

Review

Recent advances in rice biotechnology—towards genetically superior transgenic rice

Shavindra Bajaj¹* and Amitabh Mohanty²

¹Gene Technology, The Horticulture and Food Research Institute of New Zealand Limited (HortResearch) 120 Mt. Albert Road, Private Bag 92169, Auckland, New Zealand

²Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY11724, USA

Received 17 November 2004;

revised 1 February 2005;

accepted 3 February 2005.

*Correspondence: (fax +64 9 8154201;

e-mail sbajaj@hortresearch.co.nz)

Summary

Rice biotechnology has made rapid advances since the first transgenic rice plants were produced 15 years ago. Over the past decade, this progress has resulted in the development of high frequency, routine and reproducible genetic transformation protocols for rice. This technology has been applied to produce rice plants that withstand several abiotic stresses, as well as to gain tolerance against various pests and diseases. In addition, quality improving and increased nutritional value traits have also been introduced into rice. Most of these gains were not possible through conventional breeding technologies. Transgenic rice system has been used to understand the process of transformation itself, the integration pattern of transgene as well as to modulate gene expression. Field trials of transgenic rice, especially insect-resistant rice, have recently been performed and several other studies that are prerequisite for safe release of transgenic crops have been initiated. New molecular improvisations such as inducible expression of transgene and selectable marker-free technology will help in producing superior transgenic product. It is also a step towards alleviating public concerns relating to issues of transgenic technology and to gain regulatory approval. Knowledge gained from rice can also be applied to improve other cereals. The completion of the rice genome sequencing together with a rich collection of full-length cDNA resources has opened up a plethora of opportunities, paving the way to integrate data from the large-scale projects to solve specific biological problems.

Keywords: Agronomic improvement, biosafety, genetic transformation, marker-free transgenics, nutritional improvement, *Oryza sativa*, transgenic rice.

Introduction

Rice is the most important food crop in the world. Almost half of the world's population depend on rice as their staple food (Coffman and Juliano, 1987). So far, advances in breeding of rice has kept pace with the growing demand, despite losses suffered as a result of various biotic and abiotic factors. Over the next 7 to 8 years, global rice plantings are predicted to remain static, which will result in a shortfall of about 130 million tonnes (Khush, 2001, Brookes and Barfoot, 2003). Therefore, conventional breeding methods need to be supplemented with recent achievements in rice biotechnology to meet the needs of the growing world population. Rice genetic transformation has taken rapid strides since the first transgenic rice plant was produced 15 years ago. During the last 10 years, tremendous progress has been

made to develop a high-frequency, routine and reproducible genetic transformation protocol of rice either through direct DNA transfer or by *Agrobacterium*-mediated transformation technologies. Using these technologies, a number of agronomically important traits, including quality improvement and increased nutritional value, have been addressed. These improvements could not be possible through the application of conventional breeding technologies. Rice reached another milestone by becoming the first crop plant to have its genome sequenced (Sasaki and Burr, 2000; Barry, 2001; Feng *et al.*, 2002; Goff *et al.*, 2002; Sasaki *et al.*, 2002; Yu *et al.*, 2002; Delseny, 2003; The Rice Chromosome 10 Sequencing Consortium, 2003; Sasaki *et al.*, 2005). Besides its own improvement, the sequencing of rice genome opens up the possibility of improving other cereals such as maize and wheat as there is significant conservation

of genes among cereals (Devos and Gale, 2000; Tyagi *et al.*, 2004). It has been suggested that these advances in rice and other crops will realize a second green revolution through genetic engineering of food crops (Sakamoto and Matsuoka, 2004).

The commercialization of transgenic rice has lagged behind genetically modified maize, which has been grown commercially since 1996 and planted on more than 15 million hectares (James, 2003). This is not surprising given the vast commercial potential of transgenic maize in both industrial and developing world. The fact that rice is primarily grown in developing countries, where the big economic gains are unlikely to be achieved, has probably contributed to the delays in achieving advances in rice. In this context, the role of the Rockefeller Foundation programme for rice biotechnology has been the key to rice improvement (O'Toole *et al.*, 2001). Although this funding has been stopped after 16 years of support, it has positioned rice biotechnology at a stage where it can be advanced using public/government funding.

Progress on rice biotechnology has been reviewed consistently in peer-reviewed journals (Ayres and Park, 1994; Christou, 1996; Tyagi *et al.*, 1999; Giri and Laxmi, 2000; Tyagi and Mohanty, 2000). These reviews have focused on the development phase of rice transformation, including the optimization of gene delivery methods, establishment of high frequency transformation, choice of reporter or selectable marker and producing agronomic superior rice. However, in the last five years (since the publication of detailed reviews in rice biotechnology – See Giri and Laxmi, 2000; Tyagi and Mohanty, 2000; Upadhyaya *et al.*, 2000c), the focus has shifted to include use of rice as a model monocotyledon system, similar to the use of *Arabidopsis* as model for dicotyledons. This review highlights the progress on transgenic rice technology over the last five years with particular emphasis on agronomic and nutritional traits. The advances in related technologies such as new constitutive or inducible promoters to drive transgene expression, use of selectable markers other than antibiotic/herbicide selection and development of marker-free transgenic plants are also discussed. The readers are referred to reviews cited above for the background about progress made to produce genetically improved transgenic rice prior to 2000 although for the sake of continuity, some of them are discussed in this review (Table 1). The advances in structural and functional genomics of rice and related technologies such as T-DNA and transposon-mediated insertional mutagenesis are described elsewhere (Mohanty and Bajaj, in prep.). For a comprehensive review of molecular breeding of rice, including the use of molecular markers please refer to the review by Gowda *et al.* (2003). Finally, because transgenic rice field trials are being initiated, we have also discussed related

biosafety and economic concerns. We have emphasized that a pragmatic approach is required while addressing the biosafety issues so that both farmers and consumers can reap the benefit of the technology without any delay.

Rice as a model monocot system

Rice with its relatively small genome size (~430 Mb), ease of transformation, well developed genetics, availability of a dense physical map and molecular markers (Chen *et al.*, 2002; Wu *et al.*, 2002a), together with its complete genome sequence (Sasaki *et al.*, 2005) is considered a model monocot system (Goff, 1999; Shimamoto and Kyoizuka, 2002). It is being used to understand several fundamental problems of plant physiology, growth and developmental processes ranging from elucidation of a single gene function to whole metabolic pathway engineering (reviewed in Mohanty and Bajaj, in prep.). In addition, rice shares extensive synteny among other cereals thereby increasing the utility of this system (Devos and Gale, 2000). These, together with availability of ~28 000 full length cDNAs (The Rice Full Length cDNA Consortium, 2003), a large number of expressed sequence tags, yeast artificial chromosome, bacterial artificial chromosome, P1-derived artificial chromosomes libraries and rich forward and reverse genetics resources (Hirochika *et al.*, 2004) has made rice a worthy forerunner among the plants especially among the cereals. It is expected that leads obtained from rice genome would be followed for other cereals with larger genomes.

Rice transformation

Methods of transformation and characterization of transgenic rice

Recently, efforts have been focused to make different rice genotypes amenable for regeneration and transformation using *Agrobacterium* (Azhakanandam *et al.*, 2000a; Jiang *et al.*, 2000; Mohanty *et al.*, 2000; Pons *et al.*, 2000; Upadhyaya *et al.*, 2000a; Lin and Zhang, 2004; Kumria *et al.*, 2001; Rachmawati *et al.*, 2004) essentially optimizing the protocol developed by Hiei *et al.* (1994) using mature embryos as explants. Other explants such as inflorescence (Dong *et al.*, 2001) and green tissues induced from mature embryos (Cho *et al.*, 2004) have also been used as explants for transformation. More significantly, a large-scale *Agrobacterium*-mediated procedure (up to 10³ stable transformants from 100 seeds) making it realistic to perform gene-targeting in rice, has also been developed (Terada *et al.*, 2004).

Table 1 Milestones in rice biotechnology

Year	Development	Reference
1988	First transgenic rice obtained	Toriyama <i>et al.</i> , 1988; Zhang <i>et al.</i> , 1988; Zhang and Wu, 1988
1989	First fertile transgenic rice plants (japonica)	Shimamoto <i>et al.</i> , 1989
1990	First fertile transgenic rice plants (indica)	Datta <i>et al.</i> , 1990
1991	Use biolistics for generation of rice transgenics	Christou <i>et al.</i> , 1991
1993	Insect resistance rice by using δ -endotoxin (<i>Bt</i>)	Fujimoto <i>et al.</i> , 1993
	Transgenic rice with <i>bar</i> gene for sheath blight resistance	Uchimiya <i>et al.</i> , 1993
1994	First conclusive report of rice transformation by <i>Agrobacterium</i>	Hiei <i>et al.</i> , 1994
1995	Resistance against bacterial blight obtained by using cloned <i>Xa21</i> gene	Song <i>et al.</i> , 1995
1996	Insect resistance transgenic indica rice harbouring <i>cryIAb</i> gene	Wunn <i>et al.</i> , 1996
	Field trial of herbicide resistance transgenic rice	Oard <i>et al.</i> , 1996
	Insect resistance transgenic rice harbouring <i>pinII</i> gene	Duan <i>et al.</i> , 1996
1997	Use of reconstructed <i>cryIAC</i> gene for high-level resistance against pests	Nayak <i>et al.</i> , 1997
1998	Multigene transformation	Chen <i>et al.</i> , 1998
	First report of <i>Agrobacterium</i> -mediated rice transformation for agronomically important genes (<i>cryIAb</i> and <i>cryIAC</i>)	Cheng <i>et al.</i> , 1998
1999	Iron fortified transgenic rice with soybean ferritin gene	Goto <i>et al.</i> , 1999
	Resistance against rice yellow mottle virus (RYMV) derived by pathogen-derived resistance	Pinto <i>et al.</i> , 1999
2000	Field trial of hybrid rice containing <i>Bt</i> gene	Tu <i>et al.</i> 2000b
	Golden rice	Ye <i>et al.</i> , 2000
	Draft sequence of rice genome announced	Monsanto*
2002	Draft sequence of rice genome	Goff <i>et al.</i> , 2002; Yu <i>et al.</i> , 2002
	Gene targeting by homologous recombination	Terada <i>et al.</i> , 2002
	Complete sequence of rice chromosome 2	Sasaki <i>et al.</i> , 2002
	Complete sequence of rice chromosome 4	Feng <i>et al.</i> , 2002
2003	Complete sequence of rice chromosome 10	Rice chromosome 10 consortium, USA, 2003
2004	Completion of rice genome by IRGSP	Sasaki <i>et al.</i> , 2005

Abbreviations: *Bt*, *Bacillus thuringiensis*; IRGSP, International rice genome sequencing program; *pinII*, potato proteinase inhibitor II gene; *Xa*, native rice gene for resistance against bacterial blight; * Barry, 2001.

Genetic transformation either mediated by *Agrobacterium* or through particle bombardment has its own merits and limitations (Dai *et al.*, 2001b). T-DNA generally integrates into gene rich transcriptionally active regions of rice genome (Barakat *et al.*, 2000; Eamens *et al.*, 2004; Sha *et al.*, 2004). It can also cause integration of multiple elements in different patterns of inverted or tandem repeats (De Buck *et al.*, 2000; Eamens *et al.*, 2004). However, transformation through particle bombardment also results in multiple rearrangements of the transgene (Dai *et al.*, 2001b). *Agrobacterium*-mediated transformation results in lower copy transgene insertion (Dai *et al.*, 2001b), and is easy, simple and inexpensive. Where available, it is unlikely to be replaced by particle bombardment. Recently, efforts have been made to produce transgenic rice devoid of elements, which otherwise are important for transformation such as selectable marker or vector backbone, but do not impart any value to the trait. The

vector backbone sequences have been detected in transgenic plants, including rice, generated either by *Agrobacterium* (DeBuck *et al.*, 2000; Yin and Wang, 2000; Kim *et al.*, 2003b) or through particle bombardment (Kohli *et al.*, 1999). It has been argued that these sequences may have undesired effects on the transgene stability and/or expression (Breitler *et al.*, 2002). Therefore, rice transformation procedures through particle bombardment with minimal linear gene cassette (promoter, coding region of the gene and terminator) have been developed (Fu *et al.*, 2000; Breitler *et al.*, 2002; Loc *et al.*, 2002). The plants that harbour the minimal transgene cassette showed simple low-copy number integration events and fewer rearrangements than transgenic plants produced with whole plasmids (Fu *et al.*, 2000). This prompted the suggestion that particle bombardment of rice with minimal expression cassette offers a superior alternative to *Agrobacterium* to produce so called 'clean' transgenics, i.e., devoid

of any backbone sequences, of agronomic and industrial interest (Fu *et al.*, 2000). In the near future, it is expected that transformation methods will be developed that will rely minimally on the bacterial or viral sequences and instead use plant genomic elements for gene transfer. Recently, DNA sequences termed as 'P-DNA' have been identified in potato that can replace *Agrobacterium* left border (LB) and right border (RB) sequences (Rommens *et al.*, 2004). It would also be feasible to use a transformation cassette consisting entirely of plant DNA (i.e. plant DNA as source for the promoter, the gene of interest and the terminator). This cassette, when transferred either through particle bombardment or *Agrobacterium* using marker-free selection systems (discussed later), would result in a true 'clean' transgenic plant. These constructs would also be useful for multigene engineering. The examples include various abiotic stresses (Bajaj *et al.*, 1999), resistance management strategies in insect-resistant crops or for plant metabolic engineering. As a beginning, transgenic rice plants carrying multigenes have been shown to be stable in integration and expression (Gahakwa *et al.*, 2000; Wu *et al.*, 2002b) and genes of agronomic interest have been pyramided (Datta *et al.*, 2002). However, it should be noted that whatever the method of transformation is, the integration of transgenes in plants occurs through illegitimate recombination. Therefore, it is important to understand the process of integration, the state of DNA that is integrated, and the relationship between extrachromosomal integration and transgene interaction. Recent reviews (Kohli *et al.*, 2003; Somers and Makarevitch, 2004) provide excellent reading on this subject.

Novel reporter and selection marker genes

The most commonly used reporter genes used in rice transformation are the β -glucuronidase gene (*GUS*) (Jefferson *et al.*, 1987) and green fluorescent protein (*GFP*) genes (Chalfie *et al.*, 1994; Vain *et al.*, 1998) although other reporters such as maize anthocyanin, firefly luciferase (*LUC*) genes have also been used (see Tyagi *et al.*, 1999). Recently, the Centre for Application of Molecular Biology to International Agriculture (CAMBIA) has developed *GUS Plus*, which has been codon optimized for high expression in plants, and results in extremely sensitive assays (Roberts *et al.*, <http://www.cambia.org/main/r_et_vman.htm>). A synthetic sialidase reporter gene called NAN fusion has also been reported (Kirby and Kavanagh, 2002). This could be used as a sensitive and versatile partner for *GUS* for dual promoter studies as well as for internal control. More recently, a synthetic xylanase (*sXynA*) as a reporter system in rice in conjunction with *GUS Plus* for accurate and sensitive

quantification in transient gene expression studies has been developed (Vickers *et al.*, 2003) as xylanase (XYN) protein is stable, activity slopes are linear over long time periods and the assays are cost-effective over other assays such as *LUC*.

To date, *GUS* has been the choice as the reporter gene. However, the advantages of using *GFP* or variants thereof over other markers are several. First of all it provides a non-invasive way for detection of transgene product. Besides, it allows one to monitor the expression, dynamics as well as interactions of proteins *in vivo* in real time. Work by several different laboratories has resulted in panoply of different variants such as yellow fluorescent protein (YFP), citrine (a pH insensitive version of YFP), cyan fluorescent protein (CFP) and red fluorescent protein (RFP). Besides the original *GFP* from *Aequorea victoria*, fluorescent proteins have since been isolated from Anthozoan species as well as corals. Availability of such proteins allows the use of dual or multicolor imaging for analysis of fusion proteins *in vivo* thereby opening up a whole new dimensions in plant cell biology (Cutler and Ehrhardt, 2000; Jach *et al.*, 2001; Brandizzi *et al.*, 2002; Tian *et al.*, 2004).

Currently, transgenic crops rely heavily on the use of either an antibiotic resistance gene, or herbicide tolerance gene for the selection of transgenic tissues over non-transformed cells. Public apprehensions over the safety of such genes have led to research on developing new selectable markers that are more acceptable or even produce marker-free transgenic plants (Joersbo, 2001; Datta *et al.*, 2003; Rommens *et al.*, 2004).

Several selection systems successfully tested in model dicots, have now been extended to rice. Transgenic rice plants using a positive selection system based on the phosphomannose isomerase gene as the selectable gene and mannose as selection agent have been produced (Lucca *et al.*, 2001b; Datta *et al.*, 2003; He *et al.*, 2004). Similarly, a positive selection system based on galactose as selective agent using UDP-glucose:galactose 1-phosphate uridyl transferase gene as selectable marker has been described in other crops (Joersbo *et al.*, 2003). A mutant rice gene, feedback-insensitive α subunit of anthranilate synthase *OsSAID*, has been used as selectable agent in rice based on its ability to detoxify tryptophan analogue 5-methyltryptophan (Yamada *et al.*, 2004). Similarly, new negative or conditional selection systems have been developed for rice transformation (Dai *et al.*, 2001a; Upadhyaya *et al.*, 2000b; Oreifig *et al.*, 2004). A new positive/negative selection system employing a marker gene, *dao1* from yeast *Rhodotorula gracilis* has been described (Erikson *et al.*, 2004). The *dao1* product DAAO can be used as positive or negative selectable marker based on the substrate amino acid (Erikson *et al.*, 2004). It has been proposed

that DAAO could prove even more useful in combination with site-specific excision systems. After positive selection of transgenics followed by initiation of recombination, turning to negative selection would verify the removal of the now redundant selectable marker (Scheid, 2004). It will be interesting to see the application of such technology in commercial crops.

It must be noted that transgenic plants using the selectable markers described above have not yet reached the field-trial stage. Therefore, it will be several years before these become available for commercial production.

Marker-free selection

It may be desirable to remove selectable marker from transgenic crops to alleviate safety concerns (Hohn *et al.*, 2001). The simplest way to remove the selectable marker gene is by segregation, if the gene of interest and the marker gene are integrated at independent loci (Tu *et al.*, 2003). Alternatively, molecular techniques can be relied upon for the detection of transformants (de Vetten *et al.*, 2003) allowing marker-free transgenics. Techniques such as *cre/loxP* of bacteriophage P1, FLP/FRT and maize *Ac/Ds* transposon system have been used to remove the selectable marker genes in the progeny of transgenic rice and other plants (Gleave *et al.*, 1999; Cotsaftis *et al.*, 2002; Endo *et al.*, 2002). However, these methods, although useful, are time-consuming as they require crossing of the progenies to generate selectable marker free plants. A 'twin T-DNA' binary vector for the recovery of selectable marker-free plants (Komari *et al.*, 1996) was improvised by constructing a double right border (RB) binary vector (Lu *et al.*, 2001). The two RB sequences will initiate two types of T-DNA inserts, one carrying both selectable gene and gene of interest and the other carrying just gene of interest to the plant genome, which can then be segregated in subsequent generations (Lu *et al.*, 2001) to recover marker-free plants. Alternatively, a two T-DNA binary vectors harbouring the gene of interest on one and selectable marker gene on another T-DNA followed by cotransformation can also be used (Komari *et al.*, 1996; Breitler *et al.*, 2004a). Another way for producing marker-free transgenic plants was proposed by Vain *et al.* (2003) using a new dual binary vector system, pGreen/pSoup (Hellens *et al.*, 2000). The pGreen is a small Ti binary vector unable to replicate in *Agrobacterium* without the presence of another binary plasmid, pSoup in the same strain. Co-transformation with one vector (pGreen) carrying the gene of interest and the other vector (pSoup) carrying the selectable marker may lead to production of transgenic plants with multilocus unlinked integration and would allow

recovery of selectable marker-free plants in subsequent progeny (Vain *et al.*, 2003).

A system called the multi-auto transformation (MAT) vector system that makes use of the oncogenes of *Agrobacterium* as positive marker for selection of transformed tissues, has been described (Endo *et al.*, 2002). Rice calli were transformed with the MAT vector containing the chimeric isopentyl transferase (*ipt*) gene from *Agrobacterium* and *gfp* genes fused with the *CaMV 35S* promoter and site-specific recombination *R/RS* system to excise them after transformation along with other genes (*gusA*, *nptII* and *hpt*) outside the *R/RS* cassette (Endo *et al.*, 2002).

Gene silencing and ways to overcome gene silencing in transgenic rice

A stable and consistent expression of the gene of interest across generations (Kathuria *et al.*, 2003) is necessary for the successful commercialization of transgenic plants. However, gene silencing does occur in transgenic plants and is one of the limitations of the transgene technology (see Bajaj *et al.*, 1999; Iyer *et al.*, 2000). Gene silencing could be a consequence of transcription inactivation by promoter methylation, known as transcriptional gene silencing (TGS) or through post-transcriptional gene silencing (PTGS) characterized by specific reduction of cytoplasmic RNA through sequence-specific degradation, with normal rates of nuclear transcription being maintained (see Vaucheret *et al.*, 1998). Gene silencing has been observed in transgenic rice (see Iyer *et al.*, 2000; Tyagi and Mohanty, 2000). The transgenes with truncated sequences or a rearranged transgene itself, which could be transcribed into aberrant RNA species, may be responsible for silencing (Kohli *et al.*, 1999; Morino *et al.*, 1999). Wang and Waterhouse (2000) showed that the PTGS is the result of un-polyadenylated RNA and methylation is not essential for PTGS. A significant advance in the understanding of gene silencing came from Kloti *et al.* (2002) who demonstrated tissue-specific silencing of a transgene in rice spread to various organs over generations. Treatment with 5-azacytidine reversed the TGS and methylation *per se* did not affect promoter region of the protein factors, instead promoter methylation enabled the alternative binding of a protein with specificity for sequence and methylation. Thus, it was shown that TGS occurs in different stages and one of them may be involved in the attraction of a sequence and methylation-specific DNA binding protein (Kloti *et al.*, 2002). Theoretically, it is possible to engineer the transgene to minimize gene silencing (Finnegan and McElroy, 1994). Matrix attachment regions (MARs) or scaffold attachment regions (SARs) are one of such sequences that have

been used for minimizing gene silencing and increase gene expression in transgenic plants, although with variable effect (reviewed by Holmes-Davies and Comai, 1998). The positive effect of MAR sequences on higher transgene expression and stability in transgenic rice has been reported (Vain *et al.*, 1999, 2002; Cheng *et al.*, 2001). Although higher transgene expression by MAR sequences has been established, however, whether they can reduce variation or produce integration patterns of lower complexity is not very convincing with claims on both sides (James *et al.*, 2002; Van der Geest *et al.*, 2004). Variations in transgene expression can also be reduced by using *cre/lox*-mediated site-specific integration to insert the gene of interest into rice genome (Srivastava *et al.*, 2004). It has been suggested that spurious variations in transgene expression that arise during generation of transgenic plants and growth in greenhouses may also affect the analyses of transgene expression and thus need to identify the source experimental variation and develop a uniform protocol (James *et al.*, 2004a,b).

Occurrence of gene silencing and variation in the expression of transgene product is a major limitation of transgenic technology. This would likely to be further compounded with the introduction of multi-traits or stacked genes controlling the same trait. Thus, the study of factors controlling gene silencing or to avoid it, would be major focus of the research in coming years. So far, the use of MAR sequences in constructs for transformation appears promising to reduce gene silencing.

For certain traits, it is desirable that the transgene product is targeted to the organelles as it provides obvious advantages, including high expression. The rubisco small subunit gene *rbcS* is encoded in the nucleus but targeted to chloroplast by a transit peptide (TP). In transgenic rice, the presence of *rbcS* TP sequence as a plastid-targeting signal greatly enhanced accumulation of transgene products (Jang *et al.*, 2002b) in plastids. When the TP was mutated, it led to accumulation of the reporter gene (*gfp*) product in the cytoplasm although the accumulation was lower than that of control TP targeted to chloroplast (Jang *et al.*, 2002b).

Introduction of agronomically useful genes in rice

Rice production needs to increase by 755 million tonnes by 2012 to meet the predicted demands of increasing population (Brookes and Barfoot, 2003). One option is to increase the area under rice cultivation, which is getting harder as more farm areas are being converted to residential development to house the increasing population in the developing world (Fischer *et al.*, 2000). The most viable option, therefore, is to

increase productivity by breeding efforts supplemented with advances in biotechnology. Biotechnology can play a major role in reducing the crop loss caused by various biotic and abiotic factors. Further, being the staple food for most of the developing world, the nutritional improvement of rice can also help reduce malnutrition.

In this section, we will review the progress made in the new millennium to produce genetically superior rice expressing agronomically important traits. The past five years have seen tremendous achievements in producing transgenic rice that are useful to both growers as well as consumers.

Promoter analysis

The rice *actin1* or maize *ubiquitin1* gene promoters are the promoters of choice of plant origin for high constitutive expression in monocot transformation (See Tyagi *et al.*, 1999; Tyagi and Mohanty, 2000). With the demand for multigene stacking in plants, it is important to develop a range of promoters that provide high and/or regulated expression of transgenes in monocots. Moreover, a reliable monocot transformation system is necessary for promoter analysis as their activity can vary tremendously between different species (Hwang *et al.*, 2001b; Ahmadi *et al.*, 2003). In recent years, several new constitutive or tissue-specific promoters have been tested in monocots. Transient expression using rice endosperm or generating rice transformants has become a quick screening for testing new promoters (Hwang *et al.*, 2001a; Qu and Takaiwa, 2004). Now, a broad-range of promoters, constitutive or inducible, are available (Potenza *et al.*, 2004; Tang *et al.*, 2004). New promoters tested in rice during the last five years ranged from promoters isolated from monocots such as sugarcane and rice (Wang *et al.*, 2000; Al Bitar *et al.*, 2002; Jang *et al.*, 2002a; Wang and Oard, 2003; Wei *et al.*, 2003; Iwamoto *et al.*, 2004), to dicots (Mohanty *et al.*, 2000), as well as from viruses (Fukuoka *et al.*, 2000). Similarly, new tissue-specific or inducible promoters and/or elements that impart tissue-specificity to these promoters isolated from plants or viruses have been analysed in transgenic rice (Ito *et al.*, 2000; Mazithulela *et al.*, 2000; Wu *et al.*, 2000; Huang *et al.*, 2001; Su *et al.*, 2001; Fukuzawa *et al.*, 2004; Martinez-Trujillo *et al.*, 2004; Nguyen *et al.*, 2004). The presence of introns are also important for tissue-specific expression of a gene as demonstrated by (Jeon *et al.*, 2000), who showed that *intron1* of rice gene encoding for α -tubulin *OsTubA1*, directed higher expression in young tissues than mature tissues. The previous discussion reports show that promoters from different sources are functional in rice. However, it would be several years before these can be used for commercial traits.

Insect resistance

Despite significant efforts, it has not been possible to find endogenous genes for sufficient levels of resistance against a variety of insects (Schuler *et al.*, 1998). Apart from the losses and the monetary cost, there are significant health risks associated with the extensive use of agrochemicals, especially in developing countries, because of non-application of safety measures required for chemical spraying. Further, chemical insecticides have an adverse effect on beneficial insects and persistence of these chemicals in the environment is also an important issue. In this context, genetic engineering of rice for insect resistance provides a potent, cost-effective and environment friendly option. The most important source of genes to produce insect resistant transgenic plants, including rice, are the well-known group of related genes called *cry* or *Bt* genes, in different strains of bacterial species *Bacillus thuringiensis* (de Maagd *et al.*, 1999; Tabashnik *et al.*, 2003), although few non-*Bt* genes have been used to produce transgenic rice for insect resistance.

Insect resistance using *Bt* genes

The first *Bt* rice plants were produced more than 10 years ago (Fujimoto *et al.*, 1993 Table 1) and is now already under

field trials (Tu *et al.*, 2000b; Anonymous, 2001; High *et al.*, 2004; Tables 2 and 5). It has been suggested that *Bt* rice can eliminate yield loss caused by lepidopteran pests estimated at 2–10% of the total rice yield in Asia besides offering a safer option of pest control (High *et al.*, 2004). The most significant research has been conducted in China and International Rice Research Institute (IRRI) towards the field release of *Bt* rice. Shu *et al.* (2000) reported a broad spectrum of resistance of a line KMD1 of a Chinese japonica rice cultivar, Xuishi 11 transformed with a synthetic *Cry1Ab* gene. This line was resistant to eight lepidopteran pest species, including economically important *Chilo suppressalis* (striped stem borer, SSB) and *Scirpophaga incertulas* (yellow stem borer, YSB) under laboratory as well as under natural infestation. Further, field trials using two different lines from above transgenic rice plants expressing synthetic *Cry1Ab* showed high resistance against these pests (Ye *et al.*, 2001). These lines can be used as insect-resistant germplasm to cross into other important agronomic varieties as well as in hybrid rice production (Wang *et al.*, 2002a). Significantly, transgenic *Bt* hybrid rice has also been field tested in China and has been found to be highly protected against rice leaf folder (*Cnaphalocrocis medinalis*, RLF) and YSB (Tu *et al.*, 2000b). *Bt* rice has also been developed

Table 2 Examples of insect-resistant transgenic rice generated since year 2000

Gene	Target insects	Highlights	References
<i>Cry1Ab</i>	SSB, YSB	Field trials show high protection against these insects	Shu <i>et al.</i> (2000); Ye <i>et al.</i> (2001)
<i>Cry1Ab</i> , <i>Cry1Ac</i>	YSB, Leaf folder	Field-tested insect-resistant <i>Bt</i> indica hybrid rice with high insect protection and no reduction in yield	Tu <i>et al.</i> (2000b)
<i>Cry1Ab</i> or <i>Cry1Ac</i>	SSB, YSB	Transfer of insect-resistant trait into commercial varieties and/or stable expression over several generations	Shu <i>et al.</i> (2002); Wang <i>et al.</i> , (2002a); Wu <i>et al.</i> (2002c)
<i>Cry1Aa</i> or <i>Cry1Ab</i>	SSB	<i>Bt</i> rice for Mediterranean conditions; already field-tested	Breitler <i>et al.</i> (2000); Marfa <i>et al.</i> (2002); Breitler <i>et al.</i> (2004b)
<i>Cry1Ab</i> and/or <i>Cry1Ac</i>	YSB	Different varieties transformed for insect-resistance	Khanna and Raina (2002); Ramesh <i>et al.</i> (2004a,b);
<i>Cry1Ab</i>	SSB	Marker-free insect resistant transgenic rice plants	Cotsaftis <i>et al.</i> (2002)
<i>Cry1Ab</i>	SSB	Wound-inducible expression of <i>Cry1Ab</i>	Breitler <i>et al.</i> (2001)
<i>Cry1Ab</i>	YSB and leaf folder	Use of different promoters for tissue-specific <i>Bt</i> gene expression	Husnain <i>et al.</i> (2002)
<i>Cry1Ab</i> , <i>cry1Ac</i> and/or <i>gna</i>	Lepidopteran and sap-sucking insects	Gene-pyramiding for protection against broad-range of insects	Maqbool <i>et al.</i> (2001); Ramesh <i>et al.</i> (2004a)
<i>Cry</i> , <i>Xa21</i> and <i>RC7</i>	YSB, bacterial blight and sheath blight	Gene-stacking for multiple disease tolerance	Datta <i>et al.</i> (2002)
<i>gna</i>	Several homopteran, coleopteran and lepidopteran insects	Different varieties for multiple-range of insect resistance and/or no expression in seeds	Foissac <i>et al.</i> (2000); Tinjuangjun <i>et al.</i> (2000); Tang <i>et al.</i> (2001a,b); Wu <i>et al.</i> (2002d); Nagadhara <i>et al.</i> (2003; 2004)
<i>gna</i> and <i>Cry1Ac</i>	BPH and SSB	Use of linear constructs to avoid any integration of back-bone sequences in transgenic plants	Loc <i>et al.</i> (2002)
<i>ltr1</i>	Rice weevil	Constitutive or endosperm-specific expression of barley trypsin inhibitor BTI-CMe for protection against rice weevil	Alfonso-Rubi <i>et al.</i> (2003)
<i>Cry1Ac</i> ; <i>Cry2A</i>	YSB and leaf folder	Field-trial of <i>Bt</i> rice in Pakistan	Bashir <i>et al.</i> (2004)

Abbreviations: BPH, brown plant hopper; *ltr1*, *Hordeum vulgare* gene encoding for trypsin inhibitor BTI-CMe; SSB, striped stem borer; YSB, yellow stem borer.

in Pakistan (Husnain *et al.*, 2002; Bashir *et al.*, 2004) and in the Mediterranean (Breitler *et al.*, 2000; Marfa *et al.*, 2002; Breitler *et al.*, 2004b). Recent field trials in both locations suggested high tolerance of transgenic rice against YSB and RLF (Bashir *et al.*, 2004; Breitler *et al.*, 2004b). Various transgenic rice varieties resistant to YSB have been produced in India (Nayak *et al.*, 1997; Khanna and Raina, 2002; Ramesh *et al.*, 2004a,b) but so far there are no published reports of any field trials.

The field trials of *Bt* rice also revealed certain limitations of this technology. These include morphological variations in transgenic lines as compared to control plants (Shu *et al.*, 2002; Bashir *et al.*, 2004) and variations in *Bt* expression during the plant life cycle (Alinia *et al.*, 2000; Bashir *et al.*, 2004) or during several generations (Wu *et al.*, 2002c). It is very likely that these concerns will be addressed shortly.

The use of tissue-specific or wound-inducible promoters to drive expression of insect-resistant genes in transgenic plants has been suggested to reduce the biomass loss and delay resistance among the target insect population (de Maagd *et al.*, 1999; Sharma *et al.*, 2004). Breitler *et al.* (2001) demonstrated that –689/+197 region of the maize protease inhibitor gene provided high level, wound-inducible expression of *Cry1B* gene in transgenic rice plants and the plants were highly resistant to SSB damage. This report demonstrated that wound-inducible expression of a *Bt* gene could provide full protection to transgenic rice plants. Another strategy for long-term resistance against insects is the use of multiple genes with different mode of action against the same pest or a range of pests. It has been suggested that *Cry1Ab* or *Cry1Ac* could be combined with either *Cry1Ac*, *Cry2A* or *Cry9C* for long-term resistance in transgenic rice as *Cry1Ab* and *Cry1Ac* compete for the same binding site in SSB and YSB (Alcantara *et al.*, 2004). Use of *Bt* genes stacked with snowdrop (*Galanthus nivalis*) lectin gene, *gna* not only provided higher but also a broad range of resistance against pests, including non-lepidopteran sap-sucking insects (Maqbool *et al.*, 2001; Ramesh *et al.*, 2004a), on which the *Bt* genes have no effect alone (Bernal *et al.*, 2002). Stacking of genes is also an attractive strategy for multiple pest tolerance (Datta *et al.*, 2002).

The above discussion suggests that *Bt* rice technology has progressed significantly in the new millennium. It has been field-tested in several locations and as discussed later, the biosafety studies have also been initiated. It is expected that it will be commercially released in the next few years, most probably first in China.

Insect resistance with genes other than Bt

Although *Bt* genes have been most successfully and widely used for providing insect resistance in transgenic plants, it is

important to combine *Bt* with other insecticidal genes to provide durable resistance. Plants themselves may be the source of non-*Bt* insecticidal genes such as lectins, protease inhibitors or ribosome inactivating proteins (Sharma *et al.*, 2004). Plant lectins are a heterogeneous group of sugar-binding proteins that have a protective function against a range of organisms. The expression of mannose-specific lectin gene *gna* has been used extensively in transgenic rice for protection against a number of economically important homopteran, coleopteran and lepidopteran insects (Foissac *et al.*, 2000; Tinjuangjun *et al.*, 2000; Tang *et al.*, 2001b; Wu *et al.*, 2002d; Nagadhara *et al.*, 2003, 2004) (Table 2). However, protease activity, which would facilitate the degradation of GNA protein, has been detected in the gut of rice brown plant hopper (*Nilaparvata lugens*, BPH), thus limiting the activity of these proteins (Foissac *et al.*, 2002). The other important sources of genes to produce insect-resistant plants are genes encoding for proteinase inhibitors, as they are also part of plant's natural defence system against insect predation. Transgenic rice plants expressing proteinase inhibitors from various plant sources have been produced, and some have been tested under field conditions. These include synthetic gene coding for winged-bean trypsin inhibitors WTI-1B (Mochizuki *et al.*, 1999), Oryzacystatin, Cowpea trypsin inhibitors, potato proteinase inhibitors II and another class of proteinase inhibitors, the Kunitz family represented by soybean Kunitz trypsin inhibitors (reviewed in Tyagi *et al.*, 1999; Tyagi and Mohanty, 2000; Sharma *et al.*, 2004) as well as barley trypsin inhibitor BTI-CMe for resistance against rice weevil *Sitophilus oryzae* (Alfonso-Rubi *et al.*, 2003).

It is clear that research has now advanced to maximize the potential of *Bt* and to a lesser extent, lectin-producing rice plants. Advancements such as inducible expression of the transgene, pyramiding of the resistance genes to broaden the protection and delay the resistance of the insects against these genes would certainly help in the commercial application of insect resistant transgenic rice. Further, new proteins with protease inhibitor activity (Lawrence and Nielson, 2001; Hernandez *et al.*, 2003) and lectins having up to four times more binding ability to oligo/polyasaccharides than GNA have been identified (Lopez *et al.*, 2002). The cloning of genes encoding for these proteins will offer a choice of genes to produce insect-resistant transgenic rice plants.

Resistance against fungal pathogens

In rice, partial resistance to fungal diseases through conventional breeding has been achieved and could be increased further by using genetic engineering approaches.

Table 3 Recent advances in transgenic rice towards resistance against viral, fungal and bacterial diseases

Gene/function	Source	Performance of transgenic plant	References
RDV minor core protein S5 gene	RDV	Ribozyme-mediated resistance to RDV	Han <i>et al.</i> (2000)
RTSV replicase gene/RTBV RF2a and RF2b transcriptional activators	RTSV/RTBV	Tolerance to RTBV/disease-like symptoms	Huet <i>et al.</i> (1999); Petruccioli <i>et al.</i> (2001); Dai <i>et al.</i> (2003; 2004)
Gene encoding for RRSV	RRSV	Protection against RRSV infection	Chaogang <i>et al.</i> (2003)
39 kDa-viral spike protein			
RHBV nucleocapsid protein (N) gene	RHBV	Protection against RHBV	Lentini <i>et al.</i> (2003)
Pi-ta/NBS receptor class R gene	rice	Resistant to blast fungus, <i>Magnaporthe grisea</i>	Bryan <i>et al.</i> (2000)
Rir1b/defence-related gene	Rice	Resistant to blast fungus, <i>Magnaporthe grisea</i>	Schaffrath <i>et al.</i> (2000)
RC7/chitinase	Rice	Several cultivars of rice resistant to <i>Rhizoctania solani</i>	Datta <i>et al.</i> (2000); Datta <i>et al.</i> (2001)
Cry, Xa21 and RC7	YSB, bacterial blight and sheath blight	Gene-stacking for multiple disease tolerance	Datta <i>et al.</i> (2002)
C2/chalcone synthase	Maize	Expression of maize anthocyanin genes in rice for blast resistance	Gandikota <i>et al.</i> (2001)
RCH10 (chitinase)/MOD1 (maize ribosome inactivating protein)	Rice, maize	Resistance against <i>Rhizoctania solani</i>	Kim <i>et al.</i> (2003a)
chicC/chitinase	<i>Streptomyces griseus</i>	Resistant to <i>M. grisea</i>	Itoh <i>et al.</i> (2003a)
Ltp1/lipid transfer protein	Rice	Induced by pathogen attack	Guideroni <i>et al.</i> (2002)
pinA, pinB/antimicrobial/puroindoline protein	Wheat	Resistance against <i>M. grisea</i> and <i>R. solani</i>	Krishnamurthy <i>et al.</i> (2001)
Defensin/defensin	Wasabi	Resistant to <i>M. grisea</i>	Kanzaki <i>et al.</i> (2002)
TCS/trichosanthin, a ribosome inactivating protein	<i>Trichosanthes kirilowii</i>	Resistant to <i>M. grisea</i>	Yuan <i>et al.</i> (2002)
GOX/glucose oxidase	<i>Aspergillus niger</i>	Tolerant to multiple biotic stresses	Kachroo <i>et al.</i> (2003)
afp/Antifungal protein (AFP)	<i>Aspergillus giganteus</i>	Tolerant to <i>M. grisea</i>	Coca <i>et al.</i> (2004)
Gns1/1,3; 1,4- β glucanase	Rice	Resistant type lesions when infected with <i>M. grisea</i>	Nishizawa <i>et al.</i> (2003)
RBB12-3/serine protease inhibitor	Rice	Blast resistant transgenic rice	Qu <i>et al.</i> (2003)
OsSBP/selenium-binding protein homolog	Rice	Tolerance against fungal blast and bacterial blight	Sawada <i>et al.</i> (2004)
NPR1/resistance gene involved in systemic acquired resistance pathway	<i>Arabidopsis</i>	Transgenic rice tolerant to bacterial blight	Chern <i>et al.</i> (2001)
Xa21/resistance gene (leucine-rich repeat receptor kinase like protein)	Rice	Imparted bacterial blight tolerance to rice already made blast resistant by marker-aided selection	Narayanan <i>et al.</i> (2002)
Xa21	Rice	Similar expression in different transgenic lines resistant to bacterial blight	Zhai <i>et al.</i> (2004)
Xa26/leucine rice repeat receptor kinase-like protein	Rice	Another endogenous resistance gene for tolerance against bacterial blight	Sun <i>et al.</i> (2004)
cecB/cecropin B	<i>Bombyx mori</i>	Extra-cellular translocation of the transgenic protein for protection against bacterial blight	Sharma <i>et al.</i> (2000)
ap1/Ferredoxin-like protein, AP1	Sweet pepper	Tolerance against bacterial blight	Tang <i>et al.</i> (2001a)
Asthi1/thionins	Oat	Transgenic rice resistant to <i>Burkholderia plantarii</i>	Iwai <i>et al.</i> (2002)

Abbreviations: RDV, rice dwarf virus; RHBV, rice hoja blanca virus; RTBV, rice tungro bacilliform virus; RTSV, rice tungro spherical virus.

Relatively few *R* genes have been isolated in rice involved in fungal resistance. In this context, the recently cloned *Pi-ta* gene, a member of putative cytoplasmic NBS-receptor class *R* gene holds much promise for resistance against blast (Bryan *et al.*, 2000). The authors demonstrated that there was a single amino acid difference between the susceptible and resistance alleles. Presence of alanine at residue 918 correlated with gene-for-gene specificity of the *Pi-ta*/AVR-Pita system (Bryan *et al.*, 2000). The native resistance to sheath blight has also been mapped (on chromosome 5) (Che *et al.*, 2003). The linked molecular markers were also identified using a cross between one transgenic cultivar (resistant to sheath blight as a result of expression of DNA fragments of *Zizania*

caduciflora, a water-growing wild species of rice with high resistance to sheath blight) and a susceptible cultivar (Che *et al.*, 2003).

The pathogenesis-related (PR) protein genes induced by plant's defence response is one of the important and widely used genes to generate fungal resistant transgenic plants, including rice (see Tyagi and Mohanty, 2000; Muthukrishnan *et al.*, 2001; Table 3). Of the various classes of PR proteins, the important ones that have been shown to provide resistance against fungal diseases are class PR-2 proteins (β -1,3-glucanases), PR-3 (chitinases) and PR-5 proteins (thaumatin-like proteins) (Muthukrishnan *et al.*, 2001). The role of chitinases in fungal protection has also been well demonstrated in rice

(Datta *et al.*, 2000, 2001; Itoh *et al.*, 2003b; Kim *et al.*, 2003a). The progenies of indica rice expressing PR-3 rice chitinase gene showed variable levels of resistance against sheath blight fungus, *Rhizoctania solani* (Datta *et al.*, 2001). The *Rir1b* gene belongs to a family of defence-related genes (the *WIR1* family) that have been described so far only in cereals (Mauch *et al.*, 1998). Transgenic *Rir1b* over-expressing rice plants exhibited an increased but partial resistance to infection by blast fungus *Magnaporthe grisea* (Schaffrath *et al.*, 2000). Over-expression of stress-inducible PR-2 protein gene 1–3, 1–4 β glucanase gene *Gns1* of rice in transgenic plants showed resistant type lesions and activation of two defence related PR-1 and PB21 genes but also led to abnormal morphological features (Nishizawa *et al.*, 2003).

Apart from PR genes, several other gene products have been shown as good candidates to impart fungal tolerance in rice plants (Table 3). These include proteins involved in pathogen attack such as lipid transfer proteins (Guiderdoni *et al.*, 2002), selenium-binding protein homolog (Sawada *et al.*, 2004), genes involved in flavonoids pathway (Gandikota *et al.*, 2001), plant antimicrobial compounds such as puroindoline proteins that are also involved in grain texture (Krishnamurthy and Giroux, 2001; Krishnamurthy *et al.*, 2001), rice homolog of maize HC-toxin reductase (Uchimiya *et al.*, 2002), defensins (Kanzaki *et al.*, 2002), trichosanthins (Yuan *et al.*, 2002), phytoalexins (Lee *et al.*, 2004), protease inhibitors (Qu *et al.*, 2003), genes involved in cell death (Kachroo *et al.*, 2003; Matsumura *et al.*, 2003) other proteins such as antifungal protein (AFP) from *Aspergillus flavus* (Coca *et al.*, 2004) and mycotoxin detoxifying compounds (Higa *et al.*, 2003).

Transgenic rice resistant against fungal diseases is still in its developmental stage. The *R* genes for fungal resistance have been cloned recently (Bryan *et al.*, 2000). Regulated/tissue-specific expression of these genes in transgenic rice is a great prospect for imparting fungal resistance in rice.

Resistance against bacterial diseases

Earlier studies showed bacterial blight resistant transgenic rice expressing the endogenous resistance gene, *Xa21* (Song *et al.*, 1995). Since then, this gene has been introduced to several rice varieties by transformation or through conventional breeding (reviewed in Giri and Laxmi, 2000; Tyagi and Mohanty, 2000). The *Xa21* transgenic plants have also been field-tested (Tu *et al.*, 2000a; Table 5). This gene seems to be the best candidate so far to produce bacterial blight-resistant transgenic plants (Table 3) or along with other genes to provide multiple stress tolerance (Datta *et al.*, 2002; Narayanan *et al.*, 2002). Moreover, in contrast to other reports, its

expression does not seem to be variable even if integrated in different chromosomes (Zhai *et al.*, 2004). Recently, another resistance gene, *Xa26* has been isolated from rice and shown to encode a similar protein and effect in transgenic plants as *Xa21* gene (Sun *et al.*, 2004). Interestingly, the disease resistance pathway may be conserved in monocots and dicots as expression of *Arabidopsis NPR1* (a key regulator of systemic acquired resistance) in rice provided tolerance to bacterial blight (Chern *et al.*, 2001).

Apart from resistance genes, several other genes have been tested for imparting bacterial resistance in transgenic rice. Cecropins, a family of potent antibacterial peptides in the immune haemolymph of the *Cecropia* moth, have been expressed in transgenic plants but with mixed results (Florack *et al.*, 1995; Huang *et al.*, 1997). It was later known that Cecropin B transgenic plants did not express the protein at detectable levels and probably degraded by host peptidases (Mills *et al.*, 1994). The protein from *Bombyx mori* was translocated into extra-cellular spaces in transgenic rice by fusing the coding sequences of the mature protein to the signal peptide of chitinase gene. The transgenic plants showed enhanced tolerance to *Xanthomonas oryzae* infection (Sharma *et al.*, 2000). Transgenic rice plants expressing ferredoxin-like protein, AP1 from sweet pepper showed resistance to *X. oryzae* pv. *oryzae* (Tang *et al.*, 2001a). Transgenic rice plants have also been produced that are resistant to *Burkholderia plantarii* (previously classified to *Pseudomonas*) and more importantly, grain rot caused by *Burkholderia glumae*, which is an important pathogen of upland rice in Japan, by expressing one of the five oat thionins, *Asthi1* (Iwai *et al.*, 2002).

It is clear from above discussion that significant progress has been made to produce transgenic rice plants resistant to bacterial blight, mainly due to the discovery of endogenous resistance gene, *Xa21* and more recently *Xa26*. Field trials of *Xa21* transgenic rice plants suggested a significant increase in yield because of less damage caused by *X. oryzae* (Tu *et al.*, 2000a). This gene is certainly a great candidate for commercial release of bacteria-resistant transgenic rice plants.

Resistance against viruses

Rice tungro disease is the most severe viral disease of rice. It is caused by infection from two viruses. This disease is caused by rice tungro bacilliform virus (RTBV), while rice tungro spherical virus (RTSV) assists the transmission of viruses by vector green leafhopper, *Nephotettix virescens*. Earlier studies have characterized RTBV and RTSV genomes and virus-resistant transgenic rice plants have been produced using the coat protein mediated resistance strategy. Another strategy for producing virus-resistant plants is the replicase mediated

resistance (Rep-MR) strategy (Palukaitis and Zaitlin, 1997). Huet *et al.* (1999) produced transgenic rice plants containing the RTSV replicase gene in sense as well as antisense orientation. The transgenic plants with antisense sequences showed moderate resistance to RTSV. Further, the accumulation of viral RNA in sense transgenic plants suggested that RTSV-Rep-MR is RNA-mediated resistance. The RTSV-resistant transgenic plants could not assist in the transmission of RTBV (Huet *et al.*, 1999).

The elements that provide specificity to RTBV promoter are also known. These elements known as RF2a and RF2b are bZIP transcription activators and are involved in transcriptional regulation of the RTBV promoter (Petruccioli *et al.*, 2001; Dai *et al.*, 2003). Transgenic rice plants with reduced RF2b levels showed disease-like symptoms (Dai *et al.*, 2004). It has been proposed that interactions of multiple host factors including RF2a and RF2b control the phloem-specific expression of RTBV promoter and perhaps also control RTBV replication (Dai *et al.*, 2004). Resistance has also been obtained in transgenic rice against several other important viruses using various mechanisms (Zheng *et al.*, 2000; Han *et al.*, 2000; Upadhyaya *et al.*, 2001; Chaogang *et al.*, 2003; Lentini *et al.*, 2003) (Table 3).

As depicted previously, significant progress has been made in producing transgenic rice resistant to important rice pests. The need of the hour is multigene transgenics aiming towards resistance against multiple pathogens. The availability of complete rice genome sequence has opened up the way. It is expected that it would be possible to test multiple candidates for their efficacy in obtaining durable resistance derived from transgenics.

Rice genetic engineering for abiotic stress tolerance

It has been estimated that abiotic stresses such as drought, salinity, extreme temperatures, submergence and oxidative stress reduce crop yield worldwide by more than 50% (Bray *et al.*, 2000). These stresses are often interconnected, cause similar cellular damage and activate similar cell-signalling pathways (Shinozaki and Yamaguchi-Shinozaki, 2000; Zhu, 2001; Zhu, 2003). Several proteins, antioxidants and compatible solutes are induced as a result of abiotic stress responses. Many plant species have been produced over-expressing these compounds and tested for various abiotic stresses, most prominent being salt/drought tolerance (Bajaj *et al.*, 1999; Apse and Blumwald, 2002). Recently, the focus has been to engineer abiotic stress tolerance by over-expressing structural and regulatory proteins to activate the multistep

cascade involved in stress response (see Zhang *et al.*, 2004). However, most of this research has been carried out in model plants such as *Arabidopsis* and tobacco. Therefore, the challenge is to translate the information obtained from model plants into commercial crop varieties (Zhang *et al.*, 2004). We have tried to discuss transgenic rice tolerant to these stresses in separate sections but in many instances, the plants expressing one gene have been shown to be tolerant against multiple stresses (Table 4).

Tolerance to water deficiency and salinity

Drought and salt stresses are the most important factors that reduce crop yield in rice. The transgenic approach is one of the various options available to enhance salt tolerance in crop plants (Flowers, 2004). Engineering of many genes that imparted tolerance to dehydration and/or salinity tolerance in transgenic model dicot species (see Bajaj *et al.*, 1999; Tyagi and Mohanty, 2000), have been extended to crop plants such as rice (Table 4). Further, many new genes have been isolated that provide salt and water-deficit tolerance in model plants (Singla-Pareek *et al.*, 2003; Majee *et al.*, 2004; Mukhopadhyay *et al.*, 2004; Vannini *et al.*, 2004; reviewed in Flowers, 2004; Zhang *et al.*, 2004).

Salt also causes oxidative stress that results in generation of active oxygen species. Therefore, suppression of active oxygen production may result in protection against salt stress. Using this approach, Hoshida *et al.* (2000) developed transgenic rice plants expressing rice chloroplastic glutamine synthase (GS2). Transgenic plants with high GS2 levels showed increased photorespiration capacity and high tolerance to salt stress and possibly chilling stress (Hoshida *et al.*, 2000). It has been suggested that the effect of each individual gene on conferring abiotic stress tolerance would be rather small; therefore, multigene approach or activation of multiple genes through transcriptional activation of master regulatory genes can confer much greater tolerance (Jaglo-Ottosen *et al.*, 1998; Bajaj *et al.*, 1999). It is known that cytoplasmic Ca^{++} levels in plants increase rapidly in response to a variety of abiotic stresses followed by a cascade of protein phosphorylation/dephosphorylation performed predominantly by members of Ca^{++} dependent protein kinase (CDPK) family (Sanders *et al.*, 1999). The function of a rice cold and salt-inducible CDPK, *OsCDPK17* was investigated in transgenic rice plants (Saijo *et al.*, 2000). The authors demonstrated that over-expression of *OsCDPK17* enhanced induction of some stress-responsive genes including genes coding for late embryogenesis abundant (LEA) proteins in response to salt/drought stress and imparted tolerance to salt/drought stress. Xiong and Yang

Table 4 Recent advances in transgenic rice tolerant to various abiotic stresses

Gene/function	Source	Performance of transgenic plant	References
GS2/glutamine synthase	Rice	Tolerant to salt and possibly chilling stresses	Hoshida <i>et al.</i> (2000)
OsCDPK7/calcium-dependent protein kinase	Rice	Tolerant to salt and drought stresses	Saijo <i>et al.</i> (2000)
OsMAPK5/MAP kinase	Rice	Inverse modulation of biotic and abiotic stress tolerance by MAP kinase	Xiong and Yang (2003)
YK1/rice homolog of maize HC toxin reductase	Rice	Tolerant to multiple abiotic stresses including salt and submergence	Uchimiya <i>et al.</i> (2002)
OsNHX1, AgNHX1/Na ⁺ /H ⁺ antiporter HVPIP2; 1/aquaporins	Rice, Atriplex gmelini, rice Barley	Tolerance to salt stress Differential response to salt-stress	Ohta <i>et al.</i> (2002); Fukuda <i>et al.</i> (2004); Katsuhara <i>et al.</i> (2003); Lian <i>et al.</i> (2004)
Adc, Samdc/polyamine biosynthesis	Datura/oat/tritordeum	Salt/drought tolerant	Roy and Wu (2001; 2002); Capell <i>et al.</i> (2004)
HVA1/LEA proteins	Barley, wheat	Tolerant to salt and dehydration stress	Cheng <i>et al.</i> , 2002; Rohila <i>et al.</i> , 2002; Babu <i>et al.</i> (2004)
codA/glycine betaine	Arthrobacter globiformis	Tolerant to salt and/or drought stress	Sakamoto and Murata (2002); Mohanty <i>et al.</i> (2002); Sawahel (2003)
OtsA, OtsB/trehalose biosynthesis	E. coli	Multiple abiotic stress tolerance such as salt, drought and cold	Garg <i>et al.</i> (2002); Jang <i>et al.</i> (2003)
p5cs/proline biosynthesis	Mothbean	Stress induced transgene expression	Su and Wu (2004); Hur <i>et al.</i> (2004)
pdc1, adc/pyruvate decarboxylase/ alcohol dehydrogenase	Rice	Submergence tolerant	Quimio <i>et al.</i> (2000); Rahman <i>et al.</i> (2001)
AGPAT, SGPAT/fatty acids biosynthesis	Arabidopsis, spinach	Improved photosynthesis and growth at low temperatures	Ariizumi <i>et al.</i> (2002)
Cat/catalase	Wheat	Cold tolerant	Matsumara <i>et al.</i> (2002)
GST/γGlutathione S-transferase	Rice	Enhanced germination and growth under low temperature	Takesawa <i>et al.</i> (2002)
Spl7/heat-stress transcription factor	Rice	Transformation of rice spl7 mutant with Spl7 show tolerance to heat stress	Yamanouchi <i>et al.</i> (2002)
hsp101, hsp17.7/heat-shock proteins	Rice	Tolerant to high temperatures	Katiyar-Aggarwal <i>et al.</i> (2003); Murakami <i>et al.</i> (2004)
naatA, naatB/biosynthesis of muginic acid phytosiderophores	Barley	Tolerant to iron deficiency	Takahashi <i>et al.</i> (2001)

Abbreviations: *adc*, arginine decarboxylase; *codA*, choline oxidase; *hsp*, heat-shock protein; *GPAT*, glycerol-3-phosphate acyl transferase; *LEA*, late embryogenesis abundant protein; *MAPK*, mitogen activated protein kinase; *Naat*, nicotianamine aminotransferase; *OtsA* and *OtsB*, *E. coli* trehalose biosynthesis genes encoding for trehalose phosphate synthase (TPS) and trehalose phosphate phosphatase (TPP); *pdc*, pyruvate decarboxylase; *P5CS*, Δ-pyrroline-5-carboxylate synthase; *Spl*, spotted leaf.

(2003) isolated and characterized a stress responsive MAPK gene, *OsMAPK5* from rice whose expression could be induced by ABA and various biotic (fungal and bacterial) as well as abiotic (wounding, drought, salt and cold) stresses. Generation of transgenic plants either over-expressing or knockouts (using RNA interference) of *OsMAPK5* led to interesting results. The suppression of *OsMAPK5* expression resulted in activation of rice PR proteins leading to resistance to fungal and bacterial pathogens. Plants over-expressing *OsMAPK5* exhibited increased tolerance to drought, salt and cold stresses. These studies demonstrated that ABA-inducible MAPK inversely modulates broad-spectrum disease resistance and abiotic stress tolerance (Xiong and Yang, 2003). Similarly, the dehydration responsive

element (DRE) with core sequence A/GCCGAC has been identified as a *cis*-acting promoter element that activates expression of several genes under abiotic stress conditions (Yamaguchi-Shinozaki and Shinozaki, 1994). Dubouzet *et al.* (2003) isolated 5 rice *DREB* orthologs of *Arabidopsis* named as *OsDREB1A*, *OsDREB1B*, *OsDREB1C*, *OsDREB1D* and *OsDREB2A* and analysed their function in *Arabidopsis*. The authors said that they generated transgenic rice over-expressing various *OsDREB* proteins in combination with several promoters and suggested that *OsDREB1A* could be very useful for producing transgenic dicot and monocot plants with higher tolerance to drought, salt and/or cold stresses. However, it should be noted that constitutive

over-expression of these factors may cause aberrant phenotype (Shen *et al.*, 2003), so these genes need to be expressed under regulated/inducible promoters.

At the physiological level, adaptation to high salinity in plants comes from the proteins involved in ion or water transport such as Na^+/H^+ antiporter, $\text{Na}^+/\text{ATPase}$ and aquaporins (Serrano and Rodriguez-Navarro, 2001). Inducible expression of these proteins is also an attractive strategy for studying salt-tolerance in rice (Ohta *et al.*, 2002; Katsuhara *et al.*, 2003; Fukuda *et al.*, 2004; Hanba *et al.*, 2004; Lian *et al.*, 2004).

Accumulations of osmolytes or compatible solutes in plants have also been implicated in salt/drought tolerance (see Bajaj *et al.*, 1999; Wang *et al.*, 2003 for earlier references). These include LEA proteins (Xu *et al.*, 1996; Cheng *et al.*, 2002; Rohila *et al.*, 2002; Babu *et al.*, 2004), glycine betaine (Sakamoto *et al.*, 1998; Mohanty *et al.*, 2002; Sakamoto and Murata, 2002; Sawahel, 2003), Trehalose (Garg *et al.*, 2002; reviewed in Jang *et al.*, 2003; Penna, 2003) and proline (Hur *et al.*, 2004; Su and Wu, 2004). Significantly, stress-inducible or tissue-specific expression of trehalose biosynthesis in rice led to high levels of resistance to salt, drought and low temperatures (Garg *et al.*, 2002). Among the osmolytes, glycine betaine produced by choline oxidase (*codA*) from *Arthrobacter globiformis*, which catalyses conversion of choline to glycine betaine in a single step, holds lot of promise. When challenged with high salt stress, more than 50% transgenic plants harbouring *codA* gene recovered well and set seed. This yield gain is significant as under same conditions, wild-types plants failed to survive (Mohanty *et al.*, 2002).

Other compounds recently tested for salt/drought tolerance are polyamines (PAs) (Roy and Wu, 2001; 2002). PAs are low molecular weight polycationic molecules that are implicated in various growth and developmental processes including stress responses (Bajaj and Rajam, 1995, 1996; Malmberg *et al.*, 1998; Shoeb *et al.*, 2001). Recently, modulation of PA biosynthesis in transgenic rice conferred tolerance to drought stress (Capell *et al.*, 2004).

Submergence tolerance

Submergence of crops caused by flooding is also a major factor of crop loss. Engineering rice for submergence tolerance can be very useful for the crops grown in lowland areas that are vulnerable to flash flooding (Table 4). During submergence, plants are exposed to anaerobiosis and respond to it by switching from oxidative to substrate-level phosphorylation through fermentation using enzymes such as pyruvate

decarboxylase (PDC) and alcohol dehydrogenase (ADH) (Quimio *et al.*, 2000). Rice plants expressing rice *pd1* gene showed higher PDC levels and ethanol production that was positively correlated with survival after submergence (Quimio *et al.*, 2000) but not for anoxia tolerance although progenies of crossed plants overproducing both PDC and ADH did show increased anoxia tolerance and reduced acetaldehyde production (Rahman *et al.*, 2001).

Other enzymes implicated in submergence response of rice include 1-aminocyclopropane-1-carboxylic acid synthase encoded by ACS gene. By using *in situ* hybridization and histochemical analyses of GUS activity in transgenic rice expressing *OsACS5-gus* fusion, Zhou *et al.* (2002) showed that *OsACS5* mRNA is localized in specific cells and tissues both during normal development and under submergence conditions. The expression was strongly induced under complete submergence in vascular bundles of young stems and leaf sheaths, although the tolerance of *ACS5* plants to submergence stress remains to be demonstrated. Over-expression of rice *YK1* (maize homolog of HC-toxin reductase) gene in rice also resulted in increased submergence tolerance (Uchimiya *et al.*, 2002).

Tolerance to extreme temperatures

As described earlier, the pathways for adaptation to various abiotic stresses in plants are often interconnected and cause similar damage and activate similar cell signalling pathways (Shinozaki and Yamaguchi-Shinozaki, 2000; Zhu, 2003). Therefore, manipulation of these pathways in transgenic plants may provide tolerance to multiple abiotic stresses including cold stress (Kishitani *et al.*, 2000; Ariizumi *et al.*, 2002; Garg *et al.*, 2002; Matsumura *et al.*, 2002; Takesawa *et al.*, 2002; Jang *et al.*, 2003).

A heat stress transcription factor (*Sp17*) has been cloned from rice and its over-expression in *sp17* mutant suppressed the leaf-spot development induced by high temperature (Yamanouchi *et al.*, 2002). High-temperature tolerant rice plants have also been produced by over-expressing either heat-shock proteins (Katiyar-Aggarwal *et al.*, 2003; Murakami *et al.*, 2004) or enzymes involved in oxidative-stress tolerance (Kishitani *et al.*, 2000; Kouril *et al.*, 2003) (Table 4).

Mineral deficiency tolerance

Rice is extremely sensitive to low mineral concentrations, especially iron. Low iron availability to rice crops arises due to high soil pH making iron ions sparingly soluble and unavailable

to plants even though sufficient iron is present in soils (Takahashi, 2003). Interestingly, other cereals such as barley are not very sensitive to iron deficiency. They release low molecular weight compounds called phytosiderophores from their roots in response to iron deficiency. The phytosiderophores form a complex with Fe (III) in soil to solubilize iron and this complex can be taken up by grasses via a specific phytosiderophore receptor (Guerinot, 2001). The enzymes of barley phytosiderophore biosynthesis are tested in transgenic rice (Kobayashi *et al.*, 2001). Rice plants expressing iron-metabolizing genes from other cereals represent an attractive strategy to eliminate iron-deficiency (Takahashi, 2003). Therefore, two cDNAs, *naatA* and *naatB* encoding for nicotianamine aminotransferase (NAAT), one of the critical enzymes in the biosynthesis of mugenic acid phytosiderophores were introduced into rice. The transgenic plants grown in alkaline soil (pH 8.5) had higher NAAT activity, secreted larger amounts of phytosiderophores than controls and showed high tolerance to low iron. Further, the transgenic plants had more than four times greater yield than non-transgenic plants in alkaline soil (Takahashi *et al.*, 2001) (Table 4). However, the transgenic plants were not tested for iron content.

Other enzymes involved in the biosynthesis of mugenic acid phytosiderophores, nicotianamine synthase (*NAS*) gene from barley and rice have also been induced under iron-deficient conditions (Higuchi *et al.*, 2001; Inoue *et al.*, 2003; Koike *et al.*, 2004). Of these, *OsYSL2* was demonstrated to be a rice metal NA transporter and responsible for phloem-transport of iron and manganese and translocation of these metals into rice grain (Koike *et al.*, 2004). It would be interesting to see if over-expression of these genes can provide low iron tolerance to rice as well as increase iron content in transgenic rice.

Herbicide tolerance

Herbicide tolerance is an important agronomic trait that provides an efficient and flexible way of weed control. In addition, this can also be used as selectable marker in transgenic plants. Two genes commonly used to develop herbicide tolerant plants are *bar* gene isolated from *Streptomyces hygroscopicus* that detoxifies herbicide glufosinate, and *EPSPS* isolated from *Agrobacterium* strain *CP4* that detoxifies herbicide glyphosate. Herbicide tolerant transgenic rice plants expressing *bar* gene have been produced during the early stages of rice transformation research (reviewed in Tyagi and Mohanty, 2000) and have been the priority trait for field trials (Oard *et al.*, 1996, 2000).

Significant research has been done in the last five years to investigate other sources of genes that can provide herbicide

tolerance in transgenic rice. For example, transgenic rice plants expressing *Bacillus subtilis* protoporphyrinogen oxidase (protox) (a penultimate step enzyme of the branch point for the biosynthetic pathway of chlorophyll and hemes) were resistant against herbicide oxyfluorfen (Lee *et al.*, 2000; Jung *et al.*, 2003, 2004; Ha *et al.*, 2003). Transgenic rice over-expressing a human cytochrome P450s showed variable response to herbicides (Inui *et al.*, 2001; Kawahigashi *et al.*, 2002, 2003). Although as discussed later, rice expressing human genes may not be acceptable because of ethical reasons.

The previous reading shows that rice has been transformed to provide tolerance against several abiotic stresses. In few cases, the modulation of one pathway led to multiple stress tolerance (Garg *et al.*, 2002; Xiong and Yang, 2003). Further, many new proteins including structural proteins have been identified to provide stress tolerance in model plants. It would be a matter of time, when they would be introduced in commercial crops like rice. However, it would be too early to comment on the yield enhancement of rice or any other crop under abiotic stress conditions, unless they are field-tested (Flowers, 2004).

Nutritional enhancement of rice using transgenic approach

When it is said that 'there is enough food to feed the world', it probably refers to staple crops such as rice, on which most of the developing world depends as the source of their calories. While rice may be sufficient for surviving, a range of non-staple foods such as fruits, vegetables, pulses apart from animal and fish products are necessary for healthy and productive life (Zimmermann *et al.*, 2002; Bouis *et al.*, 2003). Although it can be argued that even these foods are available in plenty but their high costs prevent their access to millions in developing world. This has resulted in widespread micronutrient under-nutrition in poor countries (Bouis *et al.*, 2003). Thus, an increase in nutritive value of rice could be highly significant because of its high per capita consumption and would probably supplement the insufficient intake of fruits and vegetables by people in developing countries. Various strategies are being evaluated including breeding and genetically modified plants for the biofortification of cereals, rice in particular, for micronutrient content (Poletti *et al.*, 2004).

Provitamin A-enriched rice

It is estimated that about 3 million children of preschool age suffer from eye defects owing to vitamin A deficiency

and about 500 000 of them go blind and some of them die (Bouis *et al.*, 2003). In this context, the recent landmark development of provitamin A-enriched rice (Ye *et al.*, 2000), popularly known as 'golden rice' has immense significance. Ye *et al.* (2000) introduced entire β -carotene biosynthesis pathway into rice endosperm in a single transformation step; the genes for phytoene synthase (*psy*) and lycopene β -cyclase (*β -lcy*) came from daffodil and the gene for phytoene desaturase (*crt1*) was of bacterial origin. Later, it was observed that co-transformation and presence of *β -lcy* were not necessary and *psy* and *crt1* alone could drive β -carotene synthesis. Further advances included production of golden indica rice and use of mannose selection systems to avoid use of antibiotic selectable marker (Beyer *et al.*, 2002; Datta *et al.*, 2003; Hoa *et al.*, 2003). Nevertheless, it is estimated that if golden rice replaces the conventional rice, total provitamin A intakes would increase by 25% for adult women and preschool children (Bouis *et al.*, 2003). Thus, it should be viewed as one and not the only tool of nutritional fortification (Dawe *et al.*, 2002). Already, programmes have been initiated in developing countries such as India to breed this pathway into popular rice varieties (Potrykus, 2001; Sharma *et al.*, 2003).

Mineral enrichment

It has been estimated that over three billion people in developing countries are iron deficient (Lucca *et al.*, 2001a) and the problem is more severe in women and children, as half of this population is said to be anaemic in developing countries. Further, commercial milling removes outer layer of rice thus reducing iron in the grain. Therefore, transgenic rice with high iron content expressed in the endosperm would help to solve this problem.

Lonnerdal (2003) outlined different approaches to increase trace element nutrition using genetically modified plants. The studies that have improved rice for iron nutrition are discussed in succeeding discussions.

Increases the iron content

Human lactoferrin, the major iron-binding protein in breast milk has been over-expressed in rice either in grains or in cell cultures (Nandi *et al.*, 2002; Suzuki *et al.*, 2003). Transgenic plants with high lactoferrin levels were selected and field-tested (Lonnerdal, 2003). Thus, production of lactoferrin in rice would help as supplement in infant formula to increase iron content.

For meeting iron requirements in adults, a protein such as ferritin that could bind to more metal ions per molecule

would be required. Earlier, Goto *et al.* (1999) expressed soybean ferritin gene in rice and showed that rice iron content was doubled in the transgenic plants. Interestingly, transgenic rice expressing ferritin when fed to anaemic rats could replenish haematocrit, haemoglobin and liver iron concentrations (Murray-Kolb *et al.*, 2002). This is in contrast to earlier reports that suggested iron levels only increased sufficient enough in vegetative tissue but not in seeds in transgenic rice (Drakakaki *et al.*, 2000). Lucca *et al.* (2001a) introduced ferritin gene from *Phaseolus vulgaris* into rice and increased the iron content by 2-fold. Recently, Vasconcelos *et al.* (2003) showed that soybean ferritin gene driven by endosperm-specific glutelin promoter led to high accumulation of iron and zinc levels not only in brown rice but also in polished transgenic grains. Thus, this rice would remain rich in iron even after commercial milling.

Improves the bioavailability of iron

Phytic acid in the diet strongly reduces iron absorption. The enzyme phytase hydrolyses phosphate from phytic acid and can inhibit its activity thus, improving trace element bioavailability (Sandberg *et al.*, 1996). Lucca *et al.* (2001a) also introduced a thermotolerant phytase from *Aspergillus fumigatus* and rice cysteine rice metallothionin like protein into rice. The seeds of transgenic rice plants expressing these genes showed significant increase in phytase activity and degraded phytic acid under simulated digestion experiments. However, the phytase did not retain its property after cooking (Lucca *et al.*, 2001a). Overproduction of phytase in transgenic rice as a result of introduction of two genes *Srpfl* from bacterium *Selenomonas ruminantium* and *appA* from *Escherichia coli* resulted in high conversion of activity over broad pH ranges and hence the potential to increase phosphate availability and can be used as feed additive for improving phytate-phosphorus digestibility in monogastric animals (Hong *et al.*, 2004).

Increasing amino acid levels

As discussed previously, rice is a primary source not only of calories but also as a protein source for poor people. However, similar to seed proteins of other cereals, seed proteins of rice are deficient in some essential amino acids (Lee *et al.*, 2001). Transgenic rice with balanced amino acid composition will further help in maintaining healthy and nutritional diet. So far, transgenic rice have been produced that have increased levels of glycinins (Katsube *et al.*, 1999); lysine (Lee *et al.*, 2001; Wu *et al.*, 2003); tryptophan (Tozawa *et al.*, 2001); sulphur-rich storage seed protein such as cysteine and methionine (Hagan *et al.*, 2003; Lee *et al.*, 2003).

Interestingly, it has been shown that low-storage protein mutants can accumulate higher levels of foreign gene products than the normal host plant (Tada *et al.*, 2003). In some applications, it is desirable to reduce the level of amino acids as demonstrated by (Maruta *et al.*, 2002). Rice plants transformed with glutelin A antisense gene resulted in decrease in glutelin levels. Rice wine, 'Sake', brewed from these transgenic lines was found to contain up to 40% less amino acid and was of superior quality as excess amino acids in Sake provides rough taste and accelerates quality deterioration (Maruta *et al.*, 2002). Similarly, low protein rice cultivar can be fed to patients who require low protein intake. A mutation, low glutelin content 1 (*lgc1*) is a dominant mutation that reduces glutelin content in rice seeds. This mutation was shown as a result of 3.5 kb deletion between two highly similar glutelin gene in *lgc1* homozygotes that forms a tail-to-tail inverted repeat and could form a double-stranded RNA molecule resulting in RNA silencing (Kusaba *et al.*, 2003). This was confirmed using the same region in transgenic rice plants and provides example of natural occurrence of RNA silencing and is one of the many application of RNA interference (RNAi) technology (Kusaba *et al.*, 2003; Kusaba, 2004).

Improving oil quality in rice

Rice bran is an important oil source but lacks high levels of α -linolenic acid, which is beneficial for health. The soybean microsomal omega-3 fatty acid desaturase cDNA, the enzyme of which converts linoleic acid to α -linolenic acid driven by maize ubiquitin-1 promoter was introduced into rice (Anai *et al.*, 2003). Seed oil from transgenic rice showed increased levels of α -linolenic acid content and comprised up to 46.8% of total rice seed oil while control seeds had α -linolenic acid content of only 3.5% (Anai *et al.*, 2003).

Manipulation of starch content

Starch is one of the most important nutritional components of human food. Therefore, improvement of starch quality and quantity is very important. The major components of the starch are amylopectin and amylose; the ratios of these molecules affect the properties of processed starch in food industry. Hence, control of amylose in starch is a major objective in crop improvement (Visser and Jacobsen, 1993).

The waxy (*wx*) locus encoding for granule bound starch synthase for synthesis of amylose has been extensively studied in maize. In rice, two *wx* alleles are known to exist, *wx^a*

and *wx^b*. *Indica* rice has *wx^a* allele whereas japonica rice has *wx^b* (Hirano *et al.*, 1998). A substitution mutation at the 5' splice site of 1st intron causes *wx^b* to produce smaller mRNA and has lower amylose levels than *wx^a* (Hirano *et al.*, 1998). Using transgenic technology, the amylose content has been manipulated in both *indica* and japonica varieties (Terada *et al.*, 2000; Liu *et al.*, 2003; Itoh *et al.*, 2003a). The mechanism of *wx* transcription has been studied in rice. It has been shown that a MYC protein OsBP-5 interacts with EREBP protein (Os-EPB-89) to regulate *wx* transcription. Further, decrease in *OsBP-5* mRNA by using RNAi also lead to decline in amylose content in transgenic plants without affecting the phenotype (Zhu *et al.*, 2003). The *wx* gene has also been modified using gene targeting (Terada *et al.*, 2002). The amylopectin content in rice has been manipulated to generate novel starches (Tanaka *et al.*, 2004).

Transgenic rice for producing recombinant compounds

With genetic manipulation of several plants being routine, it is possible to express many recombinant molecules in these plants, making them a 'factory' for the production of macromolecules for pharmaceutical (such as antibodies (Torres *et al.*, 1999), nutraceutical and animal feed). These highlight the potential benefits of these plants for the production of recombinant molecules and some hold promise for large-scale commercial application (Stoger *et al.*, 2000; Herbers, 2003). It is expected that some of the plant-produced compounds will soon complement existing systems as well as add value to agriculture (Raskin *et al.*, 2002). Cereal grains offer even more advantages as the recombinant molecules driven by endosperm-specific expression can be produced in large amounts and stored safely in the endosperm cells. Considerable progress has been made to make use of transgenic rice as a 'recombinant protein' factory, the prominent examples being expression of human lactoferrin (Nandi *et al.*, 2002; Suzuki *et al.*, 2003), human lysozyme (Yang *et al.*, 2001; Huang *et al.*, 2002a,b) and recombinant pollen allergen (Okada *et al.*, 2003).

Transgenic rice cell cultures were also shown to produce recombinant human α_1 -antitrypsin (rAAT) (Terashima *et al.*, 2002; Trexler *et al.*, 2002). Similarly, biologically active human insulin-like growth factor-1 (hIGF-1) that resembles insulin has also been expressed in rice (Panahi *et al.*, 2004). Further, transgenic rice has also been explored for the production of industrial enzyme, transglutaminase by overexpression of the prostrate transglutaminase (rTGP) gene (Claparols *et al.*, 2004). However, it must be cautioned that

over-expression of such proteins may induce severe structural and biochemical changes in native plant (Yang *et al.*, 2003).

The previous discussion highlights some of the progress in the application of transgenic rice producing recombinant compounds. Since the research is still in its infancy, it would be too early to comment on its commercial feasibility.

Biosafety of transgenic rice

There are several issues related to the safe release of transgenic crops into the environment. These often led to negative perception about genetically modified organisms (GMOs) even though the transgenic crops have been commercially grown for 8 years (James, 2003). It has been suggested that failure to establish an explicit terminology to categorize various applications of gene technology in breeding is one of the reasons about the negative perception of GMOs (Nielsen, 2003). Biosafety of transgenic rice would attract more scrutiny, as it is the staple diet for half of the world's population. Moreover, rice has been modified not only for agronomic traits but also for its nutrient content leading to a situation where no precedence exists for the safety evaluation of these traits. In this section, we will address the major biosafety issues and more importantly, necessary regulatory studies required and initiated for safe release of transgenic rice. We have tried to incorporate the progress in this area with the guidelines recommended for the safe release of GMOs by international institutions such as Food and Agriculture Organisation (FAO), World Health Organisation (WHO), Organisation of Economic Cooperation and Development (OECD) and International Life Science Institute (ILSI). More detailed aspects have been covered by various reviews that have been published recently (Conner *et al.*, 2003; Taylor, 2003). Also, government regulations in individual countries to oversee the safe release of GM crops have been reviewed extensively (Nap *et al.*, 2003; Jaffe, 2004). The July 2004 issue of *Food and Chemical Toxicology* (Vol. 42, issue 7, pp. 1043–1202) extensively discusses the European legislations in various aspects of GM food.

Of all the biosafety issues being debated, the most important issues being related to rice are: (a) crop to wild gene flow (b) food safety of transgenic rice and (c) impact on non-target organisms. We feel that only certain issues may be more important while analysing a specific trait. For example, it is of less concern if golden rice genes spread to wild relatives of rice but it needs thorough food safety evaluation. On the other hand, pollen-mediated gene flow is an important issue while evaluating the bio-safety of herbicide-resistant rice. For example, the weedy rice (red rice), which often grows within

the cultivated rice field (Messeguer *et al.*, 2004), can acquire the gene for herbicide tolerance through cross-fertilization.

Crop-to-wild gene flow

It is known that many crops are sexually compatible with their wild relatives, which are often weeds. Thus, crop-to-wild gene flow can have a significant impact on the wild populations (Jarvis and Hodgkin, 1999). Although rice is primarily a self-pollinated crop but cross-pollination does occur in rice. Certain wild relatives of cultivated rice exist, grow near cultivated rice and are sexually compatible with each other (Song *et al.*, 2002). Therefore, ecological consequences of crop-to-wild gene flow in rice need to be addressed for effective conservation of genetic resources and risk assessment of transgenic crops (Song *et al.*, 2003). Recently, Song *et al.* (2003) studied the gene flow from cultivated rice to the wild species *Oryza rufipogon*, the ancestor of cultivated rice. They showed that the gene flow occurred at a considerable rate (around 3%) and distance up to 43 m. Similar results were obtained in another field trial (Chen *et al.*, 2004). However, other studies reported a low rate of gene flow from transgenic rice to red rice and conventional rice (Messeguer *et al.*, 2001, 2004) or from transgenic rice to red rice (Zhang *et al.*, 2003). Similarly, field trial of insect resistant rice also reported a low gene flow from transgenic to conventional rice (Bashir *et al.*, 2004; Table 5).

Many resistance genes have been transferred to cultivated rice through conventional breeding. It must be noted that so far there has been no known cases where wild or weedy rice biotypes of *Oryza sativa* such as red rice have become more aggressive as a result of outcrossing with cultivated rice with resistance genes (High *et al.*, 2004).

Food safety evaluation of transgenic rice

It has been generally agreed that food safety assessment of transgenic crops requires an integrated, step-wise, case-by-case approach and is directed by the results of a comparison between the genetically modified food and its conventional counterpart (WHO, 2000). The factors that is to be taken into account include the identity, source, composition, effects of processing/cooking, the transformation process itself, the recombinant DNA, expression of novel proteins, potential toxicity and allergenicity of the protein and potential intake and dietary impact of the introduction of the genetically modified food (WHO, 2000).

At this stage, it is important to discuss the concept of 'substantial equivalence' (SE), which would play an integral part

Table 5 Field-testing of transgenic rice transformed with various agronomic traits

Gene	Trait	Location	Highlights	Reference
<i>Bt</i> fusion gene derived from <i>Cry1Ab</i> and <i>Cry1Ac</i>	Insect resistance	Wuhan, China	Field-testing of transgenic hybrid rice showed high protection against YSB and leaf folder without any yield reduction	Tu <i>et al.</i> (2000b)
<i>Cry1Ab</i>	Insect resistance	Hangzhou and Hainan, China	Two rice lines KMD1 and KMD2 showed high resistance to SSB and leaf folder under laboratory as well as field conditions	Shu <i>et al.</i> (2000)
<i>Cry1Ab</i>	Insect resistance	Zhejiang, China	Further field testing of KMD1 and KMD2	Ye <i>et al.</i> (2001)
<i>Cry1Ab</i>	Insect resistance	Hangzhou, China	Transgenic rice lines further field-tested but showed high degree of variations in agronomic characteristics and quality. Insect resistance transferred to commercial rice varieties	Shu <i>et al.</i> (2002); Wang <i>et al.</i> (2002a); Wu <i>et al.</i> (2002c)
<i>Cry1Ac</i> , <i>Cry2A</i>	Insect resistance	Lahore, Pakistan	Fifth generation of indica basmati rice showed resistance to YSB in a field trial. No effect on non-target pests and gene flow up to 0.18%	Bashir <i>et al.</i> (2004)
<i>Cry1Aa</i> and <i>Cry1B</i>	Insect resistance	Catalunya, Spain	<i>Cry</i> genes under control of constitutive or inducible promoter showed high protection against SSB	Breitler <i>et al.</i> (2004b)
<i>Xa21</i>	Bacterial resistance	Wuhan, China	Excellent field-performance of transgenic indica IR72 rice variety on response to bacterial blight	Tu <i>et al.</i> (2000a)
<i>Bar</i>	Herbicide resistance	Louisiana and Arkansas, USA	Evaluation of agronomic consequences of herbicide resistance. Differences observed in plant height and maturity of transgenic plants	Oard <i>et al.</i> (2000)
<i>Bar</i>	Out-crossing using herbicide resistance as marker	Italy and Spain	Gene flow estimated to be 0.1%	Messeguer <i>et al.</i> (2001)
<i>Bar</i>	Out-crossing using herbicide resistance as marker	Louisiana, USA	Up to 0.3% out-crossing between transgenic rice and red rice	Zhang <i>et al.</i> (2003)
<i>Bar</i>	Out-crossing using herbicide resistance as marker	Amposta, Spain	Gene flow from transgenic rice to recipient red rice or-non-transgenic rice lower than previously reported. Reduction in gene flow with increase in distance	Messeguer <i>et al.</i> (2004)

Note: Reports of field trials conducted in transgenic rice published in peer-reviewed journals are included. Many field trials especially of herbicide-resistant rice have been conducted by private organizations.

in analysing the safety of transgenic rice. The term 'substantial equivalence' (SE) introduced by OECD in 1993 and elaborated in detail by WHO in 1996, is an internationally recognized standard that measures whether food derived through biotechnology shares similar health, nutrient and/or antinutrient characteristics with its conventional counterpart. As envisaged by FAO/WHO (1996), there can be three outcomes of substantial equivalence evaluations:

1. Products that are shown to be substantially equivalent to existing food or food components, it is considered as safe as its conventional counterpart and no further safety evaluation is necessary.

In terms of transgenic rice, however, no such example exists to date. Although this scenario is possible if a rice gene is over-expressed in rice carrying its own promoter and terminator lacking any vector backbone sequences.

2. Products that is substantially equivalent to existing foods or food components except for the defined differences. Then, further safety assessment should focus only on those defined differences.

Most of the transgenic rice expressing various agronomic traits such as herbicide tolerance, insect, virus, bacterial and fungal resistance would come into this category. There is a set of studies recommended by FAO/WHO to study the intended effect of the introduction of that genetic material. If the data from these studies appear insufficient for a thorough safety assessment, then there is a need for animal toxicological studies (WHO, 2000). However, it has become a norm that these studies be conducted anyway to gain regulatory approval and/or acceptance of the end users (food/feed industry). In transgenic rice, certain progress has been made to carry out a baseline study for detailed determinations of the nutritional characteristics of the grains (Azhakanandam *et al.*, 2000b). Further, 90 day feeding test on weaned male and female Sprague-Dawley rats confirmed the safety of flour obtained from transgenic rice expressing synthetic *Cry1Ab* gene (Wang *et al.*, 2002b). Interestingly, a recent report suggests that gamma irradiation of *Cry1Ab* transgenic rice could reduce *Cry1Ab* protein without altering major nutritional components (Wu *et al.*, 2004c).

3. Products that are not substantially equivalent to existing foods or food components.

Although none of the transgenic crops available commercially would come into this category at this stage but will include transgenic rice with enhanced quality of food or to prevent various human diseases. The most prominent would be golden rice, iron-rich rice or any nutritionally enhanced rice through genetic modification. Although the intended effect in this case is to provide nutritional change, there is a possibility of metabolic disturbance in the crop. It is recommended that the safety of these products be established on a case-by-case basis. Further studies, including animal feeding trials, may be required since rice modified for nutritional elements is intended to replace a significant part of the diet. Few studies have started to emerge that assess safety of nutritionally enhanced rice as in the case of amino acid-rich rice (Momma *et al.*, 1999; 2000). Feeding studies on amino acid-rich rice on rats for four weeks suggested no difference in rats fed with transgenic rice that included several parameters such as body weight, food intake, haematological analyses or histological evaluations (Momma *et al.*, 2000). Potrykus (2001, 2003) and Toenniessen *et al.* (2003) have identified the biosafety of golden rice as one of the challenges of safe transfer of this technology.

However, it must be noted that SE is only one of the several important criteria determining the safety of GM crops. But it can be used to plan the programme of safety evaluation that includes phenotypic equivalence, compositional equivalence, wholesomeness equivalence, nutritional equivalence, etc. as well as detailed analysis of potential allergenicity (Cockburn, 2002; Taylor, 2003).

Some of the studies demand even further attention like transgenic rice over-expressing sesame 2S albumin to increase methionine content (Lee *et al.*, 2003). The 2S albumin from Brazil nut has been demonstrated to be an allergen in both its purified form and in extracts of transgenic soybean seeds (Nordlee *et al.*, 1996). Interestingly, this allergen was discovered only when transgenic soybeans expressing 2S albumin from Brazil nut were tested for allergenicity, indicating the robustness of allergenicity testing protocol. Although sesame has been used as a food additive and used in various Asian countries, still it would be justified to conduct a comprehensive allergenicity testing of transgenic rice expressing sesame 2S albumin.

Impact on non-target organisms

Since transgenic rice expressing genes such as *cry* genes is not only expressed in seeds but in other parts as well, it is

important to analyse the impact of GM rice on other non-target organisms especially when it has been shown that the Cry protein is exuded roots of *Bt* rice among other plants tested (Saxena *et al.*, 2004). In case of insect-resistant rice, such studies would include the impact on insects of rice other than targeted by *cry* genes, soil flora and fauna, and various other non-targeted animals that could come into contact of GM rice. Considerable efforts have been spent on the safety studies in other *Bt* crops commercialized such as *Bt* corn and *Bt* cotton (reviewed in Shelton *et al.*, 2002) and have confirmed that these crops are safe to non-target organisms. Such studies are beginning to emerge in rice as well. Recently, it was reported that *Bt* rice straws altered some important biological properties in water-flooded soil (Wu *et al.*, 2004b). It was suggested that such an effect could be a result of shift in microbial population or a change in their metabolic activities, although later on it was shown that *Bt* rice straw is not toxic to a variety of culturable microorganisms in the flooded paddy soil (Wu *et al.*, 2004a). Similarly, *Bt* rice had no effect on non-target insects in field-conditions in Pakistan (Bashir *et al.*, 2004). Perhaps, the most convincing study on the safety of *Bt* crops on non-target organisms has been conducted in field grown *Bt* cotton. Using enzyme-linked immunosorbent assay (ELISA) and bioassays with a susceptible insect species, the tobacco budworm, Head *et al.* (2002) showed that there was no detectable *Cry1A(c)* protein in any of the soil samples collected from within and outside the fields where insect resistant cotton had been grown and subsequently incorporated into soil by postharvest tillage for the last 3–6 consecutive years. Thus, if no significant Cry protein is detected in soil, its impact, if any, should be negligible.

Other issues

There are certain ethical issues also related to the development of transgenic plants with genes from animal origin. A vast population in countries like India, Sri Lanka and Nepal are vegetarians. Certainly, vegetarians around the world would not like eating rice expressing animal genes. Thus, it would be difficult to commercialise rice expressing GFP gene or any other gene of animal or human origin. The exception would be in the use for therapeutic purposes or human lysozyme or lactoferrin in rice as a supplement in infant formula (Nandi *et al.*, 2002).

Impact of genetically modified rice

Despite being the most important crop in the world, the field release of genetically modified (GM) rice lags way behind its

cereal counterpart, maize. One reason for the delay could be that rice is staple food crop therefore several regulatory and ethical issues may be attached to the development of GM rice technology. Further, most of the rice produced in Asian countries is consumed locally and minor rice-producing countries are major rice exporters (Brookes and Barfoot, 2003). Still, commercialisation of GM rice will be a major success for transgene technology. Due to its importance in the developing world and significant part played by the public sector in developing GM rice technology, GM rice may act as a catalyst to the wider adoption and acceptance of GM crop technology (Brookes and Barfoot, 2003). However, awareness of technology among farmers of developing countries is very low. A recent exploratory study found low awareness and knowledge of golden rice among farmers in the Philippines and yield/cost savings were the first priorities of farmers (Chong, 2003). Thus golden rice must match the yield potential of existing rice. In this context, the initiatives by the Indian government on the transfer of golden trait into existing Indian varieties, and research by Datta *et al.* (2003) and Hoa *et al.* (2003) to produce golden indica rice holds great promise. It is expected that by 2012, up to seven traits of GM rice will be in the market including traits such as herbicide tolerance, disease resistance, abiotic stress tolerance and nutrition (Brookes and Barfoot, 2003). However, at this stage, the benefit of commercial release of transgenic rice can only be extrapolated using the information from other crops. For example, the gains obtained from release of *Bt* cotton in China and India can provide the information on possible benefits of *Bt* rice (High *et al.*, 2004).

Rice research in post-genomics era

The availability of a complete rice genome sequence has opened up a sea of opportunities not only for rice but for the plant community as a whole. As a direct measure, the availability of wealth of polymorphism information and molecular markers is going to make the tedious process of map based cloning a lot faster and much more efficient (Lukowitz *et al.*, 2000). It has already helped in map-based cloning of several genes. Analysis of rice genome has revealed several orthologues of *Arabidopsis* flowering time genes (Goff *et al.*, 2002) and these could have significant implications for improving yield potential of rice. Also, a number of different candidate genes now could be tested for their efficacy in a high-throughput way. More importantly, we envisage that it is going to usher in a change in our approach towards problem solving in biology. The research is going to move from a single-gene based approach to more holistic genome- and

proteome-wide translational research. Recently, a novel approach has been taken for analysis of a number of *Arabidopsis* proteins of unknown function (Tian *et al.*, 2004), which would not have been possible without the availability of the complete genome sequence. It is expected that with time, such approaches would be routine for rice as well. We realize that use of mutant resources for high throughput gene discovery (Hirochika *et al.*, 2004) would be another area of increased research focus. These mutant resources would greatly aid in the functional analysis of all the rice genes.

Another important resource is accessibility to ~28 000 full length cDNAs (The Rice Full Length cDNA Consortium, 2003). The utility of this is multifold. For *Arabidopsis*, it helped in improving annotation of more than 1/3 of the genes (Haas *et al.*, 2002). These full-length cDNAs could be utilized for transgenic analysis by over-expression or down-regulation by RNAi to decipher function of proteins. Besides, these would be used for 'OMICS'-based studies such as proteome-wide protein and localization studies with the ultimate goal of mapping and analysing complete biological networks (Brasch *et al.*, 2004). Rice research is entering an exciting era and it is expected that the information generated from such massive studies would pave the way for designing of better yielding crop plants.

Conclusions

It is clear that transgenic rice technologies have moved beyond the proof of concept to the point where it can actually supplement the breeding methods to meet the demands of growing population. Over the last five years, rice has been transformed with many new genes of both agronomic and nutritional attributes. Field trials of transgenic rice, especially with insect-resistant and herbicide tolerant rice have begun in the United States, China, South Asia and the Mediterranean (Table 5). Further, regulatory studies required for the safe release of transgenic crops have also been initiated. The development of nutritionally enriched rice has kept pace with other crops. It is expected that golden rice would initially be available for consumers of developing world within next 7 to 8 years and adopted very quickly (Brookes and Barfoot, 2003). This is not surprising given the commitment shown by the governments of developing countries such as India to take this technology forward (Potrykus, 2001; Sharma *et al.*, 2003).

The recent completion of the sequencing of the rice genome has opened up new avenues for gene regulation and transformation in rice. It has been debated that information gained from rice genome by utilizing ancestral synteny

among the cereals would help genetically improve not only rice but also other cereals such as maize, wheat and barley, which have significantly large genomes. However, it must be cautioned that recent microsynteny studies like rice-wheat or rice-sorghum revealed numerous rearrangements, which can complicate map-based cloning strategies using information from the rice genome (reviewed in Delseny, 2004).

What is certain is that rice research will undergo dramatic changes in the coming years both in terms of commercial release of genetically modified rice with existing genes, as well as discovery of new genes utilizing the advances in rice structural and functional genomics.

Acknowledgements

We are thankful to Doctors Pauline Mooney and Roger Hellens for the critical reading and two anonymous reviewers for their valuable suggestions, which helped immensely in improving the manuscript. We are also thankful to Ms. Sunita Bajaj for her help in typing and proofreading the manuscript, and Dr Rita Gandhi for valuable suggestions. AM is supported by National Science Foundation grant to David Jackson. We apologize to our colleagues whose work could not be cited due to space constraints.

References

- Ahmadi, N., Dellerme, S., Laplace, L., Guermache, F., Auguy, F., Duhoux, E., Bogusz, D., Guiderdoni, E. and Franche, C. (2003) The promoter of a metallothionein-like gene from the tropical tree *Casuarina glauca* is active in both annual dicotyledonous and monocotyledonous plants. *Transgenic Res.* **12**, 271–281.
- Al Bitar, F., Roosens, N., Van Bostel, J., Dewaele, E., Jacobs, M. and Homble, F. (2002) Expression of the rice *vdac isoform2*, histochemical localization and expression level. *Biochim. Et Biophys. Act.-Gene Struct. Expression*, **1579**, 133–141.
- Alcantara, E.P., Aguda, R.M., Curtiss, A., Dean, D.H. and Cohen, M.B. (2004) *Bacillus thuringiensis* δ -endotoxin binding to brush border membrane vesicles of rice stem borers. *Arch. Insect Biochem. Physiol.* **55**, 169–177.
- Alfonso-Rubi, J., Ortego, F., Castanera, P., Carbonero, P. and Diaz, I. (2003) Transgenic expression of trypsin inhibitor CMe from barley in indica and japonica rice, confers resistance to the rice weevil *Sitophilus oryzae*. *Transgenic Res.* **12**, 23–31.
- Alinia, F., Ghareyazie, B., Rubia, L., Bennett, J. and Cohen, M.B. (2000) Effect of plant age, larval age, and fertilizer treatment on resistance of a *cry1Ab*-transformed aromatic rice to lepidopterous stem borers and foliage feeders. *J. Econ. Entomol.* **93**, 484–493.
- Anai, T., Koga, M., Tanaka, H., Kinoshita, T., Rahman, S.M. and Takagi, Y. (2003) Improvement of rice (*Oryza sativa* L.) seed oil quality through introduction of a soybean microsomal omega-3 fatty acid desaturase gene. *Plant Cell Report*, **21**, 988–992.
- Anonymous. (2001) Consensus statement on sustainable use of transgenic rice for pest management – Bt Rice Conference report – Hangzhou, PRC, 27 November 1 December (2000). *Mol. Breed.* **7**, 99–100.
- Apse, M.P. and Blumwald, E. (2002) Engineering salt tolerance in plants. *Curr. Opin. Biotechnol.* **13**, 146–150.
- Ariizumi, T., Kishitani, S., Inatsugi, R., Nishida, I., Murata, N. and Toriyama, K. (2002) An increase in unsaturation of fatty acids in phosphatidylglycerol from leaves improves the rates of photosynthesis and growth at low temperatures in transgenic rice seedlings. *Plant Cell Physiol.* **43**, 751–758.
- Ayres, N.M. and Park, W.D. (1994) Genetic transformation of rice. *Crit. Rev. Plant Sci.* **13**, 219–239.
- Azhakanandam, K., McCabe, M.S., Power, J.B., Lowe, K.C., Cocking, E.C. and Davey, M.R. (2000a) T-DNA transfer, integration, expression and inheritance in rice, effects of plant genotype and *Agrobacterium* super-virulence. *J. Plant Physiol.* **157**, 429–439.
- Azhakanandam, K., Power, J.B., Lowe, K.C., Cocking, E.C., Tongdang, T., Bligh, H.F.J., Harding, S.E. and Davey, M.R. (2000b) Qualitative assessment of aromatic indica rice (*Oryza sativa* L.). Proteins, lipids and starch in grain from somatic embryo- and seed-derived plants. *J. Plant Physiol.* **156**, 783–789.
- Babu, R.C., Zhang, J.X., Blum, A., Ho, T.H.D., Wu, R. and Nguyen, H.T. (2004) *HVA1*, an LEA gene from barley confers dehydration tolerance in transgenic rice (*Oryza sativa* L.) via cell membrane protection. *Plant Sci.* **166**, 855–862.
- Bajaj, S. and Rajam, M.V. (1995) Efficient plant regeneration from long-term callus cultures of rice by spermidine. *Plant Cell Report*, **14**, 717–720.
- Bajaj, S. and Rajam, M.V. (1996) Polyamine accumulation and near loss of morphogenesis in long-term callus cultures of rice – restoration of plant regeneration by manipulation of cellular polyamine levels. *Plant Physiol.* **112**, 1343–1348.
- Bajaj, S., Targolli, J., Liu, L.F., Ho, T.H.D. and Wu, R. (1999) Transgenic approaches to increase dehydration-stress tolerance in plants. *Mol. Breed.* **5**, 493–503.
- Barakat, A., Gallois, P., Raynal, M., Mestre-Ortega, D., Sallaud, C., Guiderdoni, E., Delseny, M. and Bernardi, G. (2000) The distribution of T-DNA in the genomes of transgenic *Arabidopsis* and rice. *FEBS Lett.* **471**, 161–164.
- Barry, G.F. (2001) The use of the Monsanto draft rice genome sequence in research. *Plant Physiol.* **125**, 1164–1165.
- Bashir, K., Husnain, T., Fatima, T., Latif, Z., Mehdi, S.A. and Riazuddin, S. (2004) Field evaluation and risk assessment of transgenic indica basmati rice. *Mol. Breed.* **13**, 301–312.
- Bernal, C.C., Aguda, R.M. and Cohen, M.B. (2002) Effect of rice lines transformed with *Bacillus thuringiensis* toxin genes on the brown planthopper and its predator *Cyrtorhinus lividipennis*. *Entomol. Exp. Appl.* **102**, 21–28.
- Beyer, P., Al-Babili, S., YeX.D., Lucca, P., Schaub, P., Welsch, R. and Potrykus, I. (2002) Golden rice, introducing the β -carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. *J. Nutr.* **132**, 506S–510S.
- Bouis, H.E., Chassy, B.A. and Ochanda, J.O. (2003) Genetically modified food crops and their contribution to human nutrition and food quality. *Trends Food Sci. Technol.* **14**, 191–209.
- Brandizzi, F., Fricker, M. and Hawes, C. (2002) A greener world: the plant revolution in plant bioimaging. *Nat. Rev. Mol. Cell Biol.* **3**, 520–530.

- Brasch, M.A., Hartley, J.L. and Vidal, M. (2004) ORFeome cloning and systems biology: standardized mass production of the parts from the part list. *Genome Res.* **14**, 2001–2009.
- Bray, E.A., Bailey-Serres, J. and Weretilnyk, E. (2000) Responses to abiotic stresses. In: *Biochemistry and Molecular Biology of Plants* (Gruissem, W., Buchanan, B., Jones, R., eds), pp. 1158–1249. Rockville, MD: American Society of Plant Physiologists.
- Breitler, J.C., Cordero, M.J., Royer, M., Meynard, D., San Segundo, B. and Guiderdoni, E. (2001) The –689/+197 region of the maize protease inhibitor gene directs high level, wound-inducible expression of the *cry1B* gene, which protects transgenic rice plants from stemborer attack. *Mol. Breed.* **7**, 259–274.
- Breitler, J.C., Labeyrie, A., Meynard, D., Legavre, T. and Guiderdoni, E. (2002) Efficient microprojectile bombardment-mediated transformation of rice using gene cassettes. *Theor. Appl. Genet.* **104**, 709–719.
- Breitler, J.C., Marfa, V., Royer, M., Meynard, D., Vassal, J.M., Vercambe, B., Frutos, R., Messeguer, J., Gabarra, R. and Guiderdoni, E. (2000) Expression of a *Bacillus thuringiensis cry1B* synthetic gene protects Mediterranean rice against the striped stem borer. *Plant Cell Report*, **19**, 1195–1202.
- Breitler, J.C., Meynard, D., Van Bostel, J., Royer, M., Bonnot, F., Cambillau, L. and Guiderdoni, E. (2004a) A novel two tDNA binary vector allows efficient generation of marker-free transgenic plants in three elite cultivars of rice (*Oryza sativa* L.). *Transgenic Res.* **13**, 271–287.
- Breitler, J.C., Vassal, J.N., Catala, M.D.M., Meynard, D., Marfa, V., Mele, E., Royer, M., Murillo, I., Segundo, S.B., Guiderdoni, E. and Messeguer, J. (2004b) *Bt* rice harbouring *cry* genes controlled by a constitutive or wound-inducible promoter, protection and transgene expression under Mediterranean field conditions. *Plant Biotechnol. J.* **2**, 417–430.
- Brookes, P. and Barfoot, G.B. (2003) GM rice, will this be the way for global acceptance of GM crop technology. ISAAA Briefs no. 28, ISAAA, Ithaca, NY.
- Bryan, G.T., Wu, K.-S., Farrall, L., Jia, Y., Hershey, H.P., McAdams, S.A., Faulk, K.N., Donaldson, G.K., Tarchini, R. and Valent, B. (2000) A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-Ta*. *Plant Cell*, **12**, 2033–2045.
- Capell, T., Bassie, L. and Christou, P. (2004) Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. *Proc. Natl. Acad. Sci. USA*, **101**, 9909–9914.
- Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W. and Prasher, D.C. (1994) Green fluorescent protein as a marker for gene expression. *Science*, **263**, 802–805.
- Chaogang, S., Jianhua, W., Guoying, Z., Gang, S., Baozhen, P., Juanli, L., Dendi, J., Shenxiang, C., Upadhyaya, N.M., Waterhouse, P. and Zuxun, G. (2003) Ectopic expression of the spike protein of rice ragged stunt *Oryza* virus in transgenic rice plants inhibits transmission of the virus to insects. *Mol. Breed.* **11**, 295–301.
- Che, K.P., Zhan, Q.C., Xing, Q.H., Wang, Z.P., Jin, D.M., He, D.J. and Wang, B. (2003) Tagging and mapping of rice sheath blight resistant gene. *Theor. Appl. Genet.* **106**, 293–297.
- Chen, L.J., Lee, D.S., Song, Z.P., Suh, H.S. and Lu, B.R. (2004) Gene flow from cultivated rice (*Oryza sativa*) to its weedy and wild relatives. *Ann. Bot.* **93**, 67–73.
- Chen, L., Marmey, P., Taylor, N.J., Brizard, J., Espinoza, C., D'Cruz, P., Huet, H., Zhang, S., de Kochko, A., Beachy, R.N. and Fauquet, C.M. (1998) Expression and inheritance of multiple transgenes in rice plants. *Nat. Biotechnol.* **16**, 1060–1064.
- Chen, M., Presting, G., Brad Barbazauk, W., Goicoechea, J.L., Blackmon, B., Fang, G., Kim, H. and Frisch, D., YuY., Sun, S., Higingbottom, S., Phimpilai, J., Phimpilai, D., Thurmond, S., Gaudette, B., Li, P., Liu, J., Hatfield, J., Mani, D., Farrar, K., Henderson, C., Barnett, L., Costa, R., Williams, B., Walser, S., Atkins, M., Hall, C., Budiman, M.A., Tomkins, J.P., Luo, M., Bancroft, I., Salse, J., Regad, F., Mohapatra, T., Singh, N.K., Tyagi, A.K., Soderlund, C., Dean, R.A. and Wing, R.A. (2002) An integrated physical and genetic map of the rice genome. *Plant Cell*, **14**, 537–545.
- Cheng, X.Y., Sardana, R., Kaplan, H. and Altosaar, I. (1998) *Agrobacterium*-transformed rice expressing synthetic *cry1Ab* and *cry1Ac* genes are highly toxic to striped stem borer and yellow stem borer. *Proc. Natl. Acad. Sci. USA*, **95**, 2767–2772.
- Cheng, Z.Q., Targolli, J., Huang, X.Q. and Wu, R. (2002) Wheat LEA genes, *PMA80* and *PMA1959*, enhance dehydration tolerance of transgenic rice (*Oryza sativa* L.). *Mol. Breed.* **10**, 71–82.
- Cheng, Z.Q., Targolli, J. and Wu, R. (2001) Tobacco matrix attachment region sequence increased transgene expression levels in rice plants. *Mol. Breed.* **7**, 317–327.
- Chern, M.-S., Fitzgerald, H.A., Yadav, R.C., Canlas, P.E., Dong, X. and Ronald, P.C. (2001) Evidence for a disease-resistance pathway in rice similar to the *NPR1*-mediated signalling pathway in *Arabidopsis*. *Plant J.* **27**, 101–113.
- Cho, M.J., Yano, H., Okamoto, D., Kim, H.K., Jung, H.R., Newcomb, K., Le, V.K., Yoo, H.S., Langham, R., Buchanan, B.B. and Lemaux, P.G. (2004) Stable transformation of rice (*Oryza sativa* L.) via microprojectile bombardment of highly regenerative, green tissues derived from mature seed. *Plant Cell Report*, **22**, 483–489.
- Chong, M. (2003) Acceptance of golden rice in the Philippine 'rice bowl'. *Nat. Biotechnol.* **21**, 971–972.
- Christou, P. (1996) Transformation technology. *Trends Plant Sci.* **1**, 423–431.
- Christou, P., Ford, T.L. and Kofron, M. (1991) Production of transgenic rice (*Oryza sativa* L.) plants from agronomically important indica and japonica varieties via electric discharge particle acceleration of exogenous DNA into immature zygotic embryos. *Bio/Technology*, **9**, 957–962.
- Claparols, M.I., Bassie, L., Miro, B., Del Duca, S., Rodriguez-Montesinos, J., Christou, P., Serafini-Fracassini, D. and Capell, T. (2004) Transgenic rice as a vehicle for the production of the industrial enzyme transglutaminase. *Transgenic Res.* **13**, 195–199.
- Coca, M., Bortolotti, C., Rufat, M., Penas, G., Eritja, R., Tharreau, D., del Pozo, A.M., Messeguer, J. and San Segundo, B. (2004) Transgenic rice plants expressing the antifungal AFP protein from *Aspergillus giganteus* show enhanced resistance to the rice blast fungus *Magnaporthe grisea*. *Plant Mol. Biol.* **54**, 245–259.
- Cockburn, A. (2002) Assuring the safety of genetically modified (GM) foods, the importance of a holistic, integrative approach. *J. Biotechnol.* **98**, 79–106.
- Coffman, W.R. and Juliano, B.R. (1987) Rice. Nutritional quality of cereal grains, genetic and agronomic improvement. In: *Agronomy Monograph 28, ASA-CSSA-SSSA, Madison, WI, USA*.
- Conner, A.J., Glare, T.R. and Nap, J.P. (2003) The release of genetically modified crops into the environment – Part II. Overview of ecological risk assessment. *Plant J.* **33**, 19–46.
- Cotsaftis, O., Sallaud, C., Breitler, J.C., Meynard, D., Greco, R., Pereira, A. and Guiderdoni, E. (2002) Transposon-mediated

- generation of tDNA-free and marker-free rice plants expressing a *Bt* endotoxin gene. *Mol. Breed.* **10**, 165–180.
- Cutler, S. and Ehrhardt, D. (2000) Dead cells don't dance: insights from live-cell imaging in plants. *Cur Opin. Plant Biol.* **3**, 532–537.
- Dai, S., Carcamo, R., Zhang, Z., Chen, S. and Beachy, R.N. (2001a) The bacterial cytosine deaminase gene used as a conditional negative selection marker in transgenic rice plants. *Plant Cell Report*, **20**, 738–743.
- Dai, S.H., Petruccielli, S., Ordiz, M.I., Zhang, Z.H., Chen, S.Y. and Beachy, R.N. (2003) Functional analysis of RF2a, a rice transcription factor. *J. Biol. Chem.* **278**, 36396–36402.
- Dai, S.H., Zhang, Z.H., Chen, S.Y. and Beachy, R.N. (2004) RF2b, a rice bZIP transcription activator, interacts with RF2a and is involved in symptom development of rice tungro disease. *Proc. Natl. Acad. Sci. USA*, **101**, 687–692.
- Dai, S.H., Zheng, P., Marmey, P., Zhang, S.P., Tian, W.Z., Chen, S.Y., Beachy, R.N. and Fauquet, C. (2001b) Comparative analysis of transgenic rice plants obtained by *Agrobacterium*-mediated transformation and particle bombardment. *Mol. Breed.* **7**, 25–33.
- Datta, K., Baisakh, N., Oliva, N., Torrizo, L., Abrigo, E., Tan, J., Rai, M., Rehana, S., Al-Babili, S., Beyer, P., Potrykus, I. and Datta, S.K. (2003) Bioengineered 'golden' indica rice cultivars with beta-carotene metabolism in the endosperm with hygromycin and mannose selection systems. *Plant Biotechnol. J.* **1**, 81–90.
- Datta, K., Baisakh, N., Thet, K.M., Tu, J. and Datta, S.K. (2002) Pyramiding transgenes for multiple resistance in rice against bacterial blight, yellow stem borer and sheath blight. *Theor. Appl. Genet.* **106**, 1–8.
- Datta, K., Koukolikova-Nicola, Z., Baisakh, N., Oliva, N. and Datta, S.K. (2000) *Agrobacterium*-mediated engineering for sheath blight resistance of indica rice cultivars from different ecosystems. *Theor. Appl. Genet.* **100**, 832–839.
- Datta, S.K., Peterhans, A., Datta, K. and Potrykus, I. (1990) Genetically engineered fertile indica rice recovered from protoplasts. *Bio/Technology*, **8**, 736–740.
- Datta, K., Tu, J.M., Oliva, N., Ona, I., Velazhahan, R., Mew, T.W., Muthukrishnan, S. and Datta, S.K. (2001) Enhanced resistance to sheath blight by constitutive expression of infection-related rice chitinase in transgenic elite indica rice cultivars. *Plant Sci.* **160**, 405–414.
- Dawe, D., Robertson, R. and Unnevehr, L. (2002) Golden rice, what role could it play in alleviation of vitamin A deficiency? *Food Policy*, **27**, 541–560.
- De Buck, S., De Wilde, C., Van Montagu, M. and Depicker, A. (2000) T-DNA vector backbone sequences are frequently integrated into the genome of transgenic plants obtained by *Agrobacterium*-mediated transformation. *Mol. Breed.* **6**, 459–468.
- Delseny, M. (2003) Towards an accurate sequence of the rice genome. *Curr. Opin. Plant Biol.* **6**, 101–105.
- Delseny, M. (2004) Re-evaluating the relevance of ancestral shared synteny as a tool for crop improvement. *Curr. Opin. Plant Biol.* **7**, 126–131.
- Devos, K.M. and Gale, M.D. (2000) Genome relationships: the grass model in current research. *Plant Cell*, **12**, 637–646.
- Dong, J.J., Kharb, P., Teng, W.M. and Hall, T.C. (2001) Characterization of rice transformed via an *Agrobacterium*-mediated inflorescence approach. *Mol. Breed.* **7**, 187–194.
- Drakakaki, G., Christou, P. and Stoger, E. (2000) Constitutive expression of soybean ferritin cDNA in transgenic wheat and rice results in increased iron levels in vegetative tissues but not in seeds. *Transgenic Res.* **9**, 445–452.
- Duan, X., Li, X., Xue, Q., Abo, E.I., Saad, M., Xu, D. and Wu, R. (1996) Transgenic rice plants harboring an introduced potato proteinase inhibitor II gene are insect resistant. *Nat. Biotechnol.* **14**, 494–498.
- Dubouzet, J.G., Sakuma, Y., Ito, Y., Kasuga, M., Dubouzet, E.G., Miura, S., Seki, M., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2003) *OsDREB* genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. *Plant J.* **33**, 751–763.
- Eamens, A.L., Blanchard, C.L., Dennis, E.S. and Upadhyaya, N.M. (2004) A bidirectional gene trap construct for tDNA and Ds-mediated insertional mutagenesis in rice (*Oryza sativa* L.). *Plant Biotechnol. J.* **2**, 367–380.
- Endo, S., Sugita, K., Sakai, M., Tanaka, H. and Ebinuma, H. (2002) Single-step transformation for generating marker-free transgenic rice using the IPT-type MAT vector system. *Plant J.* **30**, 115–122.
- Erikson, O., Hertzberg, M. and Nasholm, T. (2004) A conditional marker gene allowing both positive and negative selection in plants. *Nat. Biotechnol.* **22**, 455–458.
- FAO/WHO (1996) *Biotechnology and Food Safety. Report of a joint Food and Agriculture Organization/World Health Organization consultation*. FAO/WHO, Rome.
- Feng, Q., Zhang, Y.J., Hao, P., Wang, S.Y., Fu, G., Huang, Y.C., Li, Y., Zhu, J.J., Liu, Y.L., Hu, X., Jia, P.X., Zhang, Y., Zhao, Q., Ying, K., Yu S.L., Tang, Y.S., Weng, Q.J., Zhang, L., Lu, Y., Mu, J., Lu, Y.Q. and Zhang, L.S., Yu Z., Fan, D.L., Liu, X.H., Lu, T.T., Li, C., Wu, Y.R., Sun, T.G., Lei, H.Y., Li, T., Hu, H., Guan, J.P., Wu, M., Zhang, R.Q., Zhou, B., Chen, Z.H., Chen, L., Jin, Z.Q., Wang, R., Yin, H.F., Cai, Z., Ren, S.X., Lv, G., Gu, W.Y., Zhu, G.F., Tu, Y.F., Jia, J., Chen, J., Kang, H., Chen, X.Y., Shao, C.Y., Sun, Y., Hu, Q.P., Zhang, X.L., Zhang, W., Wang, L.J., Ding, C.W., Sheng, H.H., Gu, J.L., Chen, S.T., Ni, L., Zhu, F.H., Chen, W., Lan, L.F., Lai, Y., Cheng, Z.K., Gu, M.H., Jiang, J.M., Li, J.Y., Hong, G.F., Xue, Y.B. and Han, B. (2002) Sequence and analysis of rice chromosome 4. *Nature*, **420**, 316–320.
- Finnegan, J. and McElroy, D. (1994) Transgene inactivation – plants fight back. *Bio/Technology*, **12**, 883–888.
- Fischer, K.S., Barton, J., Khush, G.S., Leung, H. and Cantrell, R. (2000) Genomics and agriculture – collaborations in rice. *Science*, **290**, 279–280.
- Florack, D., Allefs, S., Bollen, R., Bosch, D., Visser, B. and Stiekema, W. (1995) Expression of giant silkworm cecropin-B genes in tobacco. *Transgenic Res.* **4**, 132–141.
- Flowers, T.J. (2004) Improving crop salt tolerance. *J. Exp. Bot.* **55**, 307–319.
- Foissac, X., Du Edwards, M.G.J.P., Gatehouse, A.M.R. and Gatehouse, J.A. (2002) Putative protein digestion in a sap-sucking homopteran plant pest (rice brown plant hopper; *Nilaparvata lugens*, Delphacidae) – identification of trypsin-like and cathepsin B-like proteases. *Insect Biochem. Molec.* **32**, 967–978.
- Foissac, X., Loc, N.T., Christou, P., Gatehouse, A.M.R. and Gatehouse, J.A. (2000) Resistance to green leafhopper (*Nephotettix virescens*) and brown planthopper (*Nilaparvata lugens*) in transgenic rice expressing snowdrop lectin (*Galanthus nivalis agglutinin*; GNA). *J. Insect Physiol.* **46**, 573–583.
- Fu, X.D., Duc, L.T., Fontana, S., Bong, B.B., Tinjuangjun, P., Sudhakar, D., Twyman, R.M., Christou, P. and Kohli, A. (2000) Linear transgene constructs lacking vector backbone sequences generate low-copy-number transgenic plants with simple integration patterns. *Transgenic Res.* **9**, 11–19.

- Fujimoto, H., Itoh, K., Yamamoto, M., Kyoizuka, J. and Shimamoto, K. (1993) Insect resistant rice generated by introduction of a modified δ -endotoxin gene of *Bacillus thuringiensis*. *Bio/Technology*, **11**, 1151–1155.
- Fukuda, A., Nakamura, A., Tagiri, A., Tanaka, H., Miyao, A., Hirochika, H. and Tanaka, Y. (2004) Function, intracellular localization and the importance in salt tolerance of a vacuolar Na⁺/H⁺ antiporter from rice. *Plant Cell Physiol.* **45**, 146–159.
- Fukuoka, H., Ogawa, T., Mitsuhara, I., Iwai, T., Isuzugawa, K., Nishizawa, Y., Gotoh, Y., Tagiri, A., Ugaki, M., Ohshima, M., Yano, H., Murai, N., Niwa, Y., Hibi, T. and Ohashi, Y. (2000) *Agrobacterium*-mediated transformation of monocot and dicot plants using the NCR promoter derived from soybean chlorotic mottle virus. *Plant Cell Report*, **19**, 815–820.
- Fukuzawa, H., Yu, L., Umeda-Hara, C., Tagawa, M. and Uchimiya, H. (2004) The rice metallothionein gene promoter does not direct foreign gene expression in seed endosperm. *Plant Cell Report*, **23**, 231–235.
- Gahakwa, D., Maqbool, S.B., Fu, X., Sudhakar, D., Christou, P. and Kohli, A. (2000) Transgenic rice as a system to study the stability of transgene expression, multiple heterologous transgenes show similar behaviour in diverse genetic backgrounds. *Theor. Appl. Genet.* **101**, 388–399.
- Gandikota, M., de Kochko, A., Chen, L., Ithal, N., Fauquet, C. and Reddy, A.R. (2001) Development of transgenic rice plants expressing maize anthocyanin genes and increased blast resistance. *Mol. Breed.* **7**, 73–83.
- Garg, A.K., Kim, J.K., Owens, T.G., Ranwala, A.P., Do Choi, Y., Kochian, L.V. and Wu, R.J. (2002) Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proc. Natl. Acad. Sci. USA*, **99**, 15898–15903.
- Giri, C.C. and Laxmi, G.V. (2000) Production of transgenic rice with agronomically useful genes, an assessment. *Biotechnol. Adv.* **18**, 653–683.
- Gleave, A.P., Mitra, D.S., Mudge, S.R. and Morris, B.A.M. (1999) Selectable marker-free transgenic plants without sexual crossing, transient expression of cre recombinase and use of a conditional lethal dominant gene. *Plant Mol. Biol.* **40**, 223–235.
- Goff, S.A. (1999) Rice as a model for cereal genomics. *Curr. Opin. Plant Biol.* **2**, 86–89.
- Goff, S.A., Ricke, D., Lan, T.H., Presting, G., Wang, R.L., Dunn, M., Glazebrook, J., Sessions, A., Oeller, P., Varma, H., Hadley, D., Hutchinson, D., Martin, C., Katagiri, F., Lange, B.M., Moughamer, T., Xia, Y., Budworth, P., Zhong, J.P., Miguel, T., Paszkowski, U., Zhang, S.P., Colbert, M., Sun, W.L., Chen, L.L., Cooper, B., Park, S., Wood, T.C., Mao, L., Quail, P., Wing, R. and Dean, R., Yu, Y.S., Zharkikh, A., Shen, R., Sahasrabudhe, S., Thomas, A., Cannings, R., Gutin, A., Pruss, D., Reid, J., Tavtigian, S., Mitchell, J., Eldredge, G., Scholl, T., Miller, R.M., Bhatnagar, S., Adey, N., Rubano, T., Tusneem, N., Robinson, R., Feldhaus, J., Macalma, T., Oliphant, A. and Briggs, S. (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). *Science*, **296**, 92–100.
- Goto, F., Yoshihara, T., Shigemoto, N., Toki, S. and Takaiwa, F. (1999) Iron fortification of rice seed by the soybean ferritin gene. *Nat. Biotechnol.* **17**, 282–286.
- Gowda, M., Venu, R.C., Roopalakshmi, K., Sreerekha, M.V. and Kulkarni, R.S. (2003) Advances in rice breeding, genetics and genomics. *Mol. Breed.* **11**, 337–352.
- Guerinot, M.L. (2001) Improving rice yields – ironing out the details. *Nat. Biotechnol.* **19**, 417–418.
- Guiderdoni, E., Cordero, M.J., Vignols, F., Garcia-Garrido, J.M., Lescot, M., Tharreau, D., Meynard, D., Ferriere, N., Notteghem, J.L. and Delseny, M. (2002) Inducibility by pathogen attack and developmental regulation of the rice *Ltp1* gene. *Plant Mol. Biol.* **49**, 683–699.
- Ha, S.B., Lee, S.B., Lee, D.E., Guh, J.O. and Back, K. (2003) Transgenic rice plants expressing *Bacillus subtilis* protoporphyrinogen oxidase gene show low herbicide oxyfluorfen resistance. *Bol. Plant.* **47**, 277–280.
- Haas, B.J., Volfovsky, N., Town, C.D., Troukhan, M., Alexandrov, N., Feldmann, K.A., Flavell, R.B., White, O. and Salzberg, S.L. (2002) Full-length messenger RNA sequences greatly improve genome annotation. *Genome Biol.* **3**, 0029.1–0029.12.
- Hagan, N.D., Upadhyaya, N., Tabe, L.M. and Higgins, T.J.V. (2003) The redistribution of protein sulfur in transgenic rice expressing a gene for a foreign, sulfur-rich protein. *Plant J.* **34**, 1–11.
- Han, S.C., Wu, Z.J., Yang, H.Y., Wang, R., Yie, Y., Xie, L.H. and Tien, P. (2000) Ribozyme-mediated resistance to rice dwarf virus and the transgene silencing in the progeny of transgenic rice plants. *Transgenic Res.* **9**, 195–203.
- Hanba, Y.T., Shibasaki, M., Hayashi, Y., Hayakawa, T., Kasamo, K., Terashima, I. and Katsuhara, M. (2004) Overexpression of the barley aquaporin *HvPIP2;1* increases internal CO₂ conductance and CO₂ assimilation in the leaves of transgenic rice plants. *Plant Cell Physiol.* **45**, 521–529.
- He, Z.Q., Fu, Y.P., Si, H.M., Hu, G.C. and Zhang, S.H., Yu, Y.H. and Sun, Z.X. (2004) Phosphomannose-isomerase (*pmi*) gene as a selectable marker for rice transformation via *Agrobacterium*. *Plant Sci.* **166**, 17–22.
- Head, G., Surber, J.B., Watson, J.A., Martin, J.W. and Duan, J.J. (2002) No detection of Cry1Ac protein in soil after multiple years of transgenic *Bt* cotton (Bollgard) use. *Environ. Entomol.* **31**, 30–36.
- Hellens, R.P., Edwards, E.A., Leyland, N.R., Bean, S. and Mullineaux, P.M. (2000) pGreen, a versatile and flexible binary Ti vector for *Agrobacterium*-mediated plant transformation. *Plant Mol. Biol.* **42**, 819–832.
- Herbers, K. (2003) Vitamin production in transgenic plants. *J. Plant Physiol.* **160**, 821–829.
- Hernandez, C.A., Pujol, M., Alfonso-Rubi, J., Armas, R., Coll, Y., Perez, M., Gonzalez, A., Ruiz, M., Castanera, P. and Ortego, F. (2003) Proteolytic gut activities in the rice water weevil, *Lissorhoptrus brevirostris* Suffrian (Coleoptera, Curculionidae). *Arch. Insect Biochem.* **53**, 19–29.
- Hiei, Y., Ohta, S., Komari, T. and Kumashiro, T. (1994) Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence-analysis of the boundaries of the tDNA. *Plant J.* **6**, 271–282.
- Higa, A., Kimura, M., Mimori, K., Ochiai-Fukuda, T., Tokai, T., Takahashi-Ando, N., Nishiuchi, T., Igawa, T., Fujimura, M., Hamamoto, H., Usami, R. and Yamaguchi, I. (2003) Expression in cereal plants of genes that inactivate fusarium mycotoxins. *Biosci. Biotech. Biochem.* **67**, 914–918.
- High, S.M., Cohen, M.B., Shu, Q.Y. and Altosaar, I. (2004) Achieving successful deployment of *Bt* rice. *Trends Plant Sci.* **9**, 286–292.
- Higuchi, K., Watanabe, S., Takahashi, M., Kawasaki, S., Nakanishi, H., Nishizawa, N.K. and Mori, S. (2001) Nicotianamine synthase gene expression differs in barley and rice under Fe-deficient conditions. *Plant J.* **25**, 159–167.

- Hirano, H.Y., Eiguchi, M. and Sano, Y. (1998) A single base change altered the regulation of the *Waxy* gene at the post-transcriptional level during the domestication of rice. *Mol. Biol. Evol.* **15**, 978–987.
- Hirochika, H., Guiderdoni, E., An, G., Hsing, Y.-i., Eun, M.Y., Han, C.-D., Upadhyaya, N., Ramachandran, S., Zhang, Q., Pereira, A., Sundaresan, V. and Leung, H. (2004) Rice mutant resources for gene discovery. *Plant Mol. Biol.* **54**, 325–334.
- Hoa, T.T.C., Al-Babili, S., Schaub, P., Potrykus, I. and Beyer, P. (2003) Golden indica and japonica rice lines amenable to deregulation. *Plant Physiol.* **133**, 161–169.
- Hohn, B., Levy, A.A. and Puchta, H. (2001) Elimination of selection markers from transgenic plants. *Curr. Opin. Biotechnol.* **12**, 139–143.
- Holmes-Davis, R. and Comai, L. (1998) Nuclear matrix attachment regions and plant gene expression. *Trends Plant Sci.* **3**, 91–97.
- Hong, C.Y., Cheng, K.J., Tseng, T.H., Wang, C.S., Liu, L.F. and S.M. (2004) Production of two highly active bacterial phytases with broad pH optima in germinated transgenic rice seeds. *Transgenic Res.* **13**, 29–39.
- Hoshida, H., Tanaka, Y., Hibino, T., Hayashi, Y., Tanaka, A. and Takabe, T. (2000) Enhanced tolerance to salt stress in transgenic rice that overexpresses chloroplast glutamine synthetase. *Plant Mol. Biol.* **43**, 103–111.
- Huang, Y., Di Nordeen, R.O.M., Owens, L.D. and McBeath, J.H. (1997) Expression of an engineered cecropin gene cassette in transgenic tobacco plants confers disease resistance to *Pseudomonas syringae* pv. *tabaci*. *Phytopathol.* **87**, 494–499.
- Huang, J.M., Nandi, S., Wu, L.Y., Yalda, D., Bartley, G., Rodriguez, R., Lonnerdal, B. and Huang, N. (2002a) Expression of natural antimicrobial human lysozyme in rice grains. *Mol. Breed.* **10**, 83–94.
- Huang, N., Wu, L.Y., Nandi, S., Bowman, E., Huang, J.M., Sutliff, T. and Rodriguez, R.L. (2001) The tissue-specific activity of a rice β -glucanase promoter (*Gns9*) is used to select rice transformants. *Plant Sci.* **161**, 589–595.
- Huang, J.M., Wu, L.Y., Yalda, D., Adkins, Y., Kelleher, S.L., Crane, M., Lonnerdal, B., Rodriguez, R.L. and Huang, N. (2002b) Expression of functional recombinant human lysozyme in transgenic rice cell culture. *Transgenic Res.* **11**, 229–239.
- Huet, H., Mahendra, S., Wang, J., Sivamani, E., Ong, C.A., Chen, L., de Kochko, A., Beachy, R.N. and Fauquet, C. (1999) Near immunity to rice tungro spherical virus achieved in rice by a replicase-mediated resistance strategy. *Phytopathology*, **89**, 1022–1027.
- Hur, J., Jung, K.H., Lee, C.H. and An, G.H. (2004) Stress-inducible *OsP5CS2* gene is essential for salt and cold tolerance in rice. *Plant Sci.* **167**, 417–426.
- Husnain, T., Asad, J., Maqbool, S.B., Datta, S.K. and Riazuddin, S. (2002) Variability in expression of insecticidal *Cry1Ab* gene in indica basmati rice. *Euphytica*, **128**, 121–128.
- Hwang, Y.S., McCullar, C. and Huang, N. (2001a) Evaluation of expression cassettes in developing rice endosperm using a transient expression assay. *Plant Sci.* **161**, 1107–1116.
- Hwang, Y.S., Nichol, S., Nandi, S., Jernstedt, J.A. and Huang, N. (2001b) Aleurone- and embryo-specific expression of the β -glucuronidase gene controlled by the barley *Chi26* and *Ltp1* promoters in transgenic rice. *Plant Cell Report*, **20**, 647–654.
- Inoue, H., Higuchi, K., Takahashi, M., Nakanishi, H., Mori, S. and Nishizawa, N.K. (2003) Three rice nicotianamine synthase genes, *OsNAS1*, *OsNAS2*, and *OsNAS3*, are expressed in cells involved in long-distance transport of iron and differentially regulated by iron. *Plant J.* **36**, 366–381.
- Inui, H., Shiota, N., Ido, Y., Inoue, T., Hirose, S., Kawahigashi, H., Ohkawa, Y. and Ohkawa, H. (2001) Herbicide metabolism and tolerance in the transgenic rice plants expressing human *CYP2C9* and *Cyp2c19*. *Pest. Biochem. Physiol.* **71**, 156–169.
- Ito, H., Hiraga, S., Tsugawa, H., Matsui, H., Honma, M., Otsuki, Y., Murakami, T. and Ohashi, Y. (2000) Xylem-specific expression of wound-inducible rice peroxidase genes in transgenic plants. *Plant Sci.* **155**, 85–100.
- Itoh, K., Ozaki, H., Okada, K., Hori, H., Takeda, Y. and Mitsui, T. (2003a) Introduction of *Wx* transgene into rice *wx* mutants leads to both high- and low-amylose rice. *Plant Cell Physiol.* **44**, 473–480.
- Itoh, Y., Takahashi, K., Takizawa, H., Nikaidou, N., Tanaka, H. and Nishihashi, H. (2003b) Family 19 chitinase of *Streptomyces griseus* *HUT6037* increases plant resistance to the fungal disease. *Biosci. Biotechnol. Biochem.* **67**, 847–855.
- Iwai, T., Kaku, H., Honkura, R., Nakamura, S., Ochiai, H., Sasaki, T. and Ohashi, Y. (2002) Enhanced resistance to seed-transmitted bacterial diseases in transgenic rice plants overproducing an oat cell-wall-bound thionin. *Mol. Plant Microbe Interact.* **15**, 515–521.
- Iwamoto, M., Higo, H. and Higo, K. (2004) Strong expression of the rice catalase gene, *CatB* promoter in protoplasts and roots of both a monocot and dicots. *Plant Physiol. Biochem.* **42**, 241–249.
- Iyer, L.M., Kumpatla, S.P., Chandrasekharan, M.B. and Hall, T.C. (2000) Transgene silencing in monocots. *Plant Mol. Biol.* **43**, 323–346.
- Jach, G., Binot, E., Frings, S., Luxa, K. and Schell, J. (2001) Use of red fluorescent protein from *Discosoma* sp. (dsRED) as a reporter for plant gene expression. *Plant J.* **28**, 483–491.
- Jaffe, G. (2004) Regulating transgenic crops, a comparative analysis of different regulatory processes. *Transgenic Res.* **13**, 5–19.
- Jaglo-Ottosen, K.R., Gilmour, S.J., Zarka, D.G., Schabenberger, O. and Thomashow, M.F. (1998) *Arabidopsis* *CBF1* overexpression induces *COR* genes and enhances freezing tolerance. *Science*, **280**, 104–106.
- James, C. (2003) *Global Status of Commercialized Transgenic Crops*. ISAAA Briefs no. 30, ISAAA, Ithaca, NY.
- James, V.A., Avart, C., Worland, B., Snape, J.W. and Vain, P. (2002) The relationship between homozygous and hemizygous transgene expression levels over generations in populations of transgenic rice plants. *Theor. Appl. Genet.* **104**, 553–561.
- James, V.A., Worland, B., Snape, J.W. and Vain, P. (2004a) Strategies for precise quantification of transgene expression levels over several generations in rice. *J. Exp. Bot.* **55**, 1307–1313.
- James, V.A., Worland, B., Snape, J.W. and Vain, P. (2004b) Development of a standard operating procedure (SOP) for the precise quantification of transgene expression levels in rice plants. *Physiol. Plant.* **120**, 650–656.
- Jang, I.C., Choi, W.B., Lee, K.H., Song, S.I., Nahm, B.H. and Kim, J.K. (2002a) High-level and ubiquitous expression of the rice cytochrome c gene *OsCc1* and its promoter activity in transgenic plants provides a useful promoter for transgenesis of monocots. *Plant Physiol.* **129**, 1473–1481.
- Jang, I.C., Lee, K.H., Nahm, B.H. and Kim, J.K. (2002b) Chloroplast targeting signal of a rice *rbcS* gene enhances transgene expression. *Mol. Breed.* **9**, 81–91.
- Jang, I.C., Oh, S.J., Seo, J.S., Choi, W.B., Song, S.I., Kim, C.H., Kim, Y.S., Seo, H.S., Do Choi, Y., Nahm, B.H. and Kim, J.K. (2003) Expression of a bifunctional fusion of the *Escherichia coli* genes for trehalose-6-phosphate synthase and trehalose-6-phosphate

- phosphatase in transgenic rice plants increases trehalose accumulation and abiotic stress tolerance without stunting growth. *Plant Physiol.* **131**, 516–524.
- Jarvis, D.I. and Hodgkin, T. (1999) Wild relatives and crop cultivars, detecting natural introgression and farmer selection of new genetic combinations in agroecosystems. *Mol. Ecol.* **8**, S159–S173.
- Jefferson, R.A., Kavanaugh, T.A. and Bevan, M.W. (1987) GUS fusions: Beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.* **6**, 3901–3907.
- Jeon, J.S., Lee, S., Jung, K.H., Jun, S.H., Kim, C. and An, G. (2000) Tissue-preferential expression of a rice α -tubulin gene, *OsTubA1*, mediated by the first intron. *Plant Physiol.* **123**, 1005–1014.
- Jiang, J.D., Linscombe, S.D., Wang, J.L. and Oard, J.H. (2000) High efficiency transformation of US rice lines from mature seed-derived calli and segregation of glufosinate resistance under field conditions. *Crop Sci.* **40**, 1729–1741.
- Joersbo, M. (2001) Advances in the selection of transgenic plants using non-antibiotic marker genes. *Physiol. Plant.* **111**, 269–272.
- Joersbo, M., Jorgensen, K. and Brunstedt, J. (2003) A selection system for transgenic plants based on galactose as selective agent and a UDP-glucose, galactose-1-phosphate uridylyltransferase gene as selective gene. *Mol. Breed.* **11**, 315–323.
- Jung, S.Y., Chung, J.S., Chon, S.U., Kuk, Y.I., Lee, H.J., Guh, J.O. and Back, K. (2004) Expression of recombinant protoporphyrinogen oxidase influences growth and morphological characteristics in transgenic rice. *Plant Growth Regul.* **42**, 283–288.
- Jung, S.Y., Chung, J.S., Jang, S.M., Guh, J.O., Lee, H.J., Chon, S.U., Kim, K.M., Ha, S.B. and Back, K. (2003) Either soluble or plastidic expression of recombinant protoporphyrinogen oxidase modulates tetrapyrrole biosynthesis and photosynthetic efficiency in transgenic rice. *Biosci. Biotechnol. Biochem.* **67**, 1472–1478.
- Kachroo, A., He, Z.H., Patkar, R., Zhu, Q., Zhong, J.P., Li, D.B., Ronald, P., Lamb, C. and Chattoo, B.B. (2003) Induction of H_2O_2 in transgenic rice leads to cell death and enhanced resistance to both bacterial and fungal pathogens. *Transgenic Res.* **12**, 577–586.
- Kanzaki, H., Nirasawa, S., Saitoh, H., Ito, M., Nishihara, M., Terauchi, R. and Nakamura, I. (2002) Overexpression of the wasabi defensin gene confers enhanced resistance to blast fungus (*Magnaporthe grisea*) in transgenic rice. *Theor. Appl. Genet.* **105**, 809–814.
- Kathuria, H., Mohanty, A. and Tyagi, A.K. (2003) Analysis of inheritability and expression profile of single and multi-copy transgene(s) in rice over generations. *J. Plant Biochem. Biotechnol.* **12**, 103–107.
- Katiyar-Agarwal, S., Agarwal, M. and Grover, A. (2003) Heat-tolerant basmati rice engineered by over-expression of *Hsp101*. *Plant Mol. Biol.* **51**, 677–686.
- Katsube, T., Kurisaka, N., Ogawa, M., Maruyama, N., Ohtsuka, R., Utsumi, S. and Takaiwa, F. (1999) Accumulation of soybean glycinin and its assembly with the glutelins in rice. *Plant Physiol.* **120**, 1063–1073.
- Katsuhara, M., Koshio, K., Shibasaka, M., Hayashi, Y., Hayakawa, T. and Kasamo, K. (2003) Over-expression of a barley aquaporin increased the shoot/root ratio and raised salt sensitivity in transgenic rice plants. *Plant Cell Physiol.* **44**, 1378–1383.
- Kawahigashi, H., Hirose, S., Hayashi, E., Ohkawa, H. and Ohkawa, Y. (2002) Phytotoxicity and metabolism of ethofumesate in transgenic rice plants expressing the human *CYP2B6* gene. *Pesticide Biochem. Physiol.* **74**, 139–147.
- Kawahigashi, H., Hirose, S., Ohkawa, H. and Ohkawa, Y. (2003) Transgenic rice plants expressing human *CYP1A1* exude herbicide metabolites from their roots. *Plant Sci.* **165**, 373–381.
- Khanna, H.K. and Raina, S.K. (2002) Elite indica transgenic rice plants expressing modified *Cry1Ac* endotoxin of *Bacillus thuringiensis* show enhanced resistance to yellow stem borer (*Scirpophaga incertulas*). *Transgenic Res.* **11**, 411–423.
- Khush, G.S. (2001) Green revolution: the way forward. *Nature Rev. Genet.* **2**, 815–822.
- Kim, J.K., Jang, I.C., Wu, R., Zu, W.N., Boston, R.S., Lee, Y.H., Alm, P. and Nahm, B.H. (2003a) Co-expression of a modified maize ribosome-inactivating protein and a rice basic chitinase gene in transgenic rice plants confers enhanced resistance to sheath blight. *Transgenic Res.* **12**, 475–484.
- Kim, S.R., Lee, J., Jun, S.H., Park, S., Kang, H.G., Kwon, S. and An, G. (2003b) Transgene structures in T-DNA-inserted rice plants. *Plant Mol. Biol.* **52**, 761–773.
- Kirby, J. and Kavanagh, T.A. (2002) NAN fusions: a synthetic sialidase reporter gene as a sensitive and versatile partner for GUS. *Plant J.* **32**, 391–400.
- Kishitani, S., Takanami, T., Suzuki, M., Oikawa, M., Yokoi, S., Ishitani, M., Alvarez-Nakase, A.M. and Takabe, T. (2000) Compatibility of glycinebetaine in rice plants, evaluation using transgenic rice plants with a gene for peroxisomal betaine aldehyde dehydrogenase from barley. *Plant Cell Environ.* **23**, 107–114.
- Kloti, A., He, X., Potrykus, I., Hohn, T. and Fütterer, J. (2002) Tissue-specific silencing of a transgene in rice. *Proc. Natl. Acad. Sci. USA*, **99**, 10881–10886.
- Kobayashi, T., Nakanishi, H., Takahashi, M., Kawasaki, S., Nishizawa, N.K. and Mori, S. (2001) *In vivo* evidence that *Ids3* from *Hordeum vulgare* encodes a dioxygenase that converts 2'-deoxymugineic acid to mugineic acid in transgenic rice. *Planta*, **212**, 864–871.
- Kohli, A., Gahakwa, D., Vain, P., Laurie, D.A. and Christou, P. (1999) Transgene expression in rice engineered through particle bombardment, Molecular factors controlling stable expression and transgene silencing. *Planta*, **208**, 88–97.
- Kohli, A., Twyman, R.M., Abranches, R., Wegel, E., Stoger, E. and Christou, P. (2003) Transgene integration, organization and interaction in plants. *Plant Mol. Biol.* **52**, 247–258.
- Koike, S., Inoue, H., Mizuno, D., Takahashi, M., Nakanishi, H., Mori, S. and Nishizawa, N.K. (2004) *OsYSL2* is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. *Plant J.* **39**, 415–424.
- Komari, T., Hiei, Y., Saito, Y., Murai, N. and Kumashiro, T. (1996) Vectors carrying two separate tDNAs for co-transformation of higher plants mediated by *Agrobacterium tumefaciens* and segregation of transformants free from selection markers. *Plant J.* **10**, 165–174.
- Kouril, R., Lazar, D., Lee, H., Jo, J. and Naus, J. (2003) Moderately elevated temperature eliminates resistance of rice plants with enhanced expression of glutathione reductase to intensive photooxidative stress. *Photosynthetica*, **41**, 571–578.
- Krishnamurthy, K. and Giroux, M.J. (2001) Expression of wheat puroindoline genes in transgenic rice enhances grain softness. *Nat. Biotechnol.* **19**, 162–166.
- Krishnamurthy, K., Balconi, C., Sherwood, J.E. and Giroux, M.J. (2001) Wheat puroindolines enhance fungal disease resistance in transgenic rice. *Mol. Plant Microbe Interact.* **14**, 1255–1260.

- Kumria, R., Waie, B. and Rajam, M.V. (2001) Plant regeneration from transformed embryogenic callus of an elite indica rice via *Agrobacterium*. *Plant Cell Tiss. Org. Cult.* **67**, 63–71.
- Kusaba, M. (2004) RNA interference in crop plants. *Curr. Opin. Biotechnol.* **15**, 139–143.
- Kusaba, M., Miyahara, K., Iida, S., Fukuoka, H., Takano, T., Sassa, H., Nishimura, M. and Nishio, T. (2003) Low glutelin content1, a dominant mutation that suppresses the glutelin multigene family via RNA silencing in rice. *Plant Cell*, **15**, 1455–1467.
- Lawrence, J.C. and Nielsen, S.S. (2001) Partial isolation and characterization of a cysteine proteinase inhibitor from lima bean (*Phaseolus lunatus*). *J. Agr. Food. Chem.* **49**, 1020–1025.
- Lee, S.I., Kim, H.U., Lee, Y.H., Suh, S.C., Lim, Y.P., Lee, H.Y. and Kim, H.I. (2001) Constitutive and seed-specific expression of a maize lysine-feedback-insensitive dihydrodipicolinate synthase gene leads to increased free lysine levels in rice seeds. *Mol. Breed.* **8**, 75–84.
- Lee, H.J., Lee, S.B., Chung, J.S., Han, S.U., Han, O., Guh, J.O., Jeon, J.S., An, G. and Back, K. (2000) Transgenic rice plants expressing a *Bacillus subtilis* protoporphyrinogen oxidase gene are resistant to diphenyl ether herbicide oxyfluorfen. *Plant Cell Physiol.* **41**, 743–749.
- Lee, H.J., Lee, D.E., Ha, S.B., Jang, S.W., Lee, I.J., Ryu, S.B. and Back, K.W. (2004) The characterization of transgenic rice plants expressing a pepper 5-epi aristolochene synthase, the first committed step enzyme for capsidiol synthesis in isoprenoid pathway. *Plant Sci.* **166**, 881–887.
- Lee, T.T.T., Wang, M.M.C., Hou, R.C.W., Chen, L.J., Su, R.C., Wang, C.S. and Tzen, J.T.C. (2003) Enhanced methionine and cysteine levels in transgenic rice seeds by the accumulation of sesame 2S albumin. *Biosci. Biotechn. Biochem.* **67**, 1699–1705.
- Lentini, Z., Lozano, I., Tabares, E., Fory, L., Dominguez, J., Cuervo, M. and Calvert, L. (2003) Expression and inheritance of hypersensitive resistance to rice hoja blanca virus mediated by the viral nucleocapsid protein gene in transgenic rice. *Theor. Appl. Genet.* **106**, 1018–1026.
- Lian, H.L., YuX.YeQ., Ding, X.S., Kitagawa, Y., Kwak, S.S., Su, W.A. and Tang, Z.C. (2004) The role of aquaporin RWC3 in drought avoidance in rice. *Plant Cell Physiol.* **45**, 481–489.
- Lin, Y.J. and Zhang, Q. (2004) Optimising the tissue culture conditions for high efficiency transformation of indica rice. *Plant Cell Rep.* **23**, 540–547.
- Liu, Q.Q., Wang, Z.Y., Chen, X.H., Cai, X.L. and Tang, S.Z., Yu, H.X., Zhang, J.L., Hong, M.M. and Gu, M.H. (2003) Stable inheritance of the antisense *Waxy* gene in transgenic rice with reduced amylose level and improved quality. *Transgenic Res.* **12**, 71–82.
- Loc, N.T., Tinjuangjun, P., Gatehouse, A.M.R., Christou, P. and Gatehouse, J.A. (2002) Linear transgene constructs lacking vector backbone sequences generate transgenic rice plants, which accumulate higher levels of proteins conferring insect resistance. *Mol. Breed.* **9**, 231–244.
- Lonnerdal, B. (2003) Genetically modified plants for improved trace element nutrition. *J. Nutr.* **133**, 1490S–1493S.
- Lopez, S., Codina, C., Bastida, J., Viladomat, F., Davidson, E. and Stewart, D. (2002) Biodiversity of mannose-specific lectins within *Narcissus* species. *J. Agr. Food. Chem.* **50**, 2507–2513.
- Lu, H.J., Zhou, X.R., Gong, Z.X. and Upadhyaya, N.M. (2001) Generation of selectable marker-free transgenic rice using double right-border (DRB) binary vectors. *Aust. J. Plant Physiol.* **28**, 241–248.
- Lucca, P., Hurrell, R. and Potrykus, I. (2001a) Approaches to improving the bioavailability and level of iron in rice seeds. *J. Sci. Food Agr.* **81**, 828–834.
- Lucca, P., Ye, X.D. and Potrykus, I. (2001b) Effective selection and regeneration of transgenic rice plants with mannose as selective agent. *Mol. Breed.* **7**, 43–49.
- Lukowitz, W., Stewart Gillmor, C. and Scheible, W.-R. (2000) Positional cloning in *Arabidopsis*. Why it feels good to have a genome initiative working for you. *Plant Physiol.* **123**, 795–806.
- de Maagd, R.A., Bosch, D. and Stiekema, W. (1999) *Bacillus thuringiensis* toxin-mediated insect resistance in plants. *Trends Plant Sci.* **4**, 9–13.
- Majee, M., Maitra, S., Dastidar, K.G., Pattnaik, S., Chatterjee, A., Hait, N.C., Das, K.P. and Majumder, A.L. (2004) A novel salt-tolerant 1-myo-inositol-1-phosphate synthase from *Porteresia coarctata* (Roxb.) Tateoka, a halophytic wild rice – Molecular cloning, bacterial overexpression, characterization, and functional introgression into tobacco-conferring salt tolerance phenotype. *J. Biol. Chem.* **279**, 28539–28552.
- Malmberg, R.L., Watson, M.B., Galloway, G.L. and W. (1998) Molecular genetic analyses of plant polyamines. *Crit. Rev. Plant Sci.* **17**, 199–224.
- Maqbool, S.B., Riazuddin, S., Loc, N.T., Gatehouse, A.M.R., Gatehouse, J.A. and Christou, P. (2001) Expression of multiple insecticidal genes confers broad resistance against a range of different rice pests. *Mol. Breed.* **7**, 85–93.
- Marfa, V., Mele, E., Gabarra, R., Vassal, J.M., Guiderdoni, E. and Messegue, J. (2002) Influence of the developmental stage of transgenic rice plants (cv. Senia) expressing the *cry1B* gene on the level of protection against the striped stem borer (*Chilo suppressalis*). *Plant Cell Report*, **20**, 1167–1172.
- Martinez-Trujillo, M., Chavez-Barcenas, T., Limones-Briones, V., Simpson, J. and Herrera-Estrella, L. (2004) Functional analysis of the promoter of the rice sucrose phosphate synthase gene (*Sps1*). *Plant Sci.* **166**, 131–140.
- Maruta, Y., Ueki, J., Saito, H., Nitta, N. and Imaseki, H. (2002) Transgenic rice with reduced glutelin content by transformation with *glutelin A* antisense gene. *Mol. Breed.* **8**, 273–284.
- Matsumura, H., Nirasawa, S. Kiba, A. Urasaki, N. Saitoh, H. Ito, M. Kawai-Yamada, M. Uchimiya, H. and Terauchi, R. (2003) Overexpression of Bax inhibitor suppresses the fungal elicitor-induced cell death in rice (*Oryza sativa* L.) cells. *Plant J.* **33**, 425–434.
- Matsumura, T., Tabayashi, N., Kamagata, Y., Souma, C. and Saruyama, H. (2002) Wheat catalase expressed in transgenic rice can improve tolerance against low temperature stress. *Physiol. Plant.* **116**, 317–327.
- Mauch, F., Reimann, C., Freydl, E., Schaffrath, U. and Dudler, R. (1998) Characterization of the rice pathogen-related protein, Rirla and regulation of the corresponding gene. *Plant Mol. Biol.* **38**, 577–586.
- Mazithulela, G., Sudhakar, D., Heckel, T., Mehlo, L., Christou, P. Davies, J.W. and Boulton, M.I. (2000) The maize streak virus coat protein transcription unit exhibits tissue-specific expression in transgenic rice. *Plant Sci.* **155**, 21–29.
- Messegue, J., Fogher, C., Guiderdoni, E., Marfa, V., Catala, M.M., Baldi, G. and Mele, E. (2001) Field assessments of gene flow from transgenic to cultivated rice (*Oryza sativa* L.) using a herbicide resistance gene as tracer marker. *Theor. Appl. Genet.* **103**, 1151–1159.

- Messeguer, J., Marfa, V., Catala, M.M., Guiderdoni, E. and Mele, E. (2004) A field study of pollen-mediated gene flow from Mediterranean GM rice to conventional rice and the red rice weed. *Mol. Breed.* **13**, 103–112.
- Mills, D., Hammerschlag, F.A., Nordeen, R.O. and Owens, L.D. (1994) Evidence for the breakdown of cecropin-B by proteinases in the intercellular fluid of peach leaves. *Plant Sci.* **104**, 17–22.
- Mochizuki, A., Nishizawa, Y., Onodera, H., Tabei, Y., Toki, S., Habu, Y., Ugaki, M. and Ohashi, Y. (1999) Transgenic rice plants expressing a trypsin inhibitor are resistant against rice stem borers, *Chilo suppressalis*. *Entomol. Exp. Appl.* **93**, 173–178.
- Mohanty, A., Grover, M., Chaudhury, A., Rizwan-U., I-Haq, Q., Sharma, A.K., Maheshwari, S.C. and Tyagi, A.K. (2000) Analysis of the activity of promoters from two photosynthesis-related genes, *psaF* and *petH*, of spinach in a monocot plant, rice. *Ind. J. Biochem. Bioph.* **37**, 447–452.
- Mohanty, A., Kathuria, H., Ferjani, A., Sakamoto, A., Mohanty, P., Murata, N. and Tyagi, A.K. (2002) Transgenics of an elite indica rice variety pusa basmati 1 harbouring the *codA* gene are highly tolerant to salt stress. *Theor. Appl. Genet.* **106**, 51–57.
- Momma, K., Hashimoto, W., Ozawa, S., Kawai, S., Katsube, T., Takaiwa, F., Kito, M., Utsumi, S. and Murata, K. (1999) Quality and safety evaluation of genetically engineered rice with soybean glycinin, analyses of the grain composition and digestibility of glycinin in transgenic rice. *Biosci. Biotechnol. Biochem.* **63**, 314–318.
- Momma, K., Hashimoto, W., Yoon, H.J., Ozawa, S., Fukuda, Y., Kawai, S., Takaiwa, F., Utsumi, S. and Murata, K. (2000) Safety assessment of rice genetically modified with soybean glycinin by feeding studies on rats. *Biosci. Biotechnol. Biochem.* **64**, 1881–1886.
- Morino, K., Olsen, O.A. and Shimamoto, K. (1999) Silencing of an aleurone-specific gene in transgenic rice is caused by a rearranged transgene. *Plant J.* **17**, 275–285.
- Mukhopadhyay, A., Vij, S. and Tyagi, A.K. (2004) Over-expression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco. *Proc. Natl. Acad. Sci. USA* **101**, 6309–6314.
- Murakami, T., Matsuba, S., Funatsuki, H., Kawaguchi, K., Saruyama, H., Tanida, M. and Sato, Y. (2004) Over-expression of a small heat shock protein, *hSP17.7*, confers both heat tolerance and UV-B resistance to rice plants. *Mol. Breed.* **13**, 165–175.
- Murray-Kolb, L.E., Takaiwa, F., Goto, F., Yoshihara, T., Theil, E.C. and Beard, J.L. (2002) Transgenic rice is a source of iron for iron-depleted rats. *J. Nutr.* **132**, 957–960.
- Muthukrishnan, S., Liang, G.H., Trick, H.N. and Gill, B.S. (2001) Pathogenesis-related proteins and their genes in cereals. *Plant Cell Tiss. Org. Cult.* **64**, 93–114.
- Nagadhara, D., Ramesh, S., Pasalu, I.C., Rao, Y.K., Krishnaiah, N.V., Sarma, N.P., Bown, D.P., Gatehouse, J.A., Reddy, V.D. and Rao, K.V. (2003) Transgenic indica rice resistant to sap-sucking insects. *Plant Biotechnol. J.* **1**, 231–240.
- Nagadhara, D., Ramesh, S., Pasalu, I.C., Rao, Y.K., Sarma, N.P., Reddy, V.D. and Rao, K.V. (2004) Transgenic rice the plants expressing snowdrop lectin gene (*GNA*) exhibit high-level resistance to the whitebacked planthopper (*Sogatella furcifera*). *Theor. Appl. Genet.* **109**, 1399–1405.
- Nandi, S., Suzuki, Y.A., Huang, J.M., Yalda, D., Pham, P., Wu, L.Y., Bartley, G., Huang, N. and Lonnerdal, B. (2002) Expression of human lactoferrin in transgenic rice grains for the application in infant formula. *Plant Sci.* **163**, 713–722.
- Nap, J.P., Metz, P.L.J., Escaler, M. and Conner, A.J. (2003) The release of genetically modified crops into the environment – Part I. Overview of current status and regulations. *Plant J.* **33**, 1–18.
- Narayanan, N.N., Baisakh, N., Cruz, C.M.V., Gnanamanickam, S.S., Datta, K. and Datta, S.K. (2002) Molecular breeding for the development of blast and bacterial blight resistance in rice cv. Ir50. *Crop Sci.* **42**, 2072–2079.
- Nayak, P., Basu, D., Das, S., Basu, A., Ghosh, D., Ramakrishnan, N.A., Ghosh, M. and Sen, S.K. (1997) Transgenic elite indica rice plants expressing *CryIAC* δ endotoxin of *Bacillus thuringiensis* are resistant against yellow stem borer (*Scirphaga incertulas*). *Proc. Natl. Acad. Sci. USA*, **94**, 2111–2116.
- Nguyen, H.T., Leelavathi, S. and Reddy, V.S. (2004) Bacteriophage T7 RNA polymerase-directed, inducible and tissue-specific over-expression of foreign genes in transgenic plants. *Plant Biotechnol. J.* **2**, 301–310.
- Nielsen, K.M. (2003) Transgenic organisms—time for conceptual diversification? *Nat. Biotechnol.* **21**, 227–228.
- Nishizawa, Y., Saruta, M., Nakazono, K., Nishio, Z., Soma, M., Yoshida, T., Nakajima, E. and Hibi, T. (2003) Characterization of transgenic rice plants over-expressing the stress-inducible β -glucanase gene. *Gns1*. *Plant Mol. Biol.* **51**, 143–152.
- Nordlee, J.A., Taylor, S.L., Townsend, J.A., Thomas, L.A. and Bush, R.K. (1996) Identification of a Brazil-nut allergen in transgenic soybeans. *New Eng. J. Med.* **334**, 688–692.
- O'Toole, J.C., Toennies, G.H., Murashige, T., Harris, R.R. and Herd, R.W. (2001) The Rockefeller Foundation's International Programme on Rice Biotechnology, an experiment in international collaborative research, training and capacity building. www.rockfound.org/documents/463/O_Toole.pdf.
- Oard, J., Cohn, M.A., Linscombe, S., Gealy, D. and Gravois, K. (2000) Field evaluation of seed production, shattering, and dormancy in hybrid populations of transgenic rice (*Oryza sativa*) and the weed, red rice (*Oryza sativa*). *Plant Sci.* **157**, 13–22.
- Oard, J.H., Linscombe, S.D., Braverman, M.P., Jodari, F., Blouin, D.C., Leech, M., Kohli, A., Vain, P., Cooley, J.C. and Christou, P. (1996) Development, field evaluation, and agronomic performance of transgenic herbicide resistant rice. *Mol. Breed.* **2**, 359–368.
- Ohta, M., Hayashi, Y., Nakashima, A., Hamada, A., Tanaka, A., Nakamura, T. and Hayakawa, T. (2002) Introduction of a Na^+/H^+ antiporter gene from *Atriplex gmelini* confers salt tolerance to rice. *FEBS Lett.* **532**, 279–282.
- Okada, A., Okada, T., Ide, T., Itoh, M., Tanaka, K., Takaiwa, F. and Toriyama, K. (2003) Accumulation of Japanese cedar pollen allergen, Cry j 1, in the protein body I of transgenic rice seeds using the promoter and signal sequence of glutelin *GluB-1* gene. *Mol. Breed.* **12**, 61–70.
- Orefig, A.S., Kovacs, G., Jenes, B., Kiss, E., Scott, P. and Toldi, O. (2004) Development of a non-lethal selection system by using the *aadA* marker gene for efficient recovery of transgenic rice (*Oryza sativa* L.). *Plant Cell Report*, **22**, 490–496.
- Palukaitis, P. and Zaitlin, M. (1997) Replicase-mediated resistance to plant virus disease. *Advances in Virus Research*, **48**, 349–377.
- Panahi, M., Alli, Z., Cheng, X.Y., Belbaraka, L., Belgoudi, J., Sardana, R., Phipps, J. and Altosaar, I. (2004) Recombinant protein expression plasmids optimized for industrial *E. coli* fermentation and plant systems produce biologically active human insulin-like growth factor-1 in transgenic rice and tobacco plants. *Transgenic Res.* **13**, 245–259.

- Penna, S. (2003) Building stress tolerance through over-producing trehalose in transgenic plants. *Trends Plant Sci.* **8**, 355–357.
- Petrucelli, S., Dai, S., Carcamo, R., Yin, Y.H., Chen, S.Y. and Beachy, R.N. (2001) Transcription factor RF2a alters expression of the rice tungro bacilliform virus promoter in transgenic tobacco plants. *Proc. Natl. Acad. Sci. USA* **98**, 7635–7640.
- Pinto, Y.M., Kok, R.A. and Baulcombe, D.C. (1999) Resistance to rice yellow mottle virus (RYMV) in cultivated African rice varieties containing RYMV transgenes. *Nat Biotechnol.* **17**, 702–707.
- Poletti, S., Gruissem, W. and Sautter, C. (2004) The nutritional fortification of cereals. *Curr. Opin. Biotechnol.* **15**, 162–165.
- Pons, M.J., Marfa, V., Mele, E. and Messeguer, J. (2000) Regeneration and genetic transformation of Spanish rice cultivars using mature embryos. *Euphytica*, **114**, 117–122.
- Potenza, C., Aleman, L. and Sengupta-Gopalan, C. (2004) Targeting transgene expression in research, agricultural, and environmental applications: promoters used in plant transformation. *In Vitro Cell. Dev. Biol., Plant*, **40**, 1–22.
- Potrykus, I. (2001) Golden rice and beyond. *Plant Physiol.* **125**, 1157–1161.
- Potrykus, I. (2003) Nutritionally enhanced rice to combat malnutrition disorders of the poor. *Nutrition Rev.* **61**, S101–S104.
- Qu, L.J., Chen, J., Liu, M.H., Pan, N.S., Okamoto, H., Lin, Z.Z., Li, C.Y., Li, D.H., Wang, J.L., Zhu, G.F., Zhao, X., Chen, X., Gu, H.G. and Chen, Z.L. (2003) Molecular cloning and functional analysis of a novel type of Bowman-Birk inhibitor gene family in rice. *Plant Physiol.* **133**, 560–570.
- Qu, L.Q. and Takaiwa, F. (2004) Evaluation of tissue specificity and expression strength of rice seed component gene promoters in transgenic rice. *Plant Biotechnol. J.* **2**, 113–125.
- Quimio, C.A., Torrizo, L.B., Setter, T.L., Ellis, M., Grover, A., Abrigo, E.M., Oliva, N.P., Ella, E.S., Carpena, A.L., Ito, O., Peacock, W.J., Dennis, E. and Datta, S.K. (2000) Enhancement of submergence tolerance in transgenic rice overproducing pyruvate decarboxylase. *J. Plant Physiol.* **156**, 516–521.
- Rachmawati, D., Hosaka, T., Inoue, E. and Anza, H. (2004) *Agrobacterium*-mediated transformation of javanica rice cv. Rojolele. *Biosci. Biotechnol. Biochem.* **68**, 1193–1200.
- Rahman, M., Grover, A., Peacock, W.J., Dennis, E.S. and Ellis, M.H. (2001) Effects of manipulation of pyruvate decarboxylase and alcohol dehydrogenase levels on the submergence tolerance of rice. *Aust. J. Plant Physiol.* **28**, 1231–1241.
- Ramesh, S., Nagadhara, D., Pasalu, I.C., Kumari, A.P., Sarma, N.P., Reddy, V.D. and Rao, K.V. (2004a) Development of stem borer resistant transgenic parental lines involved in the production of hybrid rice. *J. Biotechnol.* **111**, 131–141.
- Ramesh, S., Nagadhara, D., Reddy, V.D. and Rao, K.V. (2004b) Production of transgenic indica rice resistant to yellow stem borer and sap-sucking insects, using super-binary vectors of *Agrobacterium tumefaciens*. *Plant Sci.* **166**, 1077–1085.
- Raskin, I., Ribnick, D.M., Komarnytsky, S., Ilic, N., Poulev, A., Borisjuk, N., Brinker, A., Moreno, D.A., Ripoll, C., Yakoby, N., O'Neal, J.M., Cornwell, T., Pastor, I. and Fridlender, B. (2002) Plants and human health in the twenty-first century. *Trends Biotechnol.* **20**, 522–531.
- Rohila, J.S., Jain, R.K. and Wu, R. (2002) Genetic improvement of Basmati rice for salt and drought tolerance by regulated expression of a barley *Hva1* cDNA. *Plant Sci.* **163**, 525–532.
- Rommens, C.M., Humara, J.M., Ye, J.S., Yan, H., Richael, C., Zhang, L., Perry, R. and Swords, K. (2004) Crop improvement through modification of the plant's own genome. *Plant Physiol.* **135**, 421–431.
- Roy, M. and Wu, R. (2001) Arginine decarboxylase transgene expression and analysis of environmental stress tolerance in transgenic rice. *Plant Sci.* **160**, 869–875.
- Roy, M. and Wu, R. (2002) Over-expression of S-adenosylmethionine decarboxylase gene in rice increases polyamine level and enhances sodium chloride-stress tolerance. *Plant Sci.* **163**, 987–992.
- Saijo, Y., Hata, S., Kyoizuka, J., Shimamoto, K. and Izui, K. (2000) Over-expression of a single Ca²⁺-dependent protein kinase confers both cold and salt/drought tolerance on rice plants. *Plant J.* **23**, 319–327.
- Sakamoto, T. and Matsuoka, M. (2004) Generating high-yielding varieties by genetic manipulation of plant architecture. *Curr. Opin. Biotechnol.* **15**, 144–147.
- Sakamoto, A. and Murata, N. (2002) The role of glycine betaine in the protection of plants from stress, clues from transgenic plants. *Plant Cell Environ.* **25**, 163–171.
- Sakamoto, A., Murata, A. and Murata, N. (1998) Metabolic engineering of rice leading to biosynthesis of glycinebetaine and tolerance to salt and cold. *Plant Mol. Biol.* **38**, 1011–1019.
- Sandberg, A.S., Hulthen, L.R. and Turk, M. (1996) Dietary *Aspergillus niger* phytase increases iron absorption in humans. *J. Nutr.* **126**, 476–480.
- Sanders, D., Brownlee, C. and Harper, J.F. (1999) Communicating with calcium. *Plant Cell*, **11**, 691–706.
- Sasaki, T. and Burr, B. (2000) International rice genome sequencing project: the effort to completely sequence rice genome. *Curr. Opin. Plant Biol.* **3**, 138–141.
- Sasaki, T., Matsumoto, T., Antonio, B.A. and Nagamura, Y. (2005) From mapping to sequencing, post-sequencing and beyond. *Plant Cell Physiol.* **46**, 3–13.
- Sasaki, T., Matsumoto, T., Yamamoto, K., Sakata, K., Baba, T., Katayose, Y., Wu, J.Z., Niimura, Y., Cheng, Z.K., Nagamura, Y., Antonio, B.A., Kanamori, H., Hosokawa, S., Masukawa, M., Arikawa, K., Chiden, Y., Hayashi, M., Okamoto, M., Ando, T., Aoki, H., Arita, K., Hamada, M., Harada, C., Hijishita, S., Honda, M., Ichikawa, Y., Idonuma, A., Iijima, M., Ikeno, M., Ito, S., Ito, T., Ito, Y., Iwabuchi, A., Kamiya, K., Karasawa, W., Katagiri, S., Kikuta, A., Kobayashi, N., Kono, I., Machita, K., Maehara, T., Mizuno, H., Mizubayashi, T., Mukai, Y., Nagasaki, H., Nakashima, M., Nakama, Y., Nakamichi, Y., Nakamura, M., Namiki, N., Negishi, M., Ohta, I., Ono, N., Saji, S., Sakai, K., Shibata, M., Shimokawa, T., Shomura, A., Song, J.Y., Takazaki, Y., Terasawa, K., Tsuji, K., Waki, K., Yamagata, H., Yamane, H., Yoshiki, S., Yoshihara, R., Yukawa, K., Zhong, H.S., Iwama, H., Endo, T., Ito, H., Hahn, J.H., Kim, H.I., Eun, M.Y., Yano, M., Jiang, J.M. and Gojohori, T. (2002) The genome sequence and structure of rice chromosome 1. *Nature*, **420**, 312–316.
- Sawada, K., Hasegawa, M., Tokuda, L., Kameyama, J., Kodama, O., Kohchi, T., Yoshida, K. and Shinmyo, A. (2004) Enhanced resistance to blast fungus and bacterial blight in transgenic rice constitutively expressing *OsSBP*, a rice homologue of mammalian selenium-binding proteins. *Biosci. Biotechnol. Biochem.* **68**, 873–880.
- Sawahel, W. (2003) Improved performance of transgenic glycinebetaine-accumulating rice plants under drought stress. *Bol. Plant.* **47**, 39–44.
- Saxena, D., Stewart, C.N., Altosaar, I., Shu, Q.Y. and Stotzky, G. (2004) Larvicidal Cry proteins from *Bacillus thuringiensis* are

- released in root exudates of transgenic *B. thuringiensis* corn, potato, and rice but not of *B. thuringiensis* canola, cotton, and tobacco. *Plant Physiol. Biochem.* **42**, 383–387.
- Schaffrath, U., Mauch, F., Freydl, E., Schweizer, P. and Dudler, R. (2000) Constitutive expression of the defense-related *Rir1b* gene in transgenic rice plants confers enhanced resistance to the rice blast fungus, *Magnaporthe grisea*. *Plant Mol. Biol.* **43**, 59–66.
- Scheid, O.M. (2004) Either/or selection markers for plant transformation. *Nat. Biotechnol.* **22**, 398–399.
- Schuler, T.H., Poppy, G.M., Kerry, B.R. and Denholm, I. (1998) Insect-resistant transgenic plants. *Trends Biotechnol.* **16**, 168–175.
- Serrano, R. and Rodriguez-Navarro, A. (2001) Ion homeostasis during salt stress in plants. *Curr. Opin. Cell Biol.* **13**, 399–404.
- Sha, Y., Li, S., Pei, Z., Luo, L., Tian, Y. and He, C. (2004) Generation and flanking sequence analysis of a rice tDNA tagged population. *Theor. Appl. Genet.* **108**, 306–314.
- Sharma, M., Charak, K.S. and Ramanaiah, T.V. (2003) Agricultural biotechnology research in India: status and policies. *Curr. Sci.* **84**, 297–302.
- Sharma, H.C., Sharma, K.K. and Crouch, J.H. (2004) Genetic transformation of crops for insect resistance: potential and limitations. *Crit. Rev. Plant Sci.* **23**, 47–72.
- Sharma, A., Sharma, R., Imamura, M., Yamakawa, M. and Machii, H. (2000) Transgenic expression of cecropin B, an antibacterial peptide from *Bombyx mori*, confers enhanced resistance to bacterial leaf blight in rice. *FEBS Lett.* **485**, 208–208.
- Shelton, A.M., Zhao, J.Z. and Roush, R.T. (2002) Economic, ecological, food safety, and social consequences of the deployment of *Bt* transgenic plants. *Annu. Rev. Entomol.* **47**, 845–881.
- Shen, Y.G., Zhang, W.K., He, S.J., Zhang, J.S., Liu, Q. and Chen, S.Y. (2003) An EREBP/AP2-type protein in *Triticum aestivum* was a DRE-binding transcription factor induced by cold, dehydration and ABA stress. *Theor. Appl. Genet.* **106**, 923–930.
- Shimamoto, K. and Kyoizuka, J. (2002) Rice as a model of comparative genomics of plants. *Annu. Rev. Plant Biol.* **53**, 399–419.
- Shimamoto, K., Teda, R., Izawa, T. and Fujimoto, H. (1989) Fertile transgenic rice plants regenerated from transformed protoplasts. *Nature*, **338**, 274–277.
- Shinozaki, K. and Yamaguchi-Shinozaki, K. (2000) Molecular responses to dehydration and low temperature, differences and cross-talk between two stress signaling pathways. *Curr. Opin. Plant Biol.* **3**, 217–223.
- Shoeb, F., Yadav, J.S., Bajaj, S. and Rajam, M.V. (2001) Polyamines as biomarkers for plant regeneration capacity, improvement of regeneration by modulation of polyamine metabolism in different genotypes of indica rice. *Plant Sci.* **160**, 1229–1235.
- Shu, Q.U., Ye G.Y., Cui, H.R., Cheng, X.Y., Xiang, Y.B., Wu, D.X., Gao, M.W., Xia, Y.W., Hu, C., Sardana, R. and Altaoar, I. (2000) Transgenic rice plants with a synthetic *cry1Ab* gene from *Bacillus thuringiensis* were highly resistant to eight lepidopteran rice pest species. *Mol. Breed.* **6**, 433–439.
- Shu, Q.Y., Cui, H.R., Ye G.Y., Wu, D.X., Xia, Y.W., Gao, M.W. and Altaoar, I. (2002) Agronomic and morphological characterization of *Agrobacterium*-transformed *Bt* rice plants. *Euphytica*, **127**, 345–352.
- Singla-Pareek, S.L., Reddy, M.K. and Sopory, S.K. (2003) Genetic engineering of the glyoxalase pathway in tobacco leads to enhanced salinity tolerance. *Proc. Natl. Acad. Sci. USA* **100**, 14672–14677.
- Somers, D.A. and Makarevitch, I. (2004) Transgene intergration in plants, poking or patching holes in promiscuous genomes? *Curr. Opin. Biotechnol.* **15**, 126–131.
- Song, Z.P., Lu, B.R., Zhu, Y.G. and Chen, J.K. (2003) Gene flow from cultivated rice to the wild species *Oryza rufipogon* under experimental field conditions. *New Phytol.* **157**, 657–665.
- Song, Z.P., Lug, B.R., Zhu, Y.G. and Chen, J.K. (2002) Pollen competition between cultivated and wild rice species (*Oryza sativa* and *O. rufipogon*). *New Phytol.* **153**, 289–296.
- Song, W.Y., Wang, G.L., Chen, L.L., Kim, H.S., Pi, L.Y., Holsten, T., Gardner, J., Wang, B., Zhai, W.X., Zhu, L.H., Fauquet, C. and Ronald, P. (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science*, **270**, 1804–1806.
- Srivastava, V., Ariza-Nieto, M. and Wilson, A.J. (2004) Cre-mediated site-specific gene integration for consistent transgene expression in rice. *Plant Biotechnol. J.* **2**, 169–179.
- Stoger, E., Vaquero, C., Torres, E., Sack, M., Nicholson, L., Drossard, J., Williams, S., Keen, D., Perrin, Y., Christou, P. and Fischer, R. (2000) Cereal crops as viable production and storage systems for pharmaceutical scFv antibodies. *Plant Mol. Biol.* **42**, 583–590.
- Su, P.H., Yu, S.M. and Chen, C.S. (2001) Spatial and temporal expression of a rice prolamin gene RP5 promoter in transgenic tobacco and rice. *J. Plant Physiol.* **158**, 247–254.
- Su, J. and Wu, R. (2004) Stress-inducible synthesis of proline in transgenic rice confers faster growth under stress conditions than that with constitutive synthesis. *Plant Sci.* **166**, 941–948.
- Sun, X.L., Cao, Y.L., Yang, Z.F., Xu, C.G., Li, X.H., Wang, S.P. and Zhang, Q.F. (2004) *Xa26*, a gene conferring resistance to *Xanthomonas oryzae* pv. *oryzae* in rice, encodes an LRR receptor kinase-like protein. *Plant J.* **37**, 517–527.
- Suzuki, Y.A., Kelleher, S.L., Yalda, D., Wu, L.Y., Huang, J.M., Huang, N. and Lonnerdal, B. (2003) Expression, characterization, and biological activity of recombinant human lactoferrin in rice. *J. Pediatr. Gastr. Nutr.* **36**, 190–199.
- Tabashnik, B.E., Carriere, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush, R.T., Shelton, A.M. and Zhao, J.Z. (2003) Insect resistance to transgenic *Bt* crops: lessons from the laboratory and field. *J. Econ. Entomol.* **96**, 1031–1038.
- Tada, Y., Utsumi, S. and Takaiwa, F. (2003) Foreign gene products can be enhanced by introduction into low storage protein mutants. *Plant Biotechnol. J.* **1**, 411–422.
- Takahashi, M. (2003) Overcoming Fe deficiency by a transgenic approach in rice. *Plant Cell Tiss. Org. Cult.* **72**, 211–220.
- Takahashi, M., Nakanishi, H., Kawasaki, S., Nishizawa, N.K. and Mori, S. (2001) Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. *Nat. Biotechnol.* **19**, 466–469.
- Takesawa, T., Ito, M., Kanzaki, H., Kameya, N. and Nakamura, I. (2002) Over-expression of zeta glutathione S-transferase in transgenic rice enhances germination and growth at low temperature. *Mol. Breed.* **9**, 93–101.
- Tanaka, N., Fujita, N., Nishi, A., Satoh, H., Hosaka, Y., Ugaki, M., Kawasaki, S. and Nakamura, Y. (2004) The structure of starch can be manipulated by changing the expression levels of starch branching enzyme IIb in rice endosperm. *Plant Biotechnol. J.* **2**, 507–516.
- Tang, W., Luo, X.Y. and Samuels, V. (2004) Regulated gene expression with promoters responding to inducers. *Plant Sci.* **166**, 827–834.
- Tang, K.X., Sun, X.F., Hu, Q.N., Wu, A.Z., Lin, C.H., Lin, H.J., Twyman, R.M., Christou, P. and Feng, T.Y. (2001a) Transgenic

- rice plants expressing the ferredoxin-like protein (API) from sweet pepper show enhanced resistance to *Xanthomonas oryzae* pv. *oryzae*. *Plant Sci.* **160**, 1035–1042.
- Tang, K., Zhao, E., Sun, X., Wan, B., Qi, H. and Lu, X. (2001b) Production of transgenic rice homozygous lines with enhanced resistance to the rice brown planthopper. *Acta Biotechnol.* **21**, 117–128.
- Taylor, S.L. (2003) Safety assessment of foods produced through agricultural biotechnology. *Nutr. Rev.* **61**, S135–S140.
- Terada, R., Asao, H. and Iida, S. (2004) A large-scale *Agrobacterium*-mediated transformation procedure with a strong positive-negative selection for gene targeting in rice (*Oryza sativa* L.). *Plant Cell Report*, **22**, 653–659.
- Terada, R., Nakajima, M., Isshiki, M., Okagaki, R., Wessler, S.R. and Shimamoto, K. (2000) Antisense *Waxy* genes with highly active promoters effectively suppress waxy gene expression in transgenic rice. *Plant Cell Physiol.* **41**, 881–888.
- Terada, R., Urawa, H., Inagaki, Y., Tsugane, K. and Iida, S. (2002) Efficient gene targeting by homologous recombination in rice. *Nat. Biotechnol.* **20**, 1030–1034.
- Terashima, M., Hashikawa, N., Hattori, M. and Yoshida, H. (2002) Growth characteristics of rice cell genetically modified for recombinant human α (1)-antitrypsin production. *Biochem. Eng. J.* **12**, 155–160.
- The Rice Chromosome 10 Sequencing Consortium (2003) In-depth view of structure, activity, and evolution of rice chromosome 10. *Science*, **300**, 1566–1569.
- The Rice Full-Length cDNA Consortium (2003) Collection, mapping and annotation of over 28000 cDNA clones from japonica rice. *Science*, **301**, 376–379.
- Tian, G. W., Mohanty, A., Chary, S.N., Li, S., Paap, B., Drakakaki, G., Kopec, C.D., Li, J., Ehrhardt, D., Jackson, D., Rhee, S.Y., Raikhel, N.V. and Citovsky, V. (2004) High-throughput fluorescent tagging of full-length *Arabidopsis* gene products in planta. *Plant Physiol.* **135**, 25–38.
- Tinjuangjun, P., Loc, N.T., Gatehouse, A.M.R., Gatehouse, J.A. and Christou, P. (2000) Enhanced insect resistance in Thai rice varieties generated by particle bombardment. *Mol. Breed.* **6**, 391–399.
- Toenniessen, G.H., O'Toole, J.C. and De Vries, J. (2003) Advances in plant biotechnology and its adoption in developing countries. *Curr. Opin. Plant Biol.* **6**, 191–198.
- Toriyama, K., Arimoto, Y., Uchimiya, H. and Hinata, K. (1988) Transgenic rice plants after direct gene transfer into protoplasts. *Biol. Technology*, **6**, 1072–1074.
- Torres, E., Vaquero, C., Nicholson, L., Sack, M., Stoger, E., Drossard, J., Christou, P., Fischer, R. and Perrin, Y. (1999) Rice cell culture as an alternative production system for functional diagnostic and therapeutic antibodies. *Transgenic Res.* **8**, 441–449.
- Tozawa, Y., Hasegawa, H., Terakawa, T. and Wakasa, K. (2001) Characterization of rice anthranilate synthase alpha-subunit genes *OASA1* and *OASA2*: tryptophan accumulation in transgenic rice expressing mutant of *Oasa1*. *Plant Physiol.* **126**, 1493–1506.
- Trexler, M.M., McDonald, K.A. and Jackman, A.P. (2002) Bioreactor production of human α (1)-antitrypsin using metabolically regulated plant cell cultures. *Biotechnol. Prog.* **18**, 501–508.
- Tu, J., Datta, K., Khush, G.S., Zhang, Q. and Datta, S.K. (2000a) Field performance of *Xa21* transgenic indica rice (*Oryza sativa* L.), IR72. *Theor. Appl. Genet.* **101**, 15–20.
- Tu, J.M., Datta, K., Oliva, N., Zhang, G.A., Xu, C.G., Khush, G.S., Zhang, Q.F. and Datta, S.K. (2003) Site-independently integrated transgenes in the elite restorer rice line Minghui 63 allow removal of a selectable marker from the gene of interest by self-segregation. *Plant Biotechnol. J.* **1**, 155–165.
- Tu, J.M., Zhang, G.A., Datta, K., Xu, C.G., He, Y.Q., Zhang, Q.F., Khush, G.S. and Datta, S.K. (2000b) Field performance of transgenic elite commercial hybrid rice expressing *Bacillus thuringiensis* δ -endotoxin. *Nat. Biotechnol.* **18**, 1101–1104.
- Tyagi, A.K., Khurana, J.P., Khurana, P., Raghuvanshi, S., Gaur, A., Kapur, A., Gupta, V., Kumar, D., Ravi, V., Vij, S. and Sharma, S. (2004) Structural and functional analysis of rice genome. *J. Genet.* **83**, 79–99.
- Tyagi, A.K. and Mohanty, A. (2000) Rice transformation for crop improvement and functional genomics. *Plant Sci.* **158**, 1–18.
- Tyagi, A.K., Mohanty, A., Bajaj, S., Chaudhury, A. and Maheshwari, S.C. (1999) Transgenic rice: a valuable monocot system for crop improvement and gene research. *Crit. Rev. Biotechnol.* **19**, 41–79.
- Uchimiya, H., Fujii, S., Huang, J.R., Fushimi, T., Nishioka, M., Kim, K.M., Yamada, M.K., Kurusu, T., Kuchitsu, K. and Tagawa, M. (2002) Transgenic rice plants conferring increased tolerance to rice blast and multiple environmental stresses. *Mol. Breed.* **9**, 25–31.
- Uchimiya, H., Iwata, M., Nojiri, C., Samarajeewa, P.K., Takamatsu, S., Ooba, S., Anzai, S., Christensen, A.H., Quail, P.H. and Toki, S. (1993) Bialaphos treatment of transgenic rice plants expressing a *bar* gene prevents infection by the sheath blight pathogen (*Rhizoctonia solani*). *Bio/Technology*, **11**, 835–836.
- Upadhyaya, N.M., Li, Z., Wang, M.-B., Chen, S., Gong, Z.-X. and Waterhouse, P.M. (2001) Engineering for virus resistance in rice. In: *Rice Genetics IV: Proceedings of the Fourth International Rice Genetics Symposium* (Khush, G.S., Brar, D.S. and Hardy, B., eds), pp. 405–421. Los Baños, Philippines, International Rice Research Institute.
- Upadhyaya, N.M., Surin, B., Ramm, K., Gaudron, J., Schunmann, P.H.D., Taylor, W., Waterhouse, P.M. and Wang, M.B. (2000a) *Agrobacterium*-mediated transformation of Australian rice cultivars Jarrah and Amaroo using modified promoters and selectable markers. *Aust. J. Plant Physiol.* **27**, 201–210.
- Upadhyaya, N.M., Zhou, X.R., Wu, L., Ramm, K. and Dennis, E.S. (2000b) The *tms2* gene as a negative selection marker in rice. *Plant Mol. Biol. Reporter*, **18**, 227–233.
- Upadhyaya, N.M., Zhou, X.-R., Zhu, Q.-H., Eamens, A., Wang, M.-B., Waterhouse, P.M. and Dennis, E.S. (2000c) Transgenic rice. In: *Transgenic Cereals* (O'Brien, L. and Henry, R.J., eds), pp. 28–87. AACC, Minnesota.
- Vain, P., Afolabi, A.S., Worland, B. and Snape, J.W. (2003) Transgene behaviour in populations of rice plants transformed using a new dual binary vector system, pGreen/pSoup. *Theor. Appl. Genet.* **107**, 210–217.
- Vain, P., James, V.A., Worland, B. and Snape, J.W. (2002) Transgene behaviour across two generations in a large random population of transgenic rice plants produced by particle bombardment. *Theor. Appl. Genet.* **105**, 878–889.
- Vain, P., Worland, B., Kohli, A., Snape, J.W. and Christou, P. (1998) The green fluorescent protein (GFP) as a vital screenable marker in rice transformation. *Theor. Appl. Genet.* **96**, 164–169.
- Vain, P., Worland, B., Kohli, A., Snape, J.W., Christou, P., Allen, G.C. and Thompson, W.F. (1999) Matrix attachment regions increase transgene expression levels and stability in transgenic rice plants and their progeny. *Plant J.* **18**, 233–242.

- Van der Geest, A.H.M., Welter, M.E., Woosley, A.T., Pareddy, D.R., Pavelko, S.E., Skokut, M. and Ainley, W.M. (2004) A short synthetic MAR positively affects transgene expression in rice and *Arabidopsis*. *Plant Biotechnol. J.* **2**, 13–26.
- Vannini, C., Locatelli, F., Bracale, M., Magnani, E., Marsoni, M., Osnato, M., Mattana, M., Baldoni, E. and Coraggio, I. (2004) Overexpression of the rice *Osmyb4* gene increases chilling and freezing tolerance of *Arabidopsis thaliana* plants. *Plant J.* **37**, 115–127.
- Vasconcelos, M., Datta, K., Oliva, N., Khalekuzzaman, M., Torrizo, L., Krishnan, S., Oliveira, M., Goto, F. and Datta, S.K. (2003) Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. *Plant Sci.* **164**, 371–378.
- Vaucheret, H., Beclin, C., Elmayan, T., Feuerbach, F., Godon, C., Morel, J.B., Mourrain, P., Palauqui, J.C. and Vernhettes, S. (1998) Transgene-induced gene silencing in plants. *Plant J.* **16**, 651–659.
- de Vetten, N., Wolters, A.M., Raemakers, K., van der Meer, I., ter Stege, R., Heeres, E., Heeres, P. and Visser, R. (2003) A transformation method for obtaining marker-free plants of a cross-pollinating and vegetatively propagated crop. *Nat. Biotechnol.* **21**, 439–442.
- Vickers, C.E., Xue, G.P. and Gresshoff, P.M. (2003) A synthetic xylanase as a novel reporter in plants. *Plant Cell Report*, **22**, 135–140.
- Visser, R.G.F. and Jacobsen, E. (1993) Towards modifying plants for altered starch content and composition. *Trends Biotechnol.* **11**, 63–68.
- Wang, J.L., Jiang, J.D. and Oard, J.H. (2000) Structure, expression and promoter activity of two polyubiquitin genes from rice (*Oryza sativa* L.). *Plant Sci.* **156**, 201–211.
- Wang, J. and Oard, J.H. (2003) Rice ubiquitin promoters, deletion analysis and potential usefulness in plant transformation systems. *Plant Cell Report*, **22**, 129–134.
- Wang, Z.G., Shu, Q.Y., YeG.Y., Cui, H.R., Wu, D.X., Altosaar, I. and Xia, Y.W. (2002a) Genetic analysis of resistance of *Bt* rice to stripe stem borer (*Chilo suppressalis*). *Euphytica*, **123**, 379–386.
- Wang, W., Vinocur, B. and Altman, A. (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta*, **218**, 1–14.
- Wang, Z.H., Wang, Y., Cui, H.R., Xia, Y.W., Altosaar, I. and Shu, Q.Y. (2002b) Toxicological evaluation of transgenic rice flour with a synthetic *cry1Ab* gene from *Bacillus thuringiensis*. *J. Sci. Food Agr.* **82**, 738–744.
- Wang, M.B. and Waterhouse, P.M. (2000) High-efficiency silencing of a beta-glucuronidase gene in rice is correlated with repetitive transgene structure but is independent of DNA methylation. *Plant Mol. Biol.* **43**, 67–82.
- Wei, H.R., Wang, M.L., Moore, P.H. and Albert, H.H. (2003) Comparative expression analysis of two sugarcane polyubiquitin promoters and flanking sequences in transgenic plants. *J. Plant Physiol.* **160**, 1241–1251.
- WHO (2000) *Safety Aspects of Genetically Modified Foods of Plant Origin*. Report of a joint FAO/WHO expert consultation on foods derived from biotechnology. World Health Organization, Geneva, 29 May–2 June, 2000.
- Wu, X.R., Chen, Z.H. and Folk, M.R. (2003) Enrichment of cereal protein lysine content by altered tRNA (lys) coding during protein synthesis. *Plant Biotechnol. J.* **1**, 187–194.
- Wu, J., Maehara, T., Shimokawa, T., Yamamoto, S., Harada, C., Takazaki, Y., Ono, N., Mukai, Y., Koike, K., Yazaki, J., Fujii, F., Shomura, A., Ando, T., Kono, I., Waki, K., Yamamoto, K., Yano, M., Matsumoto, T. and Sasaki, T. (2002a) A comprehensive rice transcript map containing 6591 expressed sequence tag sites. *Plant Cell*, **14**, 525–535.
- Wu, W.X., Ye, Q.F. and Min, H. (2004a) Effect of straws from *Bt*-transgenic rice on selected biological activities in water-flooded soil. *Eur. J. Soil. Biol.* **40**, 15–22.
- Wu, W.X., Ye, Q.F., Min, H., Duan, X.J. and Jin, W.M. (2004b) *Bt*-transgenic rice straw affects the culturable microbiota and dehydrogenase and phosphatase activities in a flooded paddy soil. *Soil Biol. Biochem.* **36**, 289–295.
- Wu, L.Y., Nandi, S., Chen, L.F., Rodriguez, R.L. and Huang, N. (2002b) Expression and inheritance of nine transgenes in rice. *Transgenic Res.* **11**, 533–541.
- Wu, D.X., Shu, Q.Y., Wang, Z.H., Cui, H.R. and Xia, Y.W. (2002c) Quality variations in transgenic rice with a synthetic *cry1Ab* gene from *Bacillus thuringiensis*. *Plant Breed.* **121**, 198–202.
- Wu, A., Sun, X., Pang, Y. and Tang, K. (2002d) Homozygous transgenic rice lines expressing *GNA* with enhanced resistance to the rice sap-sucking pest *Laodelphax striatellus*. *Plant Breed.* **121**, 93–95.
- Wu, D.X., Ye, Q.F., Wang, Z.H. and Xia, Y.W. (2004c) Effect of gamma irradiation on nutritional components and *Cry1Ab* protein in the transgenic rice with a synthetic *cry1Ab* gene from *Bacillus thuringiensis*. *Radiat. Phys. Chem.* **69**, 79–83.
- Wu, C.Y., Washida, H., Onodera, Y., Harada, K. and Takaiwa, F. (2000) Quantitative nature of the Prolamin-box, ACGT and AACA motifs in a rice glutelin gene promoter, minimal cis-element requirements for endosperm-specific gene expression. *Plant J.* **23**, 415–421.
- Wunn, J., Kloti, A., Burkhardt, P.K., Ghosh Biswas, G.C., Launis, K., Iglesias, V.A. and Potrykus, I. (1996) Transgenic indica rice breeding line IR58 expressing a synthetic *cry1A (b)* gene from *Bacillus thuringiensis* provides effective insect pest control. *Bio/Technology*, **14**, 171–176.
- Xiong, L.Z. and Yang, Y.N. (2003) Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid-inducible mitogen-activated protein kinase. *Plant Cell*, **15**, 745–759.
- Xu, D., Duan, X., Wang, B., Hong, B., Ho, T.H.D. and Wu, R. (1996) Expression of a late embryogenesis abundant protein gene, *Hva1*, from barley confers tolerance to water deficit and salt stress in transgenic rice. *Plant Physiol.* **110**, 249–257.
- Yamada, T., Tozawa, Y., Hasegawa, H., Terakawa, T., Ohkawa, Y. and Wakasa, K. (2004) Use of a feedback-insensitive α subunit of anthranilate synthase as a selectable marker for transformation of rice and potato. *Mol. Breed.* **14**, 363–373.
- Yamaguchi-Shinozaki, K. and Shinozaki, K. (1994) A novel cis-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature or high-salt stress. *Plant Cell*, **6**, 251–264.
- Yamanouchi, U., Yano, M., Lin, H., Ashikari, M. and Yamada, K. (2002) A rice spotted leaf gene *Spl7*, encodes a heat stress transcription factor protein. *Proc. Natl. Acad. Sci. USA* **99**, 7530–7535.
- Yang, D.C., Guo, F.L., Liu, B., Huang, N. and Watkins, S.C. (2003) Expression and localization of human lysozyme in the endosperm of transgenic rice. *Planta*, **216**, 597–603.
- Yang, D.C., Wu, L.Y., Hwang, Y.S., Chen, L.F. and Huang, N. (2001) Expression of the REB transcriptional activator in rice grains

- improves the yield of recombinant proteins whose genes are controlled by a *Reb*-responsive promoter. *Proc. Natl. Acad. Sci. USA* **98**, 11438–11443.
- Ye, X.D., Al-Babili, S., Kloti, A., Zhang, J., Lucca, P., Beyer, P. and Potrykus, I. (2000) Engineering the provitamin A (β -carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science*, **287**, 303–305.
- Ye G.Y., Shu, Q.Y., Yao, H.W., Cui, H.R., Cheng, X.Y., Hu, C., Xia, Y.W., Gao, M.W. and Altosaar, I. (2001) Field evaluation of resistance of transgenic rice containing a synthetic *cry1Ab* gene from *Bacillus thuringiensis*-Berliner to two stem borers. *J. Econ. Entomol.* **94**, 271–276.
- Yin, Z. and Wang, G.L. (2000) Evidence of multiple complex patterns of tDNA integration into the rice genome. *Theor. Appl. Genet.* **99**, 461–470.
- Yu, J., Hu, S.N., Wang, J., Wong, G.K.S., Li, S.G., Liu, B., Deng, Y.J., Dai, L., Zhou, Y., Zhang, X.Q., Cao, M.L., Liu, J., Sun, J.D., Tang, J.B., Chen, Y.J., Huang, X.B., Lin, W.YeC., Tong, W., Cong, L.J., Geng, J.N., Han, Y.J., Li, L., Li, W., Hu, G.Q., Huang, X.G., Li, W.J., Li, J., Liu, Z.W., Liu, J.P., Qi, Q.H., Liu, J.S., Li, T., Wang, X.G., Lu, H., Wu, T.T., Zhu, M., Ni, P.X., Han, H., Dong, W., Ren, X.Y., Feng, X.L., Cui, P., Li, X.R., Wang, H., Xu, X., Zhai, W.X., Xu, Z., Zhang, J.S., He, S.J., Zhang, J.G., Xu, J.C., Zhang, K.L., Zheng, X.W., Dong, J.H., Zeng, W.Y., Tao, L.YeJ., Tan, J., Ren, X.D., Chen, X.W., He, J., Liu, D.F., Tian, W., Tian, C.G., Xia, H.G., Bao, Q.Y., Li, G., Gao, H., Cao, T., Zhao, W.M., Li, P., Chen, W., Wang, X.D., Zhang, Y., Hu, J.F., Liu, S., Yang, J., Zhang, G.Y., Xiong, Y.Q., Li, Z.J., Mao, L., Zhou, C.S., Zhu, Z., Chen, R.S., Hao, B.L., Zheng, W.M., Chen, S.Y., Guo, W., Li, G.J., Liu, S.Q., Tao, M., Zhu, L.H., Yuan, L.P. and Yang, H.M. (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). *Science*, **296**, 79–92.
- Yuan, H., Ming, X., Wang, L., Hu, P., An, C. and Chen, Z. (2002) Expression of a gene encoding trichosanthin in transgenic rice plants enhances resistance to fungus blast disease. *Plant Cell Report*, **20**, 992–998.
- Zhai, W.X., Chen, C.Y., Zhu, X.F., Chen, X.W., Zhang, D.H., Li, X.B. and Zhu, L.H. (2004) Analysis of tDNA-Xa21 loci and bacterial blight resistance effects of the transgene Xa21 in transgenic rice. *Theor. Appl. Genet.* **109**, 534–542.
- Zhang, J.Z., Creelman, R.A. and Zhu, J.K. (2004) From laboratory to field. Using information from *Arabidopsis* to engineer salt, cold, and drought tolerance in crops. *Plant Physiol.* **135**, 615–621.
- Zhang, N.Y., Linscombe, S. and Oard, J. (2003) Out-crossing frequency and genetic analysis of hybrids between transgenic glufosinate herbicide-resistant rice and the weed, red rice. *Euphytica*, **130**, 35–45.
- Zhang, W. and Wu, R. (1988) Efficient regeneration of transgenic plants from rice protoplasts and correctly regulated expression of the foreign gene in plants. *Theor. Appl. Genet.* **76**, 835–840.
- Zhang, H.M., Yang, H., Rech, E.L., Golds, T.J., Davis, A.S., Mulligan, B.J. and Cocking, E.G. (1988) Transgenic rice plants produced by electroporation mediated plasmid uptake into protoplasts. *Plant Cell Report*, **7**, 379–383.
- Zheng, H.H., YuL., Wei, C.H., Hu, D.W., Shen, W.P., Chen, Z.L. and Li, Y. (2000) Assembly of double-shelled, virus-like particles in transgenic rice plants expressing two major structural proteins of rice dwarf virus. *J. Virol.* **74**, 9808–9810.
- Zhou, Z.Y., Engler, J.D., Rouan, D., Michiels, F., Van Montagu, M. and Van Der Straeten, D. (2002) Tissue localization of a submergence-induced 1-aminocyclopropane-1-carboxylic acid synthase in rice. *Plant Physiol.* **129**, 72–84.
- Zhu, J.K. (2001) Plant salt tolerance. *Trends Plant Sci.* **6**, 66–71.
- Zhu, J.K. (2003) Regulation of ion homeostasis under salt stress. *Curr. Opin. Plant Biol.* **6**, 441–445.
- Zhu, Y., Cai, X.L., Wang, Z.Y. and Hong, M.M. (2003) An interaction between a MYC protein and an EREBP protein is involved in transcriptional regulation of the rice *Wx* gene. *J. Biol. Chem.* **278**, 47803–47811.
- Zimmermann, M.B. and Hurrell, R.F. (2002) Improving iron, zinc and vitamin A nutrition through plant biotechnology. *Curr. Opin. Biotechnol.* **13**, 142–145.