Update on T-DNA Binary Vectors

T-DNA Binary Vectors and Systems

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For more than two decades, scientists have used Agrobacterium-mediated genetic transformation to generate transgenic plants. Initial technologies to introduce genes of interest (goi) into Agrobacterium involved complex microbial genetic methodologies that inserted these goi into the transfer DNA (T-DNA) region of large tumor-inducing plasmids (Ti-plasmids). However, scientists eventually learned that T-DNA transfer could still be effected if the T-DNA region and the virulence (vir) genes required for T-DNA processing and transfer were split into two replicons. This binary system permitted facile manipulation of Agrobacterium and opened up the field of plant genetic engineering to numerous laboratories. In this review, we recount the history of development of T-DNA binary vector systems, and we describe important components of these systems. Some of these considerations were previously described in a review by Hellens et al. (2000b).

Agrobacterium transfers T-DNA, which makes up a small (approximately 5%–10%) region of a resident Ti-plasmid or root-inducing plasmid (Ri-plasmid), to numerous species of plants (DeCleene and DeLey, 1976; Anderson and Moore, 1979), although the bacterium can be manipulated in the laboratory to transfer T-DNA to fungal (Bundock et al., 1995; Piers et al., 1996; de Groot et al., 1998; Abuodeh et al., 2000; Kelly and Kado, 2002; Li et al., 2007) and even animal cells (Kunik et al., 2001; Bulgakov et al., 2006). Transfer requires three major elements: (1) T-DNA border repeat sequences (25 bp) that flank the T-DNA in direct orientation and delineate the region that will be processed from the Ti/Ri-plasmid (Yadav et al., 1982); (2) vir genes located on the Ti/Ri-plasmid; and (3) various genes (chromosomal virulence [chr] and other genes) located on the bacterial chromosomes. These chromosomal genes generally are involved in bacterial exopolysaccharide synthesis, maturation, and secretion (e.g. Douglas et al., 1983; Cangelosi et al., 1987, 1989; Robertson et al., 1988; Matthesse, 1995; O’Connell and Handelsman, 1999). However, some chromosomal genes important for virulence likely mediate the bacterial response to the environment (Xu and Pan, 2000; Saenkhram et al., 2007). Several recent reviews enumerate factors involved in and influencing Agrobacterium-mediated transformation (Gelvin, 2003; McCullen and Binns, 2006).

The vir region consists of approximately 10 operons (depending upon the Ti- or Ri-plasmid) that serve four major functions.

1. Sensing plant phenolic compounds and transferring this signal to induce expression of vir genes (virA and virG). VirA and VirG compose a two-component system that responds to particular phenolic compounds produced by wounded plant cells (Stachel et al., 1986). Because wounding is important for efficient plant transformation, Agrobacterium can sense a wounded potential host by perceiving these phenolic compounds. Activation of VirA by these phenolic inducers initiates a phospho-relay, ultimately resulting in phosphorylation and activation of the VirG protein (Winans, 1991). Activated VirG binds to the vir box sequences preceding each vir gene operon, allowing increased expression of each of these operons (Pazour and Das, 1990). In addition to induction of the vir genes by phenolics, many sugars serve as co-inducers. These sugars are perceived by a protein, ChvE, encoded by a gene on the Agrobacterium chromosome. In the presence of these sugars, vir genes are more fully induced at lower phenolic concentrations (Peng et al., 1998).

2. Processing T-DNA from the parental Ti- or Ri-plasmid (virD1 and virD2). Together, VirD1 (a helicase) and VirD2 (an endonuclease) bind to and nick DNA at 25-bp directly repeated T-DNA border repeat sequences (Jayaswal et al., 1987; Wang et al., 1987). The VirD2 protein covalently links to the 5’ end of the processed single-strand DNA (the T-strand) and leads it out of the bacterium, into the plant cell, and to the plant nucleus (Ward and Barnes, 1988; Howard et al., 1992).

3. Secreting T-DNA and Vir proteins from the bacterium via a type IV secretion system (virB operon and virD4). The Agrobacterium virB operon contains 11 genes, most of which form a pore through the bacterial membrane for the transfer of Vir proteins (Christie et al., 2005). Currently, we know of five such proteins that are secreted through this apparatus: VirD2 (unattached or attached to the T-strand), VirD5, VirE2, VirE3, and VirF (Vergunst et al., 2000, 2005). VirD4 acts as a coupling factor to link VirD2-T-strand to the type IV secretion apparatus (Christie et al., 2005).

4. Participating in events within the host cell involving T-DNA cytoplasmic trafficking, nuclear targeting, and integration into the host genome (virD2, virD5, virE2, virE3, and virF). VirD2 and VirE2 may play roles in targeting the T-strand to the nucleus (Howard et al., 1992; Župan et al., 1996). In addition,
VirE2 likely protects T-strands from nucleolytic degradation in the plant cell (Yusibov et al., 1994; Rossi et al., 1996). VirF may play a role in stripping proteins off the T-strand prior to T-DNA integration (Tzfira et al., 2004).

Although *vir* genes were first defined genetically because of their importance in virulence (Koekman et al., 1979; Garfinkel and Nester, 1980; Holsters et al., 1980; DeGreve et al., 1981; Leemans et al., 1981), no gene within T-DNA is essential for T-DNA transfer. The ability to delete wild-type oncogenes and opine synthase genes from within T-DNA and replace them with genes encoding selectable markers and other goi helped initiate the field of plant genetic engineering (Bevan et al., 1983; Fraley et al., 1983; Herrera-Estrella et al., 1983).

**DEVELOPMENT OF BINARY VECTOR SYSTEMS**

Initial efforts to introduce goi into T-DNA for subsequent transfer to plants involved cumbersome genetic manipulations to recombine these genes into the T-DNA region of Ti-plasmids (co-integrate or exchange systems; Garfinkel et al., 1981; Zambryski et al., 1983; Fraley et al., 1985; Fig. 1A). This was because Ti/Ri-plasmids are very large, low copy number in *Agrobacterium*, difficult to isolate and manipulate in vitro, and do not replicate in *Escherichia coli*, the favored host for genetic manipulation. T-DNA regions from wild-type Ti-plasmids are generally large and do not contain unique restriction endonuclease sites suitable for cloning a goi. In addition, scientists wanted to eliminate oncogenes from T-DNA to regenerate normal plants. Opine synthase genes were also generally deemed superfluous in constructions designed to deliver goi to plants.

In 1983, two groups made a key conceptual breakthrough that would allow laboratories that did not specialize in microbial genetics to use *Agrobacterium* for gene transfer. Hoekema et al. (1983) and de Framond et al. (1983) determined that the *vir* and T-DNA regions of Ti-plasmids could be split onto two separate replicons. As long as both of these replicons are located within the same *Agrobacterium* cell, proteins encoded by *vir* genes could act upon T-DNA in trans to mediate its processing and export to the plant. Systems in which T-DNA and *vir* genes are located on separate replicons were eventually termed T-DNA binary systems (Fig. 1B). T-DNA is located on the binary vector (the non-T-DNA region of this vector containing origin[s] of replication that could function both in *E. coli* and in *Agrobacterium tumefaciens*, and antibiotic-resistance genes used to select for the presence of the binary vector in bacteria, became known as vector backbone sequences). The replicon containing the *vir* genes became known as the *vir* helper. Strains harboring this replicon and a T-DNA are considered disarmed if they do not contain oncogenes that could be transferred to a plant.

The utility of binary systems for ease of genetic manipulation soon became obvious. No longer were complex, cumbersome microbial genetic technologies necessary to introduce a goi into the T-region of a Ti-plasmid. Rather, the goi could easily be cloned into small T-DNA regions within binary vectors especially suited for this purpose. After characterization and verification of the construction in *E. coli*, the T-DNA binary vector could easily be mobilized (by bacterial conjugation or transformation) into an appropriate *Agrobacterium* strain containing a *vir* helper region.

Over the past 25 years, both T-DNA binary vectors and disarmed *Agrobacterium* strains harboring *vir* helper plasmids have become more sophisticated and suited for specialized purposes. Table I lists many commonly used T-DNA binary vectors (and vector series). Table II lists many commonly used disarmed *Agrobacterium* *vir* helper strains.
### Table 1. Agrobacterium T-DNA binary vectors

<table>
<thead>
<tr>
<th>Vector Series Name</th>
<th>Vector ori/ Incompatibility Group</th>
<th>Important Features</th>
<th>Gateway Compatible</th>
<th>Bacterial Selection Marker</th>
<th>Plant Selection Marker</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pGA IncPα</td>
<td>cos site ColE1 ori</td>
<td></td>
<td>No</td>
<td>Kan</td>
<td>Kan</td>
<td>An et al. (1985); An (1987)</td>
</tr>
<tr>
<td>SEV IncPα</td>
<td></td>
<td>Reconstitutes a missing T-DNA border; not a binary vector</td>
<td>No</td>
<td>Kan</td>
<td>Kan/Nos</td>
<td>Fraley et al. (1985)</td>
</tr>
<tr>
<td>pEND4K IncPα</td>
<td>cos site, mcs with blue/white selection</td>
<td></td>
<td>No</td>
<td>Kan/Tet</td>
<td>Kan</td>
<td>Klee et al. (1985)</td>
</tr>
<tr>
<td>pBI IncPα</td>
<td></td>
<td>Promoterless gusA gene for promoter studies</td>
<td>No</td>
<td>Kan</td>
<td>Kan</td>
<td>Jefferson et al. (1987)</td>
</tr>
<tr>
<td>pCIB10 IncPα</td>
<td></td>
<td>Chimeric antibiotic-resistance gene</td>
<td>No</td>
<td>Kan</td>
<td>Chimeric/Kan/Hyg</td>
<td>Rothstein et al. (1987)</td>
</tr>
<tr>
<td>pMRK63 pRi</td>
<td></td>
<td>pRi-based antibiotic-resistant gene</td>
<td>No</td>
<td>Amp/Kan</td>
<td>Kan</td>
<td>Vilaine and Casse-Delbart (1987); Becker (1990)</td>
</tr>
<tr>
<td>pCGN1547 pRi + ColE1</td>
<td>ColE1 ori for high copy no. in E. coli mcs with blue/white selection</td>
<td>No</td>
<td>Gent</td>
<td>Kan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pART IncPα + ColE1</td>
<td>ColE1 ori for high copy no. in E. coli promoter/polyA expression cassette</td>
<td>No</td>
<td>Spec</td>
<td>Kan</td>
<td></td>
<td>Gleave (1992)</td>
</tr>
<tr>
<td>pGKB5 pRiA4</td>
<td></td>
<td>Promoterless gusA gene for promoter studies</td>
<td>No</td>
<td>Kan</td>
<td>Kan/Bar</td>
<td>Bouchez et al. (1993)</td>
</tr>
<tr>
<td>pMD80 IncPα</td>
<td></td>
<td>Ω, untranslated leader</td>
<td>No</td>
<td>Kan</td>
<td>Kan</td>
<td>Day et al. (1994)</td>
</tr>
<tr>
<td>pPZP IncW</td>
<td></td>
<td>Small, stable, mcs with blue/white selection</td>
<td>No</td>
<td>Spec/Chl</td>
<td>Kan/Gent</td>
<td>Hajdukiewicz et al. (1994); van Engelen et al. (1995)</td>
</tr>
<tr>
<td>pBINPLUS IncPα</td>
<td></td>
<td>Selectable marker near LB ColE1 ori</td>
<td>No</td>
<td>Kan</td>
<td>Kan</td>
<td></td>
</tr>
<tr>
<td>pRT100 IncPα</td>
<td></td>
<td>Rare-cutting sites (NotI, AciI)</td>
<td>No</td>
<td>Kan</td>
<td>Kan/Hyg/Bar/Dhfr</td>
<td>Uberlacker and Werr (1996); Hamilton (1997)</td>
</tr>
<tr>
<td>pBIBAC</td>
<td></td>
<td>T-DNA binary vector designed to transfer large DNA fragments</td>
<td>No</td>
<td>Kan</td>
<td>Hyg</td>
<td></td>
</tr>
<tr>
<td>pCB series IncPα</td>
<td></td>
<td>Mini binary vectors small backbone, not self-mobilizable</td>
<td>No</td>
<td>Kan</td>
<td>Bar</td>
<td>Xiang et al. (1999)</td>
</tr>
<tr>
<td>pGreen IncW</td>
<td></td>
<td>ColE1 ori mcs with blue/white selection</td>
<td>No</td>
<td>Kan</td>
<td>Kan/Hyg/Sul/Bar</td>
<td>Hellens et al. (2000a)</td>
</tr>
<tr>
<td>pPZP-RCS2 pVS1</td>
<td></td>
<td>Multiple rare-cutting sites for cassette insertion. Uses pPZP200 as backbone</td>
<td>No</td>
<td>Spec</td>
<td>Kan/Gent</td>
<td>Goderis et al. (2002)</td>
</tr>
<tr>
<td>pGATEWAY destination vector pVS1</td>
<td></td>
<td>ColE1 ori. Uses pPZP200 as backbone</td>
<td>Yes</td>
<td>Spec</td>
<td>Kan/Hyg/Bar</td>
<td>Karimi et al. (2002)</td>
</tr>
<tr>
<td>pMDC pVS1</td>
<td></td>
<td>Based on pCAMBIA (except pMDC7, from PER8); Facilitates protein tagging</td>
<td>Yes</td>
<td>Kan; Spec for pMDC7</td>
<td>Kan/Hyg/Bar</td>
<td>Curtis and Grossniklaus (2003)</td>
</tr>
<tr>
<td>pRCS2 pVS1</td>
<td></td>
<td>Contains rare-cutting sites</td>
<td>No</td>
<td>Spec</td>
<td>Kan/Hyg/Bar</td>
<td>Chung et al. (2005)</td>
</tr>
<tr>
<td>pRCS2-ocs pVS1</td>
<td></td>
<td>Cloning of multiple genes</td>
<td>No</td>
<td>Spec</td>
<td>Kan/Hyg/Bar</td>
<td>Tzfira et al. (2005)</td>
</tr>
<tr>
<td>pEarleyGate pVS1</td>
<td></td>
<td>Based on pCAMBIA; Facilitates protein tagging</td>
<td>Yes</td>
<td>Kan</td>
<td>Bar</td>
<td>Earley et al. (2006)</td>
</tr>
<tr>
<td>pGWWTAC pMDC99 pRIA4</td>
<td></td>
<td>Multi-Round Gateway for cloning multiple genes</td>
<td>Yes</td>
<td>Kan</td>
<td>Hyg</td>
<td>Chen et al. (2006)</td>
</tr>
<tr>
<td>pORE IncPα</td>
<td></td>
<td>Based on pCB301 ColE1 ori FRT sites. Promoterless gusA or gfp gene for promoter studies</td>
<td>No</td>
<td>Kan</td>
<td>Kan/Pat</td>
<td>Coutu et al. (2007)</td>
</tr>
</tbody>
</table>

*Table continues on following page.*
PROPERTIES OF BINARY VECTORS

T-DNA binary vectors generally contain a number of features important for their use in genetic engineering experiments. These include the following.

(1) T-DNA left and right border repeat sequences to define and delimit T-DNA. T-DNA border repeat sequences (T-DNA borders) contain 25 bp that are highly conserved in all Ti- and Ri-plasmids examined to date (Waters et al., 1991). Nicking by the VirD1/VirD2 endonuclease occurs between nucleotides 3 and 4 (Wang et al., 1987). Thus, within Agrobacterium, nucleotides 4 to 25 remain within the T-DNA at the left border (LB), whereas at the right border (RB) nucleotides 1 to 3 remain intact. However, within the plant, the T-strand is frequently chewed back, most likely by exonucleases. Because VirD2 is linked to and therefore protects the 5' end of the T-strand, loss of nucleotides at this end is usually minimal (a few nucleotides at most). Loss of nucleotides from the unprotected 3' end occurs more frequently and is generally more extensive; deletions up to several hundred nucleotides are not uncommon (Rossi et al., 1996). Early T-DNA binary vectors contained the plant antibiotic selection marker gene near the 5' end of T-DNA (RB), and goi were placed near the 3' end (LB; e.g. Bevan, 1984). However, extensive loss of DNA from the 3' end, most likely the result of nucleolytic degradation, could result in antibiotic-resistant transgenic plants with deletions in the goi. This problem was ameliorated by placing the selection marker gene near the LB and the goi near the RB. Extensive deletion of the T-DNA from the 3' end would result in removal of the selection marker and lack of recovery of these plants. Thus, deletion of the goi was generally abrogated. Sequences near RBs (so-called overdrive sequences) can increase transmission of T-DNA (Peralta et al., 1986). These sequences are frequently incorporated into T-DNA binary vector RB regions.

(2) A plant-active selectable marker gene (usually for antibiotic or herbicide resistance). The most commonly used selection systems employ aminoglycoside antibiotics such as kanamycin or hygromycin, herbicides such as phosphinothricin/gluphosinate, or herbicide formulations such as Basta or Bialophos. Other selection systems, such as phospho-mannose isomerase, employ metabolic markers (Todd and Tague, 1996).

Table I. (Continued from previous page.)

<table>
<thead>
<tr>
<th>Vector Series Name</th>
<th>Vector ori/Incompatibility Group</th>
<th>Important Featuresa</th>
<th>Gateway Compatable</th>
<th>Bacterial Selection Markerb</th>
<th>Plant Selection Markerb</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSITE pVS1</td>
<td></td>
<td>Fluorescence protein fusion. Based on pRCS2</td>
<td>Yes</td>
<td>Spec</td>
<td>Kan</td>
<td>Chakrabarty et al. (2007)</td>
</tr>
<tr>
<td>pMSP IncPa</td>
<td></td>
<td>Super-promoter to drive expression of goi</td>
<td>No</td>
<td>Kan</td>
<td>Kan/Hyg/Bar</td>
<td>Lee et al. (2007)</td>
</tr>
<tr>
<td>pCAMBIA pVS1</td>
<td></td>
<td>Multiple vectors for cloning, expression, and tagging</td>
<td>No</td>
<td>Kan/Chl</td>
<td>Kan/Hyg/Bar</td>
<td><a href="http://www.cambia.org/daisy/cambia/materials/vectors">http://www.cambia.org/daisy/cambia/materials/vectors</a></td>
</tr>
<tr>
<td>pGD PVS1</td>
<td>Derived from pCAMBIA1301. Multiple vectors for tagging proteins with DsRed2 or GFP</td>
<td>No</td>
<td>Kan</td>
<td>Hyg</td>
<td>Goodin et al. (2002)</td>
<td></td>
</tr>
</tbody>
</table>

a: cos, Bacteriophage Φ cohesive ends; mcs, multiple cloning site; ori, vegetative origin of replication; Ω, tobacco mosaic virus translational enhancer.

b: Amp, Ampicillin; Bar, resistance to phosphinothricin; Bleo, bleomycin; Chl, chloramphenicol; Dhfr, dihydrofolate reductase; Gent, gentamicin; Hyg, hygromycin; Kan, kanamycin, Nos, nopaline synthase; Pat, resistance to phosphinothricin; Spec, spectinomycin; Sul, sulfonylurea; Tet, tetracycline.

Table II. Frequently used disarmed Agrobacterium strains

<table>
<thead>
<tr>
<th>Strain Name</th>
<th>Chromosomal Background</th>
<th>Ti-Plasmid Derivation</th>
<th>Antibiotic Resistance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGL-0</td>
<td>C58</td>
<td>pTiBo542</td>
<td>rif</td>
<td>Lazo et al. (1991)</td>
</tr>
<tr>
<td>AGL-1</td>
<td>C58</td>
<td>pTiBo542</td>
<td>rif, carb</td>
<td>Lazo et al. (1991)</td>
</tr>
<tr>
<td>CSB-Z707</td>
<td>C58</td>
<td>pTiC58</td>
<td>kan</td>
<td>Hepburn et al. (1985)</td>
</tr>
<tr>
<td>EHA101</td>
<td>C58</td>
<td>pTiBo542</td>
<td>rif, kan</td>
<td>Hood et al. (1986)</td>
</tr>
<tr>
<td>EHA105</td>
<td>C58</td>
<td>pTiBo542</td>
<td>rif</td>
<td>Hood et al. (1993)</td>
</tr>
<tr>
<td>GV3101::pMP90</td>
<td>C58</td>
<td>pTiC58</td>
<td>rif, gent</td>
<td>Koncz and Schell (1986)</td>
</tr>
<tr>
<td>LBA4404</td>
<td>Ach5</td>
<td>pTiAch5</td>
<td>rif</td>
<td>Ooms et al. (1982)</td>
</tr>
<tr>
<td>NT1(pKPSF2)</td>
<td>C58</td>
<td>pTiChry5</td>
<td>ery</td>
<td>Palanichelvam et al. (2000)</td>
</tr>
</tbody>
</table>

a: carb, carbenicillin; ery, erythromycin; gent, gentamicin; kan, kanamycin; rif, rifampicin.
Some plant species have low-level tolerance to kanamycin, and care should be taken to determine the minimum concentration of antibiotic that will completely kill nontransformed tissues. As mentioned above, early binary vectors had these markers placed near the T-DNA RB. However, because of the polarity of T-DNA transfer (RB to LB; Wang et al., 1984), recent vectors contain the selectable marker near the LB to assure transfer of the goi.

(3) Restriction endonuclease, rare-cutting, or homing endonuclease sites within T-DNA into which goi can be inserted. Early binary vectors, such as pBIN19, contained a few restriction endonuclease cloning sites in a lacZ α complementation fragment, permitting blue/white screening for the presence of the transgene insertion (Bevan, 1984). In many vectors, promoters and polyA addition signals flank these sites. More recently, binary vectors containing multiple rare-cutting restriction endonuclease or homing endonuclease sites have been developed (Chung et al., 2005; Tzifra et al., 2005). These vectors, derived from plasmids originally constructed by Goderis et al. (2002), are designed to accompany a series of satellite (pSAT) vectors. The pSAT vectors contain expression cassettes (promoter, multiple restriction endonuclease cloning sites, polyA addition signal) flanked by rare-cutting/homing endonuclease sites (Chung et al., 2005). Some of these vectors have incorporated into these expression cassettes tags to generate fluorescent fusion proteins for protein localization studies (Tzifra et al., 2005) or protein-protein interaction studies (Citovsky et al., 2006). Multiple expression cassettes from the pSAT vectors can be loaded into the cognate rare-cutting sites in the binary vectors, permitting simultaneous introduction of multiple genes into plants. Several recent binary vectors contain Gateway sites to facilitate insertion of genes or exchange of gene cassettes from other vectors. Additionally, several BAC binary vectors have been designed to clone large inserts of more than 100 kb (Hamilton, 1997; Liu et al., 1999, 2000).

(4) Origin(s) of replication to allow maintenance in E. coli and Agrobacterium. The incompatibility group of the plasmid, with function related to the specific origin of replication, can be important if several plasmids need to co-exist in the bacterium. As such, these plasmids must belong to different incompatibility groups. In some instances, origins of replication may function in both Agrobacterium and in E. coli (in which initial constructions are generally made). These broad host range replication origins include those from RK2 (incPα; e.g. pBIN19 and derivatives), pSa (incW; e.g. pUCD plasmid derivatives), and pVS1 (e.g. pPZP derivatives). Other origins of replication that function in Agrobacterium, such as those from Ri-plasmids (e.g. pCGN vectors), do not function in E. coli; thus, a ColE1 origin (such as the one used in pUC and pBluescript plasmids) is added to the vector. Different origins of replication replicate to different extents in Agrobacterium. The pSa origin replicates to two to four copies per cell (Lee and Gelvin, 2004), the RK2 (Veluthambi et al., 1987) and pVS1 (L.-Y. Lee, unpublished data) origins replicate to seven to 10 copies per cell, and the pRi origin replicates to 15 to 20 copies per cell (L.-Y. Lee, unpublished data).

(5) Antibiotic-resistance genes within the chromosome and within backbone sequences for selection of the binary vector in E. coli and Agrobacterium. Many commonly used Agrobacterium strains are resistant to rifampicin due to a chromosomal mutation (see Table II). In addition, commonly used Agrobacterium strains can be grown on Suc as the sole carbon source. Most commonly used E. coli K12 laboratory strains cannot use Suc as a carbon source. Thus, growth on minimal medium containing rifampicin and Suc generally will eliminate E. coli from Agrobacterium cultures, an especially useful selection following introduction of the binary vector into Agrobacterium by mating plasmids between E. coli and Agrobacterium (Ditta et al., 1980; Garfinkel et al., 1981).

Care must be taken in matching binary vectors with specific vir helper Agrobacterium strains. As listed in Table II, many of these strains already express genes for resistance to kanamycin, carbenicillin, erythromycin, or gentamicin. Thus, one cannot easily use binary vectors with the same selection marker in these strains. For example, many T-DNA binary vectors based upon pBIN19 utilize kanamycin-resistance as the bacterial selection marker. A. tumefaciens EHA101 is kanamycin resistant and cannot easily be used with these pBIN19 derivatives. However, one can use these binary vectors in the near-isogenic kanamycin-sensitive strain A. tumefaciens EHA105. In addition, some Agrobacterium strains are resistant to low levels of spectinomycin, an antibiotic that is used in conjunction with the pPZP plasmids and their derivatives. When using spectinomycin, the researcher should test various concentrations of the antibiotic with the vir helper strain lacking the binary vector to assure effective killing. Care must also be taken if a binary vector contains a tetracycline-resistance gene. A. tumefaciens C58 harbors a tetracycline-resistance determinant (Luo and Farrand, 1999) and is thus resistant to low levels of this antibiotic.

Although some Agrobacterium strains or binary vectors may harbor a β-lactamase gene that confers resistance to carbenicillin, it is still relatively easy to kill these bacteria following infection of plants. The β-lactam antibiotics Augmentin and Timentin contain, additionally, clavulanate, which will inhibit β-lactamases. Concentrations of Timentin ranging from 100 to 150 mg/L will completely eliminate growth of Agrobacterium C58-based strains harboring a β-lactamase gene (Cheng et al., 1998). Agrobacterium Ach5-based strains, such as LBA4404, do not express β-lactamase activity well, and thus can be killed by even lower concentrations of either carbenicillin or Timentin (Hooykaas, 1988).

ALTERNATIVE T-DNA BINARY SYSTEMS

Although T-DNA binary vector systems almost always consist of T-DNA and vir regions localized on
plasmids, it is not essential that they function this way. Replicons containing T-DNA or vir genes do not need to be plasmids. Indeed, several laboratories have shown that T-DNA can be integrated into an Agrobacterium chromosome and launched from this replicon (Hoekema et al., 1984; Miranda et al., 1992), and specialized vectors have been generated to facilitate integration of DNA into a specific neutral (i.e., not involved in virulence) region of the chromosome of A. tumefaciens C58 (Lee et al., 2001). Although launching T-DNA from the Agrobacterium chromosome can result in lower transformation frequencies, this process has the beneficial consequences of reducing integrated transgene copy number and almost completely eliminating integration of vector backbone sequences into the plant genome (Ye et al., 2007).

CONCLUSION

T-DNA binary systems have greatly simplified the generation of transgenic plants. No longer are complex, sophisticated microbial genetic regimes required to integrate into T-DNA regions located on large, cumbersome Ti- or Ri-plasmids. Along with companion vir helper strains, numerous different T-DNA binary vectors with specialized properties have been designed to facilitate such diverse activities as protein expression, activation tagging, protein localization, protein-protein interaction studies, and RNAi-mediated gene silencing. However, the ease of use of binary vectors may have come at a cost. The use of multicopy binary vectors generally results in integration of multiple copies of T-DNA into the plant genome. Multiple transgene copies have a propensity to silence to a greater extent than do single integrated copies. In addition, integration of vector backbone sequences from binary vectors into plant DNA, a potential regulatory problem, is common (Martinez et al., 1994; Kononov et al., 1997; Wenck et al., 1997). Integration of non-T-DNA region sequences when T-DNA is launched from large Ti-plasmids is relatively rare (Ramanathan and Veluthambi, 1995). Thus, the use of multicopy binary vectors may have exacerbated two common problems associated with plant transformation, multiple integrated transgene copy number and vector backbone integration. Launching T-DNA from low-copy-number T-DNA binary vectors or from the Agrobacterium chromosome may mitigate these problems (Ye et al., 2007). Such systems should greatly increase the quality of Agrobacterium-mediated transformation events.

ACKNOWLEDGMENTS

Work in the authors’ laboratory is supported by the Biotechnology Research and Development Corporation, the Corporation for Plant Biotechnology Research, and the National Science Foundation (Plant Genome grant no. 0110023).

Received November 9, 2007; accepted November 25, 2007; published February 6, 2008.

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