Technology in Horticulture

ARTICLE

Open Access

https://doi.org/10.48130/tihort-0025-0041

Technology in Horticulture **2025**, 5: e042

Evaluation and screening of fire blight resistance in *Malus* plants

Wei Shang[1], Yuan Gao[1], Xiang Lu[1,2], Xiaoli Zhang[3], Kun Wang[1], Wen Tian[1,2], Simiao Sun[1], Zhao Liu[1,2], Hanxin Guo[1], Lin Wang[1], Zichen Li[1], Lianwen Li[1] and Dajiang Wang[1*]

[1] *Research Institute of Pomology, Chinese Academy of Agricultural Sciences (CAAS), Key Laboratory of Horticulture Crops Germplasm Resources Utilization, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Xingcheng, Liaoning 125100, China*

[2] *Agricultural College of Shihezi University, Xinjiang Production and Construction Corps Key Laboratory of Special Fruits and Vegetables Cultivation Physiology and Germplasm Resources Utilization, Shihezi, Xinjiang 832003, China*

[3] *Institute of Horticulture Crops, Xinjiang Academy of Agricultural Sciences, Urumqi, Xinjiang 830091, China*

* Corresponding author, E-mail: wangdajiang@caas.cn

Abstract

Fire blight, a devastating bacterial disease, primarily infects fruit trees of Rosaceous, leading to their decline and eventual death. This study aims to evaluate and screen the fire blight resistance in 19 *Malus* plants; additionally, the effectiveness and applicability of three inoculation methods were compared. The toothpick acupuncture method in young leaves, shoots *in vitro*, and young plants *in vivo* were used in this study. The results were significantly different from the three inoculation methods for some *Malus* plants. Under the inoculation conditions of young leaves *in vitro*, only Shidong Caiping No. 1 was tolerant to fire blight, exhibiting the highest resistance level, while others showed susceptibility at graded severity levels. Under the inoculation condition of young shoots *in vitro*, nine highly resistant resources were screened, such as OT3, SH3, SH6, etc. Under the inoculation condition of young plants *in vivo*, six highly resistant resources were obtained, included E Shanjingzi No. 2, Xiaogucheng Lenggunzi No. 1, Yingyehaitang, and so on. For practical applications, the evaluation of young shoots *in vitro*, and young plants *in vivo* should be integrated to achieve a comprehensive and accurate result. A total of three resistance resources were obtained, namely E Shanjingzi No. 2, Xiaogucheng Lenggunzi No. 1, and Yingyehaitang, which can be used for the breeding of parents of fire blight resistant varieties.

Citation: Shang W, Gao Y, Lu X, Zhang X, Wang K, et al. 2025. Evaluation and screening of fire blight resistance in *Malus* plants. *Technology in Horticulture* 5: e042 https://doi.org/10.48130/tihort-0025-0041

Introduction

Fire blight, a highly destructive bacterial disease, is caused by *Erwinia amylovora*[1]. It primarily harms pome fruit trees of Rosaceous. It infects the plants through the nectaries of the flowers, and also infects host's tissues through wounds. The pathogen multiplies in large quantities in the vascular bundles after infecting the tree, and then migrates inside the tree, causing necrotic lesions on flowers, leaves, fruits, shoots, and roots. The physiological functions of the plants are impaired, and eventually, the entire plant dies as the disease progresses. It causes severe economic losses to agricultural production[2]. Fire blight was classified as a major quarantine disease in the world, as well as being categorized as a Class I crop disease in China[3].

Fire blight was first reported in North America in 1780. Currently, it has spread to over 60 regions around the world, including many countries and regions in North America, Europe, North Africa, the Middle East, Oceania, and Asia[4]. In 2016, the fire blight spread to places such as Kyrgyzstan and Kazakhstan in Central Asia, and South Korea in East Asia[5–7]. The disease was first reported in Yili Prefecture, Xinjiang (China), in May 2016[8]. As of 2023, fire blight spread to both Xinjiang and Gansu (China)[9]. Fire blight is advancing in China, and seriously endangers the production of apples, therefore, the evaluation and screening of resistant *Malus* resources to fire blight is crucial.

The methods for the evaluation of fire blight resistance mainly include field natural infection and artificial inoculation. Field natural infection is a more direct method; it reliably quantifies field resistance in the actual environment by cultivating test varieties in high-risk infection zones and monitoring natural infection progression. However, this method is greatly influenced by environmental conditions, with a high uncertainty in the occurrence of diseases and a long evaluation period, making it difficult to obtain reliable results in a short time. Artificial inoculation offers advantages such as simple operation, short cycle, and environmental independence. Common methods include the syringe inoculation of detached plant organs such as leaves, immature fruits, and young shoots[10], the soil inoculation of pot-grown seedlings with injured roots[11], as well as the spray inoculation method[12]. These methods had high repeatability and accuracy. Moreover, they were suitable for large-scale screening. However, the resistance performance under conditions *in vitro* may differ from the actual situation in the field, and cannot fully reflect the overall resistance level of the plants.

In practical applications, several methods should be combined to obtain more comprehensive and accurate results of resistance evaluation. Therefore, how to improve the accuracy of detection is an urgent problem to be solved in the evaluation and screening of fire blight resistance in *Malus* plants. In this study, the toothpick puncture method was adopted. The identification results obtained from three inoculation methods, namely, young leaves, young shoots *in vitro*, and young plants *in vivo*, were compared and analyzed. Nineteen *Malus* plants were evaluated for resistance to fire blight, and resistant resources were screened out to provide materials for fire blight-resistant breeding and related resistance gene mining.

Materials and methods

Test materials and sampling

Nineteen types of *Malus* plants were obtained from the National Pear and Apple Germplasm Repository (Xingcheng, China) (Table 1).

© The Author(s)

Table 1. Nineteen types of *Malus* plants.

Number	Resource name	Scientific name	Resource type	Origin
1	Huazhen No. 5	*Malus domestica* Borkh.	Rootstock variety	Liaoning, China
2	SH3	*Malus domestica* Borkh.	Rootstock variety	Shanxi, China
3	SH6	*Malus domestica* Borkh.	Rootstock variety	Shanxi, China
4	OT3	*Malus domestica* Borkh.	Rootstock variety	Canada
5	M9T337	*Malus domestica* Borkh.	Rootstock variety	Netherlands
6	B118	*Malus domestica* Borkh.	Rootstock variety	Russia
7	E Shanjingzi No. 2	*Malus baccata* (L.) Borkh.	Wild resources	Russia
8	Shajinhaitang	*Malus sargentii* Rehd.	Wild relatives	Japan
9	Xiaojinhaitang	*Malus xiaojinensis* Cheng et Jiang	Wild resources	Sichuan, China
10	Xiaogucheng Lenggunzi No. 1	*Malus robusta* (Carr.) Rehd.	Landraces	Hebei, China
11	Daobazui Wuxianghaitang	*Malus honanensis* Rehd.	Wild resources	Shanxi, China
12	Longdonghaitang	*Malus kansuensis* (Batal.) chneid.	Wild resources	Gansu, China
13	Balenghaitang	*Malus robusta* (Carr.) Rehd.	Landraces	Hebei, China
14	Luanzhuang Shaguo	*Malus asiatica* Nakai.	Landraces	Hebei, China
15	Zhaojue Shanjingzi	*Malus baccata* (L.) Borkh.	Wild resources	Yunnan, China
16	Xishuhaitang	*Malus prattii* (Hemsl.) Schneid.	Wild resources	Sichuan, China
17	Chuisihaitang	*Malus halliana* Koehne, Gatt.Pomac	Wild resources	Gansu, China
18	Shidong Caiping No. 1	*Malus domestica* Borkh. *Subsp. Chinensis* Li Y.N.	Landraces	Hebei, China
19	Yingyehaitang	*Malus ceracifolia* Spach.	Wild resources	Liaoning, China

In May 2019, the plants were grafted onto '*Malus baccata* (L.) Borkh.' and were sent to Korla, Xinjiang, for pot cultivation. In May 2020, leaves and shoots were collected for inoculation *in vitro*, and at the same time, inoculation was carried out on plants *in vivo* in the field.

Preparation of bacterial suspension

The preparation of the bacterial solution was carried out according to the method of Li et al.[13]. The fire blight pathogen *Erwinia amylovora* (*E.a* 6) was used. The concentration of the bacterial solution was 1×10^7 CFU·mL^{-1}, and the OD_{600} was about 0.4[14].

Inoculation of leaves *in vitro*

First, new shoots of *Malus* plants were selected, and the third or fourth fully expanded healthy young leaves from the upper part were taken. Then, these leaves were washed with ddH_2O, and their excess petioles were cut off. In the ultra-clean bench, a toothpick was dipped into the bacterial solution, with the bacterial suspension on the toothpick appearing as a hanging drop. Each sample was inoculated by piercing the main vein from the back of the young leaf. The inoculated young leaf was put on a triangular toothpick stand in a petri dish lined with sterile, moist filter paper, with its back facing up. Each petri dish contained one young leaf, then it was cultivated in a constant temperature incubator at 28 °C. The total length of the leaves and the length of the necrotic lesion were measured after 72 h.

Inoculation of shoots *in vitro*

Undamaged, healthy shoots of uniform size were selected from each *Malus* plant, and artificial inoculation conducted. First, they were washed with ddH_2O and a 0.5 cm length cut off at the bottom, in the shape of a horseshoe. A toothpick was dipped in the bacterial solution, with the bacterial suspension on the toothpick appearing as a hanging drop. Inoculation was performed by inserting the toothpick 2 mm deep into the main stem at the base of the second fully expanded leaf. The inoculated shoots were placed in an environment containing 2% sucrose water for incubation, and the sucrose water was changed every 24 h. Humidity was maintained by spraying water mist. Total length and necrotic lesion length were measured two weeks after inoculation of the shoots.

Inoculation of young plants *in vivo*

When the seedlings of each resource grew to 50 cm in height, ten plants were selected with consistent growth vigor from each. These plants were placed in screening chambers and inoculated with *Erwinia amylovora*. The inoculation method was the same as that of the inoculation of young shoots *in vitro*. The total length and necrotic lesion length of the inoculated shoots were measured two weeks after inoculation.

Resistance evaluation

The infection conditions were recorded at different time points depending on the plant material: 72 h after inoculation on young leaves *in vitro*, and two weeks after inoculation on young shoots *in vitro* and young plants *in vivo*. The infection conditions of fire blight were classified according to the classification standard[15]. To reduce the influence of uneven lengths of young leaves and young shoots, the lengths of the lesions on young leaves and young shoots were normalized by dividing them by the longest lesion length of each. The normalized ratio was used as the classification value: Grade 0: no necrotic lesion on the shoots and normal leaves. Grade 1: The length of the necrotic lesion accounted for 1% to 5% of the total length. The leaves turned blackish-brown and did not fall off. Grade 3: The length of the necrotic lesion accounted for 5.1% to 15% of the total length. The leaves turned blackish-brown, and some leaves fell off. Grade 5: The length of the necrotic lesion accounted for 15.1% to 30% of the total length, the leaves turned blackish-brown, and about one-third of the leaves fell off. Grade 7: The length of the necrotic lesion accounted for 30.1% to 50% of the total length, the leaves turned blackish-brown, and about two-thirds of the leaves fell off. Grade 9: The length of the necrotic lesion accounted for 50.1% of the total length, the leaves turned blackish-brown, and half or more of the leaves fell off.

The disease index was calculated based on the disease condition classification. The calculation method of the disease index was as follows:

$$DI = \frac{\sum(N \times G)}{T \times H} \times 100$$

where, DI = Disease Index; N = Number of diseased plants at each level; G = Corresponding disease grade; T = Total number of inoculated plants; H = The highest disease grade.

The classification criteria for the resistance of fire blight were as follows: High resistance (HR): DI was (0, 5]; Resistance (R): DI was (5, 15]; Tolerance (T): DI was (15, 30]; Moderate susceptibility (MS): DI was (30, 60]; Susceptibility (S): DI was (60, 80]; High susceptibility (HS): DI was (80, 100].

Resistance evaluation reference standards

In previous studies[16], by inoculating young plants with OT3, it was confirmed that OT3 was susceptible to infection by *Erwinia amylovora*. Therefore, the results obtained with OT3 in this study served as a reference for comparing the inoculation methods.

Data statistical analysis

Each experimental procedure was performed in triplicate. Excel (Microsoft 365) was utilized for comprehensive data processing, including organization, calculation of means, and coefficient of variation, as well as the generation of standardized column charts. Based on one-way ANOVA, Duncan's multiple range test ($p < 0.05$) in SPSS 27.0 was used for statistical analysis.

Results

Evaluation and screening of leaves inoculation *in vitro*

After 72 h of inoculation (Fig. 1a), the disease index of 19 *Malus* plants ranged from 31.11 to 100 (Table 2). It was categorized into four types: tolerant, moderately susceptible, susceptible, and highly susceptible, with a ratio of 1:1:6:11. Among them, only Shidong Caiping No. 1 was evaluated as tolerant. Luanzhuang Shaguo was moderately susceptible. A total of six resources—M9T337, Shajinhaitang, Xiaogucheng Lenggunzi No. 1, Longdonghaitang, Balenghaitang, and Yingyehaitang—were susceptible. The remaining 11 resources were highly susceptible. The variation coefficients of each replicate of each resource ranged from 0.00% to 52.70%. The one with the highest coefficient of variation was Shidong Caiping No. 1.

Evaluation and screening of shoots inoculation *in vitro*

Two weeks after inoculation, the length of the necrotic lesion on the shoots was measured and compared from 8:00 to 12:00 on the 14th day (Fig. 1b). The disease index was between 0.00 and 92.59 (Table 2). It was categorized into five types: highly resistant, resistant, tolerant, moderately susceptible, and highly susceptible, with a ratio of 9:1:3:2:4. Among them, nine resources exhibited highly resistant to fire blight: Huazhen No.5, SH3, SH6, OT3, M9T337, E Shanjingzi No. 2, Xiaogucheng Lenggunzi No. 1, Daobazui Wuxianghaitang, and Yingyehaitang. One resource was resistant to fire blight: Luanzhuang Shaguo. Three were tolerant to fire blight: Longdonghaitang, Balenghaitang, and Shidong Caiping No. 1. Two were moderately susceptible to fire blight: Shajinhaitang, and Xishuhaitang. Four were highly susceptible to fire blight: B118, Xiaojinhaitang, Zhaojue Shanjingzi, and Chuisihaitang. The coefficient of variation ranged from 0.00% to 137.88%. Among them, Longdonghaitang, Luanzhuang Shaguo, Shidong Caiping No. 1, and Yingyehaitang exhibited higher coefficients of variation.

Evaluation and screening of young plants inoculation *in vivo*

Two weeks after inoculation, the length of the necrotic lesion on the propagation seedlings after inoculation was measured (Fig.1c). The results were listed in Table 2. The disease index was between 0.00 and 88.89. It was categorized into five types: highly resistant, tolerant, susceptible, moderately susceptible, and highly susceptible, with a ratio of 6:1:4:4:4. Among them, six resources were highly resistant to fire blight: E Shanjingzi No. 2, Shajinhaitang, Xiaogucheng Lenggunzi No. 1, Longdonghaitang, Shidong Caiping No.1, and Yingyehaitang. One was tolerant to fire blight: Luanzhuang Shaguo. Four resources were susceptible to fire blight: SH6, OT3, M9T337, and B118. Four resources were moderately susceptible to fire blight: Huazhen No.5, Daobazui Wuxianghaitang, Balenghaitang, and Chuisihaitang. Four resources were highly susceptible to fire blight: SH3, Xiaojinhaitang, Zhaojue Shanjingzi, and Xishuhaitang. The coefficient of variation of each resource ranges from 0.00% to 29.59%.

Comparison of the evaluation results of the three inoculation methods

Through one-way ANOVA and Duncan's post-hoc test, the resistance evaluation results obtained by the three methods were compared and analyzed (Fig. 2). Firstly, a comparative analysis of the two inoculation methods *in vitro* revealed that among the 19 resources, only B118, Shajinhaitang, Xiaojinhaitang, Zhaojue Shanjingzi, Chuisihaitang, and Shidong Caiping No. 1 showed no significant difference in resistance outcomes under the two conditions, while others exhibited varying degrees of discrepancy. Although both methods involve inoculation *in vitro*, due to the different inoculation positions, the integrity of the materials might vary, resulting in inconsistent resistance evaluation results between young leaves inoculation and young shoots inoculation *in vitro*. Next, the two inoculation methods that were both applied to the shoots would be analyzed. Among the 19 resources, the resistance results of some resources under the two inoculation conditions showed no significant differences, including B118, Xiaojinhaitang, Xiaogucheng Lenggunzi No. 1, Longdonghaitang, Balenghaitang, Zhaojue Shanjingzi, and Yingyehaitang. However, the remaining resources showed varying degrees of differences. Although the inoculation sites of the two methods were the same, the differences in the identification results occurred due to the different physiological states.

The fundamental purpose of disease resistance identification was to accurately predict the performance of germplasm resources in their natural growth environment. Therefore, the form of young plants inoculation was more in line with the standard, as it can most realistically simulate the natural infection process and the complex physiological state of the plant as a whole. The results obtained from this were of the highest predictive value for actual agricultural production. Previous studies, using the method of young plants *in vivo* inoculation has established OT3 as susceptible to *Erwinia amylovora*. The identification results obtained in this study were consistent with previous studies. Therefore, the results obtained with OT3 in this study served as a reference for comparing the inoculation methods. At the same time, for some resources, the results of

Fig. 1 Incidence of (a) young leaves *in vitro*, (b) young shoots *in vitro*, and (c) young plants *in vivo*.

Table 2. Results of three different inoculation methods of *Malus* plants for resistance to fire blight.

Number	Resource name	Leaf inoculation			Shoot inoculation			Plant inoculation		
		Disease index	Coefficient of variation	Resistance evaluation	Disease index	Coefficient of variation	Resistance evaluation	Disease index	Coefficient of variation	Resistance evaluation
1	Huazhen No. 5	92.59a	13.85%	HS	0.00c	0.00%	HR	55.55b	0.00%	MS
2	SH3	92.59a	13.85%	HS	0.00b	0.00%	HR	87.30a	13.60%	HS
3	SH6	92.59a	13.85%	HS	0.00c	0.00%	HR	66.66b	19.24%	S
4	OT3	100a	0.00%	HS	0.00c	0.00%	HR	71.42b	29.59%	S
5	M9T337	77.78a	0.00%	S	0.00b	0.00%	HR	77.78a	18.07%	S
6	B118	92.59a	13.85%	HS	92.59a	13.85%	HS	74.60a	20.55%	S
7	E Shanjingzi No. 2	100	0.00%	HS	0.00	0.00%	HR	0.00	0.00%	HR
8	Shajinhaitang	80.00a	24.32%	S	53.33a	36.48%	MS	0.00b	0.00%	HR
9	Xiaojinhaitang	84.44a	21.67%	HS	84.44a	17.76%	HS	88.89a	24.30%	HS
10	Xiaogucheng Lenggunzi No. 1	77.78a	28.57%	S	0.00b	0.00%	HR	0.00b	0.00%	HR
11	Daobazui Wuxianghaitang	85.18a	15.06%	HS	0.00c	0.00%	HR	51.85b	17.49%	MS
12	Longdonghaitang	77.78a	35.63%	S	17.78b	109.71%	T	0.00b	0.00%	HR
13	Balenghaitang	77.78a	23.33%	S	28.89b	48.65%	T	40.00b	26.84%	MS
14	Luanzhuang Shaguo	45.56a	46.63%	MS	8.89b	114.87%	R	33.33a	0.00%	T
15	Zhaojue Shanjingzi	84.44a	21.67%	HS	84.44a	21.67%	HS	88.89a	13.18%	HS
16	Xishuhaitang	82.22a	21.32%	HS	66.67b	23.57%	MS	88.89a	17.68%	HS
17	Chuisihaitang	84.44a	21.67%	HS	91.11a	12.60%	HS	51.11b	27.50%	MS
18	Shidong Caiping No. 1	31.11a	52.70%	T	16.67a	137.88%	T	0.00b	0.00%	HR
19	Yingyehaitang	73.33a	23.90%	S	4.44b	124.71%	HR	0.00b	0.00%	HR

Different letters (a, b, c) on the same row indicated values that were significantly different ($p < 0.05$) based on one-way ANOVA, Duncan post-hoc test. And E Shanjingzi No. 2 exhibited zero variance across all inoculation methods, this extreme situation violated the basic assumptions of ANOVA. Therefore, no significance analysis was conducted on this set of data.

young shoots inoculation *in vitro* did not have a statistically significant difference from those of shoots inoculation *in vivo*. This meant that the young shoots technique *in vitro* had the potential to be used as an efficient and reliable method to replace the more time-consuming and labor-intensive inoculation method *in vivo* for the preliminary screening of large-scale germplasm resources in the early stage. By combining the ecological relevance advantages of the method *in vivo* with the efficiency advantages of the shoots method *in vitro*, more reliable identification results can be obtained. Therefore, based on the resistance evaluation obtained from young plants *in vivo* and combined with the evaluation from young shoots *in vitro* as a reference, three resistance resources have been obtained, namely E Shanjingzi No. 2, Xiaogucheng Lenggunzi No. 1, and Yingyehaitang.

Discussion

In the current prevention and control of fire blight, resistance breeding[17], physical control[8], chemical control[18], and biological control[19] were used. However, these measures did not thoroughly eliminate the damage caused by fire blight to the fruit industry. Screening for resistance resources and cultivating resistant varieties was the most fundamental and effective strategy. Resistance evaluation was the basis for pathogenicity research and resistant breeding, and methods of resistance evaluation were critical. Therefore, it was of great practical significance to study and establish a set of resistance evaluation technology systems that were reliable and accurate[20]. Fire blight mainly harmed the flowers, leaves, shoots, trunks, and rootstocks of fruit trees such as apples and pears, eventually leading to tree death and orchard yield reduction[21]. Therefore, the resistance to fire blight could be evaluated by a variety of inoculation methods, such as flowers, young leaves, young shoots, and young plants. *Erwinia amylovora* could easily infect flowers during the flowering period[22], but the incidence of the disease on flowers was difficult to evaluate. The current method for evaluating resistance to fire blight was mainly based on artificial inoculation. It could quickly and accurately screen resistant materials and was suitable for situations where there were few materials and only one disease was being evaluated[23]. Within this framework, incidence rate and lesion proportion were commonly used to evaluate host disease resistance[24]. Harshman et al. obtained 12 resources resistant to fire blight among nearly 200 samples of *Malus sieversii*, using young shoot inoculation[25]. Ozrenk et al. used young shoots inoculation to evaluate the resistance of 32 apple resources, of which five were rated as resistant, seven as moderate resistance, nine as moderate susceptibility, five as susceptibility, and six as high susceptibility[26]. Liu et al. evaluated the resistance of 54 pear resources by inoculating fruits, with resistant resources accounting for 70.4%[27]. Zhu et al. identified 28 resources with moderate resistance or higher to fire blight among 258 *Malus sieversii* samples, from seven natural populations through young shoot inoculation and field evaluation[28]. Cao et al. identified five resistant resources among 83 *Malus sieversii* samples through leaf *in vitro* and shoot inoculation, which can serve as foundation materials for breeding resistant rootstocks[29]. Consistent with the methods of predecessors, the resistance was evaluated using a combined inoculation method that targeted both young leaves and young shoots. *Erwinia amylovora* was inoculated on young leaves, shoots *in vitro*, and young plants *in vivo*, respectively. Ultimately, a total of three highly resistant resources were screened, namely E Shanjingzi No. 2, Xiaogucheng Lenggunzi No. 1, and Yingyehaitang. The three resources came from Russia and China, respectively. Among them, Xiaogucheng Lenggunzi No. 1 was a landrace, while the others were wild resources.

The 19 resources used in this experiment came from 12 different species. Among them, the three evaluated resistant resources belonged to '*Malus baccata* (L.) Borkh.', '*Malus robusta* (Carr.) Rehd.', and '*Malus ceracifolia* Spach.' respectively. Zhaojue Shanjingzi belonged to the same species as E Shanjingzi No. 2. The Balenghaitang was also part of the same species as Xiaogucheng Lenggunzi

No. 1. However, in this experiment, Zhaojue Shanjingzi and Balenghaitang were not evaluated as resistant resources. The variation in fire blight resistance among different resources of the same species was possibly due to distinct genetic backgrounds shaped by their geographical origins, and was further modulated by morphological factors. In previous studies[30,31], QTLs for resistance to fire blight have been identified in the wild species *M. baccata* and *M. robusta*. These two species were consistent with those in this experiment. However, in this experiment, some varieties were identified as resistant resources, which was not the case for others. This might be due to the differences in resistance to fire blight within the same species. It is also well-established from prior studies that resistance and susceptibility to fire blight varies within and among *Malus* species[32].

Liu et al. conducted inoculation on pear at the young fruit stage, expansion stage, and maturity stage. The results showed that the inoculation on young fruit was more accurate than the expansion and maturity stages of pear[33]. Wang et al. found five hawthorn resources showed different pathogenic responses to the fire blight by inoculation of the leaves, shoots, and young fruits *in vitro*[34]. Jing et al. found that the resistance to fire blight of different tissues of the seven crabapple varieties was different under the same experimental conditions[35]. It was found that shoots and flower inoculation were poorly correlated, indicating that the resistance of different parts were different[36]. The studies by Zhu et al. also showed that there were certain differences in the resistance to fire blight of young leaves and young shoots of the tested red flesh apples[37]. Wang et al. evaluated the resistance of 488 *Malus* resources and found that the number of resistant resources inoculated by young shoots *in vitro* was significantly more than that by young leaves[15]. All the above-mentioned studies have shown that resistance to fire blight for the same resource was different with different inoculation periods, different inoculation methods, and different inoculation sites. The same conclusion was also obtained in this study. For the same resource, when three different methods were inoculated with *Erwinia amylovora*, there were differences in the degree of resistance, and the number of resistant resources obtained varies. Therefore, in fire blight resistance evaluation, it is not advisable to rely solely on a single method. Instead, an integrated approach combining multiple techniques should be adopted, depending on the scale of screening and the specific objectives of the study.

Multiple factors contributed to such differential outcomes, as documented in prior studies. Plants formed complex defense mechanisms containing morphological structures and physiological and biochemical changes in the long-term interaction and adaptation with pathogenic bacteria. Pathogens first attached to the plant surface, the size and shape of stomata, the thickness and tightness of the palisade and spongy tissue, and the uniformity of epidermal cells may affect the invasion and spread of pathogens in terms of morphological structure[38]. Crucially, morphological traits exhibited clear correlations with disease resistance: leaves can easily be infected by other pathogens and interfere with the experimental results. At the same time, there was a significant or highly significant positive correlation between stomata density, leaf thickness, upper epidermal thickness, lower epidermal thickness, and disease index[39]. Şahin studied the relationship between the leaf characteristics and its resistance to fire blight for quince. The results showed that the resistance to fire blight of quince was strongly correlated with leaf blade: undulation of margin[40]. Therefore, in the present experiment, the leaves were more susceptible. This might be due to the relatively young cell structure of the young leaves and the incomplete development of their defense mechanisms, or because the nutrients were more easily utilized by the pathogen. Additionally, the inoculation of shoots over a longer period of time would result in wilting, and the limited time for culture *in vitro* would also affect the physiological state of the plants. However, the method *in vivo* was closer to the natural environment, but the process was more complex and had limitations. Moreover, the phenotypic evaluation relied on manual judgment of the disease condition, which was highly subjective. This inherent subjectivity inevitably introduced variability into the evaluation of disease. To objectively quantify the extent of this variation and evaluate the consistency of different leaves, shoots, or evaluators, the coefficient of variation was calculated for the disease index. It was worth noting that among the four resources of Longdonghaitang, Luanzhuang Shaguo, Shidong Caiping No. 1, and Yingyehaitang, which were inoculated with young shoots *in vitro*, the coefficient of variation of the disease index was relatively high. According to the raw data, the high coefficients of variation observed in this experiment were primarily caused by the low mean values. The primary factor was that the mean value of the data set was very low. When the mean approaches zero, even if the absolute standard deviation is small, the coefficient of variation can become very large.

Currently, molecular identification techniques for plant diseases such as fire blight and other plant pathogens, including multiplex PCR and gene chips, have become increasingly mature in various host detection practices. Based on this solid foundation, the

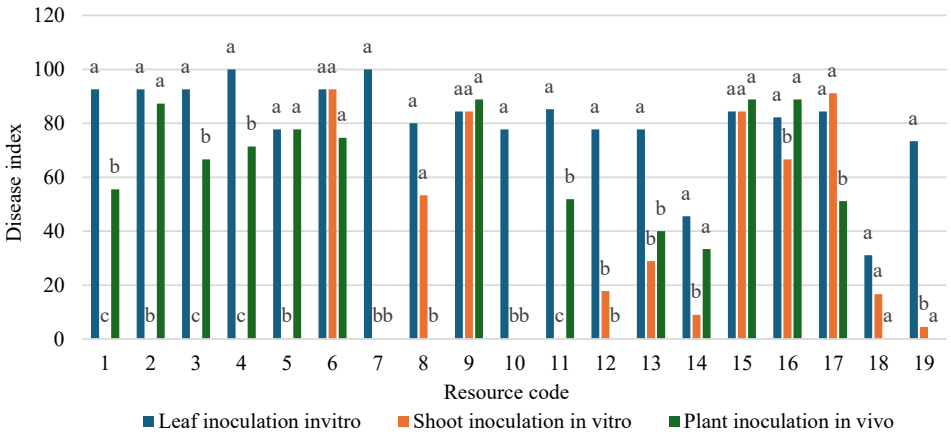

Fig. 2 Comparative analysis of the same resource using different inoculation methods (the numbers 1 to 19 on the horizontal axis represented the resource codes in Table 1). Different letters (a, b, c) on the same group indicate values that were significantly different ($p < 0.05$) based on one-way ANOVA, Duncan post-hoc test.

forefront of research is gradually shifting towards more efficient and comprehensive dimensions. Adomako et al. evaluated the resistance to *Ralstonia solanacearum* of tomato plants by using the method of molecular markers[41]. Khan et al. uncovered that the resistance of fire blight was regulated by multiple genes, and identified two novel QTLs and several functional candidate genes. It offered molecular tools and a theoretical basis for breeding resistant apple varieties[42]. Li et al. identified 125 candidate genes associated with grape white rot resistance through WGCNA analysis[43]. Fahrentrapp et al. identified the resistant genes associated with fire blight in *Malus* × *robusta* 5 through various methods, including fine mapping and gene prediction[44]. Stefano et al. showed that the expression of EFR in apple rootstock may be a valuable biotechnology strategy to improve the resistance of apple to fire blight[45]. Therefore, the combination of phenotype identification and molecular techniques should be applied to detect and evaluate fire blight resistance in the future.

Conclusions

This study evaluated fire blight resistance in 19 *Malus* plants using three inoculation methods—young leaves, young shoots *in vitro*, and young plants *in vivo*. The results demonstrated that there were significant differences in the resistance performance of different methods. The identification results established using young shoots *in vivo* served as the benchmark, supplemented by those obtained from young shoots *in vitro*. A total of three resistance resources were obtained, namely E Shanjingzi No. 2, Xiaogucheng Lenggunzi No. 1, and Yingyehaitang. Therefore, in the evaluation of fire blight resistance, it is recommended to adopt a combined approach using shoots *in vitro* and *in vivo* assays to obtain more reliable results, further integrated with molecular methods to achieve higher accuracy. The above three types of resistance resources can be used as parents for the breeding of resistant varieties. Furthermore, Xiaogucheng Lenggunzi No. 1 can also be used as a parent for the cultivation of fresh food varieties. This served as a reference for fire blight resistance breeding in *Malus* plants and the exploration of related resistance genes.

Author contributions

The authors confirm contributions to the paper as follows: study conception and design: Wang D; data collection: Lu X, Gao Y, Sun S, Zhang X, Wang K, Liu Z; analysis and interpretation of results: Shang W, Guo H, Tian W, Wang L, Li Z, Li L; draft manuscript preparation: Shang W, Wang D. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article, and are available from the corresponding author upon reasonable request.

Acknowledgments

This research was funded by the National Key Research and Development Program Project (2021YFE0104200-4), and the Science and Technology Innovation Project of Chinese Academy of Agricultural Sciences (CAAS-ASTIP-2021-RIP-02).

Conflict of interest

The authors declare that they have no conflict of interest.

Dates

Received 29 June 2025; Revised 14 November 2025; Accepted 7 December 2025; Published online 24 December 2025

References

1. Emeriewen OF, Richter K, Flachowsky H, Malnoy M, Peil A. 2021. Genetic analysis and fine mapping of the fire blight resistance locus of *Malus* × *arnoldiana* on linkage group 12 reveal first candidate genes. *Frontiers in Plant Science* 12:667133
2. Schröpfer S, Vogt I, Broggini GAL, Dahl A, Richter K, et al. 2021. Transcriptional profile of $AvrRpt2_{EA}$-mediated resistance and susceptibility response to *Erwinia amylovora* in apple. *Scientific Reports* 11:8685
3. Mansfield J, Genin S, Magori S, Citovsky V, Sriariyanum M, et al. 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular Plant Pathology* 13:614–29
4. Zhao YQ, Tian YL, Wang LM, Geng GM, Zhao WJ, et al. 2019. Fire blight disease, a fast-approaching threat to apple and pear production in China. *Journal of Integrative Agriculture* 18:815–20
5. Drenova N, Isin MM, Dzhaimurzina AA, Zharmukhamedova GA, Aitkulov AK. 2013. Bacterial fire blight in the Republic of Kazakhstan. *Карантин Растений. Наука И Практика* 1:44–48
6. Doolotkeldieva T, Bobusheva S. 2016. Fire blight disease caused by *Erwinia amylovora* on Rosaceae plants in Kyrgyzstan and biological agents to control this disease. *Advances in Microbiology* 6:831–51
7. Park DH, Yu JG, Oh EJ, Han KS, Yea MC, et al. 2016. First report of fire blight disease on Asian pear caused by *Erwinia amylovora* in Korea. *Plant Disease* 100:1946
8. Wang J, Gao JC, Bayinkexike, Muyassar M, Zhang JH, et al. 2022. Blocking field spread of fire blight by electric heating automatic disinfection pruning scissors. *Plant Quarantine* 36:25–28 (in Chinese)
9. General Office of Ministry of Agriculture and Rural Affairs. 2023. Notice of the General Office of The Ministry of Agriculture and Rural Affairs on printing and distributing the national list of administrative regions where agricultural plant quarantine pests are distributed. *Gazette of the Ministry of Agriculture and Rural Affairs of the People's Republic of China* 10:44
10. Chen LK, Xu YT, Wang YP, He LZ, Ceng B, et al. 2022. Evaluation of fire blight resistance of *Pyrus sinkiangensis* Yu germplasm resources. *China Fruits* 8:16–22 (in Chinese)
11. Lu YH, Hao JH, Luo M, Huang W, Sheng Q, et al. 2021. Screening of antagonistic bacteria against *Erwinia amylovora* and its control effect in greenhouse. *Microbiology* 48:3690–99 (in Chinese)
12. Xu LY, Gulizzier M, Han J, Jiang P, Huang W, et al. 2021. Screening of endophytic antagonistic bacteria from 'Kuerlexiangli' pear and their biocontrol potential against fire blight disease. *Acta Botanica Boreali-Occidentalia Sinica* 41:132–41 (in Chinese)
13. Li HT, Zhang JW, Sheng Q, Tang ZH, Zhang XL, et al. 2019. Resistance evaluation of 20 pear varieties (germplasms) in China to foreign strains of *Erwinia amylovora*. *Journal of Fruit Science* 36:629–37 (in Chinese)
14. He LZ, Zhang XL, Ye CX, Chen LK, Wang YP, et al. 2023. Evaluation of Resistance of Four Pear Rootstocks to Pear Fire Blight. *Acta Agriculturae Boreali-occidentalis Sinica* 32:458–67 (in Chinese)
15. Wang DJ, Gao Y, Zhang YG, Zhang XL, Sun SM, et al. 2022. Evaluation and screening of *Malus* germplasm resources with fire blight resistance. *Journal of Plant Genetic Resources* 23:1682–95 (in Chinese)
16. Norelli JL, Holleran HT, Johnson WC, Robinson TL, Aldwinckle HS. 2003. Resistance of Geneva and other apple rootstocks to *Erwinia amylovora*. *Plant Disease* 87:26–32
17. Peil A, Emeriewen OF, Khan A, Kostick S, Malnoy M. 2021. Status of fire blight resistance breeding in *Malus*. *Journal of Plant Pathology* 103:3–12
18. Sparla F, Rotino L, Valgimigli MC, Pupillo P, Trost P. 2004. Systemic resistance induced by benzothiadiazole in pear inoculated with the agent of fire blight (*Erwinia amylovora*). *Scientia Horticulturae* 101:269–79
19. Sharifazizi M, Harighi B, Sadeghi A. 2017. Evaluation of biological control of *Erwinia amylovora*, causal agent of fire blight disease of pear by antagonistic bacteria. *Biological Control* 104:28–34

20. Ji ZJ, Ge HJ, Xu XY. 2009. Identification method of tomato Helmlnthosporlum fruit rot at seedling stage and screening of resistant germplasm resources. *Journal of Northeast Agricultural University* 40:23−27 (in Chinese)
21. Van Der Zwet T, Keil HL. (Eds.) 1979. *Fire blight: a bacterial disease of rosaceous plants*. Washington: US Department of Agriculture. 195 pp
22. Farkas Á, Mihalik E, Dorgai L, Bubán T. 2012. Floral traits affecting fire blight infection and management. *Trees* 26:47−66
23. Jiang CC, Zeng SM, Chen XM, Hu NS, Huang XZ. 2022. Field resistance evaluation of 110 pear germplasm resources to major leaf diseases. *South China Fruits* 51:170−77,181 (in Chinese)
24. Korba J, Šillerová J, Kůdela V. 2008. Resistance of apple varieties and selections to *Erwinia amylovora* in the Czech Republic. *Plant Protection Science* 44:91−96
25. Harshman JM, Evans KM, Allen H, Potts R, Flamenco J, et al. 2017. Fire blight resistance in wild accessions of *Malus sieversii*. *Plant Disease* 101:1738−45
26. Ozrenk K, Balta F, Guleryuz M, Kan T. 2011. Fire blight (*Erwinia amylovora*) resistant/susceptibility of native apple germplasm from eastern Turkey. *Crop Protection* 30:526−30
27. Liu HW, Wang XM, Guo QY, Han LJ, Cao YF, et al. 2008. Study on resistance of pear germplasm to pear fire blight. *Journal of Plant Genetic Resources* 9:195−200 (in Chinese)
28. Zhu L, Ran B, Zhang SJ, Jian JN, Tang SM, et al. 2025. Evaluation and Screening of Fire Blight Resistance in 258 Accessions of *Malus sieversii* Germplasm Resources. *Journal of Plant Genetic Resources* 26:1845−57 (in Chinese)
29. Cao YZ, Chen WM, Zhang SJ, Lu B, Cui ZJ, et al. 2024. Evaluation of disease resistance of 83 *Malus sieversii* germplasm resources to *Erwinia amylovora*. *Plant Quarantine* 38:33−46 (in Chinese)
30. Peil A, Garcia-Libreros T, Richter K, Trognitz FC, Trognitz B, et al. 2007. Strong evidence for a fire blight resistance gene of *Malus robusta* located on linkage group 3. *Plant Breeding* 126:470−75
31. Peil A, Wöhner T, Hanke MV, Flachowsky H, Richter K, et al. 2014. Comparative mapping of fire blight resistance in *Malus*. *Acta Horticulturae* 1056:47−51
32. Kostick SA, Teh SL, Evans KM. 2021. Contributions of reduced susceptibility alleles in breeding apple cultivars with durable resistance to fire blight. *Plants* 10:409
33. Liu ZY, Su XL, Tang L, Lei CH, Li YP, et al. 2024. An evaluation protocol for fire blight resistance of pear cultivars. *Fujian Journal of Agricultural Sciences* 39:609−14 (in Chinese)
34. Wang JH, Han LL, Zhang SJ, Chen WM, Zhang XC. 2023. Identification and evaluation of resistance of hawthorn germplasm resources to pear fire blight in Xinjiang. *Northern Horticulture* 24:30−37 (in Chinese)
35. Jing J, Zhang SJ, Dulibibi M, Wang WJ, Wang BW, et al. 2023. Evaluation on the resistance of seven varieties of begonia to *Erwinia amylovora*. *China Fruits* 11:70−75 (in Chinese)
36. Bühlmann-Schütz S, Hodel M, Dorfmann E, Vonmetz L, Lussi L, et al. 2024. Comparison between artificial fire blight shoot and flower inoculations in apple. *Journal of Plant Pathology* 106:903−12
37. Zhu L, Zhang SJ, Ran B, Dong SL, Jian JN, et al. 2024. Identification and evaluation of resistance of 14 *Malus sieversii* f. neidzwetzkyana (Dieck) Langenf. germplasm resources to pear fire blight. *Plant Quarantine* 38:1−6 (in Chinese)
38. Bacete L, Mélida H, Pattathil S, Hahn MG, Molina A, et al. 2017. Characterization of plant cell wall damage-associated molecular patterns regulating immune responses. In *Plant Pattern Recognition Receptors: Methods and Protocols*, eds. Shan L, He P. New York, USA: Humana Press. pp. 13−23 doi: 10.1007/978-1-4939-6859-6_2
39. Wang B, Tian J. 2024. Association analysis of leaf structure and defense enzyme activity with fire blight resistance in different pear germplasms. *China Fruits* 10:113−23 (in Chinese)
40. Şahin M. 2023. Association between resistance to fire blight disease and leaf characteristics in quince progenies. *Erwerbs-Obstbau* 65:751−59
41. Adomako J, Osei MK, Prempeh RNA, Osei-Bonsu I, Gyau J, et al. 2024. Identification of *Ralstonia solanacearum* resistant solanum plants as potential rootstock to manage bacterial wilt disease in tomato production. *Technology in Horticulture* 4:e020
42. Khan MA, Zhao YF, Korban SS. 2013. Identification of genetic loci associated with fire blight resistance in *Malus* through combined use of QTL and association mapping. *Physiologia Plantarum* 148:344−53
43. Li P, Tan X, Wanghao, Sun L, Jiang J, et al. 2023. Transcriptome analysis of resistant and susceptible grapes reveals molecular mechanisms underlying resistance of white rot disease. *Horticulture Advances* 1:9
44. Fahrentrapp J, Broggini GAL, Kellerhals M, Peil A, Richter K, et al. 2013. A candidate gene for fire blight resistance in *Malus* × *robusta* 5 is coding for a CC−NBS−LRR. *Tree Genetics & Genomes* 9:237−51
45. Piazza S, Campa M, Pompili V, Dalla Costa L, Salvagnin U, et al. 2021. The Arabidopsis pattern recognition receptor EFR enhances fire blight resistance in apple. *Horticulture Research* 8:204

Copyright: © 2025 by the author(s). Published by Maximum Academic Press, Fayetteville, GA. This article is an open access article distributed under Creative Commons Attribution License (CC BY 4.0), visit https://creativecommons.org/licenses/by/4.0/.