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Harnessing Agrobacterium for Genetic Transformation in *Amaranthus* Species

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Amaranthus species are nutrient-rich, climate-resilient crops with considerable potential to improve food and nutritional security. However, genetic improvement in *Amaranthus* has been constrained by limited transformation efficiency. Agrobacterium-mediated transformation offers an effective strategy for introducing foreign genes into *Amaranthus* by exploiting the natural DNA transfer capability of *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*. This review outlines the fundamental mechanisms of T-DNA transfer and integration, key steps involved in transformation protocols, and recent advances in transformation studies across grain and leafy amaranth species. Optimized explant selection, bacterial strains, and co-cultivation conditions have enabled stable gene integration and hairy root induction. These developments highlight the potential of Agrobacterium-mediated transformation for functional genomics and genetic improvement of *Amaranthus*.

Introduction

Amaranthus, a genus within the family *Amaranthaceae*, comprises 60–70 species widely distributed across tropical and subtropical regions and valued for both grain and leafy uses (Rastogi and Shukla, 2013). These resilient C_4 annuals thrive in hot, dry and low-fertility soils, producing nutrient-rich seeds high in protein and essential amino acids as well as leaves rich in vitamins and minerals. In addition to their traditional medicinal uses and gluten-free nature *amaranthus* species are increasingly recognized as climate-resilient "future crops" capable of enhancing food and nutritional security in developing regions (FAO, 2018).

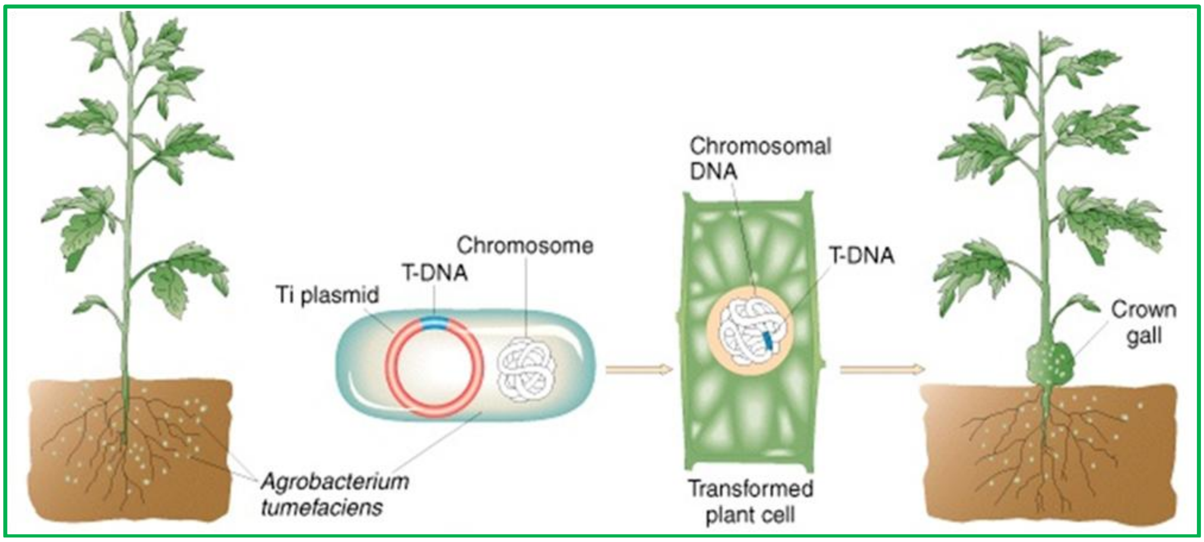
Species of Amaranth

			
Amaranthus tricolor	*Amaranthus hypochondriacus*	*Amaranthus hybridus*	*Amaranthus spinosus*

Transformation

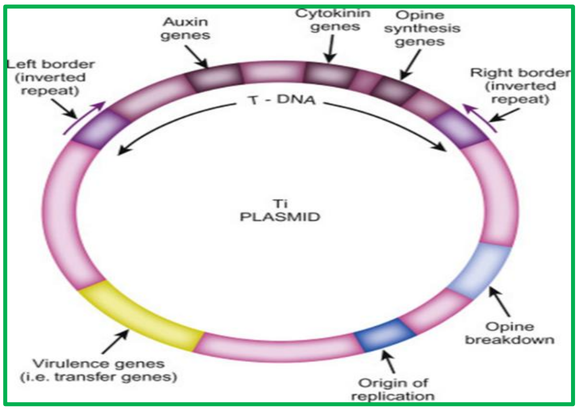
Transformation is the process by which the genetic makeup of an organism is altered through the insertion of new genes or exogenous DNA into its genome, usually by using vectors such as plasmids. The aim of producing transgenic plants is to improve crop yields, enhance varietal traits, and give cultivated plants more protection against pests, parasites, and harsh environmental conditions. Gene transfer techniques in plant genetic transformation are broadly grouped into two categories: vector-mediated gene transfer and direct or vector-less DNA transfer. One of the most important vector-mediated methods is Agrobacterium-mediated gene transfer (Primrose & Twyman, 2006)

Agrobacterium tumefaciens is a soil-borne, Gram-negative, rod-shaped and motile bacterium belonging to the family *Rhizobiaceae* and is regarded as nature's most effective plant genetic engineer. There are two main species: *A. tumefaciens*, which induces crown gall disease, and *A. rhizogenes*, which induces hairy root disease (Primrose & Twyman, 2006). Crown gall disease occurs when *A. tumefaciens* infects wounded plant tissues and releases its tumor-inducing (Ti) plasmid into the plant cell cytoplasm. A segment of this Ti plasmid, called T-DNA, is transferred into the host plant and integrated into the plant chromosome (Primrose & Twyman, 2006)



Source: Griffiths *et al*. (2000)

Organization of Ti plasmid

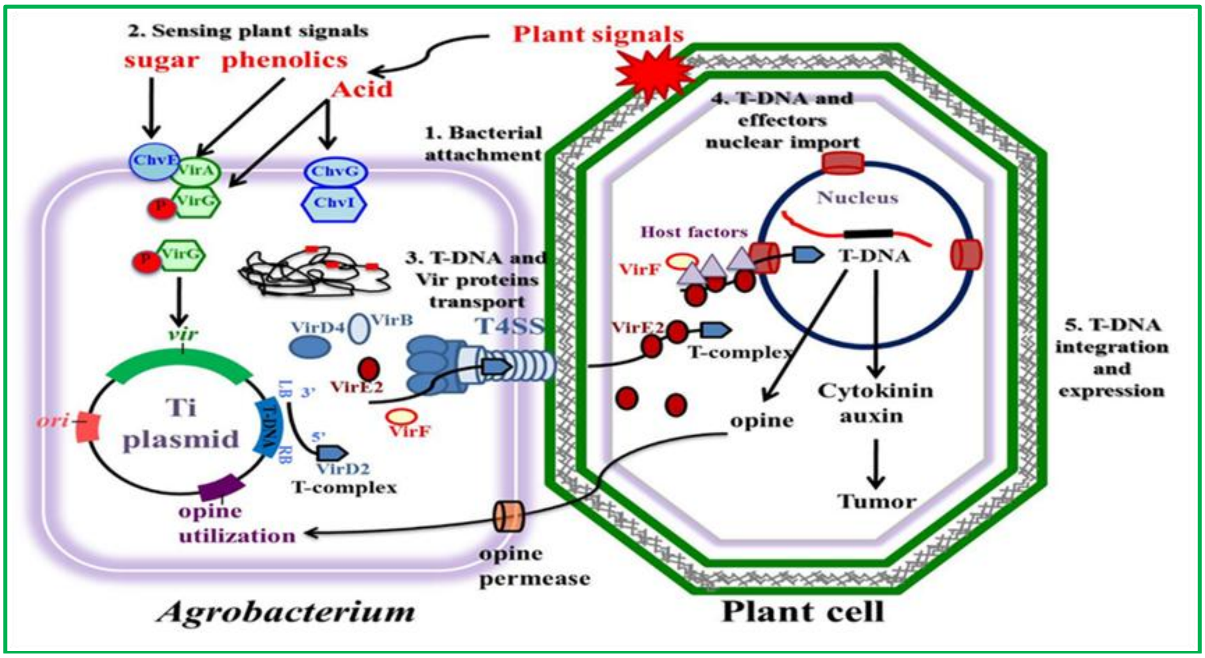


Source: Clark and Pazdernik (2013)

The Ti plasmid is organized into three regions: the T-DNA region, which contains genes for the biosynthesis of auxin, cytokinin and opine and is flanked by left and right border sequences; the virulence (vir) region, located outside the T-DNA, which contains genes involved in the transfer of T-DNA to the plant cell and includes several vir-gene operons; and the opine catabolism region, which encodes proteins for the uptake and metabolism of opines (Primrose & Twyman, 2006).

Steps of T-DNA Transfer and Integration (Gelvin, 2010)

1. **Recognition & Attachment:** Agrobacterium attaches to wounded plant cells where phenolic compounds (e.g., acetosyringone) are released.
2. **Vir Gene Activation:** Plant signals activate virulence (*vir*) genes on the Ti/Ri plasmid.
3. **T-DNA Processing:** T-DNA is cut out as single-stranded DNA and coated with VirD2 and VirE2 to form the T-complex.
4. **Nuclear Import:** Vir proteins guide the T-DNA into the plant nucleus using nuclear localization signals.
5. **Genome Integration: T-DNA integrates into the plant chromosome host recombination/repair.**
6. **Gene Expression:** Integrated T-DNA genes are expressed and producing the desired trait in the transformed plant.



Major steps of the Agrobacterium tumefaciens-mediated plant transformation process (Source: Hwang *et al.*, 2017)

Basic Protocol of Agrobacterium-mediated transformation (Gelvin, 2010)

1. Development of Agrobacterium carrying the co-integrate or binary vector with the desired gene.
2. Identification of a suitable explant e.g. cells, protoplasts, tissues, calluses, organs.
3. Co-culture of explants with Agrobacterium.
4. Killing of Agrobacterium with a suitable antibiotic without harming the plant tissue.
5. Selection of transformed plant cells.
6. Regeneration of whole plants.

Markers used in *Agrobacterium*-mediated transformation

Markers are essential in Agrobacterium-mediated plant transformation. They help identify successfully transformed plant cells. Selectable marker genes let transformed cells survive on media with antibiotics or herbicides, while non-transformed cells die. Common examples are nptII, which gives resistance to kanamycin, and bar, which offers resistance to phosphinothricin. Additionally, visual reporter genes help monitor gene expression and transformation efficiency through visible signals. Reporter genes like GUS (β-glucuronidase) create a blue color when a substrate is present, while GFP (green fluorescent protein) emits green fluorescence under certain light conditions (Gelvin, 2003).

Case Studies

Swain *et al.* (2010) developed a protocol for *Amaranthus tricolor* was optimized using stem internode explants infected with *Agrobacterium rhizogenes*. Two Agrobacterium strains LBA9402 and A4 are used. Pre-pricked explants immersed in bacterial suspension ($O.D_{660}$ = 0.6 with 100 µM acetosyringone) and co-cultivated on MS0 produced hairy roots with up to 97.22% frequency. Root clones were maintained on MS0 with cefotaxime and transformation was confirmed by opine production and PCR detection of *rolB* and *man2*. Shoots regenerated from transformed roots on MS with 2.0 mg l^{-1} zeatin. The study concludes the protocol can aid in developing amaranths with improved resistance to major biotic stresses.

Pal *et al.* (2013) have optimized a procedure for genetic transformation of a major leafy vegetable crop, *Amaranthus tricolor* L., using epicotyl explant co-cultivation with *Agrobacterium tumefaciens*. Two disarmed *A. tumefaciens* strains EHA 105 and LBA 4404, both carrying the binary plasmid p35SGUSINT harboring the neomycin phosphotransferase II gene (*npt*II) and the β-glucuronidase gene (*gus*) were evaluated as vector systems. From a total of 48 selected shoot lines derived from independent transformation events with epicotyl explants co-cultivated with EHA 105, 32 showed positive PCR amplification for both the *npt*II and *gus* genes. Transformed shoots were selected on kanamycin and confirmed through GUS staining and PCR with stable inheritance verified in the next generation.

Castellanos-Arévalo *et al.* (2020) developed an efficient *Agrobacterium rhizogenes*-mediated transformation protocol for both grain (*Amaranthus hypochondriacus*) and leafy (*Amaranthus hybridus*) amaranths. Leaf and stem are used as explants. Optimal OD_{600} bacterial cell densities were 0.4 and 0.8 for *A. hypochondriacus* and *A. hybridus* respectively. Hairy roots of both amaranth species were validated by the amplification of appropriate marker genes and monitored green fluorescent protein emission or β-glucuronidase activity. The study represents a viable protocol for plant regeneration via somatic embryo germination from grain amaranth transgenic hairy roots.

Conclusion

- Agrobacterium-mediated transformation in amaranth is achievable and efficient when key bacterial, explant and culture conditions are optimized, providing a reliable platform for genetic improvement of the species.
- An efficient *A. rhizogenes*–mediated transformation system for *Amaranthus tricolor* is established, integrating key bacterial and plant factors and enabling rapid production of hairy roots.
- A reproducible *Agrobacterium*-mediated transformation protocol for *Amaranthus tricolor* is established, enabling stable germline integration and expression of foreign genes for effective genetic improvement of the crop.
- *hypochondriacus* is the most effective species for producing transgenic hairy roots capable of exhibiting either constitutive or EtOH-inducible gene expression.

References

1. Castellanos-Arévalo, A. P., Estrada-Luna, A., Cabrera-Ponce, J. L., Valencia-Lozano, E., Herrera-Ubaldo, H., de Folter, S., Blanco-Labra, A., and Delano-Frier, J. P. (2020). *Agrobacterium rhizogenes*-mediated transformation of grain (*Amaranthus hypochondriacus*) and leafy (*A. hybridus*) amaranths. *Plant Cell Reports*, **39**(9), 1143–1160.
2. Clark, D. P., & Pazdernik, N. J. (2013). *Ti plasmids are transferred from bacteria to plants*. In *Molecular Biology*(2nd ed.). Elsevier.
3. Food and Agriculture Organization. (2018). *The future of food and agriculture: Alternative pathways to 2050*. FAO.
4. Gelvin, S. B. (2003). Agrobacterium-mediated plant transformation: The biology behind the “gene-jockeying” tool. *Microbiology and Molecular Biology Reviews*, **67**(1), 16–37.
5. Gelvin, S. B. (2010). Plant proteins involved in Agrobacterium-mediated genetic transformation. *Annual Review of Phytopathology*, **48**, 45–68.
6. Griffiths, A. J. F., Miller, J. H., Suzuki, D. T., Lewontin, R. C., and Gelbart, W. M. (2000). *An introduction to genetic analysis* (7th ed.). W. H. Freeman
7. Hwang, H. H., Yu, M., and Lai, E. M. (2017). Agrobacterium-mediated plant transformation: biology and applications. *The arabidopsis book*, **15**, e0186.
8. Pal, A., Swain, S. S., Das, A. B., Mukherjee, A. K., and Chand, P. K. (2013). Stable germ line transformation of a leafy vegetable crop amaranth (*Amaranthus tricolor* L.) mediated by Agrobacterium tumefaciens. *In Vitro Cellular & Developmental Biology-Plant*, **49**(2), 114-128.
9. Primrose, S.B. & Twyman, R.M., 2006. *Principles of Gene Manipulation and Genomics*. 7th ed. Blackwell Publishing
10. Rastogi, A., and Shukla, S. (2013). Amaranth: A new millennium crop of nutraceutical values. *Critical Reviews in Food Science and Nutrition*, 53(2), 109–125.
11. Swain, S. S., Sahu, L., Barik, D. P., and Chand, P. K. (2010). Agrobacterium × plant factors influencing transformation of ‘Joseph's coat’ (*Amaranthus tricolour* L.). *Scientia Horticulture*, **125**(3), 461-468.