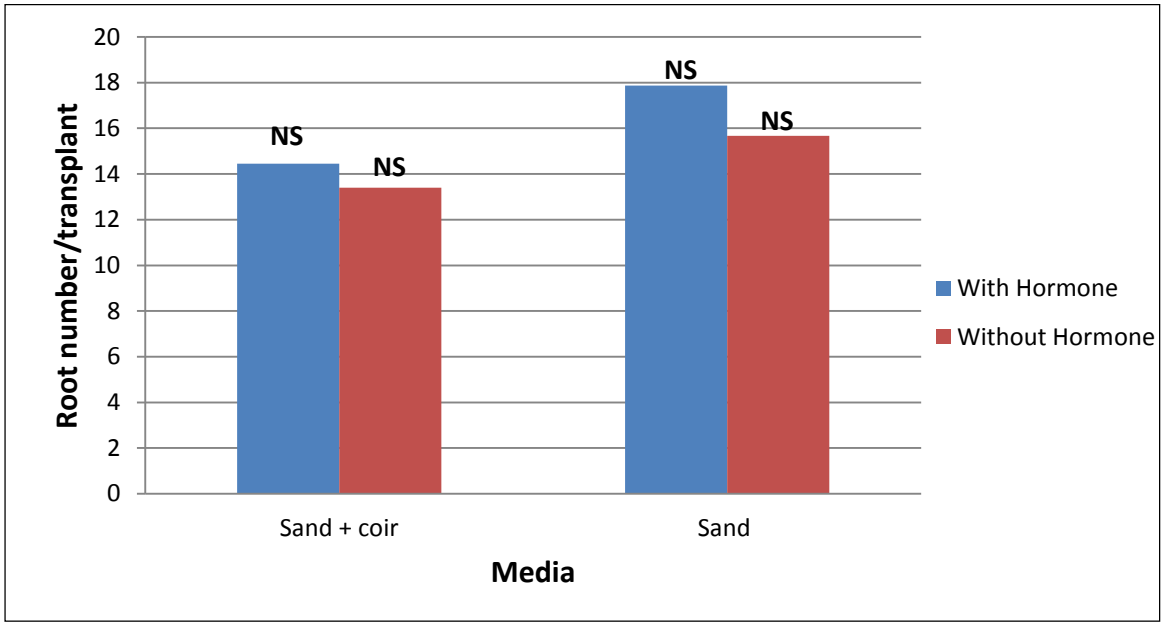

Figure 4. 22: The interaction of media and treatment on the number of roots on cuttings of *Pelargonium sidoides.* Bars bearing different letters are significantly different (Kruskal-Wallis Test, P < 0.05)

Therefore, depending on the objectives of a study, one can assess that any of the two traits and come up with reliable results.

© University of Pretoria

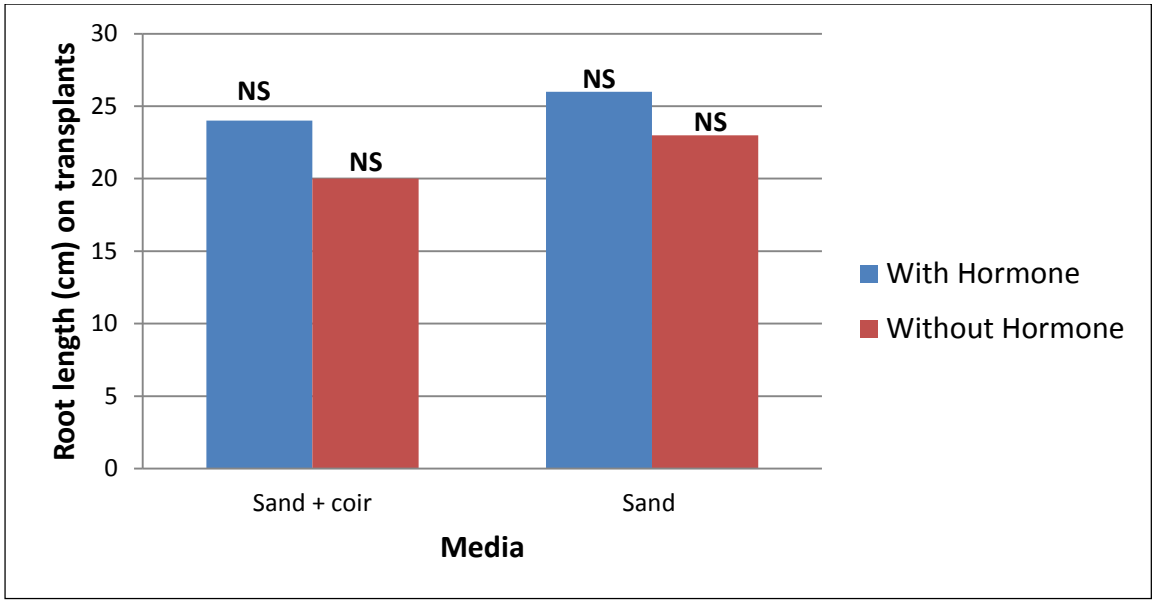

Figure 4. 23: The interaction effect of media and treatment (with or without hormone) on root length of *Pelargonium sidoides transplants.* Bars bearing different letters are significantly different (Kruskal-Wallis Test, P < 0.05)

4.10 Impacts of different treatments on morphology of *P. sidoides*

The overall goal for any plant scientist is to have plants with the best qualities for their intended purposes. For instance, a plant grown for its tubers should have good tubers and size of other parts will not matter. Traditional healers in South Africa prefer using rhizomes of *P. sidoides* in their concoctions. However, it has been shown that traditional practitioners prefer using roots but leaves have been proven to have medicinal qualities as well (Lewu *et al.,* 2006). It was therefore decided to report on how the actual plants that were given different treatments looked like. This is expected to add depth to the understanding of the impacts of the different treatments.

Transplants grown from cuttings that received hormone treatment clearly appeared healthier than transplants grown from cuttings that did not get hormone treatment (Figure 4.24). Plants grown from hormone treated cuttings had larger leaves and longer stems. It is, therefore, apparent that size and number of roots in cuttings are important even after a plant has been transplanted.

© University of Pretoria

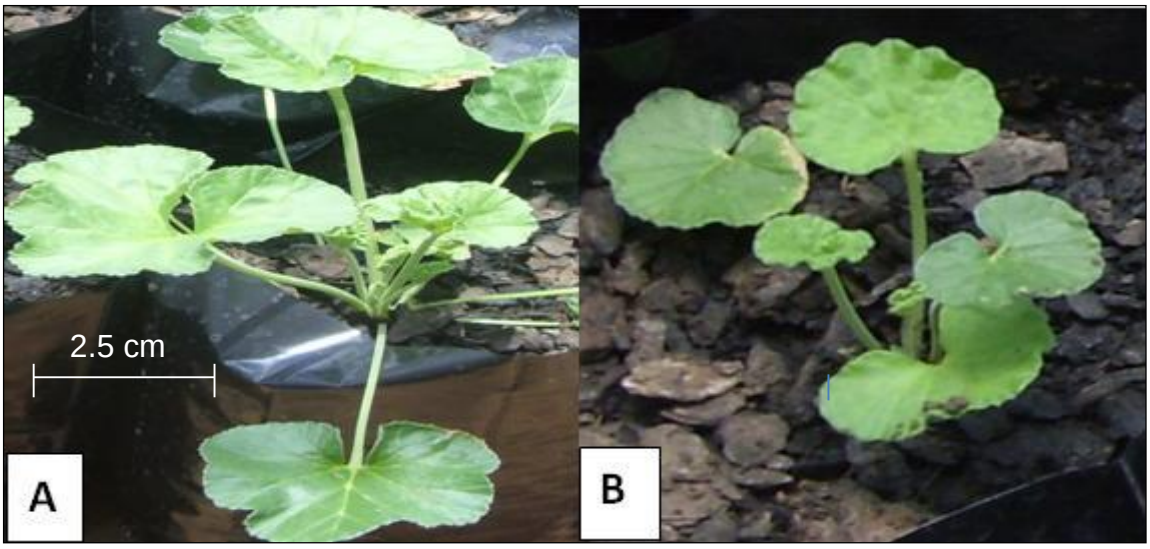

Figure 4. 24: Effect of hormone treatment on shoot length of transplanted cuttings of *Pelargonium sidoides*, (A) treated with hormone or (B) without hormone treatment.

4.11 Impacts of planting depth on rooting of *P. sidoides*

Planting depth of 3 cm resulted in higher rooting percentage (65%) and good establishment of cuttings when compared to a depth of 1.5 cm, where 35% of rooting was recorded. Cuttings planted at 1.5 cm depth wilted easily and did not have a good stand, thus resulting in poor development of the cuttings (Fig.4.25).

© University of Pretoria

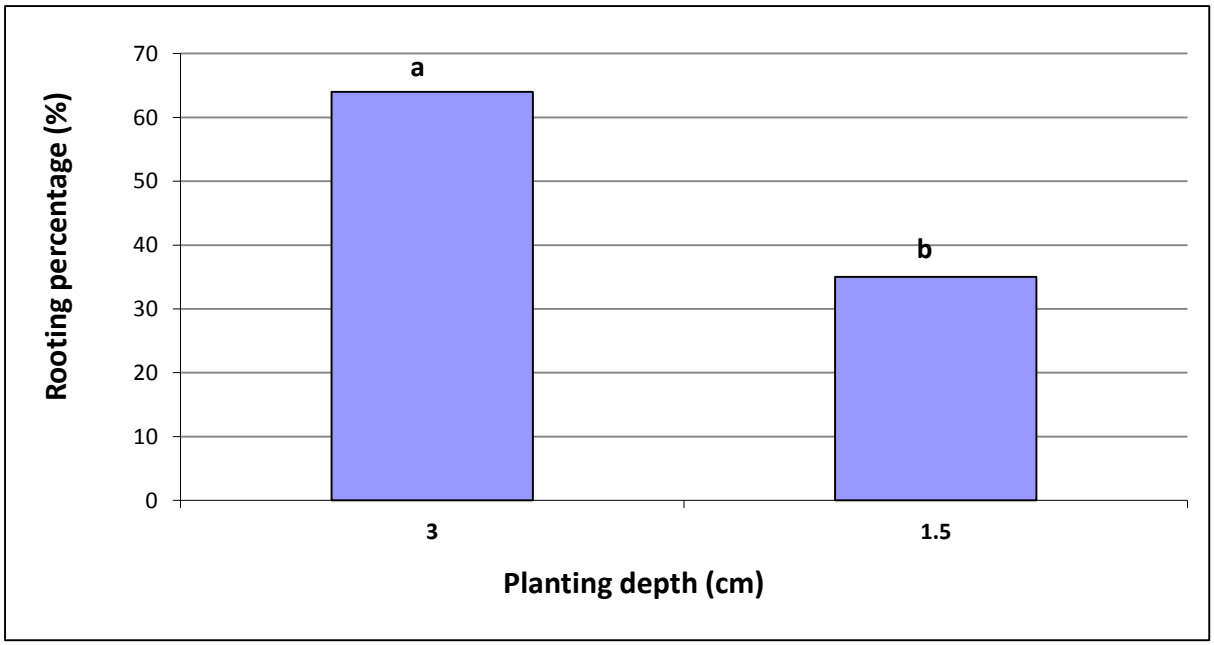

Figure. 4. 25 Effects of planting depth on rooting percentage of *P. sidoides* leaf bud cuttings. Bars bearing different letters are significantly different (Kruskal-Wallis Test, P < 0.05)

4.12 *Pelargonium sidoides* morphology

4.12.1 Leaf morphology

The leaf-bud cuttings of *P. sidoides* were observed to have two ends of the primordium, the distal (apical) end and the proximal (basal) end. Studies of the very early sequences of growth of leaf primordia indicate that the two ends of the primordium, the distal (apical) end and the proximal (basal) end, give rise to specific parts of the mature leaf (Kaplan, 1979b; Bell, 2008). A fundamental difference is found in the development of monocotyledonous and dicotyledonous leaves. In many “typical” dicotyledonous leaves the proximal ends of the primordium (lower leaf zone) develops into the leaf base, which may or may not ensheath the stem together with the stipules if present. The distal end of the primordium (upper leaf zone) develops into the dorsiventrally flattened leaf blade or laterally flattened phyllode in the case of *Acacia* species (Bell *et al.*, 2008).

© University of Pretoria

The presence of leaves on cuttings (Figure 4.26) plays a significant role in root initiation of many plants species (Hartmann *et al*., 1990). This was confirmed by Leaky and Mohammed (1985) when leafless cuttings of *Triplochiton scleroxylon* were unable to root. Similar results were also reported by Ofori *et al*. (1996) where only 30% of leafless *Milicia excelsa* cuttings rooted. Even though their presence plays an important role in rooting, there are certain factors that have to be considered as well. These include factors such as leaf-area and leaf number (Newton *et al*., 1992). This was confirmed when rooting percentage of *Shorea leprosula* (Aminah *et al*., 1997) and *Milicia excelsa* (Ofori *et al.,* 1996) were affected by the variation in leaf area.

Figure 4. 26: Leaf development and morphology of *P. sidoides* leaf bud-cutting

4.12.2 Stem morphology

The stem morphology of *P. sidoides* was observed to be a runner or creeping stem (Figure 4.27). It consists of one or more long internodes at the distal end of which a rosette of foliage leaves or a heteroblastic sequence of leaves is formed. A creeping stem is often short lived, and the production of runner and rosette represents a system of vegetative multiplication (Bell, 2008).This morphological pattern enables the plant to easily regenerate from the stem, as differentiation and bud formation also happens on the modified stem. The formation of adventitious roots and buds is dependent on

© University of Pretoria

plant cells to differentiate and develop into either root or shoot system. The process of differentiation is the capability of previously developed, differentiated cells to initiate cell divisions and form a new meristematic growing point (Hartmann *et al*., 1997).

Figure 4. 27: Stem morphology and development of *P. sidoides* leaf-bud cuttings

4.12.3 Root morphology

Almost in all monocotyledons, including *P. sidoides,* the primary root system is short lived and the whole functional root system of the plant is adventitious, with the roots arising on the stem usually near the ground level or below. This is particularly obvious in the case of rhizomatous monocotyledons. Similar elaborate adventitious root systems develop as a matter of course in many dicotyledonous plants having a rhizomatous or stoloniferous habit. In both cases, adventitious roots tend to be associated with nodes (that is, they may be termed nodal roots), and the exact positions of development of the endogenous root primordium are governed by features of the vascular tissue at the node (Yu *et al.,* 2017).

The adventitious root formation of *P. sidoides* is formed in the meristematic region of the shoot apices (Figure 4.28A). In this case, they either develop into roots immediately or possibly much later when the supporting organ is old, or they may arise in old tissue by dedifferentiation (that is the return to meristematic activity) of selected

© University of Pretoria

patches of cells (Figure 4.28B). *P. sidoides*, however, follows a different pattern of root morphology; one or more adventitious roots swell during each growing season, providing storage material for growth after the resting period. A similar development occurs in *Ranunculus ficaria*, where single adventitious roots are produced at the base of the buds on the aerial stem (Bell, 2008). The root swells to produce a detachable tubercule, which also includes the bud's apical meristem.

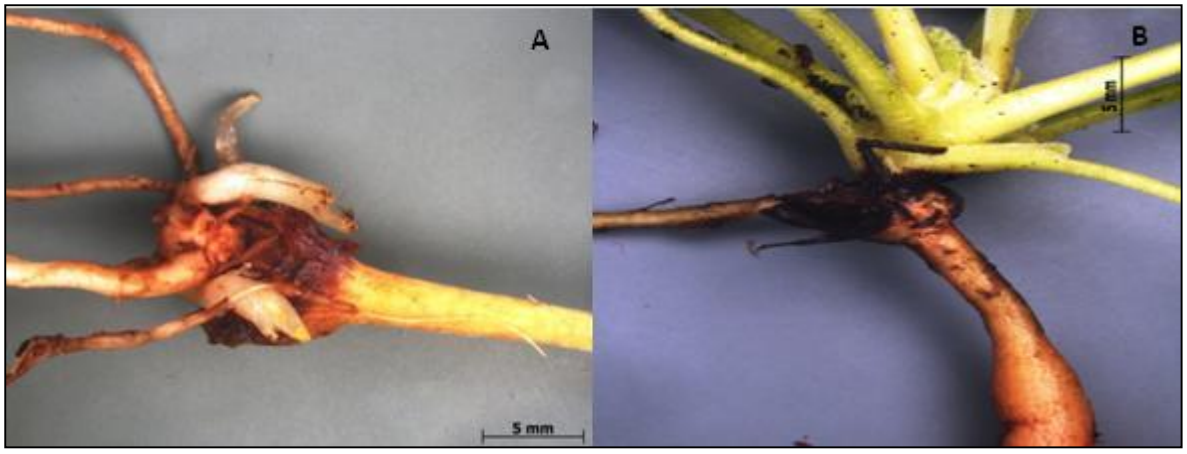

Figure 4. 28: Adventitious root formation of *P. sidoides* (A) and shoot development (B)

4.13 Chapter conclusion

The results have shown that treating cuttings with Dynaroot has significant positive impacts on the rooting capacity of the respective cuttings. In addition to that, sand was observed to be the more superior of the two media used. The robustness of the results was affected, minimally though, by rotting of some cuttings. Though cuttings grown in both media experienced some degree of rotting, the challenge was greater for cuttings grown in sand + coir medium in summer.

© University of Pretoria

CHAPTER 5

GENERAL DISCUSSION

5.1 Introduction

Rooting medium is the most important factors which determine the level of success when attempting to propagate plants using cuttings (Leakey, 1990; Berhe *et al*., 1998). In addition to the impacts of media, many other studies have also indicated that the rooting ability of many plants is affected by other factors which include presence or absence of phyto-hormones, the season or time of the year cuttings were planted (or collected) as well as the planting depth.

In summary, this study has shown that *P. sidoides* can be propagated from leaf-bud cuttings with or without the use of hormones and in a medium of either sand or a mixture of sand and coir (sand + coir). However, it was observed that the success rate of propagating the species without rooting hormone is too low to warrant any considerations by commercial growers. It was also observed that cuttings planted in sand + coir medium were more likely to rot compared to cuttings grown in a sand only medium. Results showed that autumn and spring are more ideal seasons to propagate cuttings and that 3 cm planting depth produced superior results compared to a planting depth of 1.5 cm.

This chapter will discuss these findings to explain the scientific reasons behind the observations as well as provide recommendations to plant propagators interested in growing *P. sidoides* using leaf bud cuttings. This chapter will also highlight areas or other aspects of *P. sidoides* propagation from leaf bud cuttings that may need to be researched in the future.

5.2 Effects of medium

Leakey (1990) reported that most species can be grown in a variety of media but it is important to find the medium in which a species grows best in. This is important because the mass production of any species will only be a worthwhile venture if it has both economic and conservation merits (White *et al*, 2007). In this study, it was observed that sand medium generally produced better quality roots compared to sand

© University of Pretoria

+ coir. Quality in this instance was judged by the length and number of roots on respective cuttings.

The physical properties of the two types of media can be responsible for the observed trend. Sand was observed to have lower water holding capacity compared to the mixture of sand + coir. This observation is in line with findings of Evans *et al*., (1996) who reported that coir samples they analyzed had water holding capacities which ranged from 750% to 1100% of dry weight and these magnitudes were greater than the water holding capacity of sand (Reynolds *et al*., 2000). This means that sand drains faster than sand + coir and consequently the sand medium will be better aerated (Karhu *et al.,* 2011). Aeration has been shown to be an important factor in media performance Klein *et al*., (1999), while working on *Myrtus communis,* reported that increasing aeration by adding perlite increased rooting by up to 30%. Grable and Siemer (1968) also reported that the amount of oxygen in soil have effect on the elongation of maize (*Zea mays*) roots.

In winter, *P. sidoides* cuttings grown in sand + coir exhibited longer roots than those grown in sand. This was the opposite of what had been observed in other seasons. The reason for this, again, may lie in the water content of the media. Sand + coir has a higher water holding capacity than sand, meaning it held more water than sand. Due to higher specific heat capacity of water, sand + coir medium probably lost heat at a slower rate compared to sand only medium. This means that media with more water can conserve heat better when temperatures drop (Kumar *et al*., 2015). A warmer environment, up to a certain extent, better supports the growth of plants. Ideal root-zone temperature (RZT) is important in improving plant growth and development of strawberry by impacting rate of water and nutrient uptake by roots (Kumar *et al*., 2015). However, the differences in temperatures were probably minimal hence the lack of significant differences in the length of roots. It is therefore recommended to measure the temperature of the media frequently during such experiments.

Rooting success in both media was not 100%, some of the cuttings succumbed to stem decay or rotting. It was however observed that this challenge was greater for cuttings propagated in sand + coir, especially when grown in the summer season and after 25 days after planting. This could also be a result of persistently high moisture

© University of Pretoria

content in the media coupled with high temperatures which could encourage the activity of agents responsible for the decaying of the stems. Li *et al*. (2014) stated that sustained flooding of plant roots can lead to growth inhibition and decaying of roots, this can be a result of either carbon dioxide saturation or oxygen deprivation, among other factors (Kramer and Jackson, 1954). This is not a universal rule since different species have different flood tolerance levels that are likely to be relevant in this context. This conclusion has been arrived at after looking at the fact that *P. sidoides* is endemic to a geographical location which receives an average of less than 600 mm of rainfall annually and is not prone to flooding. This line of thought is further supported by the findings of Stolzy *et al*. (1967) who concluded that inadequate oxygen supply in areas around roots is one of the most severe causes of root decay.

Coming up with better and more efficient ways of achieving goals is one of the major preoccupations of scientists. In this study, coming up with the most efficient regime of artificially propagating *P. sidoides* was the underlying goal. An efficient system minimises the inputs and at the same time maximises intended outputs. It has been reasoned that the decaying of roots could be a result or has been exacerbated by too much moisture in the rooting media. It is therefore recommended that a separate study be done to determine the optimum amount of water needed to propagate the cuttings. This is particularly important at this period of our (humans) existence as a species, which is characterized by devastating droughts, water pollution and increasing scarcity of portable water. It is therefore noble to pursue any effort to conserve this precious resource.

5.3 Effects of hormone

Dynaroot™ significantly improved root length and number of roots on cuttings in all seasons. The numbers of roots were significantly less at 25 DAP compared to 15 and 20 DAP. Summer had the lowest quantity of roots per cutting and two factors could be responsible for this. The first factor had to do with the high summer temperatures that facilitated the decaying of roots. In this study this was particularly true when the cuttings had been in the substrate the longest (25 DAP).

© University of Pretoria

The second reason may have to do with the quality of the cuttings used in the first place. In this instance, quality refers to the amount of energy resources the cuttings initially stored. Latt *et al*., (2000) reported that energy reserves in plants get used up or get depleted during winter and as such some species have the least amount of stored reserves at the beginning of summer. Low energy reserves coupled with very adverse temperatures could have resulted in the poor performance of cuttings grown in summer.

The numbers of roots were highest for cuttings propagated in winter. This is counter intuitive since winter is the leanest period in nature for plants that grow in regions which receive rainfall in summer and not much in winter. This can be explained by the realization that plants are evolutionary adapted to store reserves when water is limiting so that they will have accelerated growth when water becomes available (Aragao *et al*., 2016. This means that the parent stock could have stored starch in summer and autumn in preparation for lean winters. However, the winter in the greenhouse is not fully comparable to winter in the wild. In the greenhouse water was constantly available and soil nutrients were artificially kept high, hence the cuttings used the stored energy to establish roots. It should be stressed that these are “educated guesses” based on observed phenotypical traits. It is recommended that a study be carried out to elucidate the exact underlying physiological processes by measuring carbohydrate and sugar levels in the parent stock over time and compare then to what is obtained in the wild.

The difference between hormone treated and those without treatment was highest in autumn at 15 DAP. It can therefore be concluded that growing cuttings for 15 days and transplanting them to permanent substrate is the most efficient way of utilizing resources. If rooting hormone is not used, cuttings may need to be grown for longer in the rooting media. Based on the results of this study, it is recommended to grow *P. sidoides* cuttings for 20 days in rooting media before they are transferred to permanent substrate if no growth hormones are used. The same trend was also observed in spring even though the number of roots of *P. sidoides* cuttings in spring was less than in autumn. The reason why cuttings grown in autumn performed better could be that the cuttings had more starch reserves stored compared to those propagated in spring

© University of Pretoria

(Regier *et al.,* 2010). This is because nutrients in leaves and flowers move back to the stems and roots during late summer (Dong *et al.,* 2014).

As hinted on earlier, a number of workers have studied the dynamics of energy storage in various plants. Depending on season, some species store energy in the form of carbohydrates (Suneja *et al.,* 2015) and some in the form of water soluble sugars (Mitsui *et al*., 2015). It is therefore important to understand the process of energy storage in *P. sidoides* as it will allow one to better explain impacts of utilizing cuttings collected in different seasons.

It was also observed that cuttings from mature plants fared badly compared to juvenile plants. This agrees with what was observed by Davies *et al*., (1982) working on leaf-bud cuttings of *Ficus pumila*. They reported that mature plants can only be rooted if pre-treated with IBA, whilst leaf-bud cuttings from juvenile plants could easily be rooted without rooting hormone. This is probably due to the lignification of the walls of the sclerenchyma in nature plants, which results in a physical and physiological barrier (Davies *et al*., 1982).

5.4 Effects of planting depth

The ability to access soil moisture is extremely important for cutting and seedling survival (Padilla and Pugnaire, 2007). The more water a plant can access the greater are its chances of survival and as such drought tolerance has been observed to be positively correlated with the depth of plant roots (Pinheiro *et al.,* 2005). In this study, better results were obtained by planting cuttings at a depth of 3 cm and rooting of cuttings planted at 1.5 cm depth did not produce desired results. It is possible that the length of the cutting underground determines the amount of roots that grow from respective cuttings. Palzkill and Feldman (1993) discovered that deeper insertion of cuttings resulted in roots emerging from a greater length. It therefore follows that shallow planted cuttings will develop fewer roots which may not be adequate to satisfy the water and nutrient uptake needs of the plant, hence the significantly lower success rate. Deep rooting is seen as a strategy to avoid desiccation in plants (Hund *et al*., 2009. It can therefore be postulated that each species must have some critical amount

© University of Pretoria

of root mass for successful propagation below which rooting will be hindered because of lack of adequate water.

It is therefore not contested that the amount of water available to the cutting is important. Water is pulled towards the bottom of the media by gravity and consequently the quantity of water accessible to a cutting is related to the amount of time the cutting has access to water before it seeps down due to gravity (Steudle, 2000. It is plausible to suggest that, even though the amount of water supplied to the cuttings was the same, strictly speaking, cuttings planted at 3 cm depth had access to more water than cuttings planted at 1.5 cm.

5.5 Effects of hormone and medium on transplanted cuttings

All this research effort should culminate in the successful transplanting of the rooted cuttings to establish new plants that can be harvested for their leaves. It is therefore important to assess how the rooted cuttings behave when transplanted to permanent growing media and also to evaluate the effects of initial treatment of cuttings on productivity of the new plants. This information will enable better management of the *P. sidoides* propagation process. There is no study to the best of this author's knowledge that has assessed the survival ability of rooted *Pelargonium sidoides* cuttings when transplanted.

There were significant differences in survival percentage attributed to initial media treatment. *P. sidoides* cuttings initially propagated in sand had a higher survival percentage (78.6%) compared to those initially propagated in sand + coir. This is probably because roots initially grown in sand had more roots to begin with so they maintained their advantage when transplanted. Transplants from cuttings previously grown in sand also had significantly more roots than those initially grown in sand + coir. This trend was similar to what was observed in the original cuttings, further strengthening the argument that sand is a better medium compared to sand + coir. Root length was, however, not significantly different between the two media.

The same trend was also observed with regards to the impacts of Dynaroot. Cuttings that were initially treated with the hormone outperformed those that were never

© University of Pretoria

treated. Application of hormone (Dynaroot No.1) during propagation stage resulted in increased number of roots produced and longer roots before and 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 more vigorous growth of roots on cuttings treated with indole-3-butyric acid (IBA), a plant hormone.

The interactions amongst medium and hormone were highly significant ($p < 0.001$) for root number. The explanation could be that since roots are important in the process of taking up nutrients and water, a good root system on cuttings should translate to a healthier plant. In addition to the roots' role in resource uptake, roots also make phytohormones that are used in other parts of the plant to enhance growth. This agrees well with findings by Berhe and Negash (1998), working with *Juniperus procera*. In their study, cuttings with more roots showed better growth and establishment than those with a low number of adventitious roots. It can therefore be reasoned that the more root mass there is, the more hormones are produced and the larger leaves and shoots will be.

Transplants grown in sand and that were previously treated with hormone had larger leaves and longer stems. This is probably a direct result of larger roots due to the treatment initially received. It can therefore be concluded that hormone treatment coupled with sand medium produced the best results for propagating *P. sidiodes*.

5.6 Recommended future modifications of experiments

Each plant species has different requirements in terms of propagation. The best way is to utilize information from other researchers' publications regarding what they found out and if it is worth trying out on our species of interest. As with most research, one researcher may not cover all aspects of a subject and as such other workers can build on the foundation set. This section will therefore attempt to point out some aspects of the current experiments that add more useful information to the entire process of artificially propagating *P. sidoides* using leaf-bud cuttings.

In this study, the misting equipment was set to release water vapor whenever humidity fell below a certain pre-determined level. The function of the water vapour was to cool the plants effectively, reducing the rate of transpiration. However, the observed rotting

© University of Pretoria

of roots could be attributed to too much moisture in the media. Increasing transpiration could therefore have beneficial impacts on the health of the roots. Singh *et al*. (2015) reported that different misting intensities produced different results. There is therefore need to find the optimal mist requirements for each season while propagating *P. sidoides*. The same argument can also be put forward regarding the watering regime employed. It could be required to find the optimum soil moisture content to minimize rotting of cuttings and their roots.

It is possible that the observed rooting tendencies of the cuttings are not entirely a function of the different environmental conditions in which cuttings were exposed. Individual plants within the same species can have different rooting capabilities. Birlanga *et al*. (2015) reported that popular Carnation flower cultivars have different rooting abilities. They went on to identify some of the phenotypical traits that are linked to rooting ability. The same should be done for medicinally important species like *P. sidoides*. This enhances the efficient utilization of resources and increase production. The randomization done in this experiment guaranteed that the effects of different treatments were isolated but the maximum effects or yields achievable may be understated. This can only be addressed if high yielding plants are used.

5.8 Conclusion

The results from a series of experiments conducted during the course of this study indicated that sand was a better medium when compared to sand + coir. This contradicted a number of workers who have studied artificial propagation of plants. However, it is noted that conditions ideal for different species differ. Spring and autumn were observed to be the most ideal seasons for propagating *P. sidoides*. This is probably because of high summer temperatures which facilitated the decaying of roots in summer and low temperatures which impacted on root growth in winter.

© University of Pretoria

6. REFERENCES

Abbo, S., van-Oss, R.P., Gopher, A., Saranga, Y., Ofner, I. and Peleg, Z., 2014. Plant domestication versus crop evolution: a conceptual framework for cereals and grain legumes. *Trends in Plant Science*, *19*(6), 351-360.

Abdi, G., Hedayat, M. and Modarresi, M., 2013. In vitro micropropagation of *Aloe vera*-Impacts of plant growth regulators, media and type of explants. *J Biol Environ Sci*, *7*(19), pp.19-24.

Aminah, H., Ahmad Fauzi, M.S., Tariq Mubarak, H. and Hamzah, M., 2015. Effect of hormone and cutting length on the rooting of *Tinospora crispa*. *Int J Sci Res Pub*, *5*, 1-4.

Amri, E., 2015. Influence of arbuscular mycorrhizal fungi on rooting ability of auxin treated stem cuttings of *Dalbergia melanoxylon* (Guill and Perr.). *Research Journal of Botany*, *10*(3), 88-97.

Anekonda, T.S. and Reddy, P.H., 2006. Neuronal protection by sirtuins in Alzheimer's disease. *Journal of Neurochemistry*, *96*(2), 305-313.

Antoniadis, V., Shaheen, S.M., Boersch, J., Frohne, T., Du Laing, G. and Rinklebe, J., 2017. Bioavailability and risk assessment of potentially toxic elements in garden edible vegetables and soils around a highly contaminated former mining area in Germany. *Journal of Environmental Management*, *186*, 192-200.

Araya, H.T., Soundy, P. and du Toit, E.S. 2005. Seed germination and vegetative propagation of bush tea (*Athrixia phylicoides*). page 1-85.

Arya, S., Tomar, R. and Tokyt, O.P. 1994. Effect of plant age and auxin treatment on rooting response in stem cuttings of *Prosopis cineraria*. *Journal of Arid Environment*, 27(1): 99-103.

Bal, J.S., Kahlon, P.S., Jawanda, J.S. and Sandhu, S.S., 1991. Effect of pre-harvest spray of growth regulators at turning stage on the maturity of ber fruits (Zizyphus mauritiana Lamk.). *Frontier in Tropical Fruit Research 321*, 318-325.

© University of Pretoria

Barnes, P.M., Powell-Griner, E., McFann, K. and Nahin, R.L., 2004. Complementary and alternative medicine use among adults: United States, 2002. In: *Seminars in integrative medicine* (Vol. 2, No. 2, pp. 54-71). WB Saunders.

Bekker, F.T., Culham, A., Hettiarachi, P., Touloumenidout,T., Gibby, M., 2004. Phylogeny of *Pelargonium* (Geraniaceae) based on DNA sequences from three genomes. Taxon 53, 17-28.

Berhe, D. and Negash, L., 1998. Asexual propagation of *Juniperus procera* from Ethiopia: a contribution to the conservation of African pencil cedar. *Forest Ecology and Management*, *112*(1), 179-190.

Bertoni, G., 2011. Indolebutyric acid-derived auxin and plant development. Plant Cell 23(3), 845.

Bladt, S., 1997. Drug of African folk medicine. German pharmacist newspaper 117, 1655-1660.

Boeing, C., Deschmps, C., Filho, D.L and Scheffer, M.C. 1999. Influence of dipping time in water and of growth regulators on the guaco (*Mikania glomerata* Spreng) vegetative propagation. *Acta Horticulturae* 502, 113-115.

Bonga, J.M., 1982. Vegetative propagation in relation to juvenility, maturity, and rejuvenation. In: *Tissue culture in forestry* (pp. 387-412). Springer Netherlands.

Brendler, T. and van Wyk, B.E. 2008. A historical, scientific and commercial perspective on the medicinal use of *Pelargonium sidoides* (Geraniaceae). *Journal of Ethopharmacology* 119, 420-433.

Buta, E., Cantor, M., Hort, D. and Buta, M., 2014. Study on the vegetative propagation through cuttings of Mentha x piperita hybrid using various rooting substrates. *Bulletin UASVM Horticulture*, *71*(2), 345-346.

Ceballos, G., Ehrlich, P.R., Barnosky, A.D., García, A., Pringle, R.M. and Palmer, T.M., 2015. Accelerated modern human-induced species losses: Entering the sixth mass extinction. *Science Advances*, *1*(5),

© University of Pretoria

Copeland, L.O. and McDonald, M.B. (1994). Seed Science and Technology, 3rd edition. Chapman and Hall, 29 West 35th Street, New York, USA.

Curir, P., Sulis, S., Mariani, F., van Sumere, C.F., Marchesini, A and Dolci, M. 1993. Influence of endogenous phenols on root ability of *Chamarlaucium uninatum* Schauer stem cuttings. *Scientia Horticulturae* 55, 303-314.

da Silva, J.A.T., Dobránszki, J. and Ross, S., 2013. Phloroglucinol in plant tissue culture. *In Vitro Cellular and Developmental Biology-Plant*, *49*(1), 1-16.

Dakah, A., Zaid, S., Suleiman, M., Abbas, S. and Wink, M., 2014. In vitro propagation of the medicinal plant *Ziziphora tenuior* L. and evaluation of its antioxidant activity. *Saudi Journal of Biological Sciences*, *21*(4), 317-323.

De Castro, A., Vlok, J.H. and McCleland, W. 2010. Field survey of the distribution of *Pelargonium sidoides* and size of selected sub-populations. Resource Assessment study conducted for the South African National Biodiversity Institute.

Deen, S.E. and Mohamoud, M. 1996. Comparative study between saponin and natural auxin on root growth of rosemary *(Rosmarinus officinalis* L.) cutting. *Acta Horticulturae* 426, 635-642.

Dhiman, N., Patial, V. and Bhattacharya, A., 2016. In Vitro Approaches for Conservation and Sustainable Utilization of Podophyllum hexandrum and Picrorhiza kurroa: Endangered Medicinal Herbs of Western Himalaya. In: *Plant Tissue Culture: Propagation, Conservation and Crop Improvement* (pp. 45-69). Springer Singapore.

Dreyer, L.L. and Marais, E.M., 2000. Section Reniformia, a new section in the genus Pelargonium (Geraniaceae). *South African Journal of Botany*, *66*(1), pp.44-51.

Dreyer, L.L., Albers, F., Van der Walt, J.J.A. and Marschewski, D.E., 1992. Subdivision of *Pelargonium* sect. *Cortusina* (Geraniaceae). *Plant Systematics and Evolution*, *183*(1-2), pp.83-97.

© University of Pretoria

Duchow, S., Dahlke, R., Geske, T., Blaschek, W. and Classen, B., 2016. A new method for propagation of the medicinal plant *Pelargonium sidoides*. *Planta Medica*, *81*(S 01), P752.

Dzotam, J.K., Touani, F.K. and Kuete, V., 2016. Antibacterial activities of the methanol extracts of *Canarium schweinfurthii* and four other Cameroonian dietary plants against multi-drug resistant Gram-negative bacteria. *Saudi Journal of Biological Sciences*, *23*(5), 565-570.

Elsas, S.M., 2015. A model on how to obtain data from botanical practitioners. *Epilepsy and Behavior*, *52*, pp.333-337.

Evans, M.R., Konduru, S. and Stamps, R.H., 1996. Source variation in physical and chemical properties of coconut coir dust. *Horticultural Science*, *31*(6), pp.965-967.

Fogaça, C.M. and Fett-Neto, A.G., 2005. Role of auxin and its modulators in the adventitious rooting of *Eucalyptus* species differing in recalcitrance. *Plant Growth Regulation*, *45*(1), pp.1-10.

FOWLER, M.W. 1983. Commercial application and economic aspects of mass cell cultures. In: Mantel, S.H., Smith, H. (Eds). *Plant Biotechnology*. Cambridge University press, UK pp. 3-37.

Gafna, D.J., Dolos, K., Mahiri, I.O., Mahiri, J.G. and Obando, J.A., 2017. Diversity of medicinal plants and anthropogenic threats in the Samburu Central sub-county of Kenya. *African Journal of Traditional, Complementary and Alternative medicines (AJTCAM)*, *14*(5), pp.72-79.

Gao, Z.M., Wang, Y., Wang, L.Y. 2001.The recent development of research on propagation and breeding of Peony. J. Beijing. For. Univ. 23, 75-79. (In Chinese with English abstract).

Gehlot, A., Gupta, R.K., Tripathi, A., Arya, I.D. and Arya, S., 2014. Vegetative propagation of Azadirachta indica: effect of auxin and rooting media on

© University of Pretoria

adventitious root induction in mini-cuttings. *Advances in Forestry Science*, *1*(1), pp.1-9.

Grable, A.R. and Siemer, E.G., 1968. Effects of bulk density, aggregate size, and soil water suction on oxygen diffusion, redox potentials, and elongation of corn roots. *Soil Science Society of America Journal*, *32*(2), pp.180-186.

GRANGE, R.I and LOACH, K. (1983). Environmental factors affecting water loss from leaky cutting in different propagation systems. Journal of Horticultural Science, 58, 1-7.

GRANGE, R.I. and LOACH, K. 1983. The water economy of unrooted leafy cuttings. *Journal of Horticultural Science*, 58: 9-17.

GUO, X., FU, X., ZANG, D and MA, Y. 2009. Effect of auxin treatments, cuttings' collection date and initial characteristics on Paeonia Yang Fei Chu Yu cutting propagation. Scientia Horticulturae 119, 177-181.

Haissig, B.E. and Davis, T.D., 1994. A historical evaluation of adventitious rooting research to 1993. In *Biology of adventitious root formation* (pp. 275-331). Springer US.

HALDA, J.J., and WADDICK, J.W. 2004. The Genus *Paeonia*. Timber Press, Portland, p. 187.

HANSEN, J. 1986. Influence of cutting position and stem length on rooting of leaf-bud cuttings of *Schefflera arboricola. Scientia Horticulturae*, 28: 177-186.

Hansen, J. 1988. Effect of cutting position on rooting, auxiliary buds break and shoot growth in stephanotis floribunda*. Acta Horticulturae*, 226: 159-163.

Hao, Y., Yu, F., Lv, R., Ma, C., Zhang, Z., Rui, Y., Liu, L., Cao, W. and Xing, B., 2016. Carbon nanotubes filled with different ferromagnetic alloys affect the growth and development of rice seedlings by changing the c: n ratio and plant hormones concentrations. *PloS one*, *11*(6), p.e0157264.

© University of Pretoria

Hartmann, H.T. and Kester, D.E. 1983. *Plant Propagation: Principles and Practices*, Prentice-Hall International. London.

Hartmann, H.T. and Kester, D.E. and Davies, F.T. JR. and Genever L.R. 1997. *Plant Propagation: Principles and Practices*, 6th Edition, Prentice-Hall International Editions. Englewood Cliffs, New Jersey, USA.

Hartmann, H.T. and Kester, D.E. and Davies, F.T. JR. and Genever L.R. 2002. *Plant Propagation: Principles and Practices*, 7th Edition, Prentice-Hall International Editions. Englewood Cliffs, New Jersey, USA.

Hartmann, H.T. and Kester, D.E. and Davies, F.T., 1990. *Plant Propagation: Principles and Practices*, 5th Edition, Prentice-Hall International Editions. Englewood Cliffs, New Jersey, USA.

Helfer, M., Koppensteiner, H., Schneider, M., Rebensburg, S., Forcisi, S., Müller, C., Schmitt-Kopplin, P., Schindler, M. and Brack-Werner, R., 2014. The root extract of the medicinal plant Pelargonium sidoides is a potent HIV-1 attachment inhibitor. *PLoS One*, *9*(1), p.e87487.

Henry, P.H., F.A. Blazich, and Hinesey, L.E. 1992. Nitrogen nutrition of containerized Eastern recedar. Influence of stock plant fertility on adventitious rooting of stem cuttings. *Journal of the American Society for Horticultural Science*, 117: 568-570.

Hongmei, S., Ling, H. and Weiwei, W., 2011. Mechanism of starch-sucrose metabolism regulated by iba as well as ga3 during scale cutting propagation in *lilium*. *China Agriculture Science*.

Howard, B.H. 1996. Relations between shoot growth and rooting of cuttings in three contrasting species of ornamental shrubs. *Journal of Horticultural Science*, 71: 591-605.

© University of Pretoria

Howard, B.H. and Ridout, M.S. 1992. A mechanism to explain increased rooting in leafy cuttings of *Syringa vulgaris* 'Madame Lemoine' following dark-treatment of the stock plant. *Journal of Horticultural Science*, 67: 103-114.

Jawanda, J.S., Singh, A., Singh S. and Bal, J.S. 1991. Effect of indolebutyric acid and shoot portion on the rooting of cuttings in Japanese plum. *Acta Horticulturae*, 283: 189-197.

Karhu, K., Mattila, T., Bergström, I. and Regina, K., 2011. Biochar addition to agricultural soil increased CH 4 uptake and water holding capacity–results from a short-term pilot field study. *Agriculture, Ecosystems and Environment*, *140*(1), pp.309-313.

Khalid, N. and Tan, B.C., 2016. A to Z on Banana Micropropagation and Field Practices. In *Plant Tissue Culture: Propagation, Conservation and Crop Improvement* (pp. 101-118). Springer Singapore.

Khan, E., Siddiqui, J., Shakoor, S., Mehraj, V., Jamil, B. and Hasan, R., 2007. Dengue outbreak in Karachi, Pakistan, 2006: experience at a tertiary care center. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *101*(11), pp.1114-1119.

Klein, J. D., Cohen, S. and Hebbe, Y. 2000. Seasonal variation in rooting ability of myrtle (*Myrtus communis* L.) cuttings. *Scientia Horticulturae*, 83: 71-76.

Kolodiej, H., Burmeister, A., Trun, W., Radtke, O.A., Kiderlen A.F., Ito, H., Hatano, T., Yoshida, T., Foo, L.Y. 2005. Tannins and related compounds induce nitric oxide synthase and cytokines gene expressions in Leishmania major infected macrophage-like RAW 264.7 cells. Bio-organic and Medicinal Chemistry 13, 6470-6476.

KolodzieJ, H. and Schulz, V. 2004. Pelargonium (1) from the traditional application to modern phytotherapeutic. Swiss magazine for holistic medicine 17, 12-17.

© University of Pretoria

Kolodziej, H., Kayser, O., Woedenbag, H.T., Uden, W. and Pras, N. 1997. Structure cytotoxicity relationships of a series of natural and semi-synthetic simple coumarins as assessed in two human tumor cell lines. Zeitschrift fiir Naturforschung 52c, 240-244.

Kumar, V., Moyo, M. and Van Staden, J., 2015. Somatic embryogenesis of Pelargonium sidoides DC. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *121*(3), pp.571-577.

Kumar, V., Moyo, M., Gruz, J., Šubrtová, M. and Van Staden, J., 2015. Phenolic acid profiles and antioxidant potential of Pelargonium sidoides callus cultures. *Industrial Crops and Products*, *77*, pp.402-408.

Kumar, V., Shahid, M., Srivastava, M., Singh, A., Pandey, S. and Maurya, M.K., 2015. Screening of Trichoderma species for virulence efficacy on seven most predominant phytopathogens.*African Journal of Microbiology Research*, *9*(11), pp.793-799.

Kumari, S., Dutta, A., Farooqui, S., Naaz, M., Soren, S., Dutta, S., Prasad, A., Lal, N. and Smita, A., 2013. Nephrotoxicity induced by Pan masala in Swiss mice and its Protection By Elettaria Cardamomum (L.) Maton. *IJPBS*, *3*(1), pp.231-238.

Latt, C.R., Nair, P.K.R. and Kang, B.T., 2000. Interactions among cutting frequency, reserve carbohydrates, and post-cutting biomass production in Gliricidia sepium and Leucaena leucocephala. *Agroforestry Systems*, *50*(1), pp.27-46.

Leakey, R.R.B (1983). Stockplant factors affecting root initiation in cuttings of Trplochiton scleroxylon K.Schum, an indigenous hardwood of West Africa. J. hort.Sci, 58: 277 – 290.

Leenaars, J.G., Claessens, L., Heuvelink, G.B., Hengl, T., González, M.R., van Bussel, L.G., Guilpart, N., Yang, H. and Cassman, K.G., 2018. Mapping rootable depth and root zone plant-available water holding capacity of the soil of sub-Saharan Africa. *Geoderma*, *324*, pp.18-36.

© University of Pretoria

Lelu-Walter, M.A., Teyssier, C., Guérin, V. and Pâques, L.E., 2015. Vegetative propagation of larch species: somatic embryogenesis improvement towards its integration in breeding programs.

Lewu FB, Adebola PO, Afolayan AJ, (2007). Commercial harvesting of Pelargonium sidoides in the Eastern Cape; striking a balance between resource conservation and sustained rural livelihood. J. Arid Environ. 70(2): 380-388.

Li, Y.P., You, M.P., Colmer, T.D. and Barbetti, M.J., 2015. Effect of timing and duration of soil saturation on soilborne Pythium diseases of common bean (Phaseolus vulgaris). *Plant Disease*, *99*(1), pp.112-118.

Linder, H.P. and Hardy, C.R., 2004. Evolution of the species–rich Cape flora. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *359*(1450), pp.1623-1632.

Lo, Y.N. 1985. Root initiation of Shorea macrophylla cuttings: effects of node position, growth regulators and misting regime. Forest Ecology and management, 12: 43-52.

Loach, K. 1992. Environmental conditions for rooting cutting; importance, measurement and control. *Acta Horticulturae*, 314: 233-242.

Manning, J.C. and Goldblatt, P., 2012. Pelargonium saxatile (Geraniaceae: Section Hoarea), a new species from the southwestern Cape, South Africa, and a key to the species of the P. dipetalum group. *South African journal of botany*, *78*, pp.266-269.

Mativandlela, S.P.N., Lall, N. and Meyer, J.J.M., 2006. Antibacterial, antifungal and antitubercular activity of (the roots of) Pelargonium reniforme (CURT) and Pelargonium sidoides (DC)(Geraniaceae) root extracts. *South African Journal of Botany*, *72*(2), pp.232-237.

Moyo, M. and Van Staden, J., 2014. Medicinal properties and conservation of Pelargonium sidoides DC. *Journal of ethnopharmacology*, *152*(2), pp.243-255.

© University of Pretoria

Mpati, K.W., Soundy, P and Du Toit, E.S. 2006. Response of fever tea (Lippia javanica) to fertigation frequency, growth medium and propagation methods. Scientia Horticulturae.

Newman, D.J. and Cragg, G.M., 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010.*Journal of natural products*, *75*(3), pp.311-335.

Newman, D.J. and Cragg, G.M., 2016. Natural products as sources of new drugs from 1981 to 2014. *J. Nat. Prod*, *79*(3), pp.629-661.

Obeng-Ofori, D., Reichmuth, C.H., Bekele, J. and Hassanali, A., 1997. Biological activity of 1, 8 cineole, a major component of essential oil of Ocimum kenyense (Ayobangira) against stored product beetles. *Journal of Applied Entomology*, *121*(1-5), pp.237-243.

OuYang, F., Mao, J.F., Wang, J., Zhang, S. and Li, Y., 2015. Transcriptome analysis reveals that red and blue light regulate growth and phytohormone metabolism in Norway spruce [Picea abies (L.) Karst.]. *PloS one*, *10*(8), p.e0127896.

Palanisamy, K. and Kumar, P., 1997. Effect of position, size of cuttings and environmental factors on adventitious rooting in neem (Azadirachta indica A. Juss). *Forest Ecology and Management*, *98*(3), pp.277-280.

Pan, S.Y., Litscher, G., Gao, S.H., Zhou, S.F., Yu, Z.L., Chen, H.Q., Zhang, S.F., Tang, M.K., Sun, J.N. and Ko, K.M., 2014. Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. *Evidence-Based Complementary and Alternative Medicine*, *2014*.

Pan, S.Y., Litscher, G., Gao, S.H., Zhou, S.F., Yu, Z.L., Chen, H.Q., Zhang, S.F., Tang, M.K., Sun, J.N. and Ko, K.M., 2014. Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. *Evidence-Based Complementary and Alternative Medicine*, *2014*.

© University of Pretoria

Pandey, S., Singh, J. and Maurya, I.B., 2015. Effect of black polythene mulch on growth and yield of Winter Dawn strawberry (Fragaria× ananassa) by improving root zone temperature. *Indian Journal of Agricultural Sciences*, *85*(9), pp.1219-1222.

Pradhan, B.K. and Badola, H.K., 2015. Swertia chirayta, a Threatened High-Value Medicinal Herb: Microhabitats and Conservation Challenges in Sikkim Himalaya, India. *Mountain Research and Development*, *35*(4), pp.374-381.

Preece, J.E., 2003. A century of progress with vegetative plant propagation. *HortScience*, *38*(5), pp.1015-1025.

Priyanka, J., Hingorani, L. and Nilima, K., 2013. Pharmacodynamic evaluation for antiplasmodial activity of Holarrhena antidysentrica (Kutaja) and Azadirachta indica (Neemb) in Plasmodium berghei infected mice model. *Asian Pacific journal of tropical medicine*, *6*(7), pp.520-524.

PURI, S. and VERMAT, R.C. 1996. Vegetative propagation of Dalbergia sisso. Roxb using softwood and hardwood tem cuttings. Journal of arid environments, 34 (2): 235-245.

Rahman, M.M., Majumder, M.T.H., Mukta, M.S.H., Ali, M.E. and Mahmud, J., 2016, May. Can we predict eat-out preference of a person from tweets?. In *Proceedings of the 8th ACM Conference on Web Science* (pp. 350-351). ACM.

Röschenbleck, J., Wicke, S., Weinl, S., Kudla, J. and Müller, K.F., 2016. Genus-wide screening reveals four distinct types of structural plastid genome organization in Pelargonium (Geraniaceae). *Genome biology and evolution*, *9*(1), pp.64-76.

Saifuddin, M., Osman, N. and Motior Rahman, M., 2013. Influence of Different Cutting Positions and Rooting Hormones on Root Initiation and Root-soil Matrix of Two Tree Species.*International Journal of Agriculture and Biology*, *15*(3).

© University of Pretoria

Sale, F.A., Attah, E.S. and Yahaya, P., 2015. Influence of Soil Locations and Watering Regimes on Early Growth of Moringa oleifera Lams. *International Journal of Applied*, *5*(2).

Samie, A., Obi, C.L., Bessong, P.O. and Namrita, L., 2005. Activity profiles of fourteen selected medicinal plants from Rural Venda communities in South Africa against fifteen clinical bacterial species. *African journal of Biotechnology*,*4*(12).

Sevik, H., Güney, K., Topaçoğlu, O. and Ünal, C., 2015. The influences of rooting media and hormone applications on rooting percentage and some root characters in Schefflera arboricola. *International Journal of Pharmaceutical Science Invention*, *4*(2), pp.25-29.

Shevade, A. and Preece, J.E., 1993. In vitro shoot and floral organogenesis from stamen explants from a Rhododendron PJM group clone. *Scientia horticulturae*, *56*(2), pp.163-170.

Stolzy, L.H., Zentmyer, G.A., Klotz, L.J. and Labanauskas, C.K., 1967, January. Oxygen diffusion, water, and Phytophthora cinnamomi in root decay and nutrition of avocados. In *Proc. Amer. Soc. Hort. Sci* (Vol. 90, pp. 67-76).

Stroud, H., Ding, B., Simon, S.A., Feng, S., Bellizzi, M., Pellegrini, M., Wang, G.L., Meyers, B.C. and Jacobsen, S.E., 2013. Plants regenerated from tissue culture contain stable epigenome changes in rice. *Elife*, *2*, p.e00354.

Sucher, N.J. and Carles, M.C., 2015. A pharmacological basis of herbal medicines for epilepsy. *Epilepsy and Behavior*, *52*, pp.308-318.

Sun, L., Tian, J., Zhang, H. and Liao, H., 2016. Phytohormone regulation of root growth triggered by P deficiency or Al toxicity. *Journal of experimental botany*, *67*(12), pp.3655-3664.

Sundgren, T.K., Uhlen, A.K., Lillemo, M., Briese, C. and Wojciechowski, T., 2018. Rapid seedling establishment and a narrow root stele promotes waterlogging tolerance in spring wheat. *Journal of plant physiology*.

© University of Pretoria

Tahan, F. and Yaman, M., 2013. Can the Pelargonium sidoides root extract EPs® 7630 prevent asthma attacks during viral infections of the upper respiratory tract in children?.*Phytomedicine*, *20*(2), pp.148-150.

Theisen, L.L. and Muller, C.P., 2012. EPs® 7630 (Umckaloabo®), an extract from Pelargonium sidoides roots, exerts anti-influenza virus activity in vitro and in vivo. *Antiviral research*, *94*(2), pp.147-156.

Timofeeva, S.N., Elkonin, L.A., Yudakova, O.I. and Tyrnov, V.S., 2016. Application of Tissue Culture for Laburnum anagyroides Medik. Propagation. In *Plant Tissue Culture: Propagation, Conservation and Crop Improvement* (pp. 135-159). Springer Singapore.

Tomlinson, T.R. and Akerele, O. eds., 2015. *Medicinal plants: their role in health and biodiversity*. University of Pennsylvania press.

Topacoglu, O., Sevik, H., Guney, K., Unal, C., Akkuzu, E. and Sivacioglu, A., 2016. Effect of rooting hormones on the rooting capability of Ficus benjamina L. cuttings. *Šumarski list*, *140*(1-2), pp.39-44.

Van Der Walt, J.J., Vorster, P.J. and Ward-Hilhorst, E. 1988. Pelargonium of Southern Africa. National Botanical Gardens, Kirstenbosch, South Africa.

Van Der Walt, J.J.A. 1985. A taxonomic Revision of the type section of Pelargonium L. Herit (Geraniaceae). Bothalia 15, 345-385.

Van Wyk, B.E and Wink, M. 2004. Medicinal plants of the world: an illustrated scientific guide to important medicinal plants and their uses. Illustrated, re-print, Timber press, incorporated, ISBN: 0881926027, 9780881926026.

Veeresham, C., 2012. Natural products derived from plants as a source of drugs.

Viljoen, E., Visser, J., Koen, N. and Musekiwa, A., 2014. A systematic review and meta-analysis of the effect and safety of ginger in the treatment of pregnancy-associated nausea and vomiting. *Nutrition journal*, *13*(1), p.20.

© University of Pretoria

Watt. JM, Breyer-Brandwijk MG, (1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed. Livingstone, London.

White, A.G., Davies-Coleman, M.T and Ripley, B.S. 2008. Measuring and optimizing umckalin concentration in wild-harvested and cultivated Pelargonium sidoides (Geraniaceae). South African Journal of Botany 74, 260-267.

Wiessman-Ben, Z. and Tchoundjeu, Z. 2000. Vegetative tree-propagation for arid and semi-arid land. Internet: http://www.cgiar.org/ICRAF/res_dev/prog_s/tr_mat/course-i/t2nm ol.htm

Williams, C.A., and Harborne, J. B. 2002. Photochemistry of the genus Pelargonium in: Lis- Balchin, M(Ed), Geranium and Pelargonium: The Genera Geranium and Pelargonium. Taylor and Francis, London, p. 99.

Wilson, P.J. 1993. Propagation Characteristics of Eucalyptus globules Labill ssp. Globules stem cuttings in relation to their original position in the parent shoot. Journal of Horticultural Science, 68 (5): 715-724.

Wilson, R.S., De Leon, C.F.M., Barnes, L.L., Schneider, J.A., Bienias, J.L., Evans, D.A. and Bennett, D.A., 2002. Participation in cognitively stimulating activities and risk of incident Alzheimer disease. *Jama*, *287*(6), pp.742-748.

Witte, K., Koch, E., Volk, H.D., Wolk, K. and Sabat, R., 2015. The pelargonium sidoides extract EPs 7630 drives the innate immune defense by activating selected map kinase pathways in human monocytes. *PloS one*, *10*(9), p.e0138075.

Woodward, A.W. and Bartel, B., 2005. Auxin: regulation, action, and interaction. *Annals of botany*, *95*(5), pp.707-735.

World Health Organization, 2014. *Antimicrobial resistance: global report on surveillance*. World Health Organization.

Yu, J., Liu, W., Liu, J., Qin, P. and Xu, L., 2017. Auxin control of root organogenesis from callus in tissue culture. *Frontiers in plant science*, *8*, p.1385.

© University of Pretoria

Zenaro, E., Pietronigro, E., Della Bianca, V., Piacentino, G., Marongiu, L., Budui, S., Turano, E., Rossi, B., Angiari, S., Dusi, S. and Montresor, A., 2015. Neutrophils promote Alzheimer's disease-like pathology and cognitive decline via LFA-1 integrin. *Nature medicine*, *21*(8), pp.880-886.

Zheng, W.H., Zhuo, Y., Liang, L., Ding, W.Y., Liang, L.Y. and Wang, X.F., 2015. Conservation and population genetic diversity of Curcuma wenyujin (Zingiberaceae), a multifunctional medicinal herb. *Genet. Mol. Res*, *14*, pp.10422-10432.

© University of Pretoria