Article

Prokaryotic Expression of Coat Protein Gene of Grapevine Berry Inner Necrosis Virus and Preparation of Its Polyclonal Antibody

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Abstract: Grapevine berry inner necrosis virus (GINV) and grapevine Pinot gris virus (GPGV) are prevalent viral diseases affecting viticulture, posing significant threats in grape-producing regions of China. Previous studies have emphasized the harmful effects of grape viruses on the grape industry all over the world. However, few reports have focused specifically on GINV. In wild grapevines, GINV infection frequently leads to grapevine fanleaf degeneration disease (GFDD). GINV often co-occurs with other grape viruses, exacerbating its harmful effects on the grapevine industry in China. In this study, we collected grapevine samples from Taizhou city, Jiangsu Province, where GINV infection was confirmed. Based on the GINV coat protein (*CP*) gene, we developed a high-throughput and high-sensitivity direct antigen-coated ELISA and Dot blot assay for field diagnosis of GINV CP in grape samples. The *CP* gene was cloned from GINV-infected grape samples, and the GINV CP was expressed using the pET30(a) vector. Specific polyclonal antiserum CP^{GINV} was generated by immunizing rabbits with the purified protein, and its sensitivity was determined to be satisfactory. Leveraging the high accuracy and sensitivity of the CP^{GINV} antiserum, we developed a rapid, precise, and scalable diagnostic method for GINV in the grapevine industry. The established ELISA and Dot blot assays successfully detected GINV-infected grapevine samples. The occurrence of GINV is relatively common in China, which poses a risk of transmission and threatens the healthy development of the grape industry. Therefore, this study prepared CP^{GINV} antiserum and established an efficient, rapid, sensitive, accurate, and high-throughput diagnostic method, providing a foundational approach for the prevention and control of vitis viral diseases.

Keywords: grapevine berry inner necrosis virus; coat protein; prokaryotic; antiserum

Citation: Deng, X.; Wang, Z.; Wang, N.; Zhao, H.; Qin, L.; Jiang, R.; Yuan, M.; Chen, X.; He, Z. Prokaryotic Expression of Coat Protein Gene of Grapevine Berry Inner Necrosis Virus and Preparation of Its Polyclonal Antibody. *Agronomy* **2024**, *14*, 2594. https://doi.org/10.3390/agronomy14112594

Academic Editor: Wen-Shi Tsai

Received: 30 September 2024
Revised: 28 October 2024
Accepted: 29 October 2024
Published: 4 November 2024

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1. Introduction

Grapes are an important cash crop globally, cultivated extensively in various regions, with significant planting areas and high yields in China. Grapevine fanleaf degeneration disease (GFDD) is a prevalent viral disease affecting viticulture, particularly in grape-producing regions of China, where it poses serious threats to grape production. Infected leaves exhibit symptoms such as yellowing, chlorosis, mosaic patterns, and ring spots, while the plants also suffer from xylem depression [1]. Studies have identified several common viral pathogens in grapes, including grapevine rupestris stem pitting-associated virus (GRSPaV) [2], grapevine Pinot gris virus (GPGV) [3], grapevine virus A (GVA) [4], grapevine fanleaf virus (GFLV) [5], and grapevine berry inner necrosis virus (GINV) [6]. Furthermore, GINV can interact with other viruses to form complex infections [7]. The lack of relevant fundamental research and effective control measures for infected grapevines significantly undermines fruit quality and hinders the healthy development of the grapevine industry [8].

GINV is a member of the *Trichovirus* genus in the *Betaflexiviridae* family, characterized by a genome containing three open reading frames (ORFs) that encode the replicase, movement protein (MP), and coat protein (CP). GINV was initially discovered in Japan [9] and was first reported in China in 2016 [10]. Current research on GINV primarily focuses on virus detection and genetic variation, mainly using RT-PCR based on disease symptoms [11]. However, the RT-PCR method necessitates the isolation of total RNA from infected plant tissue and requires specialized reagents and equipment for extraction [12]. Furthermore, PCR-based methods are not suitable for processing large numbers of samples, particularly in on-site virus detection procedures [13]. Serological methods, also known as immunological techniques, rely on the specific binding of antigens to antibodies for detecting plant viruses [14,15]. The key advantages of these methods include high sensitivity, accuracy, ease of use, and straightforward observation [16]. Commonly used techniques include enzyme-linked immunosorbent assay (ELISA), dot immunobinding assay (DIBA), electrochemical enzyme-linked immunosorbent assay (ECEIA), immunofluorescence (including rapid immunofiltration paper analysis, RIPA), and immunocolloidal gold technology (ICG).

In recent years, the prokaryotic expression of fruit tree viruses and the preparation of antisera have become widely adopted [17]. However, there are currently no reports regarding the preparation of antiserum or the effective detection of GINV. In this study, the *CP* gene of GINV was amplified via RT-PCR, cloned, and expressed in *Escherichia coli*. The recombinant CP was purified and used to produce rabbit polyclonal antiserum. We established standard, sensitive, and high-throughput detection methods, including Western blot, Dot blot, and ELISA. Consequently, this study developed a routine serological detection system for GINV, which is of significant importance for the diagnosis and control of viral diseases in grape production.

2. Materials and Methods

2.1. Source of Plant Material

In 2023, we collected grapevine leaves from suspected diseased plants in vineyards located in Taizhou city, Jiangsu, China. The observed symptoms included leaf chlorosis and intra-stem necrosis. The infection of grapevines with GINV was confirmed through RT-PCR analysis.

2.2. Prokaryotic Expression and Purification of CP^{GINV} Protein

The coding sequence of *CP* gene was amplified from total RNA extracted from GINV-infected grape leaf tissue by RT-PCR using GINV-*CP*-F-EcoRI (5′-gatatcggatccgaatt catgtcaattcgtcaggaactgag-3′) and GINV-*CP*-R-XhoI (5′-ctcgagtgcggccgcaagcttttacatagtaaaag caccctcgc-3′). The amplified PCR product was inserted into the same site of the vector pET30(a), and the construct was then transformed into the *E. coli* BL21 (DE3) strain. The BL21 cells carrying pET30(a)-GINV CP were cultured in 4 mL LB liquid medium containing 50 µg/mL kanamycin at 37 °C for 8 h. The 8 h bacterial solution was then added to 500 mL of LB liquid medium containing 50 µg/mL kanamycin. After 5 h culture in an 18 °C, 200 rpm incubator (Shanghai Minquan Instrument Co., Ltd., Shanghai, China), 0.1 mM IPTG (Sangon Biotech, Shanghai, China) was added for induction, and the culture was continued under this condition for 8 h. The bacterial culture was centrifuged, and the pellet was resuspended in protein purification buffer (20 mM Tris-HCl, pH 10.3, 500 mM NaCl, 10% glycerol, 1 mM PMSF). An ultrasonic homogenizer (Thermo Fisher Scientific, Waltham, MA, USA) was used to disrupt the bacterial cells. Ultrasonic breaking time 3 s, interval 2 s, working times 90, voltage 300 V, repeated 3 times. And the resulting lysate was centrifuged to obtain the clarified supernatant. The supernatants were then incubated with Ni-NTA Sefinose™ Resin (Sangon Biotech, Shanghai, China) in a glass column, which was subsequently eluted with a stepwise increase in imidazole concentration. The eluted recombinant GINV CP was concentrated using an Ultra-15 filter unit (Millipore, Burlington, MA, USA) and was prepared for immunization of rabbits.

2.3. Production of the Polyclonal Antibody Against the His-CP^{GINV} Protein

Two New Zealand white rabbits were immunized with high-concentration recombinant His-CP^{GINV} as antigen. Healthy New Zealand white rabbits with a body weight of about 2.5 kg, glossy coat color, and free movement were selected. After selection, prebreed for 2 weeks to prevent unqualified animals. Rabbits were labeled and antigens were extracted. The concentration of the first immune antigen was 1 mg/mL, and the concentration of one rabbit was 0.5 mL. Half the amount of antigen from two to four. The adjuvant (Hangzhou HuaAn Biotechnology Co., Ltd., Hangzhou, China) was extracted, and the adjuvant and antigen were extracted at a 1:1 volume ratio. Complete adjuvants are utilized in the first phase, while incomplete adjuvants are employed in the second through fourth phases. During extraction, the adjuvant must be thoroughly mixed and evenly drawn into the syringe. The two syringes were fully emulsified after being connected with the syringe coupling tubes. The emulsification standard was that a well-emulsified immunogen should not disperse when dropped into water at 37 °C. Rabbits were injected subcutaneously at multiple sites, with each injection containing 0.2 mL. The second exemption is performed on the 14th day after the first exemption, and the interval between the second exemption and the third exemption is 7 days. A small serum sample was taken from the middle ear artery of rabbits on the 7th day after the triple exemption, and the test was qualified, and the whole blood could be collected 7 days after the addition and exemption. After venous blood collection, antibody titers were measured. The crude antiserum was obtained by centrifugation at 15-day post-immunization (DPI) intervals. The crude polyclonal antibodies were precipitated and purified with sodium sulfate (20%). After dialysis and a series of purification procedures, PAb-CP^{GINV} with high purity was successfully obtained.

2.4. RT-PCR and Western Blot Analysis

Total RNA from infected leaves was extracted by TRIzol reagent (TaKaRa, Dalian, China), and isolated RNA was generated by reverse transcription using the Takara PrimeScript RT kit (TaKaRa, Dalian, China) [18] through GINV *CP*-R (5′-ttacatagtaaaagcaccctcgc-3′). Primers GINV *CP*-F (5′-atgtcaattcgtcaggaactgag-3′) and GINV *CP*-R were used for GINV specificity detection.

The sample is ground into powder with a grinding machine (Shanghai Jingxin Industrial Development Co., Ltd., Shanghai, China). The sample was mixed with 2× SDS-PAGE loading buffer and bathed in boiling water for 10 min. The sample was put into a centrifuge (Hunan Kecheng Instrument Equipment Co., Ltd., Changsha, China) at 12,000 rpm and centrifuged for 10 min. SDS-PAGE is performed on 12.5% polyacrylamide gel. Then transferred to polyvinylidene fluoride (PVDF) membrane (Pall Corporation, Port Washington, NY, USA). PVDF membrane was placed into 10 mL of 1× TBST (20 mM Tris-HCl, 150 mM NaCl, 0.05% Tween-20, and pH 7.6) buffer solution dissolved in 0.5 g non-fat dried milk (Coolaber, Beijing, China) and sealed for 2 h. The prepared antiserum was added as primary antibody. Incubate in a shaker at room temperature (120 $r \cdot min^{-1}$) for 90 min. Wash with 1× TSBT buffer for 3 times, 10 min each time. Then AP-conjugated Goat Anti-Rabbit IgG (Sangon Biotech, Shanghai, China) was used as the second antibody. Incubate in a shaker at room temperature (120 $r \cdot min^{-1}$) for 90 min. Wash with 1× TSBT buffer for 3 times, 10 min each time. Finally, 10 mL alkaline phosphatase developing buffer (1.5 M Tris-HCl, 1 M $MgCl_2$, 5 M NaCl) was added, along with 66 µL Nitrotetrazolium Blue chloride (NBT) (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) and 33 µL 5-Bromo-4-Chloro-3-Indolyl Phosphate (BCIP) (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). After mixing, pour into the color developing box and develop the PVDF film at room temperature for 10 min away from light, scan, and save the picture. Coomassie Blue Staining Solution R250 (Coomassie Blue R250 0.25 g, methanol 45 mL, acetic acid 10 mL, ddH_2O supplemented to 100 mL) and Coomassie Blue Staining Destaining Solution (methanol 250 mL, acetic acid 80 mL, ddH_2O supplemented to 1000 mL) showed protein bands.

2.5. Dot Blot, ELISA Quantification, and Data Analysis

Fresh leaves, frozen in liquid nitrogen, were ground into a powder and dissolved in two equal volumes (*m*/*v*) of 0.02 M PBS buffer (comprising 0.02 M NaCl, 0.02 M KH_2PO_4, 0.02 M Na_2HPO_4, and 0.02 M KCl) for Dot blot analysis. A volume of 3 µL of the sample supernatant was applied to the center of each square on the nitrocellulose (NC) membrane and allowed to air dry. After sealing, the first antibody was added. The prepared antibody was diluted to various concentrations. The following steps are the same as those for Western blot.

For the ELISA assay, we first prepared 0.02 M PBST (0.02 M PBS and 0.05% Tween-20). The antigen was diluted to 1 µg/mL with coated buffer (0.01 M PBST and 5% non-fat dried milk). An amount of 50 µL antigen was added to each reaction hole, and the solution in the hole was discarded and washed once with 1× PBST buffer. Each well was enclosed with 150 µL 1% BSA (PBST) and incubated at 37 °C for 1 h. Then pour out the sealing liquid. Add 50 µL diluted sample to be tested. Positive and negative controls were set. Incubate at 37 °C for 1 h, then discard the solution and wash with 1× PBST washing buffer for 3 times, 20 s each time. 0.02% horseradish peroxidase (HRP) conjugate against rabbit immunoglobulin (HRP-A) (Sangon Biotech, Shanghai, China) as a secondary antibody. An amount of 50 µL was added to each well and incubated at 37 °C for 45 min. After abandoning the sealing liquid, wash with 1× PBST washing buffer for 3 times, 20 s each time. The temporary TMB substrate solution (42 mM TMB and 20% anhydrous ethanol) was prepared. The HRP substrate chromogenic solution (56 mM citric acid solution, 88 mM Na_2HPO_4, 1% TMB substrate solution, 0.1% H_2O_2) was added in 50 µL and reacted at 37 °C for 5 min. The reaction was terminated by adding 1 M sulfuric acid in 50 µL. Using an ELISA reader (BioTek Instruments, Murrieta, CA, USA), the results of the absorption value at OD_{450} were recorded, and the color change in the well was noted [19,20].

The ImageJ2 software was used to quantify the color in NC membrane [21]. The experimental group repeated three times, and the average value was taken as the measurement result. Difference significance analysis was performed using GraphPad Prism version 8 [22].

3. Results

3.1. Cloning, Evaluation, and Purification of the GINV CP Gene

The coding sequence of GINV *CP* was cloned from infected grapevine samples collected from Taizhou, Jiangsu (Figure 1A). The recombinant expression plasmid pET30(a)-CP^{GINV} was successfully constructed through RT-PCR, double enzyme digestion, and sequencing. The plasmid was then transferred to *E. coli* BL21 for protein expression and purification. Following a small-scale induction with 1 mM IPTG, the results showed specific bands of the expected size, approximately 27 kDa, on the nitrocellulose (NC) membrane, indicating that the target recombinant protein His-CP^{GINV} was successfully expressed (Figure 1B). Since the expression level of the target protein in sample 2# was relatively high after IPTG induction, we selected sample 2# for subsequent large-scale expression and purification. Gradient elution tests were conducted with imidazole concentrations ranging from 60 mM to 400 mM during large-scale protein expression. The maximum elution efficiency of the target protein was achieved at 80 mM, which met the requirements for polyclonal antiserum preparation (Figure 1C). The target protein eluted at this concentration was selected for subsequent experiments.

3.2. Evaluation of Prepared Anti-Body Titer for CP^{GINV}

The protein of interest eluted with 80 mM imidazole was concentrated using a protein ultrafiltration centrifuge tube, and the resulting protein concentrate was used to immunize New Zealand white rabbits. The purified His-CP^{GINV} was obtained from the affinity column, followed by dialysis, and was analyzed using SDS-PAGE. The analysis revealed that the target protein exhibited a significantly high concentration and achieved the expected level of purity (Figure 2A). The titer of the prepared His-CP^{GINV} antiserum was determined using ELISA, and the results demonstrated a consistently high titer, even when diluted

64,000-fold, as indicated by an OD_{450} reading above 0.6 (Figure 2B, red line). Additionally, the target antiserum was diluted in a series of ratios (1:10, 1:100, 1:1000, 1:10,000, 1:100,000, and 1:1,000,000) for Dot blot analysis, revealing a clear color response even at a dilution of 1:100,000 (Figure 2C). Furthermore, we incrementally increased the dilution of His-CP^{GINV} (10^3, 5×10^3, 10^4, 2×10^4, and 4×10^4) and analyzed the specificity of the target antibody using Western blot. The results showed that even when the antigen (His-CP^{GINV}) was diluted 40,000-fold and the antiserum was diluted 10,000-fold, a specific band of the expected size was still detectable (Figure 2D). These findings suggested that the His-CP^{GINV} antiserum exhibited a high titer and had the potential to detect GINV-infected specimens. In conclusion, the results from ELISA, Dot blot, and Western blot assays demonstrated that the prepared His-CP^{GINV} antiserum is of high quality and suitable for the immune detection of GINV.

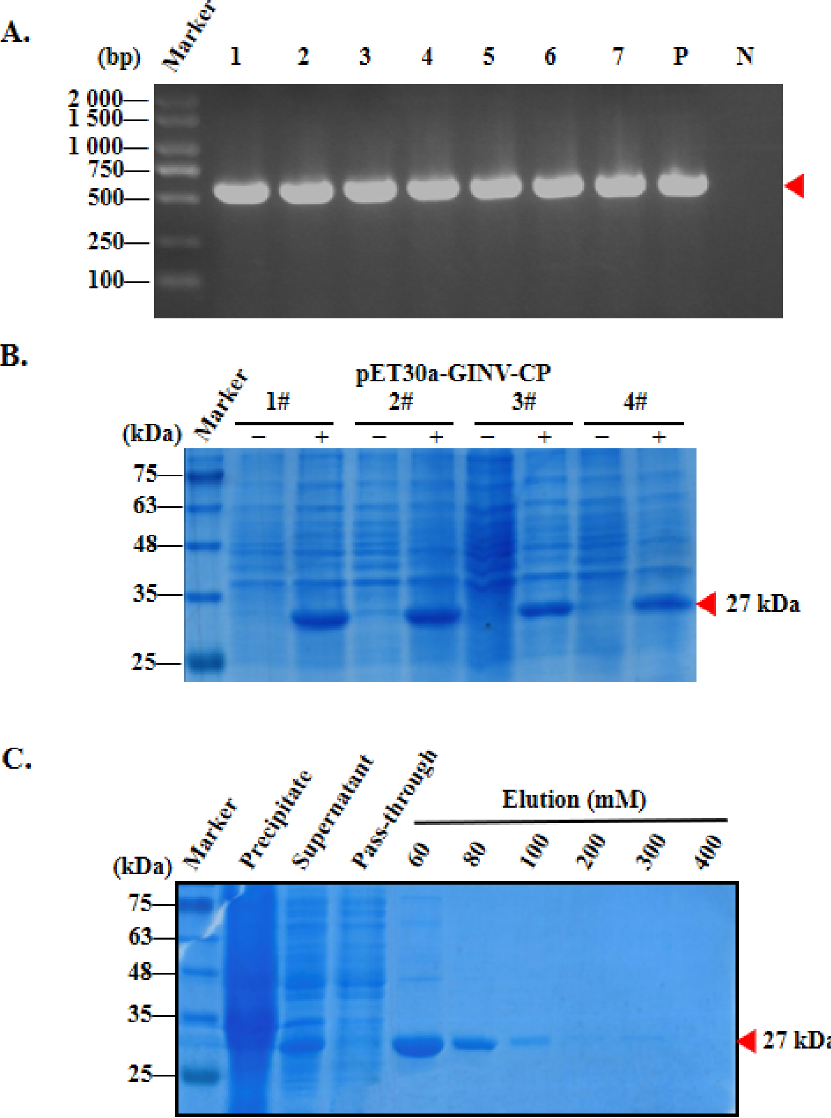
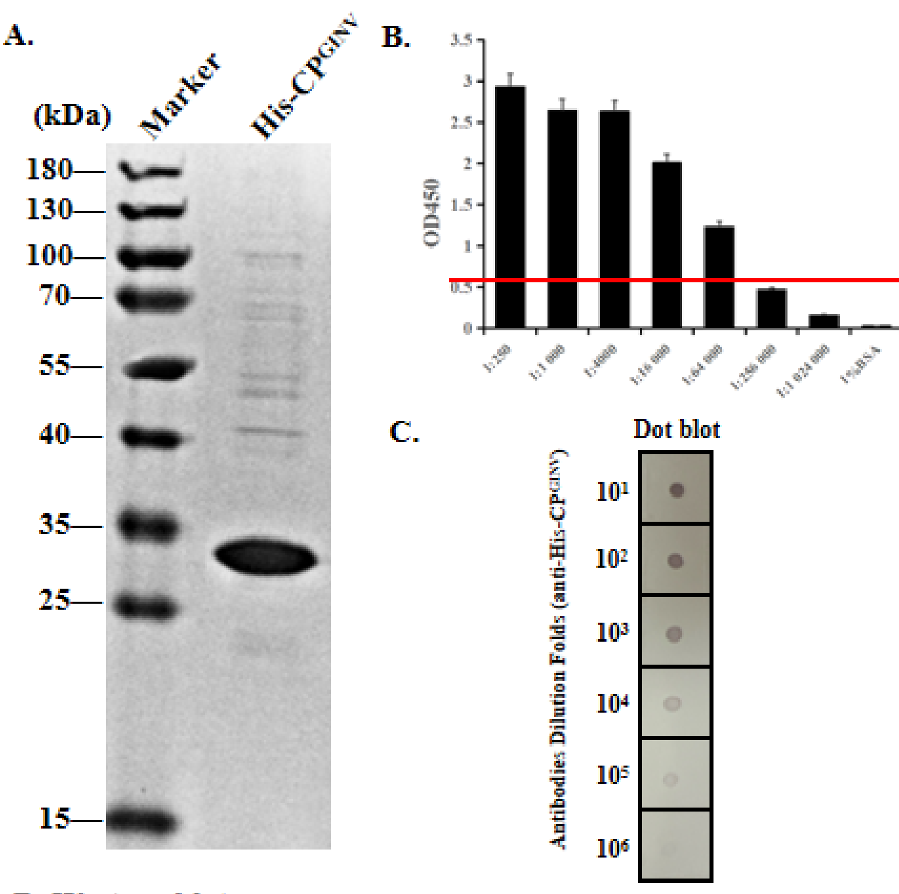


Figure 1. Prokaryotic expression and purification of the GINV CP from *E. coli.* (**A**) Agarose-gel analysis of the amplification products by RT-PCR from GINV-infected grapevine. Numbers 1–7 represent the 627 bp GINV *CP* coding region sequence in which its position is indicated by the red arrowhead. (**B**) Western blot analysis of His-CP^{GINV} protein expression induced by (+) or (−) IPTG. The numbers 1#, 2#, 3#, and 4# indicate four independent positive transformants of *E. coli* BL21 (DE3). (**C**) The prokaryotic expression of His-CP^{GINV} protein was purified by affinity chromatography. The bacterial suspension was ultrasonically broken and then centrifuged to obtain precipitate (lane 2), supernatant

(lane 3), and pass-through (lane 4). Subsequently, His-CP^{GINV} protein was bound to Ni-NTA resin by affinity binding. To eluate binding proteins, an imidazole solution with a concentration of 60 mM to 400 mM is used (lanes 5–10).



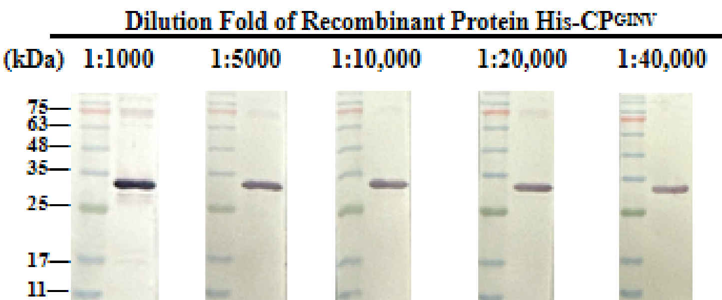


Figure 2. Evaluation of the titer of the prepared anti-body for His-CP^{GINV}, and three detection methods were used based on the His-CP^{GINV} protein. (**A**) SDS-PAGE analysis of the His-CP^{GINV} protein purified by affinity column. Marker stands for the protein marker. (**B**) Evaluation of the titer of His-CP^{GINV}-specific antiserum was performed using ELISA. For the detection of the antigen-antibody reaction, HRP-A was used as the secondary antibody. The absorption value was measured under the wavelength of 450 nm. The red line position refers to the position where the OD_{450} is 0.6. (**C**) The antiserum was evaluated by PBS at different diluted concentrations in Dot blot assay. The purified His-CP^{GINV} was taken as antigen. (**D**) The Western blot was employed to detect the purified His-CP^{GINV} protein, using varying dilutions of PAb-CP^{GINV} antibody. Marker stands for the protein markers (Lane 1, 3, 5, 7, 9) and Lane 2, 4, 6, 8, 10 refer to the PAb-CP^{GINV} with 1×10^3, 5×10^3, 1×10^4, 2×10^4, and 4×10^4 dilutions.

3.3. RT-PCR and Western Blot Detection of GINV Infection in Grape Plants

In order to establish a more specific and sensitive serological assay for GINV, we further purified the polyclonal antibody against the target His-CP^{GINV} (PAb-CP^{GINV}) in serum by salt fractionation. Most of the IgG-type antibodies were deposited in the serum, the precipitation was dissolved in normal saline, and the salt was dialyzed overnight to obtain high purity and a high concentration of PAb-CP^{GINV}.

First, fresh samples of seven grape leaves were collected in Taizhou city, Jiangsu Province. We employed the specific primers GINV *CP*-F and GINV *CP*-R for the RT-PCR detection of the GINV *CP* gene on the seven grape leaf samples suspected of being infected by GINV, including one positive control and one negative control. The RT-PCR results showed specific bands in samples 1, 2, 3, 5, and 7 (Figure 3, upper panel). To assess the quality of the prepared PAb-CP^{GINV}, we also conducted Western blot analysis of the antibodies, and the results were consistent with the RT-PCR detection, confirming that these five samples were indeed infected by GINV (Figure 3, middle panel). The total protein staining showed consistent loading of samples for each channel (Figure 3, bottom panel). This indicated that the prepared PAb-CP^{GINV} antibody was suitable for the detection of GINV.

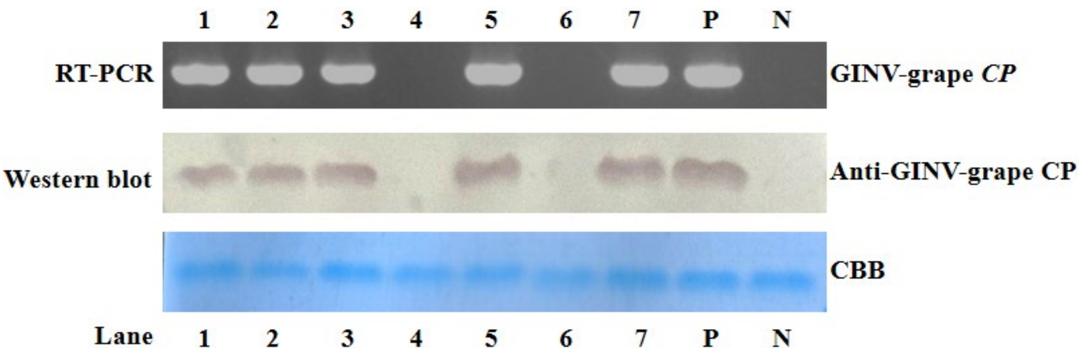


Figure 3. RT-PCR and Western blot detection of the grapevine samples in wild field. RT-PCR was used to detect 7 grapevine samples from the wild field (**upper panel**), and Western blot was used to detect 7 fresh samples from wild fields in the grapevine growing area (**middle panel**). Total protein was stained with CBB and used as control sample (**bottom panel**). P: positive sample, N: negative control.

3.4. Serological Detection of GINV by Dot Blot and ELISA

To enable the PAb-CP^{GINV} antiserum to quickly and accurately detect grape samples in the field, we established Dot blot and ELISA methods. We collected 21 grape leaf samples, extracted total protein, and conducted a Dot blot test. Purified recombinant His-CP^{GINV} protein was used as a positive control, while total protein from healthy grape samples served as a negative control. The Dot blot film revealed varying spot intensities among the tissue samples. Dot hybridization results indicated that 13 out of the 21 samples were infected by GINV (Figure 4A). ImageJ software was utilized for quantitative analysis, with the relative value of the blank processed set to 1 for normalization. The relative values of other treatments were calculated, and a histogram was created to illustrate the relative intensity of the spots (Figure 4B).

We also established an ELISA detection method for the same samples, incorporating the prepared specific antibody PAb-CP^{GINV}. During the detection, the prepared polyclonal antibody PAb-CP^{GINV} was diluted 10,000 times, and the color intensity was measured as OD_{450}. The ELISA results indicated that when using PAb-CP^{GINV}, wells containing total protein from GINV-positive samples exhibited a strong yellow color, consistent with the results from the positive control (Figure 4C). This finding aligned with the Dot blot results. Samples 1, 3, 4, 5, 7, 8, 10, 12, 13, 14, 15, 16, and 20 were identified as positive samples. The OD_{450} value of the positive control was significantly higher than that of the negative control, indicating a statistically significant difference. Subsequently, we performed quantitative analysis using ImageJ (Figure 4D). RT-PCR results corroborated the findings from both

Dot blot analysis and ELISA for these 21 samples (Figure 4E). These results all proved the successful development of the GINV detection method based on high throughput and the rapid PAb-CP^{GINV} antiserum method.

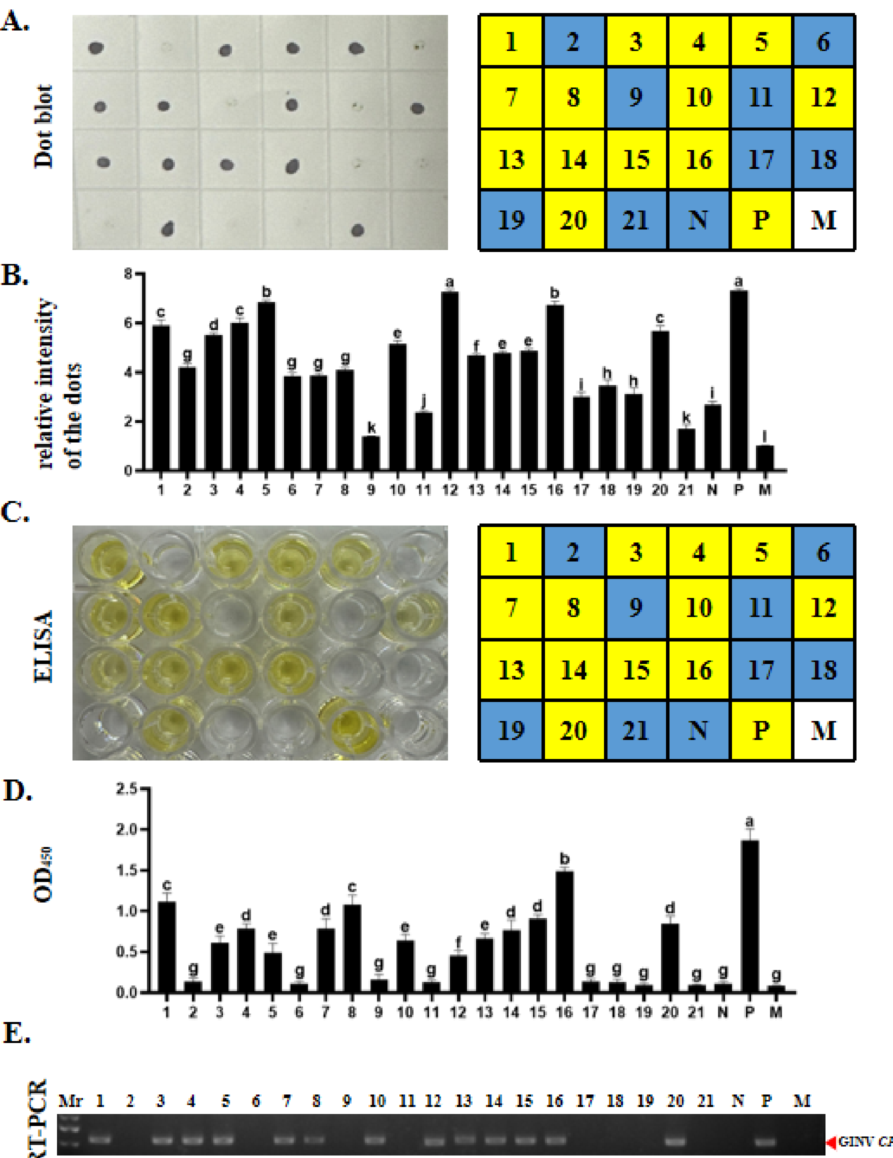


Figure 4. Dot blot and ELISA detection of the grapevine samples in wild field. (**A**) Dot blot was used to detect 21 grape samples from independent plants. Each 1 cm × 1 cm small square represents a sample, with purple indicating a positive dot hybridization result and white indicating a negative test result. In the squares of the numbers, yellow represents positive results and blue represents negative results. (**B**) Quantifying the intensity of the stain points. SD (Standard Deviation) represents the mean of independent grape samples in the same row (Student's *t* test, $p < 0.05$). N: negative sample, P: positive sample, M: blank control. Different letters obtained by the Prism 8 software analyses were marked on the top of each column, which represents the significant difference between the columns compared. (**C**) An ELISA kit was developed for specific and high-throughput diagnosis of 21 wild grapevine samples. Each hole represents a sample, with yellow indicating a positive test result and white indicating a negative test result. In the squares of the numbers, yellow represents positive results and blue represents negative results. (**D**) The sample, quantitative data analysis, and statistical methods were the same with Dot blot assay above. (**E**) RT-PCR was used to detect 21 grapevine samples.

4. Discussion

Grapes are among the oldest fruit tree species in the world, widely distributed, and holding significant economic value. In recent years, the issue of declining fruit quality due to grape viruses has become increasingly serious [23], yet reports on grape infection by GINV remain limited. Field grapes are frequently affected by GINV and other viral diseases, resulting in GFDD, which poses a significant threat to the grape industry [24]. Consequently, the sensitive and rapid detection of GINV in grape samples is of utmost importance.

However, traditional molecular detection methods, such as RT-PCR and its derivatives, require expensive reagents and are often time-consuming. This is particularly problematic for large-scale virus screening in the field, where some samples may contain high levels of the virus and can be easily detected through various serological analyses. In contrast, samples with very low virus levels may be incorrectly classified as virus-free [12]. The antibodies we prepared can avoid such problems due to their high sensitivity and specificity. At present, the serological virus detection system has the advantages of rapid, high specificity, and sensitivity in the detection of plant viruses. It has been widely used in rice [25], wheat [13], potatoes [26], and other food crops, but also widely used in apples [27], citrus [28], and other fruit trees. The accuracy of the serological detection system mainly depends on the specificity of the virus detection of polyclonal antibodies. If the antibody used has low sensitivity, it will not be able to accurately detect the virus, nor will it give definitive results. In order to overcome the limitations of GINV detection methods, we prepared highly sensitive and highly specific PAb-CP^{GINV} and developed Dot-blot and ELISA serological detection systems for high-throughput detection of GINV in the field.

Detecting plant viruses is crucial for both virus research and the development of virus-free breeding programs. GINV can be identified in many grape varieties that display symptoms. In addition, GINV is widely distributed in China, with most isolates belonging to types 1 and 2, and the Japanese isolate (D88448) belonging to type 3. Geographically, the isolates found in China and Japan are similar, which poses a risk for the further spread of GINV [8]. This virus significantly impacts the Japanese grapevine industry, as it is transmitted by mites and presents a serious threat to the development of the Chinese grape industry [29]. Previous studies have detected GINV in grapevine samples exhibiting ring spots through small RNA sequencing [29,30]. However, high-throughput sequencing technology is constrained by high costs and lengthy processing times [31]. Therefore, it is necessary to establish a serological detection method for GINV. Considering the important position of viticulture in China's agricultural production and the large area of main producing areas, better detection of GINV is conducive to preventing and controlling the spread in advance. Also, this method will also be the development direction of high-throughput detection in the future.

5. Conclusions

In summary, we prepared PAb-CP^{GINV} and established efficient, rapid, sensitive, accurate, and high-throughput detection methods, including Western blot, Dot blot, and ELISA. This study provides a series of reliable and effective methods for the detection of GINV so that GINV isolates can be identified by sequencing and other means of GINV-infected plants. The results lay the foundation for further understanding of GINV population dynamics, as well as a comprehensive insight into the genetic evolution and spread of GINV.

Author Contributions: Conceptualization, Z.W. and Z.H.; Methodology, X.D., N.W., H.Z. and L.Q.; Validation, X.D., Z.W. and R.J.; Investigation, X.D., Z.W., N.W., R.J. and M.Y.; Resources, Z.H.; Writing—original draft, X.D.; Writing—review & editing, X.D., H.Z., L.Q., X.C. and Z.H.; Visualization, M.Y.; Supervision, X.C. and Z.H.; Project administration, Z.H.; Funding acquisition, Z.H. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grants from National Key R&D Program of China (2022YFE013 0900), the National Natural Science Foundation of China (No. 32272485), "Fengchen Talent Plan" double innovation introduction special support program of Taizhou City, the High-Level Talent Support Program of Yangzhou University and Yangzhou University interdisciplinary high-level young talent Cultivation project.

Data Availability Statement: The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Acknowledgments: We thank all the other members of the Plant Virology Laboratory at Yangzhou University for their helpful suggestions and constructive criticism. We also thank each of the authors and reviewers for their contributions to this experiment. Moreover, we would like to send our gratitude to the reviewers.

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

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