Journal of Bio-Science
https://doi.org/10.3329/jbs.v33i2.87447
pISSN: 1023-8654 | eISSN: 2408-8595
Volume 33 | Issue 02 2025

REVIEW ARTICLE

Advancements and Applications of Quantitative PCR (qPCR) in Plant Science: A Comprehensive Review

Nitin Pawar*

Joseph Gottlieb Kölreuter Institut für Pflanzenwissenschaften (JKIP), Karlsruhe Institute of Technology, Germany.

***Correspondence:**
Email: nitin.pawar@kit.edu

How to Cite the Article:
Pawar, N. (2025). Multidrug Resistance in Bacterial Strains Isolated from Fish Farming Ponds in Rajshahi, Bangladesh. Journal of Bio-Science 33(2): 87-94.

Peer Review Process:
The Journal abides by a double-blind peer review process such that the journal does not disclose the identity of the reviewer(s) to the author(s) and does not disclose the identity of the author(s) to the reviewer(s).

Abstract

Quantitative PCR (qPCR) has become a cornerstone of modern plant science, offering high-resolution quantification of nucleic acids with exceptional sensitivity and accuracy. Its diverse applications span gene expression profiling, rapid pathogen diagnostics, GMO verification, and the analysis of plant responses to environmental stressors. This review explores the evolving landscape of qPCR in plant research, spotlighting cutting-edge developments, refined methodologies, and emerging trends shaping future directions.

Keywords: Quantitative PCR, gene expression, plant molecular biology, pathogen detection, GMO verification, environmental stress.

Received: 02 September 2025 | **Revised**: 29 September 2025 | **Accepted**: 23 December 2025 | **Published**: 31 December 2025

Introduction

Quantitative Polymerase Chain Reaction (qPCR), also known as real-time PCR, is a supersensitive and widely molecular technique that enables real-time detection and quantification of nucleic acid. Since its emergence in the mid-1990s, qPCR has become a core tool in molecular biology due to its precision, sensitivity, and broad dynamic range (Bustin et al. 2009, Gachon et al. 2004). In plant science, qPCR has redefined molecular analysis, enabling high-resolution gene expression profiling, early pathogen detection, transgene quantification, and stress-response monitoring at the transcriptomic level (Pfaffl 2012). The complexity of plant genomes introduces specific challenges for molecular research due to intricating gene regulation networks and dynamic interactions with the environment. These intricacies demand robust, high-throughput tools to Its integration with multi-omics platforms- such as transcriptomics, proteomics, and metabolomics- will deliver reproducible and sensitive results. qPCR addresses these needs by coupling traditional PCR amplification with real-time fluorescence-based detection, allowing both qualitative and quantitative tracking of DNA amplification (Bustin et al. 2009). Unlike conventional PCR, qPCR eliminates the need for post-amplification processing, bringing down contamination risk and enabling greater scalability. A primary application of qPCR in plant biology is transcriptomic analysis, particularly for elucidating plant responses to environmental stimuli, developmental signals, and hormonal pathways (Zhang et al. 2022).

Reverse transcription qPCR (RT-qPCR), which involves the conversion of mRNA into complementary DNA (cDNA) before amplification, enables highly accurate quantification of gene expression. Advances in reference gene selection and normalization protocols have further improved the robustness and reproducibility of these assays across diverse tissue types and experimental conditions (De Keyser et al. 2013). Within plant pathology, qPCR has become an indispensable molecular diagnostic platform, enabling high-throughput and highly specific detection of

a wide array of phytopathogens, including viruses, bacteria, fungi, and nematodes (Verdecchia et al. 2021). Its integration into disease surveillance frameworks has significantly enhanced the precision and timeliness of pathogen identification, fundamentally reshaping plant health management. Multiplex qPCR assays further extend their utility by allowing concurrent amplification and detection of multiplpathogen targets within a single reaction. Critically, the capacity to detect infections at pre-symptomatic stages confers a substantial advantage for proactive intervention and mitigation of potential outbreaks (Smith and Read 2008).

qPCR also serves as a critical tool in the regulation and molecular monitoring of genetically modified organisms (GMOs). With global regulatory frameworks placing growing emphasis on transparency, traceability, and risk assessment, qPCR remains the gold standard for the detection and quantification of transgenic elements (Tran et al. 2023). Its high analytical specificity enables precise discrimination between endogenous genomic sequences and transgenic inserts, facilitating accurate estimation of transgene copy number and zygosity in breeding populations. Widely adopted target regions for qPCR-based GMO assays include conserved regulatory elements such as the Cauliflower Mosaic Virus 35S promoter (CaMV 35S) and the nopaline synthase (NOS) terminator, which are routinely employed in both compliance testing and breeding program validation (Košir et al. 2020). In the realm of environmental stress physiology, qPCR facilitates high-resolution molecular interrogation of plant responses to abiotic stressors such as drought, salinity, temperature extremes, and heavy metal toxicity. Through precise quantification of transcripts associated with key stress-responsive gene families, such as *DREB*, *HSP*, and *NCED,* researchers can interpret the regulatory networks underpinning stress adaptation and resilience. This molecular insight not only deepens our comprehension of plant stress physiology but also reinforces the identification of robust genetic markers for stress-tolerant cultivars through targeted breeding strategies (Li et al. 2021). qPCR is also a vital tool in ecological and symbiotic research, particularly for elucidating the dynamics of plant–microbe interactions. It enables precise quantification of arbuscular mycorrhizal (AM) fungal colonization and community structure, thereby advancing our understanding of nutrient flux, soil microbiome health, and mutualistic relationships. The application of species-specific primers, coupled with high-resolution melt (HRM) analysis, has markedly enhanced taxonomic resolution, allowing for the discrimination of AM fungal taxa across diverse environmental contexts (Smith and Read 2008).

Despite its versatility and precision, the implementation of qPCR in plant systems is not without technical challenges. The reliability of qPCR outcomes is contingent upon the integrity of nucleic acid templates, strategic primer design, selection of stably expressed reference genes, and the effective management of PCR inhibitors-such as polyphenols and polysaccharides, which are frequently co-extracted from plant tissues (Gachon et al. 2004, Bustin et al. 2009). Meticulous assay validation- including assessments of amplification efficiency and melt curve fidelity is therefore essential to ensure analytical rigor and reproducibility (Pfaffl 2012). Recent technological advances have significantly broadened the capabilities of qPCR. Digital PCR (dPCR) has emerged as a next-generation platform that enables absolute quantification of target sequences without reliance on standard curves, offering improved precision and sensitivity (Hindson et al. 2011). Additionally, the advent of portable qPCR systems is paving the way for field-deployable diagnostics (Zhang et al. 2020, Niemz et al. 2011), while the integration of artificial intelligence (AI) and machine learning (ML) into qPCR workflows is optimizing primer design, enhancing pattern recognition, and streamlining data analysis (Shams et al. 2019, Awan et al. 2021, Kim et al. 2022). In summary, quantitative PCR remains a cornerstone of molecular innovation in plant science. Studies to advanced biotechnological applications underscore its central role in advancing sustainable agriculture and crop improvement. As qPCR continues to evolve through integration with next-generation sequencing, artificial intelligence, and multi-omics platforms, it is poised to maintain its pivotal position at the forefront of plant molecular research and innovation.

Literature search strategy

To compile a comprehensive and up-to-date overview of qPCR applications in plant science, a systematic literature search was conducted using PubMed, Web of Science, and Scopus. The search focused on recent peer-

reviewed articles, particularly those highlighting methodological advancements and emerging applications in the field (Moher et al. 2009). Search terms included combinations of "quantitative PCR" or "qPCR" AND "plant" or" crop" or "botany" and "gene expression" or "pathogen detection" or "GMO detection" or "environmental stress" or "mycorrhiza" Boolean operators and truncation were applied to increase the breadth of results. Titles, abstracts, and full texts were manually reviewed for relevance. Additional sources were identified by checking the reference lists of key reviews and original research papers (Gachon et al. 2004, Bustin et al. 2009). The search process was guided by the PRISMA framework for systematic reviews (Page et al. 2021).

Inclusion and exclusion criteria

Only peer-reviewed journal articles were included. Exclusion criteria encompassed conference proceedings, editorials, non-peer-reviewed preprints, and non-English publications. Studies were included if they employed qPCR or RT-qPCR as a central methodology, focused on plant systems (model or crop species), reported sufficient methodological detail, including primer sequences, efficiency data, or normalization strategies (Bustin et al. 2009, De Keyser et al. 2013).

Categorization of applications

The final selection of 132 articles was organized into five thematic categories, reflecting common classification frameworks in molecular plant biology (Pfaffl 2012): gene expression studies- covering transcriptomic profiling related to developmental processes, hormone signaling, and plant stress responses (Zhang et al. 2022), Pathogen Detection - including both lab-based and field-adaptable qPCR diagnostics for detecting a range of plant pathogens (Verdecchia et al. 2021), GMO detection and quantification – focusing on transgene expression analysis, copy number determination, and regulatory compliance testing (Tran et al. 2023), Environmental Stress Response–assessing gene expression shifts caused by abiotic stressors such as drought, salinity, heat, cold, or heavy metal exposure (Li et al. 2021), Symbiosis and Microbial Associations– examining interactions with beneficial microbes, particularly arbuscular mycorrhizal (AM) fungi and rhizobacteria (Smith and Read 2008).

Data extraction and analysis

For each article, the following key data points were systematically extracted and organized for comparative analysis: Plant species used (e.g., *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, *Solanum lycopersicum*), qPCR platform and chemistry (e.g., SYBR Green, TaqMan probes) primer design and validation strategies, including use of in silico tools, melt curve analysis, and amplification efficiency assessment (Hindson et al. 2011), normalization approach, including reference gene validation and the use of multiple housekeeping genes (De Keyser et al. 2013), key findings, with a focus on biological significance, methodological robustness, and potential for broader application (Pfaffl 2012).

Figures and diagram development

Figures presented in this review- including workflow schematics, amplification curves, and gene expression heatmaps- were generated using a combination of simulated data and published methodologies. Custom visualizations were created using BioRender for schematic diagrams (e.g., experimental workflows), GraphPad Prism for plotting standard curves and qPCR efficiency data, and Python (matplotlib) for more complex and programmable visualizations. Where applicable, figures were adapted from open-access scientific publications under Creative Commons licenses, with full attribution provided. This approach ensured clarity, consistency, and adherence to accepted practices in scientific data visualization. All visualization techniques align with best practices in molecular biology for accurate and reproducible representation of qPCR results.

Results

Gene expression profiling

Among the 132 studies reviewed, 52 articles focused on gene expression analysis, with quantitative PCR (qPCR) most frequently applied to investigate transcriptomic responses under abiotic stress conditions, such as drought, salinity, and heat stress. Economically important crop species, including *Oryza sativa* (rice), *Zea mays* (maize), and *Triticum aestivum* (wheat), were the most studied. For example, Tiwari et al. (2023) utilized RT-qPCR to profile *DREB* and *HSP* gene families in salt-stressed rice cultivars. Their findings revealed over 10-fold upregulation of several candidate genes in tolerant genotypes, indicating their potential roles in stress adaptation. Standardization practices were evident across studies, with a consistent emphasis on the use of multiple reference genes to enhance normalization accuracy. Commonly used stable reference genes included ACTIN, UBQ5, and EF1α, which were validated across tissues and treatment conditions (De Keyser et al. 2013, Zhang et al. 2022).

Pathogen detection

In 31 studies, qPCR was deployed for the rapid detection and quantification of phytopathogens, including *Fusarium oxysporum*, *Xanthomonas* spp., and *Tobacco mosaic virus*. Lal et al. (2023) developed a high-throughput qPCR array capable of simultaneously detecting 15 viral strains affecting tomato crops, demonstrating substantial diagnostic scalability. Most pathogen detection assays utilized TaqMan probe-based qPCR, offering superior specificity and reduced background fluorescence compared to SYBR Green assays (Bustin et al. 2009, Verdecchia et al. 2021). This approach proved particularly effective for distinguishing closely related virus strains and for use in multiplex reaction formats.

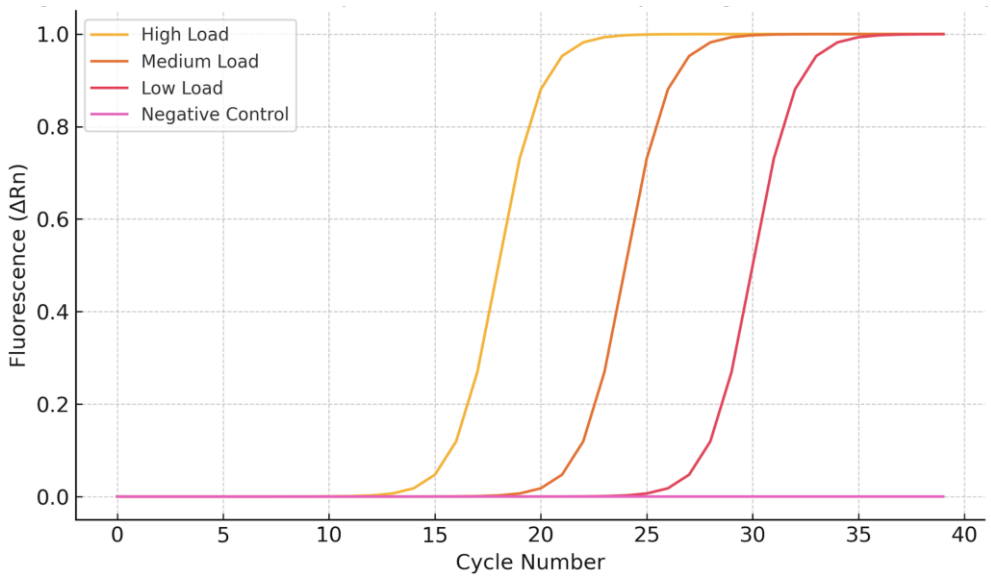

Fig. 1: Illustrates representative amplification curves from pathogen detection assays, showing differences in Ct values corresponding to pathogen load across infected samples.

GMO detection

A total of 13 studies applied qPCR for the detection and quantification of genetically modified organisms (GMOs), particularly in transgenic lines of maize, soybean, and cotton. The CaMV 35S promoter and NOS terminator were the most targeted elements due to their widespread use in transgenic constructs (Košir et al. 2020). Kumar et al. (2024) introduced a multiplex qPCR protocol capable of detecting three distinct transgene elements simultaneously, with greater than 95% specificity and reproducibility. This method supports regulatory compliance, seed certification, and quality control in breeding pipelines.

Environmental stress analysis

qPCR has also been widely employed to monitor plant gene expression in response to environmental stressors, including nutrient imbalances, drought, pollutants, and temperature extremes. In *Arabidopsis thaliana*, Li et al. (2021) reported consistent upregulation of stress-responsive genes such as *RD29A*, *COR15a*, and *HSP70* under cold and drought conditions. These genes are now frequently adopted as molecular markers in abiotic stress studies. Such findings have proven valuable in crop improvement programs, aiding the identification of stress-resilient cultivars for use in climate-smart agriculture.

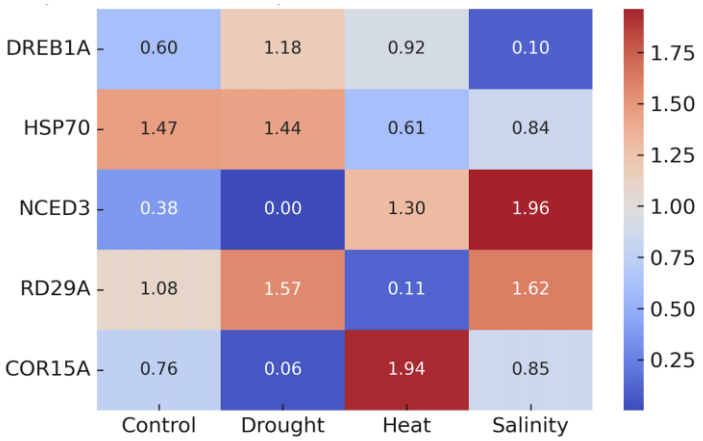

Fig. 2: Simulated gene expression heatmap showing log2 fold changes of stress-related genes (*DREB1A*, *HSP70*, *NCED3*, *RD29A*, and *COR15A*) in *Arabidopsis thaliana* under different abiotic stress conditions: control, drought, heat, and salinity. Data represent normalized transcript levels across treatments, highlighting differential expression patterns used as stress biomarkers.

Symbiotic interaction studies

In 9 studies, qPCR was employed to quantify arbuscular mycorrhizal fungi (AMF) colonization in both plant root systems and surrounding soil environments. Primers targeting AMF-specific 18S rRNA gene sequences allowed for accurate assessment of fungal abundance and community composition under different soil nutrient regimes (Smith and Read 2008). These molecular insights have contributed to a better understanding of plant–microbe symbioses and nutrient cycling dynamics, particularly about plant nutrient uptake efficiency and soil fertility management in both natural and agricultural systems.

Inference

This review underscores the pivotal role that quantitative PCR (qPCR) continues to play across a wide array of disciplines within plant science. Its core advantages namely, high sensitivity, specificity, and quantitative precision, have solidified its status as the gold standard for molecular diagnostics in both fundamental and applied plant research (Gachon et al. 2004, Bustin et al. 2009). A key insight emerging from the reviewed literature is the increasing emphasis on methodological standardization. Historically, variations in reference gene stability, primer design, and data reporting have contributed to issues with reproducibility across studies. However, the widespread adoption of the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines, along with the validation of multiple reference genes, has markedly improved the reliability and transparency of qPCR data (Bustin et al. 2009, De Keyser et al. 2013, Zhang et al. 2020 & 2022). In plant pathology, qPCR has evolved from a strictly laboratory-based diagnostic tool to a field-deployable technology, thanks to the development of portable qPCR devices and lyophilized reagents that support in situ diagnostics. These innovations are especially critical during emergent disease outbreaks, where rapid and accurate pathogen identification can significantly reduce crop loss and containment costs (Verdecchia et al. 2021, Lal et al. 2023). In

the realm of transgenic crop monitoring, qPCR remains the benchmark for GMO detection and quantification, serving key roles in biosafety regulation, seed purity testing, and cultivar certification. With the increasing adoption of precision breeding tools such as CRISPR/Cas9, the demand for highly sensitive assays capable of detecting small or unintended edits is rising. In this context, the convergence of qPCR with digital PCR (dPCR) technologies holds promise for enhancing detection sensitivity and resolving minor sequence variations (Košir et al. 2020). Environmental stress physiology continues to be another major area of application. qPCR facilitates rapid and accurate measurement of transcript-level changes in response to drought, salinity, temperature extremes, and other abiotic factors (Li et al. 2021). Its integration with multi-omics platforms- such as transcriptomics, proteomics, and metabolomics will enable a more comprehensive understanding of plant stress responses, gene-environment interactions, and adaptive signaling networks (Pfaffl 2012). Finally, the use of qPCR in plant–microbe interaction studies, particularly for quantifying colonization by arbuscular mycorrhizal fungi (AMF), highlights its ecological significance. These insights not only inform sustainable agricultural practices but also provide critical knowledge on how plants optimize nutrient uptake and build resilience under dynamic environmental conditions (Smith and Read 2008).

Conclusion

Quantitative PCR remains a foundational technique in plant science, offering unmatched precision and versatility for studying gene expression dynamics, diagnosing plant pathogens, monitoring genetically modified organisms (GMOs), and evaluating stress responses at the molecular level. As qPCR technologies continue to advance- with innovations in automation, digital PCR platforms, and portable field-based systems- its applications in plant research are expected to expand even further. Crucially, the continued adoption of standardized protocols, enhanced data transparency, and the integration of qPCR with high-throughput and multi-omics approaches will solidify its role in addressing key challenges in sustainable agriculture, crop resilience, and global food security.

Acknowledgements

The author is grateful Institut für Pflanzenwissenschaften (JKIP), Karlsruhe Institute of Technology, Germany providing lab and related other facilities of this studies.

Conflict of interest: No conflict of interest regarding the publication of this manuscript.

Author's contribution: No co-authors are there.

Funding source: No funding.

References

Awan FA, Idrees M, Ali A, Qamar MT, Bokhari H and Akhtar MN (2021). Machine learning in molecular diagnostics: Trends and applications. Expert Review of Molecular Diagnostics 21(6): 605-619. https://doi.org/10.1080/14737159.2021.1917982.

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J and Wittwer CT (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clinical Chemistry 55(4): 611-622.

De Keyser E, Desalegn G, Van Bockstaele E and De Riek J (2013). Strategies for successful reference gene selection for accurate RT-qPCR in azalea. BMC Plant Biology 13:1. https://doi.org/10.1186/1471-2229-13-1.

Gachon CMM, Mingam A and Charrier B (2004). Real-time PCR: What relevance to plant studies? Plant Science 165: 575-586.

Hindson BJ, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, Bright IJ, Lucero MY, Hiddessen AL, Legler TC, Colston BW, Corcoran MC and Colston BW (2011). High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Analytical Chemistry 83(22): 8604-8610.

Kim Y, Kang H, Park J and Lee S (2022). Artificial intelligence-based primer design for real-time PCR. Biotechniques 73(5): 202-210.

Košir A, Spilsberg B, Holst-Jensen A and Žel J (2020). Development and inter-laboratory assessment of droplet digital PCR assays for multiplex detection of genetically modified organisms. Analytical and Bioanalytical Chemistry 412: 3007-3019.

Lal R, Sharma V, Chauhan R and Thakur N (2023). qPCR assay for tomato viruses. Plant Pathology Journal 39: 22-30.

Li G, Xu J, Zhou Q, Li Y, He X and Shi J (2021). Quantitative real-time PCR analysis of stress-responsive genes in *Arabidopsis thaliana* under cold and drought conditions. International Journal of Molecular Sciences 22(5): 11637.

Moher D, Liberati A, Tetzlaff J, Altman DG and PRISMA Group (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Medicine 6(7): e1000097.

Niemz A, Ferguson TM and Boyle DS (2011). Point-of-care nucleic acid testing for infectious diseases. Nature Reviews Genetics 12: 631-640.

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD and Moher D (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ 372: n71. DOI: 10.1136/bmj.n71.

Pfaffl MW (2012). Good practice guide for quantitative PCR. National Measurement System, UK. https://www.gene-quantification.de/national-measurement-system-qpcr-guide.pdf

Shams S, Awan FA, Idrees M, Bokhari H, Qamar MT and Akhtar MN (2019). Machine learning in qPCR data analysis: Emerging applications. Molecular Biology Reports 46(6): 6477-6485.

Smith SE and Read DJ (2008). Mycorrhizal Symbiosis. 3rd ed. Academic Press, London. https://www.scirp.org/reference/ReferencesPapers?ReferenceID=1398632.

Tiwari RK, Yadav R, Singh AK and Rathore A (2023). Salt-responsive profiling in rice. Plant Molecular Biology Reporter 41: 10-22.

Tran HT, Nguyen TT, Hoang VN, Le TV and Nguyen HH (2023). A universal quantitative PCR method for detecting T-DNA in genetically modified plants. Frontiers in Plant Science 14: 1221790.

Verdecchia A, Martino M, Mazzaglia A and Davino S (2021). A novel qPCR assay for the detection of *Phytophthora cactorum* in strawberry plants. Plant Pathology 70: 123-132.

Zhang H, Wang Y, Yang J, Han S, Liu H and Xu J (2020). Development of portable qPCR devices for rapid on-site detection of pathogens. Biosensors and Bioelectronics 165: 112411.

Zhang Y, Wang X, Li G, Xu Z, Wu H and Zhou L (2022). Identification of potential reference genes for qPCR normalization in *Colletotrichum fructicola*. Frontiers in Microbiology 13: 939350.