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Yield, Flower Quality, and Photo-Physiological Responses of Cut Rose Flowers Grafted onto Three Different Rootstocks in Summer Season

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Abstract: The thermal stress caused by high temperatures on cut rose flowers grown in greenhouses is a major environmental impact that reduces the yield of growing cut rose flowers during summer. To confirm the resistance of grafted cut rose flowers to high-temperature stress, roses were grown in a greenhouse during the summer season and analyzed for yield, quality, root activity, and photo-physiological characteristics. A morphological change was observed in the stomata of the grafted cut rose flowers, which were larger in size than the scion or rootstocks. As a result of cultivating cut rose flowers by lowering the temperature of the greenhouse through shading in summer, it was confirmed that all of the scions, rootstocks, and grafted cut rose flowers were not in a stressed state by observing the maximal quantum yield of primary photochemistry (F_V/F_M) values on the chlorophyll-*a* fluorescence. However, the rate of electron transport flux from the primary acceptor (Q_A) to the secondary acceptor (Q_B) per the photosystem II reaction center (ET_0/RC) value was found to be significantly higher on grafted cut rose flowers, compared with that of the scions. The efficiencies of the photosynthesis rate, the transpiration rate, and the stomatal conductance were increased when grafted compared with non-grafted. When the root activity was confirmed by the formazan content, it was found that the root activity was improved grafting. Furthermore, when grafted, morphological changes such as flower size and the number of petals on spray roses were also observed. Although there was a difference depending on the type of rootstock, the yield of the grafted cut rose flowers increased by 11–20%, compared with the scion rose. Therefore, grafting cultivation during the summer season with high temperatures is an effective method in terms of photo-physiological response and yield.

Keywords: chlorophyll-*a* fluorescence; electron transport; heat stress; OJIP; photosynthesis; root activity

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1. Introduction

During the cultivation of horticultural crops under greenhouse conditions, abiotic stress caused by various negative environmental conditions, such as high or low temperature and high or low light intensity, is induced, resulting in reduced yield and reduced quality [1,2]. In particular, it is known that high temperature in the summer season reduces photosynthesis efficiency and increases respiration rate, thereby greatly reducing the productivity of crops [3,4]. Roses are vulnerable to high temperatures, and when they are subjected to heat stress caused by high temperature, the phenomenon of semi-dormancy results as the flower bud differentiation stops and is transformed into vegetative growth, resulting in no flowering and causing growth problems [5,6].

The occurrence of abiotic stress caused by environmental factors such as high temperature and light intensity is often diagnosed through the chlorophyll-*a* fluorescence reaction that occurs during the light reaction of photosynthesis in plants [7–9]. When the maximal

quantum yield of primary photochemistry (F_V/F_M) values is around 0.8, the plant is in a normal state, and when this value falls below 0.75, the plant is considered to be in a stress state [10,11]. In the case of strawberries in the family of *Rosaceae*, it is reported that the F_V/F_M value drops below 0.7 under extreme abiotic stress [9]. Horticultural crops grown in a greenhouse, except for those in extreme abiotic stress state where the F_V/F_M value actually falls below 0.7, are mostly irradiated with values within the normal range. On the other hand, even if the F_V/F_M value is approximately 0.8 to indicate a normal state, the rate of electron transport flux from Q_A to Q_B per the photosystem II reaction center (ET_0/RC) value and the chlorophyll-*a* fluorescence fast rise curve (OJIP) parameter can be used to judge the state of the horticultural crop with regard to the difference in photosynthesis efficiency [7,11]. In addition, an important technique employed to check the current state of various horticultural crops grown in greenhouses is to analyze the photosynthetic efficiency, such as photosynthesis rate, transpiration rate, and stomatal conductance [12,13]. Roots are generally more exposed to various abiotic stresses than shoots. Thus, the root system can be even more affected by stresses than the aerial parts of horticultural plants [14]. The triphenyl tetrazolium chloride method can be used to monitor root activity under abiotic stress conditions, and such root activity values are effective in confirming the conditions of horticultural crops [15,16].

The rose is one of the most popular ornamental plants in the world [17], and the spray-type rose (*Rosa hybrida* L.), an economically important cut flower with five or more buds per stem, is one of the most in-demand cut flowers in Korea [18]. Roses are grown all year round in greenhouses, and the high temperature in summer is a representative stress factor that reduces the flower quality and yield in Korea. A representative cultivation technique that makes it easier to grow cut rose flowers in an environment normally unsuitable for growing roses is the grafting method. Grafting is the creation of a new type of plant that combines different scion and rootstock plants into one plant. It is known that grafting is an effective way to improve rose heat resistance [5]. In addition, when a rose is grafted, the morphological characteristics of the rose are reportedly changed [19]. Guard cells are specialized cells in the leaves that produce a stomatal pore, which controls the exchange of gases such as O_2 and CO_2. In addition, the stomata are plant organs that easily undergo morphological changes according to environmental conditions [20]. The guard cells used for carbon fixation in photosynthesis are known to be related to the chlorophyll-*a* fluorescence reaction [21]. Furthermore, studies on chlorophyll-*a* fluorescence response characteristics and the morphological changes in guard cells in grafted cut flowers are rare.

Therefore, this study was conducted to confirm the morphological changes, photo-physiological responses, flower quality, and yield of grafted spray-type cut rose flowers grown in greenhouses under high-temperature conditions in the summer season.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The scion used was the spray rose cultivar *Rosa hybrida* cv. Pink Shine (PS), which is a small-flowering type of rose. The three cultivars used as rootstocks were *Rosa multiflora* cv. Hort No. 1 (N1), *Rosa canina* cv. Natal Briar (NA), and *Rosa indica* cv. Major (RI). The yield, flower quality, and photo-physiological response characteristics were confirmed using seven different individual plants, including the non-grafted scion rose cultivar (PS), three rootstock rose cultivars (N1, NA, and RI), and three grafted rose flowers (PS/N1, PS/NA, and PS/RI).

All of the rose flowers were planted in a glass greenhouse in June 2018 at the National Institute of Horticultural and Herbal Science, Korea (35°50′02″ N, 127°02′04″ E). The practical experiment to confirm the effect of grafting under high-temperature conditions in the summer season was carried out in a glass greenhouse from June to September 2019. The size of the glass greenhouse was 8 × 10 × 4 m (length × width × height). The glass greenhouse temperature was controlled by closing or opening ventilators when the temperature was below 20 °C or above 25 °C. In order to prevent a rapid temperature rise

in the glass greenhouse, a shading screen was controlled to close or open at below 30 °C or above 35 °C. The average photoperiod in the greenhouse during the experiment was 13 h in the daytime and 11 h at nighttime. The relative humidity in the greenhouse was 45 ± 10% during the daytime and 70 ± 20% during the nighttime.

The roses were planted in a rockwool medium (25 × 100 × 75 cm, Grodan, Roermond, The Netherlands) and cultivated hydroponically. During cultivation, the roses were supplied with 100 mL of a nutrient solution per one time via a drip irrigation system, up to 12 times per day. The composition of the nutrient solution from the Japan National Institute of Vegetable and Tea Science (JNIVT) was: macroelements (NO3-N:NH4-N:P:K:Ca:Mg:S = 16:1.33:4:8:8:4:4 me·L^{-1}), microelements (Fe:Mn:B:Zn:Cu:Mo = 2:0.5:0.25:0.2:0.05:0.05 mg·L^{-1}), electrical conductivity (EC) = 1.2 dS·m^{-1}, and hydrogen ion concentration (pH) = 5.8–6.0). The cultivation of the cut rose flowers was carried out using an arching cultivation technique, which explains why the basal shoots that appeared in the early stages of growth were bent in an arch. The subsequent axillary basal shoots that appeared later were harvested as cut rose flowers from the basal branching per [22]. Light intensity was recorded at 1 h intervals using LI-190 quantum sensors (Licor, NE, USA) installed 170 cm above the ground in the glass greenhouse.

During the high-temperature period from June to September 2019, the yield of the flowers and the quality of the cut flowers were investigated at weekly intervals for the entire plant for each rose experimental treatment. For the analysis of stomata, chlorophyll-*a* fluorescence, and photosynthesis, the used leaves were located in the middle of the shoot and fully unfolded at around 30 days of age.

2.2. Scanning Electron Microscopy (SEM)

SEM analysis was performed to confirm the change in the morphological character of the stomata according to the grafting. This SEM analysis was based on the Clément method [23]. For the SEM analysis, leaves from each rose plant, at around 30 days after leaf emergence (DALE), were harvested and pretreated on 5 August. The leaf tissues were fixed with 2.5% glutaraldehyde (*v*/*v* in a 0.1 M phosphate buffer) at a pH of 7.2 in the presence of 4% sucrose (*w*/*v*) for 24 h. After three rinses (15 min each) with the above buffer, the specimens were dehydrated in the alcohol series and dried in a critical point dryer (HCP-2, Hitachi, Tokyo, Japan) with carbon dioxide as the intermediate fluid. Samples were then coated with gold-palladium for 2 min (30Å) using an ion sputter (MC 1000, Hitachi, Tokyo, Japan) and examined on a SEM (SU-3500, Hitachi, Tokyo, Japan) operating at an accelerating voltage of 15 kV.

2.3. Chlorophyll-a Fluorescence Fast Rise Curve (OJIP) and Photosynthesis

The chlorophyll-*a* fluorescence fast rise curve (OJIP) test is widely used to validate the photo-physiological properties of plants, such as photo-stress and the electron transfer efficiency of photosynthesis. Fast fluorometry shows that chlorophyll-*a* fluorescence intensity rises from a minimal (O) level in less than 1 s to a maximal (P) level via 2 intermediate steps labeled J and I [24]. Table 1 shows the definitions and formulas of the OJIP test parameters used to check the grafting effect. In the OJIP test, 15 plants were analyzed by rose type, and measurements were taken three times from 3 to 5 August. For the OJIP test, leaves of roses at around 30 DALE were selected, and dark adaptation was conducted for 30 min in the morning, then measurements with a portable fluorometer (FP 100; Photon Systems Instruments, Drasov, Czech Republic) were carried out.

Table 1. Definitions and formulas of the chlorophyll-*a* fluorescence fast rise curve (OJIP) test parameters.

Parameter	Definition
F_0	Minimal fluorescence when all photosystem II reaction centers are open (at 20 μs)
F_J	Fluorescence intensity at J step (at 3 ms)
F_I	Fluorescence intensity at I step (at 30 ms)
F_M (F_P)	Maximal fluorescence intensity when all photosystem II reaction centers are closed
F_V	Maximal variable fluorescence: $F_V = F_M - F_0$
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 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: $DI_0/RC = ABS/RC - TR_0/RC$

Photosynthetic efficiency was analyzed using a portable photosynthesis system (LI-6800; LI-COR, Lincoln, NE, USA) in the morning. The leaves, at around 30 DALE, of five plants of each rose variety were randomly selected and measured from 5 to 10 August. The chamber conditions of the LI-6800 for measuring photosynthetic efficiency according to temperature change were set as follows: relative humidity (RH), 50%; CO_2, 400 μmol·mol^{-1}; and photon flux density, 800 μmol·m^{-2}·s^{-1}. The chamber conditions of the LI-6800 for measuring photosynthetic efficiency according to light intensity change were set as follows: chamber temperature, 27 °C; RH, 50%; and CO_2, 400 μmol·mol^{-1}.

2.4. Root Activity

The root activity of the roses was measured using a UV–visible spectrophotometer (Evolution 300, Thermo Co., CA, USA) at 470 nm following the method described by [25]. After washing the fresh root samples in running water, the root was cut into 2 cm lengths and mixed so that the root weight totaled 500 mg, then the mixture was placed in a glass test tube. To each sample tube was added 10 mL of a mixture composed of triphenyl tetrazolium chloride solution, 0.1 M sodium phosphate-buffered solution, and distilled water at a ratio of 1:4:5. After allowing the air bubbles to dissipate over a 10 min period, the reaction was continued in the dark for two hours in a constant temperature bath maintained at 30 °C. Next, 2 mL of 2N H_2SO_4 was added to the sample tubes, and the samples were vortexed and then rinsed with running water. An amount of 10 mL of ethyl acetate and 1 g of crushed quartz glass was then added to the reacted root samples, and the mixture was filtered through a filter paper (110 mm) (No. 2 filter paper, Advantec Mfs Inc., Dublin, CA, USA) to extract formazan. The formula for calculating the activity of roots using formazan is the amount of formazan (mg) divided by the root weight (g) times the reaction time (h).

2.5. Experimental Design and Statistical Analysis

The grafting experiment was repeated 3 times as 1 independent experiment consisting of 7 treatments (1 scion, 3 rootstocks, 3 grafted cut rose flowers) in a random block design. Five plants were planted in 1 rockwool medium for each treatment, and 7 treatments, including 35 plants in 7 rockwool media, were placed on one hydroponics gutter system and were used as 1 independent experiment. The grafting treatment results including yield, flower quality, root activity, and photo-physiological response were analyzed using descriptive statistics (mean and standard deviation) in Excel (Excel 2016, Microsoft Co., Redmond, WA, USA) and analysis of variance with Duncan's multiple range test, using a significance level of $p \leq 0.05$ in the SAS 9.4 program (SAS Institute Inc., Cary, NC, USA). Principal component analysis (PCA) was conducted to evaluate the relationships among the yield, vegetable growth, root activity, photosynthetic parameters, and chlorophyll-*a* fluorescence parameters on cut rose flowers grown in a greenhouse during the summer season using the SAS 9.4 program (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Ambient Light Intensity and Temperature in the Greenhouse

The ambient light intensity in the glass greenhouse during the experimental period, from June to September 2019, was maintained at between 500 and 900 μmol·m^{-2}·s^{-1} through shading to prevent excessive high-temperature conditions. During the experiment, the air temperature inside the glass greenhouse was controlled so as not to exceed 40 °C during the daytime through shading. However, the night-time temperature was approximately 20 °C in June and September, and there were many days when the night-time temperature rose to more than 25 °C in July and August (Figure 1).

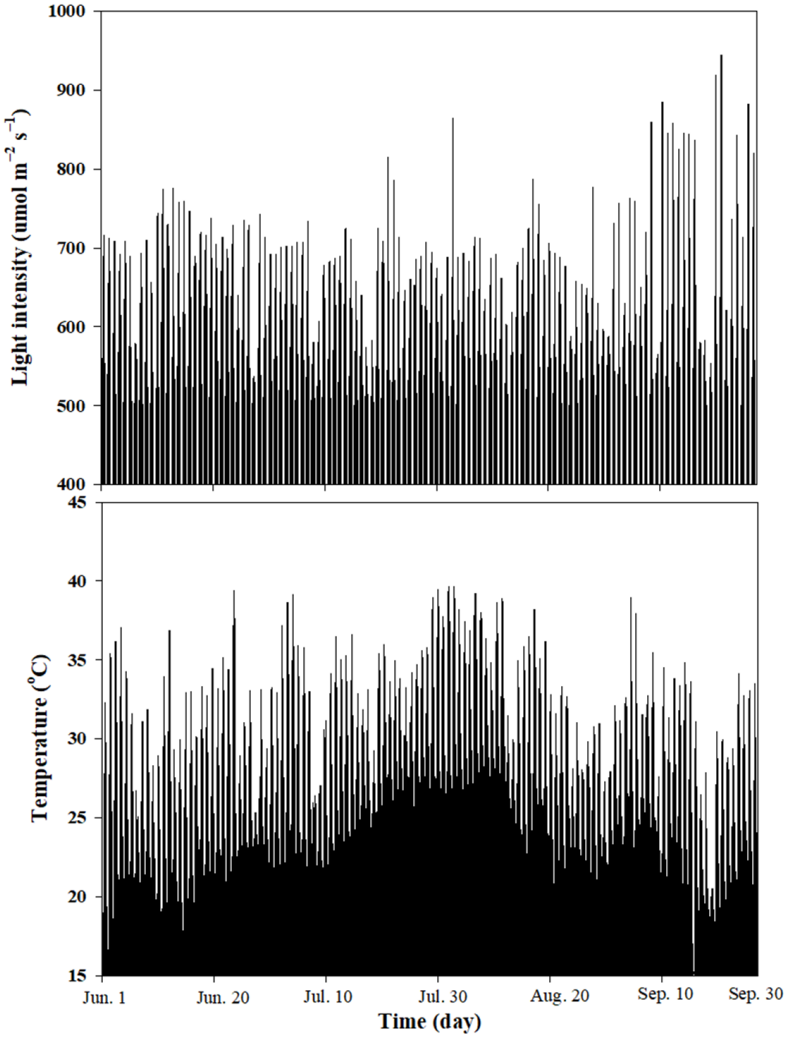

Figure 1. Ambient light intensity and air temperature in the greenhouse during cultivation of scion, rootstocks, and grafted cut rose flowers from June to September.

3.2. Stomata Images of Cut Rose Flowers

The morphological characteristics of the guard cells and stomata of the grafted cut rose flowers were changed via grafting (Table 2 and Figure 2). The stomata of the plants used as the rootstocks (N1, NA, and RI) were small in size and large in number, whereas the scion PS had larger stomata and fewer stomata, compared with the rootstocks. The grafted cut rose flowers (PS/N1, PS/NA, and PS/RI) showed morphological changes, in which the size of the guard cells and pores increased (Table 2), and the number of stomata decreased (Figure 2), compared with the scion and rootstocks.

Table 2. The size of guard cells and stoma (pore) of scion, rootstocks, and grafted cut rose flowers grown in the greenhouse during the summer season.

Treatment	Size Characteristics (μm)			
	Guard Cell Length	Guard Cell Width	Stoma (Pore) Length	Stoma (Pore) Width
PS	28.18 ± 2.26 b [z]	11.02 ± 1.12 b	17.95 ± 1.35bc	2.49 ± 0.17 b
N1	18.85 ± 1.37cd	7.42 ± 1.03cd	14.31 ± 1.16 d	2.38 ± 0.11 b
NA	17.53 ± 1.56 d	6.69 ± 1.23 d	14.16 ± 1.07 d	2.15 ± 0.23 b
RI	20.98 ± 1.48 c	8.75 ± 1.08 c	16.51 ± 1.26 c	2.46 ± 0.13 b
PS/N1	33.46 ± 1.96ab	12.24 ± 1.38 b	20.29 ± 1.42ab	3.12 ± 0.33 a
PS/NA	30.15 ± 1.56 b	12.01 ± 1.06 b	19.18 ± 1.21 b	2.97 ± 0.25 a
PS/RI	36.52 ± 1.74 a	14.37 ± 1.36 a	21.18 ± 1.35 a	3.62 ± 0.15 a

[z] Values followed by different letters within a column are significantly different (Duncan's multiple range test, $p < 0.05$. $n = 3$). PS: scion plant of spray rose *Rosa hybrida* cv. Pink Shine; N1: rootstock of *Rosa multiflora* cv. Hort No. 1; NA: rootstock of *Rosa canina* cv. Natal Briar; RI: rootstock of *Rosa indica* cv. Major; PS/N1: PS grafted onto N1; PS/NA: PS grafted onto NA; PS/RI: PS grafted onto RI.

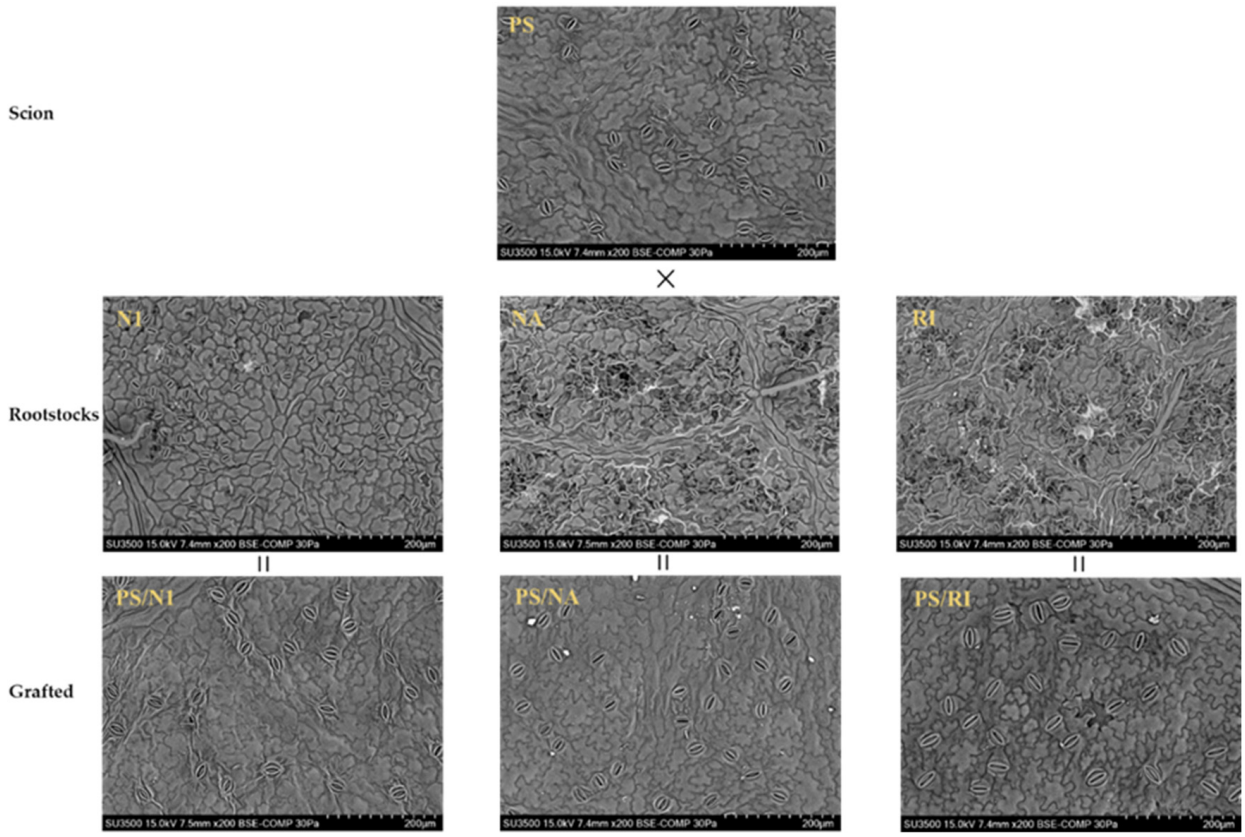

Figure 2. The guard cells and stomatal size of one scion, three rootstocks, and three grafted cut rose flowers taken with scanning electron microscopy. PS: scion plant of spray rose *Rosa hybrida* cv. Pink Shine; N1: rootstock of *Rosa multiflora* cv. Hort No. 1; NA: rootstock of *Rosa canina* cv. Natal Briar; RI: rootstock of *Rosa indica* cv. Major; PS/N1: PS grafted onto N1; PS/NA: PS grafted onto NA; PS/RI: PS grafted onto RI.3.3. OJIP and photosynthesis of cut rose flowers.

3.3. OJIP and Photosynthesis of Cut Rose Flowers

When the effect of grafting on the cut rose flowers was measured using OJIP plotting on a logarithmic time scale, it was found that there was no difference in the OJIP plotting patterns of the scion, rootstocks, and grafted cut rose flowers, and the F_V/F_M value was 0.82 or 0.83, indicating a normal state rather than stress (Figure 3A). The arbitrary unit values of the OJIP parameters, such as minimal fluorescence when all photosystem II reaction centers are open (F_O), fluorescence J step (F_J), fluorescence I step (F_I), and maximal fluorescence

intensity when all photosystem II reaction centers are closed (F_P), of the grafted cut rose flowers were higher than those of the scion and the rootstocks (Figure 3B).

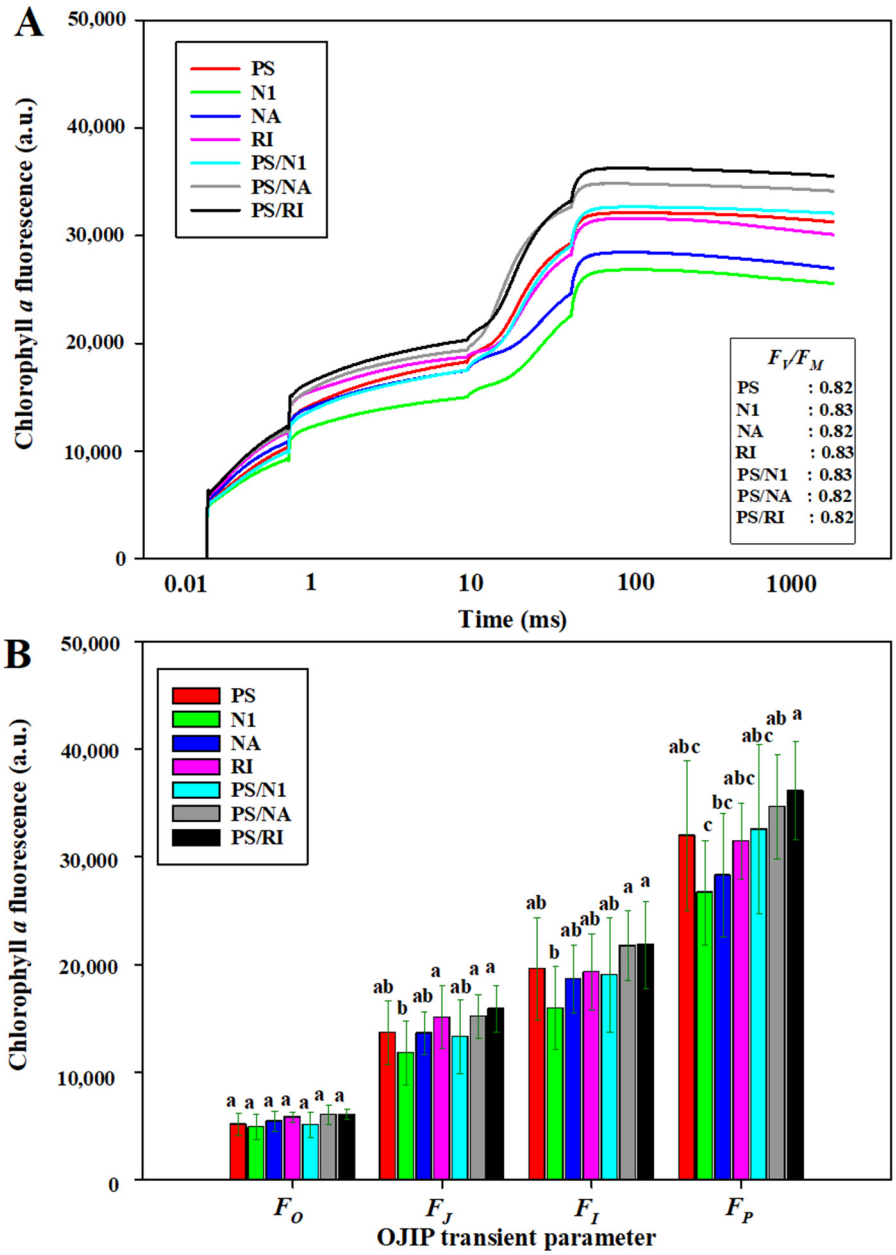

Figure 3. OJIP transient parameter plotted on a logarithmic time scale (**A**), and the parameter values (**B**) of one scion, three rootstocks, and three grafted cut rose flowers grown in the greenhouse during the summer season. PS: scion plant of spray rose *Rosa hybrida* cv. Pink Shine; N1: rootstock of *Rosa multiflora* cv. Hort No. 1; NA: rootstock of *Rosa canina* cv. Natal Briar; RI: rootstock of *Rosa indica* cv. Major; PS/N1: PS grafted onto N1; PS/NA: PS grafted onto NA; PS/RI: PS grafted onto RI. Vertical bars are standard deviations (n = 15). Small letters at the data points indicate mean separation between the values by Duncan's multiple range test at p = 0.05.

In particular, the grafted PS/NA and PS/RI were significantly higher in F_O, F_J, F_I, and F_P values. In the energy flux of the chloroplast membrane, 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), rate of electron transport flux from Q_A to Q_B per photosystem II reaction center (ET_0/RC), and dissipated energy flux per reaction center (DI_0/RC), calculated using the OJIP transient, showed the lowest in PS, a scion plant.

Among the rootstocks, the ABS/RC, TR_0/RC, and DI_0/RC values of NA were high. The grafted cut rose flowers were significantly higher in ABS/RC, TR_0/RC, ET_0/RC, and DI_0/RC values than in the scion plant (Figure 4).

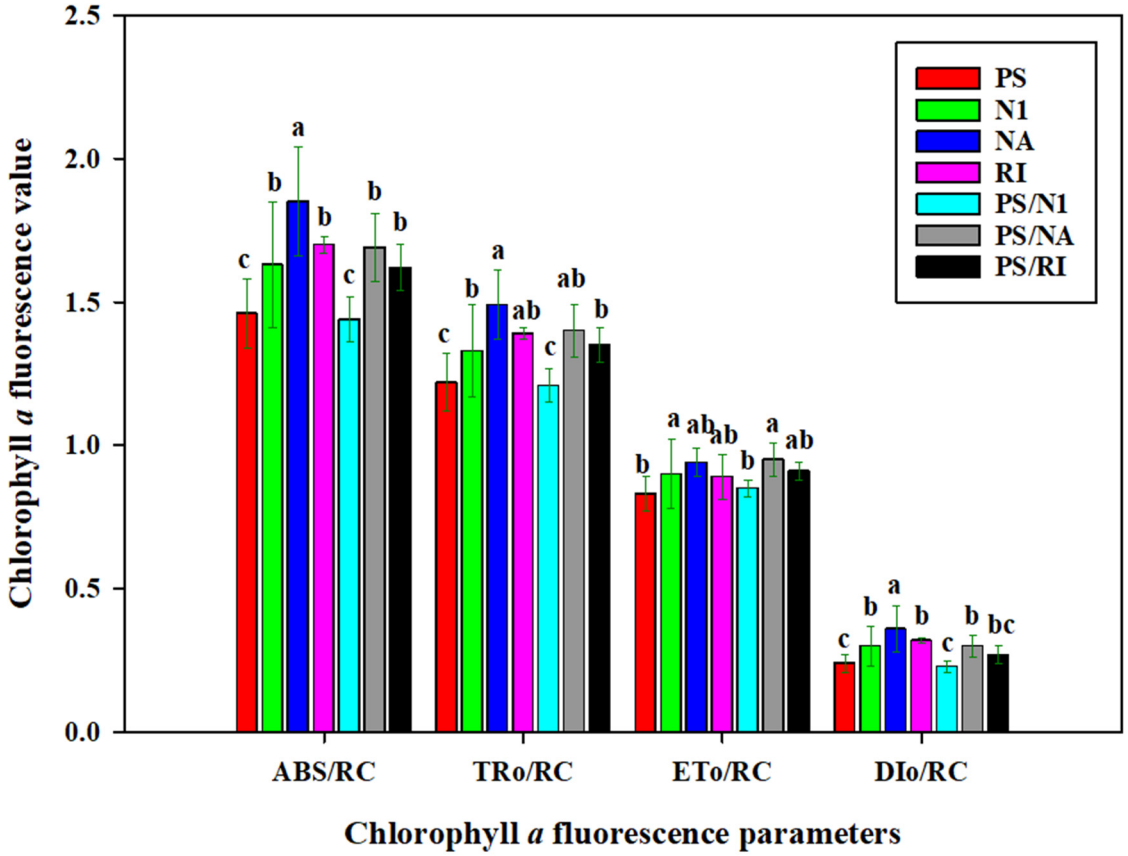

Figure 4. OJIP transient parameters absorbance unit (a.u.) of one scion, three rootstocks, and three grafted cut rose flowers grown in the greenhouse during the summer season. PS: scion plant of spray rose *Rosa hybrida* cv. Pink Shine; N1: rootstock of *Rosa multiflora* cv. Hort No. 1; NA: rootstock of *Rosa canina* cv. Natal Briar; RI: rootstock of *Rosa indica* cv. Major; PS/N1: PS grafted onto N1; PS/NA: PS grafted onto NA; PS/RI: PS grafted onto RI. Vertical bars are standard deviations (n = 15). Small letters at the data points indicate mean separation between the values by Duncan's multiple range test at p = 0.05.

The value changes in the photosynthetic parameters, such as photosynthesis rate, transpiration rate, and stomatal conductance, according to different temperatures and light intensities are shown in Figure 5. During the experimental period from June to September, the temperature in the glass greenhouse was between 30 and 40 °C, and the light intensity was between 500 and 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Figure 1). Considering the temperature and light intensity during this experiment period, the photosynthesis rate, transpiration rate, and stomatal conductance of the NA rootstock were the highest, compared with those of the other rose plants (Figure 5). Except for N1, the rootstocks (NA and RI) were higher in photosynthetic efficiency at a high temperature than PS. The grafted cut rose flowers, which inherited the characteristics of the rootstock, showed a higher photosynthesis rate, transpiration rate, and stomatal conductance efficiency at a high temperature than the scion. Interestingly, the photosynthetic efficiency of the N1 rootstock was lower than that of the scion; however, the photosynthesis rate, transpiration rate, and stomatal conductance of the grafted PS/N1 were significantly higher than those of N1 and PS. In addition, the photosynthetic efficiency of the rootstock itself was significantly higher in RI than in N1; however, in the case of grafting, PS/N1 was significantly higher than PS/RI.

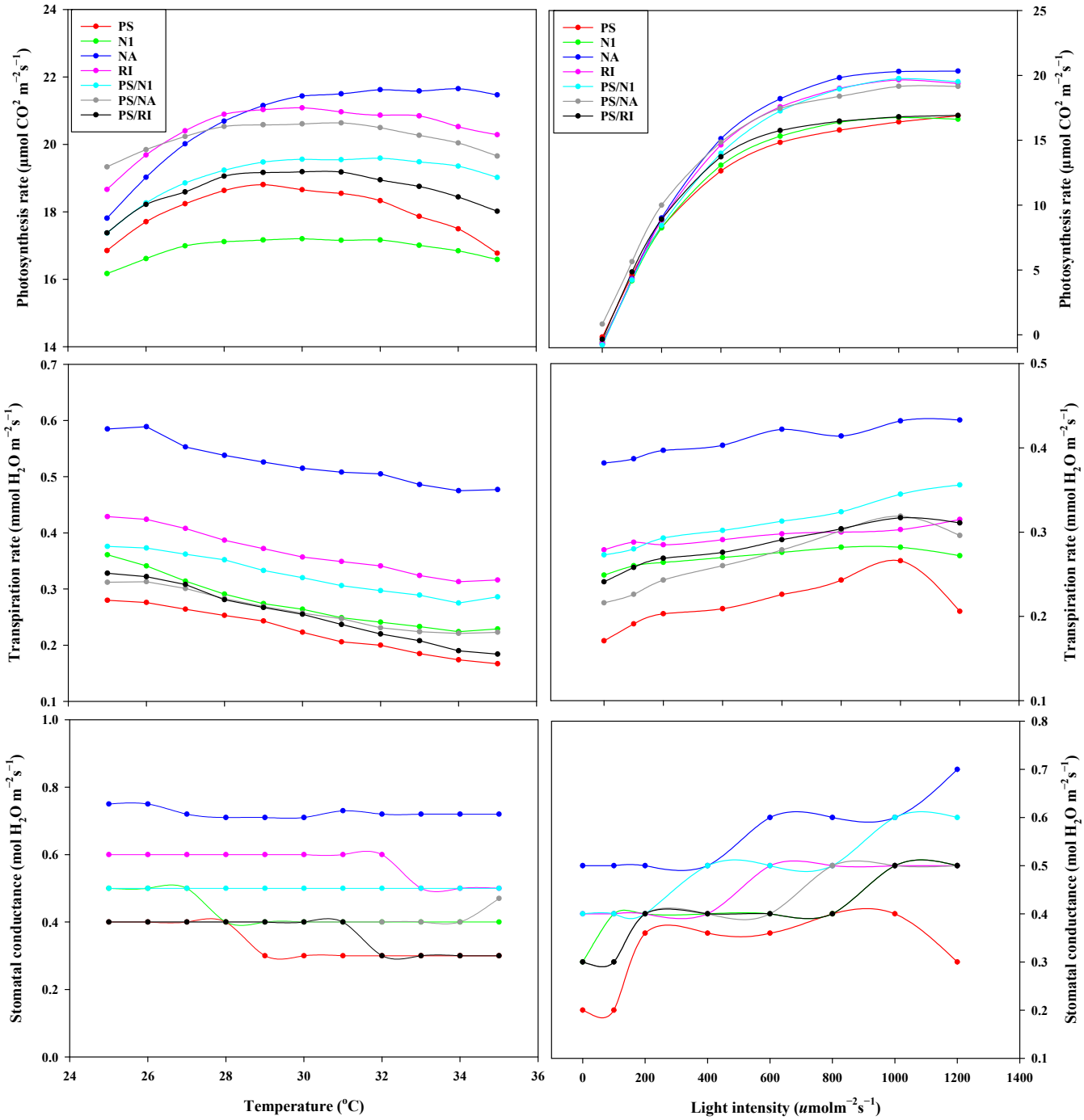

Figure 5. Photosynthetic parameters of one scion, three rootstocks, and three grafted cut rose flowers under different light and temperature conditions. PS: scion plant of spray rose *Rosa hybrida* cv. Pink Shine; N1: rootstock of *Rosa multiflora* cv. Hort No. 1; NA: rootstock of *Rosa canina* cv. Natal Briar; RI: rootstock of *Rosa indica* cv. Major; PS/N1: PS grafted onto N1; PS/NA: PS grafted onto NA; PS/RI: PS grafted onto RI.

3.4. Root Activity of Cut Rose Flowers

Regarding the results of the root activity analysis, PS was found to have the lowest, and NA had the highest. The root activity in the grafted cut rose flowers was shown according to the characteristics of the rootstock; that is, when grafted, the root activity of the roses was increased (Figure 6).

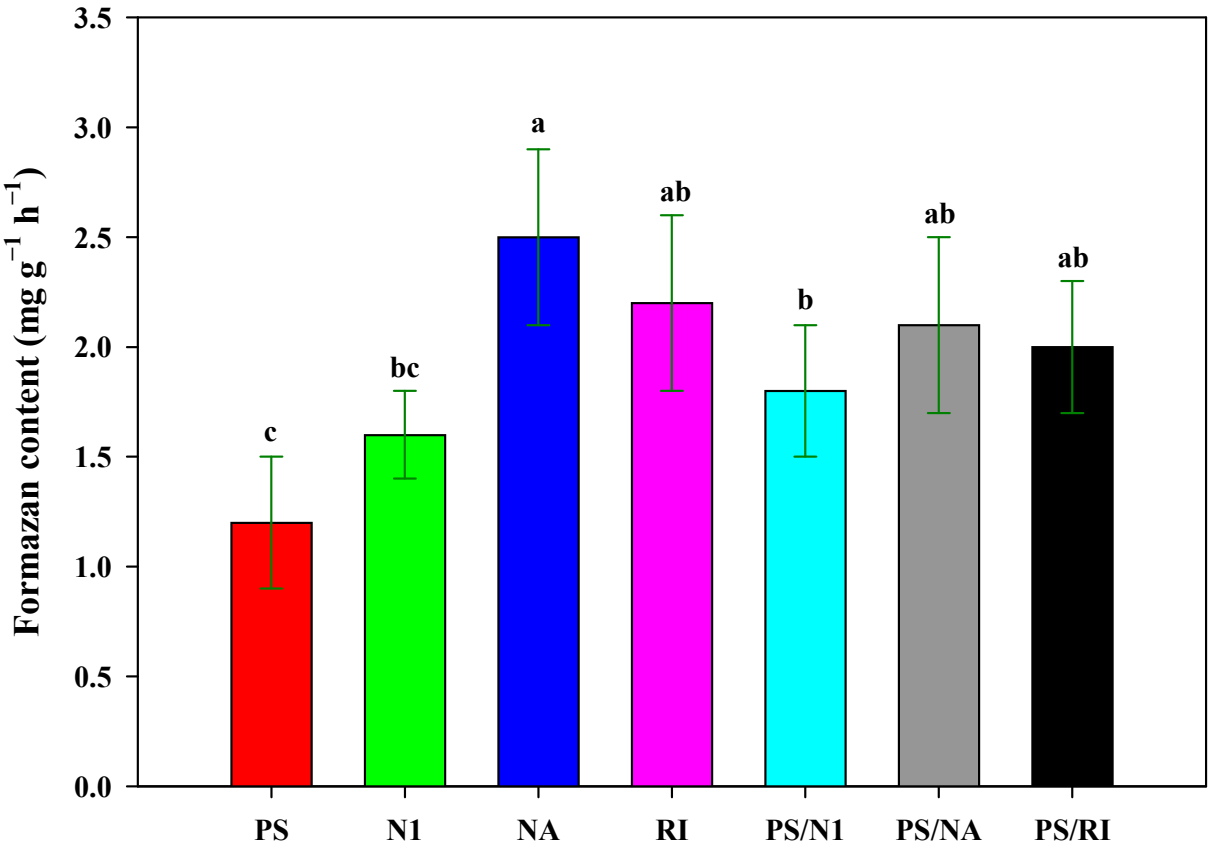

Figure 6. Root activity of one scion, three rootstocks, and three grafted cut rose flowers grown in the greenhouse during the summer season. PS: scion plant of spray rose *Rosa hybrida* cv. Pink Shine; N1: rootstock of *Rosa multiflora* cv. Hort No. 1; NA: rootstock of *Rosa canina* cv. Natal Briar; RI: rootstock of *Rosa indica* cv. Major; PS/N1: PS grafted onto N1; PS/NA: PS grafted onto NA; PS/RI: PS grafted onto RI. Vertical bars are standard deviations (n = 3). Small letters at the data points indicate mean separation between the values by Duncan's multiple range test at p = 0.05.

3.5. Yield and Flower Quality of Cut Rose Flowers

The yield of the grafted cut rose flowers was significantly increased, compared with that of the scion. In particular, the yield of PS/N1 increased the most (Table 3). There was no significant change in the stem fresh weight according to grafting. In addition, the number of leaves on the rootstocks was significantly higher than that of the scion; however, the grafted cut rose flowers did not show the characteristics of the rootstocks, so the number of leaves was similar to that of the scion. The stem length, which is one of the most important quality factors of cut rose flowers, decreased slightly in PS/N1 and PS/RI but not in PS/NA (Table 3). In terms of flower quality, when grafted, the flower width was slightly reduced. Compared with the scion, the number of petals was increased for PS/N1 and decreased for PS/NA and PS/RI (Figure 7).

Table 3. The yield and flower shoot qualities of scion, rootstocks, and grafted cut rose flowers grown in the greenhouse during the summer season.

Treatment	Stem Length (cm)	No. of Leaves (/Stem)	Fresh Weight (g/Stem)	Yield (No. of Flowers/Plant)
PS	52.0 ± 0.6cd [z]	8.4 ± 0.2 b	39.0 ± 2.6 a	4.4 ± 0.5 c
N1	99.5 ± 13.3 b	32.5 ± 4.9 a	20.0 ± 12.1 a	5.3 ± 0.6 b
NA	125.6 ± 15.8 a	34.7 ± 1.9 a	40.6 ± 12.0 a	4.8 ± 0.6 bc
RI	122.7 ± 14.4 a	33.6 ± 1.2 a	33.8 ± 4.9 a	7.0 ± 1.5 a
PS/N1	50.3 ± 0.8 d	8.2 ± 0.3 b	41.5 ± 4.1 a	5.3 ± 0.3 b
PS/NA	54.0 ± 1.9 c	8.3 ± 0.3 b	40.8 ± 3.6 a	4.9 ± 0.1 bc
PS/RI	50.2 ± 0.2 d	8.5 ± 0.2 b	41.4 ± 1.8 a	4.9 ± 0.1 bc
LSD (0.05)	14.063	3.601	11.127	1.384

[z] Values followed by different letters within a column are significantly different (Duncan's multiple range test, $p < 0.05$. $n = 15$). PS: scion plant of spray rose *Rosa hybrida* cv. Pink Shine; N1: rootstock of *Rosa multiflora* cv. Hort No. 1; NA: rootstock of *Rosa canina* cv. Natal Briar; RI: rootstock of *Rosa indica* cv. Major; PS/N1: PS grafted onto N1; PS/NA: PS grafted onto NA; PS/RI: PS grafted onto RI.

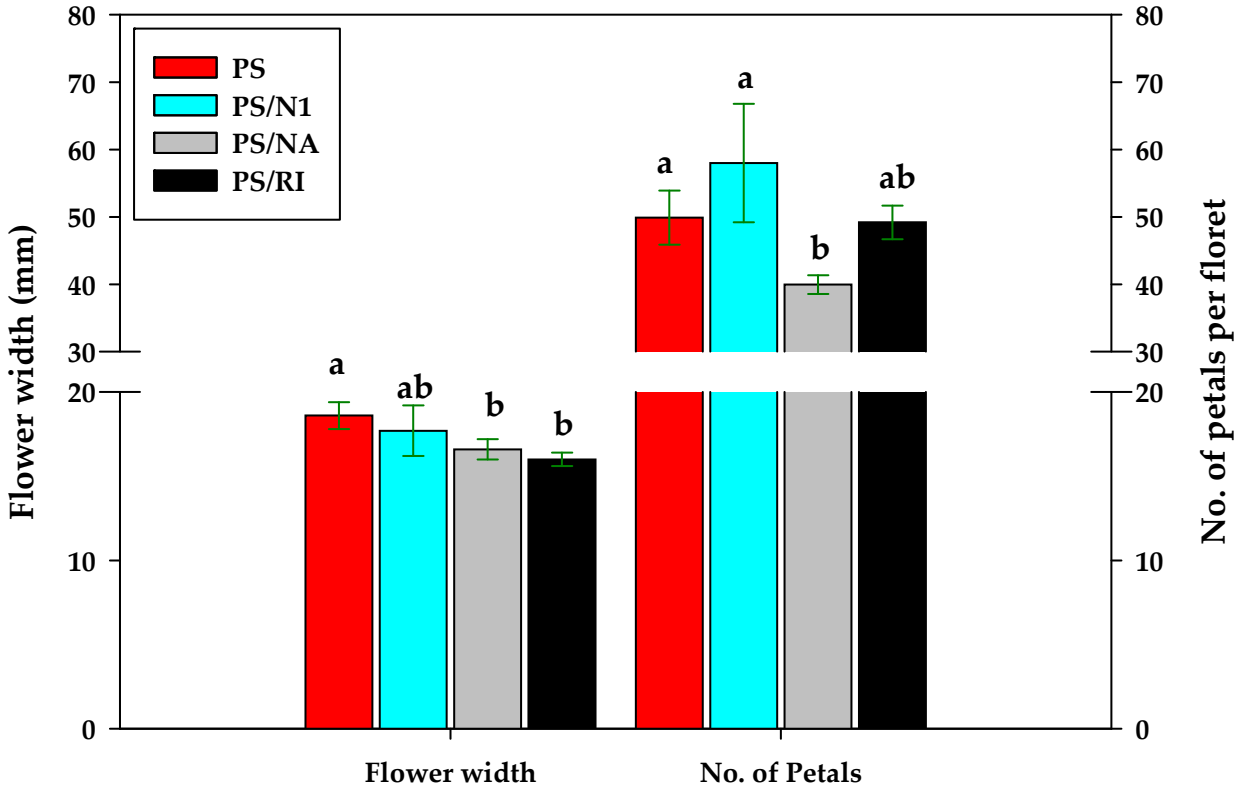

Figure 7. Flower characteristics of one scion and three grafted cut rose flowers grown in the greenhouse during the summer season. PS: scion plant of spray rose *Rosa hybrida* cv. Pink Shine; PS/N1: PS grafted onto *Rosa multiflora* cv. Hort No. 1; PS/NA: PS grafted onto *Rosa canina* cv. Natal Briar; PS/RI: PS grafted onto *Rosa indica* cv. Major. Vertical bars are standard deviations ($n = 15$). Small letters at the data points indicate mean separation between the values by Duncan's multiple range test at $p = 0.05$.

3.6. Principal Component Analysis (PCA)

Principal component analysis (PCA) was carried out to evaluate the relationships among the yield, vegetable growth, root activity, photosynthetic parameters, and chlorophyll-*a* fluorescence parameters on cut rose flowers grown in a greenhouse during the summer season under high-temperature stress conditions (Figure 8). The first two principal components (PCs) explained 70.86% of the cumulative variance, with PC1 detailing 39.22%

and PC2, 31.64%. The PC1 was positively correlated with the photosynthetic parameter, the energy flux of the photosystem parameter, root activity, and yield, while PC2 was positively correlated with the chlorophyll-*a* fluorescence OJIP parameters, guard cell size, and flesh weight.

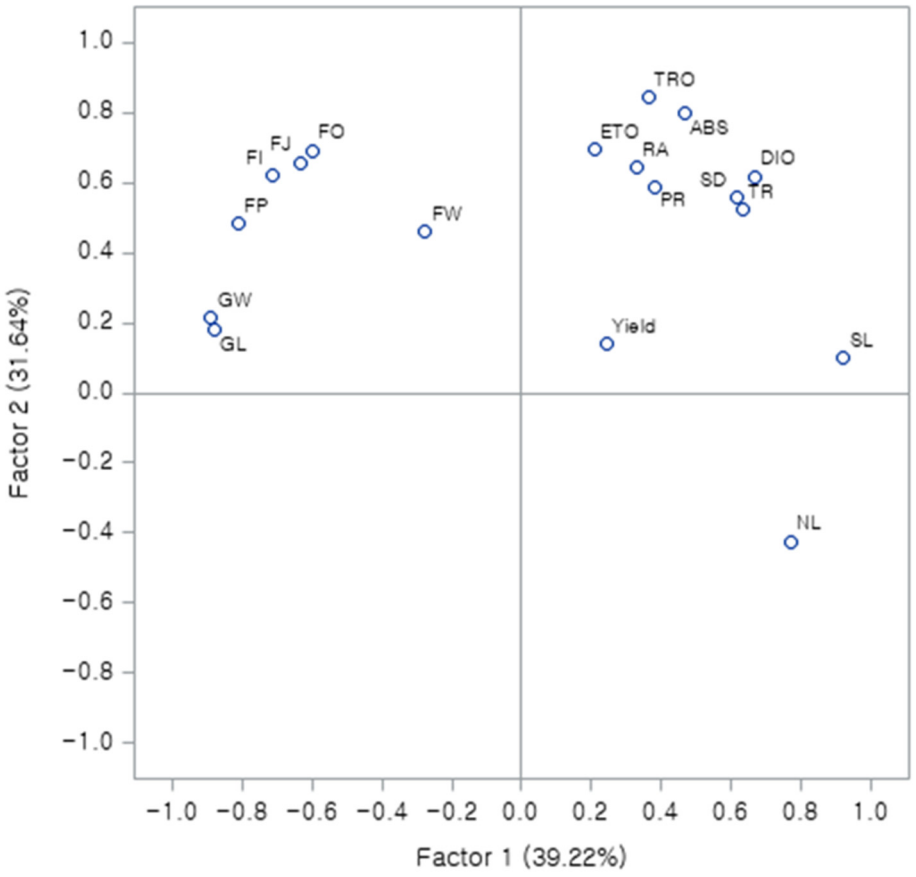

Figure 8. Biplot of the principal component analysis (PCA) of yield, photosynthetic parameters, chlorophyll-*a* fluorescence parameters, and vegetative parameters of cut rose flowers grown in the greenhouse during the summer season. The two principal components (PC1 and PC2) disclosed 70.86% of the total variation related to one scion, three rootstocks, and three grafted cut rose flowers. ABS: ABS/RC; DIO: DI_0/RC; ETO: ET_0/RC; TRO: TR_0/RC; FO: F_O; FJ: F_J; FI: F_I; FP: F_P; FW: flesh weight; GL: guard cell length; GW: guard cell width; NL: number of leaves; PR: photosynthesis rate; RA: root activity; SD: stomatal conductance; SL: stem length; TR: transpiration rate.

4. Discussion

In horticultural crops, grafting is a technique used to improve growth that is becoming more common. Grafting affects the chilling tolerance of horticultural crops [26], and physiological changes such as stomatal conductance [27] and chlorophyll-*a* fluorescence [28] have been observed with grafting in horticulture crops. In this study, as a result of observing the morphological changes in stomata when grafted, the stomatal size of all cut roses grafted onto three different rootstocks was larger, and the number of stomata decreased, which were completely different changes to the morphological characteristics of the scion or rootstocks (Table 2 and Figure 2). Stomata play important roles in transpiration control and gas exchange in the leaves [29,30]. These physiological functions depend on the density and size of stomata [31]. It is known that stomata development, such as division and size change, is caused by genetic control under abiotic stress [32]. It has been reported that stomatal size is negatively correlated with operating stomatal conductance under standard conditions and the maximum rate of stomatal opening in response to light [33], as well as the longer and wider stomatal size contributing to the enhanced plasticity of stomatal

conductance under high-temperature conditions [34]. Similar to the stomatal size and functional characteristics of the results of these previous studies, it was found that the stomatal size of the grafted cut rose flowers, to increase the efficiency of moisture and stomatal conductance, was larger than that of the scion or rootstock under high-temperature conditions during the summer season. Although the stomatal conductance of the grafted cut rose flowers was not higher than that of the rootstocks, it was higher than that of the scion. In terms of the scion and the grafted cut rose flowers having the same genetic growing point, it was discovered that the changes in the stomatal size and density of the grafted cut rose flowers is a result of the partial adoption of the characteristics of the rootstock to increase tolerance to the high-temperature stress conditions. In the case of grafted melons [35] and citrumelo [36], it was reported that the size of the stomata changed to adapt to abiotic stress, compared with the scion. In particular, Liu et al. (2016) stated that the efficiency of improving tolerance to drought stress by changing the stomata density and size of grafted cucumbers differed depending on the rootstocks [37].

The high temperatures in the summer season induce an abiotic stress state in horticultural crops grown in greenhouses [38], and it was found that the OJIP pattern significantly changed in tomatoes grown in a greenhouse under heat stress of 40 °C [39]. In horticultural crops, the F_V/F_M values calculated via chlorophyll-*a* fluorescence are used as sensitive indicators to confirm the heat stress state [40,41]. In this study, the OJIP patterns of all treated plants were similar, and the F_V/F_M value did not differ from 0.82 to 0.83 (Figure 3). This means that the plants were considered to be in a normal state, not in a stressful condition. The reason why the plants were able to avoid heat stress is presumed to be that the shading treatment of the glass greenhouse relieved the high temperature. The TR_0/RC and ET_0/RC values are useful in determining the state of plants by checking the efficiency of the photosynthetic light reaction when the stress state cannot be known via the F_V/F_M value [16]. Although it could not be confirmed whether the grafting was effective in relieving stress in summer, the TR_0/RC and ET_0/RC values indicating electron transport efficiency were higher in the grafted cut rose flowers than in the scion (Figure 4). These results suggest that grafting is effective for electron transport in the light reaction.

Photosynthesis of horticultural crops is an important growth indicator directly related to growth and yield [7]. Therefore, many grafting studies use photosynthesis parameter values to confirm grafting efficiency [42,43]. In the results of this study, the photosynthetic rate and transpiration rate of the rootstocks were significantly higher than those of the scion, and the photosynthesis parameters of the grafted cut rose flowers with the rootstock characteristics were significantly higher than those of the scion (Figure 5). It was confirmed that the improvement in photosynthetic efficiency through such grafting leads to an increase in flower yield (Table 3). The results from previous studies have shown that the grafting of horticultural crops under biotic or abiotic stress increases yield [44–46]. Furthermore, it has been reported that the photosynthetic efficiency and yield of horticultural crops are positively correlated [7]. In this study, the grafted cut rose flowers with a high photosynthetic rate generally had a higher yield than that of the scion (Table 3 and Figure 5).

Plants grow by absorbing inorganic nutrients and water through their roots, and the activity of the roots is directly related to the growth of plants [47]. Grafted melons showed significantly higher root activity than non-grafted melons [48], which can be seen as an important effect of grafting. In addition, in grafted bitter gourd, it was reported that root activity is significantly increased under waterlogging stress, compared with the scion plant. Similar to the previous results, in this study conducted at high temperatures in the summer season, the root activity of the scion was the lowest, and the root activity of the rootstocks was high. The grafted cut rose flowers with the rootstock characteristics also had increased root activity (Figure 6). Furthermore, the transpiration rate and stomatal conductance were high in the plants, such as rootstocks and grafted cut rose flowers, with high root activity. It was reported that leaf water potential decreased concomitantly due to a decrease in root hydraulic conductivity [49]. In these results, the reason why plants with high root activity also have a high transpiration rate is believed to be because it is

closely related to root water absorption and leaf transpiration. Moreover, in this study, the morphological characteristics, such as the number of petals, stem length, and flower size, showed differences in the grafted cut rose flowers, depending on the type of rootstock.

It was confirmed that there is a very high correlation between the light-reaction-related parameters (ABS/RC, TR_0/RC, ET_0/RC, and DI_0/RC), the dark-reaction-related parameters (photosynthesis rate, transpiration rate, and stomatal conductance), and root activity (Figure 8). This suggests that the rose yield is closely related to the activity of the roots absorbing water, which is the first electron donor in photosynthesis, and the light and dark reaction in the photosynthesis system. It can be deduced that the high root activity and photosynthetic efficiency of rootstocks have positive effects on the increase in yield of grafted cut rose flowers. Many studies have reported that the quality of horticultural crops depends on the rootstocks [50–52]; that is, the physiological characteristics, such as root activity and photosynthetic efficiency of rootstocks, have a significant effect on the grafted plant [53], and this ultimately increases the productivity of grafted cut rose flowers.

5. Conclusions

The conclusions regarding the grafting effect of cut rose flowers grafted onto three different rootstocks are as follows (Figure 9):

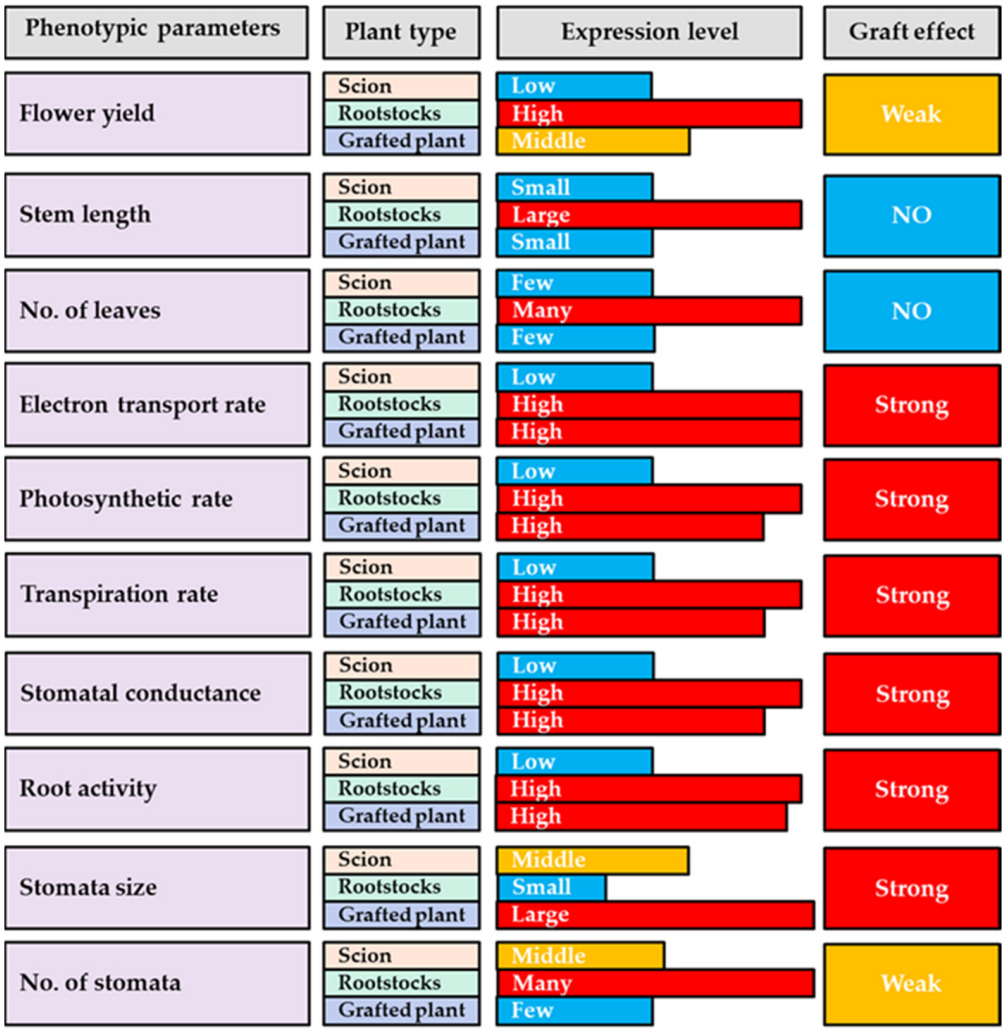

Figure 9. A summary of the phenotypical expression characteristics and grafting effects of the scion, rootstocks, and grafted cut rose flowers grown in the greenhouse during the summer season.

First, although there was a difference depending on the type of rootstock, the photophysiological response, the root activity, and the yield of grafted cut rose flowers were all improved under high temperatures in the summer season in the greenhouse. Second, the morphological characteristics, such as number of leaves, number of stomata, and stem length, of the grafted plants were hardly affected by the rootstock. Finally, the stomatal size of the grafted cut rose flowers was completely different from that of the scion or rootstocks and was in a form adapted to the high-temperature environment. The grafted plant is considered to have evolved into a new type of plant different from the scion and the rootstock with regard to specific characteristics related to environmental adaptation.

Author Contributions: O.-H.K. contributed to the data analysis, original draft, and writing, as well as to growing cut rose flowers; H.-G.C. contributed to the design of experiments, conceptualization, data analysis, data curation, and writing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available in the article.

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