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Variation in Viral Tolerance of 21 Grapevine Rootstocks

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Abstract: Grapevine is one of the most economically important fruit crops cultivated worldwide. However, grapevine is highly susceptible to virus infections and exposed to the most diverse forms of viral diseases compared to other fruit crops, and virus-induced incompatibility affects plant growth to different degrees ranging from decline to death. The influence of virus-induced incompatibility could be mitigated to an acceptable level by using appropriate rootstocks. However, the viral tolerance of various grapevine rootstocks with diverse genetic backgrounds remains unclear, along with the identification of the specific viral tolerance factors. In this study, the viral tolerance of 21 grapevine rootstocks was evaluated in a green grafting system. Cabernet Franc varieties infected with a single virus [grapevine leafroll associated virus-1 (GLRaV-1)], a co-infection of two viruses (GLRaV-1 plus grapevine virus A—GVA), and no infection were used as the scions, respectively. The vegetative growth and photosynthetic function of the grafts were analyzed 4 months after grafting. The results indicated that some rootstocks could alleviate the influence of the virus infection, with vegetative growth and photosynthetic function sustained at a normal level, whereas other rootstocks were susceptible to the virus infection, resulting in a decline in the growth and photosynthetic function of the grafts. Our research provides evidence for the existence and diversity of viral tolerance among grapevine rootstocks, offering important information for appropriate rootstock selection in the establishment of new vineyards and in the breeding of grapevine rootstocks with enhanced viral tolerance.

Keywords: graft incompatibility; rootstock; viral tolerance; *Vitis*

Citation: Zhao, M.; Peng, L.; Agüero, C.B.; Liu, G.; Zhang, Y.; Walker, A.M.; Cui, Z. Variation in Viral Tolerance of 21 Grapevine Rootstocks. *Agronomy* **2024**, *14*, 651. https://doi.org/10.3390/agronomy14040651

Academic Editor: Youssef Rouphael

Received: 12 February 2024
Revised: 5 March 2024
Accepted: 19 March 2024
Published: 23 March 2024

1. Introduction

Grapevine (*Vitis* spp.) is one of the most widespread and economically important fruit crops in the world, but it is also the host to the largest number of viruses of any cultivated plant [1]. More than 80 different viruses belonging to different genera and families have been identified in grapevines to date, and novel viruses are constantly being discovered [1]. Among them, grapevine leafroll-associated virus (GLRaV)-1 and GLRaV-3 in the genus *Ampelovirus* are the most prevalent across global grape-growing regions [2]. GLRaV-1 and GLRaV-3, either alone or in combination, are responsible for grapevine leafroll disease, which has detrimental effects on the vines, including decreased vegetative growth, reduced physiological activities, delayed fruit ripening and coloration, altered fruit chemical composition, and the altered gene expression of the hosts [2–4].

In the field, GLRaVs, especially GLRaV-1, GLRaV-2, and GLRaV-3, are frequently found to co-infect their hosts with grapevine vitiviruses, including grapevine virus A (GVA) and grapevine virus B (GVB) [5]. In addition to the detrimental effects on the scions, the

GLRaVs also cause different degrees of graft incompatibility in young grapevines, especially when they co-infect the plants with grapevine vitiviruses [6]. The reported effects of virus infection-induced graft incompatibility ranging from no noticeable signs to the death of the plant according to the different rootstocks were evaluated [7–10]. The infection with the strain RG of GLRaV-2 using the scion as the inoculum could result in a decline in growth or death 2 years post-inoculation when grafted onto the rootstocks Kober 5BB, 5C Teleki (both *V. berlandieri* × *V. riparia*), Paulsen1103 (*V. berlandieri* × *V. rupestris*), and Couderc3309 (*V. riparia* × *V. rupestris*). However, no such symptoms were observed when the scion infected with the same virus strain was grafted onto rootstock Paulsen1103 or 101-14 Mgt (*V. riparia* × *V. rupestris*) [8–10]. In combination with GVB, the infection of GLRaV-2 resulted in complete graft failure when Freedom (Champinii × 1613 C) and Harmony (Champinii × 1613 C) were used as the rootstocks, whereas infection with only one of these two viruses was not associated with vine decline or graft failure [11]. In other similar studies, the combination of GLRaV-1 and GVA infecting the scion as the inoculum caused the incomplete healing of the grafting union and a growth decline in the grafts when Freedom and 101-14 were used as the rootstocks, whereas the rootstocks St. George (*V. rupestris*) and AXR#1 (*V. vinifera* × *V. rupestris*) displayed viral tolerance in the same trial [12,13]. These findings suggest the existence of a virus–rootstock interaction that determines the fate of the grafts upon virus infection. Moreover, a synergistic effect between GLRaVs and grapevine vitiviruses could increase the virulence on grapevine grafts, indicating a further complexity underlying the virus–rootstock interaction. The genetic background determines the viral tolerance of the rootstock in the grafting system [6]. Therefore, it is necessary to comprehensively evaluate the growth behavior of grafts under virus-infected conditions with a wide range of rootstocks from different genetic backgrounds, which will provide valuable information on the association of viral tolerance with the genetic background of the rootstock. Such an evaluation could further help to identify the specific genetic determinants derived from the ancestor rootstocks with viral tolerance to facilitate the breeding of grapevine rootstocks with viral tolerance. Despite extensive research investigating the viral tolerance of grapevine rootstocks [8–10,12,13], knowledge of the genetic factors underlying the observed variation in viral tolerance is lacking, and the exploration of the molecular mechanism remains to be elucidated.

To fill this gap, in the present study, the viral tolerance of 21 grapevine rootstocks with diverse genetic backgrounds was evaluated under a single infection with GLRaV-1 or a co-infection of GRLaV-1 and GVA in a green grafting system, aiming to provide more information on the genetic mechanisms of the rootstock viral tolerance. The photosynthesis function of the scions and the rooting ability of the rootstocks under the influence of virus infection were measured and analyzed, which has not been performed in early viral tolerance testing studies. These results will therefore provide essential information for the evaluation and breeding of grapevine rootstocks with viral tolerance.

2. Materials and Methods

2.1. Plant Materials

Until now, no source of resistant-materials was found in either table or wine grapes (*Vitis vinifera*). Cabernet Franc is an important wine grape and is susceptible to grapevine leafroll disease, like other *V. vinifera* species. In this study, Cabernet Franc varieties (Franc, LR131, and LR132) were used as the scions in the grafting system. Twenty-one rootstock genotypes (see Table 1) with diverse genetic backgrounds collected from the nursery of the University of California Davis (UCD) (Davis, CA, USA) were used in the grafting system. The infection status of the collected materials was determined with the help of the UCD Foundation Plant Services (https://fps.ucdavis.edu). The Franc scion and all its rootstocks were considered to be healthy materials, as they were obtained from meristem tissue culture and tested negative for the 38 viruses of the following virus genera: *Nepovirus*, *Closterovins*, *Vitivims*, *Foveavirus*, *Maculavirus*, *Marafivirus*, *Tridovirus*, plus phytoplasmas and *X. fastidiosa*, listed in Protocol 2010 (http://fps.ucdavis.edu/fgr2010.cfm). The scion LR131 only tested

positive for GLRaV-1, while LR132 tested positive for both GLRaV-1 and GVA. Therefore, Franc served as the healthy control, and LR131 and LR132 were used as the virus inocula in the trial. All the materials with an identified infection status were carefully propagated by green cuttings Cui et al. [13], and maintained in the greenhouse in 4-L pots as stock materials for the experiment without any contamination from other plants in March 2019.

Table 1. The rootstock varieties used in this study and their parentage.

Rootstock Variety	Parentage
101-14	*V. riparia* × *V. rupestris*
LN33	Couderc 1613 × Sultanina
AXR#1	*V. vinifera* × *V. rupestris*
Freedom	Champinii × 1613 C
Schwarzmann	*V. riparia* × *V. rupestris*
Paulsen1103	*V. berlandieri* × *V. rupestris*
Ru140	*V. berlandieri* × *V. rupestris*
Couderc3309	*V. berlandieri* × *V. rupestris*
Richter 99	*V. berlandieri* × *V. rupestris*
Richter110	*V. berlandieri* × *V. rupestris*
St. George	*V. rupestris*
A.de.Serres	*V. rupestris*
TX9725	*V. rupestris*
TX9726	*V. rupestris*
TX9728	*V. rupestris*
Pump Station	*V. rupestris*
Vru147	*V. rupestris*
Vru148	*V. rupestris*
Vru123	*V. rupestris*
Vru87	*V. rupestris*
Vru110	*V. rupestris*

All the rootstocks were considered as virus-free materials.

2.2. Green Grafting

The materials used for grafting were grown in the greenhouse in 4-L pots filled with equal proportions of perlite, vermiculite, and peat. The temperature of the greenhouse was set at 30 °C/20 °C day/night and the photoperiod was 16 h. Herbaceous (in spring) shoots with a diameter of 3–5 mm were cut off from the 4-month-old pot-grown plants for grafting. For scion preparation, a shoot segment of 5 cm in length with one lateral bud and one leaf was selected, and a tapered wedge of 1 cm in length was made at the base of the segment. The leaf margins were cut off to reduce the leaf area by half. For rootstock preparation, a 1-cm-long vertical slit was made along the axis of the segment of 10 cm in length, which fit the tapered wedge of the scion. All the leaves and lateral buds of the rootstock segment were completely removed and a node point was kept at the base, which is helpful for the root initiation of the graft (Figure 1a). The scion and the rootstock segments were then cleft-grafted using parafilm (No. PM996, Laiborun, Beijing, China) to wrap the graft union, and the cut surface on the top of the scion was sealed with paraffin wax to reduce water loss. The grafting process was performed as quickly as possible, and the materials were misted with water occasionally to prevent desiccation. The half bottom of the newly grafted plant was wrapped by a wet sponge (East, Tianjing, China) and plugged into an empty plate with holes (Figure 1b,c).

The plate with grafted plants wrapped by the sponge was maintained in a mist room with 100% humidity at 30 °C and a 16-h photoperiod for 2 weeks for graft healing and root initiation. Then, the plants were transferred to the greenhouse in 1-L pots and grown under shade for 2 weeks using daily irrigation with Hoagland's solution. After the grafted plants recovered with visible graft healing and newly formed roots (Figure 1d), they

were transferred into 4-L pots without the trimming of their roots, and grown under the same greenhouse conditions as the stock materials, using daily irrigation with Hoagland's solution according to Fort and Fraga [14] (Figure 1e,f). Then, the plants were trained with bamboo sticks until the end of the trial. For the three types of scions and 21 rootstock genotypes, 63 grafting combinations were performed; 30 successfully grafted replicates were established for each grafting combination. The whole experiment was repeated three times.

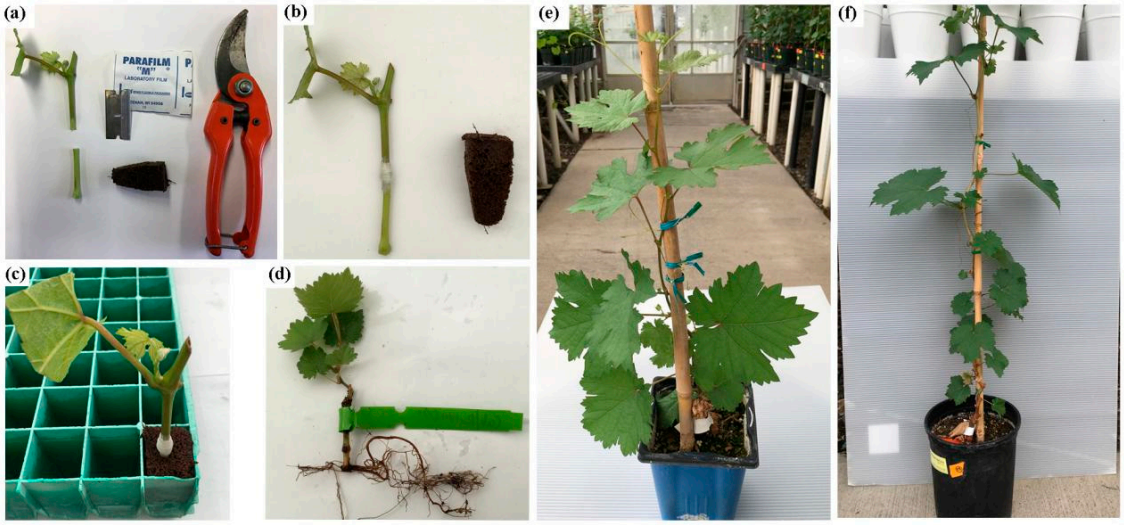

Figure 1. Illustrations of the experimental procedure. (**a**) Green cuttings and tools prepared for the grafting. (**b**) Completed graft covered in parafilm to fix the grafting union. (**c**) New graft wrapped with a sponge and plugged into a plastic plate. (**d**) Successful graft with newly grown shoots and roots 4 weeks after grafting. (**e**,**f**) Successful graft transferred into a 4-L pot trained with a bamboo stick 4 weeks after grafting..

2.3. Growth Investigation

The survival rate of the grafts was determined 4 weeks after grafting. A graft exhibiting obvious root formation and shoot elongation was considered to be a successful graft. The biomass of the grafts was measured 3 months after they were transferred to the 4-L pots. All the shoots and leaves above the graft union were taken for the measurement of scion dry weight. All the roots were taken off to analyze their dry weight. Ten plants of each grafting combination were used for the biomass analysis.

2.4. Photosynthetic Capacity Evaluation

The photosynthetic activity of the grafts was measured 3 months after they were transferred to the 4-L pots using a CIRAS-3 Portable Photosynthesis System (PP Systems, Amesbury, MA, USA) according to the method described by Dong et al. [15]. Briefly, the net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), transpiration rate (T_r), and stomatal conductance (g_s) were measured with the following settings: relative humidity, 40 ± 3%; carbon dioxide concentration, 390 μmol·mol^{-1}, light-emitting diode light source, 1200 μmol·m^{-1}·s^{-1}; red light, 90%; and blue light, 10%. After the readings became stable, the data were recorded. Three fully unfolded leaves from the bottom, middle, and top of the plants were selected for the measurements, and the experiment was performed during the day from 10:00 to 14:00. Ten plants of each grafting combination were used for the evaluation.

2.5. Virus Transmission Detection

To know if the virus was systematically transmitted from the infected scion to the healthy rootstock, the newly formed roots of the rootstock were tested for GLRaV-1 and

GVA 4 weeks after grafting. The RNA isolation and cDNA synthesis were performed according to Cui et al. [13]. The specific primers to GLRaV-1 and GVA were GAGCGACTTGCGACTTATCGA/GGTAAACGGGTGTTCTTCAATTCT (forward/reverse) and GACAAATGGCACACTACG/AAGCCTGACCTAGTCATCTTGG (forward/reverse), respectively. Grapevine 18S ribosomal RNA was used as the housekeeping gene, and its specific primer was GTGACGGAGAATTAGGGTTCGA/CTGCCTTCCTTGGATGTGGTA (forward/reverse). A real-time quantitative polymerase chain (RT-qPCR) reaction was conducted using reagent kits (RR820A, Takara Bio, Shiga, Japan) on the machine IQ5 (BIO-RAD, Hercules, CA, USA). The specificity of amplicons was verified by melting curve analysis with a range of 65–97 °C setup. Both no-template and no-RT were used as controls. The virus status was evaluated by the threshold cycles' value (C) of qPCR reactions.

2.6. Statistical Analysis

Analysis of variance was conducted for the comparison of data among groups using Duncan's multiple-range test in DPS 7.05 software (Zhejiang University, Hangzhou, China). Principal components analysis (PCA) was further performed to visualize the grouping of the data of different grafts. The loading values of the PCA plot were used to analyze the factors contributing to the group separation.

3. Results

3.1. Grafting Survival

In our grafting system, the healing of the graft union and the root initiation proceeded simultaneously. Therefore, root formation on the rootstock and wound healing at the grafting union could be observed on a successfully grafted plant. Virus infection affected graft survival for most scion–rootstock combinations (Figure 2). However, no significant difference in the survival rate was observed among the three types of scions when they were grafted onto the following five rootstocks: Paulsen1103, Couderc3309, Richter110, A.de.Serres, and Vru147. When grafted onto LN33, AXR#1, Ru140, Richter99, and TX9725, the LR131 and Franc scions showed similar survival rates, which were both significantly higher than that of LR132. When the scions were grafted onto 101-14, Freedom, and Vru148, Franc had the highest survival rate, followed by LR131, and LR132 had the lowest survival. LR132 and Franc had similar survival rates when grafted onto St. George, whereas the survival rates of both of these scions were lower than that of LR131 when grafted onto TX9728. When grafted onto Pump Station, Vru123, and Vru87, LR131 and LR132 showed similar survival rates, which were both lower than that of Franc (Figure 2).

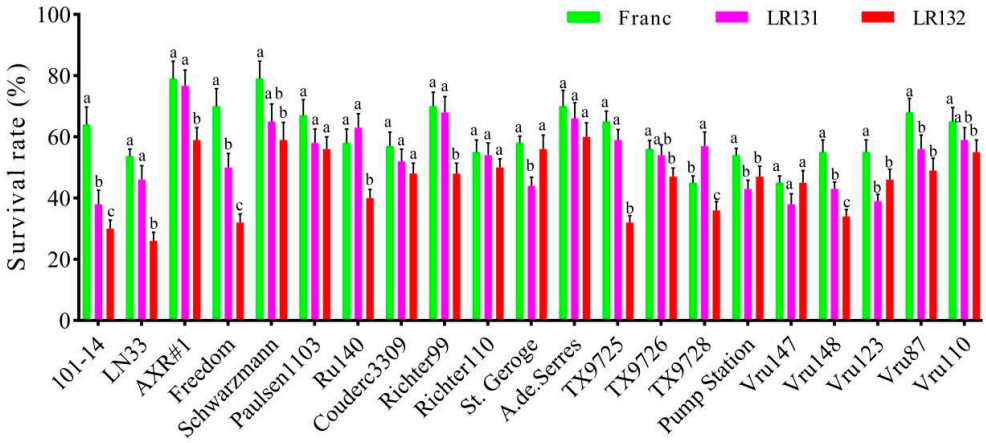

Figure 2. Comparative analysis of the survival of Franc, LR131, and LR132 when grafted onto different rootstocks. The scion Franc and the 21 rootstocks were considered as virus-free materials; LR131 was infected with GLRaV-1 only; LR132 was infected with both GLRav-1 and GVA. Different lowercase letters on top of the bars corresponding to the same rootstock indicate a significant difference in survival rates among Franc, LR131, and LR132 at $p < 0.05$ (Duncan's multiple-range test).

3.2. Biomass Analysis

After growing in the 4-L pots for 3 months, no significant difference in scion growth was observed among Franc, LR131, and LR132 when they were grafted onto Ru140, Richter110, St. George, A.de.Serres, TX9726, Vru147, and Vru110 (Figure 3). However, Franc showed a higher scion growth than LR131 and LR132 when grafted onto 101-14, Pump Station, and Vru87. When grafted onto LR33, AXR#1, Freedom, Schwarzmann, Couderc3309, Richter99, TX9725, TX9728, Vru148, and Vru123, LR131 and Franc showed similar levels of growth, while the scion growth of LR132 was much lower than that of Franc. However, the growth of LR131 was better than that of both Franc and LR132 when the scions were grafted onto Paulsen1103 (Figure 3).

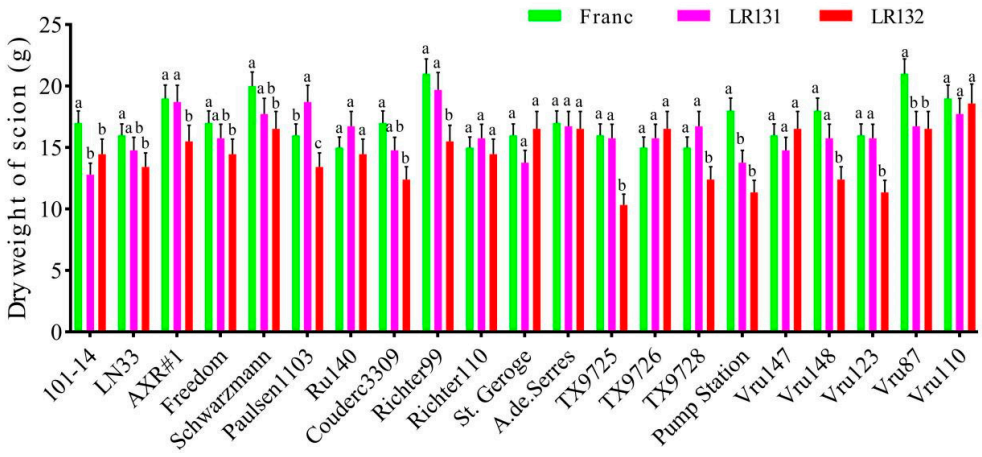

Figure 3. Comparative analysis of the scion growth of Franc, LR131, and LR132 when grafted on different rootstocks. The scion Franc and the 21 rootstocks were considered as virus-free materials; LR131 was infected with GLRaV-1 only; LR132 was infected with both GLRav-1 and GVA. Different lowercase letters on top of the bars corresponding to the same rootstock indicate a significant difference in scion growth among Franc, LR131, and LR132 at $p < 0.05$ (Duncan's multiple-range test).

When grafted by LR131 or LR132, the growth of the newly formed roots on 101-14, LN33, Freedom, Schwarzmann, Paulsen 1103, Ru140, Vru148, and Vru87 was substantially lower compared to that of the roots on rootstocks grafted by Franc after 3 months of growth in 4-L pots (Figure 4). When grafted by LR132, the rootstocks AXR#1, TX9725, and TX9728 showed markedly reduced root growth compared to that of the rootstocks grafted by LR131 or Franc. For Couderc3309, Richter110, A.de.Serres, TX9726, and Vru110, the virus infection transmitted by LR131 or LR132 did not significantly affect the growth of the new root (Figure 4).

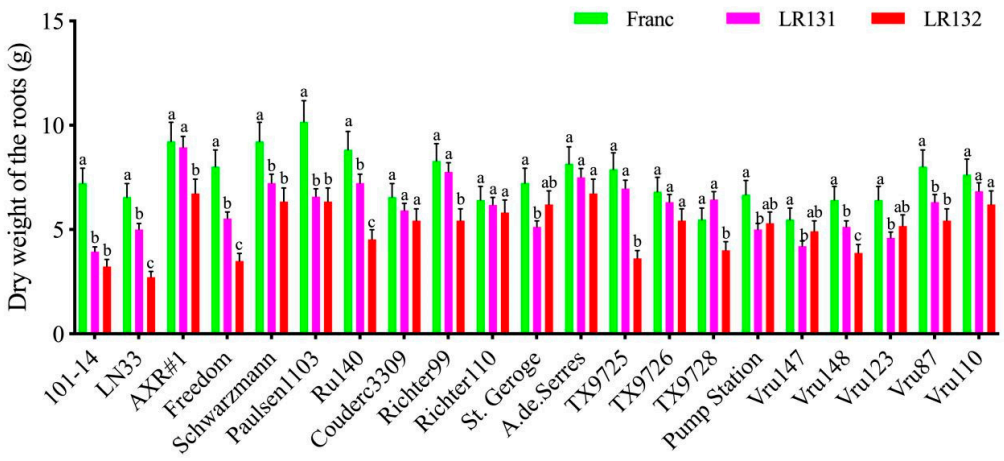

Figure 4. Comparative analysis of root growth of Franc, LR131, and LR132 grafted on different rootstocks. The scion Franc and the 21 rootstocks were considered as virus-free materials; LR131 was

infected with GLRaV-1 only; LR132 was infected with both GLRav-1 and GVA. Different lowercase letters on top of the bars corresponding to the same rootstock indicate a significant difference in root growth among Franc, LR131, and LR132 at $p < 0.05$ (Duncan's multiple-range test).

3.3. Influence of Virus–Rootstock Interactions on Photosynthetic Activity

Virus infection affected the photosynthetic capacity of most of the graft combinations based on the comparison of effects observed when LR131 or LR132 were used as the scion compared to those detected with Franc (healthy control) as the scion (Figures 5–8). Specifically, when grafted onto 101-14, Freedom, Schwarzmann, Paulsen1103, Ru140, Couderc3309, Richter99, Richter110, A.de.Serres, TX9725, TX9726, Vru147, Vru87, and Vru110, the P_n values of LR131 and LR132 were significantly lower than those of Franc. However, the P_n values of LR131 and Franc were similar when they were grafted onto LN33, AXR#1, St. George, TX9728, Pump Station, Vru148, and Vru123. TX9728 was the only rootstock that was not affected by virus infection, which produced a similar level of P_n when grafted by Franc, LR131, and LR132 (Figure 5).

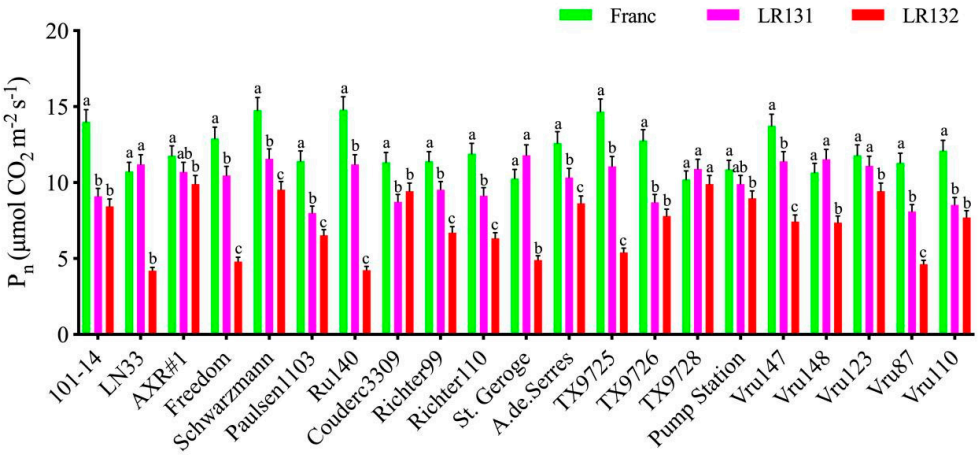

Figure 5. Comparative analysis of photosynthetic rate (P_n) for Franc, LR131, and LR132 when grafted on different rootstocks. The scion Franc and the 21 rootstocks were considered as virus-free materials; LR131 was infected with GLRaV-1 only; LR132 was infected with both GLRav-1 and GVA. Different lowercase letters on top of the bars corresponding to the same rootstock indicate a significant difference in P_n among Franc, LR131, and LR132 at $p < 0.05$ (Duncan's multiple-range test).

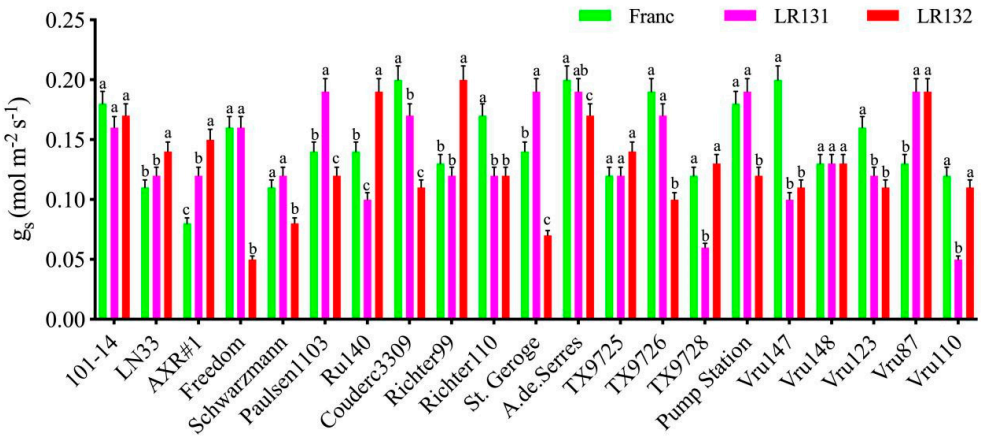

Figure 6. Comparative analysis of stomatal conductance (g_s) values of Franc, LR131, and LR132 when grafted on different rootstocks. The scion Franc and the 21 rootstocks were considered as virus-free materials; LR131 was infected with GLRaV-1 only; LR132 was infected with both GLRav-1 and GVA. Different lowercase letters on top of the bars corresponding to the same rootstock indicate a significant difference in g_s among Franc, LR131, and LR132 at $p < 0.05$ (Duncan's multiple range test).

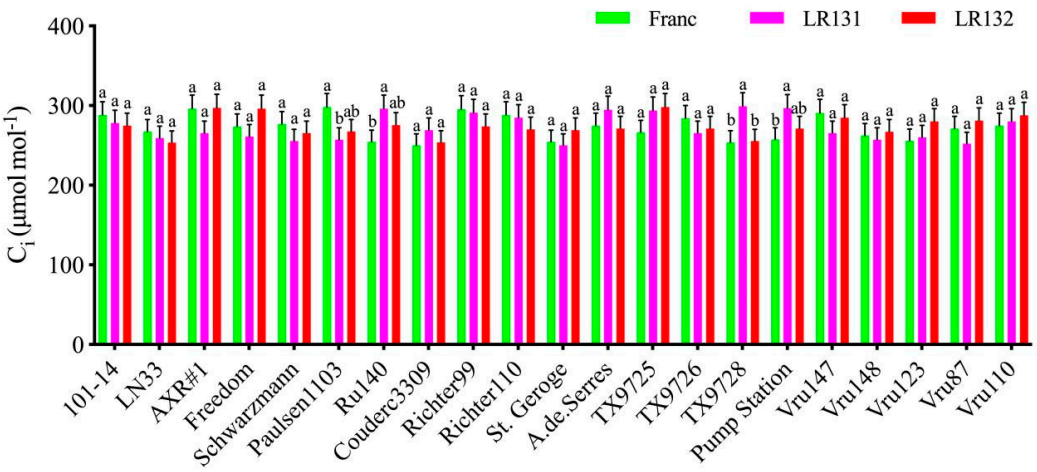

Figure 7. Comparative analysis of intercellular CO2 concentration (C_i) values of Franc, LR131, and LR132 when grafted on different rootstocks. The scion Franc and the 21 rootstocks were considered as virus-free materials; LR131 was infected with GLRaV-1 only; LR132 was infected with both GLRav-1 and GVA. Different lowercase letters on top of the bars corresponding to the same rootstock indicate a significant difference in C_i among Franc, LR131, and LR132 at $p < 0.05$ (Duncan's multiple range test).

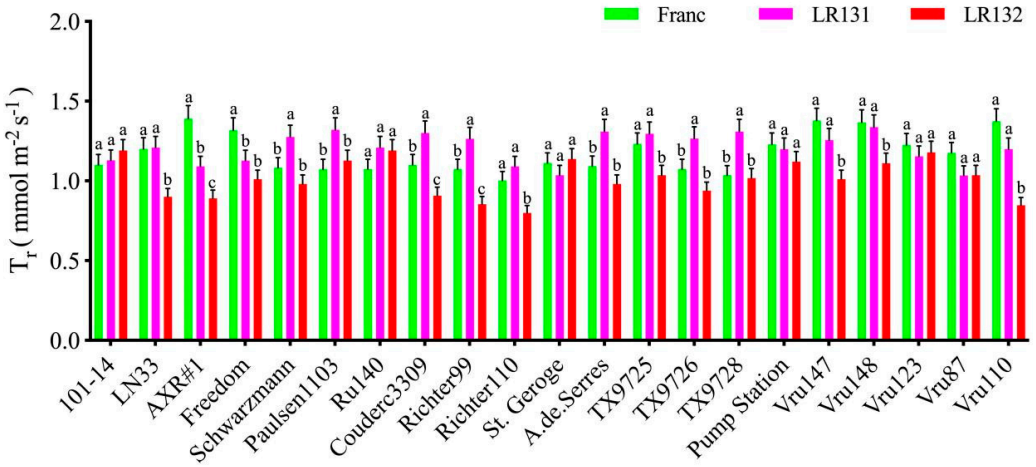

Figure 8. Comparative analysis of transpiration rate (T_r) values of Franc, LR131, and LR132 when grafted on different rootstocks. The scion Franc and the 21 rootstocks were considered as virus-free materials; LR131 was infected with GLRaV-1 only; LR132 was infected with both GLRav-1 and GVA. Different lowercase letters on top of the bars corresponding to the same rootstock indicate a significant difference in T_r among Franc, LR131, and LR132 at $p < 0.05$ (Duncan's multiple range test).

The effect of virus infection on the g_s of the grafts showed a different pattern than found for P_n (Figure 6). No significant difference in g_s was found among the Franc, LR131, and LR132 scions when they were grafted onto 101-14, TX9725, and Vru148, whereas both LR131 and LR132 showed a lower level of g_s than that of Franc when they were grafted onto Richter110, Vru87, and Vru123. When grafted onto Freedom, Schwarzmann, Paulsen1103, St. George, A.de.Serres, TX9726, and Pump Station, the g_s of LR132 was markedly reduced compared to that of Franc and LR131. However, LR132 showed a higher level of g_s than both Franc and LR131 when LN33, AXR#1, Ru140, and Ritcher99 were used as the rootstocks (Figure 6).

Virus infection did not appear to influence the value of C_i of different grafting combinations, regardless of whether LR131 or LR132 were grafted onto the rootstocks compared to Franc (Figure 7). In addition, no significant difference in the T_r was observed among LR131, LR132, and Franc when grafted onto rootstocks 101-14, Ru140, St. George, Pump Station, Vru123, and Vru87. However, when Franc was grafted onto AXR#1 and Freedom,

the value of T_r was markedly higher than that obtained with the grafting of LR131 or LR132. The T_r value of LR132 was significantly lower than those of LR131 and Franc only when grafted onto LN33, Couderc3309, Richter99, Richter110, TX9725, Vru147, Vru148, and Vru110 (Figure 8).

3.4. Cluster Analysis

PCA models with two principal components (PCs) were constructed to examine any inherent clustering using the data of the survival rate, scion growth, and root growth (Figure 9). The grafts using LR131 and Franc as the scions could not be separated under the PCA model, whereas the plants grafted by LR132 were clearly separated from those grafted by LR131 and Franc (Figure 9a). The loading plot showed that rootstocks TX9725, 101-14, St. George, Vru147, LN33, and Freedom had the greatest contribution to the group separation in the PCA model (Figure 9a,b).

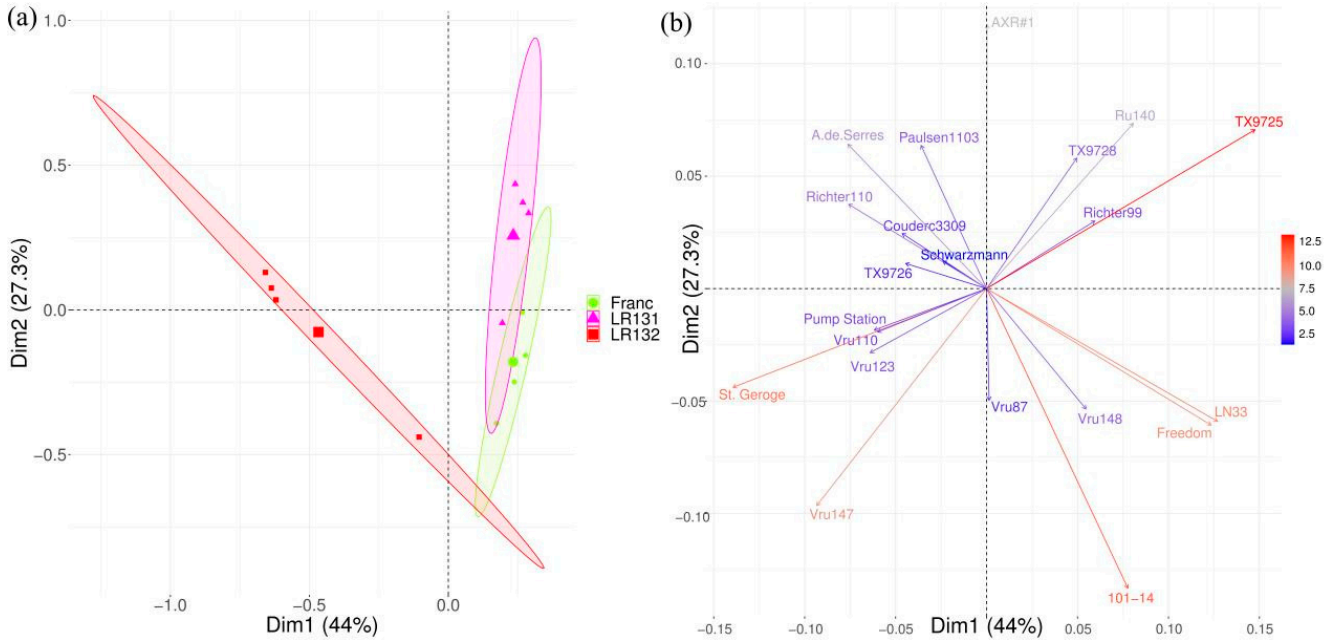

Figure 9. Clustering analysis of the growth data of the grafts, including grafting survival rate, scion growth, and root growth. (**a**) Principal component analysis score plot of the three types of scions: Franc, LR131, and LR132. Each type of scion was grafted onto the same 21 rootstocks for this analysis. (**b**) Loading plot of (**a**) according to the contribution of the variables to the model. The scion Franc and the 21 rootstocks were considered as virus-free materials; LR131 was infected with GLRaV-1 only; LR132 was infected with both GLRav-1 and GVA.

PCA models with two PCs were also constructed to examine any inherent clustering using the data of photosynthetic parameters (Figure 10). In contrast to the clear separation of the grafts of LR132 from those of Franc and LR131 based on the growth data, the grouping according to the photosynthetic data was not as clear. However, the loading plot showed that the rootstocks Vru87, TX9728, Ru140, 101-14, Vru110, Richter99, and St. George had the greatest contributions to the group separation in the PCA model (Figure 10a,b).

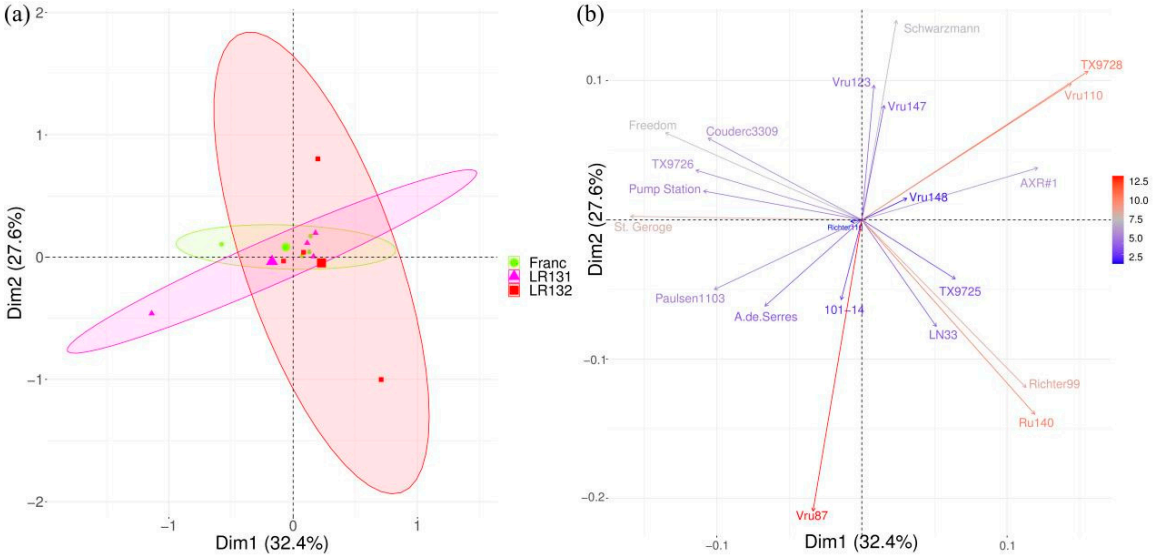

Figure 10. Clustering analysis of the photosynthetic function parameters of the grafts, including the net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), transpiration rate (T_r), and stomatal conductance (g_s). (**a**) Principal component analysis score plot of the three types of scions, Franc, LR131 and LR132, grafted on the 21 rootstocks for this analysis. (**b**) Loading plot of (**a**) according to the contribution of the variables in the model. The scion Franc and the 21 rootstocks were considered as virus-free materials; LR131 was infected with GLRaV-1 only; LR132 was infected with both GLRav-1 and GVA.

3.5. Virus Transmission from Scion to Roostock

The successful grafts with visible graft healing and newly formed roots were employed to detect the virus transmission from scion to rootstock 4 weeks after grafting. When grafted by LR131, the newly formed roots of the 21 rootstocks all tested positive for GLRaV-1 only; when grafted by LR132, the newly formed roots of the 21 rootstocks all tested positive for both GLRaV-1 and GVA; and the newly formed roots of the 21 rootstocks all tested negative for either GLRaV-1 or GVA when they were grafted by Franc (Table 2).

Table 2. The test of virus transmission from infected-scion to healthy rootstock 4 weeks after grafting. The virus status was evaluated by the threshold cycles value (Ct) of qPCR reactions.

Rootstock Variety	Grafted by		
	Franc	LR131	LR132
101-14	○	▲	★
LN33	○	▲	★
AXR#1	○	▲	★
Freedom	○	▲	★
Schwarzmann	○	▲	★
Paulsen1103	○	▲	★
Ru140	○	▲	★
Couderc3309	○	▲	★
Richter 99	○	▲	★
Richter110	○	▲	★
St. George	○	▲	★
A.de.Serres	○	▲	★
TX9725	○	▲	★
TX9726	○	▲	★
TX9728	○	▲	★
Pump Station	○	▲	★

Table 2. *Cont.*

Rootstock Variety	Grafted by		
	Franc	**LR131**	**LR132**
Vru147	○	▲	★
Vru148	○	▲	★
Vru123	○	▲	★
Vru87	○	▲	★
Vru110	○	▲	★

The newly formed roots were tested for both GLRaV-1 and GVA by RT-qPCR. ○ indicated the samples were tested negative for either GLRaV-1 or GVA with undetectable C_t values; ▲ indicated the samples were tested positive for GLRaV-1 with C_t values less than 30, but negative for GVA with undetectable C_t values; ★ indicated the samples tested positive for both GRLaV-1 and GVA with C_t values less than 30.

4. Discussion

In this study, two virus strains and 21 grapevine rootstocks were used to test the viral tolerance of diverse rootstocks in grafted plants. The results showed that virus infection induced different levels of graft incompatibility, which affected the grafting survival rate, vegetative growth, and photosynthetic function of the grafts. The combination of GVA and GLRaV-1 produced much more severe damage than a single infection of GLRaV-1 to the grafts, including the decreased vegetative growth and photosynthetic function, and the varied behaviors of the grafts using different rootstocks indicated the existence of complex interactions between the viruses and grapevine rootstocks. The same virus could be detected in the roots as found in the scions, indicating that the grafting union healed well and that the systematical virus transmission from the scion to the rootstock was successful 4 weeks after grafting using our grafting method.

Virus-induced graft incompatibility in grapevine has been confirmed in many previous studies, including tests with more than five virus species and diverse rootstocks [6,13,16]. In line with our results, more severe damage to the graft was previously reported with the co-infection of GVA and GLRaV-1 [12,13]. However, it remains unknown whether this intensified detrimental effect is due to a synergistic effect between GVA and GLRaV-1, since the single infection of GVA was found to be rare in the field and the impact of a single infection of GVA on grafts has not been reported [5]. In a study on the transmission of grapevine viruses, GVA was found to be predominantly transmitted along with GLRaV-1, whereas GLRaV-1 could be transmitted alone from single or co-infected vines [17]. Moreover, the co-infection of GLRaV-1 and GVA increased the virus titer of GVA compared to that under a single infection of GVA, whereas no changes in the titer of GLRaV-1 were found between the co-infection and single-infection conditions, indicating an asymmetric effect on the titers of these two co-infecting viruses [5]. Combining the above findings and our present results, we speculate that a synergistic effect between GLRaV-1 and GVA exists, which facilitates the transmission and replication of GVA and consequently increases the severity of a co-infection on the grapevines.

The influence of a single or mixed virus infection on the vegetative growth, physiological activity, fruit quality, and gene expression of the grapevine has been well documented [2,4,18,19]. However, the mechanism by which the rootstock can alleviate the viral impact on the scion has not been studied in depth. In our study, LR132 maintained a similar level of grafting survival as found for the healthy control scion Franc when they were both grafted onto Paulsen1103, Couderc3309, Richter110, St. George, TX9726, and Vru147. Moreover, the rootstocks Ru140, Richter140, St. George, TX9726, Vru147, and Vru110 exhibited a normal growth of the scion when grafted by LR132, similar to that for the rootstocks grafted with Franc. Similarly, some rootstocks could alleviate the impact of the virus on the net photosynthetic rate (P_n) of the scion, mainly by reducing the influence of the virus on the transpiration rate (T_r) and stomatal conductance (g_s). Even though some of the rootstocks used in our study belong to the same *Vitis* species, they exhibited diverse reactions to the virus inoculations, resulting in various impacts on the

vegetative growth and photosynthetic activity of the grafts. Early studies demonstrated that St. George and AXR#1 showed much better viral tolerance in different grafting systems compared to Freedom [12,13], indicating that *V. rupestris* may be an important source to provide viral tolerance. By contrast, an evaluation of the viral tolerance of rootstocks with diverse genetic backgrounds in a grafting system suggested that the genetic determinants of incompatibility may be mainly derived from *V. berlandieri* and *V. riparia* [9], which has not been identified. The above findings indicate that one or more viral tolerance factors exist in the rootstocks across species, posing a challenge to elucidate these complex interactions.

It is a long-held perspective that all table and wine grapes (*V. vinifera* spp.) are susceptible to grapevine viruses [20]. Therefore, using virus-free planting materials is crucial for the establishment of new vineyards as a prophylactic measure [21,22]. However, infection is ultimately inevitable due to virus transmission by mealybugs and soft scales in the field [23]; thus, the rogueing and replacement of the infected grapevines with healthy materials would become the only effective but costly management strategy [24]. Hence, it is a crucial step to select the rootstocks with a viral tolerance for the establishment of a new vineyard, which may at least minimize the virus-induced grafting incompatibility and maximize the profit of the vineyard. Combing the results of previous and our present studies, we suggest St. George, TX9725, and Vru147 to be used as rootstocks to reduce the influence of a mix-infection with GLRaV-1 and GVA, whereas Freedom, 101-14, and LN33 should be avoided in the regions with high infection with GLRaV-1 and GVA due to their susceptibility to this mix-infection. Since only one scion cultivar and two virus strains were employed in our experiment, the responses found here could be related to the scion cultivars evaluated and the virus species. In addition to virus infection, grapevines also face other abiotic and biotic threats, e.g., saline–alkaline, drought, nematode, and *Phylloxera.* Therefore, a balance of the rootstock resistance should be carefully considered to choose the most suitable rootstock for the planting regions.

5. Conclusions

Both early studies and our present study confirmed the existence and diversity of the viral tolerance of grapevine rootstocks; however, the underlying genetic mechanisms need to be identified as essential information to expedite the breeding of grapevine rootstocks with viral tolerance. The use of rootstocks with viral tolerance in the vineyard is a promising alternative tool to control or prevent virus-induced damage.

Author Contributions: M.Z. and L.P. analyzed the data and prepared the manuscript. C.B.A. provided help with materials' preparation, plant grafting, and manuscript writing. G.L. provided valuable guidance in the experiment design and writing. Y.Z. conducted the virus test work. A.M.W. and Z.C. conceived the whole experiment and provided financial support. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Natural Science Foundation of China (Grant No. 32202430), Shandong Provincial Natural Science Foundation (Grant No. ZR2021QC005), and Construction of Seedling Breeding Base and Cultivation Techniques Demonstration on Fruit Tree in Longnan (Grant No. 22-1-3-14-zyyd-nsh).

Data Availability Statement: Data are contained within the article.

Acknowledgments: Valuable help from Maher Al Rwahnih in the identification of material sanitary status was also appreciated.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

GLRaV	Grapevine leafroll-associated virus
PC	Principal components
PCA	Principal components analysis
PP	Portable Photosynthesis

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