Characteristics of Nazmieh and Spota grape rootstock resistant to crown and root gall disease

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Abstract

Crown and root gall are the most common bacterial diseases in Iranian vineyards. Commercial cultivars of *V. vinifera* spp. are highly susceptible to them. Due to the presence high lime in most of the soils of Iran, a breeding program started 14 years ago to obtain a cultivar crown and root gall disease resistant and soil lime tolerant. Cross-breeding was performed with four parents of American species (male) and four parents of Iranian seeded grape cultivars (*Vinifera* sp. – female) which made it possible to obtain 16 hybrids. Two hybrids were selected as NAZ6 (*V. vinifera* 'Jighjigha' × Riparia "Gloire") and NAZ4 (*V. vinifera* 'Qaraouzum' × Kober 5BB), respectively. In 2018, these two rootstocks received the necessary approval after being examined by the Iranian Research Institute of Registration and Certification of Seeds and Plant, and were named Nazmieh (AZ6) and Spota (NAZ4). Both *Agrobacterium vitis* resistance causing crown and root gall more than 86%, tolerance to soil lime (up to 30%) as well as cold tolerance were determined in both rootstocks. The results showed that Nazmieh induced high vigor while Spota is a dwarfing rootstock. Both rootstocks were also evaluated for their graft-compatibility and for the influence exerted on the production and quality of the bunches.

Keywords: *Agrobacterium* resistance, lime tolerance, hybridization, *Vitis vinifera*

INTRODUCTION

In grapevine, rootstocks may help to induce plant disease-resistance and to overcome extreme soil and environmental conditions (e.g., tolerance to lime, drought, high soil pH, soil excessive humidity, salts content, etc.). Crown gall caused by *Agrobacterium vitis* (*A. vitis*) (Ophel and Kerr, 1990) is a serious bacterial disease, particularly for the cultivars of *Vitis vinifera*. It survives systemically in vines, can be disseminated in propagation material and infect vineyard soils (Burr and Katz, 1983; Burr et al., 1987). Although *A. vitis*-free material can be produced through shoot-tip culture and hot water treatments (Bazzi et al., 1987; Mahmoodzadeh et al., 2003), the pathogen may survive in the grape rhizosphere and when *A. vitis*-free vines are planted on infested soil, they can become infected (Burr et al., 1998). American *Vitis* species are resistant to phylloxera 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; Süle 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 infest the whole plant, the use of resistant rootstocks may reduce infection from soil inoculum and

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prevent subsequent infection of *A. vitis*-free (Süle 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. In this research, the effects of crown gall-resistant rootstocks on the susceptibility of scions or self-rooted vines, lime tolerant and cold resistance of three grapevine cultivars were studied.

MATERIALS AND METHODS

Plant material/hybrids and evaluating their resistance to disease, tolerance to cold and soil lime and bacterial strains used

In order to create hybrids resistant to crown and root gall, based on the results of the preliminary survey, Rupestris and the rootstocks of Kober 5BB, Riparia Gloire and 110R were selected as disease resistant gene resources and chosen as male parent. Also, the cultivars of 'Jighjigha', 'Qaruzum', 'Rishbaba' and 'Alibaba' that have higher resistance to crown gall were chosen as female parents (Mahmoodzadeh et al., 2004). Sixteen crosses were made (Table 1) and 500 seeds were obtained from each crossing. Stratification of seeds was done artificially in the refrigerator and, in the spring outside and in greenhouse. Seedlings were inoculated by *Agrobacterium* suspension from different strains, based on the method suggested by Stover et al. (1997) and Süle et al. (1994) (Table 2). Pathogenic strains of *A. vitis* were isolated from sap, galls and cuttings of infected grapevine showing symptoms of crown gall disease (Mahmoodzadeh, 2001) based on the methods used by Panagopoulos and Psallidas (1973), Burr and Katz (1983), and Ophel and Kerr (1990).

Table 1. Parents and hybrids.

Hybrids	Parents
H1 (NAZ1)	*V. vinifera* 'Jighjigha' × *V. rupestris* 'Du Lot'
H2 (NAZ2)	*V. vinifera* 'Alibaba' × *V. rupestris* 'Du Lot'
H3 (NAZ3)	*V. vinifera* 'Qaraouzum' × *Vitis rupestris* 'Du Lot'
Spota (NAZ4)	*V. vinifera* 'Jighjigha' × Riparia Gloire
H5 (NAZ5)	*V. vinifera* 'Alibaba' × 110R[a]
Nazmieh (NAZ6)	*V. vinifera* 'Qaraozum' × Kober 5BB
H7 (NAZ7)	*V. vinifera* 'Rishbaba' × *V. rupestris* 'Du Lot'
H8 (NAZ8)	*V. vinifera* 'Alibaba' × Riparia Gloire
H9 (NAZ9)	*V. vinifera* 'Rishbaba' × Kober 5BB
H10 (NAZ10)	*V. vinifera* 'Rishbaba' × Riparia Gloire
H11 (NAZ11)	*V. vinifera* 'Rishbaba' ×110R
H12 (NAZ12)	*V. vinifera* 'Qaraouzum' × Riparia Gloire
H13 (NAZ13)	*V. vinifera* 'Alibaba' × Kober 5BB
H14 (NAZ14)	*V. vinifera* 'Jighjigha' × 110R
H15 (NAZ15)	*V. vinifera* 'Qaraozum' × 110R
H16 (NAZ16)	*V. vinifera* 'Jighjigha' × Kober 5BB

[a]110R was obtained (*Vitis rupestris* 'Martin' × *Vitis berlandieri* 'Ressguier N°$_2$').

Table 2. Used bacterial strains.

Strains	Characteristics	Origin
A. vitis (AT biovar 3)		
CG230	Vitopine Ti[a]	USA, T.J. Burr
AG57	Octopine Ti, LHR[b]	Crete, C. Panagopoulos
NW180	Octopine Ti	Germany, E. Bien
K1059	Octopine Ti	Australia, A. Kerr
A. tumefaciens biovar 1		
1/12	Nopalin Ti plasmid	Bulgria, I. Popova
16/6	Nopalin Ti plasmid	Hungry, S. Sule

[a]Ophel and Kerr (1990), [b]LHR = limited host range; Panagopoulos and Psallidas (1973).

Scoring gall formation, graft compatibility and effect on growth, yield and induction of disease resistance and tolerance to lime and salt

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. 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 × genotype treatment yielded a gall. Since gall size can greatly vary within a single plant, the mean largest gall plant^{-1} was evaluated (Yun et al., 2003; Goodman et al., 1993). Bacterial suspension inoculation was done on the healthy seedlings with the previous method, and finally, five hybrids selected based on the pathogenicity test in the previous stage, were evaluated for their graft compatibility, growth and yield of two commercial grape cultivars in Qazvin province (Table 3). Cuttings were prepared from healthy plants and after propagation; they were kept in the screen-house and used in the preparation of cuttings for the next stages of experiments. The evaluation of the seedlings of the hybrids was carried out for the tolerance of soil lime up to 35% of active lime and salt tolerance at a concentration of 100 mM sodium chloride.

Table 3. List of grape genotypes used. Source: Mahmoodzadeh et al. (2004).

Hybrids as rootstocks	Cultivars of *Vitis vinifera* L. as scion and own-rooted
H1 (NAZ1): *V. vinifera* 'Jighjigha' × *V. rupestris* 'Du Lot'	*V. vinifera* 'Sahebi Grmiz'
H2 (NAZ2): *V. vinifera* 'Alibaba' × *V. rupestris* 'Du Lot'	*V. vinifera* 'Sefid Bidaneh'
Spota (NAZ4): *V. vinifera* 'Jighjigha' × Riparia Gloire	
H5 (NAZ5): *V. vinifera* 'Alibaba' × 110R	
Nazmieh (NAZ6): *V. vinifera* 'Qaraouzum' × Kober 5BB	

Greenhouse experiment

One-bud scions taken from susceptible vines without visible symptoms of crown gall (Table 3) were hand-whip grafted onto 30-cm long cuttings of dormant rootstocks. Rooted cuttings of the same vines were used as control. Cuttings and scions were taken from the Viticulture Research Section of Khalat-Poushan Station, University of Tabriz, Iran. For callus formation, the scions and the cuttings were maintained in a hot-room (28°C) 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 28°C). 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^2) at the fourth to tenth internode on each plant with a scalpel and depositing 10 µL of bacterial suspension (containing about 10^{10} CFU mL^{-1}) into each wound as described by Stover et al. (1997). The experiment was carried out as factorial based on a randomized complete block design (RCBD) with four replications. 288 vines were used for the experiment. 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 $MgSO_4$ L^{-1} distilled water) (Szegedi et al., 1989; Süle et al., 1994) and on selective media of RS (Roy and 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 sterilized deionized water (SDW) and adjusted to an optical density (600 nm) of 1. After inoculation, the plants were kept at 18°C in a room for 48 h and then transferred to the greenhouse. Each vine-bacterium combination was repeated four times. After three months, gall 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 (0 to 10°C). 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 and viability. During the following years of the experiment, by seeing the first signs of maturity in healthy and infected seedlings, the yield and quality characteristics of the fruit were recorded. Data analyses were performed using SAS statistical software (SAS Institute Inc., 1992) and mean comparison was done with LSD test. It should be mentioned that during the whole period of the experiment, the seedlings were also examined as witnesses from ungrafted and grafted commercial cultivars on the mentioned bases away from contamination for comparison.

RESULTS AND DISCUSSION

The results of the pathogenicity test in the native cultivars and hybrids

The susceptibility to crown and root gall disease of 60 native cultivars of Iranian grapes was investigated during 2000-2003, based on Bliss et al. (1999) method including the inoculation of pathogenic bacterial strains (Table 2). The results showed that most of the native cultivars of Iran are highly sensitive to this disease and it is not possible to look for this resistance in the native germplasm of the country, but four cultivars including 'Jighjigha', 'Reshbaba', 'Qaraouzum' and 'Alibaba' had higher tolerance to soil lime and relative to crown gall resistance, and were selected as lime tolerant maternal parent in crossbreeding.

After crossing and creating 16 hybrids, five of them showed more resistance to the disease. The hybrids were numbered 1, 4, 2, 5 and 6 (Table 4) and selected for the next experiment steps. The test of active soil lime tolerance showed that these hybrids tolerated more than 30% of active soil lime, and also Nazimeh (hybrid 4) and Spota (hybrid 6) tolerated the salinity up to 100 mM NaCl. Nazimeh and Spota hybrids also showed higher frost damage (detected at -21°C) than the others hybrids (Table 4).

Table 4. Mean comparison hybrids resistance to crown gall, lime, cold and salinity.

Hybrids	Resistant to crown gall		Salinity tolerance (mM NaCl)	Lime tolerance[b] (% active lime/soil)	Cold damage on primary bud/low temperatures (%)				
	Susceptibility ratio[a]	% infection			-21	-19	-17	-15	-13
H1 (NAZ1)	RR	38	50	25	98	55	15	0	0
H2 (NAZ2)	RR	36	75	20	95	48	12	0	0
H3 (NAZ3)	RR	29	50	25	87	50	17	0	0
Spota (NAZ4)	R	22	100	35	15	9	3	0	0
H5 (NAZ5)	RR	35	50	25	100	60	21	0	0
Nazmieh (NAZ6)	R	24	100	35	18	10	5	0	0
H7 (NAZ7)	S	65	75	30	83	43	12	0	0
H8 (NAZ8)	VS	75	25	32	100	62	20	0	0
H9 (NAZ9)	RS	49	25	25	100	52	20	0	0
H10 (NAZ10)	S	52	25	25	89	43	13	0	0
H11 (NAZ11)	S	58	50	20	84	45	15	0	0
H12 (NAZ12)	VS	76	75	20	91	48	15	0	0
H13 (NAZ13)	S	59	25	25	57	25	15	0	0
H14 (NAZ14)	S	56	25	20	100	51	15	0	0
H15 (NAZ15)	VS	72	75	22	95	43	13	0	0
H16 (NAZ16)	S	52	50	25	82	32	8	0	0

[a]RR (relative resistance), R (resistance), S (sensitive), RS (relative sensitive), VS (very sensitive).
[b]Pavloušek (2010).

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 'Sefid Bidaneh' = 100%). Table 4 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 'Sefid 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. Comparison of average data showed that H1 had the longest growth period in one growing season, while the rate of fresh weight of vines and roots was higher than Spota (Table 5). The hybrids of Nazmieh and Spota had a shorter growth period than the others, but rooting appeared superior to others (Table 5).

Table 5. Mean comparison on vegetative growth characteristics of hybrids.

Hybrids	Growth duration time (day)	Cuttings rooted (%)	Dry weight of roots (g)	Fresh weight of vines (g)
H1 (NAZ1)	217a	75b	149.57d	428.375c
H2 (NAZ2)	212b	85a	198.75c	429.875ab
H3 (NAZ3)	213b	82.5a	185.75a	436.625 a
Spota (NAZ4)	200c	75b	176.75b	433.785b
H5 (NAZ5)	194d	75b	129.75e	423.688d
Nazmieh (NAZ6)	200c	75.25b	173.25bc	432.563b
Mean square	729.483**	1257.632*	987.325**	2549.368*
LSD 5%	4.28	5.24	5.432	3.278

Mean values within columns followed by different letters are significantly (P<0.05) different according to LSD test.
*, ** significant at P<0.05 and P<0.01 probability levels, respectively.

In the field, we observed that scions grafted on NAZ4 (Spota) were more resistant to the disease than those grafted on Kober 5 BB or other hybrids (Mahmoodzadeh, 2001). We have demonstrated that NAZ4 (Spota) and NAZ6 (Nazmieh) are resistant to *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; Süle 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 Paulsen775 were found to be resistant to crown gall (Goodman et al., 1993). Small swellings could be observed after 8 weeks but measurable tumors were observed after 3-5 months. No single strain was most pathogenic on all hybrids and cultivars.

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 'Sefid Bidaneh' grape which represents the *V. vinifera* cultivar that might benefit from being grafted to crown gall resistant rootstocks.

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. All cuttings of NAZ4 (Spota) and NAZ6 (Nazmieh) remained free from galls, but other hybrids (NAZ1, NAZ2 and NAZ5) 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 (Figure 1a) and 19.2% of self-rooted plants died by the end of the second year (Figure 1b). In the fourth year, the number of galled plants was higher for self-rooted plants and those on NAZ1, NAZ2 and NAZ5 compared to NAZ4 (Spota) and NAZ6 (Nazmieh). The survival of scions on NAZ4 (Spota) and NAZ6 (Nazmieh)

rootstocks was 78.5 and 93.2%, respectively, in contrast to 68.2% (NAZ1), 62.5% (NAZ2), 58.6% (NAZ5) and 45% (self-rooted) (Table 6; Figure 1b).

Table 6. Mean comparison on grafting success, healthy and alive vines after 3 years.

Treatments		Success grafting (%)	Healthy vines (%)	Survival vines (%)
Cultivars	Sefid Bidaneh	Owen-rooted	6c	10d
	Sahebi Germiz	Owen-rooted	5bc	9.25d
Grafting vines	(NAZ1) H1/SBC[a]	25c	17b	21c
	(NAZ1) H1/SGC	27c	26b	1905c
	(NAZ2) H2/SBC	44bc	18b	32.25c
	(NAZ2) H2/SGC	56b	28b	32b
	(NAZ3) H3/SBC	64a	29b	31.25b
	(NAZ1) H3/SGC	58b	17b	19.25c
	(NAZ4) Spota/SBC	85a	74a	82.5a
	(NAZ4) Spota/SGC	86a	78a	85.25a
	(NAZ5) H5/SBC	83a	19b	21.25c
	(NAZ5) H5/SGC	75a	12bc	15.5cd
	(NAZ6) Nazmieh/SBC	87a	77a	98.25a
	(NAZ6) Nazmieh/SGC	85a	75a	82.5a
Mean square		348.86*	4148.66*	3778.89**
LSD 5%		28.45	14.275	10.32

Mean values within columns followed by different letters are significantly (P<0.05) different according to LSD test.
SBC = 'Sefid Bidaneh'; SGC = 'Sahebi Germiz'.
*,** significant at P<0.05 and P<0.01 probability levels, respectively.

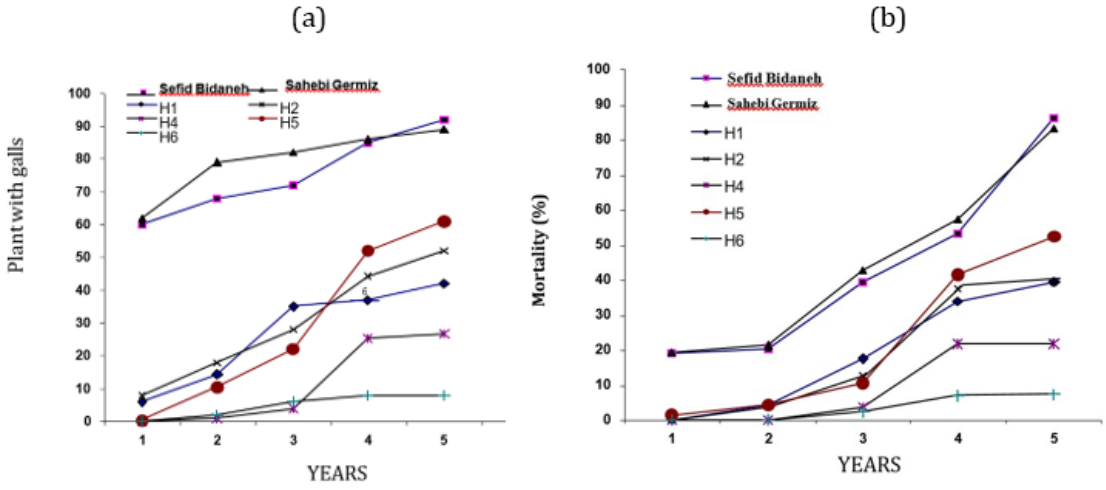


Figure 1. Effects of rootstocks on appearance of gall on the scions after grafting infected scions to healthy rootstocks (a), effects of rootstocks on survival of scions after grafting naturally infected scions to healthy rootstocks (b).

The incidence of gall formation and the death of vines increased in the following years (after the fourth year). It can be hypothesized that either the bacterial number in the vascular tissues was not high enough in the first three years to induce galling (Burr and Katz, 1983), or that the young vines were more resistant than the old ones (Burr et al., 1987; Cleveland and Goodman, 1987). Riparia "Gloire" and some interspecific hybrids, such as Teleki 5C (Süle et al., 1994), 3309C (*Riparia tomentosa* × *Rupestris martin*), 101-14 Mgt, NAZ4 (Spota) and NAZ6

(Nazmieh) 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 (Table 4). 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. At present, one possible explanation is that compounds produced by the rootstock are translocate 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 (Webster et al., 1986). 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.

CONCLUSIONS

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. In conclusion, we recommend that nurserymen should graft their scions onto resistant rootstocks to reduce the risk of crown gall.

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