Growth Performance of Agarwood Resin Producing *Gyrinops walla* Gaertn under Different Shade Conditions

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Abstract

Gyrinops walla Gaertn is the only agarwood resin producing species naturally growing in Sri Lanka, which thrives well in home gardens and forested areas of the wet zone under different light conditions. Due to the scientific discovery of its ability to produce commercially valuable agarwood resin in this species in 2012, *G. walla* became popular among many sectors. Since then, growers and investors were searching for necessary information for *G. walla* plantation establishment and sustainably managing them. Due to lack of such information, the present study aimed at finding the performance of *G. walla* under different shade levels. For this reason, 60 *G. walla* seedlings were planted in the Rubber Research Institute premises in Agalawatte, Sri Lanka in 2015. After 12 months, six plants were provided with 50% shade, and another six plants were provided with 30% shade. The rest of the plants were kept under full sun. After another 24 months, height and diameter of all plants were measured at monthly intervals. Leaf area and leaf chlorophyll content were measured at three month intervals for six plants in each shade level. Leaf nitrogen, phosphorus, potassium, and magnesium contents were analyzed at six month intervals covering both wet and dry periods for six replicates under each shade level. According to the results, height and leaf area of *G. walla* grown under full sun was significantly lower than those under 50% and 30% shade levels. Stem diameter and leaf chlorophyll contents of *G. wall* grown under different shade levels were not significant. Further, leaf nitrogen, phosphorus, potassium, and magnesium contents did not significantly vary between wet and dry seasons or between shade levels. The survival rate of the plants after 54 months after planting as 88%. The results suggest that, *G. walla* plantations can be successfully established in the low country wet zone of Sri Lanka, and plants grown initially under shade performs better than those grown under full sun.

1. Introduction

Agarwood is an extremely valuable, dark colored fragrant resin which produced by some tree members of family Thymalaeaceae as a result of a self-defense mechanism (Subasinghe and Hettiarachchi, 2013) inside the stems, branches and roots (Borris et al., 1988; Blanchette, 2003; Subasinghe and Hettiarachchi, 2015; De Alwis et al., 2016; Subasinghe et al., 2019). Due to high fragrance, agarwood is extensively used for traditional medicine, incense and perfume in cosmetic industries in many countries, especially in Asia, Europe and Middle East (Chen et al., 2011; Subasinghe et al., 2019). Some species of *Aquilaria*, *Gyrinops*, *Aetoxylon*, and *Gonystylus* are capable of producing agarwood (Blanchette, 2003; Subasinghe and Hettiarachchi, 2013). Among them *Aquilaria* and *Gyrinops* genera are widely used for the commercial-scale agarwood production in Southeast Asian countries. Those species are distributed in at least 12 countries viz., Bangladesh, Bhutan, Cambodia, India, Indonesia, Lao, Malaysia, Myanmar,

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Philippines, Thailand, Vietnam and Papua New Guinea (Gunn et al., 2004; Subasinghe et al., 2012; Subasinghe and Hettiarachchi, 2013). Among them, 21 *Aquilaria* and nine *Gyrinops* species were recorded in Asia from northern India to Vietnam and Indonesia (Subasinghe and Hettiarachchi, 2013; Rasool and Mohamed, 2016). However, only 13 *Aquilaria* species and three *Gyrinops* species are capable of producing agarwood at commercial level (Lee and Mohamed, 2016; Rasool and Mohamed, 2016). Due to the market demand of the agarwood, over-exploitation has been recorded in the natural habitats for both *Aquilaria* and *Gyrinops* species (Dharmadasa et al., 2013). Therefore, internationally they are categorised as endangered species (Saikia and Khan, 2012; Dharmadasa et al., 2013; Ahmad Zuhaidi, 2016).

When agarwood producing tree species are physically damaged, a resin is secreted into the stem to protect and to heal further damage from the external threats such as microbial invasions (Rasool and Mohamed, 2016). Therefore, it is hypothesized that agarwood resin is a product of plant defense response to the external damages (Zhang et al., 2012; Subasinghe and Hettiarachchi, 2015) caused by physical, chemical or biological stress, specifically fungal infections (Tamuli et al., 2005; Mohamed et al., 2014; Subasinghe and Hettiarachchi, 2015). However, natural formation of Agarwood resin is rare and it takes several years to produce considerable amount by trees which are about 20 years or older (Chen et al. 2011; Subasinghe et al., 2019). Therefore, artificial techniques are popular among the growers for the agarwood resin formation inducement, because it takes comparatively shorter period of time (Chen et al. 2011; Tamuli et al. 2005; Subasinghe et al., 2019). Consequently, new studies have revealed that agarwood resin formation can be artificially induced even in smaller trees which are about 3-5 years old. It is also believed that artificial inducements can yield agarwood resins 10 times faster than that is resulting from natural formation (Rasool and Mohamed, 2016; Subasinghe et al., 2019). Therefore, plantation establishment of agarwood producing species have become popular income generation method in many countries of Asia (De Alwis et al., 2016).

Among the agarwood producing species, *Gyrinops walla*, commonly known as 'Walla Patta' is the only naturally growing species found in Sri Lanka, which is distributed in the low country wet zone where temperature is around 25-27 °C and annual rainfall is around 2,000-3,000 mm (Dassanayake and Fosberg, 1981; Subasinghe and Hettiarachchi, 2016). It is also believed that *G. walla* is a shade loving species because it is mainly found in the forested areas in the country. *G. walla* is a medium tall tree which grows up to 15 m in height with straight, slender trunk with a small, rounded crown which bears several branches (Subasinghe and Hettiarachchi, 2013). Bark is thin and smooth, with varying color from grey to brownish grey to reddish brown. Leaves are alternatively arranged, oblong in shape, slightly shiny and have silky buds. Mature leaf is about 3.5×10 cm in size and with a short and bluntish acumen up to 1 cm in length. Numerous amounts of fine lateral veins and prominent midrib is characteristic to *G. walla*. Flowers are small and yellowish-white in color. Fruits are long capsule which are ovate in shape (Dassanayake and Fosberg, 1981; Jayaweera, 1982; Gunatilleke et al., 2014; Subasinghe et al., 2012; Subasinghe and Hettiarachchi 2016).

G. walla became an economically valuable tree species after discovering its ability to form agarwood resins by Subasinghe et al., (2012) and Subasinghe and Hettiarachchi (2013). These findings confirmed that the agarwood produced in *G. walla* due to natural causes are chemically similar to the commercially available agarwood of *Aquilaria* species (De Alwis et al., 2016; Subasinghe et al., 2019). Even though, plantation establishment using different *Aquilaria* species has a long history, information on the growth is limited for *G. walla* (De Alwis et al., 2016, Hossen and Hossain, 2016). However, due to the market demand of the agarwood-related products, *G. walla* can also be used to establish plantations in commercial scale in Sri Lanka and the resin formation can later be induced by artificial methods. It will open the new market opportunities to obtain considerable foreign income through plantation establishment in Sri Lanka.

However, in order to establish and manage *G. walla* plantations in sustainable and profitable manner, adequate information is required on the growth and survival of this species in response to the favorable environmental conditions. Subsequently, morphological and physiological leaf traits such as leaf chlorophyll, leaf area and leaf nutrients are useful for the tree productivity to manage sustainability (Rashid and Zuhaidi, 2011; Kenzo et al., 2019). Consequently, it is necessary to study about its growth and vigor to identify the feasibility of establishing a plantation for commercial scale. Therefore, a study was designed to identify the growth performances of *G. walla* under different shade conditions in the wet zone of the Sri Lanka to understand its growth and vigor. Then the findings can be used to establish economically profitable *G. walla* plantations for the agarwood production in Sri Lanka.

2. Materials and Method

2.1 Experimental site

The experiment site was established in May 2015, in the premises of Rubber Research Institute, Agalawatte (6°30'36.61''N and 80°10'0.01''E), which belongs to the low country wet zone of Sri Lanka. The site receives mean annual rainfall of about 2,000-3,000 mm and mean annual temperature of about 25.0-27.5 °C.

2.2 Experimental layout

The native agarwood-producing species, *G. walla* Gaertn was selected for the plantation establishment for this study. Total of 60 randomly selected six months old healthy seedlings with uniform growth were planted in the selected field in 12 rows × 5 columns under open environment condition at 3 m intervals. After 12 months of tree age, two major artificial shade setting vis., 50% and 30% shade were applied for 12 plants and the rest were kept under full sun.

2.3 Measurements

Tree growth measurements and leaf physiology measurements were made over a period of two years (May 2017 to May 2019). Tree height and stem diameter were recorded for all individual plants at monthly intervals. The height measurement was taken from the ground to the tip of the tree using a clinometer. Stem diameter was measured at 30 cm above the ground level using a diameter tape.

Leaf chlorophyll content and leaf area were measured to determine the physiological behavior of the species grown under each shade condition. Leaf chlorophyll content was measured using portable chlorophyll meter (SPAD-502DL) and leaf area was measured using leaf area (LI-3000C) meter at three months intervals using six replicates from each shade condition. Three recently matured leaves (5th or 6th leaf from the apex of a branch) were selected from each tree from the bottom of the canopy to measure leaf chlorophyll content and leaf area. Six measurements were taken from a single leaf near the midrib for leaf chlorophyll content and three measurements were taken for the leaf area.

Leaf nutrients were analyzed to identify the nutrient supplement level of the species under each shade condition. Four major nutrients; Nitrogen (N), Phosphorous (P), Potassium (K) and Magnesium (Mg) which are essential in plant productivity were analyzed at six-month intervals as wet and dry season from six replicates from each shade condition. Six recently matured leaves were sampled from the bottom canopy of a single tree. Total nitrogen content was determined using micro-Kjeldhal method. Phosphorous content was determined by colorimetric method using UV-Visible Spectrophotometer. Potassium and magnesium contents were determined using Atomic Absorption Spectrophotometer. Survival rate of the species were also recorded after 54 months of tree age.

Data were statistically analyzed after 54 months of tree age to identify the significant differences in tree growth and leaf physiology under three different shade conditions using one-way ANOVA by Minitab 17.

3. Results

3.1 Growth analysis

The results indicated that the height of *G. walla* was significantly lower (F=7.26; p=0.002) under full sun, compared to both 50% and 30% shade conditions after 54 months of tree age. However, there was no significant difference recorded for the stem diameter (F=0.29; p=0.747) under all three shade conditions. Moreover, the highest average tree height was recorded under 50% shade condition (3.94±0.49 m) for *G.*

walla (Figure 1). Even though the diameter was not significantly different for *G. walla* growing under each shade condition, the highest average stem diameter was recorded under 30% shade condition (7.3±1.6 cm) after 54 months of the age (Figure 2).

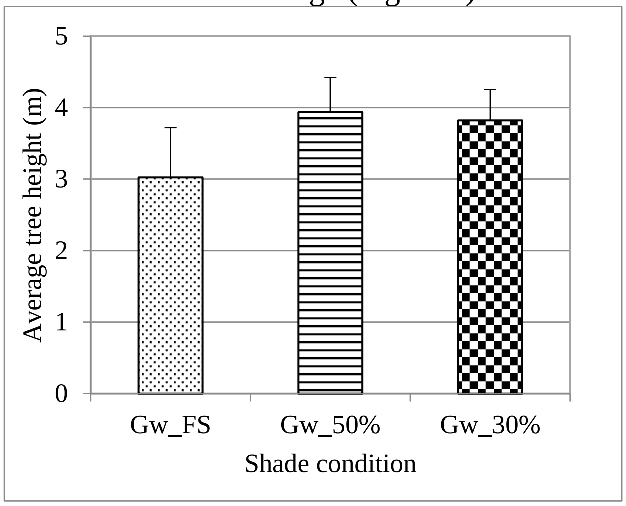

Figure 1: Average tree height of *G. walla* under different shade conditions; Gw=*G. walla*, FS=Full Sun, 50%=50% Shade, 30%=30% Shade

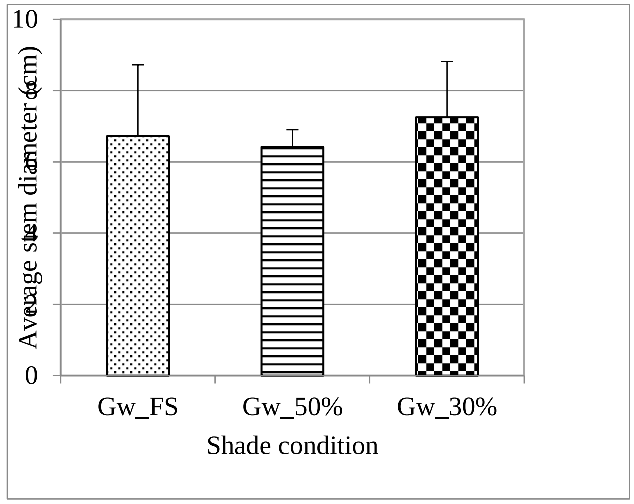

Figure 2: Average stem diameter of *G. walla* under different shade conditions; Gw=*G. walla*, FS=Full Sun, 50%=50% Shade, 30%=30% Shade

3.1 Growth analysis

3.2.1 Leaf chlorophyll

Except 39 and 54 months after planting of *G. walla*, 30% shade condition recorded comparatively high leaf chlorophyll than under full sun and 50% shade (Figure 3). However significant leaf chlorophyll contents were not recorded under each shade conditions for *G. walla* (F=2.20; p=0.115). Therefore, the effect of the different shade conditions was not significant on leaf chlorophyll content for *G. walla*.

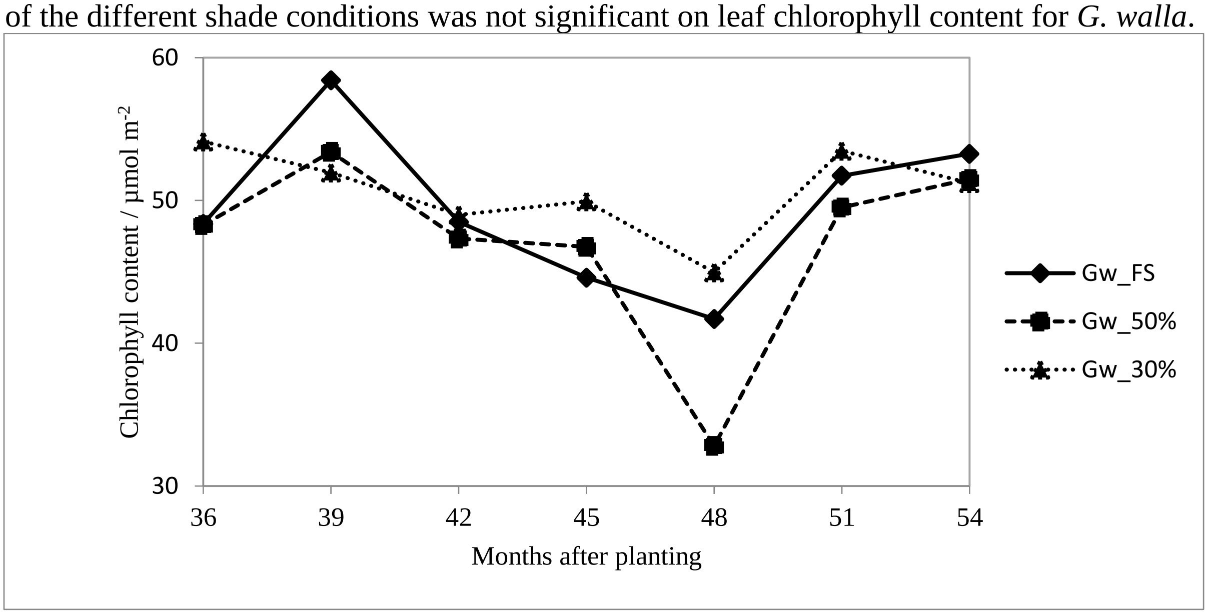

Figure 3: Variation of leaf chlorophyll content of *G. walla* grown under different shade levels with planting age; Gw=*G. walla*, FS=Full Sun, 50%=50% Shade, 30%=30% Shade

3.2.2 Leaf area

The highest and the lowest leaf area was recorded for *G. walla* under 50% shade and full sun respectively (Figure 4). Moreover, *G. walla* grown under full sun recorded significantly lower leaf area (F=11.41; p=0.000) compared to both under 50% and 30% shade conditions.

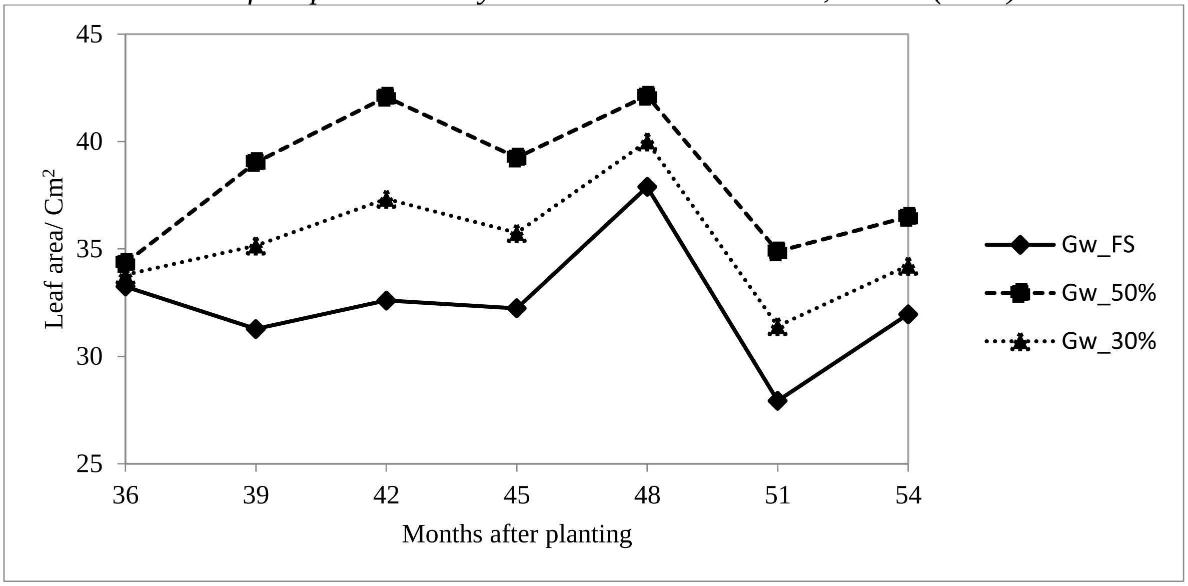

Figure 4: Variation of leaf area of *G. walla* grown under different shade levels with planting age; Gw=*G. walla*, FS=Full Sun, 50%=50% Shade, 30%=30% Shade

3.3 Leaf nature analysis

3.3.1 Variation of leaf Nitrogen (N%)

Leaf nitrogen content of *G. walla* grown under all shade conditions was comparatively low in the wet season compared to the dry season (Figure 5-a). However, there was no significant difference (F=0.81; p=0.553) recorded for the leaf nitrogen content of *G. walla* grown under each shade condition in both wet and dry seasons.

3.3.2 Variation of Leaf Phosphorous (P%)

Leaf phosphorous content of *G. walla* was comparatively high in the wet season under each shade conditions, except for the dry season under 30% shade (Figure 5b). However, leaf phosphorous content of *G. walla* under each shade conditions did not record significant differences (F=1.98; p=0.111) for both wet and dry seasons.

3.3.3 Variation of Leaf Potassium (K%)

Leaf potassium content of *G. walla* under all shade conditions was comparatively high in the wet season compared to the dry season (Figure 5-c). Moreover, leaf potassium content of *G. walla* under each shade condition did not record significant difference (F=2.29; p=0.071) in both wet and dry seasons.

3.3.4 Variation of Leaf Magnesium (Mg%)

Leaf magnesium content of *G. walla* under all shade conditions was comparatively high in the dry season compared to the wet season (Figure 5-d). However, leaf magnesium content of *G. walla* under each shade condition did not record significant difference (F=0.80; p=0.557) in both wet and dry seasons.

3.4 Survival Rate (%)

At the commencement of the study the field trial consisted of 60 *G. walla* seedlings in 2015. During the 54-month period, only 53 trees survived (88% survival rate).

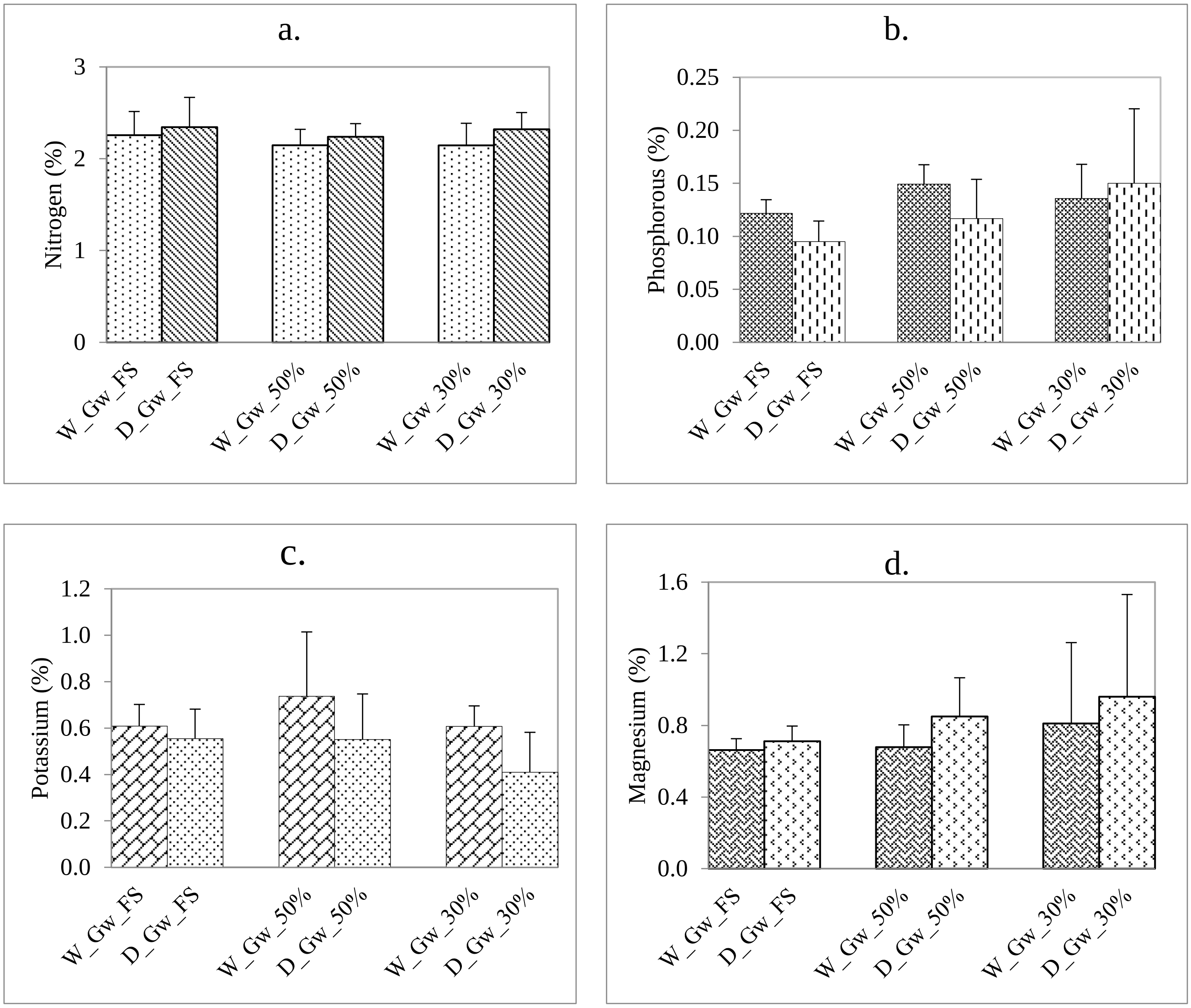

Figure 5: Leaf nutrient analysis of *G. walla* grown under different shade levels; a: Variation of leaf nitrogen, b: Variation of leaf phosphorous, c: Variation of leaf potassium, d: Variation of leaf magnesium; W=Wet, D=Dry, Gw=*G. walla*, FS=Full Sun, 50%=50% Shade, 30%=30% Shade.

4. Discussion

Plant growth can be assessed by the growth and physiological parameters because they are directly correlated with both plant development and yield outcomes (Nadeeshani et al., 2021). Availability of light plays a crucial role for regulating plant growth under different environment conditions (Hou et al., 2018). Since shade intolerant plants require more light for their growth, they exhibit increased stem and petioles lengths as well as reduced branching, leaf area and leaf thickness when they grow under low light conditions (Lambers et al., 2008). However, shade tolerant plants increase leaf area, lower leaf thickness and frequently increase leaf chlorophyll content per unit leaf area to maximize light acquisition under low light conditions (Delagrange et al., 2004; Lambers et al., 2008). According to the findings of the present study, *G. walla* recorded higher vegetative growth, particularly in terms of height when planted under both 50% and 30% shade conditions compared to those grown under full sun. Even though not significant, the highest average diameter was recorded by *G. walla* grown under 30% shade condition indicating its preference to grow under shade.

The physiological measurements such as leaf chlorophyll content, leaf area and leaf nutrients are used as indicators of determining plant growth (Lopez-Sampson et al., 2017). *G. walla* grown under both

50% and 30% shade conditions recorded significantly higher leaf area than those under full sun. Leaf chlorophyll content was not significant for the plants grown under each light condition, but plants with higher leaf area bear higher amount of chlorophyll so that they absorb more light.

Plants exhibit nutrient deficiency symptoms when available nutrients are below the required levels. All nutrients tested in this study, viz. nitrogen, phosphorous, potassium and magnesium were not significantly different for plants grown under different shade condition. According to Taji et al. (1993), the percentages nitrogen, phosphorous, potassium and magnesium content in a unit dry weight of healthy plant matter ranges from 1-4%, 0.1-0.8%, 0.5-6% and 0.1-0.8% respectively. The four nutrients tested for *G. walla* in this study during both wet and dry periods were within that range. Therefore, the reason of higher growth rates of *G. walla* grown under 50% and 30% shade levels could be due to the increased leaf area in those plants. The higher survival rate of *G. walla* recorded in this study indicated that this species is highly suitable for the commercial plantation establishment in the wet zone of Sri Lanka.

5. Conclusion

Based on the 88% survival rate of *G. walla* in the field trial, it can be concluded that this species is highly suitable for the plantation establishment in the wet zone of Sri Lanka at commercial scale. Though there were no significant differences on stem diameter, leaf chlorophyll, and leaf nutrients (N, P, K and Mg) for *G. walla* grown under different shade conditions, tree height and leaf area appeared to be the important parameters to decide the growth performance. In order to maintain a proper growth rate, shading is more preferred by *G. walla* than the full sun condition.

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