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Invited Editorial

Transgene inheritance in plants

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Abstract. The patterns of transgene inheritance in plants and the possible explanations for non-Mendelian transmission are reviewed. The non-Mendelian inheritance of a transgene has been recorded with a frequency between 10% and 50% in transgenic plants produced either by *Agrobacterium*-mediated transformation or through particle bombardment. Different effects such as deletion, duplication, rearrangement, repeated sequence recombination as well as gene interaction have been observed for transgenic loci. The nature of the recipient genome, nature of the transgene and the interactions between them seem to contribute to the non-Mendelian segregation of transgenes.

Key words: transgene inheritance, transgene interactions, transgenic plants.

Introduction

To date, various transgenes have been successfully introduced into nuclear genomes of over 120 plants species with verified methods (BIRCH 1997). Understanding of the inheritance and stability of the newly introduced transgenes is of great importance in determining the value and application of genetically engineered organisms (GMOs) in agriculture. Characterization of the transgene locus/loci on the molecular level (transmission of the transgene) as well as segregation analysis of the transgene-encoded phenotype (expression

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of the transgene) in the subsequent progenies allowed insight into the nature of transgene inheritance. Integration of transgenes at a single Mendelian locus, regardless of copy number, is typically observed in transformants produced both by direct DNA delivery (SPENCER et al. 1992, REGISTER et al. 1994) and by *Agrobacterium*-mediated transformation (DEROLES, GARDNER, 1986). Multiple complete and/or partial transgene copies inherited as digenic or multigenic Mendelian traits have also been documented (CLUSTER et al. 1996). However, the non-Mendelian segregation occurred at a frequency between 10% and 50% of lines due to either unstable transmission of the transgene or poor expression (DEROLES, GARDNER 1988, REGISTER et al. 1994, McCABE et al. 1999, LIMANTON-GREVET, JULLIEN 2001).

The regular transgene transmission as well as its expression is a main prerequisite for the production of new cultivars in generatively propagated plants. Therefore, the knowledge of distortion frequency and the sources of this phenomenon have a substantial importance for breeding of transgenic varieties. The present paper attempts to review various aspects of transgene inheritance in plants. The possible mechanisms related to the non-Mendelian inheritance of the transgene are also discussed here.

A brief review of the patterns of transgene inheritance

Agrobacterium-mediated transformation usually produces transgenic plants with a low copy number and the transgenes are transmitted to progeny according to Mendelian (HORSCH et al. 1984, BUDAR et al. 1986) and in some cases non-Mendelian inheritance (DEROLES, GARDNER 1988). The characteristic features of the transgene integration pattern resulting from DNA delivery through particle bombardment often include integration of the full-length transgene as well as rearranged copies of the introduced DNA. Copy numbers of both the transgene and rearranged fragments are often highly variable. Multiple transgene copies most frequently are inherited as a single locus. A variable proportion of the transgenic events exhibited a Mendelian ratio *vs* events exhibiting segregation distortion (PAWLOWSKI, SOMERS 1996). An overview of patterns of transgene inheritance in selected plant species is given in Table 1.

In some cases, the transgenic locus has not been stably inherited. Both deletion of a transgene locus and rearrangement of inserted T-DNA with either retention or loss of expression have been reported (POTRYKUS et al. 1985, CHYI et al. 1986, HEBERLE-BORS et al. 1988, HÅNISCH et al. 1990, MEYER et al. 1992, CHERDSHEWASART et al. 1993, SRIVASTAVA et al. 1996). Duplication or amplification of transgenes (SPENCER et al. 1992, CANNELL et al. 1999), and the epistatic interaction between different loci and/or allelic interaction within a single locus also exist (MATZKE, MATZKE, 1995, NAP et al. 1997). Furthermore, mitotic/meiotic recombination has been observed for transgenic loci in various plant species (GAL et al. 1991, ASSAAD, SIGNER 1992, TOVAR, LICHTENSTEIN 1992).

Transgene interactions

Any new transgene or transgene-associated sequence may confer or be subject to epistatic gene interaction, as reported by NAP et al. (1997). Those authors studied the interaction of the transgene alleles both within a locus (dominance) and between loci (epistasis) using six transgenic tobacco lines, each homozygous for the β-glucuronidase (GUS) gene at a different locus. Each of the four single-copy lines acted fully additively. In contrast, the two complex single-locus lines NLG-4 and NLG-47 were epistatic dominant over all other *gus* alleles. Any hybrid in which a one-copy parent was combined with either of them showed a marked decrease in GUS activity. The segregation of GUS activity in the F_2 progeny of the most extreme combination (NLG-11 × NLG-47) was consistent with the classical 12 : 3 : 1 segregation known as dominant epistasis. Lines NLG4 and NLG47 also exhibited classical single-locus overdominance or single-locus heterosis. The hemizygous NLG-47 × WT plants were significantly more active than the homozygous NLG-47, which indicated within-locus interaction between the NLG-47 alleles in homozygous form. The authors inferred that the dominant epistasis and overdominance exhibited by the NLG-4 and NLG-47 alleles seem to favour a quantitative mechanism underlying homology-dependent gene silencing. HOBBS et al. (1993) suggested that low-expressing (L) type inserts worked *in trans* to suppress *uidA* expression from high-expressing (H) type inserts in transgenic tobacco plants when both of them were present in the same genome. The *uidA* alleles on the H-type insert acted in an additive manner when no L-type inserts were present in the genome. Cross-pollination between two different transformants with H-type inserts produced F_1 progeny with GUS activity levels that were not different from the parents. The F_2 populations showed transgressive segregation with levels of GUS activity up to twice that of the parent and definite clusters of individuals around the 0, 50, 100, 150 and 200% levels.

Mitotic and meiotic recombination events for the transgene locus

Repeated sequence recombination has been studied intensively in bacteria, yeasts, animal cell cultures as well as plants (PETES, HILL 1988, BOLLAG et al. 1989, PETERHANS et al. 1990, GAL et al. 1991, TOVAR, LICHTENSTEIN 1992). In *Arabidopsis* plants, the recombination frequency between a pair of directly repeated transgenes, two different internal, non-overlapping deletion alleles of *npt* flanking an active *hpt* gene, varied from 2 to 6×10^{-5} (ASSAAD, SIGNER 1992). Recombination in the repeated sequences appears to be at most an order of magnitude (20-fold) more frequent per division in meiosis ($<2 \times 10^{-5}$) than in mitotic growth ($>10^{-6}$). In addition, simple recombination events, including simple gene conversion or simple crossover, as well as recombination products resulting from the concerted action of two or more simple events, have also been recorded. In *Brassica napus* carrying an integrated multimer of cauliflower mosaic virus, a mismatch repair in somatic recombination was evident (GAL et al. 1991). In soybean, a high frequency of recombination for the casein transgene locus was observed (CHOFFNES et al. 2001).

Table 1. Transgene inheritance in selected plant species

Plant species	Transgenic population	Transgene	Segregation ratio (transgenic:non-transgenic)		Inheritance pattern: Mendelian (M) non-Mendelian (nonM)	References
			Genotypic	Phenotypic		
1	2	3	4	5	6	7
Alfalfa	F_1 from sexual crosses between hemizygous transgenics	35S-*SOD*	–	SOD activity in F_1 individual higher than in parent	M / nonM	SAMIS et al. 2002
Asparagus officinalis L.	T_1, T_2 from crosses between transgenics and non-transgenics	35S-*uidA* *nos-npt*II	M / nonM	1:1	M / nonM	LIMANTON-GREVET, JULLIEN 2001
Barley	T_2	35S-*npt*II	83% T_2 progeny contained *npt*II gene	The transgene expressed in 82% T_2 plants	M / nonM	RITALA et al. 1994, 1995
	F_2 from crosses between homozygous transgenics and non-transgenic	α-amylase promoter-heat-stable β-glucanase gene	–	1:2:1, 3:1, distorted	M / nonM	HORVATH et al. 2001
Bean	R_1	35S-*gus*	–	3:1 or distorted	M / nonM	ARAGÃO et al. 1996
Cucumber	T_1	*nos-npt*II		3:1 or distorted	M / nonM	SZWACKA et al. 2002
Vicia narbonensis L. (grain legume)	R_1	Tr1',2'-*gus*	–	3:1	M	SAALBACH et al. 1994
Lettuce	R_1, R_2	*nos-npt*II	–	3:1, other ratios, distorted	M / nonM	MCCABE et al. 1999
Lotus corniculatus L.	F_1 from the crosses between transgenics	35S-*AS-DFR* *npt*II	Underrepresentation of transgene sequences	No transgene phenotype observed	nonM	ROBBINS et al. 1998
	T_2, T_3 from crosses between transgenics and non--transgenics	35S-*uidA*	–	1:1, distorted	M / nonM	WEBB et al. 1999

1	2	3	4	5	6	7
Maize	Backcross F_1	35S-*luc*	–	1:1	M	FROMM et al. 1990
	Backcross R_1, R_2	35S-*bar*	–	1:1	M	SPENCER et al. 1992
	Backcross or outcross R_1, R_2	35S-*hpt*	1:1, distorted	1:1, distorted	M / nonM	WALTERS et al. 1992
	Outcross progeny	35S-*npt*II 35S-*pat*	1:1, 2:1, distorted	1:1, distorted	M / nonM	REGISTER et al. 1994
	Selfed progeny	35S-*bar* 35S-*gus*	–	3:1, 1:1, 1:3	M / nonM	ISHIDA et al. 1996
	T_1, T_2, T_3 from self--pollination or cross-pollination	*act1-uidA* 35S-*bar*	–	1:1 for cross-pollination, 3:1 for self--pollination, distorted	M / nonM	ZHANG et al. 1996 ZHONG et al. 1996
Nicotiana sylvestris	Selfed progeny S_1, S_2	*nos-npt*II 35S-*CHN48*	–	1:2:1, 18% S_2 plants show silent phenotype for CHN48	M / nonM	HART et al. 1992
	Progeny from crosses between homozygous transgenics	*nos-npt*II	–	15:1, distorted	M / nonM	KUNZ et al. 1996
Oat	T_1, T_2	35S-*bar* *nos-uidA*	–	3:1, distorted	M / nonM	PAWLOWSKI et al. 1998
Petunia	Backcross progeny	*nos-npt*	–	–	M / nonM	CLUSTER et al. 1996
Rice	Pollen grains of an individual plant	*act1-gus*	–	7:1	M	ZHANG et al. 1991
	R_1, R_2	35S-*hpt* 35S-*gus*	–	3:1, 15:1, distorted	M / nonM	HIEI et al. 1994
	T_1, T_2, T_3	35S-*neo* 35S-*gusA* *act-gusA* RTBV-*gusA*	–	3:1, 1:1, 1:2, 0:all	M / nonM	PENG et al. 1995
	R_1, R_2	35S-*bar* *act1-bar*	–	distorted	nonM	PARK et al. 1996
	R_1	35S-*Btt* *cryIIIA* *ubi1-bar*	–	Under-representation of the transgenic class	nonM	KUMPATLA et al. 1997

Table 1 (cont)

1	2	3	4	5	6	7
Rice	R_1 R_2	*hph, luc, CP1, CP2, CP3, uidA, bar, RT*	3:1	3:1, distorted	M / nonM	CHEN et al. 1998
	R_1	35S-*hph*	–	3:1, distorted	M / nonM	CHENG et al. 1998
	T_1	35S-*aphIV, ubi-gusA,* 35S-*OC-I* ΔD86	3:1	3:1, distorted	M / nonM	VAIN et al. 1998
	T_1 progeny	35S-*NtFAD3*	–	–	M	WAKITA et al. 1998
	R_1	*ubi1-Cry1Ac* 35S-*Cry2A nos-gna ubi1-gna*	3:1, 1:1	–	M / nonM	MAQBOOL, CHRISTOU 1999
	Independent lines over 3 or 4 generations	35S- *gusA* 35S-*hpt*, 35S-*bar, ubi1-gna,* RSs1-*gna,* 35S-*cry2A, ubi1-cry1Ac*	–	3:1, 1:1	M / nonM	GAHAKWA et al. 2000
Soybean	T_1, T_2	soybean lectin promoter-bovine beta--casein gene	_	distorted	nonM	CHOFFNES et al. 2001
Sugar beet	Progeny from crosses between transgenics and non-transgenics	35S-*pat*	_	1:1	M	HALL et al. 1996
Tobacco	R_1, R_2	*nos-npt*II	–	75% Kan^R in R_1 92% Kan^R in R_2 90% Kan^R in selfed progeny of BC_2, 75% in backcross progeny	M / nonM	MATZKE et al. 1994
	R_1, haploids	*mas1'-npt*II *mas2'-npt*II	–	3:1, 15:1 in R_1 1:1, 3:1 in haploid	M	BEAUJEAN et al. 1998
	T_1	*nos-npt*II	–	63:1	M	BUCHERNA et al. 1999

1	2	3	4	5	6	7
Tobacco	T_1	35S-*npt*II 35S-*hph* 35S-*bar*	–	3:1, distorted	M / nonM	MCCORMAC et al. 2001
Wheat	R_1	*ubi-bar*	–	3:1, distorted	M / nonM	ALTPETER et al. 1999
	T_1, T_2, T_3	*ubi-bar* *ubi-uidA* *act-uidA* 35S-*neo*	–	5:1, 3:1, distorted	M / nonM	CANNELL et al. 1999
	BC_2, BC_1F_2	*act1D-uidA*: *npt*II	–	1:1, 15:1, distorted	M / nonM	DEMEKE et al. 1999
White clover	BC_1, BC_1F_2	35S-*uidA*	–	1:1 for backcross 3:1, 2:1 for BC_1F_2	M / nonM	SCOTT et al. 1998

Abbreviations

Promoters
35S = cauliflower mosaic virus 35S promoter
act1 = rice actin 1 promoter
act = rice actin promoter
mas1'= mannopine synthase / TR1' promoter
mas2'= mannopine synthase / TR2' promoter
nos = nopaline synthase promoter
Tr1',2' = T-DNA promoter
ubi1 = maize ubiquitin promoter
RSs1 = rice sucrose synthase-1 promoter
RTBV = rice tungro bacilliform virus promoter

Coding sequences
aphIV = hygromycin resistance gene
AS-DFR = antisense dihydroflavonol reductase gene
bar = phosphinothricin acetyltransferase gene
Btt cryIIIA = a 1794 bp synthetic *Bacillus thuringiensis* var. *tenebrionis cryIIIA* gene
CHN48 = tobacco chitinase gene
CP1 (*CP2, CP3*) = RTSV (rice tungro spherical virus) coat protein genes
cry1Ac, cry2A = *Bacillus thuringiensis* (Bt) δ-endotoxin genes
gusA = β-glucuronidase gene
gus = β-glucuronidase gene
gna = snowdrop lectin gene
hph = hygromycin phosphotransferase gene
hpt = hygromycin phosphotransferase gene
luc = firefly luciferase gene
neo = neomycin phosphotransferase II gene
npt = neomycin phosphotransferase gene
*npt*II = neomycin phosphotransferase II gene
NtFAD3 = tobacco fatty acid desaturase gene
OC-I ΔD86 = gene coding an engineered cysteine proteinase inhibitor
pat = phosphinothricin acetyltransferase gene
RT = the rice tungro bacilliform virus reverse transcriptase gene
SOD = superoxide dismutase gene
uidA = β-glucuronidase gene

In tobacco, TOVAR and LICHTENSTEIN (1992) studied meiotic and somatic chromosomal recombination events in transgenic lines carrying a functional hygromycin phosphotransferase (*hyg*) selectable marker flanked by a pair of defective neomycin phosphotransferase (*neo*) genes positioned as inverted repeats. Spontaneous somatic recombinants were recovered at frequencies between 3×10^{-5} and 10^{-6} events per cell. For meiotic recombination, kanamycin-resistant

(Km^r) seedlings were recovered with a frequency of 6.7×10^{-6}, similar to that of Km^r calli obtained during mitotic selections. Homologous recombination occurred mainly as gene conversion unassociated with reciprocal exchange. The interchromosomal associations occur at a higher frequency than intrachromosomal events. The recombination is more frequent in homozygous than in hemizygous cells.

Possible explanations for non-Mendelian inheritance of a transgene

Similarly to the aberrant phenotypic segregation observed in non-transgenic plants (BRADSHAW, STETTLER 1994), a non-Mendelian segregation of transgenes occurs after microprojectile bombardment or *Agrobacterium*-mediated transformation (BUDAR et al. 1986, CHYI et al. 1986, HEBERLE-BORS et al. 1988, TOMES et al. 1990). Effects resulting from a number of factors were thought to account for this phenomenon. These factors include the nature of the recipient genome, the nature of the transgene itself as well as the interactions between them.

Nature of the recipient genome

Genetic background

The nature of the recipient genome may influence the stability of the introduced transgenes as well as their expression. REGISTER et al. (1994) observed that the transgenes, *pat* and *npt*II genes, were not stably inherited in 6 maize transformants. The authors inferred that this might be caused by the nature of the maize genome. Examples of epigenetic control of gene expression in this species have been well documented for transposable elements (BENNETZEN 1987, CHOMET et al. 1987, FEDEROFF et al. 1989) and other epigenetic phenomena (COE et al. 1988). Tritordeum (*Hordeum chilense* × *Triticum durum*) is known to be relatively genetically unstable because it has a novel genomic combination and the transgenic loci in tritordeum lines appear less stable than in wheat lines (CANNELL et al. 1999). SCOTT et al. (1998) attribute the distorted segregation ratio observed in later generations of transgenic white clover plants to the out-breeding nature of the species, which leads to changes in the genetic background. Moreover, the same authors observed that the segregation ratio of GUS-positive to GUS-negative plants fit the 2 : 1 ratio expected for a recessive lethal. The authors indicated that the transgene has been inserted near a recessive lethal. Generally, plants homozygous for the transgene also would be homozygous for the recessive lethal and thus nonviable.

Gamete viability

CHRISTOU et al. (1989) suggested that the segregation distortion might reflect sterility in one set of gametes. The authors credited a 1 : 1 segregation ratio observed in progeny of a transgenic soybean plant to the failure of passing a transgene

to the next generation through pollen (pollen lethality). WALTERS et al. (1992) demonstrated that a site of gene insertion could affect gamete viability in some manner, leading to a lack of 1 : 1 segregation of hygromycin phosphotransferase (HPT) in transgenic maize. ARAGÃO et al. (1996) indicated that an insertional mutation of an essential gene required for ovule fecundation and/or development might account for aberrant inheritance of *gus*, *neo*, *AC123* and *BC1* transgenes. GAHAKWA et al. (2000) provided preliminary evidence to support gamete lethality as the cause for the unusual segregation in transgenic rice. Iodine staining of pollen revealed a number of intact but deformed pollen grains in some R_2 plants (GAHAKWA et al. 2000) and R_3 progeny of this line showing a distorted segregation ratio of 1 : 1 (FU et al. 2000). LIMANTON-GREVET and JULLIEN (2001) suggested that the 1 : 1 segregation of kanamycin resistance and GUS expression was due to the transmission of the transgenes through male gametes only. This could be attributed to the presence of an insertional mutation affecting the viability of the female gametes, as the transgenes were male-transmitted to the first progeny.

Chromosome abnormality

MATZKE et al. (1994) suggested that chromosomal abnormalities are a likely source of non-Mendelian inheritance of transgenes. The authors analysed a transgenic tobacco line that exhibited abnormal inheritance of marker transgenes. Some plants produced more kanamycin-resistant progenies, and some plants produced considerably less than expected on the basis of the parental transgenotype. The authors inferred that the transgene locus (*K*) was present on the chromosome responsible for the aneuploidy. However, the genetic behaviour was not completely explicable by aneuploidy. The epigenetic characteristics, including effects of *K* dosage on marker gene expression in trisomics and tetrasomics, spontaneous generation of methylated epialleles, and sensitivity to directed methylation and *trans*-inactivation in the presence of partially homologous 'silencing' loci, also contribute to the unusual inheritance pattern of the *K* locus. SPENCER et al. (1992) suggested that the T8 integration event might be linked to a deleterious chromosome abnormality, such as a small duplication or deletion. Therefore, it transmitted at a low frequency and led to the unstable integration of the *bar* gene in this maize line.

Transformation method

In addition, for the same plant species, the transformation method had a significant influence on the type and copy number of T-DNA integration events (GREVELDING et al. 1993). The authors demonstrated that most of the *Arabidopsis* transgenic plants produced by a leaf-disc method contained multiple T-DNA insertions (89%), the majority of which were organized as right-border inverted repeat structures (58%). In contrast, a root transformation method mostly resulted in single T-DNA insertions (64%), with fewer right-border inverted repeats

(38%). The multiple T-DNA insertions were known to be associated with transgene silencing or co-suppression (JORGENSEN 1991, 1993)

Nature of the transgene

Transgene silencing

Gene silencing was initially thought to contribute to non-Mendelian inheritance when segregation ratios were determined through an analysis of protein expression (FINNEGAN, McELROY 1994, McELROY, BRETTEL 1994). The presence of multiple gene copies can correlate with transgene silencing or co-suppression (JORGENSEN 1991, 1993). REGISTER (1994) suggested that silencing of *bar, pat* or *uidA* expressions in transgenic maize were associated with the presence of multiple copies of transgenes integrated at a single locus. BUCHERNA et al. (1999) found that the presence of two copies of the gene was essential for silencing, but these can be present either at the same locus (homozygous) or at different loci (double hemizygous). KUNZ et al. (1996) observed a high incidence of silencing for the *CHN48* chitinase gene in double hemizygous plants, which is comparable to that of the parents homozygous for a single insert. Some other reports suggest that a transgene becomes silenced when present in homozygous form but is continuously expressed when present in hemizygous form (De CARVALHO et al. 1992, DORLHAC de BORNE et al. 1994, KUNZ et al. 1996). DNA-methylation--induced gene silencing caused the non-Mendelian segregation of PPT resistance in the R_2 generation of transgenic rice plants (PARK et al. 1996) and an unusual segregation of the *npt*II gene in lettuce (McCABE et al. 1999). Gene silencing also contributed to the non-Mendelian inheritance of *bar* and *gusA*/*uidA* genes in rice (KUMPATLA et al. 1997, KOHLI et al. 1999, GAHAKWA et al. 2000) and wheat (CANNELL et al. 1999). PAWLOWSKI et al. (1998) suggested that transgene silencing and distortions of transgene inheritance were highly unstable in transgenic oat.

Unstable integration of a transgene

Transgene deletion, duplication or chimerism also accounted for non-Mendelian inheritance. WALTERS et al. (1992) suggested that the introduced DNA might be unstably integrated and absent from some of the gametes, resulting in a lack of 1 : 1 segregation of HPT in two transgenic maize lines. SPENCER et al. (1992) demonstrated that gene deletion or poor transmission was responsible for the aberrant segregation ratio for the *bar* gene in transgenic maize line T8. SRIVASTAVA et al. (1996) observed a deletion of *bar* and *gus* genes in R_3 plants of transgenic wheat line 2B-2. In soybean, the recombination for the casein transgene locus resulting in the loss of transgene DNA was taking place within a limited physical distance on the host chromosome (CHOFFNES et al. 2001). In some cases a duplication of the transgenes may occur. SPENCER et al. (1992) suggested that a single integration event was replicated in T9 callus, yielding T9 R_0 plants that were homozygous for *bar* and *uidA*. Possible mechanisms responsible for this

homozygosity include mitotic recombination (STERN 1936) and gene conversion (LINDEGREN 1953). REGISTER et al. (1994) reported the occurrence of a similar phenomenon where amplification of the *uidA* gene occurred in some T_3 progenies of a maize transgenic line. Experimental evidence for a duplication of the *uidA* sequence (or part of it) in some of the T_1 progenies of transgenic tritordeum line HTT2 has also been presented (CANNELL et al. 1999). Furthermore, chimerism influences transgene inheritance. HIEI et al. (1994) observed that the progenies of a limited number of rice transformants showed unusual segregation patterns of GUS expression. It appeared that the chimerism in the R_0 generation affected the segregation ratios.

Interactions between the recipient genome and the transgene

Homozygous lethality

Segregation distortion may reflect homozygous lethality (BUDAR et al. 1986, DEROLES, GARDNER 1988, SCOTT et al. 1998). LIMANTON-GREVET and JULLIEN (2001) demonstrated that a 2 : 1 segregation for kanamycin resistance and GUS expression in the T_2 progeny of transgenic asparagus was due to the lack of homozygotes. The authors suggested that the loss of homozygotes was observed when T-DNA insertion led to a lethal mutation.

Poor transmission of a transgene

ARAGÃO et al. (1996) observed that 44% of transgenic bean plants did not transfer the introduced genes *gus, neo, AC123* or *BC1* to the R_1 generation, and two plants showed poor transmission of the transgenes (1 : 10) to the R_1 generation. The authors suggested that the inserted transgenes might cause some de-stabilization of the chromosome structure and poor transgene transmission to the progeny.

Mitotic crossover / Meiotic instability

One T_0 transgenic wheat line, WT5, showed no segregation of the *neo* gene from the Southern analysis of all T_1 progenies, indicating the presence of a locus that was homozygous for the inserted *neo* gene in the T_0 generation (CANNELL et al. 1999). The most likely mechanism that would cause this is sister chromatid exchange (mitotic crossover) during the early stages of embryogenesis and regeneration of a bombarded embryo. A copy of the transgene on one chromatid could be passed to the allelic position on the opposing homologue. The daughter cell inherited a transgene-containing chromatid from each homologue would be homozygous for the transgene, whereas the other daughter cell would be null for the transgene. The former genotype would proliferate under selection, whereas the latter would die.

A low frequency of meiotic transgene instability has been reported in plants transformed via *Agrobacterium* with single-copy inserts (CHYI et al. 1986, MÜLLER et al. 1987). SCOTT et al. (1998) also reported that a homozygous

transgenic white clover plant differed from expectations in the fact that a single progeny plant lacking the *uidA* and *npt*II genes was obtained. The authors suggested that this plant might have resulted from a rare meiotic instability event that led to the loss of part or all of the T-DNA.

Conclusions

The introduction of a transgene into a recipient genome is a complex event depending on the transgene itself and the host genome. The transgene expression level may vary extremely, depending on a number of factors (HOBBS et al. 1993, MEYER 1995, KOHLI et al. 1999, YIN, MALEPSZY 2003) in which the 'positional effects' play a major role. The same is true for transgene inheritance, where the site of transgene integration determines its stability. If integration occurs in a transcriptionally active area, the resulting expression may be influenced by proximal regulatory sequences (TOPPING et al. 1991). In situations where integration occurs in the repeat-sequence regions of heterochromatin, inactivation of transgenes may result (YE, SIGNER 1996). Several other factors are also known. Generally, they are known genetic mechanisms, including transgene deletion, duplication, rearrangement, repeated sequence recombination, and gene interactions. The frequency of distortion in transgene inheritance varied between 10% and 50% of lines (independent transformants). However, these data are most frequently based on a very low number of lines analysed. The consequence of non-Mendelian inheritance for transgenic breeding is that an increasing number of lines should be produced after transformation.

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