Susceptibility of Some Grapevine Cultivars and Rootstocks to Crown Gall Disease

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Submitted for publication: May 2008
Accepted for publication: September 2008
Key words: *Agrobacterium*; *Vitis vinifera* L.; grafting; susceptibility; NAZ_4; NAZ_6

The effects of five interspecific hybrid rootstocks on the susceptibility of grafted scions to crown gall were studied for six years in field as well as greenhouse experiments. The incidence of crown gall on susceptible grape scion cultivars (*Vitis vinifera* cvs. 'Thompson seedless' and 'Red Sahebi') was not affected by their grafting onto resistant rootstocks, including NAZ_4 (*Vitis vinifera* cv. 'Jighjigha' × Riparia Gloire) and NAZ_6 (*V. vinifera* cv. 'Gharaozum' × Kober 5BB), or on self-rooted cultivars when the inoculated vines were monitored over a three-month period in a greenhouse. The weights of the galls induced on non-grafted vines by two of six strains of *Agrobacterium vitis* and *Agrobacterium tumefacience* biovar 1 were significantly different. *A. vitis* strain AG57 and *Agrobacterium tumefacience* biovar 1 strain 16/6 induced significantly larger galls (8.9 and 5.4 mm respectively) on 'Thompson seedless' and 'Red Sahebi' when these were growing as self-rooted plants than when grafted on NAZ_4 and NAZ_6. Observations over a four-year period in the field showed that there was no difference in crown gall incidence until the second year. Scions grafted onto rootstocks of NAZ_4 and NAZ_6 had a 21.5% and 6.8% incidence, compared to 55% for self-rooted vines. In another field experiment with naturally infected scions of *V. vinifera* cv. 'Asgari' (a very susceptible cultivar), crown gall was apparent on vines grafted onto all rootstocks after six years. The incidence of crown gall was 18% on NAZ_6 compared to 68% on NAZ_1. During a five-year period, many self-rooted vines died, compared to only a few scions grafted onto NAZ_4 and NAZ_6 rootstocks that died. At the end of the study, many pathogenic strains of *Agrobacterium* spp. were isolated from the roots of 'Thompson seedless' and 'Red Sahebi' vines, but not from roots or vines when NAZ_4 and NAZ_6 rootstocks were used.

Crown gall of grapevine, caused by *Agrobacterium vitis*, is a serious disease in many regions of the world. *A. vitis*, the predominant bacteria, can exist systemically in grapevines and can be disseminated in propagation material and infected vineyard soils (Burr & Katz, 1983; Burr *et al*., 1987; Bishop *et al*., 1989). Cultivars of *Vitis vinifera* are responsible for more than 90% of grape production worldwide, but more than 95% of them are very susceptible to crown gall (Bishop *et al*., 1989; Ophel & Kerr, 1990). Galls usually occur on the trunk just above the ground line, although aerial galls up to 1 m above the soil have been observed (Mahmoodzadeh, 2002). Also, *A. vitis* has been found in vineyards but not in non-vineyard soils (Burr & Katz, 1984; Ferreira & Van Zyl, 1986). When field soils are assayed for the presence of *Agrobacterium* spp., non-tumorigenic strains are often predominant (Burr *et al*., 1987). Studies have shown that some phylloxera-resistant rootstocks are also resistant to crown gall, e.g. Riparia Gloire and interspecific hybrids 3309C, 101-14 Mgt, NAZ_1, NAZ_2, NAZ_4, NAZ_5 and NAZ_6 (Goodman *et al*., 1993; Stover, 1993; Süle *et al*., 1994; Stover *et al*., 1997; Mahmoodzadeh *et al*., 2004). In this research, the effects of crown gall-resistant rootstocks on the susceptibility of scions or self-rooted vines of three grapevine cultivars were studied.

MATERIALS AND METHODS

Greenhouse experiment

Five interspecific hybrids were used as resistant rootstocks (Table 1) (Mahmoodzadeh *et al*., 2004). One-bud scions taken from susceptible vines without visible symptoms of crown gall (Table 1) were hand-whip grafted onto 30 cm long cuttings of dormant rootstocks. Rooted cuttings of the same vines were used as the control treatment. Cuttings and scions were taken from the Viticulture Research Section of Khalat-Poushan Station, University of Tabriz, Tabriz, Iran. For callus formation, the scions and the cuttings were maintained in a hot-room (28ºC) for four weeks. Grafts and cuttings were potted separately into polyethylene bags with a diameter of 15 cm containing a mixture of loam soil, perlite and compost (1:1:2 in volume) and transferred to a greenhouse (20 to 28ºC).

When the new shoots were 25 to 30 cm long (in June) and had developed a brown periderm at the lower portion, the vines were inoculated by making wounds (2 mm^3) at the fourth to tenth internodes on each plant with a scalpel and depositing 10 µL of bacterial suspension (containing about 10^{10} CFU/mL) into each wound as described by Stover *et al*. (1997). The experiment was carried out as factorial based on a randomised complete block design (RCBD) with four replications. In aggregate, 288 vines were used as the plant material for the experiment. Pathogenic strains of *A. vitis* and *A. tumefaciens* biovar 1 were selected as described by Matsumoto *et al*. (1992) (Table 2). Bacteria were grown for 48 h at 28°C on salts of yeast extract-beef (YEB) medium (1 g yeast extract, 5 g beef extract, 5 g peptone, 5 g sucrose, and 0.5 g $MgSO_4$ per 1 L distilled water) (Szegedi *et al*., 1984; Süle *et al*.,

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Acknowledgements: The authors wish to thank Dr M.R. Roozban, Y. Gholipoure and M. Golmohammadi for their technical assistance.

TABLE 1

List of the studied grapevine genotypes.

Hybrids as rootstocks*	Scion or self-rooted cultivar
NAZ_1: *V. vinifera* cv. 'Jighjigha' × *V. rupestris* cv. 'Du Lot'	*V. vinifera* cv. 'Red Sahebi'
NAZ_2: *V. vinifera* cv. 'Alibaba' × *V. rupestris* cv. 'Du Lot'	*V. vinifera* cv. 'Thompson seedless'
NAZ_4: *V. vinifera* cv. 'Jighjigha' × Riparia Gloire	*V. vinifera* cv. 'Asgari'
NAZ_5: *V. vinifera* cv. 'Alibaba' × 110R	
NAZ_6: *V. vinifera* cv. 'Gharaozum' × Kober 5BB	

*Mahmoodzadeh *et al*. (2004)

TABLE 2.

Bacterial strains used in the experiments.

Strain	Characteristic	Origin
A. vitis		
CG230	Vitopine Ti[†]	USA, T.J. Burr
AG57	Octopine Ti, LHR[‡]	Crete, C. Panagopoulos
NW180	Octopine Ti	Germany, E. Bien
K1059	Octopine Ti	Australia, A. Kerr
A. tumefaciens biovar 1		
1/12	Nopaline Ti plasmid	Bulgaria, I. Popova
16/6	Nopaline Ti plasmid	Hungary, S. Süle

[†]Ophel and Kerr (1990).
[‡]Limited host range; Panagopoulos and Psallidas (1973).

1994) and on selective media of RS (Roy & Sasser, 1983). Two days prior to inoculation, strains were streaked onto potato dextrose agar (PDA) (Difco, Germany) and grown at 28°C. Bacterial growth from the PDA was suspended in sterilised deionised water (SDW) and adjusted to an optical density (600 nm) of 1. After inoculation, the plants were kept at 18ºC in a room for 48 h and then transferred to the greenhouse. Each vine-bacterium combination was repeated four times. After three months, tumours that had formed on the scions were excised and weighted.

Field experiments

In September, four months after inoculation, the plants from the greenhouse experiment were placed outdoors and then covered with plastic in December for protection (0 to 10ºC). In May, all the canes were pruned to two buds. The thickest canes were selected and inoculated with a 10 µL bacterial suspension containing about 10^7 bacteria, based on the method used by Süle *et al*. (1994). The strains were the same as those used on the plants in the greenhouse. The inoculated plants were planted in the field in a 2×4 m spacing (four replications, four plants per bacterium). In November of each year, observations were made for the appearance of galls and for vine growth vigour and viability.

In a second field experiment, the same five rootstocks were used as earlier in the greenhouse. Scions were cut from naturally infected vines having many galls on the trunks of *V. vinifera* cv. 'Asgari' at the start of the dormant season (in December). The scions were soaked in 1% NaOCl (20% bleach) for 15 min, and rinsed in sterilised distilled water. Each cane was numbered and tissue was sampled for the presence of *A. vitis*. The remainder of the cuttings from the canes was stored at 4 to 10ºC in wet sand in plastic bags for 75 days. The presence of tumorigenic *A. vitis* was determined by incubating the basal section of the canes in wet perlite for 45 days at 28°C. Newly-formed callus and roots were excised, ground in sterile distilled water and plated on RS medium (Roy & Sasser, 1983). After incubation at 28ºC for seven to 14 days, presumptive *A. vitis* colonies were tested for pathogenicity on sunflower (*Helianthus annuus* var. Record) and carrot slices (*Daucus carota*). A heavy smear of cells from each test strain (5×10^8 CFU/mL) was spread with a sterile toothpick onto stems of two- to four-week-old seedlings of sunflower (Bishop *et al*., 1989; Mahmoodzadeh *et al*., 2004). Carrot slices were spread across each disk. The petri dishes were kept in a growth chamber in the dark at 28ºC, and the filter paper was periodically remoistened every 12 h. Plants were assessed for the presence of tumours after 1.5 months. Canes found to be infected with tumorigenic strains were used to make cuttings for grafting. Rootstock cuttings were collected from crown gall- free mother plants of hybrids, hand-whip grafted and placed in a hot-room (28ºC) for four weeks (standard heat callusing), except that only 40 randomly selected cuttings from 200 of each hybrid were tested for the presence of pathogenic bacteria. The grown grafts and ungrafted cuttings were planted into the nursery for the first growing season. One-year-old bare rooted plants were planted 2×4 m apart in four replications (10 vines per replication) in field plots. Standard pruning, training and other cultural practices were done each year based on methods described by Goodman *et al*. (1993). Observations were recorded on the appearance of galls and the vigour of growth and viability of the vine in October of each year. At the end of the experiment (end of fourth year), the vines were dug out and their roots were evaluated for the presence of pathogenic bacteria as described previously.

Statistical analysis

Analyses of variance were performed using SAS statistical software (SAS Institute Inc., 1992). Student's t least significant difference (LSD) values were calculated at the 5% significance level to facilitate comparison between treatment means.

RESULTS AND DISCUSSION

Greenhouse experiment

Visible galls developed on the inoculated shoots of two sensitive cultivars, 'Thompson seedless' and 'Red Sahebi' within six to eight weeks. Four of six bacterial strains (CG230, AG57, NW180, K1059) induced large tumours on very sensitive *V. vinifera* cultivars. The means of the size and weight of galls induced by different strains were not statistically different ($P < 0.05$) for all hybrids, except for strains 16/6 and AG57, in which the galls on scions grafted onto NAZ_4 and NAZ_6 were significantly smaller (average size for 16/6 was 0.69 mm and for AG57 1.42 mm) than those on self-rooted vines (5.53 mm). Both *A. vitis* and *A. tumefaciens* biovar 1 strains were not equally tumorigenic in all treatments (Table 3). Strain AG57 (*A. vitis)* and 16/6 (*A tumefaciens* biovar 1) induced bigger (2.86 mm) and more (38.2%) galls than

TABLE 3

Weight and size of galls formed on the grapevine scions (*Vitis vinifera* cvs. 'Thompson seedless' and 'Red Sahebi') after inoculation with different strains of *Agrobacterium**.

Cultivars rootstock	*Agrobacterium tumefaciens* biovar 1†				*Agrobacterium vitis*							
	1/12		16/6		CG230		AG57		NW180		K1059	
	GS‡	GW§	GS	GW	GS	GW	GS	GW	GS	GW	GS	GW
Red Sahebi												
Own-rooted	2.8^{dS} ± 0.20	312^{e} ± 18	5.3^{d} ± 0.22	349^{c} ±14	6.6^{d} ± 0.24	384^{d} ± 13	8.6^{d} ± 0.45	423^{d} ±14	7.8^{d} ± 0.25	398^{c} ± 14	5.30^{c} ± 0.2	384^{d} ± 14
NAZ_1	0.4^{b} ± 0.08	79^{b} ± 8	1.3^{b} ± 0.08	156^{b} ± 9	4.3^{c} ± 0.12	326^{c} ± 12	4.2^{c} ± 0.21	342^{c} ±13	2.4^{b} ± 0.09	179^{b} ± 8	1.2^{a} ±0.07	158^{b} ± 12
NAZ_2	0.8^{bc} ±0.02	148^{c} ± 12	1.9^{b} ± 0.04	198^{c} ± 11	3.2^{bc} ±0.08	298^{c} ± 8	1.7^{b} ± 0.06	195^{b} ± 8	1.2^{ab} ± 0.03	164^{b} ±7	2.4^{b} ± 0.09	225^{c} ± 16
NAZ_4	0.1^{a} ± 0.01	23^{a} ± 4	0.5^{a} ± 0.02	38^{a} ± 4	0.6^{a} ± 0.01	47^{a} ± 4	0.8^{a} ± 0.01	123^{a} ± 7	0.5^{a} ± 0.01	36^{a} ± 4	0.5^{a} ± 0.02	43^{a} ± 8
NAZ_5	1.1^{c} ± 0.09	205^{d} ±18	2.6^{c} ± 0.12	215^{bc} ± 12	2.8^{b} ± 0.07	186^{b} ± 7	2.9^{bc} ± 0.04	198^{b} ± 6	3.8^{c} ± 0.07	198^{b} ± 8	2.3^{b} ± 0.08	128^{b} ± 9
NAZ_6	0.2^{a} ± 0.03	25^{a} ± 3	0.4^{a} ± 0.07	34^{a} ± 3	0.5^{a} ± 0.02	39^{a} ± 3	1.2^{ab} ± 0.02	154^{a} ± 9	0.8^{a} ± 0.07	54^{a} ± 3	1.3^{a} ± 0.06	98^{ab} ± 6
LSD (P=0.05)	0.1	49.2	0.6	24.4	1.2	73.4	0.6	38.7	1.2	98.2	0.8	109.2
Thompson seedless												
Own-rooted	4.8^{c} ± 0.30	398^{d} ± 13	5.40^{d} ± 0.70	365^{c} ±18	5.8^{e} ± 0.12	359^{e} ± 18	8.9^{d} ±0.45	455^{d} ± 18	6.9^{c} ± 0.90	358^{c} ± 17	5.9^{c} ± 0.70	394^{d} ± 20
NAZ_1	1.4^{b} ± 0.10	123^{b} ± 8	2.80^{c} ± 0.10	265^{b} ± 14	4.8^{d} ± 0.11	324^{c} ±16	4.8^{c} ± 0.04	346^{c} ±13	3.7^{b} ± 0.60	212^{bc} ± 13	3.2^{b} ± 0.20	241^{c} ± 16
NAZ_2	1.2^{ab} ± 0.09	188^{c} ± 9	2.61^{c} ± 0.09	213^{b} ±13	4.2^{c} ± 0.09	354^{c} ±18	2.5^{ab} ± 0.03	218^{b} ± 14	1.8^{a} ± 0.08	198^{b} ± 12	2.4^{b} ± 0.10	245^{c} ±14
NAZ_4	0.4^{a} ± 0.01	39^{a} ± 4	0.54^{a} ±0.03	33^{a} ± 80	0.9^{a} ± 0.04	56^{a} ±7	1.2^{a} ±0.02	148^{a} ± 9	0.6^{a} ± 0.01	43^{a} ± 7	0.5^{a} ± 0.08	49^{a} ± 5
NAZ_5	1.1^{b} ± 0.08	218^{c} ±12	2.4 0^{c} ± 0.09	254^{b} ±14	2.9^{b} ±0.07	194^{b} ± 9	3.1^{b} ± 0.08	214^{b} ±12	3.1^{b} ± 0.04	167^{b} ± 11	3.2^{b} ± 0.20	145^{b} ±8
NAZ_6	0.8^{a} ± 0.02	42.0^{a}±3	1.22^{b} ± 0.04	58^{a} ± 40	2.1^{a} ± 0.09	98^{a} ±6	2.4^{ab} ±0.07	245^{b} ± 11	1.8^{a} ± 0.02	198^{b} ± 10	1.4^{a} ± 0.09	116^{b} ± 10
LSD (P=0.05)	0.4	80.4	0.6	145.2	0.6	98.2	1.2	60.2	2.1	139.8	1.8	69.2

*Values represent the average gall size and weight of four replications.
†Evaluations were carried out four months after inoculation.
‡Gall size (mm).
§Gall weight (mg).
$ Mean values within columns followed by different letters are significantly ($P < 0.05$) different according to LSD test.

the other strains. Analysis of variance showed differences between genotypes of grapevine and strains of *Agrobacterium* spp. and interaction between grape genotypes × strains (Table 4).

Field experiments

All the plants used in the greenhouse experiment were re-inoculated and transported to the field for further observations in the following four years. Observations showed that all of the bacterial strains induced galls on the scions, but not an equal number, size or weight (Table 3). Rootstocks impacted the incidence of root galls. All cuttings of NAZ_4 and NAZ_6 remained free from galls, but other hybrids (NAZ_1, NAZ_2 and NAZ_5) showed small or a few galls typical of *Agrobacterium* infections on their trunks.

In the first year, no significant differences were observed between treatments, i.e. galls appeared in nearly equal numbers in all seven treatments. In the second and third years, more self-rooted plants became galled than scions on rootstocks (Fig. 1) and 19.2% of self-rooted plants died by the end of the second year (Fig. 2). In the fourth year, the number of galled plants was higher for self-rooted plants and those on NAZ_1, NAZ_2 and NAZ_5 compared to NAZ_4 and NAZ_6. The survival of scions on NAZ_4 and NAZ_6 rootstocks was 78.5% and 93.2%, respectively, in contrast to 68.2% (NAZ_1), 62.5% (NAZ_2), 58.6% (NAZ_5) and 45% (self-rooted) (Fig. 2). Pieces of rootstocks and scions were indexed for the presence of pathogenic *Agrobacterium* spp prior to grafting. About 60% of the tested 'Thompson seedless' and 'Red Sahebi' led to canes that formed pathogenic colonies and only these were used to make cuttings for grafting, based on methods developed by Bazzi, Piazza and Burr (1987). The grafting success rate assessed prior to planting (200 cuttings grafted; two cultivars as scion) was 78%, 85%, 86%, 79% and 68% for the five hybrids used as rootstocks (NAZ_1, NAZ_2, NAZ_4, NAZ_5, NAZ_6) respectively. By the end of the first year in the nursery 142, 168, 161, 139 and 153 grafts remained in the nursery for transplanting. In the first year, no tumours were observed on any part of the grafts on NAZ_4 and NAZ_6. In the second year, galls were observed on many of the rootstocks (Fig. 1). In the third and fourth years, many plants developed galls, with 35% of the vines grafted on NAZ_1, 28% on NAZ_2, 4% on NAZ_4, 22% on NAZ_5 and 6% on NAZ_6 showing typical natural symptoms of crown gall disease. Over the five-year duration of the study, many galled plants died (Fig. 2).

Pathogenic isolates of *A. vitis* were recovered from the roots of NAZ_1, NAZ_2, NAZ_5 and sensitive cultivars, but not from the roots of NAZ_4 and NAZ_6. Hybrids as rootstock and scion pieces

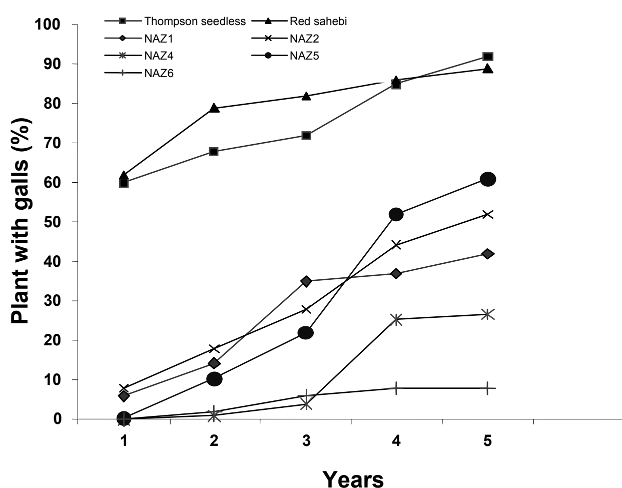

FIGURE 1

Effects of rootstocks on the appearance of gall on the scions after grafting naturally-infected scions onto healthy rootstocks.

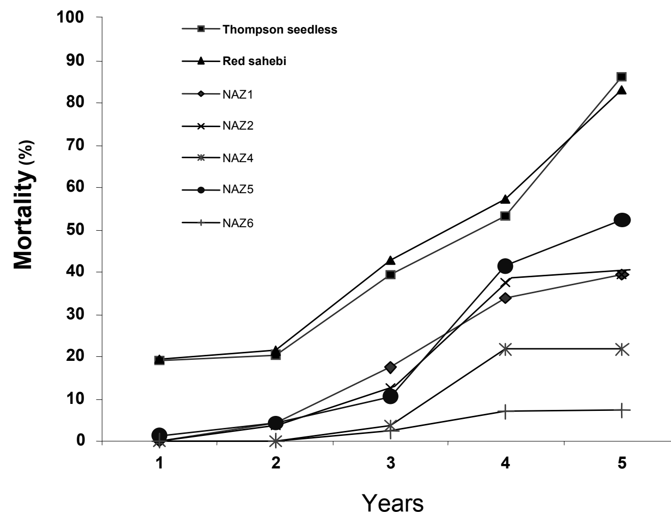

FIGURE 2

Effects of rootstocks on survival of scions after grafting naturally-infected scions onto healthy rootstocks.

TABLE 4

Analysis of variance for gall weight, size and dead plants after inoculation with different strains of *Agrobacterium* spp.

Source of variation	Degree of freedom	Mean square			
		Gall size	Gall weight	Dead plants	Plants with galls
Replication	3	25.47 †	76.41 *	4.138 †	18.287 †
Treatment	71	9.875 **	408.23 **	167.278 **	8.265**
Rootstocks (R)	5	71.89 **	3149.876**	284.625**	4.286**
V. vinifera cultivars (V)	1	14.12 †	83.26 †	18.985*	1.785 †
Agrobacterium strain (S)	5	44.76 **	263.48**	438.147**	28.475**
RV	5	10.87*	97.48 †	89.745 †	14.7858*
RS	25	18.439*	986.732**	432.58**	1.287*
VS	5	8.456 *	123.71 †	21.497 †	17.487 †
RVS	25	69.214*	846.211*	56.211 **	12.465 *
Error	213	---------	---------	---------	---------

† not significant, *,** significant at P < 0.05 and P < 0.01 probability levels respectively.

were indexed prior to grafting for the presence of pathogenic *Agrobacterium* spp. None of the indexed rootstock cuttings (40 out 200) were infested with tumorigenic strains (Lehoczky, 1968). However, about 70% of the tested *V. vinifera* cv. Asgari canes produced pathogenic (*A. vitis*) colonies (Fig. 3), and only these were used to make cuttings for grafting. Of all of the hybrids with 200 cuttings that were grafted, the grafting success rate assessed prior to planting was 56%, 36%, 74%, 81% and 79% for NAZ_1, NAZ_2, NAZ_4, NAZ_5 and NAZ_6 respectively. At the end of the first year in the nursery, 41, 26, 38, 32 and 47 grafts on hybrids remained for transplanting respectively. After transplanting, the plants were observed for six years. In the first two years, no galls could be observed on any of the rootstocks, and only a few grafts became galled in the third year. By the sixth year nearly all of the previously infected vines on NAZ_1, NAZ_2 and NAZ_5 died. No more new galls could be observed on NAZ_4 and NAZ_6 (Fig. 3). As a result of this, and the fact that most vines grafted on NAZ_4 and NAZ_6 escaped the infection, 79% of the original vines remained alive and seemingly productive. Pathogenic isolates of *A. vitis* were recovered from the roots of NAZ_1, NAZ_2 and NAZ_5, but no tumorigenic *Agrobacterium* were isolated from the roots of NAZ_4 and NAZ_6.

Although strain 16/6 of *A. tumefaciens* and strain AG57 of *A. vitis* induced galls on scions grafted on all rootstocks in this experiment, the gall weights and sizes were less (48%) on the resistant rootstocks than on self-rooted *V. vinifera* vines. The other four bacterial strain vine combinations showed no significant differences. We found that a long time (more than two months) is needed for the expression of the rootstock effect on the scion. In the first year of the experiments there were no differences in gall formation among the rootstocks after inoculating with the bacteria. However, self-rooted vines were more galled at the end of the first year and the beginning of the second year. After three years, it became clear that vines grafted on NAZ_4 and NAZ_6 rootstocks were less susceptible to crown gall. Also, the results showed that, under field conditions using naturally-infected scions, the effect of resistant rootstocks on

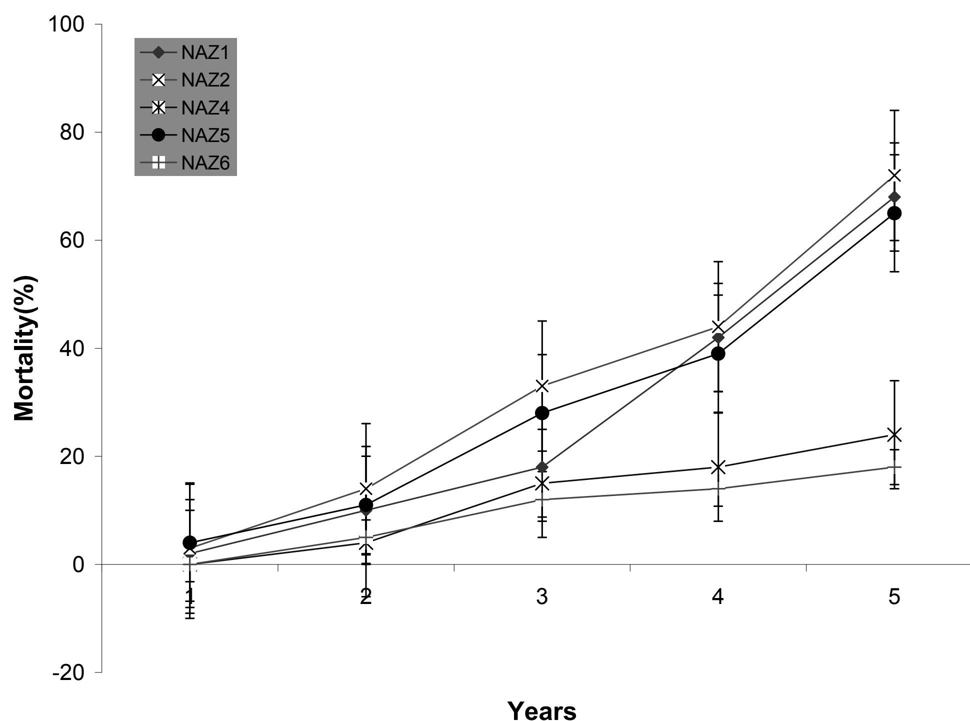

FIGURE 3
Effects of rootstocks on the survival of scions (*Vitis vinifera* cv. Asgari) after grafting Naturally-infected scions onto healthy rootstocks. Each time point represents the mean of four replicates and indicates the calculated standard deviations.

crown gall infections became highly pronounced. The incidence of gall formation and the death of vines increased in the following years (after the fourth year). It can be hypothesised that either the bacterial number in the vascular tissues was not high enough in the first three years to induce galling (Burr & Katz, 1984), or that the young vines were more resistant than the old ones (Burr *et al*., 1987). Riparia Gloire and some interspecific hybrids, such as Teleki 5C (Süle & Burr, 1998), 3309C (*Riparia tomentosa* × *Rupestris martin*), 101-14 Mgt, NAZ_4 and NAZ_6 are resistant to the crown gall pathogen (Stover *et al*., 1997; Mahmoodzadeh *et al*., 2004), and are generally resistant to cold climates and have more winter hardiness. Therefore, they can be used as grape rootstocks in cold climates as well. In most cases, the infected trunk died and the new trunk emerging from below the earlier galls remained alive without any galling. The physiological basis for this is not clear. At present, one possible explanation is that compounds produced by the rootstock are translocated to the scion and may induce a loss of pathogenicity in populations of *A. vitis* (Mahmoodzadeh *et al*., 2004) or inhibit the extent of stable T-DNA integration (Stover, 1993). Therefore, when *A. vitis* bacteria in the vascular system migrate from scion to rootstock, the rootstock may have a mechanism that reduces bacterial survival. The decline in population may not be significant in the early years after grafting, but becomes more important as the vines age. In the light of this investigation, it is clear that rootstocks can greatly affect the severity of crown gall infection of grapevine.

Further long-term experiments are required to determine if similar effects exist with other rootstocks. The available rootstocks did not prevent the disease, but significantly reduced its severity. In Iran, 100% of vineyards have been established using sensitive cultivars of *V. vinifera* as self-rooted plants, which are highly susceptible to crown gall (Mahmoodzadeh*,* 2001; Mahmoodzadeh *et al*., 2003). In conclusion, we recommend that nurserymen should graft their scions onto resistant rootstocks to reduce the risk of crown gall.

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