International Research Journal of Biotechnology (ISSN: 2141-5153) Vol. 4(2) pp. 27-33, February, 2013
Available online http://www.interesjournals.org/IRJOB

Full Length Research Paper

Serological detection of rose viruses associated with rose mosaic disease affecting Taif- rose in KSA

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Accepted February 14, 2013

Enzyme- linked immunosorbent assay (ELISA) testing of 25 rose (*Rosa multiflora*. cv Taifey) samples which exhibited virus- like symptoms of the rose mosaic disease. These samples were collected from different locations in Taif governorate, Kingdome of Saudi Arabia;. The serological assay of Double antibody sandwich indirect -enzyme linked immunosorbent (DASI-ELISA) indicated that rose mosaic disease in KSA is associated with *Prunus Necrotic Ring Spot Virus* (PNRSV, Genus *Ilarvirus*, Family *Bromoviridae* and *Apple mosaic virus* (ApMV, Genus *Ilarvirus*, Family *Bromoviridae* either as a single or mixed infection. DASI-ELISA was used to detect PNRSV and ApMV isolates by using Polyclonal antibodies (PAbs) specific to PNRSV and ApMV. In PNRSV out of 20 tested samples, 14 samples represent 70 % gave positive reactions using ELISA with values ranged between 0.504 and 2.104 compared to values ranged between 0.065 and 0.072 of the 6 negative healthy samples. In ApMV out of 20 tested samples, 7 samples represent 35 % gave positive reactions using ELISA with values ranged between 0.064 and 0,253 compared to values ranged between 0.064 and 0.218 of the 13 negative healthy samples. The sample which exhibited mixed infection of two viruses showing rose mosaic symptoms appears as chlorotic vein banding, a mosaic pattern, and a yellow net pattern on *Rosa multiflora*. cv Taifey. The viral capsid protein was estimated to be 25000 Dalton of Rose Viruses Associated with Rose Mosaic Disease. This is the first report of rose viruses in Taif- KSA.

Keywords: Taif rose, Rose mosaic disease, PNRSV, ApMV, DASI-ELISA, SDS-PAGE.

INTRODUCTION

The rose is the most popular garden plant in the world, as well as the most important cut flower. There are such a wide variety of roses available that any garden with sufficient sun should be able to grow roses. Taif has known cultivation and production of roses, which exists more in the mountains of Hada and Shafa and some other mountain areas of Taif, which is extracted attar sectarian famous shot to fame and fame in many countries of the world and the superiority of the best types of perfumes imported from abroad.

There are currently Taif specialized farms for the production of roses sectarian and transferred to industry and trade with the availability of many possibilities that have helped to increase production and distribution and to achieve international fame Lord Taif local and international level, pointing out that many of those who love with roses sectarian outside the Kingdom transferred many of the seedlings of roses to their country, especially in Morocco, Egypt, Syria, the GCC states and Yemen, Australia and some other countries in the Arab and global levels.

Virus and virus like diseases of roses have become as common as any of the other rose diseases. All species and varieties of roses are susceptible to one or more virus diseases. However, infection often goes undetected because virus and virus-like symptoms can be mild and easily overlooked (Horst, 1996).

Rose mosaic disease is the most common virus dise-

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se of roses. It can be caused by one or more of a complex of several viruses including *Prunus Necrotic Ringspot Virus* (PNRSV, Genus *Ilarvirus*, Family *Bromoviridae*) (Fulton, 1970; Moury *et.al*., 2001) and *Apple Mosaic Virus* (ApMV, Genus *Ilarvirus*, Family *Bromoviridae*) (Fulton, 1952; 1967; 1968; Johnstone *et al*., 1995; Wong and Horst, 1993).

Infected plants with rose mosaic viruses show different symptoms such as flower distortion; reduced flower production, flower size, stem caliper at the graft union and vigor; early autumn leaf drop; lower bush survival rates; increased susceptibility to cold injury; and difficulty in establishment after transplanting (Thomas, 1981, 1982; 1984; Moran *et al*., 1988; Wong *et al*., 1988).

Symptoms are highly variable and depend on the growing season, temperature, species, cultivar and type of viruses infecting the plant. (Horst, 1996).

There is no known natural vector of rose mosaic diseases. These viruses can easily be transmitted from infected mother plants to the progenies, such as roses which are vegetatively propagated by own root cuttings or grafting on rootstock (Janick, 1986) .Therefore, detection and identification of rose mosaic is necessary, particularly with regard to testing nursery materials and propagation stocks .

Advanced diagnostics are also critical to trade among countries, having great potential for importation and quarantine programs. The main objective of this study was to determine the viruses associated with rose mosaic disease in Taif region, KSA.

MATERIAL AND METHODS

The experiments of this study were conducted in the Biotechnology and Genetic engineering unit, Taif University, KSA.

Grapevine sampling

Taif- rose (*Rosa multiflora*. cv Taifey) plants were surveyed in Rose farms in Taif, KSA and symbiotic plants were collected from different locations of Hada and Shafa and were transferred to the experimental greenhouses at Taif University, KSA during the growing season. A Set of 25 leaf samples were collected and 1- 5 gm of mature and young leaves from each sample were stored at 4^{o}C.

ELISA detection

The method of detection is an Enzyme-linked immunosorbent assay (ELISA) based on Double antibody sandwich indirect (DASI) by using Polyclonal antibodies. The antigen is trapped by the Fragment F (ab) 2 and revealed by the whole IgG. Signal develops by alkaline phosphatase reaction with p-nitrophenyl phosphate; as described by Edwars and Cooper (1985) to detect PNRSV and Imed *et al*. (1997) to detect ApMV. Polyclonal antibodies (PAbs) were purchased from Agritest S.r.l., Valanzano, Italy. DASI ELISA results were taken as mean absorbance value of three replicates per sample. Positive and negative controls were supplied with the kit.

Determination of viral protein molecular weight

Using the polyacrylamide gel electrophoresis (SDS-PAGE), plant preparation of viral protein was denatured by heating in the presence of sample buffer (Lammli, 1970) and (Hill and Shepherd, 1972) and the mixture was boiled in water bath for 5 min. immediately was put in ice and loaded on the gel. The denatured gels were prepared as 12% running gel and 4.5% stacking gel. The gels were prepared form monomer solution of 30% acrylamide and 0.8% Bis-acrylamide. Ammonium persulphate and TEMED were used as initiators for cross- linking and polymerization.

Infected Taif- rose (*Rosa multiflora*. cv Taifey) samples which exhibited virus-like symptoms of the rose mosaic disease, from plant sap were prepared separately at ratio 1: 10 in 6 X sample buffer (appendix) and boiled for 5 min in water bath and clarified by centrifugation. Aliquots of 15 µl were applied per slot with low or mid standard protein markers run for 2 hr at 80 volts and a further 90 min at 120 volts. The gel was stained with Commassie Brilliant blue R 250. At the end of the run, when the dye reaches the bottom, the sandwiches were disassembled and the gel was put into staining solution. The gel was gently shaken for overnight at room temperature, then removed from the stain and washed once with water and put in destaining solution for one hr. The gel was transferred to a second container filled with destining solution and shaken for another hour. At this stage, the protein band could be visualized by naked eyes and the data could be recorded by taking a photograph.

RESULTS

ELISA detection

Rose mosaic disease is the most common virus disease of roses. It can be caused by one or more of a complex of several viruses including *Prunus Necrotic Ringspot Virus* (PNRSV, Genus *Ilarvirus*, Family *Bromoviridae*) (Fulton, 1970; Moury *et.al*., 2001; Mansour, 2006) and *Apple Mosaic Virus* (ApMV, Genus *Ilarvirus*, Family *Bromoviridae*) (Fulton, 1952; 1967; 1968; Johnstone *et al*., 1995; Wong and Horst, 1993; Mansour, 2006). The 25 rose (*Rosa multiflora*. cv Taifey) samples which

Table 1. DASI- ELISA detection of PNRSV in Roses (*Rosa multiflora*. cv Taifey) samples using the PAbs specific to PNRSV from Agritest S.r.l., Valanzano, Italy.

Samples #	ELISA detection	
	EV	R
1	0.070	-
2	0.065	-
3	0.067	-
4	0.070	-
5	0.065	-
6	0.072	-
7	2.073	+
8	1.988	+
9	2.171	+ +
10	1.838	+
11	1.989	+
12	2.072	+
13	1.686	+
14	1.789	+
15	1.749	+
16	1.710	+
17	2.092	+
18	1.919	+
19	2.402	+ +
20	2.336	+ +

Positive control: 2.145 EV: ELISA values
Negative control: 0.072 R: Result
+ : Positive - : Negative

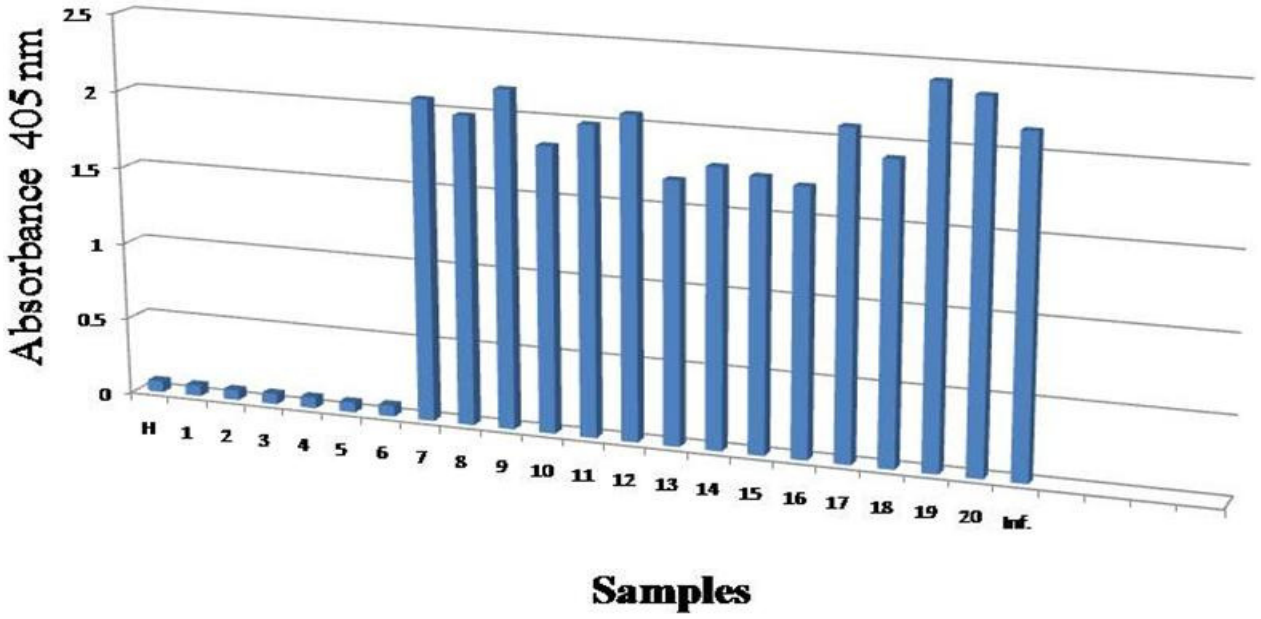

Figure 1. Histogram showing the results of the DASI- ELISA using polyclonal antibodies specific for PNRSV from Agritest S.r.l. , Valanzano, Italy, with healthy (H) as a (N.c), Infected as a (P.c.), the samples from 1 to 20 leaf samples of Roses (*Rosa multiflora*. cv Taifey) seedlings.

Table 2. DAS- ELISA detection of ApMV in Roses (*Rosa multiflora*. cv Taifey) samples using the PAbs specific to ApMV from Agritest S.r.l. , Valanzano, Italy.

Samples #	ELISA detection	
	EV	R
1	0.076	-
2	0.064	-
3	0.093	-
4	0.082	-
5	0.076	-
6	0.073	-
7	0.212	-
8	0.218	-
9	0.224	+
10	0.227	+
11	0.243	++
12	0.253	++
13	0.212	-
14	0.217	-
15	0.239	++
16	0.216	-
17	0.218	-
18	0.224	+
19	0.221	+
20	0.204	-

Positive control: 0.229 EV: ELISA values
Negative control: 0.073 R: Result
+ : Positive - : Negative

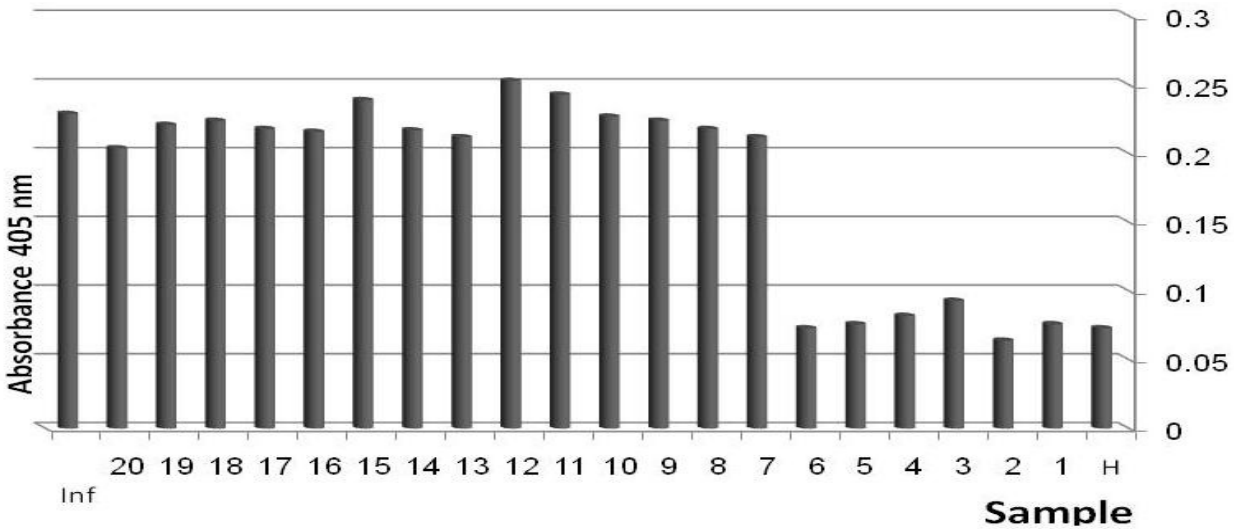

Figure 2. Histogram showing the results of the DASI- ELISA using Italian isolate of ApMV monoclonal antibodies with healthy (H) as a (N.c), Infected as a (P.c.), the samples from 1 to 20 leaf samples of Roses (*Rosa multiflora*. cv Taifey) seedlings.

exhibited virus- like symptoms of the rose mosaic disease were tested against PAbs specific for PNRSV and ApMV from Agritest S.r.l. , Valanzano, Italy. In PNRSV results in Table (1) and figure (1) showed that a number of 20 out of 14 samples represent 70% gave positive reactions using ELISA with values ranged between 0.504 and

Figure 3. Symptoms observed on Rose plants collected greenhouses the experimental farm at Taif University showed Chlorotic vein banding, a mosaic pattern, and a yellow net pattern on *Rosa multiflora*. cv Taifey.

2.104 compared to values ranged between 0.065 and 0.072 of the 6 negative healthy samples. In ApMV results in Table (2) and figure (2) showed that a number of 20 out of 7 samples represent 35 % gave positive reactions using ELISA with values ranged between 0.064 and 0,253 compared to values ranged between 0.064 and 0.218 of the 13 negative healthy samples.

Virus source

According ELISA results the mixed infection samples of two viruses were used as a source of virus infection. The highly concentrated +ve-ELISA sample (No.12) Taif- rose (*Rosa multiflora*. cv. Taifey) was showing rose mosaic symptoms appears as chlorotic vein banding, a mosaic pattern, and a yellow net pattern as shown in Figure (3).

Determination of viral protein molecular weight

The viral protein of Rose Viruses Associated with Rose Mosaic Disease migrated as a single band from plant viral preparation with a molecular mass of ~ 25 KDa as shown in Figure (4).

DISCUSSION

The survey suggested that rose mosaic disease is associated with PNRSV and ApMV either as a single or mixed infection. The results of ELISA tests clearly showed that PNRSV was the dominant virus on roses since about 70 % of infected samples were found to be PNRSV-infected either in single or mixed infection. ApMV occupied the second position with 35 %. These results are in agreement with previous reports (Cambra *et al.,* 1989; Casper, 1973; Farrar and frost, 1972; Fulton, 1968; Fulton, 1970; Moury *et al*., 2001).

ELISA was widely used for the detection of PNRSV in tissues collected early in the vegetation period in young leaves or in newly formed buds (Thresh *et al.,* 1977; Barbara *et al.,* 1978, 1979; Barbara, 1988; Thomas, 1980; Mink and Aichele, 1984 a, b; Torrance and Dolby, 1984). The sensitivity of DAS-ELISA was ten-fold increased when an amplification of the enzyme reaction was applied (Varveri, 1994). ApMV was routinely detected by ELISA (Clark *et al.,* 1976; Voller *et al.,* 1976; Thresh *et al.,* 1977; Barbara *et al.,* 1979; Korpraditskul *et al.,* 1979; Hardcastle and Gotlieb, 1980; Torrance and Dolby, 1984). ELISA detection can be done throughout the growing season in individual samples of young leaves or twigs with newly formed buds (Torrance and Dolby,

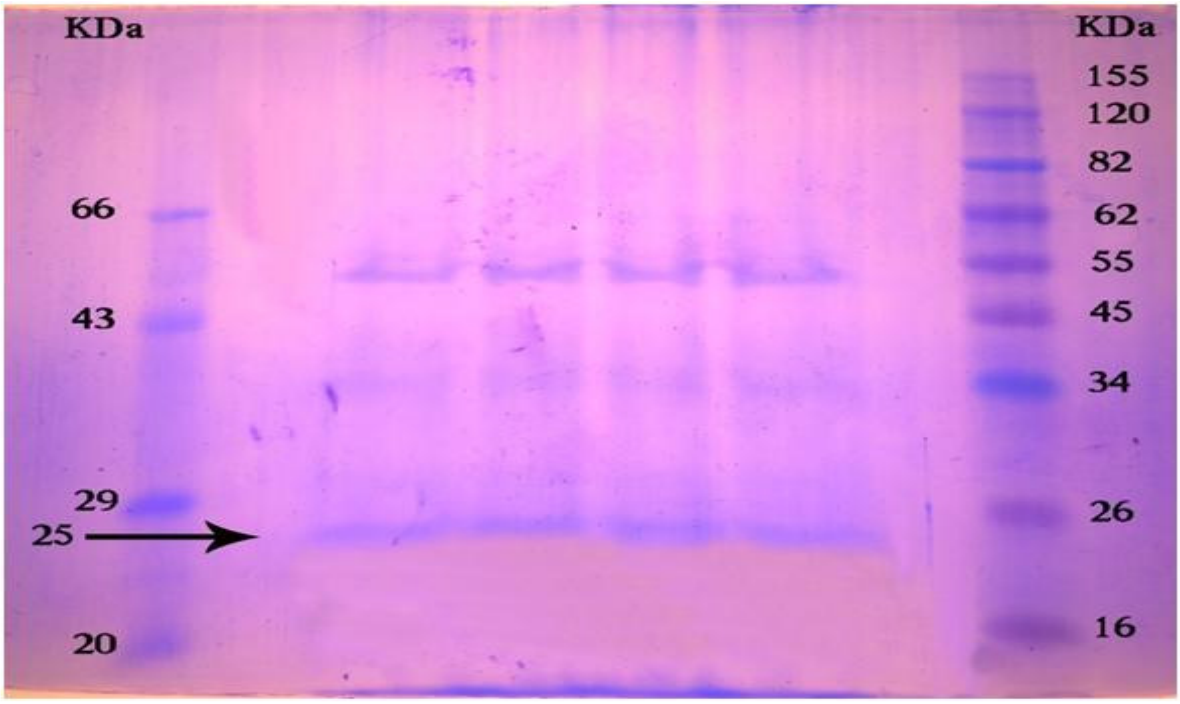

Figure 4. SDS- polyacrylamide gel electrophoresis for plant viral preparation of Rose Viruses Associated with Rose Mosaic Disease showing the viral coat protein band at MW ~ 25 KDa. Lanes 3- 6 are plant viral preparation. With two markers roti- mark standard Roth protein marker & pink plus prestained ladder GeneDirex protein marker.

1984).

Symptoms on Taif- rose (*Rosa multiflora*. cv Taifey) were similar to those on Rose trees infected with rose mosaic virus according to (Horst, 1996; Hagan and Mullen, 2000) whish reported that Rose mosaic virus has a wide range of symptoms including ring spots, wavy lines, chlorotic vein banding, an oak leaf pattern, a mosaic pattern, and a yellow net pattern.

Rose mosaic disease is the most common virus disease of roses. It can be caused by one or more of a complex of several viruses including *Prunus Necrotic Ringspot Virus* (PNRSV, Genus *Ilarvirus*, Family *Bromoviridae*) (Fulton, 1970; Moury *et.al*., 2001) and *Apple Mosaic Virus* (ApMV, Genus *Ilarvirus*, Family *Bromoviridae*) (Fulton, 1952; 1967; 1968; Johnstone *et al*., 1995; Wong and Horst, 1993). The viral protein of ApMV and PNRSV preparation migrated as a molecular mass of ~ 25 KDa. The obtained results were in agreement with previous results that obtained by Gonsalves and Fulton (1977) whish described the method of SDS-PAGE protein preparation and reported that ApMVand PNRSV capsid contains a single protein species with mol. wt. of about 25000 Dalton.

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