Agrobacterium rhizogenes mediated genetic transformation and its applications

Usha Rani T.R.
Senior Scientist, Division of Basic Sciences
ICAR-Indian Institute of Horticultural Research, Bengaluru 560089, India
usharani.tr@icar.gov.in

Agrobacterium rhizogenes mediated transformation is a powerful technique in plant biotechnology that enables the rapid and efficient production of transformed hairy roots. This process is particularly advantageous for various plant species, spanning angiosperms (both dicotyledonous and monocotyledonous plants), gymnosperms, and even moss. The key element in this transformation is the root-inducing (Ri) plasmid carried by *A. rhizogenes*. The process begins with the introduction of *A. rhizogenes* to wounded plant tissues. The bacterium, being Gram-negative, harbors the Ri plasmid, which contains the transfer-DNA (T-DNA) fragment. This T-DNA integrates into the host plant genome, triggering the formation of hairy roots. The Ri T-DNA can also transmit through meiosis, ensuring that the genetic modifications are heritable. The *A. rhizogenes*-mediated transformation system is particularly valuable for species that are challenging to transform using *Agrobacterium tumefaciens*. It offers several advantages over A. tumefaciens-mediated transformation, including a faster process and a higher transformation frequency.

The T-DNA on the Ri plasmid carries specific genes known as rol (root locus) genes, such as rolA, rolB, rolC, and rolD, which play a crucial role in inducing hairy root formation. These genes, along with auxin synthesis genes like aux1 and aux2, collaborate to facilitate the induction of hairy roots by supplying auxin. The hairy roots generated through this method serve as an excellent platform for various applications. In vitro cultivation of these hairy roots is a primary method for synthesizing secondary metabolites, particularly within medicinal plants. Additionally, hairy roots and composite plants are extensively used in scientific investigations, exploring gene functionality related to root development, wood formation, interactions between roots and soil microbes, and plant allelopathy.

The *A. rhizogenes*-mediated transformation system allows for the use of T-DNA binary vectors, enabling the insertion of genes of interest into the plant genome. Transformed hairy roots are induced rapidly and efficiently from explant tissues, displaying rapid growth and maintaining the same genetic characteristics as normal roots. The culture procedure is simple, and transformed plants can be maintained for a substantial period. This technique has been successfully applied in various plant species, including soybean, tomato, *Saussurea involucrata*, *Duboisia leichhardtii*, and *Antirrhinum*. *Agrobacterium rhizogenes*, carrying the Ri plasmid, facilitates infection through wounded plant tissue to induce the formation of hairy roots. The presence of rol genes on the Ri plasmid play a pivotal role in modifying the hormone levels of the plants, resulting in proliferation of hairy roots. This transformation process can result in plants exhibiting altered phenotypes collectively known as Ri syndrome. These altered traits may include dwarfism, altered apical dominance in both stems and roots, wrinkled leaves, and modified flower morphology.

Compared to *Agrobacterium tumefaciens*-mediated transformation, *Agrobacterium rhizogenes*-assisted transformation offers several advantages. This technique induces efficient production of transformed hairy roots from explants. The transformed clones are easily identifiable, and the process does not necessitate the use of selection marker genes.

Consequently, this approach has found applications in the study of gene functions, plant transformation, and the exploration of secondary metabolism.

Applications of *Agrobacterium rhizogenes* in plant genetic transformation

- **Gene Silencing in Composite Plants:** The *A. rhizogenes* composite plant system helps to assess the silencing phenomenon of genes in the wild-type plant. Gene silencing tend to traffic toward sinks, and as hairy roots behave as strong sinks, silencing signals do not typically move to the aerial portion of the wild-type portion of the composite plant under normal conditions.

- **T-DNA Tagging Studies:** Hairy-root cultures, derived through *A. rhizogenes*-mediated transformation, can be adapted for T-DNA tagging studies similar to *A. tumefaciens.* This technique involves the random integration of a T-DNA having constitutive promoter element into the plant genome, leading to the activation of gene. This has been successfully demonstrated in various plant species, including *Arabidopsis thaliana*, *Solanum tuberosum* and *N. tabacum.*

- **Biotic Interaction Research:** Hairy-root cultures enables gene function analysis involved in biotic interactions, without the need to produce fully transformed plants. The composite plant system allows gene validation in non-model crops without the requirement of stable transgenic plant. A. rhizogenes-derived hairy-root cultures have been applied to study interactions between roots and nematodes, mycorrhizal fungi, rhizobia in various plant species.

- **Nematode Interactions:** Hairy roots are susceptible to nematode infection, allowing the study of nematode resistance genes. The transgenic hairy roots demonstrated the silencing of specific nematode resistance genes employing dsRNA, resulting in a susceptible phenotype. This technique provides insights into pest–plant systems and offers an effective tool for understanding root biotic interactions.

- **Abiotic Stress Research:** Hairy roots are employed in developing plants with abiotic stress tolerance. They play a role in phytoremediation, eliminating contaminants from industrial effluents and contaminated soil. The hyper-accumulator plants can uptake heavy metals from polluted soil through hairy roots. The intact hairy roots associated with composite plants avoid wounding or hormone levels changes during root excision.

- **Protein Sub-cellular Localization in Soybean:** The hairy root transformation system is employed for analysing protein subcellular localization in soybean. It provides a valuable alternative to systems based on other species. This system enables the evaluation of soybean protein expression and sub-cellular localization, offering advantages in terms of clarity and ease of observation.

- ***Agrobacterium rhizogenes* as a Rooting Agent:** *A. rhizogenes* has been effectively used as a rooting inducer for plants that are problematic to propagate through stem cuttings. This application has proven remarkably effective and is suitable for a wide range of plants, enhancing root formation in commercially important tree species.

- **Genetic Engineering in Difficult-to-Transform Plants:** *A. rhizogenes* is employed as a vector for plant transformation, especially in plant species recalcitrant to *A.*

tumefaciens infection. It has the capability to infect a diverse range of plants, providing an alternative for genetically modifying the plants that are not amenable to transformation using other methods.

- **Basic Biological Research in Soybean:** *A. rhizogenes*-mediated transformation in soybean has been successfully utilized for various biological research applications, including gene function analysis, molecular analyses (such as promoter analysis, sub-cellular localization studies, ChIP-PCR, GST pull-down assays, Co-IP assays), and mutant complementation. It serves as a powerful tool for investigating fundamental biological processes in soybean.

- **Basic Biological Research in Woody Plants:** *A. rhizogenes*-assisted transformation has found extensive applications in basic biological research in woody plants. It has been used for binary vector generation of composite plants, molecular analysis, and genetic analysis in various woody plant species. This includes the study of nutrient uptake, abiotic stresses, nodule development, mycorrhizal interactions, allelopathy, biosynthesis, and wood formation. The system has been successfully applied in diverse woody plant species, demonstrating its versatility in addressing important biological questions.