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Incidence of Viruses Infecting Alfalfa in the Southeast and Central Regions of Iran

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ABSTRACT

Alfalfa crops were surveyed for the incidence of alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV), peanut stunt virus (PSV), bean leaf roll virus (BLRV), bean yellow mosaic virus (BYMV) and bean common mosaic virus (BCMV) in the major growing areas in the southeast and central regions of Iran. Samples were collected between May 2009 and March 2011 and analyzed for viral infection initially by enzyme-linked immunosorbent assay (ELISA) followed by RT-PCR using capsid protein gene specific primers. In total, 634 symptomatic leaf samples were collected in four southeastern and central provinces of Iran representing 20 regions. Our results revealed a high incidence of AMV over a wide geographical area. AMV and BLRV were identified in most regions, whereas BYMV was found only in Yazd Province. PSV was detected in three regions, but not in Sistan- Balouchestan and Hormozgan Provinces. The highest incidence of viral infection amongst the surveyed provinces was recorded in Kerman (66.8%), followed by Yazd (39%), Sistan and Balouchestan (20.8 %), and Hormozgan (4.5%). AMV, BLRV, PSV and BYMV were present in 23.3%, 12%, 0.70% and 0.28% of the samples, respectively. CMV and BCMV were not detected in any surveyed region. Multiple virus infections were recorded in 42 samples. This is the first report on the detected occurrence of BLRV, PSV and BYMV in alfalfa in the southeast and central regions of Iran.

Keywords: Alfalfa viruses, RT-PCR, Serological testing.

INTRODUCTION

Alfalfa or lucerne (*Medicago sativa* L.) is an important field crop worldwide. It is planted on over 618,000 ha under varied climate conditions in Iran (Anonymous, 2008). Kerman, Yazd, and Sistan and Balouchestan Provinces are the major alfalfa producing areas in southeast and central Iran. In all these areas, a range of locally adapted cultivars including Bami, Nik-Shahrie and Yazdi are planted in irrigated fields. Alfalfa is attacked by a wide range of pathogens including viruses (Burke, 1963; Edwardson, and Christie, 1986; Hampton, 1983; McLaughlin and Boykin, 1988; Hiruki and Hampton, 1990). It is the host of *alfalfa mosaic virus* (AMV) (Stuteville and Erwin, 1990), *bean yellow mosaic virus* (BYMV) (McLaughlin and Boykin, 1988), *peanut stunt virus* (PSV) (Hampton, 1983), *bean leaf roll virus* (BLRV) (Rahman and Peaden, 1993) and *bean common mosaic virus* (BCMV) (Alan *et al*., 1996).

Results of a study in the United Kingdom showed that AMV infections resulted in a loss of 15-23% in fresh weight and 15-18% in dry weight of the forage in five alfalfa cultivars (Bailiss and Oilennu, 1996). A study in Australia revealed that alfalfa can act as a summer refuge for viruses and for virus vectors which transmit the viruses to

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the winter legumes (van Leur and Kumari, 2011). According to Dall *et al*. (1987), infection of *Medicago truncatula* by AMV significantly reduced growth and productivity. The extent of yield reduction was dependent on the medicago cultivar and the virus isolate. AMV has been reported to reduce forage yield, crude protein content, nodulation, and winter survival in 12 cultivars of alfalfa in the field and greenhouse experiments (Tu and Holmes, 1980).

Alfalfa fields typically remain in production for 6 to 7 years, or sometimes longer, and this has possibly contributed to the spread of viral diseases. Limited information is available on the occurrence of virus infections in alfalfa from Iran. AMV has been detected in alfalfa in Khorasan Province (Zainadini *et al*., 2005). In other regions of Iran, AMV, CMV, BCMV and BYMV are known to occur in alfalfa (Bananej *et al*., 1995; Hassanalilou *et al*., 2010). However, commercial alfalfa fields in the southeast and central regions of Iran have previously not been surveyed for aphid-transmitted viruses including AMV, BLRV, PSV, BCMV and BYMV. In this study, we present our data on the occurrence and identification of viruses in alfalfa crop in southeast and central regions of Iran using ELISA and RT-PCR methods.

MATERIALS AND METHODS

Plant Samples

Samples were collected from different regions of four alfalfa producing provinces of southeast and central Iran (Figure 1). Surveys were undertaken between May 2009 and March 2011, depending on the vegetative growth in each region. Two groups of leaf samples were collected from plants in each field: (1) four to 5 symptomatic plants exhibiting mosaic, green vein banding, mild to severe mottling and leaf malformation, and (2) five to 6 plants randomly sampled irrespective of their symptoms. Young leaves from symptomatic or asymptomatic plants were stored in plastic bags and transported to the laboratory in an ice box. Samples were kept at 4ºC until tested initially by enzyme-linked immunosorbent assay (ELISA), followed by the RT-PCR assay. Virus incidence in each field was expressed as the percentage of

Figure 1. Map of Iran showing the provinces where the alfalfa crop was surveyed for viruses.

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virus positive plants relative to the total samples tested.

Virus Identification

The incidence of PSV and BYMV was determined using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977). The presence of BLRV and BCMV were determined using trapped antibody ELISA (TAS-ELISA) which was performed according to De Avila *et al*. (1990). CMV and AMV were detected by plate-trapped antigen (PTA)-ELISA (Mowat, and Dawson, 1987). The specific ELISA methods used for each virus were adopted from protocols provided by individual suppliers. Antisera, positive and negative virus controls for BLRV, BCMV, PSV and BYMV detection were purchased from DMSZ (Braunschweig, Germany). CMV and AMV antisera were kindly donated by P. Jones (IACR-Rothemsted, Harpenden, Herts, UK). We used healthy alfalfa leaf extracts as negative controls for CMV and AMV. For positive controls, we used leaf extracts of *Nicotiana tabacum* var. Samsun N., which were mechanically inoculated with Iranian isolates of AMV and CMV. The PTA-ELISA, DAS-ELISA and TAS-ELISA were used as described by Massumi *et al*. (2009).

RNA Isolation, Reverse Transcription and PCR

Total plant RNA was extracted from the leaves of each AMV, PSV and BLRV infected plant using High Pure Viral Nucleic Acid Kit (Roche Biochemical, Germany). The first strand cDNA was synthesized as described previously by Sharifi *et al*. (2008). Oligonucleotide primer pairs of AMV-F/AMV-R, PSV-F8/PSV-R8, BLRV-F1/BLRV-R2 were designed to amplify regions spanning most of the CP genes of AMV, PSV and BLRV, respectively, using Fast PCR software (Kalendal, 2005). AMV-F/AMV-R, PSV-F8/PSV-R8 and BLRV-F1/BLRV-R2 designed based on the sequence of strain 425 Madison sequence (GenBank accession # K02703), strain J2 sequence (GenBank accession number # AB360970) and BLRV-USA (GenBank accession # AF441393, respectively. The primer details and amplicon regions are provided in Table 1. The PCR reaction varied according to the primer pair used. Amplification of the CP of these three viruses was carried out in a TC-312 thermocycler (Techne, Cambridge, UK) with the following program: an initial denaturation step for 5 minutes at 94°C, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at optimized temperature for primers (Table 1) for 1 minute, and extension at 72°C for 1 minute. The final extension was performed at 72°C

Table 1. PCR primers used for the detection of AMV, PSV and BLRV in alfalfa.

Primer	Sequence (5´-3´)	Amplicon region	Annealing temperature	Size of amplicon (bp)	Polarity
AMV-F	*CATTGATCGGTAATGGGCCGT*	1137-1158	58°C	780	Sense
AMV-R	*ATCCACCCAGTGGAGGTCAGCA*	1895-1917			Antisense
PSV-F8	*TCCCTTTGGTCAATTTCGCGT*	1120-1140	52°C	932	Sense
PSV-R8	*GGGTGAGACTGACCATTTTAGC*	2030-2052			Antisense
BLRV-F1	*ATCAACGATGGTCGCTAGAGG*	3086-3106	57°C	621	Sense
BLRV-R2	*CGGAGGATCCGGTTCTCCGTC*	3687-3707			Antisense

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for 5 minutes. PCR products were separated in a 1% agarose gel stained with ethidium bromide and visualized using a UV transilluminator.

RESULTS AND DISCUSSION

In our survey, we detected AMV, BLRV, PSV and BYMV in alfalfa (Table 2). Of a total of 707 samples collected, 257 tested positive by ELISA, 23.3% were identified as AMV, 12% as BLRV, 0.7% as PSV and 0.28% as BYMV. CMV and BCMV were not detected in any sample. Of the six viruses tested, single infection with AMV was detected in all regions except in Minab-Cheraghabaad and Roudan-Kheryr Abaad. BLRV was detected in 12 out of 22 regions. PSV was detected PSV was detected only in three regions whereas BYMV in one region.

A high incidence of viral infections was detected in Kerman Province, i.e., in Zarand-Dashtkhak (29/57) and in Jiroft-Anbar abaad (23/50) for AMV and BLRV, respectively. Further, AMV was the predominant virus detected in the survey, followed by BLRV. Co-infection with both viruses was detected in 6.6% (42/634) of the samples. Double infections with AMV and PSV accounted for only 0.15% (1/634) of the collected samples. Infection of alfalfa by AMV, BLRV and PSV in ELISA-positive samples was confirmed by RT-PCR, which led to amplification of a putative 780-bp AMV CP gene fragment and a putative 621-bp BLRV CP gene fragment. Similarly, a 932-bp fragment was detected when PSV-infected samples were examined with PSV-specific primers (Figure 2). Virus-free plants grown in aphid-free greenhouses gave a negative result.

No symptoms were observed and no virus was detected by ELISA in eight fields in two regions of Hormozgan Province. A number of samples from symptomatic alfalfa plants did not react serologically with any of the available virus antisera (Figure 3, F). These symptoms may be caused by other viruses or by abiotic factors. A number of viruses including *clover vein yellow virus* (CVYV) and *pea enation mosaic virus* (PEMV) have been detected in alfalfa elsewhere in the world (Shah *et al*., 2006 and Rahman, *et al*., 1993). In this study, positive samples were tested by RT-PCR randomly. So the paragraph " Based on our previous experiences, the negative samples in ELISA test did not react in RT-PCR" should be removed.

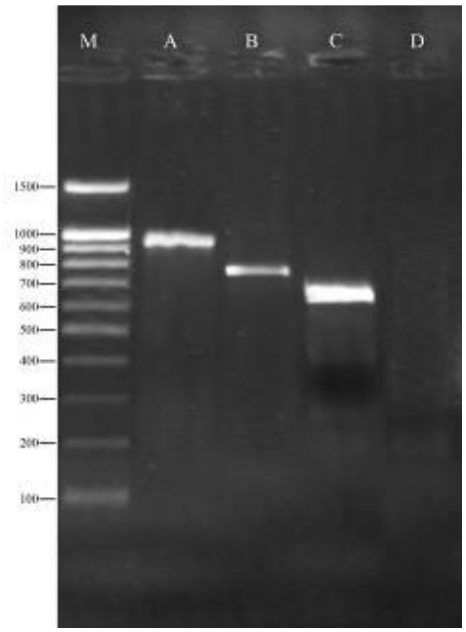

Figure 3. Reverse transcription–polymerase chain reaction used for the detection of *Alfalfa mosaic virus* (AMV), *Peanut stunt virus* (PSV) and *Bean leaf roll virus*(BLRV). (Lane M): 1-kb DNA ladder; (Lane A) Alfalfa infected with PSV; (Lane B) Alfalfa infected with AMV; (Lane C) Alfalfa infected with BLRV, (Lane D) Healthy alfalfa.

We found that AMV was the most widespread in most regions and, therefore, it may have the highest economic impact on alfalfa. The occurrence of AMV on alfalfa from Northern Khorasan, Alborz and West Azarbaijan Provinces has been reported previously (Bananej *et al*., 1995; Zainadini *et al*., 1995; Hassanalilou *et al*., 2010). Our study extends the knowledge on the natural occurrence of AMV, BLRV and BYMV in alfalfa crops in southeast and central regions of Iran. Previous studies have shown that AMV causes reduction in forage protein and yield, seed germination, regeneration, over-wintering, nodulation, and nitrogen fixation activity (Hemmati and McLean 1977; Tu *et*

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Table 2. Occurrence of viruses in alfalfa cultivars showing virus-like symptoms in southeast and central regions of Iran (January 2009 to March 2011).

Province	Region	Cultivar	Number of Samples	TAS-ELISA[a]		DAS-ELISA[b]		PTA-ELISA[c]		Double infection[d]	
				BCMV	BLRV	BYMV	PSV	CMV	AMV	AMV+ BLRV	AMV+ PSV
Yazd	Meybod	Yazdi	23	0/23	[d]3/23	0/23	0/23	0/23	6/23	1/23	0/23
“	Meybod- Hojjat abaad	Yazdi	22	0/22	0/22	0/22	0/22	0/22	3/22	0/22	0/22
“	Sadough- Ashkezar	Bami	22	0/22	4/22	0/22	0/22	0/22	7/22	2/22	0/22
“	Sadough- Rrzvanshahr	Yazdi	24	0/24	3/24	2/24	0/24	0/24	5/24	2/24	0/24
“	Mehriz	Yazdi	33	0/33	5/33	0/33	2/33	0/33	12/33	3/33	1/33
“	Yazd- Mohammad abaad	Yazdi	19	0/19	0/19	0/19	0/19	0/19	4/19	0/19	0/19
Kerman	Bardsir- Laleh zar	[??]Ranger, Hamadani	15	0/15	5/15	0/15	1/15	0/15	8/15	5/15	0/15
“	Jiroft- Ali abaad	Bami	54	0/54	8/54	0/54	0/54	0/54	6/54	0/54	0/54
“	Jiroft- Anbar abaad	Bami	50	0/50	23/50	0/50	0/50	0/50	25/50	12/50	0/50
“	Jiroft- Sarjaz	Bami	32	0/32	2/32	0/32	0/32	0/32	8/32	0/32	0/32
“	Jiroft- Bagher abaad	Bami	27	0/27	16/ 27	0/27	0/27	0/27	16/27	10/27	0/27
“	Zarand- Dashtkhak	Bami	57	0/57	8/57	0/57	2/57	0/57	29/57	4/57	0/57
Sistan and Balouchestan	Zahedan-Sharif abaad	Nik Shahri	25	0/25	0/25	0/25	0/25	0/25	3/25	0/25	0/25
“	Zahedan- Anjare	Nik Shahri	33	0/33	0/33	0/33	0/33	0/33	4/33	0/33	0/33
“	Khash	Bami	48	0/48	1/48	0/48	0/48	0/48	8/48	0/48	0/48
“	Iranshahr	Bami	43	0/43	4/43	0/43	0/43	0/43	11/43	2/43	0/43
Hormozgan	Haji abaad-shamil	Bami	35	0/35	0/35	2/35	0/35	0/35	3/35	0/35	0/35
“	Haji abaad-Jaeen	Bami	38	0/38	0/38	1/38	0/38	0/38	2/38	0/38	0/38
	Minab- Hashtbandi	Nik Shahri	33	0/33	0/33	0/33	0/33	0/33	1/33	0/33	0/33
“	Minab-Cheragh abaad	Nik Shahri	26	0/26	0/26	0/26	0/26	0/26	0/26	0/26	0/26
“	Roudan-Kheyr Abaad	Nik Shahri	31	0/31	0/31	0/31	0/31	0/31	4/31	0/31	0/31
“	Roudan	Nik Shahri	22	0/22	0/22	0/22	0/22	0/22	0/22	0/22	0/22
Total			707	0/707	85/707	2/707	5/707	0/707	165/707	41/707	1/707
Percent				0.0%	12%	0.28%	0.70%	0.0%	23.3%	5.79%	0.14%

[a]Triple antibody sandwich (TAS)-ELISA for BCMVand BLRV; [b] Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) for BYMV, and PSV; [c] Plate-trapped antigen (PTA)-ELISA for AMV and CMV, [d] Number of infected plants/number of plants sampled.

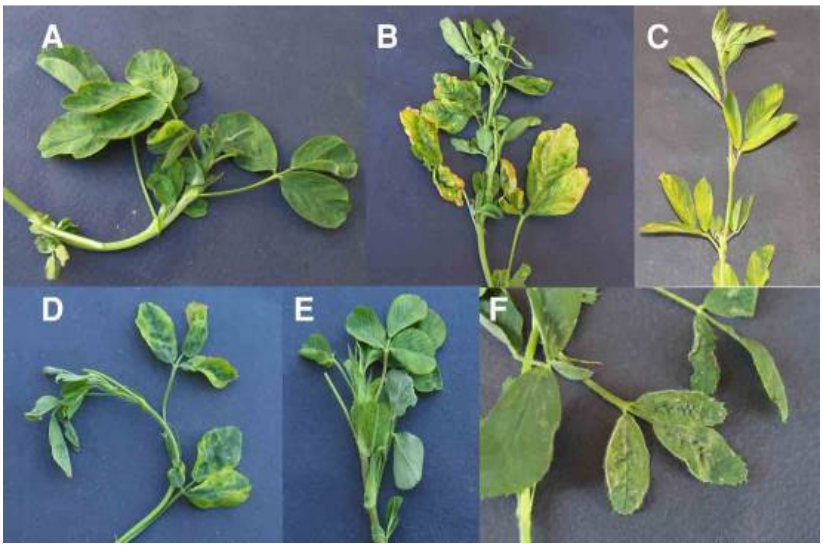

Figure 3. Alflafa cultivars infected with different viruses: (A) Cultivar Bami infected with BLRV; (B) Cultivar Bami infected with PSV; (C) Cultivar Bami infected with AMV; (D) Cultivar Hamadani infected with AMV; (E) Eultivar Ranger infected with AMV, (F) Blistering on cultivar Bami caused by an unknown virus.

al., 1980; Ohki *et al*., 1986; McLaughlin and Boykin 1988; Bailiss and Oilennu 1996). Infection of alfalfa with PSV is reported for the first time in Iran. BCMV and CMV, which have been previously reported in alfalfa, were not detected in our study. Field symptoms associated with virus infection included mosaic and vein banding for BLRV (Figure 3-A); blistering and yellowing for PSV (Figure 3, B) and mosaic, mottling, vein clearing, and vein banding for AMV (Figures 3-C, 3-D and 3-E). However, problems like mixed viral infections and occurrence of six serotypes of AMV in the surveyed areas (Massumi and Hosseini Pour, 2007), made the correlation between virus and symptom impossible. Differences in the incidence of infection of the same cultivar were observed in different geographic regions. For example, Bami cultivar had a low incidence of AMV and BLRV in Haji abaad while in Jiroft-Anbar abaad in the Kerman Province, the cultivar showed an infection rate of 72%.

The importance of alfalfa to the economy of these regions exemplifies the importance of preventive measures to viral spread and subsequent crop losses. The elimination of weeds, which can act as alternative reservoir for virus and its vector, has been recommended (Duffus, 1971; Thresh, 1981).

Breeding virus resistant or tolerant alfalfa cultivars has also been suggested (Crill and Hanson, 1969, Crill et al., 1971; Hiruki and Miczynski,1990) as an alternative.

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REFERENCES

1. Anonymous. 2008. *Annual Agricultural Statistics*. Ministry of Jihad-e-Agriculture of Iran. Available at: http://www.maj.ir.
2. Alan, B. Crabtree, K., Dallwitz, M., Gibbs, A. and Watson, L. 1996. *Viruses of Plants.*

[DOR: 20.1001.1.16807073.2012.14.5.5.3]

Description and Lists from the VIDE Database. CAB International, UK,1484 PP.

3. Bailiss, K. W. and Oilennu, L. A. A. 1996. Effect of *Alfalfa Mosaic Virus* Isolates on Forage Yield of Lucerne (*Medicago sativa*) in Britain. *Plant Pathol*., **35**: 162-168.
4. Bananej, K., Mosahebi, Gh., Okhovat, M. and Ghorbani, S. 1995. Identification of *Alfalfa Mosaic Virus* (AMV), *Cucumber Mosaic Virus* (CMV), *Bean Common Mosaic* (BCMV) and *Bean Yellow Mosaic* (BYMV) Viruses in Alfalfa Fields in Karaj, Iran. in *Proc. 12th Iranian Plant Protec. Congr*, 1995, Karaj, Iran, 95 PP.
5. Burke, D. W. 1963. Incidence of Viruses Infectious to Beans in Plants of Twelve Alfalfa Varieties. *Plant Dis. Rep*., **47**: 845-847.
6. Clark, M. F. and Adams, A. N. 1977. Characteristics of Micro Plate Method of Enzyme-linked Immunosorbent Assay for the Detection of Plant Viruses. *J. Gen. Virol*., **34**: 475-483.
7. Crill, P. and Hanson, E. W. 1969. Inheritance of Resistance to *Alfalfa Mosaic Virus* In Alfalfa. *Phytopathol.,* **59**: 1022.
8. Crill, P., Hagedorn, D. J. and Hanson, E. W. 1970. Incidence and Effect of AMV on Alfalfa. *Phytopathol.,* **60**: 1432-1435.
9. Dall, D. J., Randles, J. W. and Francki, R. I. B. 1987. The Effect of *Alfalfa Mosaic Virus* on Productivity of Annual Barrel Medic, *Medicago truncatula*. *Aus. J. Agric. Res.*, **40**: 807–815.
10. de Avila, A. C., Huguenot, C., Resende, O., Kitajima, E. W., Goldbach, R. W. and Peters, D. 1990. Serological Differentiation of 20 Isolates of *Tomato Spotted Wilt Virus*. *J. Gen. Virol*., **71**: 2801-2807.
11. Duffus, J. E. 1971. Role of Weeds in the Incidence of Virus Diseases. *Ann. Rev. Phytopathol*., **9**: 319–340.
12. Edwardson, J.R. & G. Christie. 1986. Viruses infecting Forage Legumes. Vol. 1 Monograph (14). Agricultural Experiment Stations. Institute of Food and Agricultural Sciences. University of Florida, Gainesville, Florida. 246 p.
13. Hampton, R. O. 1983. Pea Leafroll in North-Western US Pea Seed Production Areas. *Plant Dis*., **67**: 1306-1310.
14. Hassanalilou, S., Pourrahim, R., Bakhshi Khanik, Gh. R. and Farzadfar, Sh. 2010. Detection of Three Viruses Causing Mosaic Diseases in Alfalfa Fields of West Azarbaijan Province. *In Proc. 19th Iranian Plant Protec. Congr*, 2010, Tehran, Iran, 671 PP.
15. Hemmati, K. and McLean, D. L. 1977. Gamete-seed Transmission of *Alfalfa Mosaic Virus* and Its Effect on Seed Germination and Yield in Alfalfa Plants. *Phytopathol.,* **67**: 576-579.
16. Hiruki, C. and Hampton, R. O. 1990. Alfalfa Mosaic. In: "*Compendium of Alfalfa Diseases*", (Eds.): Stuteville, D. L. and Erwin, D. C.. 2nd Edition, APS Press, St. Paul, 54 PP.
17. Kalendal, R. 2005. Fast PCR Primer Design and Report Sequence Searching Program with Additional Tools for the Manipulation and Analysis of DNA and Protein. Available on Internet at: http://www.Biocenter.helsinki.fi/bi/programs/fastPCR.html (2005).
18. van Leur, J. A. G. and Kumari, S. G. 2011. A Survey of Lucerne in Northern New South Wales for Viruses of Importance to the Winter Legume Industry. *Australa. Plant Pathol*., **40**: 180-186.
19. Massumi, H., Shaabanian M., Hosseini Pour, A., Heydarnejad. J. and Rahimian, H. 2009**.** Incidence of Viruses Infecting Tomato (*Lycopersicun esculentum* Mill.) and Their Natural Hosts in the Southeast and Central Regions of Iran. *Plant Dis*., **93**: 67-72.
20. Massumi, H and Hosseini Pour, A. 2007. Serological Characterization of *Alfalfa Mosaic Virus* in alfalfa (*Medicago sativa*) in Some Regions of Iran. *J. Agri. Sci. Tech.,* **9**: 341-347.
21. McLaughlin, M. R. and Boykin, D. L. 1988. Virus Diseases of Seven Species of Forage Legumes in the Southeastern United States. *Plant Dis*., **72**: 539-542.
22. Miczynski, K. A. and Hiruki, C. 1987. Effect of Alfalfa Mosaic Virus Infection on the Yield and Regeneration of Alfalfa at Different Growth Temperatures. *Can. J. Plant Pathol*., **9**: 49-55.
23. Mowat, W. P. and Dawson, S. 1987. Detection of Plant Viruses by ELISA Using Crude Sap Extracts and Unfractionated Antisera. *J. Virol. Methods*, **15**: 233-247.
24. Ohki, S. T., Leps, W. T. and Hiruki, C. 1986. Effects of *Alfalfa Mosaic Virus* Infection on Factors Associated with Symbiotic N2 Fixation in Alfalfa. *Can. J. Plant Pathol.*, **8**: 277-281.
25. Rahman, F. and Peaden, R. N. 1993. Incidence of Viruses on Alfalfa in Western North America. *Plant Dis*., **77**: 160-162.

[DOR: 20.1001.1.16807073.2012.14.5.5.3]

26. Shah, D. A., Dillard, H. R., Mazumdar-Leighton, S., Gonsalves, D. and Nault, B. A. 2006. Incidence, Spatial Patterns, and Associations among Viruses in Snap Bean and Alfalfa in New York. *Plant Dis*., **90**: 203-210.
27. Sharifi, M., Massumi. H., Heydarnejad. J., Hosseini Pour, A. Shaabanian, M. and Rahimian, H. 2008. Analysis of the Biological and Molecular Variability of *Watermelon Mosaic Virus* Isolates from Iran. *Virus Genes*, **37**: 304–313
28. Stuteville, D. L., and Erwin, D. C. 1990. *Compendium of Alfalfa Diseases.* 2nd Edition, American Phytopathological Society Press, St. Paul, MN, 84 PP.
29. Thresh, J. M. 1981. The Role of Weeds and Wild Plants in the Epidemiology of Plant Virus Diseases. In: "*Pests Pathogens and Vegetation*", (Ed.): Thresh, J. M.. Pitman Publications, London, UK, PP. 53–70.
30. Tu, J. C. and Holmes, T. M. 1980. Effect of *Alfalfa Mosaic Virus* Infection on Nodulation, Forage Yield, Forage Protein and over Wintering of Alfalfa. *Phytopathol.,* **97**: 1-9.
31. Zainadini, A, Jafarpour, B. and Falahati Rastegar, M. 2005. Study of *Alfalfa Mosaic Virus* in Central and Northern Regions of Khorasan Province. *J. Sci. Tech. Agric. Natl. Resour.*, **12**: 147-157. (in Persian with English Abstract).

بررسی میزان گسترش ویروس های آلوده کننده یونجه در مناطق جنوب شرقی و مرکزی ایران

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چکیده

به منظور تعیین پراکنش ویروس های آلوده کننده یونجه از مناطق عمده یونجه کاری چهار استان واقع در مناطق جنوب شرقی و مرکزی ایران طی سالهای ۱۳۸۸ تا ۱۳۹۰ بازدید شد و۶۳۴ نمونه یونجه دارای علائم ویروسی جمع‌آوری گردید. آلودگی نمونه ها با آزمون های سرولوژیکی الیزا (ELISA) و RT-PCRنسبت به ویروس های موزائیک یونجه (AMV)، موزائیک خیار (CMV)، کوتولگی بادام زمینی (PSV)، پیچیدگی برگ لوبیا (BLRV)، موزائیک زرد لوبیا (BYMV) و موزائیک معمولی لوبیا (BCMV) با استفاده از آنتی سرم های چند همسانه ای و آغازگرهای اختصاصی تعیین شد. نتایج حاصل بیانگر گسترش وسیع AMV در مزارع مورد مطالعه بود. AMV و BLRV در اکثر مناطق یافت شدند، در حالیکه PSV و BYMV به ترتیب در یک (در استان یزد) و سه ناحیه (در استان های کرمان ویزد) ردیابی گردیدند. بالاترین میزان آلودگی در سطح استان های مورد بررسی به ترتیب در استانهای کرمان (۶۶/۸٪)، یزد (۳۹٪)، سیستان و بلوچستان (۲۰/۸٪) و هرمزگان (۴/۵٪) مشاهده گردید. میزان آلودگی نسبت به ویروسهای AMV, BLRV, PSV و BYMV به ترتیب ۲۳/۳، ۱۲، ۰/۷ و ۰/۲۸ در صد محاسبه گردید. در حالیکه دو ویروس CMV و BCMV در هیچکدام از مزارع مورد بررسی در این تحقیق مشاهده نگردیدند. آلودگی های مخلوط دو تائی در ۴۲ عدد از نمونه های مورد بررسی ردیابی شد. در بین ارقام مورد کاشت به ترتیب ارقام بمی، نیک شهری، یزدی، رنجر و همدانی بیشترین سطح زیر کشت را در این مناطق به خود اختصاص داده بودند. این اولین گزارش از وجود ویروس های BLRV , PSV و BYMV در مزارع یونجه واقع در تعدادی از استان های جنوب شرقی و مرکزی ایران می باشد.

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