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Photosynthetic physiology of green and red *Perilla frutescens* varieties under drought conditions

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ABSTRACT

The effects of water stress on photosynthetic pigments and secondary metabolites of two varieties of green- and purple-leaf *Perilla frutescens* plants were studied. Plants were grown in plastic pots for 14 days followed by water stress treatment for 10 days in a greenhouse. The trends and rates of soil water potential (SWP), leaf water content (LWC), and the maximum photochemical quantum yield (Fv/Fm) values in these two varieties were similar as the period of water stress increased from 1 to 8 days. The responses of SWP, LWC, and Fv/Fm values to water stress treatment of both varieties were adapted to a water stress period of 4 days. Purple plants exhibited significantly higher levels of anthocyanins and flavonoids than green plants under drought conditions. However, chlorophyll (Chl) a + b and carotenoids (Car) in the two varieties showed no significant differences in all water-depletion periods. The adjusted normalized difference vegetation index was significantly correlated with leaf Chl a+b, and the photochemical reflectance index was significantly correlated with Car in both species, and these can be used as indicators to characterize the physiology of these plants.

Key words: Chlorophyll fluorescence, drought, photosynthesis, spectral reflectance, water stress tolerance.

INTRODUCTION

Perilla frutescens is an annual belonging to the mint family Lamiaceae and is primarily cultivated in Asian countries. These herbaceous plants contain high amount of pigments and secondary metabolites in their leaves, and are considered to be medicinal important plants. On the market, *Perilla* cultivars are usually distinguished based on green and red colour of their leaves (Rouphael *et al.*, 15). The anthocyanin-rich chemotype (red *Perilla*) is an abundant source of antioxidants and food colorants (Fujiwara *et al.*, 7). The beneficial medicinal effects of *Perilla* plants result from the composition and high amount of volatile and non-volatile biologically active compounds, mainly flavonoids (Fla), anthocyanins (Ant), and carotenoids (Car) (Beta *et al.,* 4; Pintha e*t al.,* 14). The red leaves of *Perilla* (*P. frutescens* Britt. cv. Jeok Ssam Ip) contain a significantly higher amount of total carotenoids (Car, 209.4 μg/g FW), α-tocopherols (45.4), and total phenolic contents (70.6 mg gallic acid/g) compared with green leaves of *Perilla* (*P. frutescens* Britt. cv. Asia Ip) (Saini *et al.*, 16). Generally, *Perilla* plants grow rapidly and produce better biomass yields in less stressful environments, whereas under stressed conditions, these plants can respond to produce antioxidants and secondary metabolites to protect themselves from stresses (Ogawa *et al.,* 13). There is limited information available regarding the photosynthetic physiology of *P. frutescens* plants grown under water stresses. A better understanding of the photosynthetic characteristics of these plants would aid the effective cultivation of those plants on arid land.

Water stress is considered as predominant factor determining the global geographic distribution of vegetation and restriction of crop yields in agriculture. Roots suffering from periodic or prolonged drought or oxygen interfere with respiration at the level of electron transport. Symptoms of water deficiency include chlorophyll (Chl) breakdown, protein degradation, a decrease in membrane permeability, peroxidation, slower leaf expansion, petiole epinasty, and stomatal closure. Water stress also reduces the photosynthetic ability to utilize incident photons leading to photoinhibition, a concomitant reduction in the quantum yield of photochemistry, and a decrease in Chl fluorescence (ChlF) (Wu *et al.,* 19). Plants alter their photosynthesis to a certain degree in response to the prevailing environment, and the sensitivity of photosynthesis to stress varies among plant species and cultivars.

ChlF measurement, a noninvasive technique, has been widely used in a range of photosynthetic organisms and tissues to study functional changes in the photosynthetic apparatus under different abiotic-stress conditions. These measurements, such as the maximal quantum yield of PSII photochemistry (Fv/Fm), are commonly used to study the responses of

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plants to water deficits, as detailed in prior study ramie (Huang *et al.,* 9) in controlled environments and in the field under varying degrees of water stress. However, no effort has been made to study the photosynthetic pigments and secondary metabolites in response to water-depletion stress in *P. frutescens*. Reflectance spectroscopy is another underexploited, noninvasive technique that can be used in physiological studies; it is simple, rapid, and nondestructive in nature. Various reflectance spectra from leaves have been employed to calculate vegetation indices used for monitoring plant growth. Reflectance spectra are altered when stress occurs, and these alterations can be used to calculate different vegetation indices, such as the photochemical reflectance (PRI), adjusted normalized difference vegetation index (NDVI), and red-green PRI. Reflectance indices are useful for measuring leaf pigments, secondary metabolites, and water content (WC) when developing indices for nondestructive estimation (Ballester *et al.,* 3). The photosynthesis, transpiration, PRI, and NDVI closely followed relative WC of okra, and significant correlation of PRI with relative WC and photosynthesis justified the sensitivity of PRI to pigment concentrations and photosynthesis (Chaturvedi *et al.*, 5). Hence, we attempted to determine whether spectral reflectance indices of *P. frutescens* could be used as sensitive metrics for estimating leaf ChlF corresponding to leaf pigments and secondary metabolites under water-stress conditions.

It was perceived as eustress that enhances the content of secondary metabolites. The research literature lacks data on *Perilla* plants response to water stress during cultivation. In addition, higher levels of antioxidants would also provide protective mechanisms allowing *P. frutescens* plants to tolerate water stressful environments. Metabolites are important factors in the quality and antioxidant property of *Perilla* leaves. The aim of this study was to investigate the physiological performances of Chl, Ant, Car, and total Fla contents of *P. frutescens* plants in response to water-stressed conditions that can potentially be used to maximize the efficiency of the metabolic potential of *Perilla* plants grown in controlled environments for economic benefits. Moreover, spectral reflectance indices of these plants were also determined for estimating leaf water content corresponding to secondary metabolite contents under water stress conditions.

MATERIALS AND METHODS

Purple-leaf *P. frutescens* Britton var. 'acuta kudo' is an indigenous Taiwanese vegetable favoured by locals as a functional vegetable due to its medicinal properties. Green-leaf *P. frutescens* var. 'crispa forma *viridis* Makino' is a popular variety grown in Taiwan for consumption as a fresh vegetable. These two varieties of plants were obtained from local shops in Taipei, Taiwan, and grown in an open field at the Chinese Culture University from late June to early September 2018. When plants were 10~15 cm tall, they were transplanted into 12.7-cm plastic pots (917 mL) containing commercial potting soil with a 4:1 (v/v) mixture of peat moss and perlite, and were placed in an environmentally controlled greenhouse under an 8-h photoperiod at 28/22 °C day/night temperatures and a relative humidity of 80%. They were evenly spaced to encourage similar growth rates and sizes. Plants were watered twice a week, and an optimal amount of a compound fertilizer solution (20N-8.7P-16.6K water-soluble fertilizer at 0.5 g L^{-1}) was applied biweekly. Plants were grown for 14 days, and those with a uniform size were selected and randomly separated into different groups for the water stress experiments. Two hundred milliliters of distilled-deionized water was added to each plant on day 0 (control), followed by no water being given to any plant for the next 2~10 days. Each leaf sample came from seven plants, with three replicates from each plant. Three leaves from each plant were clipped daily, and immediately frozen in liquid nitrogen. They were then stored at -70 °C for subsequent analyses.

All tested plants were subjected to drought treatment by applying no water for 10 days, and the soil water potential (SWP, m^3/m^3) of each pot was measured daily with a moisture meter (ProCheck, Decagon Device, Pullman, ST, USA).

The leaf WC (LWC, %) was measured on the third leaf from the top of each plant over the 10-day duration of drought treatment. The LWC was estimated using the following equation: LWC = (FW − DW)/FW × 100%, where FW is the leaf fresh weight and DW is its dry weight.

The potted plants were moved to the shade under a cottage before sunrise at 05:30~06:00, and then the ChlF parameters of dark-adapted leaves were measured with a portable fluorometer (MINI-PAM, Walz, Effeltrich, Germany) at ambient temperature after adaptation to the dark for 20 min (Wu *et al.,* 19). The middle portions of a plant of both varieties were used for the measurements. Values of the minimal ChlF (Fo) and maximal ChlF (Fm) of dark-adapted samples were respectively determined using modulated irradiation of a weak LED beam (measuring light) and a saturating pulse. We then calculated the maximum photochemical quantum yield (Fv/Fm), where Fv, the yield of variable fluorescence, was calculated as (Fm - Fo). When measuring Fv/Fm, samples were first acclimated to dark conditions to ensure that all reaction centers were in an open state, and there was minimal non-photochemical dissipation of excitation energy. Measurements were recorded using WinControl-3 software (Heinz Walz, Effeltrich).

Spectral reflectance was measured from mature, healthy, fully expanded third leaves at wavelengths of 200~900 nm, using an integrating sphere fitted to a scanning spectrophotometer (PolyPen RP 400, Photon Systems Instruments, Prague, Czech Republic). The following indices were calculated (Weng *et al.,* 18) from the reflectance spectrum: (1) the adjusted normalized difference vegetation index (NDVI), calculated as (R750 - R705) / (R750 + R705 - 2 × R445), was used to assess the Chl content; (2) the photochemical reflectance index (PRI), calculated as (R531 - R570) / (R531 + R570), was used to assess xanthophyll cycle pigments; and (3) the red-green (R/G) ratio R500~599 / R600~699 was calculated to assess the Ant content.

Contents of Chl, Car, Ant, and Fla in tested plant leaves were determined using methods described by Djeridane *et al.* (6), and calculated with the following equations:

Chl a = (12.25 × OD663.6 - 2.55 × OD646.6) × volume of supernatant (mL)/sample weight (g);

Chl b = (20.31 × OD646.6 - 4.91 × OD663.6) × volume of supernatant (mL)/sample weight (g);

Car = [(4.69 × OD440.5 × volume of supernatant (mL)/sample weight (g)] - 0.267 × (Chl a + Chl b); and

Ant (μmol g^{-1} DW) = (A530 - 0.33 × A657/31.6) × volume of supernatant (mL) / sample weight (g), with DW being the dry weight. Quercetin was used as a reference standard, and the total Fla content was expressed as milligrams of quercetin equivalents (QE) per gram of dry weight (mg QE g^{-1} DW).

The SWP, LWC, Fv/Fm, Chl a+b, Car/Chl a+b, Ant, and Fla measurements were analyzed by a completely randomized analysis of variance (ANOVA) that compared the different water stress periods for each parameter. For significant values, means were separated by Fisher's least significant difference (LSD) test at $p < 0.05$ using SAS vers. 9 (SAS Institute, Cary, NC, USA). Data were determined in triplicate, and results are expressed as the mean ± standard deviation (SD). Regression analyses were used to examine relationships among spectral reflectance and leaf pigments, metabolites, and LWC. All graphs were created with Sigmaplot vers. 10.0 (Systat Software, San Jose, CA, USA).

RESULTS AND DISCUSSION

Fig. 1A shows that different responses and SWP levels existed each day of water stress treatment in the two varieties during the 10-day test period. The SWP significantly decreased in all plants as the period of water depletion increased, and SWP values did not significantly differ between the green- and purple-leaf plants. However, significant differences of SWP in each variety were observed after 4 days of drought treatment compared to the initial 2-d period of water stress treatment.

Non-significant differences in LWCs were found at 0 to 8 days in all plants, and values remained almost unchanged at 80% during the first 4 days

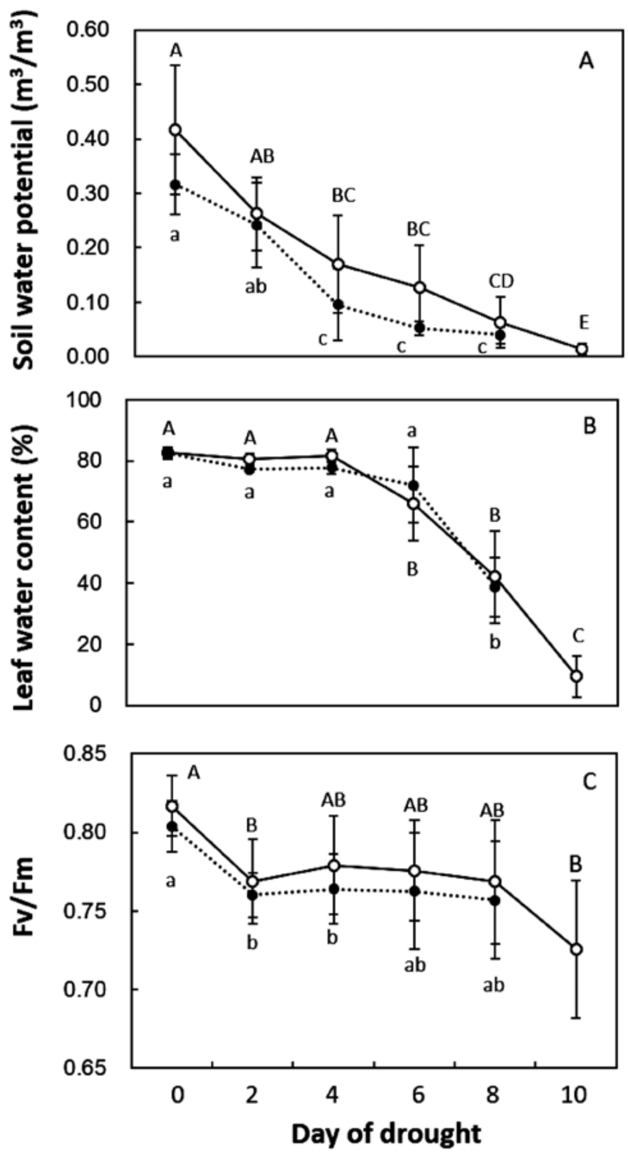

Fig. 1. Soil water potential (A), leaf water content (B), and Fv/Fm values (C) of green-leaf (white circles with a solid line) and purple-leaf (black circles with a dashed line) varieties of *Perilla frutescens* exposed to 0~10 days of drought treatment. Vertical bars indicate the standard deviation (*n* = 7). Different capital and lowercase letters among drought treatments in green and purple varieties, respectively, significantly differ at $p < 0.05$ by Fisher's least significant difference test.

of both varieties being exposed to water stress treatment. Thereafter, LWCs of purple plants sharply and significantly dropped during the 8 days of water stress and green plants dropped at the 10-day of drought treatment (Fig. 1B), indicating that long-term drought could induce a decline in the LWC of all plants. Moreover, significant differences of LWC in green variety were observed after 6 days of drought compared to the initial 4-d period of drought treatment.

Fv/Fm values showed no significant difference in either variety on different days of water-stress treatment (Fig. 1C). Although slight decreases in Fv/Fm values were noted in all plants as drought stress progressed, values of both varieties were close to 0.8 in response to water stress treatment during the 8-day period. It is noteworthy that SWP, LWC, and Fv/Fm values were detected in the purple-leaf *Perilla* variety only in the 8-day period of water stress due to drought injury to all leaves.

Chl a+b contents and Car/Chl a+b values of both varieties were slowly decrease and increased, respectively, as drought period increased (Figs. 2A, B). Ant and Fla levels in purple-leaf *P. frutescens* were significantly higher than those of the green-leaf variety at 2~4 days of drought treatment (Figs. 2C, D), whereas Chl a+b levels and Car/Chl a+b showed no significant differences between the two varieties at 0~8 days of drought treatment. Thus, different genotypes displayed variations in their metabolite contents. In addition, the pattern and trend of Ant contents of the green-leaf variety appeared similar to that of Fla contents during the 8-day period of water stress treatment. However, no phytochemical compounds of the purple-leaf *Perilla* variety were detected in the 10-d period of water stress due to drought injury to all leaves.

Fig. 3A shows relationships between PRI and Fv/Fm values from green- and purple-leaf *Perilla* plants, and correlation values (R^2) of those relationships were 0.6501 and 0.5122, respectively, indicating significant correlations between the PRI and Fv/Fm in both varieties. Fig. 3B also illustrates the significant

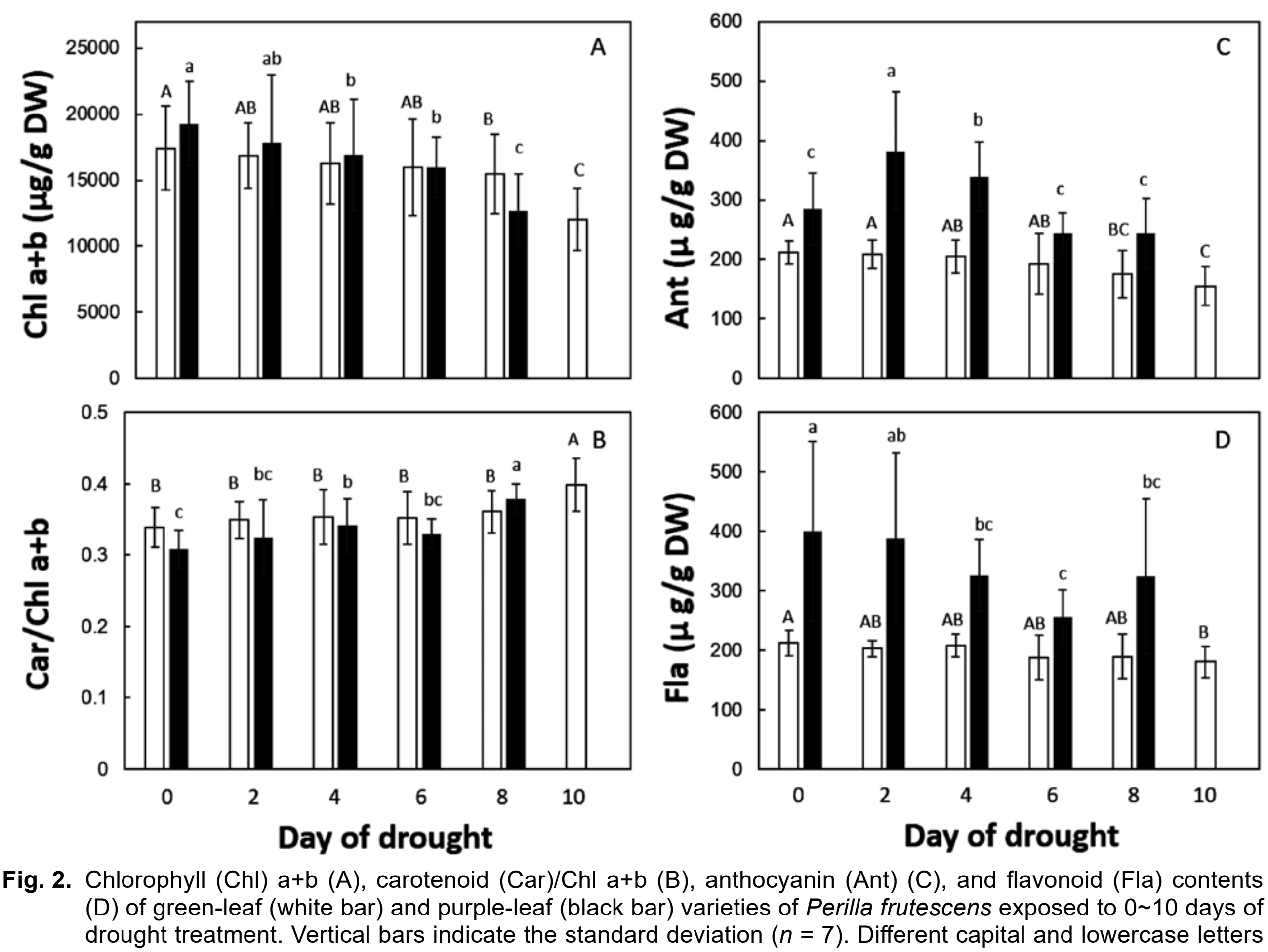

Fig. 2. Chlorophyll (Chl) a+b (A), carotenoid (Car)/Chl a+b (B), anthocyanin (Ant) (C), and flavonoid (Fla) contents (D) of green-leaf (white bar) and purple-leaf (black bar) varieties of *Perilla frutescens* exposed to 0~10 days of drought treatment. Vertical bars indicate the standard deviation (n = 7). Different capital and lowercase letters among drought treatments in green and purple varieties, respectively, significantly differ at $p < 0.05$ by Fisher's least significant difference test.

and high correlation between the PRI values and Car contents of purple- and green-leaf plants. Significant and positive correlations were observed between the NDVI values and Chl a+b contents from purple- and green-leaf *Perilla* varieties, with R^2 values of 0.8118 and 0.9508, respectively (Fig. 3C). However, there was no relationship between R/G value and Ant content of purple-leaf *Perilla* variety, whereas significant and negative relationship was detected between R/G value and Ant content of green-leaf *Perilla* variety with R^2 correlation value of 0.9152 (Fig. 3D).

Fig. 4A shows that Fv/Fm was significantly correlated with the LWC (0.6807) in green-leaf *Perilla* only but not in purple-leaf *Perilla*. When the NDVI was plotted against the LWC, purple- and green-leaf *Perilla* varieties yielded significant R^2 values of 0.5672 and 0.9847, respectively (Fig. 4B). Furthermore, in Fig. 4C, the PRI was significantly correlated with the LWC in purple-leaf (R^2 = 0.6217) and green-leaf *Perilla* (R^2 = 0.9893).

Wide variations in pigments and secondary metabolites of the two *P. frutescens* varieties were shown in Fig. 2. Purple-leaf *Perilla* had significantly higher Ant and Fla contents than did the green-leaf *Perilla* at 2~4 days of drought treatment, but Chl a+b and Car/Chl contents showed no significant differences within the initial 4-day period of water stress treatment between the two varieties. Moreover, responses of LWC and Fv/Fm values to water

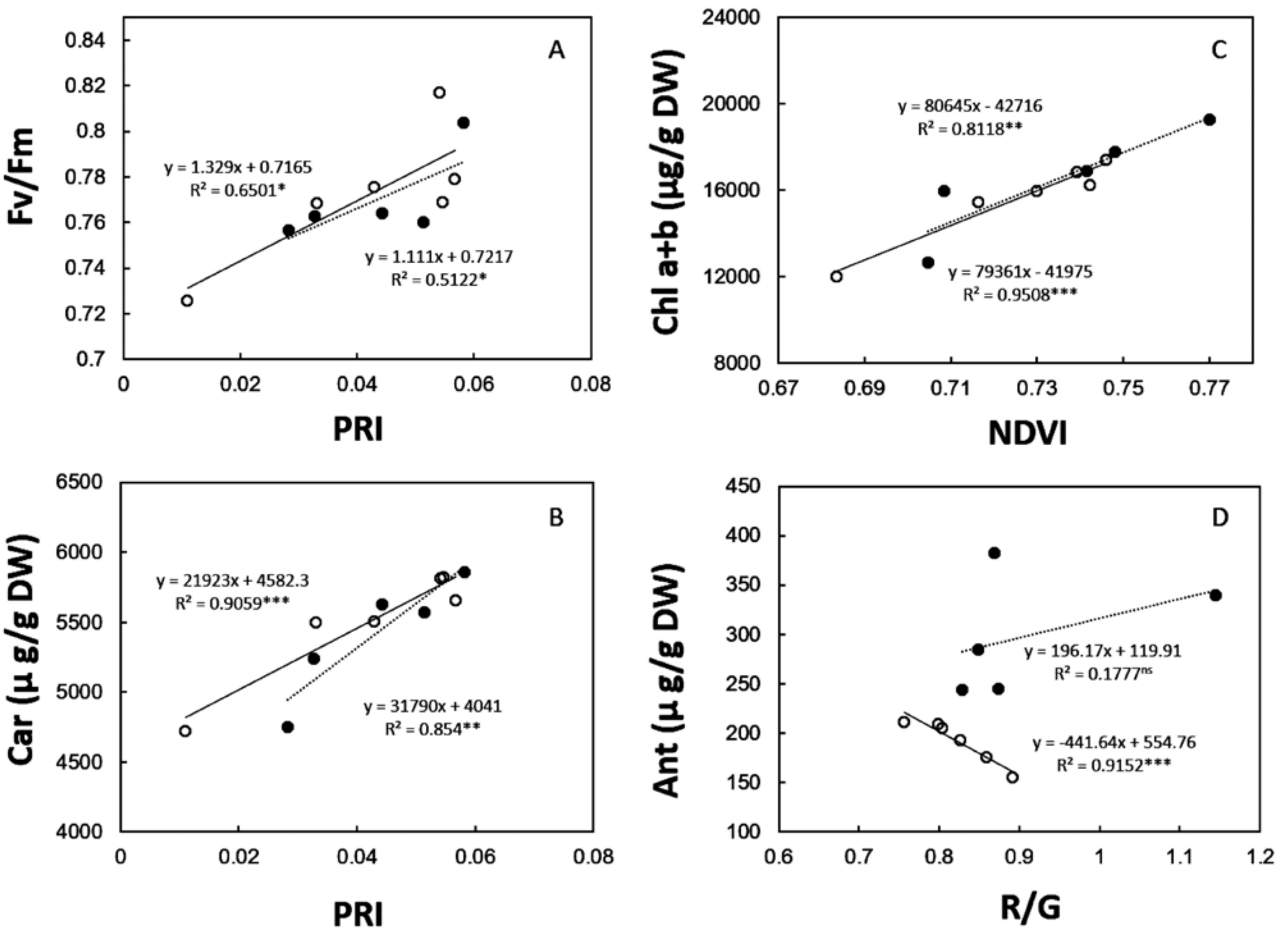

Fig. 3. Correlations between Fv/Fm values and the photochemical reflectance index (PRI) (A), carotenoid (Car) contents and the PRI (B), chlorophyll (Chl) a+b and the normalized difference vegetative index (NDVI) (C), and anthocyanin (Ant) contents and the red/green (R/G) ratio (D) of varieties of *Perilla frutescens* with different leaf colors. White and black circles represent green- and purple-leaf *P. frutescens* varieties, respectively. Each symbol represents seven plants from each water stress time period used, and Fv/Fm values, Car, Chl a+b, and Ant contents were calculated using reflectance data from the validation datasets. R^2 coefficients of the Fv/Fm values, Car, Chl a+b, and Ant contents with reflectance spectrum indices were calculated. NS, not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

stress treatment indicated that these two varieties appear to be adapted to a water stress period of 4 days. The reason for the decreased LWC after 4 days of drought treatment is that a water deficit induces stomatal closure and consequently reduces the LWC. Reductions in the LWC and Chl a+b contents as a result of drought treatment were not significant, suggesting that both *Perilla* varieties properly responded to drought stress by increasing stomatal resistance and reactive oxygen species (ROS) scavenging metabolism. Flavonoids synthesized in response to water stress are able to effectively scavenge reactive oxygen forms (Ma *et al.,* 12). Environmental conditions during plant growth may affect certain biosynthetic pathways that lead to variability in individual pigments and metabolic compounds (Lu *et al.,* 10). Many of them may play key roles in plant adaptation to abiotic stresses such as water stress. In the case of the purple-leaf *Perilla* variety, a high content of Ant or Fla that share an initial common biosynthetic pathway is normally present. Water stress can be an efficient way to maintain the sustainability of water resources, enriching pigment and metabolite contents that may be beneficial to human health.

Although the plants' visual appearance remained unchanged in the 4-day period of water stress, physiological changes had already begun. These results demonstrated that different varieties may prepare for water-stress damage by up-regulating both Ant and Fla contents, and enhancement of the production, ability, and capacity of Ant and Fla may play important roles in the ability to tolerate metabolic stress. Studies on culinary herbs showed that cultivation under water stress conditions could increase the production of phytochemicals (Azhar *et al.,* 2). The water stress level influences the growth, morphology, and photosynthetic potential of *Perilla* plants, and different responses by pigments and metabolic compounds in leaves depend on the variety of *Perilla*, which can be used to optimize growth and development of plants in water-controlled settings. Hikosaka *et al*. (8) reported that greenhouse cultivation is an effective method for the steady production of medicinal plants because environmental conditions can be suitably controlled for plant growth and quality, and it is possible that bioactive compound concentrations in medicinal plants increases with environmental control in a greenhouse. Different stress conditions might generate different photosynthetic metabolites for tolerance, and the increased Ant and Fla contents under drought stress can be considered a mechanism for overcoming such stresses (Umakanta and Oba, 17). From our observations, the lower leaves of each

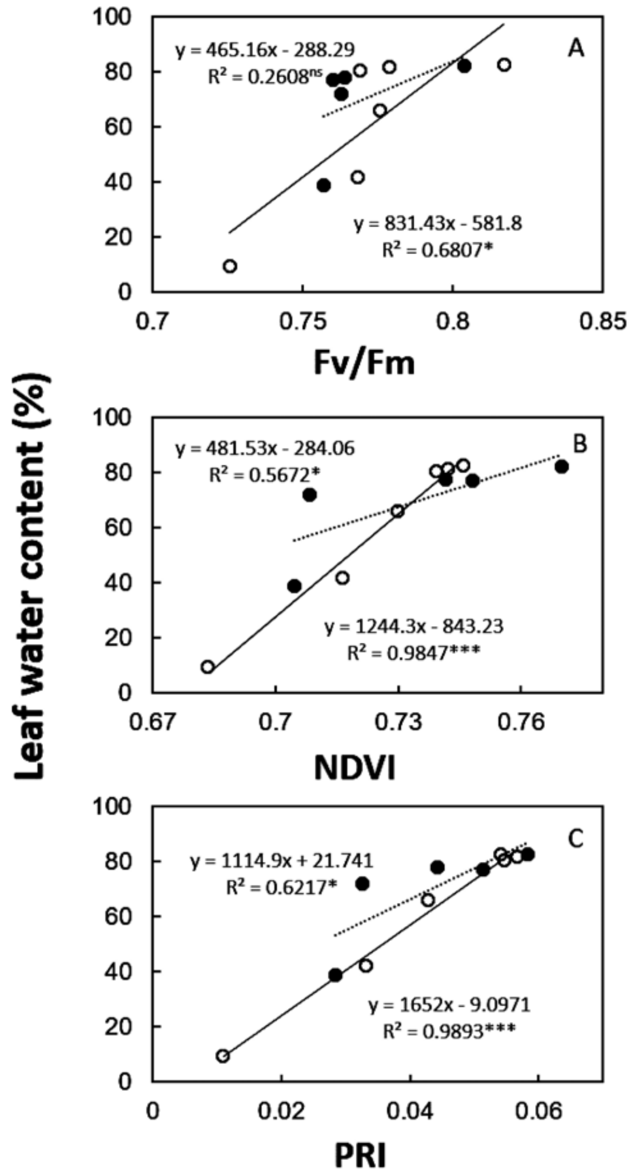

Fig. 4. Correlations between leaf water contents and Fv/Fm values (A), the normalized difference vegetative index (NDVI) (B), and photochemical reflectance index (PRI) (C) of varieties of *Perilla frutescens* with different leaf colors. White and black circles represent green- and purple-leaf *P. frutescens* varieties, respectively. Each symbol represents seven plants from each water stress time period used.

variety looked epinastic and senescent after 4 days of drought treatment. However, under 0 (control) and 1~4 days of drought treatment, most leaves appeared healthy and green. Drought stress had a harmful effect on the leaves of *P. frutescens*, and some of the damage was irreversible once drought injury occurred. The trends and rates of the decrease in SWP and LWC under drought stress were similar in both varieties. The LWC significantly decreased after 4 days of drought conditions, indicating that the water relationships of all tested plants were affected during water stress. Changes in pigment and secondary metabolite contents were related to the degree of increased chlorosis of the plants during the drought period. Once significant drought injury was evident from the appearance of the leaves, pigments and metabolites had already been affected. Ant content in purple-leaf *Perilla* under drought stress increased compared to those of green-leaf *Perilla*. The lesser degree of drought injury during 4 days of drought treatment seemed to be a result of enhancement of Ant contents in the purple-leaf *Perilla*. A better understanding of the relationships of leaf colors with Chl, Car, Fla, and Ant contents will stimulate more-efficient breeding of *Perilla* plants. These contents may be useful in screening for drought-tolerant plants, and different water stress culture systems may achieve commercial *Perilla* plant production by utilizing rapid, large-scale, precise management practices. Selection of cultivars and water practices are pre-harvest factors that significantly influence the phytochemical contents of *Perilla* plants.

Fv/Fm values of both the green- and purple-leaf plants we obtained did not display significant differences in the 8-day period of drought treatment, indicating that this parameter is not suitable for evaluating the growth of these plants under water stress. No correlation between Fv/Fm value and LWC was found for the purple-leaf *Perilla* variety, probably due to other pigment contents affecting Fv/Fm readings so that it was difficult to accurately assess its photosynthesis system (PS)II efficacy using ChlF. There is limited information available regarding the ecophysiological development of *Perilla* plants grown under water stress. One of the objectives of this study was to employ nondestructive measurements to determine leaf pigments and secondary metabolites and develop a precise, integrated, and quantitative measurement of *Perilla* species under water stress conditions. There were significant correlations of Cars with the PRI and Chl a+b with the NDVI in purple-leaf plants, indicating that these reflectance indices can be useful for nondestructive estimations of leaf Chl and Car contents in *P. frutescens*. However, no correlation of Ant with R/G in purple-leaf plants was observed. This was because the purple color of leaves affected the composition of the reflected spectrum causing the low correlations. Reflectance spectra can be affected by plant photochromes, water content, biochemical components, and tissue configuration (Zou *et al.,* 20). The PRI is highly correlated with ChlF. Under water stress, the PRI, ChlF, and photochemical reactions decline. The PRI is significantly correlated with the quantum yield of electron transfer in PSII and is indicative of xanthophyll cycle-mediated thermal energy dissipation. Indices using spectral bands (570 and 531 nm) were suggested for estimating the photosynthetic rate (Zou *et al.,* 20). The NDVI is a sensitive indicator of the canopy structure, leaf area index, and Chl content, and it offers a simple, rapid, nondestructive, and precise method to characterize the ecophysiology of plants. This index is correlated with net primary production and photosynthesis rates, and can be used to assess Chl contents and as an index for the water content. Furthermore, Liu *et al.* (11) also reported that both solar-induced ChlF and the NDVI in winter wheat under different irrigation treatments were positively and significantly correlated with the root zone soil moisture for measuring vegetation variations even when the Chl content was at a high level. Therefore, the NDVI is more comprehensively applicable to nondestructively estimate Chl contents of plant leaves and can indicate the photosynthetic capacity. Reflectance indices respond to slight changes in Car contents and also give accurate estimates of changes in the photosynthetic flux of evergreen canopies, since Cars of the xanthophyll cycle are closely related to PSII's photochemical efficiency and dissipate light energy not used in photosynthesis. The water stress level influences the photosynthetic potential of *Perilla* plants, and different responses by metabolic compounds in leaves depend on the variety of *Perilla*, which can be used to optimize the growth and development of plants in controlled water settings (Agati and Tattini, 1). This work demonstrated the feasibility of using spectral reflectance indices of water stress in *Perilla* species to determine levels of pigments and metabolites. The system may be useful when screening for drought-tolerant plants, and to develop management practices for its cultivation in fields, and enhance cultivation during scarcity of water.

In our study, the NDVI and PRI were useful for nondestructive estimation of leaf Chl a+b and Car contents, respectively, since these indices were significantly correlated with Chl a+b and Car contents in leaves of both varieties. However, the Ant content and R/G ratio can only be used as indicators to characterize the physiology of green-leaf *Perilla*. This means that many hundreds of individual plants grown under water stress can be screened per day, providing ample opportunity to discover individuals that manifest spectral reflectance indicators and exhibit greater pigments and metabolites. These results for evaluating water deficits in plants used nondestructive spectroscopic measurements that are applicable to large-scale water management of herbaceous plants, thereby enabling scarce water resources to be conserved. In addition, a better understanding of the growing characteristics of these plants would also aid their effective cultivation on arid lands or extreme climates.

REFERENCES

1. Agati, G. and Tattini, M. 2010. Multiple functional roles of flavonoids in photoprotection. *New Phytologist*, **186**: 786-93.

2. Azhar, N., Hussain B., Ashraf, M.Y. and Abbase, K.Y. 2011. Water stress mediated in growth, physiology and secondary metabolites of desi ajwain (*Trachyspermum ammi*). *Pakistan J Bot.* **43**: 15-19.

3. Ballester, C., Zarco-Tejada, P.J., Nicola, E., Alarco, J.J., Fereres, E., Intrigliolo, D. and Gonzalez-Dugo, V. 2018. Evaluating the performance of xanthophyll, chlorophyll and structure-sensitive spectral indices to detect water stress in five fruit tree species. *Precision Agric.* **19**: 178-93.

4. Beta, T., Nam, S., Dexter, J.E. and Sapirstein, H.D. 2017. Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. *LWT-Food Sci Tech*. **78**: 151-59.

5. Chaturvedi, A.K., Surendran, U., Chandran, K., Anjali, N.K. and Mohamed, C.T. 2019. Elucidation of stage specific physiological sensitivity of okra to drought stress through leaf gas exchange, spectral indices, growth and yield parameters Agricultural *Water Manag.* **222**: 92–104.

6. Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P. and Vidal, N. 2006. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chem*. **97**: 654-60.

7. Fujiwara, Y., Kono, M., Ito, A. and Ito, M. 2018. Anthocyanins in perilla plants and dried leaves. *Phyto Chem.* **147**: 158–66.

8. Hikosaka, S., Iwamoto, N., Goto, E. and Ching, H.C. 2017. Effects of supplemental lighting on growth and medicinal compounds of Japanese Honysuckle *Lonicera japonica* Thunb. *Environ. Control Biol.* **55**: 71-76.

9. Huang, C., Zhao, S. and Wang, L. 2013. Alteration in chlorophyll fluorescence, lipid peroxidation and antioxidant enzymes activities in hybrid ramie under drought stress. *Australia Journal of Crop Sci.* **7**: 594-99.

10. Lu, N., Bernardo, E.L., Tippayadarapanich, C., Takagaki, M., Kagawa, N. and Yamori, W . 2017. Growth and accumulation of secondary metabolites in perilla as affected by photosynthetic photon flux density and electrical conductivity of the nutrient solution. *Front Plant Sci.* **8**: 708.

11. Liu, L., Yang, X., Zhou, H, Liu, S, Zhou, L., Li X, Yang, J. and Wu, J. 2018. Relationship of root zone soil moisture with solar-induced chlorophyll fluorescence and vegetation indices in winter wheat: A comparative study based on continuous ground-measurements. *Ecological Indicators*, **90:** 9-17.

12. Ma, H., Johnson, S.L., Liu, W., DaSilva, N.A., Meschwitz, S., Dain, J.A. and Seeram, N.P. 2018. Evaluation of polyphenol anthocyanin-enriched extracts of blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry for free radical scavenging, reactive carbonyl species trapping, antiglycation, antiamyloid aggregation, and microglial neuroprotective effects. *Int. J. Molecular Sci.* **19**: 461.

13. Ogawa, E., Hikosaka, S. and Goto, E. 2018. Effects of nutrient solution temperature on the concentration of major bioactive compounds in red *perilla*. *J. Agri. Meteoro.* **74**: 71–78.

14. Pintha, K, Tantipaiboonwong, P., Yodkeeree, S., Chaiwangyen, W, Chumphukam, O., Khantamat, O., Khanaree, C., Kangwan, N., Thongchuai, B. and Suttajit, M. 2018. Thai perilla (Perilla frutescens) leaf extract inhibits human breast cancer invasion and migration. *Maejo Int. J. Sci. Tech*. **12**: 12-123.

15. Rouphael, Y., Kyriacou, M.C., Carillo, P., Pizzolongo, F., Romano, R. and Sifola, M.I. 2019. Chemical eustress elicits tailored responses and enhances the functional quality of novel food Perilla frutescens. *Molecules*, **24**: 185.

16. Saini, R. K., Keum Y.S. and Rengasamy K.R. 2020. Profiling of nutritionally important metabolites in green/red and green perilla (*Perilla*

frutescens Britt.) cultivars: A comparative study. *Indus. Crop Products*, **151**: 112441.

17. Umakanta, S. and Oba, S. 2018. Response of nutrients, minerals, antioxidant leaf pigments, vitamins, polyphenol, flavonoid and antioxidant activity in vegetable amaranth under four soil water content. *Food Chem.* **252**: 72-83.

18. Weng, J, Haung, J., Lin, R.J. and Chen, H.Y. 2010. Relationship between photochemical efficiency of photosystem II and the photochemical reflectance index of mango tree: Merging data from different illuminations, seasons and leaf colors. *Tree Physio.* **30**: 469-78.

19. Wu, C.W, Lin, K.H., Lee, M.C., Chou, T.Y. and Chang, Y.S. 2015. Using chlorophyll fluorescence and vegetation indices to predict the timing of nitrogen demand in *Pentas lanceolata*. *Korean J. Hor. Sci. Tech.* **33**: 845-53.

20. Zou, X., Shi, J. and Hao, L. 2011. In vivo noninvasive detection of chlorophyll distribution in cucumber (Cucumis sativus) leaves by indices based on hyperspectral imaging. *Analytica Chimica Acta*. **706**: 105-12.

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