Journal of Horticultural Research 2016, vol. 24(2): 19-24
DOI: 10.1515/johr-2016-0016

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THE FREQUENCY OF VIRAL INFECTIONS ON TWO NARCISSUS PLANTATIONS IN CENTRAL POLAND

Short communication

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Received: November 2016; Accepted: December 2016

ABSTRACT

Viral diseases in narcissus can drastically affect yields and quality of narcissus bulbs and flowers, leading even to a total crop loss. To test the frequency of viral infections in production fields in Central Poland, samples were collected over three years from two cultivars and two plantations, and tested for the presence of *Arabis mosaic* (ArMV), *Cucumber mosaic* (CMV), *Narcissus latent* (NLV), *Narcissus mosaic* (NMV) and the potyvirus group using the Enzyme Linked ImmunoSorbent Assay. Potyviruses, NLV and NMV were detected in almost all leaf samples in both cultivars, in all three years of testing. Other viruses were detected in a limited number of samples. In most cases mixed infections were present. Tests on bulbs have shown the presence of potyviruses and NMV, with the higher number of positives in cultivar 'Carlton'. In addition, for most viruses an increase in their detectability was observed on both plantations in subsequent seasons.

Key words: ELISA, flower bulbs, negative selection, viral disease

INTRODUCTION

Viral diseases can drastically affect yield as well as quality of narcissus bulbs and flowers, sometimes resulting in a total crop loss. At least 24 viruses have been reported to infect narcissus (Brunt 1995; Asjes 1996). Ten of them occur in restricted geographical locations; the rest of them are world-wide. Quite often *Narcissus* may be concomitantly infected by more than one virus. As reported by Hanks (2002), most of bulb batches of *Narcissus* in the world are heavily infested with viruses. The most widespread and dangerous are: *Narcissus yellow stripe potyvirus* (NYSV), *Narcissus degeneration potyvirus* (NDV) (Okubo & Sochacki 2012) and *Narcissus white streak potyvirus* (NWSV) (Hanks 1993). NDV is known to occur only in *Narcissus tazetta* (Brunt 1980). Very common are also: *Narcissus late season yellows potyvirus* (NLSYV), *Narcissus latent carlavirus* (NLV) and *Narcissus mosaic potexvirus* (NMV). Many of the most important viruses infecting narcissus belongs to the potyvirus group.

Asjes (1996) reported that degeneration of narcissus plants caused by viruses may decrease bulb yield by 30%. Negative iumpact of potyviruses infections on bulb and flower yield have also been described by Sochacki (2008), who reported that the yield of bulbs infected by potyviruses was 23–26% lower than that of plants from the virus-tested mother stock. Narcissus crop protection against virus infections is complicated because of the relatively large number of viruses infecting the crop, different modes of transmission (Brunt 1995; Asjes 1996) and difficulties with effective roguing in case of mild symptoms or latent infections (Asjes 1996). Symptoms on plants have limited diagnostic value as they may mimic plant ageing or sub-optimal growth conditions.

To protect crop yield it is essential to know, which viruses occur most often in the particular region

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and know their transmission routs. In Poland, the monitoring of virus infections in narcissus crops had been done to a limited extent. Random screening of several Polish plantations by the Enzyme Linked ImmunoSorbent Assay at the beginning of 21st century has shown common infections with two potyviruses – NYSV and NLSYV with a high level of detectability in cultivar 'Carlton' (Sochacki et al. 2003).

This study was undertaken to identify viruses occurring on two popular cultivars of *Narcissus* on two plantations in Central Poland.

MATERIAL AND METHODS

The studies were done in the years 2009–2011 on two narcissus (*Narcissus* L.) cultivars – 'Dutch Master' (yellow flowering, trumpet daffodil, 1Y–Y, according to Kington 1998) on two plantations (farm A and farm B) and 'Carlton' (large-cupped daffodil, 2Y–Y, according to Kington 1998) on farm B. Both cultivars are very common in the two world-leading countries in narcissus bulbs production – The Netherlands and the United Kingdom (Hanks 2002). Both cultivars are also very popular in Poland, both as the field-grown bulbs and as cut flowers.

The leaves for tests were taken before a negative selection in the field from the plants showing viral infection symptoms (date I) and after the selection, completely at random (date II). Bulb scales were taken from random samples of freshly harvested bulbs (date III). For tests, 20 leaf or bulb samples were collected on each date, from each cultivar and from each plantation.

The Enzyme Linked ImmunoSorbent Assay (ELISA) as a serological technique was used to detect the following viruses: *Arabis mosaic nepovirus* (ArMV), *Cucumber mosaic virus* (CMV), NLV, NMV and the potyvirus group. DAS-ELISA (Clark & Adams 1977) was used with specific antibodies for the detection of the NMV and NLV (from Applied Plant Research – Flower Bulbs and Nursery Stock Sector, Lisse, The Netherlands), for the detection of the ArMV (from Loewe Phytodiagnostica, Germany) and for the detection of the CMV (from the Research Institute of Horticulture, Skierniewice, Poland) (Kamińska et al. 2005). DAS-ELISA tests were performed according to the protocols of the producers, with minor modifications. Leaves were ground in phosphate buffer saline (PBS), pH 7.4, and all the conjugates were diluted in PBS-T containing 0.1% Tween 20 (w/v 1 : 5). To detect most viruses of the potyvirus group the indirect ELISA with monoclonal antibodies (Jordan & Hammond 1986; Jordan 1989; Derks 1992) was used according to the protocol provided with the commercial kit (Agdia Inc., Elkhart, IN, USA), with minor modifications described earlier (Sochacki & Orlikowska 2005).

Each sample was tested in 2–3 duplicates on each date. The absorbance A_{405} was measured with Ledetect 96 reader (Dynamica GmbH, Salzburg, Austria). The samples were recorded as infected when the value of absorbance was at least twice as high as the mean value for the negative control (according to Clark et al. 1988).

RESULTS

Potyviruses, NLV and NMV were detected in almost all leaf samples before field selection (date I) in both cultivars, during all the seasons of testing (Table 1). Predominantly, the mixed infections were present. In a limited number of samples ArMV was also present. CMV was detected in one sample of 'Carlton' originating from plantation B, only in 2011. On the date II, ELISA against potyviruses, NLV and NMV showed similar results of detectability as on the date I, in both cultivars and plantations. CMV was detected in one leaf sample of cultivar 'Dutch Master' originating from farm B, only in 2010. ArMV was detected in a limited number of samples in 2009 and 2011 but significantly more often in 2010, with the frequency between 3 and 7 positive results among 20 samples tested. On the date III, mostly potyviruses and NMV in bulbs were detected, with the highest number of positive readings in 'Carlton'. Predominantly, these viruses were detected in mixed infections. ArMV and NLV were detected sporadically, except for 2011, when 1 to 5 samples out of 20 tested were infected. CMV was not detected in bulbs. For NMV an increase in the infection rates in all dates and for potyviruses at date III was observed in the subsequent years of study.

Table 1. Viruses detected in *Narcissus* leaves and bulbs determined by ELISA in the years 2009-2011; Number of positive samples of 20 tested.

Farm	Cultivar	Viruses detected in following dates														
		Date I – leaves before selection					Date II – leaves after selection					Date III- bulbs in storage room				
		ArMV	CMV	NLV	NMV	POTY	ArMV	CMV	NLV	NMV	POTY	ArMV	CMV	NLV	NMV	POTY
2009																
A	‘Duch Master’	1	0	18	5	20	0	0	18	4	20	0	0	1	3	1
B	‘Carlton’	2	0	18	19	17	1	0	16	13	19	0	0	1	12	6
B	‘Duch Master’	1	0	19	6	19	4	0	20	9	19	0	0	0	6	7
2010																
A	‘Duch Master’	1	0	17	3	18	7	0	20	9	20	0	0	0	2	4
B	‘Carlton’	0	0	20	17	20	3	0	20	17	15	1	0	0	18	7
B	‘Duch Master’	0	0	10	14	19	7	1	20	16	20	3	0	0	9	7
2011																
A	‘Duch Master’	0	0	18	9	20	0	0	13	9	12	0	0	2	3	7
B	‘Carlton’	0	1	19	20	19	1	0	20	20	18	0	0	5	18	12
B	‘Duch Master’	1	0	18	12	19	3	0	20	20	20	1	0	1	9	8

DISCUSSION

In both tested cultivars and on both plantations, potyviruses, NMV and NLV were detected most often. This confirms our previous screening carried out on few Polish plantations of *Narcissus*, when the occurrence of two potyviruses – NYSV and NLSYV – were frequently detected, particularly in plants of 'Carlton' (Sochacki et al. 2003). This supports the wider observation that potyviruses are common in narcissus worldwide, including The Netherlands (Asjes 1996; Langeveld et al. 1997), the United Kington (Brunt 1980; Brunt et al. 1982), India (Chandel et al. 2010), Australia and China (Wylie et al. 2014). Clark and Guy (2000) reported high incidence of NYSV, *Narcissus tip necrosis carmovirus* (NTNV), and NMV and NLV in *Narcissus* in New Zealand and Ward et al. (2009) published the first report on NLSYV in New Zealand. Our results showed a very high number of virus positive readings in *Narcissus* in two Polish plantations, what reaffirms the statement of Hanks (2002), that most narcissus stocks are heavily infested with viruses. Virus infestation was one of the most important reasons of decrease of field-grown *Narcissus* in Poland – from 200 ha in 1982 (Mynett 1992) to 50 ha in 1999 (Hanks 2002).

Detection level of ArMV in our evaluation of narcissus plantations was relatively low, and detection of CMV was incidental (only one leaf sample on the date I and one on the date II during the three seasons of testing). CMV in narcissus was reported in many countries; however, this is one of the less important and harmful viruses in this crop and it is not the most common (Brunt 1995). Our earlier study on the Polish clones and cultivars was not successful to detect CMV (Sochacki 2011; Sochacki & Podwyszyńska 2012). Also Navalinskiene and Samuitiene (2001) reported that other viruses occurred more frequently than CMV in *Narcissus* and other bulbous crops in Lithuania.

In our study, leaves showing disease symptoms (date I) gave positive readings for potyviruses and NLV in all or almost all samples tested. This can suggest that these viruses are common. Mixed virus infections are very common in *Narcissus*. Such mixed infections create ambiguous symptoms and aggravate the disease and weakening of plants. Brunt (1980) reported that complexes up to five viruses were present in plants of older *Narcissus* cultivars in 1960s in the UK. Previous research and screening of *Narcissus* carried out in Poland also confirmed frequent occurrence of virus complexes (Sochacki et al. 2003; Sochacki 2011; Sochacki & Podwyszyńska 2012).

Our results show a much smaller number of positive samples from bulbs in comparison to the leaf samples. However, large-scale routine testing for many viruses in bulbous plants is doing preferentially with the bulbs after harvest, e.g., *Tulip breaking virus* (TBV) in tulips (Van Schadewijk & Eggink 1984), TBV and *Lily symptomless virus* (LSV) in lily (Van Schadewijk 1986), and *Iris mild mosaic virus* (IMMV) in Dutch bulbous iris (Van Schadewijk et al. 1988). It is known, that virus replication and movement its particles in the plant is affected by many factors, such as growing conditions and developmental stage. The effectivity of virus detection by ELISA can also be affected by time of sampling and tested plant part. The reliability of tests depends also on the particular virus. TBV is detected reliably by ELISA in homogenates of tulip and lily bulbs (Van Schadewijk & Eggink 1984). Because of unsatisfactory detection of *Tobacco rattle virus* (TRV) in tulip bulbs, Van der Vlugt et al. (1988) used to check sprouts of stored and cooled bulbs as an alternative. Results obtained by Stein et al. (1986) and by Van Schadewijk et al. (1988) showed the increase in the rate of virus detection in stored and cooled gladioli corms and bulbous iris, respectively. As reported by Mowat et al. (1988), higher absorbance readings and higher incidence of NLSYV detection in *Narcissus* leaves were noticed in the late growing season. In our case, greater number of positive samples from leaves tested in date II in comparison to bulb samples, may result from higher concentration of the virus in the leaves. This may be due to the fact that viruses from latest field infections did not reach a level of detection in freshly harvested bulbs.

Present results show a very high level of the viral infections in the studied *Narcissus* samples. This is rather obvious in tests of leaves clearly showing the symptoms (date I). However, a high

number of positive results in samples after the selection (date II) is disturbing. This may be a consequence of sporadic and inaccurate selection of the plants or no selection at all. Such low efficacy selection increases the virus infection level, which is amply demonstrated in the number of positive samples of some viruses. In consequence, it may lead to a total virus infection of the cultivars and finally degeneration of the *Narcissus*.

REFERENCES

Asjes C.J. 1996. Control situation of virus diseases in narcissus in the Netherlands. Acta Horticulturae 432: 166–174. DOI: 10.17660/ActaHortic.1996.432.21.

Brunt A.A. 1980. A review of problems and progress in research on viruses and virus diseases of narcissus in Britain. Acta Horticulturae 110: 23–30. DOI: 10.17660/ActaHortic.1980.110.1.

Brunt A.A. 1995. Bulb and corm crops. Narcissus. In: Loebenstein G., Lawson R.H., Brunt A.A. (Eds.), Virus and virus-like diseases of bulb and flower crops. John Wiley & Sons, United Kingdom, pp. 322–334.

Brunt A.A., Stone O., Phillips S. 1982. Narcissus bulbs – the quest for virus free stocks. Supplement to Grower, pp. 2–5.

Chandel V., Singh M.K., Hallan V., Zaidi A.A. 2010. Evidence for the occurrence of a distinct potyvirus on naturally growing *Narcissus tazetta*. Archives of Phytopathology and Plant Protection 43(3): 209–214. DOI: 10.1080/03235400701722145.

Clark M.F., Adams A.N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology 34(3): 475–483. DOI: 10.1099/0022-1317-34-3-475.

Clark M.F., Lister R.M., Bar-Joseph M. 1988. ELISA Techniques. In: Weissbach A., Weissbach H. (Eds.), Methods for Plant Molecular Biology. Academic Press, pp. 507–530. DOI: 10.1016/B978-0-12-743655-5.50038-5.

Clark V.R., Guy P.L. 2000. Five viruses in *Narcissus* spp. from New Zealand. Australasian Plant Pathology 29: 227–229. DOI: 10.1071/AP00044.

Derks A.F.L.M. 1992. Some unusual serological reactions among potyviruses. In: Barnett O.W. (Ed.), Potyvirus taxonomy. Archives of Virology, Supplementum 5: 77–79. DOI: 10.1007/978-3-7091-6920-9_9.

Hanks G.R. 1993. Narcissus. In: De Hertogh A.A., Le Nard M. (Eds.), The physiology of flower bulbs. Elsevier, Amsterdam, Netherlands, pp. 463–558.

Hanks G.R. 2002. Narcissus and daffodil – the genus *Narcissus*. In: Medicinal and aromatic plants – industrial profiles 21. CRC Press, Taylor & Francis, 428 p.

Jordan R. 1989. Mapping of potyvirus-specific and group-common antigenic determinants with monoclonal antibodies by Western-blot analysis and coat protein amino acid sequence comparisons. Phytopathology 79(10): 1157. DOI: 10.1094/Phyto-79-1135.

Jordan R., Hammond J. 1986. Analysis of antigenic specificity of monoclonal antibodies to several potyviruses. Phytopathology 76(10): 1091.

Kamińska M., Śliwa H., Malinowski T. 2005. Partial characterisation of cucumber mosaic virus isolate infecting *Lonicera caprifolium* L. plants. Acta Scientiarum Polonorum, Hortorum Cultus 4(2): 3–10.

Kington S. 1998. The International Daffodil Register and Classified List 1998. Royal Horticultural Society, London, 1168 p.

Langeveld S.A., Derks A.F.L.M., Konicheva V., Muñoz D., Zhin-nan C., Denkova S.T. et al. 1997. Molecular identification of potyviruses in Dutch stocks of *Narcissus*. Acta Horticulturae 430: 641–648. DOI: 10.17660/ActaHortic.1997.430.101.

Mowat W.P., Duncan G.H., Dawson Sheila. 1988. Narcissus late season yellows potyvirus: symptoms, properties and serological detection. Annals of Applied Biology 113: 531-544. DOI: 10.1111/j.1744-7348.1988.tb03330.x.

Mynett K. 1992. Floriculture in Poland: research, education and production. Acta Horticulturae 325: 29–34. DOI: 10.17660/ActaHortic.1992.325.1.

Navalinskienė M., Samuitienė M. 2001. Viruses affecting some bulb and corm flower crops. Biologija 4: 40–42.

Okubo H., Sochacki D. 2012. Botanical and horticultural aspects of major ornamental geophytes. In: Kamenetsky R., Okubo H. (Eds.), Ornamental geophytes. From basic science to sustainable production. CRC Press, Taylor & Francis Group, pp. 79–121. DOI: 10.1201/b12881-5.

Sochacki D. 2008. The influence of potyviruses infection of narcissus on the yield of bulbs and flower. Zeszyty Problemowe Postępów Nauk Rolniczych 525: 397–403. [in Polish with English abstract]

Sochacki D. 2011. The use of ELISA in the micropropagation of virus-free *Narcissus*. Acta Horticulturae

886: 253–258. DOI: 10.17660/ActaHortic.2011.886.35.

Sochacki D., Kucharska D., Orlikowska T. 2003. The occurrence of potyviruses *Narcissus yellow stripe virus* (NYSV) and *Narcissus late season yellows virus* (NLSYV) in narcissus crop in Poland. Phytopathologia Polonica 28: 75–79.

Sochacki D., Orlikowska T. 2005. The obtaining of narcissus plants free from potyviruses via adventitious shoot regeneration in vitro from infected bulbs. Scientia Horticulturae 103: 219–225. DOI: 10.1016/j.scienta.2004.04.018.

Sochacki D., Podwyszyńska M. 2012. Virus eradication in narcissus and tulip by chemotherapy. In: Van Tuyl J.M., Arens P. (Eds.), Floriculture and Ornamental Biotechnology 6, Special Issue Bulbous Ornamentals 2: 114–121.

Stein A., Salomon R., Cohen J., Loebenstein G. 1986. Detection and characterisation of bean yellow mosaic virus in corms of *Gladiolus grandiflorus*. Annals of Applied Biology 109: 147–154. DOI: 10.1111/j.1744-7348.1986.tb03193.x.

Van Schadewijk A.R., Eggink J. 1984. Detection of tulip breaking virus (TBV) in tulips by means of ELISA. Acta Botanica Neerlandica 33: 238.

Van Schadewijk A.R., Derks A.F.L.M., Lemmers M.E.C., Hollinger Th.C. 1988. Detection of iris mild mosaic virus in bulbs and leaves of bulbous iris by ELISA. Acta Horticulturae 234: 199–206. DOI: 10.17660/ActaHortic.1988.234.23.

Van der Vlugt C.I.M., Linthorst H.J.M., Asjes C.J., Van Schadewijk A.R., Bol J.F. 1988. Detection of tobacco rattle virus in different parts of tulip by ELISA and cDNA hybridisation assays. Netherlands Journal of Plant Pathology 94: 149–160. DOI: 10.1007/BF01978004.

Ward L.I., Veerakone S., Tang J., Clover G.R.G. 2009. First report of *Narcissus degeneration virus*, *Narcissus late season yellows virus*, and Narcissus symptomless virus on *Narcissus* in New Zealand. Plant Disease 93(9): 964–964. DOI: 10.1094/PDIS-93-9-0964A.

Wylie S.J., Li H., Sivasithamparam K., Jones M.G.K. 2014. Complete genome analysis of three isolates of narcissus late season yellows virus and two of narcissus yellow stripe virus: three species or one? Archives of Virology 159: 1521. DOI: 10.1007/s00705-013-1969-z.