IDENTIFICATION AND CHARACTERIZATION OF POTYVIRUSES OF *ALSTROEMERIA* SPP.

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Abstract

Several viruses have been reported to infect *Alstroemeria*. The two most important ones are considered to be *Alstroemeria* mosaic (AlMV) and *Alstroemeria* streak virus (AlSV). Both AlMV and AlSV react in ELISA with a commonly used antiserum to AlMV. There are indications that potyviruses other than AlMV and AlSV also occur in *Alstroemeria*. The present study was initiated to identify and characterise these potyviruses.

A strategy was developed that is aimed at separation of these viruses and their subsequent individual characterization. It is based on obtaining isolates of single viruses by serial passages through a local lesion host followed by RT-PCR or immuno-capture RT-PCR to obtain virus-specific cDNA fragments. The nucleotide sequence of these cDNA fragments is compared to those of other known potyviruses present in the EMBL Database. Using this identification strategy four different potyviruses were identified, three of which were not known to occur in *Alstroemeria*.

Introduction

Alstroemeria is an ornamental crop of increasing importance worldwide. It is a popular cut-flower, not only for its long vase-life, but also because its production requires relatively low energy input. In the Netherlands *Alstroemeria* is currently ranking 9th among cut-flowers with a turnover of 67,4 million Dutch guilders (Vakblad voor de Bloemisterij, 1996). The crop has been propagated vegetatively for many years and therefore often harbours several viruses at a very high incidence. A number of viruses have been reported (Hakkaart & Versluijs, 1985; Phillips & Brunt, 1986; Bellardi *et al.*, 1992), most of these can be detected by ELISA (Table 1). Based on particle morphology and serology two of the reported viruses apparently belong to the genus *Potyvirus*: *Alstroemeria* mosaic (AlMV) and *Alstroemeria* streak virus (AlSV). Additional potyviruses other than AlMV and AlSV have been reported to be present in *Alstroemeria* (Hakkaart & Versluijs, 1985; Bellardi *et al.*, 1992; Van Zaayen, 1995). Both AlMV and AlSV react strongly with a commonly used antiserum to AlMV (Van Zaayen *et al.*, 1994). However, AlSV can be distinguished from AlMV because it does not react with the monoclonal antibodies from Agdia's potyvirus group test. Obviously, this situation is not satisfactory and does not

Table 1 Viruses of *Alstroemeria* and the possibility of their specific detection by ELISA.

	ELISA
Arabis mosaic virus	+
Alstroemeria carlavirus	+
Alstroemeria mosaic virus	+
Alstroemeria streak virus	-
cucumber mosaic virus	+
tobacco rattle virus	+
tomato spotted wilt virus	+
unidentified rhabdovirus	-

provide specific detection methods for AlSV or other potyviruses suspected to be present in *Alstroemeria*. In order to clarify the virus status of *Alstroemeria* in the Member States of the European Union, the European Commission has funded a project that should provide standardized methods to clean up *Alstroemeria* germplasm and stocks, and establish detection methods for all major viruses present in this crop. These methods can be used in certification schemes by inspection services. Within this project, IPO-DLO focuses on the identification, characterization and the development of specific detection methods for the potyviruses present in *Alstroemeria*.

Results and discussion

From IEM decoration studies it became apparent that AlMV often occurs in mixtures with other unidentified potyviruses. Therefore an strategy is developed that is aimed at separation of these viruses and their subsequent individual characterization.

Chenopodium amaranticolor, *C. murale* or *C. quinoa* were identified as most suitable local lesion hosts. From the collection of virus-infected *Alstroemeria* plants present at IPO-DLO several plants were selected that were likely to contain potyviruses other than AlMV.

Samples of these plants were inoculated on the appropriate local lesion host and after four serial passages we assumed to have obtained pure isolates. Subsequently these isolates were propagated in a systemic host, in most cases *Nicotiana benthamiana*.

For further identification the virus was partially purified by centrifugation through a sucrose cushion and subjected to a RT-PCR procedure. On the partially

purified virus a cDNA synthesis was performed using an oligo-dT primer. The newly synthesized cDNA was used in a polymerase chain reaction (PCR) with a degenerate "potyvirus-specific" upstream primer. This primer is complementary to a highly conserved motif present in the coat protein cistron of all potyviruses. For the downstream primer again oligo-dT was used. The amplified cDNA-fragments cover the complete 3' non-translated region (3'-NTR) and the C-terminal part of the viral coat protein. These cDNA fragments are subsequently cloned in a pGEM-T vector (Promega) and their nucleotide sequences determined. The sequence information of these cDNA fragments was used to determine the homology with the sequence of AlMV (Schönfelder *et al.*, 1993) and those of other known potyviruses present in the EMBL Database.

Using this strategy we have been able to identify four different potyviruses from *Alstroemeria*: AlMV, lily mottle virus (LMoV), *Ornithogalum* mosaic virus (OrMV) and a sofar unidentified new potyvirus. Of these, LMoV and OrMV were until now not known to occur in *Alstroemeria*.

For each of the four potyviruses virus-specific PCR primers have been developed. These primers are able to discriminate between the individual viruses even in mixed infections in *Alstroemeria*. This virus-specific test will enable the development of a specific detection method (i.e. an ELISA grade antiserum) for the individual potyviruses. In addition it appears to be a promising technique for the identification of other potyviruses (e.g. AlSV) present in *Alstroemeria*.

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