

## SHORT COMMUNICATION

**YELLOWING DISEASE OF MELON IN SARDINIA (ITALY) CAUSED BY *BET PSEUDOEYELLOWS VIRUS***L. Tomassoli<sup>1</sup>, V. Lumia<sup>1</sup>, G.F. Siddu<sup>2</sup>, M. Barba<sup>1</sup><sup>1</sup> *Istituto Sperimentale per la Patologia vegetale, via C. G. Bertero 22, I-00156 Roma, Italy*<sup>2</sup> *ERSAT - Centro Zonale di Arborea, Corso Italia 2, I-09092 Arborea (CA), Italy***SUMMARY**

In 2001, a disease of melon characterized by yellowing of the leaves was observed in Sardinia (Italy) where it caused severe damage. Samples, collected from several melon crops, were positive in single step RT-PCR assays for *Beet pseudoyellows virus* (BPYV) when specific primers were used. An amplicon of the same size as that from BPYV controls (450 bp) was obtained from 14 out of 20 samples tested and had 98.4% nucleotide sequence identity with an authentic BPYV sequence. This is the first experimental evidence of a yellowing disease caused by BPYV in Italian melon crops.

*Key words:* Crinivirus, BPYV, melon, one step RT-PCR.

*Beet pseudoyellows virus* (BPYV), a tentative member of the genus *Crinivirus*, family *Closteroviridae*, (Martelli *et al.*, 2002), is the agent of yellowing diseases on several vegetable crops grown permanently under glass. It is a phloem-limited monopartite genome virus, transmitted by the greenhouse whitefly *Trialeurodes vaporariorum* in a semi-persistent manner (Wisler *et al.*, 1998; Caciagli, 2001). BPYV was first identified in California in sugar beet (Duffus, 1965), and was later implicated in yellows diseases in lettuce, endive and cucurbits in Europe (Van Dorst *et al.*, 1980; Lot *et al.*, 1980; Coffin and Coutts, 1990; Jorda-Gutierrez *et al.*, 1993). BPYV outbreaks have recently occurred in New Zealand (Clover *et al.*, 2002). In Italy, BPYV was provisionally identified in severely yellowed lettuce, as evidenced by symptomatology, presence of *T. vaporariorum*, and electron microscope observation of leaf dip preparations, identified BPYV as the causal agent of the disease (Ragozzino, 1998; Ragozzino *et al.* 1989). More recently Rubio *et al.* (1999) have detected BPYV in commercial Italian cucurbit crops.

During spring 2001, severe yellowing on melon was observed in some greenhouses and fields in southern Sardinia (Italy). Intermediate and lower leaves first showed angular chlorotic spots that expanded and coa-

lesced inducing a generalized yellowing of interveinal tissue while the veins remained green. Old yellow leaves were thick and brittle producing a characteristic snap when crushed, while young leaves and vegetative tips often appeared normal. In symptomatic plants, fruits stopped growing and ripening and yield were seriously compromised.

Similar but milder symptoms had earlier been observed in the same areas and were interpreted as due to nutritional deficiencies. The severity of the yellow disorder, observed in cucurbit cultivations near Cagliari, prompted us to investigate the disease.

In spring and early summer 2001, samples were collected from several greenhouse and open field crops of melon and other cucurbits that showed severe yellowing. About 0.2 mg leaf tissue was ground in liquid nitrogen and used to extract total RNA according to Celix *et al.* (1996).

A one step RT-PCR procedure was followed using BPYV-specific oligonucleotides primers: BH1 (5'-AACTCACCTTACATCCCCACTTGT-3') and BH2 (5'-AATGGCTGCTGCAGACGGTTCAAT-3') (Rubio *et al.*, 1996). Two µl of TNA were added to a 25 µl reaction mixture containing 10x PCR buffer (Promega Corp., Madison, WI, USA), 1.5 mM MgCl<sub>2</sub>, 0.5 mM each of the four dNTPs, 50 ng of each primer, 5 units of RNaseOUT, 1 unit of *Taq* DNA polymerase (Promega Corp.), 1.2 units of AMV-RT (Promega Corp.). After reverse transcription at 46°C for 30 min and a brief denaturation step at 94°C, PCR proceeded for 35 cycles, each at 94°C for 30 sec, 60°C for 30 sec and 72°C for 1 min, ending with an extension step at 72°C. PCR products were analysed on 1.5% agarose gel.

Controls consisted of sap from symptomless, and apparently healthy cucurbit plants, and TNA extracted from plants infected by BPYV or by either of two criniviruses reported from melon: *Lettuce infectious yellow virus* (LIYV) and *Cucurbit yellow stunting disorder virus* (CYSDV). All samples were also analysed by RT-PCR for the presence of CYSDV and LIYV. Specific CYSDV primers and the RT-PCR procedure were as reported (Celix *et al.*, 1996). Specific LIYV primers were designed by analysis with PILEUP, FASTA and PRIME programs (GCG, Madison, WI, USA) of the LIYV HSP70 homologue coding region, available in EMBL and Genebank databases (accession number: U15441). The sequence of the designed primers was: LIYV1

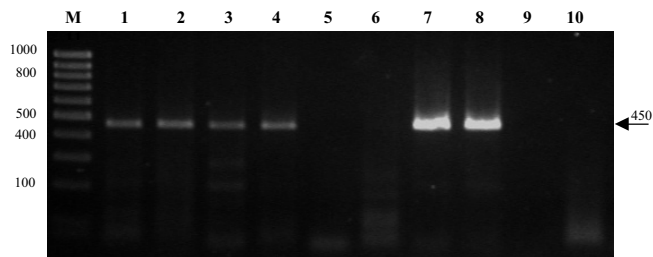
(5'- GTGGTGGTACGAAACAAG-3') corresponding to nucleotides 1608 to 1625 and LIYV2 (5'-AACGGTGTGAGTGTAAGG-3') corresponding to the sequence complementary to nucleotides 1890 to 1907. The one step RT-PCR protocol described for BPYV was also used to detect LIYV with the only exception of the annealing temperature (53°C).

Of 20 melon samples analysed (Table 1), 14 gave an amplicon of 450 bp that in agarose gels co-migrated with the amplicon from BPYV TNA extracts used as controls (Fig. 1). No products were obtained from healthy controls or samples infected by LIYV and CYS-DV. Infected melon samples were mainly from *Cucumis melo* var *reticulatus* cultivars grown in greenhouses. Four winter melon plants (*C. melo* var *inodorus*), grown in the open, were also tested and two of them proved to

**Table 1.** Occurrence of BPYV in cucurbit samples showing yellowing symptoms on older leaves, collected in different periods of the growing season 2001.

| Sample No./ Farm No. | Cucurbit species                | Cultivar     | Date  | BPYV |
|----------------------|---------------------------------|--------------|-------|------|
| 1/1                  | <i>Cucumis melo reticulatus</i> | Proteo       | April | +    |
| 2/2                  | <i>Cucumis melo reticulatus</i> | Ago          | April | +    |
| 3/3                  | <i>Cucumis melo reticulatus</i> | nd*          | May   | +    |
| 4/4                  | <i>Cucumis melo reticulatus</i> | Proteo       | May   | +    |
| 5/1                  | <i>Cucumis melo reticulatus</i> | nd           | May   | +    |
| 6/5                  | <i>Cucumis melo reticulatus</i> | Ago          | May   | +    |
| 7/6                  | <i>Cucumis melo reticulatus</i> | nd           | May   | -    |
| 8/7                  | <i>Cucumis melo reticulatus</i> | Proteo       | May   | -    |
| 9/2                  | <i>Cucumis melo reticulatus</i> | nd           | May   | +    |
| 10/6                 | <i>Cucumis melo reticulatus</i> | DRT 7622     | June  | -    |
| 11/2                 | <i>Cucumis melo reticulatus</i> | Ago          | June  | +    |
| 12/8                 | <i>Cucumis melo reticulatus</i> | nd           | June  | +    |
| 13/9                 | <i>Citrullus lanatus</i>        | nd           | June  | -    |
| 14/10                | <i>Cucumis sativus</i>          | nd           | June  | -    |
| 15/11                | <i>Cucurbita pepo</i>           | nd           | June  | -    |
| 16/12                | <i>Cucumis melo reticulatus</i> | nd           | June  | -    |
| 17/12                | <i>Cucumis melo reticulatus</i> | nd           | June  | +    |
| 18/13                | <i>Cucumis melo inodorus</i>    | Verdol       | June  | -    |
| 19/1                 | <i>Cucumis melo inodorus</i>    | Piel di Sapo | June  | +    |
| 20/14                | <i>Citrullus lanatus</i>        | Dumara       | June  | -    |
| 21/14                | <i>Citrullus lanatus</i>        | Toro         | June  | -    |
| 22/15                | <i>Citrullus lanatus</i>        | Toro         | June  | -    |
| 23/16                | <i>Citrullus lanatus</i>        | Toro         | June  | -    |
| 24/17                | <i>Cucumis melo inodorus</i>    | Piel di Sapo | July  | +    |
| 25/17                | <i>Citrullus lanatus</i>        | nd           | July  | -    |
| 26/5                 | <i>Cucumis melo inodorus</i>    | nd           | July  | -    |
| 27/18                | <i>Cucurbita pepo</i>           | nd           | July  | -    |
| 28/18                | <i>Citrullus lanatus</i>        | nd           | July  | -    |
| 29/19                | <i>Cucumis melo reticulatus</i> | nd           | July  | +    |
| 30/8                 | <i>Cucumis melo reticulatus</i> | nd           | July  | +    |
| 31/9                 | <i>Citrullus lanatus</i>        | nd           | July  | -    |
| 32/11                | <i>Cucurbita pepo</i>           | nd           | July  | -    |
| 33/20                | <i>Citrullus lanatus</i>        | nd           | July  | -    |
| 34/21                | <i>Cucumis sativus</i>          | nd           | July  | +    |
| 35/12                | <i>Cucumis sativus</i>          | nd           | July  | +    |

\*nd = not determined.



**Fig. 1.** Detection of BPYV by one-step RT-PCR. Ethidium bromide stained 1.5% agarose gel in TBE; M = 100 bp DNA ladder; lanes 1-7 = melon samples (Nos. 3-9 of Table 1); lane 8 = BPYV positive control; lane 9 = blank; lane 10 = negative control. The arrow indicates 450 bp.

be infected by BPYV. When TNAs from the melon samples were tested for CYS-DV and LIYV, the expected amplification products, respectively 465 bp and 300 bp, were not obtained.

The BPYV-specific 450 bp amplicon obtained from a melon sample (No. 29) was purified with the Strataprep PCR purification kit (Stratagene, La Jolla, CA, USA), and its nucleotide sequence was determined in both directions by GENENCO sequencing service. The sequence was compared with that of the HSP70 BPYV gene (U67447) using the LFASTA alignment programme. The sequences showed homology of 98.4%, confirming that the melon virus was indeed BPYV.

During the survey, 21 different farms were visited because of the presence of yellowing on cucurbits. BPYV was detected in ten of them (Table 1). Yellow plants belonging to other cucurbit species, collected in the same areas, were analysed, i.e. three cucumbers (*Cucumis sativus*), three zucchinis (*Cucurbita pepo*) and nine watermelons (*Citrullus lanatus*). Two of the three cucumber plants were positive for BPYV (Table 1, sample 34 and 35).

As previously mentioned, the occurrence in Italy of BPYV in lettuce was reported (Ragozzino *et al.*, 1989; Ragozzino, 1998) but identification was not based on serological or molecular assays. The presence of BPYV in Italian cucurbits was confirmed during a worldwide investigation on the geographical distribution of whitefly-borne closteroviruses (Rubio *et al.*, 1999) but the report did not specify cucurbit species affected, geographical distribution of the virus and disease severity. We now further confirm the presence of BPYV in Italy and assess the economic importance of the virus as the cause of severe yellowing disease of melon and cucumber.

The affected area of Sardinia is particularly important for melon production, either in glasshouses or in the open, and the disease causes significant losses. Moreover, the detection of BPYV in winter melon in outdoor crops indicates that the virus has potential to seriously disrupt the local cucurbit economy. The distribution of BPYV and its impact on cucurbit production in other Italian regions need to be further investigated since whitefly populations are increasing in all Italian agricultural areas (Caciagli, 2001), and may favour epidemic spread of BPYV and other criniviruses.

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