An overview of oilseed rape (canola) virus diseases in Iran

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During recent years oilseed rape (Canola-Brassica napus) cultivation has gained an importance in different geographical zones in Iran. Viral diseases of economic importance have been reported to infect canola crop and causes considerable damage to the crop and economic losses to the farmers. During different surveys, various and common symptoms of mosaic, reduction in overall plant growth, flower and pod formation disorders, necrotic spots on leaves and stem, leaf distortion and yellowing, leaf crinkling and mid rib narrowing were observed in canola diseased plants in the field. Cauliflower mosaic-CaMV, Beet western Yellow-BWYV and Turnip mosaic-TuMV viruses were accordingly the most prevalent and common viruses identified in different cultivation zone. Other viruses of less common importance were also identified. Different biological, serological and molecular approaches were applied to identify and partially characterized the viral diseases and related isolates. Comparatively Hyola 308 cultivar showed better and faster recovery from an isolates of CaMV diseased symptoms in glass house condition. The overview highlights the past and present ongoing research and achievements on status of viral diseases of canola crops in Iran.

Keywords: Canola, virus diseases, distribution, incidence

INTRODUCTION

Oilseed crops after cereals are the second sources of food energy in the world. Oilseed rape which in recent terms also known as canola is the third main sources of edible oil after soybean and cotton seeds in the world (Rimmer and. Buchwaldi, 1995; Kolte, 1985). There has been a large increase in the area of oilseed rape grown in Iran over the past decade. On the basis of 2005-2006 annual report the area under canola cultivation in Iran was estimated approximately 170,000 ha with an average total yield production of 256000 tones (Anno,1986). In Iran oilseed rape is planted in September or November and harvested in the following July or August. The crop is thus a long lived host for brassica pests and pathogens which in recent years have been increased in incidence (Shahraeen, et al., 2003; Afshariazad, 2001). The two most important aphid vector of Brassica viruses, Mayzus persicae and Brevicoryne brassicae, are known to infest and overwinter on oil seed rape in Iran (Soheri et al, 2002). More than 12 viruses from different viral groups have been reported to infect and caused different level of losses in canola production. Beet western yellows (BWYV), Cauliflower mosaic, (CaMV), Turnip mosaic (TuMV), Cucumber mosaic (CMV), and Tomato spotted wilt (TSWV) viruses has been reported from canola fields in Iran from different agricultural zones (Shahraeen et al., 2003; Farzadfar et al., 2005; Farzafar et al., 2008; Tabarestani et al., 2011; Shahraeen et al., 2006; Kamran et al., 2004; Ghaderi et al., 2009a,b,c). CaMV, BWYV and TuMV accordingly are the most abundant viruses reported to infect canola crops in Iran (Farzadfar et al., 2004; Kamran et al., 2004, Ghorbani et al., 2006; Sahandi et al., 2005; Shahraeen et al., 2003; Tabarestani et al., 2011). Different biological, serological and molecular methods were applied to investigate the viral diseases infecting canola crops in Iran. Furthermore the BWYV and CaMV infection also confirmed applying PCR and RT-PCR techniques (Tabarestani et al., 2011; Ghader et al., 2009; Farzadfar, 2005). Cruciferous crops grown in Iran is subjected to be infected by different pests and pathogens, nevertheless viral diseases of common interest and origins to these crops are widespread. TuMV, CaMV, CMV, BWYV, and TSWV have also been reported from Crucifers and other agricultural crops in Iran (Farzadfar et al., 2004, 2005, 2008, 2002; Ghotbi et
MATERIALS AND METHODS

A total of 790 samples of canola plants suspected to be infected by a viral disease were randomly collected from 11 provinces (Table 1) in different canola cultivation locations. Samples includes leaves with various and common symptoms of mosaic, reduction in overall plant growth, flower and pod formation disorders, necrotic spots on leaves and stem, leaf distortion and yellowing, leaf crinkling and mid rib narrowing were observed. The samples were placed in labeled plastic bags and brought to laboratory for further investigations. The double antibody sandwich enzyme linked immuno-sorbent assay (DAS-ELISA) (Shahraeen et al., 2003) were performed with specific polyclonal antibodies for each viruses (Table 1) provided by DSMZ Inc., Germany. Some Biological, serological and molecular properties of Cauliflower mosaic virus isolates from canola plants in Iran were previously studied by Ghaderi et al. (2009 a,b,c). Using specific primers ORF VI gene of eight selected CaMV isolates were amplified. Comparison of sequences of the amplified fragments were determined for these isolates (Figures not shown) (Ghaderi et al., 2009 a,b; Farzadfar et al., 2008).

RESULTS

Beet western yellow virus (BWYV-luteovirus), Cauliflower mosaic virus (CaMV-caulimovirus), Turnip mosaic virus (TuMV-potyviruses) Cucumber mosaic virus (CMV-cucumovirus), Tomato spotted wilt virus (TSWM-tospovirus), Tobacco mosaic virus (TMV-tobamovirus), Turnip yellow mosaic virus (TYMV-tymovirus), Broccoli necrotic yellows (BNYV-rhabdovirus), Turnip rosette virus (TRoV-sobemovirus) Turnip crinkle virus (TCV-carmovirus) and Radish mosaic virus (RMV-comovirus) have been reported to infect oilseed rape from different growing areas of the world (Walsh and Tomlinson, 1985, Smith and Hinckes, 1985, Kolte, 1985, Hardwick, et al., 1994, Rimmer and Buchwaldt, 1995, Sutic et al., 1999 and Thurston, 2001). Yields reduction of plants due to BWYV, CaMV and TuMV showing severe virus symptoms were estimated to be 70 to 79% (Hardwick et al., 1994). In China the mosaic inducing viruses of rape seed caused 30% losses of the crop which estimated 50-80% yield loss (Shen, 1965).

In Iran CaMV has been reported to be a major viral diseases infecting Cruciferous crops which includes: Brassica oleracea var. capitata, B. oleracea var. italica, B. oleracea var. botrytis, B. oleracea var. acephala and B. oleracea var. rapa. B. napus, B. pekinensis and Raphanus sativus. The virus induces mosaic and a striking veinal chlorosis in most of its hosts. A masking of symptoms may occur in chronically infected plants, particularly at high temperatures. Infected plants of turnip, Chinese cabbage, and other species tend to flower prematurely. CaMV is widely distributed in temperate regions and brassica crops tend to become infected wherever they are grown (Kolte, 1985; Sutic et al., 1999). Biological diversity of 21 CaMV isolates (Farzadfar et al., 2008) from various regions and with different symptom severity has been evaluated based on turnip (Brassica rapa), jimpson weed (Datura stramonium) and kohlrabi (B. oleracea var. gongylodes). These isolates has produced variety of symptoms on turnip. Kohlrabi plants infected by all isolates tested eventually showed recovery and became asymptomatic. All the isolates were transmitted by green peach aphid (Myzus persicae). Using specific primers ORF VI gene of nine selected CaMV were amplified. Comparison of sequences of the amplified fragments revealed a high identity (96.9-100%) (Farzadfar et al., 2008). Among Iranian isolates Turnip mosaic virus (TuMV-family potyviridae, genus potyvirus) causes important diseases of crops worldwide including in: vegetable, e.g. Brassica oleracea ssp. botrytis (Cauliflower), B. napus, B. rapa (the cabbages), Raphanus sativus (radish) Rheum rhabarbarum (rhubarb), ornamentals, e.g. Matthiola incana (stock) and Limonium vulgare (statice); and oilseeds B. napus, canola, oilseed rape) and B. junce (Mustard). It also infect a wide range of weed species naturally incuding Raphanus raphanistrum (wild radish). TuMV is transmitted non-persistently by several different aphid species. It is considered one of the most important viruses infecting field vegetable (Tomlinson, 1987). It is also damaging to B. napus field crops in England and Wales, TuMV infection was recorded in 14% of crops and in 5% of plants overall, with yield losses in infected crops of up to 70% (Hardwick et al., 1994; Coutts et al., 2007). In Western Australia, beet western yellows luteovirus (BWYV) is brought into canola crops by aphid vectors that have previously fed on infected weed host plants, such as wild radish and volunteer canola. BWYV can reach high levels in canola crops if aphids carrying virus arrive early in the growing season. In canola, infection with the virus can cause partial plant dwarfing and reddening of lower leaves but infected plants are usually devoid of obvious symptoms. BWYV symptoms tend to get confused with those of nutritional and physiological disorders. In Europe, BWYV infection results in 10-34% seed yield reductions in canola, reduced oil levels in seed and increased glucosinolate contents. (Smith and Hinckes, 1985; Walsh and Tomlinson, 1985; Sutic et al., 1999). The occurrence of viral diseases of oil seed rape in Iran has been monitored annually since 1999 as a part of the IRIPP national survey project on viral diseases of oil seed rape. During some seasons an increase in virus like symptoms in oil seed rape crops (leaf mosaic, vein clearing, leaf and stem distortion, and growth reduction was noted. Virus symptoms were most seen in the late winter and early
spring before stem extension and flowering. High infestation of the crops by rape aphid could be observed in some cultivation areas (Varamin). Selected samples representative of the observed symptoms were transferred to laboratory for testing and the results were tabulated (Table 1). Different biological serological and molecular techniques have been applied to investigate and detect oilseed rape viruses in Iran. The three main important and prevalent viral diseases of oilseed rape and other less frequent occurring canola viruses in Iran in different provinces is shown in table 1. CaMV (40.2%), BWYV (17.8%) and TuMV (6.20%) were the most common viruses in 2005/2006 survey season where the mix infection of 2 or 3 viruses in a sample could also be recorded (Farzadfar et al., 2008; Ghaderi et al., 2009 a, b, c; Ghorbani et al., 2006). Incidence and

Table 1. Natural incidence and distribution of viruses infecting oilseed rape in different areas in Iran. Survey 2005-2007

<table>
<thead>
<tr>
<th>Provinces (cities)</th>
<th>Virus/Total No of samples</th>
<th>BWYV</th>
<th>CaMV</th>
<th>TuMV</th>
<th>TCV</th>
<th>TMYV</th>
<th>RaMV</th>
<th>TSWV</th>
<th>CMV</th>
<th>Mix infections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Azarbaj an West.including: Uromieh, Mahabad, Miandoab</td>
<td>130</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Ardabil, Moghan</td>
<td>45</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>2,3,3</td>
</tr>
<tr>
<td>Fars, Shiraz</td>
<td>170</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+ (+)</td>
<td>(+)</td>
<td>1,3,4</td>
<td></td>
</tr>
<tr>
<td>Zarghan</td>
<td>(28)</td>
<td>(65)</td>
<td>(1)</td>
<td>(3)</td>
<td>(23)</td>
<td>1,2,5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ahoocar</td>
<td>2,3,8</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mazandaran</td>
<td>110</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>2,5,1</td>
<td></td>
</tr>
<tr>
<td>Sorry, Ghraghil, Vercola, Darshinaz, Behshar</td>
<td>(15)</td>
<td>(35)</td>
<td>(5)</td>
<td>(5)</td>
<td>(3)</td>
<td>1,2,5</td>
<td></td>
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<tr>
<td>Tehran, Varamin</td>
<td>150</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>(+)</td>
<td>1,2,7</td>
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<tr>
<td>Sharekord</td>
<td>60</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1,2,6</td>
<td></td>
</tr>
<tr>
<td>Lorestan, Alshtar, Brojerd</td>
<td>(13)</td>
<td>(26)</td>
<td>(7)</td>
<td>(15)</td>
<td>2,3,3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gorgan, Kordkoh, Versen</td>
<td>20</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>m</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>1,2,1</td>
<td></td>
</tr>
<tr>
<td>Isfahan</td>
<td>35</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>2,3,2</td>
<td></td>
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<tr>
<td>Zanjan</td>
<td>27</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>M</td>
<td>nt</td>
<td>+</td>
<td>+</td>
<td>1,2,1</td>
<td></td>
</tr>
<tr>
<td>Ghazvein</td>
<td>15</td>
<td>nt</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Total sample</td>
<td>790</td>
<td>141</td>
<td>318</td>
<td>49</td>
<td>23</td>
<td>4</td>
<td>35</td>
<td>3(27)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Percent Distribution</td>
<td>17/8</td>
<td>40/2</td>
<td>6/20</td>
<td>2/9</td>
<td>0/05</td>
<td>3/16</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BWYV = Beet western yellow virus, CaMV = Cauliflower mosaic virus, TuMV = Turnip mosaic virus, TCV = Turnip crinkle virus, TMYV = Turnip yellow mosaic virus, RaMV = Radish mosaic virus, TSWV = Tomato spotted wilt virus, CMV = Cucumber mosaic virus. + = positive reaction, - = negative result.

( no in brackets) = no. of infected plant samples, % = percent infectivity, nt = not tested, (+) for TSWV and CMV = recorded positive in previous surveys (shahraeen et al., 2003) but percent infectivity not included in this table.
distribution of three aphid borne canola viruses have also been reported by Tabarestani et al. (2011) in Golestan province of Iran. Evaluation of resistance to Turnip mosaic virus in Australian Brassica napus genotype has been carried out. When forty-three Australian cultivars or breeding lines and 2 cultivar of B.junce (Mustard) were inoculated with infective sap of TuMV-WA-ap isolate, different type of resistances responses occurred within 22 genotypes (Coutts et al., 2007). Reaction of few spring swan canola cultivars e.g. Hayola 401. Hayola 308, Option and PF to inoculation of an isolates of CaMV from Tehran (Varamin), Shaherkord, Shiraz and Ourmia (IOa,IOb) were evaluated in glasshouse condition. Results showed that 35 days after inoculation of the virus isolates, Hyola 308 cultivar showed better and faster recovery from the diseased symptoms as compared to Hayola 408,Option, and PF. Virus infected Hayola 308 produced more number of seed pods compare to other cultivars (Sahandi et al., 2006).

**DISCUSSION**

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 canola fields. 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 these resistance phenotypes in breeding experiments. Because of the limited host range for CaMV, crucifer growers should pay particular attention to the elimination of cruciferous weeds from around seedbed areas and production fields. This should be coupled with early aphid control on the crop and bordering areas. BWYV, CaMV and TuMV were all found infecting wild radish weeds. In general, incidences of BWYV were recorded to be more in wild radish than canola. Control of virus diseases of canola rests with breeding new cultivars with natural or genetically engineered virus resistance, insecticides applied early to BWYV-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. BWYV is not seed-borne and must survive the summer in living host plants. Whether CaMV and TuMV can be seed-borne is not known. The question arises as to how the three viruses persist over the dry summer period in the field when canola and wild radish are absent. This key epidemiological issue needs to be investigated

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