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SHORT COMMUNICATION

Determination of tolerance to *Erwinia amylovora* of intergeneric hybrid genus: *Cydomalus*

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Abstract

A greater genetic diversity is essential for disease resistance in particular, and intergeneric hybridization makes this possible. The genus *Cydomalus* (syn: *Cydolus*), which is frequently used as rootstock, was derived from intergeneric hybridization between *Cydonia oblonga* Mill. and *Malus domestica* Borkh in Moldova and Bulgaria. It is known that hybrids from this genus exhibit quince and/or apple characteristics and some of them have been identified with reddish fruit skin color in Türkiye in the last decade. *Erwinia amylovora* Burril. is the most devastating bacterial disease in the Rosaceae family, and there have been no reports of *Cydomalus.* In this study, fire blight susceptibility index of three *Cydomalus* genotypes, were collected from İzmir and Manisa provinces, and two important quince rootstocks were determined by artificial inoculations with the most virulent *Erwinia amylovora* strain of our collection, under greenhouse conditions for two consecutive years. In addition, susceptibility levels of two *Cydomalus* genotypes were observed under natural disease pressure habitats. In line with the results of artificial inoculations, two of *Cydomalus* genotypes were determined to be more tolerant to fire blight disease than quince rootstocks and these genotypes could be used in breeding studies. Moreover, *Cydomalus* genus was identified as the new host for *Erwinia amylovora.*

Keywords Red quince · Fire blight disease · Selection breeding · Rootstock

Fire blight caused by *Erwinia amylovora* Burril. is a disease that mostly affects plants of the Rosaceae family with complicated and rapidly developing pathogenicity. Especially in *Malus* spp. and *Pyrus* spp. molecular mechanisms of disease are well understood, but management remains challenging because it is controlled by multiple genes and influenced by environmental factors (Norelli et al. 2001; Sobiczewski et al. 2015; Bell 2019; Pompili et al. 2019). Studies have identified quantitative trait loci (QTLs) in various apple and pear progenies, suggesting resistance genes may be present in less susceptible parents (Calenge et al. 2005; Emeriewen et al. 2014; Bell 2019; Kostick et al. 2021). Furthermore, transgenic lines were generated using the CRISPR/Cas9-FLP/FRT-based gene editing system, resulting in the knockout of the susceptibility protein "MdDIPM4" in susceptible *Malus domestica* varieties. This suggests increased resistance and the possibility of transgenic apple varieties (Pompili et al. 2019; Peil et al. 2021). In the quince (*Cydonia oblonga* Miller.), the beginning of studies on the determination of the resistance status of genotypes, the inheritance of the disease and its connections with some morphological features date back to the recent past and molecular studies have not been done yet (Abdollahi et al. 2008; Ozrenk et al. 2012; Şahin 2017; Şahin et al. 2019a, b, 2020b).

Although the mechanism of fire blight resistance varies from species to species, it is generally controlled synergistically by multiple genes, which complicates breeding studies (Korban et al. 1988; Evrenosoğlu et al. 2020; Şahin et al. 2020b). Some breeders focus on hybridization breeding not only within species but also between species in order to obtain individuals with higher resistance (Van der Zwet et al. 1974; Gardner et al. 1980; Korban et al. 1988; Andreies 2002; Bokszczanin et al. 2012; Evrenosoğlu et al. 2020; Şahin et al. 2020b). The most significant source of genetic diversity for creating new varieties, which may be exploited to create unique morphological forms and mix traits from

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two genera, is interspecific hybridization (Kamiri et al. 2018).

When reviewing intergeneric hybridization researches on pome fruit species, numerous studies are found, primarily focusing on hybrids between *Malus x Pyrus* and *Pyrus x Cydonia*. Some of these hybrids have been subjected to testing for fire blight disease and their efficacy in breeding studies has been determined (Trabut 1916; Rudenko and Rotaru 1970; Inoue et al. 2003; Postman 2011; Fischer et al. 2014; Pasqualetto et al. 2023). In addition to these, *Cydomalus (syn: Cydolus)* genus is another important intergeneric hybridization that was obtained by crossing between *Cydonia* x *Malus*, which is known for its use as rootstock in both quince and apple species (De Paoli et al. 2002; Wertheim 2002). The breeding studies between *Cydonia* x *Malus* and *Malus* x *Cydonia* were started by Burbank in the 1950s (Burbank 1955) and then continued with Ryabov (1970). The hybrids obtained in the 1950s were determined to be sterile, and in the results of the study conducted in 1970, hybrids with weak plant growth and quince phenotypic characteristics were developed (Burbank 1955; Ryabov 1970). The most successful results were obtained from the studies conducted by Rudenko (1970) and Rudenko and Rudenko (1994) in Moldova and Bulgaria in which 47 hybrids were obtained at different ploidy levels. Morphological features and ploidy levels of hybrids in F_1, F_2 and F_3 levels were determined and it was observed that all individuals in these levels were allodiploid, allotriploid, and allotetraploid, respectivelty (Rudenko and Rudenko 1994). In this study, also Rudenko and Rudenko (1994) published some characteristics of these hybrids and mentioned that the fruit skin color turned pink in a sun-exposed part of the fruits of a hybrid determined as allotetraploid at the F_2 level. Interestingly, the existence of *Cydomalus* genotypes showing red skin color fruit characteristics in Türkiye has been mentioned in both newspaper reports and scientific publications for the last 10 years (Şahin and Mısırlı 2016). The first genotype was identified by Şahin in Seferihisar in 2012 and afterwards, two more genotypes were determined through PhD study and personal interviews (unpublished data, Şahin, M; Şahin 2017). These *Cydomalus* genotypes are thought to be hybrids obtained by Rudenko and Rudenko (1994) or new individuals formed by open pollination of these genotypes in natural conditions, but there is no conclusive evidence.

Table 1 Quince and *Cydomalus* genotypes and province of selection and/or origin

Genotype name	Field gene bank identification number	Institution registration number	Town/Province/ Country Origin*
Cydomalus genotypes			
QFBNT45-4	TUR0010619	3188	Gördes / Manisa / Türkiye
Seferihisar	TUR0010620	3189	Seferihisar / İzmir / Türkiye
Güzelbahçe	TUR0010621	3190	Güzelbahçe / İzmir / Türkiye
Quince rootstocks			
Quince A (MA)	–	–	*East Malling Research Station / U.K
Quince C (MC)	–	–	*East Malling Research Station / U.K

Through artificial inoculations and natural fire blight epidemic conditions, the current study aimed to evaluate the resistance status of three *Cydomalus* genotypes with a focus on their potential as parents in fire blight resistance breeding. Furthermore, their susceptibility levels were compared with common quince rootstocks in Türkiye because of their extensive use of the latter as a rootstock worldwide.

For this purpose, three *Cydomalus* genotypes selected from İzmir and Manisa location and two quince rootstocks (Quince A, Quince C) have been used as a plant material. Detailed information about genotypes is given in the Table 1. In artificial inoculations, ten seedlings for each genotype were used. All plant material preserved in the Aegean Agricultural Research Institute (AARI) Quince Field Gene Bank and artificial inoculations were also carried out at AARI. Since there was only one tree in the field conditions (natural habitat), scoring was made based on one tree.

The fire blight susceptibility indexes (SI) of all genotypes and rootstocks were defined by artificial inoculations in full automatic greenhouse conditions (24–27 °C temperatures, 70–75% relative humidity). Wild-type *Erwinia amylovora* strain Ea-223, determined to be the most virulent among the strains in our stock (Şahin et al. 2019a, b), was used at artificial inoculations. Inoculations were carried out on the same material for two consecutive years (2019–2020) when shoots were in an active growing period, via cutting immediately below the terminal leaves with scissors that were dipped in concentration of 10^8 cfu ml^{-1} bacterial inoculum (Şahin et al. 2020a). After inoculations, plants were wrapped in plastic bags for 72 h to maintain relative humidity levels between 95 and 100% (Şahin et al. 2020a). Measurements were done according to Şahin et al. (2020a) by proportioning the diseased shoot length to the total shoot length at the 28th day following inoculation, and classified with Gardner scale as follows: 0–10% SI, highly resistant; 11–30% SI, resistant; 31–50% SI, moderately susceptible; 51–90% SI, susceptible; and 91–100% SI, highly susceptible (Gardner et al. 1980). Disease symptoms in *Cydomalus* genotypes were also observed. Statistical analyses were conducted using two-way analysis of variance followed by Duncan's post hoc test using JMP pro 13 statistical software. Normality of residuals was investigated before analyses.

In addition to artificial inoculation, the susceptibility levels of two *Cydomalus* genotypes (Seferihisar, Güzelbahçe) to fire blight in natural field conditions was determined for two years using USDA marking system (Fig. 1) based on percentage of infected trees and tissue age (Layne and Quamme 1975). Disease observations were made on a 10-year-old tree identified an area where it was thought to have grown naturally. Scores are numbered from 10 to 1 to indicate the age of infected tissue and the overall rate of blight on the tree, with the highest score indicating the least damage. Accordingly, the classes are; highly resistant (score: 10 – 8) 0–6% wood blight; moderately resistant (score: 7 – 6) 7–25% wood blight; susceptible (score: 5) 26–50% wood blight; very sensitive (score: 4 – 1) was determined as 51–100% wood blight (Van der Zwet et al. 1974).

This is the first research to assess the levels of fire blight resistance, as well as to describe disease symptoms in *Cydomalus* genotypes with artificial inoculation and natural conditions. Moreover, *Cydomalus* genus has been identified as the new host for *Erwinia amylovora* Burril. The first symptom was observed on *Cydomalus* and *Cydonia oblonga* rootstocks nearly 90 and 72 h after inoculation with shoot tips being water soaked, respectively. The usual shepherd's crooks appearance seen in pome fruit species was also seen in *Cydomalus* (Fig. 2a). During the four-week observations, while the disease progression continued rapidly in quince rootstocks, a split point occurred at the shoot tip in the 1st week in *Cydomalus* genotypes; QFBNT45-4 and Seferihisar (Fig. 2a), and the progression of the disease was stopped. In these *Cydomalus* genotypes, new shoot growth was observed from the point where the development of the disease stopped and no disease progression was observed in

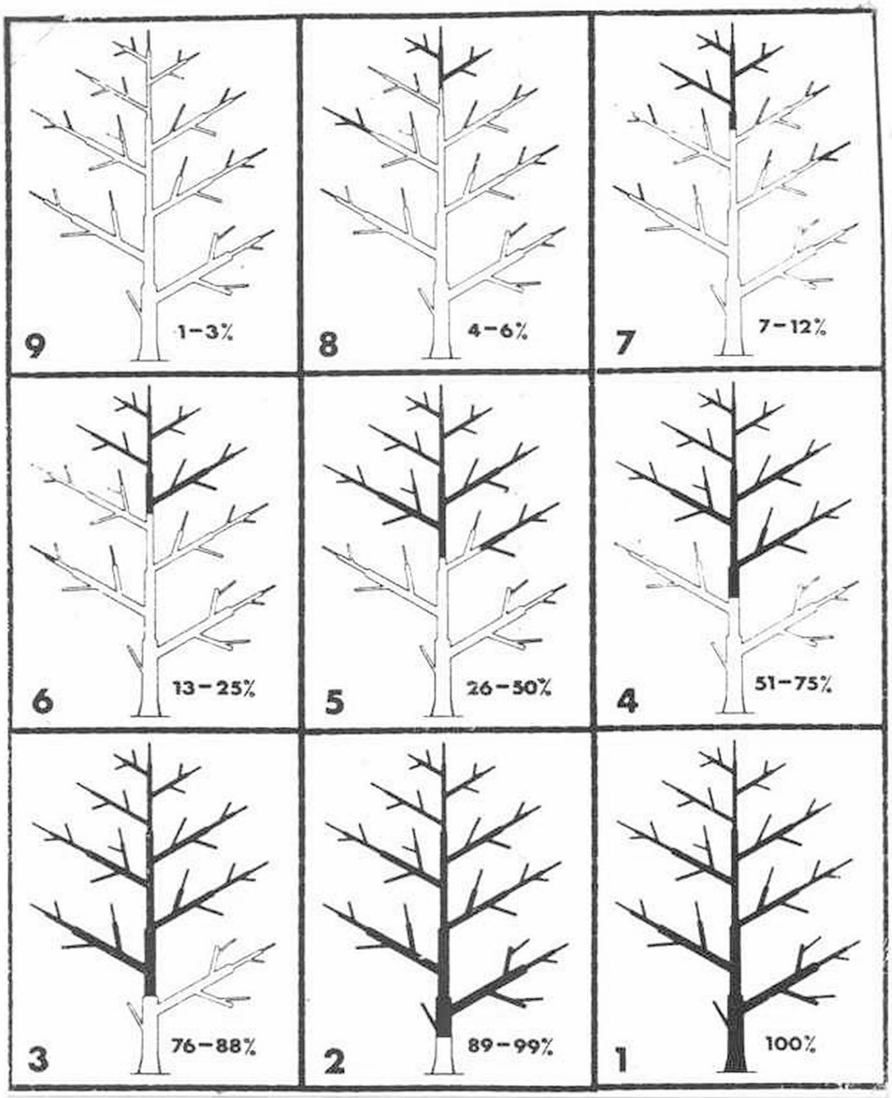

Fig. 1 USDA marking system based on percentage of infected trees and tissue age (black indicates infected tissue) (Layne and Quamme 1975)

Fig. 2 Split point (**a**) and new shoot growth (**b**) on *Cydomalus*

Table 2 Mixed factorial design showing the effect of genotype and year on SI

Source	Nparm	DF'	Sum of squares''	F Ratio'''	Prob > F
Genotype	4	4	10381.202	31.0441	<.0001*
Period	1	1	122.485	1.4651	0.2338
Genotype x Period	4	4	256.145	0.7660	0.5542

'DF refers to degrees of freedom, "F ratio represents the F-statistic used to verify whether the effect is zero, and" Nparm denotes the number of parameters linked to the effect

this new shoot (Fig. 2b). Re-growth was also observed in all plants of the two quince rootstocks, but disease development occurred on new shoots.

Disease symptoms are consistent with previous studies determined in many Rosaceae family members (Lecomte and Cadic 1992; Ozrenk et al. 2012; Şahin et al. 2020a; Pánková et al. 2023). Like QFBNT45-4 and Seferihisar *Cydomalus* genotypes, Przybyla et al. (2012) found that the Hessle pear cultivar response to pathogen infection was stable after inoculation, indicating that resistance was triggered quickly and maintained.

When the results of artificial inoculations are examined, only the difference between genotypes was statistically significant ($p \leq 0.0001$) in artificial inoculations (Table 2).

SI varied between 11.53 and 48.64 in a two-term period after artificial inoculations (Table 3). When the period averages were taken into account, the individuals with the highest resistance with 12.25% SI of QFBNT45-4 and 16.01% SI of Seferihisar, respectively in the same statistical significant level. The genotypes examined showed that both Quince A (46.96%) and Quince C (47.41%) rootstocks were susceptible at the same level of statistical significance.

The SI value of quince rootstock inoculated with a combination of the three virulent isolates was established in a previous study (Şahin et al. 2020a) as 42.68% SI as a result of the average of three periods, which is similar to this study. The SI varied between 40 and 60% in studies including the quince rootstocks BA 29 (53%), Quince C (55%), Quince A (58%), Sydo (58%), and Adam's (58%). All of these

Table 3 SI index and Gardner scale of *Cydomalus* genotypes and quince rootstocks

Genotypes	1st inoculation	2nd inoculation	Mean	Gardner scale
Cydomalus genotypes				
QFBNT45-4	11.53 ± 1.06	12.97 ± 1.41	**12.25 ± 0.84**^{c}	Resistant
Seferihisar	17.17 ± 2.29	14.85 ± 3.06	**16.01 ± 1.79**^{c}	Resistant
Güzelbahçe	39.96 ± 4.71	27.81 ± 7.15	33.89 ± 4.74^{b}	Moderately susceptible
Quince rootstocks				
Quince A (MA)	48.64 ± 0.87	45.29 ± 2.60	46.96 ± 1.41^{a}	Moderately susceptible
Quince C (MC)	47.33 ± 5.81	47.49 ± 5.97	47.41 ± 3.93^{a}	Moderately susceptible

Table 4 Field observations and disease rates of two *Cydomalus* genotypes

Genotype	Year	
	2013	2014
Seferihisar	Score 10 – 8 / 0–6% wood blight	Score 10 – 8 / 0–6% wood blight
Güzelbahçe	Score 7 – 6 / 7–25% wood blight	Score 7 – 6 / 7–25% wood blight

rootstocks have been identified in the "susceptible" group (Lespinasse and Aldwinckle 2000). It is clear that performing multiple inoculation trials is essential for evaluating fire blight resistance, as demonstrated by Barritt et al. (2004), Sobiczewski et al. (2015)d nková et al. (2023). There are studies that demonstrate the significance of year and genotype interactions in different species of pome fruit (Korba et al. 2013; Harshman et al. 2017; Şahin et al. 2020a). The inoculation period and interactions were not significant in our study, demonstrating that the greenhouse conditions were optimal.

Not only SI levels of *Cydomalus* genotypes and quince rootstocks were investigated with artificial inoculations over two term but also natural infection levels were observed on two *Cydomalus* genotypes at the selection areas. In natural infection conditions, statistical analysis was not performed, only scoring was done. Because of two-year field observations, disease rates in both types varied between 1 and 3 to 4–6% (Fig. 1; Table 4).

The genotype QFBNT45-4 was found to be highly resistant to fire blight in the field surveys done previously (Şahin 2017) in 2015 and 2016, with a disease rate of 1–3% (score 9) in both years, under natural infection conditions in the field. As a result of our observations, Seferihisar and QFBNT45-4 genotypes, which were determined to have high resistance in field conditions, obtained similar results in artificial inoculations (Tables 3 and 4). As was done in this study, after the inoculation of shoots, the susceptibility of genotypes may be measured using a variety of techniques and it seems that the shoot's level of lesion development served that objective the best (Przybyla et al. 2012).

On the other hand, evaluation of both natural and artificial inoculations is important in resistance breeding studies. Host resistance can help to promote fruit production that is resilient and sustainable. There is a chance that cultivars and rootstocks that are both tolerant to fire blight will become commercially accessible. Given that an infected scion has a lower probability of killing out the entire tree, the benefit of rootstock resistance should also be taken into account (Kellerhals et al. 2017). This work has uncovered the potential of utilizing fire blight disease-tolerant *Cydomalus* genotypes, which is widely used as rootstock worldwide. Furthermore, it emphasizes the significance of incorporating these genotypes as parents in rootstock breeding studies.

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Data Availability Data is available from the senior author upon request.

Declarations

Competing Interests The authors have no competing interests to declare that are relevant to the content of this article.

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