Matrix attachment regions as a tool to influence plant transgene expression

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Abstract

The inclusion of special regulatory sequences known as matrix attachment regions (MARs) in transgene constructs has been suggested as a possible approach to stabilise the expression of foreign heterologous genes. The present review provides a brief summary regarding the MARs that have been used in investigations studying their influence on plant transgene expression in different plants with different promoters and reporter genes, and the comparison of these investigations.

Keywords Transgenic plant · Matrix attachment regions · MAR · Transgene expression

Introduction

Plant transformation is based on the ability to integrate foreign DNA into the genomes of host plants and on the efficiency of the transformed cell to develop into the whole plant. The development of various methods for genetic transformation led to the application of transgenic plants in various spheres, such as improvement of the commercial traits of a large number of agricultural crops, high-level production of important proteins in plants and the usage of transgenic plants as an instrument for the explanation of a gene's working machinery (Lloyd 2003; Kusaba 2004). The use of transgene technology allows improvement of certain plant traits, including disease resistance, stress tolerance, enhanced nutrition and male sterility (Lanfranco 2003). However, considerable variability in transgene expression has been observed within the limits of plant populations transformed using identical expression constructs and methods under identical conditions (Peach and Velten 1991; Bennet 1993; Birch 1997; Bhat and Srinivasan 2002; Butaye et al. 2004; Dolgova et al. 2015). This variability in expression greatly complicates the analysis of phenotypical traits

as well as the development of commercially profitable crops with predictable characteristics. To reduce the cost, efforts, and number of mistakes by researchers, many approaches have been explored to achieve stable transgene expression and predict the level of this expression. The variability in transgene expression is influenced by several factors. One of these factors is the integration site, which includes the chromatin status (heterochromatic or euchromatic) and the influence of nearby regulatory sequences (enhancers or silencers). Additionally, transgene expression may be affected by the number and disposition of transgenes within the integration site (a single copy or multiple copies arranged as inverted or direct repeats).

Generally, the result of a genetic transformation is a high frequency of transgenic plants with undesirable and unpredictable levels of gene expression, along with a considerable number of transformants, in a definite proportion, with low, although expected, expression levels. For instance, this kind of result was observed in previous studies for standard reporter genes such as the gene for beta-glucuronidase protein (*uidA*) (Hobbs et al. 1993; Elmayan and Vaucheret 1996; Brouwer et al. 2002; De Bolle et al. 2003; Dolgova et al. 2015). Moreover, plants with desirable expression levels may lose these characteristics within a few generations (Scheid et al. 1991; Bhat and Srinivasan 2002; Vain et al. 2002; Butaye et al. 2004). Additionally, the variability in transgene expression raises the inconvenience of using a large number of plants for the analysis to discover individual transformants with acceptable levels of expression and desired phenotypes, and therefore further complicates

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the interpretation of outcomes of the conducted research (Birch 1997; Bhat and Srinivasan 2002; De Bolle et al. 2003). Therefore, one of the main objectives in the field of plant transformation is to design vectors and methods that can produce a high percentage of transgenic plants with the required stable phenotype and minimum differences in the expression levels among individual plants. These problems have stimulated the search for tools that would be able to reduce variations in transgene expression. The inclusion of special regulatory sequences known as matrix attachment regions (MARs) in the transgene constructs has been suggested as a possible approach to stabilise transgene expression. Since the beginning of the 1990s, several experiments have been conducted in an attempt to understand the machinery through which MARs influence the transgene expression in plants and plant cell cultures. However, to date, no complete review of these elements and the consequences of their application in plants is available. In the present review, studies (Table 1) on this subject have been summarised, and a brief overview of the MARs, model plants, promoters and reporter genes used in these studies has been provided.

MARs: what are they?

DNA sequences possessing the ability to bind to the nuclear matrix were named MARs (matrix/scaffold attachment regions), or SARs or MAR elements. These are non-transcribed, 100–3000 bp AT-rich fragments of the eukaryotic genome, which possess the ability to bind to the isolated nuclear matrix. The majority of the earlier reports that aimed at studying the effects of MAR elements were generally based on the hypothesis that the variability in transgene expression could be explained by accidental integration of the vector into the target genome. The attachment of MARs to the nuclear scaffold is supposed to protect the transgenes from the regulatory influence of neighbouring genes or chromatin. Recent research has demonstrated that plant genes containing predicted MAR sequences exhibit significantly lower differential expression profile index (DEXP) values and are likely to be expressed in one tissue, organ or developmental phase (Tetko et al. 2006). Several experiments have been conducted to identify the specific role of MAR sequences in influencing the stability of the integrated transgenes. The animal transgene expression experiments that used MAR elements demonstrated that in most cases, MARs enhanced the usual levels of expression and reduced expression variability. The investigations involving plant subjects have not been as comprehensive, as these began considerably later, and therefore, such definite resuming of expression level could not be suggested. In the initial stages, it was believed that the studies on plant systems could not be carried out as completely as the ones conducted on animals.

However, the results of the scientific investigations conducted on animals in the later stages were greatly different from the earlier animal experiments (Poljak et al. 1994; Kalos and Fournier 1995), which implies that this statement must be stricter. It may not be possible to squeeze the large amount of conflicting data from plant studies into the frames of the total contradictory data of all the biological systems.

Plants

Nicotiana tabacum has long served as a key model plant for the development of transformation technology, transgene integration and analysis of expression stability. This may be because tobacco was the first plant for which it was possible to achieve in vitro regeneration (Skoog and Miller 1957) and to develop a standard cell culture medium (Murashige and Skoog 1962). It is the most studied plant subject, and the techniques for its transformation are well established. All initial and significant modern studies regarding the MAR influence on plant transgene expression were conducted on the cultures of this plant subject. Different tobacco parts have been used in these experiments (Table 1), mostly whole plants; although, callus cultures, cells, seeds and leaves have also been used. The results of the early experiments on tobacco plant systems (Breyne et al. 1992) surprisingly differed from the majority of the data based on animal experiments published during those times. The results of a study conducted in 1993 (Schöffl et al. 1993) were somewhat closer to what was expected on the basis of animal experiments. The application of MAR elements led to a fivefold to ninefold increase in the expression of the beta-glucuronidase protein (GUS) reporter gene, while it exerted no influence on the variability in expression. However, it at least partly noted the dependence of expression on the gene copy number. Research papers published in the years 1994 and 1995 (Mlynarova et al. 1994, 1995) reported that the expression levels exhibited no dependence on the gene copy number, as opposed to the findings of the previous study (Schöffl et al. 1993), although this was in agreement with the studies conducted on animals (Poljak et al. 1994). Among the subsequent studies conducted on plants, the most similar ones to the animal experiments were those in which ballistic transformation or cell culture was used (Allen et al. 1993, 1996; Mankin et al. 2003). The results of later studies revealed that the abnormal strong effect of MARs which was observed in the tobacco suspension cell culture transformed by microprojectile bombardment (Allen et al. 1993, 1996) was most likely because of the rapidly dividing, undifferentiated cells, rather than due to certain unique advantages of the MARs or the complexity of insertion events obtained using direct DNA delivery (Ülker et al. 1999). In the experiment using T-DNA transformation, the expression levels of the reporter gene were much lower in tobacco cells compared to plants, and the influence of MAR

Table 1 Influence of MARs on transgene expression in different plant species and organ cultures

Plant system	MAR source (name)	Promoter-reporter	DNA transfer method	Influence on expression level	Influence on expression variability	Sources
Tobacco callus	Soy-bean (P1)	NOS-GUS	T-DNA	2× decrease	2× decrease	Breyne et al. (1992)
	Human (β-globin SAR)	NOS-GUS	T-DNA	Little influence	Little decrease	
Tobacco plants	Soy-bean (SAR _L)	Heat Shock-GUS	T-DNA	5–9× increase	No influence	Schöffl et al. (1993)
Tobacco cells	Yeast (ARS-1)	35S-GUS	Biolistic	12× increase	Little	Allen et al. (1993)
Tobacco plants	Chicken (A element)	Lhca3-GUS	T-DNA	3–4× increase	3–8× decrease	Mlynarova et al. (1994)
Tobacco seeds	Bean (phas MAR)	Phaseolin-GUS	T-DNA	3× increase	2× decrease	van der Geest et al. (1994)
Tobacco cells	Petunia (TBS)	35S-GUS	Biolistic	No influence	7.8–16× increase in transformation frequencies	Buising and Benbow (1994)
Maize cells					1.7–2.4× increase in transformation frequencies	
Tobacco plants	Chicken (A element)	Enh35S-GUS	T-DNA	2× increase	2–7× decrease	Mlynarova et al. (1995)
	Chicken (A element)	NOS -NPTII	T-DNA	No influence	Up to 3× decrease	
Tobacco cells	Tobacco (RB7)	35S-GUS	Biolistic	60× increase	No influence	Allen et al. (1996)
Tobacco seeds	Bean (phas MAR)	P109-GUS	T-DNA	No influence	No data	van der Geest and Hall (1997)
Tobacco leaf disk	Tobacco (RB7)	35S-GUS	T-DNA	1.6× increase	Increase of the number of GUS foci	Han et al. (1997)
Poplar explants (<i>P. tremula</i> x <i>P. alba</i> clone 717)	Tobacco (RB7)	35S-GUS	T-DNA	8.7× increase	Little	
Poplar explants (<i>P. trichocarpax</i> x <i>P. deltoides</i> clone 184-402)	Tobacco (RB7)	35S-GUS	T-DNA	7.7× increase	Little	
Tobacco plants	Pea (<i>vic</i> MAR)	35S-GUS	T-DNA	Little	Little	Liu and Tabe (1998)
Tobacco plants	Arabidopsis (<i>At</i> MAR)	35S-GUS	T-DNA	5–10× increase	No influence	
Maize cells	Chicken (A element)	35S-cabl-GUS	Biolistic	36× increase	Increase	Odell and Krebbers (1998)
Rice plants	Tobacco (RB7)	35S-GUS	Biolistic	2.5× increase	Little	Vain et al. (1999)
	Yeast (ARS-1)	35S-GUS	Biolistic	3× increase	Little	
Tobacco plants	Tobacco (RB7)	35S-GUS	Biolistic	2× increase	Little	Ülker et al. (1999)
Pine callus	Tobacco (RB7)	Enh35S-GUS:nptII	T-DNA	3× increase	Decrease of the transgene expression variability over time	Levee et al. (1999)
Rice plants	Tobacco (RB7)	Act1-GFP	Biolistic	3.3–18.4× increase	Conflict data	Cheng et al. (2001)
		Act1-GUS		376–650× increase		
Tobacco plants	Pea	35S-GUS	T-DNA	2× increase	No data	Li et al. (2001)
Tobacco plants	Synthetic (sMAR)	35S-GUS	T-DNA	2–2.5× increase	Increase	Nowak et al. (2001)

Table 1 (continued)

Plant system	MAR source (name)	Promoter-reporter	DNA transfer method	Influence on expression level	Influence on expression variability	Sources
Tobacco plants	Chicken (A element)	35S-LUC	T-DNA	Little	No influence	van Leeuwen et al. (2001)
	Chicken (A element)	35S-LUC	T-DNA	2× decrease	Increase	
Barley callus	Soy-bean (P1)	35S-GUS	Biolic	2× increase	2× decrease	Petersen et al. (2002)
	Petunia (TBS)	35S-GUS	Biolic	No influence	Increase transformation frequency	
Arabidopsis plants	Yeast (ARS-1)	APT-Lc	T-DNA	Little decrease	Little	Holmes-Davis and Comai (2002)
	Tomato (<i>HSC80</i> MAR)	APT-Lc	T-DNA	No influence	No data	
Tobacco plants	Tobacco (TM1)	35S-GUS	T-DNA	1.5× fold	No influence	Zhang et al. (2002)
	Tobacco (TM2)			5× fold		
	Tobacco (TM3)			1.35× fold		
	Arabidopsis (AM1)			1.3× fold		
	Arabidopsis (AM2)			No influence		
Tobacco cells	Tobacco (RB7)	PsFed1-GUS	Biolic	No influence	No influence	Mankin et al. (2003)
		AtAhas-GUS		No influence		
		GmHspL-GUS		2.7–3.1 increase		
		35S-GUS		4.2–5.3× increase		
		NOS-GUS		3–4.9× increase		
		OCS-GUS		8.3–15.3× increase		
Maize plants	Maize (P-MAR)	P1-rr-GUS	Biolic	No influence	No influence	Sidorenko et al. (2003)
	Maize (Adh1 5'fr-MAR)	WP-GUS				
		Rsyn7-GUS			Decrease	
Tobacco cells	Tobacco (<i>CHN50</i> MAR)	35S-GUS	T-DNA	10× increase	No data	Fukuda and Nishikawa (2003)
		35S-GUS	T-DNA	No influence		
		mini35S-GUS	T-DNA	Decrease		
Cacao plants	Tobacco (RB7)	E12-Ω- GFP	T-DNA	2.2× increase	4.5× decrease	Maximova et al. (2003)
Gene silencing Arabidopsis mutants	Chicken (A element)	35S-GUS	T-DNA	5–12× increase	No influence	Butaye et al. (2004)
Sorghum cells	Tobacco	Ubi-GUS	Biolic	2× increase	No influence	Able et al. (2004)
Maize roots	Maize (ADH1 5' MAR)	Ubi-GUS	Biolic	Increase	No data	Torney et al. (2004)
Rice plants	Synthetic (SM11)	GOS2- GUS	Biolic	1.9× increase	Little	van der Geest et al. (2004)
Arabidopsis plants		Act2- GUS	T-DNA	1.6–3.7× increase	2.3–6.6× increase	
Rice plants	Chicken (BP-MAR)	Act1-sGFP	T-DNA	Increase	Decrease	(Oh et al. 2005)
Tobacco cells	Tobacco (RB7)	TripleOp-LUC	Biolic	Increase	Percentage increase of expressing cell lines	Abranches et al. (2005)
Tobacco plants	Tobacco (RB7)	35S-TSWV-N	T-DNA	No data	1.5× percentage increase of lines with resistance to TSWV	Levin et al. (2005)

Table 1 (continued)

Plant system	MAR source (name)	Promoter-reporter	DNA transfer method	Influence on expression level	Influence on expression variability	Sources
Tobacco cells	Tobacco (RB7)	35S-GFP	T-DNA	2–3.7 × increase	No data	Halweg et al. (2005)
Rice callus	Tobacco (TM2)	35S-GUS Ubi-GUS PNZIP-GUS	T-DNA	3.2 × increase 4.1 × increase 2.3 × increase	Decrease of number of gene silencing calluses	Xue et al. (2005)
Rice plant		35S-GUS Ubi-GUS PNZIP-GUS		5–6 × increase 8–10 × increase 4 × increase	No data	
Arabidopsis plants	Chicken (A element)	35S-GUS	T-DNA	No influence	No influence	De Bolle et al. (2007)
Gene silencing Arabidopsis mutants	Tobacco (tab-MAR)	35S-GUS	T-DNA	No influence 5 × increase		
Dunaliella salina cells	D. salina (DSM 2)	RbcS-CAT	Electro-poration	4.6 × increase	2.5 × decrease	Wang et al. (2007)
Poplar plants	Tobacco (RB7)	35S-GFP RbcSTP-BAR	T-DNA	No influence 1.3 × decrease	Significantly reduced	Li et al. (2008)
Tobacco cells	Tobacco (TM2)	35S-GUS	T-DNA	5.2–6.5 × increase	No data	Zhang et al. (2009)
Tobacco plants		35S-GUS 35Smini-GUS PNZIP-GUS		4.4–5.5 × increase No influence 6.8 × increase		
Arabidopsis plants	Maize (ADH1 5' MAR)	AGIP-GUS	T-DNA	No influence	No data	Hily et al. (2009)
	Tobacco (RB7)			No influence		
	Petunia (TBS)			76 × decrease		
Tobacco plants	Rapeseed (BnMAR)	35S-GUS	T-DNA	1.5 × increase	No data	Xu et al. (2011)
Tobacco plants (cv.Samsun)	Tobacco (Mf1)	35S-Efb	T-DNA	17 × increase	No data	Festa et al. (2013)
Tobacco plants (cv.81V9)				1.5 × increase		
Tobacco plants (cv.NC89)	Tobacco (RB7) Tobacco (TM6)	35S-GUS DREB-GUS PNZIP-GUS mini35S-GUS	T-DNA	6.5 × increase 12.7 × increase 4.3 × increase 2.9 × increase 9.8 × increase	No data	Ji et al. (2013)
Tobacco leaves	Tobacco (RB7)	35S-NVCP 35S-IgG	T-DNA	1.3 × increase 3.4 × increase	No data	Diamos et al. (2016)
Tobacco plants	Petunia (Petun-SAR)	35S-GUS	T-DNA	4.3 × increase	Increase	Dietz-Pfeilstetter et al. (2016)
Tobacco leaves	Tobacco (RB7) Tobacco (TM6)	35S-GFP	T-DNA	3–40 × increase Increase	No data	Diamos and Mason (2018)

elements was approximately the same (Zhang et al. 2009). Recent studies have demonstrated that in addition to tobacco plant parts, its cultivar could also be the reason for the differences observed in MAR effects (Festa et al. 2013). The validation and confirmation of these facts require a detailed

and comprehensive study. In recent times, a method for testing different regulatory elements in transient plant expression systems has gained popularity. This technique was also used in the latest MAR experiments (Diamos et al. 2016, 2018). This approach might allow rapid and effective evaluation of the

functioning machinery of various MARs in different plant species other than tobacco.

In addition to tobacco, the most frequently used plants for MAR investigations are rice and *Arabidopsis*. Both *A. thaliana* and *N. tabacum* are the model organisms of choice for conducting research in plant biology. Currently, the transformation of *Arabidopsis* is highly reproducible and requires ordinary procedures. Moreover, a short life cycle, small plant size and efficient reproduction through self-pollination have allowed *Arabidopsis* to become an early favourite for studying induced mutations in plants (Koornneef and Meinke 2010). In the studies involving *Arabidopsis*, it was observed that the application of MARs from natural sources, such as yeast, tomato (Holmes-Davis and Comai 2002), chicken and tobacco (De Bolle et al. 2007), could not enhance transgene expression in a wild-type background, although it could be used to enhance transgene expression in a mutant impaired in gene silencing (Butaye et al. 2004; De Bolle et al. 2007). Only the synthetic MARs assembled from sequence elements recognised by the nuclear matrix proteins and containing structural features commonly present in MARs led to an increase in reporter gene expression (van der Geest et al. 2004). In contrast to the results obtained with *Arabidopsis*, all of the MARs that were used in rice plants positively affected the transgene expression (Table 1), with approximately twice the effect observed in plants compared to the callus cultures (Xue et al. 2005). The RB7 action study conducted with poplar demonstrated a limited or negative effect of MAR elements on various aspects of transgene structure and stability, including the expression levels, year-to-year variation, position effect, T-DNA configuration and copy number, along with correlation in the expressions of co-integrated transgenes in these plants (Li et al. 2008). The positive effect was observed only in immature explants (Han et al. 1997). In transgenic maize, only the chicken A element increased the reporter gene expression under the 35S promoter (Odell and Krebbers 1998), while the combinations of maize MARs with maize (P1), wheat (WP) and synthetic (Rsyn7) promoters (Sidorenko et al. 2003) or with petunia (TBS) promoter (Buisson and Benbow 1994) did not exhibit any such increase. MARs effects were also investigated in exotic cultures, such as pine callus (Levee et al. 1999), Sorghum cells (Able et al. 2004), Barley callus (Petersen et al. 2002), *Theobroma cacao* plants (Maximova et al. 2003) and *Dunaliella salina* cells (Wang et al. 2007); in all of these cases, the increase in the gene expression levels was approximately two–fourfold.

MARs

In the initial stages of investigations regarding the influence of MARs on transgene expression in plants, animal or yeast elements that had been characterised much earlier were used (Table 1). Human β -globin SAR did not result in an increase

in the reporter gene expression levels, in contrast to what was expected (Breyne et al. 1992). Yeast ARS-1 was used three times, increasing the expression levels threefold in rice plants (Vain et al. 1999) and 12-fold in tobacco cells (Allen et al. 1993) when used in combination with the 35S promoter, and exhibiting no such functioning when used with the APT (adenine phosphoribosyltransferase) gene promoter from *Arabidopsis* (Holmes-Davis and Comai 2002). Chicken 5' MAR flanking the lysozyme locus was frequently used because it has been well characterised in animal models since the late 1980s (Loc and Strätling 1988). However, in the standard model system of *N. tabacum* plus 35S promoter, the A element did not perform as expected (van Leeuwen et al. 2001; De Bolle et al. 2007). Chicken MAR resulted in a significant increase in the gene expression only in other plant species, such as maize cells (Odell and Krebbers 1998), gene silencing *Arabidopsis* mutants (Butaye et al. 2004) and rice (Oh et al. 2005), or with other gene promoters, such as Lhca3 or enhanced 35S (Mlynarova et al. 1995). Nevertheless, the A element refers to those rare MARs that are able to reduce variability in transgene expression (Mlynarova et al. 1994, 1995; Oh et al. 2005).

The most commonly used and the first-described MAR derived from plant subjects is the tobacco RB7 element from the root-specific gene (Conkling et al. 1990). This MAR operates stably in the tobacco plants, increasing the gene expression levels in conjunction with all tested promoters (Table 1), with the exception of just two—PsFed1 and AtAhas (Mankin et al. 2003). In mature poplar (Li et al. 2008) and *Arabidopsis* (Hily et al. 2009) plants, RB7 did not exert any influence on the reporter gene expression; however, in rice (Vain et al. 1999; Cheng et al. 2001) and cacao (Maximova et al. 2003), RB7 behaved the same way as in native tobacco. Thus far, RB7 is the most steadily working MAR among all the matrix attachment regions that have been tested in plant systems. Since the early 2000s, several novel tobacco MARs have been discovered (Table 1). The most promising one is tobacco MAR (TM) 2, which exhibits activity in tobacco with different promoters (Zhang et al. 2009), as well as in rice plants and callus cultures (Xue et al. 2005). MARs from other plants have not yet been widely studied.

Two *Arabidopsis* MAR sequences among the three that have been tested (Liu and Tabe 1998; Zhang et al. 2002), pea MARs (Liu and Tabe 1998; Li et al. 2001), bean beta-phaseolin MAR (van der Geest et al. 1994) and rapeseed BnMAR (Xu et al. 2011) relatively increased the expression levels of the *uidA* gene in tobacco. Petunia TBS, which was isolated 20 years ago (Meyer et al. 1988), is traditionally viewed together with the MAR elements, since its affinity towards the nuclear scaffold was established in 1995 (Galliano et al. 1995). Even so, TBS never increased the expression levels in the plants investigated (Table 1), and it possessed a set

of functions different than those of the typical plant MARs (Hily et al. 2009). However, at the same time, it highly increased the transformation frequencies in tobacco, maize (Buising and Benbow 1994) and barley (Petersen et al. 2002) and was established as an enhancer-blocking insulator in *Arabidopsis* (Hily et al. 2009). A novel petunia element, Petun-SAR, possesses properties similar to the standard MARs, and increased the transgene expression in tobacco plants fourfold (Dietz-Pfeilstetter et al. 2016). Two synthetic MAR sequences were tested, namely sMAR, exhibiting increased reporter gene expression by twofold in tobacco (Nowak et al. 2001), and SM11 in *Arabidopsis* and rice (van der Geest et al. 2004). SM11 is the only known MAR that is able to increase transgene expression in non-mutant *Arabidopsis*. Although novel MARs are continuously being discovered (Wang et al. 2007; Festa et al. 2013), researchers in recent years have been increasingly adhering to verified MARs, such as RB7, while conducting their investigations (Ji et al. 2013; Diamos and Mason 2018).

Promoter-reporter systems

Over the past 40 years, the reporter gene *uidA* has held a leading position in all experiments involving plant transgene expression. The same is true for MAR studies; only a quarter of all the scientific research investigations involving MARs did not use this particular gene. Among the studies investigating the influence of MARs, six studies used the green fluorescent protein gene (*gfp*) as a reporter (Cheng et al. 2001; Maximova et al. 2003; Oh et al. 2005; Halweg et al. 2005; Li et al. 2008; Diamos and Mason 2018), two studies used the luciferase *luc* gene (van Leeuwen et al. 2001; Abranches et al. 2005) and the other reporter genes were used only once in the studies, although the influence on the frequency of transformation was often assessed using the selective marker neomycin phosphotransferase II (NPTII). The variety of promoters used in the MARs investigations is large.

In the initial stages of plant transformation research, the promoters from tumour-inducing plasmids of *A. tumefaciens* were mainly used, such as the NOS promoter (Depicker et al. 1982). This promoter was also used in the first experiment conducted on the influence of MARs on transgene expression (Breynne et al. 1992). However, since the mid-1990s, the most commonly used promoter in MARs research is the constitutive cauliflower mosaic virus promoter 35S (Table 1). The influence of different MARs on the expression driven by different promoters has been investigated (Table 1), although the understanding of pathway patterns has encountered obstruction by different experimental parameters. In an attempt to understand the mode of MAR influence, the effect of RB7 on six different promoters was

analysed in stably transformed tobacco cell cultures (Mankin et al. 2003). The presence of MARs significantly increased the expression of transgenes under constitutive cauliflower mosaic virus (CaMV) 35S, nopaline synthase (NOS) and octopine synthase (OCS) promoters. On the other hand, the expression driven by an induced heat shock promoter (GmHspL) was increased by MAR only in the presence of heat shock. Moreover, this element did not exert any influence on the pea ferredoxin promoter (PsFed1), which is not normally expressed in this cell line, nor did it exhibit any influence on the *Arabidopsis thaliana* acetohydroxyacid synthase promoter (AtAhas). Therefore, it may be concluded that MARs increase the average GUS expression when used with an active promoter and exert little influence on the expression when applied in combination with promoters that are weak in use for a particular cell line or plant species. Similar results were obtained with another tobacco MAR, TM2, which was discovered later (Zhang et al. 2009). However, a novel MAR, TM6, enhanced transcription activation in transgenic tobacco independent of the promoters 35S, DREB, PNZIP or mini35S (Ji et al. 2013). Therefore, it is possible that MARs are a heterogeneous group of elements that share only the capacity of binding to the nuclear matrix (Holmes-Davis and Luca 1998), and their influence on transgene expression depends on both the promoter and their sequence.

Conclusion

MARs may have practical applications in plants to enhance the gene expression of commercially important proteins and facilitate the release of these proteins. Experiments have already been conducted using a target gene instead of reporter gene, such as the IgG coding gene, for which the immunogenicity of its product of expression was proven in mice models (Diamos et al. 2016), or using an MAR expression system in plants for an efficient production of bioactive *Arabidopsis thaliana* plant defensins (Sels et al. 2007). However, at this moment, there is no definite MAR element that would work in all conditions with equal effectiveness. The most universal today is tobacco RB7 MAR. From all conducted experiments, it failed to show positive activity in only two cases: mature poplar (Li et al. 2008) and *Arabidopsis* (Hily et al. 2009). In tobacco itself (Table 1), in rice plants (Vain et al. 1999; Cheng et al. 2001) and even in an exotic culture such as cocoa (Maximova et al. 2003), the use of RB7 MAR led to a steady increase in the average level of expression, and in some cases to a decrease of the expression level variability. However, this element cannot be used for all purposes. This review will help to choose the correct MAR for a specific use, depending on what kind of plant, organs and tissue cultures you want to use, and what end result

you need to achieve. Working with a certain culture, you can check the table and select the most appropriate element for required investigations or for use in biotechnology and biofarming. It is still necessary to continue research in the direction of understanding the functional concepts underlying MAR influence on transgene expression. However, right now, with the appropriate approach, you can use known MAR elements for high-efficiency transgene expression of target proteins for different usage.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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