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APPLICATIONS OF LUX-BASED BIOREPORTERS IN PLANT DISEASE DETECTION, EPIDEMIOLOGY AND BIOLOGICAL CONTROL: A REVIEW

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ABSTRACT

Plant diseases remain a major constraint to agricultural productivity worldwide, creating a need for advanced tools to study pathogen behavior and improve disease management. Among available reporter systems, bacterial bioluminescence-based lux gene technology has emerged as a powerful, non-destructive, and real-time approach for investigating plant microbe interactions. The lux operon (luxCDABE), originally identified in bioluminescent bacteria, encodes both luciferase and the enzymes required for endogenous substrate synthesis, enabling autonomous light production without external substrate addition. This unique property allows continuous monitoring of microbial populations within living plant tissues. This review summarizes the historical development, molecular biology, and bioluminescence mechanism of the lux operon, with particular emphasis on its applications in plant pathology. Lux-tagged pathogens have been extensively used to study pathogen colonization, infection dynamics, vascular movement, and disease epidemiology in economically important crops. The technology has also been applied in rhizosphere ecology for tracking plant growth-promoting rhizobacteria, endophytes, and biological control agents. Additional applications include fungicide and bactericide screening, quorum sensing analysis, biofilm studies, and biosensor development for pathogen detection. Recent advances in synthetic biology, genetic engineering, and imaging technologies have further expanded the versatility and sensitivity of lux-based systems. Despite limitations such as restricted application in fungal pathogens and limited field-scale validation, lux gene technology remains an important tool in modern plant pathology. Future integration with artificial intelligence, digital phenotyping, and multi-omics approaches is expected to enhance its role in disease diagnostics, epidemiological studies, and sustainable crop protection.

Keywords **:** Lux gene, disease, biocontrol, bioluminescence

Introduction

Plant diseases remain a major constraint to global agricultural productivity, causing substantial yield losses and threatening food security across diverse agro ecosystems. Conventional approaches in plant pathology such as visual disease scoring, pathogen isolation, and colony-forming unit (CFU) quantification are inherently destructive, time-consuming, and limited to endpoint assessments, thereby restricting the ability to capture the dynamic nature of host–pathogen interactions. Moreover, increasing emphasis on sustainable and environmentally non-polluting disease management strategies necessitates the development of advanced tools that can facilitate precise, real-time monitoring of pathogen behaviour without disrupting plant systems. In this context, reporter gene technologies have emerged as powerful tools in plant pathology, enabling in vivo tracking of microbial processes. Among these, the bacterial bioluminescence-based *lux* gene system

represents a particularly advantageous platform due to its self-sufficient light-producing capability, eliminating the need for exogenous substrates. The *lux* operon (luxCDABE), originally characterized from bioluminescent bacteria such as *Vibrio fischeri*, encodes both the luciferase enzyme and the enzymatic machinery required for substrate synthesis, allowing continuous emission of visible light proportional to cellular metabolic activity (Stewart & Williams, 1992). This intrinsic property enables non-invasive, real-time visualization of microbial populations within living plant tissues. The application of *lux* gene technology in plant pathology has significantly advanced the study of infection biology, pathogen colonization, and disease epidemiology. Lux-tagged strains of important plant pathogens, including *Ralstonia solanacearum* and *Xanthomonas campestris*, have been successfully employed to monitor spatial and temporal dynamics of infection *in planta*, providing insights into vascular colonization, systemic spread, and population fluctuations under varying environmental conditions. Unlike conventional techniques, lux-based imaging allows repeated measurements from the same biological sample, thereby enabling longitudinal analysis of disease progression without destructive sampling.

Beyond infection tracking, *lux* gene technology has found extensive application in rhizosphere ecology and biological control studies, where it facilitates the monitoring of survival, establishment, and competitive interactions of beneficial microorganisms in soil and plant-associated environments. The technology has also been utilized in screening environmentally safe disease management strategies, including biocontrol agents and reduced-risk agrochemicals, by providing rapid and quantitative assessment of pathogen viability through changes in luminescence intensity (White, 1996). In this regard, lux-based systems align closely with the principles of green and non-polluting agricultural technologies, as they minimize the need for destructive assays, chemical reagents, and labour-intensive procedures. Furthermore, the integration of *lux* reporters with promoter fusion strategies has enabled detailed investigation of virulence gene expression, quorum sensing, and stress-responsive regulatory networks within host tissues. Such applications are particularly valuable for dissecting the molecular basis of host–pathogen interactions and for identifying critical determinants of pathogenicity under *in vivo* conditions. With recent advances in imaging technologies, computational analysis, and systems biology approaches, lux-based methodologies are increasingly being incorporated into high-resolution phenotyping platforms, bridging the gap between molecular mechanisms and disease outcomes.

In this review, we critically examine the principles, methodological developments, and diverse applications of lux gene technology in plant pathology, with particular emphasis on its role in enabling sustainable, non-destructive, and real-time analysis of plant–microbe interactions.

Background of Lux Gene Technology

The phenomenon of bioluminescence has intrigued scientists for centuries and represents one of the earliest recognized examples of naturally occurring biological signal generation. Initial observations of luminous marine organisms and glowing seawater led to investigations that eventually identified bioluminescent bacteria as the source of light production in numerous marine environments. Early microbiological studies conducted during the late nineteenth and early twentieth centuries demonstrated that several marine bacterial species, particularly members of the genera *Vibrio* and *Photobacterium*, possessed the intrinsic ability to emit visible light through a genetically regulated biochemical process. Subsequent biochemical investigations established that bacterial luminescence was dependent upon oxygen and mediated by a specific enzyme system, later termed luciferase, which catalyzes the oxidation of reduced flavin mononucleotide ($FMNH_2$) and a long-chain aliphatic aldehyde, resulting in photon emission (Harvey, 1952; Meighen, 1991).

The molecular basis of bacterial bioluminescence remained largely unresolved until the advent of recombinant DNA technologies in the 1970s and early 1980s. During this period, extensive genetic analyses of *Vibrio fischeri* and *Photobacterium leiognathi* led to the identification of a cluster of genes responsible for light production, collectively designated as the lux operon (Engebrecht *et al*., 1983). This discovery represented a major milestone in microbial genetics because it revealed that bacterial luminescence was controlled not by a single gene but by a coordinated operon comprising multiple structural and regulatory elements. The canonical lux operon consists of the genes luxCDABE, where luxA and luxB encode the α and β subunits of bacterial luciferase, whereas luxC, luxD, and luxE are involved in the biosynthesis and recycling of the aldehyde substrate required for the luminescent reaction (Meighen and Dunlap, 1993). Additional regulatory genes, including luxI and luxR, were subsequently identified and shown to govern cell-density-dependent gene expression through quorum sensing mechanisms, providing one of the first

experimentally validated models of bacterial cell-to-cell communication (Fuqua *et al*., 1994). The elucidation of the lux operon coincided with rapid developments in molecular cloning, which facilitated the transfer of luminescence-associated genes into heterologous hosts. One of the most significant breakthroughs occurred when lux genes were successfully cloned and expressed in non-luminous bacterial species, demonstrating that bioluminescence could function as a genetically encoded reporter trait independent of its native host background (Engebrecht *et al*., 1985). This achievement transformed lux genes from subjects of basic biological investigation into versatile molecular tools. Unlike conventional microbiological assays that relied upon destructive sampling, colony counting, or biochemical staining, lux-mediated bioluminescence provided a rapid, non-destructive, and quantitative means of monitoring microbial populations and gene expression in real time. Consequently, lux reporters were increasingly adopted in studies investigating bacterial physiology, environmental microbiology, and microbial ecology during the late 1980s and early 1990s (Stewart and Williams, 1992).

The introduction of lux genes into plant-associated microorganisms marked the beginning of their application in plant pathology. Researchers soon recognized that lux-tagged pathogens could serve as powerful tools for studying host-pathogen interactions, enabling direct visualization of pathogen colonization, movement, and population dynamics within plant tissues. Early studies involving luminescent strains of *Pseudomonas syringae*, *Erwinia amylovora*, and *Xanthomonas campestris* demonstrated that pathogen spread could be monitored in vivo without the need for destructive tissue sampling, thereby providing unprecedented insights into infection processes and disease development (Shaw *et al*., 1992; Ried and Collmer, 1987). These developments represented a paradigm shift in plant pathology, allowing disease progression to be studied dynamically rather than through static endpoint observations. Despite these advances, early lux reporter systems were constrained by a significant limitation. Most initial applications utilized only the luxAB genes encoding luciferase, requiring exogenous addition of aldehyde substrates to generate detectable light. The necessity for substrate supplementation restricted experimental flexibility and limited the practicality of large-scale or long-term studies. To overcome this challenge, researchers increasingly employed the complete luxCDABE cassette, which incorporates both luciferase genes and the enzymatic machinery necessary for endogenous substrate synthesis. Expression of the entire lux operon enabled autonomous light production, eliminating the requirement for external substrate addition and greatly enhancing the utility of lux reporters in environmental and biological systems (Winson *et al*., 1998). The development of autonomous bioluminescent systems constituted one of the most important milestones in the evolution of lux technology. The complete luxCDABE cassette allowed transformed microorganisms to continuously emit light in response to metabolic activity, making it possible to monitor living cells over extended periods. This innovation substantially improved the sensitivity and practicality of bioluminescent imaging and facilitated applications across diverse fields, including environmental monitoring, microbial ecology, biosensor development, and plant disease research. In plant pathology, autonomous lux reporters enabled continuous tracking of pathogen populations within host tissues, assessment of disease progression, and evaluation of biological control agents under both laboratory and greenhouse conditions (White *et al*., 1996). Lux-tagged strains of beneficial rhizobacteria and antagonistic microorganisms further provided valuable insights into root colonization patterns, rhizosphere competence, and microbial interactions that were previously difficult to quantify using conventional methods. The emergence of synthetic biology and advanced genetic engineering technologies during the last two decades has further expanded the capabilities of lux-based systems. Codon optimization strategies, promoter engineering, and synthetic operon design have significantly improved expression efficiency in a wide range of bacterial and eukaryotic hosts (Close *et al*., 2012). Moreover, the integration of lux reporters with high-sensitivity charge-coupled device (CCD) imaging systems has enabled real-time visualization of microbial activity at increasingly finer spatial and temporal scales. Recent studies have combined lux technology with genome editing tools, including CRISPR-Cas systems, to investigate gene regulation, virulence mechanisms, and host–microbe interactions with unprecedented precision. These advances have transformed bacterial bioluminescence from a biological curiosity into a sophisticated imaging platform capable of addressing fundamental and applied questions in plant pathology.

Today, lux gene technology occupies a unique position among reporter systems because it permits non-destructive, real-time, and quantitative monitoring of living microorganisms without external substrate supplementation. The historical progression from the discovery of luminous marine bacteria to the development of autonomous synthetic bioluminescent systems illustrates a remarkable convergence of

microbiology, molecular genetics, and biotechnology. This evolution has established lux-based reporters as indispensable tools for investigating pathogen ecology, disease epidemiology, biological control, and plant–microbe interactions, thereby contributing significantly to modern plant pathological research.

Molecular biology of Lux Operon

The lux operon constitutes one of the most sophisticated and extensively studied prokaryotic gene regulatory systems and serves as a model for understanding transcriptional regulation, quorum sensing, metabolic integration, and bacterial communication. In naturally bioluminescent bacteria, light production is governed by a coordinated set of structural, enzymatic, and regulatory genes that collectively enable the synthesis of substrates, catalysis of the luminescent reaction, and population-dependent control of gene expression. Since its initial characterization in marine luminous bacteria, the lux operon has become a cornerstone of molecular microbiology and an indispensable tool in biotechnology, microbial ecology, and plant pathology (Meighen, 1994; Dunlap & Kita-Tsukamoto, 2006).

The genetic organization of the lux operon varies among bioluminescent bacterial species; however, the canonical arrangement typically consists of luxCDABE, which encodes all components necessary for autonomous light production (Meighen & Dunlap, 1993; Urbanczyk *et al*., 2008). Comparative genomic analyses have revealed substantial diversity in operon architecture among species of *Vibrio*, *Aliivibrio*, *Photobacterium*, and *Photorhabdus*, reflecting extensive evolutionary adaptation and horizontal gene transfer events (Dunlap, 1985; Thompson *et al*., 2005; Urbanczyk *et al*., 2008). Despite these differences, the functional conservation of the lux genes highlights their fundamental importance in bacterial physiology and ecological fitness. At the core of the lux operon are the luxA and luxB genes, which encode the α- and β-subunits of bacterial luciferase, respectively. These proteins assemble into a heterodimeric flavin-dependent monooxygenase that catalyzes the oxidation of reduced flavin mononucleotide ($FMNH_2$) and a long-chain aldehyde in the presence of molecular oxygen, producing oxidized flavin, a fatty acid, water, and visible blue-green light (Baldwin *et al*., 1984; Cohn *et al*., 1985; Fisher *et al*., 1995). Structural analyses of bacterial luciferase have demonstrated that the catalytic active site is located primarily within the α-subunit, whereas the β-subunit contributes to substrate orientation and stabilization of the enzyme complex (Fisher *et al*., 1995; Campbell *et al*., 2009). Mutational studies further revealed that highly conserved amino acid residues within LuxA are essential for substrate binding, oxygen activation, and photon emission, indicating strong evolutionary constraints on luciferase structure and function (Baldwin *et al*., 1984; Campbell *et al*., 2009).

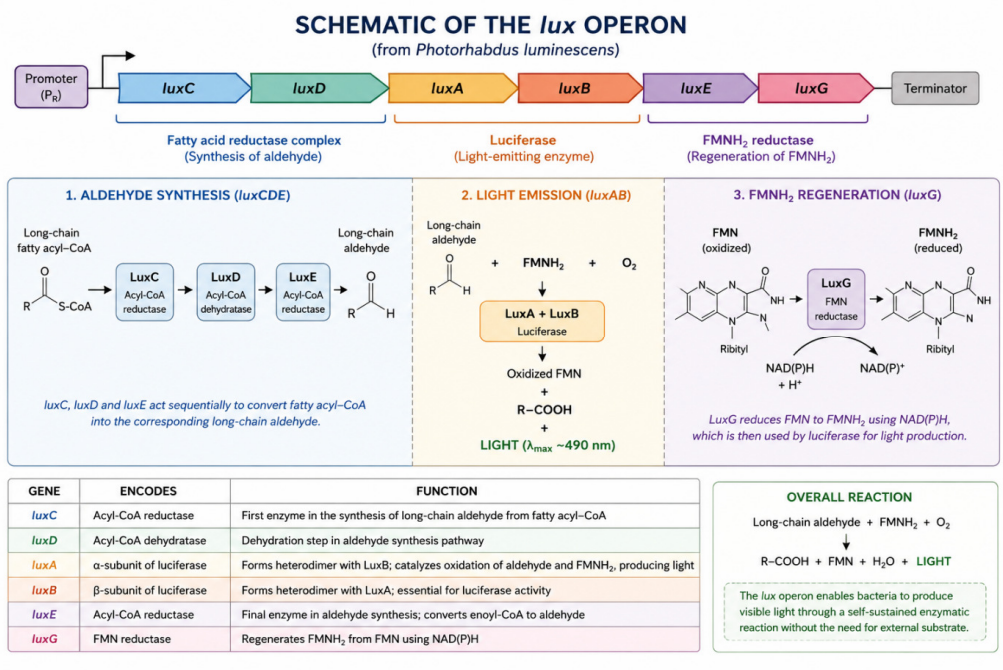

Fig. 1 : Schematic structure of Lux operon

A distinctive feature of bacterial bioluminescence is the endogenous synthesis of the aldehyde substrate required for light production. This process is mediated by the luxC, luxD, and luxE genes, which encode a multienzyme fatty acid reductase complex. LuxD functions as an acyl-transferase that transfers fatty acids from membrane phospholipids, LuxE catalyzes ATP-dependent activation of fatty acid substrates, and LuxC reduces activated fatty acids to the corresponding aldehydes utilized by luciferase (Wall *et al*., 1984; Mancini *et al*., 1988; Meighen & Dunlap, 1993). The coupling of aldehyde biosynthesis with luciferase activity allows continuous substrate regeneration and distinguishes bacterial lux systems from eukaryotic luciferase systems that require external substrate supplementation. This autonomous substrate-generation capability is one of the principal reasons for the widespread adoption of lux genes as reporter systems in microbial research. The first stage of the bioluminescent reaction involves intracellular generation of $FMNH_2$ through the action of flavin reductases that utilize reducing equivalents derived from NADH or NADPH metabolism. Because $FMNH_2$ is highly unstable in the presence of oxygen, efficient substrate channeling is necessary to ensure its rapid delivery to luciferase before spontaneous oxidation occurs. Studies employing rapid kinetic analysis demonstrated that reduced flavin initially binds to luciferase, forming a luciferase–$FMNH_2$ complex that subsequently reacts with molecular oxygen to generate a flavin-4a-hydroperoxide intermediate (Hastings *et al*., 1978; Vervoort *et al*., 1986). This hydroperoxide intermediate represents the central reactive species in bacterial bioluminescence and is considered the key oxidative agent responsible for subsequent aldehyde conversion. Formation of flavin-4a-hydroperoxide constitutes one of the most important mechanistic events in the lux system because it establishes the energetic foundation required for photon production. Spectroscopic analyses and stopped-flow kinetic studies revealed that this intermediate possesses unusual stability compared with hydroperoxide intermediates found in many other flavoprotein-catalyzed reactions (Daubner *et al*., 1987; Lei *et al*., 2004). The stabilization of this reactive intermediate by luciferase permits controlled oxidation of the aldehyde substrate while minimizing undesirable side reactions. Structural investigations have suggested that specific amino acid residues within the catalytic pocket contribute to stabilization of the hydroperoxide complex through hydrogen-bonding interactions and conformational constraints that optimize catalytic efficiency (Campbell *et al*., 2009). Following formation of the hydroperoxide intermediate, the long-chain aldehyde substrate enters the catalytic cycle. The aldehyde reacts with flavin hydroperoxide to generate a highly energetic peroxyhemiacetal intermediate, which subsequently decomposes into the corresponding fatty acid and an electronically excited flavin species. This excited-state flavin intermediate is widely regarded as the immediate precursor of photon emission and represents the critical link between chemical oxidation and visible light production (Lee *et al*., 1970; Tu, 1975). Although details of the exact reaction pathway continue to be refined, most contemporary models support the hypothesis that excitation energy originates from decomposition of oxygenated flavin intermediates generated during aldehyde oxidation. Light emission occurs when the electronically excited intermediate returns to its ground state. During this transition, excess energy is released in the form of a photon with a wavelength typically ranging between 470 and 495 nm. The precise emission spectrum varies among bacterial species and is influenced by enzyme structure, intracellular environment, substrate composition, and associated luminescence proteins (Watanabe *et al*., 1978; Baldwin & Ziegler, 1992). Marine luminous bacteria generally emit blue-green light because these wavelengths exhibit superior transmission characteristics in aquatic environments, thereby maximizing ecological visibility and signaling efficiency (Widder, 2010).

In several luminous bacteria, additional genes such as luxG and luxF contribute to optimization of the luminescent process. LuxG encodes a flavin reductase that facilitates regeneration of $FMNH_2$, thereby increasing the availability of reduced flavin for luciferase-catalyzed reactions (Brodl *et al*., 2018). Although not universally conserved, accessory genes significantly influence luminescence intensity, metabolic efficiency, and ecological adaptation. Comparative analyses indicate that variation in accessory gene content may partially explain differences in light output observed among bioluminescent bacterial species (Dunlap & Kita-Tsukamoto, 2006; Brodl *et al*., 2018). Regulation of lux operon expression is primarily mediated through quorum sensing, a population-density-dependent communication mechanism that enables bacterial populations to coordinate gene expression collectively. The LuxI–LuxR regulatory circuit of *Aliivibrio fischeri* represents the archetypal quorum-sensing system and has become the foundation for understanding bacterial cell-to-cell signaling (Fuqua *et al*., 1994; Miller & Bassler, 2001). LuxI functions as an autoinducer synthase responsible for the production of N-acyl homoserine lactone (AHL) molecules, which freely diffuse across bacterial membranes and accumulate in

the extracellular environment (Fuqua *et al*., 1994; Whitehead *et al*., 2001). As bacterial population density increases, extracellular concentrations of AHL molecules rise proportionally until a threshold concentration is reached.

Upon reaching the threshold concentration, AHL molecules bind to LuxR, a cytoplasmic transcriptional regulator belonging to the LuxR-family of DNA-binding proteins. The LuxR–AHL complex subsequently binds specific promoter regions known as lux boxes and activates transcription of the lux operon (Devine *et al*., 1989; Fuqua & Greenberg, 2002). This mechanism establishes a positive autoregulatory feedback loop in which activation of LuxR stimulates transcription of both luxCDABE and luxI, resulting in enhanced autoinducer synthesis and rapid amplification of the luminescent response (Miller & Bassler, 2001; Waters & Bassler, 2005). Such positive-feedback regulation allows bacterial populations to synchronize gene expression and has become a model system for studying cooperative microbial behavior.

The molecular basis of quorum sensing extends beyond bioluminescence and influences numerous physiological processes including virulence, motility, biofilm formation, antibiotic production, and host colonization. Consequently, investigations of lux operon regulation have profoundly influenced understanding of bacterial pathogenesis and microbial ecology (Whitehead *et al*., 2001; Ng & Bassler, 2009). In plant-pathogenic bacteria such as *Pectobacterium*, *Dickeya*, *Pseudomonas*, and *Xanthomonas*, LuxI/LuxR homologs regulate production of extracellular enzymes, toxins, and other virulence determinants, emphasizing the broader biological significance of lux-associated regulatory pathways (Venturi & Fuqua, 2013). Transcriptional regulation of the lux operon is also influenced by environmental and physiological factors. Oxygen availability is particularly important because molecular oxygen serves directly as a substrate in the luminescent reaction. Consequently, lux expression is tightly coupled with respiratory activity and cellular energy metabolism (Meighen, 1991; Hastings & Greenberg, 1999). Temperature, osmotic stress, nutrient availability, carbon source utilization, and redox state can further modulate lux expression through interactions with global regulatory networks, including cyclic AMP receptor protein (CRP), alternative sigma factors, and stress-responsive transcriptional regulators (Bassler *et al*., 1997; Dunlap, 1999). Such multilayered regulation ensures that light production occurs only under physiologically favorable conditions.

Advances in genomics and synthetic biology have substantially expanded understanding of lux operon evolution and function. Comparative phylogenetic studies indicate that horizontal gene transfer has played a major role in dissemination of lux genes among marine bacterial populations, contributing to the emergence of diverse operon architectures and regulatory mechanisms (Thompson *et al*., 2005; Dunlap & Kita-Tsukamoto, 2006). Synthetic reconstruction and codon optimization of lux operons have further enabled expression in non-native hosts, including Gram-positive bacteria, fungi, mammalian cells, and plant-associated microorganisms (Gregor *et al*., 2018; Close *et al*., 2012). These developments have transformed the lux operon from a naturally occurring bioluminescence system into a versatile molecular platform for studying gene expression, microbial ecology, and host–microbe interactions.

From a plant pathology perspective, the molecular architecture of the lux operon provides several unique advantages over conventional reporter systems. Because lux-mediated light production is directly linked to cellular metabolism and does not require exogenous substrate addition, lux-tagged pathogens can be monitored continuously within living plant tissues. Furthermore, luminescence intensity often correlates closely with microbial population density and metabolic activity, enabling quantitative assessment of pathogen colonization, disease progression, and responses to disease-management strategies (Ried & Collmer, 1987; Shaw *et al*., 1992; Close *et al*., 2012). These characteristics have established the lux operon as one of the most powerful molecular tools available for investigating plant–microbe interactions under both controlled and semi-natural conditions.

Application of Lux gene in Plant Pathology

One of the most significant contributions of lux gene technology to plant pathology has been the ability to monitor pathogen colonization, movement, population dynamics, and infection processes in living host tissues in a non-destructive manner. Traditional approaches for studying pathogen invasion largely depended on destructive sampling, dilution plating, microscopy, histological sectioning, or molecular detection techniques. Although these methods have provided valuable information regarding disease development, they are often labor-intensive, time-consuming, and incapable of continuously monitoring pathogen behavior within the same host tissue over time. The introduction of lux-tagged phytopathogens has transformed disease biology by enabling real-time

visualization of pathogen movement and proliferation inside plants without the need for tissue destruction.

Tracking and Visualization of Plant Pathogens Using Lux Gene Technology

The fundamental principle underlying lux-based pathogen tracking is that luminescence intensity correlates with metabolically active bacterial populations. Because the luxCDABE operon encodes both luciferase and the enzymes required for endogenous aldehyde synthesis, transformed bacterial pathogens emit light autonomously without exogenous substrate supplementation. Consequently, pathogen colonization can be monitored repeatedly throughout disease development, allowing dynamic observation of infection processes that were previously difficult to investigate using conventional microbiological techniques (Soldan *et al*., 2021). Bioluminescence imaging systems equipped with highly sensitive charge-coupled device (CCD) cameras can detect even relatively small bacterial populations within plant tissues, thereby providing a powerful platform for studying host–pathogen interactions under controlled and semi-natural conditions.

Among plant-pathogenic bacteria, *Pseudomonas syringae* has become one of the most extensively studied organisms using lux-based imaging approaches. As a model phytopathogen possessing a sophisticated type III secretion system and numerous virulence effectors, *P. syringae* has been widely utilized for investigating bacterial colonization and disease progression in planta (Mansfield *et al*., 2012). Lux-tagged strains have enabled direct visualization of bacterial migration through leaf tissues following entry via stomata, hydathodes, or wounds. Bioluminescence imaging demonstrated that pathogen populations are not distributed uniformly within infected tissues but instead accumulate preferentially around vascular networks and intercellular spaces where nutrient availability favors bacterial multiplication. Continuous monitoring of luminescent *P. syringae* populations has provided valuable insights into infection kinetics, spatial colonization patterns, and the temporal regulation of virulence during disease establishment (Hirano & Upper, 2000; Kennelly *et al*., 2007). Furthermore, lux-based imaging has facilitated quantitative evaluation of resistant and susceptible host genotypes by enabling direct comparison of bacterial population growth within living tissues.

Lux reporters have also proven valuable for investigating vascular pathogens such as *Clavibacter michiganensis* subsp. *michiganensis*, the causal agent of bacterial canker and wilt of tomato. This Gram-positive pathogen colonizes xylem vessels and spreads systemically throughout host plants, eventually impairing water transport and inducing wilting symptoms. Conventional assessment of pathogen movement within vascular tissues typically requires destructive stem sectioning and isolation procedures. In contrast, bioluminescent strains have enabled visualization of pathogen migration through xylem networks and have provided direct evidence regarding the dynamics of systemic colonization under different environmental conditions. Studies utilizing luminescent *Clavibacter* strains demonstrated that bacterial movement within host vascular tissues is strongly influenced by humidity, host developmental stage, and wound-mediated entry points. Bioluminescence imaging further revealed that extensive systemic colonization may occur prior to visible symptom development, highlighting the importance of latent infections in disease epidemiology (Xu *et al*., 2010; Eichenlaub & Gartemann, 2011). Such observations have important implications for understanding pathogen dissemination within transplant production systems and seedborne disease transmission.

The fire blight pathogen *Erwinia amylovora* has similarly benefited from lux-mediated tracking approaches. Fire blight represents one of the most economically destructive bacterial diseases affecting pome fruit crops, including apple and pear. Disease development involves epiphytic colonization of floral surfaces followed by invasion of nectarthodes, vascular tissues, and young shoots. Lux-tagged strains of *E. amylovora* have enabled researchers to monitor bacterial population dynamics on flower stigmas, blossoms, and internal host tissues under natural infection conditions. These studies revealed that successful disease establishment is often preceded by extensive epiphytic multiplication on floral surfaces before invasion of internal tissues occurs. Bioluminescence imaging further demonstrated that bacterial populations can persist asymptomatically within host tissues prior to symptom expression, thereby contributing to pathogen spread and epidemic development (Pusey, 2000; Sundin *et al*., 2009). Such findings have improved understanding of infection biology and facilitated development of predictive disease-management strategies.

Species belonging to the genus *Xanthomonas* have also been investigated extensively using bioluminescent reporter systems. Members of this genus cause destructive diseases in numerous economically important crops, including tomato, pepper, rice, citrus, and crucifers. Because many

Xanthomonas pathogens invade leaves through stomata or hydathodes and subsequently colonize intercellular spaces, visualization of bacterial movement within leaf tissues is essential for understanding disease progression. Recent studies employing bioluminescent *Xanthomonas hortorum* pv. *gardneri* demonstrated that luminescence intensity correlates strongly with bacterial populations measured through traditional dilution plating methods, validating the utility of lux reporters for quantitative pathogen monitoring (Bernal *et al*., 2021). Bioluminescence imaging further enabled direct observation of infection routes, colonization fronts, and spatial differences in pathogen proliferation between resistant and susceptible tomato genotypes. These findings illustrated the capacity of lux technology to bridge phenotypic disease assessment with quantitative pathogen population analysis.

Lux reporters have further facilitated studies examining bacterial movement within specific plant structures including leaves, stems, petioles, vascular bundles, roots, flowers, and fruits. In vascular pathogens, luminescent strains have provided unprecedented insights into xylem colonization patterns and systemic spread. In foliar pathogens, lux imaging has enabled visualization of bacterial aggregation around stomatal openings, hydathodes, and intercellular spaces. Such spatial information is difficult to obtain through conventional population-based assays and has substantially improved understanding of pathogen niche specialization within host tissues (Bernal *et al*., 2021; Soldan *et al*., 2021).

Rhizosphere Colonization Studies Using Lux-Tagged PGPR, Endophytes, and Biocontrol Agents

Successful colonization of the rhizosphere represents one of the most critical prerequisites for effective biological control and plant growth promotion by beneficial microorganisms. Although numerous plant growth-promoting rhizobacteria (PGPR), endophytes, and antagonistic microbes exhibit strong disease-suppressive activity under laboratory conditions, their performance in field environments is often inconsistent due to limitations in root colonization, survival, ecological fitness, and competition with indigenous microbial communities. Consequently, understanding the spatial and temporal dynamics of microbial establishment in the rhizosphere has become a major research focus in plant pathology and agricultural microbiology (Bloemberg & Lugtenberg, 2001; Wang *et al*., 2021). The development of lux-tagged beneficial microorganisms has provided a powerful approach for investigating these processes because bioluminescent strains can be monitored directly within root systems and surrounding soil environments without destructive sampling.

The rhizosphere is a highly dynamic ecological niche characterized by intense microbial competition, nutrient gradients, root exudation, and complex plant–microbe interactions. Traditional methods for studying rhizosphere colonization generally rely on dilution plating, antibiotic resistance markers, microscopy, or molecular detection techniques. While valuable, these approaches often provide only endpoint measurements and may fail to capture temporal fluctuations in microbial populations. Lux-mediated bioluminescence imaging has overcome many of these limitations by allowing continuous monitoring of metabolically active microbial populations in association with living roots. Because photon emission reflects cellular viability and metabolic activity, lux reporters provide direct information regarding microbial persistence, population dynamics, and colonization efficiency under natural conditions (Kragelund *et al*., 1997; Jäderlund, 2008).

Among PGPR, *Pseudomonas fluorescens* has become one of the most extensively studied organisms for rhizosphere colonization research. This species possesses strong rhizosphere competence and exhibits multiple plant-beneficial activities including siderophore production, phosphate solubilization, antibiotic biosynthesis, induced systemic resistance, and pathogen suppression. Lux-tagged *P. fluorescens* strains have been employed extensively to investigate bacterial distribution along root surfaces, persistence within the rhizosphere, and responses to environmental stresses. Early bioluminescence studies demonstrated that *P. fluorescens* populations are not distributed uniformly around roots but instead form localized microcolonies in regions enriched with root exudates and nutrient leakage. Such spatial heterogeneity plays a major role in determining the success of biological control activity because bacterial antagonism against pathogens is often concentrated within these colonization hotspots (Kragelund *et al*., 1997; Bloemberg & Lugtenberg, 2001).

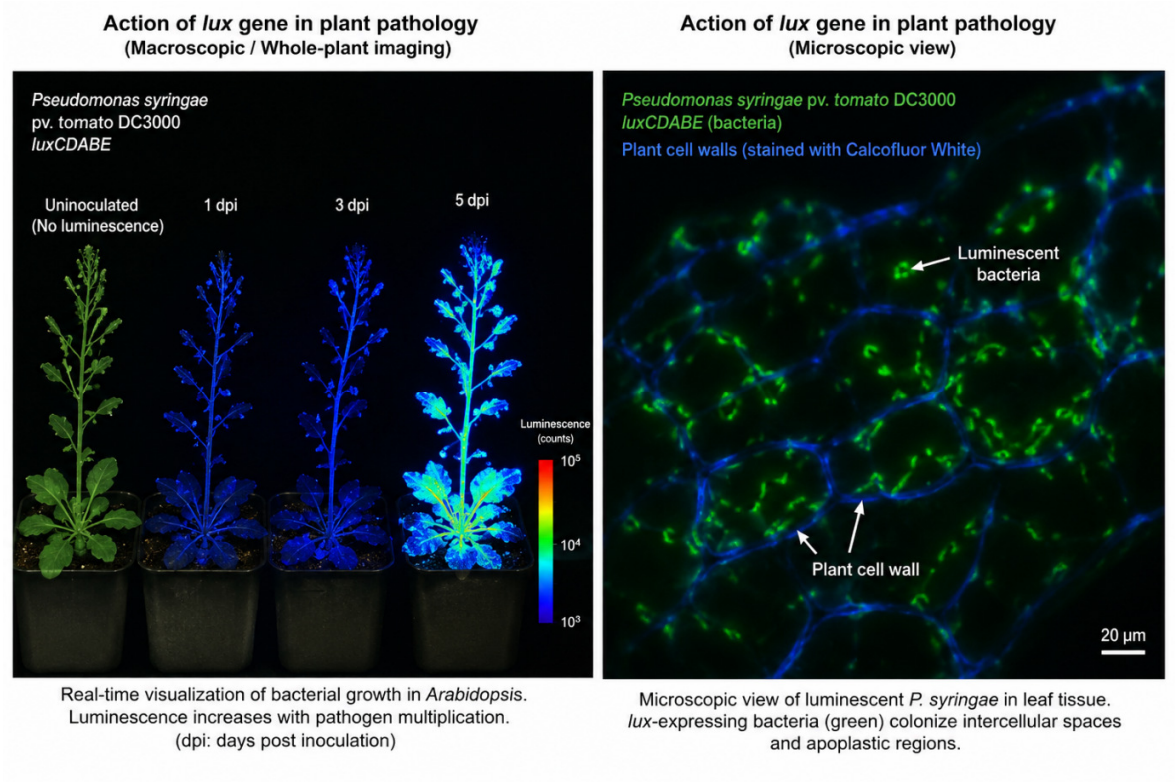

Fig. 2 : Real time visualization of bacterial growth in the leaf tissues using lux gene technology

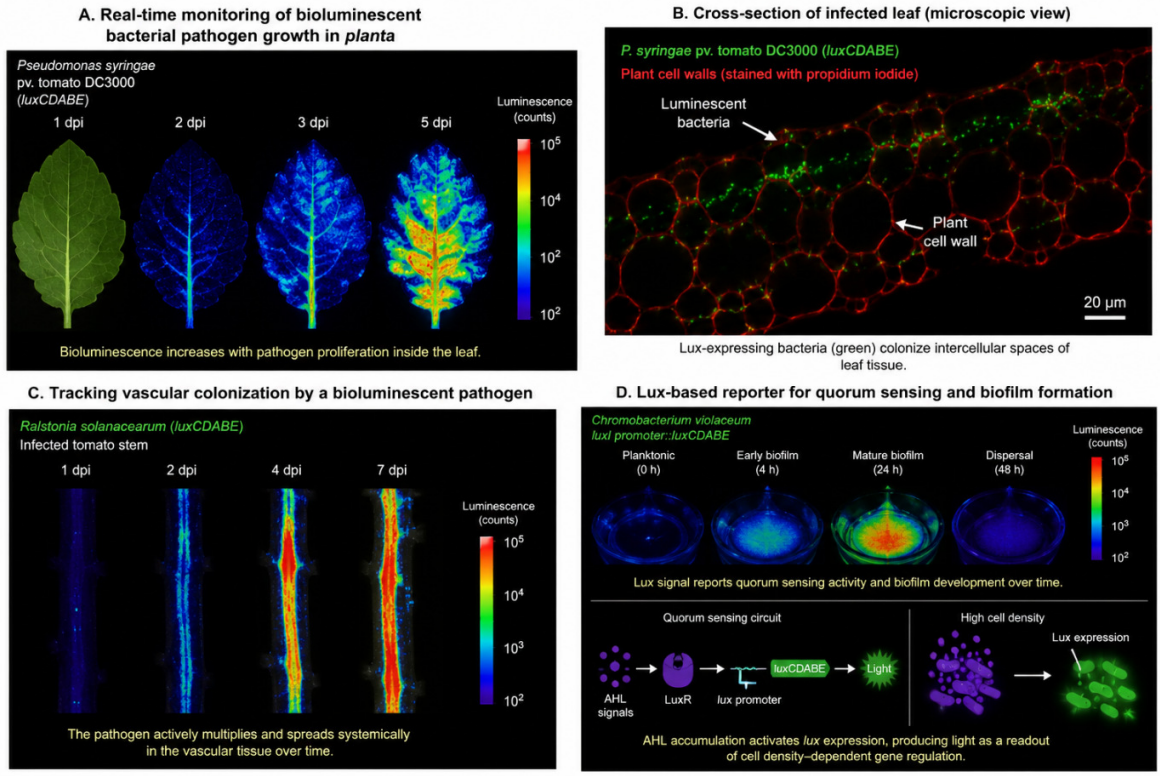

Fig. 3 : Functioning and tracking of lux genes inside plant tissues

Lux-based investigations further revealed that colonization efficiency varies considerably among plant species, root developmental stages, and soil environments. Studies utilizing lux-tagged *P. fluorescens* reporter strains demonstrated that bacterial populations frequently accumulate around root tips,

elongation zones, and emerging lateral roots where exudation rates are highest. These regions provide favorable nutritional conditions that support bacterial multiplication and biofilm formation. Bioluminescence imaging also showed that environmental stresses such as nutrient limitation, phosphate starvation, and moisture fluctuations can significantly alter bacterial metabolic activity within the rhizosphere. Reporter strains carrying lux fusions to stress-responsive promoters enabled direct visualization of physiological responses during root colonization, thereby providing important insights into microbial adaptation mechanisms under field-like conditions (Kragelund *et al*., 1997; Jäderlund, 2008).

The use of lux reporters has similarly improved understanding of *Bacillus* spp. colonization dynamics. Members of this genus are among the most commercially important biological control agents because of their ability to produce endospores, antimicrobial compounds, lipopeptides, and plant growth-promoting metabolites. Successful disease suppression by *Bacillus* largely depends upon stable root colonization and persistence within the rhizosphere. Bioluminescent tagging approaches have demonstrated that *Bacillus* populations often establish preferentially near root surfaces and can persist for extended periods despite environmental fluctuations. Monitoring of lux-marked strains has further revealed strong associations between biofilm formation, root attachment, and long-term rhizosphere competence. Such observations have contributed significantly to understanding the ecological mechanisms underlying PGPR-mediated disease suppression and plant growth enhancement (El-Saadony *et al*., 2022; Espinosa-Palomeque *et al*., 2025).

Beyond rhizobacteria, lux technology has increasingly been applied to studies involving endophytic microorganisms. Endophytes inhabit internal plant tissues without causing disease and frequently contribute to host growth promotion, stress tolerance, and pathogen suppression. Because endophytic colonization occurs within roots, stems, and vascular tissues, visualization of microbial distribution is considerably more challenging than in rhizosphere studies. Lux-tagged endophytes have enabled direct monitoring of microbial migration from root surfaces into internal tissues and have revealed that successful endophytic establishment often depends upon specific colonization routes through root hairs, lateral root emergence sites, and wounded tissues. Bioluminescence imaging has demonstrated that endophytic populations may remain localized within specific plant compartments or spread systemically through vascular networks depending upon microbial species and host genotype (Verma *et al*., 2001).

Particularly important in plant pathology are studies involving biological control agents designed to suppress soilborne pathogens. Lux reporters have allowed researchers to evaluate the persistence and ecological fitness of introduced antagonists under realistic environmental conditions. By tracking bioluminescent populations over time, investigators can determine whether inoculated strains successfully establish within the rhizosphere, compete with indigenous microorganisms, and maintain population densities sufficient for disease suppression. Such information is essential because inconsistent colonization is often a major factor limiting field performance of microbial biocontrol products (Wang *et al*., 2021; El-Saadony *et al*., 2022).

In recent years, increasing attention has been directed toward interactions between bacterial PGPR and fungal biocontrol agents, particularly *Trichoderma* spp. Although *Trichoderma* itself is generally monitored using fluorescent proteins rather than lux systems, lux-tagged bacterial partners have been used to investigate microbial interactions within the rhizosphere during combined inoculation strategies. Such studies revealed that co-inoculation with PGPR and *Trichoderma* can alter microbial spatial distribution, enhance root colonization efficiency, and improve disease suppression against soilborne pathogens. Bioluminescent monitoring further demonstrated that synergistic interactions between bacterial and fungal biocontrol agents may enhance persistence within the rhizosphere by improving nutrient acquisition, biofilm formation, and niche occupation (El-Saadony *et al*., 2022).

The integration of lux technology with modern imaging platforms has substantially improved quantitative analysis of root-associated microbial populations. High-sensitivity imaging systems can detect low-density populations directly within soil-grown plants and permit repeated observation of the same root system throughout plant development. Consequently, researchers can evaluate colonization dynamics, microbial competition, stress responses, and treatment effects with greater precision than traditional endpoint assays. Such capabilities have become increasingly important for screening PGPR strains, optimizing microbial inoculant formulations, and improving consistency of biological control programs. Overall, lux-tagged PGPR, endophytes, and biological control agents have greatly advanced understanding of rhizosphere ecology and root colonization processes. Investigations involving *Pseudomonas fluorescens*,

Bacillus spp., endophytic bacteria, and *Trichoderma*-associated microbial systems have demonstrated that successful colonization depends upon complex interactions among microbial physiology, root exudation patterns, environmental conditions, and intermicrobial competition. By enabling non-destructive visualization of microbial populations in real time, lux gene technology has become an indispensable tool for elucidating the ecological mechanisms underlying biological control and plant growth promotion.

Fungicide and Bactericide Screening Using Lux-Based Reporter Systems

The development of lux-tagged phytopathogens has significantly enhanced the efficiency and precision of fungicide and bactericide screening programs in plant pathology. Conventional evaluation of antimicrobial compounds typically relies on measurements such as radial growth inhibition, colony-forming unit (CFU) counts, disease severity ratings, or visual symptom assessment. Although these approaches remain valuable, they are often labor-intensive, destructive, and incapable of providing continuous information regarding pathogen viability and metabolic activity. The introduction of bioluminescent reporter systems has enabled rapid, real-time assessment of antimicrobial efficacy by allowing pathogen populations to be monitored continuously through light emission, thereby providing a sensitive indicator of physiological status and growth inhibition (Ried & Collmer, 1987; Shaw *et al*., 1992; van der Wolf *et al*., 2008). The underlying principle of lux-based screening is that photon emission is closely linked to cellular metabolism and viability. Since expression of the luxCDABE cassette results in autonomous light production, changes in luminescence can be monitored non-destructively following exposure to antimicrobial compounds. A decline in luminescence generally reflects reduced metabolic activity, impaired cellular function, or cell death, whereas stable or increasing luminescence indicates continued pathogen growth and survival. Consequently, lux reporters provide a rapid surrogate measure of pathogen suppression that frequently correlates with traditional microbiological assays (Close *et al*., 2012; Hakkila *et al*., 2002).

One of the major advantages of bioluminescent screening systems is their ability to generate real-time inhibition kinetics. Unlike endpoint measurements, lux-based assays allow continuous monitoring of pathogen responses following antimicrobial exposure. This capability enables researchers to distinguish between bacteriostatic and bactericidal effects, identify delayed inhibitory responses, and characterize temporal patterns of pathogen recovery following treatment. Such information is particularly valuable for understanding modes of action and optimizing application schedules for disease-management programs (Roda *et al*., 2004; Bronstein *et al*., 1994). Lux-tagged strains of *Pseudomonas syringae*, *Xanthomonas* spp., *Erwinia amylovora*, and other phytopathogens have been employed extensively for evaluating bactericide efficacy. Bioluminescence imaging permits rapid comparison of antimicrobial compounds under laboratory, greenhouse, and in planta conditions while minimizing experimental variability. Studies have demonstrated strong correlations between reductions in luminescence intensity and decreases in viable pathogen populations measured through conventional dilution plating, validating the reliability of lux reporters for antimicrobial screening (Bernal *et al*., 2021; van der Wolf *et al*., 2008). Such systems are particularly valuable for high-throughput screening programs where large numbers of candidate compounds must be evaluated efficiently.

The utility of lux technology extends beyond synthetic chemicals to biological control research. Bioluminescent pathogens have been used to quantify pathogen suppression by antagonistic bacteria, fungal biocontrol agents, bacteriophages, and plant-derived antimicrobial compounds. Continuous monitoring of luminescence enables researchers to determine not only whether pathogen suppression occurs but also the rate and duration of inhibitory effects. This temporal information is often difficult to obtain using traditional disease-assessment methods and has contributed substantially to the development of integrated disease-management strategies (Ripp *et al*., 2000; Sayler & Ripp, 2000). Recent advances in automated imaging platforms and microplate-based luminescence detection systems have further increased the utility of lux reporters in antimicrobial discovery. High-throughput assays can simultaneously evaluate hundreds of compounds against luminescent pathogens, generating quantitative data regarding inhibitory concentration, inhibition kinetics, and pathogen recovery. Such approaches have become increasingly important for identifying novel antimicrobial molecules and addressing emerging issues of resistance development among bacterial plant pathogens (Close *et al*., 2012; Brodl *et al*., 2018).

Biofilm Formation and Quorum Sensing Studies Using Lux Reporters

Quorum sensing is a cell-density-dependent regulatory mechanism that enables bacterial populations to coordinate gene expression through the

production and perception of diffusible signaling molecules. Since the discovery of the LuxI–LuxR regulatory system in bioluminescent marine bacteria, lux-based reporters have become indispensable tools for investigating bacterial communication, virulence regulation, and biofilm development. In plant-pathogenic bacteria, quorum sensing governs numerous processes including exopolysaccharide production, motility, secretion systems, extracellular enzyme synthesis, stress adaptation, and virulence expression. Consequently, lux reporter systems have played a pivotal role in elucidating the molecular mechanisms underlying bacterial pathogenicity and disease development (Fuqua *et al*., 1994; Whitehead *et al*., 2001; Venturi & Fuqua, 2013).

One of the most important applications of lux technology involves monitoring quorum-sensing activity in real time. By placing lux reporter genes under the control of quorum-sensing-regulated promoters, researchers can directly visualize signaling activity and quantify communication processes within bacterial populations. Such reporter systems have been widely employed in studies of *Pectobacterium*, *Dickeya*, *Xanthomonas*, *Pseudomonas syringae*, and other plant-associated bacteria. These investigations have revealed that quorum-sensing-regulated genes are activated only after bacterial populations reach critical threshold densities, thereby ensuring coordinated expression of energetically expensive virulence factors (Miller & Bassler, 2001; Waters & Bassler, 2005).

Lux reporters have been particularly valuable for understanding virulence regulation in plant-pathogenic bacteria. Many pathogens utilize acyl-homoserine lactone (AHL)-mediated signaling systems to regulate production of cell-wall-degrading enzymes, toxins, and secretion systems required for successful infection. Reporter-based studies demonstrated that disruption of quorum sensing frequently results in reduced virulence, impaired colonization, and diminished disease severity. Such findings have highlighted quorum sensing as a promising target for disease-control strategies aimed at interfering with bacterial communication rather than directly killing pathogens (Whitehead *et al*., 2001; Venturi & Fuqua, 2013).

Biofilm formation represents another area where lux reporters have made substantial contributions. Biofilms are structured microbial communities embedded within extracellular polymeric matrices that confer protection against environmental stresses and antimicrobial agents. In many phytopathogens, biofilm formation enhances survival on plant surfaces, promotes vascular colonization, and contributes to persistence within host tissues. Lux-based imaging systems have enabled non-destructive monitoring of biofilm development, maturation, and dispersal under diverse environmental conditions. Researchers have utilized bioluminescent strains to quantify biofilm biomass, assess gene expression during biofilm formation, and evaluate the influence of environmental factors on microbial community development (Parsek & Greenberg, 2005; Bogino *et al*., 2013). In vascular pathogens such as *Xylella fastidiosa*, biofilm formation is a critical determinant of disease development because bacterial aggregates obstruct xylem vessels and impair water transport. Lux reporters have facilitated studies of biofilm architecture and colonization dynamics within plant vascular tissues, providing important insights into mechanisms of systemic infection and pathogen persistence (Newman *et al*., 2004). Similarly, investigations involving *Pseudomonas syringae* and *Xanthomonas* spp. have revealed strong links between quorum sensing, biofilm formation, and virulence regulation, emphasizing the interconnected nature of these processes during plant disease development (Ryan *et al*., 2015).

Recent advances in synthetic biology have expanded the utility of lux reporters for investigating bacterial communication networks. Modern reporter constructs allow simultaneous monitoring of multiple quorum-sensing pathways and facilitate quantitative analysis of signaling dynamics at both population and single-cell levels. Such approaches have significantly enhanced understanding of microbial communication within complex plant-associated microbial communities and are increasingly being applied to studies of disease suppression, microbiome interactions, and ecological competition within the rhizosphere (Papenfort & Bassler, 2016; Mukherjee & Bassler, 2019).

Table 1 : List of Lux genes used so far in Plant Pathology

Pathogen / Microorganism	Lux Gene System Used	Application in Plant Pathology	Major Findings	Reference
Pseudomonas syringae pv. tomato DC3000	luxCDABE (Photorhabdus luminescens)	Quantification of bacterial growth in planta	Rapid non-destructive measurement of pathogen multiplication; luminescence correlated with bacterial counts	Fan *et al.* (2008)
Pseudomonas syringae	Chromosomal luxCDABE	Monitoring pathogen growth dynamics and host	Real-time visualization of pathogen proliferation in living	Paynter *et al.* (2006)

	integration	resistance	plants	
Pseudomonas syringae pv. tomato DC3000	Tn7-based luxCDABE tagging system	Spatial and quantitative pathogen tracking	Visualization of bacterial spread in multiple plant hosts	Matsumoto *et al*. (2022)
Ralstonia solanacearum	Tn7-based luxCDABE tagging system	Tracking vascular colonization	Monitoring bacterial wilt pathogen movement through vascular tissues	Matsumoto *et al*. (2022)
Agrobacterium tumefaciens	Tn7-based luxCDABE tagging system	Plant–microbe interaction studies	Spatiotemporal visualization of bacterial colonization and persistence	Matsumoto *et al*. (2022)
Xanthomonas hortorum pv. gardneri	luxCDABE reporter strain	Disease development and infection-route analysis	Quantification of bacterial populations and infection routes in tomato	Bernal *et al*. (2021)
Pseudomonas fluorescens	lux-tagged reporter strain	Rhizosphere colonization studies	Visualization of root colonization and phosphate-starvation responses	Kragelund *et al*. (1997)

Conclusion

Lux gene technology has become a valuable tool in plant pathology due to its ability to provide real-time, non-destructive, and quantitative monitoring of microbial populations within living plant tissues. Lux-based reporters have significantly improved our understanding of pathogen colonization, disease progression, quorum sensing, biofilm formation, rhizosphere interactions, and the effectiveness of disease-management strategies. In addition to pathogen tracking, lux systems have found applications in fungicide screening, biological control studies, biosensor development, and microbial ecology. Despite advances in genomics and other high-throughput technologies, lux reporters remain unique in their ability to directly assess microbial viability and activity in situ. Continued improvements in imaging technologies and synthetic biology are expected to further expand their applications, making lux gene technology an important component of future plant pathology research and sustainable disease management.

Research Gaps and Future Prospects

Despite significant advances in the application of lux gene technology in plant pathology, several challenges continue to limit its broader adoption. Most studies have focused on bacterial pathogens, whereas applications involving fungal and oomycete pathogens remain relatively underexplored. Furthermore, the majority of investigations have been conducted under laboratory or greenhouse conditions, with comparatively few studies validating lux-based approaches under field environments. Additional concerns include long-term stability of lux expression, limited sensitivity in dense plant tissues, and the absence of standardized protocols for imaging and data analysis.

Future research should focus on the development of brighter and more stable lux reporter systems through synthetic biology approaches, expansion of lux technology to fungal and oomycete pathogens, and integration with omics technologies for comprehensive analysis of host–pathogen interactions. The combination of lux-based imaging with artificial intelligence, digital phenotyping, and precision agriculture tools is expected to enhance real-time disease monitoring and pathogen detection. Moreover, lux-tagged beneficial microorganisms, biosensors, and multi-reporter systems offer promising opportunities for studying rhizosphere ecology, biological control, and microbial communication. Collectively, these advancements are likely to establish lux gene technology as an increasingly important tool for disease diagnostics, epidemiological studies, and sustainable plant disease management in the future.

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