J. Hortic. Sci.
Vol. 19(2), 2024

https://doi.org/10.24154/jhs.v19i2.2946

Original Research Paper

# Exploring the relationship between leaf color, canopy architecture, photosynthesis and pigment composition in guava mapping population

**Mathiazhagan M.[1], Gowtham Gowda M.D.[3], Vasugi C.[2], Shivashankara K.S.[1], Laxman R.H.[1] and Ravishankar K.V.[1*]**

[1]Division of Basic Sciences, [2]Division of Fruit Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru - 560 089, India

[3]Department of Crop Physiology, University of Agricultural Sciences, Gandhi Krishi Vigyana Kendra, Bengaluru, India

*Corresponding author Email: kv_ravishankar@yahoo.co.in

## ABSTRACT

Leaf color is a crucial parameter in determining the photosynthetic productivity of a plant. We examined 150 intervarietal guava hybrids from the cross Arka Poorna x Purple Local, that were segregating for leaf color (green and greyed purple). Variations in tree morphology, gas exchange parameters and pigment contents were investigated to understand the impact of leaf color on plant growth and physiology. Significant differences were observed in tree morphology between green and purple leaves, with green leaf plants showing better growth and vigor than purple leaf individuals. Photosynthetic rate, stomatal conductance, and transpiration rates were significantly higher in green plants than in purple leaf plants. In contrast, chlorophyll b, total chlorophyll, carotenoids, and anthocyanins were significantly higher in the purple plants. These results suggest that the presence of high anthocyanin and carotenoid pigments in purple plants have a masking effect that resulted in high chlorophyll production and low photosynthetic rates due to reduced incident light. The segregating population is an important repository for mapping QTLs associated to photosynthetic attributes.

**Keywords:** anthocyanin, canopy architecture, Guava, leaf color, photosynthesis

## INTRODUCTION

Guava (*Psidium guajava* L.) is considered as a 'Super food' because it is rich in Vitamin C, A, B, minerals, antioxidants, and dietary fibres (Mathiazhagan et al., 2023). Guava tree is small and multi-branched that grows to a height of 5-7 meters. Different canopy forms are observed as a result of branches that are drooping, spreading, or ascending in nature (Medina & Herrero, 2016). Leaf color in guava is dominated by different shades of green with a rare exception of greyed purple leaf color in the cultivar, Purple Local or Black guava or Poly guava (Mathiazhagan et al., 2024).

Leaf gas exchange parameters are affected by factors like light intensity, composition of photo-protective pigments, seasons, leaf position and its developmental stage (Zhu et al., 2018). The composition of pigments like chlorophyll, carotenoids and anthocyanins play a greater role in influencing the photosynthetic $CO_2$ assimilation in guava leaves (Idris et al., 2019). Further, higher anthocyanins and carotenoids in leaf act as photo-protective agents (Stetsenko et al., 2020) in addition to providing a shading effect on chloroplasts against high photosynthetically active radiations (PAR).

The aim of the present study was to examine the possible relationship between the leaf color, tree canopy architecture, gas exchange characters and pigment composition using 150 intervarietal $F_1$ guava mapping population. In addition, the possible influence of pigments in green and purple leaf plants on their photosynthetic machinery was examined to test the hypothesis that whether anthocyanins along with carotenoids have a shading effect in purple leaved plants.

## MATERIAL AND METHODS

### Plant material

Two years old 150 intervarietal $F_1$ segregating hybrid progenies from the cross Arka Poorna x Purple Local were used in this study. The plants were maintained in fruit breeding block of ICAR-Indian Institute of

Horticultural Research, Bengaluru, India, situated at 13°8’3,984" N latitude and 77°29’23.928" E longitude. The female parent ‘Arka Poorna’ has a green color leaf, while, the male parent Purple Local has a greyed purple leaf (Fig. 1). Out of 150 hybrid progenies, 98 progenies exhibited green leaf phenotype, while, 52 genotypes exhibited greyed purple leaf phenotype.


Fig. 1: Plant material used in the study. (a) Field view of $F_1$ intervarietal hybrids of ‘Arka Poorna’ x ‘Purple Local’ segregating for leaf color, (b) A close-up of green and greyed purple leaf, (c) Hybrid progeny with green leaf and (d) Hybrid progeny with greyed purple leaf

**Tree morphology**

Plant height (PH) was measured from the base of the stem to the top of the canopy in centimeters (cm) using a measuring tape. The stem girth (SG) was measured at the base of the trunk close to the soil surface. The canopy spread from east- west (E-W) and north- south (N-S) directions of the plants were recorded using a meter scale.

**Leaf pigment estimation**

**Chlorophyll and carotenoid content**

The chlorophyll and carotenoid content in fourth mature leaf were estimated using DMSO method (Barnes et al., 1992; Siebeneichler et al., 2019). The absorbance was read at 646 nm and 665 nm for estimating chlorophyll a and b using a UV-VIS spectrophotometer (T80+ UV/VIS Spectrophotometer, PG Instruments Ltd.). The total carotenoid content was assessed at 470 nm. The Chlorophyll a, b, a/b ratio, total chlorophyll and total carotenoid were expressed as mg/g of fresh weight.

**Total anthocyanin content**

Total anthocyanin in the fourth mature leaf was extracted in methanol : HCl (99:1 v/v) based on the method by Shivashankara et al. (2010) with slight modifications. The leaf samples were extracted in the solvent under low light and incubated overnight at room temperature. The absorbance was read at 540 nm using UV-VIS spectrophotometer. The total anthocyanin content was expressed in terms of mg/g FW using the following formula,

**Microscopic examination**

The green and greyed purple leaves of hybrid progenies were subjected to microscopic examination under a bright field microscope (Carl Zeiss, Germany, model- Axio Imager A2). Fourth mature leaves of green and purple plants were cleaned and thin cross sections were fixed on a slide using lactophenol dye. The leaf samples were visualized at 20X magnification.

**Gas exchange characteristics**

The gas exchange characteristics like net photosynthetic rate, stomatal conductance and transpiration rates were measured in the standard $4^{th}$ mature leaf. The observations were recorded using portable infra-red gas exchange analyser, LICOR LI-6400XT (LICOR, Lincon, USA) from different branches of a plant. The measurements were recorded during 9.00 to 11.00 hours to ensure that the plants were in an active photosynthetic state. The photosynthetically active radiation (PAR) was maintained at 1000 $\mu$mol $m^{-2}s^{-1}$ using the light source along with an ambient $CO_2$ concentration of around 400 $\mu$mol $mol^{-1}$.

**Statistical analysis**

All data sets, including gas exchange parameters and pigment contents of leaves in the hybrid progenies, were recorded in triplicates. The tree morphology characteristics were not replicated, as every individual $F_1$ progeny was a segregating population. Statistical tests for comparison of mean (Student t-test) and the principal component analysis were conducted using GraphPad Prism software v10.2 (www.graphpad.com). Correlation analysis was done using R program v4.3.3 (Olivoto & Lucio, 2020).

**Table 1 : Descriptive statistics of tree morphology in segregating $F_1$ guava hybrids**

| Trait | Unit | Min. | Max. | Mean | CV (%) |
|---|---|---|---|---|---|
| Plant height | cm | 95.00 | 259.00 | 186.95 | 14.92 |
| Stem girth | cm | 7.00 | 21.00 | 12.33 | 24.47 |
| EW canopy spread | cm | 23.00 | 256.00 | 126.91 | 34.77 |
| NS canopy spread | cm | 38.00 | 280.00 | 147.09 | 33.96 |

## RESULTS AND DISCUSSION

### Tree morphology

The plant height of the 150 guava hybrids ranged from 95 to 259 cm. (Table 1). The stem girth of the plants ranged from 7 cm to 21 cm. E-W canopy spread ranged from 23 cm to 256 cm, while N-S recorded 38 cm to 280 cm. Significant difference between the means of green and purple plants were observed for the plant height ($p<0.01$), stem girth ($p<0.0001$), E-W ($p<0.0001$) and N-S canopy spread ($p<0.0001$). The plants with purple leaf had a lesser stem girth, plant height and canopy spread compared to plants with green leaves (Fig. 2). Similar results were reported by Stetsenko et al. (2020), where green leaved basil plants accumulated higher biomass compared to purple leaved basil. Likewise, green perilla plants recorded better plant growth than the red perilla plants (Nguyen & Oh, 2021).

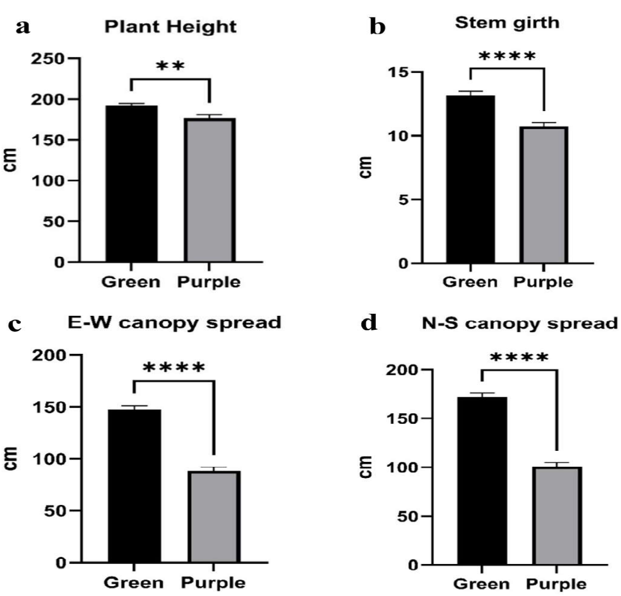


Fig. 2 : Comparison of tree morphological traits between green and purple leaf of $F_1$ segregating hybrids (a) plant height, (b) stem girth, (c) east- west canopy spread and (d) north-south canopy spread; *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$

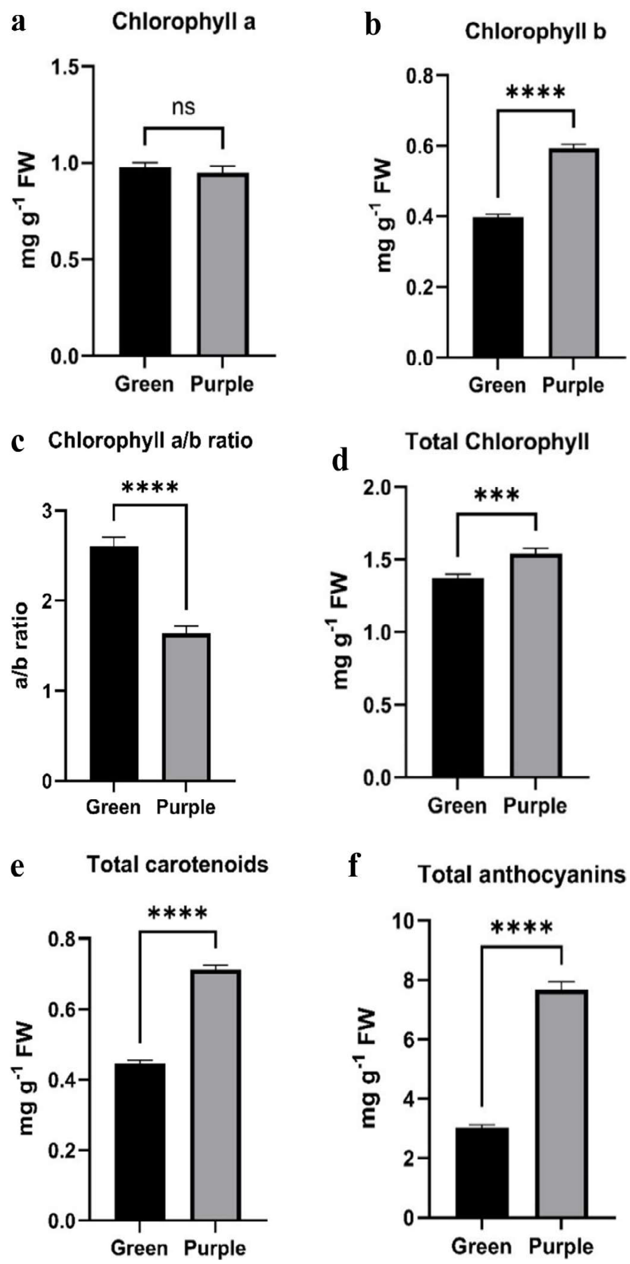


Fig. 3 : Comparison of pigment contents in green and purple leaf plants (a) chlorophyll ‘a’, (b) chlorophyll ‘b’, (c) chlorophyll a/b ratio, (d) total chlorophyll, (e) total carotenoids and (f) total anthocyanins; *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$

**Table 2 : Descriptive statistics of gas exchange parameters and pigment content in guava hybrid progenies**

| Trait | Unit | Min. | Max. | Mean | SD | SeM | CV (%) |
|---|---|---|---|---|---|---|---|
| Photosynthetic rate | µmol $CO_2$ $m^{-2}$ $s^{-1}$ | 1.40 | 17.53 | 9.11 | 2.92 | 0.24 | 32.07 |
| Stomatal conductance | mmol $m^{-2}$ $s^{-1}$ | 0.03 | 0.28 | 0.11 | 0.05 | 0.00 | 48.44 |
| Transpiration rate | mmol $m^{-2}$ $s^{-1}$ | 0.83 | 7.44 | 3.42 | 1.45 | 0.12 | 42.34 |
| Chlorophyll a | mg $g^{-1}$ FW | 0.56 | 1.80 | 0.97 | 0.25 | 0.02 | 26.03 |
| Chlorophyll b | mg $g^{-1}$ FW | 0.18 | 0.78 | 0.47 | 0.13 | 0.01 | 27.37 |
| Chlorophyll a/b ratio | - | 0.83 | 6.04 | 2.27 | 0.99 | 0.08 | 43.67 |
| Total chlorophyll | mg $g^{-1}$ FW | 0.94 | 2.49 | 1.43 | 0.27 | 0.02 | 18.77 |
| Total carotenoids | mg $g^{-1}$ FW | 0.30 | 1.03 | 0.54 | 0.16 | 0.01 | 29.59 |
| Total anthocyanins | mg $g^{-1}$ FW | 0.77 | 11.87 | 4.64 | 2.58 | 0.21 | 55.53 |

**Pigment contents in guava leaves**

Chlorophyll 'a' content ranged from 0.56 to 1.80 mg/g of FW; chlorophyll b pigment ranged between 0.18 to 0.78 mg/g of FW and leaf total chlorophyll content varied between 0.94 to 2.49 mg/g of FW (Table 2). The ratios of chlorophyll a/b were recorded between 0.83 to 6.04 mg/g of FW. The chlorophyll 'b' ($p$<0.0001) and total chlorophyll ($p$<0.001) were significantly high in purple plants compared to green leaf plants (Fig. 3b-3d). In contrast, chlorophyll a/b ratio was significantly high ($p$<0.0001) in green leaf plants than compared to purple leaf plants (Fig. 3c). Interestingly, high total chlorophyll in purple leaves did not result in higher photosynthetic rates.

Carotenoid pigments were found significantly high in purple plants ($p$< 0.0001) (Fig. 3e). Similarly, total anthocyanin content was significantly higher in purple-colored plants ($p$< 0.0001) than in green plants (Fig. 3f). It is well known that anthocyanins and carotenoids in leaves act as sunscreens or photo-protective agents against the incident light (Idris et al., 2019). It can be hypothesized that the shading effect rendered by anthocyanins in purple leaves of guava resulted in higher chlorophyll contents along with lower chlorophyll a/b ratio (Lichtenthaler & Babani, 2022).

The high anthocyanins protected the chloroplasts from solar radiations, thereby providing an edge over chlorophyll retention in purple leaves compared to green leaves. Red leaves with high anthocyanins were reported having a lower chlorophyll a/b ratio (Zeliou et al., 2022). In contrast, Manetas (2006) reported that total chlorophyll content is inversely proportional to the anthocyanin content. Additionally, it is to be mentioned that even though anthocyanins were present in green leaves, they were found in very low quantities that were not sufficient to mask the functioning of photosystems as compared to purple leaf plants.

The microscopic cross section clearly showed the presence of anthocyanin pigments in the leaf epidermal and mesophyll layers of purple plants. Anthocyanins pigments were not clearly visible in green leaf (Fig. 4). The location of foliar anthocyanins in the epidermal and/or mesophyll regions of leaf also have effect on the leaf photosynthetic rate (Boldt et al., 2014).

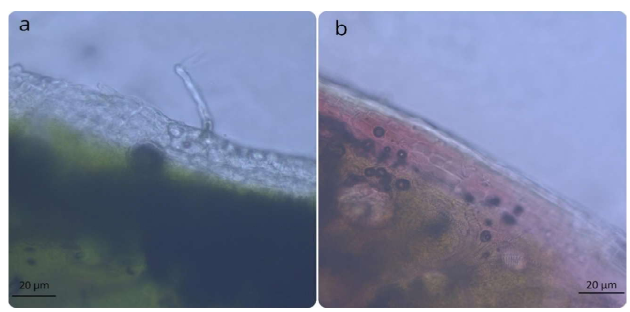


Fig. 4 : Microscopic cross sectional view of (a) green and (b) purple leaf adaxial surface (20X) showing presence of anthocyanin in mature leaf of purple plant

**Gas exchange parameters**

The net photosynthetic rates ranged from 1.40 to 17.53 µmol $CO_2$ $m^{-2}$ $s^{-1}$ (Table 2). The stomatal conductance was between 0.03 to 0.28 mol $H_2O$ $m^{-2}$ $s^{-1}$, while, transpiration rates ranged between 0.83 to 7.44 mmol $H_2O$ $m^{-2}$ $s^{-1}$. The net photosynthetic rate ($p$<0.001), stomatal conductance ($p$<0.0001) and transpiration rate ($p$<0.001) remained significantly high in green plants compared to purple plants (Fig. 5). Due to the shading effect of anthocyanins and carotenoid pigments, lower $CO_2$ assimilation was observed as less light reached the photosystems, thereby reducing their photosynthetic rate compared to green plants. Similar observations were made by Nielsen & Simonson (2011) in *Oxalis triangularis* and Borek (2016) in Coleus × hybridus.

Similar results were observed in red perilla plants where lower photosynthetic rates were recorded due to high anthocyanin pigments in leaves (Nguyen & Oh, 2021). The presence of elevated levels of anthocyanins were known to alter the photosynthetic rates in leaves by reducing the absorption of light in the uppermost mesophyll layers (Gould et al., 2000). Further, in the present study, the stomatal conductance and transpiration rates greatly varied in segregating individuals with high conductance in green leaf plants. The shaded plants were known to have low stomatal conductance compared to sun exposed leaves (Lichtenthaler et al., 2007).

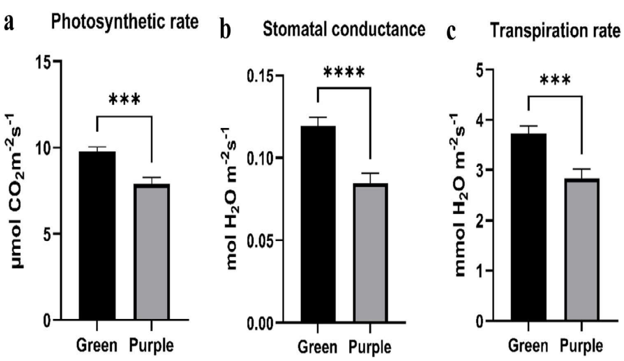


Fig. 5 : Comparison of gas exchange traits in green and purple leaf plants (a) photosynthetic rate, (b) stomatal conductance and (c) transpiration rate; *$p$<0.05, **$p$<0.01, ***$p$<0.001, ****$p$<0.0001

## Correlation analysis

The plant height, stem girth and canopy spread were positively correlated with each other; lower the stem girth lesser the plant height and canopy spread and vice versa. Similar linear relationship between plant height and stem girth was reported in sunflower (Fayyaz-ul-Hassan & Cheema, 2005). E-W ($r^2$= 0.24, $p$<0.01) and N-S canopy ($r^2$ = 0.31, $p$<0.001) had a positive significant correlation with leaf transpiration rate (Fig. 6). It can be concluded that leaf color has a profound influence on the tree morphology in our mapping population.

Photosynthetic rates showed a significant negative correlation with chlorophyll a ($r^2$= -0.25 at $p$<0.01), chlorophyll b ($r^2$= -0.18 at $p$<0.05), total chlorophyll ($r^2$= -0.32 at $p$<0.001), total carotenoids ($r^2$= -0.37 at $p$<0.001) and total anthocyanins ($r^2$= -0.47 at $p$<0.001). Similar trends were observed for stomatal conductance and transpiration rates. A similar negative association between photosynthetic rate and chlorophyll contents was observed in rice mutants with low chlorophyll content (Wang et al., 2022). But in contrary, reports on different plant species indicated a positive correlation between chlorophyll content and photosynthetic rates (Qian et al., 2021).

## PCA analysis

Principal component or factor analysis was performed to assess the pattern of contribution of various variables. The first four principal components *viz*. PC1 (32.49%), PC2 (21.97%), PC3 (13.33%), and PC4 (11.86%) together explained around 79.66 % of total cumulative variance observed in the traits. The biplot depicting the loadings of the variables in PC1 and PC2 showed that there was a negative association among the gas exchange parameters, and the pigments like anthocyanins, carotenoids and chlorophyll b. (Fig. 7). The PCA results were consistent with the correlation analysis.

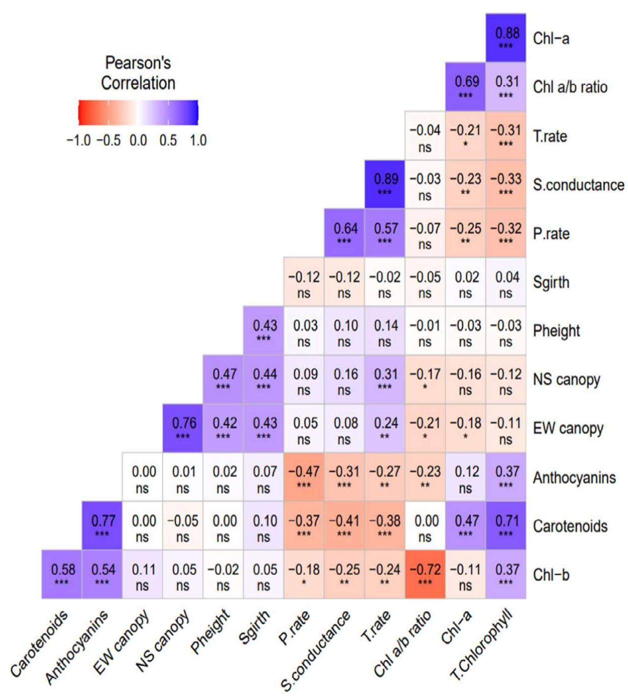


Fig. 6 : Correlation analysis using Pearson correlation coefficient for tree morphology, gas exchange and pigment contents in guava leaves (*$p$< 0.05; **$p$<0.01; ***$p$<0.001; ns $p$>= 0.05)

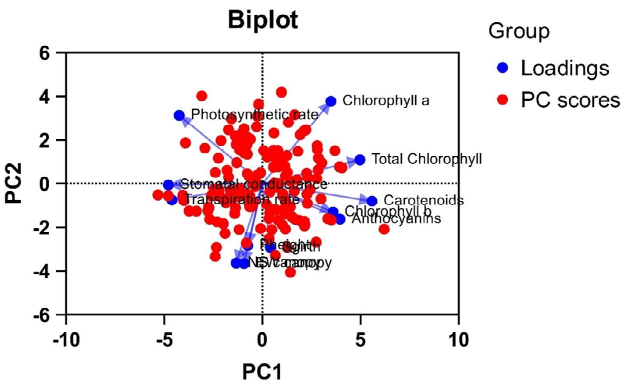


Fig. 7 : Biplot of principal component analysis for tree morphology, gas exchange and pigment contents in guava leaves. Blue lines represent loadings of the variables and red dots represent PCA scores in the PC1 (32.49% explained variance) and PC2 (21.97% explained variance)

## CONCLUSION

The color of leaf has shown profound influence on tree morphology, $CO_2$ assimilation and pigment contents in leaves of purple guava progenies. Purple leaf hybrids had a small stature due to reduced photosynthetic rate, stomatal conductance and transpiration rates. Presence of high anthocyanin and carotenoids in the purple leaf resulted in shading like effects thereby reducing PAR absorption, lowered chlorophyll a/b ratio and thus resulting in reduced $CO_2$ assimilation.

## ACKNOWLEDGEMENT

The authors acknowledge the funding agency *Rashtriya Krishi Vikas Yojana* (RKVY) and ICAR-Indian Institute of Horticultural Research, Bengaluru for providing facilities.

## REFERENCES

Barnes, J. D., Balaguer, L., Manrique, E., Elvira, S., & Davison, A. W. (1992). A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. *Environmental and Experimental Botany, 32*(2), 85-100. https://doi.org/10.1016/0098-8472(92)90034-Y

Boldt, J. K., Meyer, M. H., & Erwin, J. E. (2014). Foliar anthocyanins: A horticultural review. *Horticultural Reviews, 42*, 209-252. https://doi.org/10.1002/9781118916827.ch04

Borek, M., Bączek-Kwinta, R., & Rapacz, M. (2016). Photosynthetic activity of variegated leaves of *Coleus × hybridus* Hort. cultivars characterised by chlorophyll fluorescence techniques. *Photosynthetica, 54*, 331-339. https://doi.org/10.1007/s11099-016-0225-7

Fayyaz-ul-Hassan, G. Q., & Cheema, M. A. (2005). Growth and development of sunflower in response to seasonal variations. *Pakistan Journal of Botany, 37*(4), 859-864.

Gould, K. S., Markham, K. R., Smith, R. H., & Goris, J. J. (2000). Functional role of anthocyanins in the leaves of *Quintinia serrata* A. Cunn. *Journal of Experimental Botany*, 1107-1115.

Idris, A., Linatoc, A. C., Bakar, M. B. A., & Takai, Z. I. (2019). Effect of light intensity on the gas exchange characteristics and total pigment content of *Psidium guajava*. In *IOP Conference Series: Earth and Environmental Science, IOP Publishing*, *269*(1), 012020). https://doi.org/10.1088/1755-1315/269/1/012020

Lichtenthaler, H. K., Ač, A., Marek, M. V., Kalina, J., & Urban, O. (2007). Differences in pigment composition, photosynthetic rates, and chlorophyll fluorescence images of sun and shade leaves of four tree species. *Plant Physiology and Biochemistry, 45*(8), 577-588. https://doi.org/10.1016/j.plaphy.2007.04.006

Lichtenthaler, H. K., & Babani, F. (2022). Contents of photosynthetic pigments and ratios of chlorophyll a/b and chlorophylls to carotenoids (a+b)/(x+c) in C4 plants as compared to C3 plants. *Photosynthetica, 60*(1), 3-9. https://doi.org/10.32615/ps.2021.041

Manetas, Y. (2006). Why some leaves are anthocyanic and why most anthocyanic leaves are red? *Flora - Morphology, Distribution, Functional Ecology of Plants, 201*(3), 163-177. https://doi.org/10.1016/j.flora.2005.06.010

Mathiazhagan, M., Chinnaiyan, V., & Ravishankar, K. V. (2023). Guava: A nutraceutical-rich underutilized fruit crop. In C. Kole (Ed.), *Compendium of crop genome designing for nutraceuticals* (pp. 1-18). Springer. https://doi.org/10.1007/978-981-19-3627-2_42-1

Mathiazhagan, M., Elangovan, D., Chinnaiyan, V., Shivashankara, K. S., Sudhakar Rao, D. V., & Ravishankar, K. V. (2024). A high-density linkage map construction in guava (*Psidium guajava* L.) using genotyping by sequencing and identification of QTLs for leaf, peel, and pulp color in an intervarietal mapping population. *Frontiers in Plant Science, 15*, 1335715. https://doi.org/10.3389/fpls.2024.1335715

Medina, N. N. R., & Herrero, J. V. I. (2016). Guava (*Psidium guajava* L.) cultivars: An important source of nutrients for human health. In M. S. J. Simmonds & V. R. Preedy (Eds.), *Nutritional composition of fruit cultivars* (pp. 287-315). Academic Press. https://doi.org/10.1016/B978-0-12-408117-8.00013-1

Nguyen, T. K. L., & Oh, M. M. (2021). Physiological and biochemical responses of green and red perilla to LED-based light. *Journal of the Science of Food and Agriculture, 101*(1), 240-252. https://doi.org/10.1002/jsfa.10636

Nielsen, S. L., & Simonsen, A. M. (2011). Photosynthesis and photoinhibition in two differently coloured varieties of *Oxalis triangularis*—The effect of anthocyanin content. *Photosynthetica, 49*, 346-352. https://doi.org/10.1007/s11099-011-0042-y

Olivoto, T., & Lúcio, A. D. C. (2020). Metan: An R package for multi-environment trial analysis. *Methods in Ecology and Evolution, 11*(6), 783-789. https://doi.org/10.1111/2041-210X.13384

Qian, X., Liu, L., Croft, H., & Chen, J. (2021). Relationship between leaf maximum carboxylation rate and chlorophyll content preserved across 13 species. *Journal of Geophysical Research: Biogeosciences, 126*(2), e2020JG006076. https://doi.org/10.1029/2020JG006076

Shivashankara, K. S., Jalikop, S. H., & Roy, T. K. (2010). Species variability for fruit antioxidant and radical scavenging abilities in mulberry. *International Journal of Fruit Science, 10*, 355-366. https://doi.org/10.1080/15538362.2010.530097

Siebeneichler, S. C., Barbosa, J. S., Cruz, A. M. M., Ramos, M. A. D., Fernandes, H. E., & Nascimento, V. L. (2019). Comparison between extraction methods of photosynthetic pigments in *Acacia mangium*. *Communications in Plant Sciences, 9*(1), 1-5. https://doi.org/10.26814/cps2019001

Stetsenko, L. A., Pashkovsky, P. P., Voloshin, R. A., Kreslavski, V. D., Kuznetsov, V. V., & Allakhverdiev, S. I. (2020). Role of anthocyanin and carotenoids in the adaptation of the photosynthetic apparatus of purple-and green-leaved cultivars of sweet basil (*Ocimum basilicum*) to high-intensity light. *Photosynthetica, 58*(4), 890-901. https://doi.org/10.32615/ps.2020.048

Wang, G., Zeng, F., Song, P., Sun, B., Wang, Q., & Wang, J. (2022). Effects of reduced chlorophyll content on photosystem functions and photosynthetic electron transport rate in rice leaves. *Journal of Plant Physiology, 272*, 153669. https://doi.org/10.1016/j.jplph.2022.153669

Zeliou, K., Kyzeridou, A., & Petropoulou, Y. (2022). Exposed red leaves display adaptive adjustments in chlorophyll and photosystem ratios compatible with the shade imposed by anthocyanin accumulation. *Photosynthetica, 60*, 70-78. https://doi.org/10.32615/ps.2021.062

Zhu, H., Zhang, T. J., Zheng, J., Huang, X. D., Yu, Z. C., Peng, C. L., & Chow, W. S. (2018). Anthocyanins function as a light attenuator to compensate for insufficient photoprotection mediated by nonphotochemical quenching in young leaves of *Acmena acuminatissima* in winter. *Photosynthetica, 56*(1), 445-454. https://doi.org/10.1007/s11099-017-0740-1

(***Received : 20.5.2024; Revised : 15.7.2024; Accepted : 18.7.2024)***