Efficiency of ten populations of *Schizaphis graminum* (Rondani) in the transmission of barley yellow dwarf virus-PAV (BYDV-PAV) in Iran

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Variation in transmission of ten clones of *Schizaphis graminum* (Rondani) originating from different regions of Iran was evaluated by transmitting an isolate of barley yellow dwarf virus-PAV (BYDV-PAV) for which this species is normally an inefficient vector. After acquiring the virus, aphids of each clone-virus isolate combination were caged on 30 seedling using three forth instar nymphs per seedling. The inoculated and control plants were assayed by ELISA and assayed visually to determine the rate of transmission and the mean ELISA value for each treatment. A positive correlation was found between the rate of transmission and the mean absorbance value. Aphid clones differed in their efficiency of transmission of virus isolate. On the other hand, single clone transmitted virus isolate with varying efficiencies. The aphid clone of virus isolate origin transmitted virus isolate at the highest rate. In general, Shahre kord and Shahreza clones of aphids showed the highest efficiency of transmission and Delijan and Varamin clones had the lowest transmission efficiency between aphid clones.

Key words: Luteovirus, aphid clones, BYDV-PAV, transmission efficiency, *Schizaphis graminum*, Iran.

INTRODUCTION

Insects are vectors for a large number of plant and animal viruses and aphid are the most successful and numerous insect (Gray and Banerjee, 1999). *Schizaphis graminum* is an important insect pest of several grain crops and an efficient vector of cereal-infecting luteoviruses and poleroviruses (Porter et al., 1997; Puterka and Peters, 1995). Direct feeding injury, exacerbated by the induction of a phytotoxic response of the plant, can significantly reduce crop yield and value (Ryan et al., 1990). *S. graminum* is also a vector of several plant viruses, including the viruses that cause barley yellow dwarf disease (BYD). Barley yellow dwarf (BYD) is one of the most severe cereal diseases in the world (Lister and Ranieri, 1995). It is caused by different viruses belonging to the genera Luteovirus (BYDV-PAV and BYDV-MAV), Polerovirus (CYDV-RPV) or presently unassigned (taxa BYDV-RMV, BYDV-SGV, BYDV-GPV) of the family Luteoviridae (D’Arcy and Mayo, 1997).

The viruses are all transmitted persistently by different aphid species living on Poaceae with a variable degree of vector specificity (Rochow, 1969). The viruses are vectored by at least 25 aphid species in a persistent (circulative) manner and are limited to the phloem tissues of infected plants (D’Arcy and Burnett, 1995).

Ingested virions are actively transported across gut epithelial cell cytoplasm in vesicles and released into the hemocoel. To be transmitted into a plant, the...
virions accumulate at the surface of the accessory salivary glands and are actively transported across the cells and deposited into the salivary duct. The virus does not replicate in any tissue of the aphid (Gray and Gildow, 2003). All of the virus species were initially differentiated based on the aphid vector that transmitted them most efficiently. The species RPV, RMV, MAV and SGV are readily transmitted by *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *Sitobion* (formerly *Macrosiphum*) *avenae* (Fabricus), *Schizaphis graminum* (Rondani), respectively, while PAV is transmitted by both *R. padi* and *S. avenae* (Rochow, 1969; Johnston and Rochow, 1972; Rochow and Muller, 1971; Power et al., 1991). It is well documented that the various aphid species differ in their abilities to transmit the various variants of BYDV (Rochow, 1969; Rochow and Carmichael, 1979). Furthermore, populations within a species will differ in their ability to efficiently vector certain viruses. Intraspecific variation in vector capacity has been described for circulative plant viruses (Bencharki et al., 2000; Gray, 1999). However, little is known about intraspecific aphid variation. In USA, Rochow (1960) described clones of the greenbug *S. graminum* (Rondani) differing in their ability to transmit a BYDV-SGV isolate, and four biotypes (clones differing by biological characters) of the corn leaf aphid *Rhopalosiphum maidis* (Fitch) were reported to differ in their ability to transmit the AG-1 strain of BYDV (Saksena et al., 1964). Rochow and Eastop (1966) reported variation in the abilities of two morphologically different clones of *Rhopalosiphum padi* (L) to transmit BYDV-RMV.

Sadeghi et al. (1997 a, b) showed that two BYDV-MAV isolates were efficiently transmitted by clones of the normally inefficient vector *R. padi*. More recently, similar results have been reported for the transmission of BYDV-RMV isolates by *R. padi* in North America (Lucio-Zavaleta et al., 2001). Virus–aphid specificity seems to result from recognition between virions of a specific isolate and virus receptors in the accessory salivary glands of a particular aphid species (Gildow and Rochow, 1980; Gildow and Gray, 1993; Peiffer et al., 1997).

This vector specificity is not absolute because heteroencapsidation may enable a given aphid species to transmit a virus of which it is normally not a vector (Rochow and Pang, 1961; Rochow, 1975); also some isolates were reported to be transmitted by aphid species which are usually non vectors, as is the case for an Australian MAV serotype transmissible by *R. padi* (Lister and Sward, 1988), and for a Californian RPV serotype transmissible by both *S. avenae* and *Schizaphis graminum* (Rondani), and as by *R. padi* as well (Creamer and Falk, 1989); and finally within a given aphid species, clones could differ in their abilities to transmit viruses, as shown for both stylet-borne and circulative viruses (Sadeghi et al., 1997).

BYD was first reported in Iran in 1988, and BYDV-PAV was found to be the predominant serotype of the BYD causing viruses on both cultivated and non-cultivated cereals in all regions (Rastgou et al., 2004b). Among the aphids transmitting these viruses in Iran, *R. padi* was the predominant species followed by *S. graminum, R. maidis* and *S. avenae* (Khatabi et al., 2004b).

Aleoostoor et al. (2006) reported differences in transmission efficiency between seven clones of *R. padi* as a first intraspecific study on an aphid species in Iran. The objective of this study was to evaluate transmission efficiency of ten *Schizaphis graminum* population collected from different region of Iran in which aphid vector was inefficient vector.

This study is a pioneer work on determination of transmission efficiency in intraspecific clones which are ineffective vectors in Iran; also previous works involved only a small number of clones. The aim of the present work was to assess more widely transmission variations of a PAV isolate among a large sample of *S. graminum* clones collected in different parts of the Iran with different phenotypic characteristics. We have also sought to investigate the epidemiological role of PAV transmission by this species and finally, we have initiated a study to find contrasting variants for further investigations on the relationships between luteoviruses and putative virus receptors in their vectors.

### MATERIALS AND METHODS

#### Culture of virus isolate

One BYDV-PAV isolate was used for the transmission experiments. The virus isolate used in this investigation was obtained from Center of Cereal Pathology at Agriculture College of Shiraz University in 2008 and causes severe yellowing to barley (cv. Zar-jo). The virus isolate was transferred to a growth chamber and maintained under controlled conditions (20±2°C and 16L:8D) at the Agriculture College of University of Tehran. The isolates were maintained by repeated aphid transfers on 7-day old seedlings of barley Zar-jo cultivar at intervals of 5 to 6 weeks. The plants were inoculated with viruliferous *S. graminum* nymphs with a 3 day inoculation access period (IAP). After inoculation, plants were kept in a growth chamber at 20±2°C with 16 h illumination per day for symptoms to develop. Young leaves of the same age from infected Zar-jo plants, three weeks after the IAP, were used as a source of the virus in all experiments.

#### Collecting and maintenance of aphid clones

The aphid species used was *S. graminum*, the greenbug aphid. The aphid populations used in this work were collected during the cropping seasons of 2008 to 2009 in different regions of Iran and 10 aphid clones were prepared (Table 1). A few aphids were collected for each population. Colonies of
Table 1. Percent infection and mean ELISA value of Barley (cv. Zar-Jo) seedlings inoculated with BYDV-PAV isolate and ten S. graminum clones. In each treatment, a total 30 seedling inoculated.

| Source of aphid | Infection % By Symptom | Mean ELISA Value ± SD | By ELISA (OD<sub>450</sub> |
|-----------------|-------------------------|-----------------------|-----------------------------|
| Sg-A            | 5.0                     | 8.0                   | 0.0696 ± 0.023              |
| Sg-B            | 5.0                     | 6.0                   | 0.0761 ± 0.022              |
| Sg-C            | 10.0                    | 16.0                  | 0.0878 ± 0.016              |
| Sg-D            | 10.0                    | 13.0                  | 0.0886 ± 0.020              |
| Sg-E            | 15.0                    | 17.0                  | 0.0905 ± 0.025              |
| Sg-F            | 15.0                    | 19.0                  | 0.0906 ± 0.021              |
| Sg-G            | 20.0                    | 20.0                  | 0.0916 ± 0.017              |
| Sg-H            | 25.0                    | 21.0                  | 0.1019 ± 0.022              |
| Sg-I            | 36.0                    | 35.0                  | 0.1161 ± 0.028              |
| Sg-J            | 40.0                    | 42.0                  | 0.1233 ± 0.021              |

Testing for virus-transmission efficiency

In a first experiment, virus-free aphids of each population were removed from the rearing barley plants and given 12 h acquisition access period (AAP) on detached leaves from a barley plant infected with the PAV isolate studied. Aphids were then transferred with a fine brush to 30 barley test plants (1-leaf stage), by groups of 3 per plant and the plants were then individually caged with a cellophane bags for 5 day IAP. At the end of the IAP, plants were sprayed with the insecticide Primor and maintained in an insect-proof greenhouse. After a 15 day period of allowing virus multiplication in the plants, each plant was tested for virus content with a double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) to assess percentages of infection for each clone. Disease symptoms were scored 4 weeks after inoculation. All plants were individually tested in DAS-ELISA. Results excluded tests where the source tissue had an absorbance value lower than three times those of the healthy ones. To determine the average difference from the control group, analysis of variance (ANOVA) in Minitab software and Duncan test in SPSS software was used.

RESULTS

Our study revealed that all 10 clones tested, transmitted PAV isolate but in different amount but in low percentage (<15%) for most of them. However clones transmitted virus isolate in different efficiencies. Infection percentage by symptoms and by ELISA was evaluated. It varied from 5 to 40 and 6 to 42% respectively. The lowest absorbent rate and transmission percentage by ELISA corresponded to Sg-A population (Table 1). Plants with the highest symptoms rates were observed in Sg-J population. A positive correlation was found between the rate of transmission and the mean absorbance value (Figure 1). Infection percent for clones Sg-J and Sg-I was 42 and 35 respectively, also Duncan test showed that these two clones are in same group and they transmitted at a significantly higher level than other clones. Infection for clones Sg-1 and Sg-2 were at 8 and 6% respectively. Duncan’s range tested for these ones showed that they were in a same group and two clones transmitted virus isolate poorly (<5%) (Table 2). Between 300 inoculated seedling tested, 204 seedlings by ELISA index (X ± 3 SD) and 183 seedlings by symptoms were positive. Plants which were distinguished as infected based on symptoms also were infected by ELISA evaluation. The correlation between these indexes was 0.97.

Our results demonstrate that mean of absorbance value in ELISA had direct relation to geographical origin of viruses in which virus isolate transmitted highly with aphid population with same origin. Our results are correlated with other findings; as shown Shahre-kord population (Sg-J) transmitted virus serotype more effective than other populations; Duncan test revealed that Sg-I and Sg-J are in same group (Table 2) and transmitted virus serotype with significant difference and their mean absorbance value was 0.028 and 0.021 respectively. All aphid populations tested in the transmission efficiencies, were in four groups; Sg-A and Sg-B with 0.069 and 0.076 absorbance value were in the first group. ELISA absorbance value for Sg-C, Sg-D, Sg-E, Sg-F
and Sg-G populations was 0.087, 0.088, 0.090, 0.090 and 0.091 respectively and were in second group. The third group involved Sg-E, Sg-F, Sg-G and Sg-H. Their ELISA absorbance values were 0.090, 0.090, 0.091 and 0.101 respectively. Also Sg-I and Sg-J were in the forth group with 0.116 and 0.123 ELISA absorbance value.

**DISCUSSION**

BYDV is widespread in Iran. Among its serotypes, BYDV-PAV is the dominant serotype around the country (Afsheiifar et al., 2004). Studies on this serotype showed that there is biological and molecular diversity among serotypes of BYDV-PAV.

Table 2. Transmission efficiencies for BYD by each ten aphid clones.

| Aphid clone | Mean ± S.E. a |
|-------------|---------------|
| Group control | 0.05140 ± .001 a |
| Sg-A | 0.06967 ± .002 b |
| Sg-B | 0.07614 ± .003 b |
| Sg-C | 0.08780 ± .004 c |
| Sg-D | 0.08868 ± .004 c |
| Sg-E | 0.09050 ± .004 c |
| Sg-F | 0.09067 ± .005 c |
| Sg-G | 0.09163 ± .005 c |
| Sg-H | 0.10193 ± .006 d |
| Sg-I | 0.11610 ± .007 e |
| Sg-J | 0.12337 ± .007 e |

aMeans followed by different letters are significantly different according to Duncan’s range test.

Figure 1. Correlation between mean ELISA value and % symptoms Barley plants using BYDV-PAV isolates and ten S. graminum clones.


(Dedryver et al., 2005; Tahan 2006). *R. padi* is known to be an efficient vector of virus (Rochow, 1969). Different reports about different dispersion of this virus in fields may support the hypothesis that another virus serotypes has a major role in dispersion of virus.

Further complicating the epidemiology of BYDV-PAV is the evidence that *S. graminum* may be a more efficient vector of BYDV-PAV if the plant was originally inoculated by *S. graminum* (Rochow, 1961; Rochow, 1963).

Clones originated from regions that aphid have holocyclic life transmitted significantly better than others. Our result is similar to those of Sadeghi et al. (1997) that reported that European clones of *R. padi* transmitted BYDV-MAV significantly better than other clones in which they have holocyclic life.

There are only few reports of intraspecific variations in virus transmission by aphids and most of them compare a very small number of clones. Those on BYD-viruses mainly concern quantitative variations in transmission percentages of a given serotype by its main vector (Rochow, 1960; Saksena et al., 1964; Guo et al., 1996; Sadeghi et al., 1997).

Only Rochow and Eastop (1966) and more recently Guo (1995) reported transmission studies on serotypes for which aphid species tested are usually inefficient vectors, that is variations in transmission abilities of respectively (i) two *R. padi* clones for RMV, normally transmitted by *R. maidis* and (ii) two clones of *R. padi* for MAV transmission. We compared for the first time PAV transmission by numerous *S. graminum* clones collected from diverse geographical areas; our results show there are overall differences among isolate for transmission.

These results, which fit well with some field records and PAV transmission by *S. graminum* may have some epidemiological consequences. In France, MAV contaminations of barley fields are commonly observed in autumn, although the normal vectors of this virus (*S. avenae* and *M. dirhodum*) are absent or very scarce (Leclercq-Le Quillec, 1992). Most of the plants are infected by SGV and PAV, indicating that heterologus encapsidation could play a role in PAV transmission by *S. graminum*.

This is a direct proof that MAV transmission by *R. padi* can occur in field conditions, that all *S. graminum* clones tested transmitted at least PAV isolate in the laboratory, and that two clones of 10 were rather efficient vectors, which means that outdoor PAV transmissions by *S. graminum* could be frequent and epidemiologically important, at least when *S. graminum* populations contains many Sg-I and Sg-G.

In the aphid, the virus must cross from the hindgut into the hemocoel, then survive in the hemocoel, join the salivary glands and penetrate into the accessory salivary glands to be egested. Van den Heuvel et al. (1994) have demonstrated that symbionin, a GroEL homologous protein synthesized by endosymbiotic bacteria (genus *Buchnera*) and secreted into the aphid hemolymph, is essential for efficient luteovirus transmission. Possibly, symbionin of the inefficient aphid Sg-A and Sg-B is released at low concentrations or has low binding affinity for the virus. The later possibility is indirectly supported by the recent findings of Van den Heuvel et al. (1997), who showed that symbionin from different aphid species bound in vitro with different affinities to Luteoviridae. Also, it is likely that several barriers and therefore several genetic loci are responsible for vector competence (Dedryver, 2005).

The transmission efficiency may have been associated also to the retention and to natural losses of virus content in aphids during the feeding process. The recognition and understanding of the factors involved in the host–virus–vector interaction may lead to efficient control of those PAV isolates which provoke severe disease and aid in the breeding for resistant cultivars. (Lucio-Zavaleta et al., 2001).

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