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Determination of the Incidence Rate of Geographical Sub-groups of $PVY^{N/NTN}$ in Seed Potato Tubers in Turkey

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Abstract: In order to determine the incidence rate of tobacco veinal necrosis strain ($PVY^{N/NTN}$) and its geographical subgroups of European (EU) $PVY^{N/NTN}$ and the North American (NA) $PVY^{N/NTN}$ in seed potatoes belonging to various cultivars obtained from the main potato production areas in Turkey, a total of 320 tubers known to be infected with PVY were tested individually using S6+A primer pairs which determine in all $PVY^{N/NTN}$ regardless of their origin and type of strains. RT-PCR test result revealed that 264 out of 320 tubers were infected with $PVY^{N/NTN}$. When the samples found to be infected with $PVY^{N/NTN}$ were tested using S3+A (specific for EU-$PVY^{N/NTN}$), S4+A (specific for NA-$PVY^{N/NTN}$), S5+A (specific for NA-PVY^{NTN} and NA-PVY^{N}) primer pairs, it was determined that all positive samples (264) were found to be infected with EU-$PVY^{N/NTN}$, but NA-$PVY^{N/NTN}$ was not found in the cultivars used in this study.

Key words: Potato, PVY^{N} strain-groups, EU-$PVY^{N/NTN}$, NA-$PVY^{N/NTN}$

INTRODUCTION

Potato is affected by many pests and numerous fungal, bacterial and viral diseases. Given that potato is vegetatively propagated, viruses constitute a permanent threat for seed potato growers because virus diseases are systemic and transmitted through seed tubers. Among viruses, potato virus Y (PVY) is the type species of the genus Potyvirus, belonging to the Potyviridae family which is the largest and economically the most important group of plant viruses (Barnet, 1992). PVY has a long filamentous particle containing a single stranded, positive sense RNA genome of approximately 10 kb in length with a poly-A tail at its 3' end and it is transmitted by 50 species of aphid in a non persistent manner (Sigvald, 1984; Boiteau *et al*., 1988). PVY infects many important food and vegetable crops worldwide, including pepper, potato, tobacco and tomato (Shukla *et al*., 1994). In connection with its wide host range, PVY has high genetic variability and several distinct strain groups infecting potato crops (Kerlan *et al*., 1999). However, PVY strains are commonly subdivided into three main groups as common or ordinary strain (PVY^{O}), tobacco veinal necrosis strain (PVY^{N}) and stipple streak strain (PVY^{C}) (De Bokx and Huttinga, 1981); according to systemic and local symptoms on *Nicotiana tabacum* and *Solanum tuberosum* (De Bokx and Huttinga, 1981; Kerlan *et al*., 1999). PVY^{O}, and PVY^{N} which infect potato and tobacco are known to be spread in potato growing areas for decades (Smith and Dennis, 1940). Over recent years, a new highly virulent variant tuber necrosis strain (PVY^{NTN}), a sub-group of PVY^{N}, has been reported in most parts Europe (Romancer *et al*., 1994; Serra and Weidemann, 1997), North and South America (McDonald and Singh, 1993), Middle East (Weidemann and Maiss, 1996) and Japan (Oshima *et al*., 2000).

Diagnosis of PVY strains based on bioassay is reliable but time consuming while enzyme-linked imminosorbent assay (ELISA) is rapid but relies on the strain specific antibodies, separate tests with different antibodies and the need for sprouted rather than dormant tubers (Gugerli and Gehringer, 1980; Singh and Somerville, 1986). However, false positive results can occur with ELISA, especially for the recombinant strains, which may share PVY^{O}-like symptoms on tobacco (McDonald and Singh, 1996; McDonald *et al*., 1997; Singh *et al*., 2003). Reverse transcription polymerase chain reaction (RT-PCR) is a powerful tool for detection of PVY (Weidemann and Maiss, 1996; Singh *et al*., 1998) and for differentiation of PVY strains or isolates (Weilguny and Singh, 1998; Nie and Singh, 2002, 2003). But, primer selection is critical to the efficiency and accuracy of strain identification by RT-PCR. Differentiation of PVY strains or isolates by using specific primers was achieved by RT-PCR (Nie and Singh, 2002).

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The objective of this study was to determine the incidence rate of tobacco veinal necrosis strain (PVY^N) and its geographical subgroups of EU-$PVY^{N/NTN}$ and NA-$PVY^{N/NTN}$ in seed potatoes belonging to various cultivars obtained from the main potato production areas in Turkey by using RT-PCR.

MATERIALS AND METHODS

Plant materials and nucleic acid extraction: This study was carried out during the years of 2004 and 2005 and PVY-infected samples belonging to cvs Granola, Morfona, Pasinler 92 and Agria were obtained from our previous studies (Bostan and Haliloğlu, 2004; Bostan *et al*., 2006).

Nucleic acids from tubers were isolated as described previously (Singh *et al*., 2002). Briefly, 300 μL of extraction buffer (0.1 M Tris HCl, pH 7.4, 2.5 mM $MgCl_2$, 0.65% Na_2SO_3 containing 6 U of RNase-free DNase I) (Roshe Molecular Biochemicals) was combined with five drops (150 μL) of sap, collected from Tuber Slicer (Electrowerk, Behcke, Hannover, Germany). The mixture was vortexed for 10-15 sec and then incubated at 37°C for 10 min. Nucleic acids were extracted with an equal volume phenol:chloroform:isoamyl alcohol (24:24:1) and then precipitated with 1 vol. of isopropanol in the presence of 0.1 volume of 0.3 M sodium acetate (-20°C overnight). The precipitate was collected by centrifugation (12 000 g, 15 min, 4°C), washed with 70% ethanol, dried under vacuum and dissolved in 1000 μL (leaves) 100 μL (tubers) of distilled water.

Reverse transcription polymerase chain reaction (RT-PCR): For the reverse transcription, 2.5 μL of RNA extract was combined with 2.5 μL of water, incubated at 65°C for 5 min and then chilled on ice for 3 min to denature the RNA. The reverse transcription (RT) mixture was added to provide a final concentration of 40 ng μL of reverse primer (A), 50 mM Tris-HCl pH 8.3, 75 mM KCl, 10 mM DDT, 2.5 mM $MgCl_2$, 2.5 mM of each dNTPs (Promega), 20 U RNasin (Promega, Madison, WI) and 200 U Moleney Murine Leukemia virus-reverse transcriptase (MMLV-RT) (Invitrogen). Samples were incubated for 1 h at 42°C for reverse transcription and incubated subsequently at 95°C for 3 min to terminate the RT reaction. The antisense and sense primer pairs used RT-PCR is given in Table 1 (Nie and Singh, 2002).

PCR was carried out using 2 μL aliquots of the cDNA mixture in 23 μL containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 0.2 μM of each of antisense and sense primers and 0.625 U of Taq polymerase (Sigma). Samples were amplified in 35 cycles using a Peltier Thermal Cycler (MJ Research, Watertown, MA). Annealing temperature was 62 for the first 5 cycles, 60°C for the next cycle, 58°C for the following 10 cycles and 55°C for the last 10 cycles. Each cycles consisted of denaturation (92°C, 30°C) and primer annealing (30 sec) and primer extension at 72°C (90 sec, as well as a final extension of 10 min at 72°C. Ten microliter of amplification products were electrophoresed on a 1.5% agarose gel containing 0.2 μg mL ethidium bromide and photographed under UV illumination. In order to determine of the size of amplified products in the gel, a low mass ladder (Invitrogen) was used.

Table 1: The primer pairs used for the detection of PVY and its two geographical strain groups.

Polarity	Sequence
Antisense (A)	5'-CAT TTG TGC CCA ATT GCC-3'.
Sense (S2)	5'-AAG CTT CCA TAC TCA CCC GC-3'
Sense (S3)	5'-GCA AGA GTT TGC AAC TAT TCG A-3'
Sense (S4)	5'-GCG AGG AAA GAG AAA GAG AAG AAC AC-3'
Sense (S5)	5'-TGA ATC ACA AGT GCA GAG A-3'
Sense (S6)	5'-GGT GAA GCT AAT CAT GTC AAC-3'

RESULTS AND DISCUSSION

At firs, in order to determine PVY $^{N/NTN}$-infected samples, a total of 320 tubers known to be infected with PVY (previously determined using S2+A primer pairs amplifying a fragment of 856 bp, which detect in all PVY regardless of their strains or isolates) were tested individually using S6+A primer pairs amplifying a fragment of 443 bp, which determine in all PVY $^{N/NTN}$ regardless of their origin and type of strains. The result obtained from test showed that the average incidence rate of PVY $^{N/NTN}$ in these cultivars was determined as 82.5% (264/320).

In order to determine the incidence rate of two geographical subgroups of PVY^N, samples found to be infected with $PVY^{N/NTN}$ were tested individually with S3+A primer pairs giving a fragment of 745 bp (specific for EU-$PVY^{N/NTN}$), S4+A amplifying a fragment of 652 bp (specific for NA-$PVY^{N/NTN}$) and S5+A amplifying a fragment of 554 bp (specific for NA-PVY^{NTN} and NA-PVY ^{N}) and all positive samples (264) were found to be infected with EU-$PVY^{N/NTN}$. However, there was no incidence of NA-$PVY^{N/NTN}$ in any of those cultivars used in this study.

The reason is that the seed potatoes have been imported from European countries to Turkey. After importation the seed potatoes were multiplied and distributed to the producers by private companies. In this way, seed potato production is slow and virus diseases tend to increase with each multiplication year. On the other hand, in the presence of inoculum sources within the field, PVY can be spread in a non persistent manner within the field by colonizing or non-colonizing aphid

vectors on potato. As a matter of fact, it was reported that any aphid visiting the potato plants is a potential vector of non-persistent viruses (Harrewijn *et al*., 1981) and several aphid species have proven capable of transmitting of PVY, including many species that can not colonize potato (Boiteau *et al*., 1988). Similarly, it was reported that the infection rate of PVY within the field ranged from 6.47 to 91.0% during the three year period (Bostan *et al*., 2006). On the other hand, if PVY-infected tubers of susceptible potato cultivars are used for planting material, total yield loss can reach up to 80%, depending on cultivars in a growing season (De Bokx and Huttinga, 1981). Although virus-free seed potato is required by the potato certification programs all of countries (Slack and Sing, 1998) which grow up the crop, there is no equipped certification laboratory which tests seed potatoes in both the importation and multiplication stages in most of country. Potato is an important crop in Turkey, with an annual production of nearly 5 millions tons from 200.000 ha of arable land and needs at least 125.000 tones of seed potato per year.

As a result, we suggest that it is necessary to have the certification laboratory for the all countries which grow economically up the yield in the high amounts as well as Turkey, without losing time.

REFERENCES

Barnett, O.W., 1992. A Summary of Potyvirus Taxonomy and Definitions. In Potyvirus Taxonomy. By O.W. Barnett, (Ed.), Springer Verlag, New York, pp: 435-444.

Boiteau, G., R.P. Singh, R.H. Parry and Y. Pelletier, 1988. The spread of potato virus Y^{o} in New Brunswick potato fields: Timing and vectors. Am. Potato J., 65: 639-649.

Bostan, H. and K. Haliloglu, 2004. Distribution of PLRV, PVS, PVX, and PVY (PVY^{N}, PVY^{o} and PVY^{c}) in the seed potato tubers in Turkey. Pak. J. Biol. Sci., 7: 1140-1143.

Bostan, H. C. Guclu, E. Ozturk, I. Ozdemir and H. Ilbagi, 2006. Influence of aphids on the epidemiology of potato virus diseases (PVY, PVS and PLRV) in the high altitude areas of Turkey, Pak. J. Biol. Sci., (In Press).

De Bokx, J.A. and H. Huttinga, 1981. Potato Virus Y: Description of Plant Viruses. Version 242. Commonwealth Mycology Institute/Association of Applied Biology, Kew, England, pp: 6.

Gugerli, P. and W. Gehringer, 1980. Enzyme-linked imminosorbent assay (ELISA) for the detection of potato leafroll virus and potato virus Y in potato tubers after artificial break of dormancy. Potato Res., 23: 353-359.

Harrewijn, P., H.A. van Hoof and J.P.W. Noordink, 1981. Flight behavior of the aphid *Myzus persicae* during its maiden flight. Neth. J. Plant Pathol., 87: 111-117.

Kerlan, C., M. Tribodet, L. Glais and M. Guillet, 1999. Variability of potato virus Y in potato crops in France. J. Phytopathol., 147: 643-651.

McDonald, J.G. and R.P. Singh, 1993. Properties of strains of potato virus Y^{N} in North America. Plant J., 77: 87-89.

McDonald, J.G. and R.P. Singh, 1996. Response of potato cultivars to North American isolates of Y^{NTN}. Am. Potato J., 73: 317-323.

McDonald, J.G., E. Wng, D. Henning and T. Tao, 1997. Coat protein and 5' nontranslated region of a variant of potato virus Y. Can. J. Plant Pathol., 19:138-144.

Nie, X. and R.P. Singh, 2002. A new approach for the simultaneous differentiation of biological and geographical strains of potato virus Y by uniplex and multiplex RT-PCR. J. Virol. Meth., 104: 41-54.

Nie, X. and Singh, R.P., 2003. Specific differentiation of recombinant $PVY^{O:N}$ and PVY^{NTN} isolates by multiplex RT-PCR. J. Virol. Methods, 133: 67-77.

Oshima, K., K. Sako, C. Hiraishi, A. Nakagawa, K. Matsuo, T. Ogawa, E. Shikata and N. Sako, 2000. Potato tuber necrotic ringspot disease occurring in Japan: Its association with Potato virus Y necrotic strain. Plant Dis., 84: 1109-115.

Romancer, M.L., C. Kerlan and M. Nedellec, 1994. Biological characterization of various geographical isolates of potato virus Y inducing superficial necrosis on potato tubers. Plant Pathol., 43: 138-144.

Serra, M.C. and H.L. Weidemann, 1997. First report of potato tuber necrotic ringspot disease caused by PVY^{NTN} in Portugal. Plant Dis., 81: 694.

Shukla, D.D., C.W. Ward and A.A. Brunt, 1994. The Potyviridae. Cambridge Universty Press, Cambridge.

Sigvald, R., 1984. The relative efficiency of some aphid species as vectors of potato virus Y^{O} (PVY^{O}). Potato Res., 27: 285-290.

Singh, R.P. and T.H. Somerville, 1986. Factors affecting the detection of potato virus Y in tubers by enzyme-linked imminosorbent assay (ELISA). Ind. J. Plant Pathol., 4: 91-97.

Singh, R.P., M. Singh and J.G. McDonald, 1998. Screening by a 3-primer PCR North American PVY^{N} isolates for European-type members of the tuber necrosis-inducing PVY^{NTN} subgroup. Can. J. Plant Pathol., 20: 227-233.

Singh, R.P., X. Nie, M.R. Singh, R. Coffin and P. Duplessis, 2002. Sodium sulphite inhibition in nucleic acid extraction for virus detection by RT-PCR. J. Virol. Methods, 99: 123-131.

Singh, R.P., D.L. McLaren, X. Nie and M. Singh, 2003. Possible escape of a recombinant isolate of potato virus Y by serological indexing and methods of its detection. Plant Dis., 87: 679-685.

Slack, S.A. and R.P. Singh, 1998. Control of Viruses Affecting Potatoes Through Seed Potato Certification Programs. In: Plant Virus Diseases Control. A. Hadidi, R.K. Khetarpal, and H. Koganezawa, (Eds.). American Phytopathological Society, St. Paul, M.N., pp: 249-260.

Smith, K.M. and R.W.G. Dennis, 1940. Some notes on a suspected variant of Solanum virus 2 (potato virus Y). Ann. Applied Biol., 27: 65-70.

Weidemann, H.L. and E. Maiss, 1996. Detection of the potato tuber necrotic ringspot strain of potato virus Y (PVY^{NTN}) by reverse transcription and immunocapture polymerase chain reaction. J. Plant Dis. Prot., 103: 337-345.

Weilguny, H. and R.P. Singh, 1998. Separation of Slovenian isolates of PVY^{NTN} from the North American isolates of PVY^{N} by a 3-primer PCR. J. Virol. Methods, 70: 57-68.