Vitis **43** (2), 75–79 (2004)

Evaluation of crown gall resistance in *Vitis vinifera* and hybrids of *Vitis* spp.

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Summary

Relative levels of crown gall susceptibility were determined in 17 genotypes of *Vitis* spp. by inoculating a diverse set of *Agrobacterium vitis* strains, measuring gall size and weight, and percentage of inoculated sites with galls. Hybrids of *Vitis vinifera* cv. Jighjigha x *Vitis riparia* "Gloire" (NAZ_4) and *V.vinifera* cv. Alibaba x 110 R (NAZ_5) were the most resistant genotypes but not completely immune. No genotype of *V. vinifera* was immune to crown gall. The interactions among strain and genotype were significant. *V. vinifera* cv. White Bidaneh was especially sensitive to the limited host range strain AG57. Weight and size of galls that were induced by 4 strains of *Agrobacterium vitis* were not significantly different for all genotypes of *Vitis* spp. But susceptibility of the genotypes to individual strains of *A. vitis* were significantly different.

Key words: genotype, Vitis spp., crown gall, resistance, evaluation, intraspecific hybrid.

Introduction

Crown gall caused by *Agrobacterium vitis* (Bishop *et al*. 1989; Ophel and Kerr 1990) is a serious bacterial disease of grapes worldwide, particularly for the cultivars of *Vitis vinifera*. It survives systemically in vines, can be disseminated in propagation material and infected vineyard soils (Burr and Katz 1983; Burr *et al.* 1987; Bishop *et al*. 1988). *A. vitis*-free material can be produced through shoot-tip culture (Bazzi *et al*. 1991) and hot water treatments (Bazzi *et al.* 1991; Mahmoodzadeh *et al*. 2003) but the pathogen survives in the grape rhizosphere and when *A. vitis*-free vines are planted on infested soil they become infected (Bishop *et al*. 1988; Burr *et al*. 1987). American *Vitis* species are resistant to phylloxera (Cirami and Whiting 1991) and have been used as rootstocks for *V. vinifera* cultivars for more than one century. Studies have shown that some phylloxera-resistant rootstocks are also resistant to crown gall (Goodman *et al.* 1993; Sule *et al*. 1994). For example, Riparia Gloire, 3309 C and 101-14 Mgt are resistant (Stover *et al*. 1997). Since crown gall susceptible vines apparently permit the entry of *A. vitis* through roots and systemically infest the whole plant, the use of resistant rootstocks may reduce infection from soil inoculum and prevent subsequent infection of *A. vitis*-free, but highly crown gall susceptible scion cultivars (Sule *et al.* 1994; Stover *et al*. 1997). In this study, we evaluated crown gall susceptibility in a broader range of interspecific hybrids of *V. vinifera*, and American *Vitis* species (Mahmoodzadeh 2001) and many cultivars in Iran. Our objective was to identify high levels of resistance to crown gall in *Vitis* that may be used for development of resistant rootstocks.

Material and Methods

Plant material: Eleven commercial cultivars of *V. vinifera* and 6 interspecific hybrids of grapevines obtained from Khallatpoushan Viticulture Research Station of the University of Tabriz, Iran have been used (Tab. 1). Grape cuttings were collected in February, rooted under mist and were maintained in a mix of perlite: sand, 1:1 v/v. Actively growing young plants with long shoots (25-30 cm) were inoculated with bacterial suspensions. The hybrids and cultivars of grapevine used are listed in Tab. 1.

Bacterial isolates: Strains used are listed in Tab. 2. Pathogenic strains of *A. vitis* were isolated from sap, galls and cuttings of infected grapevine showing symptoms of crown gall disease (Mahmoodzadeh 2002) based on the methods of used by Panagopoulos and Psallidas (1973), Sule (1978), Burr and Katz (1983), Bishop *et al.* (1989), Ophel *et al.* (1990), Matsumoto *et al.* (1992) and Schulz *et al.* (1993). Bacteria were grown for 48 h at 28 ºC on yeast extract-beef (YEB) medium including 1 g of yeast extract, 5 g of beef extract, 5 g of peptone, 5 g of sucrose, and 0.5 g $MgSO_4$ per l of distilled water (Szegedi *et al*. 1988; Ophel *et al*. 1990).

Inoculation technique: Two days prior to inoculation, strains were streaked onto potato dextrose agar (PDA) (Difco) and grown at 28 ºC. Bacterial growth from PDA was suspended in sterilized deionized water (SDW) and adjusted to an optical density (600 nm) of 1 (Roy and Sasser 1983). When the new shoots were 25-30 cm long and a brown periderm developed at their basal part, fourty vines were inoculated (10 vines per replication) by making wounds (2 x 2 x 2 mm) at the 4th to 10th internodes on each plant with a lance and depositing 10 µl of bacterial suspension (containing about 10^{10} cfu·ml^{-1}) into each wound as described by Stover *et al.* (1997).

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Table 1

Grape genotypes

Hybrids	Cultivars of *Vitis vinifera* L.
NAZ_1) *V. vinifera* cv. Jighjigha x *V. rupestris* cv. du Lot	*V. vinifera* cv. Black Sardasht
NAZ_2) *V. vinifera* cv. Alibaba x *V. rupestris* cv. du Lot	*V. vinifera* cv. Ghezel
NAZ_3) *V. vinifera* cv. Gharaozum x *Vitis rupestris* cv. du Lot	*V. vinifera* cv. White Bidaneh
NAZ_4) *V. vinifera* cv. Jighjigha x Riparia Gloire	*V. vinifera* cv. Asgari
NAZ_5) *V. vinifera* cv. Alibaba x 110 R	*V. vinifera* cv. Red Sahebi
NAZ_6) *V. vinifera* cv. Gharaozum x Kober 5 BB	*V. vinifera* cv. Alhaghi
	V. vinifera cv. Fakhri
	V. vinifera cv. Shast Aros
	V. vinifera cv. White Rishbaba
	V. vinifera cv. Khalili
	V. vinifera cv. Red Bidaneh

Table 2

Bacterial strains

A. vitis strain	Characteristics	Source	Isolators	Methods
CG230	Vitopine Ti[a]	Sap, grape, Iran	MAHMOODZADEH 2002	T. J. BURR, USA
AG57	Octopine Ti, LHR[b]	Gall, White Bidaneh, Iran	MAHMOODZADEH 2002	C. PANAGOPOULOS, Greece
NW180	Octopine Ti[c]	Gall, Red Sahebi, Iran	MAHMOODZADEH 2002	E. BIEN, Germany
K1059	Octopine Ti[d]	Cutting White Bidaneh, Iran	MAHMOODZADEH 2002	A. KERR, Australia

[a] OPHEL *et al*. (1990); [b] LHR= limited host range; PANAGOPOULOS and PSALLIDAS (1973)
[c] SCHULZ *et al.* (1993); [d] MATSUMOTO *et al*. (1992)

Inoculated sites were wrapped in parafilm and plants were kept at 18 ºC for 48 h after inoculation to facilitate T-DNA transfer, then they were maintained in the greenhouse. After 4 months, inoculated sites were scored for gall formation (STOVER 1993; SULE *et al*. 1994).

Scoring gall formation: Control inoculations with sterilized deionized water did not produce any gall, but in a few cultivars hemispherical callus was produced. Each plant was scored for the number of inoculated sites producing galls, the gall size and weight. Data were analyzed for all genotypes. The mean size, weight and number of galls were determined for sites, so that a size of zero was only included in the analysis when no site in that strain x genotype treatment yielded a gall. Since gall size often varied greatly within a single plant the mean largest gall per plant was evaluated (GOODMAN *et al*. 1993).

Statistical analysis: Data were analyzed by SAS statistical software (SAS Institute, North Carolina, USA) and means compared using Duncan's multiple range test (DMRT) method. Statistical significance indicates means difference at the 5 % level (P = 0.05).

Results and Discussion

Tumor formation and the percentage of inoculated sites forming galls ranged from 0 to 100 % (Control = 0 % and very sensitive cultivars of *Vitis vinifera* such as White Bidaneh = 100 %). Tab. 3 shows the results of shoot inoculation with different strains of *A. vitis*. In all combinations, cultivars of *Vitis vinifera* were sensitive or even the most sensitive. Visible tumors developed on the inoculated shoots of sensitive cultivars within 6-8 weeks. All bacterial strains induced large tumors on White Bidaneh, average size and weight of tumors induced by different strains were not statistically different (P = 0.05) for all hybrids and cultivars in this study (Figure).

Gall formation increased on all cultivars of *Vitis vinifera* when inoculated with strain AG57 (limited host range strain) as compared to other *A. vitis* strains (Tab. 3).

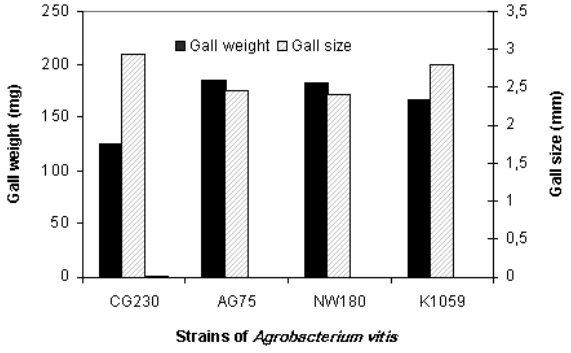

Figure: Effects of *Agrobacterium vitis* strains on gall size and weight, after 4 months.

Table 3

Response of *Vitis* genotypes to inoculation with four strains of *A.vitis*[1]

Strains	CG230			AG57			NW180			K1059		
Vitis genotype	PISWG[2] (%)	GS[3] (mm)	GW[4] (mg)	PISWG (%)	GS (mm)	GW (mg)	PISWG (%)	GS (mm)	GW (mg)	PISWG (%)	GS (mm)	GW (mg)
V. vinifera cv. Jighjigha x *V. rupestris* cv. du Lot (NAZ_1)	5.5 a[5]	0.8 a	148 ab	0 a	0 a	0 a	5.0 a	2.4 ab	156 ab	2.5 a	1.2 a	156 ab
V. vinifera cv. Alibaba x *V. rupestris* cv. du Lot (NAZ_2)	9.5 a	0.7 a	84 a	2.5 a	1.7 bc	184 bc	50.5 bc	1.2 a	143 ab	2.5 a	2.4 a	245 bc
V. vinifera cv. Gharaozum x *Vitis rupestris* cv. du Lot (NAZ_3)	8.5 a	1.5 b	263 b	5.0 a	2.5 c	163 ab	11.5 a	3.6 bc	256 bc	4.5 a	1.8 a	133 a
V. vinifera cv. Jighjigha x Riparia Gloire (NAZ_4)	0.0 a	0.0 a	0 a	2.0 a	4.3 de	184 ab	0 a	0 a	0 a	2.0 a	4.3 bc	284 bc
V. vinifera cv. Alibaba x 110 R (NAZ_5)	1.5 a	2.2 c	273 b	0 a	0 a	0 a	4.5 a	1.7 ab	230 bc	0 a	0 a	0 a
V. vinifera cv. Gharaozum x Kober 5 BB (NAZ_6)	2.5 a	1.8 bc	98 a	32.5 bc	4.8 e	199 ab	22.5 ab	3.8 bc	197 ab	5.5 a	2.3 a	109 a
V. vinifera cv. Black Sardasht	18.0 ab	4.0 ef	338 cd	75.0 e	4.2 de	338 c	32.0 b	2.2 ab	289 bc	25.0 ab	3.8 bc	247 bc
V. vinifera cv. Ghezel	22.5 abc	3.5 def	156 ab	66.0 de	3.2 cd	211 bc	21.5 a	1.8 ab	198 ab	31.0 bc	2.7 b	128 a
V. vinifera cv. White Bidaneh	100.0 d	2.6 cd	79 a	75.5 e	4.2 de	79 a	88.5 de	3.6 bc	214 bc	88.5 ef	5.3 cd	179 ab
V. vinifera cv. Asgari	85.0 de	3.1 de	214 b	65.0 de	3.2 c	221 ab	23.5 ab	1.3 a	88 a	15.5 a	5.2 cd	287 cd
V. vinifera cv. Red Sahebi	93.0 de	3.6 def	281 bc	73.5 d	1.7 b	81 a	14.0 a	2.8 b	197 ab	75.5 e	4.8 c	185 ab
V. vinifera cv. Alhaghi	34.5 bc	3.9 ef	256 b	46.5 c	4.9 e	236 bc	100 e	4.9 c	283 bc	85.5 ef	5.3 cd	189 ab
V. vinifera cv. Fakhri	85.0 de	2.4 cd	147 ab	55.5 d	1.9 bc	147 ab	12.5 a	1.4 ab	246 bc	33.0 bc	2.9 b	287 cd
V. vinifera cv. Shast Aros	75.0 d	5.1 gh	362 cd	87.5 ef	5.2 e	342 c	48.5 bc	4.2 c	234 bc	98.5 ef	4.9 cd	321 d
V. vinifera cv. White Rishbaba	12.0 a	4.8 gh	321 cd	100.0 f	3.6 cd	189 ab	85.0 d	4.8 cd	278 bc	54.0 d	2.5 b	174 ab
V. vinifera cv. White Khalili	29.0 bc	5.1 gh	298 bc	56.5 d	2.3 bc	278 bc	100.0 e	5.3 cd	336 cd	100.0 f	4.8 cd	189 ab
V. vinifera cv. Red Bidaneh	100.0 e	4.3 efg	189 ab	45.0 d	1.4 b	178 ab	75.0 d	2.8 ab	312 cd	28.5 ab	1.7 a	211 ab
Strain means[y]	34.07	2.935	125.35	46.35	2.445	158.05	34.95	2.390	182.85	32.60	2.795	166.20
LSD[6]	16.79	0.543	127.35	14.86	1.278	129.84	22.39	1.328	118.36	18.89	2.496	137.57

[1] Evaluation 4 months after inoculation; [2] Percentage of inoculated sites with galls; [3] Gall size; [4] Gall weight;
[5] Within a column, means followed by the same letter are not significantly different at P=0.05.
[6] LSD values to compare any pair of means in column; [y] No significantly differences between means strains

In our study, no genotype was found to be immune to crown gall, but the response of various genotypes to inoculation with *A. vitis* varied widely. In other words, significant differences were found among different *Vitis* genotypes with regard to their resistance to the strains of *A. vitis*. NAZ_1 (*V. vinifera* cv. Jighjigha × *Vitis rupestris* cv. Du lot) was the most resistant to AG57, NAZ_4 (*V. vinifera* cv. Jighjigha × Riparia Gloire) was the most resistant to CG230 and NW180, and NAZ_5 (*V. vinifera* cv. Alibaba × 110 R) was the most resistant to AG57 and K1059 strains. *V. vinifera* cv. White Bidaneh was the most susceptible to all strains. *V. riparia* which, according to CIRAMI and WHITING (1991) and STOVER *et al.* (1997), was found to be one of the most resistant genotypes to crown gall can be used as a rootstock for crown gall susceptible grapevine cultivars of *V. vinifera* (SZEGEDI *et al*. 1984). In the field, we observed that scions grafted on NAZ_4 were more resistant to the disease than those grafted on Kober 5 BB, NAZ_6 or other hybrids (MAHMOODZADEH 2001). We have demonstrated that NAZ_4 (*Vitis vinifera* cv. Jighjigha × *Vitis riparia* "Gloire" is resistant to most of the strains of *A. vitis.*

In this study we found that crown gall response was much greater when plants were actively growing. Other researchers (SZEGEDI *et al*. 1984; SULE *et al*. 1994; STOVER *et al*. 1997) also tested a number of parental genotypes of hybrids and reported that Riparia "Gloire" and Kober 5 BB are resistant to crown gall. Also some of interspecific hybrids between *V. rupestris* and *V. berlandieri* such as Paulsen 775 were found to be resistant to crown gall (GOODMAN *et al*. 1993).

In general, tumors formed on hybrids were smaller than those on *V. vinifera* cultivars and appeared later than these. Small swellings could be observed after 8 weeks but measurable tumors were observed after 3-5 months. The degree of pathogenicity varied for bacterial strains depending on grape cultivars. No single strain was most pathogenic on all hybrids and cultivars. The hybrids NAZ_5, NAZ_4 and NAZ_1 were the most resistant, because they were resistant to one strain of *A. vitis* (Tab. 3). The response of various genotypes was distributed homogeneously within this range. Gall size and weight also varied widely, the mean gall size over strains being 0.42-6.8 mm. The mean largest gall per plant ranged from 0.9 to 21.0 mm. The genotypes with the lowest percentage of inoculated sites forming galls were NAZ_4 (3.4 %) and NAZ_5 (6.2 %); they also had the smallest galls of all genotypes.

Four other hybrids were not significantly different from NAZ_4 and NAZ_5, in terms of inoculated sites, percent galls formed or mean largest gall per plant, but they were significantly higher in mean gall size and weight. All *V. vinifera* cultivars were among the genotypes with the smallest to largest galls at inoculated sites (the smallest gall 1.3 mm, the largest gall 5.1 mm), but the percentage of sites with gall ranged from 15.8 to 82 %. White Bidaneh was one of the most susceptible genotypes tested, forming galls at 80.3 % of the inoculated sites, with a mean gall size of 4.45 mm and a mean largest gall per plant of 18.6 mm (Tab. 3).

Analysis of variance indicated that strain x genotype interaction was highly significant. There has been considerable confusion of the identity of grape rootstocks throughout the world. Rootstock resistance to crown gall may be important in preventing passage of soil *A. vitis* into susceptible scions.

Although no genotype was found to be immune to crown gall, the most resistant selections were dramatically less susceptible than White Bidaneh which represents the *V. vinifera* cultivar that might benefit from being grafted to crown gall resistant rootstocks. Some of the most resistant genotypes examined in this work, are not established rootstock varieties. NAZ_4, NAZ_5 and NAZ_1, three of the most resistant genotypes in this study, are already recommended for use in Iran.

Acknowledgements

MAHMOUD R. ROOZBAN and SEYED K. HOSSINIBAY have provided some valuable advice on the manuscript. We are grateful for their excellent technical support. The Seed and Plant Improvement Research Institute (SPII) provided financial support.

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Received December 14, 2003