식물조직배양학회지 제24권 제4호
Korean J. Plant Tissue Culture
Vol. 24, No. 4, 225~231 (1997)

Matrix Attachment Regions (MARs) as a Transformation Booster in Recalcitrant Plant Species

HAN, Kyung-Hwan
Kumho Life and Environmental Science Laboratory, 572 Ssangamdong, Kwangsangu, Kwangju, 506-712, Korea. E-mail hanky@camd2.kkpcr.re.kr

For genetic engineering to be commercially viable, an efficient transformation system is needed to produce transgenic plants from diverse genotypes ("generalized protocol"). Development of such a system requires optimization of a number of components such as gene transfer agent, plant tissues competent for both regeneration and transformation, and control of transgene expression. Although several novel gene transfer methods have been developed for plants, a majority of stably transformed plants express the introduced genes at low levels. Moreover, silencing of selectable marker genes shortly after their incorporation into plant chromosomes may result in low recovery of transgenic tissues from selection. Matrix attachment regions (MARs) are DNA sequences that bind to the cell's proteinaceous nuclear matrix to form DNA loop domains. MARs have been shown to increase transgene expression in tobacco cells, and reduce position in mature transgenic plants. Flanking an antibiotic resistance transgene with MARs should therefore lead to improved rates of transformation in a diversity of species, and may permit recalcitrant species and genotypes to be successfully transformed. Literature review and recent data from my laboratory suggest that MARs can serve as a transformation booster in recalcitrant plant species.

Genetic engineering technique provides a means for inserting genes that confer traits not readily available in traditional gene pool. However, its use in a crop improvement program requires the development of procedures to regenerate plants from single organized tissues and to transfer genes to plant cells. These procedures must be applicable to a wide variety of species and/or genotypes to be effective. So far these prerequisites have been met in only a few crop species such as cotton, potato, and tomato. Many economically important species remain recalcitrant to tissue cultural and gene transfer manipulations. Lack of an efficient transformation protocol has been a major limitation in genetic engineering of these economically important crops.

Transformation efficiency is determined by the effectiveness of recipient plant cells, gene delivery process, and subsequent foreign gene expression. In the past, transformation efforts have been focused mainly on optimization of the first two factors, host cells and gene transfer process. As the expression of the transferred gene is unpredictable and unstable (for recent reviews, see Finnegan and McElroy, 1994: Matzke and Matzke, 1995), management of transgene expression is important not only for maintaining transgenic phenotypes but also for enhancing survival rate of the transformed cells during selection. Matrix attachment regions (MARs) are thought to protect the flanked genes from negative influences of adjacent DNA and to increase expression of the genes. Flanking selectable marker gene(s) with MARs could therefore enhance transformation rate by increasing number of transformed cells surviving the selection. In-depth review on transgene silencing and MARs *per se* is not the scope of this article. Rather, I organize this review around the relevance of MARs to practical use in plant biotechnology.

Major components of a transformation system

Transformation system is often mistaken as a mere step to introduce foreign genes into plant cells. Considering the instability of transgene expression and the time consuming transformation process, it is essential that methods for enhancing and stabilizing the expression of the introduced genes be incorporated into transformation program. Key components for an efficient transformation system are given below.

Choice of gene transfer agent to enable efficient delivery of DNA into plant cells

There are two means for transforming plants: use of biological vectors to deliver DNA to cells, primarily *via Agrobacterium*: and direct delivery of naked DNA into plant cells, primarily *via* biolistics. *Agrobacterium*-mediated transformation has been a choice of gene transfer system for many plant species including monocot (Hiei *et al.*, 1994) and offers a variety of advantages with respect to direct DNA transformation methods (for reviews, see Han *et al.*, 1996: Jouanin *et al.*, 1993).

Selection and management of plant tissues to maximize their competence for regeneration and transformation

This calls for careful selection and manipulation of explants (Han, Ma, and Strauss: in preparation) to enhance DNA uptake into cells, incorporation of DNA into chromosomes, and regeneration of transformed cells into plants. As plant regeneration is under major gene control (Han *et al.*, 1995: Koornneef *et al.*, 1993), selection of right genotype is important for transformation success. Clone-specific responses of poplar tissues to *Agrobacterium* DNA transfer have been observed (Riemenschneider, 1990: Han, unpublished results).

Management of transgene insertion, expression, and inheritance

The choice of promoters and other DNA fragments will influence expression (e.g., MARs) and allow efficient selection of transgenic cells and stable expression of transgenic phenotypes (Han *et al.*, 1996).

Transgene silencing

Many transgenes including selectable marker genes vary widely in their level of expression, and complete silencing is not uncommon. Expression of introduced genes can be unstable (Brandle *et al.*, 1995). The standard agronomic practice of seedling transplantation can cause complete loss of transgene expression (Brandle *et al.*, 1995). According to Finnegan and McElroy (1994), nearly all of the 30 plant biotechnology companies polled experienced undesired silencing of transgenes. One third of hybrid cottonwoods transformed with *rol* genes expressed no transgenic phenotypes (Han *et al.*, 1997). Phenotypic changes caused by the transgenes can be reverted back to normal (Figure 1) (Han *et al.*, 1997: Han, not published observation in transgenic black locusts: Milton P. Gordon, personal communication). Several different genetic mechanisms appear to be involved, often associated with multiple copies of transgenes and DNA methylation (Finnegan and McElroy, 1994: Flavell, 1994: Matzke and Matzke, 1995: Meyer and Saedler, 1996).

Figure 1. Pictures of transgenic plants showing reverted transgenic phenotypes. All three plants were transformed with a modified wild-type *Agrobacterium rhizogenes* strain R1601. This strain induces hairy root formation from infected plants. Typical phenotypes of hairy root transgenics include dark green and wrinkled leaves, dwarfism, shortened stem internode, etc.
Left) Lower leaves of this transgenic black locust (*Robinia pseudoacacia*) are showing a typical hairy root phenotype (dark-green and wrinkled), while the upper leaves have normal (reverted) phenotype.
Middle) a newly forming branch in this transgenic hybrid cottonwood (*Populus trichocarpa* × *P. deltoides*) shows reverted phenotype (arrow).
Right) a lateral branch in this transgenic tobacco (*Nicotiana glauca*: picture by Milton P. Gordon at University of Washington, USA) has reverted phenotype and became the leader branch (arrow).

Position effects and co-suppression

The level and specificity of expression of introduced genes is unpredictable varying with the chromosomal site of the insertion (Hobbs *et al.*, 1993: Hollick and Gordon, 1993). These "position effects" are observed in both animals and plants. Although several novel gene transfer methods have been developed for plants, a majority of detectable transformants express the introduced gene at low levels (Peach and Velten, 1991). They are attributed to variation in transcriptional activity of the genomic insertion site.

Gene silencing can often be caused by the transgenes that express at a high level (Boerjan *et al.*, 1994). Introduction of extra copies of endogenous genes and/or over expression of the introduced genes may result in the coordinate silencing of the transgenes and the homologous endogenous genes. This phenomenon, termed "co-suppression," was first reported in transgenic petunia plants transformed with a petunia chalcone synthase gene (Napoli *et al.*, 1990). Although none of them is

proven unequivocally, four categories of hypothesis have been proposed to account for this intriguing phenomenon (reviewed in Finnegan and McElroy, 1994: Flavell, 1994: Matzke and Matzke, 1995: Meyer and Saedler, 1996). The hypothesis widely discussed are: 1) competition between the genes for non-diffusible factors, essential for expression, such as nuclear matrix: 2) the formation of hybrid DNA (duplex/triplex pairing) through which different patterns of cytosine methylation can be imprinted into the participating DNA strands: 3) post transcriptional inhibition *via* production of unintended antisense RNA as the result of RNA dependent RNA polymerase activity using excess mRNA as template.

DNA methylation and demethylating agents

DNA methylation has an important role in the control of gene expression, and is usually associated with gene silencing (Matzke *et al.*, 1989). *In vitro* DNA methylation inhibited gene expression in transgenic tobacco (Weber *et al.*, 1990). The demethylating agent 5-azacytidine (*aza*C) activated silent T-DNA genes in transgenic tobacco transformed by *Agrobacterium tumefaciens* (Bochardt *et al.*, 1992). Treatment of *Agrobacterium* or leaf disks with *aza*C generated more transformants than did controls (Palmgren *et al.*, 1993).

Transgene copy number

Inserting multiple copies of a transgene may lead to co-suppression and consequent loss of gene expression. *Agrobacterium*-mediated transformation appears to result in fewer transgene copies than do direct DNA transformation methods (De Block, 1993). The stage of the cell cycle also influences transgene insertion patterns. PEG/Ca^{2+}-mediated transformation of non-synchronized protoplasts generally resulted in simple integration patterns (single copy insertions). Transformation of synchronized protoplasts in the synthetic (S) and mitotic (M) phase led to more complex patterns (Kartzke *et al.*, 1990). Root explants tend to give rise to fewer transgene copies than leaves in *Arabidopsis* (Grevelding *et al.*, 1993).

Matrix attachment regions (MARs)

MARs are DNA fragments that appear to both increase and stabilize the expression of flanked transgenes (Table 1) (for reviews, see Breyne *et al.*, 1994: Spiker and Thompson, 1996). MARs help organize chromatin into loop domains by interacting with the proteinaceous scaffold. The loops are thought to delimit differing zones of transcriptional activity, isolating flanked genes from influences of adjacent DNA.

Chromatin structure and MARs

DNA is organized into highly ordered chromatin structure in the nucleus. Several levels of chromatin organization exist in the nucleus. On a basic level, DNA is wound around histone. The DNA-histone complexes are joined by linker DNA (McGhee and Felsenfeld, 1980) and packed helically to generate a 30 nm fiber (Felsenfeld and McGhee, 1986). A variety of evidence supports the hypothesis that the 30 nm chromatin fibers are further organized into loop domains by interacting with a proteinaceous scaffold in the nucleus (Paulson, 1988). The nuclear scaffold is retained in histone-depleted DNA and is thought to bind to matrix attachment regions (MARs) present in the DNA (Laemmli *et al.*, 1992).

MARs are usually 300- to 500-bp long (Meilke *et al.*, 1990) and have a propensity to bend (Anderson, 1986: Homberger, 1989). MAR elements may function as attachment sequences bordering DNA loop domains that insulate the genes encoded

Table 1. Summary of published results showing the effects of MARs on transgene expression in plants.

Source of MAR	Plant Transformed	Transformation Method	Results	Reference
Tobacco	Tobacco	*Agrobacterium*	〉 No increase in expression and slight reduction in variability	Breyne *et al.* (1992)
Yeast	Tobacco	Biolistics	〉 12-fold increase in expression and slight reduction in variability	Allen *et al.* (1993)
Soybean	Tobacco	*Agrobacterium*	〉 5- to 9-fold increase in expression and no effect in variability	Schoffl *et al.* (1993)
Bean	Tobacco	*Agrobacterium*	〉 Modest increase in expression and modest decrease in variability	Geest *et al.* (1994)
Chicken	Tobacco	*Agrobacterium*	〉 4-fold increase in expression and 8-fold decrease in variability	Mylnarova *et al.* (1994)
Chicken	Tobacco	*Agrobacterium*	〉 Slight increase in transformation efficiency and 2- to 7-fold reduction in variability	Mylnarova *et al.* (1995)
Tobacco	Tobacco	Biolistics	〉 60-fold increase in expression and slight reduction in variability	Allen at el. (1996)
Chicken	Tobacco	*Agrobacterium*	〉 Reduced variation to the environmental variation (background) level	Mylnarova *et al.* (1996)
Tobacco	Poplar and tobacco	*Agrobacterium*	〉 2- to 10-fold increase in expression, no effect in variability, and more than 8-fold increase in transformation frequency	Han *et al.* (1997)

on that loop. MARs have been identified in many organisms including: humans, chickens, potato, soybeans, tobacco and yeast (Breyne *et al.*, 1992: Grosveld *et al.*, 1987: Hall *et al.*, 1991: Hoffman *et al.*, 1989: Meilke *et al.*, 1990: Phi-Van and Stratling, 1988). MARs from one species have been shown to bind scaffolds from other species, suggesting that they are functionally conserved during evolution (Ammati and Gasser, 1990: Cockerill and Garrard, 1986).

MARs increases transgene expression

MARs are thought to allow the transgene to form its own chromatin domain (Figure 2). Consequently, the introduced gene would be insulated from the influences of factors in the chromatin domain adjacent to its site of insertion (Hall *et al.*, 1991). Because most chromatin (about 80% in plant) is in an inactive conformation at any given time, blocking such influences may result in enhanced gene expression. With the exception of (Breyne *et al.*, 1992) who reported the lack of an effect of MARs on transgene expression, several laboratories observed an increase in expression of a reporter gene due to the flanking MARs. Geest *et al.* (1994) found that MARs flanking the bean β-phaseolin gene increased a reporter gene expression modestly. The expression of GUS gene in transgenic tobacco increased 5- to 9-fold when flanked by MARs from a clone containing soybean heat-shock gene (Schoffl *et al.*, 1993). An animal MAR (chicken lysozyme) flanking a GUS reporter gene resulted in 4-fold increase (Mlynarova *et al.*, 1995: Mlynarova *et al.*, 1994). Allen *et al.* (1993) showed that MAR-containing constructs were less sensitive to co-suppression than control constructs and had an overall positive dose effect in cell lines containing up to 40 copies of the transgene. The yeast MAR increased reporter gene expression 24-fold in tobacco suspension cell lines. Most striking results (60-fold increase) were obtained with a strong tobacco MAR in transgenic tobacco (Allen *et al.*, 1996: Spiker *et al.*, 1995). Many MARs are multi-functional and are closely associated with transcriptional enhancers.

We tested the value of a MAR fragment derived from a tobacco gene (Hall *et al.*, 1991) for increasing transgene expression in poplar and tobacco using *Agrobacterium*-mediated transformation. The binary vector that carried a GUS-intron gene and an NPTII gene was modified to contain flanking

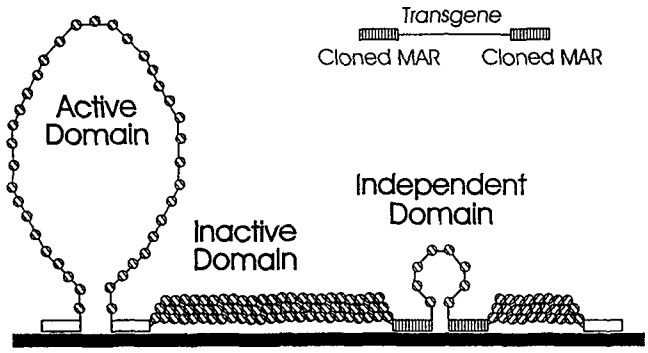

Figure 2. Model showing three different loop domains: active, inactive, and independent transgenic loop domains (proposed by Spiker and Thompson, 1996). Active domain is depicted as an 11-nm nucleosome fiber and the inactive domain as a 30-nm fiber. MARs (open box) flanking the active loop domain are believed to block the negative influences from neighboring inactive domain.

MAR elements within the T-DNA borders (Figure 3). In transgenic tobacco and two poplar clones (a readily transformable and a recalcitrant poplar clone), MARs increased GUS gene expression approximately 10-fold in the two hybrid poplar clones and 2-fold in tobacco one month after co-cultivation with *Agrobacterium*.

Although MARs clearly enhance trangene expression, whether this is due to an increased rate of transgene incorporation, an increased number of transformed cells, or an increased rate of expression per transgene was not resolved. MAR effects, while generally beneficial, differ importantly between genotypes and species. We found that, in our culture conditions optimized for poplars, the tobacco MAR was more efficient in poplars than in tobacco. This suggests that the magnitude of MAR effects may be affected by cell growth conditions. MARs seem to have little effect on transient gene expression consistent in both animal and plant systems (Allen *et al.*, 1993: Klehr *et al.*, 1991). Our data with tobacco and poplars also showed that MARs had no effect until 2-3 days after inoculation. This suggests that MARs do not work as typical enhancers and must be incorporated into the host genome to have their effect.

MARs reduce variations in transgene expression

One explanation for the variation in transgene expression level

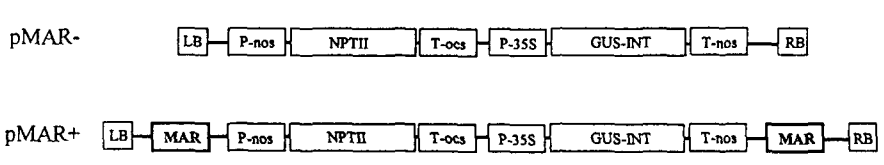

Figure 3. Schematic maps of the T-DNA region of binary vectors, pMAR+ and pMAR-. The relative positions of the GUS gene and *nptII* gene are shown with respect to the left border (LB) and right border (RB) of the T-DNA. The T-DNA regions are not drawn to scale.

may be the differences in the site of integration of the newly introduced gene ("position effect"). An introduced gene that had shown aberrant expression in a line of transgenic mice produced a full spectrum of expression when the gene was re-cloned and reintroduced into the same line of mice (Al-Shaw *et al.*, 1990). In this case, the variation seen must be related to the transcriptional potential of the genomic insertion site. In other words, the site of insertion had a dramatic influence on the level of expression.

MARs may reduce the variability of transgene expression level by alleviating the position effect. Breyne *et al.* (1992) noted slight decreases in variability in their transgenic tobacco cell lines transformed with a GUS. When the β-glucuronidase (GUS) reporter gene was flanked by chicken lysozyme matrix attachment region (MAR), variance of logarithmically transformed data was reduced 8-fold (Mlynarova *et al.*, 1995: Mlynarova *et al.*, 1994). Mylnarova *et al.* (1996) observed that chicken lysozyme MAR element lowered transgene variability to the level of environmental variation. However, there was no effect of MARs on the variability of transgene expression with a MAR from a soybean heat-shock gene (Schoffl *et al.*, 1993) and a tobacco MAR (Han *et al.*, 1997).

MARs work as a transformation booster

In plant transformation systems, one or two selectable markers (*i.e.*, antibiotic resistance genes) are co-inserted along with the gene of interest to distinguish transgenics from normal cells. Subsequently, transgenic shoots are selected based on their ability to grow in the presence of the antibiotics, against which the selectable marker genes impart resistance. From transient expression studies with a GUS-intron gene, we found that *Agrobacteria* could efficiently deliver foreign genes into selected poplar clones with high regenerability. Yet, the frequency for obtaining stably transformed plants remained very low. This finding could mean that, because of the low levels of selectable marker gene expression, many successfully transformed plant cells may be killed off during the initial selection period. Thus, the frequency of getting transgenic shoot recovery is low. This low transformation frequency in recalcitrant species may be overcome by increasing the expression level of a selectable marker gene. The use of MAR elements may increase the number of surviving transgenic cells by enhancing the marker gene expression, thus increasing the transformation frequency. The presence of chicken lysozyme MARs improved relative transformation efficiency in tobacco, indicating MAR influence on the expression of the selectable marker (NPTII) gene (Mylnaroba *et al.*, 1995). We also found that a tobacco MAR increased the frequency of kanamycin-resistant poplar shoots recovered more than 8-fold (Han *et al.*, 1997).

Genomic DNA segment that enhances transformation efficiency has been reported for *Aspergillus* (Cullen *et al.*, 1987), *Petunia* (Meyer *et al.*, 1988), and tobacco (Marchesi *et al.*, 1989). This is known as "transformation booster sequences (TBS)" and contains sequences for DNA unwinding elements, matrix attachment regions (MARs), and topoisomerase binding sites (Buising and Benbow, 1994: Galliano *et al.*, 1995). It stimulates homologous inter- and intra-molecular recombination in *Petunia*, and its 516 bp sub-fragment binds to the nuclear scaffold (Galliano *et al.*, 1995). From its strong homologies to plant retroviral elements, they concluded that the TBS is an inactive derivative of a retrovirus that still promotes DNA recombination. The mechanism(s) by which these sequences stimulate homologous recombination remains to be elucidated. Use of the petunia transformation booster sequences resulted in 7.8- to 16-fold in tobacco and 1.7- to 2.4-fold in maize using biolistics (Buising and Benbow, 1994). However, no increase in transgene expression was observed with TBS sequences. Its usefulness for *Agrobacterium*-mediated transformation has not been reported.

SUMMARY

An efficient transformation system entails control of a number of factors. Gene transfer agents and host cells must be optimized. Also, any selectable marker genes delivered must be expressed strongly and in a high proportion of transformed cells, to allow transgenic tissues to be recovered under selection. In addition, as the expression of transgenes in many recovered transformants is too low or erratic for commercial value, methods for obtaining strong and consistent expression of introduced genes is critical for successful application of biotechnology to plant improvement. Use of MARs expands the range of genotypes amenable to standard *Agrobacterium* transformation protocols, and allows scientifically and commercially valuable transformants to be generated from small transformation programs.

REFERENCES

Allen GC, Hall GJr, Michalowski S, Newman W, Spiker S, Weissinger

AK, Thompson WF (1996) High-level transgene expression in plant cells: Effects of a strong scaffold attachment region from tobacco. Plant Cell **8**: 899-913

Allen GC, Hall GE, Childs LC, Weissinger AK, Spiker S, Thompson WF (1993) Scaffold attachment regions increase reporter gene expression in stably transformed plant cells. Plant Cell **5**: 603-613

Al-Shaw R, Kinnaird J, Burke J, Bishop JO (1990) Expression of a foreign gene in a line of transgenic mice is modulated by a chromosomal position effect. Mol Cell Biol **10**:1192-1198

Ammati BB, and Gasser SM (1990) *Drosophila* scaffold-attachment regions bind nuclear scaffolds and can function as ARS elements in both budding and fission yeasts. Mol Cell Biol **10**: 5442-5454

Anderson JN (1986) Detection, sequence patterns, and function of unusual DNA structures. Nucleic Acids Research **21**: 8513-8533

Bochardt A, Hodal L, Palmgren G, Mattsson O, Okkels FT (1992) DNA methylation is involved in maintenance of an unusual expression pattern of an introduced gene. Plant Physiol **99**: 409-414

Boerjan W, Bauw G, Van Montagu M, Inze D (1994) Distinct phenotypes generated by overexpression and suppression of S-adenosyl-L-methionine synthetase reveal developmental patterns of gene silencing in tobacco. Plant Cell **6**: 1401-1414

Brandle JE, McHugh SG, James L, Labbe H, Miki BL (1995) Instability of transgene expression in field grown tobacco carrying the csr1-1 gene for sulfonylurea herbicide resistance. Bio/Technology **13**: 994-998

Breyne P, Montagu M, Gheysen G (1994) The role of scaffold attachment regions in the structural and functional organization of plant chromatin. Transgenic Research **3**: 195-202

Breyne P, Montagu Mv, Depicker A, Gheysen G (1992) Characterization of a plant scaffold attachment region in a DNA fragment that normalizes transgene expression in tobacco. Plant Cell **4**: 463-471

Buising CM, Benbow RM (1994) Molecular analysis of transgenic plants generated by microprojectile bombardment: effect of petunia transformation booster sequence. Mol Gen Genet **243**: 71-81

Cockerill PN, Garrard WT (1986) Chromosomal loop anchorage sites appear to be evolutionary conserved. FEBS Lett **204**: 5-7

Cullen D, Wilson LJ, Grey GL, Henner DJ, Turner G, Ballance DJ (1987) Sequence and centromere proximal location of a transformation-enhancing fragment ans1 from *Aspergillus* nidulans. Nucleic Acids Research **15**: 9163-9175

De Block M (1993) The cell biology of plant transformation: current state, problems, prospects and the implications for the plant breeding. Euphytica **71**: 1-14

Felsenfeld G, McGhee JD (1986) Structure of the 30 nm chromatin fiber. Cell **44**: 375-377

Finnegan J, McElroy D (1994) Transgene inactivation: plants fight back! Bio/Technology **12**: 883-888

Flavell RB (1994) Inactivation of gene expression in plants as a consequence of specific sequence duplication. Proc Natl Acad Sci U S A **91**: 3490-3496

Galliano H, Muller AE, Lucht JM, Meyer P (1995) The transformation booster sequence from *Petunia hybrida* is a retrotransposon derivative that binds to the nuclear scaffold. Mol Gen Genet **247**: 614-622

Geest AHM, Hall GEJ, Spiker S, Hall T (1994) The B-phaseolin gene is flanked by matrix attachment regions. Plant J **6**: 413-423

Grevelding C, Fantes V, Kemper E, Schell J, Masterson R (1993) Single-copy T-DNA insertions in *Arabidopsis* are the predominant form of integration in root-derived transgenics, whereas multiple insertions are found in leaf discs. Plant Mol Biol **23**: 847-860

Grosveld F, Assendelft BG, Greaves DR, Kollias G (1987) Position-independent, high-level expression of the human B-Globin gene in transgenic mice. Cell **51**: 975-985

Hall GJr, Allen GC, Loer DS, Thompson WF, Spiker S (1991) Nuclear scaffolds and scaffold-attachment regions in higher plants. Proc Natl Acad Sci USA **88**: 9320-9324

Han KH, Bradshaw HD, Gordon MP (1995) Adventitious root and shoot regeneration *In vitro* in under major gene control in an F2 family of hybrid poplar (*Populus trichocarpa* × *P. deltoides*) Forest Genetics **1**: 139-146

Han KH, Gordon MP, Strauss SH (1996) Cellular and molecular biology of *Agrobacterium*-mediated transformation of plants and its application to genetic transformation of Populus. In Biology of *Populus* and its implication for management and conservation, Edited by Stettler Jr HDB RF, Heilman PE, Hinckley TM. NRC Research Press, National Research Council of Canada, Ottawa, ON. pp. 201-222

Han KH, Gordon MP, Strauss SH (1997) High frequency transformation of cottonwoods (genus *Populus*) by *Agrobacterium rhizogenes*. Canadian Journal Forest Research, in press

Han KH, Ma C, Strauss SH (1997) Matrix attachment regions enhance transgene expression and transformation in poplar. Transgenic Research, in review

Hiei Y, Ohta S, Komari T, Kumashiro T (1994) Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. Plant J **6**: 271-282

Hobbs SLA, Warkentin TD, DeLong CMO (1993) Transgene copy number can be positively or negatively associated with transgene expression. Plant Mol Biol **21**: 17-26

Hoffman JF.X, Laroche T, Brand AH, Gasser SM (1989) RAP-1 factor is necessary for DNA loop formation *In vitro* at the silent mating type locus HML. Cell **57**: 725-737

Hollick JB, Gordon MP (1993) A poplar tree proteinase inhibitor-like gene promoter is responsive to wounding in transgenic tobacco. Plant Mol Biol **22**: 561-572

Homberger HP (1989) Bent DNA is a structural feature of scaffold-attached regions in *Drosophila* melanogaster interphase nuclei. Chromosoma **98**: 99-104

Jouanin L, Brasileiro ACM, Leple JC, Pilate G, Cornu D (1993) Genetic transformation: A short review of methods and their applications, results and perspectives for forest trees. Ann Sci For **50**: 325-336

Kartzke S, Saedler H, Meyer P (1990) Molecular analysis of transgenic plants derived from transformation of protoplasts at various stages of the cell cycle. Plant Science **67**: 63-72

Klehr D, Maass K, Bode J (1991) Scaffold-attached regions from the human interferon B domain can be used to enhance the stable expression of genes under the control of various promoters. Biochemistry **30**: 1264-1270

Koornneef M, Bade J, Hanhart C, Horsman K, Schell J, Soppe W, Verkerk R, Zabel P (1993) Characterization and mapping of a gene controlling shoot regeneration in tomato. Plant J **3**: 131-141

Laemmli UK, Kas E, Poljak L, Adachi Y (1992) Scaffold-associated regions: cis-acting determinants of chromatin structural loops and functional domains. Curr Opion Genet Dev **2**: 275-285

Marchesi ML, Castiglione S, Sala F (1989) Effect of repeated DNA sequences on direct gene transfer in protoplast of Nicotiana plumbaginifolia. Theo Appl Genet **78**: 113-118

Matzke MA, Matzke JM (1995) How and why do plants inactivate homologous (trans)genes? Plant Physiol **107**: 679-685

Matzke MA, Priming M, Trnovsky J, Matzke AJM (1989) Reversible methylation and inactivation of marker genes in sequentially transformed tobacco plants. EMBO J **8**: 643-649

McGhee JD, Felsenfeld G (1980) Nucleosome structure. Ann Rev Biochem **59**: 1115-1156

Meilke C, Kohwi Y, Kohwi-Shigematsu Y, Bode J (1990) Hierarchical binding of DNA fragments derived from scaffold-attached regions: correlation of properties *In vitro* and function in vivo. Biochemistry **29**: 7475-7485

Meyer P, Kartzke S, Niedenhof I, Heidmann I, Bussman K, Saedler H (1988) A genomic DNA segment from *Petunia hybrida* leads to increased transformation frequencies and simple integration patterns. Proc Natl Acad Sci USA **85**: 8568-8572

Meyer P, Saedler H (1996) Homology-dependent gene silencing in plants. Ann Rev Plant Physiol Plant Mol Biol **47**: 23-48

Mlynarova L, Jansen RC, Conner AJ, Stiekema WJ, Nap JP (1995) The MAR-mediated reduction in position effect can be uncoupled from copy number-dependent expression in transgenic plants. Plant Cell **7**: 599-609

Mlynarova L, Loonen A, Heldens J, Jansen RC, Keizer P, Stiekema WJ, Nap JP (1994) Reduced position effect in mature transgenic plants conferred by the chicken lysozyme matrix-associated region. Plant Cell **6**: 417-426

Mylnaroba L, Jansen RC, Conner AJ, Stiekema WJ, Nap JP (1995) The MAR-mediated reduction in position effect can be uncoupled from copy number-dependent expression in transgenic plants. Plant Cell **7**: 599-609

Mylnarova L, Keizer LCP, Stiekema WJ, Nap JP (1996) Approaching the lower limits of transgene variability. Plant Cell **8**: 1589-1599

Napoli C, Lemieux C, Jorgensen R (1990) Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous gene in trans. Plant Cell **2**: 279-289

Palmgren G, Mattson O, Okkels FT (1993) Treatment of *Agrobacterium* or leaf disks with 5-azacytidine increases transgene expression in tobacco. Plant Molecular Biology **21**: 429-435

Paulson, JR (1988) Scaffolding and radial loops: the structural organization of metaphase chromosomes. *In* Chromosome and chromatin structure, K. W. Adolph, ed. Boca Raton, Fl: CRC, pp. 3-37

Peach C, Velten J (1991) Transgene expression variability (position effect) of CAT and GUS reporter genes driven by linked divergent T-DNA promoters. Plant Mol Biol **17**: 49-60

Phi-Van L, Stratling WH (1988) The matrix attachment regions of the chicken lysozyme gene co-map with the boundaries of the chromatin domain. EMBO J **7**: 655-664

Riemenschneider DE (1990) Susceptibility of intra- and inter-specific hybrid poplars to *Agrobacterium tumefaciens* strain C58. Phytopathology **80**: 1099-1102

Schoffl F, Schroder G, Kliem M, Rieping M (1993) An SAR sequence containing 395 bp DNA fragment mediates enhanced, gene-dosage-correlated expression of a chimaeric heat shock gene in transgenic tobacco plants. Transgenic Research **2**: 93-100

Spiker S, Allen GC, Hall GEJ, Michalowski S, Newman W, Thompson W, Weissinger AK (1995) Nuclear matrix attachment regions (MARs) in plants: affinity for the nuclear matrix and effect on transient and stable gene expression. J Cell Biochem 21B, 167

Spiker S, Thompson WF (1996) Nuclear matrix attachment regions and transgene expression in plants. Plant Physiol **110**: 15-21

Weber H, Ziechmann C, Graessmann A (1990) *In vitro* DNA methylation inhibits gene expression in transgenic tobacco. EMBO J **9**: 4409-4415