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Full Length Research Paper

Natural incidence and infectivity level of three nepoviruses in ornamental crops in Iran

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Damage to ornamental crops by nepoviruses has occurred sporadically in Iran in the past. However, since 2006, outbreaks of nepoviruses have been recorded every year. The most affected ornamental crops were surveyed in two main cultivation areas in provinces of Markazi (Mahallat) and Tehran in 2006 - 2007. In all, 420 samples (with or without any conspicuous virus symptoms) were collected and analyzed by double- antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with polyclonal antibody to Tomato ring spot virus (ToRSV), Tobacco ring spot virus (TRSV) and Arabis mosaic virus (ArMV) . These viruses frequently were detected in samples of many different ornamentals (33 species) and often in mixed infections. Where as 8 samples found to be infected by one virus, 3 samples double infection and 6 samples were mix infected by three viruses. ArMV, ToRSV and TRSV were mechanically transmitted to *Vigna unguiculata*, *Nicotiana tabacum, Chenopodium amaranticolor, C. quinoa, Petunia hybrida, Datura stramonium* and *D. metel* indicator host plants and virus recovery was rechecked by ELISA. Of the total of 420 samples tested, 106 samples were reacted with the above virus antiserum. In Tehran, ToRSV was identified in 19 samples (4.52%), TRSV in 7 samples (1.66%), and ArMV in 5 samples (1.19%). In Markazi province, ToRSV was identified in 20 samples (4.76%), TRSV in 20 samples (4.76%), and ArMV in 23 samples (5.47%). In all, TRSV was shown to be prevalent nepovirus infecting ornamentals in these regions.

Key words: Tomato ring spot virus, tobacco ring spot virus, arabis mosaic virus, DAS-ELISA.

INTRODUCTION

The economic importance of ornamental horticulture is shown in a number of ways, in terms of the absolute size of the industry and world- wide sales. Many plant virus diseases cause significant losses in the production and quality of ornamental crops are difficult to control. New diseases occur as different crops are introduced or grown in new areas. Many crops are susceptible to multiple viruses, each of which may cause serious economic losses, and infected plant material may not be acceptable for export (Rakhshandehroo et al., 2006; Loebenstein et al., 1995). However, when occurring in complexes, they possibly exacerbate the symptoms induced by other in the other hand the antagonistic reactions also is not studied. At least 125 different viruses have been identified that infect and cause disease in ornamental plants. Identification of the virus and vector is important for development of practical disease prevention or control methods, and to minimize pesticide usage (Hsu and Maroon, 2003). At the final 'production' stage of growing and distributing ornamental plants, losses due to viral infections can range from 10 to 100%, depending upon the virus-host combination (Brunt et al., 1996; Loebenstein et al., 1995). Viruses of serious consequence recently identified by the floral and nur-sery industry in key ornamental crops include, but are not limited to: tospoviruses, nepoviruses, potyviruses, faba-viruses, closteroviruses, potexviruses, carlaviruses, cucu-moviruses, Caulimoviruses. There is therefore a need for research on these new and emerging virus and virus-like problems. Among these viruses, nepoviruses (Comoviri-dae) are one the main industrial group (Brunt et al., 1996; Loebenstein et al.,1995).The main nepoviruses reported to infect ornamentals are: Arabis mosaic virus, Tomato ring spot

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virus and Tobacco ring spot virus (Loebenstein et al., 1995; Card et al., 2007). ArMV is reported from *Arabis hirsute* and *Crocus* spp. in England (Smith et al., 1944). This virus is transmitted by a nematode vector (*Xiphinema and Dorylamidae spp.*), mechanical inoculation; grafting and seed (Murant, 1983) . In Iran ArMV and ToRSV from *Gladiolus* spp. were reported from ornamentals for the first time (Ghotbi et al., 2005; Ghotbi and Shahraeen, 2005; Rakhshandehroo et al., 2006; Kamran et al., 1981). ToRSV reported from America on *Nicotiana tabacum* (Price, 1936) and *Gladiolus* spp. (Leobenstein et al., 1995). ToRSV and ArMV transmitted by the vector *Xiphinema* and Dorylamidae spp. ToRSV and ArMV are transmitted by mechanical inoculation, grafting, seeds and pollen (Brunt et al., 1996; Card et al., 2007). TRSV was first reported in *Nicotiana tabacum* (Fromme et al., 1927) *Anemone* spp. In England (Hollings and Stone, 1963; Leobenstein, 1995) on *Gladiolus* spp. TRSV transmitted also, non-specifically by insects and mites, aphids and thrips. All nepoviruses produce necrotic or chlorotic local lesions following mosaic, ring spots or mottle symptoms on infected host plants but TRSV symptoms disappear soon after infection. Serological assay ,electron microscopy and RT-PCR techniques is reported to be a common tests to characterize nepoviruses including ToRSV and TRSV (Anonymous,2009) The aim of this study was to determine the prevalence and percent infection of important nepoviruses occurring on main cultivated ornamental crops in two region of Iran using routine biological and serological techniques.

MATERIALS AND METHODS

A total of 420 samples were collected in 2006 - 2007 from fields and glasshouses of mainly Tehran and Markazi Provinces. These were from 24 different family and 36 plant species. Samples, com-prised young and fresh leave and stems of each of ornamentals and flowering weeds with various symptom types including leaves and stem deformation, stunting , necrosis of stem and leaves, vei-nal discoloration, general yellowing of leaves, systemic chlorotic and necrotic spots and general mosaic mottle on leaves or without any conspicuous symptom. Samples for each plant species were selected in random and on the basis of general plant appearance at the time of sampling. In this study, nepoviruses detection in suspected plant species was carried regardless of symptoms rela-tion analysis. The number and location of the sample species are listed in Table 1.

Standard double antibody sandwich enzyme- linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977) was performed with polyclonal antiserum for ToRSV, TRSV and ArMV nepovirus species. All serological reagents against nepopoviruses used were from the Bioreba Plant Virus Antiserum collection Company, (Switzerland) including the respective positive controls for each ELISA. Absorbance at 405 nm was measured with Labsystem multiskan ELISA microplate reader (Denmark). Healthy *N. tabacum* triturated in general extraction buffer was used as negative control. A reaction was considered positive only if the absorbance was more than three times the background mean of negative control. The serological reagents used in ELISA did not reveal any con-siderable cross reactivity with other virus speciesof the genus, hence permitted an accurate species identification.

Mechanical transmissions to test plants were done for selected ELISA positives. Samples were prepared by grinding 1 g of leaf triturated in ice cold 0.1 M potassium phosphate buffer, pH 7.0 containing 0.15% of 2-mercaptoethanol. Samples were inoculated to *Nicotiana tabacum, Datura stramonium, D. metel, Petunia hybrida, Chenopodium amaranticiolor, Ch. Quinoa* and *Vigna unguiculata* (cv. local Mashad) (Table 2). The test plants were kept in an insect proof greenhouse at a constant temperature of 23 - 25°C. Symptoms on indicator hosts were recorded every two days for 14 days following inoculation and twice a week for the following 30 days. The indicator plants then tested to confirm the presence of a particular virus also to test for any symptom less infection.

RESULTS

Three nepoviruses ArMV, ToRSV and TRSV were detected infecting ornamentals in two main ornamental growing regions of Iran (Table 1, Figure 1). Of the 420 samples assayed 94 were reacted positively with ELISA. ArMV was identified in 6.66% of samples, ToRSV in 9.28% of samples and TRSV in 6.42% of samples. Besides ELISA results, symptoms descriptions of indicator test plants for these viruses are given in Table 2. ToRSV induced short flower and stunting in gladiolus crops. ArMV inoculated to *C amaranticolor* produced numerous pin point chlorotic local lesions followed by severe systemic mosaic and leaf deformation. *P. hybida* test plant reacted with mosaic and systemic brown necrotic spots on trifoliate leaves when inoculated by TRSV. Mechanically TRSV inoculated *N. glutinosa* produced systemic veinal necrosis followed by tissue wilting and plant stunting. Field collected *Gerbera sp.* with symptoms of general yellowing and leaf discoloration were shown to be TRSV infected. ToRSV infected Dahlia sp. was with symptoms of leaf discoloration mosaic and stunting (Figure 2). *C. amaranticolor* and N. glutinosa were found to be good diagnostic hosts for the nepoviruses and symptoms expressed were informative for a preliminary differentiation of the 3 nepoviruses. *Datura stramonium* and *D. metel* did not produce any symptom upon inoculation by ArMV, ToRSV and TRSV. Virus disease may cause reduction in the market value; new introduction may carry seed borne of vegetative-borne viruses. If this is combined with the economic importance of ornamental and flower production, it will explain the important of epidemiology of the viruses in ornamentals (Loebenstein et al., 1995). Seed transmission of viruses in ornamental crops is of minor economic importance, as many of these crops are vegetative propagated. Data on percent losses or estimation of reduction in true yield of ornamental crops are limited. Recent researches indicated the presence and spread of tospoviruses on ornamentals and of other agricultural crops in Iran (Farzadfar et al., 2003; Ghotbi et al., 2005). Presence of ArMV from ornamental crops and its ornamental host range from Tehran (Varamin) and Markazi (Mahallat) regions is reported for the first time from Iran (Ghotbi and Shahraeen, 2005).

DISCUSSION

On the basis of the present study ToRSV, ArMV and TRSV

Table 1. Natural distribution of nepoviruses in Markazi and Tehran provinces on important cultivated ornamental crops.

Plant species	ArMV	TRSV	ToRSV	Total tested*		Mixed infections
				Markazi	Tehran	
Amaranthacea *Amaranthus cruentus*	**1(M)	1(M)	2(M)	8	4	1M(ArMV + TRSV + ToRSV)
Amaryllidaceae *Polianthes* spp.	-	-	-	11	7	-
Araceae *Sheflera* spp.	-	1(M)	-	6	5	-
Arum oriental	2(M)	2(M)	2(M)	7	3	-
Balsaminaceae *Impatiens* spp.	-	-	12(M)	15	4	-
Caryophyllaceae *Dianthus* spp.	-	-	-	9	3	-
Centianaceae *Lesianthus* spp.	3(M)	-	-	5	7	-
Compositae *Calandula* spp.	-	-	1(T)	5	8	-
Chrysanthemum morifulium	10(M)	7(M)	11(T)	21	11	2M(ArMV + TRSV + ToRSV)
Chrysanthemum prutescene	-	1(M)	-	15	10	-
Dahlia spp.	1(T)	-	1(T)	4	8	-
Gazania spp.	-	-	-	5	4	-
Gerbera spp.	-	1(M)	-	4	9	-
Helianthus annus	3(T)	5(T)	3(T)	-	12	1T(ArMV + TRSV + ToRSV)
Rudbekia spp.	-	-	-	8	-	-
Tanaketum spp.	-	-	-	9	-	-
Zinia elegans	5(M)	-	-	7	5	-
Crucifreae *Cheiranthus cheiri*	-	-	-	-	14	-
Ericaceae *Erica carnea*	-	-	-	9	-	-
Gesneraceae *Saintpaulia ioantha*	-	-	-	-	10	-
Graniaceae *Pelargonium hortorum*	-	-	-	5	7	-
Pelargonium odoratissimum	-	-	-	9	6	-
Iridaceae *Gladiolus* spp.	1(M)	4(M)	4(M)	9	5	1M(ArMV + TRSV + ToRSV) 1M(TRSV + ToRSV)
Malvaceae *Althea* spp.	-	2(M)	-	3	8	-
Moraceae *Ficus benjamine*	-	-	-	5	8	-
Nyctaginaceae *Bougainvillea* spp.	-	-	-	-	9	-
Primulacea *Primula* spp.	1(T)	1(T)	1(T)	-	8	1T(ArMV + TRSV + ToRSV)
Rosaceae *Rosa damascena*	-	-	-	6	10	-
Scrophulariaceae *Anthirrhinum* spp.	-	-	-	-	9	-
Solanaceae *Petunia hybridagrandiflora*	-	1(T)	2(T)	5	6	1T(TRSV + ToRSV)
Tropaeolaceae *Tropaeo majus*	-	-	-	8	-	-
Umbeliferae *Tagetis patula*	1(M)	1(M)	-	8	5	1M(ArMV + ToRSV)
Violaceae *Viola* spp.	-	-	-	-	9	-
TOTAL	28 (23M,5T)	27 (20M,7T)	39 (20M,19T)	420 (206 M,214 T)		

*Total samples tested was not calculated on the basis of mixed infections
** Number of infected plant
M: Markazi provine, T: Tehran province

nepoviruses were the most prevalent viruses on ornamental crops reporting from Iran. ArMV is reported to be transmitted at low rates through seeds of *Viola tricolor* and *Petunia hybrida*. Seed transmission in this case could be of economic importance. Seed transmission of ToRSV and TRSV has been reported to occur in *Pelargonium*. TRSV and ToRSV in pelargonium species are reported as two common nepoviruses infecting green house cultivated pelargonium (Anonymous, 2009). TRSV is also transmitted in seed of *P. hybrida* and *Zinnia elegans*, at 20% and 5% respectively (Loebenstein et al., 1995; Brunt et al., 1996). ToRSV and TRSV occurred mostly on a wide range of perennial crops. TRSV and ToRSV were probably disseminated from North America to other countries in infected planting material; they were reported from Japan in imported corms (Fakomoto et al.,

Table 2. Host range studies of different nepovirus species

Host	ToRSV		TRSV		ArMV	
	L	S	L	S	L	S
Nicotiana rustica	CL	-	NL	LD	-	LD
Nicotiana glutinosa	MNL	-	-	LD, SNL	-	-
Vigna anguiculata	CL	-	NL	LD	NL	-
Datura metel	-	-	-	-	-	-
D. stramonium	-	-	-	-	-	-
Petunia hybrida	-	-	-	SNL. Mo	-	CL
Chenopodium amaranticolor	CL	SNL	CLNL	-	NL	Mo, LDMNL
Cucumis sativus	CL	LD	CL	RS LD	NL	CL

CL: chlorotic lesion, LD: leaf deformation, MNL: mild necrotic lesion, Mo: mosaic, NL: necrotic lesion, RS: ring spot, SNL: sever necrotic lesion

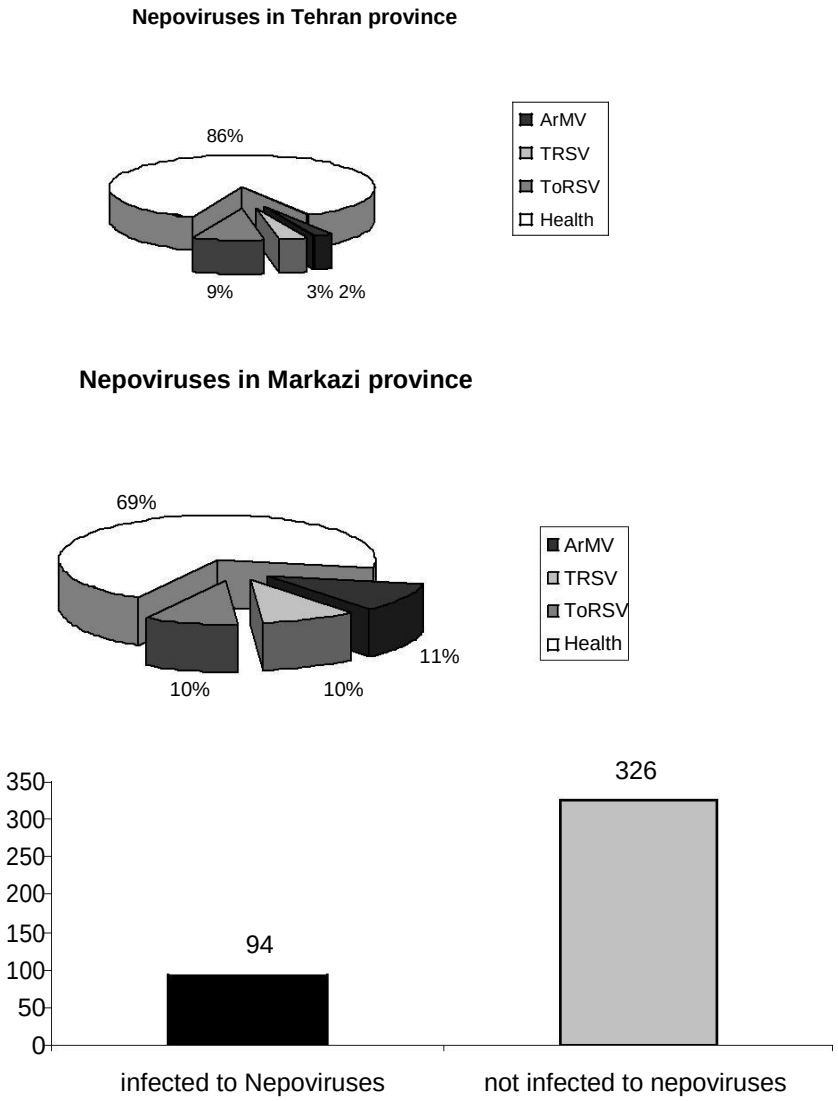

Figure 1. Percent infection of nepoviruses on ornamentals in Tehran and Markazi pro-vinces.

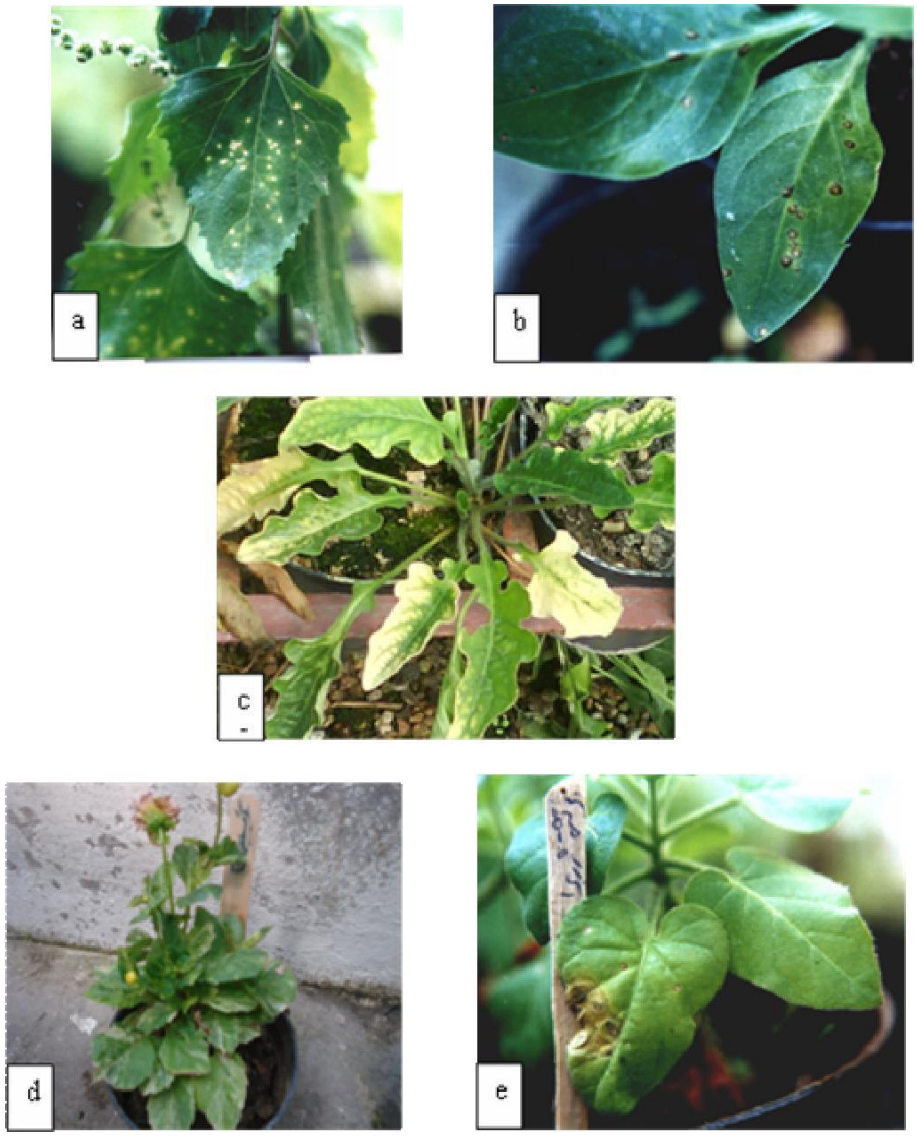

Figure 2. a: Local chlorotic pin point spot on *C. amaranticolor* infected by ArMV, b: Virus inoculated *P. hybrida* showing systemic mosaic and necrotic lesion, c: Naturally TRSV infected *Gerbera* sp. with yellowing, discoloration and growth reduction symptoms, d: ToRSV infected *Dahlia* sp. Showing mosaic, leaf discoloration and stunting, and e: ToRSV inoculated N.glutinosa indicator host plant showing mosaic, systemic veinal necrosis and stunting

1982). ToRSV was also reported from Australia (Randles and Francki, 1965) and Iran (Kamran et al., 1982). Nepoviruses is an example of seed transmitted embryoinfecting viruses, where creating loci of infection can, however cause severe damages in the field if an efficient vector is present. However as these viruses are present and reported on other cultivated plants in Iran including cucurbits (Cucurbitaceae),soybean (Fabaceae),potato (Solan--aceae) and Vitis vinifera (Vitaceae) (Farzadfar et al., 2003).The use of virus free seeds and vegetative materials for propagation and integrated pest and disease control management and breeding for virus disease resistant is recommended.

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