



# Article Changes in Yield, Quality, and Morphology of Three Grafted Cut Roses Grown in a Greenhouse Year-Round

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Abstract: Cut roses are grown throughout the four distinct seasons of spring, summer, autumn, and winter in Korea. Especially in the very hot or cold seasons of summer or winter, the temperature and light environments inside a greenhouse cause abiotic stress on the growth of horticultural crops. In a greenhouse where shade cultivation is performed in summer, the temperature is high and the light intensity is low, whereas in winter when shade cultivation is not performed, both temperature and light intensity are low. This experiment investigated the year-round growth and yield changes of cut roses grafted onto three rootstocks. The root activity of rootstocks was generally higher than that of the scion. The stomata of the grafted cut roses showed morphological changes according to the seasons. Compared with the scion, the stomata of grafted cut roses became smaller and their number increased in summer, whereas only the stomata size increased in winter. The grafted cut roses had characteristics of high photosynthetic efficiency such as photosynthesis rate, stomatal conductance, transpiration rate from rootstocks under harsh environmental conditions including temperature and light intensity, and thus the photosynthetic efficiency was higher than that of the scion. There was no significant change in the yield of grafted cut roses, but flower quality parameters such as the stem height, stem thickness, and weight of grafted cut roses were improved according to the rootstocks compared with those of the scion. In particular, in cut roses grafted with R. multiflora cv. Natal Briar and Rosa indica 'Major' rootstocks, the weight increased as the stem lengthened and thickened in spring, autumn, and winter. Therefore, grafting is effective in improving the quality of cut roses grown under abiotic stress caused by harsh temperature and light intensity conditions during winter.

Keywords: abiotic stress; photosynthesis; root activity; rootstock; scion; stomata

#### 1. Introduction

Roses are one of the most widely produced cut flowers that are grown in a greenhouse year-round, and are used as decorations and gifts on occasions such as graduations, entrance ceremonies, and coming-of-age ceremonies around the world [1–3]. Therefore, it is necessary to maintain stable flower quality and yield of cut roses year-round. However, in Korea, the highest temperature in summer is  $\geq$ 35 °C, and the lowest temperature in winter is  $\leq$ -5 °C. It is difficult to cultivate cut roses of uniform quality and yield year-round as temperature and light intensity vary greatly due to the four seasons in Korea [4]. Therefore, cut roses are grown in greenhouses where the temperature can be controlled to maintain a suitable temperature in summer or winter. Nevertheless, it is difficult to optimally control the greenhouse environment for growing horticultural crops. Therefore, in winter, the ambient light intensity and the temperature inside a greenhouse naturally decrease, whereas in summer, the ambient light intensity is artificially lowered by shaded cultivation which



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). protects the horticultural crops from high temperatures that cause abiotic stress [5]. As a result, in the greenhouse during summer, the temperature can be lowered to some extent, but the ambient light intensity is also lowered.

The temperature and light intensity in the greenhouse affect the photosynthetic efficiency [6] and growth [7,8] of cut roses. As a cut rose is a helophyte crop, the photosynthetic efficiency increases as the light intensity increases, and is best at a temperature of 27–28 °C [9]. Additionally, roses are generally cultivated in a temperature range of 20–30 °C in the daytime and 18–20 °C at nighttime [10]. High temperatures in summer induce abiotic stress, which reduces the photosynthetic efficiency and inhibits shoot growth of horticultural crops [11–13]. However, the number of days from bud break to flowering increases as temperature decreases from 30 to 15 °C [14]. Below 15 °C, cut roses can be grown but the interval between flushes becomes longer, and when roses are exposed to a low temperature of 4 °C for a cold period at 3–5 weeks, their bud growth is inhibited [8]. Additionally, even at an optimal temperature, low light intensity reduces the growth of cut roses [15].

Every year, many cut rose varieties suitable for Korea's environmental conditions are bred [16–18]. However, cut roses that are newly bred varieties are not superior in all characteristics such as stem length, stem diameter, stem weight, and flower size throughout the four seasons. Additionally, since cut rose varieties are highly heterozygous for quality traits, they must be propagated vegetatively by a scion grafted onto rootstock or rooted cutting to maintain the desired traits [19]. Rooted cuttings are the simplest cultivation technique to increase the desirable rose varieties. Grafting is used as a cultivation technique to increase adaptability to negative environmental conditions such as high or low temperature and low light intensity and to improve photosynthetic efficiency and growth [20,21]. It has been reported that the rootstock affects the photo-assimilation ability of leaves, thereby improving qualities such as the flower weight, stem length, and stem diameter of roses [22]. Grafting of melon improved the photosynthetic rate, stomatal conductance, concentration of intercellular CO<sub>2</sub>, transpiration rate and carbohydrate metabolism of its leaves [23], and it is reported that grafted plants have a higher  $CO_2$  assimilation rate and less stomatal resistance than ungrafted or autologous grafted plants [24–27]. Not only does grafting promote photosynthesis by regulating the stomata and non-stomata performance in leaves under abiotic stress [28], but also the rootstock of the grafted plants affects the growth and physiology of the scion [29]. Therefore, different types of rootstocks have different effects on grafted plants in terms of size, fruit quality, and fruit yield [30,31]. The rootstocks widely used for improving the quality and yield of cut roses in Korea are Rosa multiflora cv. Natal Briar, which is native to South Africa, Rosa indica cv. 'Major' differentiated in Israel, and Rosa multiflora cv. Hort. No. 1 selected in Korea. However, there is not enough information on the effect of rootstocks on the quality and yield improvement of cut roses in greenhouses where cut roses are grown in Korea.

Parameters related to the quality of cut roses include stem length, stem diameter, stem weight, flower size, flower color, flower shape, and disease damage. In particular, the length of the stem is an important factor in determining the quality of national agricultural products in Korea [32].

Therefore, this study was conducted to investigate the effect of the type of rootstock grafted on cut roses grown hydroponically in a greenhouse in Korea on photosynthetic efficiency, morphological change, quality, and year-around yield.

#### 2. Materials and Methods

# 2.1. Plant Materials and Grafting Procedure

### 2.1.1. Plant Material

*R. hybrida* cv. Pink Beauty (PB) was used as a scion in the experiment. The PB variety is a standard-type cut rose, and has the disadvantage of a short stem length. *R. multiflora* cv. Hort. No.1 (N1), *R. multiflora* cv. Natal Briar (NA), and *Rosa indica* 'Major' (RI) were used

as rootstocks in the experiments. Table 1 shows the characteristics of the three rootstocks used in the grafted experiment.

**Table 1.** The yield and flower shoot quality parameters of rootstocks such as *Rosa multiflora* cv. Hort. No 1 (N1), *Rosa canina* cv. Natal brier (NA), and *Rosa indica* cv. Major (RI).

Treatment	Stem Length (cm)	Stem Diameter (cm)	No. of Leaves (/stem)	Dry Stem Weight (g/Plant)	No. of Tepal (/Stem)	Yield (No. of Stem)
N1	105.4 $\pm$ 3.1 b <sup>Z</sup>	$3.7\pm0.2$ b	$29.6\pm0.4~\mathrm{c}$	$10.1\pm5.0~\mathrm{b}$	$37.4\pm2.2$ a	$5.7\pm1.2$ a
NA	$140.6\pm7.2$ a	$5.4\pm0.1$ a	$32.7\pm3.3$ a	$35.6\pm3.2$ a	$28.5\pm1.3b$	$4.3\pm1.2$ a
RI	$132.1\pm14.6$ a	$5.0\pm0.2$ a	$32.0\pm1.4b$	$32.0\pm1.4$ a	$30.9\pm0.6b$	$4.7\pm2.1~\mathrm{a}$

<sup>*Z*</sup> Values followed by different letters within a column are significantly different (DMRT, p < 0.05; n = 3).

#### 2.1.2. Cutting and Grafting Producing

The rose plants used as cutting and scion were those with a semi-hard wood material with at least three nodes and 5–8 mm diameter at a stage when the leaves are well-developed and the thorns can be broken off easily. The cuttings (PB) were inserted into rockwool medium ( $25 \times 100 \times 75$  cm, Grodan, Roermond, The Netherlands) until the roots were grown. The scions (PB) with one node and one leaf were grafted onto three rootstocks such as N1, NA, and RI with a 4–5 cm length internode. The three graft roses were designated (scion/graft) as PB/N1, PB/NA, and PB/RI according to the rootstock species. These were inserted into the rockwool medium ( $7.5 \times 7.5 \times 5$  cm, Grodan, Roermond, The Netherlands) until the stems were stenting and roots were grown.

#### 2.2. Greenhouse and Environmental Control

The experiments were carried out in a glass-covered greenhouse with a hydroponics system located in the Rural Department Administration of South Korea ( $35^{\circ}50'02''$  N,  $127^{\circ}02'04''$  E). The environmental conditions in the greenhouse, using installed equipment (consisting of convective heating, ceiling and wall windows and ventilation fans), were set to produce target day and night temperatures of below 30 °C and above 18 °C, respectively. Additionally, when the temperature was  $\geq 35$  °C in the greenhouse, the temperature was lowered by using a shading curtain. The light intensity and temperature in the greenhouse were logged at 1 h intervals using data log (WatchDog 1650; Spectrum Technologies Inc., Aurora, IL, USA).

#### 2.3. Crop Management

The rooted cuttings (PB) and three grafts (PB/RI, PB/N1, PB/NA) were planted on rockwool slabs (100 cm  $\times$  25 cm  $\times$  7 cm, Grodan, Denmark) at a density of five plants per meter in June 2018. All the plants were cultivated following the 'arching' technique by which 2–3 stems with a total of 30 leaves are bent horizontally in order to promote basal shoot formation and to decrease plant canopy and light interception [33].

The nutrient solution was composed of macro-elements (NO<sub>3</sub>-N:NH<sub>4</sub>-N:P:K:Ca:Mg:S =  $16:1.33:4:8:8:4:4 \text{ me}\cdot\text{L}^{-1}$ ) and micro-elements (Fe:Mn:B:Zn:Cu:Mo =  $2:0.5:0.25:0.2:0.05:0.05 \text{ mg}\cdot\text{L}^{-1}$ ). Table 2 shows the electrical conductivity (EC), pH, and amounts of irrigation nutrient solution supplied.

Index	Spring (19 April–May)	Summer (19 July–August)	Autumn (19 October– November)	Winter (20 January– February)
EC ( $dS \cdot m^{-1}$ )	1.5	1.2	1.5	1.8
pH	5.8~6.0	$5.8{\sim}6.0$	5.8~6.0	5.8~6.0
IANS (mL)	800	1000	800	700

Table 2. Nutrient solution supplied in the hydroponic system during the experiment.

EC: electrical conductivity; pH: an expression of hydrogen ion concentration in water; IANS: amount of irrigation nutrient solution.

# 2.4. Yield, Quality, Morphology, and Physiological Responses of Grafted Cut Roses 2.4.1. Yield and Quality

The stem length of the cut roses is an important quality standard. The quality standards for the length of cut roses is divided into a length of 80 cm or more for Grade 1, 70 cm to 80 cm for Grade 2, and 70 cm or less for Grade 3 [32]. Therefore, the cut roses with stem length over 40 cm were sequentially investigated until reaching full bloom. The yield of flowering stems and the quality of cut roses were assessed daily during the experimental period.

#### 2.4.2. Root Activity

The root activity of the rootstocks was measured using an UV–visible spectrophotometer (Evolution 300, Thermo Co., Waltham, MA, USA) at 470 nm [34]. After washing the fresh roots in running water, we cut them into 2 cm lengths, and then mixed them to a sample weight of 500 mg, and placed the mixture in a glass tube. To each tube was added 10 mL of a mixture of triphenyl tetrazolium chloride (TTC) solution, 0.1 M sodium phosphate-buffered solution, and distilled water at a ratio of 1:4:5. After allowing air bubbles to dissipate over a 10-min period, we continued the reaction in the dark for two hours in a constant-temperature bath maintained at 30 °C. We then added 2 mL of 2N H<sub>2</sub>SO<sub>4</sub> to the sample tubes, vortexed them, and rinsed the samples with running water. We subsequently added 10 mL of ethyl acetate and 1 g of crushed quartz glass to the reacted root samples and filtered the mixture through a 110 mm filter paper (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 root weight (g) multiplied by the reaction time (h).

#### 2.4.3. Photosynthesis Characteristics

Photosynthetic efficiency was analyzed using a portable photosynthesis system (LI-6800; LI-COR, Lincoln, NE, USA) in the mornings. Leaves on each rose variety at about 30 days after leaf emergence in the middle of the growing stem were randomly selected and for photosynthetic efficiency measurements from 15 to 20 June. The chamber conditions of the LI-6800 for measuring photosynthetic efficiency according to a temperature change from 25 °C to 35 °C (rise by 1 °C) were set as follows: relative humidity (RH), 50%; CO<sub>2</sub>, 400  $\mu$ mol·mol<sup>-1</sup>; photon flux density, 800  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>. The chamber conditions of the LI-6800 for measuring photosynthetic efficiency according to light intensity change (1200; 1000; 800; 400; 200; 100 and 0  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>) were set as follows: chamber temperature, 27 °C; RH, 50%; CO<sub>2</sub>, 400  $\mu$ mol·mol<sup>-1</sup>.

#### 2.4.4. Scanning Electron Microscopy (SEM)

In order to observe the morphological characters and density of stomata, tissues were fixed in 2.5% glutaraldehyde (v/v in a 0.1 M phosphate buffer) at pH 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. The samples were then coated with gold-palladium for 2 min (30 Å) using an ion sputter (MC 1000, Hitachi,

Tokyo, Japan) and examined on an SEM (SU-3500, Hitachi, Tokyo, Japan) operating at an accelerating voltage of 15 kV [35].

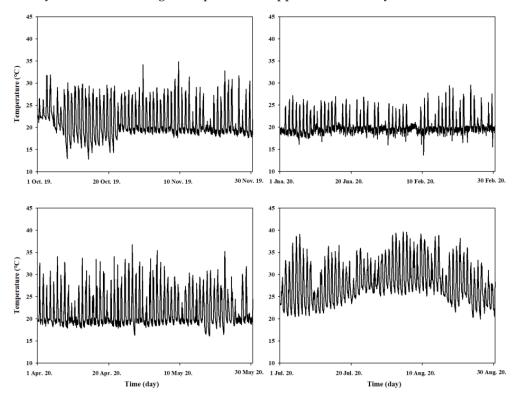
#### 2.5. Experimental Design and Statistical Analysis

This grafting experiment was replicated three times in a random block design. Each replication consisted of five plants for each treatment (one cutting and three grafts). The grafting treatment results such as yield and quality of cut roses were analyzed using analysis of variance with Duncan's multiple range test using a significance level of  $p \le 0.05$  in SAS 9.4 program (SAS Institute Inc., Cary, NC, USA).

#### 3. Results

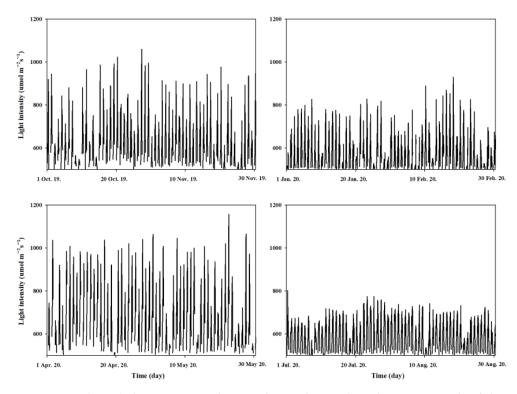
#### 3.1. Temperature and Ambient Light Intensity in the Greenhouse

The average temperature in the greenhouse over four seasons was in the order summer > spring > autumn > winter (Figure 1). The average temperature was 27.9 °C in summer, 22.9 °C in autumn, 21.8 °C in spring, and 20.2 °C in winter. In spring, autumn, and winter, the glasshouse was heated to prevent low temperatures during the night, and the temperature was maintained at 18 °C or higher. However, there was no heating at night in early autumn, so the night temperature dropped to a relatively low 14 °C.



**Figure 1.** Air temperature in the greenhouse during the cultivation periods of cuttings, rootstocks and grafts from October 2019 to August 2020.

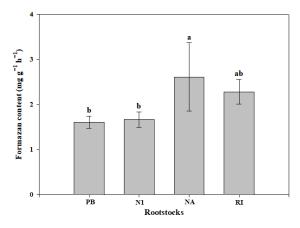
The average ambient light intensity in the greenhouse by season was in the order spring > autumn > summer > winter (Figure 2). The light intensity in spring, when the ambient light intensity was highest, was 700–1100  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>, and the ambient light intensity in summer, when the ambient light intensity was the lowest, was 500–900  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>. The light intensity in the greenhouse was low in summer because shade was applied to lower the temperature which had risen due to excessive light levels.



**Figure 2.** Ambient light intensity in the greenhouse during the cultivation periods of the scion, rootstocks and grafted roses from October 2019 to August 2020.

#### 3.2. Root Activity of Cut Roses

Hydroponics is a technique for intensively supplying nutrient solution to roots in a small-capacity medium [36]. One of the important analytical techniques for confirming whether nutrients are well-absorbed from medium to root is the measurement of root activity. According to this analysis of the root activity on the scion and three rootstocks, the NA had the highest formazan content at 2.61 mg·g<sup>-1</sup>·h<sup>-1</sup> (Figure 3). The root activity of PB and N1 was low, at 1.60 mg·g<sup>-1</sup>·h<sup>-1</sup> and 1.66 mg·g<sup>-1</sup>·h<sup>-1</sup>, respectively.



**Figure 3.** Root activity of one scion and three rootstocks by cutting: PB: scion of *Rosa hybrida* cv. Pink Beauty; N1: rootstock of *Rosa multiflora* cv. Hort No 1; NA: rootstock of *Rosa canina* cv. Natal brier; RI: rootstock of *Rosa indica* cv. Major. Vertical bars are standard deviations (n = 3). Small letters at the data points indicate mean separation between the values by DMRT at p = 0.05.

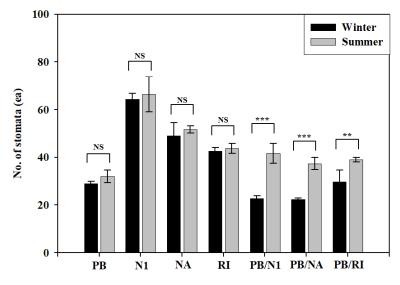
#### 3.3. Morphological Characteristics of Leaves

As a result of observing the leaves of stems generated by cutting, three rootstocks, and three grafts in summer and winter, it was confirmed that the number and size of stomata changed (Table 3, Figures 4–6). The stomata size was larger in winter than in summer. The stomata size of the three grafts was larger than that of the rootstock and smaller than or equal to that of the cutting (PB). The stomata of three grafts were smaller than those of the cuttings in summer but larger than those of the cuttings in winter. Among the three grafts, PB/N1 had the largest stomata and PB/NA had the smallest. Among the three rootstocks, the stomata size of N1 were the largest and NA were the smallest. The stomata size of the rootstocks was smaller than that of the cuttings and grafts.

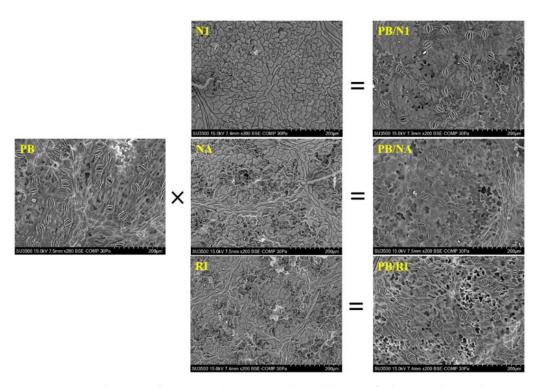
**Table 3.** The stomata size of one scion, three rootstocks, and three grafted plants measured by scanning electron microscopy in winter and summer.

Season	Win	nter	Summer		
Index	Stomata Length (µm)	Stomata Width (µm)	Stomata Length (μm)	Stomata Width (µm)	
PB	$36.7\pm2.9$ a $^{\rm Z}$	$16.7\pm2.9~\mathrm{c}$	$22.6\pm0.7b$	$9.1\pm0.5b$	
N1	$21.7\pm2.9~\mathrm{c}$	$13.3\pm2.9~\mathrm{cd}$	$16.7\pm0.3~\mathrm{c}$	$7.1\pm0.4~\mathrm{c}$	
NA	$15.8\pm1.4~\mathrm{d}$	$10.7\pm1.2~\mathrm{d}$	$14.4\pm0.2~\text{d}$	$7.2\pm0.4~\mathrm{c}$	
RI	$18.3\pm2.9~\mathrm{cd}$	$10.8\pm1.4~\text{d}$	$17.5\pm0.2~\mathrm{c}$	$7.4\pm0.9~{ m c}$	
PS/N1	$34.2\pm1.4~ab$	$25.8\pm1.4~\mathrm{a}$	$27.6\pm1.0~\mathrm{a}$	$10.2\pm0.4~\mathrm{a}$	
PS/NA	$31.3\pm2.3~\mathrm{b}$	$21.7\pm1.4~b$	$23.6\pm0.7b$	$8.9\pm0.3\mathrm{b}$	
PS/RI	$31.6\pm3.0~\text{b}$	$26.7\pm2.9~\mathrm{a}$	$22.9\pm1.0~\text{b}$	$9.1\pm0.2b$	

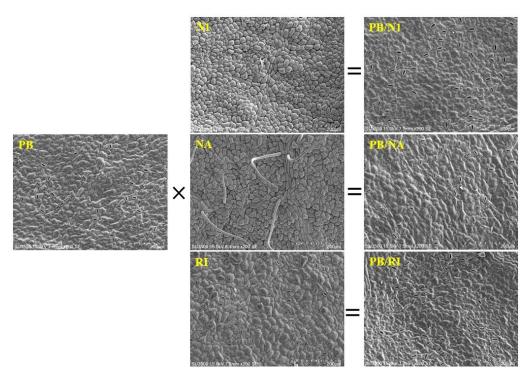
<sup> $\overline{2}$ </sup> Values followed by different letters within a column are significantly different (DMRT, *p* < 0.05. *n* = 3). PB: scion plant of spray rose *Rosa hybrida* cv. Pink Beauty; N1: rootstock of *Rosa multiflora* cv. Hort No 1; NA: rootstock of *Rosa canina* cv. Natal brier; RI: rootstock of *Rosa indica* cv. Major; PB/N1: PB grafted onto N1; PB/NA: PB grafted onto NA; PB/RI: PB grafted onto RI.



**Figure 4.** The stomatal number of one scion, three rootstocks, and three grafted plants measured by scanning electron microscopy. Seven-sample t-test for (PB): scion cut rose of *Rosa hybrida* cv. Pink Beauty; (N1): rootstock of *Rosa multiflora* cv. Hort No 1; (NA): rootstock of *Rosa canina* cv. Natal brier; (RI): rootstock of *Rosa indica* cv. Major; (PS/N1): PB grafted onto N1; (PB/NA): PB grafted onto NA; (PB/RI): PB grafted onto RI treatments with difference in winter and summer. NS, \*\*, \*\*\*: not significant or significant at *p* < 0.01 and 0.001, respectively.



**Figure 5.** Stomatal images of one scion, three rootstocks, and three grafted plants taken with scanning electron microscopy in winter. (PB): scion cut rose of *Rosa hybrida* cv. Pink Beauty; (N1): rootstock of *Rosa multiflora* cv. Hort No 1; (NA): rootstock of *Rosa canina* cv. Natal brier; (RI): rootstock of *Rosa indica* cv. Major; (PB/N1): PB grafted onto N1; (PB/NA): PB grafted onto NA; (PB/RI): PB grafted onto RI.



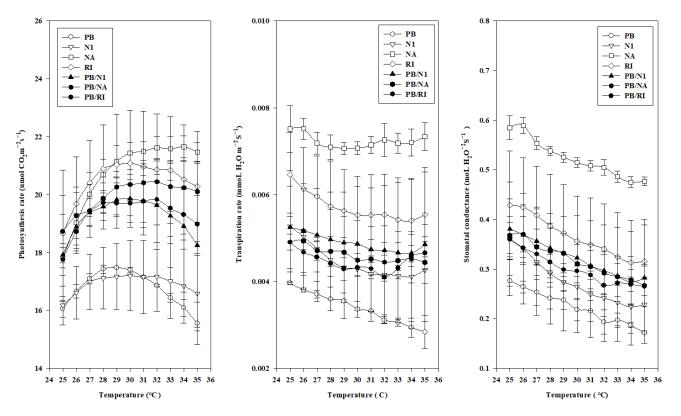
**Figure 6.** Stomatal images of one scion, three rootstocks, and three grafted plants taken with scanning electron microscopy in summer. (PB): scion cut rose of *Rosa hybrida* cv. Pink Beauty; (N1): rootstock of *Rosa multiflora* cv. Hort No 1; (NA): rootstock of *Rosa canina* cv. Natal brier; (RI): rootstock of *Rosa indica* cv. Major; (PB/N1): PB grafted onto N1; (PB/NA): PB grafted onto NA; (PB/RI): PB grafted onto RI.

The number of stomata was greater in summer than in winter. In particular, the number of stomata of the grafts was higher than that of the cuttings (PB) in summer and smaller than that of the cuttings in winter. Among the three grafts, PB/N1 had the highest number of stomata in summer and PB/RI in winter. The number of stomata of the three rootstocks was higher than that of the cuttings and three grafts. Among the three rootstocks, the number of stomata was the highest in N1.

#### 3.4. Photosynthesis of Grafted Cut Roses

#### 3.4.1. Temperature Curve

The average temperature of the greenhouse during daytime in summer was the highest at 31.7 °C (Figure 1). Additionally, the average temperature in the greenhouse during daytime in winter was the lowest at 21.5 °C. It was observed that there was little difference in photosynthetic efficiency within the temperature range of 17–25 °C [37]. Thus, we investigated the photosynthetic efficiency of one cutting, three rootstocks, and three grafted roses in the temperature range of 25–35 °C. The changes in photosynthetic parameters such as photosynthesis rate, transpiration rate, and stomatal conductance are shown in Figure 7.



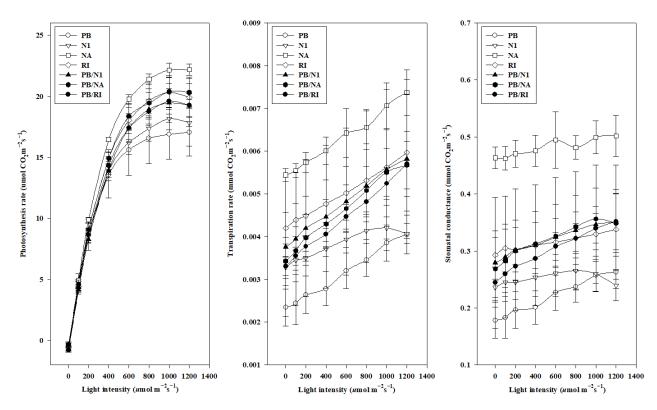
**Figure 7.** Photosynthetic parameters of one scion, three rootstocks, and three grafted plants under different temperature conditions. (PB): scion cut rose of *Rosa hybrida* cv. Pink Beauty; (N1): rootstock of *Rosa multiflora* cv. Hort No 1; (NA): rootstock of *Rosa canina* cv. Natal brier; (RI): rootstock of *Rosa indica* cv. Major; (PB/N1): PB grafted onto N1; (PB/NA): PB grafted onto NA; (PB/RI): PB grafted onto RI. Vertical bars are standard deviations (*n* = 3).

All the grafted roses (PB/N1, PB/NA, PB/RI) had higher photosynthesis rates than those of cuttings (PB). Additionally, the photosynthetic characteristics of the grafted roses were similar to those of the rootstock. Except for PB/N1, the photosynthetic efficiencies of grafts such as PB/NA and PB/RI had similar values to the rootstocks (NA and RI) and cutting (PB). In PB/NA, photosynthetic efficiency did not decrease even at high temperatures above 30 °C, similarly to NA. PB/RI had the best photosynthetic efficiency at the lowest temperature, 25 °C, similarly to RI. In PB/NA, the decrease in photosynthetic rate was large as the temperature decreased, whereas in PB/RI, the decrease was small. Although the photosynthetic rate of PB/N1 was not superior to that of other grafts (PB/NA, PB/RI), the degree of change in photosynthetic values was small in all temperature ranges, similarly to N1.

The transpiration rate of rootstocks was in the order NA > RI > N1. There was no difference in the transpiration rate among the grafted roses (PB/N1, PB/NA, PB/RI). Their transpiration rates were higher than that of PB. Although the transpiration rates of the three rootstocks and three grafted roses (PB/N1, PB/NA, PB/RI) increased at a temperature of 30 °C or higher, that of the cutting (PB) continued to decrease. Additionally, the stomatal conductance had a similar trend to the transpiration rate.

#### 3.4.2. Light Curve

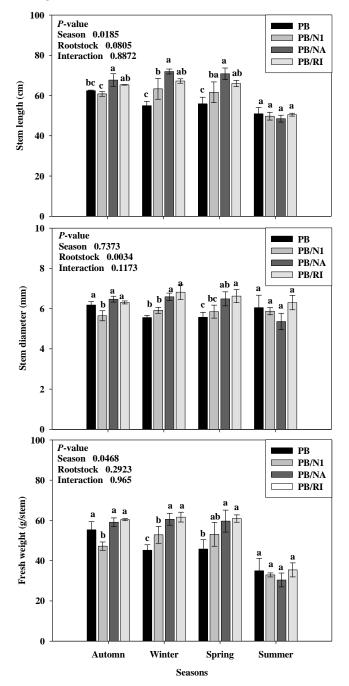
The photosynthesis rate was investigated in the light intensity range of 0–1200  $\mu$ mol·CO<sub>2</sub>·m<sup>-2</sup>·s<sup>-1</sup> that can occur in a greenhouse with cut roses cultivated (Figure 2). In the cutting (PB), rootstocks (N1, NA, RI), and grafts (PB/N1, PB/NA, PB/RI), the photosynthetic rate increased as the light intensity increased, and the saturation point was shown at the light intensity of 800–1000  $\mu$ mol·CO<sub>2</sub>·m<sup>-2</sup>·s<sup>-1</sup> (Figure 8). There was no significant difference between the cutting, three rootstocks, and three grafts for weak light of less than 500  $\mu$ mol·CO<sub>2</sub>·m<sup>-2</sup>·s<sup>-1</sup>, but as the light intensity increased, the difference in photosynthesis rate between them grew larger. At a high light intensity of 1000  $\mu$ mol·CO<sub>2</sub>·m<sup>-2</sup>·s<sup>-1</sup> or higher, the photosynthetic rate of NA was the highest of all treatments, and PB had the lowest of them. There was no significant difference in photosynthetic rate by light intensity between grafted roses. The transpiration rate and stomatal conductance increased in all the treatments as the light intensity increased, but for PB/N1, they decreased at a light intensity of 1000  $\mu$ mol·CO<sub>2</sub>·m<sup>-2</sup>·s<sup>-1</sup> or more.



**Figure 8.** Photosynthetic parameters of one scion, three rootstocks, and three grafted plants under different light intensities. (PB): scion cut rose of *Rosa hybrida* cv. Pink Beauty; (N1): rootstock of *Rosa multiflora* cv. Hort No 1; (NA): rootstock of *Rosa canina* cv. Natal brier; (RI): rootstock of *Rosa indica* cv. Major; (PB/N1): PB grafted onto N1; (PB/NA): PB grafted onto NA; (PB/RI): PB grafted onto RI. Vertical bars are standard deviations (*n* = 3).

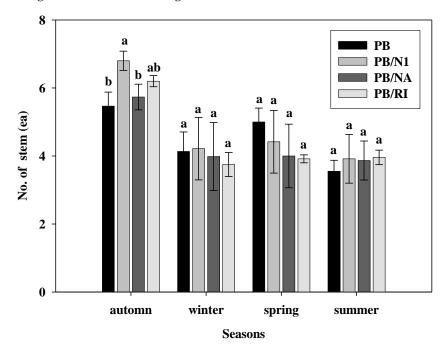
# 3.5. Yield and Quality of Cut Roses

The stem length, diameter, and fresh weight of grafted roses were significantly increased compared to those of the cutting (PB) in autumn, winter, and spring (Figure 9). In autumn, winter, and spring, the stem length of PB/N1 was longer than that of PB and other grafted roses. Additionally, the stem diameter of PB/RI was thicker than that of PB and other grafted roses.



**Figure 9.** The stem length, stem diameter, and fresh weight for scion cut rose *Rosa hybrida* cv. Pink Beauty (PB), rootstock of *Rosa multiflora* cv. Hort No 1 (N1), rootstock of *Rosa canina* cv. Natal brier (NA), rootstock of *Rosa indica* cv. Major (RI), PB grafted onto N1 (PB/N1), PB grafted onto NA (PB/NA), PB grafted onto RI (PB/RI) in four seasons. Vertical bars are standard deviations (n = 15). Small letters at the data points indicate mean separation between the values by Duncan's multiple test at p = 0.05. Values were determined by two-way ANOVA.

There was no difference in yield between any treatments in spring, summer and winter (Figure 10). However, in autumn, when the yield of cut flower roses was higher than that of other seasons, the production of cut roses produced from PB/NA was the highest among the cutting and grafted roses. Additionally, the flower height was the highest in the PB/NA among the cutting and grafted roses (Table 4). However, the number of petals of cuttings was greater than that of the grafted roses.



**Figure 10.** The yield for scion cut rose *Rosa hybrida* cv. Pink Beauty (PB), rootstock of *Rosa multiflora* cv. Hort No 1 (N1), rootstock of *Rosa canina* cv. Natal brier (NA), rootstock of *Rosa indica* cv. Major (RI), PB grafted onto N1 (PB/N1), PB grafted onto NA (PB/NA), PB grafted onto RI (PS/RI). Vertical bars are standard deviations (n = 15). Small letters at the data points indicate mean sep-aration between the values by Duncan's multiple test at p = 0.05.

Table 4. Flower characteristics of one scion and three grafted plants.

Season	Index	PB	PB/N1	PB/NA	PB/RI
Spring	Flower height (cm)	$5.5\pm0.1$ b $^{\rm Z}$	$5.6\pm0.2$ b	$5.8\pm0.1~\mathrm{ab}$	$5.9\pm0.1$
	Flower diameter (mm)	$32.5\pm1.2~\mathrm{ab}$	$33.6\pm1.0~\text{a}$	$31.4\pm1.2\mathrm{b}$	$34.4\pm0.8$
	No. of petals	$58.8\pm0.2~\mathrm{a}$	$56.2\pm1.3~\text{b}$	$48.1\pm2.1~\mathrm{d}$	$50.5\pm0.8$
Summer	Flower height (cm)	$4.6\pm0.2$ a	$4.3\pm0.1$ a	$4.7\pm0.2$ a	$4.6\pm0.4$
	Flower diameter (mm)	$23.1\pm1.3~\text{a}$	$22.7\pm0.7~\mathrm{a}$	$23.4\pm1.9~\mathrm{a}$	$23.1\pm0.6$
	No. of petals	$56.5\pm2.4~\mathrm{b}$	$64.5\pm2.3$ a	$52.2\pm0.5~\mathrm{c}$	$54.9\pm2.2$
Autumn	Flower height (cm)	$5.5\pm0.4$ a	$5.4\pm0.0$ a	$5.7\pm0.3$ a	$5.6\pm0.1$
	Flower diameter (mm)	$31.4\pm2.6~\mathrm{a}$	$31.6\pm1.7~\mathrm{a}$	$32.9\pm1.1~\mathrm{a}$	$33.1\pm0.2$
	No. of petals	$61.1\pm7.2~\mathrm{a}$	$62.3\pm0.8~\mathrm{a}$	$53.7\pm4.2~\mathrm{a}$	$55.1\pm2.6$
Winter	Flower height (cm)	$5.5\pm0.1~\mathrm{c}$	$5.5\pm0.2bc$	$6.0\pm0.1$ a	$5.8\pm0.1$ a
	Flower diameter (mm)	$33.2\pm0.3~\mathrm{a}$	$32.5\pm0.2~\mathrm{a}$	$32.2\pm1.0~\mathrm{a}$	$33.2\pm1.5$
	No. of petals	$58.5\pm1.0$ a	$56.1 \pm 2.0$ a	$48.0\pm2.3\mathrm{b}$	$49.2\pm0.3$

<sup>*Z*</sup> Values followed by different letters within a column are significantly different (DMRT, p < 0.05. n = 15). PB: scion plant of standard rose *Rosa hybrida* cv. Pink Beauty; N1: rootstock of *Rosa multiflora* cv. Hort No 1; NA: rootstock of *Rosa canina* cv. Natal brier; RI: rootstock of *Rosa indica* cv. Major; PB/N1: PB grafted onto N1; PB/NA: PB grafted onto NA; PB/RI: PB grafted onto RI.

# 4. Discussion

The light intensity due to seasonal changes affects different components of yield and quality, such as bud breaking, the rate of flower abortion, formation of renewal shoots, time period between harvests, length, weight and diameter of stem and flower buds in roses [7]. Additionally, rose plant growth parameters (bud breakage, flower abortion rates, and bud length, etc.) are affected by variations in temperature due to seasonal environmental conditions [8]. As such, light and temperature are very important environmental factors for the growth and development of roses. In addition, light intensity and temperature have a mutual relationship in rose growth and flower development. Even if the temperature is appropriate, if the light intensity is low, the growth of roses is inhibited, and even if the light intensity is high, if the temperature is high or low, the growth of roses is inhibited [37]. In this experiment, the temperature (Figure 1) and light intensity (Figure 2) in a greenhouse where grafted roses were grown changed greatly depending on the four seasons. In summer, temperature was high and light intensity was low. In winter, both the temperature and the light intensity were lower than in the spring and autumn. As such, in the results of our study, the changes in light intensity and temperature due to seasonal environmental conditions affected the photosynthesis, flower quality, quantity, and morphology of grafted roses grown in the greenhouse. Previous reports [38] stating that the seasonal changes in temperature and light environment in greenhouses in Korea affect the growth of roses are similar to our results.

Grafted plants have improved growth due to enhanced photosynthetic efficiency parameters such as photosynthesis rate, stomatal conductance, concentration of intercellular  $CO_2$  and transpiration rate [22,23]. Additionally, it is reported that the photosynthetic efficiency and growth of grafted plants vary greatly depending on the type of rootstock [30]. In our study, grafted roses improved the photosynthesis rate, stomatal conductance and transpiration rate with similar trends to those in rootstocks (Figures 7 and 8). Additionally, as the conductivity and transpiration rate of the stomata of the grafted rose increased, the shape of the stomata was observed, and it was confirmed that the stomata of the grafted roses were smaller and more numerous than those of the cuttings in summer. Additionally, the stomata of the grafted roses were larger those of the cuttings in winter (Table 3, Figures 4–6). Therefore, it is considered that grafted roses with a large amount of transpiration required more moisture, but due to the lack of the supplied nutrient solution, drying symptoms occurred, leading to smaller sized stomata in summer. Similar to our findings, it is reported that the photosynthetic efficiency of the rootstocks affected the grafted roses, just as a rose grafted with heat-resistant rootstock became heat-resistant [39,40]. In particular, there is a previous report stating that stomatal control of photosynthesis through transpiration affects plant productivity and water use efficiency [41]. According to a report investigating stomata, as the number of stomata increases, the absorption of  $CO_2$  and transpiration become smoother, and smaller stomata have a fast opening and closing speed [42]. In addition, stomata size decreases due to stress factors such as drought [43]. In particular, it has been reported that longer and wider stomata contribute to the enhanced plasticity of stomata conductance under high temperature [44], and in this study, it was confirmed that the stomatal conductivity of PB/N1 with large stomata decreases under high temperature and high light-intensity conditions (Table 3). Additionally, this is consistent with a report stating that stomata size was negatively correlated with the maximum rate at which the stomata opened in response to light [45], and stomatal conductivity increases or decreases according to the temperature increases for each crop [46]. Therefore, it is considered that the stomata conductance of grafted roses not only changes according to the temperature and the type of rootstock, but is also affected by the size and number of stomata. To sum up, it is concluded that the photosynthetic efficiency and morphological changes in grafted roses affect the quality and yield of cut roses.

Grafting benefits the growth of horticultural crops by improving their tolerance to negative environmental conditions such as high or low temperatures and low light intensity [20,21]. The benefits of grafting vary greatly depending on the type of rootstock [29,30]

and vitality of the scion or rootstock [47]. The root, which absorbs water and nutrients from the medium, is an important organ for the growth of cut roses. The root activity of the rootstocks was significantly higher than that of the scion (Figure 3). Additionally, the length, diameter, and fresh weight of the stem produced from the grafted roses was differentially increased depending on the variety of rootstocks in autumn, winter, and spring (Figure 9). In contrast, in summer, there was no effect of grafting on the quality of cut roses. This could be because the temperature in the greenhouse in summer was too high, which lowered the photosynthetic efficiency. In addition, shading to lower the high temperature in summer reduced the light intensity in the greenhouse (Figures 1 and 2). Therefore, it is considered that there was no difference in quality as the difference in photosynthesis rate due to rootstock treatments was small because in summer, the temperature in the greenhouse was too high and the light intensity of the greenhouse was lowered by shading (Figures 7, 8 and 10). The yield of cut roses was not higher in the grafted roses compared with the cuttings in spring, summer, and winter. The large yield of cut roses in autumn could be due to exposure to low temperature (14 °C) at nighttime with non-heating during 2 weeks in the early stages of rose growth (Figure 1). Our results are supported by a report that when pruned roses are subjected to low temperatures in the early stages of growth, the development of shoots is improved [8].

Among the cut roses grafted onto three rootstocks in the study, the number of petals of PB/NA decreased, but the stem length quality was good according to cut flower quality standards in Korea (Table 4). Although not decorative flowers, there is a report that the shape of grapefruit changes under grafting [48]. In addition, when a pepper with long and thin red fruit (rootstock) was grafted with a pepper with round and thick red fruit (scion), medium-length round fruit was produced [49]. As such, it was confirmed that the morphological changes in the grafted plant were due to the influence of the rootstock.

#### 5. Conclusions

The conclusions for the cut roses grafted onto three different rootstocks are as follows:

First, physiological characteristics such as root activity and photosynthetic efficiency and growth characteristics differed greatly depending on the type of rootstock widely used for grafting roses. Second, the grafted roses that received photosynthetic properties from each rootstock had improved photosynthesis parameters such as the photosynthetic rate, stomatal conductivity, and transpiration rate compared with the scion. Finally, since grafted roses have different photosynthetic efficiency and growth characteristics compared with ungrafted roses, additional research is needed to determine their optimal nutrient solution according to environmental changes (temperature and light) in the greenhouse and thereby improve the efficiency of hydroponics systems.

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