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# Molecular Analyses to Identify Gene Resistance in Pear (Pyrus Communis) Cultivars for The Fire Blight Diseases in Iraqi Kurdistan Region.

**Sabrya Jarjees Zuber [1]** **Avesta Mohammed Ali [2]**
[1] *Biology department, Collage of Science, University of Duhok, IRAQ.*
[2]*Scientific Research Center, Collage of Science, University of Duhok, IRAQ.*
*Corresponding Author: sabrya.zuber@uod.ac

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## ABSTRACT

Fire blight, a bacterial infection, is caused by *Erwinia amylovora*, is pivotal in pear *(Pyrus communis*) that is a deciduous woody fruit-bearing plant that is classified within the Rosaceae family, under the subfamily Maloideae, and is part of the *Pyrus* genus. This fruit tree is known for shedding its leaves annually and thrives in temperate regions.The pathogen *Erwinia amylovora* is widely distributed in numerous countries where apple and pear cultivation is prevalent, affecting various plant species within the Rosaceae family, especially those in the Maloideae subfamily. Cultivating resistant cultivars against fire blight is highly feasible. This study utilized seven distinct primer combinations (MdE-EaN, MdE-EaK3, MdE-EaK5, MdE-EaK4, FRMB31M87, FRMb32MO4b, and FRMb32M27) to evaluate fire blight resistance presence in pear cultivars, yielding positive results in six regions in Kurdistan, Iraq. A diverse selection of examined cultivars exhibited band sizes ranging from 135 to 200 bp. The research effectively identified distinct genetic markers linked to resistance development against fire blight within specific pear cultivars. In Duhok, Harame Sherain landraces showed positive results with all primers, while Pazie also displayed positivity except with primer MdE-EaK5, while Hawaller, Qazam, and Buharie landraces had positive outcomes in all primers except with primer MdE-EaK5, whereas Doshawe from Saylaman and Gollawie from Halbja exhibited positive results except with primer FRMB31M87. Additionally, the study recognized certain pear landraces lacking essential genes for fire blight resistance, making them vulnerable to the disease. These findings underscore the critical role of genetic factors in determining a specific level of resistance against fire blight.

***Keywords*:** Fire blight, *Pyrus communis*, Molecular investigation, rsistanse gene, *Erwinia amylovora*..



## INTRODUCTION

The pear, scientifically referred to as *Pyrus communis* L., is a deciduous woody fruit that belongs to the Rosaceae family, specifically the subfamily Maloideae and the genus Pyrus [1]. Pears have been cultivated in Europe since ancient times, as evidenced by Homer's mention of the garden of Alcinous around 1000 B.C. According to [2], the genus Pyrus species spans 81 nations across Asia, Northern Africa, and Europe, reflecting their natural dispersion. Flourishing in temperate climates, they are highly valued for their prolific fruit production and aesthetic contribution to landscaping, resulting in extensive cultivation.

[3] asserts that the pear tree carries significant importance as a prominent fruit variety in various temperate regions. However, the availability of genetic variations that bolster pear resistance to diseases is limited [4]. Pears are susceptible to numerous pests and diseases, which significantly impact fruit production and quality. The susceptibility of European pear to fire blight presents a significant risk to pear cultivation in areas where it is cultivated [5].

Fire blight (F.B.), a disease, was first detected in the United States in 1780, primarily affecting wild species. [6] found that the pathogenic bacteria causing the disease were transmitted to susceptible cultivars of pear (Pyrus communis), apple (Malus silvestris), and quince (Cydonia oblongata), which were introduced to the United States by early settlers from Eastern Europe and Southwest Asia. [7] Consider this disease to be one of the foremost challenges in worldwide pome fruit production. Earlier research suggests that some widely cultivated cultivars, esteemed for their overall quality and fruit excellence, are particularly susceptible to fire blight [8]. Erwinia amylovora, a Gram-negative bacterium, causes substantial economic losses and yield declines in pear (Pyrus communis) orchards worldwide, impacting regions in North America, Europe, North Africa, the Middle East, Oceania, and Asia. Most recently, it has reached Central and East Asia, the native region of apple germplasm. Kyrgyzstan and Kazakhstan, and Infected orchards may experience up to 60- 90% yield losses, and in Iraq, the loss may be as high as 85-88%. [9] documented its presence in 46 countries, affecting not only pears but also other plants within the Rosaceae family. Under favourable conditions, fire blight can rapidly cause significant harm. Infected blossoms become dark, wilted, and spread to clusters, resulting in spur death. The infection then advances to branches, forming dark cankers that girdle and damage tissues. On shoots, bacteria blacken the leaves and cause a "shepherd's crook" shape, creating a scorched appearance. Near the rootstock, infections lead to bark discoloration and cracking, often causing swift tree death, and this disease significantly threatens the economic

sustainability of pome fruit production. Utilizing genetic advancements to improve fire blight resistance in apple and pear trees is a highly effective strategy for mitigating the adverse impacts of this disease, such as crop loss and tree mortality [10].

Using apple cultivars that demonstrate resistance or tolerance to the effects of Erwinia amylovora could potentially serve as a strategy for controlling this disease.In addition, hybridisation is commonly employed in breeding programs because multiple genes typically influence fire blight resistance, and its mechanism is intricate [11],[12]. A breeding strategy aims to enhance fire blight resistance, expediting breeding processes, and [13] found that pear species could serve as valuable gene reservoirs for future breeding programs seeking to develop resistant varieties. One strategy to address this is genetic engineering, introducing key resistance genes into cultivars to facilitate the development of resistant plant varieties. The generation of cultivars can be achieved by this approach [14]. Other methods of resistance and control these diseases; the inherent immunity against Escherichia coli and Erwinia amylovora has undergone thorough investigation in various wild Pyrus species, and more recently, it has been incorporated into conventional breeding programs [15], also [16] reported that biological approaches involving various bacterial strains are crucial for controlling fire blight disease on pear trees, caused by Erwinia amylovora.

Managing the disease can be extremely challenging in environments conducive to its spread, Currently, disease management involves eradicating infected plants and tissues, as well as using antibiotics and copper-based compounds [17],[18]. Producing resistant cultivars has been recognized as a highly effective strategy for managing fire blight [19]. Utilizing genetic resistance offers an appealing option for disease treatment and holds potential for reducing perceived risks associated with antibiotic application in target non-target environments.

The prioritization of molecular markers has been emphasized in characterizing the genetic basis of pear resistance to major diseases and pests [20], [21],[22]. Consequently, extensive endeavors have been made to ascertain genomic regions that are pivotal in conferring resistance, leading to the discovery of numerous quantitative trait loci (QTLs) across different genetic backgrounds [23],[24],[25],[26],[27],[28],[29]. Genetic resistance research conducted on the Malus genus has been achieved by identifying many quantitative trait loci (QTLs) within apple cultivars and wild apple species accessions [30]. Furthermore, [31] have established the association between possible resistance gene markers and the fire blight resistance QTL of Mr5.

The identification of SSR markers, RGA markers, and modified AFLP markers in fire blight-resistant and tolerant pear has not yet been achieved [32]. Molecular markers have been utilized to identify apple cultivars and genotypes resistant to fire blight caused by Erwinia amylovora, as reported by [19]. Furthermore, according to [33], the use of DNA markers has shown that genetic resistance significantly contributes to fire blight management. The primary goal of this research is to achieve molecular analyses to identify genes for resistance to fire blight in sixty landrace cultivars of pear (Pyrus communis) obtained from different regions of Kurdistan

Materials and Methods

Plant Material, DNA isolation and determination of DNA concentration and purity

The current study analyzed 60 pear landraces sourced from the mountainous regions within the Kurdistan area of Iraq. These landraces were sourced from mature trees collected from six distinct geographical locations, specifically Duhok, Akri, Erbil, Sulaymaniyah, Shaqlawa, and Halabja, as outlined in Table 1.

The procedure of DNA isolation encompasses multiple sequential phases. Initially, the collection of newly growing leaves from each accession was conducted between the spring months of April and July. Subsequently, the leaves were promptly subjected to quick freezing using liquid nitrogen and subsequently preserved at a temperature of -20 °C until they were deemed necessary for subsequent examination.

In this study, the genomic DNA extraction method was based on the procedure outlined by (34). The DNA was extracted from young leaves, with an average weight of 2 g, collected from various pear trees. The leaves were later ground into a fine powder using liquid nitrogen. The fine powder underwent agitation in a water bath set at 60 °C for 30 minutes. It was achieved by utilizing a solution of 2x CTAB extraction buffer contained in beakers. The extraction buffer consisted of 2x CTAB, 5M NaCl, 1M Tris-HCl, and 0.5 M EDTA. The combination extraction was conducted using a solution comprising chloroform and isoamyl alcohol in a volumetric ratio of 24:1. Following that, the mixture was subjected to centrifugation with a force of 1400 times the acceleration due to gravity for 30 minutes. The DNA was suspended at a temperature of +4°C for twenty-four hours and, after that, stored at a temperature of -20°C. The quantification and assessment of DNA purity were conducted for each sample. The research conducted in this study includes acquiring OD260 and OD280 nanometer optical densitometry spectrophotometer NanoDrop readings for the specific objective at hand. Subsequently, each sample was diluted to achieve a final concentration of 50 nanograms per liter (ng/L).

Table 1 the name and the location of pear

| No | Name | Location | No | Name | Location |
|---|---|---|---|---|---|
| 1 | Krosk | Duhok/ Mateen | 31 | Kaske | Shaqlawa |
| 2 | Zarik | Duhok / Arza | 32 | Hara Masie | Shaqlawa |
| 3 | Hezel | Duhok / Arza | 33 | Sorke Dem | Shaqlawa |
| 4 | Gelkie | Duhok / Arza | 34 | Shenke | Shaqlawa |
| 5 | Herorie | Duhok / Arza | 35 | Spegrie | Shaqlawa |
| 6 | Rashik | Duhok / Arza | 36 | Sorke Awe | Shaqlawa |
| 7 | Krosk | Duhok/ Gara | 37 | Lasor | Shaqlawa |

| | | | | | |
|---|---|---|---|---|---|
| 8 | Harmie Sherain | Duhok/Gara | 38 | Sew Harmie | Shaqlawa/ Aqobane |
| 9 | Krosk Sherain | Duhok/ Sorie | 39 | Krosk Mazin | Shaqlawa/ Aqobane |
| 10 | Krosk | Duhok /Peda | 40 | Krosk Bechok | Shaqlawa/ Aqobane |
| 11 | Havenie | Akre/Denarte | 41 | Qalatie/ | Sulaimaniya/Khamza |
| 12 | Hezel | Akre/Denarte | 42 | Lasor | Sulaimaniya/Khamza |
| 13 | Gelase | Akre/Denarte | 43 | Balegie | Sulaimaniya/Khamza |
| 14 | Haveni Bchok | Akre/Denarte | 44 | Naske | Sulaimaniya/Khamza |
| 15 | Payzie | Akre/Denarte | 45 | Zartke/ | Sulaimaniya/Khamza |
| 16 | Buharie | Akre/Denarte | 46 | Do Shawe | Sulaimaniya/Khamza |
| 17 | Harme Sivie | Akre/Devryi | 47 | Khamza | Sulaimaniya/Khamza |
| 18 | Sana Sive | Akre/Denarte | 48 | Gollawie | Sulaimaniya/Khamza |
| 19 | Krosk Xshok | Akre/Devryi | 49 | Sew Harmie | Sulaimaniya/Khamza |
| 20 | Sorka | Akre/Denarte | 50 | Shaxawan | Sulaimaniya/Khamza |
| 21 | Qazam | Erbil/Ankawa | 51 | Sorke | Halabja/Khurmal |
| 22 | Spegre | Erbil/Ankawa | 52 | Sew Harmie | Halabja/Khurmal |
| 23 | Jafaranie | Erbil/Khanzad | 53 | Krosk | Halabja/Khurmal |
| 24 | Bar Awe | Erbil/Khanzad | 54 | Shenawie | Halabja/Khurmal |
| 25 | Naske | Erbil/ Khanzad | 55 | Kaske | Halabja/Khurmal |
| 26 | Gelke Mase | Erbil/ Khanzad | 56 | Bazinganie | Halabja/Khurmal |
| 27 | Buharie | Erbil /Khanzad | 57 | Gollawie | Halabja/Khurmal |
| 28 | Hara Masie | Erbil/ Ankawe | 58 | Khurmal | Halabja/Khurmal |
| 29 | Sorke | Erbil /Ankawe | 59 | Hawenie | Halbja/Serwan |
| 30 | Ankawe | Erbil / Ankawa | 60 | Se Bar | Halabja/Khurmal |

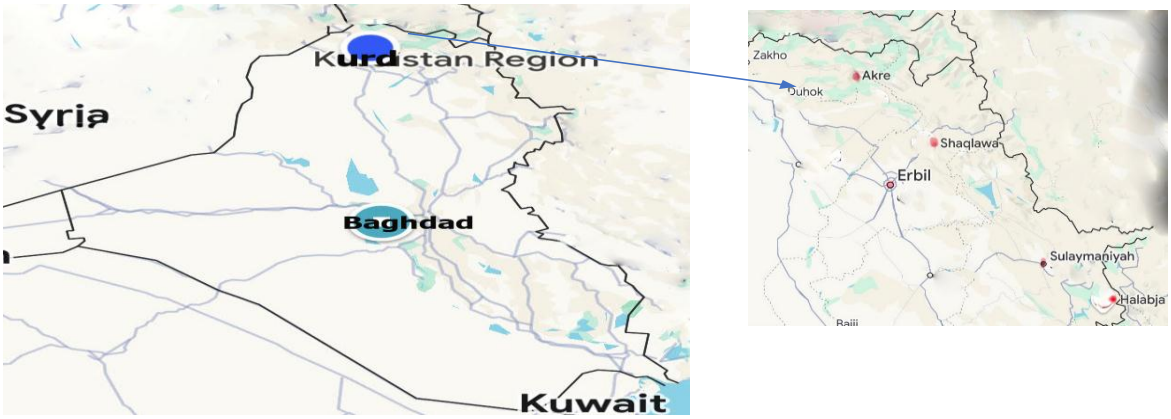


Figure 1. Geographic Distribution of Pear Landraces in the Kurdistan Region of Iraq

## PCR and electrophoresis procedures

The PCR amplification procedure was conducted using the ABI Applied Biosystems platform. Marker genotyping was performed in a multiplex format, utilizing the master mix kit specified by the manufacturer's protocol. The reaction volume was set at 15 µl, and the amplification conditions comprised an initial denaturation step at 95°C for 4 minutes. Subsequently, 30 cycles were conducted, consisting of denaturation at 95°C for 3 minutes, annealing at 48–56°C for 1 minute and 30 seconds, and extension at 72°C for 30 seconds. A final extension step was carried out for 30 minutes at 60°C. Following amplification, the DNA fragments were analyzed through agarose gel electrophoresis, employing a 1% concentration of TBE buffer. The electrophoresis procedure lasted 90 minutes at 70 volts. Subsequently, the gel was examined and photographed using ultraviolet (UV) light with minimal adjustments [35]. A total of seven out of eight primer combinations were employed for the detection of fire blight as FRMB31M87, FRMb32MO4, FRMb32M27, MdE-EaK3, MdE-EaNand, MdE-EaK5 [36]. as in Table2

Table 2: Base sequences and Tm (°C) values of primer pairs used in this study

| N | Primer name | Forward primer5-3 | Reverse primer 3-5 | Tm (°C) |
|---|---|---|---|---|
| 1 | FRMB31M87 | AAAGAGCTTTGCTTGGCTTG | TCTCAACTTTCGCACCAACC | 51 |
| 2 | FRMb32MO4 | TGGACAAATTCAGTGACACCA | CAAACCACCCCAAATTCTGT | 50 |
| 3 | FRMb32M27 | TTTAATTGGCTTTTCATTCACG | AAGGCGACTCATGATTTCGTA | 48 |
| 4 | MdE-EaK3 | AGCAGGGACGGGAGTTTAAGG | GGAAGTCGGAGAGGAAACCATTAG | 57 |
| 5 | MdE-EaK4 | GGGTTGAGGACAGGACAGACG | AGCACAGCAATACCAAACTAAGCC | 57 |
| 6 | MdE-EaN | GCAGTTAGGGAGGGAAATTGTC | CCTTTGGCTCGCTTTAGATACG | 55 |
| 7 | MdE-EaK5 | TGCCTCATCAGTGCTAAAAG | CTCAAGGAGTTGGGGACAAG | 50 |

**Data analysis**

**Results and discussion**

The study estimated 60 different pears from various regions of Kurdistan, including Duhok, Akri, Erbil, Shaqlawa, Sulaymaniyah, and Halabja, for susceptibility or resistance to fire blight. A collection of seven out of eight primers was utilized. (Fig 2, and 3).

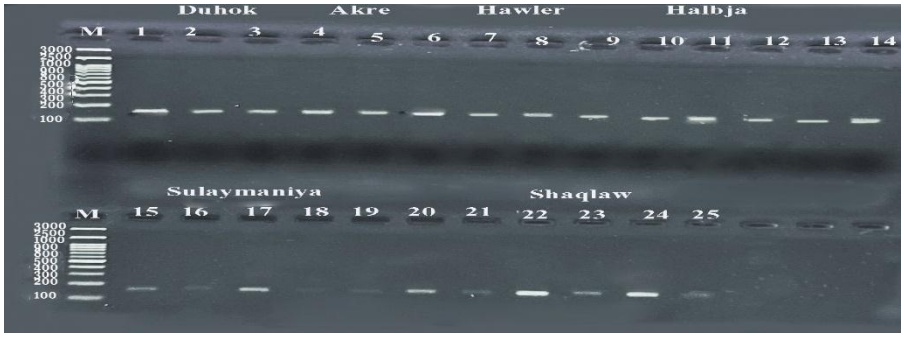


Figure (2): Amplified PCR products obtained with Microsatellite marker analysis for resistance fire bright disease the primer combination (MdE-EaK5) in pear, run on 2 % Agarose gel electrophoresis, M represents DNA Marker (100bp). from six region (Duhok) 1. Harme Sherain 2 Krosk Sherain. 3. Kroske Peda., Payzie, Buharie, and Harme Sive, (Akri) 4. Havenie 5. Payzi 6. Buharie 7. Harme Sive, Hawler )8. Qazam 9. Jufaranie 10. Gelke Mase 11. Sorke cultivars, (Halbja)12. Sorke13. Kaske 14.Gollawe,(Sulaymanlya) 15 Qalatie16. Lasor 17. Naske 18.Zartke 19. Balegie 20. Doshaw 21. Xamza 22. Shaxawan, and (shaqlaw) 23. Harme Sewe 24. Lasor 25. Krosk Bechok.

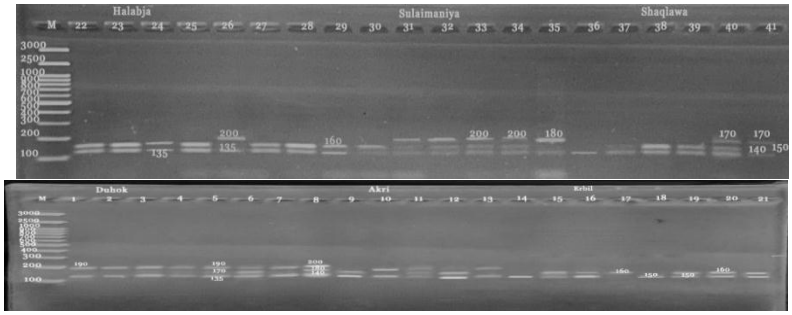


Figure (3): Amplified PCR products obtained with Microsatellite marker analysis for resistance fire bright disease the primer combination (FRMb32M27) in pear, run on 2 % Agarose gel electrophoresis, M represents DNA Marker (100bp). From six region (Duhok) 1. Zarik 2. Krosk Matenie3.Hezel 4. Herorie 5. Rashik 6. Harmie Sherain 7. Krosk Sorye, (Akri) 8. Hezel 9. Payzie10.Gelas, 11. Havine Bocho 12. Buharie13. Sana Sivi 14. Sorka, (Erbil) 15. Qazam 16. Gelke Mase 17. Spegraye 18. Jafaranie 19. BarAwe 20.Buharie 21.HaraMase, (Halbja) 22.Sorke, 23.Sew Harmie 24.Krosk 25. Shenawie 26. Bszinganie 27. Hawene, 28. Gollowie,29. Kaske, (Sulaymanlya) 30 Lasor 31 Balegie 32. Naske 33. Xamza 34. Gollawe 35. Qalate 36.Sew Harmie, and (shaqlaw) 37.Hara Mase 38.Sorka Dem, 39. Shenk 40.Spegrie 41.Seve Harmie

The MdE-EaN primers indicate limited genetic diversity in the study of ten pears from Duhok, including Krosk Mateen, Zarik,

Hezel, Gelkie, Herorie, Rashik, Krosk Sherain, Harmie Sherain, Krosk Sorya, and Krosk Peda, showing a single resistance locus at approximately 160 bp**.**

In the Akri, ten pear including Havenie Mazine, Hezel, Gelase, Havenie Bechok, Payzie, Buharie, Harme Sive, San Sivie, Krosk Xeshok, and Sorke each exhibit a single resistance locus, estimated at approximately 150 bp.In the Erbil region, nine out of ten pears, each approximately 150 bp in size, namely Qazam, Spegrie, Jifarani, Bar Awe, Naske, Gelki Masie, Buharie, Har Mase, and Sorke, exhibit a resistance locus. However, the Ankawe sample is highly susceptible. In the Halabja, seven out of ten pears like Sorke, Sew Harmie, Shenawie, Kaske, Bazinganie, Gollowie, and Khurmal demonstrate resistance to a specific locus, while the other three as Se bar, Hawenie, and Krosk are susceptible, estimated to be approximately 140 bp.

In the Sulaymaniyah, all ten pears as Qalatie, Lasor, Balegie, Naske, Zartke, Doshawe, Khamza, Gollawie, Sew Harmie, and Shaxawan have resistance loci, each approximately 170 bp.

In the Shaqlawa, all ten pears as Kaske, Harmasie, Sork Dem, Shenke, Spegrie, Sorke Awe, Lasor, Sew Harmie, Krosk Mazin, and Kroske Bchok possess single resistance loci, each around 180 bp.

The combination MdE-EaK3 showed consistent patterns across sixty pears from six regions in Kurdistan. In Duhok, two out of ten (Harmie Sherain and Krosk Sherain) demonstrated resistance at a single locus, while others were susceptible, approximately 160 bp. Similarly, in Akri, three out of ten (Payzie, Kroskie Xshok, and Sorke) exhibited resistance, while others (Havenie, Hezel, Gelase, Haveni Bchok, Buharie, Harme Sive, and Sana Sive) were susceptible, with an estimated length of 170 bp. In Erbil, three out of ten pears (Kela Mase, Buharie, and Hara Mase) showed resistance, while others (Qazam, Spegrie, Jafaranie, Bar Awe, Naske, Sorke, and Ankawe) were vulnerable, approximately 150 bp, in Halabja four out of ten pears (Sorke, Kaske, Krosk, and Sew Harmie), while others (Shenawie, Bazinganie, Gollawie, Khurmal, Hawenie, and Se Bar) were susceptible, around 160 bp. In Sulaymaniyah, four out of ten (Lasor, Balegie, Doshaw, and Khamza) displayed resistance, equivalent to about 160 bp, while the remaining five were susceptible. Lastly, in Shaqlawa, five out of ten (Hara Mase, Sorka Dem, Sorka Awe, Lasor, and Sew Harmie) showed resistance, while the others were vulnerable

The MdE-EaK5 primer reveals a consistent pattern in 60 pears across six regions in Kurdistan. In Duhok, three out of ten pears, namely Harme Sherain, Krosk Sherain, and Kroske Peda, are estimated around 170 bp and exhibit a single resistance locus, while the remaining seven are susceptible. Similarly, in the Akri , four out of ten pear, including Havenie, Payzie, Buharie, and Harme Sive, are estimated to be 160 bp and exhibit a single resistance locus, while the rest are susceptible.

In Erbil, four out of ten pears, including Qazam, Jufaranie, Gelke Mase, and Sorke, show resistance loci estimated to be around 150 bp, while the other six are susceptible. In Halabja three out of ten pears, namely Sorke, Kaske, and Gollawe, exhibit a single resistance locus with a length of 145 bp, while the rest are susceptible. Furthermore, in Sulaymaniyah's inventory, seven out of ten pears, including Qalatie, Lasor, Naske, Zartke, Balegie, Doshaw, Khamza, and Shaxawan, show single resistance loci spanning roughly 160 bp, while three are vulnerable. Shaqlawa's study identifies four out of ten resistant pears, around 150 bp, namely Kaske, Harme Sewe, Lasor, and Krosk Bechok, with the remaining six being susceptible.

The primer pair MdE-EaK4 produced consistent genetic profiles in 60 pears from all six regions of Kurdistan, in Duhok, three out of ten pears like Rashik, Harmie Sherain, and Krosk Peda exhibited a single resistance locus, while the remaining seven were susceptible, estimated to be around 170 bp. Similarly, in the Akri, seven out of ten pear like are Havenie, Hezel, Paizie, Buharie, Harme Sive, San Sivie, and Sorke , showed a single resistance locus, each roughly 160 bp. In Erbil, five out of ten pears Qazam, Jifarani, Buharie, Sorke, and Ankawe demonstrated resistance at a single locus, while the other five were susceptible, totaling approximately 160 bp.

In Halabja, seven out of ten pears' varieties, including Sorke, Sew Harmie, Kroska, and Kaske, possess resistance loci around 180 bp. In Sulaymaniyah, four out of ten pear varieties, such as Zartke, Doshaw, Khamza, and Shaxawan, exhibit resistance loci approximately 170 bp, while six other individuals are susceptible. In Shaqlawa, four out of ten, like Kaske, Shenke, Sorke, and Kroske bchok, feature loci of 160 bp. Resistance is evident at one locus in identified landraces, while susceptibility is observed at others.

The FRMb32MO4b primer combination revealed monomorphism and polymorphism among 60 pears from Kurdistan. In Duhok region , seven out of ten pears one of them exhibited two resistance loci for Zarik, approximately 190 to 250 bp, while the other is displayed a single resistance locus at approximately 190 bp like Hezel, Gelkie, Herorie, Krosk Sherain, Haeme Sherain, and Krosk peda landraces, while the remained are susceptible, Similarly, in the Akri, seven out of ten pears like, namely Havenie Mazine, Havenie Bechok, Hezel, Payzie, Hamer Seve, Sana Sivie, and Sorke, showed one resistance loci at approximately 190 bp, the other three are found to be susceptible. In the Erbil, it has been observed that five out of 10 pears, specifically Qazam, Spegrie, Buharie, and Hara Mase, have one of the resistance loci, with around 200 bp. Conversely, the remaining five were determined to be susceptible. In the Halabja, four out of ten pear landraces, two displayed a single resistance locus are about 200 bp, namely Krosk and Kaske. At the same time, the other two, Gollowie and Shenawie, have been found to contain two resistance loci (approximately 190 bp and 250 bp). In contrast, the other six pears are susceptible**.**

In the Sulaymaniyah, seven out of ten pears, namely Qalatie, Lasor, Balegie, Naske, Zartke, Doshaw, and Shaxawan, exhibit a single resistance locus at 200 bp, while the remaining three are susceptible. Within the Shaqlawa, ten distinct pears are present. Among them, Kaske and Lasor demonstrate three resistance loci, ranging from approximately 150 to 170 and 200 base pairs. Conversely, Sorke Awe, Sive Harriet, and Krosk Mazn display two resistance loci, at 150 to 200bp. However, Harmasie, Sorke, Spegrie, Shenke, and Krosk Bchoke are indicated to possess only a single resistance locus at 150 bp

The FRMB31M87 primer showed genetic similarity among sixty pears from six areas in Kurdistan.

In the Duhok, eight out of ten pears, including Krosk Maten, Zarik, Hezel, Gelkie, Herorie, Krosk Sherain, Harme Sherain, and Krosk Sorya, showed resistance loci, while the other two were susceptible, at 150 bp.

In the Akri four out of ten pear, namely Havenie Bechok, Buharie, Harme Sive, and Kroskie Xshoke, had a single resistance locus of around 150 bp, while the remaining were susceptible. In the Erbil, seven out of ten pears, namely Qazam, Spegrie, Jifarani, Bar Awe, Gelke Mase, Buharie, and Har Mase, exhibited resistance at one locus, while the other three were susceptible, at 140 bp.

In Halabja, seven out of ten pears, including Sorke, Sew Harmie, Krosk, Shenawie, Kaske, Bazinganie, Gollowie, and Khurmal, show resistance at around 145 base pairs. With individual resistance loci, while the others are susceptible.In Sulaymaniyah, among ten pear including Qalatie, Lasor, Balegie, Naske, Doshaw, Khamza, Gollawie, Sew Harmie, and Shaxawan, nine showed one resistance locus, while other one is susceptibility, with a length of around 135 bp.

Seven out of ten pears in Shaqlawa, including Harmassie, Sorka, Spegre, Sork Awe, Lasor, Krosk Mazin, and Krosk Bchoke, each exhibited a single resistance locus around 140 base pairs long. Conversely, the other landraces were susceptible.

The FRMb32M27 primer combination showed monomorphic and polymorphic patterns in sixty pears from six locales in Kurdistan. In Duhok, seven out of ten pears, such as Zarik and Krosk Matenie, displayed two resistance loci (135-190 bp), while another group, including Hezel, Herorie, Rashik, Harmie Sherain, and Krosk Sorye, had three resistance loci (135-170 and 190 bp), indicating varied genetic resistance among them, while others exhibit three susceptibility loci**.** In the Akri region, seven of ten pears display multiple resistance loci. Hezel and Payzie have three loci (approx. 140–180–200 bp), while Gelas, Havine Bochok, Buharie, and Sana Sivi show two loci (approx. 140 and 180 bp). Sorka has one locus (about 140 bp). The remaining three are susceptible.

In Erbil, researchers found eight of ten pears, like Qazam and Gelke Mase, with a single resistance locus about 150 and 160 bp long. The other six, including Spegraye, Jafaranie, Bar Awe, Buharie, Hara Mase, and Sorke, show two loci around 150 and 160 bp. The rest are susceptible.

In Halabja, eight out of ten pears, including Sorke, Sew Harmie, Krosk, Shenawie, Bszinganie, Hawene, and Gollowie, exhibit two resistance loci around 135–170 bp. Another like, Kaske, also displays two resistance loci around 135–200 bp. The remaining landraces are susceptible.

In the Sulaymanyia region, seven out of ten pears display multiple resistance loci. Five of these like Lasor, Balegie, Naske, Khamza, and Gollawe possess three resistance loci around 140 to 160 to 180 bp. Qalate has two loci around 140 and 160 bp, while Sew Harmie has a single locus at approximately 140 bp.The rest are susceptible.

In Shaqlawa, six out of ten pears show varying resistance levels. Three of them like Hara Mase, Sorka Dem, and Shenk have three resistance loci around 140-150 bp, while Spegrie, and Seve Harmie exhibit two loci around (140-150-170) and one hase one louce around 140pb, all pear remain are susceptible.

Table 3: the name, primers combination, resistant, susceptible and Allele size (bp) of pear from Kurdistan region

| no | name | primers combination, Landraces resistant (+) and susceptible (-), and allele size | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MdE-EaN | (bp) | MdE-EaK3 | (bp) | MdE-EaK5 | (bp) | MdE-EaK4 | (bp) | FRMb32MO4b | (bp) | FRMB31M87 | (bp) | FRMb32M27 | Allele size (bp) |
| 1 | Krosk Maten | + | 160 | - | - | - | - | - | - | - | - | + | 150 | + | 135-190 |
| 2 | Zarik | + | 160 | - | - | - | - | - | - | + | 190-250 | + | 150 | + | 135-190 |
| 3 | Hezel | + | 160 | - | - | - | - | - | - | + | 190 | + | 150 | + | 135-170-190 |
| 4 | Gelkie | + | 160 | - | - | - | - | - | - | + | 190 | + | 150 | - | - |
| 5 | Herorie | + | 160 | - | - | - | - | - | - | + | 190 | + | 150 | + | 135-170-190 |
| 6 | Rashik | + | 160 | - | - | - | - | + | 170 | - | - | - | - | + | 135-170-190 |
| 7 | Krosk Sherain | + | 160 | + | 160 | + | 170 | - | - | + | 190 | + | 150 | - | - |
| 8 | Harme Sherain | + | 160 | + | 160 | + | 170 | + | 170 | + | 190 | + | 150 | + | 135-170-190 |
| 9 | Krosk Sorya | + | 160 | - | - | - | - | - | - | - | - | + | 150 | + | 135-170-190 |
| 10 | Krosk Peda | + | 160 | - | - | + | 170 | + | 170 | + | 190 | - | - | - | - |
| 11 | Havenie | + | 150 | - | - | + | 160 | + | 160 | + | 180 | - | - | - | - |
| 12 | Hezel | + | 150 | - | - | - | - | + | 160 | + | 180 | - | - | + | 140-180-200 |
| 13 | Gelase | + | 150 | - | - | - | - | - | - | - | - | | - | + | 140-180 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14 | Haveni Bchok | + | 150 | - | - | - | - | + | 160 | + | 180 | + | 150 | + | 140-180 |
| 15 | Payzie | + | 150 | + | 170 | + | 160 | + | 160 | + | 180 | | - | + | 140-180-200 |
| 16 | Buharie | + | 150 | - | - | + | 160 | - | - | - | - | + | 150 | + | 140-180 |
| 17 | Harme Sive | + | 150 | - | - | + | 160 | + | 160 | + | 180 | + | 150 | - | - |
| 18 | Sana Sive | + | 150 | - | - | - | - | + | 160 | + | 180 | - | - | + | 140-180 |
| 19 | Krosk Xshok | + | 150 | + | 170 | - | - | - | - | - | - | + | 150 | - | - |
| 20 | Sorka | + | 150 | + | 170 | - | - | + | 160 | + | 180 | - | | + | 140 |
| 21 | Qazam | + | 150 | - | - | + | 150 | + | 160 | + | 200 | + | 140 | + | 150 |
| 22 | Spegrie | + | 150 | - | - | - | - | - | - | + | 200 | + | 140 | + | 150-160 |
| 23 | Jafaranie | + | 150 | - | - | + | 150 | + | 160 | - | - | + | 140 | + | 150-160 |
| 24 | Bar Awe | + | 150 | - | - | - | - | - | - | - | - | + | 140 | + | 150-160 |
| 25 | Naske | + | 150 | - | - | - | - | - | - | - | - | - | - | - | - |
| 26 | Gelke Mase | + | 150 | + | 150 | + | 150 | - | - | - | - | + | 140 | + | 150 |
| 27 | Buharie | + | 150 | + | 150 | - | - | + | 160 | + | 200 | + | 140 | + | 150-160 |
| 28 | Hara Masie | + | 150 | + | 150 | - | - | - | - | + | 200 | + | 140 | + | 150-160 |
| 29 | Sorke | + | 150 | + | - | + | 150 | + | 160 | + | 200 | - | - | + | 150-160 |
| 30 | Ankawe | - | - | - | - | - | - | + | 160 | + | - | - | - | - | - |
| 31 | Kaske | + | 180 | - | - | + | 150 | + | 160 | - | 150-170-200 | - | - | - | - |
| 32 | Hara Masie | + | 180 | + | 150 | - | - | - | - | + | 150 | + | 140 | + | 140-150 |
| 33 | Sorke Dem | + | 180 | + | 150 | - | - | + | 160 | + | 150 | + | 140 | + | 140-150 |
| 34 | Shenke | + | 180 | - | - | - | - | + | 160 | + | 150 | - | - | + | 140-150 |
| 35 | Spegrie | + | 180 | - | - | - | - | - | - | + | 150 | + | 140 | + | 140-150-170 |
| 36 | Sorke Awe | + | 180 | + | 150 | - | - | - | - | + | 150-200 | + | 140 | - | - |
| 37 | Lasor | + | 180 | + | 150 | + | 150 | - | - | + | 150-170-200 | + | 140 | - | - |
| 38 | Sew Harmie | + | 180 | + | 150 | + | 150 | - | - | + | 150-200 | - | - | + | 140-150-170 |
| 39 | Krosk Mazin | + | 180 | - | - | - | - | - | - | + | 150-200 | + | 140 | + | 140-150-170 |
| 40 | Krosk Bechok | + | 180 | - | - | + | 150 | + | 160 | + | 150 | + | 140 | - | - |
| 41 | Qalatie | + | 170 | - | - | + | 160 | - | - | + | 200 | + | 135 | + | 140-160 |
| 42 | Lasor | + | 170 | + | 160 | + | 160 | - | - | + | 200 | + | 135 | + | 140-160-180 |
| 43 | Balegie | + | 170 | + | 160 | - | -- | - | - | + | 200 | + | 135 | + | 140-160-180 |
| 44 | Naske | + | 170 | - | - | + | 160 | - | - | + | 200 | + | 135 | + | 140-160-180 |
| 45 | Zartke | + | 170 | - | - | + | 160 | - | 170 | + | 200 | - | - | - | - |
| 46 | Do Shawe | + | 170 | + | 160 | + | 160 | - | 170 | + | 200 | + | 135 | - | - |
| 47 | Khamza | + | 170 | + | 160 | + | 160 | - | 170 | - | - | + | 135 | + | 140-160-180 |
| 48 | Gollawie | + | 170 | - | - | - | - | - | - | - | - | + | 135 | + | 140-60-180 |
| 49 | Sew Harmie | + | 170 | - | - | - | - | - | - | - | - | + | 135 | + | 140 |
| 50 | Shaxawan | + | 170 | - | - | + | 160 | - | 170 | + | 200 | + | 135 | - | - |
| 51 | Sorke | + | 140 | + | 160 | + | 145 | - | 180 | - | - | + | 145 | + | 135-170 |
| 52 | Sew Harmie | + | 140 | + | 160 | - | - | - | 180 | - | - | + | 145 | + | 135-170 |
| 53 | Krosk | - | -- | + | 160 | - | - | - | 180 | + | 190 | + | 145 | + | 135-170 |
| 54 | Shenawie | + | 140 | - | - | - | - | - | 180 | + | 190-200-250 | + | 145 | + | 135-170 |
| 55 | Kaske | + | 140 | + | 160 | + | 145 | - | - | + | 190 | + | 145 | + | 135-200 |
| 56 | Bazinganie | + | 140 | - | - | - | - | - | - | - | - | + | 145 | + | 135-170 |
| 57 | Gollawie | + | 140 | - | - | + | 145 | - | 180 | + | 190 | + | 145 | + | 135-170 |
| 58 | Khurmal | + | 140 | - | - | - | - | - | - | - | - | + | 145 | - | -- |
| 59 | Hawenie | - | - | - | - | - | - | - | - | - | - | + | 145 | + | 135-170 |
| 60 | Se Bar | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

**The Topic of Discussion is Being Addressed.**

Our study results demonstrate that, for most of the genotypes analyzed, seven primers with FB resistance markers were applied to their DNA templates. As shown in Table 3, these markers were statistically significant and illustrated the frequency distribution of fire blight severity across a selection of pear trees. The configuration of an individual's alleles, influenced by the primer combination size, determines their susceptibility or resistance. The findings suggest that multiple genes contribute to fire blight resistance, with certain genomic regions and clusters of analogues specifically associated with this resistance. Furthermore, to evaluate the effectiveness and durability of this resistance trait, it is essential to understand the mechanisms

governing the interaction between the plant and the pathogen. This involves investigating the proteins responsible for pathogen recognition and the consequent defence responses activated. Once a FB resistance gene has been identified, it can be integrated into a commercial cultivar through genetic modification, aiming to develop a pome cultivar resistant to fire blight [37].

Numerous genetic maps have been constructed to establish the association between DNA markers and resistance genes in pears. [38] have identified specific genes crucial in safeguarding fruits against various diseases, such as black spot disease, scab, and fire blight.

The gene sequence was utilized in the design of primers, which were subsequently assessed as potential molecular markers. A total of sixty pear landraces were subjected to testing using these primers. Furthermore, relevant data were acquired that were hypothesised to be correlated with the presence or absence of sensitivity to fire blight**.**

Evaluating the resistance or susceptibility of pear genotypes to fire blight is a critical component of breeding programs. Like other diseases, fire blight on a particular genotype depends on several factors, including genetic makeup, tree age, environmental conditions, and soil and tree care techniques. [39],[40],[25],[41],[42] Several DNA markers have been associated with various resistance genes in pears, including those responsible for conferring protection against black spot disease, scab, and fire blight. Additionally, multiple genetic maps have been developed to aid in studying these genetic traits. [38] concluded that the findings from this study will enhance our understanding and utilization of the mechanisms involved in disease resistance and quality.

Seven primer combinations were employed in this study, five primers: MdE-EaN, MdE-EaK3, MdE-EaK5, MdE-EaK4, and FRMB31M87. 57 of 60 pear landraces as in the MdE-EaN, 21 out of 60 pear landraces as in the primer MdE-EaK3, 25 of 60 pears as in the MdE-EaK5, and 27 out of 60 pears as in FRMB31M87 which indicated monomorphic pattern. The study's findings show that the landraces being looked at have a single resistance locus, as shown in Table 3. This locus is linked to a moderate level of resistance. The study's findings by [33] indicate that certain apple cultivars with single resistance loci exhibit a modest level of resistance. These results align with previous research in the field. Previous studies have revealed the identification of apple varieties that display resilience, as evidenced by the research conducted by [43]

Similarly, utilising the two remaining primer combinations, FRMb32MO4b and FRMb32M27, unveiled a discernible pattern that exhibited a combination of fixed and variable characteristics across the cohort of 60 pear landraces. The study observed that 40 out of 60 pear samples in the FRMb32MO4b primer combination and 43 out of 60 pears in the FRMb32M27 primer combination revealed a monomorphic characteristic. The findings from combination FRMb32MO4b indicate that the landraces Zarik from the Duhok region, Shenawe from Halbja Kask, Sorke Awe, Lasor, Seve Harmie, and Krosk Mazin from the Shaqlawe region displayed monomorphic resistance loci. various landraces, including Krosk maten, Zarik, Hezel, Herorie, Rashik, Sherain, and Krosk sorya from Duhok, Hezel, Gelase, Haveni bchok, Payzie, Buharie, and Sana sive from Akri, Spegrie, Jafaranie, Buharie, Hara masie, and Sorke from Erbil , and Hara masie, Sorke dem, Shenke, Spegrie, Seve harmie, and Krosk mazin from Shaqlaw, as well as Qalatie, Lasor, Belgia, Naske, Khamza, and Gollaway from Sulamany, and Sorke, Sew Harmie, Krosk, Shenawie, Kaske, Bazinganie, Gollawie, and Hawene from Halabja region, have been identified as resistant to fire blight due to the presence of multiple resistance loci.

Furthermore, a total of 60 landraces were assessed, and it was observed that Harme Sheraina demonstrated resistance. This resistance was attributed to resistance loci in all of the employed primers. The pears described in Table 3, specifically Payzie from Akri, Qazam, and Buharie from Erbil; Sorke Dem from Shaqlawa; Lasor and Khamza from Sulaymaniyah; and Kaske and Gollawie from Halbja, are of significance within the scope of this study. the presence of resistance loci was observed in six out of seven primers across apple cultivars. Furthermore, the research undertaken by [44] provided new findings concerning the feasibility of using the RAPD marker to develop SCAR markers that can accurately identify fire blight resistance in pears.

Both wild species and domesticated cultivars can serve as sources for fire blight resistance. The initial generation of breeding outcomes may be successful with the establishment of resistant cultivars. However, integrating resistance from wild species requires numerous backcross generations to reduce the proportion of unwanted wild species in the genome [25]. In their comprehensive study, [45] examine the resistance displayed by different wild pear species. The analysis performed by the scientists encompassed a comprehensive dataset consisting of 107 selections derived from 17 separate species and 85 selections obtained from controlled interspecific crosses. Additionally, a multitude of pear species hybrids were included in the analysis [39]

Notably, both the resistance to fire blight and the level of susceptibility exhibit fluctuations, even within the same climatic conditions, throughout different years [46]. A notable degree of heterogeneity is observed in the levels of susceptibility and resistance to fire blight among wild and farmed apple cultivars. Multiple Malus species, specifically Malus robusta, Malus sublobata, Malus ×atrosanguinea, Makus prunifolia, and Malus fusca, have been recognized as prospective candidates for fire blight resistance. Moreover, specific M. Domesticated cultivars and rootstocks, namely 'Nova Easygro', 'Florina', and 'Priscilla', have exhibited noteworthy resilience. Several studies have been conducted on this topic [47],[48],[49],[50].[51] have identified genetic loci that have a heightened correlation with resistance in crossing tests involving wild and domesticated apple varieties**.** This study constitutes the primary endeavor in the Kurdistan region to evaluate the genetic resilience of indigenous varieties of pear against fire blight disease. This work provides further evidence to support the strong prediction

capabilities of marker-assisted approaches in managing fire blight.

**Conclusion**

The study analyzed pears from six areas in Kurdistan, Iraq, using seven primers to detect fire blight resistance genes, which ranged from 135 to 200 base pairs. Fire blight, caused by Erwinia amylovora, affects pears, apples, and quince, with susceptible landraces often lacking the resistance gene. This research highlights genetic diversity and resistance patterns in 60 pear landraces, offering insights to preserve and improve agricultural biodiversity

The findings highlight the critical role of genetic factors in determining resistance levels to fire blight. Various primer combinations were used to evaluate resistance, with Harme Sheraina from Dihok demonstrating resistance attributed to loci identified by all primers employed. Specific varieties, such as Payzie from Akri, Qazam, and Buharie from Erbil; Sorke Dem from Shaqlawa; Lasor and Khamza from Sulaymaniyah; and Kaske and Gollawie from Halabja, showed resistance loci in six primers, emphasizing their significance in this study. Variability was observed in other cases, with primer combinations like MdE-EaN and FRMb32M27 consistently identifying resistance loci in certain varieties across regions. In contrast, primers such as FRMB31M87 and MdE-EaK4 revealed genetic similarities among landraces within the same region.

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# التحليلات الجزيئية لتحديد جينات المقاومة في أصناف الكمثرى (Pyrus communis) ضد مرض اللفحة النارية في منطقة كردستان العراق

**صبرية جرجيس زبير[1]** **أفيسنا محمد على[2]**
[1] *قسم البايلوجى ، كلية العلوم، جامعة دهوك*
[2] *قسم مركز البحوث العلمية، كلية العلوم، جامعة دهوك*

الخلاصة
يُعد مرض اللفحة النارية، وهو عدوى بكتيرية تسببها بكتيريا *Erwinia amylovora*، من الأمراض الرئيسية التي تصيب شجرة الكمثرى *(Pyrus communis)*، وهي فاكهة مثمرة متساقطة الأوراق تُصنَّف ضمن عائلة الوردية *(Rosaceae)*، وتحديدًا ضمن الفصيلة الفرعية *Maloideae*وجنس*Pyrus* . يتميز هذا فاكهة بتساقط أوراقها سنويًا ونموه الأمثل في المناطق ذات المناخ المعتدل. تنتشر بكتيريا *Erwinia amylovora*على نطاق واسع في العديد من الدول التي تزرع فيها أشجار التفاح والكمثرى، مما يؤثر على مجموعة واسعة من النباتات ضمن العائلة الوردية، وخاصة تلك التي تنتمي إلى الفصيلة الفرعية *Maloideae* يُعد تطوير أصناف مقاومة للَّفحة النارية أمرًا بالغ الأهمية وممكنًا للغاية. اجريت في هذه الدراسة، باستخدام سبعة البادئات مختلفة *MdE-EaN, MdE-EaK3* . لتقييم مقاومة مرض اللفحة النارية في أصناف الكمثرى، حيث *FRMB31M87, FRMb32MO4b, and FRMb32M27 MdE-EaK5, MdE EaK4,* أظهرت النتائج إيجابية في ست مناطق في أقليم كردستان العراق. وقد تبيّن أن مجموعة متنوعة من الأصناف المدروسة تحتوي على شرائط جينية يتراوح طولها بين 135 و200 زوج قاعدي. وأسفرت الدراسة عن تحديد علامات جينية متميزة ترتبط بتطوير مقاومة ضدمرض اللفحة النارية في بعض أصناف الكمثرى ، في منطقة دهوك، أظهرت صنف "هه رمى شيرانه" نتائج إيجابية مع جميع البادئات، في حين أظهرت صنف بائزى نتائج إيجابية مع جميع البادئات باستثناء البادئة *MdE-EaK5* أما في منطقة أربيل، فقد أظهرت صنف "قزم" و"بهارى" نتائج إيجابية مع جميع البادئات باستثناء بادئة *MdE-EaK5*وفي المقابل، أظهرت صنف "دوشاوى" من منطقة سيليمان وصنف "كولاوى" من حلبجة نتائج إيجابية مع جميع البادئات باستثناء *FRMB31M87* . كما أظهرت الدراسة أن بعض أصناف الكمثرى تفتقر إلى جينات أساسية لمقاومة اللفحة النارية، مما يجعلها أكثر عرضة للإصابة بالمرض.وتؤكد هذه النتائج الدور الحاسم للعوامل الجينية في تحديد مستويات المقاومة ضد مرض اللفحة النارية، مما يبرز أهمية الدراسات الجينية في تطوير أصناف مقاومة للأمراض ودعم التنوع الحيوي الزراعي.

الكلمات المفتاحية: اللفحة النارية، *Pyrus communis*، التحليل الجزيئي، جين المقاومة، *Erwinia amylovora*.