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Agrobacterium-Mediated Transformation in *Oryza Sativa* (Rice) to Improve Crop Yield: A Review

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Abstract:

Since the beginning of the twentieth century, *Agrobacterium tumefaciens* and associated species of *Agrobacterium* have been referred to as plant pathogens. However, the potentiality of *Agrobacterium* to displace DNA to plant cells has been harnessed for plant genetic engineering purposes for the past two decades. For more than half of the world's population, *Oryza sativa* (rice) is the staple food and has also been a major monocot model. Plant scientists have used techniques of incorporating DNA into plant genomes to investigate gene functions. Transformation also provides an important method for enhancing breeding for crops such as rice. To explore gene functions, plant scientists have used methods of inserting DNA into plant genomes. The study explored the various restrictions that hinder the progressive transformation of genetic rice via the *Agrobacterium*-mediated method and proposed potential solutions. The pinpointing of explant, a strategy for gene exchange, and development to modify incorporation, transgene articulation without affirmation to hereditary harm and determination of transformant are among the innovative difficulties affecting rice transformation. Due to its exact T-DNA handling and simple consolidation of low copy number transgenes, the *Agrobacterium*-mediated transformation strategy was a superior decision for creating transgenic assortments of rice. This article attempts to overview the fundamental biology associated with genetic transformation promoted by *Agrobacterium*, which may perhaps be beneficial to both plant biologists and microbiologists who want a deeper perception of the expression of plant proteins, the transformation of plants, and the association between plant and microbe.

Keywords- ***Agrobacterium tumefaciens*, *Oryza sativa*, T-DNA, genetic transformation, transgene expression, transgenic rice**

I. INTRODUCTION

While considering the food crops in the entire world, *Oryza sativa* (rice) is the main one among them. For more than 33% of the total populace, rice remains the staple food. It has been calculated that to meet the total food need of the globe, total food production has to be increased by 60% at least in the coming 25 years [1]. Since the rice was weighed as one of the major refractory harvests regarding genetic manipulation long back, presently it is thinking about a significant model for contemplating different angles, for example, plant pathology, gene regulation, and expression, plant pathology, managing of the metabolic pathway, and also the inheritance, arrangement, rearrangement, and fate of transgenes [2], [3].Even though an unnatural weather change turning into a significant denunciation to the world, what's more, causes numerous swaps in the surroundings, it is a prompt objective to be considered to breed new rice cultivars with strong opposition and resilience against these changes. There exist well-executed methods for introducing a desirable gene inside the plant genome [4].There found remarkable progress in the crop yield of plants especially The studies for improving the resistance against pests that influence rice plant through acquainting genes of agronomic significance with the plant by *Agrobacterium*-mediated gene transformation is very not many as of not long ago and from the perspective of a plant breeder, it very well may be typified that the transgenic plants acquired through *Agrobacterium*-mediated gene transfer are still very low in number. In the field of plant physiology, it is a transcendent field of study where the genetic transformation in rice plant to upgrade its physiological properties and obstruction against unfavorable components and consequently the crop yield.

in rice when *Agrobacterium tumefaciences* were used to mediate the gene transformation.

For conspicuous transgenic rice (*Oryza sativa*) creation, there will be numerous specialized difficulties, for example, the creation of the desired proportions of transformants without hereditary harm and having predictable transgene expression. Rice, wheat, and maize were the monocotyledons used to change by intervening *Agrobacterium* [5].After that in 1994, it was contemplated with a great belief that rice is amendable to *Agrobacterium*-mediated transformation. As mentioned before, the *Agrobacterium*-mediated transformation system prefers as a convenient option since it troubleshoots the factors which determine transgene delivery and implant genome integration efficiently [6], [7], [8].*Agrobacterium* which comprises of tumor-inciting (Ti) plasmid moves its section of DNA to the host cell genome thus that hereditary transformation of host occurs. Bacterial, host plant and environmental origin are the key factors that determine the gene transfer arbitrated by *Agrobacterium*.

II. SIGNIFICANCE OF RICE TRANSFORMATION

The over-reliant on rice as the primary diet remains constant over the decades. Thus, increasing rice yield remains one of the vital goals in plant biological science research, including agriculture and crop production [9], particularly in the lands like Asia and Africa. A decade back, rice was well-thought-out as one of the most recalcitrant crops with genetic manipulation, it has lately risen as the model cereal for the learning of plant genomics, plant pathology, gene regulation, and expression, metabolic pathway manipulation and the inheritance, association, reorganization and destiny of transgenes [10].

With the arrival of molecular techniques, plant transformation has to turn out to be possible. The capability to control genetic material by presenting and communicating a definite novel-foreign gene in plants conveys a persuasive novel exploratory tool, allowing direct testing of speculations in the physiology of plant that have become incredibly intense to determine by customary rearing or biochemical tests. The transformation method might produce valuable plants with superior phenotypes that are unattainable by conventional breeding methods, fix faults, and advance physiological and agronomical qualities in some cultivars more professionally [11]. Fascinatingly, certain possibilities have been experienced with the age of commercial transgenic plant lines communicating transgenes presenting protection from pests, pathogenic microbes, natural pressure, and weed killers, or expanding grain gather and weight (Table 1).Moreover, the reach to which additional commercial, reasonable, or agronomically improved rice can be encounter entirely relies upon the viability of the change technique that can reap lines with no hereditary damage. Such research needs a sequence of screening of the transformants expressing the essential transgene, as well as evade misleading results from unplanned genetic modification during the process.

TABLE 1

VARIETY OF RICE, TARGET OF TRANSFORMATION, AND IMPROVEMENT IN TRANSFORMED RICE CROP BY *AGROBACTERIUM*-MEDIATED GENE TRANSFORMATION.

Sl. No	Variety of rice	Target for transformation	Advance in transformed rice crop	References
1	Pusa Basmati (*indica*)	Embryogenic callus	Expansion of transgenic fertile rice	[12]
2	IR64 (*indica*)	Shoot apex	Antibiotic-resistant plant	[13]
3	CempoIreng (*indica 2N6)*	Embryogenic callus	Early flowering growth	[14]
4	MR219 (*indica)*	Embryogenic callus	Auxin Binding Protein 57 (Abp 57), Overexpression of the stress-related gene	[15]
5	Cotton Sannal, Sambha Mahsuri *(indica N6)*	Embryogenic callus	Improvement in the resistance of drought	[16]
6	Nipponbare *(japonica)*	Embryogenic callus	Drought, salinity, and pathogen tolerance and increasing capacity for photosynthesis and tiller number	[17]
7	Nipponbare *(japonica)*	Embryo	Heat tolerance	[18]
8	Dongjin *(japonica)*	Embryo	Increase tolerance to cold stress, OsCYP19-4 gene	[19]
9	Dongjin *(japonica)*	Embryogenic callus	Promote the short-day flowering of rice	[20]
10	Taipei 309 *(japonica)*	Embryogenic callus	Enhancement of Iron Vitamins	[21]
11	Taipei 309 *(japonica)*	Embryogenic callus	Early rice growth inflorescence	[22]
12	Zhonghua 11 *(japonica)*	Immature embryo	OsELF3 floral activator monitoring heading date at the long-day condition	[23]
13	Zhonghua 11 *(japonica)*	Embryogenic callus	Increase grain yield, the height of the plant, and grain weight	[24]

Agrobacterium sp. is overwhelmingly came about soil bacterium that sources crown gall, and can bring new hereditary material inside the plant cell [7]. The genetic material which is presented is called T DNA (moved DNA/ transferred DNA) that is pinpointed on a Ti plasmid DNA. A Ti plasmid DNA is a round bit of DNA found in practically all bacteria. This normal ability to adjust the plant's genomic material was the wellspring of the transformation of plants through *Agrobacterium*. Presently, *Agrobacterium*-intervened gene transfer is the major oftentimes utilized procedure for plant genetic designing on account of its relatively high adequacy. Essentially it was felt that this bacterium can just taint dicotyledonous plants, however, it was later detailed that it can similarly be used for an alteration of monocotyledonous plants, for example, rice.

During transformation, a few parts of the Ti plasmid grant the viable exchange of the desired genes into the plant cells.

These contain:

- T-DNA outskirt successions these arrangements differentiate the DNA section (T-DNA) to be moved inside the plant genome.
- vir gene (virulence gene)- these are needed for transferring the T-DNA district to the plant yet are not themselves moved and altered.
- T-DNA locale where the genes which are answerable for the explanation for crown lesion arrangement are taken out and subbed with the ideal genes.

The *Agrobacterium*-mediated transformation procedure comprises numeral steps: (a) separation of the desired genes from the source organism; (b) improvement of a utilitarian transgenic build including the gene of desire; promoters to drive articulation; codon adjustment, whenever needed to increment fruitful protein creation; and marker genes to encourage the following of the presented genes into the host plant; (c) transgene addition into the Ti-plasmid; (d) presentation of the T-DNA-containing-plasmid into *Agrobacterium*; (e) combination of the transformed *Agrobacterium* along with the plant cells to permit the trading of T-DNA into plant chromosome (f)recovery of the changed cells into hereditarily altered (GM) plants; and (g) testing for characteristic execution or transgene articulation at the lab, greenhouse, and field level. **Figure 1** delineates the *Agrobacterium*-mediated plant transformation. The overall points of interest of utilizing *Agrobacterium*-mediated change over other change strategies are a decrease in transgene duplicate sum, and complete and steady incorporation of the recently acquainted gene with the plant genome [25]. A. tumefaciens causing crown gall/tumor disease. Maybe another rational classification system has additionally divided the genus into 'Biovars' based on the metabolic characteristics and growth mode of the organisms [26].

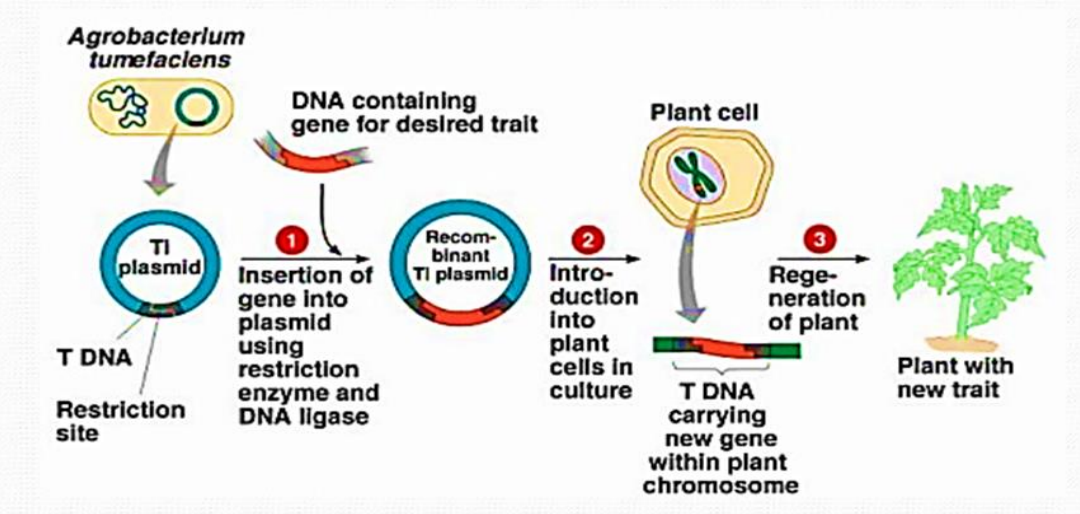

Figure 1: *Agrobacterium*-mediated plant transformation

Agrobacterium genus is a Gram-negative, soil bacterium with pathogenicity that causes the development of crown tumors in the plant genome [27]. To develop tumorigenesis and to transport in opine biosynthesis, genus Agrobacterium is required. If Agrobacterium species is taken as an example, it promotes the transmission of transferred-DNA (T-DNA) inside its host plant close to disease locales. The Agrobacterium variety can move foreign DNA by even gene exchange into the host genome during transformation, as it has a stable and effective mechanism. Agrobacterium-plant gene transfer can be divided into five main stages, including Agrobacterium virulence system activation, T-DNA complex generation, T-

DNA move to the plant cell nucleus, T-DNA incorporation into the plant genome, and T-DNA gene expression via expression of transformed plant gene [4].

The mechanism of Agrobacterium-mediated transition suggests that the Agrobacterium cell shift its T-DNA into the host plant's nuclear genome. T-DNA usually lives in plasmid-inducing bacterial growth [tumor inducing (Ti) or root inducing (Ri)], and then sliced by virulence proteins [7], [28]. The vir-genes that are found along the plasmid's T-DNA boundary are mainly polar. Vir-genes along these lines assume a fundamental part in the creation of T-strand or T-DNA by the bacterium, the progress of T-DNA or the improvement of the T-complex and its delivery to the plant cell through the conveyance of T-pilus and T-DNA to the real plant genome, just as the secretion of opines [7]. The DNA transfer method mediated by *Agrobacterium* provides specific advantages over direct gene transfer strategies as follows: ease of gene transfer, accurate foreign gene transfer and DNA sequence incorporation with specified ends, a related transfer of selectable markers along with the gene, the low copy number of the transgene, higher stable transformation frequency, relatively lower transgene-silencing rate and capability to transfer long T-DNA stretches (150 kb) [29].

In light of the forthrightness of the *Agrobacterium* Ti plasmid-based vector change and the specific joining of the single-copy number of the transgene inside the plant genome, as stated by Sood et al., (2011) [5], the technique keeps on being the most usually utilized and the best apparatus for rice transformation to date, and the adequacy of the bacterium species is acceptable [30]. Past outcomes have communicated the capability of *A. tumefaciens* to accomplish effective transgenic rice production [31], accordingly the genus *Agrobacterium* is alluded to as a regular plant genetic engineer [32], [33]. However the processes are adversely impacted by such variables as genotype, and it is remarkable to transcend such restrictions in the genetic hereditary change of rice. A schematic portrayal of rice transformation mediated by *Agrobacterium tumefaciens* is shown in **Fig. 2**.

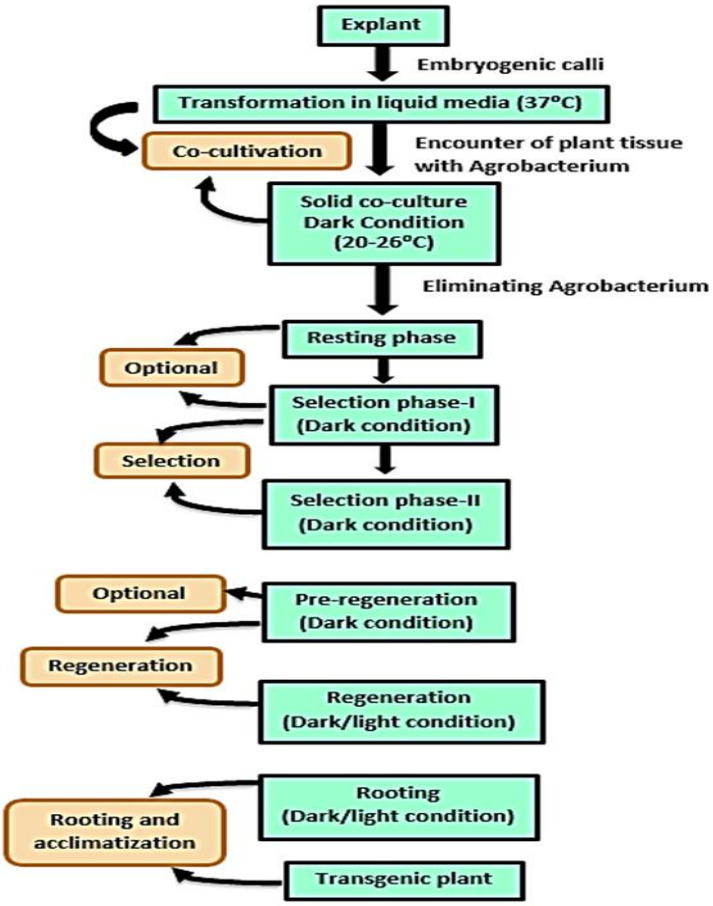

Figure 2: The graphical representation delineating the progressive advances and times needed by Agrobacterium-mediated transformation for the advancement of transgenic rice

VI. T-DNA INTEGRATION AND EXPRESSION OF TRANSGENE

Unsurprising and reproducible degrees of articulation of transgenes in designed plants is one of the principal objectives of plant sub-atomic science. Plant change doesn't generally bring about effective transgene articulation. Every so often, transgenes neglect to communicate the protein products because of silencing. This has been seen in an assortment of plant varieties. If a specific mRNA animal group isn't integrated, Silencing can occur. More than direct gene transfer the utilization of *Agrobacterium* is commonly accepted to create a less complex integration prototype. Albeit the two procedures bring about a comparative exhibit of integration episodes,the copy number and recurrence disseminations contrast [7].

Upon the Agrobacterium connection to the cells of the plant, disease action is controlled by signal transduction and vir-gene qualities just as transcriptional activation pathways and remain the components impacting the gene inclusion. Also, some of the secondary metabolites and development controllers during the Agrobacterium-disease period enact or repress the transgene incorporation. The clear T-DNA joining focusing on a particular area has kept on being a significant apparatus for resulting transgene expression, yet it is as yet unintelligible to date. Accordingly, many plants which have been transformed produce mutant aggregate because of disturbance of embryos as explants, more than 40 genotypes have been altered counting *japonica*, *indica*, and *javanica* genotypes. Transformation efficiency differs between genotypes. Meanwhile, the majority of the reports presented only the steady transformation frequency, it was not recognized whether the genotype variation was due to T-DNA transfer or the tissue culture response. Overall, for wheat, maize, barley, and sugarcane, model genotypes used in micro-projectile-mediated transformation have functioned well for Agrobacterium-mediated transformation. The origin of the explants had a significant aftermath on the transformation rate. Zhao et al. (2000) reported that immature embryos from field-grown stock plants could be transformed more efficiently than immature embryos from greenhouse-grown stock plants of sorghum [37].The influence of temperature during co-culture on T-DNA distribution was first described in dicot species. A temperature of 22˚C was obtained to be optimum for T-DNA conveyance in *Phaseolus acutifolium* callus and tobacco leaves. Though, in another report, co-culture at 258C directed to the uppermost number of transformed plants of tobacco, even though 198C was optimum for T-DNA delivery. As widely studied in dicot species, other medium components such as basal medium, sugar, plant growth regulators, and vir induction chemicals are also vital factors that affect transformation frequency.

transcription units because of T-DNA integration. Thusly, transgene integration is essential to be coordinated to a translated or even explicit district without the interference of established genes [5], [34], [35]. Vir-gene initiation for transgene joining in rice is amazingly demanding. Improving the cycle basically by streamlining utilizing phenolic aggravates like acetosyringone (AS) or vir initiating compound in rice change has been accounted for [15], [30], [36]. Conversely, lower effectiveness of rice changes has been gotten without such synthetic mixes. Subsequently, the component managing the substance's standard towards the quality combination is yet to be completely perceived. This is likewise another restriction factor causing the shortcoming of rice genetic transfer.

V. FACTORS AFFECTING *AGROBACTERIUM*-MEDIATED TRANSFORMATION

Based on several studies in rice transformation and one study in maize, five key factors were reported which are involved in *Agrobacterium*-mediated transfer of genes to rice. These five factors include vir gene initiation, active cell division in the target tissue(s), medium composition, *Agrobacterium* species and vectors, and genotype. Between all the monocotyledonous species altered to date, rice seems to be the least genotype-dependent. Via primarily embryogenic calluses or immatur

VI. FUTURE DESIRES FOR O. SATIVA TRANSFORMATION RESEARCH

The tissue culture strategy (especially *indica* subspecies) to enrich regenerative tissue/cells that might be open for gene exchange is the first restriction to be addressed by research into rice genetic engineering. The advancement of a (regenerable) selection method for valid explants is essential for efficient transformation. The basic requirement for achieving in vitro plant regeneration is the routine induction or ability to effectively develop a micro-propagation strategy for embryogenic callus and remains a prerequisite for genetic transformation, particularly in rice. The way to recalcitrant cultivars, accordingly, will in general be the creation of a strategy that will uncover the plentiful regenerable cells to compelling treatment and articulation for gene integration. Events affecting both Agrobacteria's gene transfer strategy and the direct transfer approach should be well understood. Nevertheless, there are many important questions concerning the methodology of direct gene transfer.

Optimizing the exhibition of transformation, including agro-infection, transgene T-DNA transfer integration, and regeneration of transgenic plants is currently a major concern

in rice improvement technology. The advancement of new strategies for unsurprising transgene articulation without unintended hereditary disturbance to the plant genome is another significant goal. The restricting mechanism for cultivar improvement or plant physiology in the implementation of all plant transformation is normally not transformant regeneration, yet the choice is needed to remove transgenic plants with collateral genetic damage [11]. Enlightening the procedure basically by optimization via phenolic compounds like acetosyringone (AS) or vir inducing chemical in rice transformation has been reported [15], [30], [36]. Indemnity of genetic injury and total regeneration need further thorough research into the physiology of the organism [38].

Similarly, it would be significant to classify other bacterial species (non-Agrobacterium) and will potentially improve the accomplishment of rice transformation [49]. There is interest in the use of non-Agrobacterium for genetic transformation of rice owing to freedom-to-operate subjects that reside in various jurisdictions with Agrobacterium. *Ensifer adhaerens* (OV14) bacterium for rice transformation shows maximum infection proficiencies [50]. Further analysis of the transformation induced by *Ensifer* and its function is dominant. Because of its potency, dependability, and non-pathogenic transformation, *Ensifer*-mediated transformation is proposed to be the next route for rice improvement [51]. Also, research ventures are conducted with the chance of growing useful, commercially affordable, and valuable crops, which ensures that researchers need industry funding. Similarly, for study design, scientists must unite in their aims and combine legal, societal, economic, and practical problems.

TABLE 2

THE PROCEDURE BASICALLY BY OPTIMIZATION VIA PHENOLIC COMPOUNDS LIKE ACETOSYRINGONE (AS) OR VIR INDUCING CHEMICAL IN RICE TRANSFORMATION

Sl. No.	Factors influencing *Agrobacterium*-mediated transformation	Example of Factors	References
1	Antibiotics	Cefotaxime, carbenicillin, kanamycin, timentin	[39]
2	Agrobacterium	density 1106–11010 cfu/ml pH of co-	[40]
3	Bacterial strain	LBA4404, EHA101, C58, AGL1	[41]
4	Composition of culture medium	Salt concentration, sugars, growth regulators	[42]
5	Chemicals	Acetosyringe, L-cysteine, dithiothreitol, and sodium thiosulphate	[43]
6	Cultivation medium	Acidic pH: 5.2, 5.5, 5.6, 5.8 or 6.0	[44]
7	Temperature of co-cultivation	Range: 19–30 1C; optimal temp. dicots: 19–20 1C, monocots: 24–25 1C	[45]
8	Explant type	Root, shoot, cotyledon, embryo, hypocotyl	[46], [55]
9	Surfactants	Silwet L77, pluronic acid F68, Tween20	[47]
10	Vector plasmid	pCAMBIA, pGreen, pGA, pCG, pGPTV, Bi-BAC, etc.	[48], [56]

VII. CONCLUSION

The brilliant long stretches of *Agrobacterium* research drove us to comprehend a considerable lot of the bacterium's biological process and contraption and established the framework for building up *Agrobacterium* as the significant device for plant genetic designing. The expansive investigation has revealed an enormous piece of the bacterium's exceptional and enchanting biology and a wide extent of transformation shows have been made for an incredible number of plant species, assortments, and cultivars. In light of everything, various financially significant plant species cultivars remain obstinate to *Agrobacterium* transformation and plant researchers are up 'til now confined in their capability to control the transformation cycle, even in weak plant species. Notwithstanding the less amiability showed by various rice assortments and low effectiveness of recovery after *Agrobacterium*-mediated transformation, it is as yet conceivable to change practically all rice sub-species [52], [53], [54]. Foreign DNA combination

into the reasonable plant atomic genome just as its demeanor has kept on being significant obliges. Appropriately, the determination of positive transformants and the advancement of the recuperation overlay stay significant issues in transgenic rice screening. Designing the entire biosynthetic pathway is likewise not very inaccessible. Rice with evident points of interest can assume a significant part in the coming years. It is trusted that in the coming years, rice biotechnology will lead the route for the accomplishment of practical farming and food security.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTION

All the authors have contributed equally.

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