Variation in Virus Content Among Individual Leaves and Roots of Barley and Wheat Infected with a BYDV-PAV Isolate

S.E. Sadeghi¹, C. A. Dedryver², G. Riault² and S. Tanguy²

ABSTRACT

ELISA values (O.D.s) of extracts of separately roots and individual leaves of barley and wheat plants infected by a French BYDV-PAV isolate (PAV-4), were assessed for five different virus incubation periods in controlled conditions (5, 10, 15, 20 and 25 days after inoculation). In most cases, virus contents of individual roots or leaves were not statistically different for barley and wheat. For both plant species, ELISA values peaked sooner in roots than in leaves and in most harvesting dates were higher in roots. The old leaf had a significantly lower virus titre than other leaves. Upper leaves exhibited the highest ELISA values. These results indicate that the upper part of infected plants is the most suitable for virus acquisition by aphids. A scheme for BYDV-PAV spread in young plants is proposed, and compared to the distribution of vectors on the plants.

Keywords: BYDV-PAV, Barley, Wheat, Leaf, Root, Rhopalosiphum padi.

INTRODUCTION

Barley yellow dwarf viruses (BYDV) are a group of related luteoviruses (Waterhouse et al., 1988) that infect plants in the family Graminae and are obligatory transmitted by one or more specific aphid species in a persistent, nonpropagative manner (Rochow, 1969, 1970). Viruses replicate in the phloem cells (Jensen, 1969; Gill and Chong, 1975) and are translocated along vascular bundles shortly after inoculation and first replication cycle (Gill, 1968; Jensen, 1973). Assessment of virus concentration in different parts of infected plants is of great interest for (i) transmission studies, (ii) sampling for diagnosis, (iii) studies of virus spread in plants and comparison with feeding habits of the aphid vectors. For transmission studies, virus acquisition by aphids could be done on leaves detached from the same foliar level of infected plants (Rochow, 1966; Rochow and Eastop, 1966; Gill, 1967). Feeding on detached leaves could influence virus acquisition by aphid because no sieve flood exists and early senescence of the leaves can occur (Prado and Tjallingii, 1994). Aphids can also be confined on a given non detached leaf by clip caging (Gray et al., 1991) which is closer to natural conditions but does not allow them to feed at their preferential feeding site. The most realistic method is allowing vectors to feed freely on the whole infected plant. The effect of virus titre of the feeding source leaf on subsequent virus acquisition by the vectors has been discussed: Foxe and Rochow (1975) and Pereira et al. (1989b), found no significant effect of virus concentration in the leaves on virus transmission efficiency whereas, Gray et al. (1991) found that the different virus titer in leaves of different ages was responsible for significant differences in transmission efficiencies. For methodological pur-
poses, an accurate knowledge of virus titre in every leaf of an infected plant, and dynamics with time, is needed. Also, sampling of leaves in which the highest virus titers are expected would facilitate diagnosis.

Finally, knowledge on virus titer in the different parts of the plant and its dynamic with time might help to understand transmission ability of aphid vectors, whose feeding sites differ with the aphid species involved (Dedryver and Robert, 1977). Here we describe the dynamic of the virus contents in roots and individual leaves of barley and wheat from the two-leaf to the 5-leaf growth stages of the plants. A BYDV-PAV isolate (PAV: padi-avenae-virus; Rochow, 1970) was chosen for this first set of experiment because PAV viruses are widespread on cereal crops in western Europe (Plumb, 1983; Leclercq-Le Quillec et al. 1995; Sadeghi et al. 1997b) and give severe symptoms and high yield losses on barley (Chalhoub et al. 1994b). PAV isolates are transmitted non-specifically by the aphids species, *Rhopalosiphum padi* L., *Sitobion avenae* F. and *Metopolophium dirhodum* (Walker) (Rochow, 1982).

**MATERIALS AND METHODS**

**Virus Isolate and Aphid Vector**

The isolate PAV-4 was used for the transmission experiments. It was collected in Le Rheu (western France) from barley in 1989 (Leclercq-Le Quillec, 1992). On old plants this isolate causes severe dwarf and yellowing to barley (cv Express) and reddening of the flag leaf of winter wheat (cv Arminda). On young plants, PAV-4 reduces root growth of barley and wheat, and causes yellowing of the upper leaf of barley (no foliar symptom on wheat). The virus source plants were barley seedlings cv Express, infested with viruliferous bird cherry-oat aphids *R. padi* (clone Rp1), grown at 20 °C 16h. light- 8h. dark, in 7×7×7 cm. plastic pots filled with a 50 % mixture of sand and compost, and regularly renewed. Clone Rp 1 was collected on winter wheat in 1978 at le Rheu (Simon et al., 1991) and transmits regularly PAV-4 with 90-100 % of success (Sadeghi et al., 1997a). It was used for the inoculation tests described below.

**Experimental Infections**

Two hundred twenty five seedlings - each of winter barley cv “Express” and winter wheat cv “Arminda”- were singly grown in polyethylene tubes 2×2×12 cm filled with moist vermiculite. The tubes were placed in a programme-controlled chamber at 20 °C 16h. light- 8 h dark, and irrigated twice a week with a nutritive solution (Hakaphos, BASF, Germany). When the first leaf was well developed, three apterous larvae (3rd or 4th growth) of viruliferous *R. padi* of the clone Rp 1 collected on virus source plants (see above), were transferred on each of 200 barley and 200 wheat plants and caged with a cellophane bag. After a 5 day inoculation access period (IAP), all seedlings were sprayed with a solution of Deltamethrin (Decis EC, Hoeschst, 0.2ml/l) to kill aphids and placed in the same controlled chamber for completion of virus incubation period. Control uninfested plants were put separately in the same growth conditions. Plants were sampled at five times (5, 10, 15, 20, and 25 days after the end of IAP). On each sampling time, 40 infested and 5 uninfested control plants of each plant species were harvested. Plants were pulled out, washed individually and dried by blotting. Roots and individual leaves were separated and immediately processed for ELISA. In the text, tables and figures, different leaves and roots are abbreviated as L1, L2, L3, L4, L5 and R. The leaf 1 and leaf 5 being respectively the oldest and the youngest leaf.

**Detection of Virus in the Infected Plants**

Virus content in leaves and roots of infected plants were expressed as the OD 405nm estimated by a triple antibody sandwich
Figure 1. Mean ELISA values (O.D.) for PAV-4 infection in roots, leaf 1 and leaf 2 (bars are standard errors): results of the pairwise comparison between barley and wheat. For each harvesting date and organ, histograms with different letters are significantly different (Duncan’s multiple range test).
enzyme linked immunosorbent assay, TAS-ELISA, procedure as described for BYDV by Torrance et al. (1986), Pead & Torrance (1988) and Leclereq et al. (1995). The wells of microtitre plates (Consortium de Materiel de laboratoire, France; Microtest M29-LSE) were coated with a dilution of 1:1000 polyclonal antisera, anti PAV-like produced by H. Lapierre (INRA, Laboratoire de Virologie, Versailles, France) (IgG) in 0.05 M Sodium carbonate buffer PH=9.6 (100 µl/well) incubated at 37°C for 4h. The wells were washed three times with distilled water and three times with phosphate buffer saline with Tween (PBS-T) allowing 3 min between each rinsing. Sap from 0.2g leaf or root material was extracted with 2 ml of PBST-PVP (phosphate buffered saline, 0.5% Tween 20, 2% polyvinylpyrrolidone) and the antigen incubated overnight at 4°C. Monoclonal antibody Mac91 (ADGEN diagnostic systems, Ayr, Scotland, U.K.), was added after dilution in PBST-PVP (1 µg/ml) and incubated at 37°C for 2h. IgG anti-rat (1/2000, Sigma) conjugated to alkaline phosphatase was then added and incubated at 37°C for 2h. Substrate (p-nitrophenyl phosphate) was then added and the optical density (O.D.) measured at 405nm with a Dynatech MR5000 spectrophotometer after 1-2 h incubation at room temperature. Samples were considered as positive when O.D. values were greater than three times the means of the results of uninfected control leaves. The few obviously non-infected plants from aphid infested batches were discarded and a minimum of 33 plants were taken into account in the results.

Data Analysis

Absorbance values (O.D.405) were analyzed by one-way ANOVAs based on type III, using the GLM procedure of the SAS package. Mean separation was performed by the Duncan’s multiple range tests. Virus content in roots and leaves were compared between barley and wheat at each sampling time. Also, comparisons were made for barley and wheat separately.

RESULTS

Comparative Optical Density of PAV-4 in Barley and Wheat

Results of virus content in roots and leaves of barley and wheat are shown in Tables 1 and 2, respectively. O.D. values of barley cv. Express and wheat cv. Arminda were compared for each leaf or root and harvesting time separately. Significant differences were only found for roots at 15 days after LAP (Pr = 0.0000), Li, at 5 days after LAP (Pr = 0.00033), and L2, at 10 days (Pr = 0.0000) and 15 days (Pr = 0.0000) after LAP. In these cases, O.D.s are significantly higher for wheat than for barley in roots, whereas in leaves significantly higher O.D. values were obtained for barley than for wheat (figure 1).

Comparative ELISA Values for PAV-4 in Roots and Different Leaves of Barley

As shown in table 1, virus content in roots are high during the whole growing period considered, with a maximal O.D. value 10 days after the end of LAP. Range of ELISA values shows that all roots were infected for every experiment. The older leaf (L 1), which is the inoculated one, has consistently a much lower virus content than other leaves, at every sampling time. Leaves L2 to L5 have a high virus titre as soon as they are well developed and can be harvested. In most cases O.D. values do not change significantly for each individual leaf, with time. For L1 and young L2 and L3, a great variation in O.D. values is observed between individual plants, from noninfected leaves to high virus titre. For upper leaves in the last sampling time (25 days), all sampled leaves were infected and differences in virus content were not significant.
BYDV Content of Barley and Wheat leaves and Roots

Comparative Optical Density for PAV-4 in Roots and Different Leaves of Wheat

As for barley, high virus content is observed in roots as early as 5 days after the end of IAP and maintained during the whole period. The low virus content was detected in the older leaf. The second leaf (L2) has intermediate virus titres, with O.D. values that do not vary significantly with time in most cases. The upper leaves have the highest virus content: in most cases O.D.s of L3, L4, L5 are not significantly different for a given harvesting period. Nevertheless, the range of O.D. values for individual leaves is always high, even 25 days after IAP, indicating that uninfected leaves are more frequent in wheat than in barley infected plants.

Spread of PAV-4 in Barley and Wheat

As shown in figure 2, virus moves from inoculated leaf (L1) to roots where it replicates intensively within the first days after inoculation. Virus content in roots remains high during all the 25 day period, indicating either a balance between virus translocation to upper leaves and replication, or virus survival in roots with no or few translocations. Low viral concentrations in older leaves could be due to poor replication, due to the short growing period of this leaf (2-3 days), or to translocation of virus to upper leaves. Upper leaves have the highest virus content, probably because of the cumulative effects of replication in situ and translocation from lower levels.

DISCUSSION

No significant differences were detected in the dynamic of PAV infection in barley and wheat. These results are new because

Table 1: Variations in ELISA values over a 25 day period among roots and individual leaves of barley cv Express infected with the PAV-4 isolate of barley yellow dwarf virus.

<table>
<thead>
<tr>
<th>Days after inoculation</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Access period</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>&quot;0.1312^i&quot;</td>
</tr>
<tr>
<td>10</td>
<td>0.1219^i</td>
</tr>
<tr>
<td>15</td>
<td>0.1249^i</td>
</tr>
<tr>
<td>20</td>
<td>0.1275^i</td>
</tr>
<tr>
<td>25</td>
<td>0.2254^h</td>
</tr>
</tbody>
</table>

^Mean ELISA values on a total of 33 plants for each incubation period. Values with common letter(s) are not significantly different, P=0.01, according to Duncan’s multiple range test. Mean ELISA values (S.E.) for noninfected Express barely was 0.01.
^Standard error (S.E.).
^Range of ELISA values.
^Leaf not available.
dynamics of PAV infection have only been compared between barley and oats (Pereira et al., 1989a). Therefore for early PAV infection, symptoms between barley and wheat are not linked with virus amount in either plant but with their respective susceptibility to the same viral concentration. Roots contain more virus than individual leaves, and are heavily infected 5 days after the end of inoculation period. This agrees with the results of Hammond et al. (1983), Skaria et al. (1985), and Chalhoub (1994a) who found that roots of barley or/and wheat have (i) a higher virus content per unit weight than shoots, and (ii) that virus content of root peaked sooner than that of shoots. This means that viruses migrate preferentially to roots through vascular bundles very soon after inoculation. These results are also consistent with those of Gill (1968) and Jensen (1973), who showed the evidence of systemic movement of BYDV particles 6 hours after infection and hypothesized that virus translocation could begin immediately after inoculation, because vectors feed in phloem tissues and may release virions directly into the sieve elements. Our results on virus titre at different foliar levels are consistent with those of Foxe & Rochow (1975) and Gray et al. (1991) who found a higher virus concentration in young leaves of PAV infected barley than in older ones. They agree with the hypothesis that BYDV replicates especially in phloem cells of growing tissues, so that weak virus titre found in the lower leaf could be due to the fact that leaves stop to grow very soon. Our results differ from those of Pereira et al. (1989a), which indicate that PAV distribution among individual leaves of the same barley plant is the same, and that the older leaf has virus content similar to younger ones. This discrepancy could be due to differences in barley cultivar tested or the PAV isolate used. Pereira et al. (1989a) infected test plants with a high number of virulifer-
ous aphids (8), probably resulting in a high viral concentration in the inoculated leaf.

In conclusion, the results show that, except for the oldest leaves, PAV inoculated barley and wheat plants, have comparable virus contents in the different leaves and roots, at least till 25 days after inoculation. Further studies on virus spread in older plants (till heading) are necessary before using these results in field sampling.

The dynamic of PAV spread in barley and wheat plants can be related to aphid vector feeding preferences during plant growth (Dedryver and Robert, 1977): S. avenae has a clear preference for young upper leaves, while R. padi is preferentially distributed on mature leaves of the lower levels of the plant. When comparing the ability of R. padi and S. avenae to transmit PAV after acquisition from low and upper leaves of barley, Foxe and Rochow (1975) and more recently Gray et al. (1991), found that both species transmitted better PAV after acquisition from upper leaves (that contain more virus), but that their ability to acquire is differentially affected by leaf age: differences were weak for R. padi and much higher for S. avenae. This could be due to differences in the feeding behavior of both species: R. padi that is well-adapted to feed on lower leaves, probably acquires virus at this foliar level better than S. avenae does, and both species acquire easily from the virus-rich phloem of upper leaves. Interactions between virus concentration in the plants and preferential feeding site of vectors could explain, at least in part, why R. padi is often more successful in transmitting PAV than other vectors in field conditions (Leclercq et al., 1995).

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REFERENCES


**BYDV Content of Barley and Wheat leaves and Roots**


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اندامهای مختلف گندم و جو در طول دوره کمون ویرس بطور شماتیک ارائه و با پرکش عمودی شده های ناقل بر روی گیاهان میزان مقایسه شده است.