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In vivo Evaluation of Resistance of Grape Varieties to Crown Gall Disease

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The resistance to crown gall in grape rootstocks was evaluated by inoculating cuttings from 27 grape rootstock varieties with *Agrobacterium vitis* strain Cheonan 493. Tumors were formed in all varieties of grape rootstocks tested in this study. The symptoms were observed in the stems of all plants tested including '196-17' and '41B'. Based on the measurement of tumor weight on the stems of grape rootstocks, '779P' was found to be highly susceptible to crown gall. While some varieties such as 'Gloire', '140R', '101-14M', '3309C', and '333EM' were found to be relatively resistance to crown gall. Varieties such as '99R', '1447P', 'Rupestris du lot', '110R', 'Freedom', and '41B' were also found to be susceptible to crown gall. Other varieties including '1103P', 'Teleki 5C', '420A', 'Golia', and '5BB' were found to be moderately susceptible.

Keywords **:** Grape rootstock, crown gall, *A. vitis*, resistance.

The crown gall disease in grapevines is caused by a bacterial pathogen called *Agrobacterium vitis,* (formerly *A. tumefaciens* biovar 3). The major symptoms of the disease appear on the trunk as white, fleshy, callus-like overgrowths (Ophel and Kerr, 1990). Grapevines infected with *A. vitis* show reduction of vigor with substantial loss of trunks that consequently result in reduced yield worldwide (Burr et al., 1998; Schroth and Mccain, 1988; Sule and Burr, 1998). Park et al. (2000) reported that the incidence of crown gall is high on tetraploid grape cultivars, 'Kyoho', 'Daebong', and 'Black Olympia', while it is low on diploid cultivars, 'Campbell Early' and 'Sheridan' in Korea. *A. vitis*, the causal agent of crown gall, can survive systemically in grapevines and induce galls in injuries caused by twisting and freezing (Lehoczky, 1968; Lehoczky, 1971). The strains of *A. vitis* can survive and remain tumorogenic in vine debris in the soil for years. It induces a localized necrosis instead of causing a typical crown gall in the roots and induces systemic infection of grapevines (Burr et al., 1995).

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Planting *A. vitis*-free grapevines can reduce the possibility of crown gall disease if planted on sites where grapevines were not previously planted. However, once a grapevine is planted in soils infested with *A. vitis*, the vines quickly get infected (Burr and Otten, 1999; Burr et al., 1998). Some cultural practices applied in the control of crown gall in grapevines include vineyard site selection, crop and canopy management, and trunk protection with soil during winter months. The selection of resistant rootstock and scion cultivars can also greatly influence disease development (Goodman et al., 1993; Sule and Burr, 1998). Different research groups have evaluated crown gall susceptibility among *Vitis* germplasms, grape cultivars (Stover et al., 1997; Sule et al., 1994), East-Asian *Vitis* species (Szegedi et al., 1984), and grape rootstocks (Ferreira and van Zyl, 1986; Goodman et al., 1993; Sule and Burr, 1998; Sule et al., 1994).

In Korea, Chung and Shim (1996) isolated the pathogenic *A. vitis* strains from the galls and the sap of the grapevine showing crown gall symptoms in vineyard. Park et al. (2000) surveyed the incidence of crown gall disease on grapevines in the main grape producing regions. However, there was no data showing the screening for resistance against crown gall in grape rootstock varieties. This study reports the inoculation techniques of *A. vitis* strain Cheonan 493 isolated from the infected grapevine and the efficient evaluation system for crown gall resistance in grapevines through previous work (Yun et al., 2003). In this study, we also have evaluated the resistance to crown gall of a variety of grape rootstocks using the pathogen inoculation technique.

Materials and Methods

Agrobacterium strain. *Agrobacterium vitis* Cheonan 493 was gifted by Dr. J. S. Cha from Chungbuk National University. The strain was isolated from infected grapevine stem and was maintained at -80°C in a 30% glycerol stock. Three days prior to inoculation, the pathogen was streaked on a PDA potato dextrose agar (PDA) and grown at 28°C. The single colony was then transferred into a yeast-beef extracts-peptone (YEB) medium (yeast extract 1g, beef extract 5 g, peptone 5 g, sucrose 5 g, $MgSO_4$ 0.5 g/L, pH 7.2) and allowed to grow at 28°C in a shaking incubator (140 rpm) for 18 hours (Sule et al., 1994). The bacterial

Table 1. Varieties of grape rootstocks used in this study

Cultivar	Parentage
Golia	*V. riparia* X *V. rupestris*
5BB	*V. berlandieri* X *V. riparia*
216-3	Solonis X *V. rupestris*
Cosmo 1	*V. berlandieri* X *V. riparia*
41B	*V. vinifera* X *V. berlandieri*
Harmony	Dogridge X 1613C
188-08	Monticola X *V. riparia*
110R	*V. berlandieri* X *V. rupestris*
Rupestris du lot	V. rupestris
225R	*V. berlandieri* X *V. riparia*
Teleki 5C	*V. berlandieri* X *V. riparia*
779P	*V. berlandieri* X *V. rupestris*
Freedom	1613C X Dogridge
44-53M	*V. riparia* X (*V. cordifolia* X *V. rupestris*)
99R	V. berlabdieri X V. rupestris
101-14M	*V. riparia* X *V. rupestris*
1447P	*V. berlandieri* X *V. rupestris*
SO4	*V. berlandieri* X *V. riparia*
140R	*V. berlandieri* X *V. rupestris*
3309C	*V. riparia* X *V. rupestris*
333EM	*V. vinifera* X *V. berlandieri*
8B	*V. berlandieri* X *V. riparia*
1103P	*V. berlandieri* X *V. rupestris*
420A	*V. berlandieri* X *V. riparia*
3306C	*V. riparia* X *V. rupestris*
196-17	1202C X Gloire
Riparia Gloire	V. riparia

cells were collected by centrifugation at 3,000 rpm for 15 min., resuspended in sterile distilled water and adjusted to 10^9 cfu/mL (OD600=1.0) for inoculation.

Plant materials. Grape rootstock varieties for screening the resistance to crown gall and their origins are described in Table 1. Twenty-seven grape rootstock varieties were obtained from *Vitis* germplasm repository at the National Horticultural Research Institute, Suwon in Korea. Dormant cuttings that originated from six- to seven-year old vines without visible symptoms of crown gall were collected in late autumn, stored at 4-5°C, and used for the pathogen inoculation in next spring. Thirty-centimeter-long and 8-10 mm diameter cuttings were used for pathogen inoculation.

Inoculation technique. For the inoculation of grape rootstocks with *A. vitis*, bacterial suspensions (30 μL) were injected into the holes drilled on internodes. Inoculated sites were wrapped with Parafilm tapes and plants were maintained in greenhouse (26°C). Twenty plants were inoculated for each variety along with one set inoculated with sterile distilled as control.

Scoring gall formation. Two months after the inoculation of pathogen, inoculated sites were scored for gall formation. Each variety was scored for the number of inoculated sites that produced the galls as well as the weight of galls. The data was subjected to an analysis of variance. Means were separated using pairwise t-tests.

Results

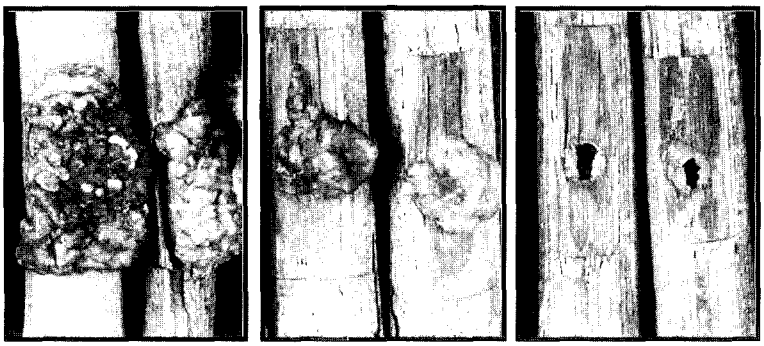
Evaluation by tumor development. The relative levels of crown gall susceptibility were determined in 27 grape rootstock varieties by the number of plants with tumors and the weight of galls formed on the stems of vines. Tumors were formed in all grape rootstock varieties inoculated with pathogen. Tumors were formed at over 60% of inoculated sites in all genotypes except '101-14M' and 'Gloire', and formed at all inoculated sites in two varieties, '196-17' and '41B' (Table 2).

Evaluation by tumor weight. The weight of galls formed on the stems ranged from 4 mg to 137 mg in the rootstock genotypes tested in this study. Based on this data, the response of the different genotypes to the pathogen was distributed evenly. There was a significant difference in the weight of galls between susceptible and resistant rootstock

Table 2. Tumor incidence and weight of galls on grapevines grown for two months after bacterial inoculation (10^9 cfu/mL) of *A. vitis* Cheonan 493

Rootstock varieties	No. of plants Tested	No. of plants with tumor	Weight of tumor (mg)[a]
779P	20	14	138.0
99R	20	16	82.4
1447P	20	18	80.0
Rupestris du lot	20	16	71.2
110R	20	18	64.3
Freedom	20	16	58.0
41B	20	20	53.7
1103P	20	19	41.4
5C	20	14	40.1
420A	20	18	35.7
Golia	20	15	32.4
5BB	20	17	30.2
8B	20	14	28.7
44-53M	20	13	28.1
Harmony	20	18	26.0
Cosmo 1	20	16	24.3
225R	20	16	23.7
196-17	20	14	22.3
SO4	20	14	21.0
216-3	20	16	20.4
188-08	20	16	17.1
3306C	20	14	16.4
333EM	20	13	12.0
3309C	20	16	8.2
101-14M	20	14	6.9
140R	20	09	5.4
Gloire	20	06	4.1

[a]The weight of tumors was measured from the grapevines grown for two months after bacterial inoculation (10^9 cfu/mL) of *A. vitis* Cheonan 493.



779P Golia 140R

Fig. 1. Different response in tumor formation among grape rootstock varieties inoculated with *A. vitis* (10^9 cfu/mL).

varieties (Table 2).

'Gloire' (*V. riparia* selection) and 'Golia', '101-14M', '3306C', '3309C', and '44-53M' (*V. riparia* × *V. rupestris* hybrids) had the smallest galls at inoculated sites. '5BB', 'Cosmo 1', '110R', '225R', '5C', '779P', 'SO4', and '420A' (*V. berlandieri* × *V. rupestris* hybrids and *V. berlandieri* × *V. riparia* hybrids) were susceptible genotypes, forming galls with a weight of 40 mg to 137 mg. Among the rootstock varieties of *V. vinifera* hybrids, '41B' was susceptible, while '333EM' was resistant to the crown gall disease. '779P', synonym of 'Paulsen 775P' and '775P' (Stover et al., 1997), was found to be the most susceptible to crown gall disease among the varieties tested in this study. Some varieties such as '99R', '1447P', 'Rupestris du lot', '110R', 'Freedom', and '41B' were found to be susceptible, while others such as '1103P', 'Teleki 5C', '420A', 'Golia' and '5BB' found to be moderately resistant to crown gall.

Discussion

Pathogen inoculation in this study showed that 'Gloire', '140R', '101-14M', '3309C', and '188-08' were resistant to crown gall and the result is consistent with the report on 'Gloire', '3309C', and '101-14M' as resistant varieties (Stover et al., 1997; Sule and Burr, 1998), and with another on 'Gloire' as the most resistant variety to crown gall (Sule et al., 1994). With '775P', it was reported that it is highly susceptible in this report, and Stover et al. (1997) also reported a similar findings. However, Ferreira and Van Zyl (1986) reported that it was resistant to crown gall.

Other varieties found to be susceptible are '99R', '1447P', 'Rupestris du lot', '110R' and 'Freedom', '1103P', 'Teleki 5C', '420A', 'Golia', and '5BB' were classified to be moderately susceptible. Although '99R' and '110R', which were found to be susceptible by Ferreira and Van Zyl (1986) and Stover et al. (1997), the 'Freedom' and '5BB', which were thought to be resistant (Ferreira and Van Zyl, 1986; Stover et al., 1997) were found to be moderately susceptible to crown gall. Burr et al. (1998) showed that '1103P' is resistant to crown gall disease in Israel, which our result contradicts. 'Teleki 5C', which was classified to be susceptible by Stover et al. (1997), Ferreira and van Zyl, 1986; Goodman et al., 1993; Sule and Burr, 1998 was found to be moderately resistant to crown gall.

There has been a considerable confusion over the identity of grape rootstocks throughout the world and it was recognized that the discrepancy in performance is due to misidentification of germplasm. The discrepancy in the resistance to crown gall can also be caused by the difference in virulence of *A. vitis* or race development in pathogen used for inoculation test in each study. Therefore, to accurately determine resistance in grape rootstocks, it is necessary to identify first the strains and race of *A. vitis* clearly and to maintain grape germplasm systematically.

There was a difference in the level of resistance to crown gall among rootstock varieties that originated from closely related hybrids. Although both '41B' and '333EM' originated from the hybridization of *V. vinifera*, one variety is susceptible, while the other is resistant to the crown gall. Although Szegedi and Kozma (1984) suggested that resistance originated from *V. amurensis* and that the segregation ratio corresponded to 1:1 among the crosses and is 3:1 (resistant to sensitive) among the selfings of resistant parents through the screening for resistance to *A. vitis* of 1,800 seedlings of 27 hybrid families, it is considered that resistance inheritance is controlled by the multiple genes in *Vitis* spp. in the study.

The breeding program for disease-resistant grapevines can help prevent soil-borne disease like crown gall in grapevines and reduce the damage or losses brought about by the disease outbreak. Resistance breeding requires the development of an efficient screening system for disease resistance and the evaluation of disease resistance in germplasm. Results showing the determination of crown gall resistance among grape rootstocks in this study are

considered to be very informative in the breeding program of grape rootstocks which can be adapted and grown in Korea.

References

Burr, T. J., Reid, C. L., Yoshimura, M., Molmol, E. A. and Bazzi, C. 1995. Survival and tumorigenicity of *Agrobacterium vitis* in living and decaying grape roots and canes in soil. *Plant Dis.* 79:677-682.

Burr, T. J., Bazzi, C., Sule, S. and Otten, L. 1998. Crown gall of grape Biology of *Agrobacterium vitis* and the development of disease control strategies. *Plant Dis.* 82:1288-1297.

Burr, T. J. and Otten, L. 1998. Crown gall of grape: Biology and disease management. *Annu. Rev. Phytopathol.* 37:53-80.

Chung, K. J. and Shim, J. S. 1996. Isolation and identification of pathogenic bacteria of grapevine crown gall in Korea. *Korean J. Plant Pathol.* 12:197-201.

Ferreira, J. H. S. and Van Zyl, F. G. H. 1986. Susceptibility of grapevine rootstocks to strains of *Agrobacterium tumefaciens* biovar 3. *South Afr. J. Enol. Viti.* 7:101-104.

Goodman, R. N., Drimm, R. and Frank, M. 1993. The influence of grape rootstocks on the crown gall infection process and tumor development. *Ame. J. Enol. Viti.* 44:22-26.

Lehoczky, J. 1968. Spread of *Agrobacterium tumefaciens* in the vessels of the grapevine after natural infection. *Phytopathol. Z.* 63:239-246.

Lehoczky, J. 1971. Further evidence concerning the systemic spreading of *Agrobacterium tumefaciens* in the vascular system of grapevines. *Vitis* 10:215-221.

Ophel, K. and Kerr, A. 1990. *Agrobacterium vitis*-New species for strains of *Agrobacterium* biovar 3 from grapevine. *Int. J. Syst. Bacteriol.* 40:236-241.

Park, K. H., Jeong, K. S. and Cha, J. S. 2000. Incidence of severe crown gall disease on tetraploid cultivars of grape in Korea. *Plant Pathol. J.* 16:290-293.

Schroth, M. N. and Mccain, A. H. 1988. Reduction in yield and vigor of grapevine caused by crown gall disease. *Plant Dis.* 72:241-246.

Stover, E. W., Swartz, H. J. and Burr, T. J. 1997. Crown gall formation in a diverse collection of *Vitis* genotypes inoculated with *Agrobacterium vitis*. *Am. J. Enol. Vitic.* 48:26-32.

Sule, S., Mozsar, J. and Burr, T. J. 1994. Crown gall resistance of *Vitis* spp. and grapevine rootstocks. *Phytopathology* 84:607-611.

Sule, S. and Burr, T. J. 1998. The effect of resistance of rootstocks to crown gall (*Agrobacterium* spp.) on the susceptibility of scions in grapevine cultivars. *Plant Pathol.* 47:84-88.

Szegidi, E., Korbuly, J. and Koleda, I. 1984. Crown gall resistance in East-Asian *Vitis* species and in their *V. vinifera* hybrids. *Vitis* 23:21-26.

Szegidi, E. and Kozma, R. 1984. Studies on the inheritance of resistance to crown gall disease of grapevine. *Vitis* 23:121-126.

Yun, H. K., Roh, J. H., Park, K. S., Cha, J. S. and Jeong, S. B. 2003. Screening system for crown gall resistance by pathogen inoculation in grapes. *Kor. J. Hort. Sci. Tech.* 21 (in press).