Available online at
http://agrospheresmagazine.vitalbiotech.org/

ISSN (E): 2582 – 7022

Agrospheres:e- Newsletter, (2025) *6*(12), 82-85

Article ID: 853

Smart Detection in the Field: RPA-CRISPR-Cas12a as a Game-Changer for Agricultural Disease Management

Kolli Leela Bhavani[1], Yogendra Singh[2]* Prashant Gigaulia[3], Reena Nair[4] and Gyanendra Tiwari[5]

[1]M. Sc. Research Scholar, Biotechnology Centre, JNKVV, Jabalpur (M.P)
[2]Assistant Professor (Senior Scale)-Biotechnology, Department of Genetics and Plant Breeding, JNKVV, Jabalpur (M.P)
[3]Guest Faculty, Molecular Biology and Biotechnology, Biotechnology Centre, JNKVV, Jabalpur (M.P)
[4]Scientist , Department of Horticulture, JNKVV, Jabalpur (M.P)
[5]Professor & Head , Department of Plant Physiology , JNKVV, Jabalpur (M.P)

Open Access

*Corresponding Author
Yogendra Singh*

Article History
Received: 11. 12.2025
Revised: 16. 12.2025
Accepted: 21. 12.2025

INTRODUCTION

Rice and Wheat are staple foods for over 1.4 billion people in Asia, particularly in India. As of 2025 estimates, India is projected to produce about 117.5 million metric tons of wheat and 149 million tons of rice, totalling around 354 million tons of food grains. However, plant diseases continue to pose a serious threat to this productivity. Diseases such as fungal rusts, rice blast, bacterial blights, and viral infections can reduce crop yields by 10–30%, or even more in severe outbreaks, leading to substantial losses for farmers and national grain reserves. Globally, disruptions in production within major agricultural regions can trigger spikes in food prices and intensify food insecurity in import-dependent countries. Any breakdown in the agricultural supply chain further strains already fragile food systems.

To address these challenges, plant breeders often rely on molecular tools like PCR and marker-assisted selection to develop disease-resistant crops. However, these methods require skilled personnel, advanced lab infrastructure, and are often expensive and time-consuming, especially in low-resource settings. Early detection remains difficult, as many pathogens lie dormant or cause overlapping symptoms. This highlights the urgent need for fast, affordable, and field-deployable diagnostics. The RPA-CRISPR-Cas12a system meets this need, offering a groundbreaking solution for real-time disease detection and smart farming.

Mechanism:

The RPA-CRISPR-Cas12a system combines two fundamental techniques in molecular biology: Recombinase Polymerase Amplification (RPA) for amplifying DNA and CRISPR-Cas12a for detecting specific targets.

1. Recombinase Polymerase Amplification (RPA):

RPA is an isothermal amplification method that functions effectively at 37–42°C, removing the requirement for thermal cycling. In contrast to PCR, which needs expensive thermal cyclers, RPA employs a recombinase enzyme to attach primers to complementary DNA sequences, a single-stranded DNA binding protein (SSB) to support the separated DNA strand, and a strand-displacing polymerase to lengthen the primers. This quick and effective amplification can produce detectable DNA levels in 10–20 minutes, even from targets with low copy numbers.

2. CRISPR-Cas12a assay:

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is an adaptive immune system found in bacteria and archaea. It protects against viruses by capturing fragments of viral DNA and inserting them as spacer sequences within the host genome. These spacers act as a molecular memory, guiding Cas proteins encoded by nearby Cas genes with helicase and nuclease activity, to recognize and destroy matching invaders upon reinfection. Cas12a, part of Class 2, Type V systems, requires only a single protein and a specific CRISPR RNA (crRNA). Once bound to the target DNA, Cas12a is activated and cleaves nearby single-stranded DNA reporters, enabling visual or fluorescent detection.

3. RPA-CRISPR/Cas12a workflow:

- Collect plant specimens (e.g., sap, leaf juice, roots, etc.) and then extract DNA from them.
- Amplify the target DNA using RPA at a consistent temperature.
- Incubate the amplified DNA with CRISPR-Cas12a and crRNA.
- When the target sequence exists, Cas12a becomes activated and cuts the reporter molecule, generating a detectable or observable signal.

The whole procedure can be finished in 20–30 minutes, needing little equipment—making it perfect for point-of-care or field diagnostics.

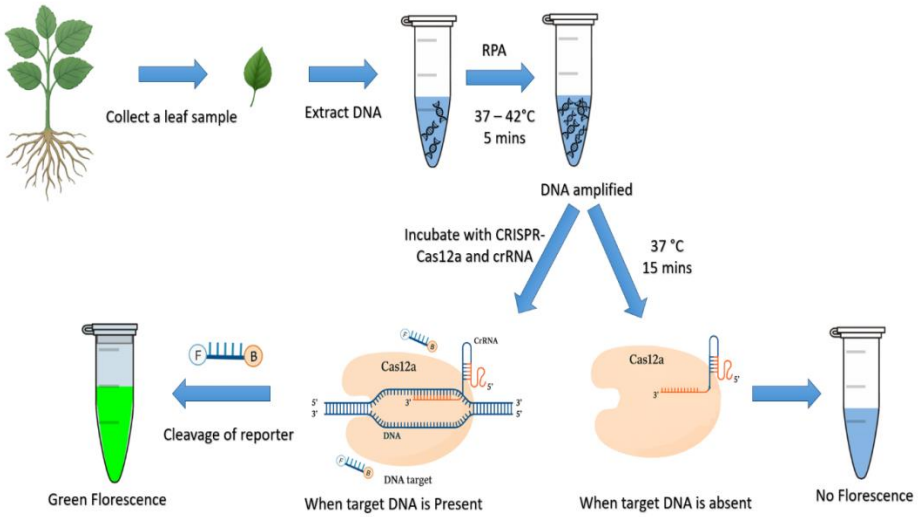

FIGURE: The workflow diagram for the RPA-CRISPR/Cas12a detection assay. Following recombinase polymerase amplification of the target DNA, Cas12a binds to a sequence-specific crRNA and activates its collateral cleavage function by binding the amplified product. When triggered, the combination cleaves neighboring FAM-labeled ssDNA reporters, resulting in a detectable green fluorescent signal at 470 nm. A bright fluorescence implies a positive detection result, while a lack of fluorescence suggests a negative result.

Advantages:

- Functions at isothermal conditions (around 37 °C), eliminating the requirement for thermocyclers or specialized temperature-control devices.
- Delivers quick diagnostic outcomes, typically within 20 to 30 minutes, significantly outpacing PCR-based approaches.
- Guarantees high specificity through dual recognition: the target attaches to RPA primers while the sequence directs Cas12a-crRNA cleavage.
- Accommodates crude plant extracts, reducing the necessity for high-purity DNA extraction.
- Adapts to various detection formats, such as fluorescence readouts and lateral flow assays, making it ideal for portable and field diagnostics.
- Is well-suited for point-of-care applications in agricultural environments with limited resources.

Limitations:

- The available orthologs of Cas12a are limited, which restricts the adaptability of target sequences due to PAM limitations.
- It is crucial to design and optimize crRNA effectively, as alterations in the scaffold can influence both the efficiency of cleavage and the accuracy of detection.
- Because RPA reagents are proprietary and relatively costly, their widespread application in the field can be challenging.
- There is a potential for non-specific amplification or primer mismatches during RPA, making it essential to meticulously optimize the assay to avoid false positives.
- Certain reagents, such as enzymes and ssDNA reporters, may require cold storage, complicating their use in the field.
- The need for additional portable equipment like dry baths, strips, and fluorescence readers may still arise, leading to logistical complications.

Applications:

- **Early Pathogen Detection** – Detects infections before symptoms appear, enabling rapid action. Zhao *et al.* (2025) used it for early-stage detection of potato late blight via microneedle sampling.
- **On-Site Field Diagnostics** – Enables portable, real-time testing directly in the field. Guo *et al.* (2023) developed a visual detection kit for *Phytophthora sojae* in soybeans.
- **Disease Surveillance Programs** – Used in large-scale monitoring and outbreak tracking. He *et al.* (2025) applied it to detect *Alternaria alternata* in yams for regional surveillance.
- **Breeding for Disease Resistance** – Accelerates screening of resistant lines in breeding programs. Anbazhagan *et al.* (2024) highlighted its role in high-throughput detection for marker-assisted selection.
- **Soil and Seed Health Testing** – Prevents disease spread through contaminated materials. Tanny *et al*. (2023) discussed seed-borne pathogen detection using CRISPR diagnostics.
- **Low-Resource Farming Solutions** – Works without cold chains or lab infrastructure. Liu *et al.* (2023) created a photothermal assay usable in rural field conditions.
- **Smart Farming Integration** – Connects with smartphones for digital crop health mapping. Zhao *et al.* (2025) integrated smartphone apps for visualizing CRISPR test results.
- **Education and Training** – Trains agricultural workers in modern diagnostics. Gong *et al.* (2025) emphasized educational use in plant pathology labs.

Future Prospects:

The future of the RPA-CRISPR/Cas12a platform relies on enhancing its stability, scalability, and suitability for field deployment. Innovations such as dehydrating reagents, employing heat-stable enzymes, and streamlining workflows could eliminate the requirement for cold storage and reduce equipment demands. Improved orthologs of Cas12a that are compatible with a broader range of PAM sequences and demonstrate higher cutting efficiency will broaden the diagnostic possibilities. Integrating smartphone imaging,

portable automated devices, and digital agriculture networks will facilitate real-time disease monitoring and early warning systems. Additionally, developing multiplex assays will enable the simultaneous detection of multiple pathogens, thereby bolstering its use in precision agriculture, biosecurity, and extensive field surveillance.

CONCLUSION

The RPA-CRISPR-Cas12a technology is revolutionary for agricultural disease control, providing quick, precise, and on-the-spot identification of plant pathogens. Its affordable, easy-to-use design enables farmers to make timely decisions, minimizing crop losses and inappropriate pesticide usage. In contrast to conventional lab-based diagnostics, it delivers molecular accuracy straight to the field. As research progresses, merging with AI, IoT, and mobile platforms will boost its effectiveness. Ongoing advancements ensure wider pathogen identification, automation, and extensive implementation for worldwide food safety.

REFERENCES

Liu, Y., Ma, L., Liu, W., Xie, L., Wu, Q., Wang, Y., Zhou, Y., Zhang, Y., Jiao, B., & He, Y. (2023). RPA-CRISPR/Cas12a combined with rolling circle amplification – enriched DNAzyme: A homogeneous photothermal sensing strategy for plant pathogens. *Journal of Agricultural and Food Chemistry*, 71(11), 4736–4744. https://doi.org/10.1021/acs.jafc.2c08866

Tanny, T., Sallam, M., Soda, N., Nguyen, N. T., Alam, M., & Shiddiky, M. J. (2023). CRISPR/Cas-based diagnostics in agricultural applications. *Journal of Agricultural and Food Chemistry*, 71(31), 11765–11788. https://doi.org/10.1021/acs.jafc.3c02206

Anbazhagan, P., Parameswari, B., Anitha, K., Chaitra, G. V., Bajaru, B., Rajashree, A., Mangrauthia, S. K., Yousuf, F., Chalam, V. C., & Singh, G. P. (2024). Advances in plant pathogen detection: Integrating recombinase polymerase amplification with CRISPR/Cas systems. *3 Biotech*, 14(9), 214. https://doi.org/10.1007/s13205-024-03816-7

Gong, X. Y., Wang, Z. H., Bashir, M., Tang, T., Gan, X., & Yang, W. C. (2025). Recent application of CRISPR/Cas in plant disease detection. *TrAC Trends in Analytical Chemistry*, 118251. https://doi.org/10.1016/j.trac.2025.11825

Guo, Y., Xia, H., Dai, T., & Liu, T. (2023). RPA-CRISPR/Cas12a mediated isothermal amplification for visual detection of *Phytophthora sojae*. *Frontiers in Cellular and Infection Microbiology*, 13, 1208837. https://doi.org/10.3389/fcimb.2023.1208837

Zhao, J., Xu, H., Xu, C., Yin, W., Luo, L., Liu, G., & Wang, Y. (2025). Smartphone-integrated RPA-CRISPR-Cas12a detection system with microneedle sampling for point-of-care diagnosis of potato late blight in early stage. *bioRxiv*, 2025-06.

He, Y., Tong, C., Chen, H., Zhao, W., Zhan, L., Wang, R., Zhang, J., Gao, J., Li, C., Li, M., & Yang, Q. (2025). A rapid and visual detection method for *Alternaria alternata*, the causal agent of leaf spot disease on yam, based on RPA-CRISPR/Cas12a. *Physiological and Molecular Plant Pathology*, 137, 102612. https://doi.org/10.1016/j.pmpp.2025.102612