Effect of Necrosis Caused by *Rhizobium vitis* on Graft Take on Widely Grown Grape Cultivars and Rootstocks and Their Reaction to Necrosis

Didem CANİK OREL[1*] Gökhan SÖYLEMEZOĞLU[2]

[1]Ankara Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü Dışkapı Ankara
[2]Ankara Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümü Dışkapı Ankara
[*]Corresponding author: D. Canik Orel, E-mail: dcanik@agri.ankara.edu.tr

Received: 1 December 2018 Accepted for publication: 8 January 2019

ABSTRACT

The effects of necrosis caused by *Rhizobium vitis* (*R. vitis*) on the graft take of some grape cultivars and American grapevine rootstocks commonly grown in our country and necrosis reactions of these cultivar/rootstock combinations were investigated. For this purpose, the performance of three different grape cultivars that is commonly growth as table, wine and dry on four different rootstocks were determined. According to the necrosis capacity on the green grapevine shoots in the *in vitro*, *R. vitis* MG2 strains were selected and inoculated to the graft surface of the cultivars and the rootstocks and basal part of the rootstocks prior to omega graft with a concentration of OD_{600}:10^5 cfu/ml. The results were evaluated after 16 weeks. The most resistant cultivar/rootstock combination was determined as Alphonse Lavallée (AL) / 5BB in terms of graft take and necrosis formation. Sultani Çekirdeksiz (SÇ) is the most susceptible cultivar in terms of graft take and 1613C is the most susceptible rootstock. It was found that 5BB was the least affected rootstock by the necrosis within the combinations and the reactions of 1103P, 110R and 1613C rootstocks to necrosis were found similar. Although root formation was generally observed in all combinations, it was determined that root formation was substantially inhibited in the combinations of AL/110R and SÇ/5BB where the roots formed were necrotic.

Keywords: *Rhizobium vitis*, grapevine, rootstock, graft take, necrosis

Rhizobium vitis'in Neden Olduğu Nekrozun Yaygın Olarak Yetiştirilen Bazı Asma Çeşit ve Anaçlarının Aşı Tutumu Üzerine Etkisi ve Bu Çeşit/Anaç Kombinasyonlarının Nekroza Karşı Reaksiyonları

ÖZ

Rhizobium vitis (*R. vitis*)'in neden olduğu nekrozun ülkemizde yaygın olarak yetiştirilen bazı üzüm çeşitlerinin ve Amerikan asma anaçlarının aşı tutumu üzerindeki etkisi ve bu çeşit/anaç kombinasyonlarının nekroza karşı reaksiyonları araştırılmıştır. Bu amaçla, ülkemizde yaygın olarak yetiştiriciliği yapılan sofralık, şaraplık ve kurutmalık üç farklı üzüm çeşidinin dört farklı anaç üzerindeki performansı belirlenmiştir. Asma sürgünleri üzerinde *in vitro*'da oluşturduğu nekroz kapasitesine göre *R. vitis*'in MG2 izolatı seçilmiş ve OD_{600}: 10^5 hücre/ml konsantrasyonda masa başı omega aşı öncesi çeşit ve anaçların aşı yüzeyi ile anaçların kök bölgesine inokule edilmiştir. Sonuçlar 16 hafta sonra değerlendirilmiştir. Aşı tutumu ve nekroz oluşumu yönüyle en dayanıklı çeşit/anaç kombinasyonu Alphonse Lavallée (AL)/5BB olarak saptanmıştır. Sultani Çekirdeksiz (SÇ)'in aşı tutumu yönüyle en hassas çeşit, 1613C'nin ise en hassas anaç olduğu; 5BB' nin ele alınan kombinasyonlarda nekroz oluşumundan en az etkilenen anaç olduğu, 1103P, 110R ve 1613C anaçlarının nekroza verdikleri reaksiyonların benzer olduğu tespit edilmiştir. Kök oluşumu genel olarak tüm kombinasyonlarda gözlemlenmiş olmakla beraber, oluşan köklerin nekrotik olduğu AL/110R ve SÇ/5BB kombinasyonlarında kök oluşumunun büyük oranda engellendiği saptanmıştır.

Anahtar kelimeler: *Rhizobium vitis,* asma, anaç, aşı tutumu, nekroz

INTRODUCTION

Rhizobium vitis (*R. vitis*) is an important bacterial pathogen of cultivars of *Vitis vinifera;* tumorigenic strains of the pathogen cause crown gall. The bacterium survives systemically and spreads in vegetative plant material and soil. Some of the grafted grapevine plants have poor growth and die after one or a few growing seasons or some do not grow after grafting. It is known that some tumorigenic (T) and non-tumorigenic (NT) strains of *R. vitis* cause not only crown gall, but also necrosis on grapevine tissues (Burr *et al*., 1987; Burr *et al*., 1988). Root decay by the T and NT strains of *R. vitis* as a pathological syndrome was first reported by Burr *et al* (1987). The necrotic lesions caused by *R. vitis* are reminiscent of disease symptoms produced by pectolytic plant pathogens (Collmer and Keen 1986). The bacterium produces *polygalacturonase A* (PG) an enzyme that catalysis the hydrolytic cleavage of picric polymers in plant cell walls and it can be isolated from necrotic lesions on grape roots (McGuaire *et al*., 1991). PG is present in both tumorigenic and non-tumorigenic *R. vitis* indicated that the enzyme is chromosomally encoded (McGuaire *et al.*, 1991). It is reported that a putative polyketide-peptide hybrid compound is produced by *R. vitis* and appears to be acting as a phytotoxin associated with grape necrosis and induction of tobacco HR (Burr and Zheng, 2013).

Restriction mapping and DNA sequence revealed that at least two genes, *tms*1 and *6b*, whose gene products are involved in the synthesis and activity modulation of auxin, are responsible for inducing necrosis (Deng *et al*., 1995). It was reported that some genes are related and essential for grape-spesific necrosis. *Lux*R homologs *avi*R, *avh*R and *avs*R, and an *lys*R-type transcriptional regulator gene, *lhn*R, in F2/5 are involved in regulation of hypersensitive reaction (HR) and necrosis (Hao and Burr, 2006; Hao *et al*., 2005; Zheng *et al*., 2003; 2012). F-avi5813 is involved in biosynthesis of polyketides and non-ribosomal peptides and F-avi3342 encodes a putative non-ribosomal peptide synthases (NRPS) that are required for grape necrosis and tobacco HR (Zheng and Burr, 2013).

Grapevine crown gall leads to poor growth, reduced yield, and possibly the death of infected vines. It occurs worldwide, but it is especially problematic where cold winter temperatures produce freeze injury that provides wounds necessary for disease induction. Grapevines are vegetative propagated and *R. vitis* survives systemically in cuttings, leading to dissemination of the bacteria from old to new vineyards. Grafting is a widely used means of grapevine propagation and growth control that is of considerable importance in the adaptation of important cultivars in different geographic regions. Presence of functional vascular connections is imported for a healthy graft take (Mosse, 1962). Incompatible graft take may cause poor plant growth and the death of the plant in long term (Creasap *et al.,* 2005).

This research is conducted to investigate the effect of necrosis caused by tumorigenic strains of *R. vitis* on graft take and root development of different cultivar/rootstock graft combinations of mainly cultivated grapevine in Turkey.

MATERIAL and METHODS

Selection of Plant Material and *R. vitis* Strain

Grapevine cultivars and rootstocks were selected from widely grown and used in Turkey. Alphonse Lavallée (AL) as table grape, Kalecik Karası (KK) as wine grape and Sultani Çekirdeksiz (SÇ) as the raisin cultivar, 5BB, 1103 P, 110R and1613 C were selected rootstocks for grafting.

Six *R. vitis* strains were selected randomly from the strain collection of our laboratory (Ankara Univeristiy Department of Plant Protection Bacteriology laboratory) which was previously identified as *R. vitis*. The necrosis capacity of the selected strains were characterized as heavy or weak necrotic strain on young grapevine green shoots as previously described by Herlache *et al.* (2001). 2-3 mm diameter young grapevine shoots were surface sterilized with 70% ethanol for 2 min and 0.5% NaOCl for 10 min by shaking and then rinsed well with sterile distilled water (Sdw). Shoots were cut into 0.5 cm pieces and dipped into water agar as 3 replicates for each strain and experiment were repeated twice. The surfaces of the shoots were inoculated with 5 µl of 10^8 cfu/ml (OD_{600} = 0.1) *R. vitis*

inoculum suspension of each strain. Sdw was used as control. Experiment petri dishes were covered with aluminum and incubated at 28°C for 5 days. Necrosis was observed 5 days after inoculation.

Grafting

Selected grape cultivars and rootstocks were inoculated with tumorigenic heavy necrosis strain of *R. vitis* MG2. Selected strain was prepared with the concentrations of about OD_{600}: 10^5 cfu/ml which might be close to the concentration on nature to inoculate grafts. Cultivars were disbudded except for the terminal bud and a graft cut with an omega graft machine was made below the terminal bud. Graft surfaces of both cultivar and rootstock were dipped in *R. vitis* suspensions for 1 hour and then inserted together and coated with grafting wax. To understand the effect of necrosis on root development basal ends of the grafted cuttings were also dipped into the same concentration of the inoculum. Twelve cuttings were used for each cultivar/variety combination and the experiment was repeated twice. Sdw was used as control for each combination. Grafted cuttings were saved in sawdust for stratification in 24°C and 85% humidity for 3 weeks in Richter boxes. Then cuttings were transferred into rooting media, perlite for planting and placed in controlled greenhouse conditions at 24-28°C and 80% humidity. Graft take, root formation and the degree of necrosis were evaluated 16 weeks after inoculation. Graft take was degreed as strong and none according to callus development and the used force to break the graft by hand.

RESULTS and DISCUSSION

Tumorigenic nopalin type *R. vitis* strain MG2 caused heavy necrosis on young green grapevine shoots and were selected as inoculum for the experiment (Figure 1). All the investigated graft combinations were inhibited by *R. vitis* strains in different degrees (Figure 2). Graft take was quite weak and the graft union was sensitive to little force on majority of the combinations. When the graft union of weak cuttings were separated, both cultivar and rootstock sides had tissue necrosis (Figure 3).

When the cultivars used in this study were evaluated, KK/1103P was the highest graft take rate followed by 110R and 5BB, respectively. The lowest graft take rate was observed on the combination of 1613C rootstock with 16.6%. The root development of the KK/1613C combination was the least with 4 rooted cuttings in each repetition. The highest graft take was observed in SÇ cultivar on 1103P and 110R rootstocks with 16.6%. 1613C combinations was follow with a rate 16%. There was not any graft take on the rootstock 5BB. In some cuttings of the SÇ/5BB combination, tissue necrosis was severe and tissue rot was observed on graft union (Figure 4). In these cuttings gall formation was slightly on the graft union and the root development was observed on only three cuttings with necrotic roots.

When the graft union was separated tissue necrosis was observed in different levels. The level of the necrosis in different combinations might be a result of cultivar/rootstock combine resistance against *R. vitis* infection. Same as roots, the gall development caused by *R. vitis* also prevented if the strain is a strong tumorigenic necrosis strain. It was observed gall development both graft union and inoculated basal ends of the cuttings as a result of tumorigenic strain and relatively low inoculum concentration. Higher concentrations of *R. vitis* causes not only causes severe necrosis but also tissue corruption and prevent callus and root formation (Canik Orel and Burr, 2015).

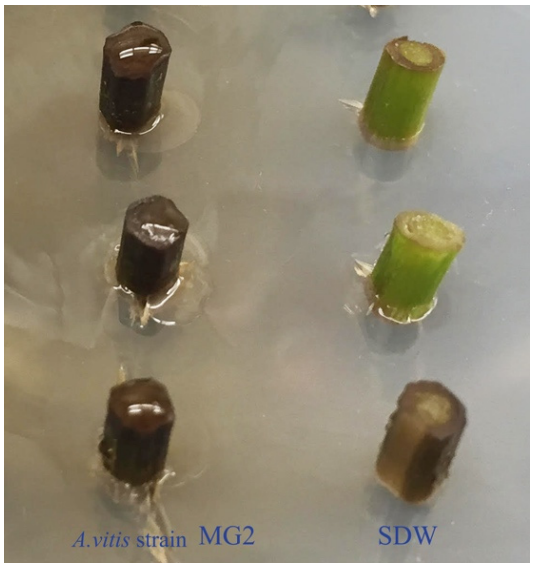

Figure 1. Tissue necrosis assay on green grapevine shoots on water agar and the necrosis caused by *R. vitis*. Left: MG2 as tumorigenic heavy necrosis strain (used in this study), right: water control (Sdw)

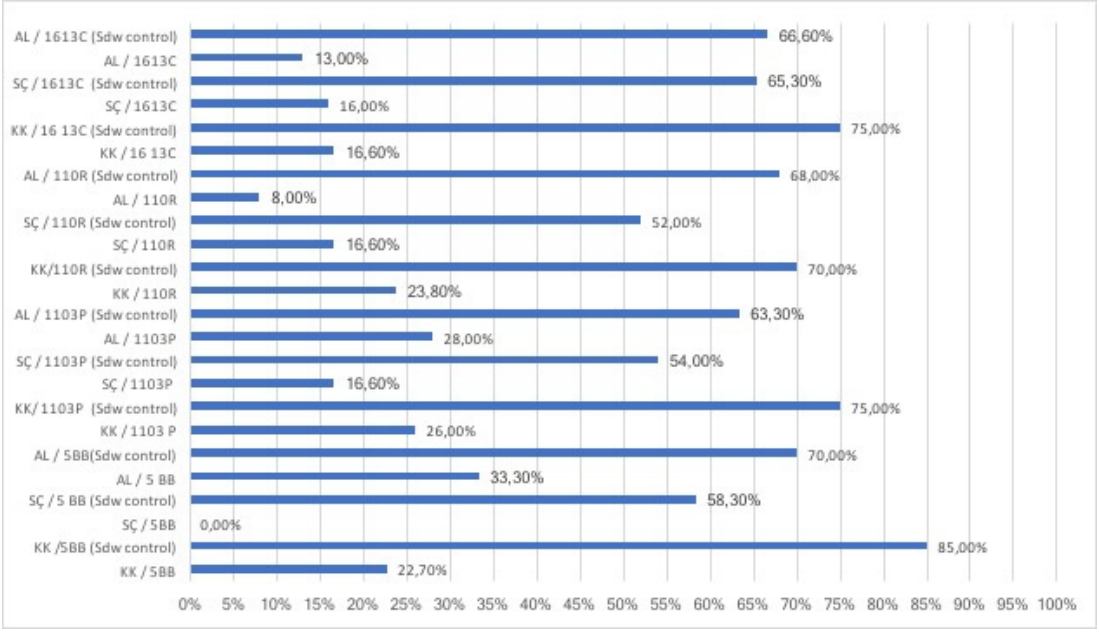

Figure 2. Graft take rates of the investigated graft combinations inoculated with TN strain MG2 of *R. vitis* with the concentration of OD_{600}: 10^5 cfu/ml (AL: Alphonse Lavallée, KK: Kalecik Karası, SÇ: Sultani Çekirdeksiz)

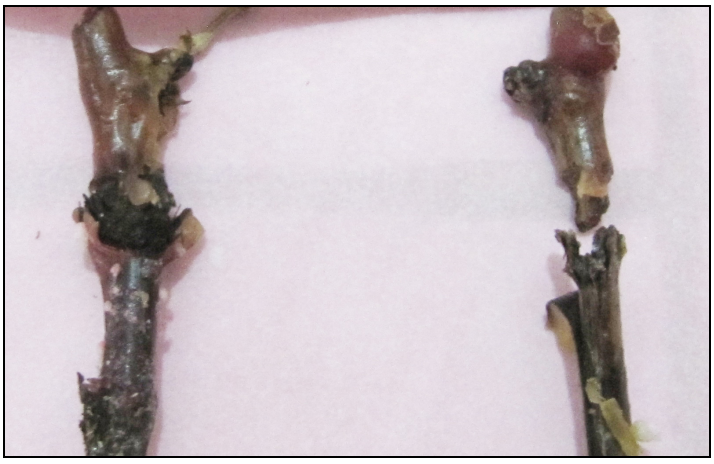

Figure 3. Tissue necrosis and corruption caused by tumorigenic heavy necrosis *R. vitis* strain MG2 at the graft union of KK/5BB combination

Figure 4. Severe necrosis and tissue rot at graft union at AL/1613C combination 16 weeks after inoculation with tumorigenic heavy necrosis *R. vitis* strain MG

AL/5BB combination was the highest graft take rate both within the AL combinations and other grape cultivars used in this study with a rate 33.3%. The tissue necrosis was also the least rate on this combination. This graft take rate was followed by 1103P and 1613C. The least was on the 110R combination with 8% and there were only two rooted cuttings with slightly and necrotic root development. Lehoczky (1968), Bur and Katz (1984), Tarbah and Goldman (1986) have shown that graft scion and rootstock cuttings used for propagation are often systemically infested with the pathogen. Burr *et al*. (1987) was reported necrotic lesions on roots and these black, sunken and dispersed along the root necrosis caused by *R. vitis*. We observed moderate necrosis on roots of majority of the combinations but the quantity of the root hairs was decreased and the root development weaker than Sdw control. When the necrosis gets severe the root development gets weaker.

It was observed that gall development causes graft take like chain between the cultivar and rootstock parts of the cuttings. This chain was a result of gall tissue unity and was not a callus development which is essential for graft take. When the gall tissue was separated, there was not any callus development on both upper and basal part of the cuttings (Figure 5).

Figure 5. The graft union of AL + 1613C combination inoculated with OD_{600}: 10^5 cfu/ml tumorigenic heavy necrosis strain MG2 16 weeks after inoculation. Severe tissue necrosis without callus development and graft take

The root development was observed in majority of the infected cuttings. The root necrosis was moderate on the all experimented cuttings but the intensity and the quantity of the roots was decreased. Some small necrotic areas were also observed on the roots of Sdw treated cuttings but the necrosis was more remarkable on the *R. vitis* inoculated cuttings when they were compared (Figure 6). The less root development was on the AL/110R, SÇ/5BB and KK/1613C combinations with two, three and four rooted cuttings, respectively. *R. vitis* caused root decay was reported as restricted to the roots of grape and Biovar 1 and Biovar 2 did not cause root decay (Burr *et al*., 1988).

Figure 6. Severe root necrosis and decresed roots and basal part of SÇ/1613C (left) combination and water control (right) 16 weeks after inoculation with tumorigenic heavy necrosis strain MG2

It is known that tumorigenic *R. vitis* strains caused gall development on wounded sites. As a great wound on the grafted cuttings inoculated with the tumorigenic heavy necrosis strain MG2 we observed severe tissue necrosis and corruption on the graft union of the most of the grafted cuttings on AL/1613C and SÇ/5BB combinations. If the necrosis severe on the graft union gall development were weak than the more tolerant combinations. In this perspective, it can be said, if the necrosis capacity of the strain is strong and the combination is susceptible, it damages the tissues around graft union and effects the integration of the pathogen to the plant genome to develop gall tissue. There should be a strong and functional vascular connection and a new callus formation between the scion and rootstock for graft take (Pina and Errea, 2005). It can be said that the tissue necrosis phenomena effect the enzyme production to produce callus and vascular connection for graft take. This severe necrosis also caused tissue rot on the graft union at the susceptible combinations. Tumorigenic and non-tumorigenic strains of *R. vitis* prevents graft take and induces necrosis on graft union by causing incompatibility and necrosis development on cambium It was also reported the normal wound healing process is prevented by *R. vitis* infection when the cuttings have *R. vitis* infection prior to grafting (Creasap *et al.*, 2005). Our results are compatible with their findings.

It is basically known that the plant, which is wounded by mechanical or freeze injury releases a chemical which stimulates certain genes in *R. vitis*. The activated genes direct the bacteria to produce a piece of T-DNA, which will be transferred from the bacteria into the plant cell. The T-DNA is incorporated into the DNA of the grapevine plant. The inserted T-DNA directs the plant to produce the hormones auxin and cytokinin which cause the plant cells to grow and divide uncontrolled and rapidly. As it is mentioned on the results, tumorigenic heavy necrosis strain MG2 caused severe necrosis and some rot on the both cultivar and rootstock parts of the grafted cuttings but was not gall formation on graft union. In this case it can be said that, on the wound, the bacteria effects auxin and cytokinin synthesis of the infected plant cell which is essential to produce gall tissue. Canik Orel and Burr (2015) reported that concentration of the bacteria is also important on the infection as much as necrosis capacity of the tumorigenic *R. vitis* strains.

Grafting is an important process to produce grapevine because rootstock can provide adaptation to different soil and environment, tolerance to abiotic factors such as salty or chalky soil, resistance to pests such as the most important soil borne pest *Phylloxera* spp. and some soil borne diseases as well. Graft take is the most important step to have the advantages of the graft. In this study we aimed to investigate how *R. vitis* caused necrosis effects graft take. When all these results were assessed it can be said that the most susceptible grape cultivar was SÇ towards the effect of necrosis caused by tumorigenic heavy necrosis strain of *R. vitis* infection for both tissue necrosis and graft take. AL and KK cultivars were found more tolerant than SÇ. When the study assessed with regard to the rootstocks, graft take was the highest rate on grafted cultivars on 5BB rootstock both inoculated cultivars and Sdw control. Tissue necrosis was also the least rate on the 5BB rootstock combinations. Graft take and root development rate of 5BB was followed by 1103P with a rate 28%. 110R and 1613C were the most susceptible rootstocks towards the effect of necrosis caused by tumorigenic heavy necrosis strain of *R. vitis* infection. Akgül *et al*. (2018) was investigated the sensitivity of most common rootstocks and cultivars to *R. vitis* infection in the Aegean region and reported Ramsey and 1613C as the most tolerant, 410A and 41B as the most susceptible rootstocks. In contrary of their results, we found 1613C as the susceptible rootstock to necrosis caused by *R. vitis*. This difference may cause of the necrosis capacity of the investigated *R. vitis* strains and their experiment was conducted as the inoculation of the cuttings. Similarly, they reported SÇ as the most susceptible cultivar in their study as ours. We reveal that when the cuttings were inoculated by a tumorigenic heavy necrosis strain of *R. vitis* prior to grafting the response of the cuttings to necrosis affect the graft take. A method to measure the graft take of autografted Cabernet Sauvignon cuttings which were inoculated with tumorigenic and non-tumorigenic strains *R. vitis* was reported by Hao *et al*. (2018) by using three-point bending reaction of infected cuttings. They reported the concentration bacterial inoculum and the necrosis capacity of the strain affect the graft take and tissue necrosis in different degrees. When the concentration of the bacteria increase the necrosis caused by *R. vitis* becomes more severe. This severe necrosis also causes severe tissue corruption that is compatible with our results in this study.

Our work reveals the effect of necrosis on graft take on different grapevine cultivar/rootstock combinations. It is clear that *R. vitis* caused necrosis effects the graft take mechanism by preventing or inhibiting some essential compounds for callus development and vascular connection for graft take. Further studies could be done to reveal the changes on essential compounds for graft take which prevented by *R. vitis* caused necrosis.

LITERATURE CITED

Akgül, S.D., Ozyılmaz, U., Onder, S., Celik, S., Oztekin, R.O. and Benlioglu, K. 2018. Susceptibility of grapevine cultivars and rootstocks to crown gall disease (*Rhizobium vitis*) in the Aegean region of Turkey. *Fresenius Environmental Bulletin*. 27(9) 6229-6238.

Burr, T.J. and Katz, B.H. 1984. Grapevine cuttings as potential sites of survival and means of dissemination of *Agrobacterium tumefaciens*. *Plant Disease* 68: 976-978.

Burr, T.J., Bishop, A.L., Katz, B.H., Blanchard, L.M. and Bazzi, C. 1987. A root-specific decay of grapevine caused by *Agrobacterium tumefaciens* and *A. radiobacter* biovar 3. *Phytopathology* 77:1424-1427.

Burr, T.J., Katz, B.H., Bishop, A.L., Meyers, C.A. and Mittak, V.L. 1988. Effect of shoot age and tip culture propagation on grapes on systemic infection by *Agrobacterium tumefaciens* biovar 3. *American Journal of Enology and Viticulture* 39: 67-70.

Canik Orel, D. and Burr, T.J. 2015. Effect of necrosis and crown gall caused by *Agrobacterium viti*s on graft take and root development of grapevine. American Phytopathological Society Annual Meeting, July 31-Aug. 05. 2015, Pasadena, California, USA.

Collmer, E. and Keen, N.T. 1986. The role of pectic enzymes in plant pathogenesis. *Annual Review of Phytopathology*. 24:383-409.

Creasap, J.E., Reid, C.L., Goffinet, M.C., Aloni, R., Ullrich, C. and Burr, T.J. 2005. Effect of wound position, auxin, and *Agrobacterium vitis* strain F2/5 on wound-healing and crown gall in grapevine. *Phytopathology* 95: 362-367.

Deng, W., Pu, X.A., Goodman, R.N., Gordon, M.P. and Nester, E.W. 1995. T-DNA genes responsible for inducing a necrotic response on grape. *Molecular Plant-Microbe Interaction* 8 (4): 538-548.

Hao, L., Kemmenoe, D.J., Canik Orel, D. and Burr, T. 2018. The Impacts of Tumorigenic and Nontumorigenic *Agrobacterium vitis* Strains on Graft Strength and Growth of Grapevines. *Plant Disease* 102(2), 375-381.

Hao, G. and Burr, T.J. 2006. Regulation of long-chain N-acyl-homoserine lactones in *Agrobacterium vitis*. *Journal of Bacteriology* 188:2173-2183.

Hao, G., Zhang, H., Zheng, D. and Burr, T.J. 2005. *luxR* homolog *avhR* in *Agrobacterium vitis* affects the development of a grape-specific necrosis and a tobacco hypersensitive response. *Journal of Bacteriology* 187(1): 185-192.

Herlache, T., Zhang, H., Reid, C., Zheng, D., Basaran, P., Thaker, M., Burr, A. and Burr, T. 2001. Mutations that affect *Agrobacterium vitis* induced grape necrosis also alter its ability to cause a hypersensitive response on tobacco. *Phytopathology* 91: 966-972.

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

McGuire, R., Rodriguez-Palenzuela, P., Collmer, A. and Burr, T.J. 1991. Polygalacturonase production by *Agrobacterium tumefaciens* biovar 3. *Applied Environmental Microbiology* 57(3): 660-664.

Mosse, B. 1962. Graft Incompatibility in Fruit Trees: With Particular Reference to its Underlying Causes. Technical Communication No. 28. Kent: Commonwealth Agricultural Bureaux, 36.

Pina, A. and Errea, P. 2005. A review of new advances in mechanism of graft compatibility-incompatibility. *Scientia Horticulturae* 106: 1-11.

Tarbah, F.A. and Goodman, R.N. 1986. Rapid detection of Agrobacterium tumefaciens in grapevine propagating material and the basis for an efficient indexing system. *Plant Disease* 70: 566-568.

Zheng, D., Zhang, H., Carle, S., Hao, G., Holden, M. and Burr, T. 2003. A *luxR* homolog, *aviR*, in *Agrobacterium vitis* is associated with induction of necrosis on grape and hypersensitive response on tobacco. *Molecular Plant-Microbe Interaction* 16(7): 650-658.

Zheng, D., Hao, G., Cursino, L., Zhang, H., and Burr, T.J. 2012. LhnR and upstream operon LhnABC in *Agrobacterium vitis* regulate the induction of tobacco hypersensitive responses, grape necrosis and swarming motility. *Molecular Plant Pathology* 13:641-652.

Zheng, D. and Burr, T.J. 2013. An Sfp-type PPTase and associated polyketide and non-ribosomal peptide synthases in *Agrobacterium vitis* are essential for induction of tobacco hypersensitive response and grape necrosis. *Molecular Plant-Microbe Interaction* 26:812-822.