Identification of Rose Viral Species Using RT-PCR Technology

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Abstract
As an important ornamental plant, rose is severely affected by virus infections, which significantly impair its growth and ornamental value. Accurate identification of virus species is crucial for the prevention and control of viral diseases. To clarify the main virus species infecting roses in Jingmen, this study employed RT-PCR technology to identify rose viruses, providing a theoretical basis for the investigation and comprehensive prevention/control of rose viruses in Jingmen. Rose petal and leaf samples suspected of viral infection were collected from four regions in Jingmen. Rose RNA was extracted using methods such as Trizol and RNA extraction kits. Specific primers were designed for common viruses including Tobacco Mosaic Virus (TMV) and Rose Mosaic Virus 2 (RIV2), followed by RT-PCR amplification. The sample bands were detected using agarose gel electrophoresis and a gel imaging analysis system to determine the virus species. Among the 40 samples, Apple Stem Grooving Virus (ASGV), Prunus Necrotic Ringspot Virus (PNRSV), Rose Cryptic Virus 1 (RoCV1), and Rose Mosaic Virus 2 (RIV2) were detected. The results showed that RT-PCR technology can efficiently identify rose virus species; RIV2 and ASGV were found to be the main viruses infecting roses in Jingmen. This study enriches the research and investigation data for the future prevention/control of rose viral diseases and the study of their pathogens.

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Introduction

Rosa chinensis Jacq., an evergreen or semi-evergreen low shrub belonging to the Rosaceae family and *Rosa* genus, is a common ornamental plant in China and is renowned as the "Queen of Flowers" [1]. Native to China, it has a cultivation history of over 2, 000 years and is mainly distributed in the mountainous areas of Hubei, Sichuan, Gansu and other provinces in China. With a long cultivation history and profound cultural heritage, the cultivation of *Rosa chinensis* began in the Tang and Song Dynasties, flourished in the Ming and Qing Dynasties, and has developed in the modern era. It is not only widely used in cut flowers and potted plants, but also plays a significant role in the market in terms of commercial, medicinal and scientific research values [2-4]. Owing to its rich flower colors and strong environmental adaptability, *Rosa chinensis* is widely regarded as one of the core flowers in the global horticultural industry. Its bright flowers and diverse varieties make it highly popular in the horticultural market, and it is very common in urban landscapes or large nurseries. With the expansion of *Rosa chinensis* cultivation area, especially the modernization of the horticultural industry, the production and management of *Rosa chinensis* are facing more and more challenges, among which viral diseases have a particularly prominent impact on the *Rosa chinensis* industry [5, 6]. Viral infections can not only significantly reduce the ornamental value of *Rosa chinensis*, but also affect the growth, flowering period and disease resistance of the plants.

As an important horticultural plant, *Rosa chinensis* plays a key role in resource development, ecological construction and economic income generation by virtue of its rich variety resources and diversified application scenarios, and has achieved remarkable results in promoting the process of national agricultural modernization and social and economic development. Hubei is one of the important *Rosa chinensis* breeding regions in China, and *Rosa chinensis* is used as a street ornamental plant in some urban areas. However, there are no reports on *Rosa chinensis* viral diseases so far. At present, the reported *Rosa chinensis* diseases in China are mainly fungal diseases such as black spot, powdery mildew and gray mold [7-9]. Viral diseases are often ignored due to their latent nature in the early stage, resulting in relatively few studies on *Rosa chinensis* viral diseases in China, and studies on mosaic virus infecting *Rosa chinensis* are even rarer at home and abroad. In response to the urgent need for the prevention and control of viral damage in *Rosa chinensis* cultivation in Jingmen, Hubei, an economical and efficient pathogen detection technology is required. RNA extraction from local *Rosa chinensis* samples should be carried out using the traditional RNA extraction method (Trizol method) or RNA extraction kits for accurate screening, so as to quickly identify the source of viral infection and provide information for the establishment of regional prevention and control strategies in the future. The results obtained from the detection experiment will be sorted out and analyzed to provide references for the detection and prevention of *Rosa chinensis* viral diseases, enrich the investigation of viral diseases in Jingmen. At the same time, it will also lay a foundation and provide certain references for clarifying the species, distribution and molecular characteristics of *Rosa chinensis* viruses in Jingmen, as well as for the research on *Rosa chinensis* viral diseases and the prevention and control of such diseases. Furthermore, it can contribute to the in-depth study of the internal structure of viruses, pathogenic mechanisms, and the prevention and control of viral diseases in horticultural plants in the next step.

Materials and methods

Collection of Rose Samples

Samples in this experiment were collected from rose plants at four locations in Jingmen City, including Kechuang City in Zhanghe New District, Yuanbo Garden in Duodao District, the Rose Garden of Jingchu University of Technology in Dongbao District, and the area near Yufeng Fishing Ground. The collection of rose samples was conducted based on morphological observation. Priority was given to collecting roses that might be infected with mosaic virus; preliminary judgment on whether the collected roses were virus-infected was made by observing the following characteristics: whether the petals showed mottled mosaic colors [10], whether there were discolored patches on the leaf surface, and whether the leaves exhibited deformation, distortion, curling, or wrinkling. After taking photos of the roses, petals and leaves were collected, labeled with information such as collection date and sample number respectively, and then packed separately.

Extraction of Total RNA from Rose

Heat Buffer RCT in a 60 °C water bath. For two samples, add 1200 μL of Buffer RCT and 12 μL of β-mercaptoethanol into a single 2 mL centrifuge tube. Weigh 500 μL of rose petals or leaves, grind them in liquid nitrogen, and transfer 200 μL of the petal/leaf powder into a pre-cooled centrifuge tube (pre-cooled in liquid nitrogen). Promptly open the tube lid after removing it from liquid nitrogen. Add 600 μL of Buffer RCT, vortex to mix thoroughly, then add 500 μL of Buffer EX and mix vigorously. Centrifuge at 12, 000 rpm for 5 minutes (set the refrigerated centrifuge to 25 °C as per the kit instructions). Aspirate 350 μL of the supernatant and transfer it to a 1.5 mL centrifuge tube. Add 350 μL of Buffer K, pipette up and down to mix, then transfer the mixture to a filter column and centrifuge at 13, 000 rpm for 1 minute. Discard the filter column. Add 700 μL of 70% ethanol to the centrifuge tube, pipette to mix, then aspirate 700 μL of the mixture and transfer it to a purification column. Centrifuge at 13, 000 rpm for 1 minute, discard the filtrate, and aspirate the remaining mixture into the same purification column. Centrifuge again at 13, 000 rpm for 1 minute. Discard the filtrate, add 500 μL of Buffer WA to the purification column, and centrifuge at 13, 000 rpm for 1 minute. For the two samples, take 10 μL of enzyme and 90 μL of RDD, pipette gently to mix. Add 50 μL of this mixture to the center of the purification column membrane, then centrifuge at 13, 000 rpm for 1 minute. Discard the filtrate, add 600 μL of Buffer WBR to the purification column, and centrifuge at 13, 000 rpm for 1 minute. Discard the filtrate and centrifuge the purification column at 14, 000 rpm for 1 minute (to remove residual liquid). Discard the centrifuge tube, place the purification column into a new 1.5 mL centrifuge tube, add RNase-free water to the center of the membrane, let it stand for 1 minute, and then centrifuge at 13, 000 rpm for 1 minute, Obtaining RNA Samples from Roses.

Identification of Rose Virus Species by RT-PCR

To design virus-specific primers, first retrieve the target virus's gene sequence from the NCBI website, then use a primer-design platform to develop a pair of specific primers, adhering to fundamental design principles: primers should typically be 18-25 base pairs (bp) in length, have a guanine-cytosine (GC) content between 40% and 60%, avoid consecutive G or C residues at the 3' end as well as self-complementary sequences and hairpin structures (to prevent primer dimer formation), and have a melting temperature (Tm) ranging from 55 ℃ to 65 ℃. Additionally, refer to literature to identify primer sequences that have already been successfully validated for virus detection, which can be kept as backups [5]. Once primer design is completed, submit the sequences to a professional biotechnology company for synthesis; synthesized primers are provided in lyophilized powder form. Upon receiving the primers, centrifuge the

primer tubes at 5000 revolutions per minute (rpm) for 1 minute, then dissolve the lyophilized primers in an appropriate volume of sterile water to achieve the required concentration, and store the dissolved primer stock solution in a -20 ℃ refrigerator for later use. To prepare the working solution, transfer 10 μL of the stock solution and 90 μL of sterile water into a 1.5 mL centrifuge tube, and mix the solution thoroughly by pipetting up and down.

Preparation of the PCR Reaction System: To prepare a 10 μL PCR reaction system, sequentially add the following reagents to a 1.5 mL centrifuge tube on ice: 5 μL of 2*One Step Mix, 0.5 μL of upstream primer, 0.5 μL of downstream primer, 0.5 μL of One Step Enzyme Mix, and 2.5 μL of sterile deionized water. Alternatively, to prepare another 10 μL PCR reaction system, sequentially add 9 μL of the working solution and 1 μL of the extracted rose RNA solution to a PCR tube.

PCR Amplification Reaction: After gently mixing the prepared PCR reaction system, perform a brief centrifugation to concentrate the reaction solution at the bottom of the PCR tube. Then place the PCR tube into a PCR gene amplification instrument and conduct the PCR amplification reaction according to the preset reaction program: reverse transcription at 50 ℃ for 30 min; pre-denaturation at 94 ℃ for 3 min; denaturation at 94 ℃ for 30 s; annealing at 58 ℃ for 30 s; extension at 72 ℃ for 5 min (repeated for 35 cycles); and final extension at 72 ℃ for 7 min. Once the PCR amplification program is terminated, remove the reaction tube from the PCR gene amplification instrument within 10 minutes, and analyze the amplification products by electrophoresis using a 10% agarose gel.

Agarose Gel Electrophoresis for PCR Product Detection: Prepare an agarose gel with a concentration of approximately 10% for the detection of PCR products. Take 8 μL of the PCR product, then use a pipette to sequentially load 5 μL of Marker and the PCR product into the sample wells of the gel. After sample loading is completed, cover the electrophoresis tank, connect the power supply, set the electrophoresis parameters to 120 V, 80 mA, and 60 minutes, and start the electrophoresis. Once electrophoresis is finished, turn off the power, take out the gel, place it in a gel imaging system, and perform imaging under ultraviolet (UV) light excitation to observe the distribution of PCR product bands. If a specific band consistent with the expected size appears on the gel, it indicates that the nucleic acid of the target virus has been detected in the rose sample; if no specific band appears, it may be because the target virus is absent from the sample.

Results

Investigation of Virus Species Infecting Roses

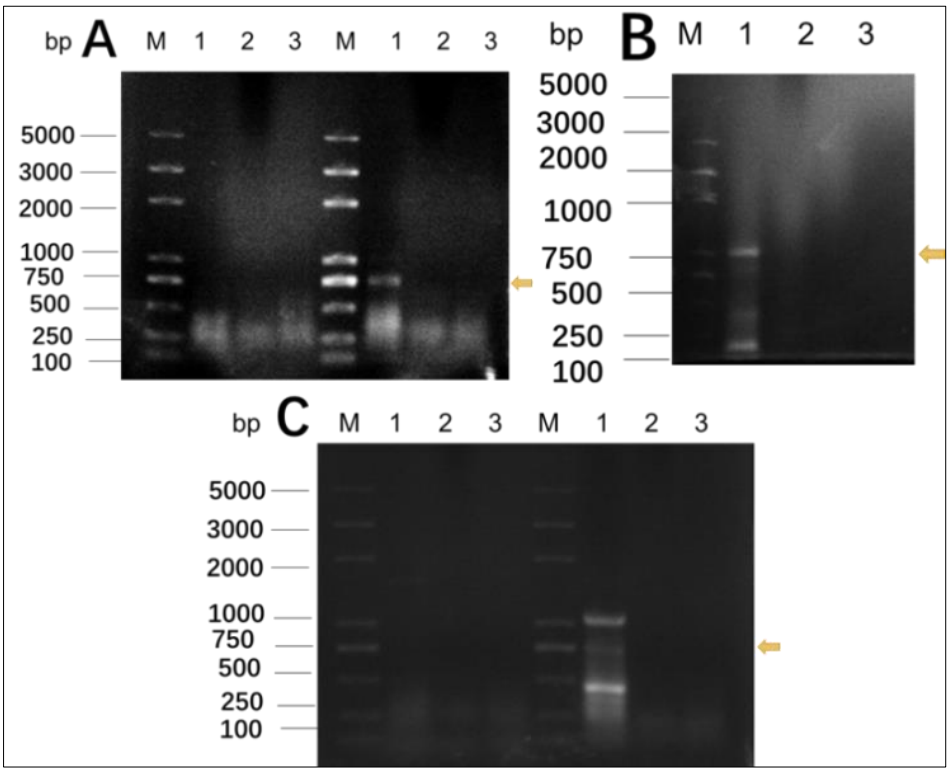
In this study, rose plants exhibiting typical phenotypic symptoms of petal mottling and mosaic (a hallmark of viral infection) were selected for sampling. These plants were hypothesized to be infected with Rose Mosaic Virus, Cucumber Mosaic Virus, and Tobacco Mosaic Virus. Additionally, common viruses with inconspicuous infection symptoms-including Prunus Necrotic Ringspot Virus, Apple Stem Grooving Virus, Rose Bushy Stunt Virus, Rose Spring Dwarf Virus, and Rose Cryptic Virus—were also targeted. Specific primers were designed based on the aforementioned hypothesized viruses to conduct detection.

Note: The first row shows samples 1-10 from Yufeng Fishing Ground; the second row shows samples 1-10 from the Rose Garden of Jingchu University of Technology; the third row shows samples 1-10 picked from Jingchu Science and Technology Innovation Park.

Fig 1: Phenotypes of rose samples

Identification of Rose Virus Species

In the experiment, Trizol reagent lysis combined with isopropanol precipitation method and RNA purification kit were used for the initial extraction of rose RNA. Subsequently, through PCR amplification and gel electrophoresis detection, four common rose viruses were detected, including Apple Stem Grooving Virus (ASGV), Prunus Necrotic Ringspot Virus (PNRSV), Rose Cryptic Virus 1 (RoCV1), and Rose Mosaic Virus 2 (RIV2).

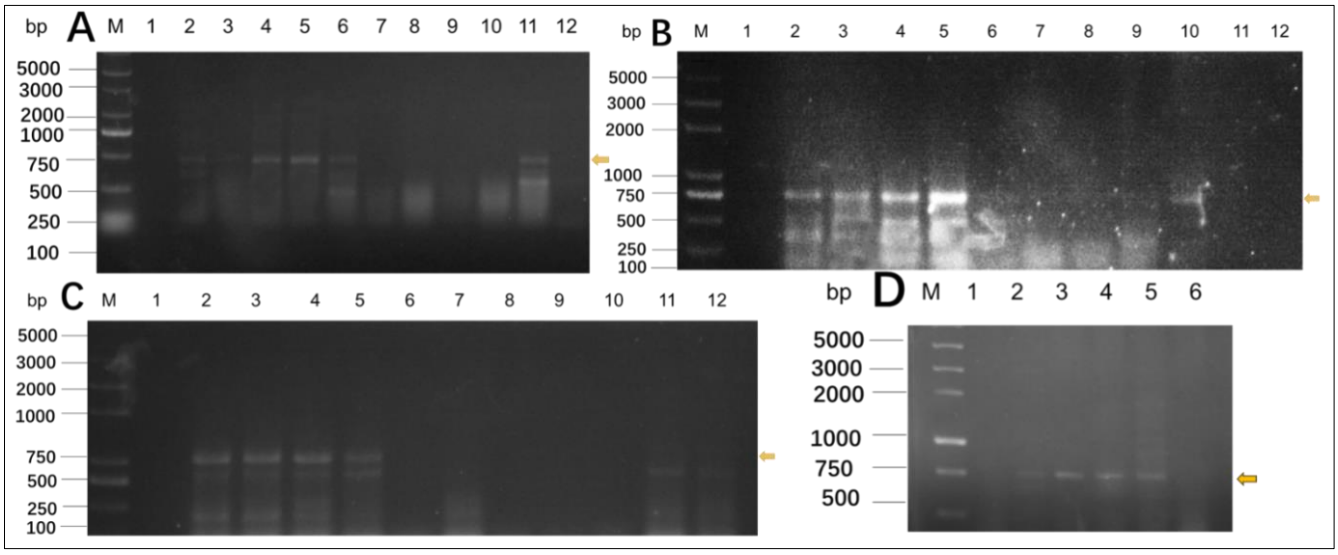


Note: A represents the mixed sample detection result of RIV2, B represents the mixed sample detection result of ASGV, and C represents the mixed sample detection result of RNRSV and RoCV1.

Fig 2: Mixed detection results of suspected rose viruses

Statistics on Rose Virus Carrier Rate

For the virus species detected in Figure 2, a separate detection method was further adopted to determine the virus detection rate. Based on the results of multiplex RT-PCR, specific viral bands were displayed in the gel electrophoresis images after PCR amplification using virus-specific primers for individual viruses.



Note: Figure 3A shows the detection result of RoCV1; Figure 3B shows the detection result of RIV2; Figure 3C shows the detection result of ASGV; Figure 3D shows the detection result of PNRSV. All Lane 1 is the negative control, and Lane 2 is the positive control.

Fig 3: Detection results of rose viruses

Discussion

This study employed RT-PCR technique to detect the species of rose viruses in the Jingmen area, which exhibited high specificity and sensitivity. The results indicated that the species of rose viruses are relatively diverse, and different viruses display distinct symptomatic manifestations. Apple Stem Grooving Virus (ASGV), Prunus Necrotic Ringspot Virus (PNRSV), Rose Cryptic Virus 1 (RoCV1), and Rose Mosaic Virus 2 (RIV2) were identified as the major viruses in this study. The analysis of these viruses can provide crucial information for the prevention and control of rose viruses.

RIV2 is one of the primary viruses causing color-breaking symptoms on rose petals [5]. Its infection significantly alters the color of rose petals, leading to mottling and color-breaking phenomena. This not only impairs the ornamental value of roses but also may affect their market economic

benefits. According to literature, the main transmission route of RIV2 is via insects such as aphids. Therefore, in rose cultivation and management, emphasis should be placed on the prevention and control of vector insects like aphids and thrips [11]. The combination of physical isolation using insect-proof nets and precise application of biological insecticides can significantly inhibit the field transmission of rose mottle viruses. Additionally, the high incidence of RIV2 suggests that future efforts should focus on developing control strategies for this virus, particularly through genetic engineering approaches or antiviral chemicals to curb its spread.

Unlike RIV2, ASGV primarily causes stem grooving lesions and plant dwarfing in rose plants. Although the symptoms of ASGV on rose petals are not obvious, its impact on plant growth cannot be ignored. ASGV is typically transmitted through grafting and can also spread via pollen and seeds, posing a high transmission risk during rose cultivation. In response to this, future research may consider developing early diagnostic methods for ASGV, especially strengthening virus detection in seedlings during the planting process to reduce its transmission in the early stages of cultivation.

Furthermore, PNRSV and RoCV1 belong to cryptic infection viruses [5]. After infection, they often do not exhibit obvious external symptoms, making early identification of the viruses more challenging. The cryptic nature of PNRSV and RoCV1 necessitates regular molecular detection during large-scale rose cultivation, rather than relying solely on visual observation of symptoms to determine virus infection. This finding highlights the importance of molecular detection methods, particularly the RT-PCR technique [12]. Through systematic regular screening, the species of viruses infecting roses can be effectively identified, enabling the early implementation of isolation and control measures and significantly reducing the risk of virus transmission. Regular detection allows for the early detection of cryptic infections, facilitating the timely adoption of prevention and control measures to prevent further spread of the viruses.

This study also found that mixed virus infections are relatively common in rose plants. In particular, the co-infection of PNRSV and RIV2 may exacerbate color variation in petals [6], resulting in more severe color-breaking. This phenomenon indicates that when preventing and controlling rose viruses, in addition to focusing on the transmission of single viruses, greater attention should be paid to the monitoring and control of mixed infections [13-15]. Future research can explore the interaction mechanisms between different viruses and how to effectively prevent the occurrence of mixed infections.

In terms of experimental methods, this study selected RNA extraction methods, namely the RNA purification kit. Experimental data showed that the RNA extracted using the RNA purification kit had higher purity [16-18], and clearer specific bands could be obtained in subsequent PCR amplification. This finding provides a strong basis for the selection of RNA extraction methods in rose virus detection.

In conclusion, this study provides important technical support for the identification of rose virus species, and the in-depth research on RIV2 in particular offers a scientific basis for the prevention and control of rose viruses. Future research can further expand the scope of virus species detection, strengthen the exploration of virus transmission routes and pathogenic mechanisms, and thus provide more comprehensive and effective strategies for rose disease prevention and control [19, 20]. Meanwhile, with the continuous advancement of technology, the early diagnosis and control of viruses will become more efficient, providing a more solid guarantee for the development of the rose industry.

Conclusion

In this study, RT-PCR technique was employed for the molecular detection of rose viruses in the Jingmen area. Four viruses were successfully identified, including Apple Stem Grooving Virus (ASGV), Prunus Necrotic Ringspot Virus (PNRSV), Rose Cryptic Virus 1 (RoCV1), and Rose Mosaic Virus 2 (RIV2).

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References

1. Wang Q, Cao X. The Queen of Flowers: The Past and Present of the Rose. Mod Hortic. 2024;2:159-63.
2. Gu RF, Bai J, Sun JW, *et al*. Effects of Cardboard Box Ventilation Hole Size During Forced-Air Precooling on Postharvest Quality and Physiological Properties in Cut Roses. Horticulturae. 2025;11:959.
3. Fu TR, Zhang YH, Cui YY, Luo P. Cloning and Functional Analysis of the RhMYB23 Gene in Rose. J Agric Biotechnol. 2025;33(8):1674-82.
4. Yuan ZH. Identification and Molecular Characterization of Lily and Rose Viruses [master's thesis]. [Place unknown]: [University unknown]; 2023.
5. Chen TC, Lin YC, Lin CC, *et al*. Rose Virome Analysis and Identification of a Novel Ilarvirus in Taiwan. Viruses. 2022;14(11):2537.
6. Zhang XQ, Liu SY, Yang YZ, *et al*. Virus Identification and Detection by High-Throughput Sequencing and RT-PCR from Rose Plants in Beijing. Acta Phytopathol Sin. 2021;51(4):525-35.
7. Xu Y, Cheng ZY, Lv HY. Common Rose Diseases and Their Control. J Henan Agric Sci. 2011;6.
8. Qing L. Occurrence and Control of Common Rose Diseases. Sichuan Agric Sci Technol. 2004;6.
9. Liu WR, Wang GQ. Occurrence and Treatment of Common Diseases in Ornamental Roses. Agric Sci Technol Equip. 2021;304(4):15-7.
10. Zhang XQ, Nie ZY, Li ML. Prokaryotic Expression and Antiserum Preparation of Coat Protein of *Rosa* Rugosa Leaf Distortion Virus. Plant Prot. 2024;50(4):230-4.
11. Zhao WH. Identification and Control of Rose Diseases and Pests. In: Proceedings of the 27th Academic Symposium of the Plant Conservation Committee, Chinese Society of Landscape Architecture; 2018.
12. Zhang LY, Wang S, Chen DL, *et al*. Establishment of a Multiplex RT-PCR System for Detection of Five Viruses/Viroids in Chrysanthemum morifolium. Chin J Virol. 2023;39(4):1045-52.
13. Li ZF, Yuan ZH, Lu YX, *et al*. Establishment and Application of Reverse Transcription Loop-mediated Isothermal Amplification for the Detection of Three Viruses Infecting *Rosa Chinensis*. J Shanxi Agric Univ. 2025;45(1):1.
14. Wang JH, Wang LH, Yang XM, *et al*. Detection and Sequencing of CP Gene of Prunus Necrotic Ring Spot Virus in Rose. Acta Phytopathol Sin. 2007;37(1):99-101.

15. Zhang XQ, Nie ZY, Li ML, *et al*. Identification and Full Genome Sequence Analysis of a Novel Carlavirus Infecting Rose. Acta Phytopathol Sin. 2021;52(4):547-54.
16. Jiang CH, Xu JY, Shi JL, *et al*. Methods Study on Extracting DNA and Total RNA from China Rose. J Anhui Agric Sci. 2008;36(21):8934-5.
17. Cao JP, Li HM, Wang WQ, *et al*. Extraction Methods of Total RNA from Some Cut Flowers. J Zhongkai Univ Agric Eng. 2011;4(1):5-9.
18. Xie JR, Cheng ZQ, Huang XQ, *et al*. Methods of Extracting Total RNA from *Rosa* Hybrida Corolla Tissue. J Yunnan Agric Univ. 2007;4(1):480-4.
19. Wu PY, Zhang YT, Wang Y, *et al*. Isolation and Identification of Pathogenic Fungi from Roses and Screening of Antagonistic Bacteria. North Hortic. 2025;11:127-39.
20. Sun LC, Yan XK, Lan YB, *et al*. Research on a Method for Identification of Chinese Rose Leaf Pests and Diseases Based on a Lightweight CR-YOLO Model. Plant Dis. 2025. doi:10.1094/PDIS-03-25-0668-RE.