The Unsung Hero: The Role of _Agrobacterium_ in Biodiversity

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Abstract

Broadly speaking, biodiversity refers to the variety of life on Earth that arises from the intricate and interdependent network of species, ecosystems, and genetic variation. Genetic recombination and mutation give birth to genetic variation even within species. Other factors that aid in environmental adaptation include genetic polymorphism, isolation of gene pools, local selection pressures, and environmental complexity. For example, many crop variations exist because of genetic variety, which enables the types to adapt to changing environmental conditions. Importantly, it is crucial to recognize the functions that microorganisms play in this adaptation. Soil bacteria belonging to the genus _Agrobacterium_ are one type of microorganism that has a significant impact on the variety of plant life on Earth. In the field of biotechnology, _Agrobacterium_ tumefaciens is a plant pathogenic bacterium that can cause Crown Gall disease by transferring a segment of oncogenic DNA, also known as T DNA (transfer DNA), to susceptible plant cells on a large plasmid known as the tumor inducing or Ti plasmid. The current article primarily focuses on the relatively underappreciated role of _Agrobacterium_ in providing much-needed plant diversity to aid in the preservation of ecological equilibrium, which benefits society, and examines this role based on literature investigations. We begin by outlining the role that underlying molecular and cellular mechanisms play in the mutualistic relationship between _Agrobacterium_ and plant species, which controls plant biodiversity. Next, we look at the crucial role that _agrobacterium_ has played in the adaptability and evolution of the plant kingdom. We have finally illuminated the critical function _Agrobacetrium_ plays in the conservation of biodiversity. We end by warning that, despite the fact that _Agrobacterium_ has been crucial to the development of biotechnology, there are a lot of challenging problems related to its unintended impacts on biodiversity. A balanced strategy that considers the possible ecological consequences of genetic engineering procedures based on _Agrobacterium_ is required to address these problems. Prioritizing sustainable approaches that reduce the risks associated with using _Agrobacterium_ is essential as technology advances and ensures the long-term conservation of biodiversity.

Introduction

Generally speaking, biodiversity is the variety of life on Earth as a result of the intricate and interwoven network of species, ecosystems, and genetic variation [1]. Biodiversity means "the variety and variability of biological organisms" according to researchers working in fields that are focused on biodiversity, such as population biology, ecology, systematics, evolution, and genetics [1]. "Variability among living organisms from all sources" is the definition of biodiversity given by the Convention on Biological Diversity [2]. Even within species, there is genetic variation. Genetic recombination or mutation, genetic polymorphism (the existence of several variants of the same gene), isolation of gene pools, local selection pressures, and habitat (environmental) complexity are a few of the factors that can cause genetic variety within a species, which helps in adaptation in the environment [3,4]. For instance, due to genetic variety that allows the kinds to adapt to changing environmental conditions, numerous crop varieties survive [5].

Varieties with little to no genetic variation, however, might become vulnerable to biotic and abiotic stressors [6]. More importantly, even though large animals like tigers

and trees are typically associated with biodiversity preservation, microorganisms also have significant roles to play [7,8]. One such microbe is the genus *Agrobacterium* of soil bacteria, which has a significant influence on the variety of plant life on Earth [9-11]. The biodiversity of plants provides people with a wealth of benefits, including resilience to climate change and other perturbations and is the basis for our current food supply, which also includes functional food and medicine derived from plants [12,13]. The current paper thus primarily focuses on the relatively underappreciated role of *Agrobacterium* in providing much-needed plant diversity to aid in the preservation of ecological equilibrium, which benefits society, and examines this role based on literature investigations.

Agrobacterium: A natural genetic engineer

In the realm of biotechnology, *Agrobacterium* is well known for its ability to introduce the Ti (tumor-inducing) plasmid—a segment of its DNA—into plant cells [14,15]. More specifically, *Agrobacterium* tumefaciens is a plant pathogenic bacterium, with the ability to deliver a segment of oncogenic DNA carried on a large plasmid called the tumor inducing or Ti plasmid to susceptible plant cells. On the other hand, nonpathogenic strains of *Agrobacterium* either lack these plasmids entirely or carry mutant forms of plasmids [14]. A strict requirement of the Ti plasmid for virulence in plants is further established by mutational analyses and the demonstration that Ti plasmids introduced into *Phyllobacterium spp.* found in soil or *Rhizobium*, a nitrogen-fixing organism, transform these nonpathogenic species into tumor-inducing pathogens in plants (a typical example is Crown Gall disease) [15,16]. Moreover, the A. tumescence-plant interactions are among the first where such host-released signals such as opines have been detected, since they are the signal transduction cascade that leads to the expression of virulence (vir) genes in response to these signals [17,18]. Notable in this context is the fact that the plant's metabolites, sugars, amino acids, and α-keto acids (collectively referred to as opines) are transformed into a range of low molecular weight compounds by the enzymes known as opine synthase, which are encoded with Ti-DNA and found in *Agrobacterium* [19,20]. These metabolites are then used by the *Agrobacterium* to continue growing. Two of the vir genes, virA and virG, are required for *A. tumefaciens* to respond to inducing conditions evoked by the plant, according to additional genetic study [21,22]. This implies that *A. tumefaciens* employs a clever two-component signal transduction mechanism, which is typically responsible for triggering complex responses to a variety of inputs, including hormonal regulation and environmental cues [23]. Moreover, Ti plasmid is generally transferred into plants by *Agrobacetrium* using the Type IV Secretion System (T4SS), which is a biological "molecular syringe" produced by many gram-negative bacteria that transports proteins or DNA-protein complexes into other cells—a necessary step for horizontal gene transfer—with the aid of the two-component regulatory system that uses virA and virG [24]. *Agrobacetrium* also employs quarum sensing, a method of cell-to-cell communication that enables bacteria to exchange information about cell density and modify gene expression appropriately, in addition to T4SS and other systems like Type VI secretion systems, etc [25,26]. This allows for the conjugative transfer of the Ti plasmid between bacteria during plant pathogenesis when specific opines (inducing factors) derived from plants are present. Because of this special quality, *Agrobacterium* is useful in genetic engineering as it allows specific genes to be inserted into plants [27]. However, because of its enormous impact on biodiversity in natural ecosystems, its significance extends beyond the lab.

Plant biodiversity is controlled by the mutualistic relationship between *agrobacterium* and plant species Numerous plant species and *Agrobacterium* have mutualistic relationships [28,29]. The plant receives genes that control its growth, development, and response to environmental stressors from *Agrobacterium* in exchange for nutrients and a safe habitat within plant tissues. The biodiversity of plants may be greatly impacted by this interaction. To elucidate, the ability of *Agrobacterium* to transfer DNA into plants for stable genetic transformation or transient expression may underpin plant diversification [30]. This allows plants to develop altered phenotypic features in response to a range of environmental stimuli. Nevertheless, even though the process of T-DNA transfer and integration has been thoroughly investigated since the early 1970s, many facets of the underlying mechanism remain unclear to date. For example, little is known about the process by which T-DNA integrates into the genome of the host [plant]. It is tempting to speculate that the variety in T-DNA integration into a particular plant genome locus may play a key role in modifying a wide range of phenotypic features in plants [31,32].

Some early research appears to indicate that integrated T-DNAs are preferentially located in transcriptionally active chromatin [33,34]. However, these investigations relied on transgenic plant analyses that were regenerated under antibiotic selection using a reporter genetic fragment that was resistant to specific antibiotics and was engineered into T-DNA [11]. Because of this, it was nearly difficult to find evidence of integration into heterochromatin, which does not support the transcription of the antibiotic-resistant reporter. Indeed, an entirely different result was observed in experiments carried out without the use of antibiotics, showing that T-DNA integrated randomly in every region of chromatin, even though specific epigenetic markers might control certain loci integration—the epigenetic signature of which may, among other things, govern the biodiversity of plants [28,35].

Moreover, it has been proposed that nucleosome histones, such as H2A, assist in the targeting of the T-DNA complex to the host chromatin upon its nuclear entry by permitting interaction between the T-DNA complex and the host chromatin prior to integration as *Agrobacterium* does not encode a dedicated integrase among its effector proteins, unlike many integrating viruses [28]. Thus, it is plausible that the class of histone proteins may control where T-DNA integration occurs within the plant genome. This could modify the plant's gene expression pattern and enable the plant to grow and survive in a particular environment with an altered phenotype as a consequence of a mutualistic relationship between *Agrobacterium* and particular plant species [36]. However, to support this assumption made based on early evidence found in the literature, more investigation is required. Alternatively, since

T-DNA is transferred to plant cells and integrates into the plant genome, it would appear that Ti plasmids can be used as a vector to introduce foreign genes into plant cells, thereby enhancing the biodiversity of the plant kingdom. A substantial body of literature describes the procedures for introducing foreign genes into plants using *Agrobacterium*, although it is outside the scope of this theme article.

Agrobacterium evades plant immunological defense system prior to the transfer of T-DNA into host cells When microorganisms like *Agrobacterium* try to invade their hosts, they typically encounter immunological resistance because host cells have what are known as "microbe- or Pathogen-Associated Molecular Patterns" (MAMPs or PAMPs) [37,38]. These so-called "non-self" molecules start PAMP-Triggered Immunity (PTI), the initial defense mechanism. In response, pathogens try to neutralize PTI activation by introducing specific effector proteins into the cytoplasm of their host [39,40]. When cognate intracellular plant proteins perceive these pathogen-encoded effectors, Effector-Triggered Immunity (ETI), the second line of defense, is activated [41,42]. Thus, in addition to limiting the spread of pathogens, plants with the ability to activate ETI can also strengthen themselves against future attacks.

Flagellin and EF-Tu are the two oligopeptides that are recognized by FLS2 and EFR, the two MAMP receptors in plants that have been studied the most [43]. These membrane-located Leucine-Rich Repeat-Receptor-Like Kinases (LRR-RLK) are able to transform and convey perceived "attack signals" into the inside of cells in order to trigger the proper defense responses because of their composite structure. On the other hand, the primary goals of pathogens like *Agrobacterium* are to take advantage of their hosts' nutrition and proliferate to a high degree. In plants, microbial PAMP recognition by plant PRRs is facilitated by Mitogen-Activated Protein Kinase (MAPK) cascades [44]. The prompt activation of genes encoding effector proteins and transcription factors in response to the pathogen's invasion is caused by this recognition.

In essence, by avoiding plant immunity and incorporating T-DNA into the plant genome, *Agrobacterium* have collectively contributed to the spread of genetic variety throughout plant populations and the evolution of plants. Plants can adapt to shifting environmental conditions because of their diversity, which serves as the foundation for evolution. New traits and attributes that give a certain plant an advantage in its natural ecosystem may result from it. As a result, new plant species may eventually appear, adding to the biodiversity.

Plant evolution and adaptation: the function of *agrobacterium*

The movement of genetic information across organisms, such as bacteria and plants, is known as Horizontal Gene Transfer (HGT) [45]. This process includes the dissemination of antibiotic resistance genes among bacteria (apart from those passed from parent to offspring), which promotes the evolution of pathogens. Bacteria and archaea are part of the microbial world, where HGT is a common occurrence. To stress this point even more, HGT mechanisms are well-characterized and thought to be essential to

the evolution of species in this field [46]. For example, there has been evidence in Nicotiana, Linaria, and, more recently, Ipomoea species, of T-DNAs from *Agrobacterium* spp. entering plant genomes and being maintained in the germline [47]. Crucially, the genes that were transferred seem to have played a part in the evolution of these plants because they do not cause the typical disease phenotype in the plant.

The impact of T-DNA acquisition on evolution, in theory primarily depends on a gene's ability to be stably integrated into the genome of a plant after transfer; the recipient plant genome containing the T-DNA must remain intact following genomic rearrangements during subsequent cell division; the transformed cell must enter the germline; and the integrated sequence must be preserved over the course of evolution [31,48]. The latter is most likely to happen if the gene confers a selective advantage on the recipient organism, enabling it to thrive in a hostile environment. But one would envision an additional specific requirement, particularly in the case of T-DNA genes integration into plant genomes, which is that the inserted genes must somehow be modified or regulated from their "natural" expression pattern which allows for vigorous cell growth, the long-term expression of which would be detrimental to the plant's survival [49,50].

As of now, it is unclear how plants have managed to evade the *Agrobacterium*-programmed expression of T-DNA sequences upon insertion into their genomes, however a couple of theories may be taken into consideration. First, it's often thought that the T-DNA may have integrated in a heterochromatin region—a transcriptionally inactive region of the genome—which is a trait that the inserted T-DNA then acquires to inhibit the expression of its genes [51,52]. Second, accumulating data appear to indicate the presence of foreign IbT-DNAs in sweet potatoes, which were initially discovered using short RNA sequencing and assembly. After being introduced into the plant genome as an inverted repeat, IbT-DNA is silenced even after it has integrated into a transcriptionally active DNA region. This is accomplished by the plant RNA silencing complex acting upon the IbT-DNA with a trans-acting effect [53,54].

The conservation of biodiversity: the role of *agrobacterium*

Agrobacterium, primarily known for its use in genetic engineering, also plays a crucial role in the conservation of biodiversity [55,56]. Its unique ability to transfer genes into plants has found application in preserving endangered species, restoring ecosystems, and enhancing agricultural sustainability. Therefore, it is essential for conservation efforts to comprehend how *Agrobacterium* contributes to plant diversity. The genetic variety and adaptability of plant species are also protected when we maintain natural habitats in which *Agrobacterium* interacts with plants. This is essential in light of environmental issues such as climate change. Essentially, *Agrobacterium* plays a multifaceted role in the preservation of biodiversity [57]. First, it helps to preserve genetic diversity. *Agrobacterium*-mediated transformation allows for the introduction of desirable traits into plants, aiding in the conservation of genetic diversity. This is particularly significant for

endangered species with limited populations, as it can help address challenges such as inbreeding depression and susceptibility to diseases [58]. Therefore, understanding the genetic composition and structure of these species in their natural habitat is essential to developing viable management methods for the protection of rare and endangered plant species.

Second, since habitat loss and changing environmental circumstances are common hazards to endangered plant species, *Agrobacterium* plays a crucial role in ecosystem restoration efforts [59,60]. *Agrobacterium* provides a tool for introducing genes that confer resistance to particular conditions, thereby offering a way to protect endangered species in their native environments [61]. Genetic variation is considered necessary for plant species to survive in their natural habitat, as the survival of these species is believed to depend on a range of environmental factors that affect the number of alleles on different loci in the genome [62,63]. Third, endangered plant species often face threats like habitat loss and changing environmental conditions. *Agrobacterium* offers a tool to insert genes that confer resistance to specific stresses, providing a means to safeguard rare species in their natural habitats [61,62,64]. Fourth, *Agrobacterium* supports sustainable agriculture in addition to conservation.

Increased resistance to pests and diseases in modified crops lowers the requirement for chemical inputs, supporting eco-friendly farming methods and preserving the biodiversity of agroecosystems [65,66]. Fifth, *Agrobacterium* is also involved in cryopreservation techniques, where plant tissues or seeds are stored at extremely low temperatures [67]. This method helps preserve the genetic material of endangered plants, serving as a backup for biodiversity conservation efforts. Finally, understanding the role of *Agrobacterium* in plant-microbe interactions is essential for maintaining healthy soils. As a natural soil bacterium, *Agrobacterium* influences plant growth and nutrient cycling, indirectly contributing to the overall biodiversity of terrestrial ecosystems [68]. Despite its potential benefits, the use of *Agrobacterium* in conservation raises ethical concerns. Striking a balance between preserving biodiversity and potential ecological risks associated with genetic modifications requires careful consideration and adherence to strict ethical guidelines [69,70].

Conclusion & Challenges

In conclusion, *Agrobacterium*'s role in biodiversity conservation extends beyond biotechnology labs. Its applications in genetic engineering contribute to the protection of endangered species, ecosystem restoration, and sustainable agriculture. However, responsible and ethical use is imperative to ensure that these interventions do not inadvertently harm the very biodiversity they seek to preserve. Essentially, *Agrobacterium* has evolved from a phytopathogen to a potent transgenic vector thanks to its remarkable ability to transfer its genetic material to host cells. For instance, for crop breeding and molecular plant biology research, stable transformation is the most extensively utilized technique for producing transgenic plants. Over time, *Agrobacterium*-mediated HGT has advanced significantly, providing a fuller understanding of the diversity within the kingdom of plants. However, the unintended consequences of its presence pose significant challenges to the conservation of biodiversity. For example, *Agrobacterium*-mediated genetic transformation can lead to unintended gene flow and hybridization between Genetically Modified Organisms (GMOs) and their wild counterparts [71].

This could lead to the extinction of native species and a reduction in biodiversity by endangering the genetic integrity of natural plant populations [72]. Moreover, the dynamics of natural ecosystems may be upset by the introduction of genetically engineered plants containing genes generated from *Agrobacterium*. These alterations could have an impact on how various species interact with one another, which could have a domino effect on the food web and affect the variety and abundance of other organisms in the environment [73,74]. Additionally, the use of *Agrobacterium* in biotechnology can inadvertently affect non-target organisms, including beneficial insects, soil microbes, and other wildlife [75]. Alterations in trophic relationships, alterations in soil microbial populations, and disturbances in pollination patterns are some of the possible ecological repercussions that could have a negative impact on biodiversity. Weeds resistant to *Agrobacterium* may evolve as a result of genetic engineering procedures that expose the organism to continuous exposure.

These resistant plants have the potential to outcompete native flora, increasing the difficulties brought on by invasive species and endangering the variety of natural ecosystems. Also, unexpected ecological repercussions could arise from the release of genetically modified organisms into the environment that carry genes produced from *Agrobacterium*. These repercussions may include adjustments to the ecological roles of certain plant species, variations to the nutrient cycling, and shifts in the interactions between pollinators and plants, all of which can have an adverse effect on biodiversity. Taken together, even while *Agrobacterium* has been essential in the advancement of biotechnology, there are a number of difficult issues associated with its unintentional effects on biodiversity. A balanced approach to resolving these concerns is necessary, taking into account the potential ecological implications of *Agrobacterium*-based genetic engineering processes. As technology continues to evolve, it is imperative to prioritize sustainable practices that minimize the risks associated with the use of *Agrobacterium*, ensuring the long-term conservation of biodiversity.

Funding

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