Vitis 23, 21—26 (1984)

Research Institute for Viticulture and Enology, University of Horticulture, Kecskemét, Hungary

Department of Plant Genetics and Breeding, University of Horticulture, Budapest, Hungary

Crown gall resistance in East-Asian *Vitis* species and in their *V. vinifera* hybrids

by

E. SZEGEDI, J. KORBULY and I. KOLEDA

Resistenz gegen Mauke bei ostasiatischen *Vitis*-Arten und ihren Kreuzungen mit *V. vinifera*

Zusammenfassung. — Ca. 50 interspezifische Kreuzungen von *Vitis vinifera* überwiegend mit *V. amurensis* wurden auf ihre Resistenz gegen Mauke untersucht. Die Resistenz gegen *Agrobacterium tumefaciens,* Stamm AT-1, manifestierte sich über 4 Generationen. Die Kreuzungen A6/1 und A5/43 waren gegen weitere 11 Stämme von *A. tumefaciens* resistent. Die resistente Kreuzung A6/1 ist unter dem Sortennamen Kunbarát bereits staatlich anerkannt. Aufgrund der vorliegenden Ergebnisse dürfte die Resistenzzüchtung eine wirksame Methode zum Schutz der Weinrebe gegen die Mauke sein.

Introduction

Agrobacterium tumefaciens induces tumors (called crown galls) on a wide range of dicotyledons including about 40 economically important plant species (DE CLEENE and DE LEY 1976, DE CLEENE 1979). The most serious damages are caused in fruit-tree, raspberry and grapevine plantations (EL-FIKI and GILES 1981, MOORE and COOKSEY 1981). Strains of *A. tumefaciens* can be divided into 3 biotypes by their chromosomal markers. Among these, the strains of biotype 3 are especially found on grapevines all over the world (KERR and PANAGOPOULOS 1977, LOUBSER 1978, SÜLE 1978, PERRY and KADO 1982, BURR and KATZ 1983).

There is no effective method of preventing crown gall disease so far. A possible way is breeding for new, resistant grapevine varieties. In the middle of the century HOERNER and decade later TAMM reported the resistance of *V. labrusca* and *V. amurensis* to one strain of *A. tumefaciens* (cited by DE CLEENE and DE LEY 1976). Some rootstock varieties have also been found resistant to virulent isolates (URBIZAGÁSTEGUI and FERNANDEZ-NORTHCOTE 1976).

A breeding programme for getting frost and downy mildew resistant varieties was started at the University of Horticulture 25 years ago (KOLEDA 1975). For these experiments *V. amurensis* has been used as source of resistance, from which a great number of different hybrid combinations could be obtained after backcrossing with *V. vinifera.* Considering the results mentioned above, it seemed promising to test these new hybrids for *Agrobacterium* susceptibility. In earlier experiments (SZEGEDI 1982), 3 resistant hybrids were found among them, which after artificial infection failed to develop tumors.

In the present study, these experiments have been extended to a wider range of interspecific hybrids obtained mainly from *V. vinifera* × *V. amurensis* and its backcrossings with *V. vinifera.* To get some information about the possibilities of resistance breeding, 4 generations were screened for susceptibility. The strain specificity of resistance has also been studied in this investigation.

Materials and methods

Bacterial strains

The *A. tumefaciens* strains used in the experiments are listed in Table 1. Each of them caused tumors on *V. vinifera* cv. Chardonnay and on *V. solonis*. In the first selection step strain AT-1, belonging to biotype 3, was used, which is pathogenic on a wide range of grapevine cultivars (SZEGEDI 1981). In the second step 8 hybrids (cultivars) — 5 resistant and 3 sensitive to strain AT-1 — were tested with every strain indicated in Table 1. This experiment aimed at answering whether susceptibility of these cultivars is strain-specific or not. The bacterial strains were chosen by their biotype and/or host range differences.

Grapevine hybrids

The tested plant materials (and their origin) are shown in Table 2. Parents were also tested in most cases. For screening, those hybrids were chosen which were considered valuable for further breeding. The hybrid C-43 was supplied by GY. KRISZTEN and RF-48 by J. FÜRI. C-43 was obtained from A2/11 × Seyve-Villard 12375, RF-48 from Pearl of Csaba × Seibel 5279 crossings (personal communication of the suppliers). Two-bud cuttings of the grapevines were forced in perlite and, after wounding, the young green shoots were infected with 48-h-old cultures of *A. tumefaciens* strains. The infected plants were incubated at 23—28 °C in greenhouse. Results were scored after 6 weeks. Each experiment was repeated 4—8 times.

Results and discussion

Susceptibility of the hybrids to *A. tumefaciens* AT-1

Similarly to earlier results (SZEGEDI 1981), each of the 6 European varieties tested proved to be sensitive (Fig. 1), while *V. flexuosa* and *V. piasezkii* did not form tumors.

Table 1

Bacterial strains used for resistance selection

Bei der Resistenzselektion verwendete Bakterienstämme

Strains	Biotype	Obtained from
Ach-5, C-58	1	L. MÁRTON
B-6, 4		S. SÜLE
AT-4		J. LEHOCZKY
1, 63	2	S. SÜLE
AT-1, AT-66	3[1])	J. LEHOCZKY
S-1, S-2		Authors' grapevine
Sz-1		isolates

[1]) Strains AT-1 and AT-66 did not cause any tumors on *Pisum sativum* and *Datura stramonium*. S-1 and S-2 were pathogenic on *D. stramonium*, Sz-1 on both plant species (SZEGEDI, unpublished).

Table 2

The expression of resistance to *A. tumefaciens* AT-1

Ausprägung der Resistenz gegen *A. tumefaciens* AT-1

Genotypes	Resistant	Sensitive
Vitis species	*V. amurensis* P-1, Pu, 34 and 124 *V. flexuosa*[1]) *V. piasezkii*[1])	*V. amurensis* 122 *V. vinifera* cvs used for crossing (see left column)
F_1 hybrids		
Portugieser[2]) × *V. amurensis*	68-2-10, 68-2-29, 68-2-30	68-2-3
Médoc Noir[3]) × *V. amurensis*	69-2-4	69-2-2, 69-2-3
Gloria Hungariae[2]) × *V. pentagona*[3])	67-39-7, 67-39-13	67-39-3, 67-39-5
BC_1 hybrids		
28/19[4]) × Muscat Thallóczy L.[2])	A3/21	A4/13
28/19[4]) × Afuz Ali[2])	A4/42	A3/42, A5/18
28/19[4]) × Italia[2])	A4/24, A5/28, A5/33, A5/43, A6/1, A6/4	A3/39, A4/18, A5/7, A5/40, A6/3
BC_2 hybrid		
Kocsis Irma[2]) × A6/1	67-28-6, 67-28-14	67-28-10
A6/1 self-pollinated	18[5])	7[5])

[1]) Plants from seeds presented by the Peking Botanical Garden.
[2]) Sensitive European variety.
[3]) Not tested.
[4]) Resistant hybrid of *V. amurensis* × *V. vinifera* F_2.
[5]) Number of progenies.

Among the 5 *V. amurensis* clones only No. 122 was sensitive. 4 of the 7 *V. vinifera* × *V. amurensis* hybrids tested also showed resistance to *A. tumefaciens* strain AT-1. 2 of the 4 hybrids from *V. vinifera* × *V. pentagona* failed to form tumors, too. The resistance was expressed in the progenies of 28/19 (*V. amurensis* × *V. vinifera* F_2) obtained from crosses with susceptible European varieties as well (Fig. 2.).

Crown gall resistance also manifested itself, when A6/1 was back-crossed to a sensitive European variety, and 18 of the 25 self-pollinated progenies of A6/1 proved also resistant (for detailed results see Table 2).

Strain specificity of susceptibility

In the second step of resistance screening 8 hybrids (varieties) were tested with 12 strains of *A. tumefaciens* representing the 3 biotypes. None of them caused tumors on

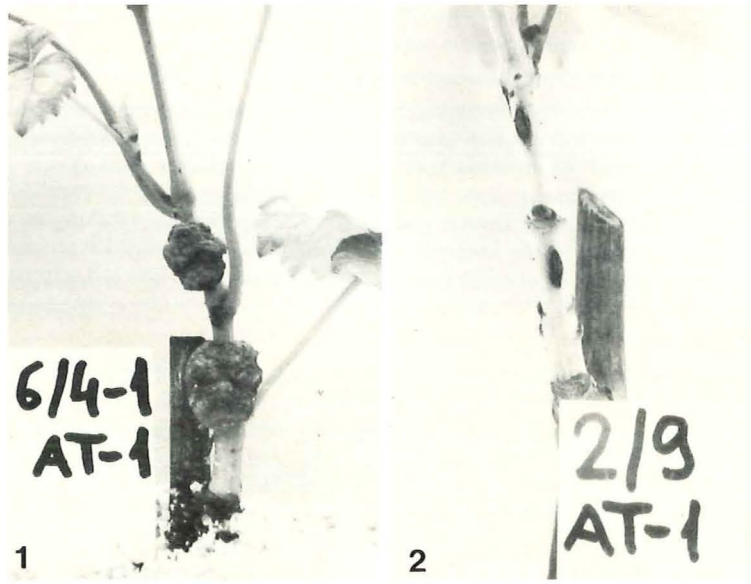

Fig. 1.: Sensitive variety (cv. Chasselas) infected with *A. tumefaciens* AT-1.
Fig. 2: Resistant hybrid (A4/42) from the crossing 28/19 × Afuz Ali.

Abb. 1: Anfällige Sorte (Gutedel) mit *A. tumefaciens* AT-1 infiziert.
Abb. 2: Resistente Hybride (A4/42) aus der Kreuzung 28/19 × Afuz Ali.

A5/43 and A6/1. The C-43, A2/11 and RF-48 hybrids gave positive response after infection with Ach-5 and B-6, but proved resistant to the other 10 strains of *A. tumefaciens.* On the other hand, Ach-5 and B-6 strains were found non-pathogenic on some European varieties which were susceptible to biotype 3 strains (Table 3).

The possibilities of resistance breeding

Resistance to a biotype 3 strain of *A. tumefaciens* was demonstrated in a great number of *V. vinifera* hybrids having *V. amurensis* in their parentage. None of the tested European varieties proved to be resistant. In addition, nearly 40 *V. vinifera* intraspecific varieties have been screened for resistance so far, and each of them showed very intensive tumor forming after being infected with *A. tumefaciens* AT-1 (SZEGEDI, unpublished results). On the basis of authors' results, *V. amurensis* obviously was the resistance source of the hybrids examined in this study, because 4 of the 5 *V. amurensis* clones tested proved to be resistant. The resistance was expressed via 4 generations:

V. amurensis		28/19	A6/1	67-28-6
×	——	——	——	——
V. vinifera		(F_2)	(BC_1)	(BC_2)

This is very important for grapevine breeding, because the resistant character of *V. amurensis* may be well selected after 4 back crossings with/to *V. vinifera.*

Table 3

Strain specificity of *Agrobacterium* resistance in different grapevine hybrids

Stammspezifität der *Agrobacterium*-Resistenz bei verschiedenen Rebkreuzungen

A. tumefaciens strains	Grapevine hybrids							
	A6/1[1])	A5/43	A2/11[1])	C-43	RF-48	A5/18[1])	A[2])	B[2])
Ach-5	–	–	+	+	+	–	–	–
B-6	–	–	+	+	+	–	–	–
C-58	–	–	–	–	–	+	+	+
AT-4	–	–	–	–	–	+	+	+
4	–	–	–	–	–	+	+	+
1	–	–	–	–	–	+	+	+
63	–	–	–	–	–	+	+	+
AT-1	–	–	–	–	–	+	+	+
AT-66	–	–	–	–	–	+	+	+
S-1	–	–	–	–	–	n. t.	+	+
S-2	–	–	–	–	–	n. t.	+	+
Sz-1	–	–	–	–	–	n. t.	+	+

[1]) A6/1 = Kunbarát, A2/11 = Alföld-100, A5/18 = Kunleány.
[2]) *V. vinifera* cvs Pannónia Kincse (A) and Narancsizü (B).
n. t. = Not tested.

Further experiments are needed — including a number of hybrids from different crossing combinations — to collect enough data for evaluation and genetical conclusions. The expression of resistance via 4 generations and an 18 : 7 ratio of segregation in the self-pollinated progenies of the hybrid A6/1 suggest that dominant factors are responsible for the resistance. Similar results were obtained when a great number of different hybrid combinations were tested with the *A. tumefaciens* strain AT-1 (SZEGEDI and KOZMA, submitted).

The strains Ach-5 and B-6 showed a host range pattern different from that of the other 10 *A. tumefaciens* strains. They caused tumors on 3 of the 5 hybrids, which had proved resistant to other strains of biotypes 1, 2 and 3. On the other hand, the Ach-5 and B-6 strains were apathogenic to 2 European varieties, which were sensitive in every other case (Table 3). Similar differences were found in host range when Greek grapevine varieties were tested (KNAUF *et al.* 1982). The biochemical background of the different host-pathogen interactions is yet unknown. The wide resistance of A6/1 and A5-43 hybrids reduces the possibility of natural infection with other strains of agrobacteria having different host range.

Resistance breeding could help to prevent crown gall disease of grapevine. According to authors' results this method may be carried out only with interspecific hybrids, using East-Asian *Vitis* species as resistance source. Breakdown of resistance has not been observed in the studied hybrids so far, but further field observations are needed to verify the results presented here.

Summary

Crown gall resistance of ca. 50 interspecific hybrids obtained from *Vitis vinifera* mainly with *V. amurensis* was tested. Resistance to *Agrobacterium tumefaciens* strain AT-1 was expressed via 4 generations. Further 11 strains representing the 3 biotypes of the pathogen did not cause tumors on the hybrids A6/1 and A5/43. A6/1 has already been certificated as variety Kunbarát. According to the results achieved, resistance breeding could provide an effective means to prevent crown gall disease of grapevine.

Literature cited

BURR, T. J. and KATZ, B. H., 1983: Isolation of *Agrobacterium tumefaciens* biovar 3 from grapevine galls and sap and from vineyard soil. Phytopathology **73**, 163—165.

CLEENE, M. DE, 1979: Crown gall: economic importance and control. Zbl. Bakt. II. Abt. **134**, 551—554.

— — and LEY, J. DE, 1976: The host range of crown gall. Bot. Rev. **42**, 389—466.

EL-FIKI, F. and GILES, K. L., 1981: *Agrobacterium tumefaciens* in agriculture and research. Intern. Rev. Cytol. Suppl. **13**, 47—58.

KERR, A. and PANAGOPOULOS, C. G., 1977: Biotypes of *Agrobacterium radiobacter* var. *tumefaciens* and their biological control. Phytopathol. Z. **90**, 172—179.

KNAUF, V. C., PANAGOPOULOS, C. G. and NESTER, E. W., 1982: Genetic factors controlling the host range of *Agrobacterium tumefaciens*. Phytopathology **72**, 1545—1549.

KOLEDA, I., 1975: Ergebnisse von Kreuzungen zwischen *Vitis amurensis* and *Vitis vinifera* in der Züchtung frostwiderstandsfähiger Reben. Vitis **14**, 1—5.

LOUBSER, J. T., 1978: Identification of *Agrobacterium tumefaciens* biotype 3 on grapevine in South-Africa. Plant Disease Rept. **62**, 730—731.

MOORE, L. W. and COOKSEY, D. A., 1981: Biology of *Agrobacterium tumefaciens:* plant interactions. Intern. Rev. Cytol. Suppl. **13**, 15—46.

PERRY, K. L. and KADO, C. I., 1982: Characteristics of Ti-plasmids from broad-host-range and ecologically specific biotype 2 and 3 strains of *Agrobacterium tumefaciens*. J. Bacteriol. **151**, 343—350.

SZEGEDI, E., 1981: Susceptibility of grapevine varieties against *Agrobacterium tumefaciens* (SMITH and TOWNSEND) (Hung.). Conn. Növényvédelem **17**, 442—450.

— —, 1982: Crown gall disease of grapevine (Hung.). Szölötermesztés és Borászat **4** (2), 7—9.

— — and KOZMA, P. JR: Studies on the inheritance of resistance to crown gall disease of grapevine. Vitis (submitted).

SÜLE, S., 1978: Biotypes of *Agrobacterium tumefaciens* in Hungary. J. Appl. Bacteriol. **44**, 207—213.

URBIZAGASTEGUI, A. E. and FERNANDEZ-NORTHCOTE, E. N., 1975: Isolation and identification of causal agent of crown gall of deciduous fruit trees in Peru. Fitopatológia (Peru) **10**, 32—40.

Eingegangen am 19. 8. 1983

E. SZEGEDI
Research Institute for Viticulture and Enology
University of Horticulture
P. O. Box 25
6000 Kecskemét
Hungary

J. KORBULY
Prof. Dr. I. KOLEDA
Department of Plant Genetics and Breeding
University of Horticulture
Ménesi ut 44
1118 Budapest
Hungary