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SCIENTIFIC COMMUNICATION

INTERCEPTATION OF VIRUSES ON FOREIGN TULIPS IN BRAZIL

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ABSTRACT

Sixty-seven tulip samples intercepted from the Netherlands by the Brazilian Agriculture Ministry, between 2004 and 2006, and two samples from São Paulo local market, Brazil, were assayed by serological and biological techniques, as well as by electron microscopy observations, for virus screening. In bulbs from the Netherlands potexviruses were detected in five samples and tobamoviruses in other three. Symptoms induced in some differential hosts were similar to those caused by *Tobacco mosaic virus* (TMV), while serological results indicated an infection by *Tulip virus X*. In two tulip samples from local flower shops, a *Potyviridae* was identified based on the presence of flexuous particles and cytoplasmic cylindrical inclusions. Mechanical transmission tests to potyvirus hosts in the Amaranthaceae, Chenopodiaceae and Solanaceae species were negative, making possible to exclude a possible infection by *Turnip mosaic viru*, a common virus species in tulips. Although TVX could be detected in intercepted tulip bulbs from the Netherlands, the virus is only reported in Scotland, Japan and USA.

KEY WORDS: Ornamental plant, *Potexvirus*, *Tobamovirus*, *Potyviridae*.

RESUMO

INTERCEPTAÇÃO DE VÍRUS EM TULIPAS IMPORTADAS PELO BRASIL. Sessenta e sete amostras de tulipas interceptadas da Holanda pelo Ministério da Agricultura, Pecuária e Abastecimento, entre os anos de 2004 e 2006, e duas amostras, adquiridas no comércio local de São Paulo, foram avaliadas para a presença de vírus por meio de ensaios biológicos, sorológicos e observações ao microscópio eletrônico. Nos bulbos provenientes da Holanda, foram detectados potexvírus em cinco amostras e tobamovírus em duas outras. Os sintomas induzidos em hospedeiras diferenciadoras indicaram a presença de *Tobacco mosaic virus* (TMV), enquanto que os resultados de testes sorológicos permitiram a identificaçãoo *Tulip virus X* (TVX). Nas duas amostras adquiridas no comércio local foram detectados *Potyviridae*, uma vez que partículas alongado-flexuosas e inclusões cilíndricas estavam presentes. Ensaios de transmissão mecânica desses vírus para espécies de Amaranthaceae, Chenopodiaceae e Solanaceae foram negativos, permitindo deste modo ser excluída uma possível infecção pelo *Turnip mosaic virus*, uma espécie frequente em tulipas. Apesar do TVX ter sido detectado em tulipas interceptadas da Holanda, esse vírus foi relatado apenas na Escócia, Japão e Estados Unidos.

PALAVRAS-CHAVE: Planta ornamental, *Potexvirus*, *Tobamovirus*, *Potyviridae*.

In order to comply with the increasing ornamental trade in the last years, Brazil has imported tulip bulbs from the Netherlands, an outstanding world producer and exporter, responsible for the production of 4.32 billion bulbs per year (Buschman, 2005).

The symptoms on tulip flowers are a landmark in plant virology for the unique flower breaking induced by viral infection. Tulip viruses which are responsible for the color break are: *Arabis mosaic virus*, *Cucumber mosaic virus*, *Lily symptomless virus*, *Tobacco rattle virus*; *Tobacco ringspot virus*; *Tomato bushy stunt virus*; *Tulip virus X* (TVX) and the potyviruses *Lily mottle virus* – LMoV (= Tulip band-breaking virus – TBBV), *Tulip breaking virus* – TBV, *Tulip mosaic virus*, Rembrandt tulip breaking virus, and *Turnip mosaic virus* (TuMV) (= Tulip chlorotic blotch virus, Tulip top breaking virus) (Dekker et al., 1993; Asjes, 1994; Mowat, 1995; Lesnaw; Ghabrial, 2000; Se; Kanematsu, 2002). Besides the petal symptoms those potyviruses can reduce the flower size, pollen production and seed number (Lesnaw; Ghabrial, 2000). Other virus species reported in tulips include: *Olive latent virus 1*, *Tobacco mosaic virus* (TMV), *Tobacco necrosis virus* (TNV) (sin. 'Augusta disease'), *Tomato black ring virus*, Tulip streak virus and *Tulip mild mottle mosaic virus* (Asjes, 1972, 1994; Kanematsu et al., 2001; Morikawa et al., 2005).

Taking into account that the introduction of tulips in Brazil is a relatively recent trade, with an annual increasing trend, it is mandatory the actual knowledge of the sanitation of tulip foreign bulbs. The present work was undertaken with the aim of detecting and identifying bulb-borne viruses in the imported material destined to the official screening analysis, and also in tulips formerly available in the local market.

The following materials were examined through biological and serological tests, and by electron microscope observations:

(i) a bulb bearing flowers with 'color break', purchased in a local supermarket – sample 1 (Fig. 1);

(ii) a flower showing 'color break' symptoms purchased in a local flower shop;

(iii) 67 tulip-bulb samples from the Netherlands and intercepted by Brazilian Agriculture Ministry belonging to 20 different cultivars: 'Ad Rem', 'Barcelona', 'Don Quixote', 'Golden Parade', 'Happy Family', 'Hollandia', 'Ile de France', 'Inzel', 'Leen van der Mark', 'Kees Nelis', 'Nairobe', 'Negrita', 'Princess Irene', 'Prominence', 'Strong Gold', 'Rococo', 'Up Star', 'Washington', 'White Dream', and 'Yokohama'.

Ten to 12 bulbs of each sample were put to germinate in sterilized soil and leaves were taken out after germination as study material.

For mechanical inoculation, leaves or tepals were ground in a virus-free mortar in 0.1 M sodium and potassium phosphate buffer pH 7.2 + 0.5% Na_2SO_3, and the extract was rubbed on previously carborundum-dusted leaves of healthy *Celosia* sp., *Chenopodium amaranticolor*, *C. murale*, *C. polyspermum*, *Datura stramonium*, *Gomphrena globosa*, *Nicotiana benthamiana*, *N. debneyi*, *N. glutinosa*, *N. rustica*, *N. sylvestris*, *N. tabacum* 'White Burley' and 'Samsun', and *Petunia* x *hybrida*. After inoculation plants were kept in greenhouse.

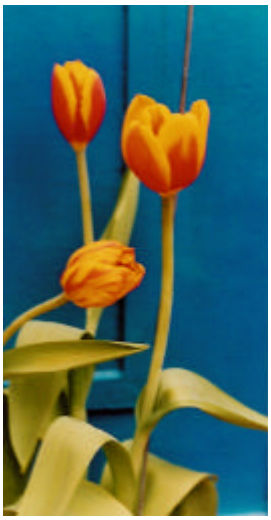

Fig. 1 - Tulip flowers showing color-break in tepals (sample 1).

For electron microscopy observations, fragments of tepals and leaves were used for negatively stained preparations and ultra thin sections (Dijkstra; De Jager, 1998).

According to the viral morphology observed in our samples and data from the literature, a set of polyclonal antisera (As), diluted at 1:50, was used targeting the serological identification of viruses in the samples, as follows: As-TMV, As-ToMV (*Tomato mosaic virus*), As-TuMV and As-TVX. 'Decoration' (Dijkstra; De Jager, 1998) was employed as serological assay.

The vegetative propagation of commercially grown tulips and the high probability for viruses to pass into the bulb progenies presents difficulties in maintaining virus-free stocks (Mowat, 1995). Although tulip leaves from some cultivars showed slight chlorosis, this symptom could not be considered exclusively associated with viral infection, since factors as genotype and physiological condition, temperature, light intensity, humidity and nutrition can also modify the phenotype of the plants.

Viruses could be detected in 10 out of 69 tested tulip samples. In two samples purchased in a local market, potyvirids were detected, being recognizable by their particle morphology and size, and by the peculiar cytoplasmic cylindrical inclusions induced (Fig. 2). They were not able to be mechanically transmitted to a set of tested host species and did not reacted to antiserum against TuMV, making possible to exclude this as the infective *Potyvirus* species.

Among the samples from the Netherlands, five showed the presence of elongated particles measuring ca. 500 nm in length in the foliar extract (Table 1). Since they were morphologically comparable to *Potexvirus* particles, the As-TVX was employed in 'decoration' tests for the samples 09, 41, 50, 53, and 66. The results showed that viruses present in these samples were serologically closely related to TVX (Fig. 3); however, different reaction intensities among the samples were observed, being the most intense observed with the sample 41. In *Gomphrena globosa*, TVX induced necrotic local lesions with reddish halo and on *C. amaranticolor* it induced chlorotic spots local lesions. *C. murale* was a differential host for TVX isolates since this host reacted with systemic necrotic symptoms only to TVX–sample 41.

Tulip virus X induces chlorotic and necrotic light brown to gray elongated streaks or elliptical markings in tulip leaves; the tepals develop narrow streaks of intensified pigmentation or with absence of pigmentation (Mowat, 1995). On our greenhouse conditions, TVX isolates did not induced color-break neither detectable symptom in tulips.

Because the peculiar reaction of local, necrotic lesions produced at 48h after inoculation with sap from samples 17, 22 and 59 on *N. glutinosa* and

Fig. 2 - Cylindrical inclusions in the tulip mesophyll cell cytoplasm from tulip sample 1. A– pinwheels (P) and laminate aggregates (L), barr = 500 nm. B– Detail of a pinwheel, barr = 300 nm.

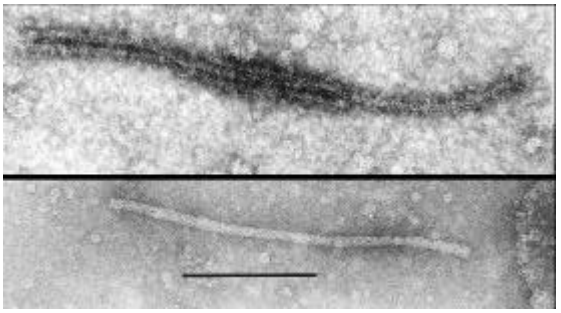

Fig. 3 - Potexvirus-like particle observed in sample 09. A– Virus particles decorate with *Tulip virus X* antiserum. B– Virus particle in negatively stained preparation. Barr = 200 nm.

Table 1 – Viruses detected in tulip samples from the Netherlands.

Year of the interception	Sample number	Virus genus	Tulip cultivar
	9	*Potexvirus*	'Barcelona'
2004	17	*Tobamovirus*	'Golden Parade'
	22	*Tobamovirus*	'Kees Nelis'
	41	*Potexvirus*	'Prominence'
2005	50	*Potexvirus*	'White Dream'
	53	*Potexvirus*	'Nairobe'
2006	59	*Tobamovirus*	'Ile de France'
	66	*Potexvirus*	'Ile de France'

D. stramonium leaves; and mosaic and malformation on *N. tabacum* 'White Burley' and 'Samsun', and the presence of rod-shaped particles with approximately 300 nm, it helped to infer the presence of *Tobamovirus*. In immunomicroscopy tests performed with the samples, virus particles appeared 'decorated', without discernible differences, with both As-TMV and As-ToMV. Thus, the identification at the species level was made by using differential plant hosts that showed necrotic local lesions on*N. sylvestris* leaves, and mosaic and systemic necrosis on *P. hybrida* and *N. rustica* . Such reactions are common in TMV infected vegetable (Bastos et al., 1999) and on ornamental infected plant (Alexandre et al., 2000). It is worth mentioning that, on tulips, TMV can induce foliar necrosis, malformation and necrotic stripes on tepals of red cultivars when planted in water soaked or poor soils, but the virus is latent in suitable crop conditions (Mokra, 1977).

Although TMV in Brazil is commonly found affecting vegetable and ornamentals, it is advisable to prevent the entry of foreign isolates. The detection of TMV and TVX on the small numbers of tested samples in this work may indicate the sporadic occurrence of these viruses in tulips in the Netherlands, despite TVX has been only reported from Scotland (Mowat, 1982), Japan (Yamaji et al., 2001), USA (Tzanetakis et al., 2005); and New Zealand (Ward et al., 2008). In the Czech Republic TMV has also been reported in tulips (Mokra et al., 1973). In Japan TVX has been reported from tulips under post-entry quarantine, from imported material from the Netherlands (Yamaji et al., 2001). Until recently, TVX was naturally restricted to the genus *Tulipa*. However, its occurrence on the dicot *Melissa officinalis* (Tzanekatis et al., 2005) indicates that the risk of TVX to affect species of economic interest in Brazil can not be neglected.

Potyviruses are frequent on tulips and are spread over the producing countries the Netherlands, Japan and USA. Among them, TBV is a prevalent and damaging virus of tulips (Mowat, 1995). So, the potyviruses detected in this work may represent those restricted to the *Liliaceae*, like TBV, or a strain of LMoV (= TBBV). The LMoV has some strains also adapted to dicotyledon hosts (Lisa et al., 2002); so, in principle it poses a potential danger to other crops indeed *Lilium* and tulips.

The present work provides information about viruses infecting tulip bulbs, which serves as warning for importers, growers and consumers who are not aware that 'tulip flower breaking' is mostly due to viruses, many of them still unknown to occur in Brazil.

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