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## Genetic transformation of rice: Current status and future prospects

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**Genetic transformation of rice has been an important area of research in the past few years. PEG, electroporation, microprojectile bombardment, and *Agrobacterium* have been used to mediate gene transfer. Genes for insect resistance, fungal resistance, virus resistance, herbicide resistance, bacterial resistance and nematode resistance have been utilized in rice transformation. The methods involved, the genes utilized and the promoters used in rice transformation along with integration and expression of transgenes are discussed in this review.**

THE ever-increasing human population especially in the developing countries and various abiotic and biotic stresses have posed a challenge to boost the rice production in limited cultivable land<sup>1,2</sup>. Genetically engineered plants with genes of direct interest can be produced in a relatively short time and can be of direct value in the agri-food industry. Recently it has been

recognized that marker-free transgenics may be more acceptable commercially<sup>3,4</sup>. Some good reviews are already available<sup>5–8</sup>. Initially rice was transformed with direct transformation methods such as particle bombardment. Recently it has been shown that *Agrobacterium tumefaciens* can efficiently transform rice also. In this review, we shall discuss the latest developments in the transformation of rice. We shall focus on advances in gene transfer techniques and we shall also mention specific genes that have recently been introduced into rice plants as well as various issues related to the integration and expression of foreign genes.

### Methods of gene delivery

#### PEG-mediated rice transformation

In 1986, Uchimiya *et al.*<sup>9</sup> first obtained transgenic calli after polyethylene glycol (PEG)-induced DNA uptake of the *nptII* gene into root-derived protoplasts, followed

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by selection on kanamycin. The first transgenic rice plants were recovered by Zhang *et al.*<sup>10</sup>. Datta *et al.*<sup>11</sup> published the first report of the recovery of transgenic indica rice plants from cultivar Chinsurah Boro II. In a systematic study of factors that influence the efficiency of PEG-mediated transformation and regeneration, Hayashimoto *et al.*<sup>12</sup> reported that increasing the PEG concentration from 0 to 30% (w/v) reduced the plating efficiency of Nipponbare protoplasts from 11 to 3% and Taipei-309 protoplasts from 14 to 5%. However, the number of hygromycin-resistant calli obtained increased with increasing concentrations of PEG in both cultivars. The transformation frequency of Taipei-309 protoplasts was also found to increase with increasing concentrations of plasmid DNA up to the levels tested (20 µg DNA per 2–5 × 10<sup>4</sup>, based on the number of protoplasts used).

Most reports of successful PEG-mediated transformation and subsequent regeneration of transgenic rice plants have used 20–25% PEG, 10–30 min incubation times, and 10–100 µg of DNA per ml protoplast. Detailed protocols have been described by Hodges *et al.*<sup>13</sup> and Li *et al.*<sup>14</sup>.

#### Electroporation-mediated gene transfer

Electroporation has also been widely used to introduce naked DNA into protoplasts. Toriyama *et al.*<sup>15</sup> first used this method for the production of Yamahoushi cultivar transgenic rice via anther culture-derived protoplasts with the aminoglycoside phosphotransferase II (*aph(3')II*) gene, conferring resistance to the antibiotics kanamycin and G418. Southern analysis showed the correct size fragment upon hybridization to the *aph(3')II* gene. In another early study, Zhang *et al.*<sup>10</sup> electroporated protoplasts from cell suspension of Taipei-309 leaf base calli with the 35S promoter fused to the *nptII* gene. Out of six regenerated plants, two were positive for NPTII activity. Biswas *et al.*<sup>16</sup> also successfully reported the recovery of transgenics from indica rice IRRI breeding line IR58 protoplasts. Zu and Li<sup>17</sup> obtained fertile transgenic indica rice plants using seed embryo cells. Chaudhury *et al.*<sup>18</sup> observed transient expression of *gus* gene in intact seed embryos of indica rice.

#### Microprojectile bombardment-mediated gene transfer

Microprojectile bombardment, also called the biolistic method or the particle gun method has been used in many laboratories. The concept has been described in detail by Sanford<sup>19</sup>. The ability to deliver foreign DNA into regenerable cells, tissues or organs appears to provide the best method for achieving genotype-independent transformation bypassing *Agrobacterium* host specificity.

In earlier experiments, Christou *et al.*<sup>20</sup> reported that immature embryos were isolated from greenhouse grown cultivars of japonica, javonica and indica rice. The scutellar region of the embryo was bombarded and transient activity for the introduced gene(s) was observed after 24 h of bombardment. Bombarded tissues were plated on regeneration media supplemented with appropriate selective agents; transformed embryos and other transgenic organized tissues, such as shoots and roots in addition to transformed plants were obtained.

Sivamani *et al.*<sup>21</sup> reported a gene transfer procedure for the model indica rice variety TN1, using bombardment of embryogenic callus. They developed a procedure which allowed generation of highly homogenous populations of embryogenic subcultured calli by selectively propagating a small number of regeneration-proficient calli derived from seed. An efficiency of 3% could be obtained on an average over a number of experiments. Zhang *et al.*<sup>22</sup> regenerated transgenic plants from bombarded embryogenic suspensions of elite indica rice varieties, using procedures which are now commonly used by many groups. They commented that this genotype and environment-independent transformation system for indica rice that uses regenerable embryogenic suspensions as targets for bombardment may now be used routinely for the introduction of useful genes into elite rice varieties. Jain *et al.*<sup>23</sup> optimized the biolistic method for transient gene expression and production of agronomically useful transgenic basmati rice plants. Many biolistic devices (e.g. particle gun) have been developed, including both commercial and lab-built models. Moreover there is a possibility to get a relatively inexpensive simple particle gun specially for rice-producing developing countries<sup>24</sup>.

#### Agrobacterium-mediated gene transfer

The transformation of dicotyledons by *Agrobacterium* is well established. But in the case of monocots it is not the general process. In the past monocots, particularly graminaceous crop plants including important cereals like rice and wheat were considered to be recalcitrant to this technology and they were outside the *Agrobacterium* host range. However, transformation methods based on the use of *Agrobacterium* are still preferred in many instances because of the following properties: (i) easy to handle, (ii) higher efficiency, (iii) more predictable pattern of foreign DNA integration, and (iv) low copy number of integration.

Raineri *et al.*<sup>25</sup> described the production of transformed japonica cultivar by cocultivation of mature embryos with *Agrobacterium*. Results of Southern blotting indicated the integration of the T-DNA into the plant genome, but no transgenic plants were regenerated. In 1992, Chan *et al.*<sup>26</sup> obtained a few transgenic

rice plants by inoculating immature embryos with a strain of *Agrobacterium*. They proved the inheritance of the transformed DNA to progeny plants by Southern blot hybridization, although they analysed the progeny of only one transformed plant. Hiei *et al.*<sup>7</sup> subsequently reported a method for efficient production of transgenic rice plants from calli of Japonica cultivars that had been cocultivated with *A. tumefaciens*. Their evidence was based on molecular analysis and genetic studies of a large number of transgenic plants and the analysis of sequence of T-DNA junctions in rice. Rashid *et al.*<sup>27</sup> reported the successful application of such a method to basmati cultivars of indica rice after only minor modifications. In the same year, Dong *et al.*<sup>28</sup> also described the successful applications of the method to Javanica rice. Aldemita and Hodges<sup>29</sup> showed that immature embryos were also good starting materials for *Agrobacterium*-mediated transformation of indica and japonica varieties. Park *et al.*<sup>30</sup> reported that the isolated shoot apices are suitable explants for successful application of this method. Uze *et al.*<sup>31</sup> and Toki<sup>32</sup> reported such types of transformation with minor modifications. Abedinia *et al.*<sup>33</sup> transformed an Australian cultivar by the *Agrobacterium*-mediated method. Mohanty *et al.*<sup>34</sup> reported the successful application of this method in an elite indica rice variety pusa basmati 1 and transmission of the transgene to the R<sub>2</sub> progeny was also demonstrated. Khanna and Raina<sup>35</sup> transformed indica rice cultivars by the *Agrobacterium*-mediated method using binary and super-binary vectors. Chen *et al.*<sup>36</sup> developed a protocol for consistent and large-scale production of fertile transgenic rice plants. Yokoi *et al.*<sup>37</sup> produced chilling tolerance of photosynthesis and unsaturation of fatty acids in rice by introducing the GPAT gene. Goto *et al.*<sup>38</sup> obtained rice seeds with iron fortification by using soybean ferritin gene. Ye *et al.*<sup>39</sup> succeeded in engineering the provitamin A ( $\beta$ -carotene) biosynthetic pathway into carotenoid-free rice endosperm.

### Desired genes introduced into rice

#### Insect resistance

In the last few years various genes have been introduced into rice, since the methods of transformation have been well established in this species. Majority of the genes that have been introduced into rice are related to disease and insect resistance (Table 1). To our knowledge, there are about eleven reports, in which *Bacillus thuringiensis* (*Bt*)-derived genes have been introduced in rice against insect resistance by electroporation<sup>40</sup>, biolistic method<sup>41-49</sup> and by *Agrobacterium* method<sup>50</sup>. Other genes such as oryzacystatin<sup>51,52</sup>, corn cystatin<sup>53</sup>, PINII<sup>54</sup>, trypsin inhibitor<sup>55,56</sup>, GNA<sup>57,58</sup> have also been added.

#### Fungal resistance

Lin *et al.*<sup>59</sup> reported the production of rice transgenic plants against a rice sheath blight pathogen (fungus) *Rhizoctonia solani*. Stark-Lorenzen *et al.*<sup>60</sup> reported the production of transgenic rice plants containing Stilbene synthase gene to enhance resistance to the fungus *Pyricularia oryzae* using PEG-mediated direct gene transfer.

#### Virus resistance

Hayakawa *et al.*<sup>61</sup> have introduced the coat protein gene of rice stripe virus into two japonica varieties of rice by electroporation of protoplasts. Recently<sup>62</sup> transgenic plants were produced showing the expression of coat protein of rice dwarf virus. Sivamani *et al.*<sup>63</sup> introduced the coat protein (CP) genes CP1, CP2 and CP3 of rice tungro spherical virus (RTSV) individually or together into indica and/or japonica rice cells by particle bombardment.

#### Herbicide resistance

Christou *et al.*<sup>20</sup> have recovered herbicide-resistant transgenic rice plants from a number of commercially important cultivars, including recalcitrant indica varieties using electric discharge particle acceleration.

Further, Cao *et al.*<sup>64</sup> have reported the production of herbicide-resistant transgenic rice plants, in which the plants were transformed by microprojectile bombardment with plasmids carrying the coding region of the *Streptomyces hygroscopicus* phosphinothricin acetyl transferase (PAT). In the same year Datta *et al.*<sup>65</sup> reported the introduction of the bar gene into protoplasts of rice (IR72) by PEG-mediated transformation. Rathore *et al.*<sup>66</sup> produced herbicide-resistant rice plants by introducing the bar gene into protoplasts derived from immature embryos by PEG-mediated DNA uptake.

Recently a successful first rice field trial using a bar gene was reported by Oard *et al.*<sup>67</sup>. They bombarded the bar gene into immature embryos of commercial cultivars 'Gulfomont', 'IR72' and 'Koshihikari'. Sankula *et al.*<sup>68,69</sup> evaluated the application of Glufosinate on rice transformed with the bar gene in a field trial study.

In one instance, introduction of the herbicide-resistant bar gene in rice also provided protection from the sheath blight pathogen, *Rhizoctonia solani*<sup>70</sup>. In several studies the bar gene was used as a selectable marker gene<sup>30,71</sup>.

#### Bacterial resistance

Zhang *et al.*<sup>72</sup> reported the introduction of the Xa21 gene into embryonic suspensions through the biolistic

Table 1. Desired genes introduced into rice

Gene of interest	Source of gene	Method	Explants/ tissue used	Marker gene	Variety	Outcome	Target organisms	Refs
$\delta$ -endotoxin-CryIA(b)	<i>Bacillus thuringiensis</i> ( <i>Bt</i> )	Electroporation	Protoplasts	<i>hpt</i>	Japonica (Nipponbare)	Stably expressed and inherited and proved to be resistant to the insects. 10–40% mortality of II instar larvae.	Insect resistance (C. <i>Chilo suppressalis</i> (SSB) ( <i>Cnaphalocrosis medialis</i> ) leaf folder)	40
Synthetic CryIA(b)	<i>Bt</i>	Biolistic	Immature embryos	<i>hpt</i>	Indica (IR 58)	100% mortality of stem borer occurred and inhibited feeding of leaf folders and inherited.	Insect resistance (Scirrophaga <i>incertulas</i> (YSB), <i>C. supressalis</i> (SSB), <i>Cnaphalocrosis medialis</i> , <i>Matasmia patinalis</i> (leaf folders))	41
CryIAc endotoxin	<i>Bt</i>	Biolistic	Embryogenic callus of scutellum	<i>hpt</i>	Indica (IR 64)	Exhibited high toxicity to yellow stem borer larvae and stably expressed.	Insect resistance ( <i>S. incertulas</i> )	42
<i>CryIA(b)</i>	<i>Bt</i>	Biolistic	Embryogenic calli of mature seed	<i>hpt</i>	Tarom Molai (aromatic rice)	Showed enhanced resistance to first instar larvae.	Insect resistance ( <i>C. supressalis</i> , <i>S. incertulas</i> )	43
Modified CryIA(b)	<i>Bt</i>	Biolistic	Immature embryo derived calli	<i>hpt</i>	Japonica (Taipei-309)	High level of expression in leaves. 100% mortality of yellow stem borer occurred.	Insect resistance ( <i>S. incertulas</i> )	44
Synthetic CryIA(b)	<i>Bt</i>	Biolistic	Embryogenic calli	<i>hpt</i>	Vaidehi (deepwater rice)	Stably expressed and inherited to F <sub>1</sub> generation; showed enhanced resistance to yellow stem borer.	Insect resistance ( <i>S. incertulas</i> ) (YSB)	45
Truncated CryIA(b)	<i>Bt</i>	Biolistic	Protoplasts	<i>hpt</i>	Several varieties of Indica and Japonica	81 plants showed 100% mortality of larvae out of 800 Southern positive plants.	Insect resistance ( <i>S. incertulas</i> )	46
Hybrid <i>Bt</i> toxin gene from CryIA(b) and CryIA(c) CryIA(b)	<i>Bt</i>	Biolistic	Immature embryos	<i>hpt</i>	IR 72	Hybrid <i>Bt</i> toxin level was 0.01–0.02%; 100% mortality of the larvae occurred.	Insect resistance ( <i>S. incertulas</i> ) (YSB)	47
Novel CryIA of <i>Bt</i> gene	<i>Bt</i>	Biolistic	Embryogenic calli	<i>hpt</i>	Indica (IR 68899B) maintainer line	Showed enhanced resistance to yellow stem borer.	Insect resistance, yellow stem borer ( <i>S. incertulas</i> )	49
Synthetic CryIAc and CryIA(b)	<i>Bt</i>	<i>Agrobacterium</i>	Scutellum-derived calli of mature seeds	<i>hpt</i>	Indica varieties (Basmati 370 and M7)	R <sub>0</sub> and R <sub>1</sub> populations confirmed stable integration and expression and C-ry 2A protein was effective for the YSB and a leaf folder.	Insect resistance ( <i>S. incertulas</i> and leaf folder)	48
			Mature or immature embryos	<i>hpt</i>	Nine varieties (Nipponbare, Zhung 8215, ZAU 16, 9 RM, T83490, T90502, Kaybonnet, 93 WA)	Stably expressed inherited to progenies. Expressed up to 3% of soluble proteins; 97–100% mortality occurred and proved to be resistant to insects.	Insect resistance ( <i>S. incertulas</i> , <i>C. supressalis</i> )	50

Oryzacystatin (OC)	Rice	Electroporation	Protoplasts	<i>hpt</i>	Japonica (Japanica)	Stably expressed; showed enhanced activity at mRNA and protein level effectively inhibits proteases of this insect.	Insect resistance ( <i>Si-top</i> , <i>Milus oryzae</i> )	51, 52
Corn cystatin (CC)	Maize	Electroporation	Protoplasts	<i>hpt</i>	Japonica (Nipponbare)	Insect resistance ( <i>S. oryzae</i> )	Insect resistance ( <i>S. oryzae</i> )	53
Pot <i>PI</i> II (Potato proteinase inhibitor II)	Potato	Biolistic	Suspension cells	<i>PAT</i>	Japonica (Nipponbare, Taiwan PI4)	Plant showed increased resistance to the insects and stably inherited up to four generations	Insect resistance ( <i>S. inferens</i> )	54
<i>CP-I</i> (Cowpea trypsin inhibitor)	Cowpea	PEG	Protoplasts	* <i>PAT</i>	Japonica (Taipei-309)	20–80% of the <i>R</i> <sub>2</sub> progeny completely resistant to insects.	Insect resistance ( <i>S. inferens</i> , <i>C. suppressalis</i> )	55
cDNA <i>SKTI</i> (Soybean Kunitz trypsin inhibitor gene)	Soybean	PEG	Protoplasts	<i>hpt</i>	Japonica	Integration, expression and inheritance of this gene was demonstrated in <i>R</i> <sub>1</sub> and <i>R</i> <sub>2</sub> generations and 0.05–2.5% of <i>SKTI</i> ; soluble proteins were detected in <i>R</i> <sub>1</sub> and <i>R</i> <sub>2</sub> plants.	Insect resistance ( <i>Nilaparvata lugens</i> , <i>Stal.</i> )	56
Snowdrop lectin	Snow drop ( <i>Galanthus nivalis agglutinin</i> )	Biolistic	Immature embryos, Protoplasts	<i>hpt</i>	Four varieties ASD16, M12, FX92 (Japonica) Radon, Norrai	GNA expressed at levels up to 2% of total soluble proteins. Survival and overall fecundity of the insect decreased	Insect resistance (brown plant hopper)	57
Snowdrop lectin	Snow drop ( <i>Galanthus nivalis agglutinin</i> )	Biolistic	Immature embryo and embryogenic calli	<i>hpt</i>	Four indica varieties ASD16, M5, M12, FX92	GNA stably expressed, plants expressed GNA at the levels up to 2% of total soluble protein.	Insect resistance ( <i>N. lugens</i> )	58
Chitinase ( <i>CHI 11</i> )	Rice	PEG	Protoplasts	<i>hpt</i>	Indica (Chinsurah Borou)	Stably expressed, showed enhanced activity and inherited up to four generations.	Fungal resistance ( <i>Rhizoctonia solani</i> )	59
Stilbene synthase gene of grapevine Coat protein gene ( <i>CP</i> ) of stripe virus	Grapevine	PEG	Protoplasts	<i>NPT II</i>	Japonica (Nipponbare)	Showed enhanced resistance.	Fungal resistance ( <i>Pyricularia oryzae</i> )	60
Rice dwarf virus (RDV) (S8) outer coat protein gene	Virus	Electroporation	Protoplasts	<i>hpt</i>	Japonica (Kinukikari, Nipponbare)	Expressed at high level (0.5% of total soluble protein also transmitted to the progeny).	Rice stripe virus (RSP)	61
Coat protein ( <i>CP</i> ) genes <i>CP1</i> , <i>CP2</i> and <i>CP3</i>	Rice dwarf virus	Biolistic	Callus of immature embryo	<i>NPT II</i>	Japonica (Zhanghua 10)	Showed enhanced resistance, stably expressed and inherited.	Rice dwarf virus resistance	62
<i>bar</i>	Rice tungro spherical virus ( <i>RSTV</i> ) <i>S. hygroscopicus</i>	Biolistic	Embryogenic calli	<i>hpt</i>	Indica (TN1) and japonica	Showed moderate levels of resistance to <i>RSTV</i> (17–73%).	<i>RSTV</i>	63
<i>bar</i>	<i>S. hygroscopicus</i>	Biolistics	Immature zygotic embryos	<i>PAT, hpt</i>	Japonica (Kushihikari, Guifmont) and Indica (IR72)	Stably expressed and showed resistance.	Herbicide resistance	20
<i>bar</i>	<i>S. hygroscopicus</i>	PEG	Suspension culture cells	<i>PAT</i>	Japonica (Taipei-309)	Stably expressed and suppressed increase of ammonia; confers resistance to <i>PPG</i> -based herbicide.	Herbicide resistance	64
<i>bar</i>	<i>S. hygroscopicus</i>	PEG	Protoplasts	<i>PAT</i>	Indica (IR72)	Plants showed complete resistance to high doses.	Herbicide resistance	65
<i>bar</i>	<i>S. hygroscopicus</i>	PEG	Protoplasts	<i>PAT</i>	Japonica (Radon)	Stably expressed and showed resistance.	Herbicide resistance	66

Contd...

Table 1. (Contd.)							
<i>bar</i>	<i>S. hygroscopicus</i>	Electroporation	Protoplasts	<i>PAT</i>	Japonica (Yamahouzi, Nipponbare) Indica varieties (IR72, Minghui 63 and BG90-2)	Plants showed complete protection from <i>R. solani</i> infection. Six of the 55 transgenic <i>R<sub>0</sub></i> plants are Southern positive and displayed high levels of resistance to the pathogen and stably inherited to the subsequent generations.	Fungal resistance ( <i>R. solani</i> ) Bacterial resistance ( <i>Xanthomonas oryzae</i> , <i>pv. oryzae</i> )
<i>Xa21</i>	Rice	Biolistic	Embryogenic suspension	<i>hpt</i>	Immature embryos	<i>aph IV</i>	70 72
Oryzacyclin ΔD86 (OC-1ΔD86)	Rice	Biolistic			African rice varieties (II, 'A212', 'DSAA6', 'LAC23', WAB56-104)	Significant reduction in egg production (50%).	73
LEA protein	Barley	Biolistic	Protoplasts	<i>bar</i>	Japonica (Nipponbare)	Transgenic plants showed tolerance to water stress and salinity.	Salinity tolerance
Phytoene synthase gene	Daffodil ( <i>N. pseudonarcissus</i> )	Biolistic	Immature embryos	<i>hpt</i>	Japonica (Taipei-309)	Accumulation of phytoene detected in endosperm.	Synthesis of provitamin - A
Antisense gene ( <i>rbcS</i> )	Rice	Biolistic	Mature embryos	<i>bar</i>	Japonica (Notokikai)	Reduction of RuBP carboxylase to a saturated level.	Use of nitrogen enhanced for efficient photosynthesis
Maize phosphoenol, pyruvate carboxylase gene	Maize	<i>Agrobacterium</i>	Scutellum-derived callus	<i>hpt</i>	Japonica (Kitakaze and Nipponbare)	Transgenic rice plants showed high level expression (up to 12% of total soluble leaf protein) and were effectively involved in photosynthesis by inhibiting O <sub>2</sub> .	To fix atmospheric N <sub>2</sub> for effective photosynthesis

method, which confers resistance to *Xanthomonas oryzae* *pv. oryzae*, a bacterial blight pathogen.

### Nematode resistance

Vain *et al.*<sup>73</sup> recovered nematode (*Meloidogyne incognita*)-resistant transgenic plants, containing genes coding for an engineered cysteine proteinase inhibitor (Oryza Cystatin-ID860; OC-I 8D86), using the particle gun method.

### Other genes

Xu *et al.*<sup>74</sup> introduced the barely *LEA3* gene into a japonica rice variety, Nipponbare, using the biolistic method and the transgenic plants showed increased tolerance to both water stress and salinity. Some other genes have been recently introduced in attempts to improve the nutritional and other qualities. Phytoene synthase from daffodil (*Narcissus pseudonarcissus*) was transferred into immature embryos of a rice model variety (Taipei-309) by microprojectile bombardment. Phytoene was unequivocally identified in several transgenic rice endosperms<sup>75</sup>. Rice mature embryos were subjected to the introduction of ribulose bisphosphate carboxylase gene. The rice plants used nitrogen with enhanced efficiency during photosynthesis at saturating levels of CO<sub>2</sub> and high irradiance<sup>76</sup>. Ku *et al.*<sup>77</sup> reported the high level expression (12% of total soluble protein) of maize phosphoenolpyruvate carboxylase (*PEPC*) gene in rice plants. The *PEPC* gene, which catalyses the initial fixation of atmospheric CO<sub>2</sub> in C<sub>4</sub> plants was introduced into the C<sub>3</sub> crop rice.

### Promoters used in rice transformation

The success of plant transformation depends very much on promoter sequences<sup>78</sup>. To express the transgenes in plant cells, appropriate promoter sequences have to be introduced alongside the gene to ensure efficient transcription of mRNA<sup>79</sup>. A large number of promoters have been used in rice transformation (Table 2) and several promoter sequences have been isolated from monocots for specific use in cereal species for the efficient expression of the transgenes. The promoter CaMV 35S or its derivatives have been used greatly<sup>7,20,27,28,64,80-83</sup>; the level of gene expression of this promoter has been reported to vary between different species of plants and different parts of the plant<sup>84</sup>. Further, it has been proved to be useful particularly in dicot species<sup>78</sup>. Unfortunately the levels of gene expression produced by this promoter in cereals are up to hundred-fold less than in dicots<sup>85,86</sup>. A number of monocot promoters (EMU<sup>87,88</sup>, Actin1<sup>30,55,64,89,90</sup>,

**Table 2.** Constitutive and non-constitutive promoter genes used in rice transformation

Promoter	Source	Gene	Pattern of gene expression	Reference
35S	Cauliflower mosaic virus	Cauliflower mosaic virus, 35S RNA transcript	All plant tissues	20, 27, 28, 64, 80-83
EMU	Maize	Modified maize alcohol dehydrogenase 1 promoter	All tissues	87, 88
Actin 1	Rice	Rice actin 1 gene	All plant tissues	30, 55, 64, 89, 98
Ubi-1	Maize	Maize ubiquitin 1 gene	All plant organs	71, 88, 91-93
Adh	Maize	Alcohol dehydrogenase II gene	Constitute root cap, anther, filament, pollen, scutellum, endosperm, embryo shoot and root, Aerobically induced in roots	94, 95
His 3	Wheat	Histone 3 gene	Dividing root cells	96
LHCP	Rice	Light harvesting chlorophyll binding gene of photosystem II	Light inducible in leaves, stems and floral organs	97
rbCS	Rice, tomato	Ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit gene	Light inducible in mesophyll	98
Pin II	Potato	Wound inducible II gene	Wound inducible	54, 55
Rol C	<i>Agrobacterium rhizogenes</i>	Open reading frame 12 (RF12) of the R <sub>1</sub> plasmid T <sub>2</sub> DNA region	Vascular tissue and embryonic tissue	88, 89
RTBV	Rice tungro bacilli form virus	Rice tungro bacilli form virus, major transcript gene	Leaf phloem tissue	100
OSg6B	Maize and rice	Glycine-rich gene	Tapetum specific expression	101
OsgrP1	Rice	Glycine-rich cell wall protein gene	Expressed in young stem, leaves, vascular bundles, epidermis, wound inducible, and in water stress conditions	102
RSs1	Rice	Rice sucrose synthase gene	Phloem-specific expression	57, 58, 103
PEPC	Maize	Phosphoenol pyruvate carboxylase gene	Green tissues, leaves and leaf blades	43, 77
Bp10 gene	Brassica	Pollen gene	Pollen-specific expression	50
GluA-2	Rice	Glutelin gene	Endosperm specific	104
GluB-1	Rice	Glutelin 1-gene	Endosperm specific	75, 104
GluA-3	Rice	Glutelin 3-gene	Endosperm specific	104
RAG-1	Rice	Albumin 1-gene	Endosperm specific	104
GLb-1	Rice	Globulin gene (Glb-1)	Endosperm specific	104
NRP33	Rice	Prolamin (NRB 33)	Endosperm specific	104
Ltp2	Barley	Lipid transfer protein	Aleurone-specific gene	105

ubiquitin-1<sup>71,91-93</sup> and Adh1<sup>94,95</sup>) have also been evaluated to enhance gene expression in cereals. Among these promoters, hpt and EMU hph constructs gave a more dramatic expression than ubiquitin gene and any of the bar containing constructs. The gene for Actin1 and ubiquitin-1 (gene of maize) has been shown to produce highest levels of expression in rice. Other promoters such as His<sup>96</sup>, LHCP<sup>97</sup>, reCS<sup>98</sup>, Pin<sup>54,55</sup>, Rolc<sup>88,99</sup>, RTBV<sup>100</sup>, O8gCB<sup>101</sup>, OsgrP1<sup>102</sup>, RSs1<sup>57,58,103</sup>, PEPC<sup>43,77</sup>, BP10<sup>50</sup>, Glu<sup>104</sup>, Ltp<sup>105</sup> have also been used successfully.

### Integration and expression of transgenes

The stable integration, expression and inheritance of transgenes are of great importance in the application of genetically transformed rice to agriculture, but they have not been extensively studied<sup>106</sup>. Only the first generation of transgenic plants (R<sub>0</sub> progeny) were analysed

in many reports. Hiei and Komari<sup>107</sup> analysed the inheritance of transgenes as far as the R<sub>4</sub> generations successfully for eighteen transgenic plants that have been produced by the *Agrobacterium*-mediated method and stable integration of transgenes in the genome of all progenies was demonstrated. In this study only a small number (fewer than three) of copies of the transgene integrated in a majority of the transformants. Due to this advantage *Agrobacterium*-mediated transformation is the method of choice. However in another study molecular analysis of genome DNA from engineered plants indicated the presence of vector sequences outside the T-DNA border<sup>108</sup>. *Agrobacterium* was also found to persist on the surface and within tissues of transformed plants in soil-grown plants up to twelve months following transformation<sup>109</sup>. These are the two potential problems in *Agrobacterium*-mediated transformation<sup>110</sup>. By contrast, direct transformation technology will result in

transgenic plants containing multiple copies of transgenes, complicated pattern of integration<sup>111</sup> and high occurrence of chimeric events<sup>112</sup>.

Takano *et al.*<sup>113</sup> analysed the transgenic plants produced from protoplasts through the calcium phosphate method. One factor that contributed to the complex patterns of integration of foreign genes in transformation is the probable activity of topoisomerase I or II. Because of these illegitimate recombinations, accomplishing rearrangements, large-scale deletions and translocations were found at the sites of integration. Further, recognition sites for topoisomerase were identified in the rearranged sequences involved in the process of gene silencing (expression of transgene and even native gene is suppressed)<sup>114</sup>. The inactivation of gene expression is known as co-suppression, when homologous coding sequences are involved. Silencing of the waxy gene was observed in rice that had been transformed with a cloned waxy gene<sup>115</sup>. This is the first study of silencing of a native gene and a transgene. A similar type of study on silencing of transgenes and 5-azacytidine-mediated reactivation of the transgene have been reported in rice<sup>116</sup>. Silencing of the genes encoding  $\beta$ -glucuronidase in transgenic rice was demonstrated by methylation<sup>117</sup>. Such type of studies probably help to understand the intrinsic mechanisms for the control of gene expression in rice and also the molecular mechanisms of plants.

## Conclusion

It is obvious that rice transformation system through both *Agrobacterium* and particle bombardment has become a routine procedure in many laboratories for the production of transgenic plants. Transgenic plants containing genes of agronomic interest including insect, herbicide, virus and nematode resistance through direct transformation techniques have been obtained. The next challenge for rice improvement via genetic engineering is to produce viral tungro disease resistance which can help in preventing the annual loss in the range of 1.5 billion US dollars in south and south-east Asia as well as to produce iron-rich rice, including introduction of the apomictic trait which will allow to fix hybrid vigour in the progeny without any further segregation.

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## RESEARCH ARTICLES

# Resolution of the nonlocality puzzle in the EPR paradox

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**Correlations of quantum-entangled multiparticle systems have been widely discussed in the context of the Einstein–Podolsky–Rosen (EPR) paradox. Bell’s theorem prohibits a local realistic description of these correlations. The standard quantum mechanical derivation as well as the interpretation of the experiments suggest that a mysterious nonlocality is a basic feature of these correlations. We show that the correlations of space-like separated entangled particles can be reproduced starting from local probability amplitudes. The use of complex number amplitudes circumvents the widely discussed Bell’s theorem. The result implies no-collapse-at-a-distance and resolves the EPR puzzle.**

THE Einstein–Podolsky–Rosen (EPR) nonlocality puzzle is one of the most discussed fundamental problems in physics<sup>1–4</sup>. EPR considered a two-particle quantum

system entangled and correlated in position and momentum and argued that under the assumption of locality and a reasonable definition of physical reality, the quantum mechanical description is incomplete. The conclusion of incompleteness was based on their reasoning that suggested the need for simultaneous existence of definite values for noncommuting observables for the individual particles before a measurement was performed. The EPR analysis was motivated by the desire to assign an objective reality to measurable properties in the microscopic world independent of the observer or apparatus. The Copenhagen interpretation of quantum mechanics rejected any such objective reality. EPR proposed that if the value of an observable could be predicted with certainty without the disturbance of a measurement, then there was an element of physical reality associated with that observable for the system. There is no way to probe experimentally such a physical reality for the single quantum, for example the reality of the spin component in a specific direction for a single

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