SEED GERMINATION AND VEGETATIVE PROPAGATION OF BUSH TEA (*ATHRIXIA PHYLICOIDES*)

By

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ABSTRACT

Bush tea (*Athrixia phylicoides*) is an herbaceous plant of the Asteraceae family used throughout history as medicinal herbal tea by the people of South Africa. Many studies stated that the plant has an ability to be commercialized as a medicinal herbal tea. But vegetative propagation of this type of plant by stem cutting, survival ability of the rooted cuttings, response to different hormone concentrations and the requirement of the seeds for germination has not been studied. In this investigation, different features aimed at effective propagation of bush tea were studied. These comprised: cutting position (apical vs. basal), media (pine bark vs. sand), hormone (Seradix No. 2), season (summer, autumn, winter and spring), transplanting survival of rooted apical and basal cuttings, response of basal cuttings to three hormone concentration levels (Seradix No. 1, 2 and 3) and light and temperature requirement for bush tea seed germination.

In vegetative propagation, apical cuttings rooted to higher percentage and produced high root number as well as longer roots than basal cuttings. Pine bark improved the number of roots developed but had no effect on rooting percentage as well as root length. Application of rooting hormone (Seradix No. 2) increased root numbers but not rooting percentage or root length. Rooting of cuttings was improved when propagated in autumn (longer roots) and spring (more number of roots) than in summer or winter.

There was higher survival percentage (67.5%), high root number as well as longer roots from apical cuttings than from basal cuttings (50%) two months after transplanting. Propagation in pine bark with hormone application increased root number after transplanting. Application of hormone also improved root and shoot length after transplanting. Apical cuttings propagated in pine bark with hormone developed more number of roots. Cuttings propagated in sand with hormone and in pine bark without hormone also produced longer shoots after transplanting.

Regarding response of basal cuttings to hormone concentration, high number of roots was produced in pine bark with Seradix No. 2 at 10 days after planting (DAP) but at 15 DAP more roots were produced in pine bark with Seradix No. 1. With sand, more roots were produced with Seradix No. 3 than Seradix No. 1 and 2. Number of roots were also higher with 0.3% IBA concentration (Seradix No. 2) and 0.1% IBA concentration (Seradix No. 1). Similarly, cuttings with lower IBA concentration (0.1%, Seradix No. 1) rooted to higher percentage followed with 0.3% IBA concentration (Seradix No. 2).

Germination percentage of bush tea seeds differed with the temperature treatments and the highest was 75.5% at 20 and 25 ^{o}C followed by 15 ^{o}C with 64.5% and low percentage at 30 and 10 ^{o}C with 36 and 47% respectively. There was a high germination percentage in constant temperatures than alternate temperatures and in continuous light than alternate light: dark or continuous dark. Germination percentage was also higher in continuous light at constant temperatures than with alternated light: dark with constant temperatures. In addition, there was more differences in germination percentage with variation in light exposure than variation in temperatures. At low temperature (10 ^{o}C), longer time was required to start germination and germination rate was high at 20 ^{o}C continuous light and low at 30:30 ^{o}C alternate light: dark.

Based on this investigation, better vegetative propagation and survival of bush tea can be attained from apical cuttings with Seradix No. 2 but basal cuttings rooted better with Seradix No. 1 in pine bark. Seeds germinated to higher percentage and rate at 20 ^{o}C constant temperature and continuous light.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The genus *Athrixia* belongs to the family Asteraceae, tribe *Inuleae* and subtribe Athrixiinae. It holds 14 species, which are found in southern Africa, tropical Africa and Madagascar and 9 of these species are distributed in southern Africa (Herman, Retief, Koekemoer & Welman, 2000). *Athrixia phylicoides* is one of the indigenous plants in South Africa; commonly known as Bushmen tea, Zulu tea or bush tea. It is an attractive shrub, about 50 cm to 1 m in height, much branched, with thin, white and woolly stems. Leaves are simple, alternate linear to broadly lanceolate, tapering to a sharp point, very shortly stalked, auriculate at the base, light grey-green, smooth on upper surface, white-woolly below, the margins are entire slightly revolute. Inflorescence head sessile or subsessile, terminal and axillary, in large subcorymbose panicles. Involucral bracts 10mm long, campanulate, straw coloured, and many ray flowers mauve, magenta or pink, the disc flowers are yellow. Based on the soil and the area where the plant grows the colour of the flowers may vary from the palest pink to all shades of pink and mauve to deep purple. It flowers from May to July in the coastal areas and from mid to late summer inland (Fox & Young, 1982; Roberts, 1990). The fruits consist of narrow, cylindrical and thin achenes of about 0.01 to 0.02 mm long and 0.03 to 0.06 mm wide; with an average of 12 pappus per seed of about 4 mm long which helps in the dispersion of the seed as a parachute.

Bush tea (*A. phylicoides*) is well known in open grassland and forest margins of the eastern part of South Africa (Limpopo Province, Free State Province, KuwaZulu-Natal and the eastern part of Eastern Cape Province) and Swaziland. The plant is commonly propagated by ripen seeds which are mostly collected at the end of the summer. For good growth, the plant needs enough space (for spreading), well-drained soils and full sun (Roberts, 1990).

The people of South Africa have used bush tea for many years as medicinal herbal tea and throughout history people gathered this plant from the mountainous regions of their homeland and used it to prepare tea. The introduction of this plant is believed to be by the Khoikhoi to the colonists and moved further inlands up to the coast with the Voortrekkers. Other people such as the San and other indigenous peoples of South Africa were taught the use of the plant from the travellers (Roberts, 1990).

Bush tea as a medicinal herbal tea, is used for cleansing or purifying the blood, treating boils, bad acne, infected wounds and cuts, for washing and as lotion on boils, skin eruptions or cuts. It is also wonderful for coughs and colds, and for loss of voice and for infected throat as a gargle. The Sotho in addition use strong brew preparation as calming wash for sore feet and then bandage the washed feet by castor oil leaves (*Ricinis communis*), since both have great treatment value and a deep acting effect on hard skin and muscles of the feet (Roberts, 1990). Furthermore the herbal tea prepared from this plant is well studied by several people to be better than low quality tea (Van Wyk & Gericke, 2001). The infusion prepared from the leaves of this plant is also pleasant to drink and it has medicinal properties, and because of this, in some areas of South Africa people grow this plant nearby for use (Fox & Young, 1982; Roberts, 1990). The stems of bush tea as well are tied up in bundles for brooms and traded on a small scale in Limpopo Province. The twigs and leaves of a related species *Athrixia elata* are also used to prepare a beverage known as *wildetee* or daisy tea (Van Wyk & Gericke, 2001).

Even though many herbs were eliminated during the early years of the twentieth century and replaced with synthetic products, in the past 20 years there has been resurgence in natural products such as herbal teas. Many health-oriented individuals with concern about the possibility of poor health effects due to consuming beverages containing caffeine such as coffee, cocoa and tea, are turning to herbal teas as an alternative (Manteiga, Park & Ali, 1997; Pietta, 2000). Today, herb tea cultivation is a big business in many parts of the world and the complex industry now produces a variety of teas. Certain areas of the earth are better known than others for producing herbal tea (PageWise**,** 2002). Among these, South Africa is well known by its indigenous herbal tea production like honeybush tea (*Cyclopia intermedia*) and rooibos tea (*Aspalathus linearis*) (Marnewick, Gelderblom & Joubert, 2000). Like honeybush and rooibos tea, bush tea (*A. phylicoides*) has been used for decades as herbal tea or medicinal tea by the peoples of South Africa. Van Wyk & Gericke (2001) also reported the suitability of this plant for domestication and development as a commercial health tea. However, vegetative propagation of this type of plant from cuttings and the effect of origin of cuttings on the rooting has not been studied. Furthermore, the germination (light and temperature) requirement of bush tea seed is not known.

The objectives of this study were to:

- Generate a protocol for the vegetative propagation of bush tea
- Compare the rooting of cuttings, that is apical and basal portion of the stem with and without rooting hormone and effect of rooting media on rooting of the cuttings
- Compare the rooting of cuttings in different seasons
- Evaluate the survival of the propagated plants
- Determine the ideal seed germination temperature and light combinations for bush tea

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

Herbs are plants that have some medicinal, cooking, or other domestic use, such as dyes, insect repellents, or aroma. Most of them are pleasingly fragrant or strongly aromatic; a few are with odour or have no odour at all. Written history from the ancient civilizations explained that plants had been used as medicine for many years (Manteiga *et al*., 1997). Manteiga *et al*. (1997) reported the first complete list, or *Material Medica,* of all known medicinal herbs was written during the Roman Empire. Based on the archaeological reports also the infusion from variety of wild plants and the traditional black tea was probably practiced for more than 500 000 years (Dufresne & Farnworth, 2001).

Many herbs were tested during the early 20th century and were eliminated as unsuccessful by synthetic products. However, a significant increase in consumption of medicinal and nonmedicinal natural products such as herbal teas has been reported during the past 20 years. Herbal teas are not true teas at all and mostly they contain mixtures of roots, berries, and other plant materials (Manteiga *et al*., 1997). They do not contain any of the leaves of the true tea plant or black tea (*Camellia sinensis*) (Trevisanato & In Kim, 2000; Phelan & Rees, 2003). Since herbal teas are caffeine free they have being used as an alternative to beverages such as coffee, cocoa and tea by many individuals (Manteiga *et al*., 1997).

Throughout history herbal teas played a great role in every day living in many societies, not for their flavour but for their medicinal value. Many of these herbal teas are flavourful; some are strong and medicinal. They were taken quite often medicinally to cure coughs, sore throats, fever and aches and headaches. A few of the more popular plants commonly used as herbal tea include chamomile, marjoram, peppermint, rosemary, sage, rose, lemon verbena and thyme (Pietta, 2000). Today due to their popularity for their flavour and medicinal properties the consumption of herbal teas is increasing all over the world. For example, Phelan & Rees (2003) mentioned that in a short period of time in the UK herbal tea consumption increased by 3 to 4%

of the total tea per year. Manteiga *et al*. (1997) also reported during 1985 that the sale of herbal teas in the United States went beyond $190 million.

Plant propagation played an important role in mankind since the beginning of civilization. Man started to grow crops, which were useful for himself and his domestic animals 10 000 years ago. But as civilization of mankind progressed, highly developed people started to grow different plants that provided fibres, medicines, ornamentals and beauty in addition to food crops. Throughout time man discovered the ability of plants to regenerate and started to use different vegetative propagation techniques to propagate a variety of plants nearby (Hartmann & Kester, 1983). Among these propagation techniques, cutting is reported to be an extensively practiced and economical means of vegetative propagation for a wide range of woody plants cultivated for use as ornamentals (for example, *Bougain villea*), fruits, agro forestry (for example, S*esbania sesban* (L.) Merrill) or for food like cassava, beverage (tea), (Leakey, 1990; Mudge, Mwaja, Itulya, & Ochieng, 1995). There are many advantages for plant species that can be propagated early by cutting. Numerous new plants can be propagated in a restricted area from a few stock plants. Unlike the other asexual methods like grafting, budding and micro propagation, cutting is easy, cheap and quick (Hartmann, Kester, Davies & Genever, 1997).

Another method of plant propagation is through seeds (sexual propagation). In this technique one of the most important steps is the germinability of the seeds. This ability of the seed to germinate is affected by environmental factors such as availability of moisture, photoperiod (light and dark), temperature and air (oxygen and carbon dioxide) and the requirement of these factors by seeds vary with plant species (Hartmann & Kester, 1983; Copeland & McDonald, 1994; Hartmann *et al*., 1997).

1.2 HERBAL TEAS

Herbal teas is amongst the most populate herbal preparations, providing healthy substitutes for tea and coffee (Jennifer, 2002). Herbal teas are different from leaves of traditional black tea and they are made from plant parts such as flowers, roots, barks and seeds. They can also be prepared from blends of different plants; due to this they exist in distinctively different flavours, colours and aromas (Phelan & Rees, 2003). Many herbal teas have a longer history of use in Europe and Far East countries (Koff, 1995). According to Robert (2002) drinking of herbal teas

was widespread in Europe long before the arrival of black tea; and some of the recurrent favourites such as chamomile, peppermint and rosehips have long been well known standards. For instance, the flowers of chamomile started to be used as a medicine by ancient Romans, and they are still used as folk medicine in Europe.

Today herbal tea cultivation is a big business in many parts of the world and the complex industry now produces a variety of teas. Certain regions of the world are better known than others for producing herbs. Hibiscus, for example, is native to Africa and Mexico, rosehips to South America and blackberry leaves to Eastern Europe (PageWise, 2002). Peter (1995) also stated that chamomile is currently grown commercially in many countries of the world such as Belarus, Slovakia, Ukraine, Finland, Moldavia, Spain, North Caucasus to South Siberia, North Africa (Egypt, Ethiopia), Asia (Turkey, Afghanistan, Pakistan, North India, Japan) and North and South America (East Coast of the USA, Cuba, Argentina, Brazil). South Africa is also well known for the production of rooibos and honeybush tea. South Africa also produces black tea but most of it is consumed locally (Africantea, 2004). Lavender, lemon verbena, lemon balm, *Lippia javanica* and mint are also the popular herbs and ingredients for making herbal tea in South Africa (Van Wijk, 1986).

1.3 MEDICINAL VALUE OF HERBAL TEA

The use of herbs is as old as mankind and people used them as medicine, cosmetic and for cooking for thousands of years. Archaeological researches also report that herbal concoctions had been used to indulgence bodily grievances many years before the writing of history. Throughout history, herbs have had their place in every civilization in the world, with their usage changing very little as the centuries passed. Ancient cultures wrote plentiful use of herbs, which included flowers, leaves, tree and bark, that were used for improving the taste of food, making medicines or preparing tea (Manteiga *et al*., 1997; Dufresne & Farnworth, 2001). One of the most popular and long-term use of herbs is the making of herbal tea. Herbal teas have a long history of helping people to stay healthy. Before the middle of the 18th century, when most people where living on farms, infusion of wintergreen, willow or birch were prepared from nearby growing herbs to calm down someone's pain. But when the old medicine men died much of our herbal heritage passed and this created an information gap for over hundred years. However, nowadays people are having knowledge with herbs and this tradition continues in

80% of the world and they are rediscovering the blessings of whole herbal medicines used throughout generations (Manteiga *et al*., 1997; John, 2003).

Many people state that taking these teas over a long period of time builds up resistance to a number of illnesses. In South Africa for example, *Athrixia phylicoides* (Zulu tea or bush tea) has been used for many decades as a medicinal tea for cleansing or purifying the blood, in treating boils, bad acne, infected wounds and cuts, and also it is excellent for coughs and colds and as a gargle for throat infections and loss of voice (Fox & Young, 1982; Roberts, 1990). Rooibos tea (*Aspalathus linearis*) and honeybush tea have also being used as health tea for many years in South Africa. People used them to relive a variety of skin irritations, digestive disorders and respiratory ailments (hay fever, asthma, etc.) (Van Wijk, 1986).

Many researches in teas have shown promising data in terms of heart disease and cancer prevention. Recent studies also showed that herbal teas might prevent stroke. The natural compounds in herbal tea that can prevent cancer or heart disease are called antioxidants (or flavonoids). Since herbal infusions come from a mixture of ingredients such as spice, fruits and berries from various plants, it is likely that some contain antioxidant components (Cao, Guohua, Emin & Ronald, 1996).

1.4 PROPAGATION

1.4.1 Vegetative propagation by stem cutting

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 can be described as a method of propagating plants by the use of detached vegetative plant parts which, when placed under conditions favourable for regeneration, will develop into a complete plant, similar in all characteristics to the parent plant (Hartmann & Kester, 1983). According to Hartmann *et al*. (1997) cuttings can be made from the vegetative portions of the plant, such as stem, modified stems (rhizomes, tubers, corms and bulbs), leaves or roots. Based on the part of the plant taken, cuttings can be classified as stem cuttings (hardwood, semi-hardwood, softwood and herbaceous), leaf cuttings, leaf-bud cuttings (single-eye or single-node cuttings) and root cuttings.

In propagation by stem cuttings, segments of shoots containing lateral or terminal buds are obtained with the expectation that under the proper conditions adventitious roots will develop and thus produce independent plants. The formation of adventitious roots and buds is dependent on 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). However, the development of adventitious roots in a variety of plant species can be influenced by different factors such as position of cutting, rooting hormone, rooting medium, environmental and physical factors (Wilson, 1993).

1.4.2 Effect of cutting position

The influence of cutting position is sometimes referred to as the influence of topophysis (Hartmann & Kester, 1983). For many years rooting ability has been known to vary between cuttings from different parts of the same plant, especially in woody species (Leakey & Mohammed, 1985) and this was correlated with structure of the stem (Hartmann *et al*., 1997) or difference in chemical composition of the plant along the stem (Hansen, 1986; Hartmann *et al*., 1997). When the stem matures and gets older a continuous sclerenchyma ring between the phloem and cortex, exterior to the point of origin of adventitious roots, occurs, and this hamper the root development. This is mostly observed in difficult-to-root species such as olive, *Hedera helix* and *Ficus pumila*, whereas easy-to-root types are characterized by discontinuity or fewer cell layers of this sclerenchyma ring (Hartmann *et al*., 1997).

Apical cuttings

Apical cuttings are mostly softwood or semi-hardwood cuttings with developing apex and associated young leaves, which may produce rooting promoters but could also be inhibitors if present in excess (Hansen, 1986; Leakey & Coutts, 1989). Many studies reported poor rooting of apical cuttings compared to that of basal ones (Leakey, 1983; Al-Saqri & Alderson, 1996). This was related to the softness of the leaves and stem, the difference in degree of maturity along the shoot, their rate of growth or relatively low structural and water-soluble carbohydrates (Hansen, 1986; Leakey & Coutts, 1989).

On the other hand, in many vegetatively propagated species, older, lignified woodcuttings are difficult to root than newly formed stems (Hartmann *et al*., 1990). This was supported in the propagation of *Grindelia chiloensis* where none of the basal cuttings rooted (Wassner & Ravetta, 2000). The same was true for apical cuttings of rosemary *(Rosmarinus officinalis* L.), which exerted more number of roots than the basal cuttings (Deen & Mahmoud, 1996). With the propagation of *Triplochiton scleroxylon* a gradual reduction in rooting percentage was recorded with distance from the apex (Leakey, 1983).

Basal cuttings

In many vegetatively propagated plants highest rooting percentage was found when cuttings were taken from the basal part of the shoot (Hansen, 1986; Hartmann *et al*., 1997). This was supported when three cultivars of high bush blueberry *(Vaccinium corymbosum*) basal cuttings produced significantly higher rooting percentage than the other types of cuttings (sub-apical and medial) (Hartmann *et al*., 1990). Similar results were also reported in *Rosa centifolia* (Al- Saqri & Alderson, 1996). Basal portion of plum also tend to root more readily as compared to sub-apical portion (Jawanda, Singh, Singh & Bal, 1991). The good rooting of basal cuttings of *Schfflera arboricola* (Leakey, 1983) and *Strephanotis floribuna* (Hansen, 1988) are in agreement with the general statement by Hartmann & Kester (1983) that the best rooting of cuttings is usually found from the basal portions of shoots and there is a gradient in rooting response from top to base.

The good rooting ability of basal cuttings could be due to higher food reserves as total sugars or it could have been due to the accumulation of natural auxins in the shoot bases or other root promoting factors with relatively low levels of rooting inhibitors (Al- Saqri & Alderson, 1996). It could also be due to juvenility factors (Jawanda *et al*., 1991) that occur along the stem of a plant (Hartmann & Kester, 1983; Hansen, 1986; Hartmann *et al*., 1990). Nautiyal, Singh, & Gurumurthi (1992) also made similar correlations in the root development of teak (*Tectonia grandis)* cuttings.

Similarly, since cuttings taken from the basal portion are known to have greater accumulation of photosynthetic products, mostly carbohydrates, it is reasonable to expect long and thick shoot, more number of roots and root weight and higher number and greater leaf area per cutting

(Jawanda *et al*., 1991). This was supported by Hansen (1988) in rooting of *Strephanotis floribunda*. In general, the ability to form roots increased with distance from the apex (Hansen, 1986).

On the other hand, the effect of position seems to be species dependent, since in some species basal or medial cuttings root best, whereas from other species apical cuttings root best (Hartmann *et al*., 1997). This was supported when the effect of position on subsequent shoot growth was observed in *Hedera canariensis*, while differences in rooting with differences in position was observed in *Hedera helix* (Hansen, 1986). The same suggestion was also made in the rooting of *Dorycnium pentaphyllum* (Table 1.1) where cutting position (apical vs. medial) did not affect rooting, root number, root length or root zone length. However, in the case of *Dorycnium hirsutum* (Table 1.2) cutting position showed a significant effect on root number and length. This showed that the rooting ability of *Dorycnium hirsutum* was clearly different from that of *Dorycnium pentaphyllum* (Alegre, Toledo, Martinez, Mora & De Andres, 1998).

Table 1.1 Mean value of rooting response of D. *pentaphyllum* cuttings (Algere *et al*., 1998)

Factors	Rooting %	Dead cuttings	Root number	Longest root (cm)	Rooting length zone (cm)
		Type of cutting			
Apical	58.4	18.4	4.74	5.64	0.47
Medial	52.9	10.5	3.97	5.14	0.47
		Environment			
Cold	42.8	11.2	3.65	3.21	0.40
Warm	68.6	17.7	5.06	7.56	0.54
		Auxin			
Control	26.2	26.5	1.94	3.25	0.24
IBA 50	73.8	8.4	5.25	7.11	0.41
IBA 200	67.0	8.4	5.87	5.79	0.76

Table 1.2 Mean value of rooting response of D. *hirsutum* cuttings (Algere *et al*., 1998)

Factors	Rooting %	Dead cuttings	Root number	Longest root (cm)	Rooting length zone (cm)
		Type of cutting			
Apical	45.4	35.0	6.48	9.45	0.59
Medial	16.7	62.1	3.55	4.27	0.67
		Environment			
Cold	38.9	28.5	4.43	6.47	0.62
Warm	23.1	68.7	5.62	7.26	0.64
		Auxin			
Control	33.6	42.9	4.61	5.80	0.59
IBA 50	33.2	48.9	5.52	7.15	0.59
IBA 200	26.2	54.0	4.87	7.63	0.72

1.4.3 Physical factors

Physical factors such as cutting length, cutting type, stem diameter, presence or absence of a leaf and stock plant from which the cuttings are taken, play an important role in rooting ability of cuttings (Leakey, 1983).

Cutting length and diameter

In addition to cutting position recent studies showed the importance of internode length on rooting ability of cuttings. Length of the cutting may vary depending on the species. Cutting length was usually strongly correlated with number of roots per rooted cutting than with rooting percentage of cuttings. The relationship between cutting length and percentage of rooting was stronger when cuttings increased in length acropetally than basipetally (Leakey, 1983; Leakey & Mohammed, 1985). Report by Leakey & Mohammed (1985) in *Triplochiton scleroxylon* and Wilson (1993) in *Eucalyptus globulus* indicated that when cutting length was greatest at apical nodes, rooting percentages and the numbers of roots per rooted cutting was higher from apical nodes than basal nodes. Palanisamy & Kumar (1997) reported similar results on rooting of neem (*Azadirachta indica*) (Fig. 1.1).

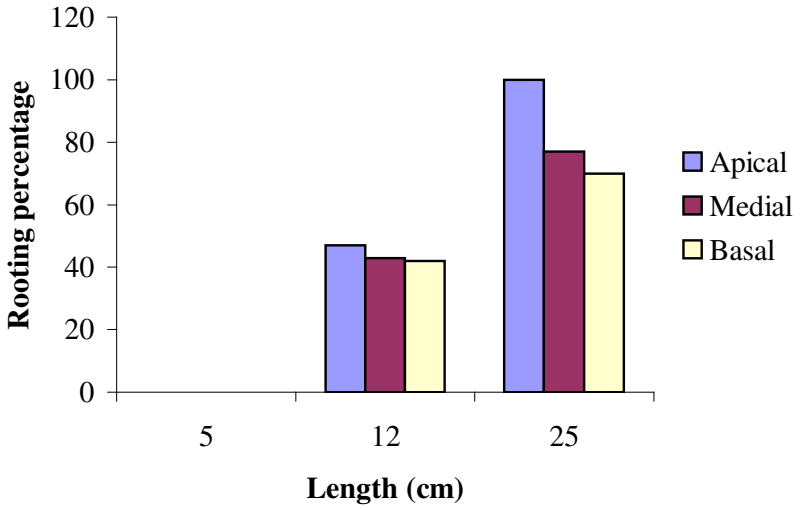


Fig. 1.1 Effect of cutting position and stem length on rooting of neem (*Azadirachta indica*) (Palanisamy & Kumar, 1997)

On the other hand, when cutting lengths of *Triplochiton scleroxylon* was greatest at basal nodes, rooting percentages and number of roots per rooted cutting was also significantly greater from basal nodes. When cuttings from all nodes were similar in length, rooting percentages and numbers of roots per rooted cutting were generally greatest from basal nodes (Leakey & Mohammed, 1985; Wilson, 1993). The effect of cutting length was related to carbohydrate accumulation at the base of the cutting and that the carbohydrate amount was more optimal for root formation in cutting with long basal compared to short ones (Hartmann *et al.*, 1990).

The size of the stem cuttings may also contribute to variation in rooting ability since diameter of the stem varies along the shoot (Wilson, 1993). These differences reflect variations in the types and variability of carbohydrate and other reserves between cuttings from different positions (Hartmann *et al.*, 1990; Leakey, 1990). For example, in *Triplochiton scleroxlon*, rooting percentage was found to decline basipetally (Leakey & Mohammed, 1985). On the other hand, the results recorded in *Cordia alliodora* is in contrast with the above result, where a lower rooting percentage was recorded from basal cuttings (Leakey, 1990). Palanisamy & Kumar (1997) reported the same result when the apical (0.5 cm), medial (1.0 cm) and basal (2.0 cm) diameter cuttings gave 100, 77 and 70 percent rooting respectively in neem (*Azadirachta indica*). A similar effect was reported in rooting of *Eucalyptus globulus* (Wilson, 1993).

A correlation between diameter and cutting length was reported by Leakey & Mohammed (1985) where thicker and longer cuttings rooted well than shorter and thinner ones, perhaps because larger cuttings contained more starch in the stem than thin cuttings. This may lead mostly to mortality of thin cuttings before getting a chance to root (Hartmann, Kester, Davies & Geneve, 2002). But sometimes thinner-stemmed cuttings also root better (Howard & Ridout, 1992).

Leaves

Nutrients and metabolites are required for both the mother plant and the cuttings for optimal growth. The metabolic process in stem cuttings takes place in the retained leaves (Wiessman-Ben & Tchoundjeu, 2000). In addition to this, their presence also has a considerable influence on rooting of cuttings, because of their ability to produce endogenous auxins, carbohydrates by means of photosynthesis and their influence on water status of the cuttings (Leakey & Coutts, 1989; Hartmann *et al*., 1990; Smalley, Dirr & Armitage, 1991; Newton, Muthoka, & Dick, 1992; Ofori, Newton, Leakey, & Grace, 1996).

The presence of leaves on cuttings plays a significant role on root initiation of many plant species (Hartmann *et al*., 1990). This was confirmed by Leakey & 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, Dick & Grace, 1997) and *Milicia excelsa* (Ofori *et al*., 1996) were affected by the variation in leaf area. Similar results were also obtained in species such as *Triplochiton scleroxyol* (Leakey & Coutts, 1989); *Cleistopholis glauca*, *Terminalia ivorensis* and *Khaya ivoresis* (Leakey & Mohammed, 1985), where optimum leaf areas for rooting were found to be 50 cm^2, 50 cm^2, 100 cm^2 and 10 to 30 cm^2 respectively. In contrast, variation in leaf area had little effect on rooting percentage of *Nauclea diderrichii* (Leakey, 1990) and *Terminalia spinosa* (Newton *et al*., 1992).

The presence of large leaves on cuttings has been found to result in increased water loss and a consequent reduction in photosynthesis activity (Leakey & Coutts, 1989; Newton *et al*., 1992).

A large number of leaves can be harmful since transpiration may lead to excessive water deficits, which impair rooting or cause mortality before rooting (Leakey & Mohammed, 1985; Hartmann *et al*., 1990; Loach, 1992). To minimise this, reducing the size of the leaves is important. But too much trimming may also reduce photosynthesis and limit rooting such as in *Eucalyptus globulus* (Wilson, 1993). Therefore, it is necessary to have a balance between photosynthesis and transpiration for optimum rooting to occur (Leakey & Coutts, 1989; Newton *et al*., 1992). Generally, Wiessman-Ben & Tchoundjeu (2000) recommend ±50 cm^2 of leaf area on a single cutting for best rooting.

1.4.4 Season

Season is one of the major factors that affect rooting success of cuttings (Klein, Cohen, Hebbe, 2000). Its effect on rooting efficiency is very common in woody plants and there is optimal time for root establishment for each species (Howard, 1996). This was confirmed when rooting of pistachio cutting from mature trees was unsuccessful without considering season (Al-Barazi & Schwabe, 1982). Similarly, Puri & Vermat (1996) stated that *Dalbergia sissoo* cuttings could be rooted in spring and monsoon seasons, while winter cuttings did not root at all. 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. In contrast, root number and rooting percentage of *Eastern redcedar* cuttings was higher throughout winter (Henry, Blazich, & Hinesey, 1992). On the other hand, rooting of Mytaceae family (*Chmaelaucium* sp) is unaffected by season (Curir, Sulis, Mariani, Van Sumere, Marchesini, & Dolci, 1993).

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 effect of season was also 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 & Dirr, 1996).

1.4.5 Rooting hormone and medium

A number of studies have demonstrated that rooting hormones and rooting medium play an important role in rooting of stem cuttings. This could either be through their direct effect on the cuttings or through their interactions (Leakey, 1990; Ofori, *et al*., 1996).

Rooting hormone

Rooting hormones are very important in the rooting process of cuttings (Wiessman-Ben & Tchoundjeu, 2000). Their beneficiary effect was also confirmed by Aminah *et al*. (1997), Arya, Tomar & Tokyt (1994), Al- Saqri & Alderson (1996) and Hartmann *et al*. (1997). According to Wiessman-Ben & Tchoundjeu (2000) hormones such as auxin (IBA, IAA, NAA) play an important role in root growth, where as hormones like gibberellins are important in the physiological process of the plants such as in stem elongation and bud development.

Among the exogenous rooting hormones, indole-3-butyric acid (IBA) and ∝-naphthaleneacetic acid (NAA) are two synthetic chemicals that have been found to be reliable in root promotion of cuttings. IBA is widely applied for general use because it can remain non-toxic within a wide range of concentrations and improves root initiation of cuttings for most plants species (Al-Barazi & Schwabe, 1982; Hartmann *et al*., 1997). Hartmann *et al*., (1997) reported that IBA might be toxic to certain cuttings taken from softwood plant species, which causes poor growth, no growth or mortality of the cuttings.

The purpose of treating cuttings with auxin is to increase the percentage of rooting, root initiation, number of roots, and uniformity of rooting (Al-Barazi & Schwabe, 1982). It also accelerates the translocation of nutrients from the upper part of the cuttings to their basal ends by increasing the activity of enzymes. This increases hydrolysis of carbohydrates by providing enough energy in rooting respond of the cells (Arya *et al*., 1994). As reported by Al-Barazi & Schwabe (1982), occasionally, IBA treatment seems to stimulate cell division in the ray cells between the primary bundles to improve root initiation and to increase uniformity of rooting. This was supported by Jawanda *et al*. (1991), when plum cuttings treated with 0.01% IBA recorded a higher rooting percentage followed by 0.005% IBA than the control. Ofori *et al*. (1996) also reported that 0.002% IBA treatment increased the final rooting percentage of *Milicia excelsa* by 9% above that of the control. Similarly, Puri, & Vermat (1996) described that rooting of *Dolbergia sissoo* cuttings was triggered and enhanced by an auxin application.

Even though auxin promote adventitious root development of stem 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 & Tchoundjeu, 2000). Application of higher concentration of auxin antagonises with the endogenous auxin of the plant. This is due to the increase of endogenous auxin in the cuttings, might have led to a level, which disturbed the hormonal metabolism and inhibited rizogenesis (Lebrun *et al*. 1998).

It is evident that the cuttings of different plants require different optimum concentrations of auxin for best rooting. As indicated in Fig. 1.2, *Cordia alliodora* was found to require a concentration of 1.6% IBA and 0.8% for optimum rooting percentage (Mesen, Newton, & Leakey, 1997). Similar results where reported by Leakey & Mohammed (1985) in rooting of *Triplochiton scloroxylon*. On the other hand, successful rooting without applied auxin has been reported in a number of other tropical tree species, such as *Nauclea diderrichii* and *Vochsia hondurensis* (Leakey, 1990), *Shorea macrophlla* (Lo, 1985) and *Milicia excelsa* Ofori *et al*., 1996). This implies that these plants are well supplied with endogenous auxin (Ofori *et al*., 1996).

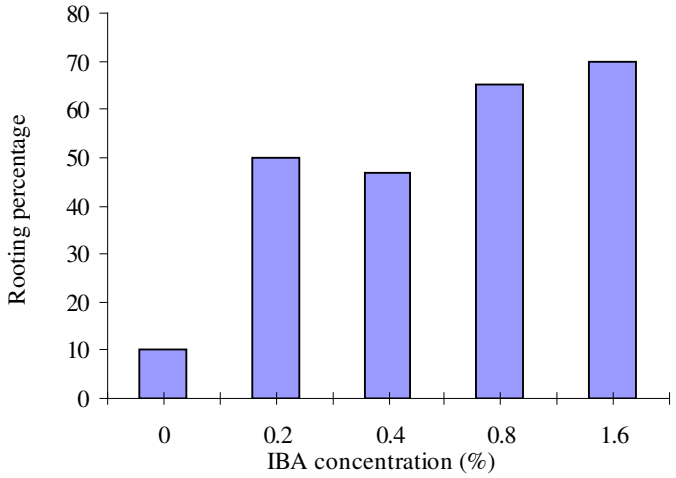


Fig. 1.2 The effect of five different concentration of IBA (0, 0.2, 0.4, 0.8 and 1.6%) on rooting percentage of *Cordia alliodora* cuttings (Mesen *et al*., 1997)

The efficiency of rooting hormone was also reported to vary when used in combination with each other (Smalley *et al*., 1991; Puri & Vermat, 1996). This was supported by Arya *et al*. (1994) when combination of NAA+IBA+thiamine and IAA+IBA+2,4-D auxins proved to be the

best auxin treatments stimulating rooting 50-60% of *P. cinararia* cuttings than when used singly. The effect of auxin also varies with time of application (Hartmann *et al*., 1997; Boeing, Deschmps, Filho, & Scheffer, 1999). Dipping the base of softwood olive cutting in solutions containing IBA in the range of 0.4 to 0.5% was an effective in stimulating root production as a 24 hours soaking treatment of 0.005%. Kromwijk & Van Mourik (1992) also reported that dipping of the *Ficus benjamina* cuttings in a 0.025% solution for 4hours was found to be more effective than using higher IBA concentrations or a rooting powder.

Rooting medium

Even though there is no ideal or universal rooting medium for cuttings (Hartmann *et al*., 1997) no propagation method is going to work if the right media for growth is not used (Berhe & 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). An ideal 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).

Although 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 & Loach, 1983; Loach, 1992). For instance, 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 (Lebrun *et al*., 1998). On another experiment, pure sawdust was found to have the highest moisture holding capacity and also the highest air content of the media tested but had a lower air/water ratio than coarse and fine sand in the propagation of *Nauclea diderrichii* cuttings (Leakey, 1990).

Temperature of the rooting medium was also found to affect the rooting of cuttings (Hartmann *et al*., 1997). Lebrun *et al*. (1998) confirmed this when cuttings of *Syzygium paniculatum* grown on a non-heated substrate (average temperature 16.0 ^{o}C) failed to root. However, when the substrate was heated (average temperature of 22±0.5 ^{o}C), the mean rooting rate exceeded 75% of the best substrates.

In addition, the type of rooting medium used is critical to the rooting process (Leakey, 1990) and rooting capacity of the cuttings (Hartmann *et al*., 1990). Cuttings of many species root successfully in a variety of rooting media but the performance of rooting both in number and percentage of rooting may be greatly influenced by the kind of rooting medium used (Leakey, 1990). This was supported by Al- Saqri & Alderson (1996) when the data collected from *Rosa centifolia* cuttings indicated that root length and rooting percentage showed some dependence on the type of medium used but not the mean root number per rooted cutting. Similar differences were also reported in rooting percentage, mean root number and leaf abscission percentage of *Milicia excelsa* with type of medium used. Type of medium used also affected the rooting of cuttings taken from different positions (Ofori *et al*., 1996). This was confirmed by Al-Saqri & Anderson (1996) when apical and basal *Rosa centifolia* cuttings responded differently with different media. Generally, the type of media used is growing region as well as requirement of species dependent. But most frequently used mediums contain combinations of sand, peat, sphagnum moss, vermiculite, pearlite, compost, and shredded bark/sawdust (Hartmann *et al*., 1997).

1.5 SEED GERMINATION

As reported by Copeland & McDonald (1994) there are different definitions on seed germination and understanding their difference is important. A seed physiologist can define seed germination as the emergence of the radical through the seed coat. A seed analyst also defines it as the emergence and development from the seed embryo of those essential structures, which for the kind of seed in question are indicative of the ability to produce a normal plant under favourable conditions. Others also consider seed germination as the active growth of the embryo, which results in the rupture of the seed coat and the emergence of young plant.

The seed remains inactive with low metabolic rate until it receives favourable environmental conditions that trigger the growth of the embryo. The response of seeds to favourable environmental conditions is different. Some seeds are capable of germinating only a few days after fertilization and long before their normal harvesting time, while others are dormant and require an extended rest period or additional development before germination can occur. But this is species dependent and it may last for only a few days or for as long as many years (Copeland & McDonald, 1994; Hartman *et al*., 1997; Pons, 2000).

Moisture

Moisture is one of the most important factors for germination (Copeland & McDonald, 1994; Baskin & Baskin, 2001). It is important for many activities in seed germination such as enzyme activation; breakdown; translocation and use of storage material. Seeds are low in moisture during their resting period and metabolically inactive. That is, they are in a quiescence state. This helps the seeds to maintain a minimum level of metabolic activities, which assures their long-term survival in the soil and during storage (Copeland & McDonald, 1994).

McLaren and McDonald (2003) reported that seeds of many tropical species mature in the dry season and are dispersed at the beginning of the rainy season when sufficient moisture is available for germination and seedling growth. In the dry tropics germination and early establishment of seedlings depend mainly upon moisture availability. During the dry season, survival requires the ability to cope with factors (or their absence) that are of direct physical origin (especially water). As a result, survival strategies involve energetically expensive physiological, morphological and anatomical adaptations directly concerned with obtaining (or retaining) water.

Many studies indicated that seeds must attain certain minimum species-specific moisture content. For example, soybean, 50%; sugar beets, 31%; corn 30.5% and rice 26%, before they germinate. Germination of seeds may be inhibited if the amount of water is too low or if it is too much (Baskin & Baskin, 2001).

Temperature

The effect of temperature on seed germination can be expressed in terms of cardinal temperature. That is, minimum optimum and maximum temperature at which germination will occur. Sometimes the minimum temperature for germination is difficult to define since germination may actually be proceeding but at such a slow rate that termination of germination is often made before actual germination is completed. Optimum temperature may be defined as the temperature giving the greatest percentage of germination within the shortest time. Maximum temperature is the temperature at which denaturation of proteins, that is essential for germination occurs (Copeland & McDonald, 1994; Hartman *et al*., 1997).

Seed response to temperature depends on species, variety, growing region, quality of the seed and duration of time from harvest. Temperate region seeds require low temperature than tropical region seeds and wild species require lower temperatures than domesticated plants. Seeds with high quality are able to germinate under a wider range of temperatures than low-quality seeds (Copeland & McDonald, 1994). The optimum temperature for most seeds is between 15 and 30^{o} C and the maximum temperature for most species is between 30 and 40^{o} C (Copeland & McDonald, 1994; Pons, 2000; Baskin & Baskin, 2001).

In a seed germination test, temperature was found to affect germination when applied in alternating or in constant fashion (Copeland & McDonald, 1994). Baskin & Baskin (2001) reported *Arthropodium cirratum* germinated to higher percentages at constant than at alternating temperatures and alternating temperatures usually are more favourable for germination than constant. There are species, however, which will germinate only when exposed to alternating temperatures. In nature, seeds are also exposed to alternating temperatures in their natural habitats (Baskin & Baskin, 2001). The requirement of alternating temperature is related to dormancy breaking and vegetation insulates the soil surface against large diurnal temperature fluctuation and in open areas the soil surface acts as an insulator (Copeland & McDonald, 1994).

Light

Since the mid-nineteenth century light has been recognized as a germination-controlling factor (Pons, 2001). Recent studies also found that light plays an important role in both dormancy induction and release. Light was also found to interact with temperature, which affects germination of seeds. The effect of light on seed germination involves both quality (wave length) and photoperiod (duration) (Hartmann *et al*., 1997).

The response to light differs with species. It was found that seeds of many species, if they are not dormant, germinate equally well in light and darkness, while others germinate to higher percentages in light than in darkness and relatively few in light (Baskin & Baskin, 2001). According to Hartman *et al*. (1997) small seeds are characterized by light sensitivity in which a shallow depth of planting would be an important factor. This is because if covered too deeply in the soil the epicotyl may not penetrate too deeply. For example, some of the important flower crops such as allussum, begonia, calceolaria, coleus, *Kalanchoe*, primrose and *Saintpaulia*

(African violet) require light for germination. On the other hand, in some species such as *Phacelia*, *Nigelia*, *Allium*, *Amaranthus* and *Phlox*, germination is inhibited by light. Some flowering crops are also listed as darkness requiring such as calendula, delphinium, pansy, annual phlox and annual verbena (Copeland & McDonald, 1994).

Baskin & Baskin (2001) reported that the light requirement of seeds might vary with temperature. This was supported when seeds of *Lactuca sativa* cv. Grand Rapids germinated to >80% in light at temperatures of 10^{o} to 30^{o} C, whereas germination in darkness exceeded 45% only at temperature of 10^{o} to about 22^{o} C; it was near 0% at 30^{o} C. Seeds such as *Bidens pilosa* require light to germinate at constant temperatures, but they require light and darkness to germinate in alternating temperature. They also reported seeds of many light requiring species germinate in the spring, after they have been exposed to low winter temperatures whereas those that germinate in autumn, do so after they have been exposed to high summer temperatures.

As suggested by Baskin & Baskin (2001) in studying the light requirement for germination, it is important to test seeds at a daily photoperiod and in continuous darkness at each of the daily alternating temperature regimes. Seeds need to be tested in light and darkness when they are freshly matured and at regular intervals during the dormancy-breaking period, because their light requirement may change as they come out of dormancy (Baskin & Baskin, 2001). As reported by Baskin & Baskin (2001) light requirement for germination of *Picea mariana* seeds was removed when exposed to low winter temperatures (cold stratification). However, seeds of *Hygrophila auriculata*, high temperature (28^{o} C, 40^{o} C) removed the requirement of light for germination.

Air (Oxygen and Carbon Dioxide)

Seed germination is also affected by another factor, which is air. The ambient air is composed of about 20% oxygen, 0.03% carbon dioxide and 80% nitrogen (Copeland & McDonald, 1994). Many studies reported the importance of oxygen in seed germination out of these gases due to its role in the oxidation process. During seed germination an increase in respiration process was reported, which implies the importance of supplementing with enough oxygen during the process (Bewley & Black, 1994; Copeland & McDonald, 1994; Corbineau & Come, 1995; Hartmann *et al*., 1997). Many studies also reported the reduction in germinability of seeds as a

result of low oxygen concentration than the surrounding air (Copeland & McDonald, 1994). This was supported by Van Toai, Fausey & McDonald (1988) when germination of corn seeds decreased with decreasing oxygen concentration than that of air. Maximum germination of crops such as wheat, sorghum, corn, soybean, and sunflower was also reported when oxygen concentration was about the atmosphere (Al-Ani, Bruzau, Raymond, Saints-ges, Leblanc & Pradet, 1985).

Even though seed germination of many plant species decreased with decreasing oxygen concentration than that of ambient air, its effect is still unknown. There are few assumptions, which has being made by different authors. This includes the increase in production of toxic substance, ethanol under anaerobic conditions (Thomson & Greenway, 1991). However, studies which were done to see this toxicity effect failed to prove this process physiologically in seeds (Van Toai *et al*., 1995; Martin, Cerevick & Reding, 1991). On the other hand, Corbineau & Come (1995) reported that agronomic crops such as lettuce and onion require less amount of oxygen than the surrounding air whereas seeds of for instance carrot, curly dock, sunflower, cocklebur, and a variety of cereals germinate better under oxygen concentration above that of air.

The effect of carbon dioxide (CO_2) in seed germination is opposite to that of oxygen. Many studies stated that increasing the concentration of CO_2 beyond that of surrounding air (0.03%) decreases the seed germination of most plant species while decreasing CO_2 has no effect (Copeland & McDonald, 1995; Corbineau & Come, 1995; Hartmann *et al*., 1997; Bewley & Black, 1994). On the other hand, no effect on the change of nitrogen concentration has been reported (Copeland & McDonald, 1995).

CHAPTER 2

INFLUENCE OF CUTTING POSITION, MEDIUM, HORMONE AND SEASON ON ROOTING OF BUSH TEA (ATHRIXIA PHYLICOIDES) STEM CUTTINGS

2.1 INTRODUCTION

Cutting is one of the extensively practised means of vegetative propagation in plants. It has many advantages such as being economical, not requiring much space and is rapid and simple. Cuttings can be made from the stem, modified stem, roots or leaves. Among these, stem cutting has been used to propagate a variety of plants. In vegetative propagation by stem, cuttings can be taken from the shoots of the plants with terminal or lateral buds, which are capable of developing adventitious roots and then to a complete plant (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 the cuttings were made as well as physical and environmental factors (Wilson, 1993). The effect of these factors on rooting of bush tea stem cuttings is not known. The aim of this experiment was to study how cutting position, rooting medium, hormone and season influenced rooting of bush tea stem cuttings.

2.2 MATERIALS AND METHODS

An experiment on vegetative propagation of bush tea (*Athrixia phylicoides*) was carried out on a mist bed in a greenhouse located at the University of Pretoria's Experimental Farm (26^{o} 12'S, 28^{o} 10' E). The propagation unit was supplemented with 24 hrs a day misting and fogging systems, which work automatically based on the humidity of the greenhouse. The used mist bed was 5 m long, 1.5 m wide and 1 m high supplied with automatic misting system operating through misting nozzles. Throughout the experimental period, the temperature of the greenhouse was measured using a thermograph. The measured mean minimum and maximum temperatures during the study period were respectively 17^{o}C and 34.7^{o}C in summer, 12.8^{o}C and 29.6^{o}C in autumn, 9^{o}C and 27.8^{o}C in winter and 13^{o}C and 34.2^{o}C in spring. A randomised complete block design (Fig. 2.1) with four blocks and ten replications was used as a design to evaluate the effect

of the source of the cuttings (position on the stem: apical or basal) with presence or absence of hormone using two different rooting media in four seasons (summer, autumn, winter and spring 2002 to 2003).

Fig. 2.1 Experimental layout used during the experimental period on the mist bed

Six mature bush tea stock plants were selected from the mountain range of Venda in the Muhuyu village, Thohoyandou District during November 2002. Selection of the mother plants was made on the basis of true-to-name and type, free of disease and insects and in the proper physiological state. As Hartmann *et al*. (1997) stated stock plants that have been injured by frost or drought, stunted by excessive flowers or fruiting or by lack of soil moisture or proper nutrition should be avoided. The selected stock plants were dug out with intact soil around the root zone and then conveyed to the propagation area. The plants were potted in large black polyethylene bags (43 cm height; 32.5 cm width) containing pine bark as propagation medium,

followed by taking them into a glasshouse with approximately (40%) shading to be raised and left for about three months until full recovery from the shock of transplanting. The plants were irrigated every day to field capacity and received in each season an application of organic foliar fertilizer (NITROSOL ®, 8% N: 2% P: 5.8% K 4 ml per L of water) as well as fungicide (Benomyl). The experiment was conducted at the end of January 2003 after the stock plants were well established.

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

Shoots of 16 to 32 cm long were cut from the stock plants early in the morning (between 06:30 and 07:30), and wrapped with wet tissue paper followed by immediately placing them in plastic bags in order to keep them cool and turgid until taken to the working area. Working on the humid misting bed, shoots were divided into a total of 320 semi-hardwood cuttings each with 8 cm length and 0.04 to 0.3 cm diameter range. Bottom leaves were stripped off, leaving only the top three followed by taking the fresh mass as well as initial circumference of each cutting. The bases of the cuttings were dipped in water and depending on the treatment type (with or without hormone) were then dipped into a rooting hormone powder (Seradix No. 2) consisting of 0.3 % IBA (4-indole-3-butyric acid) in a talc to a depth of approximately 1 cm. Excess rooting powder was tapped before planting. According to Hartmann & Kester (1983) in order to avoid brushing of the powder during planting, a trench was made in the rooting medium with a stick. The cuttings were directly planted after treatment into the pre-wetted rooting medium (pine bark and 8 mm sand) to a depth of 2 cm. Throughout the experimental period, cuttings were assessed for rooting, root number and length of the longest root and fresh and dry mass gained after 5, 10, 15 and 20 days in summer and 15, 20, 25 and 30 days in autumn, winter and spring from planting time. Data collection was done by carefully separating the rooted cuttings from the seedling trays followed by washing the root zone with water.

2.3 RESULTS

In the four seasons (summer, autumn, winter and spring) (data not shown) no rooting was commenced after 5 and 10 days from planting. However, with advancing the day after planting (DAP) to 15 and beyond, bush tea cuttings started to develop roots in all the four seasons.

2.3.1 Effect of rooting media

The data collected in all the experiments showed that the propagation media used did not affect the rooting percentage of bush tea. The two media used did not show differences in rooting percentage of both apical and basal cuttings (Fig. 2.2). Though there were no significant differences in rooting percentage for both apical and basal cuttings in the different media, cuttings performed slightly better when propagated in pine bark than sand.

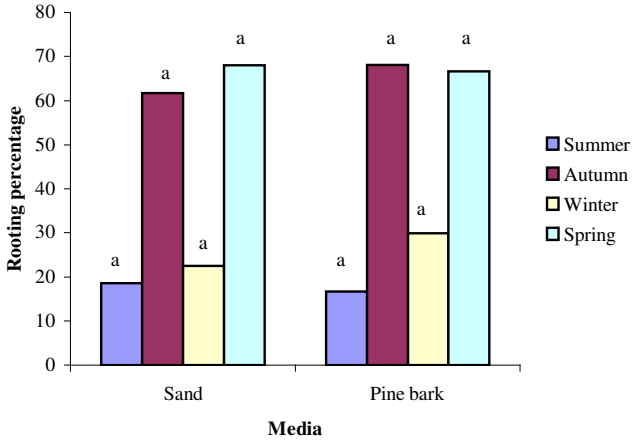


Fig. 2.2 Effect of media on rooting percentage of bush tea stem cuttings during summer, autumn, winter and spring

* Statistical comparison is between bars of the same colours

Media showed highly significant differences ($P<0.001$) in the number of roots produced during summer, autumn, winter and spring (Fig. 2.3). In summer (Fig. 2.3a), sand was better at 15 DAP but the same as pine bark at 20 DAP. In autumn (Fig. 2.3b), high number of roots was produced in pine bark than sand with the highest root number at 20 DAP. In winter (Fig. 2.3c), at 15 DAP pine bark was better but the same as sand at 20 DAP. However at 25 and 30 DAP, high number of roots was produced in pine bark than sand. In spring (Fig. 2.3d), pine bark at 20 DAP was better but the same as sand at 15 and 25 DAP. At 15 and 25 DAP number of roots produced in sand and pine bark were about the same. However, at 30 DAP sand was better than pine bark.

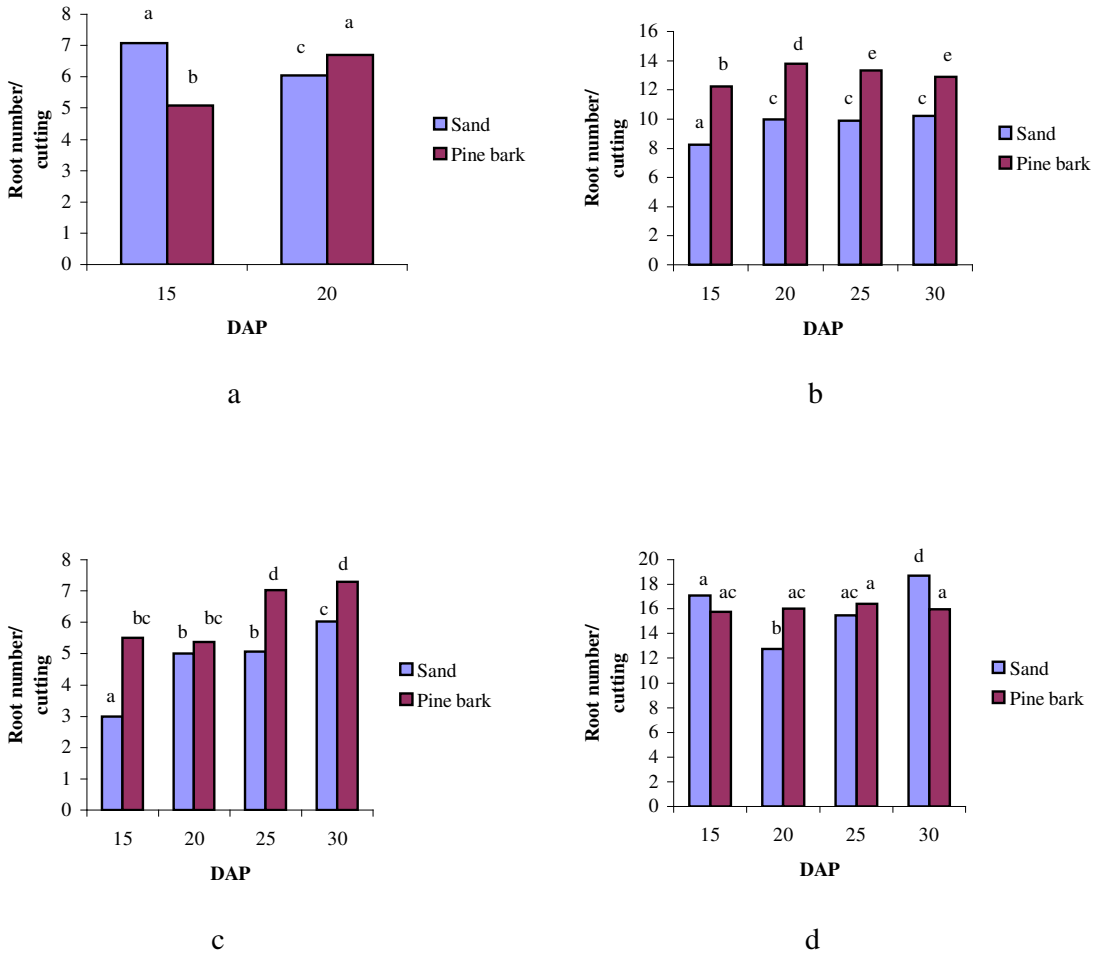


Fig. 2.3 Effect of media on the root number during (a) summer, (b) autumn, (c) winter and (d) spring of bush tea stem cuttings

Media did not show significant differences in root length during summer (Fig. 2.4a) and winter (Fig 2.4c). However, there were significant differences in autumn ($P<0.05$) and in spring ($P<0.001$). In autumn (Fig. 2.4b), longer roots were produced at 15 DAP in sand, but after 20 days and beyond length of the produced roots were about the same as pine bark. In spring (Fig. 2.4d), root length was about the same at 15, 20 and 25 DAP in sand and pine bark. However, at 30 DAP root length was better in sand than in pine bark.

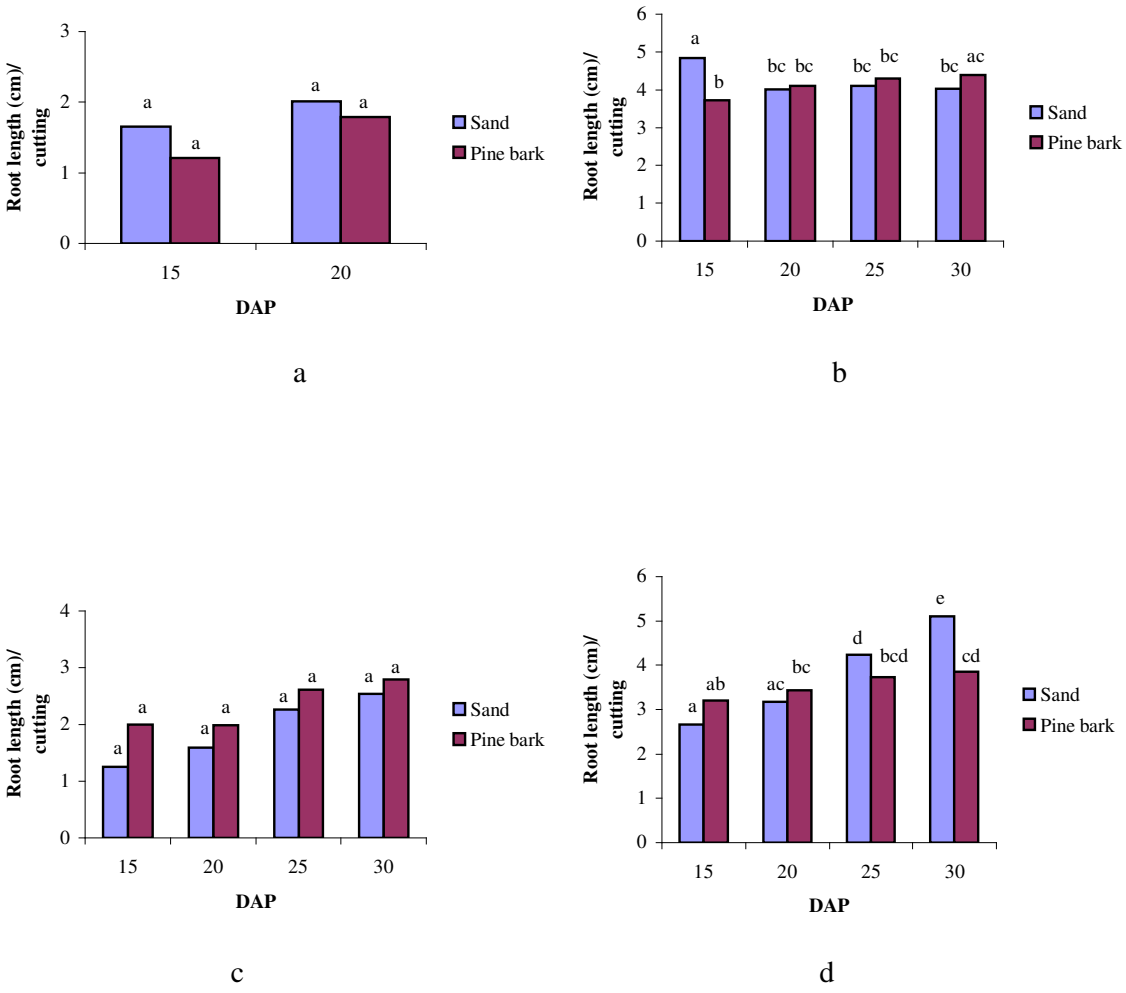


Fig. 2.4 Effect of media on the root length (cm) per cutting during (a) summer, (b) autumn, (c) winter and (d) spring of bush tea stem cuttings

2.3.2 Effect of rooting hormone

Bush tea cuttings from both cutting positions (apical and basal) did not show good response to the applied hormone (Seradix No. 2) in terms of rooting percentage during summer, autumn and spring. However, there were highly significant differences ($P<0.001$) in winter (Fig. 2.5) due to hormone application. At 15 and 20 DAP rooting percentage was about the same with and without hormone application. However, at 25 DAP cuttings with hormone rooted to higher percentage while at 30 DAP cuttings without hormone rooted to higher percentage than cuttings with hormone.

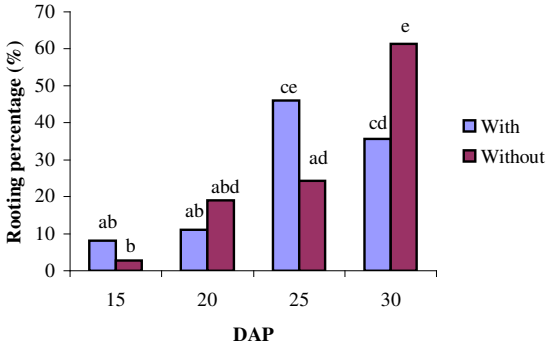


Fig. 2.5 Effect of rooting hormone (with or without) on rooting percentage of bush tea stem cuttings during winter

Number of roots per rooted cutting was affected by the application of hormone ($P<0.001$) in summer, autumn, winter and spring (Fig. 2.6). Bush tea cuttings showed positive response to the applied hormone in the number of roots produced. Except without hormone at 15 DAP in summer and 30 DAP in spring, in all four seasons (summer, autumn, winter and spring, Fig. 7a, b, c and d, respectively) cuttings with hormone produced more number of roots than cuttings without hormone. On the other hand, uniformity and more number of roots after 15 days from planting were also observed when hormone was applied than without hormone application.

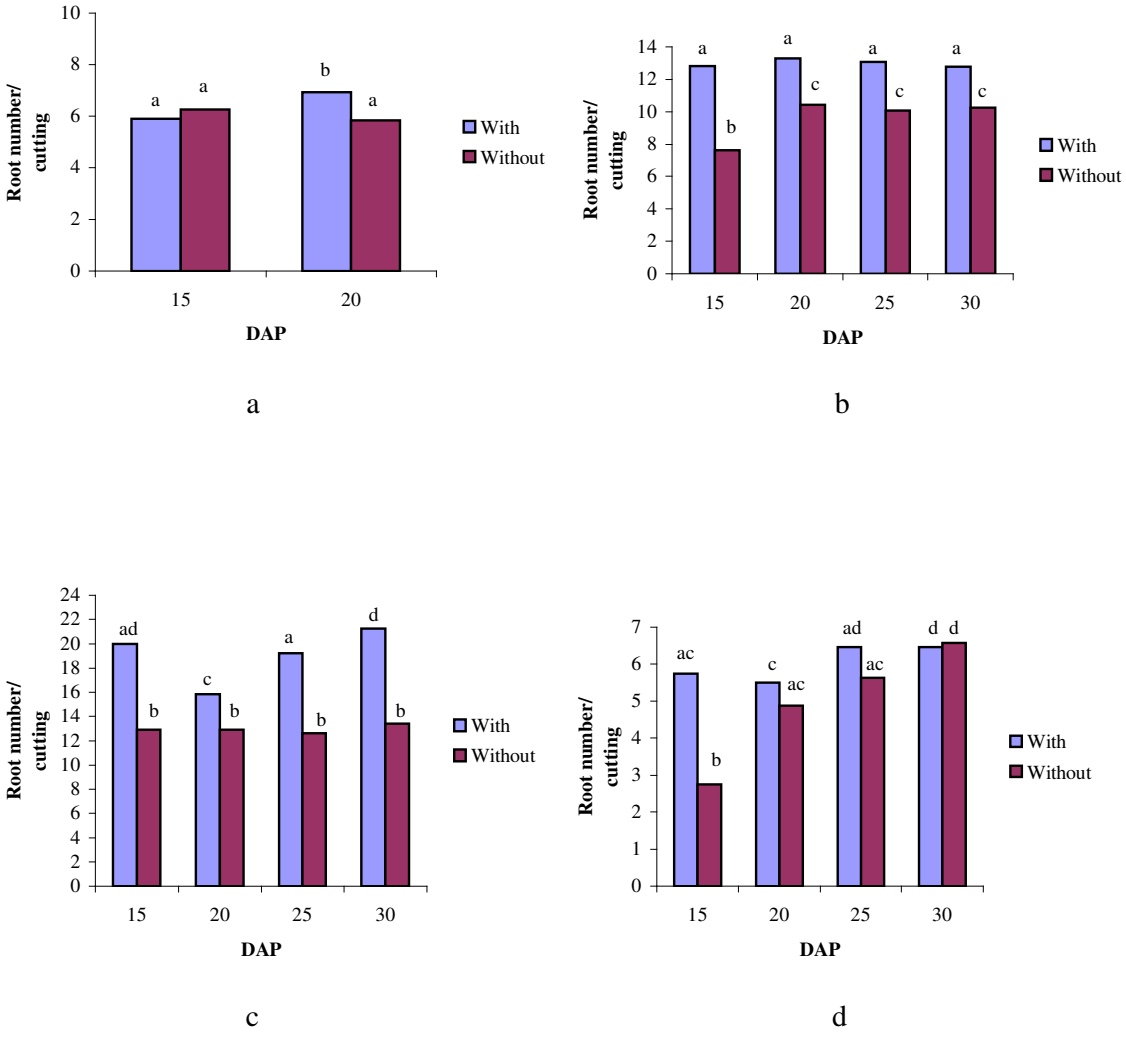
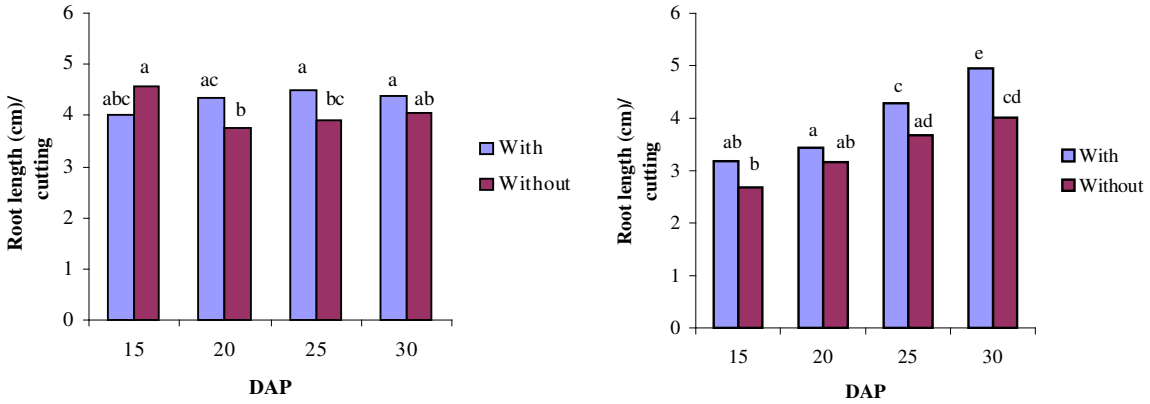


Fig. 2.6 Effect of rooting hormone (with or without) on root number during (a) summer, (b) autumn, (c) winter and (d) spring for bush tea stem cuttings

Furthermore, application of hormone (with or without) showed highly significant differences ($P<0.001$) on root length (Fig. 2.7) during autumn and spring. In autumn (Fig. 2.7a), long roots were produced without hormone after 15 days from planting than cuttings with hormone. But after 20 days and beyond, longer roots were produced from cuttings with hormone than cuttings without hormone. Similarly, longer roots were produced from cuttings with hormone in spring (Fig. 2.7b) than cuttings without hormone. However, the differences in root length were not significant in summer and winter through hormone application. Even though the differences in

root length were not significant in winter, cuttings produced longer roots with hormone than without hormone (Fig. 2.8).



a b

Fig. 2.7 Effect of rooting hormone (with or without) on root length during (a) autumn and (b) spring of bush tea stem cuttings

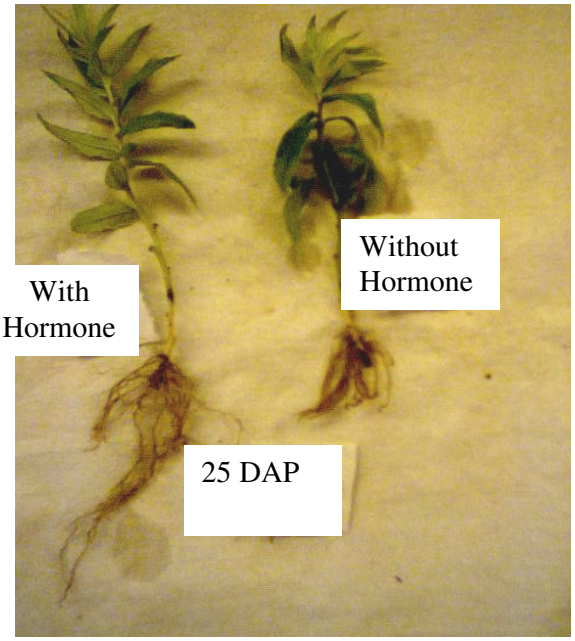


Fig. 2.8 Effect of rooting hormone (with or without) on root length of bush tea stem cutting during winter

2.3.3 Effect of cutting position

Bush tea cuttings were found to be sensitive to cutting position from the stock plant in terms of adventitious root development. There were highly significant differences (P<0.001) between the apical and basal cuttings in summer, autumn, winter and spring (Table 2.1) in rooting percentage. Apical cuttings showed higher rooting percentage than basal cuttings in the four seasons (summer, autumn, winter and spring). Fast and easy rooting ability of apical cuttings was also observed than basal cuttings.

Table 2.1 Effect of cutting position on rooting percentage, root number and root length of bush tea in summer, autumn, winter and spring

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

Cutting position (apical or basal) highly significantly (P<0.001) affected root number (Table 2.1) all through summer, autumn, winter and spring seasons. Throughout the experimental period high number of roots were produced from apical cuttings than basal cuttings. Apical cuttings also showed uniformity in the number of roots produced than basal cuttings with increasing DAP. That is, number of roots produced after 15 days from apical cuttings was about the same with number of roots produced from basal cuttings after 30 days from planting during autumn, winter and spring.

In addition, bush tea root length was also affected by cutting position from the stock plant. There were highly significant differences (P<0.001) in root length between apical and basal cuttings during summer, autumn, winter and spring (Table 2.1). Longer roots were produced from apical cuttings than basal cuttings throughout the experimental period. Length of the produced roots was observed to increase with increasing DAP from 15 to 30 days in both apical and basal cuttings.

2.3.4 Effect of season

Rooting percentage of bush tea was affected by the season when the cuttings were taken. There were highly significant differences (P<0.001) among the seasons in rooting percentage of both apical and basal cuttings (Figure 2.9).

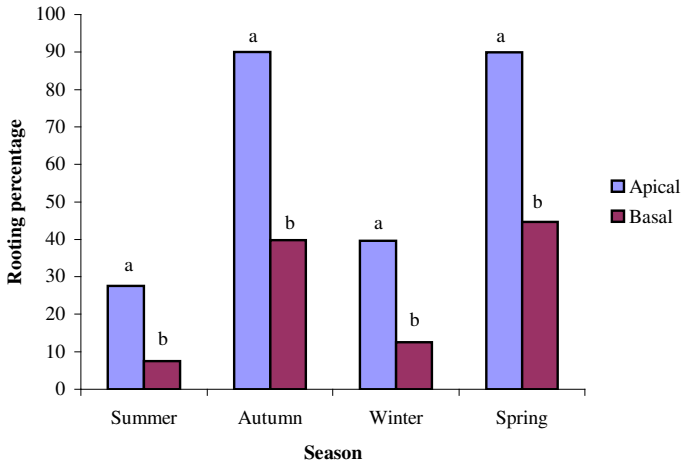


Fig. 2.9 Effect of season (summer, autumn, winter and spring) on rooting percentage of apical and basal cuttings of bush tea

* Statistical comparison is between bars of different colours per season

High rooting percentage of bush tea was recorded in autumn and spring. In both these two seasons 90 and 89.9% of the apical cuttings and 39.8 and 44.7% of the basal cuttings in autumn and spring respectively developed roots. There was low rooting in apical (39.7) and basal (12.6) cuttings in winter. But there were no significant differences between autumn and spring as well as between summer and winter in terms of rooted cuttings. Though the sampling day did not go beyond 20 days after planting in summer, low percentage (27.6%) of apical and basal (7.5%)

cuttings was recorded as compared to autumn (apical 90% and basal 40%) and spring (apical 91.9% and basal 50%) after 20 days from planting.

Rooting percentage (Table 2.2) of bush tea cuttings was increased with increasing time after planting during summer, autumn, winter and spring. Percentage of rooted cuttings was low after 15 DAP but an increase of 37.5%, 35% and 36.6% in autumn, winter and spring respectively were recorded after 30 days from planting. This increase in rooting percentage with an increase in the number of days after planting (DAP) followed the same pattern throughout the experimental period except in spring for 25 DAP. The percentage of rooted cuttings after 25 days in spring was lower (66.4%) than those after 20 days (69.7%). However, the difference between them was not significant. This low percentage of rooted bush tea cuttings was due to 12.5% mortality of the cuttings before they started developing roots. On the other hand, in summer, the DAP did not go beyond 20 days since the experiment was set to see when bush tea cuttings started developing roots.

Table 2.2 Rooting percentages of bush tea cuttings after 15, 20, 25 and 30 days from planting in summer, autumn, winter and spring

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

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

Number of roots produced per rooted cutting from both apical and basal cuttings was affected by season (P<0.001) in which the cuttings were taken from the stock plant (Table 2.3). More roots were developed during spring followed by autumn from both apical and basal cuttings. Fewer numbers of roots were produced during winter than during autumn and spring. Though DAP did not go beyond 20 days in summer, number of roots produced were not much better than number of roots produced in winter. On the other hand, root number varied with increasing DAP (Table 2.4).

Table 2.3 Effects of season and cutting position on mean root number and root length of bush tea

Cutting position	Season	Root number	Root length (cm)
		Mean ± S.E	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

Table 2.4 Mean root number and length of bush tea cuttings in summer, autumn, winter and spring after 15, 20, 25 and 30 days after planting

DAP	Season							
	Summer		Autumn		Winter		Spring	
	Root							
	Number	Length (cm)	Number	Length (cm)	Number	Length (cm)	Number	Length (cm)
15	6.08 [a]	1.43 [a]	10.21 [a]	4.28[a]	4.25[a]	1.63[a]	16.42[a]	2.93 [a]
20	6.38 [b]	1.90 [b]	11.85 [b]	4.05 [a]	5.19[b]	1.78[a]	14.39[b]	3.30 [a]
25	-	-	11.59 [b]	4.20 [a]	6.05[c]	2.44[b]	15.93[a]	3.98 [b]
30	-	-	11.53 [b]	4.20 [a]	6.68[d]	2.67[b]	17.33[a]	4.48 [c]

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

During autumn, root number was uniform from 20 DAP and beyond. In spring, number of roots produced after 30 days were not much better than number of roots produced after 15 days from planting. However, during winter and summer more roots were produced with increasing DAP.

Furthermore, root length of both apical and basal cuttings was affected ($P<0.001$) by season when the cutting was made (Table 2.3). Apical cuttings produced longest roots when the cuttings were taken in autumn than cuttings taken during winter and spring. Basal cuttings produced longer roots in autumn and spring than basal cuttings taken in winter. Root length was also affected by DAP (Table 2.4) with longer roots produced in winter and spring after 30 days than after 15 days from planting. Similarly, longer roots were produced after 20 days than after 15 days during summer. But in autumn, root length was the same at 15 and 30 DAP.

2.3.5 Interactive effect of media and rooting hormone

Highly significant differences ($P<0.001$) were recorded for root number by the interactions of media and hormone (with or without hormone) (Table 2.5) during summer, autumn and spring. Cuttings produced more number of roots in sand with hormone in summer and spring, while in autumn more roots were produced from pine bark with hormone. However, no differences were recoded in winter due to media and hormone interactions. In general, except in summer with pine bark, cuttings showed positive response to the applied hormone in producing more roots than without the hormone for both sand and pine bark.

Similarly, highly significant differences ($P<0.001$) were found through the interaction of media and hormone (with or without hormone) for mean root length of bush tea during summer, autumn and winter (Table 2.6). During summer, long roots were produced in sand with hormone, whereas in autumn cuttings produced long roots in sand without hormone and in pine bark with hormone. Similarly, in winter long roots were produced in pine bark without hormone and in sand with hormone. On the other hand, in spring this interaction did not show any significant effect in producing long roots. Though the differences were not significant better root length were produced in sand with hormone than pine bark with hormone. In general, cuttings showed good response to the applied hormone in developing longer roots throughout the experimental period.

Table 2.5 Interactive effect of media and hormone (with or without) on root number of bush tea cuttings during summer, autumn, winter and spring

Media	Hormone	Root number ± S.E			
		Season			
		Summer	Autumn	Winter	Spring
Sand	With	7.28 ± 0.15^{a}	10.19 ± 0.15^{a}	5.52 ± 0.20^{ns}	20.64 ± 0.34^{a}
	Without	5.84 ± 0.21^{bc}	8.91 ± 0.16^{b}	4.03 ± 0.21^{ns}	11.36 ± 0.33^{b}
Pine bark	With	5.54 ± 0.18^{b}	15.80 ± 0.13^{c}	6.72 ± 0.21^{ns}	17.54 ± 0.36^{c}
	Without	6.25 ± 0.19^{c}	10.28 ± 0.15^{a}	5.88 ± 0.20^{ns}	14.54 ± 0.34^{d}

Figures in a column followed by the same letter are not significantly different (P>0.05), using Tukey's comparison test; NS = non significant

Table 2.6 Interactive effect of media and hormone on mean root length of bush tea cuttings during summer, autumn, winter and spring

Media	Hormone	Root length (cm) ± S.E			
		Season			
		Summer	Autumn	Winter	Spring
Sand	With	2.47 ± 0.14^{a}	4.07 ± 0.15^{ad}	2.31 ± 0.11^{acd}	4.15 ± 0.14^{ns}
	Without	1.19 ± 0.19^{b}	4.42 ± 0.13^{ab}	1.51 ± 0.12^{b}	3.43 ± 0.13^{ns}
Pine bark	With	1.33 ± 0.17^{b}	4.53 ± 0.12^{bc}	2.10 ± 0.12^{c}	3.78 ± 0.14^{ns}
	Without	1.67 ± 0.18^{b}	3.73 ± 0.14^{d}	2.59 ± 0.11^{d}	3.34 ± 0.14^{ns}

Figures in a column followed by the same letter are not significantly different (P>0.05), using Tukey's comparison test, NS = non significant

2.3.6 Interactive effect of media and cutting position

Number of roots was affected by the interaction of cutting position and media. There were highly significant differences (P<0.001) in root number during summer, autumn and winter growing seasons (Table 2.7). Apical cuttings produced higher root number in summer, autumn and winter from in pine bark than in sand. Similarly in autumn, basal cuttings developed higher

root number in pine bark than in sand. However in summer, basal cuttings produced more roots in sand. During winter, number of roots produced in pine bark were not better than number of roots in sand. On the other hand, both apical and basal cuttings did not show differences in root number from the two media. Generally, apical cuttings produced more number of roots in pine bark than in sand.

Table 2.7 Interactive effect of media and cutting position on root number of bush tea cuttings during summer, autumn, winter and spring

Cutting position	Media	Root number ± S.E			
		Summer	Autumn	Winter	Spring
Apical	Sand	8.67 ± 0.12^{a}	12.44 ± 0.11^{a}	6.98 ± 0.18^{a}	19.29 ± 0.25^{ns}
	Pine bark	9.38 ± 0.13^{b}	17.08 ± 0.10^{b}	9.73 ± 0.15^{b}	19.66 ± 0.25^{ns}
Basal	Sand	4.46 ± 0.23^{c}	6.66 ± 0.19^{c}	2.56 ± 0.23^{c}	12.71 ± 0.40^{ns}
	Pine bark	2.42 ± 0.23^{d}	9.00 ± 0.17^{d}	2.88 ± 0.24^{c}	12.42 ± 0.43^{ns}

Figures in a column followed by the same letter are not significantly different (P>0.05), using Tukey's comparison test, NS = non significant

Interaction between cutting position and media also showed highly significant differences (P<0.001) for root length during autumn, winter and spring (Table 2.8). In summer, the interaction between cutting position and media did not show significant differences. Though the differences were not significant, both apical and basal cuttings produced long roots in sand than in pine bark. But during autumn and winter apical cuttings produced longer roots in sand and in pine bark, whereas in spring root length from pine bark was not better than root length from sand. In autumn and winter, about the same length of roots were developed for basal cuttings from the two media. On the other hand, basal cuttings produced longer roots in sand during spring. In general, apical cuttings developed longer roots in pine bark and basal cuttings in sand.

Table 2.8 Interactive effect of media and cutting position on root length of bush tea stem cuttings during summer, autumn, winter and spring

Cutting position	Media	Root length (cm) ± S.E			
		Summer	Autumn	Winter	Spring
Apical	Sand	2.12 ± 0.11^{ns}	5.39 ± 0.10^{a}	2.07 ± 0.10^{a}	4.01 ± 0.10^{a}
	Pine bark	1.96 ± 0.12^{ns}	4.97 ± 0.10^{b}	3.03 ± 0.09^{b}	4.26 ± 0.10^{a}
Basal	Sand	1.54 ± 0.21^{ns}	3.10 ± 0.17^{c}	1.76 ± 0.13^{ac}	3.57 ± 0.16^{b}
	Pine bark	1.04 ± 0.21^{ns}	3.59 ± 0.16^{c}	1.66 ± 0.14^{c}	2.84 ± 0.17^{c}

Figures in a column followed by the same letter are not significantly different (P>0.05), using Tukey's comparison test, NS = non significant

2.3.7 Interactive effect of cutting position and hormone

There were highly significant differences (P<0.01) in root number with the interactions of cutting position and hormone (with or without) (Table 2.9). Apical cuttings in autumn, winter and spring as well as basal cuttings during summer, autumn and spring produced high number of roots with hormone than without hormone. On the other hand, apical cuttings during summer and basal cuttings during winter developed high number of roots without hormone than both cuttings with hormone. But in general, more roots from both apical and basal cuttings were produced with hormone application than without hormone application.

Furthermore, interaction between cutting position and hormone (with or without) affected root length in summer (P<0.05), autumn (P<0.001) and winter (P<0.01) but there was no significance in spring (Table 2.10). Basal cuttings showed positive response to the applied hormone in producing long roots in summer and autumn. However, in winter basal cuttings with and without hormone produced about the same length of roots. Contrastingly, apical cuttings in summer and in winter produced about the same length of roots with and without hormone. On the other hand, apical cuttings produced longer roots in autumn without hormone. In general both apical and basal cuttings produced longer roots with hormone than without hormone.

Table 2.9 Interactive effect of cutting position and hormone (with or without) on root number of bush tea stem cuttings during summer, autumn, winter and spring

Cutting position	Hormone	Root number ± S.E			
		Summer	Autumn	Winter	Spring
Apical	With	8.37 ± 0.11^{a}	15.48 ± 0.10^{a}	10.17 ± 0.16^{a}	23.05 ± 0.24^{a}
	Without	9.68 ± 0.14^{b}	14.03 ± 0.10^{b}	6.54 ± 0.17^{b}	15.90 ± 0.25^{b}
Basal	With	4.46 ± 0.21^{c}	10.52 ± 0.17^{c}	2.06 ± 0.24^{c}	15.13 ± 0.43^{b}
	Without	2.42 ± 0.25^{d}	5.14 ± 0.20^{d}	3.38 ± 0.23^{d}	10.00 ± 0.40^{c}

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

Table 2.10 Interactive effect of cutting position and hormone (with or without) on root length of bush tea stem cuttings during summer, autumn, winter and spring

Cutting position	Hormone	Root length (cm) ± S.E			
		Summer	Autumn	Winter	Spring
Apical	With	2.00 ± 0.10^{a}	4.78 ± 0.10^{a}	2.54 ± 0.09^{a}	4.40 ± 0.10^{ns}
	Without	2.70 ± 0.13^{a}	5.59 ± 0.09^{b}	2.56 ± 0.10^{a}	3.88 ± 0.10^{ns}
Basal	With	1.79 ± 0.19^{a}	3.82 ± 0.17^{c}	1.88 ± 0.13^{b}	3.52 ± 0.17^{ns}
	Without	0.79 ± 0.23^{b}	2.56 ± 0.17^{d}	1.54 ± 0.13^{b}	2.88 ± 0.16^{ns}

Figures in a column followed by the same letter are not significantly different (P>0.05), using Tukey's comparison test, NS = non significant

2.3.8 Interactive effect of cutting position, hormone and media

The interactions among cutting position, media and hormone (with or without hormone) highly significantly (P<0.001) affected root number (Fig. 2.10). In summer (Fig. 2.10a), apical cuttings produced the highest number of roots in pine bark without hormone whereas basal cuttings produced the highest number of roots in sand with hormone application. Both apical and basal cuttings produced high number of roots in autumn (Fig. 2.10b) when the cuttings were treated and planted in pine bark. In winter (Fig. 2.10c), apical cuttings showed a higher root number from pine bark when treated with hormone and more roots from untreated basal cuttings

produced in pine bark treated with hormone. The effect of this interaction was also true for root number during spring (Fig. 2.10d), where more roots were produced in sand with hormone for both cutting positions (apical and basal) treated with hormone.

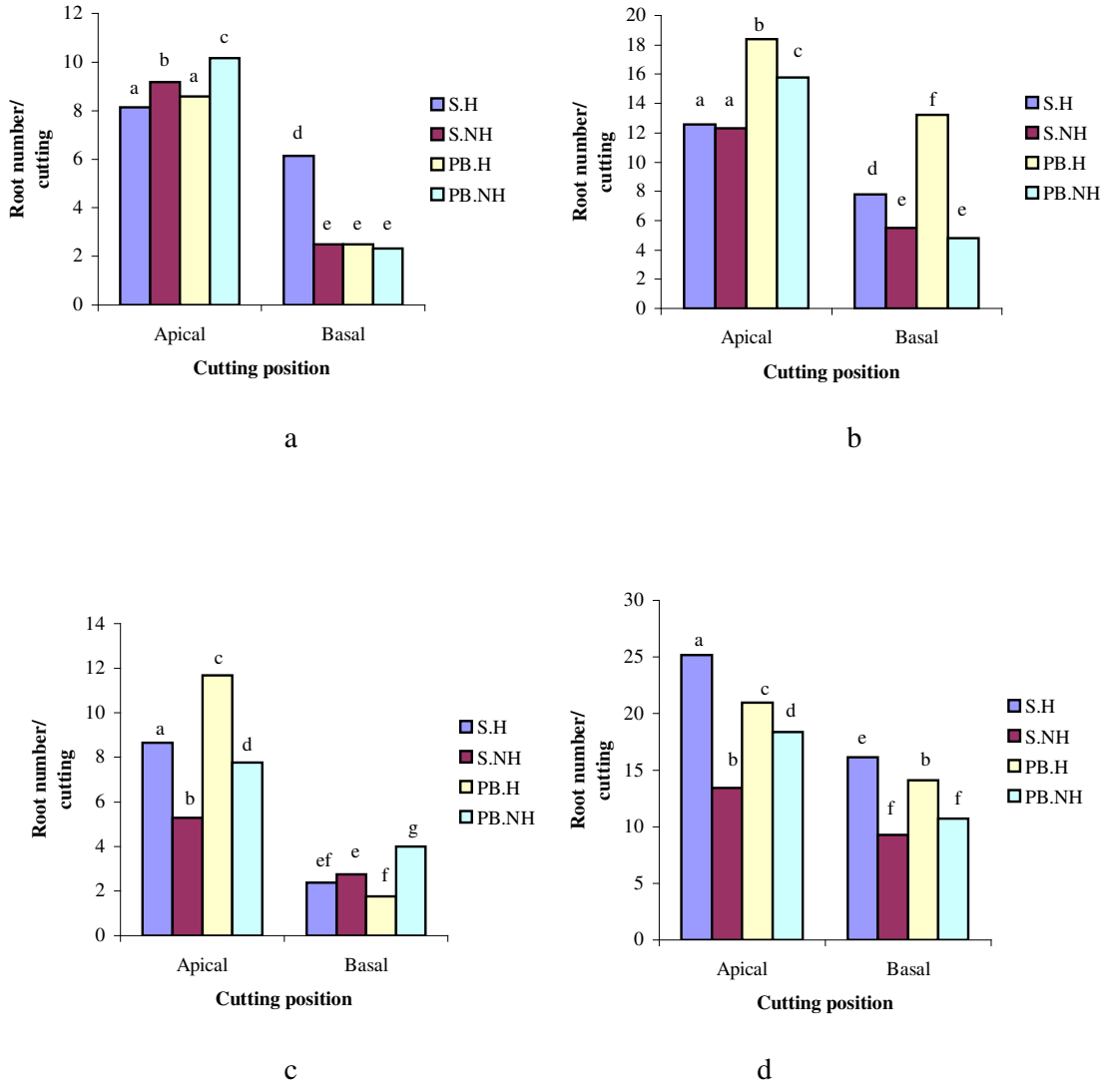
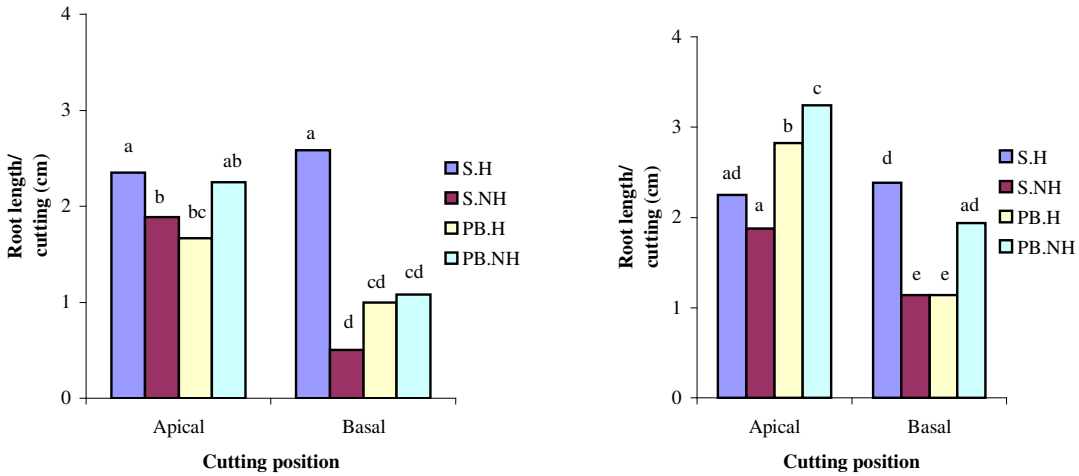


Fig. 2.10 Interactive effect of cutting position, media and hormone on root number of bush tea stem cuttings in (a) summer, (b) autumn, (c) winter and (d) spring

The interaction among cutting position, media and hormone (with or without hormone) (Fig. 2.11) was also found significant for root length of the cuttings in summer and winter ($P<0.05$

and P<0.001, respectively). In summer, (Fig. 2.11a) longer roots were produced from both apical and basal cuttings from sand treated with hormone than cuttings without hormone. In the same way, hormone treated basal cuttings from winter (Fig. 2.11b) season produced longer roots with hormone in sand. But with untreated apical cuttings, longer roots were produced in pine bark.



a b

Fig. 2.11 Interactive effect of cutting position, media and hormone on root length of bush tea stem cuttings in (a) summer and (b) winter

2.4 DISCUSSION

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

cuttings. These are position of the cuttings (apical or basal) on a plant shoot, type of the media used, presence or absence of hormones and time of the year (season) when the cuttings are taken.

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

Like many plant species the rooting ability of bush tea was sensitive to cutting position from the stock plant. In this experiment apical cutting recorded higher percentage of roots, root number and root length per rooted cuttings than the basal ones. Similar results were also reported by Palanisamy & Kumar (1997) in rooting of neem (*Azadirachta indica* A. Juss), where cutting from the upper part of the branches rooted better than the lower ones. This was supported in the propagation of *Grindelia chiloensis* where none of the basal cuttings rooted (Wassner & Ravetta, 2000).

Apical cuttings of Rosemary *(Rosmarinus officinalis* L.) rooted to higher percentage and exerted more number of roots than the basal cuttings (Deen & Mahmoud, 1996). With the propagation of *Triplochiton scleroxylon*, a gradual reduction in rooting percentage was recorded with distance form the apex (Leakey, 1983). This difference in rooting percentage of apical and basal cuttings of bush tea could be due to high concentration of endogenous root promoting substances in the apical cuttings which arise from the terminal buds and also “more cells” which are capable of becoming meristematic (Hartmann & Kester, 1983). On the other hand, basal cuttings could be too mature and highly lignified to develop roots than the apical cuttings (Hartmann *et al*., 1990).

In woody plants this difference in rooting due to cutting position can be related to the difference in the chemical composition of the shoots (position where the cuttings were taken: apical or basal), age of the stem or due to carbohydrate accumulation or due to bud growth (especially

basal cuttings may have 'root-promoting substances from buds and leaves'). If this is the case the best rooting material in such plants is the basal cuttings. In deciduous species where no carbohydrate or root promoting substances are present, cuttings from the soft shoot of the plant root best (Hartmann *et al*., 1997). Many authors (Leakey, 1983; Hansen, 1986, 1988; Jawanda *et al*., 1991; Hartmann *et al*., 1990; Al- Saqri & Alderson, 1996; Hartmann *et al*., 1997) also reported results in agreement with the general statement of Hartmann & Kester (1983) that the best rooting of cuttings is usually found from the basal portions of shoots.

The other source of variation in rooting of the cuttings could be due to the nature of the mother plants since as mentioned in the Materials and Methods, six mother plants were collected from the natural environment where the plants were grown from seeds. According to Hartmann & Kester (1983) there is variation between cuttings in rooting when cuttings are taken from mother plants that were propagated from seed. This is due to the natural variation, which exists between seeds.

Rooting media is one of the most important factors, which affect the rooting success of cuttings (Leakey, 1990; Berhe & Negash, 1998). But this experiment showed that bush tea can be propagated successfully using both sand and pine bark from the apical part of the shoots. Leaky (1990) also stated that cuttings of many species root successfully in a variety of rooting media. This result shows that there could be other factors interacting with media to affect root development. These could be the difference in moisture holding capacity and aeration between the media. In this investigation, the two media showed differences in moisture holding capacity where 48 and 39% for pine bark and sand respectively recorded. The pH was neutral for both pine bark and sand with 7 and 7.7 respectively. The soil analysis results of soil samples taken from the natural growing area of the plant also showed that the plant prefers to grow in pH with the range of 5.6 to 6.1.

Even though there were no significant differences in root development between the media, growing differences in the rooting systems were observed on the cuttings as a result of the chemical and physical property differences between the two media. Cuttings planted or propagated in sand tended to grow coarser and longer roots, whereas cuttings from the pine bark grow fine and highly branched roots. This was similar to the statement made by Hartmann & Kester (1983). Hartmann & Kester (1983) also reported that when sand is used as rooting

medium in some plant species, the root of the cuttings were long, non-branched, "coarse and brittle". However, when media such as a mixture of sand and peat moss, or perlite and peat moss were used, the cuttings had well branched, "slender" and flexible root types that were much more suitable for transplanting. This difference in rooting system or in the growth of the adventitious roots was related to the moisture holding capacity of the media.

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

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

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

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

Rooting was low in winter season for apical (39.7%) and basal (12.6%) cuttings. according to Roberts (1990) bush tea starts to produce flowers from May to July. This was the time when the cuttings were taken for the winter season and the stock plants were at flowering stage. Hartmann & Kester (1983) reported that making cuttings when the plant is at flowering stage or when flowers are present has an opposite effect on vegetative propagation of the plant. This is related to the relationship between auxins and flowers since auxins inhibit the growth of flowers. Due to

this, they recommended that for optimum rooting, cuttings should be made before or after flowering but not while the plants are at the flowering stage.

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

The influences of media and treatment on root number and root length were found to interact with season. Cuttings propagated in pine bark when treated with (Seradix No. 2) in spring produced more roots and longer roots that cuttings treated without hormone. Henry *et al*. (1992) also reported that the efficiency of auxins on rooting percentage and root number varied with season in vegetative propagation of *Eastern redcedar*. Similar effect of season was also reported in 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 & Dirr, 1996).

2.5 CONCLUSION

The results of these experiments indicated that stem cuttings on a mist bed could be used to propagate bush tea (*A. phylicoides*) successfully. Optimum rooting percentage of cuttings was recorded, which implies that effective vegetative propagation of this species would be feasible. But the vegetative propagation of bush tea was affected by factors such the cutting position, media, rooting hormone and season.

Days after planting (DAP) showed significant effect on root development of bush tea. Cuttings started to develop roots after 15 DAP which increased with increasing the DAP to 30 days. This shows the importance of giving the cuttings more time on the mist bed for successful propagation.

The major influencing factor for rooting success of bush tea cuttings was cutting position (apical or basal). It affected the root development, root number and root length of the cuttings. Throughout the experimental period apical cuttings performed better on rooting percentage, root number as well as root length than basal cuttings. Thus, compared with stem cuttings apical cuttings are better planting materials for successful vegetative propagation of bush tea.

The rooting percentage of bush tea was not significantly affected by the type of media used and by application of Seradix No. 2 hormone. This shows that the plant can be propagated either in pine bark or in sand with or without hormone. But differences in the rooting system were observed. Cuttings propagated in pine bark produced fine and highly branched root systems, which is good for transplanting success whereas sand produced long and coarse roots. The type of media also affected the number of roots produced. Compared with sand, cuttings propagated in pine bark produced a higher number of roots. Similarly, application of Seradix No. 2 hormone in pine bark increased the number of roots produced. But both media and Seradix No. 2 hormone had no effect on root length. Since root numbers are most important in the establishment of a cutting, it is recommended to propagate bush tea cuttings in pine bark with the application of Seradix No. 2 hormone.

The other major factor in rooting of bush tea was time of the year or season when the cuttings were taken. High rooting could be obtained by taking cuttings during autumn and spring. Cuttings produced more roots in spring and longer roots in autumn. Media and hormone (Seradix No. 2) also helped in producing more roots in spring than in autumn. Therefore, for successful vegetative propagation of bush tea by stem cuttings, it is recommended to take apical cuttings in spring and propagate them in pine bark with the application of Seradix No. 2 hormone so as to have successful plant establishment.

2.6 SUMMARY

The objective of this investigation was to study the effect of cutting position, rooting media and hormone on the rooting of bush tea stem cuttings. The experiment was carried out in four seasons from 2002 to 2003. The main variables studied were cutting position (apical vs. basal), rooting media (pine bark vs. sand), rooting hormone (with Seradix No. 2 vs. without Seradix No. 2), sampling days (15, 20, 25 and 30 days) and season (summer, autumn, winter and spring).

Cutting position had a highly significant effect ($P<0.001$) on rooting of bush tea; with better rooting percentage, root length and root number from apical than basal cuttings. Pine bark improved the number of roots developed but had no effect on rooting percentage as well as root length of the cuttings. Similarly, application of rooting hormone (Seradix No. 2) increased root number but not rooting percentage or root length. Season also showed highly significant differences ($P<0.001$) on rooting percentage, root number and root length. Rooting of cuttings was improved when propagated in autumn (longer roots) and spring (more number of roots) than summer or winter. The results suggested that vegetative propagation of bush tea could be attained by apical cuttings propagated in pine bark with Seradix No. 2 hormone in spring for 30 days since root number was the most important factor for successful establishment of the cuttings.

CHAPTER 3

TRANSPLANTING SURVIVAL OF ROOTED BUSH TEA (ATHRIXIA PHYLICOIDES) STEM CUTTINGS

3.1 INTRODUCTION

Survival of rooted cuttings is the second step after the development of adventitious roots by cuttings (Hartmann & Kester, 1983). Cuttings of many plant species can develop roots but do not survive for a long time after rooting. The reason for this could be due to attack by different micro-organisms after rooting or due to their inability to recover after transplanting or due to their failure to adapt to the field environment (Hartmann & Kester, 1983; Berhe & Negash, 1998; Wassner & Ravetta, 2000). The survival ability of rooted bush tea (*Athrixia phylicoides*) stem cuttings is not known. Therefore the aim of this study was to investigate the survival ability of apical and basal stem cuttings that were propagated in pine bark and in sand with or without the application of a rooting hormone (Seradix No. 2).

3.2 MATERIALS AND METHODS

An experiment was conducted to evaluate the success of rooting and transplanting survival of cuttings. To investigate this, a factorial experiment arranged in a randomised complete block design with five replications and four blocks was used. In this experiment, two cutting positions (apical and basal), two media (sand and pine bark) and two hormone treatments (with or without Seradix No. 2 which consists of 0.3 % IBA in a talc) that gave a total of 160 cuttings were used. Following similar procedures like in Chapter 2, the media and cuttings were prepared before treatment application. Following this, the cuttings were planted into seedling trays with 5 x 3 x 4.5 cm (width, breath and depth) cell size containing sand and pine bark. The experiment on bush tea rooting with respect to time when the cuttings were taken (Chapter 2) showed that successful rooting started after 30 days from planting and based on this the cuttings were left for 45 days on the mist bed. During this period “Benomyl” fungicide was applied as foliar spray to prevent cuttings from getting fungal infection.

After 45 days from planting the cuttings were assessed for number of cuttings rooted, number and length of roots, stem length and fresh mass before transplanting. Data collection was done by carefully separating the cuttings from the rooting medium. After data collection, all the rooted and non-rooted cuttings were transplanted to black polyethylene bags with 17.5 cm height and 12.5 cm width filled with pine bark. The transplanted cuttings were left in the propagation site for two weeks before moving them to a greenhouse in order to recover from the transplanting shock. After two weeks the plants were moved to the glasshouse where the stock plants were growing
(Fig. 3.1).

Fig. 3.1 Transplanted bush tea stem cuttings

During the growing period the plants were treated with fungicide and fertilized with commercially available foliar fertilizer (4 ml of 'NITROSOL ®' 8% N: 2% P: 5.8% K in 1 L of water). The plants were left to grow for two months and they were irrigated every second day based on the moisture content of the media. Second data collection was done after two months by washing the root zone of the plants in water and tangled roots were softly separated. The plants were assessed for root number, root length, stem length, leaf number, leaf area and fresh and dry mass. The collected data were subjected to ANOVA (analysis of variance) and the means were tested by confidence interval of 95% probability. Data analysis was done using SAS program (Statistical Analysis System Institute Inc, 1999-2001).

3.3 RESULTS

Survival of rooted bush tea stem cuttings followed similar pattern as that of rooting percentage of the cuttings. Like rooting percentage, survival ability of rooted apical and basal cuttings was also affected from where the cuttings were taken from on the stock plants ($P<0.05$). High survival percentage was recorded from apical cuttings (84.4%) than basal cuttings (62.5%) after two months from transplanting. However, difference in media and hormone application (with or without) used during propagation period did not show significant differences in survival percentage of the cuttings. Interactions between media and hormone application (with or without) as well as among cutting position, media and hormone treatment were also not significant on survival percentage of the cuttings.

Root number and root length of both cuttings (apical and basal) showed highly significant differences ($P<0.001$) (Table 3.1). Apical cuttings produced a higher number of roots as well as longer roots than basal cuttings. Propagation media also showed highly significant differences ($P<0.001$) in root number, but the difference in root length was not significant (Table 3.2). Cuttings propagated in sand had a high number of roots (16.41%) than cuttings propagated in pine bark (14.67%) after transplanting.

Table 3.1 Effect of cutting position on root number and root length (cm) of bush tea stem cuttings

Cutting position	Root	
	number	length
Apical	16.13[a]	22.97[a]
Basal	14.83[b]	20.26[b]

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

In addition, cuttings treated with hormone during propagation period showed beneficiary effect ($P<0.001$) in increasing the number of roots and length of roots after transplanting (Table 3.3). Cuttings propagated with hormone (Seradix No. 2) produced more roots than cuttings treated

with no hormone. Similarly, longer roots were developed from cuttings propagated with hormone treatment than those without hormone treatment after transplanting.

Table 3.2 Effect of media on root number and root length (cm) of bush tea stem cuttings

Media	Root number	Root length
Sand	16.41[a]	21.86[ns]
Pine bark	14.67[b]	21.79[ns]

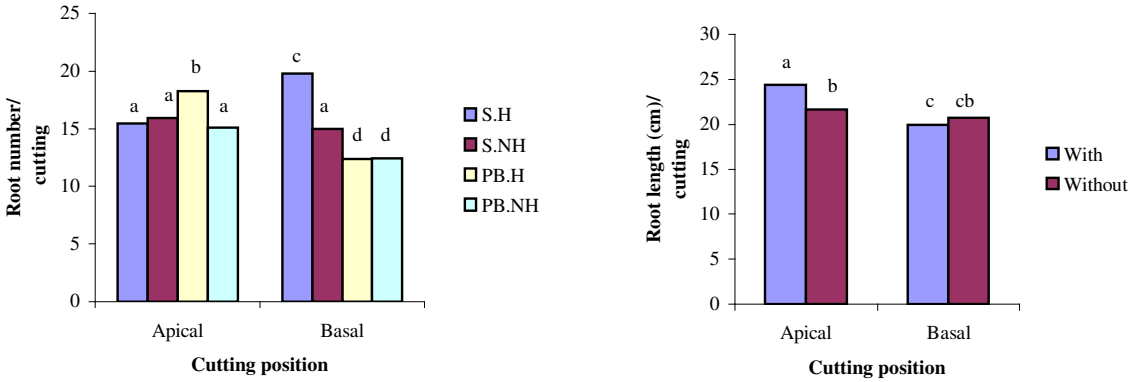
Figures in a column followed by the same letter are not significantly different (P>0.05), using Tukey's comparison test, NS. non significant

Table 3.3 Effect of hormone treatment on root number and root length (cm) of bush tea stem cuttings

Hormone	Root number	Root length
With	16.36[a]	22.38[a]
Without	14.79[b]	21.26[b]

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

The interaction among cutting position, media and hormone was found highly significant (P<0.001) for root number (Fig. 3.2a). Hormone treated apical cuttings developed more roots after transplanting when propagated in pine bark than untreated apical cuttings. While hormone treated basal cuttings produced more roots in sand than those without the hormone treatment. But this interaction did not show significant difference in root length. On the other hand, root length after transplanting was affected by the interaction of cutting position and hormone treatment (P<0.001). More roots were produced by apical cuttings (Fig. 3.2b) with hormone but basal cuttings did not show any difference to applied hormone (Seradix No. 2).



a b

Fig. 3.2 The interactive effect of cutting position, media and hormone treatment (a) on root number (b) and on root length of bush tea

After transplanting, shoot length of bush tea cuttings was found to be affected by hormone treatment used on cuttings during the propagation period ($P<0.001$). Cuttings propagated with hormone showed longer shoots as compared to those without hormone after transplanting. There were also highly significant differences ($P<0.001$) for shoot length of cuttings through the interaction of media and cutting position (Table 3.4). Apical cuttings that were propagated in pine bark produced longer shoots than those propagated in sand after transplanting. But with basal cuttings it was the opposite. Basal cuttings that were propagated in sand produced longer shoots than cuttings propagated in pine bark.

Interaction between media and hormone treatment (with or without) during propagation period affected shoot length ($P<0.001$) development after transplanting (Table 3.4). Hormone treated cuttings propagated in sand developed longer shoots than untreated cuttings propagated in sand without hormone after transplanting. Longest shoots were developed from untreated cuttings propagated in pine park than hormone treated cuttings from pine bark. On the other hand, via visual observation cuttings transplanted with well-developed adventitious roots established more successfully and grew vigorously compared to those that had fewer adventitious roots. Most of the cuttings transplanted with fewer adventitious roots failed to establish successfully.

Table 3.4 Interactive effect of media with cutting position and media with hormone treatment on shoot length of bush tea cuttings

Media	Mean shoot length (cm)			
	Cutting position		Hormone	
	Apical	Basal	With	Without
Sand	47.72[a]	50.77[b]	50.32[a]	48.18[b]
Pine bark	50.23[b]	46.62[a]	44.88[c]	51.96[a]

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

3.4 DISCUSSION

Transplanting survival of bush tea (*Athrixia phylicoides*) was affected by cutting position. Apical cuttings recorded better survival percentage than basal cuttings. Also, they resulted in more and longer roots than basal ones. This differences of apical and basal cuttings could be due to high concentration of endogenous root promoting substances in the apical cuttings which arise from the terminal buds and also "more cells" which are capable of becoming meristematic (Hartmann & Kester, 1983). This helped the apical cutting to produce more and longer roots as compared to basal cutting, which resulted to higher survival percentage of apical than the basal cuttings.

The difference in media and hormone treatment did not show any direct effect on survival of the transplanted cuttings. However, media affected root number and more roots were developed from sand than pine bark. But the difference in root length due to media was not significant. As stated in Chapter 3 pine bark had a higher moisture holding capacity than sand. According to Grange & Loach (1983) and Loach (1992) the water uptake of cuttings is directly related to the water content of the media and moisture is one of the most important factors in rooting success of cuttings.

Application of hormone (Seradix No. 2) 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 & Feldman (1993) as a result of

successful growth of the rooted cuttings. Berhe & Negash (1998) reported similar results in *Podocarpus falcatus* when plants propagated by cuttings survived well due to their better root number. The same was also true for *Juniperus procera* where cuttings with more number of roots showed better growth and establishment than those with a low number of adventitious roots (Berhe & Negash, 1998).

3.5 CONCLUSION

This experiment showed that transplanting survival of bush tea (*A. phylicoides*) was affected by cutting position. Rooted apical cuttings had better and higher survival percentage than basal cuttings. This was due to their ability to produce more roots as well as longer roots compared to basal cuttings. Similarly, cuttings with well-developed adventitious root systems established more easily and successfully than those with poor root systems. Therefore, successful transplanting survival of bush tea can be attained with apical cuttings and it is also important to transplant cuttings with a higher number of roots for successful establishment. In addition, this investigation confirmed that apical cuttings with more number of roots could be obtained by propagating them in pine bark with Seradix No. 2 hormone.

3.6 SUMMARY

Transplanting survival of rooted cuttings is the second step after the development of adventitious roots by cuttings. The survival ability of rooted bush tea stem cuttings is not known. Therefore the aim of this study was to investigate the survival ability of apical and basal stem cuttings that were propagated in pine bark and in sand with or without rooting hormone (Seradix No. 2).

There were significant differences ($P<0.05$) in survival percentage due to cutting position, with apical cuttings resulting in higher survival percentage (84.4%), high root number as well as longer roots than basal cuttings (62.5%) two months after transplanting. Media and hormone application (with or without) did not influence survival percentage. However, media showed significant differences in root number ($P<0.001$) with higher root number from pine bark than sand. Similarly, hormone application during propagation period increased root number, root length and shoot length of after transplanting.

The interactions amongst cutting position, media and hormone were highly significant ($P<0.001$) for root number. Apical cuttings treated with hormone during propagation developed more roots in pine bark with hormone while basal cuttings produced a higher root number in sand with hormone treatment. Interaction between media and cutting position and media and hormone were also significant ($P<0.001$) for shoot length of the transplanted cuttings. Apical cuttings produced longer shoots when propagated in pine bark while basal cuttings in sand. Similarly, cuttings propagated in sand with hormone and in pine bark without hormone produced longer shoots after transplanting. Therefore, higher survival percentage of bush tea can be attained from apical cuttings propagated in pine bark with Seradix No. 2 hormone.

CHAPTER 4

EFFECT OF IBA CONCENTRATION ON ROOTING OF BUSH TEA (*ATHRIXIA PHYLICOIDES*) BASAL CUTTINGS

4.1 INTRODUCTION

Rooting hormones play an important role in root development of cuttings (Wiessman-Ben & Tchoundjeu, 2000). A lot of research has confirmed their beneficiary effect in adventitious root development (Arya *et al*., 1994; Al- Saqri & Alderson, 1996; Aminah *et al*., 1997; Hartmann *et al*., 1997). Cuttings are treated with rooting hormones such as IBA, IAA and NAA in order to increase percentage of rooted cuttings, root initiation, root number, root length and uniformity of rooting on cuttings (AL-Barazi & Schwabe, 1982). Though they have this role in rooting of cuttings, using the appropriate concentration of rooting hormones is important since application of wrong concentrations can inhibit rooting or it can act as a growth retardant when applied at higher concentrations (Hartmann *et al*., 1990; Wiessman-Ben & Tchoundjeu, 2000). As stated in Chapter 3, basal cuttings of bush tea (*Athrixia phylicoides*) did not show good response to the application of Seradix No. 2 hormone in rooting percentage and the development of roots was slow compared to the apical cuttings. The response of basal cuttings to different IBA concentrations is not known. Therefore to investigate this, an experiment was set with IBA concentrations of 0.1, 0.3 and 0.8% (Seradix No. 1, 2 and 3 respectively) using pine bark and sand media. Samples were taken after 10 and 15 days from planting.

4.2 MATERIALS AND METHODS

An experiment was set up in November 2003 to investigate the effect of IBA concentration on rooting percentage of bush tea basal cuttings. A randomised complete block design was used in this experiment, with two media, three treatments and five blocks. A commercially available rooting powder named Seradix was used as a rooting hormone. The treatments were Seradix No. 1, 2 and 3 with 0.1%, 0.3% and 0.8% IBA concentrations, respectively. Eighteen replications per treatment per block were used, which gave a total of 540 cuttings for the whole experiment. Two different propagation media namely 8 mm size sand and decomposed fine pine bark were used in the experiment. The two media were randomly assigned to seedling trays with 5 x 3 x 4.5 cm (width, breath and depth) cell size. To get the medium moist before planting the filled

trays were put under a mist system set to come on at 2 min intervals for 8 seconds for a period of 48hrs. Temperature of the medium was measured prior to planting by a mercury thermometer, which had been inserted to a depth of 2 to 4 cm. The measured temperature of the two media varied between 17 and 27 ^{o}C.

On the second day after preparation of the media, cuttings were made early in the morning (between 06:30 and 7:30) from the stock plants, which had been established for one year in a greenhouse after transplanting from their natural growing area. The cuttings were then wrapped with moist tissue paper and placed in plastic bags until taken to the working area. Working in the humid propagation site, the cuttings were divided into a total of 540 cuttings each with 8 cm length and between 0.1 and 0.35 cm diameter range. The lower leaves were removed leaving only the top three leaves followed by treating the bases of the cuttings with hormones based on the treatment received by each cutting. A trench was made using a stick in the rooting media in order to avoid brushing off the rooting powder from the cuttings during planting (Hartmann & Kester, 1983). The cuttings were then planted to a depth of approximately 2 cm. Assessment for rooting was made 10 and 15 days after planting and cuttings were assessed for rooting, number of roots, root length, leaf number, leaf area, and fresh and dry mass. Data were collected by carefully separating the cuttings from the seedling trays and by washing the root zone with water.

After data collection was completed, the collected data were subjected to ANOVA (analysis of variance) and the means were tested by confidence interval at 95% of probability. Data analysis was done using SAS program (Statistical Analysis System Institute Inc., 1999-2001).

4.3 RESULTS

Bush tea basal cuttings showed differences in rooting percentage, root number and root length with days after planting (DAP) (Table 4.1). The difference in rooting percentage was highly significant (P<0.001). Percentage of rooting was increased with increasing number of DAP. Similarly, the difference in root number was also highly significant (P<0.001) with DAP. More roots were produced with increasing DAP and the number of roots produced after 15 days were higher than roots produced after 10 days. The same was true for root length (P<0.05), where longer roots were produced after 15 days from planting.

Table 4.1 Rooting percentage, mean root number and length (cm) of bush tea 10 and 15 days after planting

DAP	Rooting percentage (%) ± S.E	Mean root ± S.E	
		Number	Length (cm)
10	26.7 ± 0.06[a]	5.39 ± 0.32[a]	1.09 ± 0.26[a]
15	56.7 ± 0.06[b]	11.57 ± 0.25[b]	1.65 ± 0.21[b]
Significance	P<0.01	P<0.001	P=0.0167

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

The interaction between DAP and hormone concentrations (Seradix No. 1, 2 and 3 with 0.1, 0.3 and 0.8% IBA respectively) was found to be significant (P<0.05) for rooting percentage of bush tea basal cuttings. In the first 10 days after planting, cuttings did not show clear differences in rooting percentage (Table 4.2) with the three different hormone concentrations. But as the DAP increased to 15 days a clear difference was recorded and basal cuttings with Seradix No. 1 (0.1%, IBA concentration) rooted to the highest percentage (90%) followed by Seradix No. 2 (0.3% IBA concentration) with 55%. On the other hand, the effect of Seradix No. 3 (0.8% IBA concentrations) on rooting percentage remained the same (25%) regardless of DAP.

Table 4.2 Interactive effect of DAP with hormone concentration (Seradix No. 1, 2 and 3 with 0.1, 0.3 and 0.8% IBA respectively) on rooting percentage, mean root number and root length (cm) of bush tea basal cuttings

DAP	Seradix No.	Rooting % ± S.E	Mean root ± S.E	
			Number	Length (cm)
10	1	30 ± 0.10^{abe}	3.67 ± 0.44^{a}	1.17 ± 0.36^{ns}
	2	25 ± 0.10^{b}	7.25 ± 0.60^{b}	1.19 ± 0.49^{ns}
	3	25 ± 0.10^{b}	5.25 ± 0.60^{c}	0.90 ± 0.51^{ns}
15	1	90 ± 0.11^{c}	13.55 ± 0.31^{d}	1.97 ± 0.25^{ns}
	2	55 ± 0.10^{ad}	10.90 ± 0.32^{e}	1.47 ± 0.27^{ns}
	3	25 ± 0.10^{e}	10.25 ± 0.60^{e}	1.50 ± 0.49^{ns}
Significance		P=0.0140	P<0.001	P=0.3584

Figures in a column followed by the same letter are not significantly different (P>0.05), using Tukey's comparison test, NS= non significant

Number of roots produced were also affected by the interaction of DAP and hormone concentration (Seradix No. 1, 2 and 3) (P<0.001) (Table 4.2). In the fist 10 days, bush tea basal cuttings showed good response in root number to Seradix No. 2 but with increasing DAP its efficiency was reduced. After 15 days, cuttings produced more roots with Seradix No. 1 (0.1% IBA). On the other hand, root length did not show significant differences with this interaction.

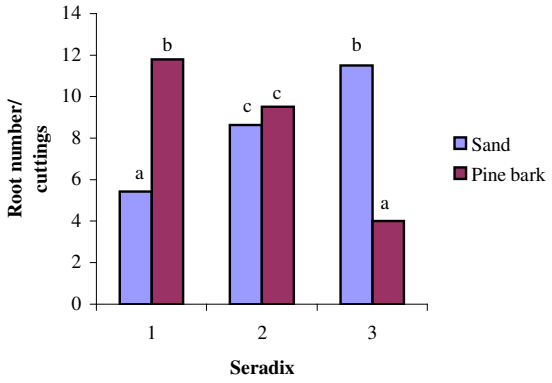
The interaction of DAP with media and hormone concentration (Seradix No. 1, 2 and 3) was highly significant (P<0.001) (Table 4.3). Cuttings grown in pine bark with Seradix No. 2 produced more roots in the first 10 days, but with increasing the number of days to 15, more roots were produced with cuttings grown in pine bark with Seradix No. 1. With sand, more roots were produced when Seradix No. 3 was used as a rooting hormone than Seradix No. 1 and 2.

Table 4.3 Interactive effect of DAP, media and hormone concentration (Seradix No. 1, 2 and 3) on mean root number of bush tea

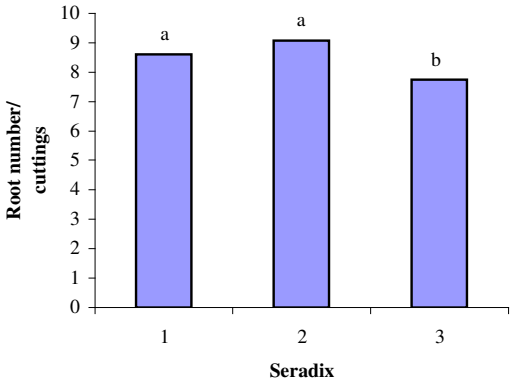
DAP	Media	Seradix No.	Mean root number ± S.E
10	Sand	1	4.33 ± 0.62^{abcei}
		2	5.50 ± 0.53^{bce}
		3	5.50 ± 0.53^{bce}
	Pine bark	1	3.00 ± 0.62^{aei}
		2	9.00 ± 1.07^{d}
		3	5.00 ± 1.07^{e}
15	Sand	1	6.50 ± 0.38^{bce}
		2	11.80 ± 0.48^{f}
		3	17.50 ± 0.53^{g}
	Pine bark	1	20.60 ± 0.48^{h}
		2	10.00 ± 0.44^{di}
		3	3.00 ± 1.07^{ej}

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

Basal cuttings showed highly significant differences (P<0.001) due to media in mean root number and root length. Cuttings grown in pine bark produced more and longer roots than cuttings grown in sand. On the other hand, the interaction between media and hormone concentration was highly significant (P<0.001) for root number (Figs 4.1a & 4.2). When cuttings were grown in pine bark, they showed beneficiary effect from Seradix No. 1 and more roots were produced than cuttings grown in the same medium but with Seradix No. 2 and 3. This shows that cuttings responded well to lower IBA concentration (0.1% in Seradix No. 1) when pine bark was used as a medium. On the contrary, when sand was used as medium, cuttings grown with higher IBA concentration (0.8%, Seradix No. 3) produced more roots than cuttings grown with 0.1 and 0.3% concentrations (Seradix No. 1 and 2 respectively).



a



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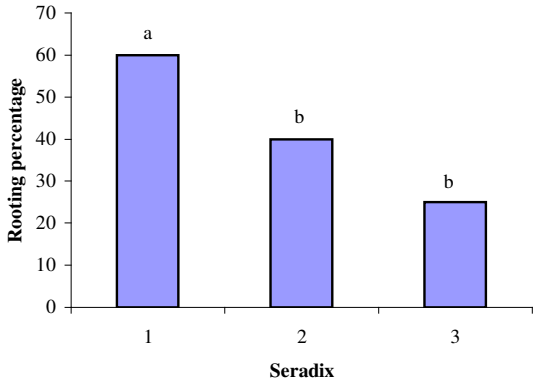
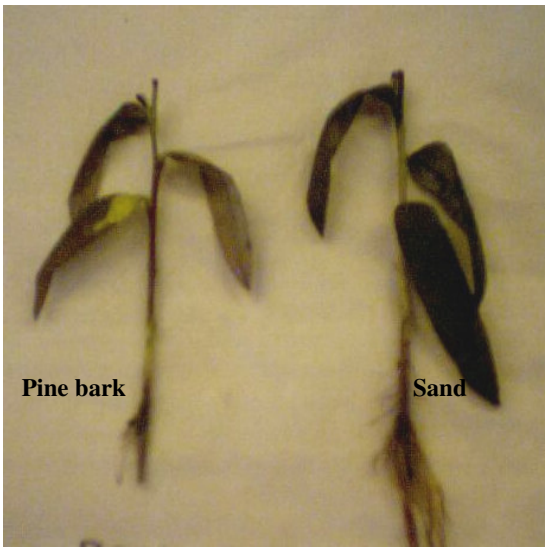


c

Fig. 4.1 (a) Interactive effect of media and hormone concentration (Seradix No. 1, 2 and 3) on mean root number, (b) effect of hormone concentration (Seradix No. 1, 2 and 3) on mean root number and (c) effect of hormone concentration (Seradix No. 1, 2 and 3) on rooting percentage of bush tea

No. 1 No. 2
a b



No. 3
c

Fig. 4.2 Interactive effect of media with (a) Seradix No 1 (0.1% IBA), (b) Seradix No 2 (0.3% IBA) and (c) Seradix No 3 (0.8% IBA) on root number of bush tea basal cuttings

Different IBA concentrations showed highly significant differences ($P<0.001$) in root number (Fig. 4.1b). Number of roots produced with 0.3% IBA concentration (Seradix No. 2) and 0.1% IBA concentration (Seradix No. 1) were about the same. Few numbers of roots were produced

with 0.8% IBA concentration (Seradix No. 3). IBA concentration also affected rooting ability ($P<0.001$) of the cuttings (Fig. 4.1c). Cuttings with lower IBA concentration (0.1%, Seradix No. 1) rooted to a higher percentage (60%) followed with 0.3% IBA concentration (Seradix No. 2). However, as IBA concentration was increased to 0.08% (Seradix No. 3), rooting percentage was reduced to 25%.

4.4 DISCUSSION

This experiment was designed to investigate factors that might improve the rooting ability of bush tea basal cuttings. As stated in "Chapter 3" basal cuttings did not show good response to rooting. Based on the collected data in the current experiment, rooting percentage, root number and root length of the basal cuttings was affected by DAP (days after planting) and more cuttings were rooted after 15 days. The same was true for root number and root length after 15 days, where more roots with longer lengths were produced. This shows that rooting percentage, number and length of roots produced are increased with increasing DAP. Ofori *et al*. (1996) reported that rooting percentage of *Milicia excelsa* was increased with increasing DAP.

Effect of hormone concentration on rooting percentage and root number showed clear differences with increasing DAP. In the first 10 days, rooting response of the cuttings to different IBA concentrations was not significant. However, with increasing the DAP to 15, cuttings showed positive response to 0.1% IBA (Seradix No. 1) and more cuttings developed roots. A similar trend was also followed with 0.3% IBA (Seradix No. 2). However, with 0.8% IBA (Seradix No. 3) percentage of rooted cuttings remained the same in both sampling dates. On the other hand, more roots were produced with Seradix No. 2 after 10 days but with increasing time to 15 days more roots were produced with Seradix No. 1. This shows that, Seradix No. 1 has a slow acting effect on bush tea basal cuttings, in enhancing root initiation as well as increasing number of roots developed.

Number of roots produced showed differences with DAP, media and hormone concentration used. More roots were produced with 0.3% IBA after 10 days but with increasing time to 15 days more roots where produced with 0.1% IBA when pine bark was used as a growing media. When cuttings grew in sand however, more roots were produced with 0.8% than 0.1% as well as 0.3% IBA concentration. This is due to slow acting effect of 0.1% IBA in increasing root number. On pine bark was also reported to have phenolic compounds and this related with the

theory reported by Hartmann & Kester (1983) that these compounds interact with auxins to promote the development of adventitious roots as well as increasing number of roots.

In this experiment, phenolic compounds required sometime before being available, but with increasing time, their availability was increased. This may have led to their antagonistic effects on the number of roots produced when IBA concentration was 0.3 and 0.8%. On the contrary, cuttings with low IBA concentration (0.1%) showed positive response to this supplement. On the other hand, cuttings grown in sand, since they do not have this supplement, showed more roots with a higher hormone concentration (0.8% IBA).

As stated in "Chapter 3" media did not show differences in rooting percentage. Cuttings could root equally well whether sand or pine bark was used as a growing medium. However, root number as well as root length were higher in pine bark. This is due to the differences in moisture holding capacity of the two media (Chapter 3). On the other hand, when cuttings were grown in pine bark, they showed beneficiary results to low IBA concentration (0.1%) and more roots were produced than with 0.3 or 0.8% IBA. This shows that cuttings respond well to a lower 0.1% IBA when pine bark was used as a medium since, the medium contained supplementary compounds (phenolic compounds) which increased root number. On the contrary, with sand, more roots were produced with higher IBA concentration (0.8%). Since sand did not have synergetic compounds such phenolic compounds, higher IBA concentration (0.8%) was required in order to produce more roots. According to Hartmann *et al*. (1997) these compounds act as co-factors and interact with endogenous auxins to promote rooting, and to increase root number and length. They also reported that these compounds reduce the destruction of auxins by indoleacetic acid oxidase enzyme.

Results of this experiment showed that rooting percentage of bush tea basal cuttings was affected by hormone concentration. Percentage of rooting was higher with 0.1% IBA than 0.3 and 0.8% IBA. The cuttings required low exogenous IBA concentration as supplement to promote rooting. In nature bush tea contains polyphenol compounds, which are believed to have beneficiary effect on adventitious root development. But as the concentration of exogenous IBA supplement was increased to 0.3 and 0.8% (Seradix No. 2) its effect on root promotion was reduced. Similarly, in "Chapter 3" both apical and basal cuttings did not show a positive response to Seradix No. 2 (0.3% IBA).

Many studies stated that even though application of exogenous auxins promote adventitious root development on stem 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 & Tchoundjeu, 2000). When high concentrations are added to the endogenous auxins of the plant, which themselves increase as a consequence of taking cuttings this, might have led to a level that disturbed the hormonal metabolism and inhibited rhizogenesis (Lebrun *et al*., 1998).

4.5 CONCLUSION

Data obtained in this experiment suggest that rooting ability of bush tea basal cuttings can be improved by using Seradix (hormone) with appropriate IBA concentration. The cuttings were found to be sensitive to IBA concentration of the applied Seradix. Higher rooting percentage and more roots can be obtained with Seradix No. 1 (0.1% IBA). Since root number is important in successful establishment of the cuttings, it is important to consider the type of media used. When pine bark is used as a growing medium, more roots and higher rooting percentage can be obtained with Seradix No. 1. If sand is used as growing medium, higher root number but with lower rooting percentage can be achieved with Seradix No. 3.

4.6 SUMMARY

Basal cuttings of bush tea did not show good response to the application of Seradix No. 2 hormone in rooting percentage and the development of roots was slow compared to the apical cuttings in Chapter 3. The response of basal cuttings to different IBA concentrations is not known. Therefore to investigate this, an experiment was set up with IBA concentrations of 0.1, 0.3 and 0.8% (Seradix No. 1, 2 and 3 respectively) using pine bark and sand media. Samples were taken after 10 and 15 days from planting.

Bush tea basal cuttings showed differences in rooting percentage ($P<0.001$), root number ($P<0.001$) and root length ($P<0.05$) with days after planting (DAP). Rooting percentage and root number were higher at 15 DAP. Similarly, long roots were produced at 15 DAP. The interaction between DAP and hormone concentrations (Seradix No. 1, 2 and 3 with 0.1, 0.3 and 0.8% IBA respectively) was significant ($P<0.05$) for rooting percentage and number of roots ($P<0.001$). At 15 DAP cuttings with Seradix No. 1 (0.1%, IBA concentration) rooted to the highest percentage (90%) followed by Seradix No. 2 (0.3% IBA concentration) with 55%. At 10 DAP cuttings treated with Seradix No. 2 produced higher number of roots but at 15 DAP higher number of roots were produced with Seradix No. 1.

The interaction of DAP with media and hormone concentration (Seradix No. 1, 2 and 3) was highly significant ($P<0.001$). Higher number of roots were produced in pine bark with Seradix No. 2 at 10 DAP but at 15 DAP more roots were produced in pine bark with Seradix No. 1. With sand, more roots were produced when Seradix No. 3 was used as a rooting hormone than Seradix No. 1 or 2. The interaction between media and hormone concentration was also highly significant ($P<0.001$) for root number. Cuttings with Seradix No. 2 in pine bark produced higher number of roots, however with Seradix No. 3 higher number of roots were produced in sand. Hormone concentration also showed highly significant differences ($P<0.001$) in root number as well as in rooting percentage. About the same number of roots were produced with 0.3% IBA concentration (Seradix No. 2) and 0.1% IBA concentration (Seradix No. 1). But cuttings with lower IBA concentration (0.1%, Seradix No. 1) rooted at a higher percentage (60%) followed with 0.3% IBA concentration (Seradix No. 2).

Therefore, high rooting percentage and root number for basal cuttings can be obtained with Seradix No. 1 in pine bark.

CHAPTER 5

BUSH TEA (*ATHRIXIA PHYLICOIDES*) SEED RESPONSE TO LIGHT AND TEMPERATURE DURING GERMINATION

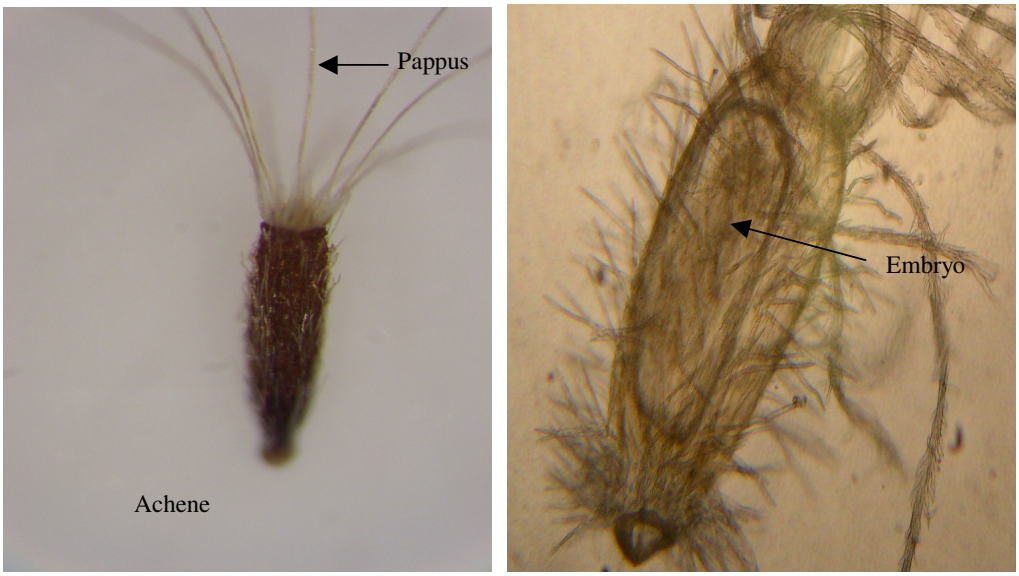
5.1 INTRODUCTION

A seed is a ripened ovule, which consists of an embryo, stored food and a seed coat (Hartmann & Kester, 1983). Propagation by seed is one means of continuation of life and assuring of plant species survival (Copeland & McDonald, 1994; Hartmann *et al*., 1997). Many authors define seed germination differently and in this study a seed is said to have germinated with the emergence of the radical through the seed coat under favourable conditions (Copeland & McDonald, 1994). Bush tea (*Athrixia phylicoides*) is commonly propagated by ripen seeds which are mostly collected at the end of summer (Roberts, 1990). However, there are certain factors that affect the germinability of bush tea seeds. Furthermore, the germination (light and temperature) requirement of bush tea seed is not known. The aim of this study was to investigate the ideal seed germination temperature and light combinations for bush tea seeds.

5.2 MATERIALS AND METHODS

Inflorescences of bush tea were collected from their natural populations at Froggy Pond (24^{o} 10′ S, 29^{o} 13′ E) in May 2002. Achenes were separated from the inflorescences, cleaned and shrivelled achenes were discarded. The seeds were dried and stored in paper bags in the laboratory at approximately 20^{o} C and 50% air humidity until used in the germination tests.

The fruit of bush tea is a narrow cylindrical thin achene of about 0.01 to 0.02 mm long and 0.03 to 0.06 mm wide and 1 000 seeds weigh 0.12 g. On average there are around 12 pappus per matured seed with the length of 4 mm, which helps in seed dispersion (Fig. 5.1a). The achene of bush tea was observed microscopically to see the presence of the embryo (Fig. 5.1b) before setting up the experiment. The germination trial started when the presence of an embryo was confirmed.



a b

Fig. 5.1 Bush tea (*A. phylicoides*) (a) achene and (b) embryo (100 x)

The study was carried out at the University of Pretoria in the Department of Botany in growth chambers (Labcon model L.T.G.C with ±0.3 ^{o}C, South Africa). Germination of bush tea was tested at constant temperature regimes (10, 15, 20 and 25 ^{o}C) with a continuous light or dark and alternate temperatures (20: 20, 20:30 and 30:30 ^{o}C) with a 12L: 12D, 8L: 16D and 16L: 8D light: dark combinations respectively. The selection of the temperature and light for germination of bush tea was done based on a preliminary study where better germination was recorded on these specific light and temperature combinations. Each observation unit (constant and alternate temperature with light and dark) consisted of four replicate petri dishes (9 cm in diameter) with at least 25 viable achenes (pappus not removed) and weight of 1000 seeds was taken using a microbalance. The achenes were placed on two layers of Whatman No.1 filter papers and prior to sowing they are moistened with distilled water, then after sowing the petri dishes were sealed using laboratory parafilm 'M' to reduce evaporation. In the dark treatment, the petri dishes were wrapped in double layer of aluminium foil according to Baskin & Baskin (2001) in order to insure 100% darkness.

The petri dishes were inspected daily or every second day over a four week period for the light treatment and germinated seeds were counted then and removed. But for the dark treatment, inspection and counting was done at the end of the experiment (after four weeks). According to

Bewley & Black (1994) a seed is considered to have germinated at emergence of the radicle. The collected data was arcsine-transformed (Gomez & Gomez, 1984) and subjected to analysis of variance (ANOVA). Tukey's LDS multi comparison was used to test the significant difference between the treatment means for germination percentage of seeds and mean germination time or germination rate.

5.3 RESULTS

Bush tea achenes started to germinate after 6 days and germinated up to 30 days from sowing. Results of the germination experiment (Fig. 5.2) showed that good germination success was obtained in most of the treatments. The achenes of bush tea could germinate in alternate and constant temperatures ranging from 10 to 30 ^{o}C.

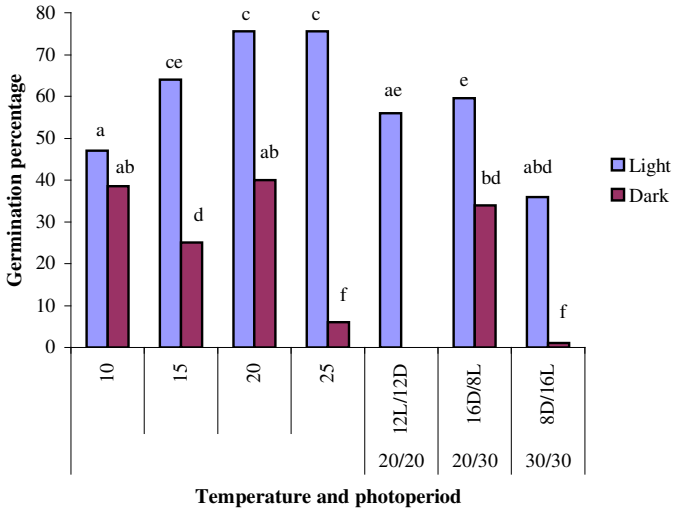


Fig. 5.2 Germination percentage of bush tea (*A. phylicoides*) at continuous and alternate light, dark, constant and alternate temperatures

There were highly significant differences ($P<0.001$) in germination percentage of the achenes through the temperature treatments. The highest germination percentage was 75.5% at 20 and 25 ^{o}C followed by 15 ^{o}C with 64.5% but the difference among them was not significant. Even though the difference was not significant, bush tea achenes had higher germination percentage

when the temperature was between 20 and 25 ^{o}C. On the other hand, percentage of the germinated achenes at 15 ^{o}C was not much better than those germinated at 20/20 (56%) and 20/30 ^{o}C (59%) alternate temperatures. Low germination percentage was recorded at 30 and 10 ^{o}C with 36 and 47% respectively.

It was also observed (Fig. 5.2) that germination percentage of bush tea increased with increasing temperatures from 10 to 25 ^{o}C but when temperature went beyond that, germination percentage was slightly reduced. The seeds also responded well to constant temperatures than alternate temperatures for germination.

Bush tea seeds responded positively to light ($P<0.001$) (Fig. 5.2) and a higher germination percentage was recorded when seeds were exposed to continuous light than alternate light or continuous dark. In continuous light, 75.5% of the seeds germinated, while in darkness 40% of the seeds germinated. Only 1% of the seeds germinated when the achenes were incubated at 30^{o}C in the dark. Even though the difference was not significant, seeds of bush tea also showed more difference in germination percentage to variation in light exposure than variation in temperatures. This was confirmed when the seeds incubated in continuous light at 20 ^{o}C gave 75.5% germination, whereas when incubated with the same temperature but a 12:12 h light: dark cycle, 56% of the seeds germinated. On the other hand, when non-germinated seeds that were incubated in the dark were exposed to light but with the same temperature, they started to germinate.

In addition, there was a highly significant difference ($P<0.001$) due to the interaction of photoperiod (light and dark) with temperature (Fig. 5.2). Seeds germinated to a higher percentage (75.5%) when incubated in continuous light at 20 and 25 ^{o}C constant temperature. But when the temperature was reduced to 10 or 15 ^{o}C or increased to 30 ^{o}C constant temperatures, germination percentage was found to be reduced. Similarly, germinating of bush tea achenes in alternate temperature also reduced their germinability.

Germination rate of bush tea seeds was affected by the individual effects of light and temperature ($P<0.001$) (Fig. 5.3). The average number of days required for a radicle to emerge or mean germination time (MGT) was higher in the low temperature (10 ^{o}C) treatment with continuous light and alternate temperature and light. In other words, when the temperature was low (10 ^{o}C) and alternate the time-required for germination to start was long. On the other hand,

at 10 °C fist germination was observed after 20 days (Table 5.1) and last germination was after 31 days. The same was true with alternate temperatures. But achenes incubated at 15, 20 and 25 °C first germination was recorded between 6 and 8 days whereas last germination was between 23 and 25 days from sowing. Overall the experimental period seeds incubated at 20 °C with continuous light recorded high germination rate (Table 5.1) and low germination rate was recorded at 30:30 °C. The radicle of seeds incubated at 10 °C was smaller in size when compared to the other temperature treatments. On the other hand, the removal of the pappus from the seeds did not affect the germination percentage as well as germination rate of bush tea seeds (data not shown).

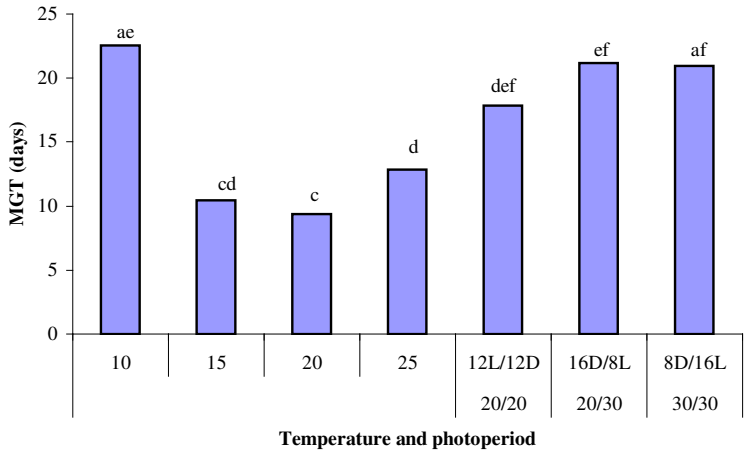


Fig. 5.3 Mean germination time (MGT) of bush tea achenes at continuous and alternate light, dark, constant and alternate temperatures

Table 5.1 Bush tea (*A. phylicoides*) germination percentage, rate and number of days for first and last germination in light at constant and alternate temperatures

Temperature °C	Number of days for		Germination	
	First germination	Last germination	%	Rate
10	20^{a}	31^{ab}	47^{cd}	4.27^{cb}
15	8^{cd}	25^{cd}	64.5^{ab}	3.79^{cb}
20	8^{bcd}	23^{d}	75.5^{a}	5.03^{a}
25	6^{b}	24^{bcd}	75.5^{a}	4.19^{b}
20:20 12:12L	8^{d}	23^{abc}	56^{cb}	3.73^{cb}
20:30 8L:16D	8^{bc}	31^{a}	59.5^{cb}	2.59^{cb}
30:30 16L:8D	9^{b}	31^{a}	36^{d}	1.64^{c}
Significance	P<0.0001	P<0.0001	P<0.0001	P<0.0001

5.4 DISCUSSION

Bush tea flowers from May to July (Fox & Young, 1982; Roberts, 1990). The fruit is a narrow cylindrical thin achene of about 0.01 to 0.02 mm long and 0.03 to 0.06 mm wide. It has an average of 12 pappus per seed of about 4 mm long that helps in the dispersion of the seed. Farmers collect mostly these seeds at the end of summer (Roberts, 1990) for propagation of this species.

In a seed germination test, temperature was found to affect germination when applied in either alternating or in a constant fashion (Copeland & McDonald, 1994). For most plant species the optimum temperature for germination is between 15 and 30 °C (Copeland & McDonald, 1994; Pons, 2000; Baskin & Baskin, 2001). In this study, germination percentage and germination rate of bush tea seeds were affected by temperature. The seeds of bush tea germinated better at constant 20 and 25 °C than at the alternate temperatures. This could be due to the preferences of the chemical reactions inside the seed for constant rather than alternate temperatures. Similarly, Copeland & McDonald (1994) reported that *Arthropodium cirratum* seeds prefer to germinate at constant than alternate temperatures. However, most plants prefer to germinate under alternate than constant temperatures and this is because plants in nature are exposed to alternate and not to constant temperatures (Baskin & Baskin, 2001).

The requirement for alternating temperature is related to dormancy breaking and vegetation insulates the soil surface against large diurnal temperature fluctuation and in open areas the soil surface acts as an insulator (Copeland & McDonald, 1994). This was supported by Rossello & Mayol (2002), where germination percentage of *Lysimachia minoricensis* was high when incubated at alternate than constant temperatures. The seeds of bush tea germinated at a wide range of temperatures (10 to 30 ^{o}C). Rossello & Mayol (2002) reported this is the characteristic of plant species where moisture is the main factor for germination in the field.

The requirement of light in seed germination varies with species. Seeds that are not dormant germinate equally in light and dark (Baskin & Baskin, 1988). However, others might germinate to a higher percentage in light, whereas others will germinate in the dark (Baskin & Baskin, 2001). The seeds of bush tea responded positively to light and seeds, which were incubated in continuous light than in dark, recorded a higher germination percentage and germination rate. Similar results were also reported by Schutz, Milberg and Lamont (2002), where *Millotia myosotidifolia* germinated better when they were incubated in light than in the dark. Some flower crops like allussum, begonia, calceolaria, coleus, *Kalanchoe primrose* and *Saintpaulia* require light for germination (Copeland & McDonald, 1994). Seeds that require light for germination are said to be photodormant (Salisbury & Ross, 1992). Most of the time this is the characteristic of small seeds where shallow planting is important since if they are planted too deep in the soil they might not have enough food reserves for the epicotyl to penetrate the soil surface (Hartmann *et al*., 1997). The same is true for bush tea seeds since the seeds are small in size. In additions, Salisbury & Ross (1992) stated that most plant species that require light for germination are undomesticated plants and rich in a fat but this is not the characteristics of most of the cultivated seeds because of human selection for light exclusion.

The dark incubated seeds of bush tea started to germinate when exposed to light. Similar results were also reported by Rossello & Mayol (2002) when dark incubated seeds of *Lysimachia minoricensis* started to germinate after they were exposed to light. This suggested that the seeds were introduced to secondary dormancy when incubated in darkness. In addition, removal or the presence of pappus on the seeds of bush tea did not affect the germination percentage and germination rate. This shows that the importance of pappus in this plant species is only for dispersion but they do not have a role in seed germination.

Sensitivity of lettuce seed to light in germination is also cited by Copeland & McDonald (1994). They stated that this is due to the presence of a light sensitive pigment (phytochrome) in the seed. This pigment has two phases where one is P_{FR} and when exposed to red light (660 nm) it is converted to a physiologically active, far-red absorbing form, which helps germination to proceed and the other is P_R where exposure to far-red (730 nm) converts it to a physiologically inactive red light absorbing form, which inhibit seed germination. Only brief exposure to far-red or red light can convert this pigment to its opposite form (Copeland & McDonald, 1994). According to Salisbury & Ross (1992) seeds that require light for germination are also mature seeds with embryo covered with materials that contain high amount of chlorophyll. These seeds require light for germination but not seeds that are covered by tissue with little or no chlorophyll. The reason given for this is that during maturity stage of the seeds, the chlorophyll absorbs the red light and prevents the formation of P_{FR} in the mature seeds. Due to this, they require light to start germination. Hartmann & Kester (1983) also suggested that seeds that germinate in dark are seeds that have sufficient phytochrome in the form of P_{FR} in the mature seeds to promote germination.

Seeds of many light-requiring species germinate in the spring, after they have been exposed to low winter temperatures (Baskin & Baskin, 1988), whereas those that require darkness germinate in autumn, after they have been exposed to high summer temperatures (Baskin & Baskin, 1988). The same is true for the seeds of bush tea, since this species start to disperse mature seeds between late winter and early spring.

An additional feature of light on germination of bush tea is through its interactive effect with temperature in which the seeds are incubated. High germination percentage and rate was recorded when seeds were incubated in continuous light at constant temperature (20 and 25 ^{o}C continuous light). Germination percentage and rate decreased at alternate and low temperatures with alternate and continuous light. In dark 38.5, 40 and 36% germinated at 10 and 20^{o}C continuous light and 20:30 ^{o}C with 8:16 h light: dark cycle and only 1% of the seeds germinated at 30^{o}C darkness.

The requirement of light for germination at 10 ^{o}C was reduced but the seeds could not germinate at 30 ^{o}C without light. Similar results were also reported by Baskin & Baskin (2001), where low winter temperatures (cold stratification) removed the light requirement for the germination of *Picea mariana*. The same was true for seeds of Callow Grand Rapids lettuce seeds, where they

germinated well without light requirement when incubated at 10 to 15 ^{o}C (Salisbury & Ross, 1992). Baskin & Baskin (2001) also reported that seeds of *Lactuca sativa* cv. Grand Rapids germinated to >80% in light at temperatures of 10 to 30 ^{o}C, whereas germination in darkness exceeded 45% only at temperatures of 10 to 22 ^{o}C; it was near 0% at 30 ^{o}C. Seeds such as *Bidens pilosa* require light to germinate at constant temperatures but they require light and darkness to germinate in alternating temperatures. Salisbury & Ross (1992) also reported the interaction of light and temperature on germination. They also stated that temperature has almost no effect on photochemical interconversions of P_R and $P_{FR,}$ but the chemical reactions controlled by P_{FR} and those influencing its distraction are quite temperature sensitive.

Mean germination time (MGT) of bush tea achenes was also affected by the interaction of temperature and light. The germination was slow when seeds were incubated at 10^{o}C continuous light and when exposed to both alternate temperature and light. This could be due to the effect of low and alternate temperatures on the chemical relations of the seeds. On the other hand the seeds of bush tea also germinated to higher percentages when incubated at 20^{o}C continuous light than the same temperature but at 12:12 h light: dark cycle. This showed that the seeds are more sensitive to differences in light than differences in temperature.

5.5 CONCLUSION

The germination percentage and germination rate of bush tea (*A. phylicoides*) was affected by photoperiod (light and dark) and temperature both under constant and alternate conditions. Seeds germinated better when incubated under continuous light and constant temperature than alternate conditions of both. This is related to the size of the seeds and their weight. Since small size and light seeds do not have enough stored food to support growth of the embryo and they prefer to germinate on the surface of the soil after dispersion where there is enough light.

Germination of this species is more dependent on the variation of photoperiod (light and dark) than variation in temperature. Requirement for light was reduced when seeds were incubated at 10 ^{o}C but the rate of germination was very slow. A similar case was also true when seeds were exposed to alternate light and dark periods. Under dark conditions at 30 ^{o}C there was nearly zero germination. However, when these seeds were exposed to light they started to germinate. This showed that the seed of bush tea were introduced to secondary dormancy. That is, they became photodormant (light requiring) when incubated at 30 ^{o}C.

The presence or absence of pappus during seed germination did not show any effect on germination percentage and germination rate of the seeds. This indicates that the role of pappus in this plant species is only for dispersion but has no effect on germination. Based on this investigation better germination of this species can be obtained by germinating seeds under continuous light at 20 or 25 ^{o}C constant temperature.

5.6 SUMMARY

Seed germination is the emergence of the radical through the seed coat under favourable conditions. Bush tea is commonly propagated by ripen seeds which are mostly collected at the end of summer. The germination (light and temperature) requirement of bush tea seed is not known. The aim of this study was to investigate the ideal germination temperature and light combinations for bush tea seeds. Germination of bush tea was tested at constant temperature regimes (10, 15, 20 and 25 ^{o}C) with a continuous light or dark period and at alternate temperatures (20: 20, 20:30 and 30:30 ^{o}C) with a 12L: 12D, 8L: 16D and 16L: 8D light: dark combinations respectively.

Bush tea achenes started to germinate after 6 days and germinated up to 30 days from sowing. They could germinate in alternate and constant temperatures ranging from 10 to 30^{o}C. However, there were highly significant differences ($P<0.001$) in germination percentage due to temperature treatments and the highest was 75.5% at 20 and 25 ^{o}C followed by 15 ^{o}C with 64.5% but the difference among them was not significant. Low germination percentage was recorded at 30 and 10 ^{o}C with 36 and 47% respectively. The seeds responded well to constant temperatures than alternate temperatures. Similarly, they responded positively to light ($P<0.001$) with a higher germination percentage when seeds were exposed to continuous light than alternate light: dark or continuous dark. There were highly significant differences ($P<0.001$) due to the interaction of photoperiod (light and dark) with temperature. Seeds germinated at a higher percentage with continuous light at constant temperatures than with alternated light: dark with constant temperatures. In addition, seeds of bush tea showed more difference in germination percentage to variation in light exposure than variation in temperatures.

The average number of days required for a radicle to emerge or mean germination time (MGT) was high in the low temperature (10 ^{o}C) treatment with continuous light and in alternate of both temperature and light. Germination rate was high at 20 ^{o}C with continuous light and low at

30:30 ^{o}C. On the other hand, removal of pappus did not affect germination percentage and germination rate of bush tea. Based on this investigation better germination of this species can be obtained by germinating seeds under continuous light at 20 or 25 ^{o}C constant temperature.

GENERAL DISCUSSION AND CONCLUSIONS

A study was carried out to investigate vegetative propagation of bush tea (*A. phylicoides*) by stem cutting and light and temperature requirement of bush tea seed for germination. This study was conducted at Hatfield Experimental Farm on mist bed and Department of Botany growth chambers, University of Pretoria. The investigated features were: cutting position (apical vs. basal), media (pine bark vs. sand), hormone (Seradix No. 2), season (summer, autumn, winter and spring), transplanting survival of rooted apical and basal cuttings, response of basal cuttings to three hormone concentration levels (Seradix No. 1, 2 and 3). For seed germination, continuous light, continuous dark, alternate light: dark, constant and alternate temperatures were studied.

Vegetative propagation of bush tea by stem cutting is feasible. Optimum rooting of this species can be achieved if factors that affect rooting are considered. These are, days after planting, cutting position, media, rooting hormone and season when the cuttings were taken. More rooting, high number of roots and long roots were recorded after 30 days from planting. Apical cuttings root to higher percentage than basal cuttings. The same is true for root number and root length. Differences in growing media did not show an effect on rooting percentage and root length but more roots were produced with pine bark. On the other hand, fine highly branched roots were observed when cuttings grown in pine bark and cuttings planted or propagated in sand tended to grow coarser and longer roots. Seradix No. 2 (hormone) increased root number but had no effect on root length and rooting percentage.

Higher rooting percentage was achieved in autumn and spring. However, the number of roots was higher in spring, whereas in autumn long roots were obtained. Low rooting was recorded in winter season, since the plants were at flowering stage and therefore, low physiological process. It is not recommended therefore, to take cuttings during this season. Successful rooting, higher root number as well as longer roots of bush tea can be obtained from apical cuttings taken in spring, grown in pine bark with Seradix No.2 for 30 days.

Transplanting survival of rooted cuttings was also affected by cutting position, media and hormone treatment (Seradix No. 2). Higher survival percentage was recorded with apical than basal cuttings. This was due to their ability to produce more roots as well as longer roots as compared to basal cuttings. Apical cuttings with more roots can be achieved by growing them in

pine bark with Seradix No. 2 hormone. Cuttings with a well-developed adventitious root system established more easily and successfully than those with a poor root system. Therefore, successful field and transplanting survival of bush tea can be achieved with apical cuttings and it is also important to transplant cuttings with more roots for successful establishment.

Rooting ability of basal cuttings can be improved when the right hormone concentration is used. Since bush tea in nature contains polyphenol compounds, it was sensitive to higher IBA concentrations in terms root of initiation and root number. Higher rooting percentage and more roots were obtained with Seradix No. 1 (0.1% IBA). It is also important to consider the type of rooting and growing media used. When pine bark is used as a growing medium, more roots and a higher rooting percentage can be obtained with Seradix No. 1. If sand is used as growing medium, a higher root number but with a lower rooting percentage can be achieved with Seradix No. 3.

The germination percentage and germination rate of bush tea was affected by photoperiod (light and dark) and temperature both under constant and alternate conditions. Seeds germinated better when incubated under continuous light and constant temperature than alternate of both conditions. This is related to the size of the seeds and weight. Since small size and light seeds do not have enough stored food to support growth of the embryo, they therefore, prefer to germinate on the surface of the soil after dispersion where there is enough light.

Germination of this species is more dependent on the variation of photoperiod (light and dark) than variation in temperature. Requirement for light was reduced when seeds were incubated at 10 ^{o}C but the rate of germination was very slow. A similar case was also true when seeds were exposed to alternate light and dark conditions. Under dark conditions at 30 ^{o}C, seeds germinated to nearly zero percent. But when these seeds were exposed to light, they started to germinate.

The presence or absence of pappus during seed germination did not show any effect on germination percentage and germination rate of the seeds. This indicates that the role of pappus in these plant species is only for dispersion but has no effect on germination. Based on this investigation, better germination of this species can be obtained by germinating them under continuous light at 20 or 25 ^{o}C constant temperatures.

SUMMARY

A study on bush tea (*A. phylicoides*) vegetative propagation by stem cutting and response of seeds to light and temperature difference for germination was conducted at Hatfield Experimental Farm on a mist bed and in the Department of Botany growth chambers, University of Pretoria, respectively. The effects of cutting position (apical vs. basal), media (pine bark vs. sand), hormone (Seradix No. 2) and season (summer, autumn, winter and spring) on vegetative propagation were studied. Transplanting survival of rooted apical and basal cuttings and response of basal cuttings to three-hormone concentration levels (Seradix No. 1, 2 and 3) were also studied. For seed germination, continuous light, continuous dark, alternate light: dark, constant and alternate temperatures were studied.

In vegetative propagation of bush tea stem cuttings, cutting position showed significant effect on rooting percentage, root number and root length of the cuttings. Apical cuttings rooted to a higher percentage and produced more roots as well as longer roots than basal cuttings. Pine bark improved the number of roots developed but had no effect on rooting percentage as well as on root length of the cuttings. Similarly, application of rooting hormone (Seradix No. 2) increased the root number but not the rooting percentage nor root length. Season also showed highly significant differences for rooting percentage, root number and root length. Rooting of cuttings was improved when propagated in autumn (longer roots) and spring (more number of roots) than in summer or winter.

Transplanting survival of rooted bush tea stem cuttings was investigated. It was found that there was a higher survival percentage (84.4%), more roots as well as longer roots from apical cuttings than basal cuttings (62.5%) two months after transplanting. Media and hormone application (with or without) did not influence survival percentage. However, cuttings in sand produced a higher number of roots and cuttings with hormone produced a higher root number, longer roots and longer shoot that those without hormone. In addition, apical cuttings developed more roots in pine bark with hormone application while basal cuttings produced higher a root number in sand regardless of hormone treatment. Similarly, cuttings propagated in sand with hormone and in pine bark, without hormone appilication produced longer shoots after transplanting.

Basal cuttings of bush tea did not show a good response to the application of Seradix No. 2 hormone on rooting percentage and the development of roots was slow compared to apical cuttings. Therefore to investigate this, an experiment was set up with IBA concentrations of 0.1, 0.3 and 0.8% (Seradix No. 1, 2 and 3 respectively) using pine bark and sand media. Higher number of roots was produced in pine bark with Seradix No. 2 at 10 DAP but at 15 DAP more roots were produced in pine bark with Seradix No. 1. With sand, more roots were produced when Seradix No. 3 was used as a rooting hormone than Seradix No. 1 and 2. Hormone concentration also showed differences in root number as well as in rooting percentage. Higher number of roots were produced with 0.3% IBA concentration (Seradix No. 2) and 0.1% IBA concentration (Seradix No. 1), but the differences between them was not significant. Similarly, cuttings with lower IBA concentration (0.1%, Seradix No. 1) rooted at a higher percentage (60%) followed with 0.3% IBA concentration (Seradix No. 2). Therefore, when pine bark is used as growing medium, more roots and higher rooting percentage can be obtained with Seradix No. 1. If sand is used as growing medium, higher root number but with lower rooting percentage can be achieved with Seradix No. 3.

Bush tea is commonly propagated by ripen seeds which are mostly collected at the end of summer. The germination (light and temperature) requirement of bush tea seed is not known. Germination of bush tea was tested at constant temperature regimes (10, 15, 20 and 25 ^{o}C) with a continuous light or dark and alternate temperatures (20: 20, 20:30 and 30:30 ^{o}C) with a 12L: 12D, 8L: 16D and 16L: 8D light: dark combinations respectively. The achenes started to germinate after 6 days and germinated up to 30 days from sowing. There were differences in germination percentage due to temperature treatments and the highest was 75.5% at 20 and 25 ^{o}C, followed by 15 ^{o}C with 64.5% but the difference among them was not significant. Low germination percentage was recorded at 30 and 10 ^{o}C with 36 and 47% respectively. The seeds responded well to constant temperatures than alternate temperatures. Similarly, they responded positively to light with a higher germination percentage when seeds were exposed to continuous light than alternate light: dark or continuous dark. There were also differences due to the interaction of photoperiod (light and dark) with temperature. Seeds germinated at a higher percentage with continuous light at constant temperatures than with alternated light: dark with constant temperatures. In addition, seeds of bush tea showed more difference in germination percentage to variation in light exposure than variation in temperatures.

The average number of days required for a radicle to emerge or mean germination time (MGT) was high in the low temperature (10 ^{o}C) treatment with continuous light and in alternate of both temperature and light. Germination rate was high at 20 ^{o}C with continuous light and low at 30:30 ^{o}C. On the other hand, removal of pappus did not affect germination percentage and germination rate of bush tea. Based on this investigation better germination of this species can be obtained by germinating seeds under continuous light at 20 or 25 ^{o}C constant temperature.

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APPENDIX

SUMMARY OF ANALYSIS OF VARIANCE (ANOVA)

Table A1 Analysis of variance for root number and root length of bush tea in autumn

Source of variation	DF	Mean Squares	
		Root number	Root length (cm)
Day (D)	3	10.74**	1.78*
Cutting (C)	1	1557.77**	144.33**
DxC	3	3.87**	1.23 NS
Media (M)	1	814.05**	0.51 NS
DxM	3	5.43**	1.93*
CxM	1	34.61**	11.61**
DxCxM	3	2.60*	1.05 NS
Treatment (T)	1	314.06**	1.04 NS
DxT	3	2.30*	3.09**
CxT	1	123.00**	45.80**
DxCxT	3	16.26**	1.20 NS
MxT	1	142.89**	9.60**
DxMxT	3	2.60*	1.33 NS
CxMxT	1	28.04**	0.43 NS
DxCxMxT	3	0.67NS	4.45**
Error	174	0.71	0.61

F-value significant (*), highly significant (**) or NS: not significantly different at 5% level probability

Table A2 Analysis of variance for root number and root length of bush tea in winter

Source of variation	DF	Mean Squares	
		Root number	Root length (cm)
Day (D)	3	22.47**	2.80**
Cutting (C)	1	397.05**	4.60**
DxC	3	15.72**	2.93**
Media (M)	1	68.95**	6.92**
DxM	3	3.22**	0.18NS
CxM	1	9.14**	3.43**
DxCxM	3	5.30**	0.08NS
Treatment (T)	1	75.84**	0.00NS
DxT	3	1.33NS	0.14NS
CxT	1	83.52**	1.61**
DxCxT	3	1.19NS	0.53*
MxT	1	1.54NS	5.48**
DxMxT	3	1.87*	0.18NS
CxMxT	1	11.11**	2.52**
DxCxMxT	3	8.11**	1.33**
Error	48	0.50	0.16

F-value significant (*), highly significant (**) or NS: not significantly different at 5% level probability

Table A3 Analysis of variance for root number and root length of bush tea in spring

Source of variation	DF	Mean Squares	
		Root number	Root length (cm)
Day (D)	3	113.90**	22.78**
Cutting (C)	1	2282.81**	46.68**
DxC	3	62.74**	2.15*
Media (M)	1	0.63NS	1.08NS
DxM	3	142.07**	10.04**
CxM	1	0.54NS	5.91**
DxCxM	3	50.19**	3.78**
Treatment (T)	1	2115.24**	15.10**
DxT	3	80.53**	1.18NS
CxT	1	57.27**	0.11NS
DxCxT	3	16.53**	0.86NS
MxT	1	674.06**	2.20NS
DxMxT	3	92.00**	2.05*
CxMxT	1	98.67**	0.31NS
DxCxMxT	3	14.17*	1.97*
Error	170	4.11	0.67

F-value significant (*), highly significant (**) or NS: not significantly different at 5% level probability

Table A4 Analysis of variance for root number and root length of bush tea after transplanting

Source of variation	DF	Mean Squares	
		Root number	Root length (cm)
Cutting (C)	1	39.11**	168.72**
Media (M)	1	63.38**	1.90NS
CxM	1	213.16**	0.13NS
Treatment (T)	1	60.78**	36.02**
CxT	1	7.34NS	72.43**
MxT	1	0.01NS	13.13NS
CxMxT	1	103.12**	13.13NS
Error	86	2.12	5.09

F-value significant (*), highly significant (**) or NS: not significantly different at 5% level probability

Table A5 Analysis of variance for root number and root length of bush tea basal cuttings with three level hormone concentrations

Source of variation	DF	Mean Squares	
		Root number	Root length (cm)
Day (D)	1	500.28**	4.97*
Media (M)	1	39.79**	9.59**
DxM	1	32.79**	0.00NS
Treatment (T)	2	23.39**	0.28NS
DxT	2	28.97**	0.83NS
MxT	2	189.04**	1.51NS
DxMxT	2	156.96**	1.32NS
Error	33	1.14	0.78

F-value significant (*), highly significant (**) or NS: not significantly different at 5% level probability

Table A6 Analysis of variance for mean germination time (MGT) and germination percentage of bush tea seeds due to temperature and photoperiod treatments

Source of variation	DF	Mean Squares	
		MGT	Germination %
Temperature (T)	6	206.14**	2465.47**
Photoperiod (P)	4	2655.20**	15364.75**
TxP	2	111.91**	1915.88**
Error	33	11.51	154.70

F-value significant (*), highly significant (**) or NS: not significantly different at 5% level probability