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Determining the Performance of Plantation Grown Young *Santalum album* L. with Different Host Species

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Abstract

Sri Lanka is one of the pioneer countries to adopt the green economy concept and thereby promotes the establishment of forest plantations with the trees of high value. The essential oil produced in the heartwood of *Santalum album*, native to India, Indonesia and Sri Lanka fetches a very high price and it has been used for perfumery, medicinal, religious and cultural purposes over centuries of years. Due to the over-exploitation, *S. album* resource in the wild is diminishing and therefore the government implemented strict laws to protect it. Although plantation establishment has been recently started, management became difficult due to lack of technical information. *S. album* is an obligate hemi-parasite, and obtains certain nutrients from potential host trees via root connections called haustoria. Therefore identifying the most suitable host species in *S. album* plantation establishment is essential. In order to test the impacts of different host species, a field trial of two hosts per *S. album* was established and maintained for three years for this study. *S. album* growth under 21 combinations of six host species including legumes (*Acacia auriculiformis*, *Calliandra calothyrus*, *Gliricidia sepium*, *Sesbenia grandiflora*) and non-legumes (*Coffea arabica*, *Gravellia robusta*) were examined. Height of *S. album* was measured at two week intervals. Leaf nitrogen, phosphorous, potassium and magnesium of *S. album* grown with 21 host combinations were also analyzed at regular intervals. Height of *S. album* was significantly different when planted with different hosts. *Calliandra calothyrus* was found to be the best host species in increasing height of *S. album*. It was followed by *Acacia auriculiformis* and *Sesbenia grandiflora*. The nitrogen, phosphorus, potassium and magnesium contents of *S. album* leaves were not significant when planted with the tested host combinations. Therefore the significant height growth of *S. album* could be caused due to the absorption of water at different levels from different host species.

Keywords: Santalum album, obligate parasitism, host species, haustoria, nutrient uptake

1. Introduction

Many species of the genus *Santalum* of family Santalaceae produces a highly fragrant essential oil that can be extracted from the heartwood. This oil has been used for perfumery, medicinal and cultural purposes over centuries of years (Joulain, et al., 2000). The aroma of the oil and the wood is also used by people belonging to three major religions of the world, i.e., Hinduism, Buddhism and Islam (Kumar et al., 2012). The heartwood itself is also used for carvings and making incense sticks known as agarbhatti (Fox, 2000).

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Although the sapwood of *S. album* is white or yellow and not scented, it is also used in preparing turnery items (Kumar et al., 2012) and manufacturing of incense sticks, toys and souvenirs. However, the oil produced by *Santalum album* L., native to India, Indonesia and Sri Lanka is known to have the best quality than oils extracted from any other *Santalum* species. Therefore, *S. album* is regarded as one of the most valuable trees in the world (Sundararaj and Muthukrishnan, 2011) and it can bring promising economic prospects for the countries through properly managed plantations.

S. album is a small, evergreen tree which can grow to a height of 20 m and attain a girth of over 1.5 m. It grows at a slow rate (Brand et al., 2012) and the average annual growth rates of diameter and height in Sri Lanka are approximately about 0.3 to 1.0 cm and 0.5 to 1.0 m respectively at the young stage (Subasinghe et al., 2013). The trees in natural forests of Karnataka, India shows even much lesser diameter increment averaging to about 0.3 cm per year (Rai, 1990).

S. album is an obligate hemi-parasite, so that it is capable of obtaining certain nutrients from the host trees via root connections known as haustoria (Brand et al., 2004; Fox, 2000). Structurally, hosts also provide protection from sun, wind and grazing. It is believed that nutrients such as calcium and iron (Rao and Bapat, 1995), nitrogen and phosphate (Barrett, 1998), potassium and magnesium (Rangaswamy et al., 1986) are supplied by the hosts to *S. album*. The presence of basic amino acids indicates that leguminous plants are more efficient hosts for sandalwood growth than non-leguminous plants. According to Radomiljac et al. (1998) and Nagaveni and Vijayalakshmi (2003), the growth performances of *S. album* vary when planted with different leguminous species. However, there was no considerable variation in the micronutrient contents of *S. album* due to the presence or absence of host species (Rocha et al., 2014). Although *S. album* is capable of making haustoria with a wide variety of plant species, a comprehensive understanding of suitable host species in Sri Lankan context is still lacking. Determination of suitable host species plays a vital role in the establishment of plantations to maximise the commercial benefits.

Natural sandalwood resources are diminishing in all countries irrespective of the species and therefore, strict rules were implemented over harvesting and exporting sandalwood products. However, this may have severely reduced the sandalwood product supply to the market causing rapid increase of price over a short period of time.

In Sri Lanka, *S. album* has been and harvested from the wild for traditional medicine since the known history (Subasinghe, 2013). Before a few decades, this species had been commonly found in the drier areas of the hilly intermediate zone of Sri Lanka. It was also found in smaller numbers in many other areas belonging to different climatic zones throughout the country. Due to the high value of heartwood and oil, this species has been illegally harvested in large scale and therefore has been listed as a protected species in Sri Lanka under the Flora and Fauna Act of 1964 and again with the recent amendment of the year 2009 (Fauna and Flora Protection Amendment Act No. 22, 2009). Despite with all the protection efforts, illegal harvesting of *S. album* still continues and therefore it is becoming scarce even in the hilly intermediate zone of the country where *S. album* had been commonly found in the past. Commercial plantation establishment can, however, be one of the most effective ways to protect the *S. album* resource exist in the wild. *S. album* plantation establishment has recently been started in Sri Lanka with the involvement of the private sector. However, the lack of technical knowledge at the local conditions has become a major barrier for the management of those plantations. Therefore the present study was conducted with the objective of identifying the most suitable host species for the young stage of *S. album* grown as plantations in Sri Lanka.

2. Materials and methods

2.1 Establishment of the host trial

A field trial of two host species per *S. album* plant was established in May, 2011 at the location called Amupitiya (6º40/51// N, 80º43/31// E) close to Balangoda town of Rathnapura administrative district in Sri Lanka in May 2011 to test the impact of different host species. Climate of Amupitiya lies in between wet and intermediate. Average annual rainfall of this area is >1,270 mm and average temperature varies from 25° to 30° C. There are about five dry months prevail per year. Elevation of the research site is 590 m above the mean sea level. Soil type of Balangoda area is reddish-brown latosols and the terrain is steeply dissected, hilly rolling and undulating (Somasekaran, 1997).

S. album seeds were collected from healthy mother trees in March 2011 and those were pooled and germinated in a nursery. Once the germinated *S. album* seedlings produced 4-6 leaves, those were transplanted into 17×30 cm polythene bags filled with top soil (1): compost (1) and sand (1). *Alternanthera variegata*, a small plant of Family Amaranthaceae, has been recorded as the most commonly used host species for *S. album* when grown in pots at the nursery stage (Annapurna et al., 2006; Loung, 2008). Therefore one month before transplanting of *S. album* seedlings, two cuttings of *A. variegata* were planted as seedling hosts in the polythene pots. After transplanting, those pots were kept in a 70% shade house until transported to the selected site for filed planting.

Based on the literature (Radomiljac et al., 1998; Nagaveni and Vijayalakshmi, 2003) priority was given to the legumes in selection of the suitable host species for this study. Following a preliminary survey conducted by the authors of this paper, six multi-purpose plant species (Table 1) were selected as suitable hosts based on their high survival rate and the ability of growing in different climatic conditions. Other than legumes, *Coffea arabica* (Family: Rutaceae) was selected as a suitable agroforestry species and *Gravellia robusta* (Family: Proteaceae) was selected due to the timber value. Due to the recognised values as timber, fuel or beans, all selected species can successfully be used in agroforestry systems with *A. album*.

Seedlings of the selected host species were raised in the same nursery where *S. album* seedlings were raised. Those host species were planted in the field six months, i.e., May 2011 before planting *S. album* seedlings. The early planting of the host species provided adequate time for the root growth so that young *S. album* plants were able to form haustoria within a short time period.

Table 1: Host species selected for the host trial of the present study.

Species	Code	Family	Use
Acacia auriculiformis	*Aa*	Fabaceae	Timber, firewood
Calliandra calothyrus	*Cc*	Fabaceae	Fodder, firewood
Coffea arabica	*Ca*	Rutaceae	Coffee beans
Gliricidia sepium	*Gs*	Fabaceae	Fodder, mulch, firewood
Gravellia robusta	*Gr*	Proteaceae	Timber, firewood
Sesbenia grandiflora	*Sg*	Fabaceae	Food, fodder, firewood

Planting holes were marked for *S. album* seedlings at 4 m distances in the selected field. Then two hosts of various combinations were planted along north-south direction at 1.2 m away from the marked hole for *S. album*. Altogether, 21 combinations of host species were used as *Aa*×*Aa*, *Aa*×*Cc*, *Aa*×*Ca*, *Aa*×*Gs*, *Aa*×*Gr*, *Aa*×*Sg*, *Cc*×*Cc*, *Cc*×*Ca*, *Cc*×*Gs*, *Cc*×*Gr*, *Cc*×*Sg*, *Ca*×*Ca*, *Ca*×*Gs*, *Ca*×*Gr*, *Ca*×*Sg*, *Gs*×*Gs*, *Gs*×*Gr*, *Gs*×*Sg*, *Gr*×*Gr*, *Gr*×*Sg*, *Sg*×*Sg*. Eight replicates were used for each combination and *S. album* was introduced six months after planting the host combinations, i.e., December 2011. This trial was maintained for three years until December 2014.

2.2 Measurements

Height of *S. album* plants was measured at two-week intervals for three years. *S. album* leaves were collected at six month intervals. Total nitrogen, phosphorous, potassium and magnesium contents per 1 g of oven dried *S. album* leaves were analysed to examine the nutrient supply of different host spices. Once the leaves of *S. album* were collected at six month intervals, those were air dried to remove the excess moisture and then oven dried at 72° C for 48 hours before analysis. Total nitrogen content was determined using Micro-Kjeldhal method. Potassium and magnesium levels were determined using Atomic Absorption Spectroscopy and phosphorous content was analysed by colorimetric determination using a Spectrophotometer.

The difference of the height growth of *S. album* plants grown with different host combinations were analysed by one-way ANOVA using MINITAB© 14 software. One-way ANOVA was used to identify the differences of leaf nutrient levels determined for 1 g of oven dried leaf samples.

3. Results

3.1 Impacts of different host combinations on S. album height growth

Height of *S. album* planted with different host combinations indicated significant differences at α=0.05 (F=6.38; p=0.000). However, among all the host combinations tested, *S. album* planted with *Cc*×*Cc* indicated the best height growth. *Aa*×*Sg*, *Cc*×*Sg*, *Cc*×*Gs* and *Aa*×*Cc* were the other host combinations which were not significantly different with *Cc*×*Cc*. Figure 1 illustrates the height growth of *S. album* grown with the above five host combinations for a period of three years. Height of *S. album* planted with the rest of the host combinations were significantly lower than the combinations given in Figure 1.

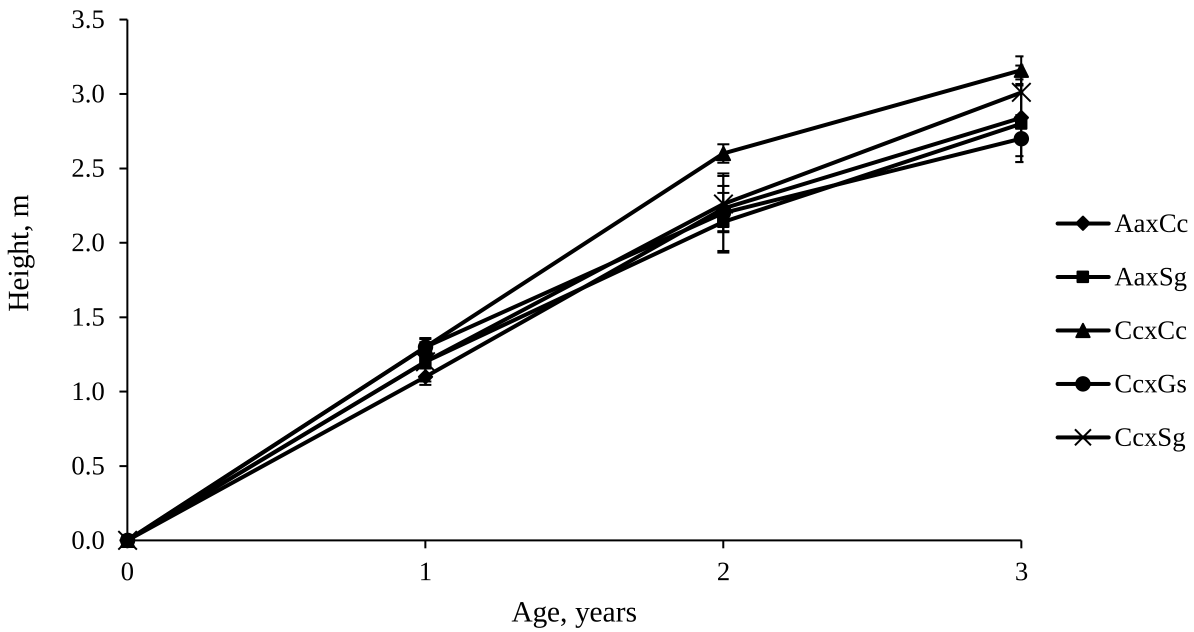

Figure 1: *S. album* height growth with the best non-significant host combinations (±SE).

Among the best five host combinations, *Calliandra calothyrus* (*Cc*) was observed in four combinations (Figure 1) proving its ability to enhance the growth of *S. album*. Moreover, *Acacia auriculiformis* (*Aa*) and *Sesbenia grandiflora* (*Sg*) were found in two host combinations. Both non legume species, i.e., *Coffea arabica* (*Ca*) and *Gravellia robusta* (*Gr*) were not included in the best host combinations.

3.2 Variation of S. album leaf nutrient contents

All four nutrients tested, viz, nitrogen, phosphorus, potassium and magnesium did not show significant differences at α=0.05 among the leaves of *S. album* grown with all 21 host combinations ($F_{nitrogen}$=1.35, $p_{nitrogen}$=0.172; $F_{phosphorus}$=1.50, $p_{phosphorus}$=0.102; $F_{pottasium}$=1.28, $p_{pottasium}$=0.217 and $F_{magnesium}$=0.64, $p_{magnesium}$=0.870).

Due to the above results, significant differences of the nutrient contents were neither observed among the host species selected from the legume family, nor between legumes and the two non-legume host species.

Per 1 g of oven-dried sandalwood leaves, the nitrogen content (Figure 2) varied from 0.0084 to 0.0138 g per 1 g of leaves (0.84% to 1.38%) for different host combinations. Variation of the phosphorus content (Figure 3) was 0.0024 to 0.0036 g (0.24% to 0.36%); potassium content (Figure 4) was 0.0060 to 0.0128 g (0.60% to 1.28%) and the variation of magnesium content was (Figure 5) 0.0004 to 0.0010 g (0.04% to 0.10%).

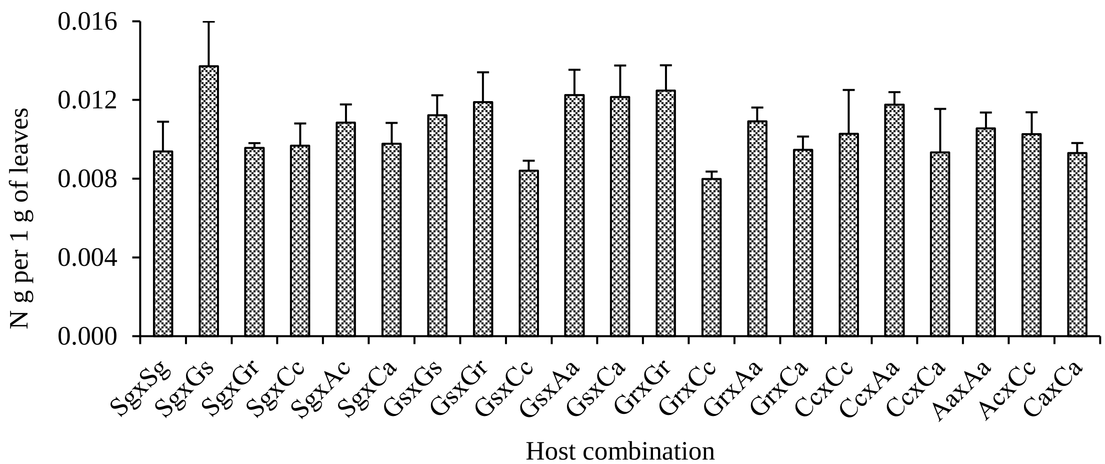

Figure 2: Mean nitrogen content (g) per 1 g of leaves of *S. album* grown with different combinations (+SE).

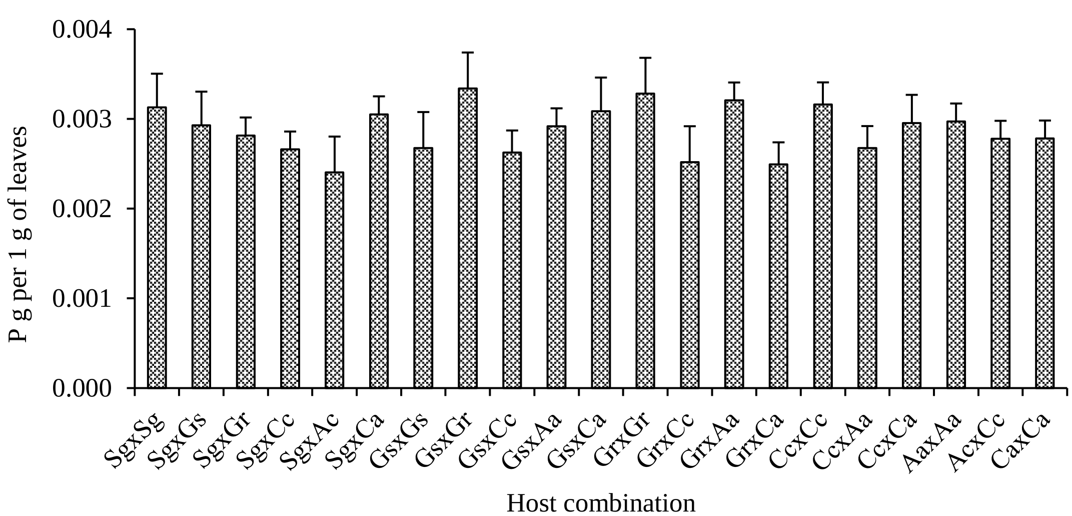

Figure 3: Mean phosphorus content (g) per 1 g of leaves of *S. album* grown with different combinations (+SE).

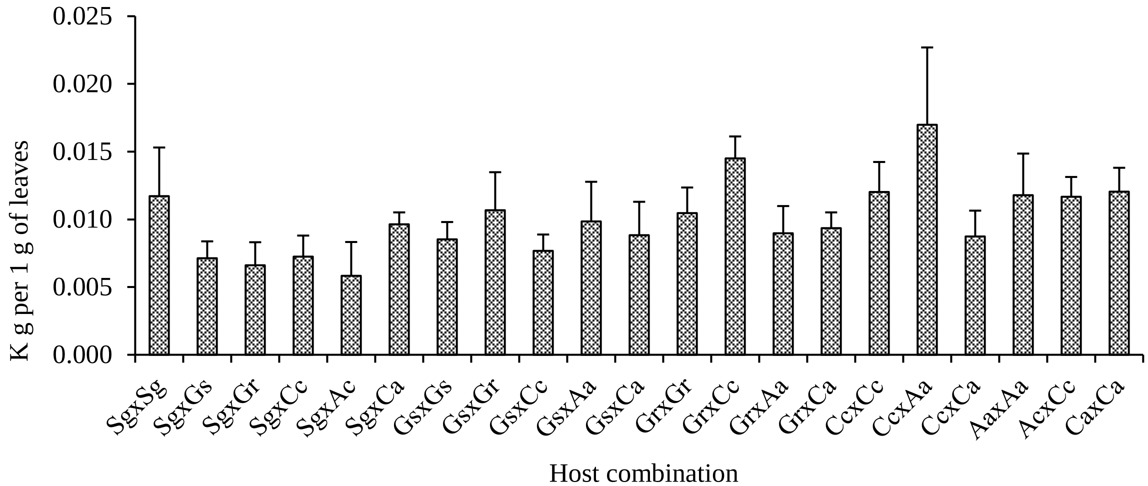

Figure 4: Mean potassium content (g) per 1 g of leaves of *S. album* grown with different combinations (+SE).

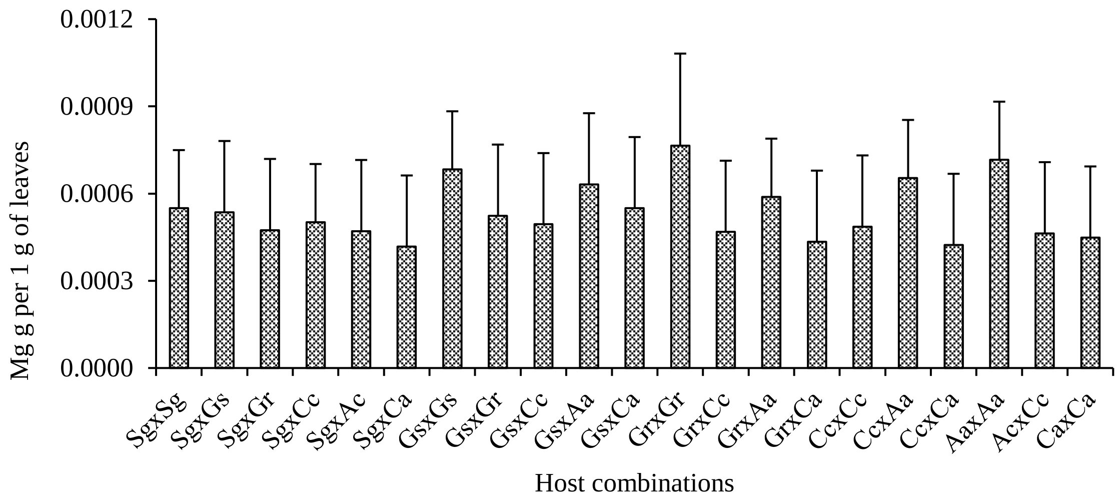

Figure 5: Mean magnesium content (g) per 1 g of leaves of *S. album* grown with different combinations (+SE).

Results revealed that the height growth enhancement of *S. album* is significantly different with different host combinations. The leaf nitrogen, phosphorus, potassium and magnesium contents of *S. album* grown with different hosts indicated no significant difference. The significant height growth of *S. album* could therefore be due to the absorption of water from the host trees via haustoria.

4. Discussion

S. album is an aggressive hemi-parasite with 70% of seedlings able to generate haustoria within 30 days from germination (Nagaveni and Srimathi, 1985). *Pongamia pinnata*, *Casuarina equisetifolia* and *Azadirachta indica* were good hosts for *S. album* in India (Nagaveni and Vijayalakshmi, 2003). Based on the results of this study, however, *Calliandra calothyrus*, *Sesbenia grandiflora* and *Acacia auriculiformis* were identified as the most suitable host species that can be used in *S. album* plantation establishment in Sri Lanka.

Both legumes and non-legumes were selected as potential host species in this study based on the importance and their ability of surviving under different climate conditions. However, Extensive trials have shown that *Santalum* species show improved growth and vigor when cultivated with leguminous hosts (Annapurna et al., 2006; Radomiljac et al., 1998; Brand et al., 2000). This study also proved that the selected leguminous species, viz, *C. calothyrus*, *S. grandiflora* and *A. auriculiformis* bear the ability of enhancing the *S. album* growth over the other species used in the field trial.

The presence of basic amino acids indicates that leguminous plants are more efficient hosts for sandalwood growth than non-leguminous plants. However, as Radomiljac et al. (1998) and Rocha et al. (2014) stated, the growth performances of *S. album* showed a variation in this study when planted with different legume host species. Results of the present study also proved this because one legume, *Gliricidia sepium* did not increase the *S. album* height as other legumes did.

*S. album*s grown with host plants have shown higher contents of nitrogen, phosphorous and potassium in their leaves (Rocha et al., 2014). In Australia, *S. spicatum* growing near *Acacia acuminata* had significantly shown higher foliar concentrations of nitrogen and potassium, and greater potassium to calcium ratio. Increased concentrations of elements in the foliage of *S. spicatum* may relate to greater nutrient movement from *A. acuminata* to the parasite (Brand et al., 2004). The nutrient analysis of the leaves of S. album conducted in this study indicated no significant difference when grown with legume and non-legume host species though Nagaveni and Vijayalakshmi (2003) stated that legume host species were capable of providing more of those nutrient contents than that of non-legumes.

S. album absorbs water from the host species other than the nutrients (Brand et al., 2000; Tennekoon, 2000). Water movements in three year old *S. album* planted in different regions of Western Australia were different with different host species (Brand et al., 2000). This could be the reason of observing significant height growth increase of *S. album* in this study due to some host species over the others.

5. Conclusion

This study found that performance of *S. album* height growth is better when planted with legumes over the non-legumes although a significant difference of nitrogen, phosphorus, potassium and magnesium contents were not observed in leaves. Therefore it can be suggested that the reason of significant height growth of *S. album* planted with some host species could be due to the absorption of the water in high quantities from those host species.

Among the tested host species, *Calliandra calothyrus* was found to be the best followed by *Acacia auriculiformis* and *Sesbenia grandiflora.*

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