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Research Note

A quick-test for screening resistance to transmission of grapevine fanleaf virus by *Xiphinema index*

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Key words: grapevine fanleaf virus, resistance to transmission of GFLV, resistance to Xiphinema index, Vitis species, breeding.

Introduction: Nematode resistance has been defined by Kunde *et al.* (1968) as any characteristic of *Vitis* plants or nematode-plant interaction which retards or prevents development and/or reproduction of the parasites. Spreading of virus from one plant to another can only be controlled by plants which are either resistant to the transmission of virus by nematodes or that are resistant to the virus itself.

Boubals and Pistre (1978) were the first to show resistance to transmission of grapevine fanleaf virus, GFLV, by *Xiphinema index* in several Vitaceae. Since they could not apply modern methods of virus identification they had to wait from inoculation until visible symptoms had developed, i. e. investigations took up to 5 years.

The aim of the present paper is to propose a quick test to investigate resistance of germplasm to the transmission of viruses by nematodes. The test has been already successfully used (Staudt and Kassemeyer 1990; Staudt and Weischer 1992; Staudt 1993).

Materials and methods: Plants: Virus-tested plants of the cultivar Siegfried (Riesling x chance seedling of Oberlin 595) were used as test plants and to maintain the nematodes. The plants were propagated *in vitro* to avoid any infestations by viruses. After transfer to the greenhouse, plants were cultivated in 200 ml pots in a mixture of loess, sand and styromoll (2:1:1,v:v:v) sterilized by autoclaving (20 min, 1.5 bar). To prevent nematode contamination, pots were kept on plastic supports. Plants were grown on heated greenhouse benches (20-25 °C). During winter additional light was supplied by Fluora lamps, minimum daylength: 16 h.

Nematodes and extraction: Virus-free *X. index*, obtained from INRA, Station de Recherche Vigne et Vin, Laboratoire de Pathologie, Colmar, were maintained on *Ficus carica*. GFLV-infested nematodes used for inoculation were obtained by feeding for at least 3 months on Siegfried vines which were systemically infested by the viruses.

A modified decanting and sieving technique (Flegg 1967; Bleyer and Kassemeyer 1992) was used to extract the nematodes. The soil of each pot was soaked and washed with 250 ml water. The root system was carefully washed with a hand shower. After 25 s of settling the supernatant was decanted over a bank of 4 sieves (1 mm up to 150 µm mesh). The remaining soil was stirred again and after 25 s of settling the supernatant decanted through the sieves. This procedure was repeated three times. Extraction was carried out without using a Baermann funnel to count active and inactive nematodes as well. The nematodes and debris which remained on the 150 µm sieves were rinsed with water and collected. Aliquots of 10 x 1 ml were gathered from the suspension under continuous stirring with a magnetic stirrer. Counting was carried out in a Hawksley counting chamber and the number of nematodes per pot was calculated. To avoid false positive ELISA readings by accidental contamination, samplings of washed roots were microscopically inspected for adhering nematodes. No nematodes were found on the roots after the above mentioned washing procedure.

Virus: GFLV originated from *V. vinifera* cv. Weißer Burgunder grown at Ortenberg, Baden, showing severe fanleaf symptoms. The virus was transmitted to cv. Siegfried by grafting and confirmed by ELISA.

Inoculation: Well-established plants with an average height of ca. 30 cm were selected for inoculation. They always showed a well-growing root system. The suspension of viruliferous nematodes in tap water was diluted to a titer of ca. 200 adult nematodes per 3 ml. 1 ml each was pipetted into 3 holes around the test plants, 5 cm deep. The high number of nematodes per pot was applied to increase the likelihood for a successful inoculation. Temperature was maintained at 20-25 °C, i. e. at the upper end of the favorable range for transmission (Das and Raski 1968).

Roots: For the extraction of nematodes and further investigations the root system was carefully washed. Thereafter the roots were inspected and rated according to the following scheme: (1) Root system well established throughout the pot, growing root tips abundant. (2) Root system not filling up the whole pot; growing root tips present. (3) Root system slender but still some growing root tips present. (4) Root system scanty and/or mostly rotten; no growing root tips present. Only plants which showed growing root tips still 3 months after inoculation, i.e. ratings 1-3, were considered for further investigations.

The reactions of the plants to nematode infection, i.e. curvation of root tips, formation of swellings or galls, growth retardation of root tips and formation of multiple laterals was rated as follows: (0) Normal root growth, no signs of swellings and/or galls; (1) a few galls per root system; (2) several galls throughout the root system; (3) parts of the root system heavily galled; (4) total root system heavily galled.

ELISA: Roots, especially from those parts showing galling, multiple laterals and other malformations were tested. If no signs of nematode feeding were found the sample was taken from different parts of the root system. A total of 0.5 g of root tissue was ground in the presence of 5 ml extraction buffer. ELISA was performed using Bioreba reagents (Bioreba Basel, Switzerland) according to Clark and Adams (1977) and customers instructions.The ELISA reaction was measured photometrically at 405 nm using a

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Microplatereader MR 600 (Dynatech). Each sample was repeated twofold and compared with the substrate, extracts from healthy controls (cv. Siegfried) and GFLV-infected plants as reference. Samples with twice the ELISA-reading of the healthy control were considered as infected by GFLV.

Results and Discussion: Successful testing of grapevine accessions from various geographical regions to attract nematodes and to be infested with viruses by transmission during feeding, depends on a number of factors which have carefully to be considered (TRUDGILL *et al.* 1983, BROWN *et al.* 1988).

A source of *X. index* has to be selected which aggressively attacks the roots of various accessions in a comparable manner. Since considerable differences in the vector efficiency between populations from different geographical origin can be expected (CATALANO *et al.* 1989), different populations of nematodes should be tested, especially with the resistant accessions.

Viruliferous *X. index* have to be produced which are able to readily transmit viruses to the accessions. If nematodes which were reared for at least 3 months on systemically infested plants were used for inoculation, transmission was observed in a fair number (Table).

Table

Effect of GFLV carrying *X. index* on cv. Siegfried, 3 months after inoculation with 200 adult viruliferous nematodes per pot

Date of inoculation	Number of plants	Transmission %	Number of nematodes per pot (mean)	Root sensitivity*)
17.03.1989	19	89	22,293±3,518	3.00±.15
19.10.1989	7	100	11,440±2,602	2.55±.24
07.12.1989	5	40	14,354±2,999	2.60±.25
13.03.1990	20	85	12,590±1,315	2.40±.11
28.02.1990	15	80	20,833±2,331	2.40±.11
09.04.1990	10	10	5,244± 552	1.65±.15
18.09.1990	5	80	8,595±2,197	2.08±.45

*) 0 = no signs of swellings and/or galls, 1 = a few galls per root system, 2 = some galls throughout the root system, 3 = parts of the root system heavily galled, 4 = total root system heavily galled.

Although nematode multiplication was measured under stardardized conditions the replications varied up to 4 times. Similar results were obtained by KUNDE *et al.* (1968). This variation cannot be explained by seasonal influences and needs additional investigations. Nevertheless, the virus-nematode-plant interaction was satisfactory under the conditions of the test. No essential differences have been observed regarding the percentage of infected plants after 3 and 10 months of access of viruliferous *X. index*.

Investigation of transmission of viruses by nematodes depends on normal root growth and on a sufficient parasite-plant interaction. Test plants should be grown in a soil mixture which is suitable to the requirements of the various accessions and of the nematodes. The soil mixture should be suitable to the extraction procedure.

The spread of viruses after inoculation by nematodes is relatively slow. It took at least 5 months until viruses had spread into the shoot and leaves of potted plants (STAUDT 1991). However, still after that time no symptoms were observed macroscopically. Therefore, transmission of viruses was investigated in the root system by ELISA 3 months after inoculation. Although transmission was observed already 2 weeks after inoculation, the viruses were allowed to multiply and spread throughout the root system and reach a concentration which can be detected easily by ELISA.

As can be seen from the Table, the average transmission was ca. 80 % with cv. Siegfried. However, in some tests transmission was considerably lower. This may be due to the relatively small number of plants in these tests or to insufficient root growth. Therefore, it is necessary to grow a reasonable number of control plants. The reliability of the rating of the resistance to virus transmission by nematodes depends on the number of plants tested. A minimum of 20 plants should be investigated.

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