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ORIGINAL ARTICLE

Occurrence of Root Gall Disease on Hazelnut (*Corylus avellana* L.) in Iran

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K E Y W O R D S

Agrobacterium tumefaciens;
Corylus avellana;
Hazelnut;
Root gall

A B S T R A C T

Hazelnut (*Corylus avellana* L.), one of the world's major tree nut crops, is widely produced in Iran, with an annual yield of more than 21,500 tons of hazelnuts. A survey of hazelnut orchards in Iran was conducted during 2021-2022, revealing the presence of root tumors on hazelnut trees in Guilan (northern Iran), Mazandaran (northern Iran), Qazvin (northwestern Iran), and Qom (north-central Iran) provinces. Samples of these root tumors were collected for further analysis. Isolations from fresh galls on SNA, D1M, IA, LB, and SC culture media yielded predominant colonies similar to Agrobacterium, which were subsequently purified and characterized. All 29 strains isolated were Gram-negative, rod-shaped, obligate aerobic, motile, and tested positive for oxidase, catalase, arginine dihydrolase, and urease. However, they did not hydrolyze starch, gelatin, or esculin, but did hydrolyze Tween-80 and urea. They exhibited growth on 2% NaCl and at 35°C, and were able to produce 3-ketolactose but not indole, DNase, pectinase, levan, or reduce nitrate. Furthermore, 11 representative strains were randomly selected for PCR amplifications of T-DNA genes using specific primers At1/At2, F8360/F8361, and tms2F1/tms2R2, resulting in 338, 453, and 617 bp amplicons, respectively. In pathogenicity tests, bacterial strains were inoculated into tomato, hazelnut, sunflower seedlings, and carrot discs, leading to the formation of tumors on plant stems and callus on carrot discs. Based on phenotypic, physiological, and biochemical properties, pathogenicity tests, and molecular methods, the bacterium responsible for causing root gall in hazelnut trees was identified as *A. tumefaciens* biovar 1. This bacterial root gall appears to be widespread among various local hazelnut cultivars (Gerd-e-Eshkavar, Alamout, Tarom, etc.), with a higher prevalence in old hazelnut orchards with stony soils. This study represents the first report of root gall on hazelnut trees grown in Iran. It was observed that the number of sites where tumors have developed and the number of galls present in old hazelnut orchards with stony soils are higher.

Introduction

Hazelnut (*Corylus avellana* L.), is the common name for the flowering plant genus *Corylus*, which is usually classified in the Betulaceae family. However, some botanists consider it to be a distict family, Corylaceae (Cotini *et al.*, 2011). Hazelnut is a tree that is deciduous and native to Europe and Asia, where it is commonly seen as an understory species in mixed forests. Hazelnut is the fifth most important

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tree nut in the world, with a total cultivated area of about 1,027,000 ha and a global production of 1.1 million metric tons (FAO 2020). Iran, with about 25,500 ha of hazelnut growing area and annual production of about 21,550 tons is the seventh largest hazelnut producer in the world. Guilan (Northern Iran), Mazandaran (Northern Iran, 1,400 ha), Qazvin (Northwestern Iran, 3,000 ha), Ardabil (Northeastern Iran, 315 ha), Zanjan (Northwestern Iran, 400 ha), and Qom (north-central Iran, 170 ha) provinces are five regions where hazelnut are grown in Iran (Salimi and Hoseinova, 2012). Eshkevarat region (including Roudsar, Amlash, and Syahkal counties, Guilan province) with high rainfall and relative humidity and 73% of Iran's total hazelnut production is the major producer of the country producing about 15,300 tons and occupying an area of about 16,000 hectares.

Anomalies caused by biotic and abiotic (water stress and salinity) factors may be limiting factors for the growth and development of nut trees such as in hazelnut production areas in the Guilan province (Arab *et al.*, 2020; Houshyarfard 2020; Akca and Sahin, 2022; Vahdati *et al.*, 2023). The expanding trend of establishing hazelnut orchard, and hazelnut growing in sloping lands, and renovating traditional hazelnut orchards in Guilan province made the need for research on the limiting factors of hazelnut production, including fungi, bacteria, and root nematodes. Die-back, slow growth, loss of vigor, decline of the hazelnut trees, and reduction of yield can be attributed to the galls on the hazelnut roots(Houshyarfard, unpublished data). *Agrobacterium tumefaciens*, that affect the hazelnut (*C. avellana*) crown and roots has been reported (Reed *et al*., 1998; Pscheidt and Stone 2001; Guerrero *et al*. 2012; Sheikh Beig Goharrizi *et al.*, 2016). It was declared that, the main cause of the spread is primarily due to the commercialization of diseased plants and cutting jobs as manual clearings of weeds and branches (Figs. 1 & 2).

Fig. 1. Aerial symptoms of a hazelnut trees with root gall infection including chlorosis, loss of vigour, die-back, and decline due to obstruction of water and nutrients transport.

Fig. 2. Galls on the roots of hazelnuts from Guilan province, root galls frequently appear along the roots and near the soil line. Galls could consist of irregular phloem tissue or deformed cells.

The family *Rhizobiaceae* (order *Rhizobiales*) of the *Alphaproteobacteria* containsbacteria that areassociated with plants and have significant rolesin both ecology and agriculture. Many strains that are

designated as *Agrobacterium* are plant pathogens within *Rhizobiaceae*. *Agrobacterium tumefaciens* is a Gram-negative bacterium. *A. tumefaciens* causes crown gall on dicotyledonous plants, such as nut trees and, stone fruit; *Agrobacterium* strains are categorized into three biovars based on their physiological and biochemical properties. Research has revealed that after the introduction of the bacteria into the plant tissue and its T-DNA incorporation into the plant genome, there is no longer a need for the presence of bacterial cells to develop and grow gall (Fortin *et al*., 1983; Agrios, 1988; Webster *et al*., 1988). There are very few publications about *Agrobacterium* on hazelnut. Also, there is no accurate information about the impact and causal agent of root gall-like disorder on hazelnut, as well as its extent of distribution and damage in the world.

To know phytosanitary aspects of the hazelnut commercial orchards according to different regions and climatic conditions is a basic and fundamental aspect in decision taking in a context of phythosanitary integrated plant management.

Materials and Methods

Sampling regions

Guilan (northern Iran) is the site of implementation and the hazelnut orchards of Mazandaran (northern Iran), Qazvin (northwest of Iran), and Ardabil (northwest of Iran) provinces, in addition to Guilan, are only considered as hazelnut root gall sampling regions. During 2021-2022, the hazelnut orchards of Guilan, Qazvin, Mazandaran, Zanjan, Ardabil, and Qom provinces were surveyed and the roots with gall (tumor) symptoms were collected. Samples were immediately transferred to the laboratory.

Determining the percentage of infected trees

To determine the percentage of orchards and/or hazelnut trees with root galls, we move along the width of the infected orchards and randomly select 30 trees and examine their root system for the presence or absence of symptoms of gall-like disorders on the roots. The number of trees with galls (tumors) on the their roots and aerial symptoms such as decline and die-back are recorded and marked. Then the infected trees (%) in each orchard is calculated from the relation:

$$\text{Infected trees} = \frac{\text{Number of infected trees}}{\text{Total number of trees}} \times 100$$

Laboratory studies

Plant samples

Root gall samples were collected from hazelnut orchards in Guilan, Mazandaran, Qazvin, and Qom provinces (Table 1). At least five samples of roots with galls are collected and placed in plastic bags. After recording their characteristics, they are transferred to the laboratory. The samples, galls of samples were stored in a refrigerator to reduce contamination until gall extraction. The galls were cut out from the roots with sharp knives dipped in 70% ethanol, and placed in sterile containers containing sterilized distilled water (SDW) (Moore, 1988).

Gall extraction

Samples were rinsed with tap water to remove soil and hazardous materials. Galls were surface sterilized with 2% NaOCl for 30 min by soaking the galls. After washing galls with SDW three times, They were finely chopped, immersed in distilled water, and incubated for an overnight period at room temperature.

Isolation and purification of Agrobacterium isolates from root and sap

Some of the prepared extracts were added to the selective and differential culture media plates including of Nutrient agar + 1% Yeast extract (YNA, Sigma-Aldrich), sucrose nutrient agar (SNA, Merck) D1M agar contained (per liter) 5 g of cellobiose, 3 g of K_2HPO_4, 1 g of NaH_2PO_4, 1 g of NH_4Cl, 0.3 g of

$MgSO_4 \cdot 7H_2O$, 10 mg of malachite green, and 15 g of agar, LB IA (lysogeny broth, alternatively known as Luria-Bertani media or Luria Broth, ibresco co.), SC agar (synthetic complete agar, Merck), YMA (yeast mannitol agar, Fluka), and MacConkey agar (Sigma-Aldrich) (Moore *et al*., 1988; Moore, 1989; Schaad *et al*., 2001). The culture plates are incubated at 25–28°C for 3-7 days. Pure cultures were obtained by adopting subculture method. The purified cells were maintained on YMA that contained 0.5% $CaCO_3$ at 4°C.

Pathogenicity test

Tumor forming ability of the 29 *Agrobacterium* isolates was tested on seedlings of tomato (*Lycopersicon esculentum L*.), hazelnut (*C. avellana*), and sunflower (*Helianthus annus* L.) plants under greenhouse conditions using stem-wound inoculation method (Ridé *et al*., 2001; Mafakheri *et al*., 2019). First, the inoculation site was disinfected with 96% ethyl alchol, and then with a strile scalpel, a wound of 1-2 mm depth was created in the stem of test plants. A fresh bacterial culture was placed on the wound, then wet cotton and parafilm were used to cover the area. The control plants were also inoculated with SDW (- control), and a standard strain of *A. tumefaciens* (+ control) (Fahy and Peresley, 1983; Schaad, 1988). The inoculated plants were examined for the appearance of young galls (tumors) after 21-28 days. No symptoms developed on plants with negative control (- control). The bacteria were re-isolated from inoculated plants.

Carrot disc test

Pathogenicity of the strains was tested on sterilized 5 mm carrot (*Daucus carota*) root discs, by needle inoculation of 100 µl bacterial suspensions ca. 1×10^9 CFU ml^{-1} in sterile petri plates (Ridé *et al*., 2001; Gasky *et al*., 1980). After 1-2 weeks of incubation at 27±1°C temperature and dark conditions, the carrot discs are checked for the appearance of microtumors (MTs). The Agrobacterium strains were demonstrated to be virulent by producing MTs in the center of carrot pieces.

Identification of bacterial strain(s)

Morphological, physiological and biochemical tests

The bacterial strains were identified using appearance characteristics, phenotype, biochemical, physiological tests, and PCR analyses (Schaad *et al*., 2001; Moore *et al*., 1988; Bernaerts and de Ley, 1963).

PCR tests using specific primers

Representative bacterial strains whose pathogenicity was confirmed on test plants such as tomato seedlings and carrot discs, polymerase chain reaction (PCR) were used to proving the genus *Agrobacterium* and presence of Tumor inducing plasmid (Ti plasmid). The evaluation of the T-DNA gene fragment amplifications was conducted through PCR method using the specific primers At1/At2 (Pulawska and sobiczewski, 2005; Hass *et al*., 1995), F8360/F 8361 (Shams *et al*., 2013), and tms2F1/tms2R2 (Mafakheri *et al*., 2019) that amplify DNA fragments of 338 bp, 453 bp and, 617 bp, respectively. The reference strain *A. tumefaciens* and sterile distilled water were used as positive and negative controls, respectively. At the end, the agarose gel was stained with DNA Green Safe Stain for 30 minutes, and it was evaluated in the gel documentation device (Bio Rad Co.) under UV light with a wavelength of 364 nm (Cubero *et* al. 1999; Mazzola *et al*., 1992). In each set of electrophoresis, along with the main samples and + control and - control, a DNA marker with a molecular weight of 100 bp was used. All bands at positive control level were deemed to be positive treatments.

Results

Distribution of root gall disease

In this study, we attempted to isolate *A. tumefaciens* strains from the root galls of hazelnut

trees. In the summer of 2021 and 2022, nearly 5-20% of >15-year-old hazelnut trees (*C. avellana*) in 47 commercial orchards in four regions of Iran, namely Guilan, Qazvin, Mazandaran, and Qom were observed with root gall symptoms (Table 1). Galls, or tumors, were frequently found on the roots of trees at or just below the soil surface. The disease caused by root gall disease affected the majority of hazelnut local cultivars (Gerd-e-Eshkavar, Tarom, Alamut, Zar Abadi, etc.) in the surveyed areas, and no differences were observed among different hazelnut local cultivars. To our knowledge, Guilan (Eshkevarat) is where the hazelnut cuttings in all orchards came from.It's possible that, the pathogen was introduced into hazelnut growing areas of Iran with the cuttings. In Guilan, hazelnut orchards in Roudsar (15-20%), Amlash (10%), and Syahkal (4%) had higher root gall incidences, respectively, compared to all other regions. .Galls are most frequently found on the hazelnut roots. Hazelnut root galls are scattered along the entire length of the roots in different sizes and shapes. At first, the galls appear light-colored and spongy. As the galls mature, they turn dark brown and woody. Older trees and orchards with a more stony soil texture had a higher incidence of hazelnut root galls. The size of root galls increased from the end of the root towards the soil surface, and had woodier textures.

Isolation of causal agent of root gall

In the present research, the causal *Agrobacterium* strains were isolated from a number of galls. The causal *Agrobacterium* strain was not isolated in several root gall samples, so it seems that they did not contain the bacterium.Twenty-nine *Agrobacterium* strains were isolated on SNA, IA, LB, and SC culture media and pathogenicity tests were performed.

Pathogenicity of bacterial strains

The results of this research revealed *Agrobacterium* strains were different in their pathogenicity on two week old tomato plants, and carrot root discs. So, some of the bacterial strains produced tumors only on tomato and/or on hazelnuts and/or tomato or sunflower plants. (Fig. 3).

Biochemical and phenotypic characteristics of Agrobacterium strains

The isolated strains were confirmed as *Agrobacterium* using morphological, biochemical, physiological analyses; and pathogenicity tests. All pathogenic *Agrobacterium* strains were Gram (-ve), rod shape, obligate aerobic, motile, oxidase, catalase, arginine dihydrolase, and urease positive. Strains didn't hydrolyze starch, gelatin and esculin but hydrolyzed Tween-80 and urea. They grew on 2% NaCl and at 35°C, and were able to produce 3-ketolactose. None were capable of producing a fluorescent pigment on King's B medium nor were able to reduce nitrate, and produce indole, levan, H_2S from cysteine and peptone. Strains didn't use citrate, L-lysine, or glucose in anaerobic conditions. Although, sometimes *Agrobacterium* strains reacted differently to a specific antibiotic,the antibiotics Kanamycin, Gentamicin, and Streptomycin remained effective in preventing bacterial growth.

Molecular identification

Electrophoresis of PCR products using two pairs of specific primers showed that, these primers were able to amplify fragments of 453 bp and 617 bp. The amplification of 453 bp DNA fragment using primer pair F8360/F8361, confirming them as *Agrobacterium* sp. (Shams *et al.*, 2013). Also, amplification of 617 bp DNA fragment in 6 bacterial strains using the primer pairs tms2F1/tms2R2 revealed these strains were carrying the Ti-plasmid. The bands formed on the gel completely corresponded to the bands of the positive control (standard strain of *A. tumefaciens*) (Fig. 4).

Table 1. Studied area of hazelnut plantings.

Province	Sampling site (infected orchard)	Hazelnut region (District)	Infected tree (galls on the roots)
Guilan	1) Kojid,Estakhr Sar, Dimajan Kesh, Somam, Garmabsar, Taresh, Shooleh, Matla Kooh	Eshkavarat:	+
	2) Lima Govabar, Sajiran, Niloo, Kakrood, Jir Kol, Tiola, Lasboo, Sharm Dasht	1)Amlash 2)Rudsar 3)Siahkal	+
	3)Pirkooh		+
Qazvin	Hir, Talator,Viar,Por Rud, Akoo Jan, Yarud	Alamut-e Gharbi (Rudbar-e Shahrestan)	+
	Zar Abad, Zavar Dasht	Alamut	+
Mazandaran	Do Hezar	Tonekabon Ramsar	+
Zanjan	Shirmisheh	Tarom county (Chavarzaq)	-
Ardabil	Fandoghloo	Fandoghloo jungle	_
Qom	Veshnaveh	Kahak	+
	Ghahan	Khalajestan	+

Fig. 3. Pathogenicity tests. Formation of galls on tomato stem three weeks after inoculation (left); Formation of callus on carrot disc (right) as a result of inoculation with root gall Agrobacterium strain.

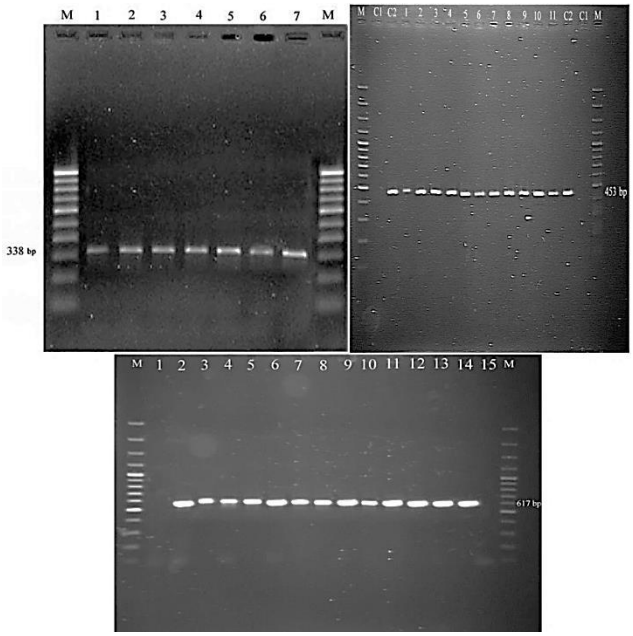

Fig. 4. Agarose gel electrophoresis of PCR products. Electrophoresis of PCR products of *Agrobacterium tumefaciens* with specific primers At1 and At2; M, 100 bp DNA marker; Lane 7 is positive control (standard strain *A. tumefaciens*) showing the amplification of 318-bp DNA fragment in length; Lanes 1 to 6, strains of *Agrobacterium tumefaciens* isolated from hazelnut (up-left), Electrophoresis of PCR products of *Agrobacterium* sp. with primer pair F8360/F8361; M, 100 bp DNA marker; Lane C1 is negative control (distilled water); Lane C2 is positive control (standard strain *A. tumefaciens*) showing the amplification of 453-bp DNA fragment in length; Lane 1 to 11, strains of *Agrobacterium* sp. isolated from hazelnut (up-right); Electrophoresis of PCR products of *A. tumefaciens* with primer pair tms2F1/tms2F1; M, 100 bp DNA marker; Lanes 1and 15 are negative controls (distilled water); Lanes 2 and 14 are positive controls (standard strain *A. tumefaciens*) showing the amplification of the DNA fragment approximately 617-bp in length; Lanes 3 to 13, strains of *A. tumefaciens* were carrying the Ti-Plasmid, isolated from hazelnut (down).

Discussion

During a survey of the hazelnut orchards in Guilan, Qazvin and Mazandaran provinces, it was found that hazelnut trees had gall-like disorders on their roots that were 1-15 cm in diameter. These galls were almost spherical, and initially soft, and fleshy, and the large galls became dry and wooden. Large numbers of small galls appeared on the ends of the roots.

Orchardists reported that the yield of hazelnuts was somewhat lower than before. Infected trees didn't appear relatively normal. Low growth, yield reduction, die-back, chlorosis and general weakness and etc. are symptoms of root infection that it's not easily distinguishablefrom other pathogenic factors, like root rot pathogens. Freshness of tumors, presence of Agrobacterium in the gall, concentration of Agrobacterium in the root tissue, andsynthetic culture media are factors that influenced the success of isolations (Sigee, 1993).

Within 4-7 days after culturing of root gall extract on 1A culture medium, *A. tumefaciens* (biovar 1) colonies grew well with a white border, and brown center. The use of 1A culture medium was introduced by Schaad et al. (2001) to isolate and diagnose *A. tumefaciens* (biovar 1) rapidly. Also, the predominant bacterial colonies similar to *Agrobacterium* on sucrose nutrient agar (SNA) and D1M plates were isolated 2–3 days after incubation at 25–28 °C. The D1 culture medium has been introduced as a selective culture medium for differentiating *Agrobacterium* from *Acidovorax*, *Burkholderia*, and *Ralstonia* (Kado and Heskett, 1970; Schaad 2001), usually *A. tumefaciens* bacterium grow as round and convex colonies with a metallic green color on this medium. Additionally, other bacteria with similar characteristics grew on the D1 medium as well. Therefore, it is not possible to distinguish *A. tumefaciens* based only on the color of the colony on this culture medium. The *A. tumefacience* Biovar I could be identified by using selective culture media LB, 1A, and SC (Moore *et al*., 2001). All the bacterial strains made Litmus milk to alkaline, but the color change and formation of sediment by the strains in this test were different. This indicated that *Agrobaterium* strains were not homogeneous and differed in various tests.

The results of morphological, biochemical, and nutritional tests on *Agrobacterium* strains, obtained from hazelnut trees, were confirmed using PCR method, and specific primers. PCR primer pair F8360/F8361 amplifying a 453 bp DNA fragment in the recA gene and confirmed as *Agrobacterium* sp. (Shams *et al*., 2013). The PCR test yielded results that were comparable to those obtained using common and traditional methods, including culture isolation, biochemical, and physiological characteristics.. It was confirmed that, the *Agrobacterium* (*Rhizobium*) *radiobacter* (*Agrobacterium tumefaciens*). biovar 1 associated with root gall disease in hazelnut growing areas of Iran. The isolation and characterization of *A. tumefaciens* biovar 1 strains from root gall of hazelnut have not been carried out worldwide. The *A. tumefaciens* biovar 1,causal agent of root bacterial gall disease of hazelnut (*C. avellana*), hasn't been previously reported from Iran. This appears to be the first report on the natural occurrence of root gall disease on hazelnuts and the first record of a hazelnut disease caused by *A.tumefaciens* biovar 1.

The *A.tumefaciens* biovar 1 has a wide host range and produces galls in several plants (Thamashow, *et al*., 1980; Loper and Kado, 1979). The *A. tumefaciens* biovar 1 was introduced as the causal agent of bacterial root and crown gall on cherry, plum, peach and nectarine trees (Marefat, 2000). *A. tumefaciens* biovar 1 and *A. vitis* were identified as the causal agent of grapevine (*Vitis vinifera* L.) gall in Qazvin province (Salehi *et al*. 2004).A. tumefaciens host range encompasses 650 species from 90 plant families included herbaceous and dicotyledonous plants (Snare, 2006; Furuya *et al*., 2004; Niknejad, 2002; Ogawa *et al*., 1995; Tamashow *et al*., 1980; Loper and Kado, 1979).

s. The pathogen name has been under dispute for decades, and the pathogen traditionally known to cause crown gall in most plants is *A. tumefaciens* (*Rhizobium radiobacter*) (Flores-Flix *et al*., 2020; Young *et al*., 2003). It is known that *A. tumefaciens* is a diverse group of 11 different genomospecies.

All of the *A. tumefaciens* strains were positive for tumor-forming ability in pathogenicity tests on carrot discs. It is well-known that the pTi plasmid, which contains vir genes, is responsible for the pathogenicity of virulent *A. tumerfaciens*. (Gohlke *et al*., 2014). This result showed that one or two plants should not be sufficient to prove the virulence of *A. tumefaciens* isolates. In the past, the sucker emissions were considered of economic value as they were functional to the gradual renewal of the hazelnut orchards and they allowed on-farm supply self-rooted plants to be used to establish new hazelnut orchards (Koksal *et al*., 2008; Cristofori *et al*., 2014; Rovira *et al*., 2014). Isolation of the *A. tumefaciens*from the vascular sap of hazelnut roots with gall symptoms showed that the vascular system of the hazelnut contains bacterial cell, which can also infect the suckers. It was assumed that the suckers of hazelnut trees that are used to propagate the plants, from the hazelnut trees affected by root gall disease are systemically infected to *A. tumefaciens*. It is important to consider systematic infections in several cultivated hazelnut trees when designing strategies for controlling root gall disease.. Therefore, one should avoidance from preparing seedlings and/or cuttings of infected hazelnut trees to root gall for establishing new hazelnut orchards.

Wounds are the primary location of *A. tumefaciens* entry into the host plant. Wounds are caused by the emergence of lateral roots, frost injury, insect and nematode feeding(Agrios 2005; Creasap *et al*., 2005). In recent years, root-chewing insects such as *Melolontha melolontha* L. (Coleoptera, Scarabaeidae) is among the pest insects damaging hazelnut (Froschle 1994; Luisa and Mauro 1996; Vlug 1996; Sezen 2004). The pest's larvae of various ages settle and consume nutrients from host plants' roots in the soil., which may lead to the cutting of a part of the root, general weakness, and ultimately the death of the hazelnut tree. *M. melolontha* is a pest that has the potential to play a more significant role in the root gall disease complex. Several factors including Ti plasmid, bacterial chromosomal background and host genetic diversity are involved in gall disease occurrence (Zhu *et al*., 2000; Knauf *et al*., 1982; Perry and Kado, 1982). *Agrobacterium* enters the roots from the wound site due to damage to the root colonizes the wound, attaches firmly to damaged plant cells, and transfers part of its DNA into the DNA of the plant cells (De Costa *et al*., 2001). In the later growing season, latent infections often develop into galls. Pathogenic

A. tumefaciens can be shed from the gall into the surrounding soil or water where they colonize or infect new plant tissues (De Costa *et al*., 2001).

Conclusions

In recent years, the cultivation of hazelnut (*Corylus avellana*) has expanded in several areas of Iran. The *A. tumefaciens* biovar 1 was identified and characterized through the use of multiple morphological, physiological, biochemical, and phytopathogenic tests, as well as molecular analysis. *A. tumefaciens*-induced bacterial disease poses a significant threat to hazelnut farmers.To clarify the economic impact of *A. tumefaciens* on the hazelnut industry throughout the country, further surveys and samplings are needed. Hazelnut root gall disease, caused by *A. tumefaciens*, is easy to identify based on symptomology alone. The control of root gall disease is through preventive actions especially the selection of plants free of galls. Integrated management measures for bacterial plant pathogens include resistant cultivars, bacteria-free propagation materials, preventing wounds that permit the entrance of bacteria into the inner tissues, and propagating only bacteria-free nursery stock. We found the role of systemic *A. tumefaciens* populations in root **g**all **i**ncidence on the hazelnut seedlings

obtained from infected hazelnut trees. Probably, infected plant suckers from infected trees and/or soil and humidity play an important role in the transmission of pathogen causing the root gall throughout the hazelnut production regions. Systemic movement of *A. tumefaciens* in symptomless sucker and/or cutting tissue of hazelnut is a complex phenomenon. PCR was the most efficient technique for detecting the movement of *Agrobacterium* spp. within the plants.

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Conflict of interests

The author declare no conflict of interest.

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