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A Brief Review on Soybean Infecting Virus Diseases in Iran

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Abstract: Viral diseases of economic importance have been reported to infect soybean crop and causes considerable damage to the crop and economic losses to the farmers. During different surveys, various and common symptoms of mosaic, vein banding, reduction in overall plant growth, flower and pod formation disorders, necrotic spots on leaves and stem, leaf deformation and yellowing, leaf crinkling and mid rib narrowing were observed in soybean diseased plants in the field. Totals of 3,110 random leaf samples were collected during the growing season of 2018 and 2019 in five provinces of Iran, where commercial soybean is grown, and tested by Enzyme-linked immunosorbent assay (ELISA) using specific polyclonal antibodies. Serological diagnoses were confirmed by electron microscopy and host range studies. The highest virus incidence among the surveyed provinces was recorded in Mazandaran (18.6%), followed by Golestan (15.7%), Khuzestan (14.2%), Ardabil (13.9%), and Lorestan (13.5%). Incidence of viruses in decreasing order was SMV (13.3%), TSWV (5.4%), TRSV (4.2%), TSV (4.1%), PEMV (2.9%), BYMV (2.2%), ToRSV (2.1%), AMV (1.3%), BCMV (0.8%), and CMV (0.6%). Additionally, 1.5% of collected leaf samples had positive reactions in ELISA with antiserum to TMV, indicating the possible infection of soybeans in Iran with a tobamovirus that is related serologically to TMV. In Dot-Elisa, BICMV and PeMoV was also shown to be present in the collected samples. Soybean mosaic potyvirus (SMV) and the other seed transmissible viruses, are the most prevalent viruses in soybean fields in Iran. The overview highlights the past and present ongoing research and achievements on status of viral diseases of soybean crop in Iran.

Keywords: Viral agents, survey, infection percentage, soybean, detection

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INTRODUCTION

The top five superior countries with the highest rate of soybean production consists of United states, Brazil, Argentina, China and India with total production area under cultivation of 129,523,964 ha and production rate of 353,370,766 t. Among them, USA has become the major soybean production region by rate of 87,896,985 t, yearly (Fao,2020). Cultivation of soybean in Iran could be traced back 90 years ago, when some soy varieties were imported to Iran from China by soybean dealer's (Majidian et. al.,2024). Based on the report, the trend of soybean plantation didn't show the balanced process during 1994–2021 emphasizing the area harvested of 82,000 ha and production level of 200,000 t in 2021 (lower than recent decades), (Anonymous,2006; FaoStat,2020). Generally, soybean cultivation is distributed in five different provinces of the country, including north (Ardabil, Golestan, and Mazandaran), west (Lorestan), and south (Khuzestan) with productivity range of about 2.5 and 1.5 t/ha under irrigated and rain-fed conditions, respectively (Sattari and Khalilian,2020; Majidian et. al.,2024). Soybean cultivation and production is one of the priorities of the country (Anonymous,2006; Afshari-Azad et. al.,2021; Majidian,2024). One of the important factors limiting the increase in the production of soybean is viral diseases. These diseases cause a reduction in yield and a decrease in the quantity and quality of seed and plant products (Wrather and Koenning,2009). Soybean viral diseases can cause severe damage, especially many of

these viruses are seed transmitted, seed borne in soybean and thus spread and cause primary focus of contamination (Hartman *et al.*,2015; Afshari Azad *et al.*,2021; Heydari *et al.*,2012). Depending on the time of infection and cultivar, the location of the plant and environmental conditions, for example, tobacco ring spot virus (TRSV) can be transmitted between 40 and 100 percent through contaminated seeds (Bashir, et.al.,2000; Hartman et. al.,2015; Anonymous,1991; Sutic, et. al.,1999;). Soybean mosaic virus (SMV) is one of the members of the genus potyvirus, this virus and related isolates /strain is of special importance because of the damage it causes to soybean fields every year, so that sometimes the damage caused by the virus is estimated to be more than 50%. The damages caused by viruses varies depending on the soybean variety, virus strain, farming managements and environmental conditions. SMV has been reported between 1-68% of seed production and is widespread in all soybean growing regions of the world (Goodman and Oard,1980; Hartman *et al.*,2015; Shahraeen,1989; Wrather and Koenning,2009). SMV and other important viral agents has also been reported from most of the soybean growing areas in northern Iran (Table. 1, Fig. 1 and 2), (Karimi and Noaparad, 1989; Rahimian et. el. 1995; Gholnaraghi et.al.,2004,2002e). Usually, the reduction of soybean yield per unit area due to the viral factors is under estimated by farmers although the annual damage can amount to billions in any domestic currency (Shameli et.al.,2023; Sattari and khalilian,2020; Hartman et. al.,2015).

Table 1: Natural incidence and distribution of viruses infecting soybean in different soybean growing areas in Iran. Survey 2005-2007

Distribution Area *	Soybean Virus Disease
Gol, Maz, Lor, Ard, Khu	Soybean mosaic virus- SMV (Potyvirus)
Ard, Maz, Khu, Lor, Gol	Tobacco ring spot virus -TRSV(Nepovirus)
Ard, Gol, Khu, Lor, Maz	Bean yellow mosaic virus -BYMV(Potyvirus)
Ard, Maz, Khu, Lor, Gol	Tomato ring spot virus-TRSV(Nepovirus)
Ard, Maz, Khu, Lor, Gol	Tomato spotted wilt virus-TSWV(Orthospovirus)
Ard, Maz, Khu, Lor, Gol	Tomato yellow fruit ring virus-TYRV(Orthospovirus)
Gol, Gil	Peanut mottle virus-PeMoV(Potyvirus)
Ard, Maz, Khu, Lor, Gol	Tobacco streak virus-TSV(Illarvirus)
Maz, Kho, Lor, Gol	Bean common mosaic virus-BCMV(Potyvirus)
Ard, Maz, Khu, Lor, Gol	Alfalfa mosaic virus -AMV(Alfamovirus)
Ard, Maz, Khu, Lor, Gol	Pea enation mosaic virus-PEMV(Enamovirus)
Gol	Bean pod mottle virus-BPMV(Comovirus)
Khu, Gil, Maz	Cowpea mild mottle virus-CpMMV(Carlavirus)
Gol	Groundnut bud necrosis virus-GBNV (Orthospovirus)
Maz, Khu, Lor, Gol	Black eye cowpea mosaic virus-BICMV(Potyvirus)
Ard, Maz, Khu	Cucumber mosaic virus-CMV(Cucumovirus)

*Locations/Area, Ard=Ardabil, Maz = Mazandaran, Kho = Khuzestan, Lor = Lorestan, Gol = Golestan, Gil = Gillan

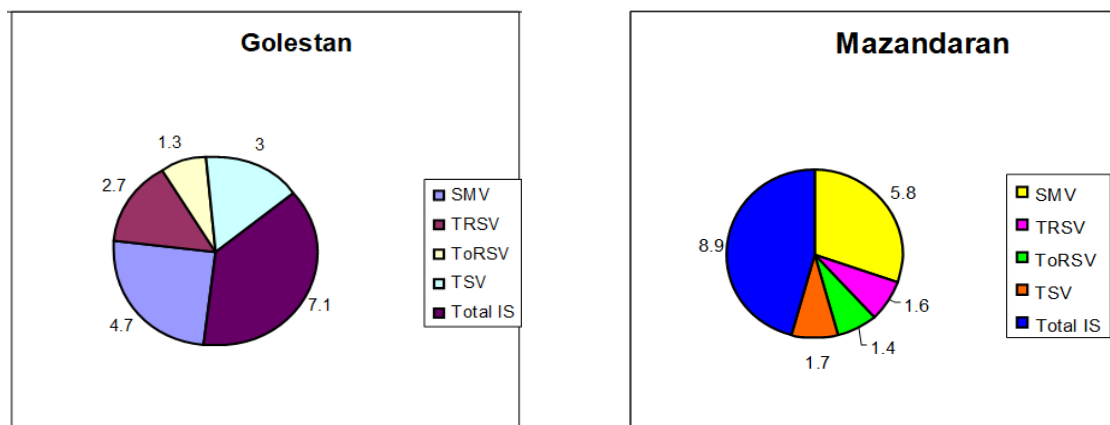


Figure 1: Percentage curve of soybean seed contamination to some important virus factors of soybean seed breeding in Golestan and Mazandaran

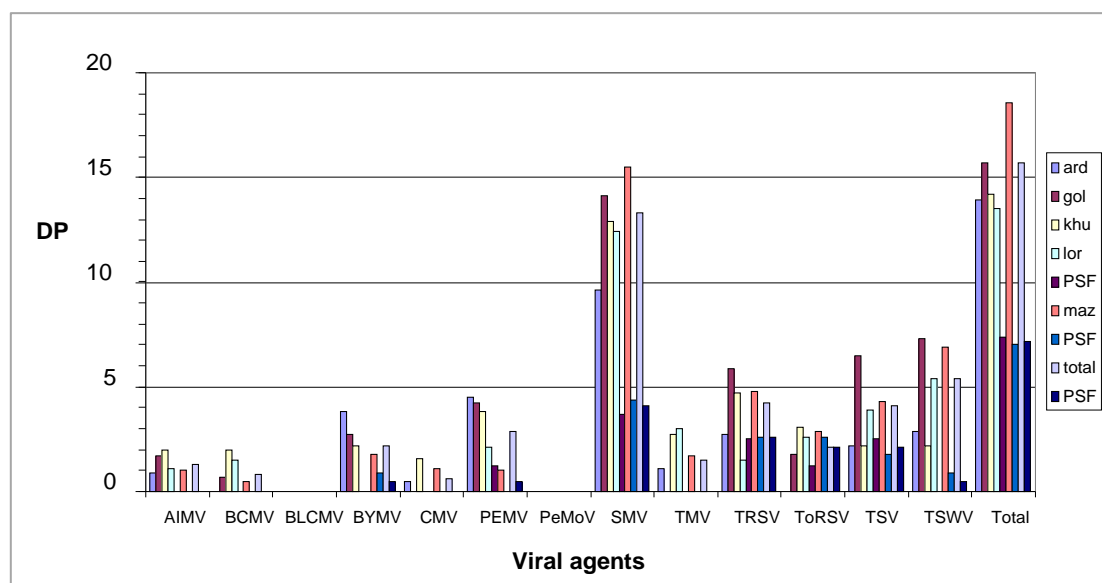


Figure2: Vertical curve showing the distribution percentage (DP) of soybean virus agents. *(PSF= Pod Set Failure Syndrome)

MATERIALS AND METHODS

Soybean samples suspected of infection by viral agents were collected from important soybean planting areas (Table 1, Figure.1 and 2). Green house experiments were carried out to investigate viral agents and to establish some of these agents for further studies in the greenhouse (Naghavi, et.al., Golnaraghi, et.al., 2004, 2002).

Serological Methods

Suspected soybean samples for viral agents were tested mostly by using ELISA method and in some cases by dot blot assay (Tissue blot) based on descriptive method (Lister, 1978; Shahraeen et.al.,2005; Ali,2017) and also in some cases by using transmission electron microscope, TEM method using antisera purchased and donated from various foreign sources (DSMZ Center, and Bioreba Co) against important viral agents contaminating soybean. In order to examine and observe the morphology of viral particles, electron microscope grids were prepared by leaf dip method. TEM - Phillips 301 and also LEO- 906 model electron microscope were used for particle observation in infected plant materials (Winter, et. el.,2006; Milne and Lesemann, 1984; Ghorbani, et. al.,2007).

Soybean Virus Disease Control Management

Considering the importance of soybeans and its increasing cultivation level in different regions of the country, the study to identify viral agents infecting soybeans, especially soybean seed viruses in one region, will be of special importance. The speed of spread of many soybeans viral pathogens depends on the existence of other heterogeneous crops, weeds adjacent to the field and the population of pests and vector insects. Seed transmission from infected soybean plants is one of the most important methods of virus transmission. Although soybean diseases and viral agents can be transmitted by a number of insect vectors, particularly aphids, beetles, whiteflies, thrips and nematodes in soybean fields. The results of researches have shown the difference in the level of contamination in different cultivars (Bashir,2000; Zamharir and Aldaghi,2018; Hartman et. el.,2015; Shameli et.al.,2023; Heydari et. el.,2012). Therefore, the use of multi-breeding methods is recommended to increase yield in more resistant cultivars, by considering the difference in the level of contamination in different farms, in order to reduce the contamination of farms, healthy and certified seeds should be used. Also, due to the effect of weather conditions on the activity of vectors and the reduction of the activity of virus vectors in colder areas, soybean seeds should be procured from the proper areas as much as possible. Considering that a number of soybean viral diseases, including mosaic virus and ringspot virus and some other viral agents are primarily transmitted through infected soybean seeds (from infected soybean plants in the field) and can be the primary source of contamination in soybean farm and then the secondary infection is transferred from infected young plants and through

various insects carrying viral diseases to other plants in the farm, so it is recommended to reduce the damage to the crop and prevent the spread of viral disease in the soybean farm to observe the following tips for soybeans cultivators are essential in selecting seeds for planting (Jener, *et al.*,2006; Hartman et. al., 2015). In general, incidences of soybean viral diseases in many cultivation areas in Iran were recorded to be more in an abandoned an unmanaged farm. Control of virus diseases of soybean rests with breeding new cultivars with natural or genetically engineered virus resistance, insecticides applied early to soybean-infected crops, removal of weed host reservoirs and, potentially, cultural control measures that limit virus spread, such as those used against non-persistently aphid-transmitted viruses. Numbers of viral diseases of soybean are seed borne, seed transmitted and few survive in living host plants. And there are numbers were can be seed-borne is not known. The question arises as to how these viruses persist over in the field when soybean and other nonhosts are absent. This key epidemiological issue needs to be investigated. It should be noted that in some provinces and planting areas, soybeans are planted in mixtures near other crops, including alfalfa, tomatoes, legumes, and squashes (Hartman, et. el.,2015; Golnaraghi, et. el.,2004; Naghavi *et al.*,2008). Due to various climatic issues, insect activity, and the type of variety planted, these contamination percentages may differ for each region (Majidian, et.al., 2024).

CONCLUSION

Our survey provides preliminary information from which integrated pest and crop diseases management strategy could be made in the events of new crop/variety introductions and virus spread. These surveys were snap shoot of over all diseases problem in soybean fields in Iran. Regular surveys in different growing stages of the plant will produce more and an accurate situation of the viral diseases in the field. Further research to identify the resistance gene involved would establish the potential usefulness of resistance phenotypes in breeding experiments. Because of the limited host range for number of viral diseases, soybean growers should pay particular attention to the elimination of nonhosts and weeds from around seedbed areas and production fields. This should be coupled with early pests specially aphid control on the crop and bordering areas. While significant differences were observed in the incidence of contamination in farms within a province. It seems that the presence of the primary source of contamination (especially contaminated seeds) is more important in terms of the spread of contamination in an area within the province than the location of the farm and the area of contamination. The incidence of contamination in large-area farms that used certified and healthy seeds was lower than that of small-area and scattered farms that used uncertified seeds (Fig.1 and 2). In a research survey, observations showed that about 90-100 percent of some small soybean fields in the Mazandaran region were contaminated with symptoms

of viral diseases. Viral agents transmitted through contaminated soybean seeds were more widespread than non-seed-borne viral agents infecting soybeans. The results also indicate that the spread of viral agents that are transmitted by only one insect vector (or one mode of transmission), for example, only by aphids, is lower than viral agents that are transmitted by two or more different modes (transmission mechanisms) SMV, TRSV, ToRSV, TSV or transmission by thrips such as TSWV, which are also capable of infecting a wide range of hosts (Golnaraghi et al., 2004, 2002a,e,c; Naghavi et al., 2008; Shameli, et al., 2023; Shahraeen, et al., 1995, 2005). High populations of Thrips tabaci Lindeman (Jenser, et al., 2006, Alavi, 2003) were observed in fields where soybean samples were infected with TSWV, and a number of these thrips samples also reacted positively to antiserum to a tospovirus in the ELISA assay. Based on serological and molecular characterization, BICMV is currently recognized as a strain of BCMV (Brunt, et al., 1995), so the observation of cross-reaction of these two putative viruses in ELISA assays would be justified. *Peanut mottle virus* was first reported from Golestan region on peanut (Shahraeen and Bananej, 1996). This virus is transmitted latently by a number of aphid vectors and has been reported to infect up to 8% of seed in some peanut cultivars (Sutic et al., 1999). There is a great similarity in terms of host range between the isolates of this virus from peanut and soybean (Shahraeen and Bananej 1996) from the Golestan region, and it seems that the virus has a common origin in the region, which can be proven by using molecular methods and comparing the sequences of the strains and virus isolates from each other. The host range of the tospoviruses isolated from the Golestan, Mazandaran, and Lorestan regions was similar, but significant differences were observed in the occurrence of symptoms and the ability of each isolate to infect different plants (Golnaraghi et al., 2002a, 2004). However, the possibility of the presence of different and unknown strains and genera of tospoviruses in soybean fields in Iran cannot be ruled out. Extensive and detailed studies using monoclonal antibody and molecular tools and comparing virus strains will clarify this hypothesis better. In recent years, contamination of soybean plants with various agents of the genus Tospovirus has been reported in Iran (Golnaraghi, et al., 2001, 2002a; Pourrahim, et al., 2001., Ghotbi, et al., 2005). Tospoviruses TSWV and Impatiens necrosis spot (INSV) are among the most widespread and emerging viruses for many agricultural crops in most regions of the world (Sutic et al., 1999; Hatman et al., 2015) and may be of particular importance in regions where soybean is also grown (Hartman, et al., 1999). Natural contamination of soybean with GBNV and GRSV of the genus tospoviruses has been reported from South Africa and also from India (Brunt, et al., 1995). Recently, natural contamination of soybean with viral agents of the genus Geminiviruses has also been reported from China and India (Li, et al., 2022; Singh, et al., 2024; Hartman, et al., 2015). Since the inoculation of each of the 13 viral agents isolated from soybean under

greenhouse conditions into healthy soybean plants of the Williams cultivar did not produce symptoms similar to the of pod set failure -PSF disease (Fig.1) (Golnaraghi et al., 2004), the involvement of other pathogenic and non-pathogenic factors, including environmental stresses (Faragi et al., 2023) in the occurrence of this disease (PSF) and in susceptible cultivars cannot be ruled out. They suggested with appropriate crop management particularly effective insect pest control, adequate potassium fertilizers application and adequate number of irrigations, the soybean proliferation effects / damages can be narrowed and even closed. The results of the two-year field study showed that under the conditions of Golestan province, it is possible to significantly increase soybean seed yield by application and adoption of appropriate crop management strategies and techniques (Faragi, et al., 2023). Recently, Zamharir, et al. (2022a and b, 2018) reported the Phytoplasma associated with pod set failure (PSF/soybean proliferation) of soybean in Iran. The *Hemiptera creontiades* Pallidus also known as cotton shredder bug, transmitted *Ca. P. trifolii* to healthy soybean plant under insect proof condition. Although in Iran and in the past, some reports have indicated the association of a number of viral agents with this disease (Rahimian et al., 1995). In a few sources, the disease of green stem of soybean (Shahraeen, et al., 2005; Schwenk and Nickell, 1980) has been reported in the field after the end of the plant growth period, which sometimes causes problems in harvesting soybeans in susceptible cultivars. This degree of greenness has been reported in different soybean cultivars. This disease is caused by the *bean pod mottle virus* -BPMV, which is transmitted mechanically with infected seeds and also by grasshoppers (Shahraeen, et al., 2005., Schwenk and Nickell, 1980, Hartman, et al., 2015). Based on the results obtained from seed testing and the presence of seed-borne viral agents including SMV, TSV, TRSV, ToRSV, and with a significant percentage in soybean fields and in seeds sown from infected plants (Table 1, Fig 1 and 2), the importance of using healthy and certified seeds for planting and preventing the spread of these damaging agents is more evident. In this survey, natural contamination of soybean with viral agents AMV, BCMV, BICMV, BYMV, CMV, PEMV, PeMoV, TSWV, and also a virus of the genus tobamovirus that has a close serological relationship to TMV is reported. Some of these agents have been previously reported from other hosts (Golnaraghi, et al., 2004a and e; Tavassoli, et al., 2007; Ghotbi, et al., 2005; Ghotbi and Shahraeen, 2025a and b).

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