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Research Article

Developing of specific antibody against *chickpea chlorotic dwarf virus* (CpCDV) through recombinant coat protein

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Abstract: The legume crops such as chickpea and lentils are mainly cultivated in semi-arid tropical lands. *Chickpea chlorotic dwarf virus* (CpCDV) causes major losses to legumes throughout the world. Producing of specific antibody against this virus is crucial for surveys of disease in the fields and assessment of vial resistance in plant cultivars. Present article describes developing of specific antibody against the CpCDV virus by applying recombinant protein. In this study, coat protein of CpCDV was selected as a target for detection and preparation of polyclonal antibody. To achieve this aim CP gene encoding coat protein of CpCDV was initially PCR-amplified and inserted into bacterial expression vector. Expression of recombinant protein was performed in Bl21 strain of *Escherichia coli*. Purification was carried out under native conditions and the accuracy of recombinant protein production was confirmed by electrophoresis. The purified recombinant coat protein of CpCDV was used for immunization of rabbit. Purification of immunoglobulin molecules was performed by affinity chromatography using protein A column followed by conjugating of IgG to alkaline phosphatase enzyme. The capability of purified antibodies and conjugates for efficient detection of infected plants was assessed by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), western blotting and dot immunosorbent assay (DIBA). These results proved that prepared IgG and conjugate are able to distinguish with high efficiency CpCDV infected plants. To the best of our knowledge, this is the first report for production of anti-CpCDV antibodies raised through recombinant protein technology.

Keywords: antibody, chickpea, CpCDV, ELISA, recombinant protein

Introduction

The chickpea (*Cicer arietinum*) plant as a legume belongs to family Fabaceae. Different types of chickpeas are currently cultivated throughout the world. *Chickpea chlorotic dwarf virus* (CpCDV) is a monopartite virus of the genus Mastrevirus (family Geminiviridae) that is naturally transmitted by the leafhopper *Orosius orientalis* (Horn *et al*, 1993). The genus CpCDV contains circular single-stranded DNA (ssDNA) genomes of approximately 2.7 kb. The disease is assumed to make yield losses in chickpea around 75-100 % that is strictly related to the time of infection (Horn *et al*, 1995).

Although most viruses of the genus mastrevirus infect monocotyledonous plants, a

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small number of this group are able to infect dicotyledonous plants (Muhire *et al*, 2013). CpCDV is a dicot-infecting mastrevirus that was first identified in India (Horn *et al*, 1993) and later reported to affect legume crops in Egypt, Sudan, Syria, Iran, Iraq, Burkina Faso, Pakistan and Yemen (Ali *et al*, 2004; Kumari *et al*, 2004; Makkouk *et al*, 1998; Makkouk *et al*, 2001; Makkouk *et al*, 2003; Ouattara *et al*, 2017).The virus causes severe disease in chickpea with symptoms of yellowing, plant dwarfing, inter-node shortening, reduced seed production, phloem browning in the collar region and chlorosis or reddening of leaves, depending on the chickpea variety (Horn *et al*, 1993).

In the field, CpCDV naturally infects some species such as chickpea (Horn *et al*, 1993), lentil (*Lens culinaris*) (Kraberger *et al*, 2013), faba bean (*Vicia faba*), field pea (*Pisum sativum*), French bean (*Phaseolus vulgaris*) (Kraberger *et al*, 2015), pepper (*Capsicum annum*) (Akhtar *et al*, 2014) and cotton (*Gossypium sp*.) (Manzoor *et al*, 2014).

Several viruses such as *Bean leaf roll luteovirus* (BLRV), *Beet western yellows luteovirus* (BWYV), *Subterranean clover red leaf luteovirus* (SCRLV), *Faba bean necrotic yellows virus* (FBNYV) and *Soybean dwarf virus* (SbDV) cause symptoms in chickpea that are indistinguishable from those of CpCDV (Bosque-Perez and Buddenhagen, 1990; Horn *et al*, 1996; Kumari *et al*, 2006; Reddy *et al*, 1979). Early infection with these viruses normally leads to rapid decline and few plants survive. Applying of diagnostic tools, such as enzyme-linked immunosorbent assay (ELISA) and PCR, is indispensable for distinguishing CpCDV from above-mentioned viruses, which cannot be distinguished by the symptoms they induce. Access to rapid and efficient assay for identification of CpCDV is crucial in disease management, developing of resistance through conventional breeding or genetic engineering and conducting field surveys. Production of specific antibody against CpCDV would be the first step for improving diagnostic approach. There is another report for production of polyclonal antibodies against Syrian isolate of CpCDV through conventional methods (Kumari *et al*, 2006). The present article describes developing of specific antibodies against CpCDV virus through recombinant coat protein approach.

Materials and Methods

Plant materials and detection of infected plants:

In March 2016, chickpea plant samples with yellowing and stunting symptoms (Fig. 1) were collected from fields in Kermanshah province of Iran. Detection of CpCDV infected plants was made through PCR analysis. For this aim, total plant DNA was initially extracted from healthy and infected chickpea leaves by applying GF-1 Plant DNA extraction kit Vivantis (Subang Jaya, Malaysia). Around 100mg of leaves was initially frozen with liquid nitrogen then scraped into a chilled mortar and ground to powder. DNA purification was performed as described by manufacturer. The integrity and purity of extracted DNAs was visualized on agarose gel. The concentration of purified DNAs was calculated by OD260/280 measurements on a spectrophotometer (Eppendorf, Germany).

The presence of CpCDV in the plants was confirmed through polymerase chain reaction (PCR) by using specific forward (CDV-s2710: 5'-GAGAGGCACGTTCAGTGACT-3') and reverse (CDV-as710: 5'-TGAGCTTCATCAAGATGGCA-3') primers binding to C1 and C1/C2 genes of CpCDV genome, respectively. PCR reaction was performed in 25µl volume containing 1 ng of extracted template DNA, 2.5µl PCR buffer (10 ×), 0.75µl $MgCl_2$ (25mM), 1µl deoxynucleotide triphosphates (10 mM), 1µl of each primers (CDV-s2710, CDV-as710) and 1µl *Taq* DNA polymerase (5U/µl) (Fermentas, Vilnius, Lithuania). PCR reaction was carried out as follows: initial denaturation at 95 °C for 5min, followed by 30 cycles of denaturing at 95 °C for 30 sec, annealing at 53 °C for 30 sec and extension at 72 °C for 2min and a final extension at 72 °C for 5min. The PCR product was visualized by agarose gel electrophoresis.

Figure 1 Yellowing and stunting symptoms of CpCDV-infected chickpea plants.

Cloning of CpCDV CP gene

To isolate the gene encoding CpCDV coat protein from infected plant, a primer set was designed according to the sequence of CpCDV-CP (Accession No. KR014247.1). The gene encoding coat protein of CpCDV (735 bp) was PCR-amplified using *Sal*I tailed forward (CDV-F-882: 5´ GTCGACAATCAACTTCTACGTGGGG 3´), and *Xho*I tailed reverse primer (CDV-R-882: 5´CTCGAGTTATTGATTTCCAACGGAC 3´) pairs. PCR reaction was performed in 25µl of volume containing 1 ng of extracted template DNA, 2.5µl PCR buffer (10 ×), 0.75µl $MgCl_2$ (25 mM), 1µl deoxynucleotide triphosphates (10 mM), 1µl of each primers (CDV-s2710, CDV-as710) and 1µl *Taq* DNA polymerase (5U/µl) (Fermentas, Vilnius, Lithuania). PCR reaction was carried out as follows: initial denaturation at 95 °C for 5min, followed by 30 cycles of denaturing at 95 °C for 1min, annealing at 53 °C for 1min and extension at 72 °C for 90 sec and a final extension at 72 °C for 5min. The PCR product was visualized by agarose gel electrophoresis. The amplified fragment was picked up from gel and recovered by using GF-1 gel DNA recovery kit Vivantis (Subang Jaya, Malaysia) followed by ligation into PTG19-T cloning vector (SinaClon, Iran). The new construct was transformed into DH5α strain of *Escherichia coli* by heat-shock transformation (Sambrook *et al*, 1996). Selection of intact clones containing right sequence was initially performed through restriction enzyme digestion (with *SalI* and *Xho*I enzymes). To avoid un-wanted mutation during amplification, the selected clones were sequenced by Macrogen (Korea) using universal primers for PTG19-T.

Preparation of recombinant CP

The pET28a bacterial expression vector was used for large scale production of recombinant coat protein of CpCDV. The cloned CP gene was inserted into pET28a via *Sal*I/*Xho*I restriction reaction and new construct, pET28-CpCDV-CP, was transformed to BL21 (DE3) strain of *E. coli*.

Expression of the CpCDV-CP in the bacterial host was induced through Isopropyl β-D-1-thiogalactopyranoside (IPTG). Extraction and purification of recombinant CP were performed under native conditions at 4 °C. The bacterial cells were harvested after overnight by centrifugation. The cells were re-suspended in lysis buffer (50 mM NaH_2 PO_4, 300 mM NaCl, Imidazole) and disrupted by sonication. Debris was removed by centrifugation at 12,000 x g for 30min. Purification of recombinant protein was carried out through Immobilized metal ion affinity chromatography (IMAC) (Qiagen, Hilden, Germany) following the manufacturer's instructions.

The purity and integrity of the produced recombinant CpCDV-CP was evaluated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (stacking gel 4%, pH 6.8; separating gel 12%, pH 8.8) post stained with Coomasie

Briliant Blue (Ausubel *et al*, 1995). Protein concentration was estimated using bovine serum albumin (BSA) as standard.

Preparation of antibody

Two white inbred rabbits (2-2.5 kg, 10-12 months old) were used for immunization. Six subcutaneous injections were given fortnightly in two sites per injection. Injections performed over the back of the rabbits 0.5ml per site, 2 sites per rabbit. Each injection contained about 100µg of CpCDV recombinant coat protein as antigen. In the first injection the antigen was emulsified in complete Freund's adjuvant (Sigma, Deisenhofen, Germany) and in subsequent injections in incomplete Freund's adjuvant. After gaining sufficient antibody response (14 days after 5^{th} immunization) blood was drawn by intra-cardiac(IC) method under anesthesia. Rabbits were euthanized without recovery from anesthesia. The collected blood was let stands one hour at room temperature then placed in 4 °C overnight. The clot was removed and it was centrifuged in 1500g for 10 minutes and the The serum fraction was collected and stored at -20 °C.

Titration of antibody

To determine the antibody titer, small quantities (500µl) of blood samples were taken after each boosting from marginal ear vein and analyzed for binding against CP by Indirect-ELISA as described earlier (Safarnejad *et al*, 2008). A Nunc-Immuno™ MaxiSorb™ 96-wells microtiter plate (Thermo Fisher Scientific Inc.) was initially coated with 10µg ml^{-1} of purified recombinant CpCDV-CP diluted in carbonate coating buffer (15mM Na_2CO_3, 35mM $NaHCO_3$, pH 9.6) and plate was incubated overnight at 4 °C.

To prevent non-specific binding, blocking reagent (2% (w/v) skim milk (Fluka, Neu-Ulm, Germany) in 1 × PBS) was added into the wells. Serial dilutions of serum (1/512-1/262144) prepared in 1 × PBS was added to the coated plate and incubated for 2 hours at 37 °C. Detection of bound antibody was done by addition of 1/3000 diluted alkaline phosphatase-conjugated goat anti-rabbit IgG (Abcam, UK) for 2 hours at 37 °C. Finally, *p*-nitrophenyl phosphate (pNPP) as substrate (Sigma, Deisenhofen, Germany) was added and the plate was placed at room temperature for 20 - 60min and every 15 minutes the absorbance values at 405 nm was measured using a micro plate reader (EL_X808 Absorbance Microplate Reader, Winooski, VT, USA).

Purification of IgG and Conjugation with Alkaline Phosphatase

Antibody purification from serum was performed using Protein A spin column according to the manufacturer's manual (AbD Serotec kit, UK). The concentration and the purity of antibodies were determined by SDS-PAGE. Purified antibodies were conjugated to alkaline Phosphatase in a one-step procedure using the homo-bifunctional reagent glutaraldehyde (Wisdom, 2005).

Detecting of infected plant

Double-antibody sandwich ELISA: Specificity of the prepared antibody against CpCDV infected plant was determined through double antibody sandwich-ELISA (DAS-ELISA) as described by Clark and Adams (1977). A Nunc-Immuno™ MaxiSorb™ 96-wells microtiter plate (Thermo Fisher Scientific Inc.) was initially coated with purified anti-CpCDV IgG (dilution of 1:500) and incubated overnight at 4 °C. Plant extraction from leaves of healthy and infected chickpea was performed in a plastic bag with a roller.

The extraction was completed by adding 1:20 (w/v) in extraction buffer (1 × PBS pH 7.5, 5 mM EDTA, 5mM β-mercaptoethanol or 2% (v/v) polyvinylpyrrolidone, molecular weight 25000 (PVP-25). The plant extracts were added to the immune-plate and incubated overnight at 4 °C. After that, the alkaline-phosphatase–conjugated anti-CpCDV IgG (dilution of 1: 500) was added and incubated 3 hours at 37 °C. Finally, the substrate (pNPP) was added and after 30min absorbance values were read at 405nm. The sample was identified as positive if the mean DAS-ELISA (A_{405}nm) value of sample exceeded at least twice that of the healthy control(s).

Blotting analysis

Capability of prepared AP-conjugated IgG for detection of infected plants was further evaluated by dot-blot immunobinding assay (DIBA) (Makkouk *et al* 1993) and western blot analysis (Ausubel *et al*., 1995). In DIBA assay, the extraction was performed by adding 1: 10 (w/v) in extraction buffer (1 × PBS pH 7.5, 5mM EDTA, 5mM β-mercaptoethanol). 5µl of each were disposed on nitrocellulose membrane.

In western blot assay, the samples were initially mixed with loading buffer and boiled for 3min. the protein samples were separated on a 12% SDS-PAGE. The protein bands were transferred onto PVDF membrane.

In both DIBA and western blot assay, the membrane was blocked with PBS buffer containing 2% skimmed milk powder (w/v). The target protein was detected by 1: 500 diluted AP-labeled anti-CpCDV antibody. The bound antibody was revealed by addition of substrate NBT/BCIP.

Results

Isolation and cloning of CpCDV-CP gene

Total DNA was initially extracted from CpCDV chickpea plants showing symptoms with yellowing and stunting (Fig. 1). The infected plants were detected through PCR analysis with specific CpCDV primers (Fig. 2A). The gene encoding CpCDV-CP was PCR-amplified by using the total DNA extracted from infected plants. The amplified fragment with a size around 600 bp (Fig. 2B) was recovered from the gel and cloned into the pTZ57R/T vector. The accuracy of cloning of CpCDV-CP gene in obtained clones was assessed by PCR amplification, restriction analysis and sequencing. The right clone containing the intact sequence was selected for sub-cloning in pET28a bacterial expression vector. The nucleotide sequencing data confirmed correct in-phase insertion of the full-length CP gene in the expression vector in frame upstream of a 6 × His-tag at the C-terminal. Blasting analysis of CpCDV-CP gene in NCBI databases indicated that this gene encodes a protein of 172aa which has the most similarity with coat proteins of some geminiviruses such as; CpCDV, *Chickpea redleaf virus*, *Chickpea yellows virus* and *Tobacco yellow dwarf virus-A.*

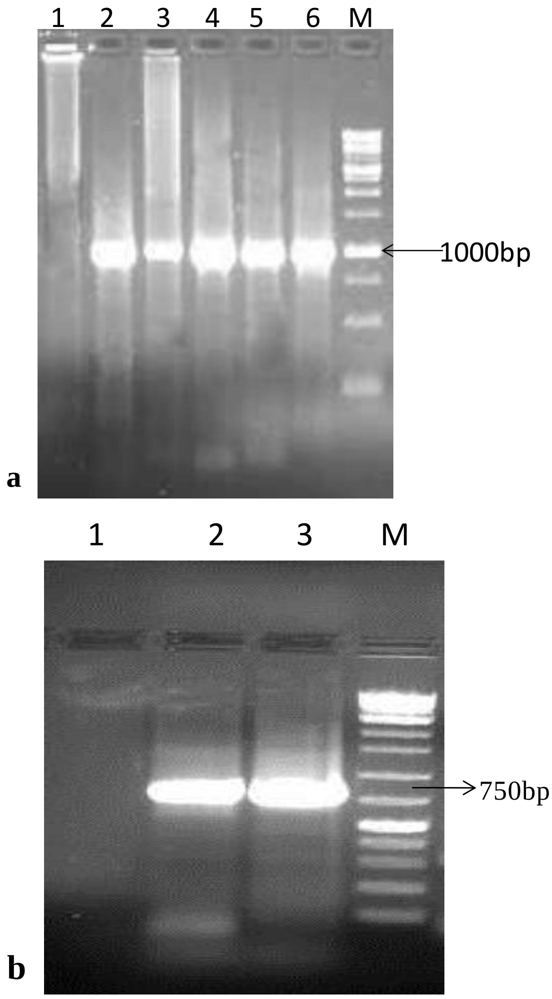

Figure 2 Electrophoresis of PCR product on 1% agarose gel. **A)** The PCR analysis for detection of infected chickpea plants by specific CpCDV primers 1: healthy chickpea plant; 2-6: infected plant samples; M: 1kbp DNA ladder. **B)** The PCR amplification for isolation of CpCDV-CP gene. M: 1kbp DNA ladder; 1: healthy chickpea plants; 2-3: chickpea infected plants**.**

Expression and purification of CpCDV-CP
Samples from different steps of expression and purification were prepared and subjected to SDS-PAGE analysis. These results revealed presence of CP protein with high purity and integrity with a size of about 31 kDa, which roughly corresponded to the predicted size of the complete CpCDV CP (27kDa) plus the fused N-terminal His-Tag/thrombin/T7-Tag (4kDa) (Fig. 3A). Complementary western blot analysis using anti His-tag monoclonal antibody revealed presence of a distinct band at the expected position. Thus, it was concluded that the exposed band is related to CpCDV-CP recombinant protein (data not shown here). Concentration of the purified CP was estimated to be about 500µg/ml, well above the optimal concentration of antigen for immunization of rabbit.

Antibody preparation and characterization
The purified recombinant coat protein was used for raising rabbit polyclonal antibodies. At first, around 100µg of purified recombinant CP was injected subcutaneously into rabbits. After each boosting, the antibody titer was determined by indirect ELISA. When the raw CpCDV antiserum (sixth bleeding) produced had a titer of 1/65000, blooding was performed and whole serum was isolated from other blood cells (Fig. 4). No significant reaction was observed in extracts from negative control samples. The IgG molecules were affinity purified from serum by using staphylococcus protein A. The purity of prepared polyclonal antibodies was visualized by SDS-PAGE electrophoresis which appeared as approximately 25kDa and 50kDa bands corresponding to light and heavy chains (Fig. 3B). The concentration of antibody was estimated around 1mg. ml^{-1}, by comparison to known amount of BSA, as a standard protein. The purified IgG molecules were used for conjugation with alkaline phosphatase enzyme.

Immunoassay analysis
Specificity and efficacy of produced polyclonal antibodies against recombinant CP was confirmed by DAS-ELISA as well as DIBA and western blotting analysis. In DAS-ELISA approach, the prepared IgG and conjugate were used for detection of CpCDV within plants. This technique prepares a scaffold for making direct comparison between infected plants and to quantify the pathogen.

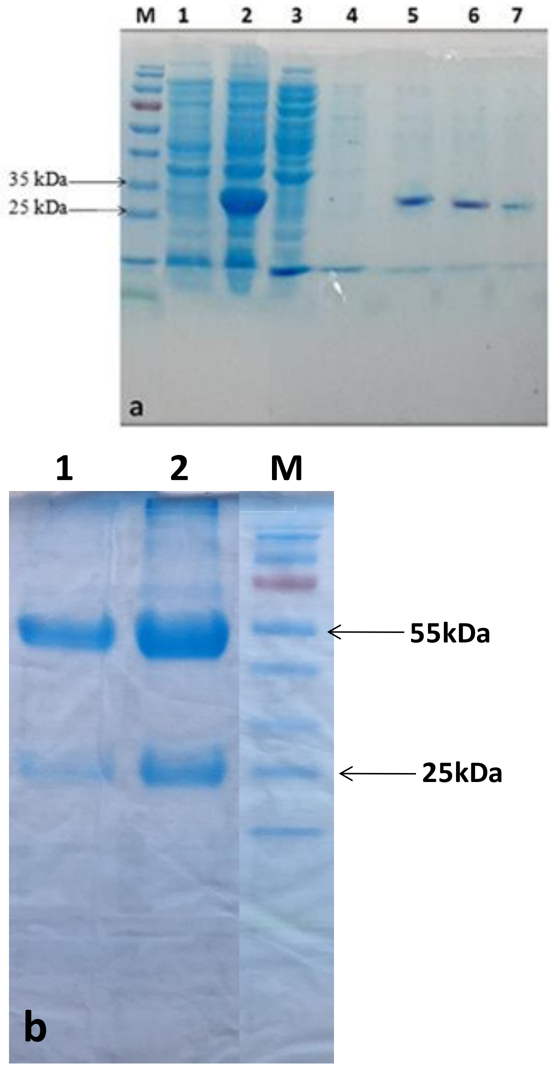

Figure 3 A: SDS-PAGE analysis of the expressed CpCDV coat protein gene in *E. coli*. Proteins were separated on 12% polyacrilamide gels and stained with Coomassie brilliant blue. M: pre-stained protein marker, 1: non- induced bacterial cells, 2: IPTG-induced cells, 3: flow through of column, 4: after washing, 5-7: elution steps. **B:** SDS-PAGE analysis of the affinity purified rabbit IgG. 1 and 2) purified IgG obtained in elution steps No. 1 and 2; M) M: pre-stained protein marker

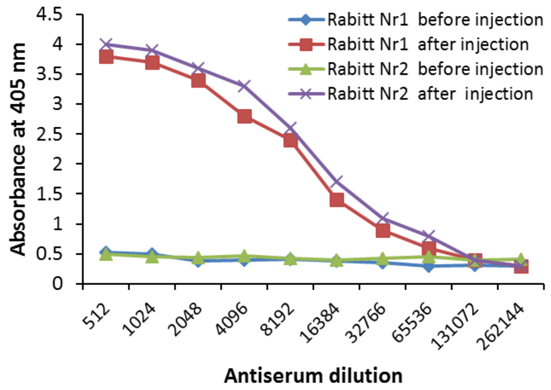

Figure 4 Determination of polyclonal antibody titer obtained from rabbits immunized by recombinant coat protein of CpCDV estimated by indirect ELISA. Absorbance values were read at 405 nm after 30 min incubation with AP substrate. Each value represents the mean absorbance value of two replicate wells.

Based on the results obtained in DAS-ELISA, prepared antibody and conjugate are able to detect CpCDV in chickpea leaf extracts (Fig. 5). As a parallel, the results obtained from PCR analysis on same plant samples were used in ELISA and proved presence of virus in positively detected plants (data not shown).

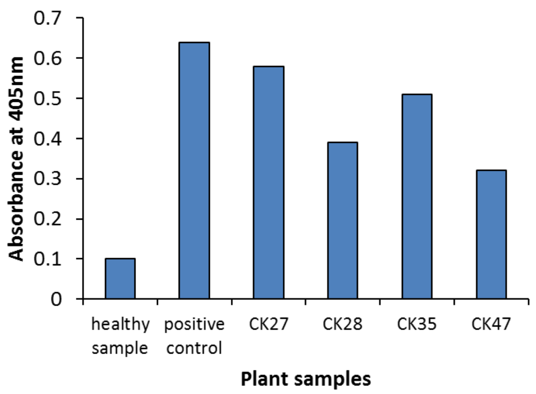

Figure 5 Detection of infected plant samples using DAS-ELISA using purified IgG and AP-conjugated IgG prepared against recombinant CP protein, at a dilution 1:500. CK27, CK28, CK35 and CK47 represent different chickpea samples collected from fields in Kermanshah region, Iran.

Complementary DIBA analysis was performed to evaluate the specificity of prepared conjugate antibody against recombinant and native CP protein. The results proved the binding ability of antibody against CpCDV present in infected plant and recombinant CP (Fig. 6A).

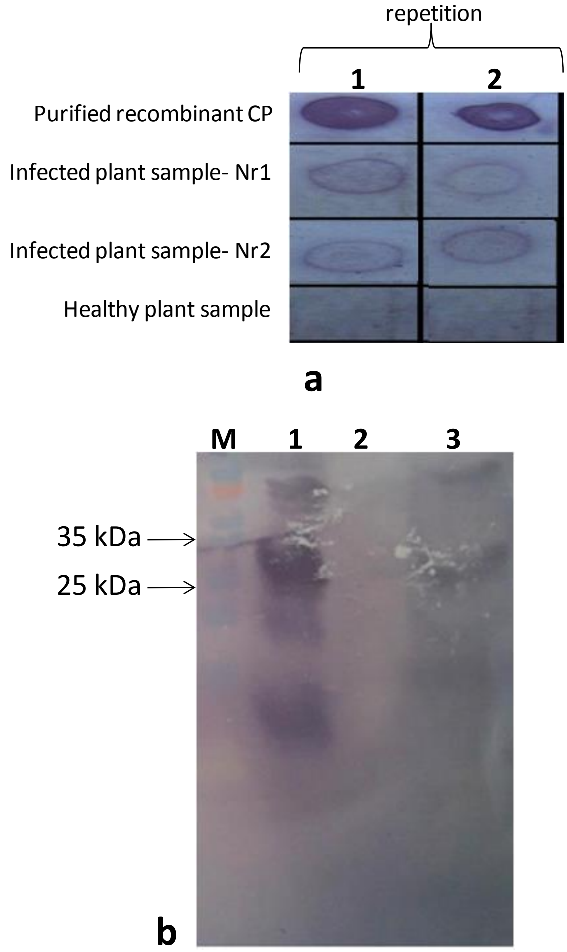

Figure 6 Blotting analysis by the use of anti-CpCDV coat protein IgG conjugated to Ap (diluted 1:500). The bound antibody was revealed by addition of substrate NBT/BCIP. **A:** detection of CpCDV infected plants through dot blot imuunoassay. **B:** Detection of infected plants by western blot analysis. Lane M, pre-stained marker; lane 1, bacterial expressed recombinant coat protein of CpCDV; lane 2, healthy plant; lane 3, CpCDV-infected chickpea plant.

The western blot analysis showed presence of a unique band in the position expected for CpCDV coat protein (Fig. 6B). In blotting analysis, no positive reaction was observed in healthy plant samples. These results re-confirm capability of AP-conjugated IgG for detection of virion particles within infected plants

Discussion

The chickpea chlorotic dwarf is an important disease causing major losses in legume crops throughout the world. Developing of diagnostic approach for efficient detection of virus within the infected plant is important for early detection and monitoring presence of virus in plant cultivars to define sources of resistance against the disease (Al-Moslih, 2012; Kumari *et al*, 2006). Up to now, several laboratory approaches have been developed for efficient detection of plant viruses. These assays are mainly based on physical, biological, cytological, serological, and molecular properties of viruses. Among these methods, the serologically based approaches, such as ELISA and DIBA, are user-friendly, rapid, and precise for detection of viruses within plants (Al-Moslih, 2012; Astier *et al*, 2001). To produce specific antibodies against obligate parasites, the major problem is preparing of pure materials from pathogen that is actually needed for immunization of the animals. Present article describes developing of specific antibody against CpCDV through recombinant coat protein approach. Due to difficulty for purification of viral particles from infected plants, expression of structural viral genes in bacterial host is an alternative strategy for the preparation of antibodies against viruses (Shahmirzaie *et al*, 2019). However, owing to difference in folding of native and recombinant proteins, application of this approach may lead to production of antibodies which are unable to recognizing native epitopes (Koolivand *et al*, 2016; Korimbocus *et al*, 2002). The results obtained here prove efficacy of raised antibody for efficient detection of infected chickpea plants through DAS-ELISA and blotting analysis. In a similar approach, other investigations have proven feasibility of recombinant expressed viral proteins to prepare antibodies for the detection of a number of plant viruses (Abou-Jawdah *et al*, 2004; Cerovska *et al*, 2012; Čeřovská *et al*, 2003; Iracheta‐Cárdenas *et al*, 2008; Kumari *et al*, 2001; Lee and Chang, 2008).

The gene encoding coat protein of CpCDV was expressed as a fusion with His Tag/thrombin/T7 Tag in bacterial host. The SDS-PAGE analysis shows that the produced CpCDV coat protein size is around 31 kDa which matched the deduced amino acids expected by using clone manager software. About 4 kDa of this mass is due to the fused tag and the remaining, that is around 27 kDa, stands for CP mass which is similar to earlier reports (Horn *et al*, 1993; Kumari *et al*, 2006). Nevertheless a little amount of expressed protein was detected in the pellet fraction, but a considerable part of the expressed recombinant protein was in the soluble fraction which is supported by many authors (Koolivand *et al*, 2016; Lee and Chang, 2008; Raikhy *et al*, 2007). There are many other reports expressing that the recombinant coat protein of plant viruses are insoluble within the bacterial cells and are localized in inclusion bodies (Galluzzi *et al*, 2007; Hema *et al*, 2003; Jain *et al*, 2005).

Furthermore, the results obtained in dot-blot assay proved its efficacy for rapid detection of infected plants while no background was observed in healthy samples. Practically, this test requires 3 h to be completed and compared with 2 days for DAS-ELISA, it is much more rapid. Thus, dot-blot assay seems to be very useful for CpCDV detection in dry tissue extracts in laboratories where ELISA readers are not available.

In conclusion, results obtained in present study prove that the antibody prepared against Iranian isolate of CpCDV is highly specific and can be used for surveys of chickpea fields and monitoring of virus multiplication in sources of resistance to this virus in chickpea breeding programs. Likewise, it provides necessary biological component that is actually needed for developing of much more sensitive biosensors against the disease.

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تولید آنتی‌بادی اختصاصی ویروس *Chickpea chlorotic dwarf virus* (CpCDV) با روش مبتنی بر پروتئین نوترکیب

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چکیده: حبوبات از قبیل نخود و عدس عمدتاً در مناطق نیمه‌خشک دنیا کشت می‌شود. ویروس کوتولگی سبزرد نخود (*Chickpea chlorotic dwarf virus*, CpCDV) از عوامل مهم ایجادکننده زردی، کوتولگی و کاهش شدید میزان محصول نخود در دنیا و ایران می‌باشد. تولید آنتی‌بادی‌های اختصاصی این ویروس اهمیت زیادی در راستای انجام مطالعات میدانی و ارزیابی مقاومت ارقام به این بیماری دارد. هدف از اجرای این پژوهش تولید آنتی‌بادی اختصاصی علیه ویروس CpCDV با استفاده از پروتئین پوششی نوترکیب می‌باشد. برای این‌منظور ابتدا ژن تولیدکننده پروتئین پوششی ویروس CpCDV با استفاده از پرایمرهای اختصاصی تکثیر و جداسازی شد و سپس در ناقل بیانی باکتریایی همسان‌سازی صورت گرفت. بیان و تولید پروتئین نوترکیب در سویه BL21 باکتری *Escherichia coli* انجام شد. خالص‌سازی پروتئین نوترکیب با روش کروماتوگرافی تمایلی و میل ترکیبی پروتئین نوترکیب حاصله با ستون حاوی یون نیکل صورت پذیرفت. کیفیت پروتئین نوترکیب خالص با روش الکتروفورز پروتئین بررسی شد. پروتئین نوترکیب جهت ایمنی‌زایی به خرگوش تزریق شد. خالص‌سازی آنتی‌بادی با روش کروماتوگرافی تمایلی و با استفاده از ستون پروتئین A صورت پذیرفت. از آنتی‌بادی خالص شده برای تهیه کانژوگه (آنزیم آلکالین فسفاتاز-آنتی‌بادی) استفاده شد. قابلیت و کارآیی آنتی‌بادی و کانژوگه جهت شناسایی نمونه‌های گیاهی آلوده با آزمون‌های سرولوژیک داس-الیزا، وسترن بلات و دات‌بلات مورد بررسی قرار گرفت. نتایج حاصل از آزمون‌های سرولوژیک حاکی از قابلیت بالای آنتی‌بادی حاصله جهت تشخیص نمونه‌های آلوده به ویروس می‌باشد. براساس اطلاعات موجود این اولین گزارش از تولید آنتی‌بادی اختصاصی ویروس CpCDV با استفاده از روش‌های مبتنی بر پروتئین نوترکیب می‌باشد.

واژگان کلیدی: آنتی‌بادی، نخود، ویروس، الیزا، پروتئین نوترکیب