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Wheat Dwarf Virus Detection and its Possible Entrance in Georgia

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ABSTRACT. Wheat dwarf virus, which strongly affects crop development, is discovered in Turkey. As Georgia is a neighboring country of Turkey. There is a threat of virus entrance. So, in the present study, we offer most cheap and sensitive methods for detection of the virus in wheat and *Psamottetix alienus*. *© 2008 Bull. Georg. Natl. Acad. Sci.*

Key words: wheat dwarf virus, *Psammotettix alienus, PCR, RCA.*

Wheat dwarf virus is the genus *Mastrevirus* in the family *Geminiviridae*. It has a monopartite genome, containing one single stranded DNA packaged in geminate particles. The size of the DNA varies between 2.700kb to 3.000kb [1]. The virus disease of wheat often occurs in patches in the field, but in the worst case, infection may result in crop failure. The symptoms are dwarfing, mottling and yellowing. Suppressed heading in the plant can be the reason of low yield. The characteristic red or yellow leaf symptoms were attributed to limited nutrient uptake by plants in soils. However, Vacke [2] from the former Republic of Czechoslovakia described the disease and gave it the name *Wheat Dwarf Virus* (WDV). It was proved that the virus was transmitted from infected plants to healthy ones, by specific vectors. Transmission of WDV is a circulative, nonpropagative type of transmission and is characterized by the circulation of virions throughout *Psammotettix alienus* body suspended in haemocoel.

The disease was relatively uncommon, but during the last 20 years it has occurred in Sweden, Hungary, France, Germany and Turkey [3]. Total worldwide damage, due to this virus, is as high as 60 USD billion per year. In "developing countries", where maximum crop yields are often required to maintain basic food supplies, even a relatively small or complete loss of crop will have disastrous consequences. As Turkey lies next to Georgia, there is a great risk of virus entrance. Bearing in mind the above mentioned, the object of our research was to find the cheapest and most sensitive methods for WDV detection.

Materials and methods. Experiments were conducted on infected wheat and leafhoppers (*P.alienus*). The DNA extraction was performed by Nucleospin plant DNA isolation kit (Machery- Nagel), by Phenol [5] and modified method of Dorochov [4] as follows: The samples were homogenised in 50μl PBS buffer in an 1.5 Eppendorf tube. Homogenisation was followed by the addition of 85ml extraction buffer (200mM Tris-HCL, pH 7.5, 250mM NaCl, 25mM EDTA). Afterwards, 15 μl of extraction buffer (200mM Tris – HCL, pH 7.5, 250mM NaCl, 0.1% sodium dodecyl sulphate [SDS] and 25mM EDTA) was added to the homogenate. The content was mixed by inversion and incubated for 15 min at 65°C. Then 50μl phenol and 50 ml chloroform were added and the sample was vortexed for 1 min. The mixture was centrifuged at 13.000 rpm for 10 min at room temperature. The aqueous phase was transferred to a fresh tube and DNA was precipitated by adding 10μl of 5M potassium-acetate and 150μl iso-propanol. The mixture was centri-

Table 1.

Primers used for viral DNA amplification

Name	Sense[a]	sequence [5´-3`]	Genome position[b]	Tm [°C] [c]	PCR Fragment size
WDV3-1750	C	5`-AACAGGAAAGACTTCCTGGGCA-3`	1730-1760	59.1	465
WDV5- 1285	V	5`-GGCTACGAGCAAAGAYAAACCAAA-3`	1230-1260	55.8	
WDV5 - 700	V	5`-AATAATCGGCATACAAATCAGACC -`3	650-700	53.3	550
WDV3 -1250	C	5´- TTTRTCTTTGCTCGTAGCCGAGC-3	1200-1270	58.8	
WDV5 -1240	V	5`- GCCGACGAACCACTTCCAGTT- 3	1200-1230	57.7	1220
WDV3- 20	C	5´- CTCACGAAAAGCCGTGTGCGC-3`	10-25	62.4	

a V viral strand, C complementary strand
b with reference to WDV-EN , AM296018, or BaW1..
c calculated with primer Express 2,0 (Applied Biosystems)

fuged at 13.000 rpm for 10 min at room temperature and DNA pellet was collected. DNA was washed with 70% ethanol and dried for 3-4 minutes under vacuum. Then it was redissolved in 1/10 TE buffer (10mM Tris HCI, pH 7.5), 1mM EDTA, 1µg/µl RNase A and stored at -20^{0}C.

Polymerase chain reaction (PCR) for partial genome sequence was achieved in a volume of 25 ml. Each reaction contained the DNA template (approximately 100 ng, measured with Nano-Drop spectrophotometer), virus specific reverse and forward primers (0.2 mM each), diluted 10 times in concentrated reaction buffer (provided by supplier of the Taq polymerase, containing 1.5 mM MgCl2, 0.2mM dNTPs, 1U of Taq polymerase (Promega, GoTaq A). The thermocycling conditions were: 96°C 2 min; 94°C 30s, 62°C 30s, 72°C 1 min, 31 cycles, final extension at 72°C for 10 min. The expected bands were analysed on a 1% agarose gel in TAE buffer, including ethidium bromide (EthBr) and viewed under UV illumination using a digital imaging system (INTAS UV system P93DW). Primers (see Table 1) were developed by the alignment of WDV sequences with the help of software DNAMAN (Lynnon, Biosoft, vers 5.0). Sequences were obtained in www.NCB.com or provided by J. Schubert (personal communication).

Rolling circle amplification of circular DNA was performed following the manufacturer's protocol (GE.Healthcare, Templiphi). 10 to 20 ng of total nucleic acids were dissolved in 5ml of sample buffer, denatured for 3 min at 95°C and cooled down to room temperature. After adding 5ml reaction buffer and 1U enzyme mix, the reaction was run for 18–20 h at 30°C and stopped by heating for 10min at 65°C. Aliquots corresponding to 5ml of RCA amplified nucleic acids in 30ml volume containing EcoRI restriction buffer with BSA (provided by the manufacturer of the enzyme) and 10U restriction endonuclease EcoRI were digested at 37°C for 90min. Restriction products were analysed on a 1% agarose gel and stained with EthBr and viewed under UV illumination using a digital imaging system (Intas UV system P93DW).

Statistical analysis of the data was performed with software "Statistical Analyses System (Sas. Proc. Freq Version 9.1).

Results and discussion. DNA was extracted from plants and *P. alienus* by Machery-Nagel Kit method. Nucleic acids were readily affordable to be amplified with PCR application. As shown in Fig. 1 in all cases lanes 1-4 and lanes 4-8 DNA had the required accuracy to be amplified.

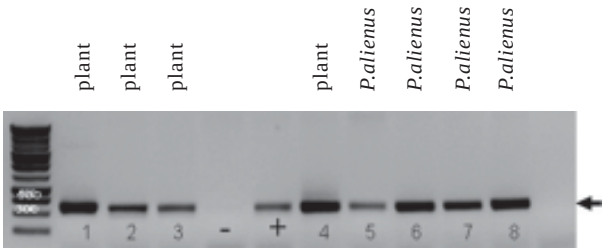

Fig. 1. Representative agarose gel of DNA extraction with the Machery-Nagel Kit and its amplification with PCR: Lanes 1-4, plant DNA, Lanes 5 till 8 DNA extracted from *P.alienus*. Arrow indicates expected bands of 500bp

By the method of Haible [5] it was possible to amplify DNA from plants (Fig. 2) lanes 5 to 8 but amplification of DNA, extracted from *P. alienus* (Lanes 1-4), was impossible due to some residual contamination.

With the modification of Dorochov [4] method, as described earlier (Fig. 3), it was possible to amplify DNA

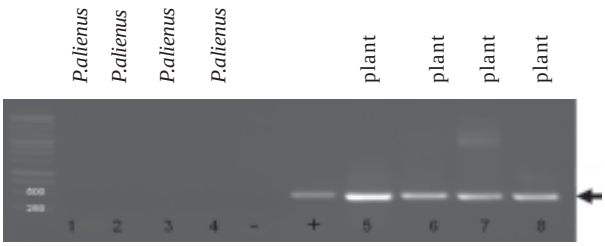

Fig. 2. Representative agarose gel of DNA extraction with phenol extraction method of Haible (5) and its further amplification with PCR. Lanes 1-4 DNA of *P. alienus*, lanes 5 to 8 DNA from plants. Arrow indicates expected 500bp bands.

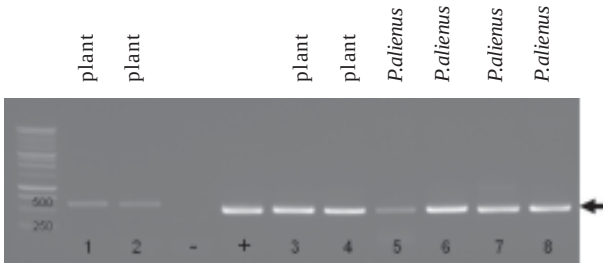

Fig. 3. Representative agarose gel of virus detection in plants and *P.alienus* by means of PCR. DNA extracted by modified method of Dorochov. The arrow indicates expected 500bp bands

with PCR application in all samples (plant and *P. alienus*).

The RCA technique for defining actively replicating *Geminiviruses* is ideal for virus detection [4]. On the other hand, the application of PCR has gained prominence as a sensitive assay method for tracking *Geminiviruses*. When both methods were compared , it was found that in our case the assay by PCR is more sensitive than RCA (Fig. 4). With the method of RCA (A lanes 1-5) it was impossible to detect expected bands of 1250 bb (A Lanes 1-5) However, in the same samples (B lanes 1-5) by PCR we detected expected bands of 500bp which indicated the presence of WDV.

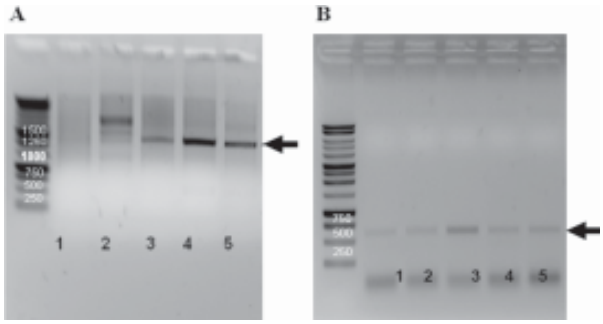

Fig 4. WDV detection in plant by RCA(A) and by PCR (B). The arrows indicate expected bands in the case of RCA ~1250 bp, and in PCR ~500bp

To assess the risk of virus spread it is necessary to determine whether the insects/plants are viruliferous or not. In order to identify them in a cheap and easy way, we have compared the RCA method with PCR application. The results showed that PCR application is a more sensitive method for *Geminivirus* detection in single insects and plants than RCA. Moreover, PCR application is a cheaper method for *Geminivirus* detection than RCA. The price of one sample PCR is 50 cents when one probe of RCA costs USD 5.

These results are not in contrast with the research of Haible [5], reporting that RCA method is ideal for detection of *Geminiviruses;* this method permits detection of *Geminiviruses* in plants but not in insects. One of the drawbacks of this method is that they are sensitive to impurities in the sample DNA. Thus, we eliminated contamination by nucleic acid purification. A number of different methods are available in the literature for isolation of nucleic acids. We compared DNA extraction by means of a Nucleospin plant DNA isolation Kit (Machery-Nagel), extraction by phenol [5] and DNA extraction according to Dorochov [4] modified by us.

By phenol extraction according to Haibles [5] method it was not possible to amplify DNA from insects, it does not eliminate residual contamination from insects (Fig. 1). Modified method of Dorochov [4] and DNA extraction Kit (Figs 1, 3) permits amplification of DNA in both insects and in plant. However, advantage of the method, developed by us is that it is much cheaper than DNA extraction with Machery - Nagel Kit. The price of Machery Nagel Kit for DNA extraction is nearly 3 USD, while the method modified by us costs not more than 10 cents per sample and its results are equal.

Conclusion

A new cheapest method of DNA extraction and WDV detection in plants and *P. alienus* is offered. The use of this method is advantageous for the Georgian agrarian sector to predict and prevent the disastrous consequences of virus spread in Georgia.

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მოლეკულური ბიოლოგია

ხორბლის dwarf virus-ის აღმოჩენა და საქართველოში მისი შემოჭრის საშიშროება

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შემუშავებულია დნმ-ის გამოყოფისა და ხორბლის dwarf virus-ის აღმოჩენის იაფი და მაღალ მგრძნობელობის მეთოდი, რაც უზრუნველყოფს საქართველოში ხორბლის მაზიანებელი ვირუსის შემოჭრის თავიდან აცილებას და ადრეულ ეტაპზე პრევენციული ზომების მიღებას.

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