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ORIGINAL ARTICLE

Environmental Influences on SPAD Values in *Prunus mume* Trees: A Comparative Study of Leaf Position and Photosynthetic Efficiency Across Different Light Conditions

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Abstract

Prunus mume is a culturally significant fruit tree in East Asia that is widely used in traditional foods and medicines. The present study investigated the effects of sunlight exposure and leaf position on the photosynthetic efficiency of *P. mume* using SPAD values. The study was conducted at Cheongju National University of Education, Korea, under contrasting conditions between sunny (Site A) and shaded (Site B) areas on *P. mume* trees. Over three days, under varied weather, photosynthetic photon flux density (PPFD) and SPAD measurements were collected using a SPAD-502 plus chlorophyll meter and a smartphone PPFD meter application. The SPAD values of the 60 leaves were measured in triplicate for each tree. The results indicated that trees in sunny locations consistently exhibited higher SPAD values than those in shaded areas, implying greater photosynthetic efficiency. Moreover, leaves positioned higher in the canopy showed increased photosynthetic efficiency under different light conditions, underscoring the significance of leaf placement and light environment in photosynthetic optimization. Despite the daily sunlight variability, these factors maintained a consistent influence on SPAD values. This study concludes that optimal leaf positioning, influenced by direct sunlight exposure, significantly enhances photosynthetic efficiency in *P. mume.* These findings highlight the potential of integrating smart farming techniques, especially open-field smart farming technology, to improve photosynthesis and, consequently, crop yield and efficiency. The findings also highlight the need for further exploration of environmental factors affecting photosynthesis for agricultural advancement.

Key words: Environmental control, Light condition, Photosynthesis, Smart farming, SPAD, Species, Weather

1. Introduction

The appropriate environment for photosynthesis is essential for the plant's survival, and the light environment is especially important. For instance, shade-intolerant plant species require high levels of light and activate shade-avoidance responses that concentrate the resources for growth while reducing the energy of the defense system when the light is limited (Ballaré, 2014). Light availability in the shaded environment, including the understory, is essential to seeding survival and plant growth in forest ecosystems (Lin et al., 2014). Within a single plant, the light environment can vary depending on the position of each leaf or the surrounding environment.

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Zhen et al.(2022) reported that far-red photons could account for over 50% of the total incident photons between 400-750 nm in vegetation shade, and it could contribute 10-14% of leaf gross photosynthesis in a tree and an understory species in deep shade.

As highlighted, it is imperative to find ways to utilize light because of its importance to plants effectively. Before studying the light environment, it is important to consider that the light environment is constantly changing. The sun's position shifts over time, and weather changes affect the amount of daylight, influencing the light environment crucial for plant survival. Leaves in strong light environments develop a thicker palisade parenchyma layer to utilize high light intensities more effectively than those in shaded areas (Arrigoni-Blank et al., 2022; Yang et al., 2023). Additionally, leaves exposed to light tend to have higher nitrogen and chlorophyll content compared to those in shaded areas (Arrigoni-Blank et al., 2022). The nitrogen-to-phosphorus ratio is higher, and leaf mass per area is greater in high-light environments than in shaded places (Arrigoni-Blank et al., 2022; Yang et al., 2023).

The position of individual leaves significantly influences photosynthetic efficiency by determining their exposure to sunlight in terms of both duration and intensity. Leaves located higher within the canopy are more likely to receive direct sunlight for extended periods, enhancing their photosynthetic capacity. Leaf angles and density adjust to optimize light capture as the sun's position changes (de Casas et al., 2011). The leaf angle strategy also varies depending on the leaf's position, with more horizontal leaf angles observed in low-light environments to maximize light capture (Migliavacca et al., 2017). These traits are different from plant species to species. Each plant species adapts their leaf traits differently based on its light environment (de Casas et al., 2011; May and Oberbauer, 2021). These adaptations affect photosynthetic efficiency, with some species adapting to a wide range of environments and others to more specific conditions.

In this study, *Prunus mume* is used as plant material. The *P. mume* originated in China and is an important fruit crop in the subtropical region (Shi et al., 2020). *P. mume* is a culturally significant tree valued for its flowers; in East Asia, the fruits of this plant are extensively utilized in traditional foods and medicines and are recognized for their nutritional benefits, including vitamins, minerals, and antioxidants such as phenolic compounds (Ali et al., 2017). In Korea, the application of an open-field smart farm system to *P. mume* is being promoted (Lee et al., 2020), and for this purpose, research on the light environment of plum plants is needed. Aside from this, light environment research to increase the efficiency of fruit production is being conducted all over the world. The light intensity and exposure during fruit development significantly impact many traits of the fruit that are related to the final quality and price (Ismail et al., 2009). Also, the leaf condition directly related to photosynthetic capacity can impact the supply of carbohydrates and other nutrients to the quality of the developing fruits (Pino et al., 2023). Therefore, the light environment of the leaf is an important factor in fruit.

Soil plant analysis development (SPAD) measures leaves' greenness or relative chlorophyll content by measuring the leaf's transmittance in the red and near-infrared regions of the electromagnetic spectrum (Zhang et al., 2022). The SPAD meter provides a quick and non-destructive way to estimate chlorophyll content. It is widely used in agriculture to Estimate nitrogen status and fertilizer requirements (Vishwakarma et al., 2023). It is also used to monitor plant health and stress levels. Han et al.(2022a) report that the blueberry plant changes the SPAD values over time and differs from cultivars. Also, it was reported that the blueberry's planted place (pot vs ground) affects the SPAD value changes in a day (Han et al., 2022b).

In this study, we confirm the change in the SPAD value of *P. mume* in a planted place with a different light environment. One area is sunny, while the other is shaded. We also investigate the SPAD value differences of each leaf based on canopy height and time. This comprehensive analysis provides insights for optimizing the light environment to improve plant growth and fruit quality.

2. Materials and Methods

2.1. Character of the experiment area

The trees of *P.mume* located at Cheongju National University of Education in Sugokdong, Cheongju City, Korea, were measured to conduct this study. Fig. 1 represents Site A, the sunny area, and Site B, the shaded area. Table 1 represents the photosynthetic photon flux density (PPFD) value of sites A and B for three days, from 11:00 to 11:30 and from 16:00 to 16:30. The first two days were sunny, and the last day, 26 July, was rainy and cloudy during measurement (Table 1).

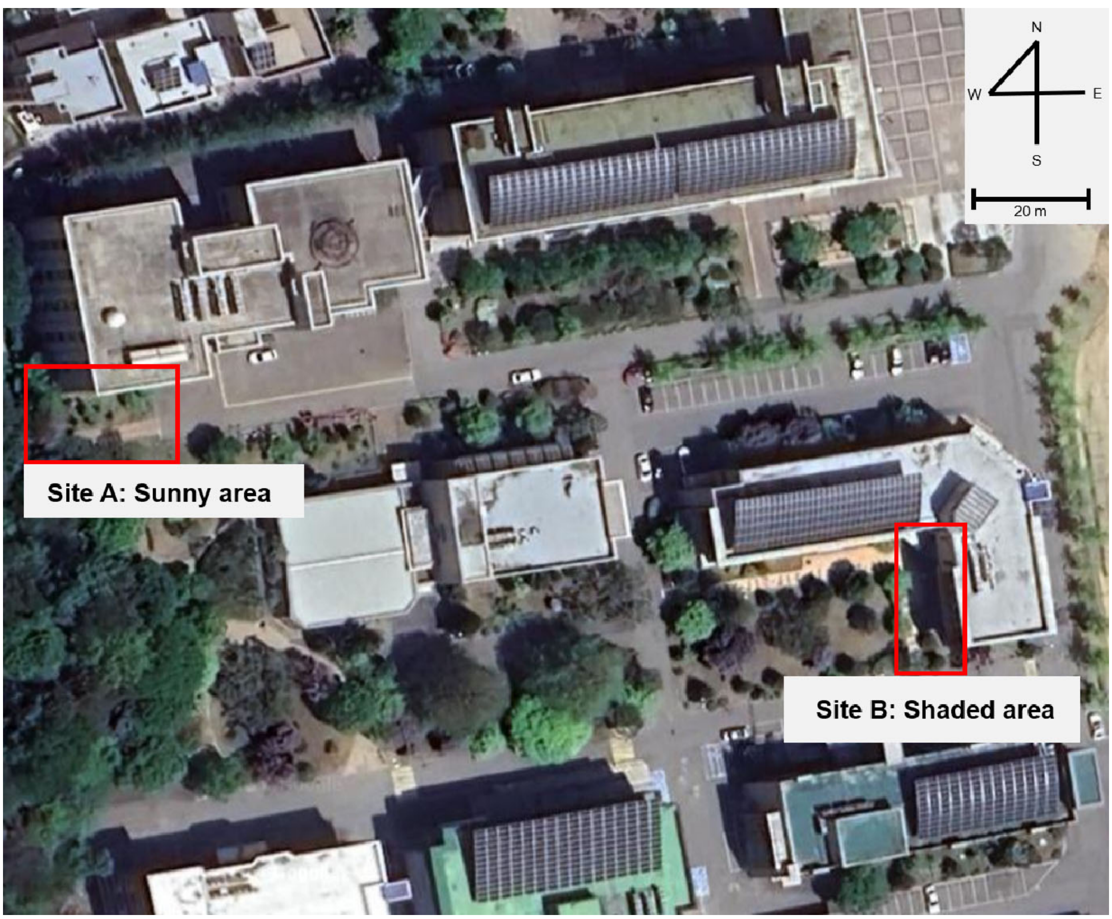

Fig. 1. Experiment areas. Site A is a sunny area, and site B is a shaded area. The image is from Google Maps.

Table 1. The day envelopment of each measuring day (2023 July 24 – 26)

	July 24	July 25	July 26
Sunrise time (hh: mm)[a]	05:29	05:30	05:31
Sunset time (hh: mm)	19:44	19:43	19:42
Aver temp. (℃)	27.4	26.8	27.3
Max temp. (℃)	30.8	30.0	32.0
Min temp. (℃)	24.5	25.0	24.5
Mean cloud amount[b]	8.0	9.4	7.1
Precipitation (mm)	6.6	3.0	7.3
Weather features	Rainy Mist	Rainy	Rain shower Mist Rainbow

[a] Data for Cheongju City obtained from the Korea Meteorological Administration
[b] Observe the sky with the naked eye and set it as 10 when it is all clouds, and give a number between 0 and 10 depending on the proportion of the sky covered by clouds

2.2. Plants materials

The trees of *P. mume* were cultivated in Cheongju. These trees were planted at the sites as saplings in 2010. Since then (to 2023), it has grown without any additional management, such as fertilizing or pruning, which affects the growth of the trees. The height of *P. mume* tree 1-6 was 230-250 cm, and the width was similar.

2.3. SPAD and PPFD measurement

A SPAD-502 plus chlorophyll meter (Konica Minolta Sensing, Japan) was used to measure the light intensity transmitted through the plant leaves at two wavelengths (650 nm and 940 nm) in a non-destructive manner (Minolta, 1989). To confirm photosynthesis efficiency, the SPAD value is measured (Han et al., 2022a, 2022b). Each *P. mume* tree was divided into two parts: Higher than 180 cm and lower than 180 cm. The fully grown leaves were measured from each part. Also, the SPAD values were measured two times a day, at 11:00 and 16:00. A Galaxy Note 10 Plus smartphone (Samsung, Korea) that uses the PPFD meter android application (Homestudio, US) measured the PPFD value simultaneously during the SPDA value measurement. Each leaf was measured three times, and 30 leaves for each height level of the tree were measured.

2.4. Statistical analysis

Excel (Microsoft, US) was used to organize and record the SPAD value data. Using the Excel file, statistical analysis was done using R software. T-test, ANOVA, and Tukey test were used. For the PPFD value, the minimum, maximum, and average values of the time were used.

3. Results and Discussion

The trees of *P. mume* planted at site A are in the sunny area in front of the building, and the direction of the building is on the north side of the trees. In contrast, the trees planted at site B are in the shaded area on the side of the building. The direction of the building is east of the *P. mume* trees; Because of the height of the building, the trees are shaded most of the day (Fig. 1).

Table 2 presents the PPFD measurements at sites A and B over three days, specifically during the peak sunlight hours of 11:00 to 11:30 at site B and the late afternoon period of 16:00 to 16:30, marking the end of significant photon availability from the sun.

On July 24, 11:00, the PPFD values of min, max, and average were higher than those in site B. Also, from 16:00 to 16:30, the values in site A were higher than those in site B (Table 2). This

Table 2. PPFD value of each day during measuring SPAD value (from 11:00 to 11:30 and 16:00 to 16:30)

Time	Place	Value	24 July	25 July	26 July
11:00	Site A (Sunny)	Min	176[a]	147	137
		Max	2223	1751	3214
		Average	560	345	655
	SiteB (Shaded)	Min	101	83	165
		Max	1675	1682	2481
		Average	280	212	2285
16:00	Site A (Sunny)	Min	180	150	12
		Max	1200	1124	221
		Average	456	240	161
	SiteB (Shaded)	Min	48	44	46
		Max	455	90	342
		Average	165	69	141

[a] unit = μmol m^{-2} sec^{-1}

Table 3. ANOVA result of SPAD value difference in a day between three *P. mume* trees

Place	Tree number	24 July	25 July	26 July	Total
Site A (Sunny)	Tree 1	35.88 ± 4.66 b [a]	38.37 ± 6.18 a	34.83 ± 5.45 b	36.36 ± 5.65 b
	Tree 2	40.80 ± 5.42 a	38.87 ± 5.91 a	38.00 ± 6.48 a	39.22 ± 6.05 a
	Tree 3	30.94 ± 3.96 c	32.00 ± 3.80 b	31.41 ± 4.26 c	31.45 ± 4.02 c
		$p < 0.001^{***,b}$ n = 360 df = 2	$p < 0.001^{***}$ n = 360 df = 2	$p < 0.001^{***}$ n = 360 df = 2	$p < 0.001^{***}$ n = 1080 df = 2
SiteB (Shaded)	Tree 4	31.43 ± 3.81 a	31.25 ± 3.97 a	30.92 ± 4.21 a	31.20 ± 3.99 a
	Tree 5	27.66 ± 4.26 b	29.26 ± 3.62 b	26.92 ± 3.31 b	27.95 ± 3.87 b
	Tree 6	28.00 ± 2.30 b	27.89 ± 2.13 b	27.58 ± 2.24 b	27.82 ± 2.22 b
		$p < 0.001^{***}$ n = 360 df = 2	$p < 0.001^{***}$ n = 360 df = 2	$p < 0.001^{***}$ n = 360 df = 2	$p < 0.001^{***}$ n = 1080 df = 2

[a] Mean ± standard deviation followed by different letters within columns significantly different by the Tukey test
[b] The statistical significant of *p*-value; $p < 0.05^{*}$, $p < 0.01^{**}$, $p < 0.001^{***}$

trend was maintained the next day; All PPFD values were higher at site A than at B. However, on day 3, on July 26, this trend changed, caused by the cloudy and rainy weather. This weather can be confirmed in Table 1, which is the record of a rain shower. On that day, at 11:00, the maximum PPFD value was higher at site A, but the other values were higher at site B. Similarly, at 16:00, only average PPFD values were higher at site A, and the others were higher at site B (Table 2).

Table 3 illustrates the ANOVA results for SPAD values among trees at sites A and B. On 24 July, a significant difference in SPAD values was observed between all trees at both sites ($p < 0.001$). This significant variation persisted throughout the observation period (24 July to 26 July), consistently showing higher SPAD values at site A, a sunny area, compared to site B, a shaded area,

Table 4. T-test result of SPAD value of *P. mume* at 11 AM and 4 PM for three days

Tree number [a]	Time	24 July	25 July	26 July	Total
Tree 1	11:00	34.96 ± 5.16	40.30 ± 7.24	35.53 ± 4.62	36.92 ± 6.23
	16:00	36.81 ± 3.93	36.45 ± 4.13	34.12 ± 6.13	35.79 ± 4.95
	p-value	$p < 0.05^{*, b}$ n = 120	$p < 0.001^{***}$ n = 120	$p = 0.16$ n = 120	$p = 0.056$ n = 360
Tree 2	11:00	40.31 ± 5.93	38.54 ± 5.79	39.48 ± 5.69	39.44 ± 5.81
	16:00	41.29 ± 4.87	39.22 ± 6.07	36.52 ± 6.93	39.01 ± 6.29
	p -value	$p = 0.33$ n = 120	$p = 0.532$ n = 120	$p < 0.05^{*}$ n = 120	$p = 0.50$ n = 360
Tree 3	11:00	30.49 ± 4.77	32.39 ± 3.93	31.83 ± 3.94	31.57 ± 4.29
	16:00	31.40 ± 2.90	31.61 ± 3.66	30.99 ± 4.56	31.33 ± 3.75
	p -value	$p = 0.21$ n = 120	$p = 0.265$ n = 120	$p = 0.28$ n = 120	$p = 0.58$ n = 360
Tree 4	11:00	31.72 ± 3.08	30.65 ± 3.52	30.32 ± 4.27	30.89 ± 3.69
	16:00	31.17 ± 4.43	31.86 ± 4.32	31.53 ± 4.09	31.51 ± 4.27
	p -value	$p = 0.40$ n = 120	$p = 0.10$ n = 120	$p = 0.12$ n = 120	$p = 0.15$ n = 360
Tree 5	11:00	26.79 ± 4.16	28.82 ± 3.60	27.48 ± 3.28	27.69 ± 3.77
	16:00	28.52 ± 4.22	29.70 ± 3.61	26.36 ± 3.28	28.19 ± 3.96
	p -value	$p < 0.05^{*}$ n = 120	$p = 0.19$ n = 120	$p = 0.07$ n = 120	$p = 0.22$ n = 360
Tree 6	11:00	27.90 ± 2.21	28.08 ± 1.89	28.02 ± 2.47	27.99 ± 2.19
	16:00	28.10 ± 2.40	27.70 ± 2.35	27.15 ± 1.90	27.65 ± 2.25
	p -value	$p = 0.63$ n = 120	$p = 0.33$ n = 120	$p < 0.05^{*}$ n = 120	$p = 0.14$ n = 360

[a] Tree 1-3 are planted in Site A (sunny area), and tree 1-6 are planted in Site B (shaded area)
[b] The statistical significant of p-value; $p < 0.05^{*}$, $p < 0.01^{**}$, $p < 0.001^{***}$

despite the trees being of similar age (Table 3).

The variation in SPAD values over time was analyzed, with Table 4 detailing the differences between 11:00 and 16:00 measurements. On 24 July at Site A, SPAD values for Trees 2 and 3 showed no significant difference ($p > 0.05$), whereas Tree 1 exhibited a significant difference ($p > 0.05$). This pattern persisted on 25 July. On 26 July, Trees 1 and 3 showed no significant difference, contrasting with Tree 2, which did significantly differ. These discrepancies could potentially be attributed to daily environmental variations or differing conditions. Consequently, the three days of merged data reveal that time does not significantly impact SPAD values for any trees at Site A ($p > 0.05$). This tendency in SPAD values was consistent across Site B as well. The statistical analysis result of Site B is similar to that of trees in sunny areas; there were no significant differences ($p > 0.05$) in SPAD values over time (Table 4).

Table 5 shows the difference in SPAD values between leaves positioned above and below 180 cm (Table 5). At Site A, significant differences ($p < 0.001$) in SPAD values were observed between higher and lower leaves for all trees, except for Tree 2 on 26 July. On that day in Tree 2, The SPAD value by the position of the leaves is not significantly different ($p > 0.05$). This anomaly may be attributed to daily environmental

Table 5. T-test result of SPAD value of *P. mume* at over 180 cm and under 180 cm height for three days

Tree number	height	24 July	25 July	26 July	Total
Tree 1[a]	> 180 cm	34.70 ± 4.88	35.23 ± 3.55	33.42 ± 5.32	34.45 ± 4.86
	< 180 cm	37.07 ± 4.13	41.51 ± 6.66	36.23 ± 5.25	38.27 ± 5.89
	p-value	$p < 0.01^{**, b}$ n = 120	$p < 0.001^{***}$ n = 120	$p < 0.01^{**}$ n = 120	$p < 0.001^{***}$ n = 360
Tree 2	> 180 cm	38.56 ± 3.86	36.51 ± 4.53	38.51 ± 4.95	37.86 ± 4.55
	< 180 cm	43.03 ± 5.85	41.25 ± 6.21	37.49 ± 7.73	40.59 ± 7.00
	p-value	$p < 0.001^{***}$ n = 120	$p < 0.001^{***}$ n = 120	$p = 0.39$ n = 120	$p < 0.001^{***}$ n = 360
Tree 3	> 180 cm	32.22 ± 3.85	34.21 ± 3.20	33.84 ± 3.98	33.42 ± 3.77
	< 180 cm	29.66 ± 3.67	29.78 ± 3.01	28.98 ± 2.96	29.48 ± 3.23
	p-value	$p < 0.001^{***}$ n = 120	$p < 0.001^{***}$ n = 120	$p < 0.001^{***}$ n = 120	$p < 0.001^{***}$ n = 360
Tree 4	> 180 cm	32.39 ± 3.29	32.34 ± 3.67	32.91 ± 3.66	32.55 ± 3.53
	< 180 cm	30.47 ± 4.07	30.17 ± 4.00	28.93 ± 3.79	29.85 ± 3.99
	p-value	$p < 0.01^{**}$ n = 120	$p < 0.01^{**}$ n = 120	$p < 0.001^{***}$ n = 120	$p < 0.001^{***}$ n = 360
Tree 5	> 180 cm	26.71 ± 3.38	27.99 ± 2.86	26.27 ± 3.60	26.99 ± 3.35
	< 180 cm	28.60 ± 4.84	30.52 ± 3.88	27.57 ± 2.88	28.90 ± 4.11
	p-value	$p < 0.05^{*}$ n = 120	$p < 0.001^{***}$ n = 120	$p < 0.05^{*}$ n = 120	$p < 0.001^{***}$ n = 360
Tree 6	> 180 cm	28.18 ± 2.25	28.19 ± 1.97	27.31 ± 1.84	27.89 ± 2.06
	< 180 cm	27.81 ± 2.36	27.59 ± 2.26	27.86 ± 2.56	27.76 ± 2.38
	p-value	$p = 0.39$ n = 120	$p = 0.13$ n = 120	$p = 0.18$ n = 120	$p = 0.561$ n = 360

[a] Tree 1-3 are planted in Site A (sunny area), and tree 1-6 are planted in Site B (shaded area)
[b] The statistical significant of *p*-value; $p < 0.05^{*}$, $p < 0.01^{**}$, $p < 0.001^{***}$

variations. Merged data from the three-day period across all trees at Site A confirmed significant differences in SPAD values based on leaf position ($p < 0.001$). A similar trend was noted at Site B, except for Tree 6. For Trees 4 and 5, SPAD values significantly differed by leaf position on all measurement days, a finding that was consistent in the aggregated data ($p < 0.001$). However, for Tree 6, no significant difference was found in SPAD values between the positions of the leaves ($p > 0.05$).

In summary, after 13 years of acclimatization to their environments, *P. mume* trees displayed distinct adaptive responses. Significantly, trees situated in Site A, a sunny area, consistently registered higher SPAD values than those in Site B, a shaded region. It was observed that SPAD values remained consistent throughout the day for each tree, regardless of the light conditions at their respective sites. This indicates that diurnal shifts in sunlight intensity, associated with the sun's movement, do not markedly affect SPAD values. However, it is important to note that the data for this study were collected over only three days. Despite the short observation period, the reliability of the findings is enhanced by the high number of data points (1800 single data points per tree) used in the analysis. This large sample size provides a reliable basis for the conclusions. A similar observation was reported in blueberry;

However, in that plant, the SPAD value was differently affected by sun to blueberry cultivars (Han et al., 2022a). Moreover, our research decisively shows that leaf position within the canopy significantly influences SPAD values, with a notable statistical variance in SPAD readings based on the vertical position of leaves. This finding emphasizes the importance of leaf placement in optimizing photosynthetic efficiency. While these outcomes underscore the multifaceted factors impacting SPAD values in *P. mume*, the specific influence of leaf position is identified as a pivotal factor. Notably, the efficiency of different leaf positions did not present a uniform pattern, potentially due to varying daylight intensity or duration. An expanded sample size would be beneficial to obtain more definitive data. In addition, Wang et al.,(2021) reported that open-field smart farming has developed to a practical level, making it possible to control cultivation environment parameters such as light intensity. Combined with our findings, it could increase the efficiency of photosynthesis in orchards with open-field smart farming.

4. Conclusion

In this study, we found that the light environment and the height of the leaf position could affect photosynthetic efficiency. Furthermore, the impact of the environment varies, as each plant and each leaf adjusts with different reactions. In the past, due to a lack of technology, optimizing every leaf's photosynthetic potential was difficult. However, with the advent of smart farming technology that senses and analyzes complex factors, it is now possible to optimize the photosynthetic potential of most leaves, even in open-field conditions. This study suggests that even under field environment conditions, open-field smart farming technology can control and optimize the photosynthetic potential of plants. Therefore, further research should consider larger sample sizes and a detailed examination of environmental variables to ascertain the precise elements affecting leaf position efficiency. Identifying these factors could enable the integration of this knowledge into smart farming practices, potentially enhancing crop yield and system efficiency.

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