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Abstract

Plant genetic engineering has become one of the most important molecular tools in the modern molecular breeding of crops. Now a days, production of transgenic plants is a routine process in some crop species. Transgenes are delivered into plants to confer novel traits such as improving nutritional qualities, resistance to pests. It is possible to insert genes from plants at evolutionary distant from the host plant, as well as from fungi, viruses, bacteria and even animals. Genetic transformation requires penetration of the transgene through the plant cell wall, facilitated by ELRORJLFDO RU SK\VLFDO PHWKRGV 2YHU WKH ODVW IHZ GHFDGHV D VLJQL¿FDQ RI QHZ DQG HI¿FLHQW WUDQVIRUPDWLRQ PHWKRGV 'HVSLWH D YDULHW\ RI DYDL and Biolistic mediated transformation remain two predominantly applied approaches. The objective of this article is to review the currently used methods for genetic plant transformation, their biological requirements and critical parameters.

Keywords Genetic transformation rice; Agrobacterim; Biolistic transformation.

Introduction

Cereals are the most important source of calories to humans since rice, wheat and maize provide 23%, 17% and 10% calories globally [1]. Rice (

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genes varies among individual transformants. erefore, a relatively large number of transgenic plants must be developed in order to select desirable transformants as well as to study the expression of introduced genes [21].

e most commonly used method for transformation are Biolistic approach and Agrobacterium mediated transformation. is review will summarise various gene delivery methods applied to improve rice traits. Subsequent molecular analysis of the transgenic rice will also be discussed. Additionally, it will consider the future prospects of transgenic researches on the crop.

Genetic Transformation

Purpose of genetic transformation

Main purpose of genetic transformation is to generate plants with useful phenotypes i.e. unachievable by conventional plant breeding, to correct faults in cultivars more e ciently than conventional breeding and to allow the commercial value of improved plant lines to be captured by those investing in the research more fully than is possible under intellectual property laws governing conventionally bred plants. Some reasons for genetic modi cations are yield improvement, more resistant to disease and pest resistance, herbicides tolerance, better nutritional value, increased shelf life, better climatic survival by increasing tolerance to drought, ood or frosty conditions to allow the use of previously inhospitable land, higher crop yields, reduced farm costs, increased farm pro t and improvement in health and environment..

Biological requirements for transformation

e essential requirements in a gene transfer system for production of transgenic plants are availability of a target tissue including cells competent for plant regeneration, a method to introduce DNA into those re-generable cells and a procedure to select and to re-generate transformed plants at a satisfactory frequency.

Methods of Genetic Transformation

Agrobacterium mediated genetic transformation

e soil pathogen Agrobacterim tumefaciens has been extensively studied since 1907, when it was identi ed as the causative agent of crown gall disease [22-24] Braun initially proposed the Agrobacterium as a source of a 'tumor inducing principle', possibly DNA, that permanently transformed plant cells from a state of quiescence to active cell division.

A. tumefaciens is a soil dwelling bacteria that naturally infect dicots and causes tumorous growth resulting in crown gall disease. Tumor formation results from incorporation of T-DNA (transfer DNA), a part of small independent DNA molecule outside the bacterial genome called Ti (tumor inducing) plasmid. Phenolic compounds exuded from plant wounds that stimulate the expression of vir genes, located on Ti

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so transgenes of any size and arrangement can be introduced, and multiple gene co-transformation is straightforward. It has bigger advantage that the delivered DNA can be manipulated to in uence the quality and structure of the resultant transgene loci. is approach can be used for transfer of more than one gene simultaneously in a host plant. As many as 14 genes have been co-introduced in rice by this approach [29]. Nowadays, particle bombardment is the most e cient way to achieve plastid transformation in plants and is the only method so far used to achieve mitochondrial transformation [30]. A genotype independent method for rice transformation was originally reported by Christou et al. [31] and it has been widely used throughout the world. Researchers at the International Rice Research Institute, Philippines, have used particle bombardment successfully to transform over 20 di erent cultivars adapted to di erent eco-geographic conditions. ese cultivars have been transformed with a range of agronomically important genes like psy, crt1, cry, ferritin, FRO2, Xa21, Bt, Chitinase,

Genotype	Explants	Promoters	Strain	Plasmid	Transgene	Marker gene	Transformation Efficiency	Transgenic Analysis	References
Ratna (IET411)	Seeds	35S	LBA4404	pCAMBIA1301	hpt	Kanamycin GUS	47%	PCR Analysis	Basu et al. [33]
IRGA424	Seeds	Mpi ubi	LBA4404	pX2.H.C1mpi Cry1Bgene pC1300.ubi Cry1B.nos	Cry1Aa Cry1B	GFP hph	86.4%	PCR Analysis	Pinto et al. [34]
IR36	Mature seeds	35S	EHA105AL	pCAMBL 1301	hpt	Uid A	98% callus induction	PCR Analysis	Krishnan et al. [35]
Indica rice	Mature seeds	35S	LBA4404	pKhg4	Cry1 Ac	Hygromycin resistance gene	-	-	Guruprasad et al. [36]
IR64 Swarna CSR10 PB1	Mature dry seeds	35S	LBA4404	pCAMBL A1304	Glyoxylase 1 (Bigly1)	nptII hptII uidA GFP	Approximately 45%	PCR Analysis	Sahoo et al. [37]
Bg 250	Mature seeds	35S	GV3101	pCAMBL 1303	hptIV	Hygromycin GUS	20%	GUS Analysis	Ratnayaka et al. [38]
Pusa Basmati 1	Mature seeds	35S	LBA4404	pCAMBIA 1301	Am-SOD	Kanamycin resistance genes Hygromycin resistance genes	Very good	PCR Analysis Southern blotting	Sarangi et al. [39]
Kalizira Radhunipagol Tulsimala Pusa basmati-1	Mature embryo with endosperm	35S	EHA105	pIG121-Hm	Uid hpt	nptII	40-75%	GUS assay	Hossain et al. [40]
Heugnam—byeo Daesanbyeo	Scutellum of mature rice seeds	OsCc1 35S	LBA4404	pMJC-GB pMJC-GH	Isoflavone synthase 2 Chalcone reductase	bar hpt	12.8% (Hygromycin At Callus proliferation stage And Phosphinothricin At Shoot regeneration stage) 100% (for vice versa)	RT-PCR	Sohn et al. [41]
HKR-46 HKR-126	Seeds	EHA105		pCAMBIA 1301	Uid Hygromycin resistance gene Carbenicillin resistance gene	Hygromycin resistance gene Carbenicillin resistance gene	28.9%- 44.4%	GUS Assay	Saharan et al. [42]
Taipei 309	Mature zygotic embroys	Glutelin 35S	LBA4404	pAGt1Fe pAGt1Me	Ferritin (pte) Metallathionein-like (rgMT) Phytase (phyA)	hptII hptIV		Western blot Northern blot	Lucca et al. [43]
Senia Tebre Bahia	Mature seeds		LBA4404	pTOK233	GUS gene	hpt npt	5% (Tebra) 3% (Bahia, Senia)	Southern blot	Pons et al. [44]
Taipei 309 Pusa basmati 1 Tinawen	EC		LBA4404	pTOK233		hpt		16-31%(Taipei309) 12-21%(Pusa basmati1) 10-19%(Tinawen)	Azhakanandam et al. [45]

regenerated in a whole plant. However, the tissue culture approach causes somaclonal variation due to both epigenetic e ects and chromosomal rearrangements [66,67]. e in planta transformation method overcomes the disadvantages of the conventional in vitro Agrobacterium-mediated transformation method. e latter requires sterile condition, that is time consuming and causes somatic mutation or somaclonal variation in plant cells during in vitro culture, and some plants are recalcitrant to regeneration. In contrast, in planta transformation involves no in vitro culture of plants cells or tissue, which is its greatest advantage.

Floral dip transformation

Clough and Bent [68] modi ed the Agrobacterium vacuum in ltration method to transform Arabidopsis thaliana. is process was eliminated in favor of simple dipping of developing oral tissues into a solution containing Agrobacterium tumefaciens, 5% sucrose and 500 µlL

77]. PCR analysis showed various range of transformation e ciency

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research, as well as on favorable regulatory guidelines and public acceptance. us, all the strategies discussed in the present review will de nitely contribute to biotechnological breeding programs of rice for its improvement.

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