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Management and biotechnological applications of crown gall disease: A mini review

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Abstract: Crown gall disease, caused by *Agrobacterium tumefaciens*, is a significant issue for global agriculture. This bacterium is unique because it transfers a portion of its Ti plasmid DNA, known as T-DNA, into plant cells. The T-DNA integrates into the plant genome, leading to uncontrolled cell growth and the formation of tumours known as crown galls. Infected cells also produce opines, which provide nutrients for the bacterium. Crown gall affects plant development, reducing plant yield, quality, and market value. During the infection, the bacterium enters plant cells through wounds and activates virulence genes to transfer T-DNA. Key proteins, such as VirD2, VirE2, and VirB/D4, facilitate the transfer of the T-DNA from *Agrobacterium* into the plant nucleus. Managing crown gall is particularly challenging because *Agrobacterium* can persist in soil for long periods and infect a wide range of plant hosts. To reduce infection, integrated strategies are needed, including cultural practices and biotechnological approaches such as biological control using *Agrobacterium radiobacter* strain K84. In addition to its role as a plant pathogen, disarmed strains of *Agrobacterium* are widely used in genetic engineering to introduce foreign genes into plants, enabling the development of crops with improved traits such as pest resistance, herbicide tolerance, and enhanced nutritional quality. Consequently, studying *Agrobacterium* not only supports effective disease management but also improves genetic transformation and innovative applications in modern biotechnology.

Keywords: crown gall, *Agrobacterium* sp., plant disease, genetic engineering, disease control

Introduction

Bacterial diseases in plants are serious problems for global agriculture. The pathogens cause yield loss, low crop quality, and higher production costs. One well-known bacterial disease is crown gall, which is primarily caused by *Agrobacterium tumefaciens*. This bacterium is important because it can transfer its DNA into plant genomes. Unlike other plant pathogens, which use toxins or enzymes to cause disease, *Agrobacterium* causes tumours by inserting a portion of its plasmid DNA (T-DNA) into plant cells (Gelvin, 2000). This causes plant cells to grow uncontrollably and to produce special compounds called opines. These opines give benefits to the bacterium (Gelvin, 2000).

Taxonomically, *Agrobacterium tumefaciens* is classified within the family *Rhizobiaceae* and the genus *Agrobacterium*, a group characterized by a polyphyletic lineage (Waldburger et al., 2023; Yang et al., 2023). The *A. tumefaciens* species comprises several biovars, distinguished by physiological and biochemical characteristics (Tiwari et al., 2022; Waldburger et al., 2023). Among the biovars, biovar 1, associated with *A. tumefaciens*, is recognized for its broad pathogenicity toward numerous dicotyledonous plant species (Tiwari et al., 2022; Waldburger et al., 2023; Vargas et al., 2024). Advances in whole-genome sequencing and comparative genomics have further clarified the taxonomic structure by identifying multiple genomospecies, thereby improving the precision and resolution of its classification (Mafakheri et al., 2022; Vargas et al., 2024).

The study of crown gall disease has a unique place in plant pathology and biotechnology. On the one hand, it represents a serious agricultural problem, affecting hundreds of plant species and resulting in economic losses (Burr et al., 1998). On the other hand, it becomes a foundation for developing tools in modern plant biotechnology, such as Agrobacterium-mediated genetic transformation (Broothaerts et al., 2005; Zupan and Zambryski, 1995; Li et al., 2020). Understanding the mechanisms of Agrobacterium and disease provides deeper insights into plant-microbe interactions, host defence responses, and innovative agricultural applications.

The transfer of T-DNA from *Agrobacterium tumefaciens* to plant cells is initiated by bacterial attachment, followed by the activation of virulence genes that mediate T-DNA excision and its subsequent transport into the plant nucleus (Sunday et al., 2024). During the T-DNA transfer, central virulence genes of *Agrobacterium tumefaciens*, including *virD2* and *virE2*, facilitate

the formation of the T-complex, a nucleoprotein structure that shields single-stranded T-DNA during intracellular transport to the host nucleus (Li et al., 2020). After nuclear entry, the T-DNA is integrated into the plant genome with the assistance of host factors, such as VirE2-interacting proteins and plant histones, which enhance integration efficiency (Subramoni et al., 2014).

The aim of this literature review is to provide an overview of *Agrobacterium* and crown gall disease, its biology and taxonomy, mechanisms of pathogenesis, ecological and agricultural impacts, molecular processes underlying DNA transfer, strategies for disease management, and its applications in biotechnology.

Biology and taxonomy of Agrobacterium

The classification of *Agrobacterium* has undergone numerous changes. Scientists still debate its taxonomic status, especially when compared with the closely related genus, *Rhizobium* (Kuzmanović et al., 2024). Recent genomic studies show that *Agrobacterium* is polyphyletic (Naranjo et al., 2023). This conclusion is supported by multilocus sequence analysis (MLSA) and genome-based phylogenetic methods (Gan and Savka, 2018).

Phylogenomic studies have expanded the genus to include several new species of *Agrobacterium*. Both ecological traits and genomic differences identify these new species (Delamuta et al., 2020; Castellano-Hinojosa et al., 2021). For example, *Agrobacterium leguminum* and *Agrobacterium arsenijevicii* have been assigned to novel species in the genus of *Agrobacterium* (Delamuta et al., 2020; Castellano-Hinojosa et al., 2021; Kuzmanović et al., 2015).

Core genome analysis has revealed that many genes in *Agrobacterium* strains are conserved, providing a strong foundation for comparative genomic studies (Lassalle et al., 2017). These analyses have helped identify distinct genomospecies, such as *Agrobacterium genomovar* G8 (Lassalle et al., 2011).

Mechanisms of crown gall disease

Crown gall disease is demonstrated as tumor-like growths, typically at the crown region near the soil line, but also on stems, roots, or branches, depending on the host and the infection site (Hooykass, 2023). The disease cycle begins when *Agrobacterium* enters plant tissues through wounds caused by cultivation, grafting, insects, and/or natural injuries (Fig. 1; Stachel et al., 1985). Once inside, the bacterium detects phenolic compounds and sugars released by wounded cells, which serve as chemical signals that activate virulence (vir) genes of *Agrobacterium* (Stachel et al., 1985; Hooykass, 2023).

The key pathogenic event is the transfer of T-DNA from the Ti plasmid of *Agrobacterium* into the plant host genome (Teo et al., 2022; Hu et al., 2024; Gelvin, 2021). Following recognition of wound signals of the plant house, the virA–virG (e.g. two-component regulatory system) in *Agrobacterium* is activated, inducing the expression of other vir genes that mediate T-DNA processing and transfer (Fig. 2; Singer et al., 2022; Stachel et al., 1985; Teo et al., 2022; Xu et al., 2023). The T-DNA is excised as a single-stranded DNA molecule and guided into plant cells via a type IV secretion system, which resembles bacterial conjugation machinery (Christie, 2001; Huang et al., 2021).

Once inside the host cell nucleus, the T-DNA integrates into the plant genome, leading to the expression of genes encoding enzymes for auxin and cytokinin biosynthesis, as well as for opine synthesis (Dessaux et al., 1998; Faist et al., 2023; Kuzmanović et al., 2024; Veselov et al., 2003). Elevated levels of auxins and cytokinins lead to uncontrolled cell proliferation, resulting in gall formation (Veselov et al., 2003; Frolova et al., 2025). Opines, such as nopaline or octopine, serve as unique carbon and nitrogen sources for Agrobacterium but are not metabolized by most other organisms, giving the bacterium an ecological niche advantage (Dessaux et al., 1998; Frolova et al., 2025).

The tumor development caused by T-DNA integration is stable and heritable, as the inserted DNA is replicated along with the plant genome (Azhakanandam et al., 2000; Hooykass, 2023). This natural genetic engineering event distinguishes *Agrobacterium* from other plant pathogens.

Host range and ecological impact

Agrobacterium has an extensive host range, infecting plant species across dicots, some gymnosperms, and even certain monocots under experimental conditions (Agrawal and Rami, 2022; Azhakanandam et al., 2000; Burr and Otten, 1999; Gelvin, 1998; Gohlke and Deeken, 2014). Economically essential hosts include grapevine, rice, tobacco, and many ornamental plants (Agrawal and Rami, 2022; Azhakanandam et al., 2000; Burr and Otten, 1999; Gelvin, 1998; Saeger et al., 2021). In vineyards, *A. vitis* infections can be devastating, reducing vine vigor, yield, and longevity (Burr and Otten, 1999; Kuzmanović et al., 2022; Faist et al., 2023). In nurseries, crown gall reduces market value and increases plant mortality, posing significant challenges for the horticultural industry (Burr and Otten, 1999).

The ecological impact of the *Agrobacterium* infection extends beyond crop losses. Crown galls interfere with nutrient and water transport, weaken structural stability, and predispose plants to secondary infections (Gohlke and Deeken, 2014; Galieni et al., 2021). Moreover, because *Agrobacterium* stays in soil and plant debris, eradication from infested fields is challenging (Burr and Otten, 1999). The ability of *Agrobacterium* to colonize root surfaces and survive independently of tumors means *Agrobacterium* can survive even in the absence of susceptible crops (Anand and Mysore, 2007). Despite its destructive potential, crown gall disease rarely leads to total crop failure; instead, the crown gall reduces plant vigor and productivity over time (Agrawal and Rami, 2022; Burr and Otten, 1999; Gohlke and Deeken, 2014). This chronic nature of the disease complicates management and requires long-term strategies rather than short-term strategies.

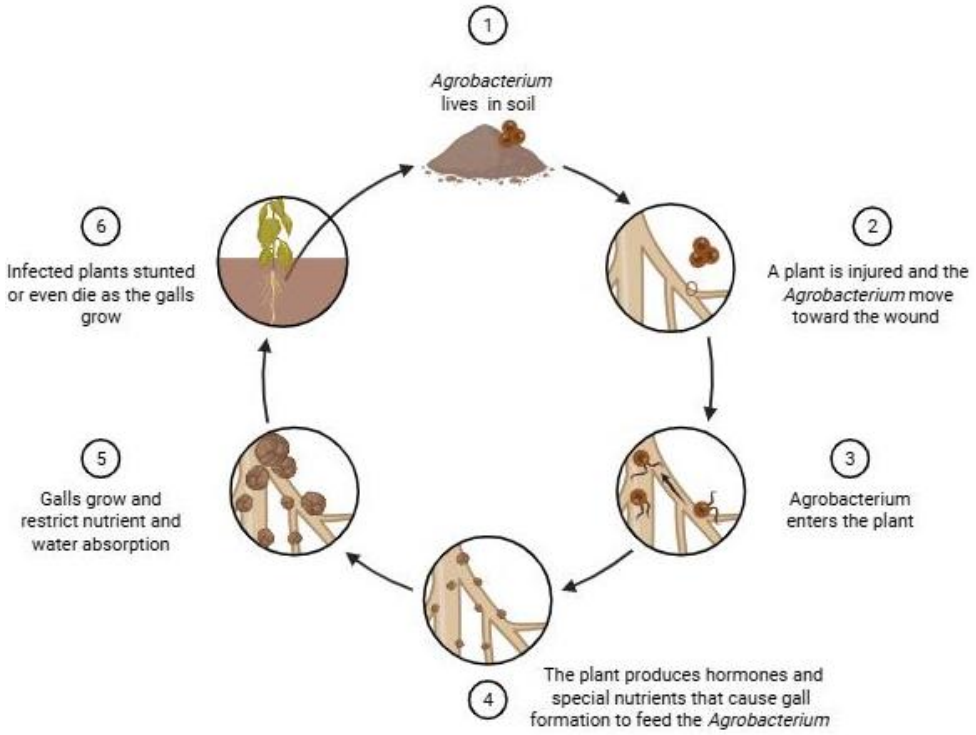

Fig 1. The life cycle of Agrobacterium. Agrobacterium lives in the soil and waits for plants to get wounded (1). When a plant is injured, the bacteria move toward the wound and attach to the plant cells (2). The plant releases chemical signals that activate the bacteria, enabling them to enter the plant (3). The plant then produces hormones and special nutrients that cause gall formation and feed the bacteria (4). After the gall develops, the bacteria multiply and return to the soil, where they can infect new wounded plants (5-6). (Created in https://www.biorender.com/).

Molecular basis: Ti plasmid and gene transfer

The Ti plasmid is a type of DNA that exists outside the bacterial chromosome, ranging in size from 180 to 250 kilobases (Broothaerts et al., 2005). The Ti plasmid is organized into distinct functional regions, including the T-DNA, vir genes, opine catabolism genes, and replication/maintenance sequences (Fig. 2; Gelvin, 2003; Tiwari et al., 2022).

The T-DNA region is the portion of the T-DNA that is transferred into the plant genome (Fig. 2). Genes within this region encode enzymes for auxin and cytokinin synthesis, which drive tumorigenesis, and for opine synthesis, which ensures a nutrient source for the bacterium (Gelvin, 2003; Veselov et al., 2003). The vir region, located outside the T-DNA, encodes proteins that process and transport the T-DNA (Tzfira et al., 2004; Hu et al., 2024).

Among the most critical virulence proteins are VirD1 and VirD2, which nick the T-DNA borders and covalently bind to the single-stranded T-DNA, commonly called the T-strand (Gelvin, 2003). VirE2 is another important protein; it binds along the T-strand, protecting it from degradation and helping guide it into the plant nucleus (Gelvin, 1998). The VirB/D4 complex forms the type IV secretion system, which functions like a molecular syringe to transport the T-DNA complex into host cells (Gelvin, 2003; Christie, 2004). These proteins coordinate the transfer of T-DNA during the infection process (Christie, 2004). Combined activity is essential for successful tumor formation and the pathogenicity of *Agrobacterium* (Christie, 2004). Moreover, the T-DNA integrates into the plant genome at double-strand break sites by hijacking the DNA of plant host repair machinery through illegitimate recombination (Tzfira et al., 2004).

Management and control strategies

Biological control has emerged as the most promising strategy (Liang et al., 2020; Wang et al., 2021; Wu et al., 2021). *Agrobacterium radiobacter* strain K84, a nonpathogenic relative, produces the antibiotic agrocin 84, which inhibits pathogenic strains carrying the Ti plasmid (Anand and Mysore, 2007; Burr et al., 1998). The application of K84 to plant roots before planting has been widely adopted and is considered one of the most successful examples of bacterial biocontrol (Burr et al., 1998; Burr and Otten, 1999). However, resistant strains that can inactivate agrocin 84 have occasionally been reported, necessitating continued monitoring and improvement of biocontrol approaches.

Cultural practices involve minimizing wounding, disinfecting tools, avoiding infested soils, and using certified pathogen-free planting material; however, these measures are often insufficient in high-pressure environments (Asghari et al., 2020; Burr et al, 1998; Oksel et al., 2024; Tiwari et al., 2022). Pest management, such as the combination of soil fumigation and biocontrol (HLB-2), has been reported to be effective in reducing the visible galls by ~90% in field experiments (Burr et al., 1998). However, this fumigation has been avoided due to growing environmental concerns.

Genetic resistance in plants remains limited, although some wild species and cultivars exhibit tolerance (Burr et al., 1998; Tiwari et al., 2022). Advances in biotechnology may enable the introduction of resistance genes or the use of gene editing to

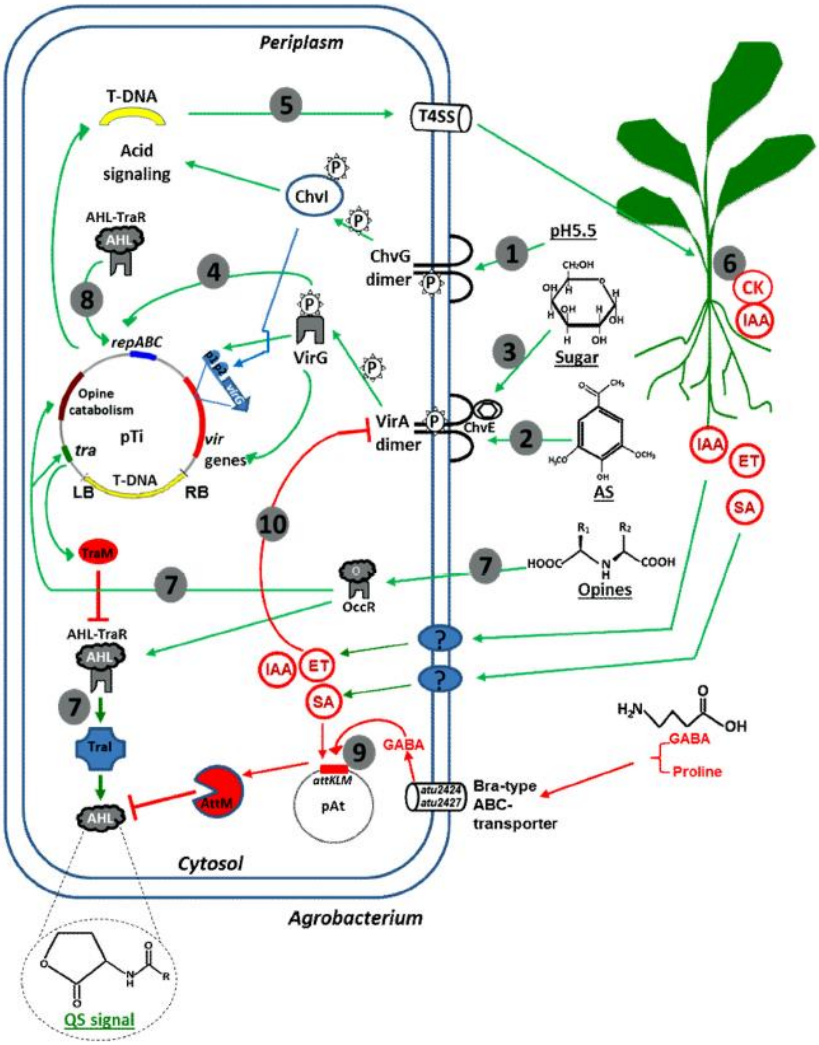

Fig. 2. Agrobacterium response to host plant signal. (1) Upon perception of acidic conditions in the rhizosphere, the ChvG/I two-component system activates the expression of several virulence genes, including chvI and virG. (2) Upon sensing plant-derived phenolic compounds, the VirA/G two-component system activates all vir genes, including virG, to further enhance vir gene expression. (3) ChvE binds plant-released sugars and interacts with VirA to enable maximal vir gene expression. (4) The copy number of the Ti plasmid in Agrobacterium is upregulated in response to phenolic compounds. (5) Vir gene products process and transfer T-DNA into the plant cell nucleus. (6) Expression of T-DNA–encoded genes in plant cells leads to the production of IAA, CK, and opines. (7) Opines activate Agrobacterium genes involved in opine metabolism, as well as the TraR/TraI quorum-sensing (QS) system, which subsequently induces Ti plasmid conjugation. (8) The QS system also up-regulates Ti plasmid copy number to maximize pathogenicity. (9) Agrobacterium quorum quenching via the att KLM operon is activated by plant-derived GABA and SA, thereby down-regulating QS. (10) Agrobacterium additionally exploits plant-derived SA, IAA, and ET to suppress vir gene expression. (Adopted from Subramoni et al., 2014).

create resistant cultivars in the future (Burr and Otten, 1999; Liang et al., 2020). Integrated management approaches combining cultural, biological, and biotechnological strategies currently offer the best prospects for sustainable control.
Managing crown gall disease remains challenging due to the persistence of *Agrobacterium* in soils and its broad host range (Liang et al., 2020; Wu et al., 2021). Traditional approaches include biological, cultural, and chemical methods, which can be combined to provide a comprehensive control of Crown gall disease.

Agrobacterium in biotechnology and genetic engineering

One of the most significant outcomes of crown gall research has been the development of *Agrobacterium* as a tool for plant genetic engineering (Li et al., 2020; Sunday et al., 2024; Tiwari et al., 2022). By disarming the Ti plasmid, which removes tumour-inducing genes while retaining the DNA transfer functions, researchers created binary vector systems that enable the stable introduction of foreign genes into plants (Anand and Mysore, 2007; Broothaerts et al., 2005; Christie, 2001; Stachel et al., 1985; Sunday et al., 2024). This innovation revolutionized plant biotechnology, enabling the production of genetically modified crops with desirable traits such as pest resistance, herbicide tolerance, and enhanced nutritional quality.
Compared to other transformation methods, such as particle bombardment, *Agrobacterium* mediated transformation offers several advantages, including higher efficiency, more predictable integration patterns, and lower copy number insertions (Teo et al., 2022; Vargas et al., 2024). Moreover, T-DNA transfer mediated by *Agrobacterium* has been successfully applied

across a wide range of crops, including staple cereals, high-value fruits, and ornamentals (Azhakanandam et al., 2000; Gelvin, 2003).
Beyond its role in crop improvement, *Agrobacterium* provides a valuable model for studying horizontal gene transfer, plant defence responses, and host–microbe interactions. Its natural genetic engineering capabilities not only illuminate evolutionary processes but also inspire innovative approaches in synthetic biology.

Conclusion

Crown gall disease, caused by *Agrobacterium tumefaciens*, is a significant challenge for agriculture. The bacterium transfers T-DNA into plant cells, resulting in tumor formation and the production of opines of a host plant. This infection reduces crop yield, quality, and market value. Managing the disease is difficult due to the persistence of *Agrobacterium* in soil and its wide host range. Biological control, cultural practices, and biotechnological approaches can help reduce infections. Research on *Agrobacterium* has also led to the development of essential tools in plant biotechnology. Disarmed strains are used to introduce foreign genes, enabling the development of crops with improved traits, such as pest resistance, herbicide tolerance, and enhanced nutrition. Studying *Agrobacterium* improves understanding of plant-microbe interactions, natural genetic engineering, and innovative agricultural applications. Integrated management and biotechnological innovations offer promising prospects for controlling crown gall disease and supporting sustainable agricultural practices.

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