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Effect of TiO₂ Nanoparticles on the Yield and Photophysiological Responses of Cherry Tomatoes during the Rainy Season

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Abstract: The rainy season occurs mainly from June to July in Korea, and this season causes insufficient ambient light intensity for the growth of cherry tomato in a greenhouse. Titanium dioxide (TiO₂), as a photocatalyst, is known to affect photosynthesis in plants. This study was carried out to investigate the influence of TiO₂ foliar spray application on the yield and photophysiological responses of cherry tomato under low ambient light intensity during the rainy season in a greenhouse. Cherry tomato plants were treated with 100 mg·L⁻¹ TiO₂ (T1) or 200 mg·L⁻¹ TiO₂ (T2) nanoparticle suspension on 26 June. The control group was not treated with TiO₂. In the O–J phase of the OJIP transient under a cloudy day (2 July), the slope in the control and T1 groups rose more sharply than that in the T2 group. Conversely, on a clear day (10 July), the J–I phase of the T2 group sharply increased compared to that of the control and T1 groups. On a cloudy day with low ambient light intensity, the rate of electron transport flux from QA to QB per photosystem II reaction center (ET₀/RC) and carbon dioxide (CO₂) fixation of TiO₂-treated plants were increased compared to those of the control. However, on a clear day of high light intensity, the ET₀/RC and CO₂ fixation of the T2 group were lower than those of the control and T1 groups. The yield of fruit was increased in the T1 group over that in other treatments. TiO₂ treatment reduced the size of the fruit and delayed the ripening time, but greatly increased the fruit hardness. These results suggest that setting the concentration and supply amount of TiO₂ nanoparticles suitable for various environmental conditions should be prioritized in order to improve the effect of TiO₂ nanoparticles in tomato cultivation.

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1. Introduction

Due to the increase in CO₂ emissions, abnormal weather phenomena have been increasing worldwide in recent years. This abnormal weather negatively affects agricultural productivity in Korea [1]. An increase in the number of cloudy days, a type of abnormal climate phenomenon, increases agricultural risk in terms of solar energy [2]. Also, low-light-intensity conditions due to cloudy days are one of the limiting environmental factors that negatively affect the photophysiological response of horticultural crops during cultivation [3]. In Korea, where the rainy season occurs in June and July, the ambient light intensity temporarily decreases significantly. Recently, abnormal weather phenomena have been added to this period, resulting in a significant decrease in light intensity and increased risk to crops [4,5]. During these low-light-intensity periods, useful technologies that can reduce the risk to horticultural crops are urgently needed [6].

TiO₂, a representative photocatalyst, has been studied for application to agriculture [7], and research on its agricultural use is still in progress in many countries. TiO₂ is reported to be nontoxic to tomato plants and effective in increasing their yield in terms of leaves and fruits [8]. Zahra et al. (2017) reported that TiO₂ promotes rice growth, and there is no risk of TiO₂ intake following soil application of TiO₂ [9]. It has been reported that the application of TiO₂ nanoparticles is effective in protecting stevia plants under salt stress conditions by increasing their growth, photosynthetic rate, and antioxidant activity and reducing hydrogen peroxide, malondialdehyde, and electrolytes [10]. In the case of red beans, it

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is reported that TiO_2 is beneficial for growth by promoting physiological activity [11]. In addition, the application of TiO_2 nanoparticles is reported to be effective for strawberry cultivation in the low-light season by increasing the chlorophyll content, fruit yield, and fruit hardness of strawberries in winter when ambient light is insufficient [12]. On the other hand, there have been opposing results that TiO_2 nanoparticles decrease the electron transport rate and photosynthetic efficiency of chloroplasts when exposed to grapevine leaves [13]. In Korea, it is necessary to examine the effect of TiO_2 when horticultural crops receive insufficient light intensity due to cloudy weather during the rainy season.

Cherry tomato is a popular horticultural crop that is low in calories and consumed raw, and it is also a major vegetable grown in greenhouses in Korea [14]. Cherry tomato is greatly affected by the environment in the greenhouse during cultivation. Photosynthesis efficiency and chlorophyll *a* fluorescence test are the main analysis methods used to check the physiological response according to the environmental stress acting on tomato growing in a greenhouse [15,16]. In particular, the OJIP test related to electron transfer in the chloroplast can determine horticultural crops' rapid stress response to the environment [17]. Currently, studies on the phenotypic characteristics of cherry tomato fruit under TiO_2 application are rare. Therefore, this study was conducted to determine the yield, fruit phenotypic characteristics, and photophysiological responses of cherry tomato treated with TiO_2 foliar spray in a greenhouse during the rainy season in Korea.

2. Materials and Methods

2.1. Cherry Tomato Plant Cultivation

Cherry tomatoes (*Lycopersicon esculentum* Mill. cv. Berry King) were cultivated from spring to summer in a plastic greenhouse at Kongju National University, Korea. One greenhouse with a size of $25 \times 10 \times 3.5$ m (length $\times$ width $\times$ height) was used in this experiment. Seeds were sown on 10 April 2018, and seedlings with 4 to 5 leaves grown for 50 days were planted in the greenhouse on 30 May. For soil cultivation, the greenhouse soil was fertilized with nitrogen, phosphorus, potassium, and calcium elements at 24, 16, 24, and $150 \text{ kg} \cdot 10\text{a}^{-1}$, respectively. Irrigation was started at a soil water tension of -25 kPa or less by drip irrigation to grow cherry tomatoes in the greenhouse. The light intensity data in the greenhouse were measured using a photosynthetically active radiation sensor (S-LIA-M003; Onset Computer Corp., Bourne, MA, USA), which was installed 1.5 m from the ground, and stored in a data log (H21-USB; Onset Computer Corp., Bourne, MA, USA) (Figure 1).

Figure 1. Ambient light intensity in the greenhouse used to cultivate cherry tomatoes from June to July.

2.2. TiO₂ Treatment

Based on the results of studies that confirmed the effect of TiO₂ treatment at a concentration of 0–200 ppm in various crops, in this experiment, the plants were treated with a similar TiO₂ concentration [9,10,12]. TiO₂ foliar spray materials were prepared by mixing 100 mg (T1) or 200 mg (T2) of TiO₂ nanoparticles in 1 L of distilled water containing 1% glycine and stirring for 2 h. TiO₂ solution (T1 or T2) was applied to the foliage at 200 mL per plant on June 26. The control group was not treated with TiO₂. The absorbance at each wavelength (from 300 nm to 900 nm) of the TiO₂ treatment and control groups was measured (Figure 2A), and cherry tomato leaves after TiO₂ treatment are shown in Figure 2B.

Figure 2. Absorbance (A) and foliar application (B) of TiO₂ nanoparticle suspension.

2.3. Chlorophyll *a* Fluorescence OJIP and Photosynthesis

The OJIP test is widely used to validate the photophysiology of plants; fast fluorometry shows that the chlorophyll *a* fluorescence intensity rises from a minimal (O) level in less than 1 s to a maximal (P) level via two intermediate steps, labeled J and I [18]. The definitions and formulas of the OJIP test parameters used in this experiment are shown in Table 1. In the OJIP test, 10 plants were analyzed for each treatment, and measurements were taken three times on 26 June, 2 July, and 10 July. For the OJIP test, the leaf above the first flower cluster of cherry tomato plants was selected and allowed to adapt to the dark for 20 min in the morning and then measured with a fluorometer (FP 100; Photon Systems Instruments, Drasov, Czech Republic).

Table 1. Definitions of the OJIP test parameters and formulas selected using data from the fast fluorescence transient.

Parameter	Definition
F_0	Minimal fluorescence when all photosystem II reaction centers are open (at 20 μ s)
F_J	Fluorescence intensity at step J (at 3 ms)
F_I	Fluorescence intensity at step I (at 30 ms)
F_M (F_P)	Maximal fluorescence intensity when all photosystem II reaction centers are closed
F_V	Maximal variable fluorescence: $F_V = F_M - F_0$
V_J	Relative variable Chl fluorescence (at 2 ms): $V_J = (F_{2ms} - F_0)/(F_M - F_0)$
F_V/F_M (ϕP_0)	Maximal quantum yield of primary photochemistry. Expresses the probability that an absorbed photon leads to a reduction in Q_A : $\phi P_0 = TR_0/ABS = F_V/F_M$
M_O	Initial slope of the induction curve: $M_O = 4(F_{300\mu s} - F_0)/(F_M - F_0) = TR_0/RC - ET_0/RC$
ABS/RC	Average absorbed photon flux per photosystem II reaction center: $ABS/RC = M_O(1/V_J)(1/\phi P_0)$
TR_0/RC	Trapped energy flux leading to a reduction in Q_A : $TR_0/RC = M_O(1/V_J)$
ET_0/RC	Rate of electron transport flux from Q_A to Q_B per photosystem II reaction center: $ET_0/RC = M_O(1/V_J)(1 - V_J)$
DI_0/RC	Dissipated energy flux per reaction center: $DI_0/RC = ABS/RC - TR_0/RC$

Photosynthesis was analyzed using a portable photosynthesis system (LI-6400; LI-COR, Lincoln, NE, USA) on the same day by randomly selecting 4 plants from among those plants for which the OJIP was measured in the morning. The chamber conditions of the LI-6400 were set as follows: chamber temperature, 30 °C; RH, 50%; CO_2 , 400 μ mol·mol⁻¹; photon flux density, 1000 μ mol·m⁻² s⁻¹.

2.4. Yield and Fruit Characteristics

On the afternoon of July 10, both mature and immature fruits were harvested, and the weight, sugar content, and hardness of the fruits were measured. The sugar content of the fruit was measured using a pocket refractometer (PAL-1, ATAGO Co. Ltd., Kyoto, Japan), and the hardness of the fruit was measured using a fruit hardness tester (FHM-1, Takemura Industry Co. Ltd., Kyoto, Japan).

2.5. Experimental Design and Statistical Analysis

This experiment was repeated 3 times with a random block design. Each repeat consisted of 20 plants treated with the assigned TiO_2 concentration. The TiO_2 treatment results were analyzed via analysis of variance with Duncan's multiple range test using a significance level of $p \leq 0.05$, and correlation analysis using significance levels of $p < 0.01$ and $p < 0.001$, in the SAS 9.4 program (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Ambient Light Intensity in the Greenhouse and TiO_2 Absorbance

The ambient light intensity in the greenhouse from 20 June to 11 July is shown in Figure 1. From 20 to 26 June, the light intensity was over 1400 μ mol·m⁻²·s⁻¹, and from 27 June to 2 July, the light intensity dropped below 600 μ mol·m⁻²·s⁻¹ due to rainy or cloudy days. After 3 July, sunny days continued again, and the light intensity increased to over 1200 μ mol·m⁻²·s⁻¹. The absorbance for each wavelength increased proportionally as the content of TiO_2 increased (Figure 2A). When TiO_2 suspension was sprayed on the leaves,

white water droplets were formed, after which the moisture evaporated, and only the white TiO_2 nanoparticles remained on the leaves (Figure 2B).

3.2. OJIP and Photosynthesis of Cherry Tomato

When the effect of TiO_2 foliar spray was investigated by plotting the OJIP on a logarithmic time scale, the J–P phase of the chlorophyll *a* fluorescence OJIP transient fell significantly under the low-light-intensity conditions compared to that under the high-light-intensity conditions (Figure 3). In the control and T1, the I peak under low light intensity was lower than the J peak. Contrariwise, in T2, the I peak was higher than the J peak (Figure 3B). When the ambient light environment changed from low light intensity (from 27 June to 2 July) to high light intensity (after 3 July), the OJIP curve recovered to increasing order of the OJIP peaks for T1 and the control. On the contrary, in T2, the J peak was higher than the I peak (Figure 3C).

Figure 3. OJIP transient plotted on a logarithmic time scale for tomatoes grown under foliar spray treatments of different TiO_2 concentrations with changes in ambient light intensity. (A): measured on June 26; (B): measured on July 2; (C): measured on July 10.

In all treatments, the values of average absorbed photon flux per photosystem II reaction center (ABS/RC), trapped energy flux leading to a reduction in Q_A (TR_0/RC), and dissipated energy flux per reaction center (DI_0/RC) of cherry tomato leaves tended to decrease as the light intensity increased, whereas in T1 and the control, the rate of electron transport flux from Q_A to Q_B per photosystem II reaction center (ET_0/RC) value increased as the light intensity increased. For T2, the ET_0/RC value continued to decrease regardless of the light intensity (Figures 1 and 4).

Figure 4. Energy fluxes per reaction center calculated using data on the OJIP transients of tomato plants grown under foliar spray treatments of different TiO_2 concentrations with changes in ambient light intensity. Vertical bars are standard deviations ($n = 10$).

The value changes for photosynthetic parameters such as the photosynthesis rate (Pr), transpiration rate (Tr), and stomatal conductance (Sc) are shown in Figure 5. The Pr , Tr , and Sc values were mainly affected by the light intensity rather than TiO_2 treatment and increased in proportion to the light intensity. For the TiO_2 treatments under low light intensity, the Pr and Tr values were significantly higher in T1. On the other hand, when the light intensity increased again on 10 July, T1 continued to have a higher Pr value compared to other treatments, but the Pr value of T2 was the lowest. There was no significant difference in the Sc values according to TiO_2 treatment.

Figure 5. Photosynthetic parameters of tomato leaves grown under foliar spray treatments of different TiO₂ concentrations with changes in ambient light intensity. Vertical bars are standard deviations ($n = 4$). Small letters at the data points indicate mean separation between the values on the same date by Duncan's multiple range test at $p = 0.05$.

The correlation coefficients between the photosynthesis and OJIP parameters of cherry tomato plants treated under different TiO₂ concentrations with changes in light intensity are shown in Table 2. Pr showed a positive correlation of 99.9% with Tr (0.825), Sc (0.714), and ET₀/RC (0.641), but a negative correlation of 99.9% with ABS/RC (−0.758), TR₀/RC (−0.544), and DI₀/RC (−0.827). Among the OJIP parameter values, ABS/RC, TR₀/RC, and DI₀/RC showed a negative correlation with ET₀/RC. In these results for TiO₂ treatments according to changes in light intensity, there was a high positive correlation between the ET₀/RC value, representing the electron transfer efficiency of photosystem II, and the Pr value, representing the efficiency of CO₂ fixation.

Table 2. Correlation coefficients between photosynthetic parameters and chlorophyll fluorescence parameters of cherry tomato plants treated with different TiO₂ concentrations with changes in ambient light intensity.

Index	Pr	Tr	Sc	ABS/RC	TR ₀ /RC	ET ₀ /RC	DI ₀ /RC
Pr	1						
Tr	0.825 ***						
Sc	0.714 ***	0.887 ***					
ABS/RC	−0.758 ***	−0.827 ***	−0.890 ***				
TR ₀ /RC	−0.544 ***	−0.676 ***	−0.790 ***	0.908 ***			
ET ₀ /RC	0.641 ***	0.595 ***	0.545 **	−0.473 **	−0.092		
DI ₀ /RC	−0.827 ***	−0.842 ***	−0.85 ***	0.948 ***	0.729 ***	−0.704 ***	1

, * Significant correlation at the 99% and 99.9% level ($n = 36$), respectively, using Pearson correlation coefficients. Pr: photosynthesis rate; Tr: transpiration rate; Sc: stomatal conductance.

3.3. Yield and Fruit Characteristics of Cherry Tomato

Table 3 shows the yield of cherry tomatoes according to the TiO₂ foliar spray application used. The total fruit yield of T1, including ripe and unripe fruits, was significantly higher than those of T2 and the control. The weight per fruit decreased with increasing TiO₂ concentration in the order of control (10.7 g) > T1 (8.3 g) > T2 (7.6 g).

Table 3. The yields of cherry tomatoes grown under foliar spray treatments of different TiO₂ concentrations.

Treatment	Ripe Fruit Weight (g/Plant)	Unripe Fruit Weight (g/Plant)	Total Fruit Weight (g/Plant)	Average Fruit Weight (g/Fruit)
Control	46.7 ± 2.5 a ^z	149.9 ± 2.4 b	195.7 ± 4.9 b	10.7 ± 0.3 a
T1	49.3 ± 3.7 a	200.3 ± 7.8 a	249.7 ± 11.4 a	8.3 ± 0.1 b
T2	38.3 ± 1.2 b	162.0 ± 4.3 b	200.3 ± 5.6 b	7.6 ± 0.2 c

^z Values followed by different letters within a column are significantly different (DMRT, $p < 0.05$, $n = 3$).

The solid soluble content of ripe fruits was significantly higher in the T2 (8.8 brix) group than in the control (8.2 brix) and T1 (8.1 brix) groups. However, there was no significant difference between the treatments in terms of the solid soluble content of unripe fruits. In terms of fruit hardness, both ripe and unripe fruits were significantly harder in T1 and T2, treated with TiO₂, than in the control group (Table 4).

Table 4. The soluble solid content and fruit hardness of tomatoes grown under foliar spray treatments of different TiO₂ concentrations.

Treatment	Ripe Fruit Soluble Solid Content (Brix%)	Unripe Fruit Soluble Solid Content (Brix%)	Ripe Fruit Hardness (kg·m ^{−2})	Unripe Fruit Hardness (kg·m ^{−2})
Control	8.20 ± 0.43 ab ^z	6.20 ± 0.19 a	25.68 ± 2.27 b	35.27 ± 1.06 b
T1	8.08 ± 0.28 b	6.30 ± 0.18 a	28.62 ± 1.69 a	38.42 ± 1.57 a
T2	8.82 ± 0.49 a	6.58 ± 0.50 a	29.01 ± 1.00 a	38.42 ± 1.44 a

^z Values followed by different letters within a column are significantly different (DMRT, $p < 0.05$, $n = 20$).

4. Discussion

The rainy season is a meteorological phenomenon that occurs periodically from June to July every year in Korea [19]. By measuring the light intensities in this study, it was observed that the light intensity required for the growth of cherry tomatoes in a greenhouse decreased sharply between the end of June and the beginning of July due to rainy and cloudy days (Figure 1). In this study, the reduction in light intensity had a negative effect on the photophysiological response and yield of cherry tomatoes. According to

Fuijuan and Cheng (2012), tomatoes grown at a light intensity of $1150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ yielded more than 150% the amount of fresh fruit compared to tomatoes grown at a light intensity of $368 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ [20]. In tomatoes, the difference in photosynthesis efficiency between light intensities of $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ is very large [21]. To reduce the damage caused by low light intensity, TiO_2 suspension was applied to the leaves of cherry tomatoes, as shown in Figure 2B. The EI_0/RC value measured in the low-light-intensity condition increased with TiO_2 treatment, which is considered to indicate that TiO_2 improved the rate of electron transport flux on photosystem II reaction centers. Additionally, DI_0/RC , the dissipated energy flux per reaction center of TiO_2 -treated plants, was decreased, indicating that TiO_2 contributed to the photosynthetic light reaction (Figure 4). Shabbir et al. (2019) reported that the photochemical efficiency of PSII was increased when an appropriate amount of TiO_2 ($90 \text{ mg}\cdot\text{L}^{-1}$) was applied to vetiver plants [22]. However, in the T2 group in this study, for which a high TiO_2 content was used, both EI_0/RC and DI_0/RC fell sharply when the weather conditions changed to a high light intensity (Figure 4). Teszlák et al. (2018) reported that when TiO_2 was applied to grapevines growing in field conditions, lower electron transport rate and lower nonphotochemical quenching were observed in the process of photoprotection of leaves from photoinhibition [13]. From these results, it is concluded that photoinhibition occurred due to long-term exposure to high light intensity after TiO_2 treatment of horticultural crop leaves. In addition, it was confirmed that photoinhibition occurred when a TiO_2 concentration higher than an appropriate amount was applied to the leaves of plants. TiO_2 foliar spray affected the CO_2 assimilation and chlorophyll *a* fluorescence of cherry tomatoes under different light intensities (Figure 5). With results similar to those of this study, Gao et al. (2006) reported that when spinach was treated with TiO_2 , a complex of Rubisco and Rubisco activase was induced to promote Rubisco carboxylation and eventually improved photosynthetic efficiency [23]. When TiO_2 was applied to cherry tomato during the low-light-intensity period, the fruit yield was increased but the average fruit weight was decreased compared to those of the control (Table 3). This result is considered to be due to the increase in the number of flowers in the flower cluster by TiO_2 treatment. In addition, TiO_2 treatment delayed fruit maturation and increased fruit hardness in tomatoes (Tables 3 and 4). With results similar to those of this experiment, when TiO_2 was applied to strawberry leaves, the yield of fruit was increased and the fruit hardness was improved [12]. Azmat et al. (2020) reported that when spinach was treated with TiO_2 nanoparticles, the starch content was increased due to its photocatalytic properties [24]. Additionally, Khater (2015) reported that the production and antioxidant content of coriander plants treated with TiO_2 were increased [25]. According to Waani et al. (2021), the effect may be good or bad depending on the concentration of TiO_2 applied in rice cultivation [26]. Based on these results, TiO_2 is effective agriculturally. However, further studies on TiO_2 application are needed because the effect on crops may vary depending on the concentration and method of TiO_2 application [27–29].

5. Conclusions

In this study, as a result of applying TiO_2 foliar spray to tomatoes under changing light environment conditions during the rainy season, the photosynthetic rate and the electron transfer rate of tomatoes were improved in the $100\text{mg}\cdot\text{kg}^{-1}$ TiO_2 , and thus the fruit yield was increased. On the other hand, in the $200\text{mg}\cdot\text{kg}^{-1}$ TiO_2 , the photosynthetic rate of tomatoes decreased under high light intensity conditions, and there was no effect of increasing the fruit yield. In contrast to fruit yield, the fruit hardness was increased and the average fruit weight was decreased under all treated TiO_2 concentrations.

In conclusion, the use of TiO_2 nanoparticles in agricultural application can be beneficial or harmful depending on various situations. Therefore, in order for TiO_2 to have a positive effect, it is necessary to clarify the various conditions in terms of low and high light intensities, the concentration of applied TiO_2 , and crop types. In addition, I think that

research on the effect of TiO₂ under various conditions is continuously needed in the application of greenhouse cultivation.

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