**BEAN YELLOW MOSAIC VIRUS ON GLADIOlus IN BRAZIL**

**ABSTRACT**

Gladioli are affected by a large number of viruses which may cause extensive losses, but Bean yellow mosaic virus (BYMV) and Cucumber mosaic virus (CMV) have been reported to be the most prevalent. Forty-five gladiolus cultivars were submitted to serological tests and electron microscopic observations. Indirect-ELISA was performed on leaf and corm extracts for detection of BYMV or CMV. The only positive reaction was for BYMV, and this only from the leaves. Filamentous particles in leaf dip preparations and cytoplasmic cylindrical inclusion bodies in thin sections were visualized. Due to the intense commercialization of the gladiolus crop and the practice of vegetative propagation, the presence of BYMV indicates a high degree of risk to this and other crops. For this reason its presence continues to act as a barrier to international trade in gladiolus for many countries.

**INTRODUCTION**

Gladiolus (Gladiolus x grandiflorus L.) is an appreciated garden plant and a commercial cut flower crop grown in many countries all over the world (Stein, 1995). In Brazil, it is among the five most important cut flowers (Paiva et al., 2000). Gladioli are affected by a large number of viruses. Bridgmon & Walker (1952) described gladiolus as a virus “reservoir” in the sense of being
a host of four different viruses and a virus source for other crops. Since then, the “reservoir” has increased to include no fewer than 14 viruses which have been noted to occur on gladioli, some of them of minor importance. None of the viruses found on gladioli was specific to the crop species or to the Iridaceae. All of them have a wide host range as well as geographic distribution and can be transmitted by external vectors (Stein, 1995).

Bean yellow mosaic virus (BYMV) and Cucumber mosaic virus (CMV) have been reported to be the most prevalent viruses infecting gladiolus, though not always associated with mosaic and white-break symptoms on leaves and color-break symptoms on flowers. It is worth mentioning that these viruses may cause extensive losses in the quality and quantity of flowers and corms. Furthermore, BYMV represents a serious problem which precludes international trade in gladiolus for several countries.

Our work aims at identifying the two main viruses that affect several gladiolus cultivars grown for inner and foreign markets.

MATERIAL AND METHODS

Virus source.

Forty-five gladiolus cultivars were obtained from commercial sources in Holambra and in the Estação Experimental de Capão Bonito, both in São Paulo state. Their corms were put to germinate under greenhouse conditions for indexing to BYMV and CMV.

Serological tests.

Leaf and corm extracts from gladioli were used in indirect-ELISA (Van Regenmortel, 1982) using antiserum to CMV isolated from Commelinaceae (Duarte et al., 1994) and to BYMV isolated from bean. Extracts were prepared at a dilution of 1:10 in coating buffer and the antisera diluted at 1:1000 in PBSTpo. All samples with values 3x the negative control values were considered as positive.

Electron microscopy.

Formvar-coated carbon-stabilized copper grids were floated for 5 min on a drop of crude sap extracted from gladioli leaves. Then, grids were washed with distilled water and stained with 2% uranyl acetate.

For in situ studies, small pieces of healthy or infected foliar tissue were fixed overnight at 4°C in 2.5% glutaraldehyde in 0.1M phosphate buffer pH 7.0, postfixed in 1% osmium tetroxide in the same buffer and stained overnight in 1% uranyl acetate. Then pieces were dehydrated in graded dilutions of acetone, embedded in Spurr’s medium, sectioned with an LKB III ultratome, and stained with uranyl acetate and lead citrate before being examined in a 208 EM Philips electron microscope.

RESULTS AND DISCUSSION

Glasshouse observation.

Diseased plants were observed among 38 cultivars, whose foliar mosaic symptoms varied from mild to severe. Zettler & Abo El-Nil (1977)
reported foliar symptoms, generally inconspicuous, consisting primarily of mild mosaic which was most evident on leaf sheaths and leaves developing just after emergence.

**Electron microscopy observations.**

Leaf dip preparations from symptomatic leaves of gladiolus consistently showed the presence of filamentous virus particles with sizes typical for potyviruses (Fig. 1), which are thought to be associated with the disease. However, symptomless plants of cv. Goldfield and Peters Pears contained similar Potyviridas-like particles. It is already known that the symptoms caused by BYMV tend to be masked when high temperatures occur.

Ultra-thin sections of gladiolus leaves revealed the presence of cytoplasmic cylindrical inclusion (CI) bodies in the form of laminated aggregates and pinwheels (Figs. 2 and 3), which are exclusive of the *Potyviridae* family (Berger et al., 2000). Amorphous inclusion bodies present in the cytoplasm (Fig. 3) were similar to those described for BYMV infection (Edwardson, 1974). Crystalline, cytoplasmic or nuclear inclusions may occur in both nucleous and cytoplasm of the BYMV infected cells (Riedel et al., 1998). However, such structures were not found in our gladiolus samples.

**Serological tests.**

Samples from corms and symptomatic or symptomless leaves were tested by indirect-ELISA with polyclonal antibodies for BYMV and CMV. No sample showed positive reaction to CMV, although the literature reports that CMV is easily detectable by ELISA from various parts of gladiolus plants, especially from corms (Stein et al., 1979). BYMV was detected in 88% of the 45 gladiolus cultivars assayed when extracts were used from the leaves and not from the corms. The results indicate that BYMV is widespread in gladiolus crops and that well-expanded leaves are required for BYMV detection due to an uneven distribution of the virus. Assay of BYMV by ELISA has been possible from leaves and petals, but not from corms. Apparently, in corms BYMV either occurs in a extremely low concentration or a substance in the corms alters BYMV so that infection by mechanical inoculation and serological reaction are inhibited. (Stein et al., 1979).

In most symptomless samples no virus was found except for Goldfield and Peter Pears which harbored BYMV.

It is particularly difficult to eradicate viruses from bulb crops that are vegetatively propagated year after year (Kamo, 1998). Up to now no chemical cure is available for virus infection, so prevention and sanitation are management tools available.
Figures 1 to 3 - Electron micrographs from naturally infected gladiolus. Fig. 1 - Elongated virus particle observed from leaf-dip preparation. Figs. 2 and 3 - Ultra-thin sections from infected gladiolus mesophyll cells: 2 - Laminar aggregates (LA), pinwheel (P) and scroll (S) in the cytoplasm; note thin circular structures along the LA, apparently formed by lamellar material (LS). 3 - Dense, amorphous inclusions (Ai). Bars = 300 nm
REFERENCES


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