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Review

Agrobacterium-mediated transformation of plants: emerging factors that influence efficiency

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Despite production of fertile transgenic plants through transformation mediated by Agrobacterium tumefaciens, transformation efficiency is still low. Apart from plant genotype, Agrobacterium strains, plasmid vectors, virulence (vir) gene inducing compounds, medium composition and tissue specific factors, some other factors are becoming important for improving transformation efficiency of plant species. Sucrose treatment of explant increased T-DNA delivery in rice while desiccation improved the T-DNA delivery and stable transformation of sugarcane, maize, wheat and soybean. Silver nitrate suppresses the Agrobacterium growth and facilitates plant cell recovery that resulted in increased efficiency of transformation in wheat. Inclusion of thiol compounds, L-cysteine, dithiothreitol and sodium thiosulphate in co-cultivation medium increased transformation efficiency as high as 16.4% in soyabean. A temperature of 22^{0}C was found to be optimal for T-DNA delivery in tobacco. The optimal temperature for both T-DNA delivery and stable transformation was 23-25^{0}C for wheat and ~23^{0}C for maize. Surfactants Silwet 77, pluronic acid F68, Tween 20 enhanced T-DNA delivery in wheat. Evidence that Agrobacterium density, co-culture medium, antibiotic and selectable marker influence T-DNA delivery and integration and stable transformation of plants were also presented.

Key words: Agrobacterium, stable transformation, T-DNA delivery, T-DNA integration, transformation efficiency.

INTRODUCTION

Agrobacterium tumefaciens causes crown gall disease of a wide range of plants, especially members of the rose family such as apple, pear, peach, cherry, almond, raspberry and roses. The discovery of the bacterial origin of crown gall disease (Smith and Townsend, 1907) sparked a number of studies with understanding the mechanisms of oncogenesis in general and applied it to study of cancer disease in animals and humans as objectives. The elegant work of Binns and Thomashaw (1988) which revealed that A. tumefaciens is capable of transferring a particular DNA segment Transfer (T)-DNA of the tumour-inducing (Ti) plasmid into the nucleus of infected cells where it is subsequently integrated into the host genome, changed the objectives of research on A. tumefaciens to transformation of plants. Early realization of this goal was brighten with the report that the T-DNA contains two types of genes: the oncogenic genes, encoding for enzymes involved in the synthesis of auxins and cytokinins and responsible for tumour formation; and the genes encoding for the synthesis of opines, a product resulted from condensation between amino acids and sugars, which are produced and excreted by the crown gall cells and consume by A. tumefaciens as carbon and nitrogen sources. Outside the T-DNA, are located the genes for the opine catabolism, the genes involved in the process of T-DNA transfer from the bacterium to the plant cell and for the bacterium-bacterium plasmid conjugative transfer genes (Zupan and Zambrysky, 1995).

Virulent strains of A. tumefaciens contain a large megaplasmid (more than 200 kb) that plays a key role in tumour induction and for this reason it was named Ti plasmid. The transfer is mediated by the co-operative action of proteins encoded by genes determined in the Ti plasmid virulence region (vir genes) and in the bacterial chromosome. The 30 kb virulence (vir) region is a region organised in six operons that are essential for the T-DNA

Table 1. *Agrobacterium*-mediated transformation of some dicots plants

Host plant	Strain plasmid	marker	Explant	TF(%)	Reference
Pigeon pea (*Cajanus cajan* L.)					
ICP787	LBA4404 (pdhdps-GUS)	*npt*II	CN	93.2	Thu et al.2002
Broad bean *Vicia faba* L.)					
Lobab lippoi	C58C1 (pArA4b)	none	IS	92.5	Jelenic et al.2000
Canola (*Brassica napus* L.)					
Westars	GV3850 (pBinmGFP5-ER	*npt*II	H	17.0	Cardoza and Stewart 2003
Maplus	GV3850 (pNK55-Resy.KCS)	*npt*II	MP	25.0	Wang et al.2005
Chickpea (*Cicer arientum* L.)					
Semsen	AGL1 (pRM50)	*npt*II	CN	0.5	Sarmah et al 2004
Soybean (*Glycine max* L. Merill)					
Lambart	LBA4404 (pCAMBIA 1303)	*hpt*	CN	16.4	Olhoft et al 2003
Cotton (*Gossypium hirsutum*)					
Ekang 9	LBA4404 (pBin438)	*npt*II	EC	33.0	Wu et al.2005

TF-Transformation frequency; *npt*II-neomycin phosphotransferase;CN-Cotyledonary node;EC-embryonic calli ;MP-MesopyhII protoplast; H-Hypocotyl; IS-Internodal segment

Table 2. *Agrobacterium*-mediated transformation of some monocot plants

Host plant	Strain (plasmid)	Marker	Explant	TF(%)	Reference
Banana(*Musa* spp.)					
Grand Nain (AAA)	LBA4404 (pBI141)	*npt*II	MCS	2.0	May et al. 1995
Barley (*Hordeum vulgare* L.)					
Winter (igri)	LBA4404 (pSBI: VG35PAT)	hpt	PC	2.2	Kumlehn et al.2006
Rice (*Oryza sativa* L.)					
Indica (basmati 370)	EHA101 (pIGI21Hm)	hpt	EC	22	Rashid et al.1996
Japonica (Taipei 309)	LBA4404 (pTOK233)	hpt	PCIE	3.0	Uze et al.1997
Rye (*Secale cereale* L.)					
Spring (L22)	AGLO (pJFnptII)	*npt*II	PCIE	3.5	Popelka and Altpeter,2003
Sugarcane (*Saccharium officinarium* L.)					
Ja60-5	LBA4404 (pBI141)	*hpt*	SC	0.94-1.15	Arencibia et al.,1998
Sorghum *(Sorghum bicolor* L.)					
C401	EHA101 (pPZP201)	*pmi*	IE	3.3	Gao et al. 2005
Pioneer 8505	EHA101 (pPZP201)	*pmi*	IE	2.8	Gao et al.2005
Maize (Zea mays L.)					
A188	EHA101 (pTF102)	Bar	FIIE	5.5	Frame et al.2002
A188	LBA4404 (pTOK233)	*hpt*	FIIE	11.8-30.6	Ishida et al.1996
Wheat (*Triticum aestivum* L.)					
Spring(Bobwhite)	ABI (pMON18365)	*npt*II	EC	10.5	Cheng et al.2003
Winter(Candenza)	AGLI (pAL151)	Bar	IE	1.7	Wu et al.2003

TF-Transformation frequency; *npt*II-neomycin phosphotransferase;
hpt-hygromycin phosphotranferase;pmi-phosphomannose isomerase
Bar-bialaphos-resistant gene;PCIE-Precultured immature embryo
EC-Embryogenic calluses;FIIE-Freshly isolated immature embryo;SC-suspension culture;IE-Immature embryo;MCS-Meritem corm slices
PC-pollen culture

transfer (virA, virB, virD, and virG) or for the increasing of transfer efficiency (virC and virE) (Zupan and Zambrysky, 1995; Jeon et al., 1998).

The initial results of the studies on T-DNA transfer process to plant cells demonstrate three important facts for the practical use of this process in plants transformation. Firstly, the tumour formation is a transformation process of plant cells resulted from transfer and integration of T-DNA and the subsequent expression of T-DNA genes. Secondly, the T-DNA genes are transcribed only in plant cells and do not play any role during the transfer process. Thirdly, any foreign DNA placed between the T-DNA borders can be transferred to plant cell, no matter where it comes from. These well es-

tablished facts, allowed the construction of the first vector and bacterial strain systems for plant transformation (Rival et al., 1998; Opabode 2002)

The first record on transgenic tobacco plant expressing foreign genes appeared at the beginning of the last decade. Since that crucial moment in the development of plant science, a great progress in understanding the Agrobacterium-mediated gene transfer to plant cells has been achieved. However, Agrobacterium tumefaciens naturally infects only dicotyledonous plants and many economically important plants, including the cereals, remained accessible for genetic manipulation by other methods. For these cases, alternative direct transformation methods have been developed such as polyethyleneglycol-mediated transfer, microinjection, protoplast and intact cell electroporation and gene gun technology (Rival et al., 1998). However, Agrobacterium-mediated transformation has remarkable advantages over direct transformation methods, including preferential integration of defined T-DNA into transcriptionally active regions of the chromosome (Czernilofsky et al., 1986; Koncz et al., 1989, Le et al., 2001; Olhoft et al., 2004) with exclusion of vector DNA (Hiei et al., 1997; Fang et al., 2002), unlinked integration of co-transformed T-DNA (McKnight et al., 1987; Komari et al., 1996; Hamilton, 1997; Olhoft et al., 2004). The transgenic plants are generally fertile and the foreign genes are often transmitted to progeny in a Mendelian manner (Rhodora and Thomas, 1996).

Agrobacterium-mediated gene transfer into monocotyledonous plants was not possible until recently, when reproducible and efficient methodologies were established on rice, banana, corn, wheat, and sugarcane (Hiei et al., 1994; Cheng et al., 1998; May et al., 1995; Ishida et al., 1996; Enriquez-Obregon, 1998; Arencibia et al., 1998). Reviews on plant transformation using Agrobacterium tumefaciens and the molecular mechanisms involved in this process have been published during the last years (Hooykas and Schilperoort, 1992; Zupan and Zambrysky, 1995; Rival et al., 1998; Zupan et al., 2000; Cheng et al., 2004).

The transfer of T-DNA and its integration into the plant genome is influenced by several *A. tumefaciens* and plant tissue specific factors. These include plant genotype, explant, vectors-plasmid, bacteria strain, addition of vir-gene inducing synthetic phenolics compounds, culture media composition, tissue damage, suppression and elimination of *A. tumefaciens* infection after co-cultivation (Alt-morbe et al., 1989; Bidney et al., 1992; Hoekema et al., 1993; Hiei et al., 1994; Komari et al., 1996; Nauerby et al., 1997; Klee, 2000). Some of these factors are summarized in Tables 1 and 2 for selected plant species. Recently, some other factors have been found important in influencing the efficiency of Agrobacterium -mediated genetic transformation of crops. This review shall summarize those factors for further ptimization of existing transformation protocols and establishment of new ones for recalcitrant plant species.

OSMOTIC TREATMENT OF EXPLANT

After the explant is chosen, in vitro manipulation of the explant may be necessary to enhance competency of plant cells to T-DNA delivery, and to facilitate plant cell recovery after infection. Unlike biolistic-mediated transformation, osmotic treatment enhancement of Agrobacterium-mediated transformation largely depends upon species. Supplementation of co-culture medium with 68.5 gl^{-1} (200 m M) sucrose and 36 gl^{-1} (200 mM) glucose was extensively used in rice and maize transformation (Hiei et al., 1994; Zhao et al., 2001; Frame et al., 2002). However, the effect of osmotic medium on T-DNA delivery and stable transformation was not described. Uze et al., (1997) observed that plasmolysis with 65 gl^{-1} (292 mM) sucrose improved T-DNA delivery into precultured immature embryos rice. This treatment was extensively used to produce large numbers of transgenic plants for various projects (Ye et al., 2000; Lucca et al., 2001). However, osmotic treatment was not effective with precultured immature embryos of wheat (Uze et al., 2000). Osmotic treatment did not have a beneficial effect on T-DNA delivery in wheat (Cheng et al., 2003).

PRECONDITIONING, CO-CULTIVATION TIME AND *A. TUMEFACIENS* DENSITY

Optimizing the preconditioning time to 72 h and co-cultivation time with *A. tumefaciens* to 48 h provided an increase in the transformation efficiency from a baseline 4% to 25% in canola (Cardoza and Stewart, 2003). Zhang et al. (2000) reported that in Chinese cabbage, co-cultivation for 72 h yielded the highest transformation frequency. Co-cultivation of explants with *A. tumefaciens* has made possible the use of some explants, which were hitherto recalcitrant for transformation experiment. Canola was transformed by co-cultivation of mesophyll protoplast with a strain of *A. tumefaciens* carrying nptII and KCS genes (Wang et al., 2005). Similarly, high efficient transformation of cotton was achieved by co-cultured embryonic calli with *A. tumefaciens* (Wu et al., 2005). Hiei et al. (1997) reported that transformation of rice was possible when the Agrobacterium density was between 1.0 x 10^6 and 1.0x10^{10} colony-forming units (cfu) ml^{-1}, and the optimal concentration was approximately 1.0 x 10^{10} $cfuml^{-1}$ (Hiei et al., 1994). The same density of *A. tumefaciens* was successfully used later in maize (Ishida et al., 1996) and adopted by many other laboratories for various genotypes and explants in rice. A. tumefaciens densities higher or lower than 1.0 x 10^{10} $cfuml^{-1}$ were evaluated systematically with N_6-based medium in maize (Zhao et al., 2001), transient GUS activity increased with

higher A. tumefaciens density, but the callus initiation frequency was reduced and peak transformation frequency was achieved with A. tumefaciens at 0.5 x 10^{10} cfuml^{-1}. Similar results were reported with sorghum immature embryos (Zhao et al., 2000). Experiments with various explants of wheat showed that higher A. tumefaciens density could increase transient GUS expression, but was not correlated with higher stable transformation frequency (Cheng et al., 1997). With wheat suspension cells as a model system, an optimal A. tumefaciens density of around 0.5 x 10^{10} cfu ml^{-1} was identified. With higher or lower A. tumefaciens density, both transient and stable transformation decreased. A. tumefaciens density higher than 1 x 10^{10} cfu usually damaged the plant cells, and resulted in lower cell recovery that ultimately reduced the stable transformation frequency. Nevertheless, when a higher density of A. tumefaciens is necessary for recalcitrant explants or species, transformation frequency can be improved by a short inoculation time, gently rinsing the explants after inoculation with fresh inoculation medium as performed in dicot transformation, or addition of a bactericide agent such as silver nitrate in the co-culture medium (Zhao et al., 2000; 2001; Zhang et al., 2003).

Although efficient T-DNA delivery is a prerequisite for achieving efficient stable transformation in most cases, under many conditions increased T-DNA delivery has not resulted in increased stable transformation. For example, when surfactant was included in the inoculation medium for freshly isolated immature embryos of wheat, T-DNA delivery (as measured by transient gene expression) was increased, but stable transformation frequency was not improved. The likely reason for the lack of correlation between T-DNA delivery and stable transformation in this case was the detrimental effect of surfactant on plant cell/tissue recovery (Cheng et al., 1997). T-DNA delivery has correlated well with stable transformation frequency inoculation and co-culture conditions favour both T-DNA delivery and plant cell recovery. One example is the desiccation treatment post A. tumefaciens infection for precultured immature embryos or embryogenic calluses of wheat (Cheng et al., 2003). When T-DNA delivery is not rate-limiting for a given explant, adjust the transformation parameters to favour plant cell recovery has been an effective means of achieving efficient stable transformation.

DESICCATION OF EXPLANTS

A significant factor that enhances transformation of crop species is dessication of explants prior to, or post, A. tumefaciens infection. Arencibia et al. (1998) reported that air-drying sugarcane suspension cells prior to inoculation under laminar flow conditions for 15-60 min slightly improved T-DNA delivery and subsequently increased transformation efficiency, but the actual desiccation stringency was not defined in this report. Similarly, air-drying calluses derived from rice suspension cultures for 10-15 min increased the transformation efficiency 10-fold or more as compared to the control without air-drying (Urushibara et al., 2001). It is unclear to the investigators what factors were affected by air-drying, but it is possible that plasmolysis or wounding may be important. The effect of air-drying on other explants of rice such as embryonic calluses and precultured immature embryos was not evaluated. Using the same air-drying conditions, it was shown that air-drying precultured immature embryos and embryogenic calluses in wheat prior to inoculation did not have the same effect as in sugarcane and rice. However, Cheng et al. (2003) reported that desiccation of precultured immature embryos, suspension culture cells, embryonic calluses of wheat, and embrogenic calluses of maize greatly enhanced T-DNA delivery and plant tissue recovery after co-culture, leading to increased stable transformation frequency. This treatment was not only effective in monocot species, but also improved T-DNA delivery in recalcitrant dicot species such as soybean suspension cells based on our preliminary study (Cheng and Fry, 2000). Although the molecular mechanism of desiccation during co-culture remains unclear, it is known that desiccation suppresses the growth of Agrobacterium similar to the effect observed with silver nitrate. In addition, maize embryogenic calluses from the desiccation treatment recovered better than explants co-cultured under non-desiccation conditions (with H_2O), when co-culture plates were supplemented with 20 µM silver nitrate. Furthermore, osmotic treatments and abscisic acid (ABA) treatment before and during inoculation, and during co-culture, did not have the same effect on T-DNA delivery as the desiccation treatment.

ANTINECROTIC TREATMENTS

With respect to pretreatments, antinecrotic mixtures for pre-induction were shown to be important for reducing oxidative burst. Enrique-Obregon et al. (1998) treated merismatic spindle sections of sugarcane with a medium containing 15 mgl^{-1} (0.09 µM) ascorbic acid, 40 mgl^{-1}(0.33 µM) cysteine, and 2 mgl^{-1}(0.01µM) silver nitrate. An efficient transformation system was developed using this pretreatment in sugarcane. Transformed calluses were obtained only when the mixture of these antinecrotic compounds was added in their previous study (Enrique-Obregon et al., 1998). A similar protocol was applied to rice transformation using seedling explants (Enrique-Obregon et al., 1999). Explant viability was significantly improved when the plantlet explant were treated with this mixture of compounds. Inclusion of cysteine in the co-culture medium led to an improvement in both transient β-glucuronidase (GUS) expression in target cells and a significant increase in stable transformation frequency in

maize. In Olhoft and Somers (2001) and Olhoft et al. (2003), T-DNA transfer into cotyledonary-node cells and genomic integration were increased through the inclusion of thiol compounds in the solid co-cultivation medium, resulting in an increased production of transgenic plants. Hygromycin B selection combined with the inclusion of the thiol compounds L-cysteine, dithiothreitol (DDT) and sodium thiosulphate in the co-cultivation medium, further improved the production of transgenic plants, with transformation efficiencies as high as 16.4% of independent Southern-positive T_o plants produced per explants treated (Olhoft et al., 2003). Inclusion of silver nitrate in co-culture medium enhanced stable transformation in maize (Armstrong and Rout, 2001; Zhao et al., 2001). Silver nitrate significantly suppresses the Agrobacterium growth during co-culture without compromising T-DNA delivery and subsequent T-DNA integration. The suppressed Agrobacterium growth on the target explants could facilitate plant cell recovery and result in increased efficiency of transformation (Cheng et al., 2003).

TEMPERATURE

The effect of temperature during co-culture on T-DNA delivery was first reported in dicot species. A temperature of 22^{o}C was found to be optimal for T-DNA delivery in tobacco leaves (Dillen et al., 1997). However, in another report, co-culture at 25^{o}C led to the highest number of transformed plants of tobacco, even though 19^{0}C was optimal for T-DNA delivery (Salas et al., 2001). These results indicate that the optimal for stable transformation with a given species and explant. The optimal temperature for stable transformation should be evaluated with each specific explant and Agrobacterium strain involved (Salas et al., 2001). In monocots, the co-culture temperature for most of the crops ranged from 24 to 25^{0}C, and in some cases, 28^{0}C was used for co-culture (Rashid et al., 1996; Arencibia et al., 1998; Enriquez-Obregon et al., 1998; Hashizume et al., 1999). The effect of lower temperature (≤ 23^{0}C) on T-DNA delivery and stable transformation was also evaluated. Kondo et al. (2000) tested the effect of four temperatures, namely 18, 20, 22 and 24^{0}C on T-DNA delivery with garlic calluses. The highest transient GUS expression was observed at 22^{0}C, in which 64% of the total calluse showed GUS activity. The ratio of GUS-stained calluses decreased by 85% at 20^{0}C and by 69% at 24^{0}C. Higher transformation frequency was observed in maize immature embryo transformation at 20^{0}C than at 23^{0}C when using a standard binary vector (Frame et al., 2002).Transgenic maize plants have also been obtained from elite inbred lines PHP38 and PHN46 by co-culture of the immature embryos at 20^{0}C followed by 28^{0}C subculture (Gordon-Kamm et al., 2002). The effect of temperature on both transient and stable transformation was extensively studied in other laboratories using suspension-cultured wheat (cv. Mustang) and maize (cv. BMS) cells as model systems. The optimal temperature for both T-DNA delivery and stable transformation was 23-25^{0}C for wheat and ~23^{0}C for maize (Rout et al., 1996).

SURFACTANTS

Including surfactants such as Silwet L77 and pluronic acid F68 in inoculation medium greatly enhanced T-DNA delivery in immature embryos of wheat (Cheng et al., 1997). Surfactants may enhance T-DNA delivery by aiding A. tumefaciens attachment and or by elimination of certain substances that inhibit A. tumefaciens attachment. The surfactant Silwet L77 was also shown to be useful to the success of the floral dip method of Arabidopsis thaliana transformation. Surfactant added to the inoculation medium may play a role similar to vacuum infiltration, facilitating the delivery of A. tumefaciens cells to closed ovules, the primary target for A. tumefaciens during in planta transformation of A. thaliana (Ye et al., 1999; Bechold et al., 2000; Desfeux et al., 2000).

INOCULATION AND CO-CULTURE MEDIUM

Medium component, sugar, plant growth regulators, and vir induction chemicals are also important factors that affect transformation frequency. The modified N6 medium (Chu et al., 1995) containing 2,4-dichlorophenoxyacetic acid (2,4-D) and casamino acids was shown to be suitable for co-culture in rice. Several laboratories with different genotypes and explants adopted a similar medium recipe. MS (Murashige and Skoog, 1962) or a modified MS-based medium was shown to be suitable for inoculation and co-culture in several report of rice transformation (Dong et al., 1996; Enriquez-Obregon et al., 1999; Mohanty et al., 1999; Luca et al., 2001). Ishida et al. (1996) reported transformation of maize immature embryo using LS-based (Linsmaier and Skoog, 1965) medium, and N6-based medium failed to generate transformed plants. With additional component added in the mixture such as silver nitrate. Zhao et al. (2001) showed that N6-based medium was also suitable for inoculation and co-culture of immature maize embryos, resulting in transgenic plants. Similarly, the addition of $CaCl_2$ in the medium increased transformation efficiency in barley (Kumlehn et al., 2006).

Reducing the salt strength in the inoculation and co-culture media was reported as beneficial for transformation of canola (Fry et al., 1987). Medium with reduced salts enhanced T-DNA delivery in wheat (Cheng et al., 1997). This treatment was used to regenerate stable transformed wheat plants from embryogenic callus with a superbinary vector in a recent study (Khanna and

Daggard, 2003). Medium with reduced salts also enhanced T-DNA delivery in maize (Armstrong and Rout, 2001), and half-strength MS salts in both inoculation and co-culture media have been used in maize transformation (Zhang et al., 2003). The impact of salt strength within the inoculation and co-culture medium on transient GUS expression was extensively assessed in barley with immature embryos as the target explants (Ke et al., 2002). One-tenth MS salt strength enhanced transient GUS expression 10-fold over full-strength salts. Furthermore, the distribution of cells expressing the GUS gene within each set of immature embryos was clearly altered, showing significantly more cells on the scutellar surface expressing GUS.

Chemicals such as acetosyringone for vir induction are recommended in most of crops transformation protocols (Hiei et al., 1994; Ishida et al., 1996; Cheng et al., 1997; Tingay et al., 1997; Zhao et al., 2000; Kumlehn et al., 2006). When acetosyringone was omitted, the level of transient GUS expression was low and stable transformed plants could not be regenerated in rice, onion or barley (Rashid et al., 1996; Hiei et al., 1997; Zheng et al., 2001; Kumlehn et al., 2006). However some explants of monocot species could be efficiently transformed without the aid of external vir induction chemicals for special treatment. For example, meristematic sections of sugarcane pretreated with an antinecrotic mixture (Enriquez-Obregon et al., 1999), and precultured immature embryos and embryogenic calluses of wheat co-cultured under desiccation conditions could be efficiently transformed (Cheng et al., 2003).

ANTIBIOTICS

Antibiotics such as cefotaxime, carbenecillin and timentin have been used regularly in Agrobacterium-mediated transformation of crops following co-culture to suppress or eliminate Agrobacterium (Cheng et al., 1996; Bottinger et al., 2001; Sunikumar and Rathore, 2001). Although cefotaxime worked will in Agrobacterium-mediated transformation of rice and maize initially, it was later found that cefotaxime at a concentration of 250 mgl^{-1} (Ishida et al., 1996) had a detrimental effect to maize Hi II callus, Callus formation was greatly reduced when cefotaxime (50 or 250 mgl^{-1}) was added in the callus induction medium, and consequently transformation frequency was reduced 3-fold compared to that with carbenicillin (100 mgl^{-1}). Carbenicillin at 100 mgl^{-1} was used for all the subsequent experiments (Zhan et al., 2001). Carbenicillin has been the antibiotic of choice in reports of Agrobacterium-mediated transformation of wheat and maize (Cheng et al., 1997, 2003; Zhang et al., 2003). On the other hand, 100 mg^{-l} kanamycin was economical and improved the transformation efficiency in white spruce by enrichment of transformed tissue in bud-forming callus (Le et al., 2001) and increased the proportion of positively transformed shots during subculture on kanamycin containing medium in peanut and pigeon pea (Sharma and Anjaiah, 2000; Thu et al., 2003).

SELECTABLE MARKER

The most widely used selectable markers for transformation of crops are genes encoding hygromycin phosphotransferase (hpt), phosphinothricin acetyltransferase (pat or bar), and neomycin phosphotransferase (nptII). Use of these marker genes under the control of constitutive promoters such as the 35S promoter from cauliflower mosaic virus, the ubiquitin promoter from maize, works as efficiently for selection of Agrobacterium-transformed cells as for biolistics-mediated transformation. For Asparagus and banana, the npt II gene under the control of the nopaline synthase promoter has been used to successfully select stable transformants with kanamycin (May et al., 1995; Limanton-Grevet and Jullien, 2001). The positive selectable marker phosphomannose isomerase was first used for Agrobacterium-mediated transformation of sugar beet and was recently used to enhance transformation of sorghum (Joersbo et al., 1998; Lucca et al., 2001; Gao et al., 2005). To improve selectable marker genes for crops, Wang et al. (1997) inserted introns into the coding region of hpt as the strategy used in enhancing transgene expression in monocot species (Simpson and Filipowics, 1996). The intoduction of introns into the hpt not only improved transformation frequency in rice Agrobacterium-mediated transformation due to the elevated hpt expression, but also reduced copy numbers of the marker gene. Furthermore, inserting the introns into the marker gene also enabled better control of Agrobacterium growth during the transformation process (Wang et al., 1997). This modified selectable marker enhanced stable transformation with elite rice and barley cultivars as well (Upadhyaya et al., 2000; Wang et al., 2001). Glyphosate-insensitive plant 3-enolpyruvylshikimate-5-phosphate synthases (EPSPS) genes, the bacterial CP4 gene or a bacterial gene that degrades glyphosate, i.e. glyphosate oxidoreductase (GOX) gene, have been used in some laboratories to generate transgenic plants in wheat and maize with biolistics-mediated transformation approaches (Armstrong et al., 1995; Zhou et al., 1995; Russell and Fromm, 1997; Howe et al., 2002). One of these genes, CP4, has been successfully used in Agrobacterium transformation of wheat (Cheng et al., 2003; Hu et al., 2003).Transformation frequency was comparable to biolistics-mediated transformation in wheat (Hu et al., 2003) when a desiccation-based protocol was used.

CONCLUSION

Efficient transformation systems using readily available explants are in high demand for agronomically important plants. Though fertile transgenic plants have been

generated from more than a dozen plants, yet the transformation frequency for most species is still low. In some cases, only a few transformed plants have been regenerated. Further optimizing the transformation parameter such as inoculation, co-culture condition and selectable marker could increase transformation frequency. Since indication that explant competency to Agrobacterium infection using techniques such as desiccation, antinecrotic mixture for pre-induction as well as plant growth regulation treatment is emerging. Understanding the molecular basis of several factors such as desiccation and antinecrotic treatments affecting both T-DNA delivery and stable transformation may facilitate application of these treatments to other species or transformation systems to further improved many published protocols.

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