Study on the Resistance of ‘Cabernet Sauvignon’ Grapevine with Different Rootstocks to *Colomerus vitis*

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Abstract: In recent years, *Colomerus vitis* has caused serious economic losses due to reduced grape production in Xinjiang (northwest China). Several rootstock varieties have been reported to improve the resistance of Cabernet Sauvignon to *Colomerus vitis*. This study explored the influence of Cabernet Sauvignon with different rootstocks on the resistance to *Colomerus vitis*. In particular, Cabernet Sauvignon/Cabernet Sauvignon (CS/CS) was selected as the control, and Cabernet Sauvignon grafted with five resistant rootstocks (3309C, 1103P, 140R, SO4, and 5C) was employed as the treatment. The infestation rate and injury index of *Colomerus vitis* to grapevines was investigated, and insect-resistant types of grapevines with different rootstocks were determined. The resveratrol (Res) content, the gene expression of resveratrol synthase (*RS*), and the activities of peroxidase (POD), polyphenol oxidase (PPO), catalase (CAT), and superoxide dismutase (SOD) in the leaves of each rootstock grapevine were measured. The activity of the four enzymes and the content of Res were negatively correlated with the injury index. The results revealed the ability of the rootstock to improve the resistance of grapevines to *Colomerus vitis* by increasing the enzyme activity or Res content. In particular, 140R, SO4, and 5C rootstocks can be employed as rootstocks of the ‘Cabernet Sauvignon’ grapevine with resistance to *Colomerus vitis*. The contents of Res and the four resistance enzymes studied here can be used as indexes to evaluate the insect resistance of rootstock–scion combinations.

Keywords: rootstock; grapevines; *Colomerus vitis*; oxidase; resveratrol; mite

1. Introduction

The widespread use of resistant rootstocks of grapevines (*Vitis* spp.) began in the 19th century during the fight against the grape phylloxera [1]. This, consequently, prompted the beginning of research on grapevine resistant rootstocks [2]. Previous work has reported that rootstocks can improve the resistance of grapevine scions to Bois noir (BN) disease [3] and Pierce’s disease (PD) [4,5]. As a high-quality grape production base, Xinjiang (northwest China) has unique environmental and geographical advantages. In addition, the grape industry has developed rapidly in recent years. ‘Cabernet Sauvignon’ is a high-quality red grape of the wine-making variety, which has become the main wine-making grape variety in Xinjiang. However, the increase in grapevine planting area has caused a rise in diseases and insect pests, which negatively impacts grape production, as well as the quality and yield of grapevines. Using rootstocks can effectively improve the stress and disease resistance of grapevine scions [3]. Current research on rootstocks focuses on the resistance to root aphids and nematodes, while advancements have also been made in screening rootstocks with salt, alkali, drought, and cold resistance [6,7]. However, reports on the resistance of grafted grapes with different rootstocks to gall mites are limited.

*Colomerus vitis* (Pagenstecher) (Acari: Eriophyidae) (Grape erineum mite, GEM) eats grapevine leaves, leading to leaf deformities and grape production losses [8]. GEM-infested
grapevine leaves initially show white spots on the back. This is followed by the appearance of a bubble bulge. The leaves then twist, become brittle, and experience the formation of a layer of white fluff plaque at the back. Similar to rusts, brown, felt-like patches form in the later period, which is commonly known as ‘Mao Zhanbing’. When GEM infestation is serious, the grapevines cannot regenerate and grow new shoots, which inhibits the light and ability of leaves and reduces the quality and yield of berries [9]. GEM infestation in grapevine leaves facilitates the spread of grape berry necrosis virus [10] or grapevine Pinot gris virus [11]. Furthermore, GEM, as a common gall mite in wine grape plantations in Xinjiang, has proved to be serious in numerous years, causing great production losses. Therefore, the control of grape gall mites in Xinjiang should not be underestimated.

When plants are under stress and infected by pathogens, their disease resistance systems start to respond. The accumulation of reactive oxygen species (ROS) is closely related to the initial defense response of plants. For example, H$_2$O$_2$, as a signal of plant-induced defense responses, can activate the disease-related protein (PR) gene and induce phytoalexin or other defense responses [12]. In order to protect cells and reduce the pressure of reactive oxygen species, the activities of SOD, CAT, POD, and other enzymes increase [13–16]. Moreover, resveratrol (Res) is a phytoalexin produced by plants under stress and is widely studied due to its key role in grapevine disease resistance. In the response of grapevines to downy mildew, the accumulation of Res and H$_2$O$_2$ is related to programmed cell death (PCD), and the faster the response speed, the stronger the resistance of the plant to downy mildew [17]. Grapevines are a key source of natural Res, a stilbene compound that usually exists as a trans-structure in nature and is typically synthesized by phenylalanine metabolism in plants [18]. Res is also one of the main substances related to the health care function of wine. The content of Res in grapes gradually increases with ripening after it enters the ripening stage, with significant differences among grapevine varieties [19]. At present, breeding experts improve the disease resistance of grapevines through intraspecific or interspecific breeding, including increasing the Res content of plants [20,21]. Related studies have found that the content of Res in scions can also be increased by grafting [22,23]. However, research shows that resveratrol itself does not have a high antimicrobial activity, and its polymers, such as Pterostilbene, are the main substances that can resist microbial infection. Res is usually accumulated at a high concentration in response to induction or pathogen infection [24]. Stilbene compounds may be involved in cell wall strengthening through peroxidase-mediated cross-linking with cell wall components [25]. After the leaves of grapevines are infected by fungal diseases, Res is rapidly synthesized in large quantities at the infected site and is then synthesized by the rootstock to be transported upward to the infected site through the phloem. Moreover, large amounts of Res produced by infected grapevine varieties will be quickly glycosylated to form polydatin (Pd) with less toxicity, while large amounts of Res produced by infected resistant varieties are rapidly oxidized into more toxic viniferin [26,27]. Therefore, the Res content can be used as a reference index to measure the disease or insect resistances of grafted grapevines.

This study explores the effects of different rootstocks on the resistance of ‘Cabernet Sauvignon’ vines to GEM. In particular, ‘Cabernet Sauvignon’ grapevines with different rootstocks were used as the experimental material, and GEM was inoculated artificially in the field in order to investigate the infestation rate and infestation index of new shoot leaves infested with GEM. The activity of related resistance enzymes, Res content, and RS gene expression in the leaves of different ‘Cabernet Sauvignon’ grapevine rootstocks with different GEM infestation grades were measured. The correlation between the infestation index and enzyme activity and the Res content of scion grapevine leaves at the initial stage of infestation (infestation grade 1) was analyzed. The resistance of ‘Cabernet Sauvignon’ grapevines with different rootstocks to GEM was then evaluated. This work provides a reference to explore the mechanism of GEM resistance in ‘Cabernet Sauvignon’ grapevines with different rootstocks and acts as a theoretical basis for the selecting of grapevine rootstocks with GEM resistance in Xinjiang.
2. Materials and Methods

2.1. Field Experiments

The test site is located in the experimental station of the Agricultural College of Shihezi University, Xinjiang, China. Six-year-old grapevine combinations with different rootstocks of ‘Cabernet Sauvignon’ were used as the experimental material. ‘Cabernet Sauvignon’ grafted with ‘Cabernet Sauvignon’ (CS/CS) was used as the control (CK). The planting row spacing was set as 1 m × 3 m, with hedgerow cultivation. Rootstocks and scions were all from the Zhengzhou Fruit Research Institute, Chinese Academy of Agr-cultural Sciences. The selected rootstocks were 3309C (V. riparia × V. rupestris), 1103P (V. berlandieri × V. rupestris), SO4 (V. berlandieri × V. riparia), 140R (V. berlandieri × V. rupestris), and 5C (V. berlandieri × V. riparia). Six grapevine plants with similar growth were selected from each rootstock–scion combination in the experimental station as the experimental material. Each rootstock–scion combination was isolated from other plants in the experimental station using a 300-mesh (84.6 µm aperture) nylon gauze. In 2019, abamectin was used to control mites across the entire experimental station (including the experimental plants) to ensure that there were no GEM and predatory natural enemies on the grapevines in the experimental station. In April 2020, the experimental plants isolated by nylon netting were unearthed separately and placed on shelves. On 3 May, a chemical mixture (abamectin and mancozeb) was used to ensure that there were no pests or injuries on the experimental plants. Following this, the field trimming, soil, fertilizer, and water management of the test materials were standardized and unified. On 30 May, the main shoot was cored, while the auxiliary shoot was not cored.

On 15 June, at 20:30 (Beijing time), the branches of GEM-infected leaves were collected in the ‘Cabernet Sauvignon’ Vineyard, Shihezi City (13 km away from the experimental station) and kept fresh at 25 °C in a sampling box. Whole leaves exhibiting felted injury spots were used as the GEM inoculation material for inoculation. In the evening, the GEM inoculation material and young leaves of the secondary shoots of the experimental plants were fixed back-to-back with paper clips at the experimental station. The total area of the GEM-infected leaves of each grapevine was approximately 10 cm². On 30 June, the grapevine plants exhibited symptoms of GEM infestation. Follow-up management ensured that the nylon net was in a closed state, with the exception of the tester entrance and exit. The resistance of ‘Cabernet Sauvignon’ grapevines with different rootstocks to gall mites was then investigated in the field.

In 2020, 36 grapevine plants of a similar growth stage were selected as the test material. Identical rootstocks were combined into pairs and considered as a single treatment. All treatments were repeated three times. At the peak period of the GEM damage (18 August), five accessory shoots (new shoots) were selected from each grapevine, and five functional leaves larger than 1/3 of the accessory shoot and above (Upper morphology) were selected from each new shoot. A total of 50 leaves were selected to investigate the GEM damage and related indexes. As no leaves with a GEM infestation level of 4 were identified in the statistical analysis, the activities of SOD, CAT, PPO, and POD, the contents of Res and Pd, and the relative expression of the RS gene in the leaves were measured using functional leaves with infestation levels of 0, 1, 2, and 3. Leaves of the same rootstock and scion combinations and the same infestation grade were collected and mixed for sampling. Each test index was repeated three times, and the significant differences of related indexes of different rootstock and scion combinations under the same injury grade were analyzed. The index measured at the earliest stage of worm development following the appearance of the GEM infestation symptoms (i.e., at the level 1 infestation stage) was selected for the correlation analysis with the injury index.

2.2. Infestation Evaluation

The damage degree of GEM to grapevine leaves can be divided into different injury grades based on the following criteria: level 0, no bubble bulge; level 1, bubble bulge area accounts for less than 1/4 of leaf area; level 2, bubble bulge area accounts for 1/4–1/2 of
whole leaf area; level 3, bubble bulge area accounts for 1/2–3/4 of whole leaf area; and level 4, bubble bulge area accounts for more than 3/4 of whole leaf area. Leaf area surveying was performed with a single-lens reflex (SLR) camera, and the affected area was calculated with Photoshop CC2017 (Adobe Inc., San Jose, CA, USA) as follows:

\[
\text{Incidence} = \frac{\text{Incidence}}{\text{Investigation}} \times 100\% \tag{1}
\]

\[
\text{Injury index} = \sum (\text{number of cases at all levels} \times \text{value at all levels})/(\text{total number of investigations} \times \text{highest grade value}) \times 100 \tag{2}
\]

The types of grapevine resistance to gall mites were divided into five levels: (i) immunity (IM), with an injury index of 0; (ii) high resistance (HR), with an injury index of 0.1–5.0; (iii) injury resistance (R), with an injury index of 5.1–25.0; (iv) injury (I), with an injury index of 25.1–50.0; and (v) high injury (HI), with an injury index greater than 50.0.

2.3. Biochemistry Methods

The enzyme activities of SOD, CAT, PPO, and POD in scion leaves with different injury grades were determined according to [28]. Three technical repetitions were performed. The Res and Pd contents were quantified via HPLC (high-performance liquid chromatography) using Shimadzu High Performance Liquid Chromatography (Kyoto, Japan) (LC-2010AHT) of Japan. The equipment included: an ultraviolet detector, a HPLC 2D workstation, the Kunshan ultrasonic instrument, a freezing centrifuge, the SEG ProteCol-PC18 reverse chromatographic column, etc. The chromatographic bottle used for the high-efficiency liquid phase was Agilent Technology Co., Ltd. (Santa Clara, CA, USA). The specific process employed is described as follows: take a 1 g sample and grind it into powder in liquid nitrogen; transfer to a centrifuge tube with methanol to a constant volume of 10 mL; swirl for 2 min; extract with ultrasonic for 30 min; centrifuge it at 12,000 rpm for 10 min; repeat twice; filter the supernatant with a 0.45 µm filter membrane (organic phase) in the chromatographic bottle (Agilent); and place it in a 4 °C refrigerator for later use. The mobile phase was selected as acetonitrile/0.2% phosphoric acid = 45/55, the detection wavelength as 306 nm, and the flow rate as 0.8 mL/min. Column temperature, sample volume, and elution condition were set as: 25 °C, 10 µL, and low elution, respectively.

The related primers were designed according to the literature on the synthesis pathway of resveratrol from a grapevine (Table 1). The primers of the RS gene and internal reference gene (actin1 [29]) were designed with Primer Premier 6.0. The designed primers were synthesized by Shanghai Shenggong Co., Ltd. (Shanghai, China).

Table 1. Primer Sequences.

| Genes | Forward Primer | Reverse Primer |
|-------|----------------|---------------|
| actin1 | CTTCATCTCTACCTAGTGATGCA | TCCTGTGGACAAATGGATGGA |
| RS | GCTATGCGGTTGGAACGTGTCCTTC | CTCAGAGCACACCACAAGAAGTCG |

An RNA extraction kit (Meiji Biotechnology Co., Ltd., Guangzhou, China) was used for the total RNA extraction. The quality of the RNA was identified by a micro-ultraviolet spectrophotometer and agarose gel electrophoresis. An RT-5-UHUUK reverse transcription kit (Shanghai Yisheng biotechnology Co., Ltd., Shanghai, China) was employed to synthesize cDNA strands by reverse transcription, and was stored in the refrigerator at −20 °C.

qRT-PCR reactions were performed with the Green Realtime PCR Master Mix kit (Toyobo, Japan). Each sample was repeated three times. The total volume of the amplification system was 20 µL, with a cDNA 2 µL template, SYBR mixture of 10 µL, ddH2O of 7.2 µL, and forward and reverse primers of 0.4 µL each. The amplification procedure is described as follows: pre-denaturation at 95 °C for 5 min; denaturation at 95 °C for 5 s; annealing
at 60 °C for 10 s; extension at 72 °C for 15 s; and a duration of 40 cycles. The relative expression of genes was calculated using the $2^{-\Delta\Delta Ct}$ method.

2.4. Data Analysis

The experimental data were analyzed and visualized using Photoshop CC2017, Excel 2010 (Microsoft Corp., Redmond, WA, USA), SPSS 19.0 (IBM), and GraphPad Prism 9 (GraphPad Software, Inc., San Diego, CA, USA). Among them, SPSS 19.0 was used for Tukey’s HSD test, and GraphPad Prism 9 was used for the normal distribution test and Peel correlation analysis.

3. Results

3.1. GEM Field Identification Results of ‘Cabernet Sauvignon’ with Different Rootstocks

We investigated the damage of GEM to ‘Cabernet Sauvignon’ grapevines with different rootstocks in the field (Figure 1). GEM was generally observed to damage the new shoots and leaves of grapevines and occurred in the new shoots and leaves of ‘Cabernet Sauvignon’ under all rootstock–scion combinations. The GEM damage rates of leaves in all rootstock combinations were compared (Table 2). The CS/3309C, CS/1103P, and CK combinations exhibited the highest incidence. Compared with the CK, there were no significant differences in the GEM resistance of 3309C and 1103P rootstocks to the ‘Cabernet Sauvignon’ grapevine, and the incidence of GEM infestation in the three combinations was much higher than that of other rootstock combinations. The incidence of the CS/5C combination was the lowest, reaching just 9%. The injury index reflects the severity of the injury. Comparing the injury index of leaves in each rootstock–scion combination identifies the CK as the most seriously damaged, and the resistance type of the variety is injury (I). The SO4, 5C, and 140R rootstocks were able to significantly improve the GEM resistance of the ‘Cabernet Sauvignon’ grapevine, and the corresponding morbidity and injury indexes were much lower than those of the CK. The SO4 and 140R varieties were determined to have injury resistance (R). The CS/5C combination had the lowest infestation degree and injury index (4.89), and the strongest resistance to GEM, with a high resistance (HR) type.

![Figure 1. GEM infection on leaves of different rootstocks of ‘Cabernet Sauvignon’ (18 August 2020).](image)
Table 2. GEM field identification results of different rootstocks of ‘Cabernet Sauvignon’ grapevine leaves. The letters above infestation rate and injury index denote significant differences (p < 0.05) attested using Tukey’s HSD test.

| Processing Combination | Infestation Rate | Injury Index | Injury Resistance Degree |
|------------------------|-----------------|--------------|-------------------------|
| CS/CS                  | 68% c           | 39.78 d      | I                       |
| CS/3309C               | 65% c           | 33.11 cd     | I                       |
| CS/1103P               | 66% c           | 34.22 d      | I                       |
| CS/SO4                 | 44% b           | 23.01 bc     | R                       |
| CS/140R                | 29% b           | 14.00 ab     | R                       |
| CS/5C                  | 9% a            | 4.89 a       | HR                      |

3.2. Effects of Different Rootstocks on Activities of SOD, PPO, CAT, and POD in Leaves of ‘Cabernet Sauvignon’ under Different Levels of GEM Injury

3.2.1. Analysis of SOD Activity in Leaves of ‘Cabernet Sauvignon’ with Different Rootstocks and GEM Injury Levels

CS/140R, CS/5C, CS/SO4, and CS/1103P can significantly increase the SOD activity of scion leaves compared with CS/CS (Figure 2). The SOD activity of the CS/5C and CS/140R combinations with different rootstock and scion combinations was higher in three injury grades. Compared with CS/CS, the combinations CS/1103P and CS/3309C were unable to significantly improve SOD activity. At the injury level of 1, the SOD activity of each rootstock–scion combination increased rapidly, with CS/CS (49.03 U/(g·min)) and CS/1103P (4.81 U/(g·min)) exhibiting the highest increase and activity (27%). With the exception of CS/CS, the activities of SOD in other rootstock and scion combinations increased again at the injury level of 2. Following this, the SOD activity of each rootstock–scion combination decreased in the third stage of injury.

Figure 2. Analysis of SOD activity in leaves of different rootstocks of a ‘Cabernet Sauvignon’ grapevine in different injury grades of GEM. Lowercase letters above the bars denote significant differences (p < 0.05) attested using Tukey’s HSD test. Error bars represent SE (n = 3).

3.2.2. Analysis of PPO Enzyme Activity in Leaves of ‘Cabernet Sauvignon’ with Different Rootstocks and GEM Injury Grades

Significant differences were observed in the PPO activity of ‘Cabernet Sauvignon’ grapevine leaves with different rootstocks (Figure 3). The PPO activity of the same rootstock–scion combination under different injury grades also exhibited differences. Each rootstock–scion combination exhibited significantly higher PPO activity than that of CS/CS in different injury grades, and the CS/5C activity was the highest among the three injury grades, followed by CS/140R. The PPO activity of leaves of the same rootstock–scion combination increased rapidly after being infested with GEM, and subsequently decreased
with the increasing injury grade. In the healthy period, the PPO enzyme activities of CS/5C and CS/140R were higher in six combinations (the enzyme activities were all over 100 U/(g·min)), and in particular, they were 128.61% and 86% higher than those of CS/CS, respectively. At the GEM injury level 1, the PPO enzyme of CS/5C was significantly higher than other rootstock–scion combinations of the same level, reaching 209.85 U/(g·min). Compared with the healthy period, the PPO activity of CS/5C and CS/140R in the six rootstock combinations at level 1 did not exceed 100% (43% and 62%, respectively), yet their enzyme activities were higher in all rootstock combinations (at 209.85 U/(g·min) and 193.17 U/(g·min), respectively). CS/SO4 exhibited the highest increase (109%), with an enzyme activity that ranked third among the six combinations in the same grade. At the injury level of 2, CS/140R, CS/5C, and CS/3309C presented the lowest decrease in enzyme activity, while those with the highest activity were CS/5C, CS/140R, and CS/309C. At the grade 3 injury level, the enzyme activities of CS/CS, CS/140R, and CS/5C decreased the least compared with those of grade 2, while the top three combinations for PPO enzyme activity in descending order were CS/5C (166.44 U/(g·min)), CS/140R (152.44 U/(g·min)), and CS/3309C (120.48 U/(g·min)).

![Figure 3. Analysis of PPO enzyme activity of different rootstocks of ‘Cabernet Sauvignon’ grapevine leaves in different injury grades of GEM. Lowercase letters above the bars denote significant differences (p < 0.05) attested using Tukey’s HSD test. Error bars represent SE (n = 3).](image)

### 3.2.3. Analysis of CAT Enzyme Activity in Leaves of ‘Cabernet Sauvignon’ with Different Rootstocks and GEM Injury Grades

Prior to the GEM injury, CS/SO4 could significantly improve CAT enzyme activity compared with CS/CS, while other rootstocks did not have any obvious effects on the CAT enzyme activity of the scion (Figure 4). However, following the infestation, the CAT activity increased greatly, with varying degrees of differences among the rootstock–scion combinations. Compared with the control, different rootstocks improved the CAT enzyme activity of the scion at varying degrees. After the infestation, the CAT activity of each rootstock–scion combination increased rapidly and subsequently decreased with the increasing injury grade. The CS/5C enzyme activity was the highest among all rootstock and scion combinations across the three injury grades. Compared with the healthy period, the CAT activity of each rootstock and scion combination increased by: (i) 81.19 U/(g·min) (22.14 times) for CS/CS; (ii) 140.97 U/(g·min) (32.75 times) for CS/3309C; (iii) 152.44 U/(g·min) (30.01 times) for CS/1103P; (iv) 206.82 U/(g·min) (47.65 times) for CS/5C; (v) 245.83 U/(g·min) (41.95 times) for CS/140R, and (vi) 245.83 U/(g·min) for CS/5C, which was the highest CAT activity.
Figure 4. Analysis of CAT enzyme activity of different rootstocks of ‘Cabernet Sauvignon’ grapevine leaves in different injury grades of GEM. Lowercase letters above the bars denote significant differences (p < 0.05) attested using Tukey’s HSD test. Error bars represent SE (n = 3).

3.2.4. Analysis of POD Enzyme Activity in Leaves of ‘Cabernet Sauvignon’ with Different Rootstocks and GEM Injury Grades

CS/SO4, CS/5C, and CS/140R were observed to affect the POD enzyme activity of scion (CS) leaves to different degrees compared with the control (Figure 5). In addition, the POD enzyme activity of CS/5C was significantly higher than that of other rootstock combinations at all levels of GEM injury, with a maximum enzyme activity during the healthy period (105.09 U/(g·min)) between groups and within groups. The POD activity in each combination decreased with the increasing injury grade. The POD enzyme activities of CS/CS, CS/3309C, and CS/1103P initially increased at the injury level of 1, and subsequently decreased as injury level increased. CS/SO4 and CS/140R did not exert any obvious changes in the POD activity at the injury level of 1 compared with the healthy period. The POD activity for these two combinations also exhibited a downward trend with the increasing injury level.

Figure 5. Analysis of POD enzyme activity of different rootstocks of ‘Cabernet Sauvignon’ grapevine leaves in different injury grades of GEM. Lowercase letters above the bars denote significant differences (p < 0.05) attested using Tukey’s HSD test. Error bars represent SE (n = 3).
3.3. Effects of Different Rootstocks on the Relative Expression of the Res and RS Genes in Leaves of ‘Cabernet Sauvignon’ Grapevine under Different GEM Injury Levels

3.3.1. Changes of Res Content in GEM Leaves of ‘Cabernet Sauvignon’ with Different Rootstocks and Injury Grades

The rootstocks increased the contents of Res and Pd in ‘Cabernet Sauvignon’ leaves by varying degrees (Figure 6). At the grade 1 infection level, the Res content in the leaves of each rootstock–scion combination increased rapidly, with increasing rates of 486% (CS/CS), 476% (CS/3309C), 422% (CS/1103P), 321% (CS/5C), 256% (CS/SO4), and 195% (CS/140R). CS/140R exhibited the highest Res content, with a value that was significantly different from other rootstock combinations, followed by CS/5C. At the injury grade of 2, the Res content of CS/SO4 increased greatly for the second time, reaching 12.83 μg/g, which is 3.60 μg/g higher than that of the first grade (by 39%), and significantly different from other rootstock and scion combinations in the same injury grade. The Res content of other combinations decreased or remained stable. The Res content of CS/3309C increased slightly at the injury grade of 3, exceeding the grade 2 value by 0.65 μg/g to reach 10.31 μg/g (increase of 7%). This was significantly different from other rootstock combinations at the same grade.

![Figure 6](image)

**Figure 6.** Changes of Res and Pd contents in GEM leaves of different rootstocks of ‘Cabernet Sauvignon’. Lowercase letters above the bars denote significant differences (p < 0.05) attested using Tukey’s HSD test. Error bars represent SE (n = 3).

The Pd content exhibited marked changes in different rootstock–scion combinations and injury grades. At the injury level of 1, the Pd content in leaves of ‘Cabernet Sauvignon’ grapevine with different rootstocks increased greatly. At grade 2, the changes in Pd content for different rootstock and scion combinations began to show variations. The CS/5C content was the highest (22.12 μg/g).

3.3.2. Expression of the RS Gene under Different GEM Injury Grades in the ‘Cabernet Sauvignon’ Leaves of Different Rootstocks

Resveratrol synthase, as the last enzyme in the pathway of Res synthesis, has a strong relationship with the content of Res. Thus, we measured the relative expression of the RS gene in different samples (Figure 7). During the healthy period, the rootstocks could increase the relative expression of the RS gene in scion leaves to different degrees, with CS/140R exerting the most significant impact. The relative expression of the RS gene in each rootstock combination increased rapidly at the injury level of 1, and CS/140R exhibited the highest (and significantly different) expression level among all rootstock combinations,
followed by CS/5C. As the injury grade increased, the relative expression of the RS gene in different rootstock–scion combinations varied. The relative expression of the RS gene in the same rootstock combination is not completely consistent with the change in Res content (cf. Figure 6).

Figure 7. Relative expression of the RS gene of GEM in different injury grades of different rootstocks of ‘Cabernet Sauvignon’ grapevine leaves. Lowercase letters above the bars denote significant differences ($p < 0.05$) attested using Tukey’s HSD test. Error bars represent SE ($n = 3$).

3.4. Correlation Analysis between Res Content, Resistance Enzyme Activity, and Injury Index of ‘Cabernet Sauvignon’ Grapevine Leaves with Different Rootstocks

Correlation analysis of the Res content, injury index and SOD, POD, PPO, and CAT activities of each rootstock–scion combination sample under injury grade 1 was performed (Figure 8). The distribution of the data was tested, and all the data combinations exhibited a skewness $<2$ and kurtosis $<8$, thus conforming to the normal distribution. The content of Res, Pd, and SOD in the leaves of each rootstock–scion combination was moderately negatively correlated with the injury index, with correlation values of $-0.66$, $-0.83$, and $-0.68$, respectively. There was a high negative correlation between POD activity and injury index ($r = -0.93$). The PPO and CAT activities were significantly negatively correlated with the injury indexes ($-0.96$ and $-0.96$, respectively), reaching significant levels. Thus, combinations with high CAT and PPO activity in scion grapevine leaves can effectively resist GEM infection.

Figure 8. Cont.
The grape is an important economic berry. In the process of studying the injury and insect resistance of grapevine rootstocks, it is indispensable to identify the injury and insect resistance of rootstocks. Field identification can accurately reflect the natural injury and insect resistance of grapevines. In this experiment, ‘Cabernet Sauvignon’ grafted with different rootstocks was investigated under the condition of artificial inoculation of GEM in the field. The incidence and injury index of the CS/5C, CS/140R, and CS/SO4 combinations caused by GEM infection were lower than those of other combinations. 5C, 140R, and SO4 rootstocks can significantly improve the GEM resistance of ‘Cabernet Sauvignon’ grapevines. Through the determination of the activity of four enzymes and the content of resveratrol in different GEM injury grades, it was confirmed that grafted rootstocks could indirectly affect the resistance of scions to GEM through secondary metabolites and enzyme activities.

Previous research shows that the activities of SOD, CAT, POD, and other enzymes are up-regulated after plants are subjected to abiotic or biotic stress [30–32]. The current study also found that the activities of four protective enzymes increased rapidly after being injured by GEM, but there were significant differences in enzyme activities at different stages of injury. The process adopted by sap-sucking pests when injuring plant leaves with probe mouthparts is similar to that of the invasion of plants by pathogens [33]. Therefore, there may be some similarities between the grapevine defense response activated by GEM invading grapevine leaves and the induced results of fungal injuries. After mites feed, leaves are damaged and reactive oxygen species (ROS) begin to accumulate. In order to prevent lipid peroxidation, SOD, CAT, and POD begin to participate in the reaction and finally convert O$_2^-$ into H$_2$O and O$_2$ [34,35]. In addition, POD can thicken the cell wall by participating in the cork and lignification of plant cells [36]. As the first barrier against pathogens, the change in the cell wall polymer can strengthen the structure of the cell wall [37]. This process can make it more difficult for gall mites to pierce cells. In addition, grapevine varieties with a thick waxy layer and high waxy content also have high resistance to GEM [38]. After infestation, plants will also increase the content of phenolic compounds in the feeding substances of gall mites, thus reducing the palatability of leaves and preventing gall mites from eating [39]. PPO can oxidize polyphenols in plants into quinones, which can inhibit the growth of pathogenic bacteria. Moreover, quinones and phenolic compounds have inhibitory, antifeedant, and toxic effects on gall mites [40]. In the current study, the PPO activity of CS/5C and CS/140R maintained a high level at all stages, which may be related to the low injury index of the two combinations. We found the CAT activity of each rootstock–scion combination to be low in the healthy period, and the enzyme activities were the
highest in the tested rootstock-scion combinations or had no significant difference with the highest combination. The strong correlation between CAT and the injury index may be a result of \( \text{H}_2\text{O}_2 \) accumulation. The activities of CAT, SOD, POD, etc., have been reported to increase significantly or more rapidly when varieties insensitive to GEM are infected by GEM [28]. This agrees with our results. In addition, we also observed the activities of POD, CAT, and PPO gradually decrease with the development of GEM infection.

Previous studies have revealed the impact of rootstocks on the Res content of ‘Cabernet Sauvignon’ [41,42]. In this study, we also determined significant differences in the Res content of healthy leaves among several rootstock-scion combinations. The content of Res in leaves of all combinations increased rapidly after being injured by GEM. The combination with the top three Res contents is considered to have a high resistance to GEM. Therefore, we speculated that the content of resveratrol in plants is related to GEM resistance. This was confirmed by the correlation analysis. Res, as a type of plant protection element with a high content in grapevine, plays an important role in the injury resistance of grapevines, and acts as a signal molecule in plant injury resistance. Res is accumulated in high concentrations in response to pathogen infection, and its derivatives are related to POD through peroxidase-mediated cell wall strengthening [17,24]. Studies have also shown that calmodulin synergistically regulates cell division and proliferation in plant tissues, for example, the root gall of Arabidopsis thaliana caused by the parasitic root nematode M. incognita infection. The root gall of a grapevine injured by grape phylloxera is related to this pathway [43,44]. Therefore, the blistering of leaves caused by gall mites may be related to calmodulin, and whether Res is involved in this process as a signal molecule requires further research. The infestation of pathogens can induce the change in antimicrobial active substances in plants, such as Erwinia carotovora, which increases the mustard oil content in Arabidopsis thaliana [45]. Fungal infection can induce the synthesis of Res in grapevines [46]. In this experiment, GEM infestation also induced a rapid increase in the Res content of leaves. The contents of Res in the CS/140R, CS/5C, and CS/SO4 combinations at a low infestation rate were all high. This indicates the influence of Res on the resistance of grapevines to GEM. Res was observed to be negatively correlated with the injury index. At present, there is no direct research evidence on the toxic effect of Res GEM, yet a series of injury-related responses mediated by Res may play a role in the resistance to GEM. The derivatives of Res, such as Pterostilbene, have a higher toxicity than Res, and may also play a toxic role in GEM. When a scion is infected with powdery mildew, the Res in the infected region is rapidly synthesized and accumulated. Res in rootstocks can be polarly transported through the phloem to the injured site of the scion [20]. Therefore, we speculate that the change in Res content in CS/3309C scion leaves is inconsistent with the change in the relative expression of the RS gene. This may be due to the upward transportation of Res in rootstock, which enhances the Res content in the scion. The relative expression of the RS gene is different among rootstock and scion combinations at the same injury level, which may also be affected by rootstocks. CS is a susceptible variety, and the Pd content in CS/SO4 and CS/5C increased rapidly when the injury grade was 2. This may be attributed to the glycosylation of Res provided by rootstocks into Pd by the scions. This implies that the source of Res in the scion leaves of each rootstock combination is not only the synthesis of the leaves themselves, but also the contribution of rootstocks. According to the changes of the relative RS expression in scion leaves with different grades of GEM injury in each rootstock combination, the rootstocks are able to regulate the expression of the RS gene through several pathways during GEM development.

5. Conclusions and Prospects

In this experiment, the GEM resistance of ‘Cabernet Sauvignon’ grafted with different rootstocks was investigated. The activities of SOD, CAT, POD, and PPO, and the content of resveratrol in grapevine leaves of different GEM injury grades were determined. The results reveal the ability of GEM to induce changes in resistance-related factors in grapevine (Cabernet Sauvignon) leaves, such as the Res and Pd contents, the relative expression of the
RS gene, and the increase in SOD, CAT, POD, and PPO enzyme activities. In the current study, the activities of the four enzymes, and the Res and Pd contents were negatively correlated with the injury index of the GEM infection. This can be used as the basis for judging the rootstock resistance to GEM. This work clarifies the injury resistance mechanism of the rootstock–scion interaction, and also provides a theoretical basis for selecting GEM-resistant rootstocks of grapevines in Xinjiang.

At present, although the varieties of grapevine resistant rootstocks are gradually increasing, the breeding and excavation of the composite resistance of rootstocks are not at the required level. This leads to limitations and selectivity in the related applications. In recent years, the incidence of grapevine injuries and insect pests in various regions has become more and more complicated, which has brought severe challenges to the sustainable development of the industry. As an organic whole, the resistance of grafted plants to injuries and pests is a result of rootstocks and scions. This offers a powerful approach to reduce the use of fertilizer and medicine, improve plant quality, and employ the resistance of rootstock varieties to injuries and pests by selecting the best rootstock–scion combination with multiple resistances for key grapevine varieties. In the follow-up study, we will combine scanning electron microscopy, transmission electron microscopy, and metabolomics to conduct in-depth research.

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