Journal of BioSustainability
ISSN 3063-2129 (Online)
Journal homepage: https://ejournal.upi.edu/index.php/JoBS/

Identification of the Effect of Leaf Age on the Photosynthesis Rate of the Guava (*Psidium guajava*)

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ABSTRACT

The primary factor influencing photosynthesis is the presence of chlorophyll pigment. The objective of this study was conducted to identify the impact of leaf age on the rate of photosynthesis and to elucidate the correlation between the level of chlorophyll content in leaves of varying ages and the aforementioned rate. This was achieved through the utilisation of the FLDA (Floating Leaf Disk Array) method. Furthermore, the chlorophyll content measurement method was employed. The FLDA test results demonstrated that old leaves exhibited a higher rate of photosynthesis, potentially due to the elevated chlorophyll level in old leaves relative to young leaves, which averaged ±4,791 mg/ml and ±1,345 mg/ml, respectively. These findings substantiate the existence of a correlation between leaf age and the rate of leaf photosynthesis.

ARTICLE INFO

Article History:
Submitted/Received: 01 September 2024
First Revised: 30 October 2024
Accepted: 20 December 2024
First Available Online: 27 December 2024
Publication Date: 27 December 2024

Keyword:
chlorophyll level,
leaf age,
photosynthesis rate,
Psidium guajava.

1. INTRODUCTION

As the primary source of life on Earth, plants play a vital role in supporting the life activities of all living organisms. For this reason, plants possess unique capabilities that differentiate them from other living organisms. One such capability is photosynthesis, a biological process through which plants synthesize their own food, deriving energy from various natural components (Mattoli, *et al*., 2023). This process involves the conversion of carbon dioxide, water, and sunlight into organic compounds with the assistance of chlorophyll (Habibillah, 2023).

The primary factor influencing photosynthesis is the pigment chlorophyll, which is responsible for absorbing, transmitting, and processing light, thereby facilitating the photosynthesis process (Widawati, *et al*., 2022). Chlorophyll has a phytyl chain that can turn into phytol when exposed to water, with the assistance of a chlorophyllase catalyst (Guyer, *et al.,* 2017). A deficiency in chlorophyll can impede the reaction and subsequent production of carbohydrates. Consequently, the distribution of chlorophyll pigments is of paramount importance in ensuring the optimal efficiency of photosynthesis in plants (Nugroho, *et al*., 2021).

The distribution of chlorophyll pigments in leaves commences at the base, where the highest concentration of chlorophyll b is observed. As the leaves mature, the chlorophyll undergoes synthesis into chlorophyll a, which coincides with a color change from light green to dark green (Naikofi, 2023). Dark green leaves exhibit 72% more chlorophyll content than light green leaves (Songke, et al., 2019; Sigala, et al., 2019).

Guava leaves (Psidium guajava) play a significant role in photosynthesis, which is crucial for the plant's growth and fruit production. The age of guava leaves significantly influences their photosynthetic rate (dos Santos, et al., 2023). Fully mature leaves, which have reached their maximum size, display a significantly higher photosynthetic rate as they contain more chlorophyll and are better adapted for light absorption (Nava, et al., 2014). The increase in chlorophyll concentration in mature leaves correlates with enhanced light absorption and carbon fixation capabilities (dos Santos, et al., 2023). Higher chlorophyll levels are associated with increased rates of photosynthesis, which is crucial for overall plant health and fruit yield (Singh, *et al.,* 2017). Furthermore, this study aims to examine the effect of leaf age on the rate of photosynthesis and to determine the relationship between the level of chlorophyll in leaves of different ages.

2. METHODS

This study employs a quantitative experimental methodology with the objective of measuring the impact of independent variables, specifically young and old leaves, on dependent variables, namely the rate of photosynthesis (Dharma, 2008). The data has been presented in tabular form, derived from the results of the FLDA (Floating Leaf Disk Assay) test. The FLDA method is a straightforward technique that can be employed to quantify the rate of photosynthesis in plants (William, 2017). The FLDA method is conducted with the use of 10 leaf disks for each repetition, selected from areas distant from the leaf veins and subsequently inserted into the syringe. Subsequently, the syringe is filled with a baking soda solution until it is nearly full. The syringe tip should then be sealed with the finger and the plunger pulled back to create a vacuum for a period of 10 to 15 seconds. This will replace the air in the leaf tissue with the baking soda solution, causing the disk to sink. Then, the infiltrated leaf disk is transferred into a transparent glass containing baking soda solution

and is under a fairly bright light source. The subsequent observation period allows for the measurement of the time taken for the leaf discs to rise to the surface, along with the number of discs involved (Lohner, 2020).

Moreover, the leaves utilized for FLDA testing will be extracted and subsequently analyzed for their chlorophyll content through the use of spectrophotometry. This approach enables the investigation of potential correlations between chlorophyll levels and variations in photosynthesis rates across young and old leaves. The test was conducted using a spectrophotometer with wavelengths of 645 and 663 nm (Kamagi, *et al*., 2017).

3. RESULTS AND DISCUSSION

The results of the Floating Leaf Disk Assay (FLDA) test showed that the floating speed of the old leaves was significantly higher than that of the young leaves, after being tested for 16 minutes and 30 seconds (**Table 1**). The floating speed of the leaf disk is directly correlated with the rate of photosynthesis, as evidenced by the observation that the faster the leaf disk floats, the more oxygen is produced through the process of photosynthesis. Higher mean values of photosynthesis rate were found in older leaves of guava (**Table 2**). This indicates that the rate of photosynthesis in old leaves is higher than in young leaves, which is likely due to the development of a more optimal leaf structure and function in old leaves (Hu, *et al.,* 2020).

Table 1. Comparison of the number of FLDA disks in young and old leaves of guava.

No.	Repetition	Young Leaves	Old Leaves
1	1	No floating leaves	3 Units
2	2	3 Units	7 Units
3	3	5 Units	2 Units
	Total	8 Units	12 Units

Table 2. Comparison of photosynthesis rate in young and old leaves of guava.

No.	Repetition	Photosynthesis rate (m/s)	
		Young leaves	Old Leaves
1	1	0	0.0002
2	2	0.0002	0.0004
3	3	0.0003	0.0001
	Average	0.000167	0.000233

The results of the chlorophyll content test demonstrate that the chlorophyll levels in old leaves are markedly higher than those in young leaves, with a average of 4.791 mg/ml and 1.345 mg/ml, respectively (**Table 3**). The nearly 3.5-fold discrepancy in chlorophyll levels

substantiates the positive correlation between chlorophyll levels and the photosynthesis rate. This can be explained by the fact that chlorophyll is the main pigment involved in the capture of light energy for the photosynthesis process (Tambaru, 2024). The higher chlorophyll content, the greater the amount of light energy that can be captured and converted into chemical energy in the photosynthesis process, which ultimately results in the production of more oxygen and glucose (Li, *et al*., 2021). This finding is in accordance with the theory that photosynthetic efficiency is directly proportional to chlorophyll concentration in leaf tissue.

Table 3. Chlorophyll levels in young and old leaves of guava.

No.	Repetition	Chlorophyll level (mg/l)	
		Young leaves	Old leaves
1	1	0.2915	4.274
2	2	3.2235	3.473
3	3	0.522	6.627
	Average	1.3456	4.7913

The reduced chlorophyll content observed in younger leaves is attributed to the diminished formation of this pigment, which results in a slight greenish hue. In comparison, the chlorophyll content in older leaves is typically higher, leading to a more vibrant green color. In young leaves, chlorophyll has not yet been formed, but is still present in the form of protochlorophyll (Maryamah, *et al.,* 2021). Following the transformation of protochlorophyll into chlorophyll, the leaves will become green (Khafid, *et al*., 2021). In accordance with leaf growth, the capacity for photosynthesis will increase until the leaves are fully developed, after which it will begin to decline at a gradual rate. Old leaves that are nearing the end of their lifespan will turn yellow and become unable to photosynthesize due to damage to chlorophyll and the loss of chloroplast function (Zakiyah, *et al*., 2018).

4. CONCLUSION

In conclusion, the results of the Floating Leaf Disk Array (FLDA) test clearly indicate that the rate of photosynthesis in older leaves is higher than in younger ones. This difference can be attributed to the higher concentration of chlorophyll present in older leaves, which enhances their ability to absorb light and perform photosynthesis more efficiently.

5. AUTHORS' NOTE

The authors certify that there are no conflicts of interest pertaining to the publication of this article. Furthermore, the authors confirm that the paper is free of plagiarism.

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