Critical Reviews in Plant Sciences
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bpts20

Selectable Markers and Reporter Genes: A Well Furnished Toolbox for Plant Science and Genetic Engineering

Daniele Rosellini [a]
[a] Department of Applied Biology, University of Perugia, Italy
Published online: 27 Sep 2012.

To cite this article: Daniele Rosellini (2012) Selectable Markers and Reporter Genes: A Well Furnished Toolbox for Plant Science and Genetic Engineering, Critical Reviews in Plant Sciences, 31:5, 401-453, DOI: 10.1080/07352689.2012.683373

To link to this article: http://dx.doi.org/10.1080/07352689.2012.683373

Critical Reviews in Plant Sciences, 31:401–453, 2012

ISSN: 0735-2689 print / 1549-7836 online
DOI: 10.1080/07352689.2012.683373

Taylor & Francis
Taylor & Francis Group

Selectable Markers and Reporter Genes: A Well Furnished Toolbox for Plant Science and Genetic Engineering

Daniele Rosellini
Department of Applied Biology, University of Perugia, Italy

Table of Contents

Marker genes (MGs) are essential tools for plant research and biotechnology. Positive selectable marker genes (SMGs) are used in genetic transformation to allow only transgenic cells to grow and develop and are necessary for efficient transformation. Negative SMGs confer a selective disadvantage to the cells that express them, and have several uses in both basic and applied research. Reporter genes (RGs) make it possible to easily screen cells or tissues for their expression. Several tens of different genes from bacteria, fungi, plants, and animals have been demonstrated to function as SMGs. Here, SMGs are classified based on the mechanism of action of the gene products. To provide the readers with practically useful information, details on transformation and selection efficiency are given. RGs are the object of intense research. Refinement of existing RGs and development of new ones is constant, and has provided powerful aids for fine studies on cell biology and more efficient genetic engineering. They are classified as vital and non vital, depending on the possibility to screen their expression in living cells. The effect of MG expression on the phenotype and their safety in crops is briefly discussed. The picture emerging from this literature review is that a plentiful array of powerful and versatile tools for basic and applied research is available.

Address correspondence to Daniele Rosellini, Department of Applied Biology, University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy. E-mail: roselli@unipg.it

Keywords fluorescent proteins, genetic transformation, negative selection, plant biotechnology, positive selection

I. GLOSSARY

Bioluminescence. Emission of light by a living organism as a consequence of a chemical reaction.

Escape. In plant transformation technology, it is a non-transgenic plants obtained in the presence of positive selection. The percentage of escapes, or 'escape rate' is calculated as the difference 100-Selection Efficiency.

Fluorescence. Emission of light by a substance after absorbing light, or other electromagnetic radiation, usually at higher wavelength.

Negative selectable marker gene. Gene that confers a selective disadvantage to the cells expressing it.

Positive selectable marker gene (SMG). Gene used in genetic transformation to ensure that only transgenic cells can grow preferentially over the non-transformed cells.

Reporter gene (RG). Marker gene that, when introduced, makes it possible to visually identify the cells or tissues expressing it.

Resistance. In plant transformation technology, resistance to phytotoxins is realized by chemical modification of the substance or by transport to a cell compartment where it is no longer deleterious.

Secondary selectable marker gene. SMG that confer resistance to a phytotoxin at the tissue level but not at the single cell level; it is not suitable to obtain transgenic plants.

Selection efficiency (SE). The number of independent transgenic events in 100 regenerated events. The difference 100-SE is often referred to as the escape rate.

Sensitivity. When referred to RGs, it is the ratio between reporter and background signal.

Tolerance. In plant transformation technology, tolerance to a phytotoxin is attained by substituting the target molecule that is inactivated by the toxin with an insensitive, or less sensitive molecule.

Transformation efficiency (TE). The number of independent transgenic events obtained from 100 explants. In biolistic transformation, TE is often given as the number of transgenic events per bombarded plate. This definition of TE cannot be applied to transformation of protoplasts or cells obtained from suspension cultures, for which efficiency can be measured on the basis of the fresh weight of treated cells.

ABBREVIATIONS

AA = aminoacid or aminoacid analog
AB(s) = antibiotic(s)
CH = carbohydrate
E35S = Double enhancer cauliflower mosaic virus (CaMV) 35S promoter
GE = genetically engineered
GR(s) = growth regulator(s)
H = herbicide
HR = herbicide resistance
MG(s) = marker gene(s)
RG(s) = reporter gene(s)
SE = selection efficiency
SMG(s) = selectable marker gene(s)
TE = transformation efficiency
35S = cauliflower mosaic virus (CaMV) 35S promoter

II. CLASSIFICATION OF MARKER GENES

Marker genes (MGs) are commonly classified into two main groups: selectable marker genes (SMGs) and reporter genes (RGs). In turn, SMGs can be positive or negative.

Positive SMGs are used in genetic transformation to ensure that only transgenic cells, which are always a small minority of the cells that are subjected to the transformation treatment, can grow preferentially over the non-transformed cells.

Negative SMGs are used in basic research to kill specific cells or tissues (selective ablation) and in strategies to obtain marker-free plants, to reduce the chimerism of transgenic plants, or to improve the efficiency of gene-targeting in plants.

Reporter genes (RGs), sometimes referred to as 'screenable genes,' make it possible to visually identify cells or tissues that express them. They are invaluable tools to study promoter activity, as well as cell structure and function. In plant genetic engineering, they are used to monitor the efficiency of transformation and sometimes for the manual selection of transgenic tissue.

In their comprehensive review, Miki and McHugh (2004) adopted, after Babwah and Waddel (2000), a classification that clarified some confusing terminology of SMGs. Here, a partly new classification of SMGs is presented to better accommodate many new SMGs that appeared in recent years (Table 1).

A. Positive Selectable Marker Genes (SMGs)

The following seven types of positive SMGs can be identified based on the selection strategy (Table 1):

1. genes acting through chemical detoxification of a phytotoxin;
2. genes acting through removal or exclusion of a phytotoxin from the affected cell compartment;
3. genes acting through overexpression of the phytotoxin-sensitive, or expression of an insensitive target molecule;
4. genes that confer pathogen resistance;
5. genes that confer heat tolerance;
6. genes that confer to the plant cells the ability to utilize as carbon sources carbohydrates that otherwise cannot be metabolized; in some cases these carbohydrates also exert phytotoxic effects;
7. genes that confer to the plant cell the ability to autonomously produce the growth regulators necessary for regeneration.

TABLE 1
Classification of marker genes

Marker gene type	Selection type	Selection strategy/ screening tool	Resistance mechanism/ cell viability	Average maximum TE[1]	Average maximum SE[1]
Selectable marker genes	**Positive**	**Resistance or tolerance**	**Chemical detoxification of phytotoxin** (Type 1)	24[2]	73[2]
Genes that allow *in vitro* selection of transgenic cells	Genes that confer selective advantage to transgenic cells	Confer resistance or tolerance to a phytotoxin, to biotic or abiotic stresst	**Removal of phytotoxin from the sensitive cell compartment** (Type 2)	43	61
			Overexpression of the sensitive, or expression of an insensitive target molecule (Type 3)	29	78
			Pathogen resistance (Type 4)	–	–
			Heat tolerance (Type 5)	–	–
		Carbohydrate metabolism (Type 6) Confer the ability to utilize a carbohydrate that otherwise is not metabolized		15	75
		Growth regulator metabolism or regulation of the cell cycle (Type 7) Confer the ability to produce, or increase sensitivity to cytokinin or auxin		23	60
	Negative Genes that confer selective disadvantage to transgenic cells or plants	**Cell death**		na	na
Reporter genes Genes whose expression in transgenic cells, tissues or plants is easy to screen		**Pigmentation or fluorescence**	**Vital** **Non vital**	na	na

[1]Average of the maximum values of Transformation Efficiency (TE) or Selection Efficiency (SE) of all markers of each group. Data from *A. thaliana* transformation and plastid transformation experiments are not included.

[2]Data on the *nptII, hpt*, and *pat* genes are not included.

na: not applicable.

TABLE 2
Positive selectable marker genes

Gene (wt/mutant)	Marker type and use[1]	Source organism	Promoter	Selection[2]	Plant species transformed	TE%[3]	SE%[3]	Associated phenotype	References
aac(3)-III (wt)	1, n	*Klebsiella pneumoniae* or *Serratia marcescens*	*35S*	Gentamicin and other aminoglycosides (AB)	*Arabidospsis thaliana*, Petunia	nr	nr	nr	Hayford *et al.*, 1988
aac(3)-IV (wt)	1, n	*Salmonella*			*A. thaliana*, Petunia, and others	nr	nr	nr	
aac(6′) (wt)	1, n	*Shigella*	*35S*	Kanamycin (AB)	Tobacco	nr	90	nr	Gossele *et al.*, 1994
aadA (wt)	1, p	*E. coli*	Tobacco rRNA operon (*prrn*)	Spectinomycin (AB)	*A. thaliana*	0.01	–	Sterile	Sikdar *et al.*, 1998
	p		*prrn*	Spectinomycin and Streptomycin (AB)	*Sugar beet*	2.5	–	nr	De Marchis *et al.*, 2009
	n		*Octopine synthase*	Spectinomycin (AB)	*Canola*	2–10	93	nr	Schroder *et al.*, 1994
	p		*prrn*	Spectinomycin (AB)	*Canola*	25	–	Heteroplasmy	Cheng *et al.*, 2010
					Cabbage	na	–	nr	Nugent *et al.*, 2006
	p			Spectinomycin and Streptomycin (AB)	*Brassica capitata*	13–20	–	None	Liu *et al.*, 2007
	p		*prrn*	Spectinomycin (AB)	Carrot	14	–	None	Kumar *et al.*, 2004a
	p		*prrn*		Soybean	0.07–2	–	None	Dufourmantel *et al.*, 2004; 2005; 2007
	p		*prrn, Lactuca prrn*	Spectinomycin (AB)	Lettuce	na	–	nr	Lelivelt *et al.*, 2005
			Lactuca prrn			1	–	None	Kanamoto *et al.*, 2006
	p		*PrrnLrbcL*	Spectinomycin and Streptomycin (AB)	*Lesquerella fendleri*	0.04	–	nr	Skarjinskaia *et al.*, 2003
	p		*prrn*		Tobacco	1–14	–	None	Svab and Maliga 1993 Too many to list
	n		*35S*	Streptomycin (AB)	Rice	0–39 (+)	70–100	nr	Oreifig *et al.*, 2004
	p		*prrn*		Rice	nd	–	Heteroplasmy	Khan and Maliga 1999
						0.02	–	Heteroplasmy	Lee *et al.*, 2006
	p		*Canola prrn*	Spectinomycin and Streptomycin (AB)	Petunia	0.1	–	None	Zubko *et al.*, 2004
	p		*prrn*	Spectinomycin (AB)	Poplar	0.3	–	None	Okumura *et al.*, 2006
	p		*prrn*		Tomato	0.05–0.15	–	None	Ruf *et al.*, 2001
	p		*psbA*	Spectinomycin and Streptomycin (AB)	Eggplant	nr	–	nr	Singh *et al.*, 2010
	p		*prrn, psbA*		Potato	0.03–0.17	–	nr	Sidorov *et al.*, 1999
			35S	Spectinomycin (AB)	Potato	0.2	nr	nr	
ak (wt)	3, n	*E. coli*	*35S*	Lysine and threonine (AA)	Potato	nr	100	nr	Perl *et al.*, 1993

ak (lysC) (mutant)	3, n	*E. coli*	*35S*		Chikpea	nr	1	nr	Tewari-Singh *et al.*, 2004
alr (wt)	1, n	*Corynebacterium glutamicum*	nr	D-alanine (AA)	*A. thaliana*	nr	nr	Many traits affected	Thiruvengadam *et al.*, 2010
ALS (*AHAS*) (mutant)	3, n	*A. thaliana*	*E35S*	Bispyribac-sodium or Pyrithiobac-sodium (H)	*A. thaliana*	1.1–1.2	nr	HR	Kaway *et al.*, 2010
			35S	Chlorsulfuron, imazethapyr (H)	*Brassica juncea*	4–10 (+)	100	HR	Ray *et al.*, 2004
			Native	Imazapyr (H)	Soybean	4–20	8–20	HR	Aragao *et al.*, 2000
			Native	Chlorsulfuron (H)	Linseed	na	na	HR	McHughen 1989
			Native		Tobacco	nr	nr	HR	Olzewski *et al.*, 1988; Gabard *et al.*, 1989
			Native/*35S*		Tobacco	3–7(−)	nr	HR	Charest *et al.*, 1990
			Native/*35S*		Rice	na (protoplast) (−)	nr	HR	Li *et al.*, 1992
			Native/*E35S*		Hybrid poplar	22–77 (=)	88–100	HR	Brasileiro *et al.*, 1992
			Native/*nos*	Imazamox (H)	Potato	100	87–100	HR	Andersson *et al.*, 2003
			35S	Chlorsulfuron (H)	Maize	nr	nr	HR	Fromm *et al.*, 1990
						nr	100	HR	Howe *et al.*, 2002
			35S			nr	16–24	HR	Zhang *et al.*, 2005
		Cotton	Native	Imazethapyr/imazaquin (H)	Cotton	6–12	100	HR	Rajasekaran *et al.*, 1996
		Tobacco	*Maize Ubi*	Chlorsulfuron (H)	Banana	na (cell suspension)	100	HR	Ganapathi *et al.*, 2001
			Native		Tobacco	na (protoplast)	21–100	HR	Lee *et al.*, 1988
		Rice	*E35S*	Bispyribac-sodium or Pyrithiobac-sodium (H)	*A. thaliana*	0.7–0.8	100	HR	Kaway *et al.*, 2010
			E35S	Bispyribac-sodium (H)	Soybean	0.3–1.2	31–64	HR	Togou *et al.*, 2009
			Native		Rice	nr	nr	HR	Osakabe *et al.*, 2005
			Maize Ubi			nr	nr	HR	Okuzaki *et al.*, 2007
			Rice Ubi			2–30	nr	HR	Taniguchi *et al.*, 2010
			CSP	Pyriminobac (H)	Rice	10–30	85–94	HR	Wakasa *et al.*, 2007
			Native	Bispyribac-sodium (H)	Bread wheat	6–17	40–92	HR	Ogawa *et al.*, 2008
apha6 (wt)	1, p	*Acinetobacter baumannii*	*prrn*	Kanamycin (AB)	Tobacco	0.5	–	nr	Huang *et al.*, 2002
		E. coli	*Phage T7 gene 10*		Cotton	5	100	None	Kumar *et al.*, 2004b
ASA (mutant)	3, n	*A. thaliana*	*pyk10*	5-methyl-tryptophan (AA) + sucrose	*A. thaliana*	nr	100	nr	Nonomura *et al.*, 2009
	n	Tobacco	*35S*	4-methylindole (AA)	Tobacco	14	21	Higher tryptophan	Barone and Widholm 2008
				7-methyl-DL-tryptophan (AA)		7	22		
	p		*Tobacco 16S rRNA*	4-methylindole (AA) 7-methyl-DL-tryptophan (AA)	Tobacco	3.8–4.3	20	Higher free tryptophan	Barone and Widholm 2009
	n	Rice	*Z. mays Ubi*	5-methyl-tryptophan (AA)	Rice	0–7 (=)	100	Higher tryptophan	Yamada *et al.*, 2004
			35S		Potato	25–70 (=)	37-100	Higher tryptophan; anthocyanin pigmentation *in vitro*	

(*Continued on next page*)

TABLE 2
Positive selectable marker genes (*Continued*)

Gene (wt/mutant)	Marker type and use[1]	Source organism	Promoter	Selection[2]	Plant species transformed	TE%[3]	SE%[3]	Associated phenotype	References
atlD (wt)	6, n	*E. coli* (plant optimized)	D-Arabitol	*35S*	Rice	9 (=)	nr	None	LaFayette *et al.*, 2005
BADH (wt)	1, p	Spinach	*prrn*	Betaine aldehyde	Tobacco	2.5 (+)	73 (+)	None	Daniell *et al.*, 2001
BBM (wt)	7, n	Canola	*35S*	None	Poplar	3	100	Dwarfism	Deng *et al.*, 2009
			35S	None	Sweet pepper	0.6–1	nr		Heidmann *et al.*, 2011
ble (wt)	1, n	*E. coli*, *Streptoalloteichus hindustanus*	*35S*	Bleomycin and phleomycin (AB)	Tobacco	nd	nd	nd	Perez *et al.*, 1989
bnx (wt)	1, n	*Klebsiella pneumoniae*	nd	Oxynils (H)	Canola	nd	nd	nd	Freyssinet *et al.*, 1996
cah (wt)	1, n	*Myrothecium verrucaria*	*Maize UBI1*	Calcium cyanamide (H)	Bread wheat	0.2	nr	HR	Weeks *et al.*, 2000
cat (wt)	1, n	*E. coli*	*nos*	Chloramphenicol (AB)	Tobacco	na	na	None	DeBlock *et al.*, 1984
	p		*prrn*		Tobacco	0.6–1.0 (−)	80–100 (+)	None	Li *et al.*, 2011
CYP (wt)	1, n	*Homo sapiens*	*35S*	Various herbicides	*A. thaliana*	0.05–0.50	nr	nr	Inui *et al.*, 2005
DAAO (wt)	1, n	*Rhodotorula gracilis*	*35S*	D-alanine, D-serine (AA)	*A. thaliana*	1.7–2.1	100	nr	Erikson *et al.*, 2004
deh1 (wt)	1, n	*Pseudomonas putida*	*nd*	2,2 dichloropropionic acid (Dalapon, H)	nd	nd	nd	nd	Buchanan-Wollaston *et al.*, 1992
DEF2-D (wt)	3, n	*A. thaliana*	*MMV M24*	Actinonin (AB)	Tobacco	nr (=)	nr	nr	Hou *et al.*, 2007
dhdps (wt)	3, n	*E. coli*	*35S*	S-Aminoethyl l-cysteine (AA)	Potato	nr	100	nr	Perl *et al.*, 1993
Dhfr (wt)	3, n	*E. coli*	*35S*	Methotrexate (AB)	*A. thaliana*	nd	nd	nd	Kemper *et al.*, 1992
		Mouse	nd		Petunia	nd	nd	nd	Eichholtz *et al.*, 1987
		E. coli	*nos*		Tobacco	na	na	None	DeBlock *et al.*, 1984
		Candida albicans	Native		Tobacco	nr	nr	nr	Irdani *et al.*, 1998
		Mouse	*35S*		Tobacco (male germline or pollen transformation)	na	na	nr	Aionesei *et al.*, 2006

DOG^{R}1 (wt)	1, n	*Saccharomyces cerevisiae*	*35S*	2-Deoxyglucose (CH)	Tobacco	48 (−)	36–63	None	Kunze *et al.*, 2001
					Potato	53 (−)	20–50 (−)	None	
DREB2A (wt)	2, n	Rice	*Rice 4ABRC*	NaCl	Rice	13 (−)	78	nr	Zhu and Wu 2008
dsdA (wt)	1, n	*E. coli*	*Maize UBI1*	D-serine (AA)	*A. thaliana*	0.4 (=)	100	None	Erikson *et al.*, 2005
					Maize	15–41	100	None	Lai *et al.*, 2011
epsps (wt) (*aroA*)	3, n	*A. tumefaciens* (strain *CP4*)	Sugarcane bacilliform virus, *E35S, A. thaliana ACTIN1*	Gliphosate (H)	Bread wheat	3–4	100	HR	
			Figwort mosaic virus *35S*		Soybean	nr	nr	HR	
EPSPS (mutant)	3, n	Rice	*35S*	Glyphosate (H)	Rice	9 (=)	100	HR	Charng *et al.*, 2008
		Maize	*E35S*		Maize	nr	100	HR	Howe *et al.*, 2002
galT (wt)	1, n	*E. coli*	Double enhancer *35S* (*E35S*)	Galactose (CH)	Canola	nr	4	nr	Joersbo *et al.*, 2003
					Potato	nr	5–35	Transient growth rate reduction	
gox (wt) (with *epsps*)	1, n	*Ochrobactrum anthropi*	nd	Glyphosate (H)	Bread wheat	nd	nd	HR	Zhou *et al.*, 1995
GPT (wt)	3, n	*A. thaliana*	*35S*	Tunicamycin (AB)	*A. thaliana*	1[(2)]	100	None observed	Koizumi 2003
GSA (mutant)	3, n	Alfalfa	*E35S*	Gabaculine	Alfalfa	43	94	None observed	Ferradini *et al.*, 2011a
					Tobacco	47	100		
					Durum wheat	1 (=)	80 (−)		Gadaleta *et al.*, 2012
hemL (mutant)	3, n	*Synechococcus*	*E35S*	Gabaculine	Alfalfa	82–88 (+)	60–65 (+)	None observed	Rosellini *et al.*, 2007
			35S	Gabaculine	Tobacco	nr	nr	nr	Gough *et al.*, 2001
hpt (*hph, aphIV*) (wt)	1, n	*E. coli*	Various	Hygromycin B (AB)	Many	Variable	Variable	None	Waldron *et al.*, 1985 Too many to list

(*Continued on next page*)

TABLE 2
Positive selectable marker genes (*Continued*)

Gene (wt/mutant)	Marker type and use[1]	Source organism	Promoter	Selection[2]	Plant species transformed	TE%[3]	SE%[3]	Associated phenotype	References
HSP101 (wt)	5, n	Rice	*35S*	Heat	Tobacco	nr	nr	Thermotolerance	Chang *et al.*, 2007
IaaH + *IaaM* (wt), with *ipt*	7, n	*A. tumefaciens*	Native	Lack of growth regulators	Citrus, Citrange	4–9	5–14	Cytokinin overproduction	Ballester *et al.*, 2008
					Tobacco	90–95	45–49		Endo *et al.*, 2002a
ilvA (mutant)	1, n	*E. coli*	*35S*	L-O-methylthreonine (AA)	*A. thaliana*	0.7–6.2 (−)	<10–55	Can be severe	Ebmeier *et al.*, 2004
ipt (wt)	7, n	*A. tumefaciens*	*Potato Ubi3, Ubi7*	Lack of growth regulators	Canola	2–4	nr	Cytokinin overproduction	Richael *et al.*, 2008
			Native	Lack of cytokinin	Citrus, Citrange	5–9 (−)	14–16 (−)		Ballester *et al.*, 2007
			Native	Lack of growth regulators	*Kalanchoe blossfeldiana*	76	79		Thirukkumaran *et al.*, 2009
			Native		Cassava	6–9	100		Saelim *et al.*, 2009
			35S		Tobacco	6	100		Ebinuma *et al.*, 1997a
			Native		Tobacco	12	nr		Ebinuma and Komamine 2001
			Potato Ubi3, Ubi7		Tobacco	13–19	nr		Richael *et al.*, 2008
			35S		*Nierembergia caerulea*	40	46		Khan *et al.*, 2006
			35S		Hybrid poplar	6	100		Ebinuma *et al.*, 1997
			Native, *35S, rbcS*			0.7–4	11–53		Ebinuma and Komamine 2001
			rbcS			6	100		Matsunaga *et al.*, 2002
			Native		Apricot	4–9	3–14		Lopez-Noguera *et al.*, 2009
			35S		Rice	27	37		Ebinuma and Komamine 2001
						9.5	30		Endo *et al.*, 2002b
			Potato Ubi3, Ubi7		Tomato	4–5	nr		Richael *et al.*, 2008
			Native		Potato	20	nr		Khan *et al.*, 2011
			Potato Ubi3, Ubi7		Potato	2–8	nr		Richael *et al.*, 2008
KN1 (wt)	7, n	Maize	*35S*	Lack of growth regulators	Tobacco	> 100 (+)	nr	Shooty	Luo *et al.*, 2006
lyr (wt)	1, n	Soil metagenome	*35S*	L-lysine (AA)	*A. thaliana*	2.1–2.7 (+)	95–100	None	Chen *et al.*, 2010

					Tobacco	21–28	88–100		
manA/pmi (wt)	6, n	*E. coli*	*CMPS*	Mannose	Onion	23–24	nr	nr	Aswath *et al.*, 2005
			35S		*Agrostis stolonifera*	0.7 (−)	na	None	Fu *et al.*, 2005
			A. thaliana Ubq3		*A. thaliana*	2.5 (=)	100	nr	Todd and Tague 2001
			35S		Sugar beet	0–1 (+)	1–97 (+)	None	Joersbo *et al.*, 1998
			Several			0.3–0.9 (+)	nr	None	Joersbo *et al.*, 2000
			nd		*Brassica campestris ssp. Pekinensis*	nd	nd	nd	Min *et al.*, 2007
			*A. thaliana Ubq*3			1–3 (−)	nr	None	Ku *et al.*, 2006
			Cestrum yellow leaf curling virus (*CMPS*)		Canola	0–24 (=)	10–86 (=)		Wallbraun *et al.*, 2009
			*A. thaliana Ubq*3		Papaya	21 events per gram callus (+)	77 (−)	nr	Zhu *et al.*, 2005
			CMPS		Chikpea	3 (−)	nr	nr	Patil *et al.*, 2009
			A. thaliana Act2		Citrus	3–23	31–75	nr	Boscariol *et al.*, 2003
			CMPS		Citrus, Citrange	0–31	0–61 (+)	nr	Ballester *et al.*, 2008
			E35S			15–45	nr	None	Dutt *et al.*, 2010
			nr		Water melon	2	nr	nr	Reed *et al.*, 2001
			35S		Kewcumber	0–23	0–100	nr	He *et al.*, 2006
			nr		Barley	3–4	100	nr	Reed *et al.*, 2001
			CMPS		Lettuce	0–25 (=)	0–67 (−)	nr	Bříza *et al.*, 2010
			CMPS		Linseed	0.8–6 (=)	100	nr	Lamblin *et al.*, 2007
			CMPS		*Liquidambar formosana*	0–9	61–85	nr	Qiao *et al.*, 2010
			CMPS		Apple	1–24	100	nr	Degenhardt *et al.*, 2006
			35S		Cassava	0.32–28	100	None	Zhang and Puonti-Kaerlas 2000
						12	100	None	Zhang *et al.*, 2000
			35S		*Oncidium* sp	0–21	10–78	None	Thiruvengadam *et al.*, 2011
			CMPS		Rice	nr	20–90	None	Datta *et al.*, 2003
			35S			0–6	50–100	nr	He *et al.*, 2004
			CMPS			37–44 (+)	84–91	None	Lucca *et al.*, 2001
			35S			16 (−)	57 (=)	nr	Zai-Song *et al.*, 2006
			Maize ubi		Pearl millet	0–3 (+)	20	None	O'Kennedy *et al.*, 2004
			CMPS		Plum	<0.1	nr	nr	Mikhailov *et al.*, 2007
			CMPS		Apricot	7 (+)	39	nr	Ramesh *et al.*, 2006
			CMPS		Hybrid poplar	nr	12	nr	Qiao *et al.*, 2011
			Maize ubi		Sugar cane	Approx. 1 (per 100 calli)	56 (+)	None	Jain *et al.*, 2007
			CMPS		Tomato	2–15 (−)	nr	nr	Sigareva *et al.*, 2004
						0–4 (−)	20–100	nr	Bříza *et al.*, 2008
						0–53	3–100	nr	Bříza *et al.*, 2008

(*Continued on next page*)

TABLE 2
Positive selectable marker genes (*Continued*)

Gene (wt/mutant)	Marker type and use[1]	Source organism	Promoter	Selection[2]	Plant species transformed	TE%[3]	SE%[3]	Associated phenotype	References
			Maize ubi	Mannose	Potato	3	100	None	Gao *et al.*, 2005
			35S			5–10	100	nr	Li *et al.*, 2007
			Maize ubi			20 (+)	nr	nr	Wright et al 2001
			CMPS			1 (=)	90 (+)	nr	Gadaleta *et al.*, 2006
			Maize ubi			0.7–32	72–100	nr	Negrotto *et al.*, 2000
						45 (+)	nr	nr	Wright et al 2001
MAR1 (RTS3) (wt)	2, n	*A. thaliana*	*35S*	Kanamycin (AB)	*A. thaliana*	nr	nr	None observed	Aufsatz *et al.*, 2009
MPR1 (wt)	1, n	*S. cerevisiae*	*E35S*	L-Azetidine-2-carboxylic acid (AA)	Tobacco	21 (−)	23 (−)	None	Tsai *et al.*, 2010
M6PR (wt)	6, n	Celery	Mannose	*35S*	*A. thaliana*	0.68 (=)	79	nr	Song *et al.*, 2010
NiR (wt)	1, n	Rice	Native/*35S*/*Rice Actin1*	None / $NaNO_2$	Rice	8 (−)	nr	Higher nitrite reductase activity	Nishimura *et al.*, 2005
						43	>80		Ozawa and Kawahigashi 2006
nptII (wt)	1, n, p	*E. coli*	Various	Kanamycin, G418, paramomycin (AB)	Too many to list	Variable	Variable	None	Too many to list
			prrn	Kanamycin (AB)	Tobacco	4	2–4	None	Carrer *et al.*, 1993
nptII (wt) + *aphA6*	1, p		*psbA*	Kanamycin (AB)	Cotton	42	100	None	Kumar *et al.*, 2004b
OMR1 (mutants)	1, n	*A. thaliana*	*35S*	L-O-methylthreonine (AA)	*A. thaliana*	na	na	None to severe	Garcia and Mourad 2004
pat/bar (wt)	1, n	*Streptomyces hygroscopicus* *S. viridochromo-genes*	Various	Phosphinothricin (AA, H)	Many	Variable	Variable	Transcriptome changes observed	Too many to list
PDS (mutant)	3, n	*Hydrilla verticillata*		Norflurazon (H)	*A. thaliana*	nr	nr	None	Arias *et al.*, 2006
PFLP (wt)	4, n	Sweet pepper	*35S*	*Erwinia carotovora*	*Oncidium sp.*	3–4 (=)	100	Soft rot resistance	You *et al.*, 2003
PPO1 (mutant)	2, n	*A. thaliana*	Native	Butafenacil (H)	*A. thaliana*	nr	nr	HR	Li *et al.*, 2003
			Maize Ubi		Maize	2–33 (=)	nr	HR	
Rol ABCD (wt)	7, n	*A. rhizogenes*	Native	Lack of growth regulators	Tobacco	nr	15	Shooty	Ebinuma *et al.*, 1997b
					Hybrid poplar	20	27		Ebinuma and komamine 2001
			35S		Snapdragon	17–67	40–100		Cui *et al.*, 2000; 2001
			35S	None	Petunia	17	100		Khan *et al.*, 2010
rrnS (*16S RNA*) (mutant)	3, p	Tobacco	Native	Spectinomycin and streptomycin (AB)	Tobacco	0.02	100	nr	Svab *et al.*, 1990
		Tobacco or *Solanum nigrum*			Tomato	na	na	nr	Nugent *et al.*, 2005

rstB (wt)	2, n	*Sinorhizobium fredii*	*35S, E35S*	NaCl	Tobacco	0–>100	80–83 (=)	Salt tolerance	Zhang *et al.*, 2009
sat3 (wt)	1, n	*E. coli*	*35S*	Streptothricin (AB)	Carrot, Birdsfoot trefoil, L. *japonicus* Tobacco	nr	90–100	nr	Jelenska *et al.*, 2000
SOS1 (wt)	2, n	*A. thaliana*	*4ABRC*	NaCl	Rice	74 (−)	52	nr	Zhu and Wu 2008
spt (mutant)	1, n	*E. coli*	*A. tumefaciens 2′*	Streptomycin (AB)	Tobacco	Na (=)	na	nr	Maliga *et al.*, 1988
sulI (mutant)	3, n	*E. coli*	*E35S*	Asulam, sulfadiazine (H)	Tobacco	nr	89	None	Guerineau *et al.*, 1990
				Sulfanilamide, sulfadiazine (H)	Potato	nr	18	None	Wallis *et al.*, 1996
				Asulam, sulfadiazine (H)		nr	nr	None	Surov *et al.*, 1998
TDC (wt)	1, n	*Catharanthus roseus*	*35S*	4-methyl tryptophan (AA)	Tobacco	nr	80 (=)	Aminoacid profile change	Goddijn *et al.*, 1993
tflA (wt)	1, n	*Paenicillum polymixa*	*35S*	Toxoflavin (AB) + light	*A. thaliana*	0.6 (−)	50	None	Koh *et al.*, 2011
					Rice	7–8 (=)	81-89		
TPS1 (wt)	1, n	*A. thaliana*	*35S*	Glucose (CH)	*A. thaliana*	1	80	None	Leyman *et al.*, 2006
					Tobacco	nr	94		
TSB1 (wt)	3, n	*A. thaliana*	*35S*	5-methyltryptophan (AA) $CdCl_2$	*A. thaliana*	0.11–0.14[2]	100	Higher free tryptophan	Hsiao *et al.*, 2007
TUB1 (mutant)	3, n	*Eleusine indica*	*Maize Ubi*	Trifluralin (H)	Finger millet Soybean, N. *plumbaginifolia N. sylvestris,* Linseed	nr	nr	nr	Yemets *et al.*, 2003
UidA (*gusA*) (wt)	7, n	*E. coli*	nd	Benzyladenine-N-3-glucuronide	Tobacco	nd	nd	Cytokinin overproduction	Joersbo and Okkels, 1996
WBC19 (wt)	2, n	*A. thaliana*	*35S*	Kanamycin (AB)	Melon	20–91	nr	nr	Bombale *et al.*, 2010
					Tobacco	~ 40 (+)	73 (=)	None observed	Mentewab and Stewart 2005
					Hybrid poplar	7 (=)	20 (=)	nr	Kang *et al.*, 2010
xylA (wt)	6, n	*Thermoanaero-bacterium sulfurogenes*	d-Xylose	*E35S*	Tobacco	7–13 (−)	nr	None	Haldrup *et al.*, 1998a
					Tomato	5–9 (=)			
					Potato	29 (+)			
						3–12	1–11	None	Haldrup *et al.*, 2001
		Streptomyces rubiginosus			Potato	3–32 (+)	nr	None	Haldrup *et al.*, 1998b
		T. sulfurogenes	L-rhamnose		Tobacco	3	4	None	Haldrup *et al.*, 2001
					Potato	5	12		
		S. rubiginosus	L-rhamnose		Potato	0–3	7–83		

[1] 1–7: Marker type (see Table 1); n: nuclear genome transformation; p: plastid genome transformation.

[2] AA: aminoacid or aminoacid analog; AB: antibiotic; CH: carbohydrate; H: herbicide.

[3] (+), (−), (=): better, worse, equal performance with respect to conventional selection system. For the *ipt* and *Rol ABC* genes, efficiencies are calculated considering only the phenotypically normal (marker-free) transgenic events.

na: not applicable; this applies to transformation of protoplast or cell suspension cultures.

nd: no data available.

nr: not reported.

The positive SMGs are listed alphabetically in Table 2. Most belong to Type 1. It should be noted that some SMGs that confer resistance to a phytotoxin in adult plants, do not permit to select T_0 transgenic cells (Day and Goldschmidt-Clermont, 2011); they have been designated 'secondary SMGs' in contrast to 'primary' SMG that are used for cell selection during the regeneration phase. Secondary SMG are probably not effective enough at the single-cell level, but become effective at the tissue level. They are discussed in the text, but not listed in Table 2.

A few Type 2 SMGs have been reported, mostly encoding membrane transporter proteins. In the *A. thaliana* genome there are hundreds of such genes, belonging to different families, and many have been linked to Multiple Drug Resistance (MDR) (reviewed by Conte and Lloyd, 2011). Two of these have been used as SMG with kananycin selection (see below), but many others that may confer resistance to other phytotoxic compounds could be tested as SMGs.

Genes encoding transporter proteins that mediate the entrance of phytotoxins in the cytosol (for instance, Xi *et al.*, 2011) may also be exploited as SMGs by inhibiting them during tissue culture. Manipulation of transporters can also be useful for phytoremediation of contaminated soils, for example in the case of antibiotics present in soils fertilized with livestock waste (Conte *et al.*, 2009).

Type 3 SGMs include several genes encoding enzymes or other essential cell components that are insensitive to an inhibiting substance: they are either mutated forms of the sensitive enzyme, or isoenzymes from a different organism, naturally resistant to the inhibitor. One SMG codes for a mutated plastid ribosomal RNA, and one for a mutated α-tubulin protein.

Type 4 and Type 5 SMGs comprise one gene each.

Type 6 genes rely on the inability of many plant species to metabolize certain carbohydrates, at least *in vitro*. By partly or totally replacing the conventional sugar (most often sucrose) in tissue culture media with a sugar that cannot be utilized, the tissues are starved and regeneration does not occur. Starvation is sometimes accompanied by a toxicity effect of the non conventional carbohydrate, due to interference with the cell metabolism; this effect is usually modulated by mixing the selective and the normal carbohydrates at rates that have been optimized empirically.

Type 6 SMGs have been developed from bacterial or plant genes that endow the transgenic plant cell with an enzyme that converts the unusable into a usable carbohydrate. In general, higher transformation efficiencies (TE) have been reported with selection based on starvation than with toxicity-based selection. This may be attributed to the fact that, contrary to phytotoxins, starvation does not immediately produce necrotic cells and tissue releasing inhibiting substances into the growth medium. Another possible explanation is that the cells killed by phytotoxins may constitute a barrier between the medium and the transgenic cells, preventing or slowing nutrient uptake (Joersbo, 2001). A limitation of these systems lies in the capacity of certain plant species to metabolize some of the non conventional carbohydrates (for instance grapevine, Kieffer *et al.*, 2004).

A terminological confusion was introduced with this type of selection, termed 'positive' by their proponents (Joersbo and Okkels, 1996), as opposed to the classic selection systems, termed 'negative,' in that they utilize phytotoxins. In reality, the two systems are both positive, as defined above; moreover, in some cases the distinction between starvation and toxicity is subtle.

Type 7 SMGs control the cell cycle genes and regeneration ability. Arias *et al.* (2006b) pointed out that the process of making a transgenic plant can stall at various points, including the acquisition of totipotency, the entry into the S phase of the cell cycle (the transformation-competent phase), cell fate, and regeneration. Several genes affecting these phenomena have been described, most in *A. thaliana* (reviewed by Zuo *et al.*, 2002; Arias *et al.*, 2006b). These genes are implicated in phenomena such as chromatin remodeling, growth regulator (GR) biosynthesis and signaling.

Like the *Agrobacterium* genes controlling GR biosynthesis in the crown gall cells (*ipt, iaaM/H,* see below), several plant genes have the potential to become effective SMGs. For example, the *Arabidopsis IPT8/PGA22* gene, a cytokinin pathway gene similar to *ipt* (Sun *et al.*, 2003), and the *CYTOKININ-INDEPENDENT 1* (*CKI1*) gene, encoding a putative cytokinin receptor (Kakimoto, 1996), both enhance *in vitro* regeneration via organogenesis in the absence of GRs. Another example is the *Arabidopsis ENHANCER OF SHOOT REGENERATION1* (*ESR1*) gene, encoding a transcription factor that likely changes lateral root meristems to shoot apical meristems in tissue culture, and causes the regeneration of shoots from hypocotyl or root explants in *A. thaliana* (Matsuo *et al.*, 2011, and references therein). Fine up- or downregulation of some of these genes may markedly improve TE, particularly in recalcitrant crops (Arias *et al.*, 2006b): their potentiality has to be demonstrated in crop species. A key limitation of these cytokinin production genes is that they do not work in regeneration systems that do not depend on the use of exogenous cytokinins.

A general problem in the use of Type 7 SMGs lies in the fact that they regulate key developmental steps, and therefore their constitutive expression or silencing causes phenotypic abnormalities (discussed below).

1. List of Positive SMGs

Several reviews on SMGs (e.g., Miki and Mc Hugh, 2004; Sundar and Sakthivel, 2008; Rosellini, 2011) appeared in the last decade. Here, the genes that have been used as SMG are discussed with particular attention to their ease of use in the laboratory and acceptability outside the laboratory, and are listed alphabetically in Table 2. Only the peer-reviewed literature is considered, whereas patent applications are not. Whenever available, comparative data on transformation efficiency (TE) and selection efficiency (SE) are provided. Admittedly, TE and SE figures reported in this work in some cases required calculations based on a degree of interpretation of the data. An

important caveat is that, in *A. thaliana* floral dip (*in planta*) transformation, selection is not exerted on single cells, but on embryos, so SMGs that are effective in *A. thaliana* will not necessarily function in crop plants, which are transformed *in vitro*.

aac(3)-III, *aac(3)-IV*, *aac(6′)*—Aminoglycoside-N-acetyltransferases

The *aac(3)-III* and *aac(3)* bacterial genes were among the first SMGs shown to confer resistance to several aminoglycoside AB in *Petunia, Arabidopsis* and other plants at Monsanto (Hayford *et al.*, 1988); *aac(6′)* was used for tobacco transformation with kanamycin selection (Gossele *et al.*, 1994). These genes did not become popular SMG.

aadA — Aminoglycoside 3″-adenylyiltransferase

The *aadA* enzyme detoxifies spectinomycin and streptomycin by acetylation. The *aadA* gene has been used to a limited extent for the transformation of the plant nuclear genome. Grasses are not very sensitive to these two antibiotics: in rice, Oreifig *et al.* (2004) reported that selection with streptomycin resulted in green transgenic and chlorotic non transgenic rice plants. In alfalfa, a leguminous species, even very high concentrations of the two antibiotics combined did not suppress regeneration by somatic embryogenesis (D. Rosellini, unpublished).

The usefulness of *aadA* as a SMG comes from its application for plastid transformation; in fact, it was the key to 100-fold increased TE in tobacco, and it has allowed plastid genome (plastome) transformation in several other plant species (Table 2; reviewed by Day and Goldschmidt-Clermont, 2011). The regeneration of spectinomycin-resistant 16S rRNA mutants is a common feature of plastid transformation with the aadA SMG, but streptomycin can be used for secondary screening of the mutants; therefore, the two antibiotics are both used often together for transformation (Table 2).

ak — Aspartate kinase

The aspartate amino acid family comprises lysine, threonine, methionine and isoleucine. The biosynthetic pathway is feedback-regulated by lysine and threonine, and these two amino acids, together, strongly inhibit growth in many plants, even at low concentration, due to methionine starvation. The enzyme AK, the first enzyme of the pathway, is one of the targets of feedback regulation. Potato was efficiently transformed in the presence of lysine and threonine, with the *E. coli* gene encoding ak, because the bacterial enzyme is less sensitive to feedback inhibition (Perl *et al.*, 1993). More recently, a feedback-desensitized mutant ak enzyme encoded by the *E. coli lysC* gene was introduced into chickpea, and some transgenic plants were regenerated using lysine and threonine for selection, but with low efficiency (Tewari-Singh *et al.*, 2004).

alr — Alanine racemase

Plants are very sensitive to several amino acids isomers that feedback-inhibit endogenous synthesis (Erikson *et al.*, 2004; Chen *et al.*, 2010). The expression of enzymes capable of converting these isomers into non-toxic forms was exploited to develop novel SM systems. In particular, plants can generally use L-amino acids, whereas high concentrations of D-amino acids inhibit growth (reviewed by Pollegioni and Molla, 2011).

D-alanine is toxic to several plant species, and expression of the *Corynebacterium glutamicum alr* gene converts it to L-Alanine. *A. thaliana* transgenic seedlings were obtained with a twofold higher efficiency using *Alr* compared with kanamycin selection (Thiruvengadam *et al.*, 2010). However, the overexpression of the gene leads a major phenotypic impact on the plants and affects the concentration of several aminoacids, so *alr* is not suitable for crop transformation.

ALS — Acetolactate synthase

The use of *ALS* (also known as acetohydroxy acid synthase, *AHAS*) as a SMG has been recently reviewed (Rosellini, 2011). It is an enzyme of the biosynthetic pathway of the branched-chain amino acids isoleucine, leucine, and valine, and is the target of several classes of herbicides: pyrimidinylcarboxylates, sulfonylureas, imidazolinones, triazolopyrimidine sulfonamides, sulfonylaminocarbonyltriazolinones, and pyrimidinyl oxybenzoates. Several of these herbicides are useful as selective agents in tissue culture.

Single or double amino acid substitutions can make ALS herbicide-tolerant, and the herbicidal system *ALS*/imazethapyr is proprietary (Pursuit™, BASF Corp, Research Triangle Park, NC, USA).

ALS has been tested as a SMG since the advent of plant genetic engineering era, and several sources of mutated, herbicide-resistant ALS enzymes were found or created by site-specific mutagenesis, and many plant species have been transformed using them as SMGs (Table 2).

The *ALS* locus has been modified by homologous recombination. Gene targeting is difficult to accomplish in plants, and the *ALS* locus is an ideal candidate for basic studies and attempts to improve gene-targeting efficiency. At first, chimeric DNA-RNA oligonucleotides were employed to obtain herbicide resistance by ALS-targeting in tobacco (Beetham *et al.*, 1999) and maize (Zhu *et al.*, 2000). Endo and co-workers then demonstrated the feasibility (2006) and improved the efficiency (2007) of ASL-targeting in rice. Recently, gene-targeting of *ALS* was accomplished efficiently by means of artificial zinc finger nucleases in tobacco (Townsend *et al.*, 2009).

ALS has no mammalian toxicity and has a favorable environmental profile. Possible alteration of amino acid metabolism due to ALS overexpression has to be checked. Mutated, HR *ALS* genes are present in several commercial crops (Table 5).

aphA-6 — Aminoglycoside phosphotransferase

The *E. coli aphA-6* is one of the genes encoding phosphotransferase enzymes that can detoxify aminoglycoside antibiotics. It was used for plastome transformation of tobacco (Huang

et al., 2002 and cotton (Kumar *et al.*, 2004b) with kanamycin selection, but its application has been limited.

ASA — Anthranilate synthase α subunit

Plant anthranilate synthase is a heterotetrameric enzyme that catalyzes the first step of tryptophan (Trp) biosynthesis by converting chorismate to anthranilate. The α subunit (ASA) alone can synthesize anthranilate using ammonia as the amino donor. It is feedback-inhibited by tryptophan and its analogs such as 5-methyltryptophan: this compound binds specifically to an allosteric site of ASA and therefore interferes with cellular Trp synthesis; it is the most effective selective substance for plant transformation with this system. Some examples exist of the use of mutant, feedback insensitive *ASA* genes from tobacco and rice as SMGs (reviewed by Rosellini *et al.*, 2011; Table 2). The efficiency of this selection system is generally lower than that of the classical SMGs, but some optimization work may improve its performance. *ASA*-based selection was extended to plastid transformation (Barone and Widholm, 2009), thus providing a very useful addition to the toolbox for plastome engineering, which almost exclusively relies on the bacterial antibiotic resistance *aadA* gene discussed above. Recently, an interaction between the effect of increased sucrose concentration and Trp starvation was exploited to improve transgenic seedling screening at the cotyledonary stage in *A. thaliana* transformed with a synthetic mutant *ASA* gene, driven by a seedling root-specific promoter (Nonomura *et al.*, 2009).

The interest for mutant *ASA* genes also comes from the possibility of increasing free Trp, an essential aminoacid, in plant products. In fact, higher Trp concentration is a common feature of transgenic plants harboring mutant ASA genes: free Trp was increased up to 7-fold in tobacco (Barone and Widholm, 2008) and 28-fold in transgenic potato tubers (Yamada *et al.*, 2004).

Recently, ASA was used as the target for homologous recombination in rice (Sayka *et al.*, 2011). *Agrobacterium* transformation was carried out with vectors carrying three different mutant ASA sequences conferring 5-methyltryptophan resistance, which were not functional unless integrated at the homologous genomic site. Several resistant lines were regenerated, showing higher free tryptophan content, up to 7-fold in leaves and 230-fold in grains.

AtlD — Arabitol dehydrogenase

The bacterial D-arabitol dehydrogenase enzyme converts arabitol into xylulose. In plants, xylulose is an intermediate of the oxidative pentose phosphate pathway and is metabolized as a carbon source *in vitro* (Haldrup *et al.*, 1998b). A synthetic *atl*D gene, sequence-optimized for expression in rice, was tested as an SMG. The efficiency was the same as hygromycin selection (LaFayette *et al.*, 2005). *In silico* examination of allergenicity potential of the atlD protein showed a six contiguous amino acid match with two allergens; however it was completely degraded in an *in vitro* digestibility test. No other reports of this selection system were found.

BADH — betaine aldehyde dehydrogenase

Betaine aldehyde is an osmoprotectant produced in chloroplasts of a few plant species adapted to dry and saline conditions. It is phytotoxic to some plant species, but can be converted to non-toxic glycine betaine by the BADH enzyme. The spinach *BADH* gene was used to obtain transplastomic tobacco, with a SE 25-fold higher than with conventional spectinomycin selection (Daniell *et al.,* 2001). Unfortunately, no other reports using this system were found. We have tested betaine aldehyde as a selective agent in alfalfa, but it did not inhibit somatic embryogenesis. The same may occur in other species.

BABY BOOM (*BBM*)

This gene, isolated from canola, encodes an *AP2/ERF* transcription factor that allows exogenous growth regulator-free regeneration in *Arabidopsis* and *Brassica* (Boutilier *et al.*, 2002). *BBM* promotes cell proliferation and morphogenesis during embryogenesis, and its overexpression brings about strong phenotypic alterations. *BBM* has enhanced transformation and regeneration in poplar and can be subsequently excised by heat shock-induced FRT/FLP recombination, to obtain phenotypically normal plants (Deng *et al.*, 2009). *BBM* transient activation was exploited for sweet pepper transformation (Heidmann *et al.*, 2011), by fusing the BBM protein to the ligand binding domain of the rat glucocorticoid receptor, and using dexamethasone (DEX) to activate the protein transiently during the *in vitro* regeneration phase. Two recalcitrant varieties were successfully transformed, with an efficiency that, while low, was higher than had been previously realized. In this work, however, kanamycin selection was used in conjunction with *BBM*.

At a recent meeting (http://www.sivb.org/2011MeetingPDFs/AddendumBooklet.pdf), three abstracts were presented the use of a maize BBM ortholog to promote transformation in several maize genotypes.

ble — bleomycin resistance

The antibiotics bleomycin and phleomycin can cause DNA damage and interfere with tobacco *in vitro* regeneration. Hille *et al.* (1986) demonstrated that bleomycin-resistant calli of tobacco and tomato can be obtained by introducing the *E. coli ble* gene. Perez *et al.* (1989) were then able to obtain transgenic plants by overexpressing *E. coli* or *Streptoalloteichus hindustanus ble*.

bnx — bromoxynil nitrilase

The enzyme 3,5-dibromo-4-hydroxybenzonitrile aminohydrolase, encoded by the *bnx* gene from *Klebsiella pneumoniae* detoxifies the herbicide bromoxynil by hydrolyzation. Only oilseed rape was transformed using this SMG (Freyssinet *et al.*, 1996).

cah – Cyanamide hydratase

Calcium cyanamide is used as a fertilizer, but it also has herbicide activity, because it inhibits seed germination. In wheat, it prevents regeneration from embryo-derived callus (Weeks *et al.*, 2000). The enzyme encoded by the *Cah* gene from the soil fungus *Myrothecium verrucaria* hydrates the nitrile group of cyanamide to form urea, which can be used by the plant as a nitrogen source. This gene was introduced into wheat, and used to select for two transgenic events (Weeks *et al.*, 2000). A colorimetric essay based on trisodium pentacyanoammineferroate made it possible to quantify *Cah* expression in transgenic plant tissue. Despite the fact that expression of this gene may allow transgenic crops to convert phytotoxic cyanamide into a source of nitrogen, no other reports of *Cah* as SMG were found. In tobacco, cyanamide resistance was obtained, but the gene was introduced by kanamycin selection (Maier-Greiner *et al.*, 1991).

cat — Chloramphenicol acetyltransferase

Chloramphenicol blocks the formation of the peptide bond by binding to the 23S rRNA of the bacterial and plastid ribosome. The Cat enzyme attaches an acetyl group from acetyl-CoA to the antibiotic, which is then unable to bind to the ribosome. The nuclear genome was transformed in a few species using the *cat* gene from different organisms at the beginning of the plant genetic engineering era (DeBlock *et al.*, 1984).

Very recently, *cat* was used for plastid transformation in tobacco (Li *et al.*, 2011), thus adding a new SMG to the short list of those used for the transformation of the plastome. It was less efficient than the conventional *aadA*/spectynomycin-streptomycin selection, but allowed a quicker establishment of the homoplastomic state and avoided the regeneration of antibiotic-resistant mutants. The authors, based on the phenotype of transplastomic seedlings *in vitro,* suggest that chloramphenicol is not only toxic to plastids but to mitochondria as well, and are attempting to use *cat* for the transformation of the mitochondrial genome, which has not yet been achieved in higher plants.

CYP — cytochrome P450 monooxygenases

CYP are heme proteins that use electrons from NADPH to catalyze the activation of molecular oxygen. They show overlapping and broad substrate specificity and confer the ability to metabolize diverse chemicals, including herbicides. Herbicide detoxification by human P450 enzymes involves aryl- or alkyl-hydroxylation, or *N*-, *S*-, or *O*- dealkylation; human *CYP* genes have been used to generate herbicide-tolerant tobacco, potato, and rice (Inui *et al.*, 2005, and references therein). Overexpression of human *P450* enzymes allowed the selection of transgenic *Arabidopsis* seedlings cultured in the presence of different herbicides. In particular, the combinations of *CYP1A1*-chlorpropham, *CYP2B6*-pendimethalin, and *CYP2C19*-amiprophos-methyl showed the highest transformation efficiency (Inui *et al.*, 2005). Confirmation of these results would be useful. Implementing plant-derived *P450* as SMGs and HR genes is preferable because the presence of human or other animal genes in crops contribute to negative public perceptions.

DAAO — D-amino acid oxidase

DAAO, a flavoenzyme, catalyzes the oxygen-dependent oxidative deamination of amino acid D-isomers, producing α-keto acids, ammonia and hydrogen peroxide (Pollegioni and Molla, 2011). DAAO has absolute stereo selectivity, that is, L-amino acids are not DAAO substrates or inhibitors. Plants do not possess DAAO, which reflects the low occurrence of D-amino acids in nature. The mechanism of toxicity exerted by some D-amino acids is not known, but may involve competitive binding to tRNA.

Erikson *et al.* (2004) overexpressed DAAO from the yeast, *Rhodotorula gracilis*, in *A. thaliana*, demonstrating that it allows early and clear-cut selection of T_1 seeds when D-alanine or D-serine are used as the selective substances. However, selection of primary transformants was by kanamycin. D-alanine or D-serine can be used as foliar sprays for progeny screening. Additionally, DAAO can act as a negative SMG (see below).

deh1 — dehalogenase

A dehalogenase gene isolated from *Pseudomonas putida* can metabolize 2,2 dichloropropionic acid (dalapon), a chlorinated aliphatic acid herbicide. It has been reported to permit efficient selection of transgenic plants *in vitro* and *in vivo* (Buchanan-Wollaston *et al.*, 1992), but no details were available.

DEF2-D – Peptide deformylase

Peptide deformylase (DEF) catalyses the hydrolysis of the *N*-formyl group from the initiating methionine in newly translated proteins, and is essential for all subsequent N-terminal protein processing (Hou *et al.*, 2007, and references therein). In all plants in which DEF has been investigated, there are two peptide deformylase genes, *DEF1* and *DEF2;* DEF2 exhibits a strong polypeptide sequence preference for the N-terminus of the D1 polypeptide subunit of the photosystem II complex. Peptide deformylase inhibitors, such as the AB actinonin, are lethal to plants, probably *via* incomplete and/or incorrect co-translational processing of the D1 polypeptide.

Overexpression of either *DEF1* or *DEF2* from *A. thaliana* conferred resistance to actinonin, providing the basis for developing novel broad-spectrum herbicides, through the design of inhibitors specific to the plant forms of DEF. *DEF2-D* permitted to regenerate transgenic tobacco plants using actinonin for selection (Hou *et al.,* 2007). Actinonin resistance was also obtained by plastid transformation with the same gene (named *PDF1B*) in tobacco, but the gene was not suitable as a SMG in this case (Fernandez-San Millan *et al.*, 2011).

dhdps — Dihydrodipicolinate synthase

Like AK (see above), plant DHDPS is an enzyme of the aspartate aminoacid family pathway, and is feedback-inhibited by lysine or its toxic analogue S-aminoethyl l-cysteine, which

competes with lysine in protein synthesis. The *E. coli* dhdps enzyme is not inhibited by these compounds and allowed to regenerate transgenic potato plants using S-aminoethyl l-cysteine for selection (Perl *et al.*, 1993). Overproduction of lysine or threonine had been observed in plants accumulating lysine (Shaul and Galili, 1992a,b).

Dhfr — Dihydrofolate reductase

Methotrexate is an antibiotic that binds to the active site of Dhfr, an essential enzyme of the adenine, histidine, methionine and thimidilate metabolic pathways. Together with *nptII*, *dhfr* was among the first genes to be transferred to plant callus via *Agrobacterium* (Herrera-Estrella *et al.*, 1983). Transgenic plants were generated by the *dhfr*/methotrexate selection system in *A. thaliana*, tobacco and petunia using mouse or yeast *Dhfr* genes (see refs. in Table 2). More recently, tobacco immature pollen transformation by particle bombardment with this SMG permitted to obtain transgenic plants with very low efficiency (Aionesei *et al.*, 2006).

DOG^{R}1 — 2-deoxyglucose-6-phosphate phosphatase

2-Deoxyglucose (2-DOG) undergoes hexokinase-mediated phosphorylation in the plant cytosol and is converted to 2-DOG-6-phosphate (2-DOG-6-P), a competitor of glucose-6-phosphate. The 2-DOG-6-P severely impairs plant growth by inhibiting glycolysis and protein synthesis, and by interfering with the glycosylation of proteins and the synthesis of cell wall polysaccharides. Tobacco and potato were transformed using the yeast DOG^{R}1 gene and 2-DOG as the selective compound (Kunze *et al.*, 2001).

DREB2A — dehydration-responsive element binding protein

The rice *DREB2A* gene codes for a dehydration-responsive element (DRE) binding protein involved in tolerance to dehydration, salt (NaCl) and other abiotic stress factors. *DREB2A* was tested by Zhu and Wu (2008) as a SMG for rice *Agrobacterium*-mediated transformation, using the stress-responsive promoter ABRC, repeated four times, to drive its expression. In rice transformation, using NaCl as the selection agent, the efficiency of selection was reported to be half that of the conventional hygromycin selection (Zhu and Wu, 2008). Even though the way it alleviates salt toxicity has not been characterized, it is listed in the group of SMGs acting by removal of phytotoxins, because this is the likely final outcome of its expression. The added value of stress tolerance provided by such SMGs makes these attractive, once their efficacy is confirmed and possibly improved.

dsdA — D-serine ammonia lyase

D-serine, like D-alanine (see the *DAAO* gene above), inhibits the growth of maize embryogenic callus *in vitro*; in *E. coli,* the *dsdA* enzyme breaks down D-serine to pyruvate, ammonia, and water. Erikson *et al.* (2005) were the first to demonstrate that *dsdA* can be a SMG in *A. thaliana*. They obtained an efficiency similar to that of kanamycin selection, with more unambiguous and rapid seedling selection. Furthermore, D-serine was selective not only in solidified growth media, but also in foliar sprays or in liquid culture.

Transgenic maize plants were also obtained in the presence of D-serine by expressing *dsdA,* with no escapes (Lai *et al.*, 2011). No effect of *dsdA* overexpression were noticed on phenotypic traits and agronomic performance. Furthermore, no significant change was noticed in seed protein content, starch, fatty acids, fiber, phytic acid, and free amino acids. Allergenicity and toxicity evaluations have not evidenced any allergies or toxic effects from the dsdA protein.

EPSPS — Enolpyruvylshikimate-3-phosphate synthase encoding genes

EPSPS is an enzyme of the shikimate pathway for the biosynthesis of the aromatic amino acids. In plants, this enzyme is encoded in the nucleus but functions in the plastid. Glyphosate (*N*-phosphonomethylglycine) is a broad-spectrum herbicide that inhibits plant and microbial EPSP. Glyphosate resistance is the most widespread trait in commercial transgenic plants, and was obtained by two genes: mutant forms of bacterial or plant *EPSPS* genes with or without the glyphosate oxidoreductase (GOX) gene (see below).

The use of mutant *EPSPS* genes as SMGs has been limited, probably due to the high toxicity exerted by glyphosate *in vitro*, even at low concentration. Armstrong *et al.* (1995) and Russell and Fromm (1997) mentioned glyphosate selection for maize transformation but no details were given. The *aroA epsps* gene from *A. tumefaciens* strain CP4 was applied as SMG in soybean (Clemente *et al.*, 2000) and wheat (Hu *et al.*, 2003). In this paper, a large-scale transformation experiment for wheat is reported, in which glyphosate selection showed good efficiency.

Plant EPSPS genes where also implemented as SMGs. Howe *et al.* (2002) isolated a maize EPSPS gene and, by changing two amino acids, produced a glyphosateresistance marker that was efficient as a SMG in maize. Similar results were obtained in rice by Charng *et al.* (2008), who isolated and mutated the rice *EPSPS* gene for glyphosate resistance, and used it as SMG in rice transformation.

galT — UDP-glucose:galactose-1-phosphate uridyltransferase

In plants, galactokinase converts galactose to galactose-1-phosphate, the accumulation of which is toxic, probably due to inhibition of phosphoglucomutase, which interconverts glucose-1-phosphate to glucose-6-phosphate. The *galT* gene is the next enzyme in galactose metabolism, converting it to UDP-galactose; the fact that galactose-1-phosphate is toxic to many plant species indicates that the endogenous *galT* activity is not sufficient for its rapid metabolization.

Joersbo *et al.* (2003) estimated the *in vitro* toxicity of galactose to potato, oilseed rape, wheat, sugar beet, sunflower and pea. Pea was found to be relatively insensitive, whereas the other species were sensitive. Potato and oilseed rape were then transformed using the *E. coli galT* gene and transgenic plants were obtained in both species, with variable efficiency, using galactose at different concentrations. Rather than providing transgenic cells with the ability to metabolize galactose, the *galT* gene converts galactose to a less toxic compound. The authors conclude that this selection system needs to be optimized for each plant species by finding the best concentration of galactose and sucrose/carbohydrate source.

go — Glycine oxidase

A glycine oxidase (*go*) gene from *Bacillus subtilis* was engineered with three *in silico*-designed amino acid substitutions to increase its substrate affinity with glyphosate over its natural substrate, glycine (Pedotti *et al.*, 2009). It efficiently degrades the herbicide into aminomethylphosphonic acid (AMPA), glyoxylate and hydrogen peroxide. In the author's laboratory, this mutated gene was tested for the recovery of transgenic alfalfa; modest *in vitro* and *in vivo* tolerance was obtained when a plastid transit peptide was used, but not with cytoplasmic expression (Nicolia *et al.*, unpublished); it was not functional as a SMG, but optimization might result in glyphosate resistance both *in vitro* and *in vivo*.

gox – Glyphosate oxidoreductase, and *gat* - glyphosate acetyltransferase

Like the *go* gene, the *gox* gene from *Ochrobactrum anthropi* degrades glyphosate to glyoxylate and AMPA. Alone, it is not effective as a SMG, but was used with the glyphosate resistant *epsps* gene, in canola (http://www.monsanto.com/products/Documents/safety-summaries/canola_pss.pdf) and wheat (Zhou *et al.*, 1995) transformation. Another gene used to develop glyphosate-resistant crops is a synthetic glyphosate acetyltransferase (Delaney *et al.*, 2008), but it is not known if it is effective as a SMG.

GPT — UDP-N-acetylglucosamine:dolichol phosphate N-acetylglucosamine-1-P transferase

GPT catalyzes the initial reaction for the synthesis of asparagine-linked glycans, essential for correct protein glycosylation. AB tunicamycin inhibits GPT and is lethal for plant cells at high concentration. When overexpressed, the *A. thaliana* gene encoding GPT was capable to confer tunicamycin tolerance in *Arabidopsis* transformation (Koizumi, 2003). Selection occurred at an earlier seedling germination stage than is customary with kanamycin. No phenotypic effects of GPT overexpression were observed.

GSA — Glutamate 1-semialdehyde aminotransferase

The GSA enzyme catalyzes the conversion of glutamate-1-semialdehyde into aminolevulinic acid (ALA), a regulatory step in the synthesis of heme and all tetrapyrrolic compounds, including chlorophyll and phytochromes. Heme is essential for all aerobic organisms, and chlorophyll is necessary for green photosynthetic organisms. GSA is irreversibly inhibited by gabaculine (3-amino-2,3-dihydrobenzoic acid).

The *Medicago sativa* GSA cDNA was cloned, and a point mutation introduced in the attempt to induce gabaculine resistance (Ferradini *et al.*, 2011a). This mutated gene (*MsGSAgr*) was assessed as an SMG in tobacco and alfalfa transformation, in comparison with the wt gene and *nptII*. *MsGSAgr* performed better than *nptII*, especially for a very low escape rate, whereas the wt gene did not confer gabaculine resistance. The efficacy of gabaculine selection may be due to the type of *in vitro* phytotoxicity of this chemical, which does not cause necrosis but prevents the formation of green embryos (in alfalfa) or green shoots (in tobacco). The same construct was tested for durum wheat transformation, with good results (Giancaspro *et al.*, 2012), suggesting that it is a versatile system.

GSA is involved in only one biosynthetic, non-regulatory reaction, and ALA synthesis is feedback-inhibited, so unintended effects of its overexpression are highly unlikely. Because GSA functions in the plastid, *MsGSAgr* can be engineered for plastid transformation. This selection system, both with the bacterial *hemL* (see below) and the plant gene, is not patented, and the gabaculine resistance mutation should be easily reproducible in GSA genes of any plant species.

GST — glutathione *S*-transferase

GST is involved in the detoxification of xenobiotics via glutathione conjugation. The maize gene encoding the GST-27 subunit, under the control of the maize ubiquitin promoter, was introduced into wheat, and shown to confer *in vitro* and *in vivo* resistance to the chloroacetanilide herbicide, alachlor, and other herbicides of the same family (Milligan *et al.*, 2001). However, the *pat* gene was used for selection in this work, so the efficacy of *GST* as a SMG remains to be demonstrated.

hemL — Glutamate 1-semialdehyde aminotransferase

A mutant *hemL* gene from the photosynthetic cyanobacterium, *Synechococcus,* encoding a gabaculine-insensitive GSA (see above) has been demonstrated to be an efficient SMG in tobacco and alfalfa (Gough *et al.*, 2001; Rosellini *et al.*, 2007).

HOL1 — Harmless to ozone layer 1

The *HOL1* gene is involved in metabolism of glucosinolate hydrolysis products, such as thiocyanate, and in emission of methyl halide in *A. thaliana*. HOL1 overexpression confers potassium thiocyanate resistance to *A. thaliana* seedlings, and the conditions for its use as an SMG were investigated (Midorikawa *et al.*, 2009). Seven days in the dark during the initial seedling growth are necessary for efficient selection, probably due to suppression of endogenous HOL1 expression in these conditions. No direct use as a SMG has been reported.

hpt (*hph, aphIV*) — Hygromycin phosphotransferase

This gene is one of the most popular SMGs, that is mentioned in over 490 abstracts of the plant science sector (ISI Web of Science, October 2011). The HPT enzyme detoxifies by phosphorylation hygromycin B, an antibiotic that potently inhibits protein synthesis. It has been used to transform many diverse plant species, especially grasses and those in which *nptII* does not perform well (reviewed by Miki and Mc Hugh, 2004).

HSP101 – Heat shock protein 101

HSP101 is involved in heat tolerance in plants, probably by interacting with other molecular chaperones to refold aggregated, denatured proteins. Chang *et al.* (2007) showed that overexpression of rice *HSP101* confers resistance to heat stress in tobacco, and that this resistance can be exploited for *in vitro* selection of transgenic cells by placing leaf explants at 47°C for 60′ after five days of co-culture with *Agrobacterium.* This selection system, the only exploiting abiotic stress tolerance (type 5) may be useful in in crops due to the possible added heat tolerance trait.

iaaH, iaaM — Indoleacetamide hydrolase and tryptophan monooxygenase

These genes are present in the *A. tumefaciens* T-DNA, and catalyze auxin synthesis in the crown gall tumors; like *ipt* (see below), they were removed from binary vectors for plant transformation, in order to control GR concentration for plant regeneration. However, in tobacco transformation, the combination of the *ipt* gene and the *iaaM/H* genes was found to improve regeneration of transgenic shoots compared with the *ipt* gene alone (Endo *et al.*, 2002a). These three genes are present in the MATIMH transformation vectors, and are removed after regeneration using a R/RS recombination system under the control of the inducible GST-II-27 promoter, in order to regenerate phenotypically normal plants. The MATIMH vector was used to transform *Citrus* (Ballester *et al.*, 2008) with good results, and a significant proportion of the transgenic plants were marker-free as a consequence of correct functioning of the site-specific recombination system.

ilvA and *OMR1* — Threonine deaminase

This enzyme converts threonine to α-ketobutyrate, the initial step in the synthesis of isoleucine (Ile). Threonine deaminase (TD) is feedback-regulated by Ile in both bacteria and plants. A structural analog of Ile, L-O-methylthreonine (OMT), competes with Ile during translation and is toxic. Either the wild-type *E. coli ilvA* gene, encoding TD, or the mutant, feedback insensitive, *ilvA*-466, fused to a plastid transit peptide were introduced into tobacco (Ebmeier *et al.*, 2004) and were able to detoxify OMT. The transformation efficiency of the OMT-*ilvA* selection system was much lower than that of *nptII*-kanamycin, and a subset of the *ilvA*- and a majority of the *ilvA-466*-transgenic lines, showed stunted growth, delay in flowering, leaf curling and slight chlorosis. Free isoleucine content was increased, and other amino acids also appear to have altered concentrations in transgenic lines. These two *ilvA* alleles cannot be efficiently used as SMGs.

ipt — Isopentenyl transferase

Ipt, the first enzyme of the cytokinin biosynthetic pathway, is encoded by the *ipt* gene in *A. tumefaciens* T-DNA. Ebinuma *et al.* (1997) have been the first to demonstrate the application of *ipt* as a SMG to tobacco transformation: autonomous cytokinin synthesis allowed shoot regeneration in the absence of added growth regulators. However, constitutive cytokinin production causes an abnormal phenotype, known as 'shooty' or 'bushy' (in general, lack of apical dominance, formation of multiple shoots and inability to form roots) which does not allow the regeneration of normal plants.

To recover phenotypically normal plants, two strategies have been devised: post-transformation excision and inducible expression. Initially, a transposon-based system was used for excision (Ebinuma *et al.*, 1997). Then the so called *ipt*-type multi auto transformation (MAT) vectors, based on yeast site-specific recombination systems were implemented (Ebinuma and Komamine, 2001). Kunkel *et al.* (1999) demonstrated that *ipt* overexpression under the control of a dexamethasone-inducible promoter was possible in tobacco. The Safener-inducible *glutathione S-transferase* (*GST*) promoter was later implemented for the same purpose (Endo *et al.*, 2002a).

Ipt was used to transform several crops (Table 2). In some cases, transformation was accomplished but *ipt* excision did not occur, indicating that the R/RS site-specific recombination system is not functional in all plant species (Scaramelli *et al.*, 2009). It has been suggested that the MAT system may not be suitable for plant species that regenerate through auxin-dependent somatic embryogenesis (Endo *et al.*, 2002a). For instance, in white poplar (*Populus alba*), *ipt* was not able to promote regeneration in growth regulator-free medium, but was used as a visual selection marker thanks to the shooty phenotype (Zelasco *et al.*, 2007). In Table 5, only the crops in which phenotypically normal plants have been obtained by *ipt*-based selection are listed, and the efficiencies refer to the reported numbers of normal, useful transgenic events.

Ipt was exploited in a potentially interesting way by Mihálka *et al.* (2003). With systematic screening of several 'shooty' mutant strains of *A. tumefaciens*, that is, strains lacking at least one of the auxin biosynthesis genes, but having an intact *ipt* gene, two strains (GV3170 and ShooterG) were found that produced phenotypically normal shoots at a high frequency when used to transform tobacco in GH-free medium. The normal shoots did not contain the *ipt* gene, demonstrating that it was either transiently expressed in the cells originating these regenerants, or that these cells were fed with exogenous cytokinins synthesized by neighboring *ipt*-transformed cells. These strains also allowed regeneration of transgenic shoots in sweet pepper and muskmelon. When a binary vector was

introduced in these strains, T-DNA transfer was observed in some *ipt*-free regenerants. Marker-free transformation of diverse crop species may be possible by optimizing the use of these *A. tumefaciens* strains, as long as they are primarily organogenic.

The *Agrobacterium vitis ipt* gene was found to enhance the efficiency of *A. tumefaciens* transformation of sunflower, by bombarding it into embryonic axes before co-culture with the bacterium (Molinier *et al.*, 2002). Richael *et al.* (2008) used *ipt* in an original approach aimed at obtaining marker-free and vector backbone-free transgenic plants. *Ipt* was cloned in binary vectors just outside the left border, and transformation of potato, tomato, tobacco and canola was performed. In this way, transgenic plants that integrate the vector backbone would display the shooty phenotype and be easily selected against. Even though selection was applied by using cytokinin-free medium, *ipt*-free events were obtained at frequencies between 2 and 6%, depending on the species and vector used. This showed that, during regeneration, cells able to produce cytokin (i.e., cells that had integrated the backbone or transiently expressing *ipt*) provided cytokinin to neighboring cells that had not integrated the vector backbone and therefore did not contain *ipt*. When kanamycyn selection was combined with the screening of *ipt*-expressing events (because the *nptII* gene was present in the T-DNA), the frequency of backbone-free events was increased from 6 to 45% in potato. Even though the efficiency was not high, this selection system appears very useful to obtain marker-free and backbone-free transgenics in all the species that can be transformed with the *ipt* SMG.

KN1 - KNOTTED1

The maize homeobox gene *KN1* is involved in cytokinin function; it is expressed and controls their initiation and maintenance. Transgenic plants overexpressing *KN1* exhibit morphological alterations, including changes in leaf shape, loss of apical dominance, and production of ectopic meristems, similarly to *ipt*-expressing plants. The suitability of *KN1* for tobacco transformation was investigated, as opposed to *ipt* and *nptII* (Luo *et al.*, 2006). *KN1* efficiency was slightly higher than with *ipt,* and much higher than with *nptII* selection. However, its constitutive expression generally resulted in the typical 'shooty' or 'bushy' phenotype, which did not allow the regeneration of normal plants.

lyr — Lysine racemase

L-lysine was found to be toxic to plants, probably via feedback inhibition. Overexpression of bacterial *lyr* relieves toxicity, likely by isomerization to D-lysine. Transgenic tobacco and *A. thaliana* plants were successfully transformed on regeneration medium containing L-lysine as the sole nitrogen source, with very good efficiencies (Chen *et al.*, 2010). Considering that L-lysine is inexpensive and that no effect of *lyr* overexpression has been observed for several vegetative and reproductive traits, this SMG appears very promising. The aspartic acid content should be analyzed *in vivo*, because a concentration change was observed *in vitro*.

ManA — Phosphomannose isomerase (*pmi*)

If plant cells are cultured *in vitro* in the presence of mannose, they convert it to mannose-6-phosphate thanks to an endogenous exokinase enzyme. This carbohydrate is not metabolized by most plant species, and accumulates into the cells with a concomitant depletion of the phosphate and ATP pools. Inhibition of phosphoglucose isomerase activity and blockage of glycolysis, transcriptional repression of the promoters of genes involved in photosynthesis and the glyoxalate cycle, and apoptosis, have been associated with mannose (reviewed by Stoykova and Stoeva-Popova, 2011).

The *E. coli* gene encoding phosphomannose isomerase (PMI) has been first used for sugar beet transformation by partially or totally replacing sucrose with mannose in the culture medium (Joersbo *et al.*, 1998). PMI isomerizes D-mannose, converting it into D-fructose-6-P, that can be metabolized.

There is a long list of species that were transformed with this selection system, proof of its versatility (Table 2). In hemp, transgenic callus, but not plants, was obtained with PMI selection (Feeney and Punja, 2003), and reports of transformation of sweet pepper (Kim *et al.*, 2002) and *Torenia* (Seitz *et al.*, 2007) were found, but with no details.

A key step to obtain good *pmi* performance as SMG is establishing the optimal concentration ratio of mannose to the conventional carbohydrate used in tissue culture (depending on the species, sucrose, glucose, maltose, or sorbitol). In fact, 100% mannose throughout the regeneration protocol cannot generally be used, and mannose concentrations must be modulated during the tissue culture steps case by case (Stoykova and Stoeva-Popova, 2011). In some species, mannose is toxic and this allows the use of a mannose solution as a spray to select transgenic progenies *in vivo* (Todd and Tague, 2001). Some plants, like soybean (Chiang and Kiang, 1988), are naturally capable of metabolizing mannose and cannot be transformed with this selection system. A fusion of *manA* with the egfp RG (see below) was realized (Dutt *et al.*, 2010) and used as an efficient bifunctional selectable-reporter system for *Citrus* transformation.

Phenotypic neutrality and safety of PMI in food and feed has been demonstrated (reviewed by Miki and Mc Hugh, 2004). This selection system was patented by Syngenta under the name Positech™. Lastly, plants able to metabolize mannose may be sensitive to overexpression of mannose biosynthesis genes. For example, celery M6PR inhibited shoot regeneration in tobacco, in the presence of mannose, probably due to a negative interaction between the endogenous mannose biosynthetic pathway and the introduced M6PR enzyme. This may provide a new negative selection marker (see below) for 'mannose tolerant' crops (Song *et al.*, 2010).

MAR1 – Multiple antibiotic resistance1

Kanamycin resistance, allowing seedling selection, can be induced in *A. thaliana* by knocking out or silencing the *MAR1* gene (originally named *RTS3,* Aufsatz *et al.*, 2009). Further research on this gene has indicated that it encodes a plastid transporter protein localized in the chloroplast envelope; it is probably involved in iron homeostasis, but it would allow opportunistic entry of kanamycin (but not gentamycin and hygromycin, Aufsatz *et al.*, 2009) into the plastids (Conte *et al.*, 2009). This selection system is the first based on RNA-medited silencing of a plant gene. In experimental conditions, no phenotypic effect of silencing was noticed in *A. thaliana*, but the downregulation of a chloroplast transport protein could have side effects.

merA – Mercuric ion reductase

In bacteria, merA reduces toxic mercury Hg(II) to the volatile and less toxic metallic mercury molecule, Hg(0) and confers mercury resistance. A modified bacterial *merA* gene (*merApe9,* from the gram-negative transposon Tn21*)* was introduced into *A. thaliana* by kanamycin selection, and $HgCl_2$ resistance was obtained (Rugh *et al.*, 1996). T_2 seeds were screened efficiently *in vitro* with $HgCl_2$, indicating that *merA* might be used as a SMG. However, when the gene was transferred to yellow poplar (Rugh *et al.*, 1998) or to soybean (Yang *et al.*, 2003) $HgCl_2$ resistance was acquired, but transformants were selected on kanamycin, and it was not possible to regenerate transgenic plants under $HgCl_2$ selection. In the latter experiment, the authors hypothesize that the *ACT2* promoter was not suitable due to insufficient expression in the tissue used for transformation (embryo axes). High toxicity of $HgCl_2$ may limit the adoption of this selection system.

MPR1 — Sigma1278b gene for L-proline analog resistance

L-Azetidine-2-carboxylic acid (A2C) is a proline analog that can be incorporated into proteins and, consequently, is toxic to bacterial, yeast, animal and plant cells due to its alteration of protein structure. A2C is found in many members of the *Liliaceae* family, presumably playing a role in defense. These plants selectively use L-proline, not its analogs, for protein synthesis.

Zhang *et al.* (2004) demonstrated that A2C resistance could be engineered in tobacco with the yeast *MPR1* gene. It encodes an acetyltransferase that acetylates A2C to N-acetyl-A2C, which is no longer recognized by L-prolyl-tRNA synthetase. However, *MPR1* was not effective as a SMG. A second, recent attempt using the *E35S* promoter was successful (Tsai *et al.*, 2010). Gas chromatography–mass spectrometry (GC-MS) analysis showed that there was no significant difference between *MPR1* and control plants for metabolite composition. Because *MPR1* can prevent ROS-induced cell death in oxidative stress conditions in yeast, the responses of suspension cells to H_2O_2 was investigated, but the results were inconclusive. This selection system employing an eukaryotic gene does not seem to affect the plant phenotype and awaits testing in crops.

M6PR — Mannose-6-phospate reductase

In some plant species, the *M6PR* enzyme can metabolize mannose, by converting mannose-6-P into mannitol-1-P, which is then converted to fructose. In a recent work, the M6PR gene from celery was used as an SMG in *A. thaliana*, using a mannose-supplemented culture medium (Song *et al.*, 2010). Therefore, a plant gene can now be tested for mannose-based selection in crops.

*NiR - N*itrite reductase

Plant cells in tissue culture reduce nitrate to nitrite, and then to ammonium. Because nitrite can be toxic to plant cells, the efficiency of nitrite reduction can influence the ability of the cells to regenerate. In rice, the *NiR* genes, encoding a ferredoxin nitrite reductase, were isolated from genotypes that show high and low regeneration capacity through map-based cloning (Nishimura *et al.*, 2005; Ozawa and Kawahigashi, 2006). A much higher transcription level was found in the highly regenerating genotype.

The NiR genes of highly regenerating varieties were introduced into the variety Koshihikari, that has very low regeneration capacity, and abundant regeneration was obtained in non-selective conditions. For transformation of recalcitrant rice varieties, therefore, Nir is a non-conditional SMG. However, for transformation of highly regenerating rice genotypes that have high endogenous NiR activity, it can be used as a conditional marker by adding phytotoxic $NaNO_2$ (Ozawa and Kawahigashi, 2006). A protocol for transformation of rice genotypes with different regeneration potential based on NiR was published (Nishimura *et al.*, 2006). It would be interesting to test this gene for transformation of recalcitrant cereal varieties. The influence of NiR overexpression on nitrogen assimilation and nitrogen content of transgenic plants will need to be determined.

nptII - Neomycin phosphotransferase

The *E. coli nptII* gene (also known as *neo*), is by far the most popular SMG. It encodes one of the bacterial enzymes able to detoxify aminoglycoside ABs, of which the most utilized is kanamycin, through phosphorylation: the phosphorylated antibiotics can no longer bind the plastid ribosomes. *NptII* is found in over 1100 plant science papers (ISI Web of Science, as of October 2011), a testimony of its vast use in basic and applied research; most transgenic plants on the market contain it (Table 5). It has been reviewed extensively from different points of view, including safety for crops and food (Miki and McHugh, 2004, and references therein). The *E. coli nptI* gene is also able to confer aminoglycoside AB resistance to callus of *Petunia* and other species (Fraley *et al.*, 1983) but not as much as *nptII*, so it did not become a popular SMG.

The performance of the *nptII*-kanamycin selection system is almost always the term of comparison for new marker systems. Nonetheless, recently, the efficacy of *nptII*-based selection was reexamined by Padilla and Burgos (2010), who argued that it

could be further improved by considering factors such as explant complexity, timing of exposure to antibiotics and growth medium composition.

NptII is the first SMG that was investigated using profiling techniques (see below) for the presence of unintended effects in plants.

OMR1. — threonine deaminase

In *A. thaliana*, threonine deaminase (TD, see above the *ilvA* SMG) is encoded by the single gene *OMR1*. The spontaneous double mutant of the *A. thaliana OMR1* gene, *omr1-1*, encoding feedback-insensitive forms of threonine dehydratase/deaminase, and three new mutants obtained by site-directed mutagenesis, *omr1-5*, *omr1-7*, and *omr1-8* with single point mutations in separate regulatory regions within the carboxy allosteric end, were transformed into *A. thaliana* and assessed for their ability to confer resistance to L-O-methylthreonine. While *omr1-1* caused an abnormal phenotype and a marked alteration of free aminoacid profile (especially a strong increase of free isoleucine), *omr1-5, omr1-7*, and *omr1-8* transgenic plants have a normal phenotype and are proposed as SMG. However, no direct selection on L-O-methylthreonine was performed (Garcia and Mourad, 2004).

oph — Organophosphate hydrolase

The gene encoding the organophosphate hydrolase (OPH) enzyme from *Pseudomonas diminuta* was recently shown to function as a dual-purpose MG in maize (Pinkerton *et al.*, 2008). OPH is a dimeric metalloprotein that hydrolyzes a wide range of organophosphorus compounds, including pesticides and herbicides. Pinkerton *et al.* (2008) expressed a plant-optimized *oph* gene in maize under the maize ubiquitin and globulin promoters, with the barley α-amylase apoplastic signal. Significant levels of enzymatic activity were stably produced in both callus tissue and mature seed. Its usefulness for scoring transgenic plant tissue was assessed. The herbicide haloxon greatly inhibited non transgenic callus, whereas it even stimulated transgenic callus growth, indicating that it may function for *in vitro* selection in maize transformation; however, *pat* was used as SMG in this work. Moderate *in vivo* resistance to the Bensulmec-4LF (bensulide) herbicide was observed. *Oph* could also be a viable RG for maize (see below).

pat – Phosphinothricin N-acetyl transferase

Phosphinothricin (PPT) is an analog of L-glutamic acid and competitively inhibits glutamine synthetase; this results in the inability to convert ammonia into glutamic acid, causing ammonia accumulation and toxicity. PPT is the active ingredient of several herbicides, including Basta. Two similar bacterial genes have been isolated that confer PPT resistance by acetylation: *pat* from *Streptomyces viridochromogenes* and *bar (*Basta resistance) from *S. hygroscopicus.* This selection system is versatile and mentioned in over 1000 plant science papers (ISI web of Science, October 2011). Safety was assessed and field release granted in the 1990s (reviewed by Miki and McHugh, 2004).

L-methionine sulfoximine, a glutamate analog inhibitor of glutamine synthetase, like PPT, has been used as a selection agent with the *pat* gene (Maughan and Cobbett, 2003) and found to be particularly suitable for orchid transformation (Chai *et al.*, 2007). Recently, *pat* was used to set up a screening tool for rare homologous recombination (HR) events in *A. thaliana*: two mutated *pat* genes give origin to a functional gene only in case of HR induced by double strand breaks, created by the *I-SceI* meganuclease (Wehrkamp-Richter *et al.*, 2009).

PDS — phytoene desaturase

Carotenoids are essential components of the photosynthetic apparatus, in that they protect the chloroplasts from the harmful effect of singlet oxygen formed during photosynthesis. PDS converts phytoene to ζ-carotene and is an essential enzyme in the carotenoid pathway. PDS-inhibiting herbicides prevent the formation of carotenoids, resulting in the degradation of chlorophyll and the destruction of chloroplast membranes and photobleaching of green tissue.

Spontaneous mutations at the amino acid 304 confer resistance to fluridone and other *PDS*-inhibiting herbicides in *Hydrilla verticillata,* an acquatic weed (Arias *et al.*, 2006a). In this work, the amino acid 304 was substituted with each of the other 19 amino acids, and the activity of the enzymes was tested *in vitro* against fluridone. Four of these mutations were further characterized. Cross-resistance was tested against seven PDS-inhibiting herbicides, and cases of decreased or increased sensitivity were observed. The mutant Arg304Thr was successfully used as SMG for *Arabidopsis* transformation. An interest aspect of this selection system is that it may confer high resistance to some herbicides, such as fluridone, while making the genetically modified plants more susceptible to other PDS inhibitors, thus providing both HR and a tool for efficient clean up of volunteer transgenic plants from the field (Arias *et al.*, 2006a).

PFLP — Ferrodoxin-like protein

The sweet pepper PFLP gene was used for orchid transformation (You *et al.*, 2003). It is the only type 4 SMG, exploiting *in vitro* infection with *Erwinia carotovora* for selection. The efficiency of selection appeared to be slightly higher than that of hygromycin selection in both *Agrobacterium* and biolistic transformations. In addition, the transgenic plants were resistant to soft-rot disease, at least *in vitro*. No phenotypic effects of PFLP overexpression were observed. This selection system is attractive because of the added value that comes from bacterial resistance. The need for *Erwinia* infection complicates the transformation protocol, which may not be easily transferable to other species.

PPO1 — Protoporphyrinogen Oxidase

PPO (or hemG) is a key enzyme in the heme biosynthetic pathway, catalyzing the oxidation of protoporphyrinogen IX to protoporphyrin IX, precursor of both heme and chlorophyll. PPO is the target of PPO-inhibiting herbicides, belonging to various chemical families.

An *Arabidopsis* double mutant *PPO1* gene, among several obtained by *in vivo* and site-directed mutagenesis, was used to develop a SMG for *Agrobacterium* transformation of maize (Li *et al.*, 2003). Butafenacil was used as the selective substance, but several PPO inhibitors, belonging to various chemical families, exist. *PPO1* was first applied successfully to *Arabidopsis* transformation using its native promoter and shown to confer tolerance to different classes of PPO-inhibiting herbicides, at different levels. For maize transformation, the maize *UBI1* promoter was adopted. Transformation frequency (transgenic events over the number of cultured embryos) was comparable to those obtained with *pat* or *manA* (see below) selection. Interestingly, the selection pressure could be augmented by exposing the callus to light, and modulated by increasing light intensity or duration of light exposure, or both, probably due to the formation of singlet oxygen in the presence of the PPO inhibitor and light. More stringent selection saved time and labor during *in vitro* regeneration. The authors report the isolation and sequencing of several plant PPO genes, all of which can be mutated to develop both herbicide tolerance (branded Acuron^{TM} Technology) and efficient SMG systems for any crop of interest. Lastly, *PPO1* was one of the first plant loci to be successfully targeted by homologous recombination (Hanin *et al.*, 2001).

rolABCD

Agrobacterium rhizogenes infects plants at wound sites causing the differentiation of adventitious roots, referred to as 'hairy roots.' This is due to the expression of four bacterial genes, the so called 'root loci' (*rolABCD*), carried by the *A. rhizogenes* T-DNA, that increase auxin sensitivity of the transgenic cells. Consequently, transgenic plants regenerated from hairy roots exhibit abnormal phenotypes such as wrinkled leaves, shortened internodes, and reduced apical dominance. In *rol*-type MAT vectors (Ebinuma *et al.*, 1997b), the *R* recombinase and the *rol* genes are flanked by the *RS* recognition sequences of the recombinase. Spontaneous excision of the sequence gives the transgenic tissue the chance to regenerate into normal plants.

Many plant species were transformed using *rol* genes, but phenotypically normal plants were produced only with *rol*-type MAT vectors (Table 2). GRs may be necessary to regenerate plants from isolated hairy roots, but sometimes this is accomplished without them. Like *ipt, rol* is not effective as a SMG in white poplar (Zelasco *et al.*, 2007). Use of this system is limited to plants able to regerate from roots.

rrnS – 16S plastid ribosomal RNA

Spectinomycin and streptomycin bind the 16S plastid ribosomal RNA, thereby impairing protein syntesis. Point mutations in this RNA induce resistance to these antibiotics, and a mutant *rrnS* gene was the first SMG that allowed transformation of the higher plant plastome (Svab *et al.*, 1990). Such markers have been described as recessive, meaning that they are relatively ineffective until they approach the homoplastomic state. With the development of dominant plastid SMGs (such as the *aadA* gene, see above) plastome transformation became much more efficient, and rRNA mutant genes are no longer popular for plastome transformation. Mutations in other plastid ribosomal genes are of the basis for antibiotic resistance in plants (Dix and Kavanagh, 1995). Recently, the tobacco plastid genome was transformed efficiently by the combined use of a mutation in the plastid *rrnS* gene conferring spectinomycin resistance and a mutation in the plastid ribosomal protein gene *rps12* gene conferring streptomycin resistance (Craig *et al.*, 2008).

RstB — Rhizobium salt tolerance B

The *rstB* gene was cloned from *Sinorhizobium fredii* strain RT 19, isolated from the root nodules of salt-tolerant soybean, and putatively encodes a glycosyltransferase that regulates the synthesis of extracellular polysaccharides. Its overexpression made it possible to recover transgenic tobacco plants in the presence of 170 mM NaCl with a very high efficiency (Zhang *et al.*, 2009). The regenerated plants displayed salt tolerance both *in vitro* and *in vivo*, and no phenotypic abnormality was observed. The metabolic consequences of *rstB* expression in plants are not known.

sat3 — Streptothricin acetyltransferase

Streptothricins produced by *Streptomyces* spp. are antimicrobial compounds that consist of gulosamine, streptolidin and a peptide chain of 1–6 residues that differentiates types of streptothricins. They inhibit protein synthesis by binding to the ribosomal small subunit. The *E. coli sat3* gene codes for an acetyl transferase activity that inactivates streptothricins. After showing that several plant species are sensitive to streptothricin, Jelenska *et al.* (2000) employed *sat*3 as a SMG for the transformation of tobacco, carrot, and *Lotus*, but no details on efficiency were given.

SOS1 — Salt Overly Sensitive 1

Alongside *DREB2A* (see above), Zhu and Wu (2008) also tested the *SOS1* gene of *A. thaliana*, encoding a putative Na+/H+ antiporter, controlled by the ABRC promoter. In rice *Agrobacterium* transformation with NaCl selection, the efficiency was one third relative to hygromycin selection.

spt — Streptomycin phosphotransferase

A mutant form of the *E. coli spt* was used to transform the nuclear genome of tobacco using streptomycin for selection (Maliga *et al.*, 1988). Despite the fact that it was reported as efficient as *nptII*-based selection, no other papers using this selection system were found.

sulI — dihydropteroate synthase (DHPS)

The enzyme DHPS of the folic acid (vitamin B9) pathway is the target of asulam and other sulfonamide-type herbicides, that prevent the synthesis of the vitamin. The *E. coli sulI* gene, coding for an asulam-insensitive DHPS, was successfully applied to tobacco (Guerineau *et al.*, 1990) and potato (Wallis *et al.*, 1996) transformation with asulam, sulfanilamide or sulfadiazine selection.

Asulam is effective against the parasitic weed *Orobanche spp.,* but has limited selectivity, so it is not widely used. Because the transgenic plants do not degrade the herbicide, it gets translocated to *Orobanche* from the treated potato plants via the roots, and effectively kills the parasitic weeds (Surov *et al.*, 1998).

TDC — Tryptophan decarboxilase

Tryptophan is at the base of the synthesis of all the compounds containing the indole ring, such as auxins, glucosinolates, nicotinic acid, phytoalexins, and alkaloids. A few genes of the tryptophan biosynthetic pathway have been tested as SMGs by exploiting the phytotoxic activity of tryptophan analogs.

In plant species that have *TDC*, the encoded enzyme converts L-tryptophane into tryptamine, a precursor of terpenoid indole alkaloids; it can also decarboxylate phytotoxic derivatives of tryptophan, so it was tested as a SMG in plants that do not have TDC activity. The *Catharanthus roseus TDC* gene was used in conjunction with phytotoxic 4-methyl tryptophan as the selective agent (Goddijn *et al.,* 1993).

Transgenic tobacco seedlings overexpressing TDC displayed altered aromatic and non-aromatic amino acid pools and a root-curling phenotype associated with the depletion of the tryptophan pool (Guillet *et al.*, 2000); similar alterations of amino acid levels occurred in potato tubers (Yao *et al.*, 1995). Leaf necrotic lesions were associated with chloroplast-targeted TDC expression and consequent high tryptamine accumulation (Di Fiore *et al.*, 2002). Because of unexpected effects of its expression on plant metabolism and/or phenotype, the use of TDC as an SMG is questionable.

tflA – Toxoflavin A gene

Toxoflavin is a virulence factor of the bacterium *Burkholderia glumae,* which causes bacterial rice grain rot. In the presence of oxygen and light, toxoflavin produces superoxide and H_2O_2 that have phytotoxic effects. Koh *et al.* (2011) tested toxoflavin on leaf tissue on several plant species and found that, in the light, toxicity was manifested within two days at various degrees, whereas no toxicity was observed if the tissue was incubated in the dark. The authors went on to isolate microorganisms able to grow in the presence of toxoflavin, and succesfully isolated a *Paenibacillus polymyxa* strain (JH2) from rice seeds, from which the toxoflavin A (*tflA*) gene was cloned.

Under the control of the *35S* promoter, *tflA* was assessed as a SMG in rice and *Arabidopsis*. The efficiency of *tflA* in rice was comparable to that of *hpt*; in *A. thaliana* it was less efficient, but permitted easier screening due to strong seed germination inhibition. No obvious phenotypic effect of *tflA* constitutive expression was observed. This selection system is potentially applicable to diverse plant species.

TPS1 — Trehalose-6-phosphate synthase

Glucose can be toxic to plant cells at high concentrations because it switches off the photosynthetic machinery. In *A. thaliana*, germinating seed in the presence of high glucose produces chlorotic plantlets that do not grow, whereas overexpression of TPS1 makes plants less sensitive to glucose (Avonce *et al.*, 2004). In fact, TPS1 and the endogenous enzyme threalose-6-phosphate phosphatase, together, convert glucose into non-toxic trehalose. The *A. thaliana* TPS1 gene was introduced in *A. thaliana* and tobacco, and permitted to obtain transgenic plants in the presence of glucose as the selective agent. TPS1 is a regulator of glucose, ABA, and stress signaling (Avonce *et al.*, 2004); even though no phenotypic change was noticed in TPS1 transgenic plants, the effects of its overexpression will need to be checked under a variety of growth and stress conditions.

TSB1 — Tryptophan synthase beta 1

Plant tryptophan synthase is made of two subunits: the α-subunit catalyzes the formation of indoleglycerol phosphate to indole, whereas the β subunit synthesizes tryptophan from indole and serine. A mutant *Arabidopsis TSB1* gene, encoding a β subunit insensitive to the tryptophan analog 5′ methyltryptophan, was introduced by floral dip into *Arabidopsis*, resulting in seedling resistance to 5-methyl-tryptophan and to $CdCl_2$ (the role of tryptophan in $CdCl_2$ resistance is not known; Hsiao *et al.*, 2007). The plants accumulated up to 15-fold more free tryptophan than non-transgenic plants but, maybe surprisingly, appeared phenotypically normal, to the extent they were evaluated. The efficiency of the selection system employing *TSB1* with 5-methyl-tryptophan or $CdCl_2$ selection appeared to be comparable to that of hygromycin-based selection. Since TSB1 functions in the plastid, this selection system may be suitable for the transformation of the plastome.

TUB1 — α-tubulin 1

In goosegrass (*Eleusine indica*), a point mutation in the *TUB1* gene confers a high level of tolerance to antimicrotubule herbicides of the dinitroaniline and phosphoroamidate families (Yemets *et al.*, 2008). The transformation of four crops, finger millet, soybean, flax, and tobacco was possible by overexpressing the mutant α-tubulin (*TUB1*) gene and using the herbicide trifluralin as the selective agent. The barley β tubulin gene was co-transformed with *TUB1* to achieve a balance of both microtubule subunits. No details on the efficiency of the system were provided.

***uidA/gusA* — Beta-glucuronidase**

The *uidA* gene is a RG (see below) that was also adapted as a SMG by substituting benzyladenine-N-3-glucuronide for cytokinin in the growth medium. Upon hydrolysis by GUS, active cytokinin is released allowing the transformed cells to regenerate (Joersbo and Okkels, 1996). In tobacco leaf disc transformation, this selection system resulted in 1.7-2.9 fold higher transformation frequencies compared to kanamycin selection. The possibility of using a single gene as both a selectable and a reporter gene is useful. A limitation of this method may be the commercial availability of benzyladenine-N-3-glucuronide.

***WBC19* — ATP-binding cassette (ABC) transporter**

The *A. thaliana WBC19* gene, encodes an ATP-binding cassette (ABC) transporter likely localized in the tonoplast (Mentewab and Stewart, 2005). The mechanism of resistance is probably based on active transport of kanamycin into the vacuole. After demonstrating its efficiency in *A. thaliana*, it was used to transform two crop plants, hybrid aspen (Kang *et al.,* 2010) and muskmelon (Bombale *et al.*, 2010), but does not appear to be suitable for alfalfa transformation (unpublished data from the author's lab).

***XylA* — Xylose isomerase**

Xylose isomerase catalyzes the reversible isomerization of D-xylose to D-xylulose. The former sugar cannot be generally utilized as carbon source by plant cells *in vitro*, probably because xylose isomerase is very weakly expressed, whereas the latter can be metabolized.

Two *xylA* genes were developed as SMGs, one from *Thermoanaerobacterium sulfurogenes* (Haldrup *et al.*, 1998a) and one from *Streptomyces rubiginosus* (Haldrup *et al.*, 1998b). The sucrose/xylose concentration ratios were optimized, and the concentration of growth regulators needed some modification for *xylA*-based selection. Optimization of these selection systems led to a very high TE in potato and tomato. A report of maize transformation with this system was found as well (Guo *et al.*, 2007).

XylA also catalyzes the reversible isomerization of glucose into fructose, and therefore is often referred to as glucose isomerase. Its most important use in the food industry is to enhance the sweetness of hydrolyzed starch; several glucose isomerase products are commercially available and generally recognized as safe (GRAS) (Haldrup *et al.*, 2001). In plants, xylose isomerase activity is found at variable levels, further indicating that this enzyme is safe in food.

Expression of E. coli *xylA* in potato tubers resulted in a shift in the glucose to fructose ratio, in small changes in other metabolites, and in a stimulation of the glycolytic and sucrose synthetic pathways (Urbanczyk-Wochniak *et al.*, 2003). Goldenkova *et al.* (2002) found important phenotypic effects of overexpression of the same gene in tobacco. These reports indicate that significant changes in plant metabolism may occur in transgenic plants overexpressing *xylA*.

Antibodies for phytotoxin resistance

Herbicide resistance was obtained in tobacco by expressing a single-chain antibody fragment (scFv) with specific affinity to the herbicide picloram (Almquist *et al.*, 2004). This approach could be used to develop new SMGs.

B. Negative SMGs

These genes can be classified as conditional or non conditional: the former encode enzymes that metabolize a non toxic substrate into a phytotoxin; the latter directly encode a phytotoxic protein, and their expression results in immediate lethality unless they are driven by a developmentally regulated or inducible promoter. Non-conditional negative SMGs are useful for selective genetic ablation experiments, whereby their expression is controlled by promoters that are only active in specific cell or tissue types and/or developmental stages. In these studies, cells and tissues are killed to shed light on their role in plant development, but practical applications exist, as in the case of engineered male sterility, or transgene containment, i.e., the so-called genetic use restriction technologies. Conditional negative SMGs are usually placed under the control of constitutive promoters because their lethality is only expressed when their substrates are provided.

In plant transformation, negative selection has been used to eliminate unwanted vector backbone integration, by placing a negative SMG outside the T-DNA LB border. It is also useful to efficiently obtain marker-free plants by eliminating the transgenic events in which site-specific recombinase-mediated SMG excision has not occurred (see below).

Negative SMGs have also been applied to gene targeting. In fact, the success rate of gene targeting in plants is very low, due to the low frequency of somatic homologous recombination; negative selection is employed in the so-called positive-negative selection systems, that allows to counter-select illegitimate recombination events and isolate the rare gene targeting events. Accordingly, gene targeting constructs carry negative SMGs outside the homologous recombination sequences. If integration of the construct occurs by homologous recombination, the negative SMG is lost, whereas in case of random integration it integrates in the genome. Treatment with the negative SMG substrate results in killing the unwanted random integration events so that the targeting events can be efficiently isolated (for example, see Risseeuw *et al.*, 1997; Thykjaer *et al.*, 1997; Terada *et al.*, 2002, 2007, 2010). Several negative SMGs have been found effective in plants (Table 3).

1. List of Negative SMGs

***ADH* - Alcohol dehydrogenase**

Negative selection with the *ADH* gene is possible using allyl alcohol, which is not toxic to plants, but is converted by

TABLE 3
Negative selectable marker genes

Gene	Source	Promoter	Substrate	References
ADH	*A. thaliana*	*Cab3*	Allyl alcohol	Li *et al.,* 1995
anti-*nptII*	*E. coli*	*35S*	Requires a *nptII*-expressing plant	Xiang and Guerra, 1993
aux2	*A. rhizogenes*	*35S*	Naphthalene acetamide	Beclin *et al.,* 1993
codA	*E. coli*	*35S, A. tumefaciens 2′ prrn*	5-Fluorocytosine	Stougaard 1993 Serino and Maliga 1997
CYP105A	*Streptomyces griseolus*	Various	Sulfonylurea R7402	O'Keefe *et al.,* 1994
DAAO	*Rhodotorula gracilis*	*35S*	D-isoleucine, D-valine	Erikson *et al.,* 2004
dhlA	*Xanthobacter autotrophicus*	*35S*	Dihaloalkanes	Naested *et al.,* 1999
DIANTHIN	Carnation	*35S*	None	Shah and Veluthambi 2010
dtA	*Corynebacterium diphteriae*	*Tobacco TA29*	None	Koltunow *et al.,* 1990
Exotoxin A chain	*Pseudomonas aeruginosa*	Canola Napin	None	Koning *et al.,* 1992
HSVtk	*Homo sapiens*	*35S*	Ganciclovir	Czakó and Márton 1994
RNase	*Bacillus amyloliquefaciens Aspergillus oryzae*	*Tobacco TA29*	None	Mariani *et al.,* 1990
tms2	*A. tumefaciens*	Native	Indole-3-acetamide	Budar *et al.,* 1986
TrAP	*Mungbean yellow mosaic virus*	*35S*	None	RamannaRao and Veluthambi, 2010

ADH into the phytotoxic aldehyde acrolein (Li *et al.*, 1995). An *Arabidopsis ADH* knockout mutant line was transformed with the *Arabidopsis ADH* gene, and allyl alcohol selection successfully applied. It is not known whether endogenous ADH activity would prevent the use if this negative SMG in plants. Mammalian toxicity of allyl alcohol must be considered.

Anti-*nptII*

A construct harboring both a sense and an antisense *nptII* gene was introduced into tobacco, demonstrating that silencing *nptII* by RNAi can restore kanamycin susceptibility in a *nptII*-transgenic plant, and function as a negative SMG (Xiang and Guerra, 1993).

aux2 – Auxin 2

The *A. rhizogenes aux2* gene encodes an enzyme that converts naphthalene acetamide into naphtalene acetic acid, an auxin that interferes with normal development and can be lethal to plants. Cabbage plants expressing *Aux2* develop abnormal roots and grow slowly in the presence of naphthalene acetamide (Beclin *et al.*, 1993). However, this substrate also reduced growth of non transgenic plants at high concentrations and it was not possible to use this system for discriminating transgenic from non transgenic segregants growing on selective medium, probably due to secretion of auxin by transgenic seedlings in the medium. Better results were obtained with the orthologous gene from *A. tumefaciens* (*tms2*, see below). Hamza *et al.* (1993) used this negative SMG to select tomato haploids from crosses between a male sterile line and an *aux2* homozygous transgenic plant; diploid *aux2* hemizygous progenies were eliminated by naphtalene acetamide treatment.

codA — cytosine deaminase A

The *E. coli cod*A gene encodes a cytosine deaminase that converts non-toxic 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU), which inhibits nucleic acid synthesis and is cytotoxic. The expression of *cod*A confers sensitivity to 5-FC to plant and mammalian cells (Mullen *et al.,* 1992; Stougaard, 1993), and has been used for negative selection in *A. thaliana* (Perera *et al.*, 1993; Kobayashi *et al.*, 1995), *Lotus japonicus, N. sylvestris* (Stougaard, 1993), tobacco (Risseeuw *et al.*, 1997; Schlaman and Hooykaas, 1997), barley (Koprek *et al.*, 1999), canola (Babwah and Waddell, 2000), and other species. The *codA* system can be applied to all plant species that lack endogenous cytosine deaminase activity (Stougaard, 1993). This negative SMG is functional when introduced in the plastid genome, so it can be a tool to identify nuclear genes controlling expression of plastid genes (Serino and Maliga, 1997).

Gleave *et al.* (1999) were the first to employ this system to obtain marker-free plants, by selecting kanamycin-resistant transgenic tobacco plants in which *lox* sequences flanked *npt*II and *cod*A. Transient expression of the *Cre*-recombinase gene made it possible to select marker-free transgenic plants on 5-FC-containing medium. Park *et al.* (2004) demonstrated the use of *codA* for elimination of MGs and selection of marker-free tobacco plants with a co-transformation approach.

By combining the open reading frames of *cod*A and *npt*II into a hybrid enzyme, a new bifunctional selectable marker was created by Schaart *et al.* (2004). This *cod*A-*npt*II positive/negative MG was tested in transformation experiments in potato and then used to obtain marker-free transgenic strawberry with the aid of inducible site-specific recombination: positive kan selection was applied to select transgenic cells; then expression of the recombinase caused the excision of the MG and the recombinase genes; lastly, negative selection with 5-FC was used to eliminate the regenerants that still had the MG (Schaart *et al.*, 2004).

A similar strategy was adopted in the so called PROGMO binary vector (Kondrák *et al.*, 2006), that is based on the *Zygosaccharomyces rouxii* R/*Rs* recombination system and the *codA-nptII* MG. The vector carries only the right border of the T-DNA, and consequently the whole plasmid will be inserted into the plant genome. However, the recombinase recognition sites are located at such positions that enzyme activity will only leave the useful gene in the plant genome. Marker- and backbone-free state was demonstrated in a significant percentage of the transgenic plants (Kondrák *et al.*, 2006).

CYP — Cytochrome P450

It has been demonstrated that CYP enzymes, presented above among positive SMGs, can also be used as negative SMGs because they can metabolize certain compounds to phytotoxins. For instance, human CYP2B6 converts benfuresate and ethofumesate to more toxic compounds (Kawahigashi *et al.*, 2002).

The *CYP105A1* from *Streptomyces griseolus* was efficient as a negative SMG in tobacco: the encoded cytochrome, targeted to plastids, activated the sulfonylurea pro-herbicide R7402 by dealkylation (O'Keefe *et al.*, 1994). When the gene was expressed under a tapetum-specific promoter, treatment of immature flower buds with R7402 resulted in much lower *in vitro* pollen germination. This gene allowed screening transgenic barley plants in the greenhouse by spraying with R7402 (Koprek *et al.*, 1999).

DAAO — D-Aminoacid oxydase

Erikson *et al.* (2004) demonstrated that *Rodothorula gracilis* DAAO, besides functioning as positive SMG (see above), can act as a negative SMG if D-isoleucine or D-valine are supplied in the culture medium. These D-aminoacids have low toxicity, but are converted by DAAO into toxic 3-methyl-2-oxo pentanoic acid and 3-methyl-2-oxo butanoic acid, respectively.

DAAO has recently been applied to develop a male sterility system (Hawkes *et al.*, 2011): when expressed in tobacco under the control of the Snapdragon *TAP1* tapetum-specific promoter, it converts the non-toxic D-enantiomer of glufosinate into the toxic L-enantiomer; as a consequence, the tapetum is selectively destroyed and no pollen is produced. A mutant form of the enzyme was also created to improve efficiency. Male sterility persisted up to two weeks after treatment with the glufosinate D-enantiomer, then fertility recovered: this transient nature of male sterility appears a unique feature and a very interesting one for plant breeders, provided it can be strictly controlled in the field. Glufosinate resistance was obtained in the same plants with the *pat* gene, by expressing it in vegetative tissue only. Exclusion of *pat* expression from the anthers was obligatory, in order to prevent the detoxification of the L-enantiomer produced by DAAO.

dhlA — dehalogenase A

The *dhlA* gene of *Xanthobacter autotrophicus* encodes a dehalogenase which hydrolyzes dihaloalkanes, such as 1,2-dichloroethane, to a halogenated alcohol and an inorganic halide. The alcohol is then dehydrogenated to aldehyde and a hydroxyacid that are much more toxic than the haloalkane. After showing that several plant species do not possess detectable haloalkane dehalogenase activity, Naested *et al.* (1999) overexpressed *dhlA* in *A. thaliana;* dichloroethane was then added in solution to liquid culture medium, or simply placed as a volatile liquid, in a container close to the plants, and severe bleaching of the transgenic plantlets was observed, both *in vitro* and in soil. It was suggested that *dhlA* transgenic plants might also prove useful for ground water and soil remediation of toxic dihaloalkanes, such as dichloroethane, dichloropropene and dibromoethane. This gene is also functional in rice callus (Moore and Srivastava, 2008).

DIANTHIN — ribosome-inactivating protein

The *DIANTHIN* gene of carnation encodes a ribosome-inactivating protein (RIP). These proteins, present in many plant species (ricin from castor bean is the best known) are N-glycohydrolases that exert toxicity by removing one or more adenines from ribosomal rRNA. Recently, evidence of the toxicity of expression of DIANTHIN in tobacco, but not in rice, was presented (Shah and Veluthambi, 2010). RIP genes may be used in agriculture for pathogen resistance, and in human therapy of viral and other diseases (reviewed by Stirpe, 2004), but the very high mammalian toxicity of certain RIPs requires careful assessments.

dtA — Difteria toxin A chain

The diphtheria toxin is encoded by a phage infecting *Corynebacterium diphteriae,* the bacterium that causes diphteria. It catalyzes the ADP-ribosylation of eukaryotic elongation factor 2, and thus inhibits translation. The A-chain, encoding the toxic portion of the protein, was used in tobacco for cell

ablation in anthers, resulting in male sterility (Koltunow *et al.*, 1990). Under the control of the developing seed-specific pea vicilin promoter, *dtA* resulted in a dominant, seed-lethal factor in *Arabidopsis* and tobacco (Czakó *et al.*, 1992). It was also used to accomplish vegetative cell-specific ablation in pollen by driving it with the tomato *lat* 52 late-pollen promoter (Twell, 1995). Due to high toxicity of *dtA*, this system cannot be used in agriculture.

exotoxin A

The *Pseudomonas aeruginosa* exotoxin A gene, like the difteria toxin A chain (see above), catalyzes ADP ribosylation of the protein elongation factor 2. The ADP ribosylating domain of the gene was transiently expressed in tobacco protoplasts, which it inhibited the expression of a RG (Koning *et al.*, 1992). Under the control of the napin promoter, it arrested embryo development in the seeds of transgenic *B. napus*. The same construct was expressed in tobacco, in which it conferred both seed and male sterility. Despite low mammalian toxicity of the engineered toxin, it is unlikely that this SMG it can be used in crops.

HSVtk — human herpes simplex virus thymidine kinase type 1

The HSVtk enzyme phosphorylates certain nucleoside analogs (e.g. ganciclovir), converting those to toxic DNA replication inhibitors. Transgenic *Arabidopsis* plants expressing *HSVtk* were normal, but treatment with ganciclovir drastically reduced shoot regeneration of root explants and callus formation of leaf explants (Czakó and Márton, 1994). In tobacco, high variability of *HSVtk* effectiveness was reported, especially when viral 5′ and 3′ untranslated regions were present in the construct (Czakó *et al.*, 1995). Because it encodes a viral toxin, *HSVtk* has been only utilized under laboratory conditions.

RNase

Mariani *et al.* (1990) developed a tissue ablation system by expressing two RNAse genes, one from *Bacillus amyloliquefaciens* RNase (barnase) and one from *Aspergillus oryzae* (RNase T1). By using the tobacco tapetum-specific *Ta29* promoter, the expression of both enzymes resulted in RNA degradation in the tapetum and consequent disruption of pollen development and male sterility tobacco and canola. The *barnase* gene was used to engineer male sterility in other crop species (Rosellini *et al.*, 2001, and references therein).

An inhibitor of the barnase enzyme, *barstar*, can restore male fertility when co-expressed in the anthers (Mariani *et al.*, 1992), thus providing an efficient male sterility system for plant breeding. Placed in binary vectors outside the T-DNA borders, it was also exploited to eliminate vector backbone-containing transgenic plants (Hanson *et al.*, 1999). The use of *barnase* as a tool to prevent transgene flow from genetically engineered crops was proposed (Kuvshinov *et al.*, 2001).

The barnase enzyme was engineered by splitting it into two subunits that spontaneously reassociate to restore enzyme activity when plants expressing the single subunits are crossed (Burgess *et al.*, 2002). Therefore, the genes encoding the subunits can be bred to homozygosity, each in one of two breeding lines that, when crossed, will produce a sterile hybrid. Control of these genes by male and female organ-specific promoters could allow the engineering of both male and female sterility in a hybrid crop, thus preventing seed and pollen transgene dispersal. Due to ubiquity of Rnase, this gene has passed safety assessment in crops (Table 5).

tms2 — Indoleacetamide hydrolase

The *A. tumefaciens tms2* gene encodes the indoleacetamide hydrolase (iaaH) enzyme, able to convert naphthaleneacetamide to naphthaleneacetic acid (NAA), a potent auxin that can inhibit normal plant growth. This gene was used as negative SMG in tobacco (Budar *et al.*, 1986; Depicker *et al.*, 1988; Eklof *et al.*, 2000) *Arabidopsis* (Karlin-Neuman *et al.*, 1991; Sundaresan *et al.*, 1995) and rice (Upadhyaya *et al.*, 2000).

TrAP — Transcriptional activator protein

The mungbean yellow mosaic virus (MYMV) *TrAP* gene encodes a protein that suppresses post transcriptional and transcriptional gene silencing in the host plant cell, and has been shown to be toxic in tobacco (RamannaRao and Veluthambi, 2010). *TrAP* was used in a co-transformation strategy to generate marker-free T_0 transgenic tobacco plants using a selectable T-DNA harboring the *bar* and the *TrAP* genes. Transient positive selection with phosphinothricin and the negative selection exerted by *TrAP* expression made it possible to produce marker-free plants, but with low frequency (RamannaRao *et al.*, 2010).

C. Reporter Genes (RGs)

RGs encode proteins whose activity can be easily quantified *in vitro,* or imaged *in planta* (de Ruijter *et al.*, 2003). Sensitivity of RGs depends on plant pigments and fluorescent molecules, endogenous enzymes that may compete with the reporter protein for substrates, or light emission from endogenous reactions. RG choice strongly depends on sensitivity that can be obtained in the host species and experimental conditions of interest (see below).

An important distinction is between vital and non vital RGs: the former require killing the cells to image gene expression, whereas the latter can be monitored in living cells, and obviously have more versatility and wider application. Some RGs can behave as vital or non vital depending on the technique adopted. RGs can also be classified as conditional or non-conditional: conditional RGs depend on an exogenous a substrate; non-conditional RGs do not. RGs have a large and increasing range of applications that are briefly discussed here.

Characterizing and isolating transcription control sequences. One of the main uses of RGs is to characterize promoter activity. In this application, their coding sequences are transcriptionally fused to the promoter of interest. It should be kept in mind that promoter activity as estimated by the activity

of the protein product is a result of several factors: mRNA transcription, processing, transport, translation, and post-translation events. The stability of the gene product, both the mRNA and protein, strongly influence the performance of the reporter system in correctly estimating transcriptional activity of a promoter: stable products are not suitable. For this purpose, destabilized versions of RGs have been created (reviewed by de Ruijter *et al.*, 2003; Mirabella *et al.*, 2004).

Recently, a vital RG (GFP, see below) was exploited for large scale promoter screening in soybean (Hernandez-Garcia *et al.*, 2010), and in *A. thaliana* (Xiao *et al.*, 2010). The latter work revealed the expression patterns of many previously uncharacterized genes. The performance of chimeric inducible promoters can also be easily examined, as recently demonstrated in rice for an estrogen-inducible promoter (Okuzaki *et al.*, 2011).

Promoter and enhancer trapping experiments have exploited promoter-less RG constructs to identify sequences controlling gene expression in plant genomes: RG expression occurs only when the RG construct integrates in proximity of an endogenous promoter or enhancer (see, for instance, Kerbundit *et al.*, 1991; Sundaresan *et al.*, 1995; Campisi *et al.*, 1999; Yamamoto *et al.*, 2003; Alvarado *et al.*, 2004; Koo *et al.*, 2007).

Studying gene silencing mechanisms. A constitutively expressed, vital RG provides a tool for monitoring the silencing of a gene of interest: if a silencing construct is introduced that targets both the RG and the gene of interest, the disappearance of the RG phenotype indicates that the gene of interest is also silenced (Ruiz *et al.*, 1998; Birch *et al.*, 2010), Accordingly, RGs has been implemented to control the efficacy of virus-induced gene silencing (VIGS) in *A. thaliana* and tomato (Chen *et al.*, 2004; Orzaez *et al.*, 2009; Quadrana *et al.*, 2011).

Studying transposon activity. Transposon activity has been monitored with RGs that become expressed upon excision (for example, Charng and Pfitzner, 1994; Goldsbrough *et al.*, 1996), making it possible to detect somatic excision events in transgenic plants.

Marking specific proteins, cells, and tissues. Genetically encoded markers have been created by placing RGs under promoters, or translationally fusing them to proteins, which mark specific cellular components. In particular, live imaging techniques have been developed using vital RGs, which help to shed light on many phenomena like patterns of cell division, cell polarity acquisition in the apical meristems, vascular network development, polar auxin transport, auxin distribution, mitotic events, endoplasmic reticulum dynamics, tonoplast and vacuolar sorting signals, stromule functions, reproductive development, and signal transduction (Okada and Toriyama, 2001; Snap *et al.*, 2006; Fobis-Loisy *et al.*, 2007; Hunter *et al.*, 2007; Sawchuk *et al.*, 2007; Sijacic and Liu, 2010, Ckurshumova *et al.*, 2011, and reference therein; Shaw and Gray, 2011).

Recently, new techniques have been developed to image nucleic acids in live plant cells. The localization of RNA of interest can be realized by fusing it to an RNA stem-loop sequence that is recognized by viral RNA binding proteins. Two such proteins, MS2-CP and λN_{22}, were then fused to several fluorescent proteins (FPs, see below) and proved to be able to visualize different RNAs simultaneously in *N. benthamiana* epidermis cells, after transient transformation by *Agrobacterium* infiltration (Schönberger *et al.*, 2012). Fusion of a FP to histone H2B permitted to monitor the DNA content and endoreduplication dynamics (Wozny *et al.*, 2012).

Aiding selection of transgenic cells and tissues. RGs can significantly improve plant transformation protocols (Pang *et al.*, 1996; Vain *et al.*, 1998; Elliott *et al.*, 1999; Ghorbel *et al.*, 1999; Escobar *et al.*, 2000; Fleming *et al.*, 2000; Jordan, 2000; Kaeppler *et al.*, 2000, 2001; Richards *et al.*, 2001; Fang *et al.*, 2002; Huber *et al.*, 2002; Bellucci *et al.*, 2003; Maximova *et al.*, 2003; El-Shemy *et al.*, 2004; Pérez-Clemente *et al.*, 2004; Zhu *et al.*, 2004; Fu *et al.*, 2005; Gao *et al.*, 2005; Joshi *et al.*, 2005; Duque *et al.*, 2007; Omar and Grosser, 2008; Yang *et al.*, 2009; Leclerq *et al.*, 2010; Selvaraj *et al.*, 2010). In particular, vital RGs allow early visual selection of transgenic tissue, thus reducing the amount of material and work. However, as in the case of SMGs, when the RG and the useful genes are linked, the transgenic plants will permanently carry the RG, and this may not be the ideal final outcome, given the regulatory need to keep genome modification at a minimum. RGs have also been used to demonstrate that transgenic plants can be obtained by transformation of pollen or microspores (Stöger *et al.*, 1995; Fukuoka *et al.*, 1998; Carlson *et al.*, 2001; Aziz and Machray, 2003).

An innovative fluorescence-based RG system, named fluorescence accumulating seed technology (FAST), capable of facilitating transformation in *A. thaliana* was implemented by Shimada *et al.* (2010). The FAST marker is a fusion of a FP with the *Arabidopsis* oleosin *OLE1* gene, under the control of the OLE1 promoter. The marker is only expressed in dry seeds, and makes it possible to unequivocally identify transgenic seed in T_1 and the following generations by quick examination with a fluorescence steromicroscope, without the need for *in vitro* seed germination on selective medium. Furthermore, fluorescence intensity correlated very well with zygosity, allowing easy identification of homozygous transgenic progeny seeds. Convenient, dual purpose, selectable-reporter genes have been constructed by fusing RGs with SMGs, useful for nuclear (Li *et al.*, 2001; Dutt *et al.*, 2010) or plastid (Khan and Maliga, 1999) transformation.

Transgene monitoring. The use of FPs for monitoring transgenes in living plants in the environment has been discussed by Stewart (2005). Either linkage of transgene(s) and the RG or translational fusion make it possible to trace transgenes. Seeds expressing FPs can be easily be detected, for example, in *Arabidopsis* (Shaked *et al.*, 2005; Shimada *et al.*, 2010) and oat (Kaeppler *et al.*, 2000), demonstrating that it could be feasible to sort transgenic versus non transgenic seeds automatically in large samples. This application appears particularly interesting for transgenic plants producing pharmaceutical proteins that should be kept segregated from the food chain.

Screening tools for homologous recombination. RGs have been implemented to identify rare homologous recombination events in plants (Schuermann *et al.*, 2005). In particular, RGs have been used to prove the effectiveness of artificial zinc-finger nuclease-mediated gene targeting: rare gene targeting events can be selected by first transforming plants with a construct in which RG expression is disrupted by a deletion; then, transformation with a second construct can restore the RG sequence and functionality only in case of homologous recombination (Wright *et al.*, 2005). Marton *et al.* (2010) applied RGs to *in situ* mutagenesis mediated by zinc-finger nucleases.

A fusion of GFP with *A. thaliana* cruciferin, a seed protein, has been recently used as a tool to efficiently screen large numbers of *A. thaliana* seeds for rare homologous recombination (HR) events (Shaked *et al.*, 2005). Transformation with a promoterless construct induced fluorescent in seeds only if integration had occurred downstream of the endogenous cruciferin promoter by HR. This vital RG allowed HR events to be isolated and characterized.

Plant pathology and symbiosis studies. Introducing RGs in viruses, bacteria or fungi and monitoring their expression after plant infection is a very powerful tool for plant-microbe interaction studies (Liljeroth *et al.*, 1993; Buell and Anderson, 1993; Baulcombe *et al.*, 1995; Bergero *et al.*, 2003; Kooshki *et al.*, 2003; Liu *et al.*, 2010; Rochat *et al.*, 2010). The ability of chemicals to elicit defense responses can also be tested by expressing RGs from promoters of pathogenesis related genes (Narusaka *et al.*, 2009).

In Table 4, RGs used in plants are listed. The SMGs *cat, lacZ* and *nptII* can also function as RGs (discussed by Jefferson, 1987), but they were superseded by other RGs, more sensitive and easy to essay.

1. List of Reporter Genes

aadA^{au}* and *bar^{au}

A visual marker capable of aiding plastid transformation was recently developed by the Pal Maliga laboratory (Tungsuchat-Huang *et al.*, 2011). In the most effective construct, the antibiotic resistance gene *aadA* coding sequence was fused with the 5′ UTR and the first 14 codons of the tobacco plastid *clpP* gene. The expression of this chimeric gene interferes with the production of the endogenous clpP protease and causes a reduction of plant growth and a distinctive light-green leaf color (the *aurea* phenotype). This phenotype makes it possible to identify the transplastomic leaf sectors in chimeric plants having both wild type and transformed plastids, and makes it possible to select homoplastomic plants. The advantage with respect of the previously used *bar^{au}* gene (Kittiwongwattana *et al.*, 2007), also conferring the aurea phenotype, is that *aadA^{au}* is both a selectable and a visual marker, whereas *bar^{au}* is not selectable in the transformation process. These RGs are a step forward relative to conventional visual reporters based on mutations in plastid genes and causing pigment deficiency (see below), because homoplastomic plants are essential for trait stability. *Bar^{au}* and *aadA^{au}* are useful for basic research on plastid sorting in heteroplastomic cells, or for monitoring plastid genome instability as the consequence of the introduction of direct repeats or site-specific recombination sites in the plastome.

ALS – Acetolactate Synthase

ALS has been discussed above as a SMG. The activity of mutated, herbicide-resistant *ALS* genes can be evidenced in plant extracts, *in vitro,* with the method Shimizu *et al.* (2008), which exploits herbicide inhibition of the wt ALS genes to carry out a colorimetric reaction that estimates the activity of the introduced, mutated *ALS*. The method is laborious and appears only useful in testing ALS expression in transgenic plants to select for herbicide resistance.

Anthocyanin biosynthesis transcription factors

Transcription factors (TFs) belonging to the *MYB* superfamily that control the anthocyanin biosynthetic pathway have been proposed as RGs since the 1990s (Ludwig *et al.*, 1990; Chawla *et al.*, 1999). This group includes many genes, such as maize *C1* (the first transcription factor isolated in plants), and *P1*, petunia *anthocyanin2* (*AN2*), *Arabidopsis MYB75* and *MYB90*, grapevine *VvMYB5a* and *MybA1,* sweet potato *IbMYB1*, and apple *MYB10* (Kim, 2010, and references therein; Kortstee *et al.*, 2011; Li *et al.*, 2011).

TFs belonging to the basic helix-loop-helix DNA binding/dimerization domain found in the myC oncoproteins (Grotewold *et al.*, 2000), such as the maize *Lc* gene, are also implicated, and interact with MYB proteins to induce anthocyanin synthesis. Anthocyanin pigmentation is stable and is easily detected both visually and by imaging devices that can give quantitative data. Toxicity to plant cells has been reported (reviewed by Miki and McHugh, 2004) that may limit the use of these genes as RGs.

In Table 4, the anthocyanin synthesis TFs are listed in alphabetical order among the other RGs. Several examples are found of their use to identify transgenic events. Two TFs from maize, ***C1*** and ***Bp***, appeared useful in maize transformation, when jointly expressed under the maize globulin promoter (Shen and Petolino, 2006). These genes did not perform as well in transgenic wheat and triticale embryos under the control of the barley embryo-specific *Ltp1* promoter, due to inconsistent expression (Doshi *et al.*, 2007).

Recently, sweet potato ***MYB1,*** isolated from a purple-fleshed variety, was assessed as a potential RG, as well as for increasing anthocyanin content, by transient expression in *N. benthamiana* using the 35S or sweet potato-derived promoters, with promising results (Kim *et al.*, 2010).

A mutant allele of the apple ***MYB10*** gene that induces anthocyanin production throughout the plant, was used as a RG for visual selection in transformation of apple, strawberry, and potato (Kortstee *et al.*, 2011). The *MYB10* genomic fragment, including the control sequences, was introduced. After apple

TABLE 4
Reporter genes

Gene	Source	Screening	Substrate	Plant species	References
aadA^{au} and *bar*^{au}	*E. coli, Streptomyces viridochromogenes*	*Aurea* phenotype of leaves	None	Tobacco	Tungsuchat-Huang *et al.,* 2011
ALS	*A. thaliana* Rice	Colorimetric reaction (pink color) in the presence of *ALS*-inhibiting herbicide	Creatine + 1-Naphthol	Tobacco (plastid) Bread wheat	Shimizu *et al.,* 2008 Ogawa et al 2008
β-glucuronidase	Rat	Histochemical reaction - Blue precipitate	X-Gluc	Tobacco	Di Sansebastiano *et al.,* 2007
CHS$^{(1)}$	Petunia	Disappearance of anthocyanin pigmentation	None	Petunia	Chen *et al.,* 2004
C1 + Bp	Maize	Anthocyanin pigmentation	None	Maize Bread wheat, Triticale	Shen and Petolino 2006 Doshi *et al.,* 2007
Delila+Rosea$^{(1)}$	Snapdragon	Disappearance of anthocyanin pigmentation	None	Tomato	Orzaez *et al.,* 2009
GFP and derivatives (*YFP, BFP,* CFP)	*Aequorea victoria*	Fluorescence	None	Many Tobacco (plastid)	Sheen *et al.,* 1995 Too many to list Khan and Maliga 1999
GusA (*uidA*)	*E. coli*	Histochemical reaction - Blue precipitate	X-gluc, MUG	Many	Jefferson *et al.,* 1987 Too many to list
HaloTag	Procariotic	Fluorescence	Different fluorophores	Tobacco, *N. plumbaginifolia, Populus tremula x Populus alba*	Lang *et al.,* 2006
LacZ	*E. coli*	Histochemical reaction - Blue precipitate	X-Gal	Tobacco, Sunflower	Helmer *et al.,* 1984
Lc1	Maize	Anthocyanin pigmentation	None	Maize	Ludwig *et al.,* 1990

(*Continued on next page*)

TABLE 4
Reporter genes (*Continued*)

Gene	Source	Screening	Substrate	Plant species	References
licB	*Clostridium thermocellum*	Detection of reducing sugars	Lichenan	Tobacco	Piruzian *et al.,* 1998
LUC	*Photynus pyralis (ffLUC)*	Histochemical reaction - light emission	Luciferin	Many	Ow *et al.,* 1986 Too many to list
	Pyrophorus plagiophthalamus			Onion Spinach	Ogura *et al.,* 2005
LuxA, B, F	*Vibrio harveyi*	Histochemical reaction - light emission	Decanal	Tobacco, Carrot	Koncz *et al.,* 1987
Mutant plastid genes *rpoA, petA, ycf3*	Tobacco	Recovery of photoautotrophy	Requires *rpoA, petA, ycf3* deficient genotype	Tobacco (plastid)	Klaus *et al.,* 2003
MYBA1	Grape	Anthocyanin pigmentation	None	Tobacco	Li *et al.,* 2011
MYB1	Sweet potato	Anthocyanin pigmentation	None	Sweet Potato, *N. benthamiana*	Kim *et al.,* 2010
MYB10	Apple	Anthocyanin pigmentation	None	Strawberrry, Apple, Potato	Kortstee *et al.,* 2011
nanH	*Clostridium perfringens*	Histochemical reaction - Blue precipitate	X-NeuNAc	Onion, Tobacco	Kirby and Kavanagh 2002
oph	*Pseudomonas diminuta*	Fluorescence	*Coumaphos, Coroxon, Haloxon*	Maize	Pinkerton *et al.,* 2008
OxO	Bread wheat	Histochemical reaction - Blue-purple precipitate	4-chloro-1-naphtol	Several	Simmonds *et al.,* 2004
pmi	*E. coli*	*in vitro* pH indicator color shift	Mannose + chlorophenol red	Bread wheat, Maize	Wright *et al.,* 1996
RFP and related fluorescent proteins	*Anemonia sulcata, Anemonia majano, Discosoma* sp, *Heteractis crispa, Zoanthus* sp	Red to green fluorescence	None	Several	Jach *et al.,* 2001; Mirabella *et al.,* 2004; Nishizawa *et al.,* 2006; Forner and Binder 2007; Scabone *et al.,* 2011
xynA	*Neocallimastix patriciarum*	Colorimetric reaction (blue color)	Azurine-crosslinked xylan	Bread wheat	Vickers et al., 2003

(1) Reporters used only for gene silencing studies.

transformation, red colored calli, shoots and plants were scored. All the red apple shoots were transgenic. Strawberry did not accumulate anthocyanin in callus, but did so in leaves and roots, whereas no visible accumulation of anthocyanin could be observed in potato, unless explants were incubated in acid methanol. TE was 6-9% using anthocyanin-based visual selection, which was higher than GUS-based selection but lower than kanamycin-based selection. Visual selection would require supplementary work, especially in strawberry and potato in which selection at the callus stage is not possible. In apple, this RG can be used for cisgenic transformation; however, the issue of possibly unwanted anthocyanin coloration of plants and/or fruits was not discussed.

MybA1 regulates the final step of the biosynthesis pathway in grapes, and its potential as a quantitative RG for promoter studies was demonstrated by Li *et al.* (2011), who expressedit in grape and tobacco under three promoters (E-35S, enhanced CsVMV or the bi-directional dual promoter BDDP). After establishing a linear relationship between the anthocyanin derived color and optical density using a dilution series, a non-destructive test was developed for the assessment of transcriptional activity through anthocyanin accumulation measurement. Computer analysis of digital images of transformed grapevine somatic embryos and various tissues of tobacco showed a consistent six- to sevenfold activity difference between the three promoters. This observation was verified by spectroscopic measurement of anthocyanin concentrations in sepal tissue of transgenic tobacco plants, and confirmed previous findings on the strength of the three promotes based on a GUS fluorometric assay. The authors concluded that *MybA1* could offer a new and reliable alternative for vital, optical quantitative promoter analysis in plants. The proposed use of this RG as an aid in plant transformation would be possible if anthocyanin coloration of the transgenic plant is not a problem.

Transgene-induced anthocyanin coloration has been implemented as an internal visual reference for VIGS in the tomato fruit. This system uses transgenic tomato expressing the snapdragon ***Delila*** and ***Rosea1*** transcription factors under the control of the fruit-specific E8 promoter, showing purple fruits. *Delila* encodes a basic helix-loop-helix transcription factor and *Rosea1* encodes a *MYB*-related transcription factor; together, they induce anthocyanin biosynthesis in flowers. A modified tobacco rattle virus VIGS vector incorporating partial *Rosea1* and *Delila* sequences restores the red-fruited phenotype upon agroinjection in the purple fruits. Silencing of the gene of interest is accomplished by incorporating a specific silencing sequence in the viral vector. Three tomato genes were used to demonstrate the utility of this silencing RG system (Orzaez *et al.*, 2009). Lastly, anthocyanin biosynthesis genes have been expressed in forage legumes with the aim to improve forage quality (Damiani *et al.*, 1999; Ray *et al.*, 2003): they may also be useful as RGs in these species.

CHS – Chalcone synthase

CHS is a key enzyme of the anthocyanin biosynthetic pathway in plants. In *Petunia hybrida*, infection with tobacco rattle virus (TRV) containing a *CHS* fragment resulted in silencing of anthocyanin production in flowers (Chen *et al.*, 2004). The silencing phenotype ranged from white spots on purple petals to completely white flowers. Infection with TRV containing a tandem silencing construct of phytoene desaturase (*PDS*) and *CHS* resulted in leaf photobleaching (the *PDS*-silenced phenotype) and white patterns on the flowers, with the two phenotypes overlapping in affected leaf sectors due to simultaneous silencing of both genes. When a fragment of a petunia gene encoding for 1-aminocyclopropane-1-carboxylate oxidase, an enzyme of the ethylene biosynthesis pathway, was introduced with the CHS fragment, the white flowers or flower sectors produced less ethylene and senesced later than the purple ones, demonstrating again the simultaneous silencing of the two genes. The authors concluded that *CHS* is a suitable visual RG for studying the function of floral-associated genes by transgenic silencing (Chen *et al.*, 2004).

GFP – Green fluorescent protein, and its derivatives

GFP from the jellyfish *Aequorea victoria* is a 27 kD protein able to transduce UV or blue light to green light (507 nm). GFP fluorescence in transgenic plants is monitored by shining a ultraviolet light on cultured plant cells and tissue or whole plants in the dark. It does not necessitate cofactors or substrates, and can be detected in intact plant organs.

GFP has been widely used as a versatile RG for a variety of applications in many plant species. Over 2000 plant science papers in the ISI Web of knowledge contain the keywords ‘green fluorescent protein’ (October 2011). The discovery and development of GFP earned the Nobel prize award for chemistry in 2008 to O. Shimomura, M. Chalfie and R. Y. Tsien. GFP and its derivative fluorescent proteins (FPs) have been reviewed (Leffel *et al.*, 1997; Tsien, 1998; Stewart, 2001; Zhang *et al.*, 2002; Millwood *et al.*, 2003).

GFP is a small protein, and can passively diffuse through plasmodesmata from expressing to non expressing cells. This limitation was addressed by expressing GFP as a series of multimerized repeats or by incorporating a specific subcellular localization signal such as HDEL/KDEL for endoplasmic reticulum targeting, or a nuclear localization signal (discussed by Ckurshumova *et al.*, 2011).

GFP has been targeted efficiently to subcellular compartments including mitochondria (Koehler *et al.*, 1997a) and chloroplasts (Koehler *et al.*, 1997b). This makes it a valuable marker for plastome transformation: a high level of expression in potato chloroplasts was obtained (5% of total soluble protein, Sidorov *et al.*, 1999), and suitability for plastid gene expression studies was demonstrated (Tangphatsornruang *et al.*, 2011).

GFP has also been modified for increased expression and optical properties. The codon usage was adapted to plant

expression to avoid recognition of a cryptic intron (Haseloff *et al.*, 1997). Then modifications were introduced to improve stability, brightness, and protein maturation, thus obtaining the enhanced GFP (EGFP) version. Technical improvements in fluorescence detection, such as pH optimization and microplate reader adoption, may help overcome some limitations of GFP performance in plants (Wu *et al.*, 2011). A wide range of spectral variants has also been selected or generated, and currently, GFP mutants emitting blue (BFP), cyan (CFP) and yellow (YFP) light are available (Cubitt *et al.*, 1995; Ckurshumova *et al.*, 2011). Cyan fluorescent protein (CFP) mutants are well enough spectroscopically separated from YFP to be easily distinguishable, making them useful for studies requiring two markers (Griesbeck *et al.*, 2001).

YFP was improved for faster maturation (Venus YFP, Nagai *et al.*, 2002), stability and better expression in organelles (Citrine, Griesbeck *et al.*, 2001). A recent innovation was introduced by mutating citrine to a reversibly switchable form, named Dreiklang (Brakemann *et al.*, 2011), that will facilitate *in vivo* protein-tracking and single-molecule observations in mammalian cells and may be applied to plant science. These improved FPs are at the base of genetically encoded markers (discussed above).

Hundreds of different proteins can be fused to GFP on the N- or C-termini with no loss of function, but the functionality of new fusion proteins would need to be examined case by case. A useful fusion of GFP with another RG, GUS (see below) was created and tested in transient and stable expression in *A. thaliana* and *L. japonicus,* thus combining the high sensitivity of GUS staining and the GFP vital marker in one RG (Qaedvlieg *et al.*, 1998).

GFP has been used as a tool in protein glycosylation studies in plants, an important issue in molecular pharming, due to the necessity to 'humanize' plant glycosylation patterns (Paris *et al.*, 2010, and references therein). In order to endow GFP with an efficient glycosylation tag, three different GFPs differing to the extent that the glycosylation site is at positions 80, 133, or 172, were created. The best glycosylation site was at position 133, as it appears to be protected from undesired processing, when transiently expressed in tobacco. This new GFP form may help understand the sites of glycan modification within the secretory pathway of plants (Paris *et al.*, 2010).

Ghosh *et al.* (2000) demonstrated that GFP could be expressed in two halves that are not fluorescent individually but form a fluorescent molecule when combined. This technique became known as bimolecular fluorescence complementation (BiFC, Hu *et al.*, 2002). Split GFP was refined to be self-associating and soluble in living systems (Cabantous *et al.*, 2005) and has been applied to studies of protein-protein interactions (Kerppola, 2008).

Ozawa *et al.* (2007) applied BiFC for live-cell imaging of a mitochondrial RNA. They exploited the sequence-specific RNA-binding properties of human protein *Pumilio,* modified to bind to two adjacent motifs on the target RNA. Two Pumilio RNA-binding polypeptides were engineered to recognize two closely adjacent eight-nucleotide sequences in the mitochondrial RNA, and were fused to either the N- or C-terminal halves of split EGFP. Binding of the two *Pumilio* proteins to their target RNA sequences brought the split EGFP into close proximity, allowing BiFC to occur and revealing the localization of the target RNA, thus highlighting the subcellular localization of the RNA within plant mitochondria. Recently, an improved citrine (mCitrine), split between amino acids 173 and 174, was used to detect tobacco mosaic virus RNA within plant cells with the same approach (Tilsner *et al.*, 2009).

By driving GFPs expression using cell type-specific promoters and taking advantage of quick preparation of protoplasts, high-throughput purification of GFP-labelled plant cells of a specific cell type has become possible. This technique was named Fluorescence Activated Cell Sorting (FACS). The full transcriptome of individual cell types or of functional zones can then be determined through microarray analysis or RNA sequencing (Bargmann and Birnbaum, 2010).

The pH sensitivity of GFP fluorescence has led to the creation of GFP-derived pH indicators (pHluorins; Schulte *et al.*, 2006, and references therein) to monitor cellular pH in response to a variety of conditions, such as anoxia and salt-stress. They are derived from *Aequorea victoria,* or, more recently from the orange seapen *Ptilosarcus gurneyi* (*Pt*-GFP). A *Ptilosarcus*-derived YFP was implemented to study programmed cell death (PCD) in plant cells, demonstrating that YFP fluorescence is lost during the plant PCD process due to acidification (Young *et al.*, 2010). The effect of PCD on YFP fluorescence should be taken into account when using this RG: a pH-insensitive reporter may be preferable to avoid misinterpretation of results.

A specific application of a GFP fusion is the construction of fluorescent Ca^{2+} indicator proteins by translationally fusing GFP, CFP or YFP with calmodulin (Miyawaki *et al.*, 1997; Tian *et al.*, 2009; Muto *et al.*, 2010; Zhao *et al.*, 2011). These RGs, the so called genetically encoded Ca^{2+} indicators (GECIs), and also named yellow cameleons, were used to study Ca^{2+} dynamics in plant cells. For example, guard cell-specific Ca^{2+} dynamics were investigated with this tool (Allen *et al.*, 1999).

Krebs *et al.* (2011) created a set of vectors for Ca^{2+} studies in plants using two yellow cameleons driven by the the *Arabidopsis UBQ10* promoter. Targeting of the Ca^{2+} reporters to the cytoplasm, the nucleoplasm, the plasma membrane and the tonoplast was demonstrated; different plant selection markers are available, and constructs for N- and C-terminal fusions with proteins or tags of interest were also prepared.

In jellyfish, the chemiluminescent calcium binding protein aequorin is associated with GFP and a fluorescence signal is emitted upon Ca^{2+} stimulation. This chemiluminescence resonance energy transfer between the two molecules has been exploited to create calcium-sensitive fluorescent RGs by fusing GFP and aequorin. These fusion proteins have been assessed in plant cells (Kiegle *et al.*, 2000).

TABLE 5
Selectable marker or reporter genes present in the GM Crop Database (CERA 2010) as of October 2011

Marker gene	Source	Crop	No. of events
aadA[1]	*E. coli*	Cotton, Potato, tomato	10
als	Tobacco, Soybean	Carnation, cotton, maize, soybean[2]	7
aphIV	*E. coli*	Cotton	2
barnase	*Bacillus amyloliquefaciens*	Canola, chicory, maize	8
Bla[1]	*E. coli*	Linseed, maize, soybean	13
bxn	Klebsiella pneumoniae	Tobacco, cotton, canola	4
csr1-2	*A. thaliana*	Soybean	1
dhdps	*Corynebacterium glutamicum*	maize	2
epsps	*A. tumefaciens* or *Maize*	Creeping bentgrass, sugar beet, canola, soybean, cotton, alfalfa, Potato, wheat, maize	41
gat	*Bacillus licheniformis*	Soybean, maize	2
gox	*Ochrobactrum anthropi*	Brassica rapa, canola, maize, sugar beet	8
gus/uidA	*E. coli*	Sugar beet, papaya, cotton, plum, soybean	9
nptII	*E. coli*	Sugar beet, canola, cotton, maize, papaya, chicory, melon, squash, flax, tomato, plum, tobacco, Potato	41
pat/bar	*Streptomyces sp.*	Sugar beet, canola, cotton, maize, chicory, rice, soybean	48
pmi	*E. coli*	maize	9

The genes are listed irrespective of their role as gene of interest and/or SMG. Not all the events listed are presently on the market. Overlap exists between events containing each marker, due to stacked events or presence of more than one MGs in the same event.

[1] bacterial SMG present due to vector backbone integration.

[2] Other crops species that contain ALS mutant genes obtained by chemical mutagenesis or selection of natural or somaclonal mutations, that were introduced by crossing, are present in the database, totaling 20 events.

gusA (*uidA*) — Glucuronidase

This RG derives from the *gus* (*uid*) operon of *E. coli* and encodes for a 68 kD protein (GUS) that assembles into a homotetrameric enzyme that catalyzes the hydrolysis of many β-glucuronides and β-galacturonides (reviewed by de Ruijter *et al.*, 2003). GUS from *Bacillus, Staphylococcus* sp. have also been codon-optimized and used in plants (Miki and McHugh, 2004; Broothaerts *et al.*, 2005). The substrates used with this reporter systems are 4-methyl umbelliferyl β-D-glucuronide (MUG) for measurements of specific activity, and 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-gluc) for histological localization (Jefferson, 1987).

GUS activity can also been measured with a non destructive fluorescence-based assay, in which the product released by hydrolysis of MUG, 4-methyl-umbelliferone, is a fluorescent molecule (ImaGene Green, Molecular probes). In this case the essay can be non-destructive (Fleming *et al.*, 1996). This technique was recently employed to monitor the stability of gene expression as affected by micropropagation in the rubber tree (*Hevea brasiliensis,* Lardet *et al.*, 2011). A significant improvement of fluorometric measurement of GUS activity was recently introduced (Fior *et al.*, 2009b) allowing continuous measurement at the pH optima of the enzyme.

There are many reports of the use of *gusA* as a selection tool to obtain transgenic plants by post-hoc selection of GUS-positive plants (for example, Graham *et al.*, 1990; Aziz and Machray, 2003; Zheng *et al.*, 2004; Ballester *et al.*, 2008). This approach generally has low efficiency.

This RG has been very popular in plant science and genetic engineering and is present in commercial crops (Table 5). Limited intrinsic glucuronidase activity was evidenced in several plant species, especially in embryos, fruit walls, seed coats, and endosperm (Hu *et al.*, 1990), but did not limit *uidA* application as a RG. The use of *gusA* has decreased since the advent of vital, fluorescent RGs.

Fior *et al.* (2009a) have recently shown that inhibitors of GUS activity are present in *Arabidopsis*, tobacco and rice, which create confounding artifacts in the quantitative measurement

of GUS activity and may cause bias in studies of promoter activity. They propose that, in order to obtain reliable results, the inhibitory effects of plant extracts should be tested when performing GUS assays, and present a method for correction of inhibitor-induced artifacts.

E. coli gus is a cytosolic enzyme, and therefore is not to be a good reporter for secretion studies in plants. A rat β-glucuronidase enzyme was modified to obtain secreted and vacuolar variants (Di Sansebastiano *et al.*, 2007). Five different C-termini were produced: the original C-terminus of the rat enzyme, a 19-codon deletion, a 15-codon deletion and fusions of the deleted termini with the last 6 or 7 codons of the vacuolar sorting determinant of tobacco chitinase A, respectively. The signal sequence of the rat β-glucuronidase polypeptide was replaced by the signal sequence of tobacco chitinase A. In a transient expression system, the best enzymatic activity was found with β-glucuronidase having the 15-codon deletion. This construct, with and without the chitinase signal, proved to be a good tool for studies with tobacco protoplasts, but slow enzymatic activity was observed in transgenic plants (Di Sansebastiano *et al.*, 2007).

HaloTag

HaloTag^{TM} Interchangeable Labeling Technology (Promega, Mannheim, Germany), was developed for mammalian cells and recently tested in plants. This system allows different fluorescent colours to be used to visualize the localization of the non-fluorescent bacterial HaloTag protein within living cells. HaloTag, an engineered derivative of a procaryotic hydrolase gene, binds covalently to a ligand that carries a flexible reporter group, such as a fluorophore. The ligand is supplemented to the HaloTag expressing cells, then unbound ligand is washed off and fluorescence can be detected in living or fixed cells.

A vector for HaloTag overexpression was tested in transient assays by transforming tobacco protoplasts and by biolistic delivery into tobacco and poplar leaf cells (Lang *et al.*, 2006). After incubation with ligands carrying two different fluorochromes, fluorescence was visualized by confocal laser scanning microscopy. Fluorescence was observed in protoplasts and in different types of plant cells, demonstrating that no significant disturbance is caused by the cell wall. This system appears flexible and powerful, allowing the use of different ligand colors with one cloning procedure. The characteristics of the HaloTag Protein should make it easy to realize protein fusions (Lang *et al.*, 2006).

lacZ — β-Galactosidase

The *E. coli* gene encoding β-galactosidase has been widely used as a RG for gene cloning in bacteria, and demonstrated to stain transgenic tobacco and sunflower cells treated with the substrate 5-bromo-4-chloro-3-indoyl- β-D-galactosylpyranoside (X-Gal) (Helmer *et al.*, 1984).

LicB — Lichenase

A thermostable β-1,3;1,4-glucanase (lichenase) gene from *Clostridium thermocellum* was tested as a RG in tobacco (Piruzian *et al.*, 1998). The enzymatic activity is measured via the release of reducing sugars from the substrate lichenan, with a 3,5-dinitrosalicylic acid reagent. Staining the enzyme for activity in SDS-polyacrylamide gels containing lichenan is also possible. It appears that, despite high stability of the enzyme, this RG system is too laborious for practical use in plants. Interestingly, however, transgenic leaf explants were able to regenerate on media containing lichenan instead of glucose, indicating that *licB* can be used as SMG.

LUC — Luciferases

Proteins that have bioluminescence activity are generally referred to as luciferases, but they are of different nature, and their substrates, all named luciferins, are also different. Luciferase from the firefly *Photynus pyralis* (ff-LUC) is the most used in plants, but luciferases from the click beetle, *Pyrophorus plagiophthalamus* (CbLUC), the bacterium *Vibrio harveyi* (lux) and from the coral (*Anthozoa*) *Renilla* (RLUC) have also been expressed in plants (Mudge *et al.*, 1996).

ff-LUC encodes a single polypeptide that oxidizes its substrate (firefly luciferin) in the presence of ATP and oxygen, releasing a photon at 562 nm. Ow *et al.* (1986) were the first to demonstrate the functionality of this RG in plants. The coding sequence was optimized for expression in plant cells obtaining an increase in activity (*LUC+*, Lonsdale *et al.*, 1988). This version was thoroughly assessed in rice and several other plant species (Baruah-Wolff *et al.*, 1999, and references therein). Bourdon *et al.* (2004) improved the performance of this RG in wheat by introducing additional introns.

The application of *ff-LUC* to promoter activity characterization has been reviewed by de Ruijter *et al.* (2003), in comparison with other RGs. It is suitable to monitor promoter dynamics because the enzyme forms an inactive complex with oxyluciferin-luciferase; this complex releases luciferase efficiently only in the presence of CoA, which is available in limited amounts in plant cells. Therefore, when luciferin is supplied, after an initial peak of activity due to the accumulated enzyme, light emission becomes proportional to the newly produced luciferase, which reflects promoter activity. However, ff-LUC activity depends not only on luciferin, but also on oxygen and ATP, so not only the amount of enzyme but the availability of three substrates influences light emission. Luciferin can be applied by spraying or via root absorption.

Fusion of *ff-LUC* with nuclear or plastid signals were successfully realized, and a C-terminal fusion with *nptII* was also functional (de Ruijter *et al.*, 2003). A fusion of ff-LUC with GUS was used to create a gene-trapping vector: LUC activity is utilized as a vital and highly sensitive reporter to identify tagged genes, and GUS as a histochemical reporter to analyze the cellular expression patterns of the trapped genes (Koo *et al.*,

2007). Thousands of *Arabidopsis* lines with tagged genes were isolated with this system.

Recently, a study demonstrated that *ff-LUC* transcriptional and translational fusions can be a tool to monitor RNA-mediated gene-silencing in tobacco suspension cells (Birch *et al.*, 2010), using a technique termed *in vivo* transient expression analysis of RNAi target and trigger efficacy (InVITE) essay. In this work, target-reporter gene fusions were co-transformed with constructs designed to silence the target sequence. The efficacy of the silencing (antisense or ribozyme) constructs against several target sequences from transgenes and endogenous genes (β-glucuronidase, glucanase, vacuolar invertase, cucumber mosaic virus, watermelon mosaic virus and tobacco anionic peroxidase) was tested by quantification of transient RG activity. In some cases target-luciferase fusions had to be optimized for performance, but this tool appears applicableto assess the effectiveness and specificity of RNA-mediated silencing of plant genes.

FfLUC has some limitations because light emission is weak and requires sensitive equipment for detection. It should also be considered that luciferin may be phytotoxic at high concentrations and may induce pathogen defense responses (de Ruijter *et al.*, 2003).

CbLUC. Luciferases with different colors were isolated from the bioluminescent click beetle, *Pyrophorus plagiophthalamus,* and have been developed as a reporter vector systems (Chroma-Luc™ reporter system, Promega). The click beetle luciferase genes encode proteins that emit red (CBRluc) or green light (CBG99luc, CBG68luc). These enzymes differ for only a few amino acids, which are responsible for the different colors. They use the same substrate as ff-LUC. Ogura *et al.* (2005) tested these RGs in transient expression tests after biolistic delivery using the 35S promoter, showing that they are functional in spinach and onion leaf cells.

lux A, B. The *lux A* and *B* genes from the bacterium *Vibrio harveyi* encoding a two-subunit luciferase were expressed in plants (Koncz *et al.*, 1987). The two genes were fused with a peptide linker and optimized for plastid expression in *Chlamydomonas* (Mayfields and Schultz, 2004). This synthetic protein, luxCt, reacted with the substrate decanal and proved to be a useful *in vivo* reporter. No example of its use in higher plants was found.

RLUC. *Renilla reniformis LUC* (*RLUC*) and an intron-containing version (*RiLUC*) were tested in transiently and stably transformed tobacco (Cazzonelli and Velten, 2003). *RiLUC* has the advantage of lack of expression in *Agrobacterium*, making it possible to monitor luciferase expression in transient and stable transformation experiments without interference from contaminating *Agrobacterium.*

Mutant plastid genes as RGs to aid tobacco plastid transformation.

Mutations in the plastid *petA, rpoA and ycf3 genes* (Klaus *et al.*, 2003) were introduced by site-directed mutagenesis in tobacco. The resulting lines were heterotrophic and had clear, distinctive, pigment deficiency phenotypes.

These mutants were used to efficiently reintroduce the wt genes, along with a SMG in the tobacco plastome. Transplastomic eventscould be selected efficiently based on the green color obtained upon the restoration of photosynthetic capacity, and also prevented the selection of antibiotic-resistant mutants and nuclear transformants, and accelerated the process because the homoplastomic state was reached without the need of multiple regeneration cycles. These mutant lines can ease plastid transformation in tobacco.

***nanH* — Sialidase (neuraminidase)**

Sialidases catalyze the cleavage of terminal N-acylneuraminate (sialic acid) residues from glycoproteins, glycolipids and polysaccharides. They are found in most animals and in some microorganisms. The *nanH* gene, encoding a cytoplasmic sialidase of *Clostridium perfringens,* was codon-optimized for plants and expressed transiently in tobacco and onion leaf epidermal cells, and stably in transgenic tobacco (Kirby and Kavanagh, 2002). Nan and GUS activities were visualized using the substrates X-NeuNAc and X-GlucM, respectively: cells expressing *nan* stained blue, those expressing *GUS* stained magenta, using both substrates in the same buffer. Therefore, *nan* can be used as a highly sensitive RG, both alone and as a partner of *GUS*, when the combined use of two RGs is needed.

***oph* — Organophosphate hydrolase**

This gene, isolated from *Pseudomonas diminuta*, besides showing promise as a SMG (see above), can function as a useful RG (Pinkerton *et al.*, 2008). The organophosphate compounds, coumaphos, haloxon and coroxon, were readily hydrolyzed by the secreted gene product in transgenic maize, resulting in the production of chlorferon, which fluoresced brightly under UV illumination.

The substrates and the fluorescent product were not toxic to callus tissue, which continued to grow after the screening test. Coroxon was used to score *oph* expression in leaf tissue and seeds, demonstrating the versatility of this reporter system as an aid for screening transgenic maize.

***OxO* — Oxalate oxidase**

A wheat germin gene with OxO activity was demonstrated to be a useful RG in monocot and dicot species by Simmonds *et al.* (2004), who expressed the genomic clone of the gene with constitutive promoters in maize, soybean, tobacco, tomato and canola. For detection, the assay buffer containing oxalate is supplemented with 4-chloro-1-naphtol that is oxidized by the H_2O_2 generated by OxO. The oxidized substrate precipitates and confers intense blue-purple coloration visible without chlorophyll clearing. It remains associated with the cell wall, with no apparent leakage, which is an advantage with respect to GUS. Both quantitative assays and fast qualitative essays were developed.

Native OxO activity was found in wheat and maize seedlings, but not in soybean, and did not interfere with RG detection. It should be considered, however, that endogenous H_2O_2

formation can result in weak coloration, making the use of controls for native or non specific OxO activity necessary. A possible toxic effect of the released peroxide should perhaps be taken into account. Interestingly, transgenic soybean plants expressing OxO showed improved resistance to *Sclerotinia sclerotiorum*, a fungal pathogen that secretes oxalate, and no yield loss was observed in the absence of *Sclerotinia* infestation.

Pmi – Phosphomannose isomerase

Pmi, a SMG, can be also a RG because its expression can be assessed in plant tissues by means of a convenient *in vitro* essay (Wright *et al.*, 1996). The incubation of PMI-expressing maize and wheat tissue for 2–5 days in the dark in liquid growth medium supplemented with mannose and chlorophenol red results in a color shift from red or purple to yellow: this is due to acidification of the medium due to the ability of the transgenic cells to metabolize mannose.

RFP — Red Fluorescent Proteins and related FPs

Several genes encoding FPs have been cloned from non-fluorescent species of reef coral organisms (*Anthozoa*), and are collectively called reef coral fluorescent proteins (RCFP): *AmCyan* from *Anemonia majano*, *DsRed1* and *DsRed2* from *Discosoma* sp., *ZsGreen1* and *ZsYellow1* from *Zoanthus* sp. (Matz *et al.*, 1999), *AsRed1* and *AsRed2* from *Anemonia sulcata* (Lukyanov *et al.*, 2000), *HcRed* from *Heteractis crispa* (Gurskaya *et al.*, 2001). These and other FPs are available in a variety of constructs from Clontec. These proteins have been expressed transiently or stably in wheat, maize, barley, rice, banana, onion, soybean, cotton, tobacco, potato and tomato (Wenk *et al.*, 2003). Transient expression allowed clear visualization of individual cells. Transgenic plants were phenotypically normal, showing a wide range of fluorescence levels, and were fertile. Expression of *AmCyan*, *ZsGreen* and *AsRed* was visible in maize and rice T_1 seeds, allowing accurate visual discrimination of transgenic from non transgenic seeds. Callus tissue was colored by *AsRed* and *DsRed* under white light. The excitation and emission wavelengths of some of these proteins are different, so that the simultaneous visualization of cells transformed with more than one of the fluorescent proteins should be possible.

Jach *et al.* (2001) presented accurate data on transient and stable DsRed expression in tobacco after targeting to the cytosol, the endoplasmic reticulum or the vacuole in tobacco BY2 suspension culture cells. Cytosolic expression produced readily detectable levels of fluorescence, whereas expression in the endoplasmic reticulum was poor, and vacuolar expression was not detectable. In transgenic tobacco plants, it was possible to block the red chlorophyll fluorescence, allowing specific dsRed detection. In the plants, the same pattern of expression in the three cell compartments observed in suspension cells was found.

DsRed has the disadvantage of forming multimeric proteins. The formation of multimers makes transgenic protein fusions problematic and can also cause solubility and aggregation problems. Monomerization was accomplished (Campbell *et al.*, 2002; Shaner *et al.*, 2004); furthermore, the protein brightness was increased, the range of emission extended from yellow to far red, and fluorescence lifetime extended (Miyawaki, 2002).

RFPs are interesting for plant research plants because there is not much fluorescence in plants in the red wavelengths when excited by green-to-red wavelengths. Secretion and targeting to vacuoles of a monomeric RFP demonstrated its ability to fluoresce at the low pH of vacuoles (Scabone *et al.*, 2011).

DsRed2 was used in combination with antibiotic selection for genetic transformation of soybean. Transient and stable expression in somatic embryos in leaves and seeds was easily detected by fluorescence microscopy. In contrast to seeds expressing GFP, those expressing DsRed2 were readily identifiable even under white light by the red color of the cotyledons, visible even through the seed coat. The protein composition of seeds was not affected (Nishizawa *et al.*, 2006).

DsRed2 has been recently employed to create a plant transformation vector that enables high throughput, non-destructive selection of Agrobacterium rhizogenes-mediated 'hairy-root' transformation (Lin *et al.*, 2011). The applicability of this reporter system to the study of root traits, including nodulation, was demonstrated in soybean.

The high stability of DsRed (half-life of approximately 4-5 days) makes them of little use for visualizing dynamic processes, such as transient changes in gene expression. The DsRed-E5 variant forms a green fluorescent intermediate before maturing to the red fluorescent configuration, but compared to DsRed – that also has a green intermediate– it has a higher intensity of green fluorescence that can be easily detected. This shift from green to yellow to orange to red during protein maturation makes it possible to determine the age of DsRed-E5, which could facilitate the study of gene expression dynamics (Terskikh *et al.*, 2000). The utility of this RG in plants was demonstrated by transient expression in *Vigna unguiculata* mesophyll protoplasts, and by expressing it in *Medicago truncatula* transformed roots under promoters that are either constitutive or developmentally regulated in root hairs (Mirabella *et al.*, 2004).

A fluorescent protein from the stony coral *Lobophyllia hemprichii* (EosFP) can be photoconverted: it changes color from green to red when exposed to near-UV light. A monomeric EosFP form, and provedes a tool study cell dynamics (Mathur *et al.*, 2010).

New RFPs were recently isolated from the sea anemone *Entacmaea quadricolor* (Merzlyak *et al.*, 2007; Forner and Binder, 2007, and references therein). One of these, eqFP611, was expressed in the native form and successfully targeted to mitochondria and peroxisomes in tobacco, and proved to be suitable for dual-labeling experiments with GFP. No detrimental effect of its expression was noticed (Forner and Binder, 2007).

***XynA* — Xylanase A**

This RG derives from the second catalytic domain of the *xynA* gene isolated from the anaerobic fungus, *Neocallimastix patriciarum* (Vickers *et al.*, 2003). It can hydrolyze the substrate azurine-crosslinked xylan, forming a blue pigment. A codon-optimized version was transiently expressed in wheat endosperm tissues using different promoters, and it showed very good sensitivity as compared with GUS and GFP. However, the protein extract was used for the analysis, and *in planta* expression detection has not been demonstrated.

III. PHENOTYPIC EFFECTS

SMGs and RGs should only allow selection or screening of transgenic cells, tissues or plants, without interfering with the phenotype in other ways. Such 'phenotypic neutrality' of MGs is required for basic studies and to make transgenic crop plants acceptable to both the farmer and the consumer.

A. SMGs

The specificity of the selective chemical and its derivatives and of the SMG-encoded enzymes is key to the absence of pleiotropic effects (Rommens, 2006; Miki *et al.*, 2009). Probably because AB metabolism is alien to the plant cells, no phenotypic effect is generally reported from the expression of the AB resistance SMGs.

For some MGs, on the contrary, phenotypes are expected because they exploit important metabolic pathways and have been reported (see above; Table 2). For instance, amino acid concentration changes were observed with SMGs that exploit amino acid analogs for selection, such as *TDC* and *TSB1*.

A special case is that of regeneration-promoting and cell cycle genes (type 7). Their constitutive expression causes characteristic abnormal phenotypes (see above) and prevents the recovery of phenotypically normal plants. Therefore, their expression must be stopped after the *in vitro* regeneration step. This can be accomplished either by post-transformation marker removal (discussed in paragraph V), or by inducible gene expression, or by transient expression. Strict control of inducible promoters is difficult to realize and often makes use of chimeric transcription factors composed of viral, bacterial, and eukaryotic elements (Zuo *et al.*, 2000; Padidam, 2003) that may not be allowed in commercial crops. Transient expression during tissue culture was accomplished (Heidman *et al.*, 2011, see above).

Profiling ('omic') technologies have been applied to study the effects of MG expression in *A. thaliana*. El Ouakfaoui and Miki (2005) compared *nptII*-transgenic *A. thaliana* lines with the non transgenic line under controlled growth conditions *in vitro*, and found no reproducible mRNA level changes for the approximately 24,000 genes screened. By comparison, abiotic stresses (salt, drought, cold, and heat) changed expression of approximately 8,000 genes (35% of the genome) in both wild-type and transgenic plants.

For the *bar* SMG, the same group did find significant effects: Abdeen and Miki (2009) observed changes in transcription levels of a few genes in three *bar*-transgenic lines, four of which were common to all lines. This study was conducted *in vitro* so it is not known whether any difference between *bar*-containing and non transgenic crop plants or products would be found in field conditions. Bregitzer *et al.* (2007) found that, in some barley varieties, *bar* expressing lines had lower performance in the field that non transgenic counterparts but the genes underlying these differences are not known.

Ren *et al.* (2009b) examined the metabolome of four transgenic *Arabidopsis* lines expressing the *bar* gene, and found differences for the levels of alanine and tryptophan. The authors attributed this difference to the *bar* gene, carried by all the lines. In another study, proteomic analysis on 12 *bar*-containing lines showed no consistent differences (Ren *et al.*, 2009a). In another work, the expression of *Csr1* in *A. thaliana* was examined, and no perturbation of the transcriptome was evidenced (Manabe *et al.*, 2007).

Ricroch *et al.* (2011) reviewed the unintended effect of transgenesis in plants as assessed by 'omic' technologies, and concluded that the alterations were consistently within the range found between the conventional varieties. Since most, if not all, the GMPs contain a SMG linked to the useful gene, the conclusions indirectly demonstrate that the expression of those SMGs does not significantly alter the plant phenotype. Only a thorough assessment of MG effects *per se* would make it possible to separate the effect of the gene of interest from that of the MG (Miki *et al.*, 2009). Replacement of well studied SMGs with new marker systems in crops demands the assessment of their effects on the phenotype.

B. RGs

Few studies addressed the pleiotropic phenotypic effects of RGs in plants, probably because they are mostly used for basic and applied research and are not often present in commercial transgenic plants. Two RGs are found in crops: *GFP* and *gusA* (Table 5).

There are reports of no significant effect of GFP expression in plants (reviewed by Stewart, 2001) or in pollen (Hudson and Stewart, 2004). However, it has been reported that high expression of GFP may affect plant morphology (Haseloff and Siemering, 1998). In *A. thaliana,* high accumulation in the cytoplasm and nucleoplasm appeared to inhibit plant regeneration (Haseloff *et al.*, 1997), but targeting GFP to the endoplasmic reticulum restored normal regeneration. In plastids, high expression did not cause visible abnormalities (Sidorov *et al.*, 1999). An association between GFP and apoptosis in mammalian cells was reported (Liu *et al.*, 1999), but not confirmed.

Page and Angell (2002) employed the cDNA-AFLP technique to examine the transcriptional changes in response to transient expression (agroinfiltration) of *GFP* and *gusA* in *N. benthamiana*. They tested an endoplasmic reticulum-targeted GFP (GFP5) and a non-targeted form (GFP4), and an ER-targeted and

non-targeted *gusA* gene. Sixty cDNA-AFLP fragments showed altered expression profiles in response to ER-targeted GFP expression. Only two genes showed expression changes in response to GFP4, and one gene did so in response to both GFP4 and GFP5. It is not known if such effects were transient or long-lasting.

No changes in gene expression were observed in response to agroinfiltration of the *gus*A gene, unless it was targeted to the ER, in which case a subset of the GFP-induced genes were also induced by *gusA*. The GFP5-induced genes were not upregulated in transgenic plants constitutively expressing GFP5 at a low level. Sequencing of the cDNA-AFLP fragments showed that many of the induced genes showed homology to genes involved in plant defense, suggesting that the plant response to ER-targeted reporter proteins can mimic the response to attack by pathogens.

The effect of accumulation of DsRed on morphogenesis, development and fertility was investigated in tobacco (Jach *et al.*, 2001). No abnormalities were observed during transformation and regeneration. The transgenic plants expressing the protein in the cytosol, endoplasmic reticulum or plastid were fertile, the seed germinated normally although they showed red fluorescence. DsRed expression in soybean also did not alter seed protein composition, but the seed showed red coloration even in white light (Nishizawa *et al.*, 2006).

El Ouakfaoui and Miki (2005) applied the microarray technology to assess gene transcription alteration induced by *gusA* in *A. thaliana,* and found no perturbation of transcription.

IV. SAFETY AND ACCEPTANCE

As pointed out by Miki and McHugh (2004), the perception of risk has become one of the guiding criteria for the development SMGs for crop genetic engineering, because it affects the legislation and the regulatory process of GE crops.

Even though 'classic' MGs such as *nptII* or *GUS* have been demonstrated to be safe for use in crops, alternatives will be useful while public perception of the presence of AB resistance genes, and bacterial genes in general, in crop plants is negative. It appears that the development of SMG that not only are safe, but are perceived as safe is not merely "cosmetic research" (as argued by Ramessaar *et al.*, 2007), because it could help to streamline the adoption of biotech crops. In particular, the use of plant, rather than bacterial genes could be more acceptable in terms of public opinion (Lusk and Sullivan, 2002). Moreover, some of the new selection systems are found to be more efficient than the conventional ones, and deserve large adoption. A wide choice of such markers can be very useful for transgene stacking, an increasing trend in the commercial arena.

Thirteen SMGs are found in commercial transgenic plants (Table 5). In particular, *nptII*, *bar/pat* and *hpt* genes are individually present in 130 commercial events. However, antibiotic resistance genes in transgenic plants elicit considerable public concern, despite the absence of scientific evidence of any significant risk for human health or the environment (Miki and McHugh, 2004; Ramessar *et al.*, 2007, and references therein). Consequently, FDA (2009) and EFSA (2004) published guidelines against using genes for resistance to medically important antibiotics.

Public opposition has stimulated intense research on AB-free selection systems. Accordingly, the use of plant genes as SMGs is seen favorably as more acceptable.

It has been observed that SMGs that act by accumulation of phytotoxins in cell compartments where they are not toxic (Type 2) can present hazards due to their general lack of substrate specificity (Conte and Lloyd, 2011): as pointed out by Rommens (2006), accumulation of heavy metals, herbicides, fungicides, or pesticides present in the soil may occur in the plant. Therefore, SMGs encoding transporter proteins may need to be restricted to non-food species.

Recently, the 'intragenic' and 'cisgenic' engineering approaches have been proposed to avoid gene transfer between kingdoms or sexually incompatible species. The intragenic approach uses genetic elements from sexually compatible species that are assembled with conventional gene cloning methods (Nielsen, 2003; Rommens *et al.*, 2004b). The cisgenic approach only allows the isolation of genomic fragments containing one or more genes with their native regulatory sequences and their insertion into sexually compatible species (Jacobsen and Schouten, 2007).

The idea behind these approaches is that, in Europe, future legislation may ease the regulatory burdens for intragenic and cisgenic crops. These techniques can only use of SMGs isolated from species that are sexually compatible with the host crop plant. Plant-derived SMGs have been reviewed recently (Rosellini, 2011), and a few more are listed here (refer to the Source organism column in Table 2). Some of these SMGs exhibit versatility and phenotypic neutrality and, based on the available genomic sequence knowledge, they can be easily developed from any crop species of interest.

No studies have been found regarding safety of negative SMGs. Some of them encode proteins that are known toxins for mammals, and their use is obviously confined to the lab. The only negative SMG in commercial crops is *barnase*, as a component of a male sterility system (Table 5).

RGs are a research tool and their presence in crops is limited (Table 5). There are few studies addressing safety of *GUS* and *GFP* (Metz and Nap, 1997; Richards *et al.*, 2003) and no significant risk was evidenced.

V. MARKER REMOVAL

SMGs are necessary for efficient plant genetic engineering to avoid the necessity of screening large numbers of regenerated plants in order to identify the transgenic ones. Otherwise, marker-less transformation (deVetten *et al.*, 2003; Popelka *et al.*, 2003; Doshi *et al.*, 2007; Jia *et al.*, 2007; Weeks *et al.*, 2008; Bhatnagar *et al.*, 2010; Ferradini *et al.*, 2011b), that is,

transformation with only the gene of interest, requires the identification of the transgenic among the regenerated plants by PCR or high-throughput sequencing. This procedure is labor-intensive, and will remain so until very high transformation efficiencies are realized.

More practically, markers can be removed after use. Marker removal can be accomplished in different ways: co-transformation (Ebinuma *et al.*, 2001; Miller *et al.*, 2002; Park *et al.*, 2004; Dutt *et al.*, 2008; Ferradini *et al.*, 2011b), post-transformation excision using recombinase systems (reviews by Miki and McHugh, 2004; Wang *et al.*, 2011), or preventing their integration by only allowing transient expression (Rommens *et al.*, 2004b).

Marker removal techniques have limitations: they necessitate either considerable more time and work, or the preparation of more complex genetic constructs, or more difficult regeneration protocols. In particular, those based on microbial recombination systems may cause chromosomal alterations and phenotypic aberrations (Srivastava and Gidoni, 2010). Therefore, SMGs are still very useful.

VI. CONCLUSION: SELECTING A MARKER GENE

Versatility of a marker gene is an important factor when a new host species is involved, because it increases the chances of success. For SMGs, it can be immediately appreciated by counting the number of crop species that have been successfully transformed with each SMG. Apart from the 'classic,' universally used AB and HR markers (*hpt, nptII, pat/bar*), only a few (*ALS*, *ipt, pmi*) have been tested in a significant number of species, so it would be very useful to widen the species range of other SMGs.

A second factor in SMG choice is their performances in terms of TE and SE, which vary widely: there are examples of new SMGs with higher efficiencies than traditional systems. Table 1 presents calculations for the average maximum TE and SE of the SMG types for nuclear transformation of crop plants. The results are a mere indication of the comparative efficiencies of the positive selection mechanisms, because different species, and different numbers of species contribute to the means. It seems that type 2 SMGs acting by removal of phytotoxins from the sensitive cell compartment, though few, perform better than the others. This lends credence to the claims that the selection mechanisms that are not based on chemical detoxification may be generally more efficient because the phytotoxin remains present and active during tissue culture, reducing the regeneration of escapes. Although higher efficiencies of SMGs based on carbohydrate metabolism relative to AB selection have been reported, overall, they do not seem to perform better.

A third factor that may be important is availability: most selection systems are patented, and consequently not readily available for commercial applications, but a few freely available SMGs exist. Availabilty varies country by country, depending whether patent coverage was sought in specific countries or not. In any case, once a patent expires, it enters the public domain.

A specific field of application of SMGs is that of plastid genome transformation. Transplastomic plants have been obtained in several species, but many remain recalcitrant, particularly grasses, which appear to be naturally resistant to spectinomycin and streptomycin, the ABs to which the *aadA* gene confers resistance. Extending plastid transformation to new species has been a slow process.

As pointed out by Clarke and Daniell (2011) the choice of selection markers has been shown to be crucial for success in plastome transformation. New markers are being developed (Barone and Widholm, 2009; Li *et al.*, 2011) that may help to increase the number of species amenable to plastid genetic engineering. Several SMG systems used for nuclear transformation that exploit plastid functions are candidates to become plastome transformation tools.

The promoter driving the SMG can affect the transformation outcome. In that large majority of the studies, SMGs have been expressed using constitutive promoters. Overexpression can alter the phenotype to the point of lethality, and in these cases, either post-transformation excision, inducible expression, or *in vitro*-specific expression are necessary. Prakash *et al.* (2008) compared the rice *Actin1* with the *35S* promoter driving *nptII* and found that the former was more efficient for corn transformation, but resulted in a higher transgene copy number. They suggest that the promoter is an important factor to consider for the establishment of high throughput transformation methods.

The toxicity and cost of the selection agent used in the lab has relevance: some SMGs employ substrates that are costly, or not commercially available, or very highly toxic, whereas others use inexpensive or non toxic chemicals.

Negative selection can be performed with several genes (13 of them were reviewed here), and some of them have been essential tools for basic and applied studies. One negative SMG, *barnase,* has served practical purposes.

Many RGs are available for plants: more than 20 of them were found in the literature. Fluorescent proteins have become by far the most successful RG type, and the scope of their applications is very large. They have been isolated from many organisms and mutated versions, adapted to specific objectives and experimental systems, continue to appear.

The picture emerging from this literature review is that a vast array of MGs is available for diverse uses. New MG systems need further refinement and better knowledge of their phenotypic effects, but there is a plentiful set of tools for basic research and efficient and safe genetic engineering.

ACKNOWLEDGMENTS

I am grateful to Wayne Parrott (University of Georgia, Athens, USA) for his insightful and accurate review and to Fabio Veronesi and Alessandro Nicolia (University of Perugia, Italy) for their critical reading.

REFERENCES

Abdeen, A. and Miki, B. 2009. The pleiotropic effects of the bar gene and glufosinate on the *Arabidopsis* transcriptome. *Plant Biotec. J.* **7**: 266–282.

Aionesei, T., Hosp, J., Voronin, V., Heberle-Bors, E., and Touraev, A. 2006. Methotrexate is a new selectable marker for tobacco immature pollen transformation. *Plant Cell Rep.* **25**: 410–416.

Allen, G. J., Kwak, J. M., Chu, S. P., Llopis, J., Tsien, R. Y., Harper, J. F., and Schroeder, J. I. 1999. Cameleon calcium indicator reports cytoplasmic calcium dynamics in *Arabidopsis* guard cells. *Plant J.* **19**: 735–747.

Almquist, K. C., Niu, Y. Q., Mclean, M. D., Mena, F. L., Yau, K.Y.F., Brown, K., Brandle, J. E., and Hall, J. C. 2004. Immunomodulation confers herbicide resistance in plants. *Plant Biotechnol. J.* **2**: 189–197.

Alvarado, M. C., Zsigmond, L. M., Kovács, I., Cséplö, Á., Koncz, C., and Szabados, L. M. 2004. Gene trapping with firefly luciferase in *Arabidopsis*. Tagging of stress-responsive genes. *Plant Physiol.* **134**: 18–27.

Andersson, M., Trifonova, A., Andersson, A.B., Johansson, M., Bulow, L., and Hofvander, P. 2003. A novel selection system for potato transformation using a mutated AHAS gene. *Plant Cell Rep.* **22**: 261–267.

Aragao, F. J. L., Sarokin, L., Vianna, G. R., and Rech, E. L. 2000. Selection of transgenic meristematic cells utilizing a herbicidal molecule results in the recovery of fertile transgenic soybean [*Glycine max* (L.) Merril] plants at a high frequency. *Theor. Appl. Genet.* **101**: 1–6.

Arias, R. S., Dayan, F. E., Michel, A., Howell, J., and Scheffler B. E. 2006. Characterization of a higher plant herbicide-resistant phytoene desaturase and its use as a selectable marker. *Plant Biotechnol. J.* **4**: 263–273.

Arias, R. S., Filichkin, S. A., and Strauss, S. H. 2006. Divide and conquer: development and cell cycle genes in plant transformation. *Trends Biotechnol.* **24**: 267–273.

Armstong, C. L., Parker, G. B., Pershing, J. C., Brown, S. M., Sanders, P. R., Duncan, D. R., Stone, T., Dean, D. A., DeBoer, D. L., Hart, J., Howe, A. R., Morrish, F. M., Pajeau, M. E., Petersen, W. L., Reich, B. J., Rodriguez, R., Santino, C. G., Sato, S. J., Schuler, W., Sims, S. R., Stehling, S., Tarochione, L. J., and Fromm, M. E. 1995. Field evaluation of European corn borer control in progeny of 173 transgenic corn events expressing an insecticidal protein from *Bacillus thuringiensis*. *Crop Sci.* **35:** 550–557.

Aswath, C. R., Mo, S. Y., Kim, D. H., and Park S. W. 2005. *Agrobacterium* and biolistic transformation of onion using non-antibiotic selection marker phosphomannose isomerase. *Plant Cell Rep.* **25**: 92–99.

Aufsatz, W., Nehlin, L., Voronin, V., Schmidt, A., Matzke, A. J., and Matzke, M. 2009. A novel strategy for obtaining kanamycin resistance in *Arabidopsis thaliana* by silencing an endogenous gene encoding a putative chloroplast transporter. *Biotechnol. J.* **4**: 224–229.

Avonce, N., Leyman, B., Mascorro-gallardo, O., Dijck, P. V., Thevelein, J., and Iturriaga, G. 2004. The *Arabidopsis* trehalose-6-Psynthase *AtTPS1* gene is a regulator of glucose, ABA and stress signalling. *Plant Physiol.* **136**: 3649–3659.

Aziz, N. and Machray, G. C. 2003. Efficient male germ line transformation for transgenic tobacco production without selection. *Plant Mol. Biol.* **51**: 203–211.

Babwah, A. V. and Waddell, C. S. 2000. Cytosine deaminase as a substrate-dependent negative selectable marker in *Brassica napus*. *Theor. Appl. Genet.* **100:** 802–809.

Ballester, A., Cervera, M., and Pena, L. 2007. Efficient production of transgenic citrus plants using isopentenyl transferase positive selection and removal of the marker gene by site-specific recombination. *Plant Cell Rep.* **26**: 39–45.

Ballester, A., Cervera, M., and Pena, L. 2008. Evaluation of selection strategies alternative to nptII in genetic transformation of citrus. *Plant Cell Rep.* **27**: 1005–1015.

Bargmann, B. O. R. and Birnbaum, K. D. 2010. Fluorescence activated cell sorting of plant protoplasts. *J. Vis. Exp.* **18** (36): 1673.

Barone, P. and Widholm, J. M. 2008. Use of 4-methylindole or 7-methyl-DL-tryptophan in a transformant selection system based on the feedback-insensitive anthranilate synthase alpha-subunit of tobacco (ASA2). *Plant Cell Rep.* **27**: 509–517.

Barone, P., Zhang, X.-H., and Widholm, J. M. 2009. Tobacco plastid transformation using the feedback-insensitive anthranilate synthasee [alpha]-subunit of tobacco (ASA2) as a new selectable marker. *J. Exp. Bot.* **60**: 3195–3202.

Baruah-Wolff, J., Harwood, W. A., Lonsdale D. A., Harvey, A., Hull, R., and Snape, J. W. 1999. Luciferase as a reporter gene for transformation studies in rice (*Oryza sativa* L.). *Plant Cell Rep.* **18**: 715–720.

Baulcombe, D. C., Chapman, S., and Santa Cruz, S. 1995. Jellyfish green fluorescent protein as a reporter for virus infections. *Plant J.* **7**: 1045–1053.

Beclin, C., Charlot, F., Botton, E., Jouanin, L., and Dore, C. 1993. Potential use of the aux2 gene from *Agrobacterium rhizogenes* as a conditional negative marker in transgenic cabbage. *Transgenics Res.* **2**: 48–55.

Beetham, P. R., Kipp, P. B., Sawycky, X. L., Arntzen, C., and May, G. D. 1999. A tool for functional plant genomics: Chimeric RNA/DNA oligonucleotides cause *in vivo* gene-specific mutations. *Proc. Natl. Acad. Sci. U.S.A.* **96**: 8774–8778.

Bellucci, M., De Marchis, F., Mannucci, R., and Arcioni, S. 2003. Jellyfish green fluorescent protein as a useful reporter for transient expression and stable transformation in *Medicago sativa* L. *Plant Cell Rep.* **22**: 328–337.

Bergero, R., Harrier, L. A., and Franken, P. 2003. Reporter genes: applications to the study of arbuscular mycorrhizal (AM) fungi and their symbiotic interactions with plant roots. *Plant Soil.* **255**: 143–155.

Bhatnagar, M., Prasad, K., Bhatnagar-Mathur, P., Narasu, M. L., Waliyar, F., and Sharma, K. K. 2010. An efficient method for the production of marker-free transgenic plants of peanut (*Arachis hypogaea* L.). *Plant Cell Rep.* **29**: 495–502.

Birch, R. G., Shen, B., Sawyer, B. J. B., Huttner, E., Tucker, W. Q. J., and Betzner A. S. 2010. Evaluation and application of a luciferase fusion system for rapid *in vivo* analysis of RNAi targets and constructs in plants. *Plant Biotechnol. J.* **8**: 465–475.

Bombale, S. L., Gowda, P. H. R., Gunnaiah, R., Malatheshaiah, N. T., Salome, T., and Swamidatta, S. H. 2010. *In vitro* regeneration and transformation of muskmelon (*Cucumis melo* L.) with alternative AtWBC19 marker gene. *Intl. J. Biotechnol. Biochem.* **6**: 1–12.

Boscariol, R. L., Almeida, W. A. B., Derbyshire, M., Mourao, F. A. A., and Mendes, B. M. J. 2003. The use of the PMI/mannose selection system to recover transgenic sweet orange plants (*Citrus sinensis* L. Osbeck). *Plant Cell Rep.* **22**: 122–128.

Bourdon, V., Wickham, A., Lonsdale, D., and Harwood, W. 2004. Additional introns inserted within the luciferase reporter gene stabilise transgene expression in wheat. *Plant Sci.* **167**: 1143–1149.

Boutilier, K., Offringa, R., Sharma, V. K., Kieft, H., Ouellet, T., Zhang, L., Hattori, J., Liu, C. M., van Lammeren, A. A., Miki, B. L., Custers, J. B., and van Lookeren Campagne, M. M. 2002. Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. *Plant Cell* **14**: 1737–1749.

Brakemann, T., Stiel, A. C., Weber, G., Andresen, M., Testa, I., Grotjohann, T., Leutenegger, M., Plessmann, U., Urlaub, H., Eggeling, C., Wahl, M. C., Hell, S. W., and Jakobs, S. 2011. A reversibly photoswitchable GFP-like protein with fluorescence excitation decoupled from switching. *Nat Biotech.* **29**: 942–947.

Brasileiro, A. C. M., Tourneur, C., Leplt, J.-C., Combes, V., and Jouanin, L. 1992. Expression of the mutant *Arabidopsis thaliana* acetolactate synthase gene confers chlorsulfuron resistance to transgenic poplar plants. *Transgenic Res.* **1**: 133–141.

Bregitzer, P., Cooper, L. D., Hayes, P. M., Lemaux, P. G., Singh, J., and Sturbaum, A. K. 2007. Viability and bar expression are negatively correlated in Oregon Wolfe barley dominant hybrids. *Plant Biotechnol. J.* **5**: 381–388.

Bříza, J., Pavingerová, D., Přikrylová, P., Gazdová, J., Vlasák, J., and Niedermeierová, H. 2008. Use of phosphomannose isomerase-based selection system for *Agrobacterium*-mediated transformation of tomato and potato. *Biol. Plant.* **52**: 453–461.

Bříza, J., Růžičková, N., Niedermeierová, H., Dusbábková, J., and Vlasák, J. 2010. Phosphomannose isomerase gene for selection in lettuce (Lactuca sativa L.*) transformation*. *Acta Biochim. Polonica* **57**: 63–68.

Broothaerts, W., Mitchell, H. J., Weir, B., Kaines, S., Smith, L. M. A., Yang, W., Mayer, J. E., Roa-Rodriguez, C., and Jefferson, R. A. 2005. Gene transfer to plants by diverse species of bacteria. *Nature*. **433**: 629–633.

Buchanan-Wollaston, V., Snape, A., and Cannon, F. 1992. A plant selectable marker gene based on the detoxification of the herbicide dalapon. *Plant Cell Rep.* **11**: 627–631.

Budar, F., Deboeck, F., Van Montagu, M., and Hernalsteens, J.-P. 1986. lntroduction and expression of the octopine T-DNA oncogenes in tobacco plants and their progeny. *Plant Sci.* **46**: 195–206.

Buell, C. R. and Anderson, A. J. 1993. Expression of the *aggA* locus of *Pseudomonas putida in vitro* and *in planta* as detected by the reporter gene, *xylE*. *Mol. Plant Microbe Interact.* **6**: 331–340.

Burgess, D. G., Ralston, E. J., Hanson, W. G., Heckert, M., Ho, M., Jenq, T., Palys, J. M., Tang, K. L., and Gutterson, N. 2002. A novel, two-component system for cell lethality and its use in engineering nuclear male-sterility in plants. *Plant J.* **31**: 113–125.

Cabantous, S., Terwilliger, T. C., and Waldo, G. S. 2004. Protein tagging and detection with engineered self-assembling fragments of green fluorescent protein. *Nat Biotech.* **23**: 102–107.

Campbell, R. E., Tour, O., Palmer, A. E., Steinbach, P. A., Baird, G. S., Zacharias, D. A., and Tsien, R. Y. 2002. A monomeric red fluorescent protein. *Proc. Natl Acad. Sci. USA*. **99**: 7877–7882.

Campisi, L., Yang, Y., Yi, Y., Heilig, E., Herman, B., Cassista, A. J., Allen, D. W., Xiang, H., and Jack, T. 1999. Generation of enhancer trap lines in *Arabidopsis* and characterization of expression patterns in the inflorescence. *Plant J.* **17**: 699–707.

Carlson, A. R., Letarte, J., Chen, J., and Kasha, K. J. 2001. Visual screening of microspore-derived transgenic barley (*Hordeum vulgare* L.) with green-fluorescent protein. *Plant Cell Rep.* **20:** 331–337.

Carrer, H., Hockenberry, T. N., Svab, Z., and Maliga, P. 1993. Kanamycin resistance as a selectable marker for plastid transformation in tobacco. *Mol. Gen. Genet.* **241**: 49–56.

Cazzonelli, C. I. and Velten, J. 2003. Construction and testing of an intron-containing luciferase reporter gene from *Renilla reniformis*. *Plant Mol. Biol. Reporter.* **21**: 271–280.

CERA. 2010. *GM Crop Database. Center for Environmental Risk Assessment (CERA)*, ILSI Research Foundation, Washington DC. Available at: http://cera-gmc.org/index.php?action=gm_crop_database, accessed February 2012.

Chai, D., Lee, S. M., Ng, J. H., and Yu, H. 2007. l-Methionine sulfoximine as a novel selection agent for genetic transformation of orchids. *J. Biotechnol.* **131**: 466–472.

Chang, C.-C., Huang, P.-S., Lin, H.-R., and Lu, C.-H. 2007. Transactivation of protein expression by rice HSP101 *in planta* and using Hsp101 as a selection marker for transformation. *Plant Cell Physiol*. **48**: 1098–1107.

Charest, P. J., Hattori, J., DeMoor, J., Iyer, V. N., and Miki, B. L. 1990. *In vitro* study of transgenic tobacco expressing Arabidopsis wild type and mutant acetohydroxyacid synthase genes. *Plant Cell Rep.* **8**: 643–646.

Charng, Y.-C., Li, K.-T., Tai, H.-K., Lin, N.-S., and Tu, J. 2008. An inducible transposon system to terminate the function of a selectable marker in transgenic plants. **21**: 359–368.

Charng, Y.-C. and Pfitzner, A. J. P. 1994. The firefly luciferase gene as a reporter for *in vivo* detection of Ac transposition in tomato plants. *Plant Sci*. **98**: 175–183.

Chawla, H. S., Cass, L. A., and Simmonds, J. A. 1999. Developmental and environmental regulation of anthocyanin pigmentation in wheat tissues transformed with anthocyanin regulatory genes. *In Vitro Cell. Devel. Biol. - Plant.* **35**: 403–408.

Chen, I.-C., Thiruvengadam, V., Lin, W.-D., Chang, H.-H., and Hsu W.-H. 2010. Lysine racemase: a novel non-antibiotic selectable marker for plant transformation. *Plant Mol. Biol.* **72**: 153–169.

Chen, J.-C., Jiang, C.-Z., Gookin, T., Hunter, D., Clark, D., and Reid, M. 2004. Chalcone synthase as a reporter in virus-induced gene silencing studies of flower senescence. *Plant Mol. Biol.* **55**: 521–530.

Cheng, L., Li, H.-P., Qu, B., Huang T., Tu, J.-X., Fu, T.-D., and Liao, Y.-C. 2010. Chloroplast transformation of rapeseed (*Brassica napus*) by particle bombardment of cotyledons. *Plant Cell Rep.* **29**: 371–381.

Chiang, Y. C. and Kiang, Y. T. 1988. Genetic analysis of mannose-6-phosphate isomerase in soybeans. *Genome* **30**: 808–811.

Ckurshumova, W., Caragea, A. E., Goldstein, R. S., and Berleth, T. 2011. Glow in the dark: fluorescent proteins as cell and tissue-specific markers in plants. *Mol. Plant.* **4**: 794–804.

Clarke, J. L. and Daniell, H. 2011. Plastid biotechnology for crop production: present status and future perspectives. *Plant Mol. Biol.* **76**: 211–220.

Clemente, T. E., LaValle, B. J., Howe, A. R., Conner-Ward, D., Rozman, R. J., Hunter, P. E., Broyles, D. L., Kasten, D. S., and Hinchee, M. A. 2000. Progeny analysis of glyphosate selected transgenic soybeans derived from *Agrobacterium*-mediated transformation. *Crop Sci.* **40**: 797–803.

Conte, S. S. and Lloyd, A. M. 2011. Exploring multiple drug and herbicide resistance in plants-Spotlight on transporter proteins. *Plant Sci.* **180**: 196–203.

Conte, S., Stevenson, D., Furner, I., and Lloyd, A. 2009. Multiple antibiotic resistance in arabidopsis is conferred by mutations in a chloroplast-localized transport protein. *Plant Physiol*. **151**: 559–573.

Cubitt, A. B., Heim, R., Adams, S. R., Boyd, A. E., Gross, L. A., and Tsien, R. Y. 1995. Understanding, improving and using green fluorescent proteins. *Trends Biochem. Sci.* **20**: 448–455.

Cui, M., Takayanagi, K., Kamada, H., Nishimura, S., and Handa, T. 2000. Transformation of *Antirrhinum majus* L. by a rol-type multi-auto-transformation (MAT) vector system. *Plant Sci.* **159**: 273–280.

Cui, M., Takayanagi, K., Kamada, H., Nishimura, S., and Handa, T. 2001. Efficient shoot regeneration from hairy roots of *Antirrhinum majus* L. transformed by the rol type MAT vector system. *Plant Cell Rep.* **20**: 55–59.

Czakó, M., Jang, J.-C., Herr, J. M. J., and Márton, L. 1992. Differential manifestation of seed mortality induced by seed specific expression of the gene for Diphtheria toxin A chain in *Arabidopsis* and tobacco. *Mol. Gen. Genet*. **235**: 33–40.

Czakó, M., Marathe, R. P., Xiang, C., Guerra, D. J., Bishop, G. J., Jones, J. G., and Márton, L. 1995. Variable expression of the herpes simplex virus thymidine kinase gene in *Nicotiana tabacum* affects negative selection. *Theor. Appl. Genet.* **91**: 1242–1247.

Czakó, M. and Márton, L. 1994. The herpes simplex virus thymidine kinase gene as a conditional negative-selection marker gene in *Arabidopsis thaliana*. *Plant Physiol*. **104**: 1067–1071.

Damiani, F., Paolocci, F., Cluster, P. D., Arcioni, S., Tanner, G. J., Joseph, R. G., Li, Y. G., de Majnik, J., and Larkin, P. J. 1999. The maize transcription factor *Sn* alters proanthocyanidin synthesis in transgenic *Lotus corniculatus* plants. *Aust. J. Plant Physiol*. **26**: 159–169.

Daniell, H., Muthukumar, B., and Lee, S. B. 2001. Marker free transgenic plants: engineering the chloroplast genome without the use of antibiotic selection. *Curr. Genet.* **39**: 109–116.

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.

Day, A. and Goldschmidt-Clermont, M. 2011. The chloroplast transformation toolbox: selectable markers and marker removal. *Plant Biotechnol. J.* **9**: 540–553.

DeBlock, M., Herrera-Estrella, L., Van Montagu, M., Schell, J., and Zambryski, P. 1984. Expression of foreign genes in regenerated plants and in their progeny. *EMBO J*. **3**: 1681–1689.

Degenhardt, J., Poppe, A., Montag, J., and Szankowski, I. 2006. The use of the phosphomannose-isomerase/mannose selection system to recover transgenic apple plants. *Plant Cell Rep*. **25**: 1149–1156.

Delaney, B., Zhang, J., Carlson, G., Schmidt, J., Stagg, B., Comstock, B., Babb, A., Finlay, C., Cressman, R. F., Ladics, G., Cogburn, A., Siehl, D., Bardina, L., Sampson, H., and Han, Y. 2008. A gene-shuffled glyphosate acetyltransferase protein from *Bacillus licheniformis* (GAT4601) shows no evidence of allergenicity or toxicity. *Toxicol. Sci.* **102**: 425–432.

De Marchis, F., Wang, Y., Stevanato, P., Arcioni, S., and Bellucci, M. 2009. Genetic transformation of the sugar beet plastome. *Transgenic Res.* **18**: 17–30.

Deng, W., Luo, K., Li, Z., and Yang Y. 2009. A novel method for induction of plant regeneration via somatic embryogenesis. *Plant Sci.* **177**: 43–48.

Depicker, A. G., Jacobs, A. M., and Van Montagu, M. C. 1988. A negative selection scheme for tobacco protoplast-derived cells expressing the T-DNA gene 2. *Plant Cell Rep.* **7**: 63–66.

deRuijter, N. C. A., Verhees, J., Leeuwen, W., and Krol, A. R. 2003. Evaluation and Comparison of the GUS, LUC and GFP reporter system for gene expression studies in plants. *Plant Biol*. **5**: 103–115.

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.

Di Sansebastiano, G. P., Ul Rehman, R., and Neuhaus, J.-M. 2007. Rat β-glucuronidase as a reporter protein for the analysis of the plant secretory pathway. *Plant Biosystems* **141**: 329–336.

Dix, P. and Kavanagh, T. 1995. Transforming the plastome - genetic-markers and DNA delivery systems. *Euphytica.* **85**: 29–34.

Doshi, K. M., Eudes, E., Laroche, A., and Gaudet, D. 2007. Anthocyanin expression in marker free transgenic wheat and triticale embryos. *In Vitro Cell. Dev. Biol.-Plant* **43**: 429–435.

Dufourmantel, N., Dubald, M., Matringe, M., Canard, H., Garcon, F., Job, C., Kay, E., Wisniewski, J. P., Ferullo, J. M., Pelissier, B., Sailland, A., and Tissot, G. 2007. Generation and characterization of soybean and marker-free tobacco plastid transformants over-expressing a bacterial 4-hydroxyphenylpyruvate dioxygenase which provides strong herbicide tolerance. *Plant Biotechnol. J.* **5**: 118–133.

Dufourmantel, N., Pelissier, B., Garcon, F., Peltier, G., Ferullo, J. M., and Tissot, G. 2004. Generation of fertile transplastomic soybean. *Plant Mol. Biol.* **55**: 479–489.

Dufourmantel, N., Tissot, G., Goutorbe, F., Garçon, F., Muhr, C., Jansens, S., Pelissier, B., Peltier, G., and Dubald, M. 2005. Generation and analysis of soybean plastid transformants expressing *Bacillus thuringiensis* Cry1Ab protoxin. *Plant Mol. Biol*. **58**: 659–668.

Duque, A. S., Sousa Araújo, S., Cordeiro, M. A., Santos, D. M., and Fevereiro, M. P. 2007. Use of fused gfp and gus reporters for the recovery of transformed *Medicago truncatula* somatic embryos without selective pressure. *Plant Cell Tiss. Organ Cult.* **90**: 325–330.

Dutt, M., Lee, D. H., and Grosser, J. W. 2010. Bifunctional selection-reporter systems for genetic transformation of citrus: mannose- and kanamycin-based systems. *In Vitro Cell. Dev. Biol.-Plant.* **46**: 467–476.

Dutt, M., Li, Z. T., Dhekney, S. A., and Gray, D. J. 2008. A co-transformation system to produce transgenic grapevines free of marker genes. *Plant Sci.* **175**: 423–430.

Ebinuma, H. and Komamine, A. 2001. Mat (Multi-Auto-Transformation) vector system. The oncogenes of *Agrobacterium* as positive markers for regeneration and selection of marker-free transgenic plants. *In Vitro Cell. Devel. Biol.-Plant.* **37**: 103–113.

Ebinuma, H., Sugita, K., Endo, S., Matsunaga, E., and Yamada, K. 2005. Elimination of marker genes from transgenic plants using MAT vector systems. *Methods Mol. Biol.* **286**: 237–254.

Ebinuma, H., Sugita, K., Matsunaga, E., and Yamakado, M. 1997a. Selection of marker-free transgenic plants using the isopentenyl transferase gene. *Proc. Natl Acad. Sci. USA*. **94**: 2117–2121.

Ebinuma, H., Sugita, K., Matsunaga, E., Yamakado, M., and Komamine, A. 1997b. Principle of MAT vector system. *Plant Biotechnol.* **14**: 133–139.

Ebmeier, A., Allison, L., Cerutti, H., and Clemente, T. 2004. Evaluation of the *Escherichia coli* threonine deaminase gene as a selectable marker for plant transformation. *Planta*. **218**: 751–758.

EFSA. 2004. Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. *The EFSA Journal* **48:** 1–18.

Eichholtz, D. A., Rogers, S. G., Horsch, R. B., Klee, H. J., Hayford, M., Hoffman, N. L., Braford, S. B., Fink, C. F., Flick, J., O'Connell, K. M., and Fraley, R. T. 1987. Expression of mouse dihydrofolate reductase gene confers methotrexate resistance in transgenic petunia plants. *Som. Cell Mol. Genet.* **13**: 67–76.

Eklof, S., Astot, C., Sitbon, F., Moritz, T., Olsson, O., and Sandberg, G. 2000. Transgenic tobacco plants co-expressing *Agrobacterium* iaa and ipt genes have wild-type hormone levels but display both auxin and cytokinin-over producing phenotypes. *Plant J.* **23**: 279–284.

Elliott, A., Campbell, J., Dugdale, B., Brettell, R., and Grof, C. 1999. Green-fluorescent protein facilitates rapid *in vivo* detection of genetically transformed plant cells. *Plant Cell Rep*. **18**: 707–714.

El Ouakfaoui, S. and Miki, B. 2005. The stability of the *Arabidopsis* transcriptome in transgenic plants expressing the marker genes nptII and uidA. *Plant J.* **41**: 791–800.

El-Shemy, H. A., Teraishi, M., Khalafalla, M. M., Katsube-Tanaka, T., Utsumi, S., and Ishimoto, M. 2004. Isolation of soybean plants with stable transgene expression by visual selection based on green fluorescent protein. *Mol. Breed.* **14**: 227–238.

Endo, M., Osakabe, K., Ichikawa, H., and Toki, S. 2006. Molecular characterization of true and ectopic gene targeting events at the acetolactate synthase gene in *Arabidopsis*. *Plant Cell Physiol.* **47**: 372–379.

Endo, M., Osakabe, K., Ono, K., Handa, H., Shimizu, T., and Toki, S. 2007. Molecular breeding of a novel herbicide-tolerant rice by gene targeting. *Plant J.* **52**: 157–166.

Endo, S., Kasahara, T., Sugita, K., and Ebinuma, H. 2002a. A new GST-MAT vector containing both ipt and iaaM/H genes can produce marker-free transgenic tobacco plants with high frequency. *Plant Cell Rep.* **20**: 923–928.

Endo, S., Kasahara, T., Sugita, K., Matsunaga, E., and Ebinuma, H. 2001. The isopentenyl transferase gene is effective as a selectable marker gene for plant transformation in tobacco *(Nicotiana tabacum* cv. Petite Havana SR1) *Plant Cell Rep.* **20**: 60–66.

Endo, S., Sugita, K., Sakai, M., Tanaka, H., and Ebinuma, H. 2002b. Single-step transformation for generating marker-free transgenic rice using the *ipt*-type MAT vector system. *Plant J.* **30**: 115–122.

Erikson, O. and Hertzberg, M. 2004. A conditional marker gene allowing both positive and negative selection in plants. *Nature Biotechnology*. **22**: 455–458.

Erikson, O., Hertzberg, M., and Näsholm, T. 2005. The *dsdA* gene from *Escherichia coli* provides a novel selectable marker for plant transformation. *Plant Mol. Biol.* **57**: 425–433.

Escobar, M. A., Park, J. I., Polito, V. S., Leslie, C. A., Uratsu, S. L., McGranahan, G. H., and Dandekar, A. M. 2000. Using GFP as a scorable marker in walnut somatic embryo transformation. *Ann. Bot.* **85**: 831–835.

Fang, Y. D., Akula C., and Altpeter, F. 2002. *Agrobacterium*-mediated barley (*Hordeum vulgare* L.) transformation using green fluorescent protein as a visual marker and sequence analysis of the T-DNA: barley genomic DNA junctions. *J. Plant Physiol.* **159**: 1131–1138.

FDA. 2009. Use of antibiotic resistance marker genes in transgenic plants. http://www.fda.gov/food/guidancecomplianceregulatoryinformation/guidancedocuments/biotechnology/ucm096135.htm, accessed February 2012.

Feeney, M. and Punja, Z. K. 2003. Tissue culture and *Agrobacterium*-mediated transformation of hemp (*Cannabis sativa* L.). *In Vitro Cell. Devel. Biol.-Plant*. **39**: 578–585.

Fernandez-San Millan, A., Obregon, P., and Veramendi, J. 2011. Over-expression of peptide deformylase in chloroplasts confers actinonin resistance, but is not a suitable selective marker system for plastid transformation. *Trangenic Res.* **20**: 613–624.

Ferradini, N., Nicolia A., Capomaccio S., Veronesi F., and Rosellini D. 2011a. A point mutation in the *Medicago sativa* GSA gene provides a novel, efficient, selectable marker for plant genetic engineering. *J. Biotechnol.* **146**: 147–152.

Ferradini, N., Nicolia, A., Capomaccio, S., Veronesi, F., and Rosellini, D. 2011b. Assessment of simple marker-free genetic transformation techniques in alfalfa. *Plant Cell Rep*. **30**: 1991–2000.

Fior, S. and Gerola, P. D. 2009a. Impact of ubiquitous inhibitors on the GUS gene reporter system: evidence from the model plants *Arabidopsis*, tobacco and rice and correction methods for quantitative assays of transgenic and endogenous GUS. *Plant Methods*. **5**: 19.

Fior, S., Vianelli, A., and Gerola, P. 2009b. A novel method for fluorometric continuous measurement of β-glucuronidase (GUS) activity using 4-methylumbelliferyl-β-d-glucuronide (MUG) as substrate. *Plant Sci.* **176**: 130–135.

Fleming, A. J., Manzara, T., Gruissem, W., and Kuhlemeier, C. 1996. Fluorescent imaging of GUS activity and RT-PCR analysis of gene expression in the shoot apical meristem. *Plant J.* **10**: 745–754.

Fleming, G. H., Olivares-Fuster, O., Del-Bosco, S. F., and Grosser, J. W. 2000. An alternative method for the genetic transformation of sweet orange. *In Vitro Cell Devel. Biol. Plant* **36**: 450–455.

Fobis-Loisy, I., Chambrier, P., and Gaude, T. 2007. Genetic transformation of *Arabidopsis lyrata*: specific expression of the green fluorescent protein (GFP) in pistil tissues. *Plant Cell Rep.* **26**: 745–753.

Forner, J. and Binder, S. 2007. The red fluorescent protein eqFP611: application in subcellular localization studies in higher plants. *BMC Plant Biol.* **7**: 28.

Fraley, R. T., Rogers, S. G., Horsch, R. B., Sanders, P. R., Flick, J. S., Adams, S. P., Bittner, M. L., Brand, L. A., Fink, C. L., Fry, J. S., Gallupi, G. R., Goldberg, S. B., Hoffman, N. L., and Woo, S. C. 1983. Expression of bacterial genes in plant cells. *Proc. Natl. Acad. Sci. U.S.A.* **80**: 4803–4807.

Freyssinet, G., Pelissier, B., Freyssinet, M., and Delon, R. 1996. Crops resistant to oxynils: from the laboratory to the market. *Field Crops Res.* **45**: 125–133.

Fromm, M. E., Morrish, F., Armstrong, C., Williams, R., Thomas, J., and Klein, T. M. 1990. Inheritance and expression of chimeric genes in the progeny of transgenic maize plants. *Bio/Technology* **8**: 833–839.

Fu, D., Xiao Y., Muthukrishnan, S., and Liang, G. H. 2005. *In vivo* performance of a dual genetic marker, manA-gfp, in transgenic bentgrass. *Genome*. **48**: 722–730.

Fukuoka, H., Ogawa, T., Matsuoka, M., Ohkawa, Y., and Yano, H. 1998. Direct gene delivery into isolated microspores of rapeseed (*Brassica napus* L.) and production of fertile transgenic plants. *Plant Cell Rep.* **17**: 323–328.

Gabard, J. M., Charest, P. J., Iyer, V. N., and Miki, B. L. 1989. Cross-resistance to short residual sulfonylurea herbicides in transgenic tobacco plants. *Plant Physiol*. **91**: 574–580.

Gadaleta, A., Giancaspro, A., Blechl, A., and Blanco, A. 2006. Phosphomannose isomerase, pmi, as a selectable marker gene for durum wheat transformation. *J. Cereal Sci.* **43**: 31–37.

Ganapathi, T. R., Higgs, N. S., Balint-Kurti, P. J., Arntzen, C. J., May, G. D., and Van Eck, J. M. 2001. *Agrobacterium*-mediated transformation of embryogenic cell suspensions of the banana cultivar Rasthali (AAB). *Plant Cell Rep.* **20**: 157–162.

Gao, Z. S., Jararaj, J., Muthukrishnan, S., Claflin, L., and Liang, G. H. 2005. Efficient genetic transformation of sorghum using a visual screening marker. *Genome* **48**: 321–333.

Gao, Z., Xie X., Ling, Y., Muthukrishnan, S., and Liang, G. H. 2005. *Agrobacterium tumefaciens*–mediated sorghum transformation using a mannose selection system. *Plant Biotechnol. J.* **3**: 591–599.

Garcia, E. L. and Mourad, G. S. 2004. A site-directed mutagenesis interrogation of the carboxy-terminal end of *Arabidopsis thaliana* threonine dehydratase/deaminase reveals a synergistic interaction between two effector-binding sites and contributes to the development of a novel selectable marker. *Plant Mol. Biol.* **55**: 121–134.

Ghorbel, R., Juarez, J., Navarro, L., and Pena, L. 1999. Green fluorescent protein as a screenable marker to increase the efficiency of generating transgenic woody fruit plants. *Theor. Appl. Genet.* **99**: 350–358.

Ghosh, I., Hamilton A. D., and Regan L. 2000. Antiparallel leucine zipper-directed protein reassembly: application to the green fluorescent protein. *J. Am. Chem. Soc.* **122**: 5658–5659.

Giancaspro, A., Rosellini, D., Blanco, A., and Gadaleta, A. 2012. Gabaculine selection using bacterial and plant marker genes (GSA-AT) in durum wheat transformation. *Plant Cell Tiss. Organ Cult.*, **109**: 447–455.

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.

Goddijn, O., Schouten P., Schilperoort, R., and Hoge, J. 1993. A chimeric tryptophan decarboxylase gene as a novel selectable marker in plant cells. *Plant Mol. Biol.* **22**: 907–912.

Goldenkova, I., Musiychuk, K., Abdeev, R., and Volkova, L. 2002. The expression of the bacterial gene for xylose (glucose) isomerase in transgenic tobacco plants affects plant morphology and phytohormonal balance. *Russ. J. Plant Physiol.* **49**: 524–529.

Goldsbrough, A. P., Tong, Y., and Yoder, J. I. 1996. Lc as a non-destructive visual reporter and transposition excision marker gene for tomato. *Plant J*. **9**: 927–933.

Gossele, V., van Aarssen, R., and Cornelissen, M. 1994. A 6′-gentamicin acetyltransferase gene allows effective selection of tobacco transformants using kanamycin as a substrate. *Plant Mol. Biol.* **26**: 2009–2012.

Gough, K. C., Hawes, W. S., Kilpatrick, J., and Whitelam, G. C. 2001. Cyanobacterial GR6 glutamate-1-semialdehyde aminotransferase: a novel enzymebased selectable marker for plant transformation. *Plant Cell Rep*. **20**: 296–300.

Graham, J., McNicol, R., and Kumar, A. 1990. Use of the GUS gene as a selectable marker for *Agrobacterium*-mediated transformation of *Rubus*. *Plant Cell Tiss. Organ Cult.* **20**: 35–39.

Griesbeck, O., Baird, G. S., Campbell, R. E., Zacharias, D. A., and Tsien, R. Y. 2001. Reducing the environmental sensitivity of yellow fluorescent protein. *J. Biol. Chem*. **276**: 29188–29194.

Grotewold, E., Sainz, M. B., Tagliani, L., Hernandez, J. M., Bowen, B., and Candler, V. L. 2000. Identification of the residues in the Myb domain of maize C1 that specify the interaction with the bHLH cofactor R. *Proc. Natl Acad. Sci. USA* **97**: 13579–13584.

Guerineau, F., Brooks, L., Meadows, J., Lucy, A., Robinson, C., and Mullineaux, P. 1990. Sulfonamide resistance gene for plant transformation. *Plant Mol. Biol.* **15**: 127–136.

Guillet, G., Poupart, J., Basurco, J., and De Luca, V. 2000. Expression of tryptophan decarboxylase and tyrosine decarboxylase genes in tobacco results in altered biochemical and physiological phenotypes. *Plant Physiol.* **122**: 933–944.

Guo, X.-M., Zhang, X.-D., Liang, R.-Q., Zhang, L.-Q., and Chen, Y.-F. 2007. Maize transformation using xylose isomerase gene as a selection marker. *J. Plant Physiol. Mol. Biol.* **33**: 547–552.

Gurskaya, N. G., Fradkov, A. F., Terskikh, A., Matz, M. V., Labas, Y. A., Martynov, V. I., Yanushevich, Y. G., Lukyanov, K. A., and Lukyanov, S. A. 2001. GFP-like chromoproteins as a source of far-red fluorescent proteins. *FEBS Letters*. **507**: 16–20.

Haldrup, A., Noerremark, M., and Okkels, F. T. 2001. Plant selection principle based on xylose isomerase. *In vitro Cell. Devel. Biol.-Plant* **37**: 114–119.

Haldrup, A., Petersen, S. G., and Okkels, F. T. 1998a. The xylose isomerase gene from *Thermoanaerobacterium thermosulfurogenes* allows effective selection of transgenic plant cells using D-xylose as the selection agent. *Plant Mol. Biol.* **37**: 287–296.

Haldrup, A., Petersen, S. G., and Okkels, F. T. 1998b. Positive selection: a plant selection principle based on xylose isomerase, an enzyme used in the food industry. *Plant Cell Rep.* **18**: 76–81.

Hamza, S., Camilleri, C., Pollien, J., Vaucheret, H., Bourgin, J., and Chupeau, Y. 1993. Selection for spontaneous tomato haploids using a conditional-lethal marker. *Theor. Appl. Genet.* **86**: 657–664.

Hanin, M., Volrath, S., Bogucki, A., Briker, M., Wark, E., and Paszkowski, J. 2001. Gene targeting in *Arabidopsis*. *Plant J*. **28**: 671–677.

Hanson, B., Engler, D., Moy, Y., Newman, B., Ralston, E., and Gutterson, N. 1999. A simple method to enrich an *Agrobacterium*-transformed population for plants containing only T-DNA sequences. *Plant J.* **19**: 727–734.

Haseloff, J. and Siemering, K. R. 1998. The uses of GFP in plants. In: *Green fluorescence protein: strategies, applications and protocols* (Chale, M., and Kain, S., eds.). New York, Wiley-Liss, pp. 191–220.

Haseloff, J., Siemering, K. R., Prasher, D. C., and Hodge, S. 1997. Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic *Arabidopsis* plants brightly. *Proc. Natl. Acad. Sci. U.S.A.* **94**: 2122–2127.

Hawkes, T., Pline-Srnic, W., Dale, R., Friend, E., Hollinshead, T., Howe, P., Thompson, P., Viner, R., and Greenland, A. 2011. D-glufosinate as a male sterility agent for hybrid seed production. *Plant Biotechnol. J.* **9**: 301–314.

Hayford, M. B., Medford, J. I., Hoffman, N. L., Rogers, S. G., and Klee, H. J. 1988. Development of a plant transformation selection system based on expression of genes encoding gentamicin acetyltransferases. *Plant Physiol.* **86**: 1216–1222.

He, Z., Duan, Z., Liang, W., Chen, F., Yao, W., Liang, H., Yue, C., Sun, Z., Chen, F., and Dai, J. 2006. Mannose selection system used for cucumber transformation. *Plant Cell Rep*. **25**: 953–958.

He, Z. Q., Fu, Y. P., Si, H. M., Hu, G. C., 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.

Heidmann, I., de Lange, B., Lambalk, J., Angenent, G. C., and Boutilier, K. 2011. Efficient sweet pepper transformation mediated by the BABY BOOM transcription factor. *Plant Cell Rep*. **30**: 1107–1115.

Helmer, G., Casadaban, M., Bevan, M., Kayes, L., and Chilton, M.-L., 1984. A new chimeric gene as a marker for plant transformation: the expression of *Escherichia coli* β-galactosidase in sunflower and tobacco cells. *Biotechnology* June: 520–527.

Hernandez-Garcia, C. M., Bouchard, R. A., Rushton, P. J., Jones, M. L., Chen, X., Timko, M. P., and Finer, J. J. 2010. High level transgenic expression of soybean (*Glycine max*) GmERF and Gmubi gene promoters isolated by a novel promoter analysis pipeline. *BMC Plant Biol*. **10**: 237.

Herrera-Estrella, L., De Block, M., Messens, E., Hernalsteen, J.-P., Van Montagu, M., and Schell, J. 1983. Chimeric genes as dominant selectable markers in plant cells. *EMBO J.* **2**: 987–995.

Hille, J., Verheggen, F., Roelvink, P., Franssen, H., vanKammen, A., and Zabel, P. 1986. Bleomycin resistance: a new dominant selectable marker for plant cell transformation. *Plant Mol. Biol.* **7**: 171–176.

Hood, E. E. 2003. Selecting the fruits of your labors. *Trends Plant Sci*. **8**: 357–358.

Hou, C.-X., Dirk, L. M. A., Pattanaik, S., Das, N. C., Maiti, I. B., Houtz, R. L., and Williams, M. A. 2007. Plant peptide deformylase: a novel selectable marker and herbicide target based on essential cotranslational chloroplast protein processing. *Plant Biotechnol. J.* **5**: 275–281.

Howe, A. R., Gasser, C. S., Brown, S. M., Padgette, S. R., Hart, J., Parker, G. B., Fromm, M. E., and Armstrong, C. L. 2002. Glyphosate as a selective agent for the production of fertile transgenic maize (*Zea mays* L.) plants. *Mol. Breed*. **10**: 153–164.

Hsiao, P., Sanjaya, Su. R.-C., da Silva, J. A. T., and Chan, M.-T. 2007. Plant native tryptophan synthase beta 1 gene is a non-antibiotic selection marker for plant transformation. *Planta*. **225**: 897–906.

Hu, C.-D., Chinenov, Y., and Kerppola, T. K. 2002. Visualization of interactions among bZIP and Rel family proteins in living cells using bimolecular fluorescence complementation. *Mol. Cell* **9**: 789–798.

Hu, C.-yeh., Chee, P., Chesney, R., Zhou, J., Miller, P., and O'Brien, W. T. 1990. Intrinsic GUS-like activities in seed plants. *Plant Cell Rep.* **9**:1–5.

Hu, T., Metz, S., Chay, C., Zhou, H. P., Biest, N., Chen, G., Cheng, M., Feng, X., Radionenko, M., Lu, F., and Fry, J. 2003. *Agrobacterium*-mediated large-scale transformation of wheat (*Triticum aestivum* L.) using glyphosate selection. *Plant Cell Rep.* **21**: 1010–1019.

Huang, F. C., Klaus, S. M., Herz, S., Zou, Z., Koop, H. U., and Golds, T. J. 2002. Efficient plastid transformation in tobacco using the aphA-6 gene and kanamycin selection. *Mol. Genet. Genomics* **268**: 19–27.

Huber, M., Hahn, R., and Hess, D. 2002. High transformation frequencies obtained from a commercial wheat (*Triticum aestivum* L. cv. 'Combi') by microbombardment of immature embryos followed by GFP screening combined with PPT selection. *Mol. Breed*. **10**: 19–30.

Hudson, L. C. and Stewart, C. N., Jr. 2004. Effects of pollen synthesized green fluorescent protein on pollen grain fitness. *Sex. Plant Reprod.* **17**: 49–53.

Hunter, P. R., Craddock, C. P., Di Benedetto, S., Roberts, L. M., and Frigerio, L. 2007. Fluorescent reporter proteins for the tonoplast and the vacuolar lumen identify a single vacuolar compartment in *Arabidopsis* cells. *Plant Physiol*. **145**: 1371–1382.

Inui, H., Yamada, R., Yamada, T., Ohkawa, Y., and Ohkawa, H. 2005. A selectable marker using cytochrome P450 monooxygenases for *Arabidopsis* transformation. *Plant Biotechnol*. **22**: 281–286.

Irdani, T., Bogani, P., Mengoni, A., Mastromei, G., and Buiatti, M. 1998. Construction of a new vector conferring methotrexate resistance in *Nicotiana tabacum* plants. *Plant Mol. Biol.* **37**: 1079–1084.

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.

Jacobsen, E. and Schouten, H. J. 2007. Cisgenesis strongly improves introgression breeding and induced translocation breeding of plants. *Trends Biotechnol.* **25**: 219–223.

Jain, M., Chengalrayan, K., Abouzid, A., and Gallo, M. 2007. Prospecting the utility of a PMI/mannose selection system for the recovery of transgenic sugarcane (*Saccharum* spp. hybrid) plants. *Plant Cell Rep*. **26**: 581–590.

Jefferson, R. A., Kavanaugh, T. A., and Bevan, M. W. 1987. GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.* **6**: 3901–3907.

Jelenska, J., Tietze, E., Tempe, J., and Brevet, J. 2000. Streptothricin resistance as a novel selectable marker for transgenic plant cells. *Plant Cell Rep*. **19**: 298–303.

Jia, H., Liao, M., Verbelen, J.-P., and Vissenberg, K. 2007. Direct creation of marker-free tobacco plants from agroinfiltrated leaf discs. *Plant Cell Rep.* **26**: 1961–1965.

Joersbo, M., Donaldson, I., Kreiberg, J., Petersen, S. G., Brunstedt, J., and Okkels, F. T. 1998. Analysis of mannose selection used for transformation of sugar beet. *Mol. Breed.* **4**: 111–117.

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 uridyltransferase gene as selective gene. *Mol. Breed* **11**: 315–323.

Joersbo, M., Mikkelsen, J. D., and Brunstedt, J. 2000. Relationship between promoter strength and transformation frequencies using mannose selection for the production of transgenic sugar beet. *Mol. Breed.* **6**: 207–213.

Joersbo, M. and Okkels, F. T. 1996. A novel principle for selection of transgenic plant cells: Positive selection. *Plant Cell Rep.* **16**: 219–221.

Jordan, M. C. 2000. Green fluorescent protein as a visual marker for wheat transformation. *Plant Cell Rep*. **19**: 1069–1075.

Joshi, M., Niu, C., Fleming, G., Hazra, S., Chu, Y., Nairn, C. J., Yang, H., and Ozias-Akins, P. 2005. Use of green fluorescent protein as a non-destructive marker for peanut genetic transformation. *In Vitro Cell. Dev. Biol.-Plant.* **41**: 437–445.

Kaeppler, H. F., Carlson, A. R., and Menon, G. K. 2001. Routine utilization of green fluorescent protein as a visual selectable marker for cereal transformation. *In vitro Cell. Devel. Biol.-Plant* **37**: 120–126.

Kaeppler, H. F., Menon, G. K., Skadsen, R. W., Nuutila, A. M., and Carlson, A. R. 2000. Transgenic oat plants via visual selection of cells expressing green fluorescent protein. *Plant Cell Rep*. **19**: 661–666.

Kakimoto, T. 1996. CKI1, a histidine kinase homolog implicated in cytokinin signal transduction. *Science*. **274**: 982–985.

Kanamoto, H., Yamashita, A., Asao, H., Okumura, S., Takase, H., Hattori, M., Yokota, A., and Tomizawa, K. 2006. Efficient and stable transformation of *Lactuca sativa* L. cv. Cisco (lettuce) plastids. *Transgenic Res.* **15**: 205–217.

Kang, B.-guk., Ye, X., Osburn, L. D., Stewart, C. N. Jr., and Cheng, Z.-M. 2010. Transgenic hybrid aspen overexpressing the Atwbc19 gene encoding an ATP-binding cassette transporter confers resistance to four aminoglycoside antibiotics. *Plant Cell Rep.* **29**: 643–650.

Karlin-Neumann, G. A., Brusslan, J. A., and Tobin, E. M. 1991. Phytochrome control of the tms2 gene in transgenic *Arabidopsis*: a strategy for selecting mutants in the signal transduction pathway. *Plant Cell* **3**: 573–582.

Kawahigashi, H., Hirose, S., Hayashi, E., Ohkawa, H., and Ohakwa, Y. 2002. Phytotoxicity and metabolism of ethofumesate and in transgenic rice plants expressing the human *CYP2B6* gene. *Pestic. Biochem. Physiol.* **74**: 139–147.

Kawai, K., Kaku, K., Izawa, N., Shimizu, M., Kobayashi H., and Shimizu, T. 2010. Transformation of *Arabidopsis* by mutated acetolactate synthase genes from rice and *Arabidopsis* that confer specific resistance to pyrimidinylcarboxylate-type ALS inhibitors. *Plant Biotechnol*. **27**: 75–84.

Kemper, E., Grevelding, C., Schell, J., and Masterson, R. 1992. Improved method for the transformation of *Arabidopsis thaliana* with chimeric dihydrofolate reductase constructs which confer methotrexate resistance. *Plant Cell Rep.* **11**: 118–121.

Kerppola,T. K. 2008. Bimolecular Fluorescence Complementation: Visualization of molecular interactions in living cells. *Methods Cell Biol.* **85**: 431–470.

Kertbundit, S., De Greve, H., Deboeck, F., Van Montagu, M., and Hernalsteens, J. P. 1991. *In vivo* random beta-glucuronidase gene fusions in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U.S.A.* **88**: 5212–5216.

Khan, M. S. and Maliga, P. 1999. Fluorescent antibiotic resistance marker for tracking plastid transformation in higher plants. *Nat. Biotechnol.* **17**: 910–915.

Khan, R. S., Chin, D. P., Nakamura, I., and Mii, M. 2006. Production of marker-free transgenic *Nierembergia caerulea* using MAT vector system. *Plant Cell Rep*. **25**: 914–919.

Khan, R. S., Ntui, V. O., Chin, D. P., Nakamura, I., and Mii, M. 2011. Production of marker-free disease-resistant potato using isopentenyl transferase gene as a positive selection marker. *Plant Cell Rep*. **30**: 587–597.

Khan, R. S., Thirukkumaran, G., Nakamura, I., and Mii, M. 2010. Rol (root loci) gene as a positive selection marker to produce marker-free *Petunia hybrida*. *Plant Cell Tiss. Organ Cult.* **101**: 279–285.

Kieffer, F., Triouleyre, C., Bertsch, C., Farine, S., Leva, Y., and Walter, B. 2004. Mannose and xylose cannot be used as selectable agents for *Vitis vinifera* L. transformation. *Vitis* **43**: 35–39.

Kiegle, E., Moore, C., Haseloff, J., Tester, M., and Knight, M. 2000. Cell-type-specific calcium responses to drought, salt and cold in the *Arabidopsis* root. *Plant J*. **23**: 267–278.

Kim, C. Y., Ahn, Y. O., Kim, S. H., Kim, Y.-H., Lee, H.-S., Catanach, A. S., Jacobs, J. M. E., Conner, A. J., and Kwak, S.-S. 2010. The sweet potato IbMYB1 gene as a potential visible marker for sweet potato intragenic vector system. *Physiol. Plant.* **139**: 229–240.

Kim, J. Y., Jung, M., Kim, H. S., Lee, Y. H., Choi, S. H., Lim, Y. P., Min, B. W., Yang, S. G., and Harn, C. H. 2002. A new selection system for pepper regeneration by mannose. *J. Plant Biotechnol.* **4**: 129–134.

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.

Kittiwongwattana, C., Lutz, K. A., Clark, M., and Maliga, P. 2007. Plastid marker gene excision by the phiC31 phage site-specific recombinase. *Plant Mol. Biol.* **64**: 137–143.

Klaus, S. M., Huang, F. C., Eibl, C., Koop, H. U., and Golds, T. J. 2003. Rapid and proven production of transplastomic tobacco plants by restoration of pigmentation and photosynthesis. *Plant J.* **35**: 811–821.

Kobayashi, T. H. S., Stougaard, J., and Ichikawa, H. 1995. A conditional negative selection for *Arabidopsis* expressing a bacterial cytosine deaminase gene. *Jpn. J. Genet.* **70**: 409–422.

Koehler, R. H., Cao, J., Zipfel, W. R., Webb, W. W., and Hanson, M. R. 1997b. Exchange of protein molecules through connections between higher plant plastids. *Science* **276**: 2039–2042.

Koehler, R. H., Zipfel, W. R., Webb, W. W., and Hanson, M. R. 1997a. The green fuorescent protein as a marker to visualize plant mitochondria *in vivo*. *Plant J.* **11**: 613–621.

Koh, S., Kim, H., Kim, J., Goo, E., Kim, Y.-J., Choi, O., Jwa, N.-S., Ma, J., Nagamatsu, T., Moon, J. S., and Hwang, I. 2011. A novel light-dependent selection marker system in plants. *Plant Biotechnol. J.* **9**: 348–358.

Koizumi, N. 2003. A chimeric tunicamycin resistance gene as a new selectable marker for *Arabidopsis thaliana*. *Plant Biotechnol*. **20**: 305–309.

Koltunow, A. M., Truettner, J., Cox, K. H., Wallroth, M., and Goldberg, R. B. 1990. Different temporal and spatial gene expression patterns occur during anther development. *Plant Cell* **2**: 1201–1224.

Koncz, C., Olsson, O., Langridge, W. H. R., Schell, J., and Szalay, A. A. 1987. Expression and assembly of functional bacterial luciferase in plants. *Proc. Natl. Acad. Sci. U.S.A.* **84**: 131–135.

Kondrák, M., Meer, I. M., and Bánfalvi, Z. 2006. Generation of marker- and backbone-free transgenic potatoes by site-specific recombination and a bifunctional marker gene in a non-regular one-border agrobacterium transformation vector. *Trangenic Res*. **15**: 729–737.

Koning, A., Jones, A., Fillatti, J. J., Comai, L., and Lassner, M. W. 1992. Arrest of embryo development in *Brassica napus* mediated by modified *Pseudomonas aeruginosa* exotoxin A. *Plant Mol. Biol.* **18**: 247–256.

Koo, J., Kim, Y., Kim, J., Yeom, M., Lee, I. C., and Nam, H. G. 2007. A GUS/Luciferase fusion reporter for plant gene trapping and for assay of promoter activity with luciferin-dependent control of the reporter protein stability. *Plant Cell Physiol.* **48**: 1121–1131.

Kooshki, M., Mentewab, A., and Stewart, C. N., Jr. 2003. Pathogen inducible reporting in transgenic tobacco using a GFP construct. *Plant Sci.* **165**: 213–219.

Koprek, T., McElroy, D., Louwerse, J., Williams-Carrier, R., and Lemaux, P. G. 1999. Negative selection systems for transgenic barley (*Hordeum vulgare* L.): comparison of bacterial codA- and cytochrome P450 gene-mediated selection. *Plant J.* **19**: 719–726.

Kortstee, A. J., Khan, S. A., Helderman, C., Trindade, L. M., Wu, Y., Visser, R. G. F., Brendolise, C., Allan, A., Schouten, H. J., and Jacobsen, E. 2011. Anthocyanin production as a potential visual selection marker during plant transformation. *Transgenic Res.* **20**: 1253–1264.

Krebs, M., Held, K., Binder, A., Hashimoto, K., Den Herder, G., Parniske, M., Kudla, J., and Schumacher, K. 2012. FRET-based genetically encoded sensors allow high-resolution live cell imaging of Ca2+ dynamics. *Plant J.* **69**: 181–192.

Ku, J.-J., Park, Y.-H., and Park, Y.-D. 2006. A non-antibiotic selection system uses the phosphomannose-isomerase (PMI) gene for *Agrobacterium*-mediated transformation of Chinese cabbage. *J. Plant Biol.* **49**: 115–122.

Kumar, S., Dhingra, A., and Daniell, H. 2004a. Plastid expressed betaine aldehyde dehydrogenase gene in carrot cultured cells, roots and leaves confers enhanced salt tolerance. *Plant Physiol.* **136**: 2843–2854.

Kumar, S., Dhingra, A., and Daniell, H. 2004b. Stable transformation of the cotton plastid genome and maternal inheritance of transgenes. *Plant Mol. Biol.* **56**: 203–216.

Kumar, S. and Thompson, W. F. 2009. Simultaneous excision of two transgene flanking sequences and resolution of complex integration loci. *Plant Mol. Biol.* **69**: 23–32.

Kunkel, T., Niu, Q. W., Chan, Y. S., and Chua, N. H. 1999. Inducible isopentenyl transferase as a high-efficiency marker for plant transformation. *Nat. Biotechnol.* **17**: 916–919.

Kunze, I., Ebneth, M., Heim, U., Geiger, M., Sonnewald, U., and Herbers, K. 2001. 2-Deoxyglucose resistance: a novel selection marker for plant transformation. *Mol. Breed.* **7**: 221–227.

Kuvshinov, V., Koivu, K., Kanerva, A., and Pehu, E. 2001. Molecular control of transgene escape from genetically modified plants. *Plant Sci.* **160**: 517–522.

LaFayette, P. R., Kane, P. M., Phan, B. H., and Parrot, W. A. 2005. Arabitol dehydrogenase as a selectable marker for rice. *Plant Cell Rep.* **24**: 596–602.

Lai, F.-M., Privalle, L., Mei, K., Ghoshal, D., Shen, Y., Klucinec, J., Daeschner, K., Mankin, L. S., Chen, N., Cho, S., and Jones, T. 2011. Evaluation of the *E. coli* d-serine ammonia lyase gene (*Ec. dsdA*) for use as a selectable marker in maize transformation. *In Vitro Cell. Dev. Biol.-Plant.* **47**: 467–479.

Lamblin, F., Aime, A., Hano, C., Roussy, I., Domon, J.-M., Van Droogenbroeck, B., and Laine, E. 2007. The use of the phosphomannose isomerase gene as alternative selectable marker for *Agrobacterium*-mediated transformation of flax (*Linum usitatissimum*). *Plant Cell Rep.* **26**: 765–772.

Lang, C., Schulze, J., Mendel, R.-R., and Hänsch, R. 2006. HaloTag: a new versatile reporter gene system in plant cells. *J. Exp. Bot.* **57**: 2985–2992.

Lardet, L., Leclercq, J., Bénistan, E., Dessailly, F., Oliver, G., Martin, F., and Montoro, P. 2011. Variation in GUS activity in vegetatively propagated *Hevea brasiliensis* transgenic plants. *Plant Cell Rep.* **30**: 1847–1856.

Leclercq, J., Lardet, L., Martin, F., Chapuset, T., Oliver, G., and Montoro, P. 2010. The green fluorescent protein as an efficient selection marker for *Agrobacterium tumefaciens*-mediated transformation in *Hevea brasiliensis* (Müll. Arg). *Plant Cell Rep.* **29**: 513–522.

Lee, K. Y., Townsend, J., Tepperman, J., Black, M., Chui, C.-F., Mazur, B., Dunsmuir, P., and Bedbrook, J. 1988. The molecular basis of sulfonylurea herbicide resistance in tobacco. *EMBO J.* **7**: 1241–1248.

Lee, S. M., Kang, K. S., Chung, H., Yoo, S. H., Xu, X. M., Lee, S. B., Cheong, J. J., Daniell, H., and Kim, M. 2006. Plastid transformation in the monocotyledonous cereal crop, rice (*Oryza sativa*) and transmission of transgenes to their progeny. *Mol. Cells* **21**: 401–410.

Leffel, S. M., Mabon, S. A., and Stewart, C. N., Jr. 1997. Applications of green fluorescent protein in plants. *BioTechniques* **23**: 912–918.

Lelivelt, C. L. C., McCabe, M. S., Newell, C. A., deSnoo, C. B., van Dun, K. M. P., Birch-Machin, I., Gray, J. C., Mills, K. H. G., and Nugent, J. M. 2005. Stable plastid transformation in lettuce (*Lactuca sativa* L.). *Plant Mol. Biol.* **58**: 763–774.

Leyman, B., Avonce, N., Ramon, M., Van Dijck, P., Iturriaga, G., and Thevelein, J.M. 2006. Trehalose-6-phosphate synthase as an intrinsic selection marker for plant transformation. *J. Biotechnol.* **121**: 309317.

Li, H.-M., Culligan, K., Dixon, R. A., and Chory, J. 1995. *CUE1*: a mesophyll cell-specific positive regulator of light-controlled gene expression in *Arabidopsis*. *Plant Cell* **7**: 1599–1610.

Li, H.-Q., Kang, P.-J., Li, M.-L., and Li, M.-R. 2007. Genetic transformation of *Torenia fournieri* using the PMI/mannose selection system. *Plant Cell Tiss. Organ Cult.* **90**: 103–109.

Li, W., Ruf, S., and Bock, R. 2011. Chloramphenicol acetyltransferase as selectable marker for plastid transformation. *Plant Mol. Biol.* **76:** 443–451.

Li, X. G., Volrath, S. L., Nicholl, D. B. G., Chilcott, C. E., Johnson, M. A., Ward, E. R., and Law, M. D. 2003. Development of protoporphyrinogen oxidase as an efficient selection marker for *Agrobacterium tumefaciens*-mediated transformation of maize. *Plant Physiol.* **133**: 736–747.

Li, Z., Hayashimoto, A., and Murai, N. 1992a. A sulfonylurea herbicide resistance gene from *Arabidopsis thaliana* as a new selectable marker for production of fertile transgenic rice plants. *Plant Physiol.* **100**: 662–668.

Li, Z., Jayasankar, S., and Gray, D. J. 2001. Expression of a bifunctional green fluorescent protein (GFP) fusion marker under the control of three constitutive promoters and enhanced derivatives in transgenic grape (*Vitis vinifera*). *Plant Sci.* **160**: 877–887.

Li, Z. T., Dhekney, S. A., and Gray, D. J. 2011. Use of the VvMybA1 gene for non-destructive quantification of promoter activity via color histogram analysis in grapevine (*Vitis vinifera*) and tobacco. *Trangenic Res.* **20:** 1087–1097.

Li, Z. Y., Upadhyaya, N. M., Meena, S., Gibbs, A. J., and Waterhouse, P. M. 1997. Comparison of promoters and selectable marker genes for use in Indica rice transformation. *Mol. Breed.* **3**: 1–14.

Liljeroth, E., Jansson, H., and Schafer, W. 1993. Transformation of *Bipolaris sorokiniana* with the gus gene and use for studying fungal colonization of barley roots. *Phytopathology* **83**: 1484–1489.

Lin, M.-H., Gresshoff, P. M., Indrasumunar, A., and Ferguson, B. J. 2011. pHairyRed: a novel binary vector containing the DsRed2 reporter gene for visual selection of transgenic hairy roots. *Molecular Plant* **4**: 537–545.

Liu, C.W., Lin, C. C., Chen, J. J., and Tseng, M. J. 2007. Stable chloroplast transformation in cabbage (*Brassica oleracea* L. var. capitata L.) by particle bombardment. *Plant Cell Rep*. **26**: 1733–1744.

Liu, H.-S., Jan, M.-S., Chou, C.-K., Chen, P.-H., and Ke, H.-J. 1999. Is green fluorescent protein toxic to the living cells? *Biochem. Biophys. Res. Commun.* **260**: 712–717.

Liu, T., Liu, L., Hou, J., Li, G., Gao, S., and Chen, J. 2010. Expression of green fluorescent protein in *Curvularia lunata* causing maize leaf spot. *Can. J. Plant Pathol.* **32**: 225–228.

Lonsdale, D. M., Lindup, S., Moisan, L. J., and Harvey, A. J. 1998. Using firefly luciferase to identify the transition from transient to stable expression in bombarded wheat scutellar tissue. *Physiol. Plant.* **102**: 447–453.

Lopez-Noguera, S., Petri, C., and Burgos, L. 2009. Combining a regeneration-promoting ipt gene and site-specific recombination allows a more efficient apricot transformation and the elimination of marker genes. *Plant Cell Rep.* **28**: 1781–1790.

Lucca, P., Ye, X., and Potrykus, I. 2001. Effective selection and regeneration of transgenic rice plants with mannose as selective agent. *Mol. Breed.* **7**: 43–49.

Ludwig, S. R., Bowen, B., Beach, L., and Wessler, S. R. 1990. A regulatory gene as a novel visible marker for maize transformation. *Science* **247**: 449–450.

Lukyanov, K. A., Fradkov, A. F., Gurskaya, N. G., Matz, M. V., Labas, Y. A., Savitsky, A. P., Markelov, M. L., Zaraisky, A. G., Zhao, X., Fang, Y., Tan, W., and Lukyanov, S. A. 2000. Natural animal coloration can be determined by a nonfluorescent green fluorescent protein homolog. *J. Biol. Chem.* **275**: 25879–25882.

Luo, K., Zheng, X., Chen, Y., Xiao, Y., Zhao, D., McAvoy, R., Pei, Y., and Li, Y. 2006. The maize Knotted1 gene is an effective positive selectable marker gene for *Agrobacterium*-mediated tobacco transformation. *Plant Cell Rep*. **25**: 403–409.

Lusk, J. L. and Sullivan, P. 2002. Consumer acceptance of genetically modified foods. *Food Technol*. **56:** 32–37.

Maier-Greiner, U. H., Klaus, C. B. A., Estermaier, L. M., and Hartmann, G. R. 1991. Herbicide resistance in transgenic plants through the degradation of the phytotoxin to urea. *Angew. Chem.* Int. Ed. Engl. **30**: 1314–1315.

Maliga, P., Svab, Z., Harper, E. C., and Jones, J. D. G. 1988. Improved expression of streptomycin resistance in plants due to a deletion in the streptomycin phosphotransferase coding sequence. *Mol. Gen. Genet.* **214**: 456–459.

Manabe, Y., Tinker, N., Colville, A., and Miki, B. 2007. CSR1, the sole target of imidazolinone herbicide in *Arabidopsis thaliana*. *Plant Cell Physiol*. **48**: 1340–1358.

Mariani, C., De Beuckeleer, M., Truettner, J., Leemans, J., and Goldberg, R. B. 1990. Induction of male sterility in plants by a chimaeric ribonuclease gene. *Nature* **347**: 737–741.

Mariani, C., Gossele, V., De Beuckeleer, M., De Block, M., Goldberg, R. B., De Greef, W., and Leemans, J. 1992. A chimaeric ribonuclease-inhibitor gene restores fertility to male sterile plants. *Nature* **357**: 384–387.

Marton, I., Zuker, A., Shklarman, E., Zeevi, V., Tovkach, A., Roffe, S., Ovadis, M., Tzfira, T., and Vainstein, A. 2010. Nontransgenic genome modification in plant cells. *Plant Physiol.* **154**: 1079–1087.

Mathur, J., Radhamony, R., Sinclair, A. M., Donoso, A., Dunn, N., Roach, E., Radford, D., Mohaghegh, P. S. M., Logan, D. C., Kokolic, K., and Mathur, N. 2010. mEosFP-based green-to-red photoconvertible subcellular probes for plants. *Plant Physiol.* **154:** 1573–1587.

Matsunaga, E., Sugita, K., and Ebinuma, H. 2002. Asexual production of selectable marker-free transgenic woody plants, vegetatively propagated species. *Mol. Breed.* **10**: 95–106.

Matsuo, N., Makino, M., and Banno, H. 2011. *Arabidopsis* ENHANCER OF SHOOT REGENERATION (ESR)1 and ESR2 regulate *in vitro* shoot regeneration and their expressions are differentially regulated. *Plant Sci*. **181**: 39–46.

Matz, M. V., Fradkov, A. F., Labas, Y. A., Savitsky, A. P., Zaraisky, A. G., Markelov, M. L., and Lukyanov, S. A. 1999. Fluorescent proteins from non bioluminescent *Anthozoa* species. *Nat. Biotechnol.* **17**: 969–973.

Maughan, S. C. and Cobbett, C. S. 2003. Methionine sulfoximine, an alternative selection for the bar marker in plants. *J. Biotechnol.* **102**: 125–128.

Maximova, S., Miller, C., de Mayolo, G. A., Pishak, S., Young, A., and Guiltinan, M. J. 2003. Stable transformation of *Theobroma cacao* L. and influence of matrix attachment regions on GFP expression. *Plant Cell Rep*. **21**: 872–883.

Mayfield, S. P. and Schultz, J. 2004. Development of a luciferase reporter gene, luxCt, for *Chlamydomonas reinhardtii* chloroplast. *Plant J*. **37**: 449–458.

McHughen, A. 1989. *Agrobacterium*-mediated transfer of chlorsulfuron resistance to commercial flax cultivars. *Plant Cell Rep*. **8**: 445–449.

Mentewab, A. and Stewart, C. 2005. Overexpression of an *Arabidopsis thaliana* ABC transporter confers kanamycin resistance to transgenic plants. *Nat. Biotechnol*. **23**: 1177–1180.

Merzlyak, E. M., Goedhart, J., Shcherbo, D., Bulina, M. E., Shcheglov, A. S., Fradkov, A. F., Gaintzeva, A., Lukyanov, K. A., Lukyanov, S., Gadella, T. W. J., and Chudakov, D. M. 2007. Bright monomeric red fluorescent protein with an extended fluorescence lifetime. *Nat. Methods* **4**: 555–557.

Metz, P. L. J. and Nap, J. P. 1997. A transgene-centred approach to the biosafety of transgenic plants: Overview of selection and reporter genes. *Acta Bot. Neerland.* **46**: 25–50.

Midorikawa, K., Nagatoshi, Y., and Nakamura, T. 2009. A selection system for transgenic Arabidopsis thaliana using potassium thiocyanate as the selective agent and AtHOL1 as the selective marker. *Plant Biotechnol*. **26**: 341–344.

Mihálka, V., Balázs, E., and Nagy, I. 2003. Binary transformation systems based on «shooter» mutants of *Agrobacterium tumefaciens*: a simple, efficient and universal gene transfer technology that permits marker gene elimination. *Plant Cell Rep.* **21**: 778–784.

Mikhailov, R. V., Muratova, S. A., and Dolgov, S. V. 2007. Production of transgenic plum plants from vegetative tissues by means of positive selection. *Acta Hort.* **734**: 129–138.

Miki, B., Abdeen, A., Manabe, Y., and MacDonald, P. 2009. Selectable marker genes and unintended changes to the plant transcriptome. *Plant Biotechnol. J.* **7**: 211–218.

Miki, B. and McHugh, S. 2004. Selectable marker genes in transgenic plants: applications, alternatives and biosafety. *J. Biotechnol.* **107**: 193–232.

Miller, M., Tagliani, L., Wang, N., Berka, B., Bidney, D., and Zhao, Z. Y. 2002. High efficiency transgene segregation in co-transformed maize plants using an *Agrobacterium tumefaciens* 2 T-DNA binary system. *Transgenic Res.* **11**: 381–396.

Milligan, A. S., Daly, A., Parry, M. A. J., Lazzeri, P. A., and Jepson, I. 2001. The expression of a maize glutathione S-transferase gene in transgenic wheat confers herbicide tolerance, both *in planta* and *in vitro*. *Mol. Breed.* **7**: 301–315.

Millwood, R. J., Halfhill, M. D., Harkins, D., Russotti, R., and Stewart, C. N., Jr. 2003. Instrumentation and methodology for quantifying GFP fluorescence in intact plant organs. *BioTechniques*. **34**: 638–643.

Min, B.-W., Cho, Y.-N., Song, M.-J., Noh, T.-K., Kim, B.-K., Chae, W.-K., Park, Y.-S., Choi, Y.-D., and Harn, C.-H. 2007. Successful genetic transformation of Chinese cabbage using phosphomannose isomerase as a selection marker. *Plant Cell Rep.* **26**: 337–344.

Mirabella, R., Franken, C., van der Krogt, G. N. M., Bisseling, T., and Geurts, R. 2004. Use of the fluorescent timer DsRED-e5 as reporter to monitor dynamics of gene activity in plants. *Plant Physiol*. **135**: 1879–1887.

Miyawaki, A. 2002. Green fluorescent protein-like proteins in reef Anthozoa animals. *Cell Struct. Funct.* **27**: 343–347.

Miyawaki, A., Llopis, J., Heim, R., McCaffery, J. M., Adams, J. A., Ikura, M., and Tsien, R. Y. 1997. Fluorescent indicators for Ca^{2+} based on green fluorescent proteins and calmodulin. *Nature* **388**: 882–887.

Molinier, J., Thomas, C., Brignou, M., and Hanhe, G. 2002. Transient expression of ipt gene enhances regeneration and transformation rates of sunflower shoot apices (*Helianthus annuus* L.). *Plant Cell Rep*. **21**: 251–256.

Moore, S. K. and Srivastava, V. 2008. A bacterial haloalkane dehalogenase (dhlA) gene as conditional negative selection marker for rice callus cells. *In vitro Cell. Devel. Biol.-Plant* **44**: 468–473.

Mudge, S., Lewis-Henderson, W., and Birch, R. 1996. Comparison of *Vibrio* and firefly luciferases as reporter gene systems for use in bacteria and plants. *Functional Plant Biol*. **23**: 75–83.

Mullen, C. A., Kilstrup, M., and Blaese, R. M. 1982. Transfer of the bacterial gene for cytosine deaminase to mammalian cells confers lethal sensitivity to 5-fluorocytosine: a negative selection system. *Proc. Natl Acad. Sci. USA* **89**: 33–37.

Muto, A., Ohkura, M., Kotani, T., Higashijima, S.-ichi., Nakai, J., and Kawakami, K. 2011. Genetic visualization with an improved GCaMP calcium indicator reveals spatiotemporal activation of the spinal motor neurons in zebrafish. *Proc. Natl Acad. Sci. USA* **108**: 5425–5430.

Naested, H., Fennema, M., Hao, L., Andersen, M., Janssen, D. B., and Mundy, J. 1999. A bacterial haloalkane dehalogenase gene as a negative selectable marker in *Arabidopsis*. *Plant J.* **18**: 571–576.

Nagai, T., Ibata, K., Park, E. S., Kubota, M., Mikoshiba, K., and Miyawaki, A. 2002. A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. *Nat. Biotechnol.* **20**: 87–90.

Narusaka, Y., Narusaka, M., Abe, H., Hosaka, N., Kobayashi, M., Shiraishi, T., and Iwabuchi, M. 2009. High-throughput screening for plant defense activators using a BETA-glucuronidase-reporter gene assay in *Arabidopsis thaliana*. *Plant Biotechnol.* **26**: 345–349.

Negrotto, D., Jolley, M., Beer, S., Wenck, A. R., and Hansen, G. 2000. The use of phosphomannose-isomerase as a selectable marker to recover transgenic maize plants (*Zea mays* L.) via *Agrobacterium* transformation. *Plant Cell Rep.* **19**: 798–803.

Nielsen, K. M. 2003. Transgenic organisms – time for conceptual diversification? *Nat. Biotechnol.* **21**: 227–228.

Nishimura, A., Aichi, I., and Matsuoka, M. 2006. A protocol for *Agrobacterium*-mediated transformation in rice. *Nat. Protocols* **1**: 2796–2802.

Nishimura, A., Ashikari, M., Lin, S., Takashi, T., Angeles, E. R., Yamamoto, T., and Matsuoka, M. 2005. Isolation of a rice regeneration quantitative trait loci gene and its application to transformation system. *Proc. Natl. Acad. Sci. U.S.A.* **102**: 11940–11944.

Nishizawa, K., Kita, Y., Kitayama, M., and Ishimoto, M. 2006. A red fluorescent protein, DsRed2, as a visual reporter for transient expression and stable transformation in soybean. *Plant Cell Rep.* **25**: 1355–1361.

Nonomura, T., Komaki, S., Xu, L., Moriura, N., Ioroi, H., Takashima, S., Kakutani, K., Takikawa, Y., Matsuda, Y., and Toyoda, H. 2009. Tryptophan synthesis block-associated sugar response, a physiological marker for rapid selection of *Arabidopsis thaliana* transformants, marked with feedback-inhibition-insensitive anthranilate synthase gene. *Plant Breed.* **128**: 282–289.

Nugent, G. D., Coyne, S., Nguyen, T. T., Kavanagh, T. A., and Dix, P. J. 2006. Nuclear and plastid transformation of *Brassica oleracea* var. botrytis (cauliflower) using PEG-mediated uptake of DNA into protoplasts. *Plant Sci*. **170**: 135–142.

Nugent, G. D., Ten Have, M., van der Gulik, A., Dix, P. J., Uijtewaal, B. A. and Mordhorst, A. P. 2005. Plastid transformants of tomato selected using mutations affecting ribosome structure. *Plant Cell Rep*. **24**: 341–349.

O'Keefe, D. P., Tepperman, J. M., Dean, C., Leto, K. J., Erbes, D. L., and Odell, J. T. 1994. Plant expression of a bacterial cytochrome P450 that catalyzes activation of a sulfonylurea pro-herbicide. *Plant Physiol.* **105**: 473–482.

O'Kennedy, M. M., Burger, J. T., and Botha F. C. 2004. Pearl millet transformation system using the positive selectable marker gene phosphomannose isomerase. *Plant Cell Rep*. **22**: 684–690.

Ogawa, T., Kawahigashi, H., Toki, S., and Handa, H. 2008. Efficient transformation of wheat by using a mutated rice acetolactate synthase gene as a selectable marker. *Plant Cell Rep*. **27**: 1325–1331.

Ogura, R., Matsuo, N., Wako, N., Tanaka, T., Ono, S., and Hiratsuka, K. 2005. Multi-color luciferases as reporters for monitoring transient gene expression in higher plants. *Plant Biotechnol.* **22**: 151–155.

Okada, T. and Toriyama, K. 2001. Pollen vegetative cell-specific expression of Bra r 1: useful tool for observation of the vegetative nucleus and identification of transgenic pollen by nuclear-targeted GFP. *Sex. Plant Reprod.* **13**: 301–307.

Okumura, S., Sawada, M., Park, Y. W., Hayashi, T., Shimamura, M., Takase, H., and Tomizawa, K. 2006. Transformation of poplar (*Populus alba*) plastids and expression of foreign proteins in tree chloroplasts. *Transgenic Res*. **15**: 637–646.

Okuzaki, A., Konagaya, K.-ichi., Nanasato, Y., Tsuda, M., and Tabei, Y. 2011. Estrogen-inducible GFP expression patterns in rice (*Oryza sativa* L.). *Plant Cell Rep.* **30**: 529–538.

Okuzaki, A., Shimizu, T., Kaku, K., Kawai, K., and Toriyama, K. 2007. A novel mutated acetolactate synthase gene conferring specific resistance to pyrimidinyl carboxy herbicides in rice. *Plant Mol. Biol.* **64**: 219–224.

Olszewski, N. E., Martin, F. B., and Ausubel, F. M. 1988. Specialized binary vector for plant transformation: expression of the *Arabidopsis thaliana* AHAS gene in *Nicotiana tabacum*. *Nucleic Acids Res.* **16**: 10765–10782.

Omar, A. A. and Grosser, J. W. 2008. Comparison of endoplasmic reticulum targeted and non-targeted cytoplasmic GFP as a selectable marker in citrus protoplast transformation. *Plant Sci.* **174**: 131–139.

Oreifig, 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 Rep*. **22**: 490–496.

Orzaez, D., Medina, A., Torre, S., Fernandez-Moreno, J. P., Rambla, J. L., Fernandez-del-Carmen, A., Butelli, E., Martin, C., and Granell, A. 2009. A visual reporter system for virus-induced gene silencing in tomato fruit based on anthocyanin accumulation. *Plant Physiol.* **150**: 1122–1134.

Osakabe, K., Endo, M., Kawai, K., Nishizawa, Y., Ono, K., Abe, K., Ishikawa, Y., Nakamura, H., Ichikawa, H., Nishimura, S., Shimizu, T., and Toki, S. 2005. The mutant form of acetolactate synthase genomic DNA from rice is an efficient selectable marker for genetic transformation. *Mol. Breed.* **16**: 313–320.

Ow, D. W., Wood, K. V., DeLuca, M., De Wet, J. R., Helinski, D. R., and Howell, S. H. 1986. Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. *Science* **234**: 856–859.

Ozawa, K. and Kawahigashi, H. 2006. Positional cloning of the nitrite reductase gene associated with good growth and regeneration ability of calli and establishment of a new selection system for *Agrobacterium*-mediated transformation in rice (*Oryza sativa* L.). *Plant Sci.* **170**: 384–393.

Ozawa, T., Natori, Y., Sato, M., and Umezawa, Y. 2007. Imaging dynamics of endogenous mitochondrial RNA in single living cells. *Nat. Methods.* **4**: 413–419.

Padidam, M. 2003. Chemically regulated gene expression in plants. *Curr. Opin. Plant Biol.* **6**: 169–177.

Padilla, I. M. G. and Burgos L. 2010. Aminoglycoside antibiotics: structure, functions and effects on *in vitro* plant culture and genetic transformation protocols. *Plant Cell Rep.* **29**: 1203–1213.

Page, A. and Angell, S. 2002. Transient expression of reporter proteins can alter plant gene expression. *Plant Sci.* **163**: 431–437.

Pang, S., DeBoer, D. L., Wan, Y., Ye, G., Layton, J. G., Neher, M. K., Armstrong, C. L., Fry, J. E., Hinchee, M. A. W., and Fromm, M. E. 1996. An improved green-fuorescence protein gene as a vital marker in plants. *Plant Physiol.* **112**: 893–900.

Paris, N., Saint-Jean, B., Faraco, M., Krzeszowiec, W., D'alessandro, G., Neuhaus, J.-M., and Di Sansebastiano, G. P. 2010. Expression of a glycosylated GFP as a bivalent reporter in exocytosis. *Plant Cell Rep.* **29**: 79–86.

Park, J., Lee, Y. K., Kang, B. K., and Chung, W. I. 2004. Co-transformation using a negative selectable marker gene for the production of selectable marker gene-free transgenic plants. *Theor. Appl. Genet.* **109**: 1562–1567.

Patil, G., Deokar, A., Jain, P. K., Thengane, R. J., and Srinivasan, R. 2009. Development of a phosphomannose isomerase-based Agrobacterium-mediated transformation system for chickpea (*Cicer arietinum* L.). *Plant Cell Rep.* **28**: 1669–1676.

Pedotti, M., Rosini, E., Molla, G., Moschetti, T., Savino, C., Vallone, B., and Pollegioni, L. 2009. Glyphosate resistance by engineering the flavoenzyme glycine oxidase. *J. Biol. Chem.* **284**: 36415–36423.

Perera, R. J., Linard, C. G., and Signer, E. R. 1993. Cytosine deaminase as a negative selective marker for *Arabidopsis*. *Plant Mol. Biol.* **23**: 793–799.

Perez, P., Tiraby, G., Kallerhoff, J., and Perret, J. 1989. Phleomycin resistance as a dominant selectable marker for plant cell transformation. *Plant Mol. Biol.* **13**: 365–373.

Perez-Clemente, R. M., Perez-Sanjuan, A., Garcia-Ferriz, L., Beltran, J. P., and Canas, L. A. 2004. Transgenic peach plants (*Prunus persica* L.) produced by genetic transformation of embryo sections using the green fluorescent protein (GFP) as an *in vivo* marker. *Mol. Breed*. **14**: 419–427.

Perl, A., Galili, S., Shaul, O., Ben-Tzvi, I., and Galili, G. 1993. Bacterial dihydrodipicolinate synthase and desensitized aspartic kinase: two novel selectable markers for plant transformation. *Biotechnology* **11**: 715–718.

Pinkerton, T. S., Howard, J. A., and Wild, J. R. 2008. Genetically engineered resistance to organophosphate herbicides provides a new scoreable and selectable marker system for transgenic plants. *Mol. Breed.* **21**: 27–36.

Piruzian, E. S., Monzavi-Karbassi, B., Darbinian, N. S., Goldenkova, I. V., Kobets, N. S., and Mochulsky, A. V. 1998. The use of a thermostable β-glucanase gene from *Clostridium thermocellum* as a reporter gene in plants. *Mol. General Genetic*s **257**: 561–567.

Pollegioni, L. and Molla, G. 2011. New biotech applications from evolved D-amino acid oxidases. *Trends Biotechnol.* **29**: 276–283.

Popelka, J. C., Xu, J., and Altpeter, F. 2003. Generation of rye (*Secale cereale* L.) plants with low transgene copy number after biolistic gene transfer and production of instantly marker-free transgenic rye. *Transgenic Res.* **12**: 587–596.

Prakash, N. S., Prasad, V., Chidambram, T. P., Cherian, S., Jayaprakash, T. L., Dasgupta, S., Wang, Q., Mann, M. T., Spencer, T. M., and Boddupalli, R. S. 2008. Effect of promoter driving selectable marker on corn transformation. *Transgenic Res*. **17**: 695–704.

Qiao, G., Zhou, J., Jiang, J., Sun, Y., Pan, L., Song, H., Jiang, J., Zhuo, R., Wang, X., and Sun, Z. 2010. Transformation of *Liquidambar formosana* L. via *Agrobacterium tumefaciens* using a mannose selection system and recovery of salt tolerant lines. *Plant Cell Tiss. Organ Cult*. **102**: 163–170.

Qiao, G., Zhuo, R., Liu, M., Jiang, J., Li, H., Qiu, W., Pan, L., Lin, S., Zhang, X., and Sun, Z. 2011. Over-expression of the *Arabidopsis* Na+/H+ antiporter gene in *Populus deltoides* CL x P. euramericana CL "NL895′ enhances its salt tolerance. *Acta Physiol. Plant.* **33**: 691–696.

Quadrana, L., Rodriguez, M. C., López, M., Bermúdez, L., Nunes-Nesi, A., Fernie, A. R., Descalzo, A., Asis, R., Rossi, M., Asurmendi, S., and Carrari, F. 2011. Coupling virus-induced gene silencing to exogenous green fluorescence protein expression provides a highly efficient system for functional genomics in Arabidopsis and across all stages of tomato fruit development. *Plant Physiol*. **156**: 1278–1291.

Quaedvlieg, N. E., Schlaman, H. R., Admiraal, P. C., Wijting, S. E., Stougaard, J., and Spaink, H. P. 1998. Fusions between green fluorescent protein and beta-glucuronidase as sensitive and vital bifunctional reporters in plants. *Plant Mol. Biol.* **38**: 861–873.

Rajasekaran, K., Grula, J. W., Hudspeth, R. L., Pofelis, S., and Anderson, D. M. 1996. Herbicide-resistant Acala and Coker cottons transformed with a native gene encoding mutant forms of acetohydroxyacid synthase. *Mol. Breed.* **2**: 307–319.

Ramana Rao, M. V. and Veluthambi, K. 2010. Selectable marker elimination in the T-0 generation by *Agrobacterium*-mediated co-transformation involving Mungbean yellow mosaic virus TrAP as a non-conditional negative selectable marker and bar for transient positive selection. *Plant Cell Rep.* **29**: 473–483.

Ramesh, S. A., Kaiser, B. N., Franks, T., Collins, G., and Sedgley, M. 2006. Improved methods in *Agrobacterium*–mediated transformation of almond using positive (mannose/*pmi*) or negative (kanamycin resistance) selection-based protocols. *Plant Cell Rep.* **25**: 821–828.

Ramessar, K., Peremarti, A., Gómez-Galera, S., Naqvi, S., Moralejo, M., Muñoz, P., Capell, T., and Christou, P. 2007. Biosafety and risk assessment

framework for selectable marker genes in transgenic crop plants: a case of the science not supporting the politics. *Transgenic Res*. **16**: 261–280.

Ray, H., Yu, M., Auser, P., Blahut-Beatty, L., McKersie, B., Bowley, S., Westcott, N., Coulman, B., Lloyd, A., and Gruber, M. Y. 2003. Expression of Anthocyanins and Proanthocyanidins after transformation of Alfalfa with Maize Lc. *Plant Physiol*. **132**: 1448–1463.

Ray, K., Jagannath, A., Gangwani, S. A., Burma, P. K., and Pental, D. 2004. Mutant acetolactate synthase gene is an efficient in vitro selectable marker for the genetic transformation of *Brassica juncea* (oilseed mustard). *J. Plant Physiol*. **161**: 1079–1083.

Reed, J., Privalle, L., Powell, M. L., Meghji, M., Dawson, J., Dunder, E., Suttie, J., Wenck, A., Launis, K., Kramer, C., Chang, Y. F., Hansen, G., and Wright, M. 2001. Phosphomannose isomerase: An efficient selectable marker for plant transformation. *In vitro Cell. Devel. Biol.-Plant* **37**: 127–132.

Ren, Y., Lv, J., Wang, H., Li, L., Peng, Y., and Qu, L. J. 2009a. A comparative proteomics approach to detect unintended effects in transgenic *Arabidopsis*. *J. Genet. Genomics* **36**: 629–639.

Ren, Y., Wang, T., Peng, Y., Xia, B., and Qu, L. J. 2009b. Distinguishing transgenic from non-transgenic *Arabidopsis* plants by (1)H NMR-based metabolic fingerprinting. *J. Genet. Genomics* **36**: 621–628.

Richael, C. M., Kalyaeva, M., Chretien, R. C., Yan, H., Adimulam, S., Stivison, A., Weeks, J. T., and Rommens, C. M. 2008. Cytokinin vectors mediate marker-free and backbone-free plant transformation. *Trangenic Res*. **17**: 905–917.

Richards, H. A., Han, C.-T., Hopkins, R. G., Failla, M. L., Ward, W. W., and Stewart, C. N. 2003. Safety assessment of recombinant green fluorescent protein orally administered to weaned rats. *J. Nutrition.* **133**: 1909–1912.

Richards, H. A., Rudas, V. A., Sun, H., McDaniel, J. K., Tomaszewski, Z., and Conger, B. V. 2001. Construction of a GFP-BAR plasmid and its use for switchgrass transformation. *Plant Cell Rep*. **20**: 48–54.

Ricroch, A. E., Berge, J. B., and Kuntz, M. 2011. Evaluation of genetically engineered crops using transcriptomic, proteomic, and metabolomic profiling techniques. *Plant Physiol*. **155**: 1752–1761.

Risseeuw, E., FrankevanDijk, M., and Hooykaas, P. 1997. Gene targeting and instability of *Agrobacterium* T-DNA loci in the plant genome. *Plant J.* **11**: 717–728.

Rochat, L., Péchy-Tarr, M., Baehler, E., Maurhofer, M., and Keel, C. 2010. Combination of fluorescent reporters for simultaneous monitoring of root colonization and antifungal gene expression by a biocontrol Pseudomonad on cereals with flow cytometry. *Mol. Plant-Microbe Interactions.* **23**: 949–961.

Rommens, C. M. 2004. All-native DNA transformation: a new approach to plant genetic engineering. *Trends Plant Sci.* **9**: 457–464.

Rommens, C. M. 2006. Kanamycin resistance in plants: an unexpected trait controlled by a potentially multifaceted gene. *Trends Plant Sci*. **11**: 317–319.

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.

Rosellini, D. 2011. Selectable marker genes from plants: reliability and potential. *In vitro Cell. Devel. Biol.-Plant* **47:** 222–233.

Rosellini, D., Capomaccio, S., Ferradini, N., Savo Sardaro, M. L., Nicolia, A., and Veronesi F. 2007. Non-antibiotic, efficient selection for alfalfa genetic engineering. *Plant Cell Rep*. **26**: 1035–1044.

Rosellini, D., Pezzotti, M., and Veronesi, F. 2001. Characterization of transgenic male sterility in alfalfa. *Euphytica* **118**: 313–319.

Ruf, S., Hermann, M., Berger, I. J., Carrer, H., and Bock, R. 2001. Stable genetic transformation of tomato plastids and expression of a foreign protein in fruit. *Nat. Biotechnol.* **19**: 870–875.

Rugh, C. L., Senecoff, J. F., Meagher, R. B., and Merkle, S. A. 1998. Development of transgenic yellow poplar for mercury phytoremediation. *Nature Biotechnol.* **16**: 925–928.

Rugh, C. L., Wilde, H. D., Stack, N. M., Thompson, D. M., Summers, A. O., and Meagher, R. B. 1996. Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial *merA* gene. *Proc Natl Acad Sci USA* **93**: 3182–3187.

Ruiz, M. T., Voinnet, O., and Baulcombe, D. C. 1998. Initiation and maintenance of virus-induced gene silencing. *Plant Cell*. **10**: 937–946.

Russell, D. A. and Fromm, M. E. 1997. Tissue-specific expression in transgenic maize of four endosperm promoters from maize and rice. *Transgenic Res*. **6**: 157–168.

Saelim, L., Phansiri, S., Suksangpanomrung, M., Netrphan, S., and Narangajavana, J. 2008. Evaluation of a morphological marker selection and excision system to generate marker-free transgenic cassava plants. *Plant Cell Rep*. **28**: 445–455.

Saika, H., Oikawa, A., Matsuda, F., Onodera, H., Saito, K., and Toki, S. 2011. Application of gene targeting to designed mutation breeding of high-tryptophan rice. *Plant Physiol*. **156**: 1269–1277.

Sawchuk, M. G., Head, P., Donner, T. J., and Scarpella, E. 2007. Time-lapse imaging of Arabidopsis leaf development shows dynamic patterns of procambium formation. *New Phytol.* **176**: 560–571.

Scabone, C. M., Frigerio, L., and Petruccelli, S. 2011. A fluorescent reporter protein containing AtRMR1 domains is targeted to the storage and central vacuoles in *Arabidopsis thaliana* and tobacco leaf cells. *Plant Cell Rep*. **30**: 1823–1833.

Scaramelli, L., Balestrazzi, A., Bonadei, M., Piano, E., Carbonera, D., and Confalonieri, M. 2009. Production of transgenic barrel medic (*Medicago truncatula* Gaernt.) using the ipt-type MAT vector system and impairment of recombinase-mediated excision events. *Plant Cell Rep.* **28**: 197–211.

Schaart, J. G., Krens, F. A., Pelgrom, K. T. B., Mendes, O., and Rouwendal, G. J. A. 2004. Effective production of marker-free transgenic strawberry plants using inducible site-specific recombination and a bifunctional selectable marker gene. *Plant Biotechnol. J.* **2**: 233–240.

Schlaman, H. R. M. and Hooykaas, P. J. J. 1997. Effectiveness of the bacterial gene *codA* encoding cytosine deaminase as a negative selectable marker in *Agrobacterium*-mediated plant transformation. *Plant J*. **11**: 1377–1385.

Schönberger, J., Hammes, U. Z., and Dresselhaus, T. 2012. *In vivo* visualisation of RNA in plants cells using the λN22 system and a GATEWAY-compatible vector series for candidate RNAs. *Plant J.* **71**: 173–181.

Schroder, M., Dixelius, C., Rahlen, L., and Glimelius, K. 1994. Transformation of *Brassica napus* by using aadA gene as selectable marker and inheritance studies of the marker genes. *Physiol Plant.* **92**: 37–46.

Schuermann, D., Molinier, J., Fritsch, O., and Hohn, B. 2005. The dual nature of homologous recombination in plants. *Trends Genet.* **21**: 172–181.

Schulte, A., Lorenzen, I., Böttcher, M., and Plieth, C. 2006. A novel fluorescent pH probe for expression in plants. *Plant Methods* **2**: 7.

Seitz, C., Bruna, S., Li, H., Hauser, B., and Forkmann, G. 2007. Establishment of a mannose-based selection system for the transformation of *Torenia* hybrids. *Acta Hort.* **743:** 139–142.

Selvaraj, N., Kasthurirengan, S., Vasudevan, A., Manickavasagam, M., Choi, C. W., and Ganapathi, A. 2010. Evaluation of green fluorescent protein as a reporter gene and phosphinothricin as the selective agent for achieving a higher recovery of transformants in cucumber (*Cucumis sativus* L. cv. Poinsett76) via *Agrobacterium tumefaciens*. *In Vitro Cell. Devel. Biol.-Plant*. **46**: 329–337.

Serino, G. and Maliga, P. 1997. A negative selection scheme based on the expression of cytosine deaminase in plastids. *Plant J.* **12**: 697–701.

Shah, J. M. and Veluthambi, K. 2010. DIANTHIN, a negative selection marker in tobacco, is non-toxic in transgenic rice and confers sheath blight resistance. *Biol. Plant.* **54**: 443–450.

Shaked, H., Melamed-Bessudo, C., and Levy, A. A. 2005. High-frequency gene targeting in *Arabidopsis* plants expressing the yeast RAD54 gene. *Proc. Natl Acad. Sci. USA* **102**: 12265–12269.

Shaner, N. C., Campbell, R. E., Steinbach, P. A., Giepmans, B. N. G., Palmer, A. E., and Tsien, R. Y. 2004. Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein. *Nat Biotechnol.* **22**: 1567–1572.

Shaul, O. and Galili, G. 1992a. Increased lysine synthesis in transgenic tobacco plants expressing a bacterial dihydrodipicolinate synthase in their chloroplasts. *Plant J*. **2**: 203–209.

Shaul, O, and Gallli, G. 1992b. Threonine overproduction in transgenic tobacco plants expressing a mutant desensitized aspartate kinase from *Escherichia coli*. *Plant Physiol*. **100**: 1157–1163.

Shaw, D. J. and Gray, J. C. 2011. Visualisation of stromules in transgenic wheat expressing a plastid-targeted yellow fluorescent protein. *Planta* **233**: 961–970.

Sheen, J., Hwang, S. B., Niwa, Y., Kobayashi, H., and Galbraith, D. W. 1995. Green fluorescent protein as a new vital marker in plant cells. *Plant J*. **8**: 777–784.

Shen, L. Y. and Petolino, J. F. 2006. Pigmented maize seed via tissue-specific expression of anthocyanin pathway gene transcription factors. *Mol. Breed.* **18:** 57–67.

Shimada, T. L., Shimada, T., and Hara-Nishimura, I. 2010. A rapid and non-destructive screenable marker, FAST, for identifying transformed seeds of *Arabidopsis thaliana*. *Plant J.* **61**: 519–528.

Shimizu, M., Goto, M., Hanai, M., Shimizu, T., Izawa, N., Kanamoto, H., Tomizawa, K.-I., Yokota, A., and Kobayashi, H. 2008. Selectable tolerance to herbicides by mutated acetolactate synthase genes integrated into the chloroplast genome of tobacco. *Plant Physiol*. **147**: 1976–1983.

Sidorov, V. A., Kasten, D., Pang, S., Hajdukiewicz, P. T. J., Staub, J. M., and Nehra, N. S. 1999. Stable chloroplast transformation in potato: use of green fluorescent protein as a plastid marker. *Plant J*. **19**: 209–216.

Sigareva, M., Spivey, R., Willits, M., Kramer, C., and Chang, Y. F. 2004. An efficient mannose selection protocol for tomato that has no adverse effect on the ploidy level of transgenic plants. *Plant Cell Rep*. **23**: 236–245.

Sijacic, P. and Liu Z. 2010. Novel insights from live-imaging in shoot meristem development. *J. Integrative Plant Biol.* **52**: 393–399.

Sikdar, S. R., Serino, G., Chaudhuri, S., and Maliga, P. 1998. Plastid transformation in *Arabidopsis thaliana*. *Plant Cell Rep*. **18**: 20–34.

Simmonds, J., Cass, L., Routly, E., Hubbard, K., Donaldson, P., Bancroft, B., Davidson, A., Hubbard, S., and Simmonds, D. 2004. Oxalate oxidase: a novel reporter gene for monocot and dicot transformations. *Mol. Breed.* **13**: 79–91.

Singh, A. K., Verma, S. S., and Bansal, K. C. 2010. Plastid transformation in eggplant (*Solanum melongena* L.). *Transgenic Res*. **19:** 113–119.

Skarjinskaia, M., Svab, Z. and Maliga, P. 2003. Plastid transformation in *Lesquerella fendleri*, an oilseed *Brassicacea*. *Transgenic Res.* **12**: 115–122.

Snapp, E. L., Sharma, A., Lippincott-Schwartz, J., and Hegde, R. S. 2006. Monitoring chaperone e*n*gagement of substrates in the endoplasmic reticulum of live cells. *Proc. Natl. Acad. Sci. U.S.A.* **103**: 6536–6541.

Song, G.-qing., Sink, K. C., Ma, Y., Herlache, T., Hancock, J. F., and Loescher, W. H. 2010. A novel mannose-based selection system for plant transformation using celery mannose-6-phosphate reductase gene. *Plant Cell Rep.* **29**: 163–172.

Srivastava, V. and Gidoni, D. 2010. Site-specific gene integration technologies for crop improvement. *In vitro Cell. Devel. Biol.-Plant* **46**: 219–232.

Stewart, C. N. 2001. The utility of green fluorescent protein in transgenic plants. *Plant Cell Rep.* **20**: 376–382.

Stewart, C. N., Jr. 2005. Monitoring the presence and expression of transgenes in living plants. *Trends Plant Sci*. **10**: 390–396.

Stirpe, F. 2004. Ribosome-inactivating proteins. *Toxicon* **14**: 371–383.

Stöger, E., Fink, C., Pfosser, M., and Heberle-Bors, E. 1995. Plant transformation by particle bombardment of embryogenic pollen. *Plant Cell Rep*. **14**: 273–278.

Stougaard, J. 1993. Substrate-dependent negative selection in plants using a bacterial cytosine deaminase gene. *Plant J.* **3**: 755–761.

Stoykova, P. and Stoeva-Popova, P. 2011. PMI (manA) as a nonantibiotic selectable marker gene in plant biotechnology. *Plant Cell Tiss. Organ Cult.* **105**: 141–148.

Sun, J., Niu, Q., Tarkowski, P., Zheng, B., Tarkowska, D., Sandberg, G., Chua, N., and Zuo, J. 2003. The *Arabidopsis* AtIPT8/PGA22 gene encodes an isopentenyl transferase that is involved in de novo cytokinin biosynthesis. *Plant Physiol.* **131**: 167–176.

Sundar, I. K. and Sakthivel, N. 2008. Advances in selectable marker genes for plant transformation. *J. Plant Physiol.* **165**: 1698–1716.

Sundaresan, V., Springer, P., Volpe, T., Haward, S., Jones, J. D., Dean, C., Ma, H., and Martienssen, R. 1995. Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements. *Genes Devel*. **9**: 1797–1810.

Surov, T., Aviv, D., Aly, R., Joel, D. M., Goldman-Guez, T., and Gressel, J. 1998. Generation of transgenic asulam-resistant potatoes to facilitate eradication of parasitic broomrapes (*Orobanche* spp.), with the sul gene as the selectable marker. *Theor. Appl. Genet*. **96**: 132–137.

Svab, Z., Hajdukiewicz, P., and Maliga, P. 1990. Stable transformation of plastids in higher plants. *Proc. Natl Acad. Sci. USA* **87:** 8526–8530.

Svab, Z. and Maliga, P. 1993. High-frequency plastid transformation in tobacco by selection for a chimeric aadA gene. *Proc. Natl Acad. Sci. USA* **90**: 913–917.

Tangphatsornruang, S., Birch-Machin, I., Newell, C. A., and Gray, J. C. 2011. The effect of different 3′ untranslated regions on the accumulation and stability of transcripts of a gfp transgene in chloroplasts of transplastomic tobacco. *Plant Mol. Biol.* **76**: 385–396.

Taniguchi, Y., Kawata, M., Ando, I., Shimizu, T., and Ohshima, M. 2010. Selecting genetic transformants of indica and indica-derived rice cultivars using bispyribac sodium and a mutated ALS gene. *Plant Cell Rep*. **29**: 1287–1295.

Terada, R., Johzuka-Hisatomi, Y., Saitoh, M., Asao, H., and Iida, S. 2007. Gene targeting by homologous recombination as a biotechnological tool for rice functional genomics. *Plant Physiol* **144**: 846–856.

Terada, R., Nagahara, M., Furukawa, K., Shimamoto, M., Yamaguchi, K., and Iida, S. 2010. Cre-loxP mediated marker elimination and gene reactivation at the waxy locus created in rice genome based on strong positive-negative selection. *Plant Biotechnol*. **27**: 29–37.

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.

Terskikh, A., Fradkov, A., Ermakova, G., Zaraisky, A., Tan, P., Kajava, A. V., Zhao, X., Lukyanov, S., Matz, M., Kim, S., Weisman, I., and Siebert, P. 2000. "Fluorescent timer": protein that changes color with time. *Science* **290**: 1585–1588.

Tewari-Singh, N., Sen, J., Kiesecker, H., Reddy, V. S., Jacobsen, H.-J., and Guha-Mukherjee, S. 2004. Use of a herbicide or lysine plus threonine for non-antibiotic selection of transgenic chickpea. *Plant Cell Rep*. **22**: 576–583.

Thirukkumaran, G., Khan, R. S., Chin, D. P., Nakamura, I., and Mii, M. 2009. Isopentenyl transferase gene expression offers the positive selection of marker-free transgenic plant of *Kalanchoe blossfeldiana*. *Plant Cell Tiss. Organ Cult*. **97**: 237–242.

Thiruvengadam, M., Hsu, W.-H., and Yang, C.-H. 2011. Phosphomannose-isomerase as a selectable marker to recover transgenic orchid plants (*Oncidium* Gower Ramsey). *Plant Cell Tiss. Organ Cult*. **104**: 239–246.

Thiruvengadam, V., Chen, I.-C., Lin, W.-D., and Hsu, W.-H. 2010. Alanine racemase as a novel positive selectable marker for plant transformation. *J. Biotechnol.* **150**(Suppl. 1): S476.

Thykjaer, T., Finneman, J., Schauser, L., Christensen, L., Poulsen, C., and Stougaard, J. 1997. Gene targeting approaches using positive-negative selection and large flanking regions. *Plant Mol. Biol.* **35**: 523–530.

Tian, L., Hires, S. A., Mao, T., Huber, D., Chiappe, M. E., Chalasani, S. H., Petreanu, L., Akerboom, J., McKinney, S. A., Schreiter, E. R., Bargmann, C. I., Jayaraman, V., Svoboda, K., and Looger, L. L. 2009. Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. *Nat Meth*. **6**: 875–881.

Tilsner, J., Linnik, O., Christensen, N. M., Bell, K., Roberts, I. M., Lacomme, C., and Oparka, K. J. 2009. Live-cell imaging of viral RNA genomes using a Pumilio-based reporter. *Plant J*. **57**: 758–770.

Todd, R. and Tague, B. W. 2001. Phosphomannose isomerase: A versatile selectable marker for *Arabidopsis thaliana* germ-line transformation. *Plant Mol. Biol Reporter*. **19**: 307–319.

Tougou, M., Yamagishi, N., Furutani, N., Kaku, K., Shimizu, T., Takahata, Y., Sakai, J.-ichi., Kanematsu, S., and Hidaka, S. 2009. The application of the mutated acetolactate synthase gene from rice as the selectable

marker gene in the production of transgenic soybeans. *Plant Cell Rep*. **28**: 769–776.

Townsend, J. A., Wright, D. A., Winfrey, R. J., Fu, F. L., Maeder, M. L., Joung, J. K., and Voytas, D. F. 2009. High-frequency modification of plant genes using engineered zinc-finger nucleases. *Nature* **459**: 442–445.

Tsai, F.-Y., Zhang, X.-H., Ulanov, A., and Widholm, J. M. 2010. The application of the yeast N-acetyltransferase MPR1 gene and the proline analogue L-azetidine-2-carboxylic acid as a selectable marker system for plant transformation. *J. Exp. Bot.* **61**: 2561–2573.

Tsien, R. Y. 1998. The green fluorescent protein. *Ann. Rev. Biochem.* **67**: 509–544.

Tungsuchat-Huang, T., Slivinski, K. M., Ramona Sinagawa-Garcia, S., and Maliga, P. 2011. Visual spectinomycin resistance (aadA (au)) gene for facile identification of transplastomic sectors in tobacco leaves. *Plant Mol. Biol.* **76**(3–5): 453–461.

Twell, D. 1995. Diphtheria toxin-mediated cell ablation in developing pollen: Vegetative cell ablation blocks generative cell migration. *Protoplasma*. **187**: 144–154.

Upadhyaya, N. M., Zhou, X.-R., Wu, L., Ramm, K., and Dennis, E. S. 2000. The tms2 gene as a negative selection marker in rice. *Plant Mol. Biol Reporter.* **18**: 227–233.

Urbanczyk-Wochniak, E., Leisse, A., Roessner-Tunali, U., Lytovchenko, A., Reismeier, J., Willmitzer, L., and Fernie, A. R. 2003. Expression of a bacterial xylose isomerase in potato tubers results in an altered hexose composition and a consequent induction of metabolism. *Plant Cell Physiol*. **44**: 1359–1367.

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.

Vickers, C. E., Xue, G. P., and Gresshoff, P. M. 2003. A synthetic xylanase as a novel reporter in plants. *Plant Cell Rep.* **22**: 135–140.

Wakasa, Y., Ozawa, K., and Takaiwa, F. 2007. *Agrobacterium*-mediated transformation of a low glutelin mutant of 'Koshihikari' rice variety using the mutated-acetolactate synthase gene derived from rice genome as a selectable marker. *Plant Cell Rep.* **26**: 1567–1573.

Waldron, C., Murphy, E. B., Roberts, J. L., Gustafson, G. D., Armour, S. L., and Malcolm, S. K. 1985. Resistance to hygromycin B. *Plant Mol. Biol.* **5**: 103–108.

Wallbraun, M., Sonntag, K., Eisenhauer, C., Krzcal, G., and Wang, Y. P. 2009. Phosphomannose-isomerase (pmi) gene as a selectable marker for *Agrobacterium*-mediated transformation of rapeseed. *Plant Cell Tiss. Organ Cult.* **99**: 345–351.

Wallis, J. G., Dziewanowska, K., and Guerra, D. J. 1996. Genetic transformation with the sull gene; A highly efficient selectable marker for *Solanum tuberosum* L cv 'Russet Burbank.' *Mol. Breed*. **2**: 283–290.

Wang, Y., Yau, Y.-Y., Perkins-Balding, D., and Thomson, J. G. 2011. Recombinase technology: applications and possibilities. *Plant Cell Rep*. **30**: 267–285.

Weeks, J. T., Koshiyama, K. Y., Maier-Greiner, U., Schäeffner, T., and Anderson, O. D. 2000. Wheat transformation using cyanamide as a new selective agent. *Crop Sci*. **40:** 1749–1754.

Weeks, J. T., Ye, J., and Rommens, C. M. 2008. Development of an in planta method for transformation of alfalfa (*Medicago sativa*). *Transgenic Res.* **17**: 587–597.

Wehrkamp-Richter, S., Degroote, F., Laffaire, J.-B., Paul, W., Perez, P., and Picard, G. 2009. Characterisation of a new reporter system allowing high throughput *in planta* screening for recombination events before and after controlled DNA double strand break induction. *Plant Physiol. Biochem*. **47**: 248–255.

Wenck, A., Pugieux, C., Turner, M., Dunn, M., Stacy, C., Tiozzo, A., Dunder, E., van Grinsven, E., Khan, R., Sigareva, M., Wang, W. C., Reed, J., Drayton, P., Oliver, D., Trafford, H., Legris, G., Rushton, H., Tayab, S., Launis, K., Chang, Y. F., Chen, D. F., and Melchers, L. 2003. Reef-coral proteins as visual, non-destructive reporters for plant transformation. *Plant Cell Rep*. **22**: 244–251.

Wozny, M., Schattat, M. H., Mathur, N., Barton, K., and Mathur, J. 2012. color recovery after photoconversion of H2B::mEosFP allows detection of increased nuclear dna content in developing plant cells. *Plant Physiol.***158**: 95–106.

Wright, D. A., Townsend, J. A., Winfrey, R. J., Irwin, P. A., Rajagopal, J., Lonosky, P. M., Hall, B. D., Jondle, M. D., and Voytas, D. F. 2005. High-frequency homologous recombination in plants mediated by zinc-finger nucleases. *Plant J.* **44**: 693–705.

Wright, M., Dawson, J., Dunder, E., Suttie, J., Reed, J., Kramer, C., Chang, Y., Novitzky, R., Wang, H., and Artim-Moore, L. 2001. Efficient biolistic transformation of maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) using the phosphomannose isomerase gene, pmi, as the selectable marker. *Plant Cell Rep*. **20**: 429–436.

Wright, M. S., Launis, K., Bowman, C., Hill, M., Dimaio, J., Kramer, C., and Shillito, R. D. 1996. A rapid visual method to identify transformed plants. *In vitro Cell. Devel. Biol.-Plant* **32**: 11–13.

Wu, J.-J., Liu, Y.-W., and Sun, M.-X. 2011. Improved and high throughput quantitative measurements of weak GFP expression in transgenic plant materials. *Plant Cell Rep*. **30**: 1253–1260.

Xi, J., Xu, P., and Xiang, C. Loss of AtPDR11, a plasma membrane-localized ABC transporter, confers paraquat tolerance in *Arabidopsis thaliana*. *Plant J*. **69**: 782–791.

Xiang, C. and Guerra, D. J. 1993. The anti-nptll gene: a potential negative selectable marker for plants. *Plant Physiol.* **102**: 287–293.

Xiao, Y.-L., Redman, J. C., Monaghan, E. L., Zhuang, J., Underwood, B. A., Moskal, W. A., Wang, W., Wu, H. C., and Town, C. D. 2010. High throughput generation of promoter reporter (GFP) transgenic lines of low expressing genes in *Arabidopsis* and analysis of their expression patterns. *Plant Meth*. **6**: 18.

Yamada, T., Tozawa, Y., Hasegawa, H., Terakawa, T., Ohkawa, Y., and Wakasa, K. 2004. Use of a feedback-insensitive at subunit of anthranilate synthase as a selectable marker for transformation of rice and potato. *Mol. Breed.* **14**: 363–373.

Yamamoto, Y. Y., Tsuhara, Y., Gohda, K., Suzuki, K., and Matsui, M. 2003. Gene trapping of the *Arabidopsis* genome with a firefly luciferase reporter. *Plant J.* **35**: 273–283.

Yang, A., Su, Q., and An, L. 2009. Ovary-drip transformation: a simple method for directly generating vector- and marker-free transgenic maize (*Zea mays* L.) with a linear GFP cassette transformation. *Planta* **229**: 793–801.

Yang, H. Y., Nairn, J., and Ozias-Akins, P. 2003. Transformation of peanut using a modified bacterial mercuric ion reductase gene driven by an actin promoter from *Arabidopsis thaliana*. *J. Plant Physiol*. **160**: 945–952.

Yao, K., De Luca, V., and Brisson, N. 1995. Creation of an artificial metabolic sink for tryptophan modifies the accumulation of phenylpropanoid compounds in potato and causes altered susceptibility to *Phytophtora infestans*. *Plant Cell* **7**: 1787–1799.

Yemets, A., Radchuk, V., Bayer, O., Bayer, G., Pakhomov, A., Vance Baird, W., and Blume, Y. B. 2008. Development of transformation vectors based upon a modified plant alpha-tubulin gene as the selectable marker. *Cell Biol. Int.* **32**: 566–570.

You, S., Liau, C., Huang, H., Feng, T., Prasad, V., Hsiao, H., Lu, J., and Chan, M. 2003. Sweet pepper ferredoxin-like protein (pflp) gene as a novel selection marker for orchid transformation. *Planta* **217**: 60–65.

Young, B., Wightman, R., Blanvillain, R., Purcel, S. B., and Gallois, P. 2010. pH-sensitivity of YFP provides an intracellular indicator of programmed cell death. *Plant Meth*. **6**: 27.

Zai-Song, D., Ming, Z., Yu-Xiang, J., Liang-Bi, L., and Ting-Nin, K. 2006. Efficient *Agrobacterium*-mediated transformation of rice by phosphomannose isomerase/mannose selection. *Plant Mol. Biol. Rep.* **24**: 295–303.

Zelasco, S., Ressegotti, V., Confalonieri, M., Carbonera, D., Calligari, P., Bonadei, M., Bisoffi, S., Yamada, K., and Balestrazzi, A. 2007. Evaluation of MAT-vector system in white poplar (*Populus alba* L.) and production of ipt marker-free transgenic plants by «single-step transformation. *Plant Cell Tiss. Organ Cult.* **91**: 61–72.

Zhang, J., Campbell, R. E., Ting, A. Y., and Tsien, R. Y. 2002. Creating new fluorescent probes for cell biology. *Nat. Rev. Mol. Cell Biol.* **3**: 906–918.

Zhang, P., Potrykus, I., and Puonti-Kaerlas, J. 2000. Efficient production of transgenic cassava using negative and positive selection. *Transgenic Res.* **9**: 405–415.

Zhang, P. and Puonti-Kaerlas, J. 2000. PIG-mediated cassava transformation using positive and negative selection. *Plant Cell Rep*. **19**: 1041–1048.

Zhang, W.-J., Yang, S.-S., Shen, X.-Y., Jin, Y.-S., Zhao, H.-J., and Wang, T. 2009. The salt-tolerance gene *rstB* can be used as a selectable marker in plant genetic transformation. *Mol. Breed*. **23**: 269–277.

Zhang, Y. S., Yin, X. Y., Yang, A. F., Li, G. S., and Zhang, J. R. 2005. Stability of inheritance of transgenes in maize (*Zea mays* L.) lines produced using different transformation methods. *Euphytica* **144**: 11–22.

Zhao, Y., Araki, S., Wu, J., Teramoto, T., Chang, Y.-F., Nakano, M., Abdelfattah, A. S., Fujiwara, M., Ishihara, T., Nagai, T., and Campbell, R. E. 2011. An expanded palette of genetically encoded Ca^{2+} indicators. *Science* **333**: 1888–1891.

Zheng, S.-J., Henken, B., Kyun, Ahn Y., Krens F. A., and Kik, C. 2004. The development of a reproducible *Agrobacterium tumefaciens* transformation system for garlic (*Allium sativum* L.) and the production of transgenic garlic resistant to beet armyworm (*Spodoptera exigua* Hübner). *Mol. Breed.* **14**: 293–307.

Zhou, H., Arrowsmith, J. W., Fromm, M. E., Hironaka, C. M., Taylor, M. L., Rodriguez, D., Pajeau, M. E., Brown, S. M., Santino, C. G., and Fry, J. E. 1995. Glyphosate-tolerant CP4 and GOX genes as a selectable marker in wheat transformation. *Plant Cell Rep.* **15:** 159–163.

Zhu, T., Mettenburg, K., Peterson, D. J., Tagliani, L., and Baszczynski, C. 2000. Engineering herbicide-resistant maize using chimeric RNA/DNA oligonucleotides. *Nature* **18**: 555–558.

Zhu, Y. J., Agbayani R., McCafferty H., Albert H. H., and Moore P. H. 2005. Effective selection of transgenic papaya plants with the PMI/Man selection system. *Plant Cell Rep*. **24**: 426–432.

Zhu, Y. J., Agbayani, R., and Moore, P. H. 2004. Green fluorescent protein as a visual selection marker for papaya (*Carica papaya* L.) transformation. *Plant Cell Rep*. **22**: 660–667.

Zhu, Z. and Wu, R. 2008. Regeneration of transgenic rice plants using high salt for selection without the need for antibiotics or herbicides. *Plant Sci*. **174**: 519–523.

Zubko, M. K., Zubko, E. I., van Zuilen, K., Meyer, P., and Day, A. 2004. Stable transformation of petunia plastids. *Transgenic Res.* **13**: 523–530.

Zuo, J., Niu, Q.-W., and Chua, N. H. 2000. An estrogen receptor-based transactivator XVE mediates highly inducible gene expression in transgenic plants. *Plant J*. **24**: 265–273.

Zuo, J., Niu, Q.-W., Ikeda, Y., and Chua, N.-H. 2002. Marker-free transformation: increasing transformation frequency by the use of regeneration-promoting genes. *Curr. Opin. Biotechnol.* **13**: 173–180.