European Journal of Plant Pathology **103:** 481–484, 1997.

Short communication

Molecular evidence for the occurrence of beet western yellows virus on chickpea in Morocco

M. Fortass[1,*], F. van der Wilk[2], J.F.J.M. van den Heuvel[2] and R.W. Goldbach[1]
[1]*Department of Virology, Wageningen Agricultural University, P.O. Box 8045, 6700 EM Wageningen, The Netherlands;* [2]*DLO-Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands;* [*]*Permanent address: Department of Plant Pathology, Ecole Nationale d'Agriculture, B.P. S/40, Meknes, Morocco*

Accepted 10 January 1997

Key words: food legumes, *Cicer arietinum* L., luteovirus, PCR, nucleotide sequence, coat protein gene

Abstract

A luteovirus isolate infecting chickpea in Morocco was experimentally transmitted by *Myzus persicae* to *Physalis floridana*, on which it produced mild symptoms. When tested in western blots against antisera to known legume luteoviruses, this isolate reacted strongly to beet western yellows virus (BWYV) antiserum, moderately to bean leafroll virus antiserum, while no reaction was recorded with the antiserum against subterranean clover red leaf virus. In PCR, a fragment of ca. 950 bp was amplified, comprising the 3′ end of the open reading frame (ORF) 3, the complete coat protein gene (ORF 4), and the non-translated region in between these ORFs. The nucleotide sequence of the amplified fragment showed high similarity with BWYV (approximately 96%), and lower (50–60%) with other luteoviruses reported to infect legumes. On the basis of these data, the Moroccan isolate was identified as BWYV. This is the first molecular evidence for the occurrence of BWYV on chickpea in Morocco, and on food legumes in general in North Africa.

Early surveys of faba bean (*Vicia faba* L.) and chickpea (*Cicer arietinum* L.) for viruses in Morocco have revealed that species of the genus *Luteovirus* are economically important and widely spread in the country (Fortass and Bos, 1991). Their effect on yield is dramatic; early infections often lead to crop failure. Thus far, none of the faba bean-derived luteoviruses could be clearly identified by serology alone as one of the known luteoviruses that infect legumes (Fortass et al., 1996). The different serological reaction patterns suggested the involvement of different luteoviruses or luteovirus strains. Molecular approaches are required to properly identify luteovirus species since members of the genus *Luteovirus* seem to form a continuum and exhibit varying degrees of serological inter-relatedness (Randles and Rathjen, 1995). Here we report on the identification of a beet western yellows virus (BWYV) isolate from chickpea in Morocco, on the basis of the nucleotide sequence of the coat protein gene and its upstream non-translated region.

Leaf material was collected from a single chickpea plant exhibiting symptoms indicative of a luteovirus infection. Symptomatic leaf material (0.5 g) was homogenized in 1.0 ml of 0.1 M citrate buffer pH 6.0 and centrifuged at 10000 g for 15 min at 4 °C. Approximately 20 nl of the supernatant was injected into four-day-old *Myzus persicae* nymphs. The aphids were transferred to young *Physalis floridana* plants, and allowed to feed for a period of 96 h. Three weeks later, test plants were screened for luteovirus infection using the antisera mentioned below in a double antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA)(Clark and Adams, 1977). Five to eight weeks after inoculation the virus (designated Y3) was purified from these plants according to Van den Heuvel et al. (1990). Purified virus preparations were

Table 1. Reactions in western blots of the Moroccan isolate Y3 to antisera to legume luteoviruses

Virus	Polyclonal Antisera		
	BWYV-CP	BLRV	SCRLV
BWYV	++*	+	–
BLRV	+	++	++
Isolate Y3	++	+	–

* Intensity of the reaction (++: strong, +: moderate, –: no reaction).

subjected to SDS-PAGE followed by Western blotting essentially as described by Fortass et al. (1991) using polyclonal antisera to the coat protein of BWYV (Brault et al., 1995), to bean leafroll virus (BLRV; supplied by L. Katul, Institute for Biochemistry and Plant Virology, Braunschweig, Germany), and subterranean clover red leaf virus (SCRLV; supplied by G.J. Johnstone, Tasmanian Department of Agriculture, Tasmania, Australia). Goat anti-rabbit gamma-globulins conjugated to alkaline phosphatase (Sigma) were used as secondary antibodies.

The virus isolate Y3 was transmitted by *M. persicae* and not by *Aphis fabae* to all *Physalis floridana* plants tested, on which it showed a very mild interveinal chlorosis. The virus was also back-transmitted from *P. floridana* to chickpea, and luteovirus-like symptoms were reproduced. The reactivity of the purified isolate Y3 on Western blots is summarised in Table 1. The pattern of reaction is similar to that of BWYV, i.e. a strong reaction to BWYV-coat protein antiserum, moderate reaction to BLRV antiserum, and no reaction with the antiserum to SCRLV. Serological cross-reactivities between BWYV and BLRV were seen which is in line with earlier conclusions that the two viruses have epitopes in common (Waterhouse et al., 1988; Van den Heuvel et al., 1990). As a consequence, no definite conclusion could be drawn as to whether Y3 is BWYV or BLRV. It is not a mixed infection of both viruses as evidenced by the migration pattern of their coat proteins in SDS-PAGE. This shows that earlier conclusions concerning serological studies on the identification of luteoviruses infecting legumes based on serology alone are premature. Molecular approaches are undoubtedly required to identify the nature of these viruses.

Therefore, polymerase chain reaction (PCR) was performed and a pair of nucleotide primers were designed to amplify in PCR a product of ca. 950 bp. This fragment comprises the coat protein gene, its upstream non-translated region, and 130 nucleotides of ORF 3. The downstream primer Lu4 (5′ GTCTACC-TATTTGG 3′) is complementary to nucleotides 4084 to 4097 of BWYW-RNA (Veidt et al., 1988). The upstream primer BL (5′ ATGGTCGCTAGAGG 3′) is identical to nucleotides 3139 to 3152 of BWYV-RNA. RNA was extracted from purified virus by phenol/chloroform extraction and subsequent ethanol precipitation. Complementary DNA synthesis and DNA amplification were carried out as described by Robertson et al. (1991). The PCR product was cloned using a pGEM-T vector kit from Promega, and according to the manufacturer's instructions. The nucleotide sequence was determined from three clones, in both directions, using an automatic (ABI 373A) DNA sequencing system. The sequences were analyzed and the phylogenetic tree was drawn using the University of Wisconsin GCG program[MF] package version 8 (Genetic Computer Group, Madison, Wisconsin, USA).

In PCR tests, a fragment of the predicted size (ca. 950 bp) was obtained from both BWYV and isolate Y3. The nucleotide sequence of the obtained product from Y3 as well as its deduced amino acid sequence are presented in Figure 1. The PCR product comprises 130 bp from ORF 3, the complete non-translated region downstream of this ORF, and the coat protein gene (ORF 4). Sequence comparison with other luteoviruses known to infect legumes, i.e. BWYV, BLRV, and soybean dwarf virus (SDV), showed that Y3 is 96% similar to BWYV (Table 2). The phylogenetic tree based on the coat protein nucleotide sequences (Figure 2) shows that the isolate Y3 clearly clusters with BWYV isolates (De Miranda et al., 1995). The similarities between Y3 and other luteoviruses in the non-translated region are lower than the similarities at the coat protein gene level. The diversity in length and sequence in this area of the genome makes the comparisons difficult. The molecular data clearly indicate that Y3 is an isolate of BWYV.

In our previous virus surveys of food legumes in Morocco, a large number of collected samples reacted similarly as isolate Y3: a strong reaction with BWYV antiserum and a moderate or weak reaction with BLRV antiserum. It is likely that these plants were infected with BWYV, either alone or in mixed infection with BLRV; and that BWYV is more important in the disease syndrome previously ascribed to BLRV only. Currently we are developing diagnostic tools, based on PCR, to permit differentiation of these two luteo-

Table 2. Nucleotide and deduced amino acid sequence similarity between the Moroccan isolate Y3 and other luteoviruses that infect legumes at the levels of the open reading frames 3 and 5 (ORF 3 and ORF 5), the non-translated region (NT region), and the coat protein (CP) gene

	Nucleotide similarity (%)			Amino acid similarity (%)	
Virus	ORF 3	NTregion	CP	CP	ORF 5
BWYV-FL1[a]	96.1	63.3	96.1	96.5	96.6
BLRV	n.a.[b]	n.a.	63.3	63.0	n.a.
SDV[c]	39.8	51.6	64.2	64.7	60.0

[a] BWYV-FL1 (Veidt et al., 1988), BLRV (Prill et al., 1990), SDV (Rathjen et al., 1994).
[b] Data not available.
[c] SDV is synonymous with SCRLV (Francki et al., 1991).

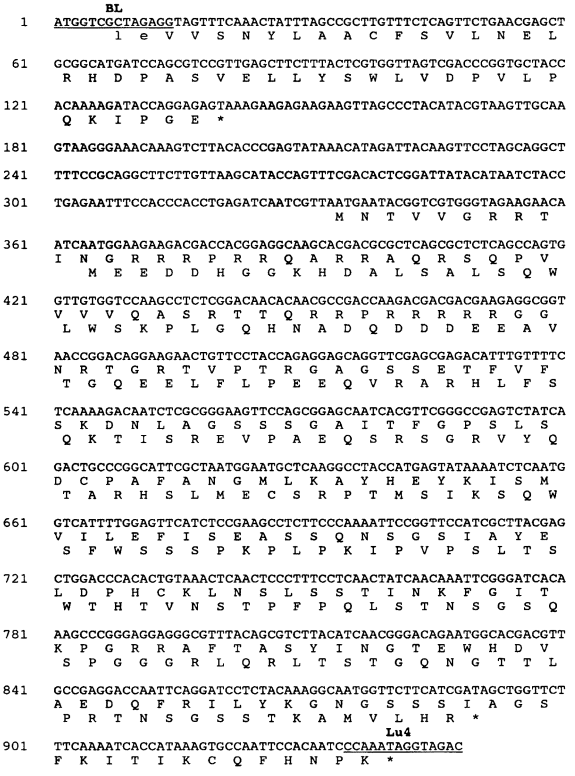

Figure 1. Nucleotide sequence of part of the ORF 3, the non-translated region, and the coat protein gene of the Moroccan luteovirus isolate Y3. The deduced amino acid sequence is shown on the top line. The internal ORF 5-encoded sequence is shown on the second line. The underlined nucleotide sequences indicate positions of the primers (BL and Lu4).

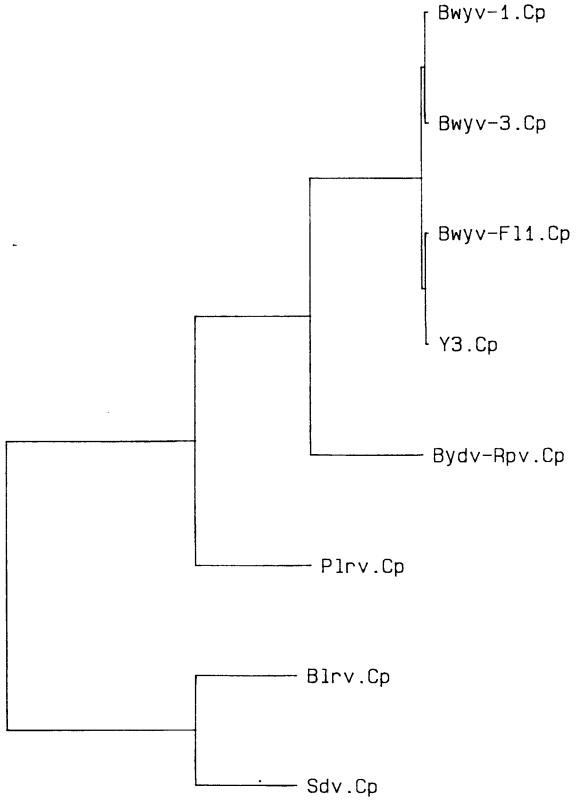

Figure 2. UPGMA phylogenetic tree based on the alignments of the coat protein (CP) nucleotide sequences of different luteoviruses and Y3. Sources of sequences: BWYV-1 and BWYV-3 (de Miranda et al., unpublished data), BWYV-FL1 (Veidt et al., 1988), BLRV (Prill et al, 1990), SDV (Rathjen et al., 1994), BYDV-RPV (Larkins et al., 1991), and PLRV (Van der Wilk et al., 1989).

viruses. Proper identification and assessment of the relative importance of either virus is indispensable for resistance breeding using classical approaches or novel strategies e.g. pathogen-derived resistance. Furthermore, proper characterization of a large number of BWYV isolates occurring in legumes in Morocco is required to gain more insight in the variability of this virus.

Acknowledgements

M. Fortass is the recipient of a research grant of the Graduate School Experimental Plant Sciences in which – among others – the Department of Virology of the Wageningen Agricultural University, and IPO-DLO participate. The authors wish to thank L. Bos for critically reading the manuscript.

References

Brault V, Van den Heuvel JFJM, Verbeek M, Ziegler-Graff V, Reutenauer A, Herrbach E, Garaud J-C, Guilley H, Richards K, and Jonard G (1995) Aphid transmission of beet western yellows luteovirus requires the minor capsid read-through protein P74. The EMBO J 14: 650–659

Clark MF and Adams AN (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J Gen Virol 34: 475–483

De Miranda JR, Stevens M, De Bruyne E, Smith HG, Bird C and Hull R (1995) Beet luteovirus coat protein sequence variation. Ann appl Biol 127: 113–124

Fortass M and Bos L (1991) Survey of faba bean (*Vicia faba* L.) for viruses in Morocco. Neth J Plant Path 97: 369–380

Fortass M, Bos L and Goldbach RW (1991) Identification of potyvirus isolates from faba bean (*Vicia faba* L.); and the relationships between bean yellow mosaic virus and clover yellow vein virus. Arch Virol 118: 87–100

Fortass M, van der Wilk F, Goldbach RW, Bos L and van den Heuvel JFJM (1996) Diversity of luteoviruses infecting faba bean in Morocco and their detection by the polymerase chain reaction. Agronomie 16: 61–68

Francki RIB, Fauquet CM, Knudson DL and Brown F (1991) Classification and nomenclature of viruses. Fifth report of the International Committee on Taxonomy of Viruses. Arch Virol [Suppl 2]: 309–311

Prill B, Maiss E, Katul L and Casper R (1990) Nucleotide sequence of the bean leafroll luteovirus coat protein gene. Nucl Acids Research 18: 5544

Randles JW and Rathjen JP (1995) Genus *Luteovirus*. In: Murphy FA, Fauquet CM, Bishop DHL, Ghabrial SA, Jarvis AW, Martelli JP, Mayo MA and Summers MD (eds) Virus Taxonomy, Sixth Report of the International Committee on Taxonomy of Viruses (pp 379–383) Springer-Verlag, Wien New York

Rathjen JP, Karageorgos LE, Habili N, Waterhouse PM and Symons RH (1994) Soybean dwarf luteovirus contains the third variant genome type in the luteovirus group. Virol 198: 671–679

Robertson NL, French R and Gray SM (1991) Use of group-specific primers and the polymerase chain reaction for the detection and identification of luteoviruses. J Gen Virol 72: 1473–1477

Van den Heuvel JFJM, De Blank CM, Goldbach RW and Peters D (1990) A characterization of epitopes on potato leafroll virus coat protein. Arch Virol 115: 185–197

Van der Wilk F, Huisman MJ, Cornelissen BJC, Huttinga H and Goldbach RW (1989) Nucleotide sequence and organization of potato leafroll virus genomic RNA. FEBS Lett 245: 51–56

Veidt I, Lot H, Leiser M, Scheidecker D, Guilley H, Richards K, and Jonard G (1988) Nucleotide sequence of beet western yellows-virus RNA. Nucl Acids Research 16: 9917–9932

Vincent JR, Lister RM, and Larkins BA (1991) Nucleotide sequence analysis and genomic organization of the NY-RPV isolate of barley yellow dwarf virus. J Gen Virol 72: 2347–2355

Waterhouse PM, Gildow FE and Johnstone GR (1988) Luteovirus group. CMI/AAB Descriptions of Plant Viruses. No. 339