RNA-3 PCR IDENTIFICATION OF UKRAINIAN ISOLATE OF BEET NECROTIC YELLOW VEIN VIRUS

K. GRYNCHUK[1], I. ANTIPOV

The bioinformative analysis of the nucleotide sequences of RNA-3 virus isolates of beet necrotic yellow vein (BNYVV) was made. The conservative sequences of a gene that encodes a protein 25 (P25) have been shown. Primers design for identification of RNA-3 has been made. The PCR system for RNA-3 identification was optimized by temperature rates of primer annealing.

Key words: *beet necrotic yellow vein virus, polymerase chain reaction, identification, gene, primer*

Introduction. Beet necrotic yellow vein virus is belonging to the genus *Benyvirus*. BNYVV consists of 4-5 rod-shaped particles, which encapsidate 4-5 genomic ss(+) strand RNAs, depending on the isolates [4, 6]. BNYVV causes proliferation of roots from the main root, reducing the size of root and leaf yellowing of veins. RNA-3 [1175 nucleotide in length excluding the poly (A)-tail) codes protein 25 kDa, (P25, 445-1102 nucleotide) is important for the symptoms formation [1].

The RNA-3 is responsible for symptom development in sugar beet roots and the formation of local lesions in experimental hosts like *Chenopodium quinoa*. On different susceptible and partial resistant sugar beet leaves observed the formation of necrotic lesions [5]. RNA-3 formed more pronounced local affected at a time when the absence of RNA-3 observed more soft signs of injury. RNA-3 is needed for virus multiplication and movement in the roots [2, 3].

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[1] Supervisor – Ph.D of philosophy I. Antipov

The purpose. Development of PCR system for RNA-3 of BNYVV detection and identification.

Materials and methods.

BNYVV RNA-3 nucleotide sequences were searched with NCBI database (National Center for Biotechnological Information) [9]. Nucleotide sequences alignment was performed using the software «MultAline» (Multiple sequence alignment) [7]. Design of primers was created with the software «Primer3» [8].

Extraction of RNA was performed using a commercial kit «RiboSorb» (AmpliSens, Russia), reverse transcription reaction was performed using a commercial kit «Reverta-L-100» (AmpliSens, Russia), according to recommendations.

15 ml of reaction mixture for the polymerase chain reaction (PCR) consists of: 1x PCR buffer with 1,5 mM $MgCl_2$ (AmpliSens, Russia), 0,2 mM deoxynucleoside triphosphate (dNTF) (AmpliSens, Russia), 1 pkmol each of oligonucleotide primers, 10-40 ng cDNA, 0,5 U Taq-polymerase (AmpliSens, Russia). The amplification reaction of DNA was performed in thermocycler «Tertsyk» TP4-PCR 01.

After amplification PCR products were separated by horizontal electrophoresis in 1,5% agarose gel, which was prepared using TBE buffer with a concentration of 0,5 mg/ml ethidium bromide. PCR results were visualized UV rays (T-312-C).

Results. An alignment of the nucleotide sequences of BNYVV RNA-3 was made. The analysis involved 266 fragments of nucleotide sequences BNYVV of RNA-3 of that are registered in the genetic database NCBI. The consensus nucleotide sequence of full RNA-3 of BNYVV analysis was performed (Figure 1).

The conservative (uppercase letters) and polymorphic (small letters) fragments of genomic RNA-3 of BNYVV are shown. Strictly conservative fragments were selected for further primers design. Polymorphic sites of the genome were excluded from the analysis. This ensures that a universal system

for identifying all existing patotypes and potential isolates of RNA-3 of BNYVV.

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AAATTCAAAA TTTACCATTA CATATTGGTA TtTATTTACC CTCAGTTGGT GATATATGTG AGGACGCTAG
CCTGTTGGGT TTCCTGACCG ACCAAATCCA AGCGAGCTTA ATCCAAGTAC CTCGTCTCAA ATTGAGTGTC
AAGTGAATAA GCATAGTGAC cCCATCGTTT CAGGGTAGTT gACGGCTATT AATAGAcATA tTACaAACGC
TTCTCTTTAT TTATCaCCAA CATGGGATGT AATGTTTATG CGTGAGCcTA CGGCCGCATT GTAAAATTAG
TGGTTTTGAA TTTCTATTCT tCGGAATATa CAAGGTTTAA AAGACCAgCa TTTGGGTTAA AAATTTTTAA
ACCTTACTAT CtTTaACTAG tAACTcGAAc TCGATTTATA TTCAGATTTT aaataTCaaG TTGTTGTGTT
tTCTGAtCAT CATTAAGTGa CCGtCATGGG TGATATATTA GGCGCAGTTT ATGATTTAGG GCACAGACCT
TACCTAGCAC GGCGTAcGGT TTATGAGGAT CGTTTGAtTC TTAGcACAcA TGGTAATaTC TGTCGgGCTA
TTAACTTGtT AACTCACGAT AATCGtACTa CACTGGTGTA TCACAATAAt ACTAAACGCA TAAGGTTTCG
TGGaTTATTG TGTgcTtaTC aTggGCCTTA TTGTGGGTTT CGTGCCTTAT GTAGAGTAAT GTTATGTTCt
cTACCTCGTT TGTGTgACAT CCCTATCAAT GGATCTCgCG aCTTTGTtGc aGATCCTACC AGACTCGACA
GCTCTGtTAA TGAGTTGcTG GTTTCTAatG GTCTCGTCAt CCACTATGAT CGTGtTCATc ATGtTCCCtT
ACAcACTGAt GGTTtTGAAG TTGTAGAtTT cACGACTGTC TTtCGTGGTC CtGGAAAcTT TCTTTTGCCT
AATGCAACAA ATTTCCCTCG GcCaACCACA ACCGATCAGG TTTACATGGT gTGTTTGgTA AACACGGTTa
ATTGTGTGTT ACGTTTTgAG TCcGaACTTa cAGTGTGGgT TCACTCTGGT TTGTATaCAG GtGATGTTTT
AGATGTGGAT AATAATGTTA TTCAAGCCCC TGaCGGTGTT GATGATgATG aTTAGAGTTa TCACAATTTC
AACAACACAC TTaTTGGTGT GTTGTTCTGT TACACCATTT GAAAGTTTAA TAATTgTCTC AaTtCGATtG
TTGATCTGGT TGGGACAATT ATTTTATTTT CTTTTGGTgt AATCGTCCGA AGACGTTAAA CTACACGTGA
TTTCACGGTG TTCGATGAGA AGATTGTTTA ACGGTGTtAC GTTGTgtAcc TTTAAGCtTT CTTCTCaTTT
tACCACATGT GATGATTGTA GCCTGTGGGT tGTTATGTGG ACAATTATGG TTACTTATTT GTAAAtGaTA
AAGAGTGTGC gGTAGCcGAC TTTATgCGAG TGGGAGTAGT TGTGTTATTA CTACTATTCT GGTtCGTATA
AAGATCCTTG ACGGCGGCAT CGTGGGTTCC ACAGCCGGTT ACATGgTGTT CCCGTCCGTT TACGAAGGTT
TAACTGTGAG CCTTGTATTT TACaAATACA CAGTTTTTAT CtTAACAGGC TCGTTCACAA GCCTCCTTTT
ACATTAAGTT TAAAGGtTTA TGTGGACACA AAAATATGGC TTATTGGTTA TGCTAAACCT CATATCATGT
TATAaTATTc GTTtcATaTT ATAATTAAgG tTAAGATGTA CTGaCTGGGT GtGAAATGTA CCAGTCCTTG
TAGGGTTCTT TGTCAGTATA TTGACAAAAA AAAAAAAAAA
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Fig.1. The consensus sequence of RNA-3

Primer design with optimum characteristic was made. For synthesis was chosen primers Forward 5'-TGTGGGTTTCGTGCCTTATG-3 'and Reverse 5'-CGTCAGGGGCTTGAATAACATT-3' with the names of P25-F and P25-R. The calculated optimum temperature primers annealing for Forward primer is $58{,}47^0$ C and for Reverse primer is $58{,}98^0$ C, percentage deoxyguanosine-5'-phosphate and deoxycytidine-5'-phosphate for Forward primer 50%, and Reverse 45,45% for the primer that are the best characteristics (Table. 1).

1. Molecular and biological primers characteristics for the identification of gene P25 of Ukrainian isolate of BNYVV

Gene	Possition on matrix	Nucleotide sequence 5'-3'	Number of nucleotides	Annealing temperature, ^{0}C	GC-composition, %	Product size, n.p.
P25	Forward	tgtgggtttcgtgccttatg	20	58,47	50,00	424
	Reverse	cgtcaggggcttgaataacatt	22	58,98	45,45	

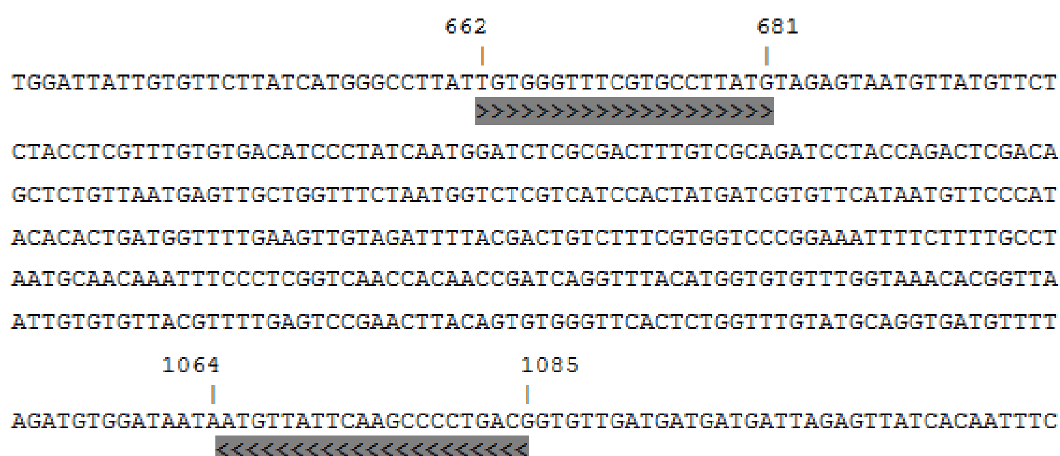

Fig. 2. Localization of primers sites hybridization on the DNA matrix of P25 BNYVV consensus sequence

The PCR was performed using cDNA of Ukrainian isolate of BNYVV under the following conditions: initial denaturation 5 min – 94^{0} C; 30 cycles: denaturation 30 sec – 94^{0} C, annealing of primers 30 sec – 60^{0} C, elongation 30 sec – 72^{0} C, 72^{0} C final synthesis – 7 min.

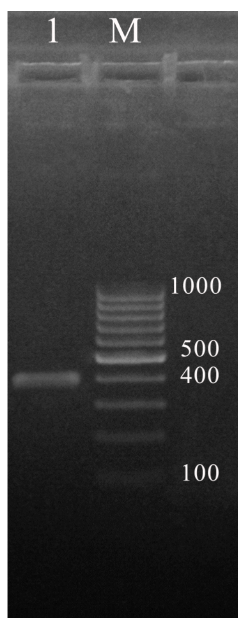

Fig. 3. The electrophoregram of PCR product analysis for the determination of RNA-3 of BNYVV: M (GeneRuler 10 bp DNA Lader 0241) – lengths of fragments (base pairs) marker

As a result, the visualization of PCR amplification products revealed fragments of size – 424 pairs of nucleotides. Presence of expected reaction product on the electrophoregram indicates that the developed system for the identification of RNA-3 of BNYVV is effective (Figure 3). During the next stage of our research we were supposed to figure out the operating temperature of primers annealing. Made a series of reactions and multi-temperature annealing of primers from 50^0 C to 64^0 C. PCR was performed under the following conditions: initial denaturation 5 min – 94^0 C; 20 cycles: denaturation 30 sec – 94^0 C annealing of primers with 30 sec – 50^0 C – 64^0 C, 30 sec elongation – 72^0 C, 72^0 C final synthesis – 7 min.

Although primers annealing temperature did not affected significantly the amplification reactions efficiency conditions, we have discovered that the most optimal temperature is in the range of 50^0 C – 60^0 C.

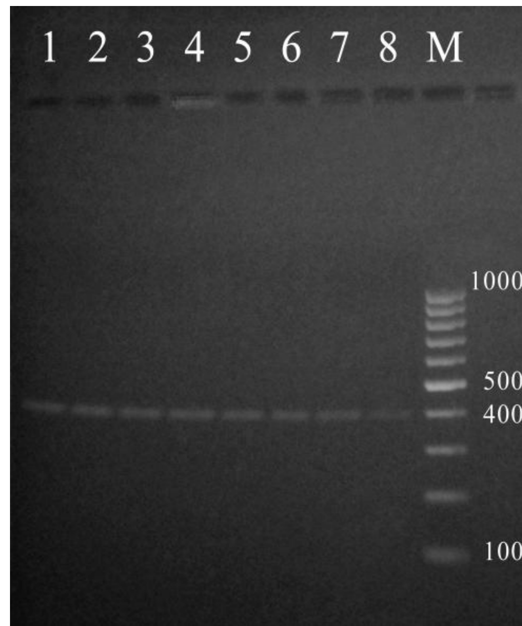

Fig.3. The electrophoregram of optimization of primers annealing temperature for the identification of gene P25: 1 – 50^0 C, 2 –52^0 C, 3– 54^0 C, 4 – 56^0 C, 5 – 58^0 C, 6 – 60^0 C, 7 – 62^0 C, 8 – 64^0 C, M (GeneRuler 10 bp DNA Lader 0241)

Under these conditions nonspecific amplification products were not observed, and the amount of amplicon was sufficient for clear visualization (Figure 4).

Conclusions. 1. A bioinformative analysis of nucleotide sequences of RNA-3 of BNYVV was done. 2. Conserved sequences of the gene encoding protein P25 of BNYVV were shown and implemented design primers for identification of RNA-3 of BNYVV was made. 3. PCR system for the identification of RNA-3 of BNYVV for temperature annealing primers was optimized.

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ПЛР ІДЕНТИФІКАЦІЯ РНК-3 УКРАЇНСЬКОГО ІЗОЛЯТУ ВІРУСУ НЕКРОТИЧНОГО ПОЖОВТІННЯ ЖИЛОК БУРЯКУ

К. В. ГРИНЧУК, І. О. АНТІПОВ

Проведено біоінформативний аналіз нуклеотидних послідовностей РНК-3 ізолятів вірусу некротичного пожовтіння жилок буряку (ВНПЖБ). Встановлено консервативні послідовності гена, що кодує протеїн P25 ВНПЖБ та розроблено дизайн праймерів для ідентифікації РНК-3. Оптимізовано ПЛР систему ідентифікації РНК-3 за температурними показниками відпалу праймерів.

Ключові слова: *вірус некротичного пожовтіння жилок буряку, полімеразна ланцюгова реакція, ідентифікація, ген, праймер*

ПЦР ИДЕНТИФИКАЦИЯ РНК-3 УКРАИНСКОГО ИЗОЛЯТА ВИРУСА НЕКРОТИЧЕСКОГО ПОЖЕЛТЕНИЯ ЖИЛОК СВЕКЛЫ

ГРИНЧУК К. В., АНТИПОВ И. А.

Проведен биоинформативный анализ нуклеотидных последовательностей РНК-3 изолятов вируса некротического пожелтения жилок свеклы (ВНПЖС). Показаны консервативные последовательности гена, который кодирует протеин P25 ВНПЖС и разработан дизайн праймеров для идентификации РНК-3 ВНПЖС. Оптимизирована ПЦР система идентификации РНК-3 ВНПЖС по температурным показателям отжига праймеров.

Ключевые слова: *вирус некротического пожелтения жилок свеклы, полимеразная цепная реакция, идентификация, ген, праймер*