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Propagation of *Antidesma alexiteria* and *Syzygium caryophyllatum*, two underexploited fruit plants in Sri Lanka: Effect of cutting types, potting media and auxin application

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Abstract: *Antidesma alexiteria* and *Syzygium caryophyllatum* are two fruit species found in Sri Lanka of which the commercial development potential has not been fully identified. To bring these fruit species back into cultivation, establishment of suitable propagation systems for mass propagation is important. Therefore, this research was designed to identify suitable potting media, stem cutting types and requirement of auxin for successful vegetative propagation of the two fruit species. Softwood, semi-hardwood and hardwood cuttings of the fruit trees were planted in three potting media (M_1 – topsoil, sand 1:1, M_2 – topsoil, sand, compost 1:1:1 and M_3 – topsoil, sand, coir dust 1:1:1) under two treatments; T_1 – with plant growth regulator indole-3-acetic acid (IAA) and T_0 – controls without IAA. Each treatment was conducted with 15 replicates. The cuttings were planted in individual propagators and were kept in a net house of 50% shading under room temperature. Growth performance of the cuttings was monitored in regular intervals (every 28 days) for a period of six months. According to sprouting, survival, and growth performance, both softwood and semi-hardwood cuttings are suitable for vegetative propagation of *A. alexiteria*. The M_3 medium showed 100% sprouting and survival but with respect to overall growth performance, the M_1 medium was the most suitable for softwoods of *A. alexiteria* without auxin. Sprouting and survival of semi-hardwood cuttings were 100% with high values of growth parameters in M_3 under T_1 treatment. The M_1 medium without auxin can be recommended for better performance of softwoods while $M_3$with auxin is for semi-hardwoods of *A. alexiteria*. Softwoods, semi-hardwoods and hardwoods can be recommended for vegetative propagation *S. caryophyllatum* in M_1 medium. Both softwood and semi-hardwood cuttings under T_1 treatment expressed higher sprouting and survival percentages while semi-hardwood cuttings under T_1 treatment have shown the highest sprouting and growth performance. Hence, semi-hardwood cuttings with auxin can be considered as the best planting material for vegetative propagation of *S. caryophyllatum*while M_1 is the best medium. The vegetative propagation of *S. caryophyllatum* and *A. alexiteria* through stem cuttings can be recommended as suitable mass propagation method for the purpose of commercialization.

Keywords: *Antidesma alexiteria*, Auxin, Potting media, Stem cuttings, *Syzygium caryophyllatum*

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Introduction

Underutilized fruits can be described as "species with under-exploited potential". These species were grown widely in the past, but are less consumed nowadays due to various agronomic, genetic, economic and cultural factors (Padulosi & Hoeschle-Zeledon, 2004). They can play a significant role in food security, health, income generation and environmental conservation (Mitra et al., 2011). Currently more than 60 underutilized fruit crop varieties are identified in Sri Lanka (Dahanayake, 2015). These plants grow naturally in wild habitats, and they have the potential for commercialization because most of the tropical fruits are well known for having high nutritive values and medicinal properties (Khoo et al., 2016). With urbanization, land degradation and deforestation some of those valuable fruit crop species are critically endangered and facing the state of speedy disappearance from wild habitats (Dahanayake, 2015). In addition, research on propagation techniques suitable for mass propagation of underutilized fruit plants, which is essential for their commercialization, is also lacking (Singh et al., 2021).

Most of the fruit crops have a heterozygous genotype, therefore vegetative propagation is the scientifically proven best method to obtain uniform produces (Sanjay & Singh, 2006; Singh & Singh, 2007). Mostly, unavailability of clonal planting material of a recommended cultivar is the main barrier for the cultivation and popularization of the underutilized fruit crops. Vegetative propagation of plants through stem cuttings could overcome the limitations of propagation through seeds, such as changes of superior characters, seed dormancy and poor viability of the seeds with lengthy time for maturation (Somashekar & Sharma, 2002). The plants, which are raised through vegetative stem cuttings genetically resemble the mother plant and help in maintaining the essential qualities (Henrique et al., 2006). However, vegetative method for commercial multiplication has not been standardized for many underutilized fruits (Ghosh & Bera, 2015).

Syzygium caryophyllatum and *Antidesma alexiteria* are two underutilized fruits crops found in Sri Lanka. *S. caryophyllatum* is a tropical fruit plant belongs to family Myrtaceae and found in intermediate and wet low lands in Sri Lanka. *A. alexiteria* is a plant of family Phyllanthaceae, naturally grown in dry and wet woodlands in low country of Sri Lanka (Corner & Ashton, 1977). Even though fruits are rich in nutrients, these plants species are rarely found in home gardens and in the market. Their commercial development potential has not been fully identified, hence, promoting the cultivation of these fruits will be useful for better utilization of these species.

In vegetative propagation, sections of stems, leaves and roots are used from the mother plant and plant growth promoters like indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) are applied to the propagules to induce the formation of adventitious roots or shoots under controlled environmental conditions (Leakey & Simons, 2000; Hartmann et al., 2002; Fowler & Chaffee, 2010). In this process, softwood, semi-hardwood or hardwood stem cuttings could be used and it may vary with respect to the nature of chosen plant species. Softwoods are present at the tip of the branches and are green in colour with soft and succulent tissues and tender small leaves. They are delicate and sensitive to drying out. Partially matured, slightly woody cuttings with mature leaves are known as semi-hardwoods whereas brown, fully matured woody cuttings with fully grown leaves are known as hardwoods (Kontoh, 2016). The present study aimed to establish vegetative propagation systems suitable for two underutilized fruits grown in Sri Lanka; *Antidesma alexiteria* and *Syzygium caryophyllatum*. Vegetative propagation potential of these selected underutilized fruit crops using stem cuttings were evaluated as that can easily be used for large-scale production.

Methods

Sampling of plant materials

The experiment was conducted in a shade house (50% shading) located in the Gampaha district (7.13714° N, 80.04748° E), Sri Lanka. Stem cuttings; softwood, semi-hardwood and hardwood cuttings were selected as planting materials from naturally grown, young and healthy plants, for the research. Plants in flowering and fruiting stages were not selected to obtain cuttings. Healthy shoots were collected from the selected fruit plants growing in the wild habitats and were immediately placed in a water-filled container to prevent cavitation and dehydration and transported to the shade house. Softwood, semi-hardwood and hardwood cuttings were selected and they were trimmed from the shoots using a sharp blade. Average lengths of softwood and semi-hardwood cuttings were 12 cm with 3–4 nodes and the average length of hardwood cuttings were 15 cm with 4–5 nodes. The trimmed cuttings were immediately placed in a container filled with water, separately, to avoid desiccation. In softwood and semi-hardwood cuttings 1/4 of the top two leaves were trimmed and all the other leaves were removed. All the leaves of the hardwood cuttings were removed except for *S. caryopyllatum* (Fig. 1). Bottom ends of the cuttings were cut as slant ends and were briefly immersed in a fungicide solution (Captan).

Fig. 1. Softwood, semi-hardwood and hardwood stem cuttings of *A. alexiteria* (A, B, C) and *S. caryophyllatum* (D, E, F)

They were then kept under shade for 2–3 minutes and thereafter commercially available plant growth regulator (IAA, powdered form, Uni power Pvt Ltd, Sri Lanka) was applied at the bottom end of the stem cuttings. A set of similar stem cuttings were used as the controls without the treatment of IAA. Bottom ends of the cuttings were buried up to approximately 5 cm in the potting mixture. Individual propagators (Fig. 2) were made by covering the planted stem cuttings with transparent polythene and were placed in the shade house (50% shade) under room temperature.

Fig. 2. Individual poly-propagator

Preparation of potting media: topsoil + sand M_1 (1:1,), topsoil + sand + compost M_2 (1:1:1) and topsoil + sand + coir dust M_3 (1:1:1)

Stem cuttings were planted in black polythene bags (15 cm width × 20 cm height). Three potting media were selected for planting the stem cuttings using different compositions of four potting ingredients; topsoil, sand, compost and coir dust. Prior to the experiment all the potting media were soil solarized and topsoil and sand were sieved through a mesh to remove gravel. Three types of potting mixtures were prepared from the four potting ingredients; topsoil + sand (1:1), topsoil + sand + compost (1:1:1) and topsoil + sand+ coir dust (1:1:1). Polythene bags were filled up to 12–15 cm from the bottom with different potting media and watered prior to planting of the stem cuttings.

Experimental design

The experiment was conducted according to a randomized complete block design. The main plot factor was the type of potting medium (topsoil + sand, topsoil + sand + compost, topsoil + sand + coir dust). The sub plot factor was the plant growth regulator (T) (treated with plant growth regulator and control). The type of the stem cuttings was considered as the sub-sub plot factor. A total number of 270 stem cuttings of each species were grown under 18 treatments (Table S1) with 15 replicates for each treatment.

Nursery management practices

All propagators were observed daily and dead cuttings were removed. The fungicide (Captan) was applied to the stem cuttings from the second month, until the fourth month in two-week intervals to prevent

fungal diseases. The polythene covering of the stem cuttings with newly formed buds were removed after three months of planting. They were watered in two-day intervals and the weeds were removed.

Data collection and analysis

The numbers of sprouted and survived cuttings per treatment and the number of shoots, shoot length and the number of leaves per cutting were measured every 28 days for six months. After six months, three plants were randomly selected from each treatment and the length of the roots and the number of roots per cutting were recorded by considering the tap root and lateral roots that are longer than 0.5 mm. The length of the longest root of each plant was measured and the length of the longest root was calculated for each treatment. Fresh weight of plants, shoots and roots, and dry weight (kept 48 hours at 80 °C oven) of shoots and roots of the uprooted plants were measured. The percentages of sprouting and survival per treatment were calculated using the equations given below.

$$\text{Sprouting Percentage} = \frac{\text{Number of sprouted cuttings after 6 months}}{\text{Total number of cuttings per treatment}} \times 100$$

$$\text{Survival Percentage} = \frac{\text{Number of survived cuttings after 6 months}}{\text{Total number of cuttings per treatment}} \times 100$$

Data were analyzed using the Minitab 17 software. The mean values of number of shoots, number of leaves, shoot length, root length, the length of the longest root, number of roots and fresh and dry weights of plants, shoots and roots of the cuttings were subjected to Two-way Analysis of Variance (ANOVA, 0.05 probability) and Tukey's pair wise comparison to find the significant differences among the means of the treatments.

Results

A. alexiteria

Softwood and semi-hardwood cuttings of *A. alexiteria* were able to propagate through M_1, M_2 and M_3 potting media (Fig. 3). None of the hardwood cuttings of *A. alexiteria* perform successfully in the evaluated vegetative propagation methods (sprouting and survival 0%). The highest sprouting and survival

Fig. 3. Growth of shoots and roots of *A. alexiteria* softwood and semi-hardwood cuttings, after 6 months in M1, M2 and M3 media with and without IAA

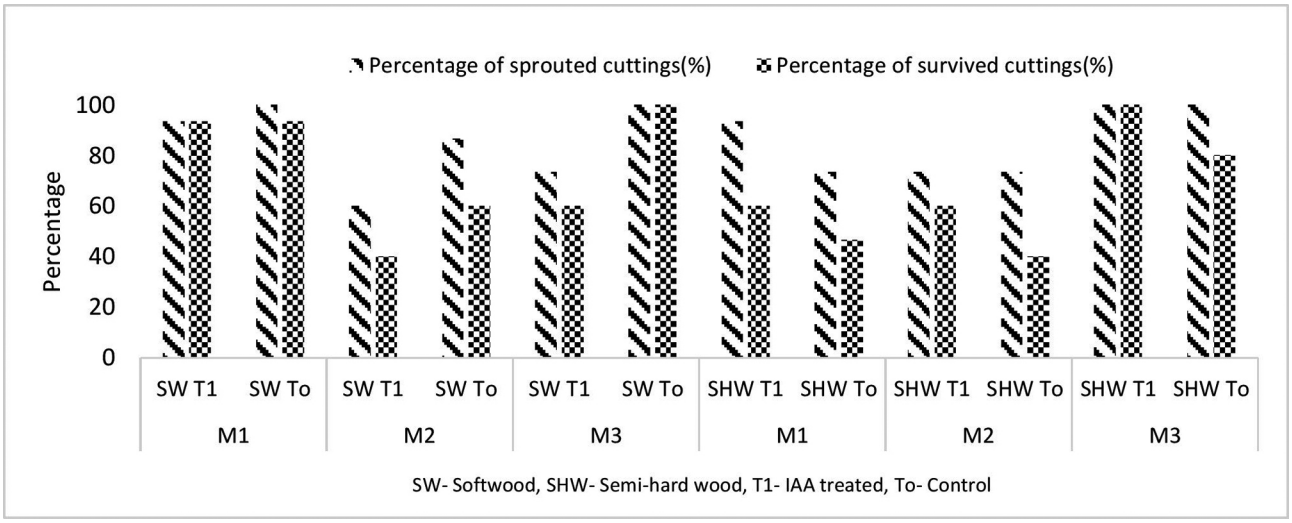

Fig. 4. Sprouting and survival of softwood and semi-hardwood stem cuttings of *A. alexiteria* in topsoil + sand, M1 (1:1,), topsoil + sand + compost, M2 (1:1:1) and topsoil + sand + coir dust M3 (1:1:1), with IAA treatment (T1) and without IAA (To)

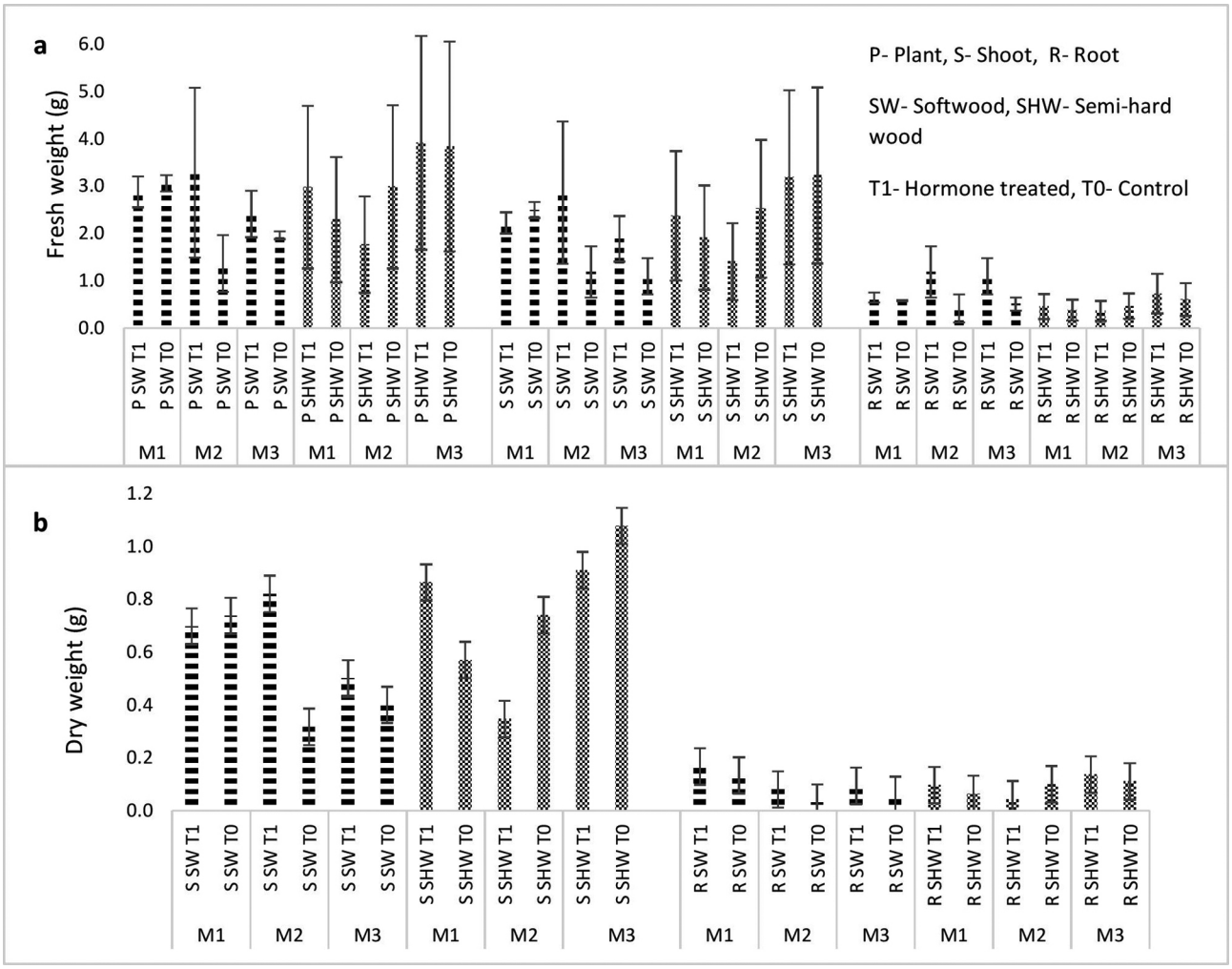

Fig. 5. (a) Fresh and (b) dry weights of softwood and semi-hardwood stem cuttings of *A. alexiteria*. Softwood and semi-hardwood cuttings were statistically analyzed separately for fresh and dry weights. Among the treatments (interaction between potting medium and auxin application) there was no significant difference in fresh and dry weights of wood cuttings. Each treatment consisted of three replicates. IAA treatment (T1) and without IAA (To)

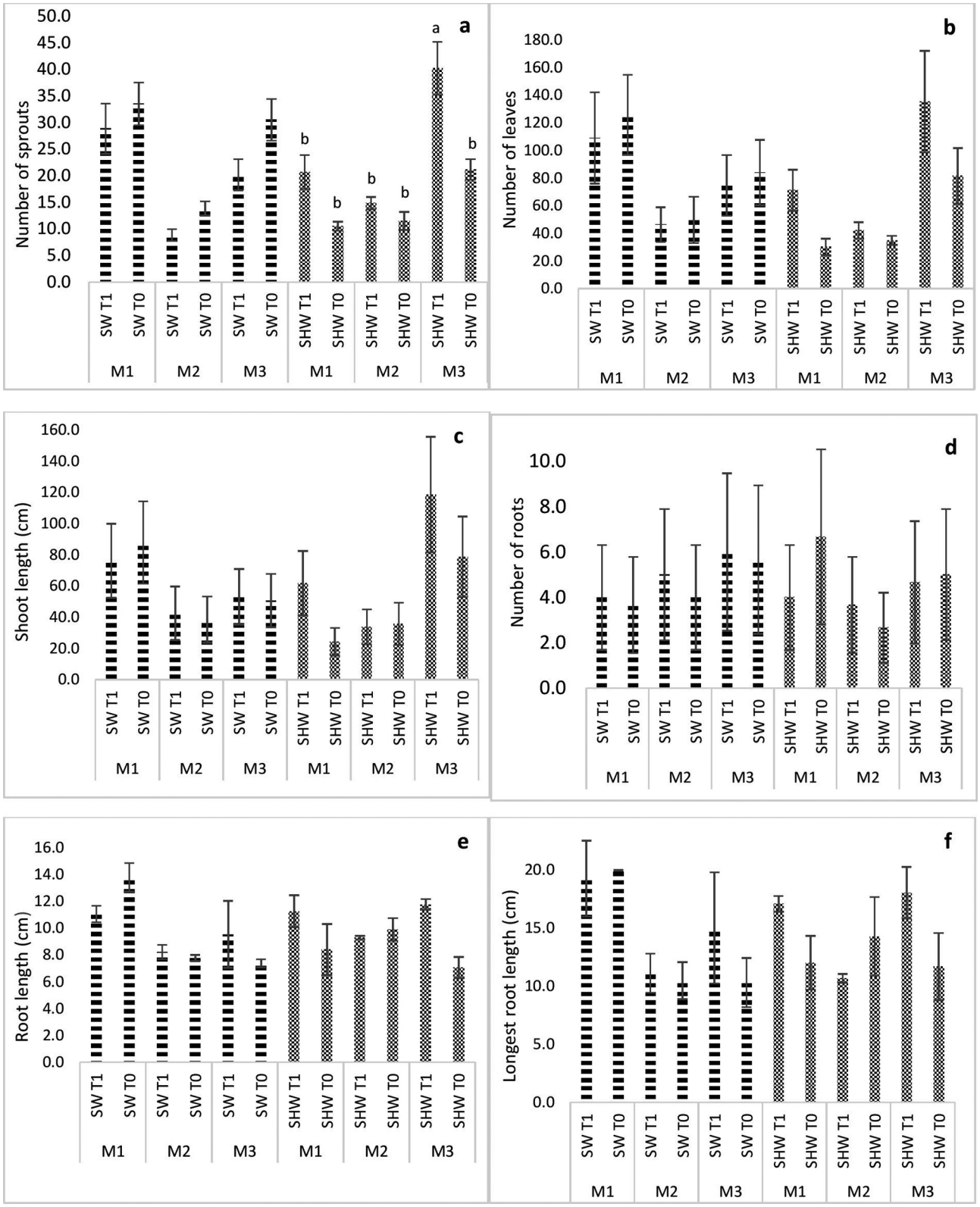

Fig. 6. Number of sprouts (a), number of leaves (b), shoot length (c) number of roots (d), root length (e) and the length of the longest root (e) of softwood and semi-hardwood cuttings of *A. alexiteria*. Softwood and semi-hardwood cuttings were statistically analyzed separately for each growth parameter. Number of sprouts of semi-hardwood cuttings were significantly different among treatments. Means with the same letters are not significantly different at $P<0.05$ in Tukey's test for interaction of two factors (potting medium and auxin application). Panels a, b and c – Each treatment consisted of 15 replicates. Panels d, e and f – Each treatment consisted of three replicates

percentages (100%) for softwood cuttings were spotted in M_3 medium followed by softwood cuttings in M_1medium, both without auxin application (100% sprouting and 93.3% survival). The lowest values for sprouting (60%) and survival (40%) percentages were recorded from auxin treated softwood cuttings in M_2 medium. Semi-hardwoods of *A. alexiteria* marked the highest sprouting and survival percentages (100%) from M_3 medium with auxin and the lowest sprouting (73.3%) and survival percentages (40%) from M_2 medium without auxin (Fig. 4).

All evaluated growth parameters of softwood cuttings were not significantly different ($P > 0.05$) according to the potting medium and auxin application(-Fig. 5&6). Even though M_3 potting medium showed 100% sprouting and survival, with respect to the overall growth performance of softwoods, the highest values for the growth parameters were recorded from the control cuttings grown in M_1 medium. Therefore, potting medium of topsoil and sand (M_1) 1:1 can be considered as the most suitable potting mixture for softwoods of *A. alexiteria* without auxin (Fig. 7).

The number of sprouts produced by auxin treated semi-hardwood cuttings grown in M_3medium was significantly higher than the cuttings of the other treatments. None of the other growth parameters of semi-hardwood cuttings show a significant difference ($P > 0.05$) with the interaction of potting medium and auxin application. Semi-hardwood cuttings grown in M_3 medium with auxin, which recorded 100% sprouting and survival, showed the highest values for most growth parameters (Fig. 5 & 6). Therefore, the potting medium of topsoil, sand and coir dust (M_3) 1:1:1 with the auxin application could be considered as the best medium for propagation of semi-hardwood cuttings of *A. alexiteria* (Fig. 8).

Fig. 7. Growth of *A. alexiteria* softwood cuttings without IAA, in M1 medium (3–6 months)

Fig. 8. Growth of *A. alexiteria* semi-hardwood cuttings in M3 medium with IAA (3–6 months)

S. caryophyllatum

Softwood, semi-hardwood and hardwood cuttings of *S. caryophyllatum* were successfully propagated only in the M_1 potting medium. M_2 and M_3 media were not suitable for vegetative propagation of *S. caryophyllatum* by stem cuttings. Semi-hardwood cuttings with auxin treatment have shown the highest sprouting (93%) and survival (87%) percentages followed by softwood cuttings (80% sprouting and 67% survival). The lowest sprouting and survival percentages were spotted from hardwood cuttings under auxin treatment (Fig. 9).

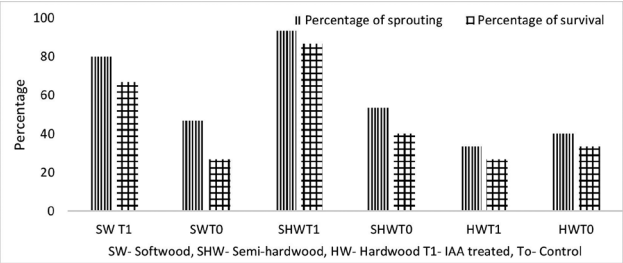

Fig. 9. Sprouting and survival of softwood, semi-hardwood and hardwood stem cuttings of *S. caryophyllatum* in M1 medium with and without IAA, after 6 months

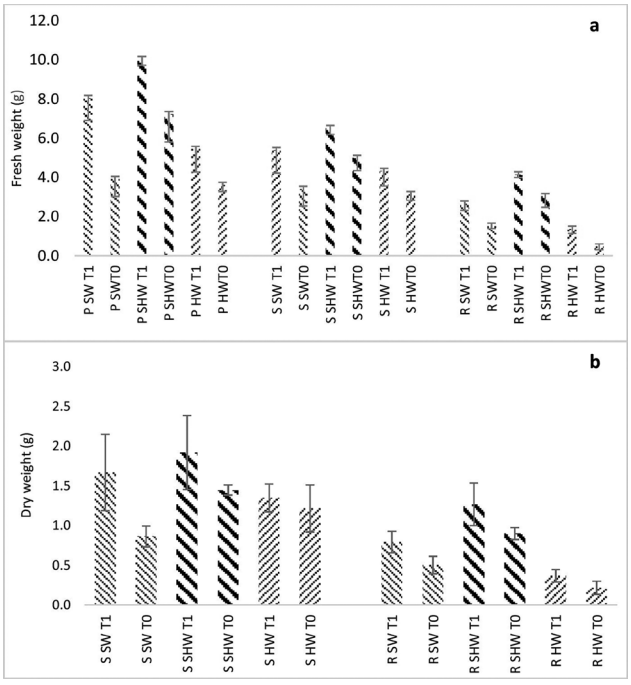

Fig. 10. Fresh weights (a) and dry weights (b) of softwood, semi-hardwood and hardwood stem cuttings of *S. caryophyllatum* grown in M1 medium, with IAA (T1) and without IAA (To) application. Softwood, semi-hardwood and hardwood cuttings were statistically analyzed separately for fresh and dry weights. Among the treatments (interaction between potting medium and auxin application) there was no significant difference in fresh and dry weights of wood cuttings. Each treatment consisted of three replicates. T1: IAA treatment and To: without IAA

All growth parameters except the number of sprouts were not significantly different($P < 0.05$) with the interaction of stem cutting type and the auxin application. The treatment with the highest sprouting and survival percentages; the auxin treated semi-hardwood cuttings, produced the maximum number of sprouts and leaves and the longest shoots. Similarly, when the root parameters were compared, the highest number of roots and the longest roots were produced by semi-hardwood cuttings treated with auxin (Fig. 10 & 11). The highest fresh and dry weights of the plants were also recorded from this treatment. Hence semi-hardwood cuttings with auxin application can be considered as the best planting material for vegetative propagation of *S. caryophyllatum* whereas M_1topsoil:sand (1:1)is the best potting medium (Fig. 12).

Discussion

Generally, vegetative propagation methods are used in the tropics for the establishment of commercial plantations of timber and fruit tree species (Ngwuli et al., 2019). Compared with the other vegetative propagation techniques, use of cuttings as planting material, can be considered as an inexpensive, rapid, efficient and the simplest method of plant propagation (Leaky, 2014). Cuttings are commonly used for large-scale production of plants with superior genotypes (Abdullah et al., 2006; Singh et al., 2012).

The suitability of the medium depends on the species, type of cutting, season, propagation system used as well as cost and availability of the medium components (Macdonald, 1986; Hartmann et al., 2002; Ýsfendiyaroðlu et al., 2009). An ideal rooting medium provides sufficient porosity to allow good aeration, has a good water holding capacity, well drained and free from fungi and bacteria (Hartmann et al., 1997). Therefore, the potting mixtures selected for this study consisted of sand to provide aeration and compost or coir dust as a water holding medium (Leakey, 2014; Kontoh, 2016). *A. alexiteria* stem cuttings successfully propagated in M_1, M_2 and M_3 potting media while *S. caryophyllatum* stem cuttings propagated only in M_1 potting medium. M_2 medium contained compost which is rich in nutrients and the results of *A. alexiteria* softwood and semi-hardwood cuttings showed poor growth performance in this medium. *S. caryophyllatum* also failed to grow in M_2 medium. Few numbers of softwood, semi-hardwood and hardwood cuttings of both species planted in M_3 medium showed the emergence of buds but perished within 3 to 7 days. These results indicated that a potting medium rich in nutrients was not suitable for the growth of *A. alexiteria* and *S. caryophyllatum* and

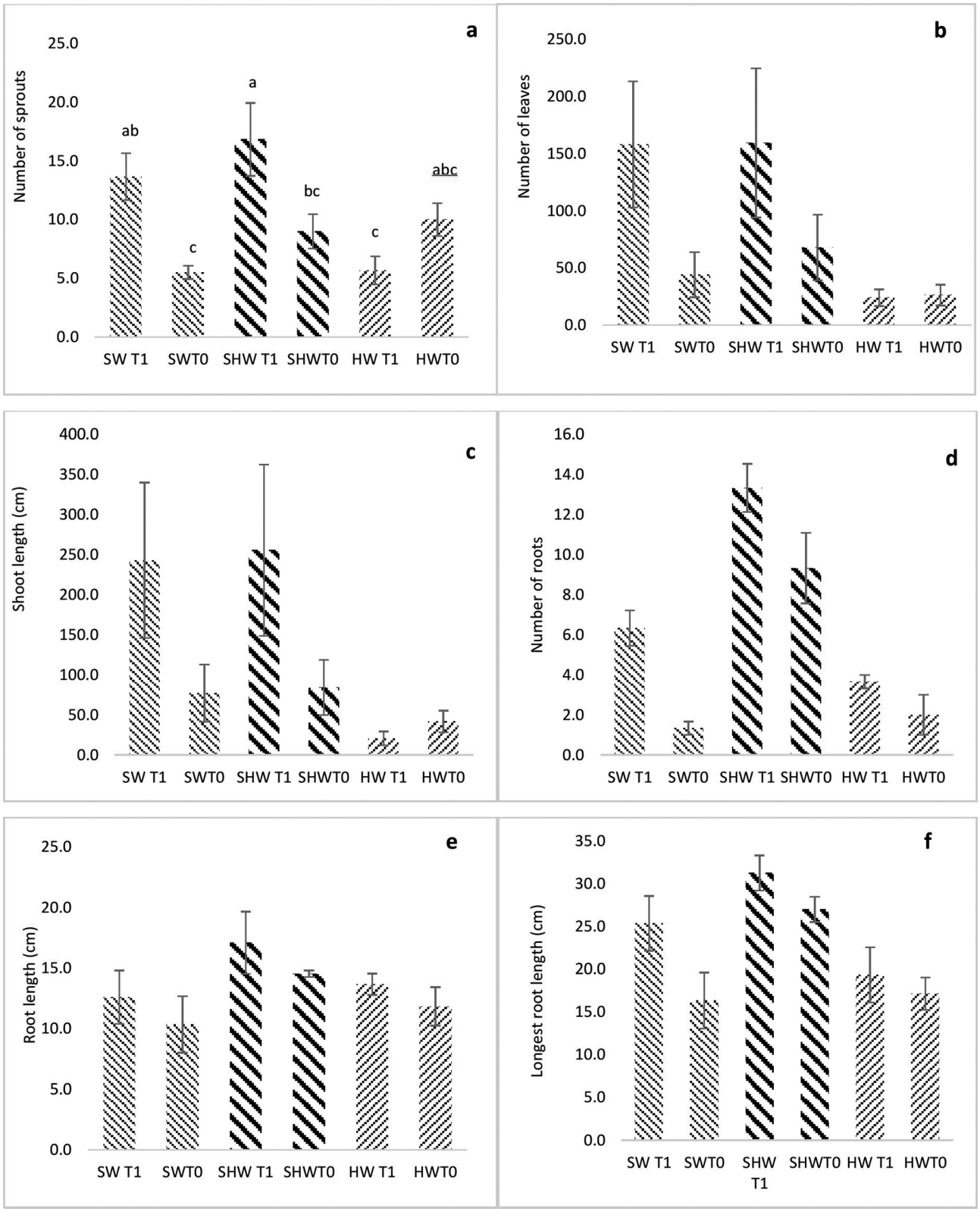

Fig. 11. Number of sprouts (a), number of leaves (b), shoot length (c) number of roots (d), root length (e) and the length of the longest root (f) of softwood, semi-hardwood and hardwood stem cuttings of *S. caryophyllatum*. Means with same letters are not significantly different at $P<0.05$ in Tukey's test for interaction of two factors (stem cutting type and auxin application). Panels a, b and c – Each treatment consisted of 15 replicates. Panels d, e and f – each treatment consisted of three replicates. T1: IAA treatment and To: without IAA

Fig. 12. Growth of *S. caryophyllatum* semi-hardwood cuttings in M1 medium with IAA (3–6 months)

such media could be successful in the marginal lands with low nutrients. *Voacanga africana* indigenous tree in Ghana developed more sprouts and roots in a mixture of topsoil and sand than other soil media used (Kontoh, 2016). Rooting media with excessive amounts of fertilizers damaged or inhibited the formation of new roots (Relf & Ball, 2009). Similarly in the present study, all three potting media contained topsoil. Topsoil is rich in organic matter. M_3 medium with topsoil and coir dust is moderately rich in nutrients and fertility of M_2 medium with topsoil and compost must be too high, hence it was not a successful potting mixture for both *A. alexiteria* and *S. caryophyllatum*.

Based on the results of the present study, *A. alexiteria* can be propagated through softwood and semi-hardwood stem cuttings and *S. caryophyllatum* can be propagated through softwood, semi-hardwood and hardwood stem cuttings. Sprouting and rooting of wood cuttings depend on the type of the cutting used for propagation and leafy softwood cuttings are the best for vegetative propagation as they are dependent on current photosynthates for rooting (Leakey, 2014; Kouakou et al., 2016). The results also proved that leafy stem cuttings are more suitable for vegetative propagation. Both *A. alexiteria* and *S. caryophyllatum* showed better sprouting and survival percentages and growth performances from soft and semi-hardwood cuttings than hardwoods. The stem cuttings of juvenile stages have less lignified tissues and synthesize a smaller number of rooting inhibitors compared to the stem cutting of mature stages of plants (Leopold, 1975; Hartmann et al., 1997; Castellanos-Castro & Bonfil, 2013). Hence juvenile shoot materials cultivated had found to be conducive to good rooting in enclosed humid environment, low temperature and radiant energy (Leakey, 1983; Newton & Jones, 1993; Mesén et al., 1997). All the hardwood cuttings of *A. alexiteria* used for the study died within the first month. Generally, hardwood cuttings have shown poor propagation ability due to the presence of rooting inhibitors and leafless hardwood cuttings may have to depend on the carbohydrates stored within the stem tissues (Dao et al., 2020).

Auxin is a growth promoting hormone which stimulates cell division, cell elongation and cell differentiation. Development of shoots and roots depends on the application of exogenous Indole Acetic Acid (IAA), Indole Butyric Acid (IBA), Naphthalene Acetic Acid (NAA) and 2,4-Dichloropheoxy Acetic Acid (2,4-D) (Schroeder & Walker, 1990; Quainoo et al., 2014; Jan et al., 2015). The responses to different types and concentrations of auxins vary among plant species and are affected by genotypes (Guo et al., 2009). The softwood and semi-hardwood cuttings of guava cv. '*Safeda*' have shown the highest sprouting and survival percentages with IAA compared to NAA and IBA (Zamir et al., 2017). The effect of various concentrations of IBA and NAA on rooting of hardwood stem cuttings of *Morus alba* L. was tested by Singh et al., (2014) and IBA (2000 ppm) was found to be the most effective for rooting. The auxin type used in the present research was IAA which was available commercially to the growers.

In this study, individual poly-propagators were used as they are simple to build and use. They are designed to reduce the post-severance physiological stress (wilting and leaf abscission) that results from water loss through transpiration, by keeping the cuttings cool, moist, and turgid and enable rooting

Table 1. Cost of production of one plant

Plant species	Best stem cutting type, potting medium and requirement of auxin	Cost of propagating one plant (USD)
Antidesma alexiteria	Softwood, M_1, without auxin	Less than 0.14 USD
	Semi-hardwood, M_3, with auxin	About 0.17 USD
Syzygium caryophyllatum	Semi-hardwood, M_1, with auxin	About 0.14 USD

(Leakey, 2014). The cost of propagation of one plant can be calculated as in Table 1. These underutilized fruits can be propagated easily using poly-propagators as it is not expensive. Farmers can sell the plants for a fair marketable price. Findings of this study can play key role in rapid supply of quality planting material for commercialization. It will help to ensure food security and nutrient deficiencies among people who live in developing tropical countries like Sri Lanka which is susceptible to climatic changes and natural disasters.

Conclusions

The methods for vegetative propagation of the two underutilized fruit crops; *A. alexiteria* and *S. caryophyllatum* were recognized for mass propagation using stem cuttings. M_1 potting medium (topsoil + sand 1:1) was more suitable for softwoods of *A. alexiteria* without auxin. M_3 medium(topsoil + sand + coir dust 1:1:1) with auxin application was the best treatment for propagation of semi-hardwood cuttings of *A. alexiteria*. *A. alexiteria* can be propagated even without auxin at farmer level with a low cost using softwood stem cuttings. Semi-hardwood cuttings were the best cutting type for the vegetative propagation of *S. caryophyllatum*, with auxin treatment and the best potting medium for *S. caryophyllatum* was M_1 (topsoil: sand 1:1).

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