



ARTICLE

SPAD Value Difference between Blueberry Cultivar ‘STAR’ by Planted Ground and Pot

Gyung Deok Han¹, Dae Ho Jung², Seong Heo^{3,*} and Yong Suk Chung^{1,*}

¹Department of Plant Resource and Environment, Jeju National University, Jeju, 63234, Korea

²Division of Smart Horticulture, Yonam College, Cheonan, 31005, Korea

³Department of Horticulture, Kongju National University, Yesan, 32439, Korea

*Corresponding Authors: Seong Heo. Email: heoseong@kongju.ac.kr; Yong Suk Chung. Email: yschung@jejunu.ac.kr

Received: 30 March 2022 Accepted: 05 May 2022

ABSTRACT

In the smart farm, we can control every detail for production. Collecting every factor that affects the crop's final yield is necessary to optimize its efficiency. The SPAD values were observed in the ‘Star’ cultivar blueberry (*Vaccinium darrowii*) three times a day and at three different plant heights. The pattern of SPAD value change was different by the planting position. Ground planted blueberry (*V. darrowii*) represented a stable SPAD value during the day and at the different heights. However, the SPAD value was increased by time in pot-planted blueberry (*V. darrowii*). Also, the SPAD value of pot-planted blueberry was lower than ground planted blueberry (*V. darrowii*). Even when plants were of the same cultivar and age, planting conditions affected the changing pattern of SPAD in a day. Each planting condition had merit. Therefore, proper management is needed to compensate SPAD value in pot-planted blueberry (*V. darrowii*). This study suggests that environmental conditions like planting factors affect the final products. Therefore, to maximize the efficiency at the smart farm, the factors that could affect the final yield should be investigated and accumulated.

KEYWORDS

Cultivation; chlorophyll; fruit; greenhouse; light; smart farm

1 Introduction

Smart farming with the sensor in future agriculture maximizes efficiency and decreases labor to increase benefits for the agricultural industry [1,2]. With the support of sensors, artificial intelligence, and machine learning, we can accumulate formidable data about crops. For successful smart farming, securing each crop's phenotype information is essential. After accumulating the factors that affect the final yield, the smart farming system could support the disadvantage of each crop to maximize its product's quality and quantity.

Many factors affect crop yield quality and quantity. Of the factors, photosynthetic efficiency is one of the most considerable elements. Because the daylight time is limited, it is important to optimize every environmental condition during the time that enables photosynthesis to maximize the final yield. Fortunately, our smart farm system allows controlling environmental conditions, including light. Therefore, fast determination of leaf status is needed to respond to rapid and frequent environmental changes in actual farming. SPAD (Soil Plant Analysis Development) value is a possible available



indicator for determining leaf condition for photosynthesis [3]. The SPAD value is measured by leaf transmittance in the red (650 nm; the measuring wavelength) and infrared (940 nm; a reference wavelength used to adjust for non-specific differences between samples) regions of the electromagnetic spectrum [4]. The SPAD is correlated with grain yield and nutritional status in fruit trees [5,6]. Also, the SPAD value is impacted by environmental factors like time in a day or leaf characteristics [7]. In addition, it is possible to estimate the SPAD value from RGB (Red, Green, Blue) images [8].

This study uses blueberry (*Vaccinium darrowii*) as plant material. Blueberry (*V. darrowii*) is an evergreen tree. Blueberry (*V. darrowii*) cultivar “Star” in this study is southern highbush blueberry [9]. It has many health-beneficial ingredients such as flavonols [10] and many other advantages that attract people worldwide. Because of its popularity and usefulness, this crop has been started cultivated worldwide recently [10]. For the growth of these plants, low substrate pH, high organic matter, and good drainage are needed [11–13]. Also, each cultivation region has a different environment; various cultivation strategies have been applied to adapt to each environmental condition. Now, standardization of cultivation methods is necessary to optimize the product qualities and quantities. For that, getting at each cultivation method’s characteristic, especially related to photosynthesis, is needed. These confirm the character of the blueberry photosynthetic characteristics such that each cultivation methods help develop the standardization of the cultivation process. Also, we compared each SPAD value between ground and pot-planted blueberry. Therefore, this study confirmed the SPAD value change in a day by height from ground and pot-planted blueberry. Also, we compared each SPAD value between ground and pot-planted blueberry.

2 Materials and Methods

2.1 Plant Material

This experiment used the blueberry (*V. darrowii*) cultivated in the rain-shelter house at Gosan, Jeju, for five years. The age of blueberry was five. The spacing in the plant row was 2.0 m, and the spacing of each row was 1.5 m. For ground-planted blueberry, the ground was mulched with wood chips. For potted blueberry, a 180 L round pot was used, and the soil was composed of 130 L of peatmoss and 40 L of perlite mixture and mulched with wood chip. The average temperature during the daytime in winter was 4°C–7°C, and in summer was 35°C–41°C. From April to June, the crop was fertilized. Considering the age of blueberry plants, the amount of nitrogen fertilization was 100 kg/ha. The fertilizer was determined based on pH 5; when the pH was higher than pH 5, ammonium sulfate was used, and urea fertilizer was used when the pH was lower than pH 5. The pH of the soil was 5.0 to 5.5. The SPAD value was measured at 8 AM, 1 PM, and 6 PM. The leaf directly contacted with sunlight and positioned at 90%, 50%, and 10% of plant height were randomly selected and measured. It was repeated five times for each height.

2.2 Measurement of Chlorophyll Content

SPAD-502 chlorophyll meter (Konica Minolta Sensing, Japan) was used for measuring the SPAD value. It measured two wavelengths (650 and 940 nm) of light intensity through the leaf, a non-destructive method. SPAD reading value was calculated from the logarithm of the transmittance at 650 nm related to that at 940 nm [14].

2.3 Statistical Analysis

Collected data were organized by Microsoft Excel software (Microsoft Corporation, Redmond, WA). For the regression, R statistical software (R Core Team, Vienna, Austria) was used. Some data did not satisfy normality; we used the Wilcoxon Mann-Whitney test [15] and the Kruskal Wallis test [16]. For the post-doc of the Kruskal Wallis test, the ‘agricolae’ package was installed, and the Dunn test with Bonferroni adjustment was used. The scatter plot and three-dimensional mesh plot tool in SigmaPlot10 software (Systat Software, Inc., San Jose, California) were used for drawing figures in this study.

3 Results

Table 1 shows the SPAD value changes at the same height between different times in a day. In-ground planted blueberry's SPAD values by times are not significantly different at the same height. In contrast, in the pot-planted blueberry, the SPAD values at 90% and 10% of the height were significantly different by time only at 90% plant height. Furthermore, the SPAD values increased while observing time in pot-planted blueberry. At SPAD values compared at the same observing time by plant height, the ground-planted blueberry's results are similar to **Table 1**. It is not significantly different by the height at the same observing time. However, not like **Table 1**, the SPAD values in pot-planted blueberry (*V. darrowii*) are not significantly different by the height at the same observing time, although the *P*-value is 0.08716 and 0.07085 at 8 AM and 6 PM, close to 0.05 (**Table 2**). SPAD values are significantly different in observed plant heights between ground and pot-planted blueberry (*V. darrowii*) at 8 AM and 1 PM (**Table 3**). Ground planted blueberry's SPAD value's mean observed in this study is larger than that of pot-planted blueberry (*V. darrowii*). However, at 6 PM in all observed plant heights, the SPAD values are not significantly different statistically (**Table 3**).

Table 1: Kruskal-Wallis test result compares SPAD values between height planted at ground and pot

Height (%)	Time	Ground (SPAD)	Pot (SPAD)
90% of plant height	8 AM	33.58 ± 0.85 a	28.66 ± 1.08 ab
	1 PM	32.26 ± 0.59 a	28.12 ± 0.83 b
	6 PM	33.72 ± 0.61 a	33.02 ± 0.92 b
	<i>P</i> -value	0.1872 NS, a	0.02194**
50% of plant height	8 AM	34.62 ± 0.53 a	28.56 ± 1.35 a
	1 PM	32.94 ± 0.72 a	27.52 ± 0.44 a
	6 PM	32.66 ± 0.80 a	31.14 ± 0.86 a
	<i>P</i> -value	0.1584 NS	0.08046 NS
10% of plant height	8 AM	35.40 ± 0.42 a	25.26 ± 1.40 a
	1 PM	33.10 ± 0.73 a	26.68 ± 1.21 ab
	6 PM	33.98 ± 1.06 a	29.70 ± 0.69 a
	<i>P</i> -value	0.1604 NS	0.04489**

Notes:

^a NS, nonsignificant at $P > 0.05$, * significant at 0.05, and ** significant at 0.01.

^b Means of ± standard errors followed by different letters within columns are significantly different by Dunn test with Bonferroni adjustment. Non-parametric rank data were used for statistical analysis; however, untransformed data are presented.

Figs. 1A to 1C represent each regression by time and SPAD value. The ground planted blueberry's regression is not significant, and the R square is low. Nevertheless, the scatter plot and slope represent the changes of SPAD value of ground planted blueberry (*V. darrowii*) in a day is not larger than that of pot-planted blueberry (*V. darrowii*). Similar results are found in height. **Figs. 1D to 1F** showed the regression by height and SPAD value. The scatter plot and slope showed that the changes are not larger at ground planted blueberry compared with pot-planted blueberry (*V. darrowii*).

In **Fig. 2**, three-dimensional mesh graphs are visualized the SPAD value changes of ground and pot-planted blueberry (*V. darrowii*) in a day. As mentioned above, the ground-planted blueberry (*V. darrowii*) maintains a stable and high SPAD value for a day, while the SPAD value is changed by time and height in pot-planted blueberry (*V. darrowii*).

Table 2: Kruskal-Wallis test result compares SPAD values between time planted at ground and pot

Time	Height (%)	Ground (SPAD)	Pot (SPAD)
8 AM	90% of plant height	33.58 ± 0.85 a	28.66 ± 1.08 ab
	50% of plant height	34.62 ± 0.53 a	28.56 ± 1.35 a
	10% of plant height	35.40 ± 0.42 a	25.26 ± 1.40 a
	<i>P</i> -value	0.1599 NS, a	0.08716 NS
1 PM	90% of plant height	32.26 ± 0.59 a	28.12 ± 0.83 a
	50% of plant height	32.94 ± 0.72 a	27.52 ± 0.44 a
	10% of plant height	33.10 ± 0.73 a	26.68 ± 1.21 a
	<i>P</i> -value	0.43 NS	0.4311 NS
6 PM	90% of plant height	33.72 ± 0.61 a	33.02 ± 0.92 a
	50% of plant height	32.66 ± 0.80 a	31.14 ± 0.86 a
	10% of plant height	33.98 ± 1.06 a	29.70 ± 0.69 a
	<i>P</i> -value	0.6188 NS	0.07085 NS

Notes:

^a NS, nonsignificant at $P > 0.05$.^b Means of ± standard errors followed by different letters within columns are significantly different by Dunn test with Bonferroni adjustment. Non-parametric rank data were used for statistical analysis; however, untransformed data are presented.**Table 3:** Welch's *t*-test result compares SPAD value between the blueberry planted in the ground and pot

Height (%)	Time	Ground (SPAD)	Pot (SPAD)	<i>P</i> -value
90% of plant height	8 AM	33.58 ± 0.85	28.66 ± 1.08	0.03175 *,a
	1 PM	32.26 ± 0.59	28.12 ± 0.83	0.007937**
	6 PM	33.72 ± 0.61	33.02 ± 0.92	0.8413 NS
50% of plant height	8 AM	34.62 ± 0.53	28.56 ± 1.35	0.01193*
	1 PM	32.94 ± 0.72	27.52 ± 0.44	0.007937**
	6 PM	32.66 ± 0.80	31.14 ± 0.86	0.4206 NS
10% of plant height	8 AM	35.40 ± 0.42	25.26 ± 1.40	0.007937**
	1 PM	33.10 ± 0.73	26.68 ± 1.21	0.01193*
	6 PM	33.98 ± 1.06	29.70 ± 0.69	0.007937 NS

Note:

^a NS, nonsignificant at $P > 0.05$, * significant at 0.05, and ** significant at 0.01.

4 Discussion

The SPAD values are related to the products of crops, and the cultivar-specific SPAD values can accurately estimate the final yields [17,18]. SPAD values of blueberry (*V. darrowii*) leaves were closely related to blueberry growth and dry matter accumulation [19]. Furthermore, a strong significant positive correlation was found between grain yield and SPAD values [18]. The blueberry (*V. darrowii*) planted in the pot represents significantly different SPAD values by time changes in a day (Table 1). However, the blueberry (*V. darrowii*) showed no significantly different SPAD values at the same height during a day (Table 2). Leaf position is related to the maturity of the leaf, which is apical positioned leaves are relatively immature, and basal position leaves are comparably old [20,21]. Also, the chlorophyll levels are increased on the age of leaf tissue and N status [4]. However, this study represents that basal leaf

SPAD value in pot-planted blueberry is lower than apical positioned leave of ground planted blueberry. This result indicated that the environmental factors essentially affect the SPAD value of blueberry (*V. darrowii*). Photosynthesis is affected by the environment and has a daily rhythm [22,23].

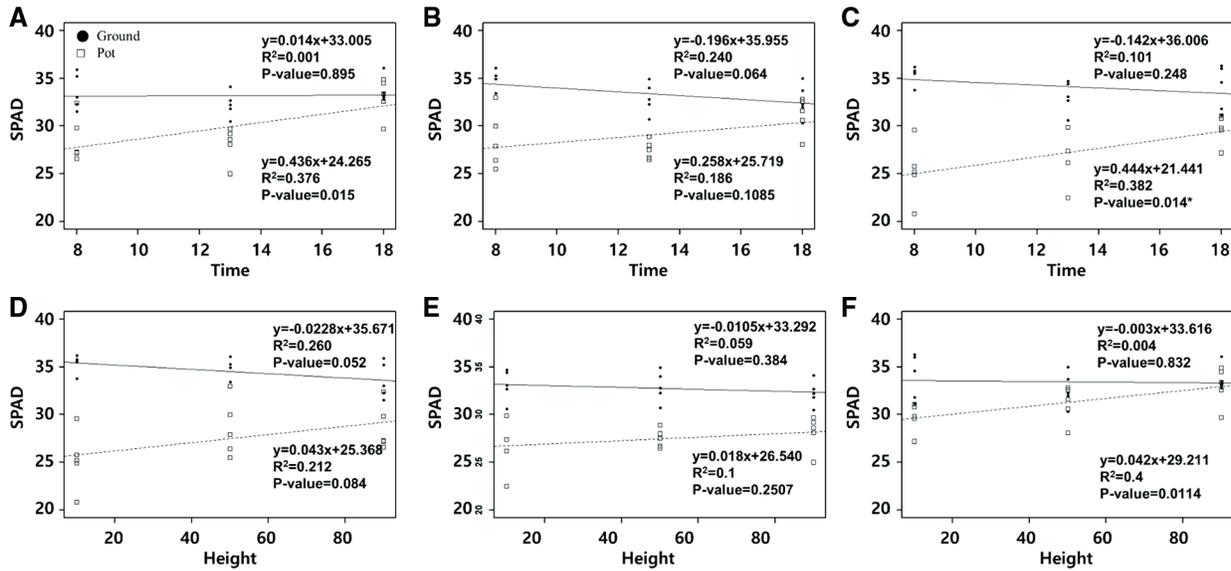


Figure 1: Scatter plot and regression of SPAD value from blueberry planted on ground and pot. A, B, and C. The SPAD value were collected three times a day at 90%, 50%, and 10% of plant height, respectively. D, E, and F. The SPAD value was collected from three heights at 8 AM, 1 PM, and 6 PM in a day, respectively. Black circle and white square mean ground and pot-planted blueberry’s SPAD values, respectively

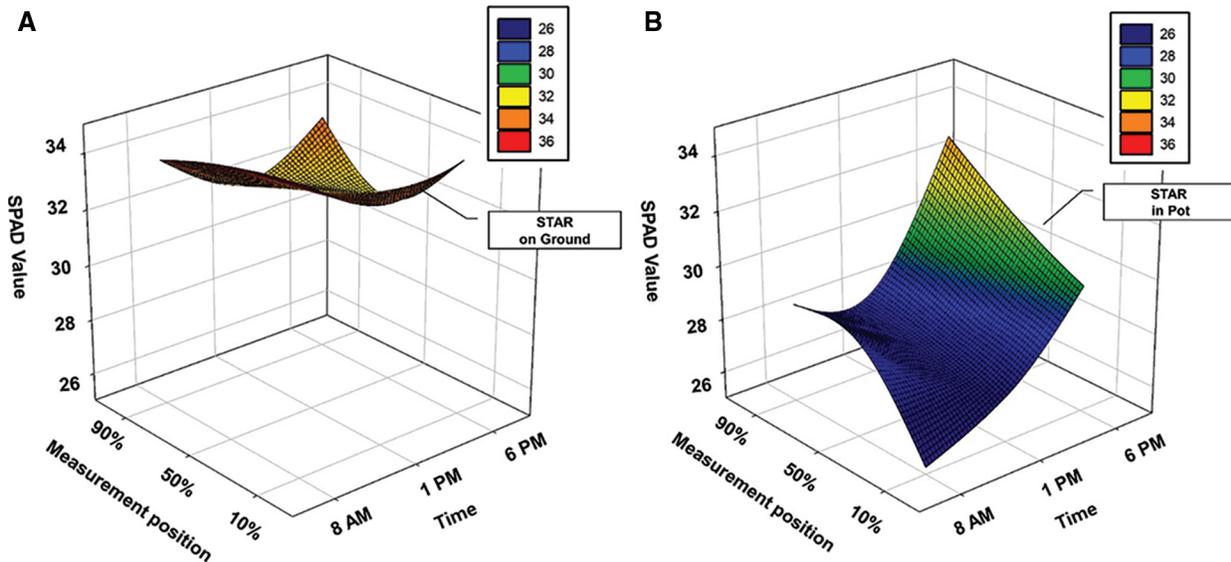


Figure 2: Three-dimensional mesh graph of SPAD value by blueberry height and time. A. Ground planted blueberry. B. Pot-planted blueberry

A hypothesis might explain each SPAD value pattern difference because evergreen trees do not maximize instantaneous photosynthesis for enduring water and nutrient stress [24]. In addition, the drought condition decreases the SPAD value in plants [25,26]. The pot size might not be enough, which causes drought or nutrient stress to the blueberry (*V. darrowii*). That could be the reason for the SPAD value pattern of pot-planted blueberry (*V. darrowii*).

Based on Figs. 1 and 2, we estimate that the SPAD value is affected by the environment, such as planted condition or time in the day. Also, the SPAD value in blueberry (*V. darrowii*) might have a daily rhythm.

In addition, this study found that the planted condition affects SPAD values (Table 3). The same plant height's SPAD value of ground planted blueberry (*V. darrowii*) was higher, while 14 h that observed than pot-planted blueberry (*V. darrowii*). These results suggest that ground planted blueberry (*V. darrowii*) has advantages that maintain SPAD value during the day. However, pot planting has other merits to management than ground planting. Therefore, increasing the SPAD value in the morning by substitute light would be required for pot-planted blueberry (*V. darrowii*) to maximize the photosynthetic rate for a higher yield. In smart farming, controlling the environment could optimize the SPAD values. Maintaining lighting, controlling light intensity, or other environmental factor management could improve the SPAD value of crops [27–29].

The current study confirms that the SPAD values represent differences in planted conditions. The ground planted blueberry maintained a stable and high SPAD value from 6 AM to 8 PM, 14 h. Unlike the ground planted blueberry, the pot-planted blueberry (*V. darrowii*) represents not have stable SPAD values during the day. It could affect the final yield of blueberry. To summarize, this study found that the same cultivar crop showed differences that could affect final yields by cultivating conditions. It suggests that even with the same cultivar, to maximize final yield, different management is needed. Future studies would confirm many factors affecting crop growth and yield to establish an efficient smart farming system.

Acknowledgement: We thank Sustainable Agriculture Research Institute (SARI) at Jeju National University for providing the experimental facilities.

Author Contributions: Yong Suk Chung and Gyung Deok Han designed the experiments. Gyung Deok Han and Dae Ho Jung conducted the experiments and analyzed the data. Gyung Deok Han wrote the manuscript. Yong Suk Chung and Seong Heo reviewed and edited the manuscript.

Funding Statement: Basic Science Research Program supported this research through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (2019R1A6A1A11052070).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. Balafoutis, A. T., Evert, F. K. V., Fountas, S. (2020). Smart farming technology trends: Economic and environmental effects, labor impact, and adoption readiness. *Agronomy*, 10(5), 743.
2. Jagannathan, S., Priyatharshini, R. (2015). Smart farming system using sensors for agricultural task automation. *2015 IEEE Technological Innovation in ICT for Agriculture and Rural Development (TIAR)*, pp. 49–53.
3. Sim, C., Zaharah, A., Tan, M., Goh, K. (2015). Rapid determination of leaf chlorophyll concentration, photosynthetic activity and NK concentration of *Elaeis guineensis* via correlated SPAD-502 chlorophyll index. *Asian Journal of Agricultural Research*, 9(3), 132–138.
4. Ling, Q., Huang, W., Jarvis, P. (2011). Use of a SPAD-502 meter to measure leaf chlorophyll concentration in *Arabidopsis thaliana*. *Photosynthesis Research*, 107(2), 209–214.

5. Islam, M. R., Haque, K. S., Akter, N., Karim, M. A. (2014). Leaf chlorophyll dynamics in wheat based on SPAD meter reading and its relationship with grain yield. *Journal of Scientia Agriculture*, 8(1), 13–18.
6. Porro, D., Dorigatti, C., Stefanini, M., Ceschini, A. (2000). Use of SPAD meter in diagnosis of nutritional status in apple and grapevine. *IV International Symposium on Mineral Nutrition of Deciduous Fruit Crops*, 564, 243–252.
7. Xiong, D., Chen, J., Yu, T., Gao, W., Ling, X. et al. (2015). SPAD-based leaf nitrogen estimation is impacted by environmental factors and crop leaf characteristics. *Scientific Reports*, 5(1), 1–12. DOI 10.1038/srep13389.
8. Liu, Y., Hatou, K., Aihara, T., Kurose, S., Akiyama, T. et al. (2021). A robust vegetation index based on different UAV RGB images to estimate SPAD values of naked barley leaves. *Remote Sensing*, 13(4), 686. DOI 10.3390/rs13040686.
9. Lyrene, P. M., Sherman, W. B. (2000). ‘Star’ southern highbush blueberry. *HortScience*, 35(5), 956–957. DOI 10.21273/HORTSCI.35.5.956.
10. Rodriguez-Saona, C., Vincent, C., Isaacs, R. (2019). Blueberry IPM: Past successes and future challenges. *Annual Review of Entomology*, 64(1), 95–114. DOI 10.1146/annurev-ento-011118-112147.
11. Retamales, J. B., Hancock, J. F. (2018). *Blueberries*. USA: CABI.
12. Whidden, A. (2008). Commercial blueberry production methods in Hillsborough County. *Proceedings of the Florida State Horticultural Society*, USA.
13. Milivojević, J. M., Radivojević, D. D., Maksimović, V. M., Dragišić-Maksimović, J. J. (2020). Variation in health promoting compounds of blueberry fruit associated with different nutrient management practices in a soilless growing system. *Journal of Agricultural Sciences*, 65(2), 175–185.
14. Nauš, J., Prokopová, J., Řebíček, J., Špundová, M. (2010). SPAD chlorophyll meter reading can be pronouncedly affected by chloroplast movement. *Photosynthesis Research*, 105(3), 265–271.
15. Wilcoxon, F. (1992). *Individual comparisons by ranking methods*. USA: Springer.
16. Kruskal, W. H., Wallis, W. A. (1952). Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*, 47(260), 583–621.
17. Costa, F. D. S., Lima, A. S. D., Magalhaes, I. D., Chaves, L. H. G., Guerra, H. O. C. (2018). Fruit production and SPAD index of pepper (*Capsicum annum* L.) under nitrogen fertilizer doses. *Australian Journal of Crop Science*, 12(1), 11–15.
18. Monostori, I., Árendás, T., Hoffman, B., Galiba, G., Gierczik, K. et al. (2016). Relationship between SPAD value and grain yield can be affected by cultivar, environment and soil nitrogen content in wheat. *Euphytica*, 211(1), 103–112.
19. Yang, H., Wu, Y., Zhang, C., Wu, W., Lyu, L. et al. (2022). Growth and physiological characteristics of four blueberry cultivars under different high soil pH treatments. *Environmental and Experimental Botany*, 197, 104842.
20. Ernest, K. A. (1989). Insect herbivory on a tropical understory tree: Effects of leaf age and habitat. *Biotropica*, 21(3), 194–199.
21. Xie, S., Luo, X. (2003). Effect of leaf position and age on anatomical structure, photosynthesis, stomatal conductance and transpiration of Asian pear. *Botanical Bulletin of Academia Sinica*, 44, 297–303.
22. Kaiser, E., Morales, A., Harbinson, J., Kromdijk, J., Heuvelink, E. et al. (2015). Dynamic photosynthesis in different environmental conditions. *Journal of Experimental Botany*, 66(9), 2415–2426. DOI 10.1093/jxb/eru406.
23. Pallas Jr, J. (1973). Diurnal changes in transpiration and daily photosynthetic rate of several crop plants. *Crop Science*, 13(1), 82–84. DOI 10.2135/cropsci1973.0011183X001300010025x.
24. Warren, C. R., Adams, M. A. (2004). Evergreen trees do not maximize instantaneous photosynthesis. *Trends in Plant Science*, 9(6), 270–274. DOI 10.1016/j.tplants.2004.04.004.
25. Nemeskéri, E., Neményi, A., Bócs, A., Pék, Z., Helyes, L. (2019). Physiological factors and their relationship with the productivity of processing tomato under different water supplies. *Water*, 11(3), 586. DOI 10.3390/w11030586.
26. Rahimi, A., Hosseini, S. M., Pooryoosef, M., Fateh, I. (2010). Variation of leaf water potential, relative water content and SPAD under gradual drought stress and stress recovery in two medicinal species of *Plantago ovata* and *P. psyllium*. *Plant Ecophysiol*, 2, 53–60.

27. Kasim, M. U., Kasim, R. (2017). While continuous white LED lighting increases chlorophyll content (SPAD), green LED light reduces the infection rate of lettuce during storage and shelf-life conditions. *Journal of Food Processing and Preservation*, 41(6), e13266.
28. Lee, B. J., Won, M. K., Lee, D. H., Shin, D. G. (2001). Changes in SPAD chlorophyll value of chrysanthemum (*Dendranthema grandiflora* Tzvelev) by photoperiod and light intensity. *Horticultural Science & Technology*, 19(4), 555–559.
29. Raza, M. A., Feng, L. Y., Iqbal, N., Ahmed, M., Chen, Y. K. et al. (2019). Growth and development of soybean under changing light environments in relay intercropping system. *PeerJ*, 7, e7262.