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Sharka (*Plum pox virus*): A forgotten disease in Iran

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Abstract
Plum pox or sharka, a viral disease caused by *Plum pox virus* (PPV), severely affects the production of *Prunus* species in Europe. The first evidence of sharka was reported in Iran in 2000 . Due to the economic impact of this disease on crop production, recent advances in the term of biology, epidemiology, and disease management are provided in this paper to assure awareness among growers and professionals involved in *Prunus* production. This study will provide fundamental knowledge about this virus to guaranty the successful detection and controlling of sharka disease in Iran.

Keywords: PPV, potyvirus, *Prunus,* sharka disease, detection, control, resistance, breeding

Introduction

Stone fruit trees are affected by a large number of diseases of viruses leading to the strong economic losses (Rubio et al., 2017). These viruses either alone or in combination with other viruses, affects plant growth, fruit maturity and yield. However sharka disease, caused by *Plum pox virus* (PPV), is the most important virus causing huge yield loss in plums (*Prunus domestica* L.), apricots (*P. armeniaca* L.) and peaches [*P. persica* (L.) Batsch] because of the reduced fruit quality, premature fruit drop and rapid natural virus spread by aphid vectors (Scholthof et al., 2011).

Although the impact of this virus on stone fruit production is huge, evaluation of the economic impact is still under debate. The impact of the sharka disease over 30 years was estimated to be over €10,000 million (Cambra et al., 2006). Furthermore, PPV was included among the top 10 plant viruses in terms of scientific and economic importance worldwide (Scholthof et al., 2011).

The purpose of this research is to provide a general information of the status of this disease in Iran with emphasis on strains, detection methods and epidemiological factors involved in disease expansion.

The virus

Plum pox symptoms were first observed in plums in Bulgaria between 1915 and 1918, although few reports indicated that these symptoms were seen in Macedonia as early as 1910. However the first paper describing the viral nature of the disease did not appear until 1932 when Atanosoff (1932) presented it as "Sarka po slivite" meaning "Pox of Plum" (=Sharka). Since then, PPV has spread throughout Europe, the Mediterranean, the Middle East (Egypt and

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Syria), India and South and North America (Table 1; Figure 1). In Europe, estimation of the European and Mediterranean Plant Protection Organization (EPPO) indicated that more than 100 million stone fruit trees have been infected by PPV (https://www.eppo.int/). The constant progress of Sharka in Europe and the severity of the disease led to the development of the Sharka International Working Group in the 1970's within the framework of the EPPO allowing to coordination of researches and a free flow of information among countries (https://www.eppo.int/).

Plum pox virus (PPV) belongs to the genus Potyvirus in the family of Potyviridae. PPV is a RNA virus with flexuous filamentous particles approximately 750 nm in length x 15 nm in diameter. PPV particles are composed of one molecule of RNA (positive sense, single-stranded RNA) and a protein envelope. The total protein size which is expressed by viral genome is a 350 kDa polyprotein precursor that is proteolytically processed by viral and host proteases into ten smaller functional proteins include: P1, HCPro, P3, 6K1, CI, 6K2, VPg, NIapro, NIb and CP. P1 and HC, responsible of processing themselves at their respective C-termini, and NIa which performs the rest of cleavages within the polyprotein. The cylindrical inclusion (CI) protein of potyviruses is involved in virus replication and cell-to-cell movement. The interaction of the chloroplast PSI-K protein with potyviral CI protein has been found during viral infection which is negatively affected by PSI-K protein.(García et al., 2014) (Figure 2).

Table 1. Distribution, isolate and year of detection of sharka by countries.

Year	Country	Year	Country
1917	Bulgaria (D, M)	1986	Syria (D, M)
1935	Serbia (D, M, Rec)	1987	Egypt (EA); Slovenia (D, M)
1938	Hungary (D, M, C)	1988	Croatia (D, M)
1941	Romania (D, M, C, Rec)	1992	Chile (D)
1947	Albania (D, M, Rec)	1994	Estonia (D); Georgia (D, M)
1950	Slovakia (D, M)	1995	Lithuania (D); India (D)
1952	Check Republic (D, M)	1998	Norwich (D)
1956	Germany (D, M)	1999	USA (D, Pen)
1961	Austria (D); Poland (D, M)	2000	Canada (D, W); China (D), Iran (D, M)
1962	Moldavia (C); Russia (D, C, CR)	2001	Jordan (M)
1965	Holland (D); United Kingdom (D)	2002	Tunisia (D)
1967	Greece (D,M); Switzerland (D); Ukraine (D, M)	2004	Bosnia-Herzegovina (D, M, Rec); Kazakhstan (D); Argentina(D)
1968	Turkey (D, M, Rec, T, An)	2006	Pakistan (D, Rec)
1970	France (D, M); Sweden (D)	2007	Montenegro (D, M)
1973	Italia (D, M, C, Rec)	2010	Japan (D)
1974	Belgium (D)	2011	Byelorussia (D, M, Rec)
1982	Cyprus (M)	2015	Finland (D)
1984	Portugal (D); Spain (D)	2016	South Korea (D)

Similar to other plant viruses, *Plum pox* is comprised of several strains based on biology, serological reactions, and molecular and biological data. To date, nine PPV strains including PPV-D, PPV-M, PPV-Rec, PPV-EA, PPV-C, PPV-T, PPV-W, PPV-CR, and PPV-An have been recognized, among them M (Marcus) is introduced as a very aggressive, and D (Dideron) is representiveof a less aggressive strains. However another isolate namely El Amar, was further reported which some researchers categorized it within M strain and others categorized it as a D type strain. Recently, another isolate has been reported from cherry in Moldavia (PPV-SC), and its genome has been sequenced, characterized and

classified as a new type C. These new types show the dangerous capacity of the virus to mute and change independently. However, these strains are partially differentiable their biological or epidemiological properties. To date, PPV-D and M are the most widely distributed strains of plum pox worldwide. While both of these strains can infect peach, nectarine, plum, and apricot, M is much more aggressive in peach and spreads rapidly in orchards via aphids. PPV-M is also the only strain reported capable of infecting the seed (García et al., 2014; Sihelska et al., 2017) (Table 2).

PPV symptoms and transmission

Plum pox virus has been transmitted by at least 20 different aphid species, although only 4-6 species are considered important inoculants. Notably, due to the long-distance distribution of this virus controlling the biological material in the frame of human horticulture practices which its potential to the infection is highly important (García et al., 2014). The efficiency of transmission is dependent on the virus strain, host cultivars, age of the host cultivars, aphid species, and time of year. Virus infection can cause considerable losses with characteristics symptoms in leaves and fruits, and in the case of apricots in the stone fruits (Figure 3).

Sharka is particularly detrimental in apricots, European plums, peaches and Japanese plums because it can seriously reduce yield and fruit quality. Losses in susceptible cultivars may reach 100% in some cases. PPV symptoms may appear on leaves, shoots, bark, petals, fruits and even stones. The symptoms change according to the *Prunus* species and cultivar, PPV strain, season and location. Infected leaves show chlorotic spots or lightly pigmented yellow rings or line patterns. Fruits may become deformed or irregular in shape, and may develop brown or necrotic areas under the discoloured rings, patterns, and chlorotic bands or blotches. Some peach cultivars may show colour-breaking symptoms on the flower petals. In addition, some Iranian plum trees show premature fruit drop (Mohammadi et al., 2002). Infected almond trees generally show either not or less leaf symptoms. Generally, the fruits of early maturing cultivars of all susceptible species show strong symptoms in compare with those of late maturing cultivars (Myrta et al., 2003) (Figure 3).

Table 2. Main PPV isolates described at this moment.

Isolate Type	Description
PPV-D (Dideron)	Originally described in apricot in France in 1995, it is the most abundant isolate, characterized by a lower aggressiveness and a low speed of diffusion. It affects the apricot tree, plum tree and, to a lesser extent, the peach tree. It is believed to be the original isolate detected in Bulgaria in 1917.
PPV-M (Marcus)	Described in 1995 in peach orchard in Greece, it presents high aggressiveness and speed of diffusion. Affects peach, apricot and plum.
PPV-EA (El Amar)	Originally described in apricot tree in Egypt in 1987, it has high aggressiveness. Affects peach, apricot and plum.
PPV-C (Cherry)	Originally described in cherry tree in Moldavia in 1994. It is the unique PPV that affects the cherry tree in the nature, although also it affects the peach tree, apricot tree and plum tree. It is located in centre Europe.
PPV-Rec (Recombinant)	Recombinant isolate between PPV-M and PPV-D with a breakpoint at the carboxyl-terminal end of the NIb gene described in 2004.
PPV-T (Turkey)	It is a new strain originated in Turkey in 2009.
PPV-W (Winona)	It is a new strain, apparently originated in Eastern Europe and Russia in 2011.
PPV-Pen (Pennsylvania)	It is a new strain described in Pennsylvania (United States) in 2011.
PPV-An (Anatolia)	It is a new strain described in Turkey in 2012.
PPV-CR (Cherry Russian)	It is a new strain, apparently originating in Eastern Europe and Russia in cherry in 2013

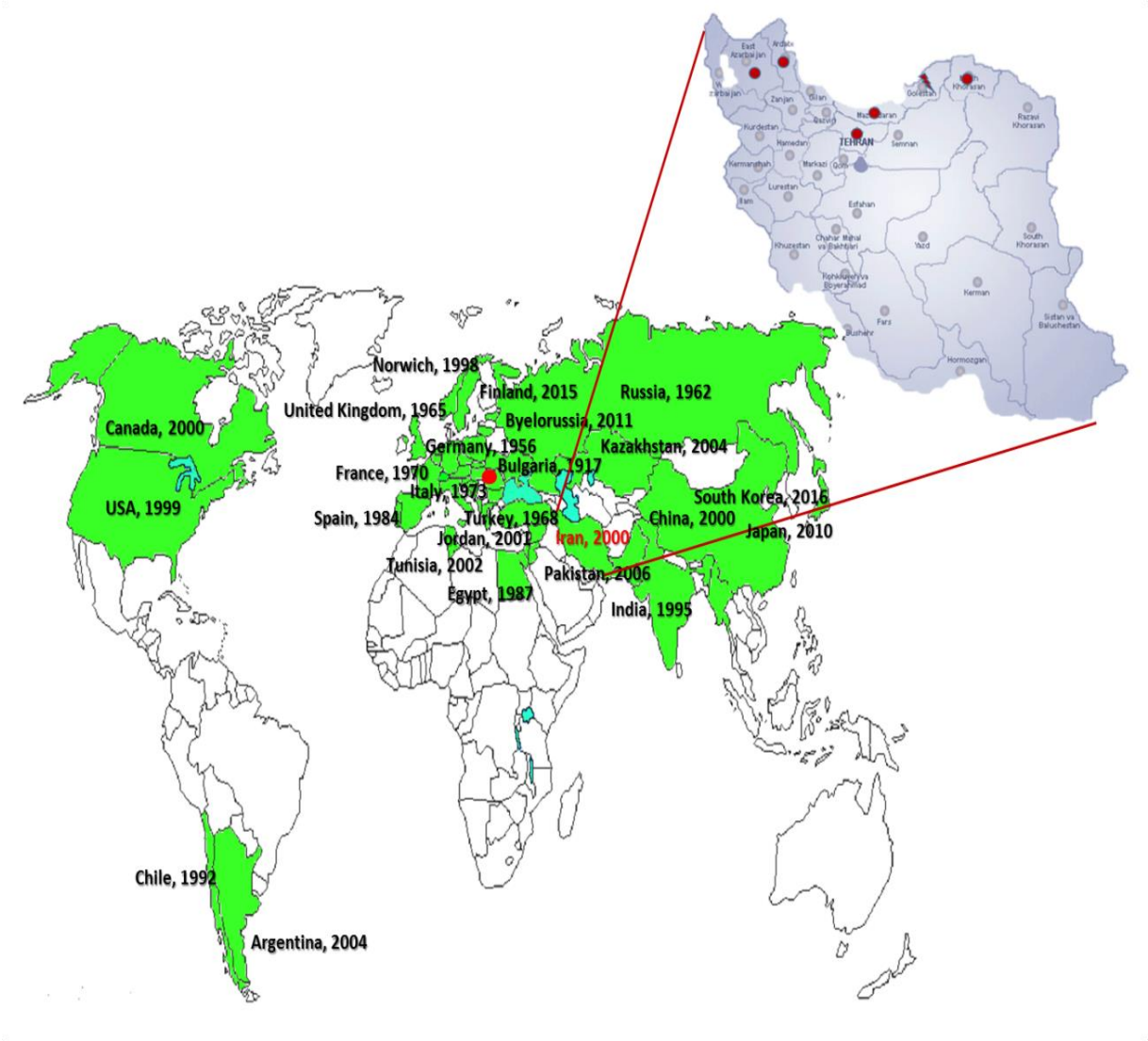

Fig. 1. *Plum pox virus* (sharka) distribution around the world including the year of the first detection in the most important countries is also indicated. Affected areas in Iran are also showed in detail.

PPV can affect most species of the *Prunus* genus. Although germplasm evaluation studies carried out for more than 20 years in Virology Laboratory of CEBAS (Centro de Edafología y Biología Aplicada del Segura) in Murcia (Spain) about 4,000 different genotypes have been evaluated, mainly of apricot but also 18 different species of the *Prunus*. Genomic analysis reveals that MATH gene(s) is a candidate(s) for *Plum pox virus* (PPV) resistance in apricot (*Prunus armeniaca* L). This finding together with the results published by different groups, mainly from France and central Europe, reveal a great heterogeneity in the intensity of the symptoms of the disease and the specificity of the isolates which can infect the different species according to their taxonomic classification.

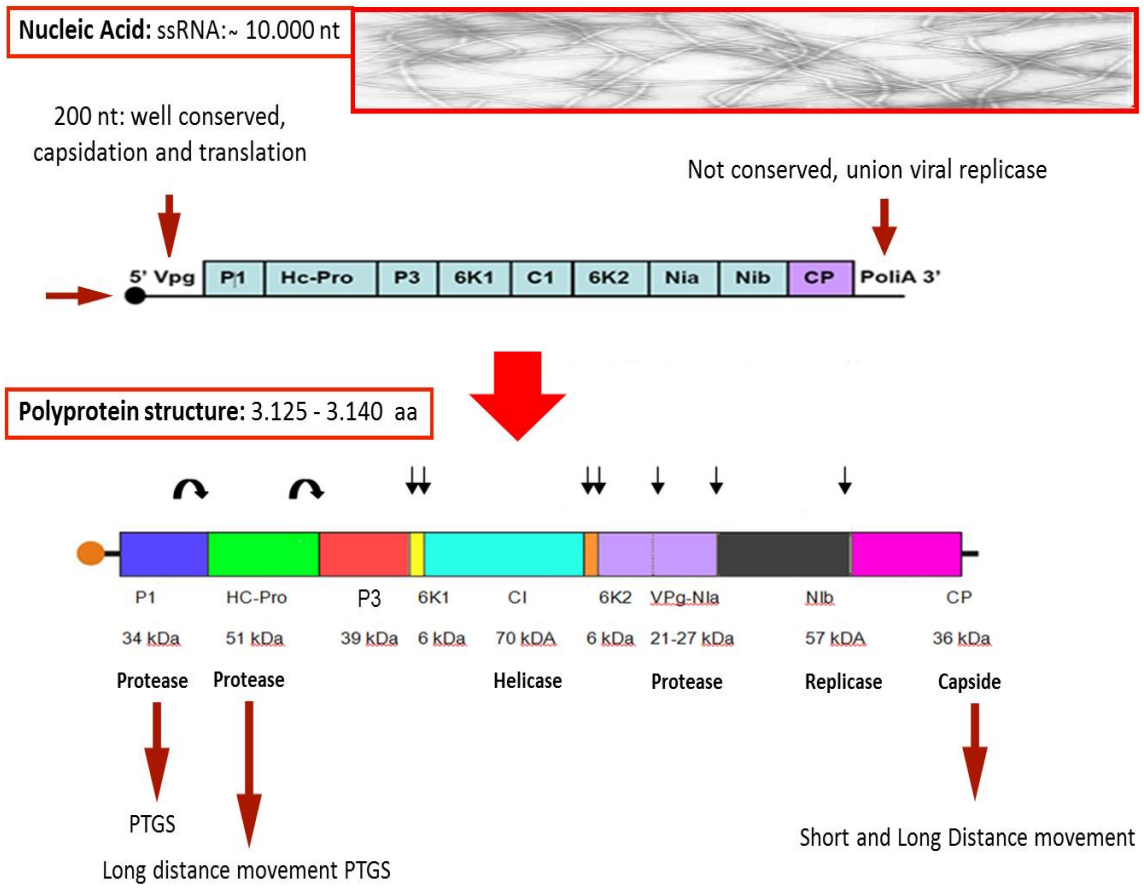

Fig. 2. Structure of the polyprotein generated by the direct translation of the ssRNA (+), the different protein regions are represented by the cut-off points: P1 first protein, Hc-Pro helper factor protein, P3 third protein, 6K1 first peptide 6K, C1 cell inclusion protein, 6K2 second 6k peptide, NI nuclear inclusion protein "a", NIb nuclear inclusion protein "b", CP capsid protein (Adapted from García et al., 2014).

Within the genus *Prunus*, the subgenus *Prunus*, the apricot and plum region, shows the least specificity for all PPV isolates and greater aggressiveness with high symptom severity. Conversely the subgenus *Amygdalus* with species such as peach and almond (*P. dulcis* Mill., DA Webb) represent the lower intensity of symptoms (particularly in almond), however, specificity of isolates is greater. In an intermediate situation is the group of cherry trees (*Cerasus* subgenera) (Martínez-Gómez et al., 2000; 2004; Rubio et al., 2003; 2012).

Sharka detection and control

Detection of PPV can be achieved by using of a biological, serological or molecular researches. However, a serological or molecular test is the minimum requirement to detect and identify the PPV. Further investigation is required to identify the strain of PPV . The simplest method for detection of plum pox is using of biological index hosts. PPV can be detected in herbaceous indicator hosts by mechanical inoculation in diagnostic hosts like *Chenopodium foetidum* and several *Nicotiana* species. The virus is also detected reliably in woody indicator plants by chip budding to the hosts such as GF305 peach (Sochor et al., 2012).

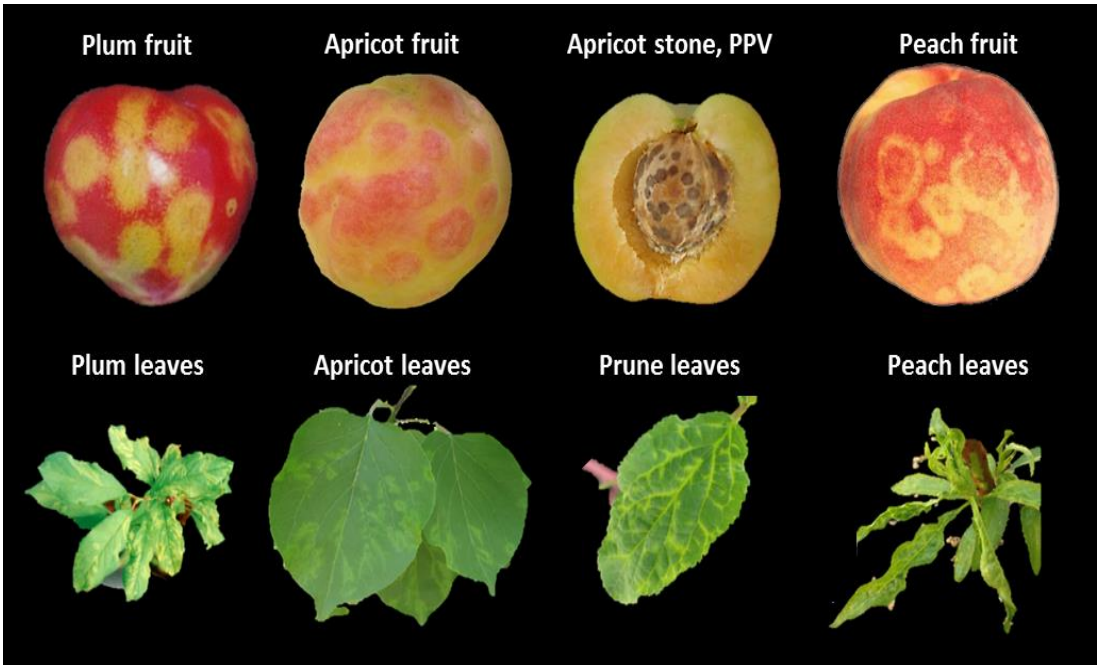

Fig. 3. *Plum pox virus* (sharka) symptoms in fruits, endocarps and leaves of different *Prunus* species including plum, apricot, peach and prune.

The first, but highly important step in progress of PPV diagnostics was based on the serological investigations which were based on the Enzyme-Linked Immunosorbent Assay (ELISA). *Plum pox virus* detection by ELISA was done by Clark and Adams when they applied this technology for the detection of plant viruses. Similar to other tree fruit viruses, in some *Prunus* cultivars, PPV concentration is reduced at certain times of the year. In 2010, plum, peach and nectarine samples, that show mosaic, chlorosis, necrosis and ring pattern, were collected and evaluated by using of a DAS-ELISA (Double Antibody Sandwich-ELISA) and a polyclonal antibody methods. The results of this research indicated that among all samples, none of them showed positive reaction in DAS-ELISA test. Since in this study most samples were collected from an infected province (Golestan), further studies on more samples with more sensitive methods was suggested (Hosseinzadeh et al., 2012).

More sensitive and accurate detection of plum pox became possible in the 1980's through the application of cDNA and cRNA probes, which helped to overcome the difficulty in detection of low concentration of the virus. By introducing of polymerase chain reaction technology to plant virus detection in the early 1990's, *Plum pox virus* was among the first viral targets. . However, molecular methods, especially real-time PCR technique or quantitative PCR (qPCR), which are generally more sensitive than serological techniques, have been suggested for detection of viral infection. Application of qPCR represented more advantages such as avoiding of any post-amplification processing (e.g. gel electrophoresis) reducing contamination level in comparison with conventional PCR. With the exception of immunocapture (IC)-RT-PCR (for which RNA isolation is not required), RNA extraction should be done using appropriately validated protocols. The samples should be placed in individual plastic bags to avoid cross-contamination during extraction (Martínez-Gómez et al., 2003).

Although, to avoid of PPV spread over long distances by plant material translocation, reliable methods are needed for the appropriate detection of the virus in

symptomless nursery plants and propagative material (Scholthof et al., 2011; Sochor et al., 2012).

PPV can be managed by multiple approaches such as quarantine and management activities, certification programs, vector control system and use of resistant varieties. It has been proven that *Plum pox virus* is a disease with difficulties for its control. An applicable anti-virus treatment in trees or orchids has not been introduced so far. The best approach in controlling of PPV is to prevent spread of the virus to new fruit growing areas. Management strategies of PPV control are aimed primarily at preventing introduction by use of virus-tested clean nursery stock. Since infected plants will be never free of the disease, strict quarantine, eradication and ongoing surveys are the examples of useful strategies in controlling of the PPVs..

Reducing of aphid populations by applying insecticide management strategies will help to prevent from PPV translocation to the areas with low population of PPVs. Chemical treatments cause winged forms of the aphids capable of transferring the virus and infect new hosts. Alternatively, the destruction of infected trees can be considered as a fast acting controlling strategy in infected areas. It is worth nothing that a single infected tree in an orchard would serve as a virus source and infection foci for all surrounding trees and for adjacent orchards (Sochor et al., 2012).

An ideal strategy in controlling of PPV is using resistant plants. Limited naturally occurring resistance genes are available for use in developing highly resistant stone fruit through conventional breeding techniques. Few naturally occurring resistant genes are available for plant breeders to use in developing highly resistant fruit varieties (Martínez-Gómez et al. 2000, 2004, Rubio et al. 2003, 2012). Hybrid plum cultivars have been identified that respond to PPV by a hypersensitive response thus preventing the systematic infection. Transferring of resistant genes to stone fruit crops is challenging strategy and rather time consuming compare with the conventional breeding techniques. Genetic engineering and the use of biotechnology approach in plants will help to development of sharka resistant cultivars (Ilardi and Di Nicola-Negri, 2011).

Presence of sharka in Iran

Economically stone fruits havs an important role in Iranian horticulture sector. Due to suitable conditions, Iran is one of the most important regions for planting stone fruit trees. The first strain of PPV in Iran was detected in Dasht-e-Moghan in 2000 (Moeini and Izadpanah 2000). Later, Ghayeb Zamharir et al (2006) observed PPV for the first time in Iranian stone fruit trees. PPV has been reported from Mazandaran, Khorasan, Tehran, Tabriz and Ardebil regions (Figure 1).

After 2010, PPV-D was introduced as the only strain detected in the Iran but Mohammadi et al. (2012) found PPV-M for the first time in Mazandaran province. They collected leaf and shoot plum samples based on typical PPV symptoms and tested by RT-PCR, DAS-ELISA and IC-RT-PCR and found infected samples with PPV-M however in their study no samples were positive for PPV-D. Nowadays, the presence of PPV-M and PPV-D strains has been confirmed in Iran. To date, the virus is distributed in different Iranian regions in the north of the country (Hosseinzadeha et al., 2012; Jafarpour et al., 2013). PCR amplicons belongs to the different regions were sequenced and compared with the corresponding worldwide strains available in NCBI. Comparisons showed the close similarity between the M6 isolate and 15S and 10s isolates with the D (99.4%) and M (99.3% and 99.4%) strains of PPV (Shirazi et al., 2014).

These results strongly suggest that the risk of PPV distribution in Iran is extremely high . Therefore, Some approaches such as plant quarantine laws

and avoidance of infected plant material entrance are strongly recommended. In addition, evaluation of PPV resistance in the traditional cultivars and the introduction of new resistant cultivars will be the definitive solution.

Concluding remarks and prospects

Plum pox virus (PPV, sharka), was detected in 2000 in Iran. This disease is causing an important economic impact in this country where it is prevalent. Nowadays the availability of high-throughput sequencing (NGS) technologies offers new opportunities for the in-depth characterisation of the PPV isolated affecting *Prunus* species in Iran. This includes the characterisation of as-yet-unknown agents or variants and improved descriptions of the variability of all viruses needed. Another interesting application of NGS is the massive detection of multiple pathogens (including viruses). These NGS detection techniques possibly would be included in quarantine and *Prunus* certification programmes in the near future. Alternatively, massive detection of multiple infections, including the viral diseases that affect *Prunus*, by means of spectral imaging can be considered as a strong detention techniques for future researches. Furthermore, NGS also represents the most powerful technology for analysing *Prunus*/virus interaction at the RNA level. The use of RNA-Seq to study a wide range of *Prunus*/virus interactions will produce new insights in virus pathogenesis and control of a wide range of *Prunus*/virus interactions in genome level. New resistant varieties adapted to Iranian climatic conditions are strong candidates for controlling of PPV. Genome editing technique known as CRISPR-Cas (Clustered regularly interspaced short palindromic repeats and their associated Cas proteins) can play powerful role in plant virology in the future. In the near future, this technology will help researchers develop new genotypes resistant to both existing viruses and viroids and those that are yet to be discovered in the uncertain scenario of climate change.

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