plasmid enables the effective transfer of genes of interest into the plant cells such as T-DNA border sequences, vir genes; for transferring T-DNA region to the plant genome but are not transferred themselves. The advantage of using this method over other methods are reduction in transgene copy number; intact and stable integration of transgene into the plant genome **(Bartlett *et al*., 2008).**

Seven steps make up the protocol, which can be summed up as follows: Stage (I) involves thepreparation of sterilised seed or samples and inoculum; Stage (II) entails explant preparation, infection, and cocultivation with *A. tumefaciens*; Stages (III) and IV involve selection; Stage (V) involves acclimatisation and molecular identification of T_0; Stage (VI) involves cultivation and self-crossing of T_0; and Stage (VII) involves T1 plant analysis **(Pratiwi and Surya., 2020)**.

Also, to cause crown gall formation, T-DNA encodes genes for the production of **auxin or indole-3-acetic acid** via the IAM pathway. This biosynthetic pathway is not used in many plants for the production of auxin, so it means the plant has no molecular means of regulating it and auxin will be produced constitutively. Genes for the production of **cytokinin** are also expressed. This stimulates cell proliferation and gall formation.

Example of T-DNA genes in Ti plasmids

Gene	Product	Function
ocs	octopine synthase	opine synthesis
nos	nopaline synthase	opine synthesis
tms1	tryptophan-2-mono-oxygenase	auxin synthesis
tmr	isopentyl transferase	cytokinin synthesis
ags	agropine sunthase	opine synthesis

T-DNA transfer and integration into the genome is mediated by various vir genes such as vir A and vir G both expresses constitutively. Vir A codes for kinase spans the bacterial membrane and for phenolic molecules (acetosyringone) it acts as receptor, released by wounded plant cells. This binding of acetosyringone causes vir A to get autophosphorylated on histidine residue. This phosphate group transferred to aspartate residue in vir G. Both vir A and vir G genes acts as activator for other vir genes. T-DNA transfer occurs through a conjugative pilus formed as a result of vir gene expression, initiated by the products of vir D1 gene (helicase) and vir D2 gene (act as endonuclease). The representation of *Agrobacterium* T-DNA transfer is given below:

In planta transformation

Since the first time *Agrobacterium* was used to transform plant explants, there has been a lot of interest in creating transformation techniques that do not rely on tissue culture regeneration, can provide high throughput transformation, require little to no labour, money, or expertise, and lower the rate of unintended mutagenesis and somaclonal variations induced by in vitro culture. Feldman first created an *in planta* transformation procedure in the A. thaliana model plant **(Ziemienowicz., 2014)**.

Role of gene transfer methods in crop improvement

- Genetic engineered wheat has led to increase the grain yield and minimize the crop loss due to unfavourable weather conditions and has developed resistance in crop against various pathogen and pests. The first transgenic wheat plants were produced by microprojectile bombardment as a method of DNA delievery. Genetic transformation with a single target gene has been used for the production of transgenic wheat expressing tolerance to herbicide, resistance to fungal and viral diseases.
- A coat protein-mediated resistance to viruses, introduced into rice via protoplast transformation, was transferred then to maize and barley via particle gun bombardment. The resistance to sulfonylurea (herbicide) conferred by the *als* gene of *Arabidopsis thaliana* was also transferred to maize by particle gun technology. Maize has been reported to get transformed by Silicon carbide fiber-mediated DNA delivery system. Whiskers-mediated maize transformation has also been reported.
- Transformation in rice leads to double the food supply and improved the quality as well as quantity. Biolistic was successfully used for transformation of immature embryos of rice . Reports were also maderegarding the transformation of indica and javanica rice in addition to other japonica rice. Fujimoto et al. were the first to engineer japonica rice through electroporation with modified d-endotoxin gene (*cry*) from *Bacillus thuringiensis* (**Khan., 2009)**.

3. Conclusion

One of the most difficult elements of plant science is arguably the development of gene transfer systems in plants. *Agrobacterium*-mediated and biolistic-mediated DNA delivery technologies are now the two best options. Two decades ago, the primary goal of the development of transformation technology was the creation of transgenic crops with enhanced agronomic properties for increased crop productivity.

The challenge of applying transformation strategies to higher plants with a higher frequency to produce GT events has not yet been solved. The transgene-carrying *Agrobacterium* strains can easily transform *Arabidopsis* plants by dipping the plants in the solution. It takes a lot of work to alter and screen higher plants for GT events. By using gene knocking, the creation of the GT technique represents a significant advance in furthering our understanding of how a single gene functions in the context of its genome. It also has the potential to boost public acceptability of molecularly-based plant gene manipulation.

Conflicts of Interest

The authors declare no conflict of interest.

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