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# Modern technologies to detect fungi that cause diseases to agricultural crops: a review

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## ABSTRACT

**Background:** Fungal species that parasitize crops cause significant losses annually as they affect the quality and quantity of crops. Infected crops are considered a poor product that negatively affects the local and international agricultural sector. Therefore, the rapid and accurate early detection of the pathogen is crucial for the crop and to avoid losses. A loop-mediated isothermal amplification (LAMP) assay is used to detect about 100 pg fragments of DNA genes per reaction, which accurately detects fungal infections. This assay is used after isothermal amplification to detect genes without the use of PCR. In addition, the analysis of the whole genome (AGE), a global method for determining micro genes, includes experimental practice analysis and bioinformatics testing. Recent tests to detect pathogenic fungi include isothermal amplification analysis, polymerase chain reaction (PCR) techniques, overlapping, biomagnetic, and quantum biometric methods. One of the modern methods is the bioinformatics approach, which involves comparing the entire gene to identify distinctive and specialized areas of the fungus and designing polymer interaction tests for the species and genus to be diagnosed. **Objective:** The current study aims to survey accurate and rapid methods for diagnosing fungi that infect local crops, with the goal of controlling these infections, finding solutions, and preventing crop damage. **Conclusion:** Due to the increase in pathogenic fungi that cause plant diseases, there is a need to dedicate efforts to following developments in modern biological and molecular techniques and to work on the diagnosis of new fungal species. The traditional detection of pathological fungi yields dubious and inaccurate results, which do not facilitate effective treatment against this pathogenic fungus. It has proven its efficiency in accurately and rapidly diagnosing fungal species and diseases that do not exhibit symptoms. Although many assays focus on polymer chain reaction, most recent studies tend to use quantitative polymer chain reaction on a vast scale for quantitative measurement and differentiation between the factors causing fungal pests on plants, especially when the sample quantity is too small to detect, where the Lamb technique has succeeded in discovering many fungal species such as *Alternaria* spp., *Fusarium* spp., *Puccinia* spp., *Colletotrichum* spp., and *Foma* spp.. By amplifying the genomes of samples, modern genetic techniques have also succeeded in identifying fungal species and comparing them with the dolphin data without prior knowledge.

Keywords: Crops, pathogenic fungi, LAMP, genome.
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## INTRODUCTION

The fungus can spread widely in nature due to the spores it forms and has the ability to grow in various ecological zones. Fungi are considered organisms that feed on numerous nutrients in different ways, depending on their vital requirements. They can be saprophytic, living on other organisms, or parasitic on animals and plants (1). It is characterized by rapid adaptation in all soil and water environments due to its ability to decompose large molecules and convert them into absorbable fine molecules such as carbohydrates and amino acids (2)**.** Fungi play a crucial role in maintaining the ecological balance of the soil due to their vast numbers and diverse species, with approximately 3,000,000 species identified (3). About 8000 types cause different plant diseases and are considered one of the most dangerous types of microorganisms that cause economic losses and the inefficiency of local economic crops due to their ability to infect the roots, as they cause rotting diseases, as well as infecting some types of seeds and affecting the plant's production of fruits (4,2). Fungal spores are spread in the soil, which can survive and withstand the harsh conditions for an entire agricultural season and return to growth in the new season, where they are endemic to the farming area, which negatively affects production, especially with the lack of efficiency of commercial fungicides used and the ability of fungi to resist. In recent years, numerous modern techniques have been developed for the early detection of fungal infections in crops, both in the laboratory and in the field. The issue of plant disease control is of great importance to biotechnologists, as understanding the mechanisms that have rapid diagnostic power and the future of sustainable agriculture will depend on the future of modern technology, in addition to traditional agricultural practices (5). The current study aims to explore modern techniques, particularly genetic techniques, for the early detection and protection of local crops, to obtain high-yielding plants that are free from fungal infections, which cause significant losses to the country.

## 1- Molecular methods of detecting fungi

### 2.1 Steps of molecular diagnosis of infectious fungi of plants

The discovery and diagnosis of pathogenic fungi on plants using modern molecular techniques involves several steps before carrying out the analysis process to detect fungi. These steps include the extraction of fungal DNA and the quantification of the DNA in these samples. Several protocols are available for extracting DNA from infectious plant fungi (1,6); however, laboratories rely on methods such as freezing fungal filaments and breaking the cell wall that contains chitin by grinding, which allows the nuclear material to come out, removing proteins by adding a mixture of phenols and chloroform, then precipitating with propanol. Other modern DNA isolation methods include the pelletizing method, spin filter, silica membrane, and silica-coated magnetic particle separation (7,8). Finally, the standard concentration of DNA can be determined using ultraviolet light and a spectrophotometer. Additionally, sterile, ion-free distilled water can be added to achieve the necessary nuclear concentration, allowing for the completion of the remaining techniques for the early detection of infectious plant fungi (9).

### 2.2 Loop-Mediated Amplification (LAMP)

It is considered one of the essential analyses for diagnosing fungal plant diseases, as this analysis has a huge potential for early detection of fungi, as well as plant protection and plant disease management (10). The reaction includes three main phases: the initial step, the cycle amplification process, and the elongation step. For the (LAMP) assay, two sets of prefixes are used: the first is the front internal, the second is the inner posterior, and the third set of outer prefixes is the two outer and the rear B-loop primer. Exponential and isothermal amplification produce twice as much DNA at 60-65 °C, making this production ideal for promoting the activity of polymerase Bst (DNA polymerase, a polymerase enzyme derived from *Bacillus stearothermophilus*). An important application of this technique was its ability to diagnose the cytochrome within record time, specifically within 30 minutes, and target the fungal DNA sequence, thereby enabling targeted and accurate analysis. It was also revealed that the development of systems for analysis and quantitative analysis using code for *Magnaporthe oryzae* enabled the immediate and precise detection of rice plant explosion disease (11, 12).

### 2.3 Analysis of Whole Genome (AGE)

The basic working idea of an AGE analysis is based on the fact that different types of fungi have complete genes. The analysis consists of two steps: the first is the analysis of life informatics, and the second is experimental practice (13, 14). The target genetic sequences, which are called targets from the whole genome, are examined, and the targets for this whole genome are determined and compared with other genes of other species. To ensure the identification of the specific target, we first enrich the sequences of the identified target, known as the target DNA, in the genomic DNA, using specific elementary pairs (15). Different applications of these techniques were used to identify the specific targets of the species, including genetic sequencing and the CRISPR-Cas12A system; regarding sequencing, the enlarged genes were sequenced, the sample of the species of the plant pathogenic fungi was identified and compared with the target sequences, and thus the rapid detection of the sample (16). As for the CRISPR-Cas12a system, after the corresponding crRNA is synthesized, the Cas12a protein is incubated to form the Cas12a-crRNA compound. After this, fluorescence signals are transmitted through the separators to ssDNA and recorded by the instant microplatelet analyzer, corresponding to (17).

### 2.4 End-Point PCR

This technique revolutionized the early detection of fungal plant pathogens. In this technique, a piece of DNA is amplified many times through repeated cycles of denaturation, annealing, extension, and stabilization reactions at different temperatures, using specific primers. In this reaction, it is possible to design a small number of specific target nucleotides targeting specific fungal types for plant disease, or to use a primary universal to amplify specific fungal species with a specific genetic sequence. Then it can be compared with an NCBI GenBank database to confirm the diagnosis of the type causing the disease (18).

### 2.5 Quantitative PCR

This analysis enables the detection of a specific genetic sequence of DNA or RNA from pathological fungi, which is measured by a polymerase chain reaction device in record time. The relative number of copies of the target DNA sequence is calculated by dropping the cycle threshold value of fungal species samples using sequence activation (19). Special fluorescent dyes are also used to monitor the reaction during the amplification period. The basic principle of this technique involves fluorescent indicators that are commensurate with the amount of amplification. This approach has been successfully applied to diagnose many highly virulent plant fungi, including blight, bark ulcers, leaf wilting, root rot, and other diseases (20).

## CONCLUSION

The rise in pathological fungi causing plant diseases necessitates a commitment to advancing modern biological and molecular techniques, as well as diagnosing new fungal species, as shown in Figure (1) (21).

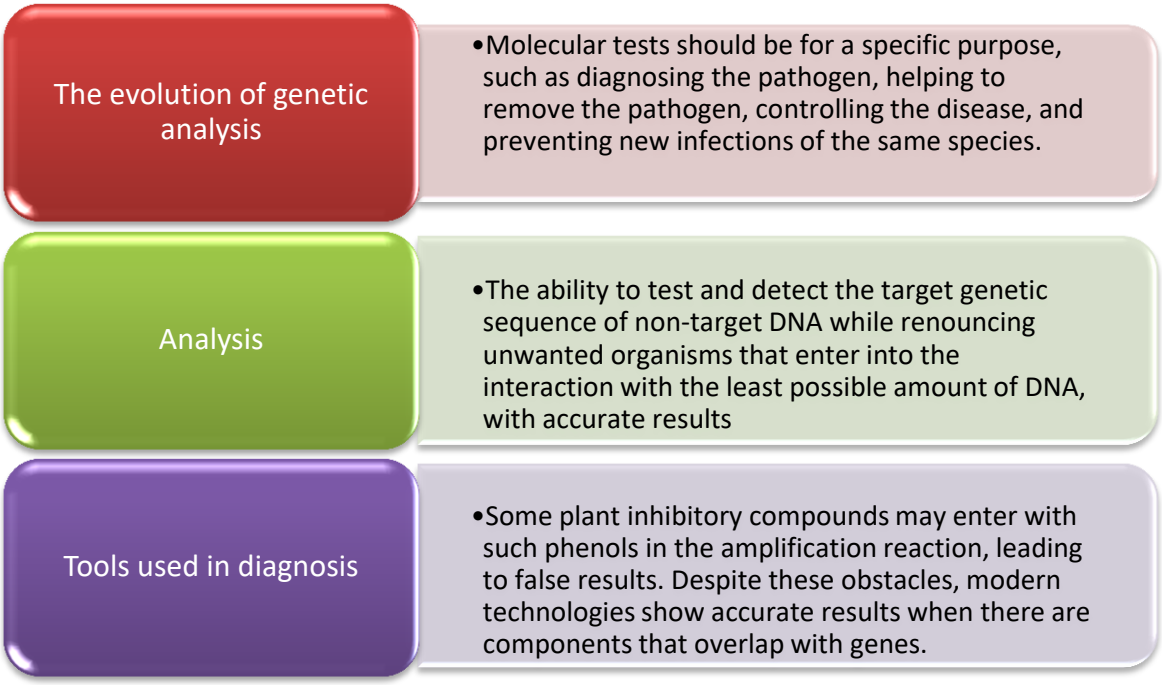


**Figure (1): Molecular assay on pathogenic fungi development (21)**

The traditional detection of pathological fungi yields dubious and inaccurate results, hindering effective treatment against this pathogen. New methods based on modern technologies, such as Loop Mediated Amplification (LAMP), Genome (AGE) assay, End-Point PCR, and Quantitative PCR, are being developed. It has proven its efficiency in the accurate and rapid diagnosis of fungal species and fungal diseases that also do not exhibit symptoms (22). Although many assays focus on polymer chain reaction, most recent studies tend to use quantitative polymer chain reaction on a massive scale for quantitative measurement and differentiation between the factors causing fungal pests on plants, especially when the sample quantity is too small to detect, where the Lamb technique has succeeded in discovering many fungal species such as *Alternaria* spp., *Fusarium* spp., *Puccinia* spp., *Colletotrichum* spp., and *Foma* spp. (15, 19). By amplifying the genomes of samples, modern genetic techniques have also succeeded in identifying fungal species and comparing them with the dolphin data without knowing them in advance, thanks to these techniques (7).

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## التقنيات الحديثة للكشف عن الفطريات التي تسبب الامراض للمحاصيل الزراعية في العراق: مراجعة

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**الخلاصة**
**خلفية عن الموضوع:** تتسبب الأنواع الفطرية التي تتطفل على المحاصيل في خسائر كبيرة سنويا لأنها تؤثر على نوعية وكمية المحاصيل. تعتبر المحاصيل المصابة منتجا رديئا يؤثر سلبا على القطاع الزراعي المحلي والدولي. لذلك ، فإن الكشف المبكر السريع والدقيق لمسببات الأمراض مهم للمحصول وتجنب الخسائر. يتم استخدام مقايسة التضخيم متساوي الحرارة بوساطة حلقية (LAMP) للكشف عن حوالي 100 جزء من جينات الحمض النووي لكل تفاعل ، والذي يكتشف بدقة الالتهابات الفطرية. يستخدم هذا الاختبار بعد التضخيم متساوي الحرارة للكشف عن الجينات دون استخدام تفاعل البوليميراز المتسلسل بالإضافة إلى ذلك ، فإن تحليل الجينوم بأكمله (AGE)، وهي طريقة عالمية لتحديد الجينات الدقيقة ، يشمل تحليل الممارسة التجريبية واختبار المعلوماتية الحيوية. الاختبارات الحديثة للكشف عن الفطريات المسببة للأمراض وتحليل التضخيم متساوي الحرارة ، وتقنيات تفاعل البوليمر المتسلسل (PCR) ، والقياس الحيوي المتداخل والمغنطيسي الحيوي والكمي. إحدى الطرق الحديثة هي طريقة اتباع المعلومات الحيوية والقياس الحيوي الكمي. من الطرق الحديثة هي طريقة استخدام المعلوماتية الحيوية والتي تقوم على مقارنة الجين بأكمله لتحديد المناطق المميزة والخاصة للفطر وتصميم اختبارات تفاعل البوليمر للأنواع والجنس المراد تشخيصها. **الهدف من الدراسة:** تهدف الدراسة الحالية إلى الكشف عن طرق دقيقة وسريعة لتشخيص الفطريات التي تصيب المحاصيل المحلية للسيطرة وإيجاد الحلول وتجنب تلف المحاصيل.
**الاستنتاج:** نظرا لتزايد الفطريات المرضية المسببة للأمراض النباتية ، مما دعى التفاني في متابعة التطورات في التقنيات البيولوجية والجزيئية الحديثة والعمل على تشخيص أنواع فطرية جديدة. يعطي الكشف التقليدي عن الفطريات المرضية نتائج مشكوك فيها وغير دقيقة ، والتي لا تسمح بالعلاج الفعال ضد هذه الفطريات المرضية. لقد أثبتت كفاءتها في التشخيص الدقيق والسريع للأنواع الفطرية والأمراض الفطرية التي لا تتوقع أعراضا أيضا. على الرغم من وجود العديد من المقايسات التي تركز على تفاعل البوليمر المتسلسل ، إلا أن معظم الدراسات الحديثة تميل إلى استخدام تفاعل البوليمر المتسلسل الكمي على نطاق واسع جدا للقياس الكمي والتمايز بين العوامل المسببة للآفات الفطرية على النباتات ، خاصة عندما تكون كمية العينة صغيرة جدا بحيث لا يمكن اكتشافها ، حيث نجحت تقنية الخروف في اكتشاف العديد من الأنواع الفطرية مثل *Alternaria* spp. ، *Fusarium* spp.، *Puccinia* spp.، *Colletotrichum* spp. ، و *Foma* spp. من خلال تضخيم جينومات العينات ، نجحت التقنيات الجينية الحديثة أيضا في معرفة الأنواع الفطرية ومقارنتها ببيانات الدلافين دون معرفتها مسبقا بفضل هذه التقنيات.

**الكلمات المفتاحية:** المحاصيل ، الفطريات المسببة للامراض ، LAMP ، الجينوم.