

Inheritance of Resistance to Beet Western Yellows Virus in *Lactuca virosa* L.

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Abstract. Beet western yellows virus (BWYV), a member of the Luteovirus group, causes severe losses in many lettuce-growing areas. Attempts to identify complete resistance to BWYV in lettuce cultivars (*Lactuca sativa* L.) or in *L. serriola* L. and *L. saligna* L. were unsuccessful. Among three accessions of *L. virosa* tested, one, IVT 280, appeared to be extremely resistant to BWYV. Heredity of this resistance was studied in crosses with susceptible *L. virosa* IVT 1398. The results were compatible with the hypothesis of one gene, with resistance being dominant. The symbol Bw for Beet western is proposed for this gene.

Beet western yellows virus (BWYV) incites a yellowing disease of lettuce in most countries where lettuce is grown. In recent years, its incidence has become very severe in Europe, especially in the Mediterranean countries. This virus, a member of the Luteovirus group, is confined to the host phloem tissue. It is transmitted by at least 10 aphid species in a persistent manner (Duffus, 1973). In France, *Myzus persicae* (Sulz.) and *Macrosiphum euphorbiae* (Thos.) are the most efficient vectors under natural conditions (H. L., unpublished data). BWYV strains isolated from lettuce in France and Spain can

infect a large range of hosts, including oil-seed rape, cabbage, radish, pea, fodder and table beet, and numerous wild species (Lot et al., 1989). The interveinal yellowing symptom of the older leaves results in appreciable losses to field-grown butterhead lettuce; symptoms appear 12 to 25 days after inoculation, depending on plant age and light intensity.

The control of vectors with insecticides is ineffective, and the small size of lettuce fields in Europe is favorable for maintaining some host plants as reservoir for the virus. Initial attempts to identify BWYV resistance in lettuce cultivars were unsuccessful (Watts, 1975).

Recently, significant differences in symptom severity were observed in lettuce cultivars, especially among butterhead and "Batavian" types (Lot et al., 1989; Walkey and Pink, 1990). Some cultivars exhibited only late mild yellowing, but all tested cultivars multiplied the virus to the same level according to ELISA tests (Lot et al., 1989). Thus, our activity was directed toward a search in wild *Lactuca* spp. for sources of heritable resistance to use in the development of BWYV resistant cultivars.

In preliminary tests, we included four accessions of *L. serriola* L. (two from INRA, LS 162 and LS 169, collected in France, and two others from D. Globerson, Volcani Center, Israel, supplied by B. Moreau, Graines Caillard, France), three accessions of *L. saligna* L. (one collected in France by B. Moreau, one other, P33, from D. Globerson, and IVT 688 from Centre for Plant Breeding Research, formerly IVT, Wageningen, Netherlands, kindly supplied by A.H. Eenink) and three accessions of *L. virosa* (IVT 280, IVT 1145, and IVT 1398 provided by A.H. Eenink). These accessions were observed in a field trial. They were grown in two replicate blocks of 30 plants each. Experimental plants were surrounded by a row of lettuce plants artificially inoculated before

Table 1. Reaction to BWYV-FL₁ of *Lactuca virosa* parents and segregating generations of the cross between IVT 280 and IVT 1398.¹

Progenies	No. of plants			Expected ratio	χ ² Value	Prob.
	Resistant ²	Susceptible	Total			
IVT 280	25	1*	26			
IVT 1398	0	18	18			
(IVT 1398 × IVT 280)F ₁	5	0	5			
(IVT 280 × IVT 1398)F ₁	15	0	15			
(F ₁ × IVT 280)	7	0	7			
(F ₁ × IVT 1398)	12	8	20	1 : 1	0.80	0.37
(IVT 280 × IVT 1398)F ₂	65	28	93	3 : 1	1.29	0.26

¹Combined results from three separate experiments with susceptibility evaluated ≈3 weeks after inoculation.

²Resistance determined by ELISA.

*A₄₀₅ slightly above threshold.

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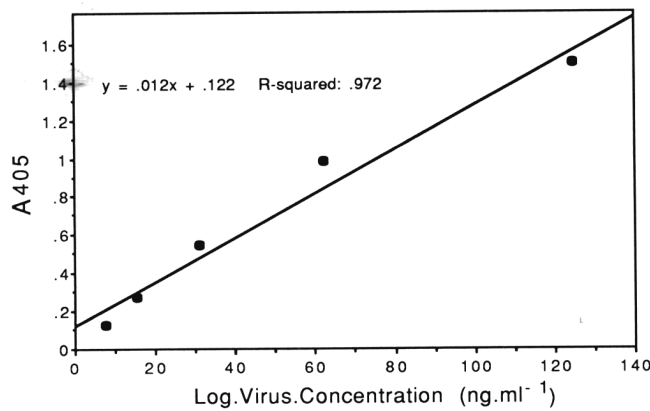


Fig. 1. Reaction in ELISA to various concentrations of BWYV-FL. Purified virus was diluted in virus-free lettuce extract (1:4). Healthy lettuce sap diluted 1:4 gave A_{405} of 0.005. Absorbance values were measured 2 h after adding substrate.

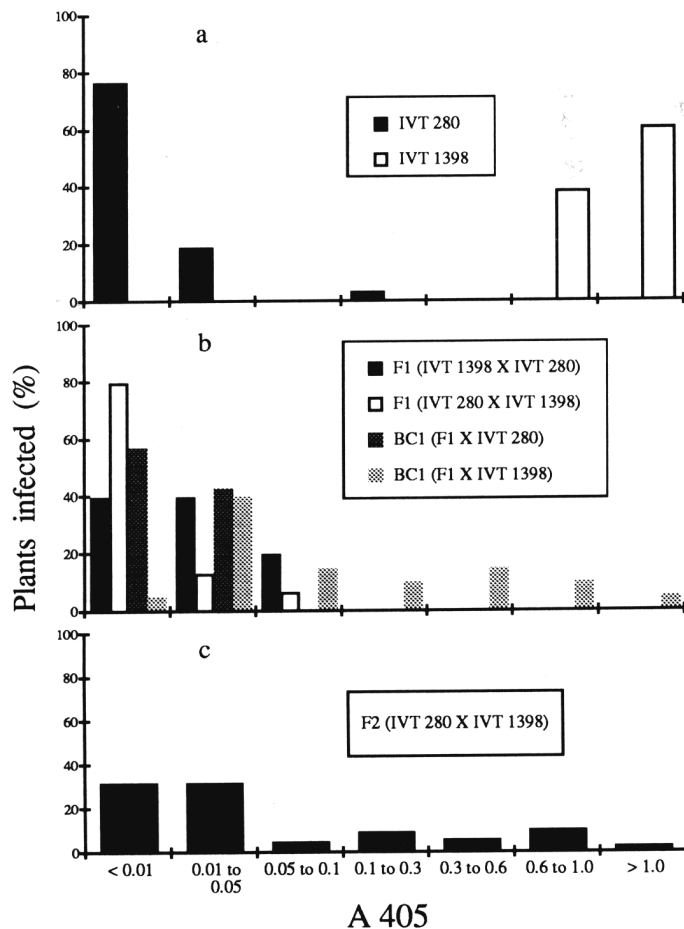


Fig. 2. Frequency of individual plants infected with BWYV-FL, according to their absorbance values (A_{405}). (a) *L. virosa* parents, IVT 280 and IVT 1398. (b) F_1 and BC_1 between IVT 280 and IVT 1398. (c) F_2 (IVT 280 x IVT 1398).

transplanting at the three- to four-leaf stage with BWYV-FL1, using viruliferous aphids as described below. Seedlings were transplanted 10 May and plants observed for symptoms twice a week until the end of June (mean day temperature of 26C; high light intensity). Symptoms were assessed by counting the number of leaves with interveinal yellowing. Under these conditions, all

accessions of *L. serriola* and *L. saligna*, and all but one *L. virosa* exhibited severe symptoms on three to six leaves. *L. virosa* IVT 280 did not show any symptoms.

These accessions were also tested in a greenhouse. The lettuce strain BWYV-FL1, isolated in 1982 Provence (southern France), was maintained, after inoculation by *M. persicae*, on *Physalis floridana* L. grown in a

growth room (22C day/18C night, 16-h day-length with 50 $W \cdot m^{-2}$). In these conditions, apterous aphids multiplied well. Inoculations were made using small pieces of infected leaves carrying viruliferous aphids (10 to 30 larvae and adults), that were placed for 48 h on each *Lactuca* plant. A first inoculation was made at the two- to three-leaf stage and one to two others were made subsequently, 2 and 4 days after the first. Then, aphids were allowed to feed for at least 48 h before being sprayed with pyrimicarb (0.75 $g \cdot liter^{-1}$). Inoculated and control plants were incubated in an insect-free greenhouse (18 to 25C day/14 to 18C night). Plants were grown in peat in 1500 cm^3 pots. About 3 weeks after inoculation, symptoms were visually assessed and ELISA tests were carried out. Coating globulin was applied at 0.5 $\mu g \cdot ml^{-1}$ and conjugate at 1/2000. Absorbance values (A_{405}) were measured with a Titertek Multiskan Plus reader (Flow Laboratories, Finland). Two-gram samples of leaves (old, middle aged, young) were homogenized in phosphate buffer 0.1 M, pH 7.2, containing 0.2% DIECA and 2% skimmed milk (1 g leaf to 4 ml solution). In these conditions, by use of the DAS-ELISA standard procedure and antibodies prepared against BWYV-FL1, it was possible to detect 2 to 3 ng virus/ml lettuce extract. A good linear relationship was observed between the log of the virus concentration diluted in virus-free lettuce extract and A_{405} (Fig. 1).

The results were essentially similar in numerous greenhouse tests and confirmed the data obtained under field conditions. All *L. serriola* and *L. saligna* accessions were susceptible. Among three *L. virosa*, IVT 280 did not show any symptoms on either inoculated or noninoculated leaves and the virus did not multiply in any plants as judged by ELISA in several trials conducted at different seasons. In one trial performed during a summer with exceptionally high temperature, one out of 10 plants had an absorbance value above the threshold corresponding to a concentration of 3 to 4 $ng \cdot ml^{-1}$; but the virus was detected only in the inoculated leaves. IVT 1398 was susceptible to BWYV, showing severe yellowing symptoms on three to five leaves and high virus concentration, i.e., 30 to 100 $ng \cdot ml^{-1}$ (Fig. 2a). The response of IVT 1145 was not consistent; some plants appeared completely resistant to BWYV, but others multiplied the virus without symptom expression. Therefore, IVT 280 was selected for use as a resistant parent.

The inheritance of resistance to BWYV-FL1 was studied in intraspecific crosses between IVT 280 and susceptible *L. virosa* IVT 1398. Crosses were made by manual pollination after flowers were emasculated with forceps and then washed. The reciprocal F_1 s and the BC_1 to the resistant parent were resistant (Fig. 2b). There was segregation for resistance in the BC_1 to the susceptible parent and in the F_2 . The majority of plants in the F_2 were resistant (Fig. 2 b and c). If we consider as susceptible the plants that give an A_{405} value > 0.1 (no plant gave a value between 0.075, the actual threshold, and 0.1),

the segregation observed was compatible with the hypothesis that there is one gene, with resistance dominant (Table 1). We propose that this gene be named *Beet western* (symbol: *Bw*) in accordance with the rules given by Robinson et al. (1983). However, a small proportion of BC₁ and F₂ plants were intermediate between both parents. An explanation for this observation could be an interaction between some minor genes unidentified in IVT 280 and the genetic background of the susceptible parent.

L. virosa IVT 280 was reported as completely resistant to *Nasonovia ribis nigri* Mosley (Eenink et al., 1982) and partially resistant to *M. persicae* (Eenink, 1984). In our experiments, *M. persicae* was used as vector. Thus, the resistance could be either to the vector or to the virus itself. Two reasons favor resistance to virus multiplication. First, the population of *M. persicae* transferred to IVT 280 appeared active and feeding, since many aphids stayed alive on the plants for 2 days. Second, some IVT 280 plants inoculated with other lettuce BWYV isolates multiplied the virus slightly, as shown with ELISA. In that case, after recovery by healthy aphids and multiplication on *P. floridana*, it was demonstrated that the virus was confined to lower leaves and never became systemic; none of these inoculated IVT 280 plants showed yellowing symptoms (V.C. and H.L., unpublished data). Thus, we conclude that IVT 280 has resistance to the virus in addition to tolerance to *M. persicae*.

Interspecific crosses between *L. virosa* and *L. sativa* are difficult to obtain. The hybrids are either not viable or the adult plants are completely sterile (Lindqvist, 1960). In our laboratory, a method for producing these interspecific crosses by in vitro embryo culture has been developed (Maisonneuve, 1989). Thus, resistance to BWYV from IVT 280 is being transferred into *L. sativa*. In the early generations, i.e., BC₁ to *L. sativa* selfed one to three times, the results were not consistent with the hypothesis of a single dominant gene. This deviation could be attributed to abnormal meiosis resulting from the low fertility of these plants. The inheritance of genetic factors transmitted to *L. sativa* needs to be studied in fixed lines with high fertility.

The resistance to BWYV from *L. virosa* IVT 280 should be transferred to various types of field-grown lettuce and be combined with BWYV tolerance as found in some commercial cultivars and with tolerance to lettuce mosaic virus, another major lettuce virus in the field.

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