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Comparing Morphological Traits and Chlorophyll Fluorescence in Sun and Shade Leaves of *Eucalyptus* hybrid in an Ex-Tin Mine

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ABSTRACT

Selecting suitable tree species is one of the most important factors determining the success of rehabilitation initiatives or projects in degraded areas such as ex-mining sites. The ability of selected trees to survive and establish in these areas can be explained physiologically through examination of their leaves. Among four species planted in May 2022 in an ex-tin mining area in Malim Nawar, Perak, this study has chosen to focus on the *Eucalyptus* hybrid due to its superior survival and growth. A total of four *E.* hybrid trees were selected with an average height of 2.71 ± 0.65 m and collar diameter of 36.23 ± 8.75 mm. Five sun leaves and another five shade leaves were sampled from each tree for measurements of leaf area, moisture content, chlorophyll and plant efficiency. Sun leaf was found to have significantly higher ($p < 0.01$) length and width compared to shade leaf which contributed to a larger area for the sun leaf (28.40 ± 3.98 cm^2) than that of the shade leaf (22.88 ± 5.16 cm^2). Sun leaf also had higher chlorophyll content compared to shade leaf ($p < 0.05$). The Chl-a fluorescence analysis results indicated better photosynthetic performance in sun leaves with higher PI_{ABS} and PI_{total} than shade leaves. Our findings thus showed the morphological adaptation as well as physiological responses of sun and shade leaves of *Eucalyptus* hybrid when planted in an open degraded area.

Keywords: Electron transport, *Eucalyptus*, light, photochemical efficiency, rehabilitation.

INTRODUCTION

Anthropogenic footprints on the earth's surface have led to land degradation resulting in serious environmental and economic losses. Land degradation is the progressive deterioration or loss of the productive capacity of soils for the present and future (GEF Secretariat, 2023). Among the activities that contribute to land degradation include unsustainable farming, overgrazing, industrialisation, urbanisation, resource exploitation and mining. The effects vary from loss of soil fertility and loss of biodiversity to erosion and contamination, among others, adversely affecting livelihoods and food security. Across the world, land degradation and desertification have caused huge economic losses estimated at USD6.3 to 10.6 trillion or USD870 to 1,450/person/year (Noel et al., 2015). It was reported that the costs of land degradation due to land use and land cover change are about USD231 billion per year or about 0.41% of the global gross domestic product of USD56.49 trillion in 2007 (Nkonya et al., 2011). In Malaysia, among the culprits of land degradation are harvesting of forests, agriculture in hilly areas and mining of ores (Ibrahim Abdullahi and Teh, 2023). Degraded areas are usually nutrient-impoverished, devoid of vegetation and lacking valuable tree seed sources. Repeated disturbances in these places make it more difficult for natural regeneration and succession to take place, thus requiring human intervention. Planting trees to green these harsh environments is thus a challenge. The IPCC Guidelines define afforestation as the "planting of new forests on lands which, historically, have not contained forests" (Allwood et al., 2014). Due to the limitations in these areas, it is important to select suitable species that are tolerant to poor environments and ameliorate soil to improve the physical and chemical properties of soil as well as to promote the growth of plants (Lim et al., 2022). The introduction of indigenous species or plants that are native to the area is generally preferred for any restoration project. However, the use of exotic fast-growing species along with native species is also an acceptable alternative, particularly in dry areas (Foroughbakhch et al., 2017).

To develop adaptation to climate change, several species of *Eucalyptus* have been studied and results showed plasticity exhibited in physiological traits such as maximum photosynthetic rate, transpiration rate, and measures of water use efficiency (Stylianou, 2011; McLean et al., 2014). Additionally, wood density increased in dry sites (Freeman et al., 2013) while stem biomass increased with warming in cool-origin provenances (Drake et al., 2015). To understand why exotic species survive better and faster compared to native Malaysia species when planted in degraded areas begins with an investigation into the physiological responses of these plants. Each species responds to abiotic stresses including heat, salinity and drought differently. These factors will cause distinct changes to their development and physiology (dos Santos et al., 2022). The physiological response mechanism towards abiotic stresses will trigger a string of molecular events, resulting in various levels of physiological, metabolic and developmental responses (Bhargava and Sawant, 2013). Their ability and plasticity to adapt, including the development of leaves, will determine their survival, growth and subsequent productivity. Sun leaves develop in a way to minimise carboxylation limitations while shade leaves adapt to minimise light limitation (Martin et al., 2020).

This paper presents part of the findings from a study where the *Eucalyptus* hybrid was one of the four species planted for afforestation of an ex-tin mine. Four species were planted over one hectare in the ex-tin mine and these were *Eucalyptus* hybrid, *Anthoshorea roxburghii*, *Dipterocarpus oblongifolius* and *Dryobalanops rappa*. Among the four species, *Eucalyptus* hybrid showed the best growth and survival compared to the other three native species at 10 months after planting. *Eucalyptus* has been shown to have good survival, growth and ability to adapt besides having a good form (Ahmad Zuhaidi, 2020). With the rising demand for wood, the expansion of *Eucalyptus* plantations is expected to reduce pressure on natural forests by providing resources for veneer, plywood as well as engineered wood products like particleboard among others (Lee et al., 2023). *Eucalyptus* hybrid was thus chosen to study its ability to acclimatise and adapt to the harsh environment when planted on an ex-tin mine. Therefore, only the results of this species will be discussed and presented here. This paper aimed to discuss the morphological and physiological responses of *Eucalyptus* hybrid to environmental stresses when planted in a degraded ex-tin mine.

MATERIALS AND METHODS

Study Site

The study site was located in an ex-tin mine, Stesen Ujian dan Galian (SUGA), Malim Nawar in a research plot measuring 0.5 ha. The site was planted with four species in May 2022 including *Eucalyptus* hybrid whereby all planting holes were added with topsoil and Christmas Island Rock Phosphate (CIRP). The soil was sandy and the average pH was 4.12. Measurements were conducted in March 2023 with air temperature that ranged from the lowest of 20.72 °C up to the highest of 40.30 °C.

Measurement of Leaf Traits and Physiology

A total of four *Eucalyptus* hybrid trees were chosen for this study. The *Eucalyptus* hybrid were plantlets produced by the Tissue Culture Laboratory of Forest Research Institute Malaysia (FRIM). All measurements were carried out in March 2023 including height and diameter at breast height (dbh). Five fully matured sun leaves and another five shade leaves were sampled from each tree for measurements of chlorophyll and plant efficiency. Sun leaves were harvested from those growing on exterior portions of the tree canopy where the leaves were fully exposed to sunlight. Meanwhile, shade leaves were selected from leaves that were growing in interior parts of the tree canopy. All chosen leaves were dark-adapted for 30 min using white leaf clips in the middle of each leaf before measurement of leaf chlorophyll fluorescence (ChlF) was done using Pocket PEA (Plant Efficiency Analyser) (Hansatech Instruments, King's Lynn, UK) with a single strong light pulse (3,500 $\mu mol/m^2/s$) for 1 s. All parameters obtained using Pocket PEA are in relative units.

The leaves were also measured for chlorophyll content using a SPAD-502 Plus Chlorophyll Meter (Konica-Minolta, Osaka, Japan). After all physiological measurements were completed, the same leaves were harvested for the determination of moisture content and leaf dimensions (length and width). Leaf length and width were measured at the longest and widest parts of the leaf. Leaf area was measured using LI-3100C Area Meter (LI-COR, Nebraska, USA). Specific leaf area (SLA) was calculated using the ratio between leaf area and leaf dry weight.

Statistical Analysis

Results were analysed using a Paired-sample T-test, Pearson correlation and analysis of variance (ANOVA) followed by Tukey's HSD test ($p = 0.05$) by IBM SPSS Statistics Version 26.

RESULTS AND DISCUSSION

Figure 1 illustrates the distribution of length and width of the *Eucalyptus* hybrid. The two distinct groups in continuous and dashed lines represent sun and shade leaves, respectively. Both the length and width of leaves were significantly ($p < 0.05$) higher than shade leaves thus contributing to higher leaf area (Table 1). The dry biomass of sun leaves was also higher than leaves that were shaded. A high Pearson correlation coefficient value ($r = 0.930$, $p < 0.05$) confirmed that leaf dry weight has a strong positive relationship with leaf area (Figure 2).

SLA is one of the morphological traits in the leaf-economics spectrum and represents the amount of leaf area for light capture per unit of biomass invested (Torrez et al., 2013; Gratani, 2014). The plasticity of SLA indicates the morphogenetic control of leaves whereby leaf area increases in shade leaf to capture more light (Puglielli et al., 2017). Evans and Poorter (2001) showed that the acclimation process to different light environments requires variation in SLA by modification of leaf anatomy. Results indicated that the shade leaf, concerning the sun leaf, had significantly higher SLA as shown by other studies too (Pilegaard et al., 2003; Lombardini et al., 2009; Puglielli et al., 2017). This morphological adaptation allows better chances of light interception by shade leaf despite the investment of a much lower leaf biomass.

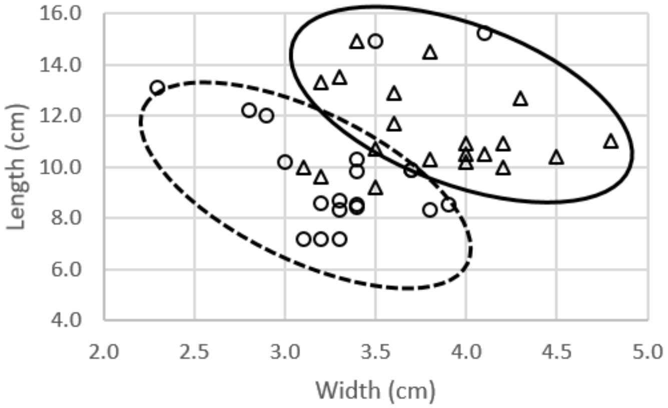

Figure 1. Length and width of sun (Δ) and shade (o) leaves of *Eucalyptus* hybrid

Table 1. Morphological traits of sun and shade leaves of *Eucalyptus* hybrid

Parameter	Sun	Shade
Length (cm)	11.39 ± 1.66^a	9.92 ± 2.40^b
Width (cm)	3.81 ± 0.47^a	3.34 ± 0.41^b
Water content (%)	0.56 ± 0.02^a	0.57 ± 0.02^a
Area (cm^2)	28.40 ± 3 .98^a	22.88 ± 5.16^b
Dry weight (g)	0.30 ± 0.04^a	0.21 ± 0.07^b
SLA (cm^2/g)	96.68 ± 7.39^a	112.38 ± 16.85^b

Note: Mean value ± standard deviation is shown. Means in the same row with different letters are significantly different at $p < 0.05$ level according to Paired-samples t-test.

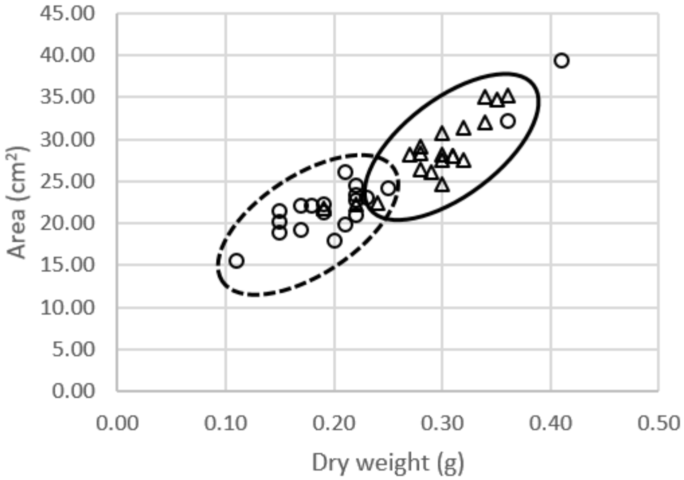

Figure 2. Relationship between leaf area and dry weight of *Eucalyptus* hybrid

Chlorophyll content in the sun leaf (41.78 ± 4.43) was found to be significantly higher than that of the shade leaf (38.75 ± 4.47). The normalized chl-*a* fluorescence transient revealed a typical OJIP curve shape in both sun and shade leaves (Figure 3). There were no clear differences in the relative fluorescence of each step between sun and shade leaves which indicated that electron transfer between PSII and PSI of these leaves was similar. Parameters (in relative units) that describe energy absorbed (ABS/RC) and trapping of light energy (TR_0/RC) per active reaction centre (RC) were found to be significantly higher in the shade leaf than in the sun leaf ($p < 0.05$), as was the dissipation of excess energy (DI_0/RC) (Figure 4). The capability of both leaf types for electron transport beyond the primary quinone electron acceptor of photosystem II (Q_A) as represented by ET_0/RC was, however, similar.

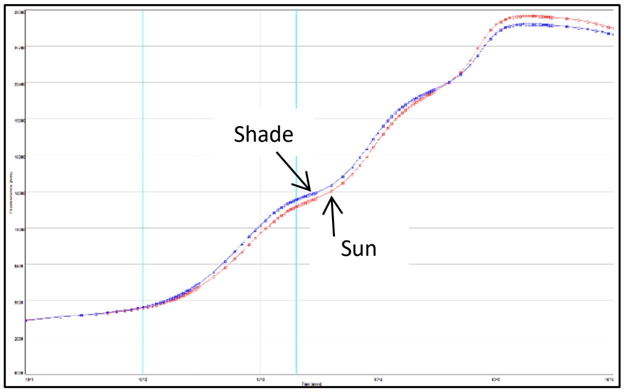

Figure 3. The polyphasic chlorophyll-*a* fluorescence transient (OJIP) exhibited by sun and shade leaves of *Eucalyptus* hybrid.

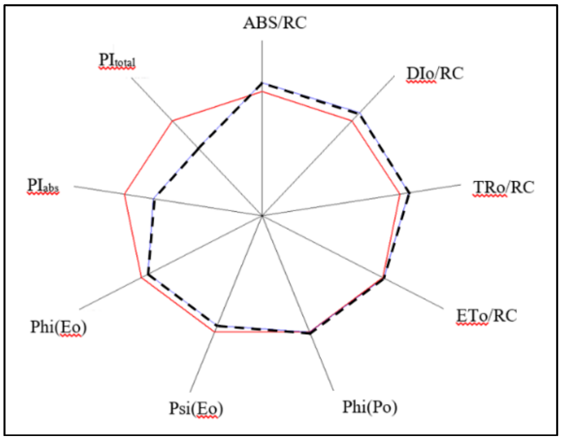

Figure 4. Spider plot JIP-test parameters of sun (continuous line) and shade (dashed line) leaves of *Eucalyptus* hybrid. *(Note: Phi represents φ; Psi represents Ψ)*

This study found that the higher average functional antenna size (ABS/RC) along with a lower density of active RCs (RC/CS_0) showed the presence of non-QA-reducing RCs in shade leaves. These non-QA-reducing RCs are also referred to as silent RCs and act as heat sinks (Strasser et al., 2004). As the electron transport beyond Q_A^- (ET_0/RC) did not differ between sun and shade leaves, there was an increase in the dissipation of excess energy (DI_0/RC) by shade leaf to avoid oxidative damage of photosystem II (PSII). Reaction centres (RCs) are where the primary steps of photosynthesis occur which include the absorption of light energy and the conversion of it into chemical energy in the form of ATP and NADPH. As such, higher active RCs in sun leaf indicated higher efficiency at utilising available light energy which can contribute to better growth and biomass production.

PI_{ABS} is a consolidated parameter which involves the energy cascade processes from the first absorption events until the reduction of plastoquinone (Martinazzo et al., 2012; Stirbet et al., 2018). The three parameters that describe PI_{ABS} are the apparent antenna size of an active PSII (ABS/RC), the possibility that an absorbed photon can be trapped by PSII RCs ($Fv/Fm = \varphi_{Po}$), and the efficiency of electron transfer beyond Q_A in the electron transport chain (Ψ_{Eo}) (Khan et al., 2020). Significantly higher Ψ_{Eo} and φ_{Eo} were observed in sun leaf whereby φ_{Eo} represents the probability that an absorbed photon moves an electron into the electron transport chain (Hao et al., 2021). Sun leaf had about 24% higher PI_{ABS} than shade leaf. Sun leaf also possesses higher number of active RCs (Mathur et al., 2018) and the ability to transfer electrons beyond QA which increases its overall photosynthetic capacity (PI_{ABS}). While PI_{ABS} is the process where the electron is transported to the plastoquinone pool, PI_{total} is the performance index of overall photochemistry. PI_{total} was about 38% higher in sun leaf showing higher electron transfer from PSII to the final electron acceptors of PSI. With higher PI_{ABS} and PI_{total}, sun leaf thus has better overall photosynthetic performance compared to shade leaf.

CONCLUSION

This study provides an understanding of the morphological traits and physiological responses of sun and shade leaves of *Eucalyptus* hybrid planted for rehabilitation in a degraded environment. This species invested a significant amount of biomass into the sun leaf enabling it to maximise photosynthesis. Findings from this study thus concluded that the sun leaf has better adaptability and plasticity in terms of light absorption and photosynthetic performance compared to the shade leaf of *Eucalyptus* hybrid when grown in an open area of an ex-tin mine with high air temperature. Further to this, an investigation of the physiological performance comparing *Eucalyptus* hybrid and other native species planted in this study is recommended to ascertain the reason for the better growth of *Eucalyptus* hybrid.

ETHICAL APPROVAL

This study was part of a project conducted with the approval of both Forest Research Institute Malaysia (FRIM) and Mineral and Geoscience Department Malaysia (JMG). *Eucalyptus* hybrid seedlings were obtained from the Tissue Culture Lab of FRIM and approval was obtained before the planting of this species.

AUTHORS CONTRIBUTION

This study was designed and conceptualised by HWM who was also the principal investigator. MZA, DID, SB and NA executed the planting and maintenance of the research site. HWM, FNS and RK collected data and performed the analysis. HWM wrote the manuscript which was reviewed by MZA.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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