An investigation on Rose Mosaic Disease of Rose in Hatay-Turkey

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Abstract

Field inspections were carried out to investigate *Apple mosaic virus* (ApMV), *Arabis mosaic virus* (ArMV) and *Prunus necrotic ringspot virus* (PNRSV) which are associated with rose mosaic disease (RMD) during the years of 2008 and 2009. Characteristic symptoms, including chlorotic line patterns (zigzag pattern), vein-banding and mottles in leaves were observed during spring. Symptoms were also evident during summer on leaves produced until early summer. Flower abnormalities as phyllody were also exhibited during autumn. Distortion and reduction in flower size and early leaf drop have been observed on symptomatic plants in winter. Leaf samples taken from 15 rose plants from 'Rosa hybrida L.' neighboring stone fruit orchards were tested by mechanical inoculation to herbaceous plants and enzyme-linked immunosorbent assay (DAS-ELISA) for the presence of ApMV, ArMV and PNRSV, which are the viruses related to RMD. *Catharanthus roseus* L. G. Don, *Chenopodium amaranthicolor* Coste and Reyn., *C. quinoa* Wild, *Cucumis sativus* L., *Gomphrena globosa* L., *Lagenaria siceraria* (Mol.) Standl, *Nicotiana benthamiana* L., *N. clevelandii* L., *Nicotiana glutinosa* L., *Phaseolus vulgaris* L., *Vigna unguiculata* L. test plants were incubated after mechanical inoculation for symptom appearance at 25°C±2 and 16:8 h photoperiod (day:night) conditions in an insect-proof room. Symptoms including chlorotic local lesions, systemic necrosis, stunting and yellow mottling began to appear on *C. quinoa* and *C. sativus* in 2-3 weeks after sap inoculation. Serological tests of test plants are in progress. The rose plants showing symptoms in home gardens were re-tested for the viruses in spring by ELISA. According to the results of the Bioassay by sap inoculation and ELISA on symptomatic rose plants, the causal agent of RMD is PNRSV. The viruses affecting rose plants spread through cuttings from a diseased plant because new plants are generally produced by the rooting of cuttings in home gardens in Hatay. Further detailed investigations are necessary to find out the causal agent/s of RMD in rose in the region, because infected rose plants can be an important factor in the epidemiology of virus diseases caused by these agents in rose plantations.

Keywords: ApMV, Bioassay, ELISA, Oil Rose, PNRSV, virus

Introduction

Rose mosaic disease (RMD) is the one of most important and widespread virus diseases of rose plants. RMD is caused by infection with any of a number of different viruses. RMD is associated especially with *Prunus necrotic ringspot virus* (PNRSV), *Apple mosaic virus* (ApMV), *Arabis mosaic virus* (ArMV) and *Strawberry latent ringspot virus* (SLRV). The most important of these viruses in the United States and United Kingdom is PNRSV, a common disease of stone fruit trees (Thomas; 1981; 1982; 1984; Horst, 1983; Manners, 1985). PNRSV), a member of the genus *Ilarvirus* in the family *Bromoviridae*, occurs worldwide and is a serious pathogen of many plant species, including rose, *Prunus* spp.(Barbara et al., 1978; Barbara,1980; Thomas, 1980; Cambra et al., 1982). The new virus,related most closely to *blackberry chlorotic ringspot virus* was reported to be isolated from rose and is considered a strain of that virus (Tzanetakis et al., 2006).

There has been much opinion and research conducted on the means of transmission of RMD in roses. It was suggested that the Rose mosaic was probably transferred to roses originally from one of the stone fruits, by graftage (Cochran, 1984). It then spread from one rose cultivar to another through infected rootstocks (Manners, 1985). PNRSV and ApMV transmission by seed, pollen, on cutting implements and by root grafting from infected plants to healthy plants has been reported and the results showed that root grafting is involved in the natural spread of the virus in roses (Golino et al., 2005). Rose mosaic viruses cause symptoms on leaves that include ringspots, line patterns (zigzag pattern), mosaics, distortion and puckering. Serological procedures have been used more than other methods for the detection of PNRSV (Mink and Aichele, 1984).

RMD has been shown to cause flower distortion, reduced flower production and flower size, stem caliper at the graft union and reduction in vigor, early autumn leaf drop, lower bush survival rates, increased susceptibility to cold injury and more difficult establishment after transplanting (Cochran, 1972; 1982; 1984; Secor et al., 1977; Thomas, 1982; 1984). The symptoms are highly variable among rose cultivars and are strongly influenced by weather and growing conditions. Infected plants may appear to be quite healthy for much of the year, and any symptoms which do appear may be attributed to other causes, such as spray burn, nutrient deficiencies, high temperature, or poor horticultural

practices. It has been suggested that the "deterioration" which often occurs in rose cultivars several years after their introduction may be a result of virus infection (Allen, 1984).

Material and methods

Leaf samples taken from 15 *Rosa hybrida* plants neighboring stone fruit orchards and showing symptoms associated with virus diseases were tested by Bioassay-sap inoculations on herbaceous plants and enzyme-linked immunosorbent assay (DAS-ELISA) for the presence of ArMV, ApMVand PNRSV in both autumn and spring. For attempted sap inoculations of the viruses to herbaceous test plants, young leaves were homogenized in 0.1 M phosphate buffer (pH 7.2) in a pestle and mortar, and the sap extracts inoculated onto Celite-dusted leaves of herbaceous virus indicator plants: *Catharanthus roseus* L. G. Don, *Chenopodium amaranthicolor* Coste and Reyn., *C. quinoa* Wild, *Cucumis sativus* L., *Gomphrena globosa* L., *Lagenaria siceraria* (Mol.) Standl, *Nicotiana benthamiana* L., *N. clevelandii* L., *Nicotiana glutinosa* L., *Phaseolus vulgaris* L., *Vigna unguiculata* L.. Test plants were incubated after mechanical inoculation for symptom appearance at 25°C±2 and 16:8 h photoperiod (day:night) conditions in an insect-proof room. Four plants from each of the herbaceous indicator species were mechanically inoculated with the sap of a rose sample. All rose samples and inoculated test plants were tested for the presence of the viruses by ELISA as described by Clark and Adams (1977). Antiserum kits from Bioreba AG (Switzerland) were used in standard DAS-ELISA. Four indicator plants of each species used for testing of each samples in Bioassays were pooled together as one samples for ELISA. Four asymptomatic rose seedlings taken from a nursery were also inspected visually as control plants and tested serologically.

Eight to ten single-node cuttings (approximately 15-20 cm long) of symptomatic rose plants were excised. Basal ends of the cuttings were dipped into 0.5% Indole butyric acid (IBA),rooted in pots containing a peat:perlite (1:1) mixture and kept in insect-proof growing room at 25°C±2 and 16:8 h photoperiod (day:night) in the autumn of 2008 for symptom observation. Young shoots grown from the axillary buds of each cutting were tested by ELISA when they were approximately 3 to 5 cm long.

Ten oil rose (*Rosa damascena*) seedlings obtained from a nursery in Isparta province (where the main oil rose production area in the Lakes region of Turkey is) and indexed by grafting and ELISA. Five healthy oil rose plants were graft-inoculated with buds from the PNRSV-rose source in autmn of 2008. The inoculum consisted of three buds per each seedling. Three plants negative for virus by ELISA was used as the healthy control.

Results and discussion

Although, Rose mosaic disease (RMD) is caused by a complex of several viruses in rose plants, PNRSV is the most common agent of RMD (Thomas; 1982; 1984; Horst, 1983). Initially the symptoms were thought to be caused by PNRSV. For this reason, the presence of PNRSV and ArMV were mainly detected in the samples taken from symptomatic rose plants by Bioassay sap inoculations and double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA).

Although symptoms often are evident in spring and early summer but may not be on leaves produced in summer, chlorotic-zigzag or oak leaf patterns, leaf distortion and puckering were noted on rose plants in September and all kinds of symptoms were very abundant during the autumn season of 2008 (Figure 1). However, ringspot patterns and vein clearing symptoms associated with rose mosaic were not observed during field inspections in both years. All of the PNRSV-infected (15) samples taken from young leaves of symptomatic shoots reacted positively for PNRSV by DAS-ELISA and were used in Bioassay sap inoculations. Basal leaves were more symptomatic, but apical leaves gave a more definitive result by ELISA. RMD is known to cause increasing susceptibility to cold injury (Secor et al., 1977), and early autumn leaf drop (Thomas, 1982). Also in the present work, reducing number of leaves and early leaf drop were observed in symptomatic plants compared with asymptomatic ones in the gardens in autmn of 2008. An influence of variety and environmental conditions is suggested. *R. damascena* seedlings obtained from a nursery in Isparta showed no characteristic symptoms related to virus diseases and seemed to be healthy. However, one out of ten *R. damascena* plants was also found to be infected with PNRSV.

Inoculation with extracts from 15 symptomatic *R hybrida* plants and one assymptomatic *R. damascena* seedling mainly produced systemic mosaic, stunting, vein banding on *C. sativus*, chlorotic local lesions on *V. unguiculata*, chlorosis with reducing of the leaves on *C. roseus*, and mosaics and chlorosis on *R. damascena* (Figure 1 and Table 1). These symptoms were generally similar to those that were described previously for these viruses (Boulila and Marrakchi, 2001; Salem et al., 2004; Rakhshandehroo et al., 2006). Only one PNRSV-infected rose sample exhibited symptoms of chlorotic local lesions and top necrosis on three *P. vulgaris* test plants. Except for this sample, no symptoms were observed in *Phaseolus vulgaris* inoculated with extracts from the other 14 rose samples. Single or mixed infections in

combination with ApMV and/or ArMV were not detected by ELISA in this study. According to the results of Rakhshandehroo et al. (2006), mixed infections of PNRSV and ArMV were found in all rose samples tested by sap inoculations and ELISA in Iran. However, PNRSV was reported to be mostly distributed through the red rose varieties (*Rosa × damascena, R. chinensis, R. canina,* and *R. multiflora*) and ArMV was within the white varieties (*R. canina, R. indica,* and *R. multiflora*) by serological tests. A survey for viruses in rose propagated in Europe resulted in the detection of only *Prunus necrotic ringspot virus* (PNRSV) among seven viruses screened by Moury et al. (2001).

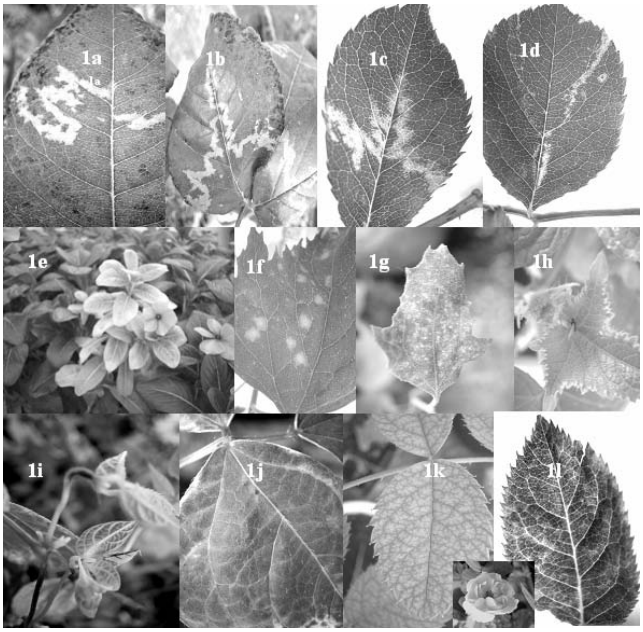

Fig. 1 Symptoms of PNRSV on naturally infected rose and inoculated test plants: 1a-d: Chlorotic zigzag patterns appeared on naturally infected rose leaves, 1e: Severe chlorosis and leaf reducing on *Catharanthus roseus,* 1f-g: Chlorotic spots on *Chenopodium amaranticolor* and *C. quinoa,* 1h: Chlorosis and mosaic symptoms on *Cucumis sativus,* 1i-j: Chlorosis and necrosis on *Phaseolus vulgaris* and *Vigna unguiculata,* 1k-l: Chlorosis and mosaics on artificially infected leaves of *Rosa damascena* (Oil rose)

Using the *P. persica* clone GF 305 it was possible to differentiate rose PNRSV isolates, and the *P. avium* clone F12/1 was also reported to be a new host-plant for differentiating pathogenicity of PNRSV rose isolates ((Moury et al., 2001; Paduch-Cichal et al., 2007). During inspections on new plants in 2009, except leaf deformation and mosaics, no symptom has been observed on rose plants obtained by rooting of cuttings. Further studies are also necessary to investigate the status of other virus diseases of Rose in Turkey. Indexing of PNRSV-infected source plants by using woody indicators such as the *P. persica* clone GF 305 and almond (*P. dulcis*) seedlings are in progress.

Tab. 1 Symptomatology of test plants inoculated with PNRSV mechanically or by tissue grafting

Indicator Plants	Symptoms	ELISA test
Family: Amranthaceae		
Gomphrena globosa L.	0	-
Family: Apocynaceae		
Catharanthus roseus L. G. Don.	Cl, L.R.	-
Family:Chenopodiaceae		
Chenopodium amaranticolor Coste and Reyn.	C.L.L.	+
Chenopodium quinoa Wild	C.L.L.	+
Family:Cucurbitaceae		
Cucumis sativus L. cv. Cemre F1	M, Cl and/or Vb	+
Lagenaria siceraria (Mol.) Standl	0	-
Family: Fabacceae		
Phaseolus vulgaris L.	(C.L.L. and TN)[b]	-
Vigna unguiculata L.	C.L.L	-
Family:Rosacae[a]		
Rosa damascena (Oil rose)	M and Cl	-
Family: Solanmceae		
Nicotiana benthamiana L.	0	-
Nicotiana clevelandii L.	0	-
Nicotiana glutinosa L.	0	-

(+ Positive, - Negative) in ELISA test. C.L.L.=Chlorotic Local Lesion, Cl.=Chlorosis, L.R.=Leaf reducing, M=Mosaic, N.L.L.=Necrotic LL, TN.=Top necrosis, Vb=Vein banding, 0 = no symptoms. [a] : inoculation by grafting, [b]: only for one rose sample infected with PNRSV.

Because the only proven means of transmission of RMD in roses is through vegetative propagation of infected buds, scion or root stocks, the use of clean and virus-tested production material is essential to improve productivity in gardens. The lack of certified virus free plants is one of the main problems in commercial rose production, which includesoil rose, *R. damascena* cultivation in Turkey.

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