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Development of a polyclonal antibody against the coat protein of *Prunus necrotic ring spot virus*

Sara Ebrahimi[1], Davoud Koolivand[1*]

[1]Department of Plant Protection, Faculty of Agriculture, University of Zanjan, Zanjan, Iran.
[*]Corresponding author, 0000-0002-5863-2591. Email: koolivand@znu.ac.ir.

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ABSTRACT

The *Prunus necrotic ring spot virus* (PNRSV) poses a significant threat to the global stone fruit industry. In this study, samples suspected of PNRSV infection were gathered from Iran's primary stone fruit-growing areas. The coat protein (CP) gene of the isolates was amplified, and the nucleic acid and amino acid sequences of the PNRSVs were determined. Phylogenetic analysis revealed that these isolates belonged to the PV-96-II, a prominent group of PNRSVs found worldwide. An isolate from this clade was chosen to express the CP gene in *Escherichia coli*. The CP gene of this isolate was cloned into the pET28 vector for expression, and the resulting coat protein was purified as a native protein using Ni-NTA agarose. The purified protein served as a recombinant antigen to generate anti-PNRSV-CP antiserum in rabbits. Purified anti-PNRSV-CP IgG and conjugated IgG showed specificity and sensitivity, successfully detecting expressed CP and PNRSV isolates in infected stone fruit trees through various serological and sero-molecular techniques such as plate-trapped antigen enzyme-linked immunosorbent assay (PTA-ELISA), Double Antibody Sandwich-ELISA (DAS-ELISA), and western blotting. These antibodies can be valuable in virus and plant screening studies, as well as serological and sero-molecular tests. This study represents the first documentation of a polyclonal antibody preparation against the CP of an Iranian PNRSV isolate, offering potential assistance in controlling and preventing this economically significant virus in the future.

***Key words*:** Antibody, Competent cell, Purification, Polymerase chain reaction, Prokaryotic system.

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INTRODUCTION

Prunus necrotic ring spot virus belonging to the *Ilavirus* genus in the *Bromoviridae* family (King *et al.,* 2011; Pallas *et al.,* 2012), it is a global viral pathogen that affects various commercial fruit trees, such as peach, cherry, plum, apricot, almond, apple, and hazelnut, as well as ornamentals such as roses. Generally, viral infections lead to a reduction in fruit quality and growth, along with delayed fruit maturity (Hammond and Crosslin, 1995; Pallas *et al.,* 2012). PNRSV spreads through budding and grafting, seeds, pollen, infected vegetative propagation materials, and insects carrying contaminated pollen (Milne and Walter, 2003; Fiore *et al.,* 2008; Oliver *et al.,* 2009).

PNRSV has a tripartite single-stranded RNA genome and is encapsulated in quasi-isometric particles (King *et al.,* 2011), RNA1 and RNA2 are monocistronic, encoding P1 and P2 replicase, respectively, whereas RNA3 is bicistronic, containing two open reading frames (ORFs) (King *et al.,* 2011). The 5'-proximal ORF encodes the movement protein (MP) and the 3'-proximal ORF encodes the coat protein (CP). CP is expressed through the transcription of a subgenomic messenger RNA, named RNA4, derived from RNA3 (Pallas *et al.,* 2012). All genomic RNAs have cap structures at the 5' ends and lack a poly (A) tail at the 3' end. Numerous PNRSV isolates from different *Prunus* species and geographical regions have been characterized based on the coat protein gene and are classified into three phylogenetic subgroups: PV32-I, PV96-II, and PE-5-III (Hammond and Crosslin, 1995; Aparicio *et al.,* 2003; Sokhandan-Bashir *et al.,* 2017; Mansoue *et al.,* 2023). The phylogenetic subgroups have been established based on the whole genome of PNRSV (Noorani *et al.,* 2023).

PNRSV symptoms vary depending on the subgroup and host species, ranging from asymptomatic or latent to a diverse array of symptoms (Fiore *et al.,* 2008; Oliver *et al.,* 2009). PNRSV can lead to bud death in trees, chlorotic to yellow line patterns, shot holes in leaves, discolored rings or spots in fruits (e.g., apricot), and severe stunting (Hammond and Crosslin, 1995; Pallas *et al.,* 2012). In cherry-infected trees, one may observe chlorotic to yellow line pattern mosaics, foliar mosaics, rings, chlorotic areas, or severe necrotic spots with a shot hole (Oliver *et al.,* 2009). Ring necrosis primarily impacts leaves but also affect branches and emerging buds in aggressive strains. Initially chlorotic foliar rings progress to necrosis eventually causing leaf perforation (Hammond and Crosslin, 1995; Milne and Walter, 2003). Symptoms typically manifest in the first year after post-infection often becoming asymptomatic thereafter, though certain strains may cause recurrent symptoms annually (Glasa *et al.,* 2002).

The most efficient way to control this viral disease involves removing severely infected trees from orchards and refraining from using infected scions during grafting. Therefore, having a reliable method for detecting plant viruses is essential for managing viral diseases, particularly in virus-infected trees. Various techniques have been developed for virus detection, including molecular methods like Reverse Transcription Polymerase Chain Reaction (RT-PCR). However, screening large numbers of samples using these methods can be challenging due to the costs and execution complications (Fajardo *et al.,* 2007). As an alternative, serological assays, such as Enzyme-linked Immunosorbent Assay (ELISA), are commonly employed for testing numerous samples simultaneously because of their cost-effectiveness, accuracy, sensitivity, and reliability (Lima *et al.,* 2012). ELISA is a sensitive, specific, affordable, robust, and suitable for testing multiple samples at once (Abou-Jawdah *et al.,* 2004; Lima *et al.,* 2005; Lima *et al.*, 2012; Mohammadloo *et al.,* 2023). The primary component of ELISA is an antibody derived from antiserum and targeted against purified virus (Fajardo *et al.,* 2007). Traditional methods for producing antibodies against purified viruses are often inefficient due to low viral titer in plant tissues, particle instability, and antibody specificity issues (Fajardo *et al.,* 2007). Moreover, there can be serological cross-reactivity among closely related viruses using these antibodies (Lima *et al.,* 2012). Additionally, virus purification from plant tissues necessitates specialized equipment like high-speed and ultra-centrifuges, which may not be available in laboratories of less developed countries. Hence, utilizing viral gene expression in *E. coli* to generate recombinant proteins as immunogens can address these challenges (Barbieri *et al.,* 2004; Fajardo *et al.,* 2007). CP is a promising candidate for use as a recombinant antigen due to its immunogenicity and a high degree of genetics conservation (ČEřovská *et al.,* 2006). The rapid production, cost-effectiveness, and high specificity of antibody production without cross-reaction to plant proteins are key advantages of recombinant protein technology. This approach has been successful in generating recombinant antibodies against various plant viruses which are extensively utilized in serological methods for virus detection (Abou-Jawdah *et al.,* 2004; Barbieri *et al.,* 2004; Shams-Bakhsh and Symon, 2004; Amiri Sadeghan *et al.,* 2013; Koolivand *et al.,* 2014; Bashir *et al.,* 2015; Koolivand *et al.,* 2016; Koolivand *et al.,* 2017; Masoudi *et al.,* 2018; Antony

et al., 2021; Dhir *et al.,* 2021). Additionally, these antibodies are used in highly sensitive techniques such as immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) (Fajardo *et al.,* 2007; Koolivand *et al.,* 2014; Afrashteh *et al.,* 2023).

The objectives of this study were to identify widely spread isolate of PNRV from Iran, and express the recombinant CP gene in *E. coli* for large-scale production of PNRSV-specific polyclonal antibodies.

MATERIALS AND METHODS

Sampling

Approximately two hundred leaf and fruit samples showing symptoms of infection by Prunus necrotic ring spot virus were collected based on virus symptom description, such as growth reduction, purple spots, necrosis, chlorosis, and leaf perforation, during spring, summer, and early autumn. The samples were transported to the laboratory in an ice bag and kept at -80 °C until needed.

RNA extraction, cDNA synthesis, and PCR amplification

Total RNAs were extracted from leaf and fruit samples using an RNA extraction Kit (RNX PLUS) per the manufacturer's instructions. cDNAs were synthesized with Random Hexamer primers following the guidelines of 2X HyperScript™ (GenAll, South Korea). The polymerase chain reaction was optimized for amplifying the complete coat protein gene with specific primers (forward primer, 5' ATG GGA TCC ATG GTT CGA ATT TGC 3'; reverse primer, 5' ACA TAA GCT TCT AGA TCT CAA GCA GGTC 3'). A 12.5 µL PCR reaction mix included 3.75 µL ddH_2O, 2 µL cDNA, 2X PCR Master mix (Ampliqon, Denmark), and 10 pmol of each primer. The thermoprofile comprised one cycle of 94 °C for 5 min, 35 cycles of 94 °C for 45 s, 48 °C for 45 s, 72 °C for 60 s, and a final cycle of 72 °C for 10 min.

Cloning, sequencing, and phylogenetic analysis

The CP genes were amplified, inserted into the cloning vector pTG19, and transformed into *E. coli* strain DH5α via heat shock. The transformed constructs (pTG19-PNRSV-CP) were then cultured in LB medium supplemented with ampicillin (100 µg/ml). Clone sequencing utilized general plasmid primers. The obtained sequences were aligned with those of previously reported isolates in GenBank using the Clustal W algorithm in MEGA X software. Subsequently, a phylogenetic tree was constructed through the neighbor-joining method after determining the best DNA model in MEGA X software, focusing on the CP region with 1000 bootstrap replicates.

Expression of the construct in *E. coli*.

In *E. coli*, a confirmed recombinant pTG19-PNRSV-CP and pET28a plasmids were digested with *Bam*HI and *Hind*III. The CP gene was then ligated into the bacterial expression vector pET28a, creating a new construct (pET28-PNRSV-CP) that was introduced into *E. coli* strain BL21 (Chung *et al*., 1989). Transformant colonies containing pET28-PNRSV-CP were cultured overnight in LB medium with kanamycin. The culture was grown until reaching an optical density of 0.4-0.6. A non-induced control was prepared, and different concentrations of isopropyl-β-D-1-thiogalactopyranoside (IPTG) were added to induce the T7 promoter. Samples were analyzed at 4-, 6-, and 16-hours post-induction. Cell suspensions were centrifuged, and the resulting pellets were suspended in a lysis buffer. The supernatant containing soluble proteins was then analyzed. Protein samples were loaded onto a polyacrylamide gel, stained, and destained. An unstained gel was blotted onto a nitrocellulose membrane for western blotting. The membrane was incubated with an anti-His antibody, followed by a goat anti-mouse IgG-alkaline phosphatase conjugate. Nitro Blue Tetrazolium and 5-bromo-4-chloro-3-indolylphosphate were used as substrates for staining. The reaction was stopped, and the membrane was photographed after air-drying.

Purification of recombinant PNRSV CP

To purify PNRSV-CP, *E. coli* BL21 with pET28-PNRSV-CP was cultured in 100 ml LB medium with kanamycin. The induced culture was centrifuged, and the protein was purified using a Ni-NTA agarose Kit. The soluble extract was loaded onto Ni-NTA columns for purification. Purified protein samples were analyzed by SDS-PAGE, and 10 µg of each sample was used for further testing. Electroblotting onto a nitrocellulose membrane was performed, followed by incubation with specific antibodies. Commercial anti-PNRSV antibodies were used to assess the purification and antigenicity of PNRSV-CP through PTA-ELISA, DIBA, and western blotting.

Immunization of rabbits and purification of IgGs

Recombinant CP was purified and used to immunize two rabbits. The injection schedule involved an initial injection with 100 µg/ml of purified protein mixed with complete Freund's adjuvant, followed by three booster injections with 50 µg/ml of PNRSV CP mixed with incomplete Freund's adjuvant. Two weeks post final injection, rabbits were bled, and

serum fractions were collected and stored. Antiserum titration using PTA-ELISA at dilutions from 1:512 to 1:32768 was conducted. IgG purification utilized a protein A purification kit, with antibody concentration estimated at 280 nm wavelength. Furthermore, IgG was conjugated to alkaline phosphatase using an Easy Link Alkaline Phosphatase Conjugation Kit per the manufacturer's protocol.

Efficiency of anti-PNRSV CP antibodies

Polyclonal antibodies against recombinant PNRSV CP were evaluated for sensitivity and specificity using various methods. Western blotting (1:500), double antibody sandwich (DAS)-ELISA (1:1000, 1:200, 1:3000), and plate-trapped antigen (PTA)-ELISA (1:1000, 1:2000, 1:3000) were utilized. The calibration of antibodies involved crude leaf extracts from healthy tissue, three PNRSV-infected leaf tissue samples, and purified CP protein expressed in *E. coli*. Results were assessed by adding the universal conjugate antibody AP and measuring light absorption at 405 nm. SDS-PAGE analysis was conducted on crude protein extract (30 µl) and purified PNRSV-CP (10 µg/ml). The nitrocellulose membrane was probed with an anti-PNRSV CP dilution of 1:500, followed by the addition of a secondary antibody (universal alkaline phosphatase-conjugated IgG) at a dilution of 1:500. Target proteins were detected using the substrates 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitro blue tetrazolium (NBT).

RESULTS

Sequencing and phylogenetic analysis

A 675 bp fragment was amplified in 38 DNA samples taken from trees exhibiting viral symptoms. No amplification was observed in DNA from healthy control tree samples. Sequence analysis on BLAST indicated that the CP product sequences (16 isolates) bore a strong resemblance to previously reported isolates from Iran, Poland, and India. Alignment of the newly characterized isolate sequences with those of known PNRSV isolates revealed a match with the CP gene of PNRSV. Phylogenetic analysis showed that the newly identified isolates formed a cluster with PNRSV isolates from various countries including Iran, Poland, Uruguay, China, Chile, Montenegro, and the USA. The identities between the PNRSV isolates from Iran and other isolates ranged from 82% to 99%. Furthermore, comparison of deduced amino acid (AA) sequences indicated identities between the new Iranian isolates and others ranging from 70% to 99%. The identities among the new isolates themselves were approximately 95% to 100% at the nucleotide level and 97% to 100% at the amino acid level. A lower level of similarity was observed between a strain from the USA (L38823) classified under the PE-5 group and two counterparts from different groups (PV-96 and PV-32), with around 83% and 74% similarity based on nucleotide (NT) and amino acid (AA) data, respectively. Phylogenetic trees constructed using NT or AA sequences of the coat protein gene from 38 PNRSV isolates, including 16 new isolates from Iran, indicated that all the fresh Iranian isolates, along with isolates from Bulgaria (MT009388), USA (FJ231733), Brazil (KX574326), Italy (AJ133199), Chile (EF565251), China (HQ833192), and Iran (KY484022, KY484014, KX353932), formed a distinct subclade within the PNRSV PV-96 group (Figure 1). BLASTN analysis confirmed that the sequenced fragments corresponded to the PNRSV coat protein gene. Phylogenetic assessment demonstrated that the Iranian isolates in this research belonged to the PV-96-II group (Figure 1).

Expression of PNRSV CP

PNRSV CP expression was successfully confirmed through plasmid sequencing, validating the insertion of the CP gene sequence into the vector. Sequencing data also verified the accurate genetic coding of the full-length CP gene in the expression vector (pET28a). SDS-PAGE analysis of the total protein extracted from bacterial cells carrying pET28-PNRSV-CP demonstrated efficient expression of CP (~27 kDa) upon IPTG induction, with no protein band observed in non-induced cells. Optimal CP gene expression was observed four hours post-induction, prompting cell harvest at this time point following induction with 1mM IPTG (Figure 2). Western blotting with an anti-His-tag antibody confirmed the identity of the expressed protein, showing one distinct band at the expected molecular weight (Figure 3).

Purification and characterization of PNRSV-CP

Analysis of the native recombinant purified protein via SDS-PAGE revealed an expected 27 kDa band, indicative of PNRSV-CP (Figure 4), along with fusion tags. Furthermore, western blotting demonstrated a strong band post BCIP/NBC addition.

Antibody efficiency

The antibody's efficiency was demonstrated through titration results using PTA-ELISA, indicating an approximate titer of 1:16000 for the anti-PNRSV CP antisera (Figure 5). The anti-PNRSV-CP serum effectively reacted with the purified expressed CP. Conversely, there was no significant reaction in the extractions of the non-induced bacteria, serving as the negative control. Additionally, the DAS-ELISA results,

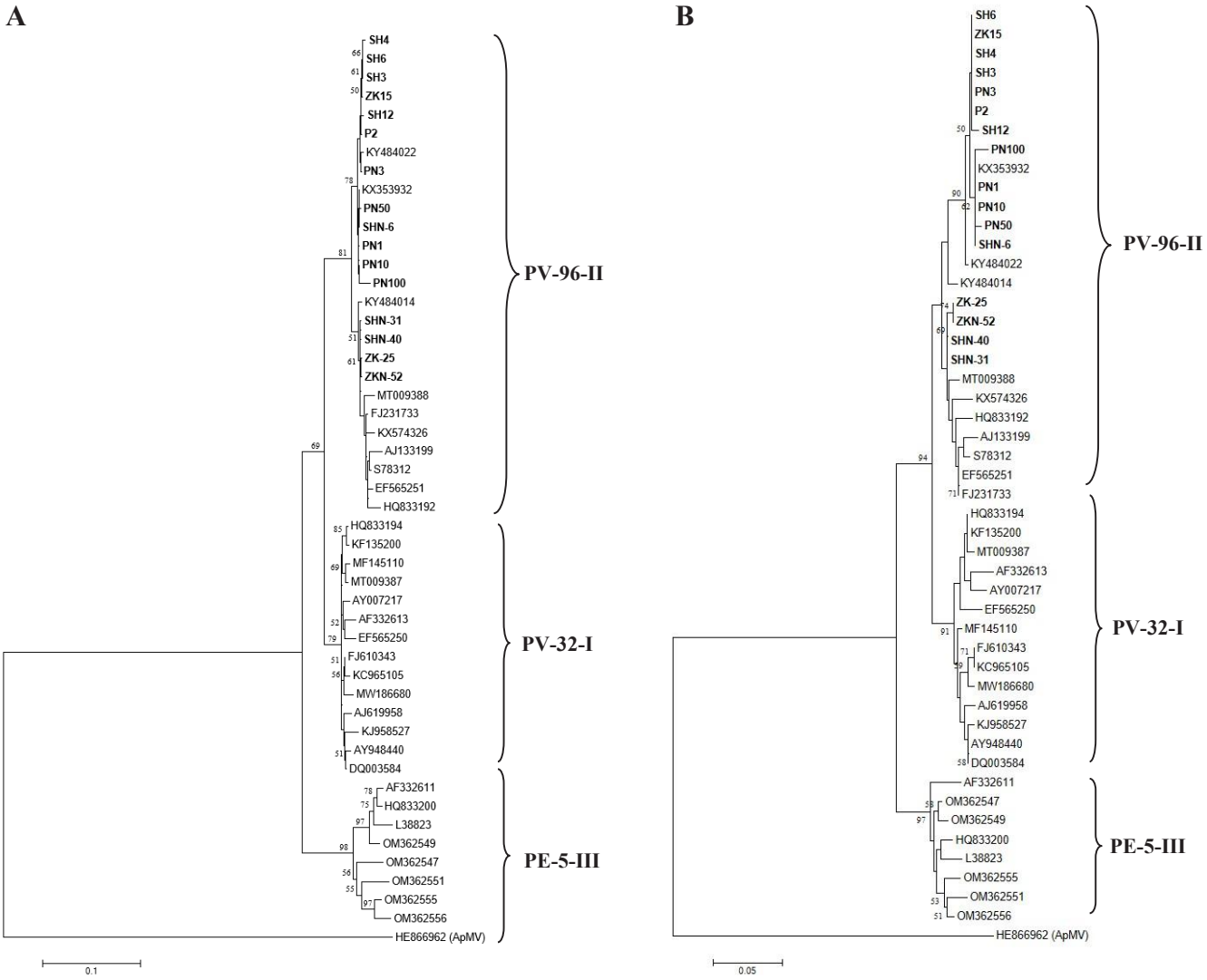

Figure 1. Neighbor joining phylogenetic trees generated using Mega X program based on **A:** nucleic acid and **B:** amino acid sequences of PNRSV coat protein. Branch lengths are proportionate to genetic distances and the scale bar represents 0.05 genetic distance. Branches with bootstrap value of <50% are unresolved. *Apple mosaic virus* (ApMV) is assigned as the outgroup species.

evaluating the effectiveness of the prepared anti-PNRSVCP IgG (1:1000, 1:2000, and 1:3000 dilutions) and the conjugated IgG (1:2000 dilution) for antigen detection, revealed efficient reactions with both IgG and the conjugated antibody to the PNRSV-infected leaf tissue and purified CP (Figure 5). Furthermore, western blotting results demonstrated that the anti-PNRSVCP antibody (1:1000 dilution) effectively reacted with the expressed protein and the PNRSV-infected leaf extracts (Figure 6).

DISCUSSION

Stonefruit trees can be affected by a various plant viruses. *Prunus necrotic ring spot virus* is a notable virus that results in significant harm and substantial losses to commercial stone fruits like peach, nectarine, sweet cherry, sour cherry, almond, apricot, and plum. PNRSV poses a significant risk to the fruit industry due to its broad host range (Pallas *et al.,* 2012; Pallás *et al.,* 2013). Phylogenic analysis in this study aided in determining the predominant PNRSV group in Zanjan province, and sequencing of the PNRSV-CP gene showed that majority of isolates were part of the PV-96-II group (Figure 1). These isolates, mainly spread through pollen, resulted in shot holes in the leaves. The most effective management of the disease is to remove severely infected trees from orchards and avoid the use of infected scions when grafting. Therefore, disease control depends on an efficient method of virus detection.

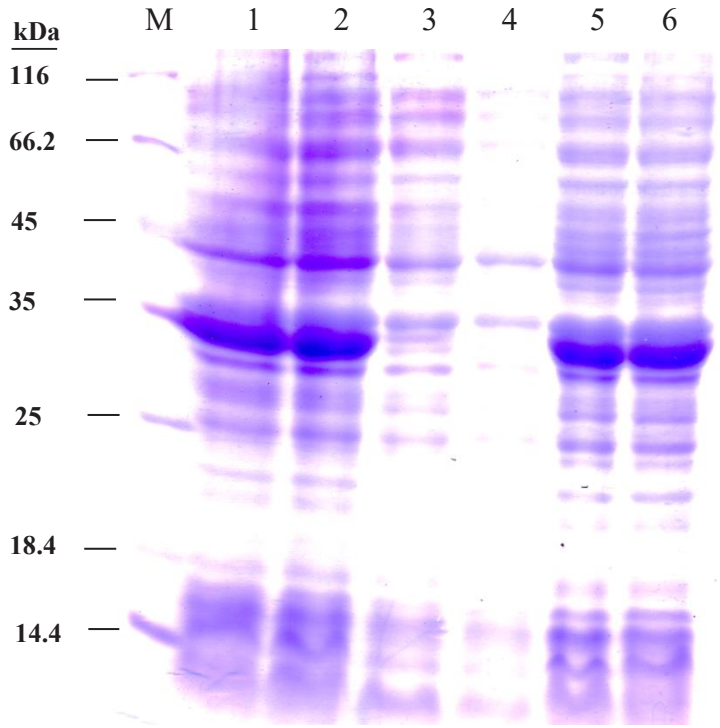

Figure 2. SDS-PAGE of the expressed PNRSV-CP in *E. coli* strain BL21. Lanes: M) Unstained protein marker; 1, 2, and 5) total protein from induced *E. coli* BL21 (DE3) with pET28-PNRSV-CP after 1mM IPTG induction for 4, 6, and 16 hours. 6) total protein from induced *E. coli* BL21 (DE3) with pET28-PNRSV-CP after 2mM IPTG induction for 4 h. 3) total protein from uninduced *E. coli* BL21 (DE3) with pET28-PNRSV-CP. 4) total protein from *E. coli* with pET28 only.

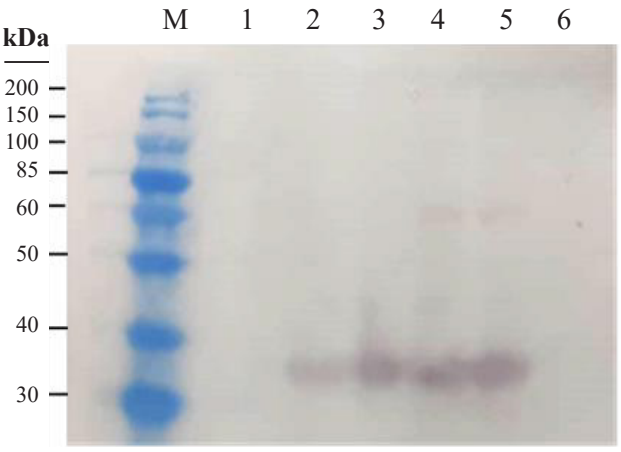

Figure 3. Western blotting of PNRSV-CP on nitrocellulose membrane. Lanes: M. Prestained protein marker, 2, 3, 4, and 5) expressed PNRSV-CP after three, four, six, and 16 hours of induction by 1mM of IPTG; c, d. expressed PNRSV-CP; 6) protein extracted from non-induced *E. coli.* 1) empty.

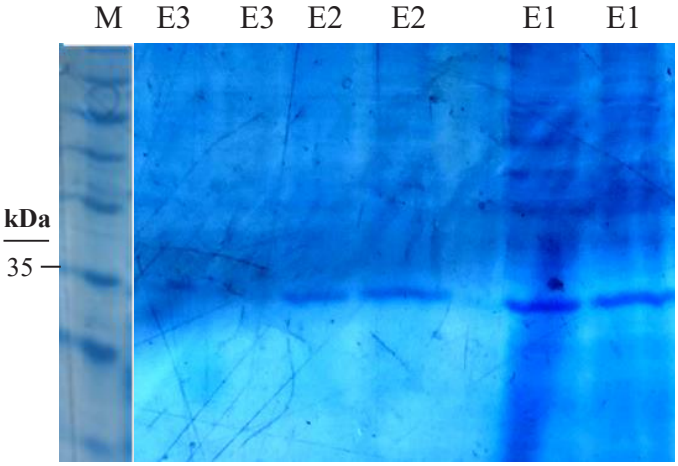

Figure 4. SDS-PAGE of purified protein from expressed protein in *E. coli* BL21 (DE3) containing pET28-PNRSV-CP. Lanes: M. protein size marker; E1, E2 and E3. purified protein (E1 has the highest concentration and E3 has the lowest concentration.

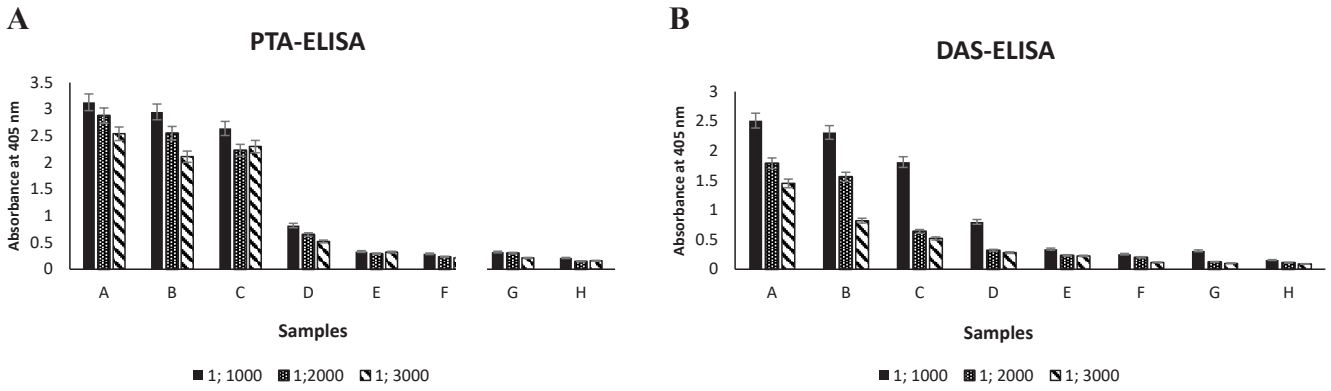

Figure 5. Absorbance coefficients in **A:** PTA-ELISA and **B:** DAS-ELISA used to evaluate the efficiency of recombinant antibodies developed against PNRSV-CP. A (purified coat protein), B (protein expressed in bacteria), C (leaf sample infected with PNRSV), D (protein expressed pre-induction), E (healthy leaf sample), F (protein extracted from bacteria with plasmid but no CP insert), G (protein extraction buffer), H (substrate).

In recent decades, laboratory-based techniques have advanced significantly, leading to the detection of a wide array of plant viruses. These methods encompass the analysis of physical, biological, cytological, and molecular characteristics of viruses. Progress in virus research has enabled the utilization of recombinant proteins as antigens for antibody production in plant virus diagnostics. The capacity to generate recombinant viral CP in bacteria holds various implications. By creating antigenic recombinant CP, the production of antibodies against the virus becomes feasible, facilitating their use in serological tests. This development is particularly beneficial in regions where importing antibodies is expensive and time-consuming. Serological approaches like ELISA stand out as highly effective and reliable techniques for swift and accurate virus identification (Lima *et al.,* 2012), with antibodies serving as crucial indicators in ELISA assays (Fajardo *et al.,* 2007).

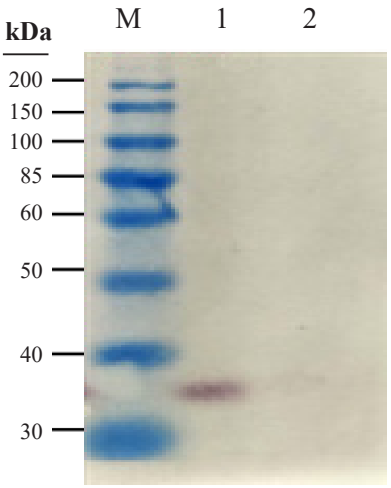

Figure 6. Western blotting by the use of anti-PNRSV CP IgG (diluted 1:500). M: Prestained Protein Marker, 1) injected purified PNRSV CP protein, 2) protein sample from a colony containing pET28a.

Using recombinant viral CPs expressed in bacterial cells as immunogenic materials can be an efficient alternative for antibody preparation against viruses found in low concentrations in infected plants or that are challenging to purify. These antibodies can be produced in large quantities and easily manipulated if necessary. The pET vector is widely utilized for expressing recombinant protein. This system has been employed for gene expression in various plant viruses (ČEřovská *et al.,* 2006; Folwarczna *et al.,* 2008; Cerovska *et al.,* 2012; Amiri *et al.*, 2013; Koolivand *et al.,* 2014; Lotfi *et al*., 2015; Bashir *et al.,* 2015; Koolivand *et al.,* 2016; Koolivand *et al.,* 2017; Masoudi *et al.,* 2019). Other expression vectors like pGEX, pTrcHis (Shams-bakhsh and Symons, 2004; Lee and Chang, 2008), and pTBG (H) (Cerovska *et al.,* 2012), also have the potential to express foreign genes in *E. coli*. *E. coli*, being a prokaryotic system, offers numerous advantages, including efficient and rapid generation time, fast high-density cultivation, high expression capacities, and cost-effectiveness. CP genes from various plant viruses have been successfully expressed in *E. coli* (Abou-Jawdah *et al.,* 2004; Chen *et al.,* 2006; Fajardo *et al.,* 2007; Plchova *et al.,* 2011; Afrashteh *et al.,* 2023).

In the current study, the phylogenetic relationships of the detected isolates were examined to identify the dominant group and produce the desired recombinant PNRSV CP isolate in *E. coli*. The large-scale production of PNRSV-specific polyclonal antibodies was also carried out. The anti-PNRSVCP antibodies generated

can be utilized in serological and sero-molecular tests like ELISA, western blot analysis, and IC-RT-PCR. This method of antibody production can be particularly beneficial in developing countries where importing antibodies can be expensive. The expressed and purified proteins were analyzed using SDS-PAGE, revealing a molecular mass of around 27 kDa. The identity of the proteins was confirmed through western blot analysis. Different IPTG concentrations and durations were tested for the expression of genes of interest. The study found that 1 mM concentration and 4 h of induction led to sufficient expression level, consistent with previous research (Koolivand *et al.,* 2017; Masoudi *et al.,* 2018). While IPTG concentration may vary based on the gene of interest or other conditions; expression for 3-4 h at 37 °C has been identified as optimal (Bragard *et al.,* 2000; Liu *et al.,* 2001; Jacob and Usha, 2002; Saini *et al.,* 2003).

The impact of the T7 peptide tag and His tag fused in the CP on antibody specificity may be a concern, however, previous studies have indicated that tags do not have notable immunogenic properties on CP expressed in *E. coli* (Mutasa-Gottgens *et al.,* 2000; Kumari *et al.,* 2001; Gulati-Sakhuja *et al.,* 2009). In addition, tags at the N- and C-termini do not exhibit significant immunogenic properties (Mutasa-Gottgens *et al.,* 2000; Kumari *et al.,* 2001). The recombinant coat protein was used to generate antibodies in mice or rabbits, tested in different serological assays, and ultimately employed in virus diagnosis (Carvalho *et al.,* 2013; Hamdayanty *et al.,* 2016; Sharma *et al.,* 2016; Koolivand *et al.,* 2017; Antony *et al.,* 2021; Dhir *et al.,* 2021). Various dilutions of polyclonal antibodies were assessed to determine the optimal concentration for virus detection using anti-PNRSVCP antibodies. The results indicated detectable CP in both PNRSV-infected leaves and purified *E. coli* preparations using PTA-ELISA (Figure 5). The optimal diagnostic conditions included a 1:10 dilution of the leaf extract and a 1:1000 dilution of IgG in the PTA-ELISA (Figure 5). Furthermore, anti-PNRSVCP IgG (dilution: 1:1000) and conjugated IgG (dilution: 1:1000) efficiently detected CP in the DAS-ELISA (Figure 5) and western blot analysis (Figure 6). However, there have been reports that antibodies developed against recombinant viral proteins may not detect the associated virus in DAS-ELISA (Korimbocus *et al.,* 2002; ČEřovská *et al.,* 2006; Folwarczna *et al.,* 2008; Cerovska *et al.,* 2012), due to the inability of the coated antibodies to interact with native viral epitopes, although this was not observed in this study. It is worth noting that nonspecific background reactions are common in western blot analysis when using polyclonal antibodies prepared against recombinant viral proteins (Kumari *et al.,* 2001; Abou-Jawdah *et al.,* 2004; Xu *et al.,* 2006; Gulati-Sakhuja *et al.,* 2009; Cerovska *et al.,* 2012; Mohammadloo *et al.,* 2023).

This research demonstrated the effective expression of the PNRSV-CP open reading frame, under the T7 promoter's control in *E. coli* BL21 strain. The resulting recombinant coat protein serves as a potent immunogen for generating anti-PNRSV-CP antibodies that exhibit strong reactivity with the CP and virus particles, contributing significantly to the management of viral diseases.

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