Journal of Plant Physiology 168 (2011) 30–44

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Review

Transgene expression systems in the *Triticeae* cereals

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ARTICLE INFO

Article history:
Received 17 April 2010
Received in revised form 19 July 2010
Accepted 21 July 2010

Keywords:
Gene expression
Genetic engineering
Promoter
Transgenic
Transient expression
Transformation
Triticeae

SUMMARY

The control of transgene expression is vital both for the elucidation of gene function and for the engineering of transgenic crops. Given the dominance of the *Triticeae* cereals in the agricultural economy of the temperate world, the development of well-performing transgene expression systems of known functionality is of primary importance. Transgenes can be expressed either transiently or stably. Transient expression systems based on direct or virus-mediated gene transfer are particularly useful in situations where the need is to rapidly screen large numbers of genes. However, an unequivocal understanding of gene function generally requires that a transgene functions throughout the plant's life and is transmitted through the sexual cycle, since this alone allows its effect to be decoupled from the plant's response to the generally stressful gene transfer event. Temporal, spatial and quantitative control of a transgene's expression depends on its regulatory environment, which includes both its promoter and certain associated untranslated region sequences. While many transgenic approaches aim to manipulate plant phenotype via ectopic gene expression, a transgene sequence can be also configured to down-regulate the expression of its endogenous counterpart, a strategy which exploits the natural gene silencing machinery of plants. In this review, current technical opportunities for controlling transgene expression in the *Triticeae* species are described. Apart from protocols for transient and stable gene transfer, the choice of promoters and other untranslated regulatory elements, we also consider signal peptides, as they too govern the abundance and particularly the sub-cellular localization of transgene products.

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Introduction

The *Triticeae* tribe within the *Pooideae* subfamiliy of the grass family *Poaceae* includes ca. 350 annual and perennial species assigned to ca. 30 genera out of which only about half are commonly accepted among taxonomists (Barkworth and von Bothmer, 2009). Thanks to their outstanding importance as food, feed and industrial raw material, the small grain cereals bread wheat (*Triticum aestivum*), durum wheat (*Triticum turgidum* ssp. *turgidum*), barley (*Hordeum vulgare*), rye (*Secale cereale*) and triticale (*x Triticosecale*) are the most prominent representatives of the *Triticeae*. Over the last decade, technological developments have moved the genetic transformation of the *Triticeae* cereals from being difficult

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doi:10.1016/j.jplph.2010.07.007

to achieve to becoming routine. The pressure of continued human population growth and the probability of non-beneficial climate change only serve to increase the need to increase the supply of plant-based food and energy, and transformation technology will play a part in this effort—not just in the generation of superior cultivars, but also in the elucidation of gene function. In plant cells which are directly accessible to transformation, both transient over-expression (TOX) and transient-induced gene silencing (TIGS) approaches have been elaborated to provide rapid screening methods for large numbers of genes. As a contrast, some viral vectors can be exploited to ensure the systemic dispersal of a transiently expressed transgene beyond the site of primary infection. Promoter sequences are the key to the spatial, temporal and quantitative pattern of transgene expression, particularly in stable transformants, where the transgene is ubiquitously present.

A major limiting factor inhibiting advances in *Triticeae* transgene technology is that many of the best characterized plant promoters have been developed in dicotyledonous species; many of these have proven to be inefficient or ineffective in a monocotyledonous environment, for reasons which remain ill understood. Nevertheless, the number of functional transgene promoters in the *Triticeae* has been increasing with time. Transgene expression, and the subsequent site of transgene product accumulation can further be manipulated by the judicious choice of non-promoter regulatory elements such as introns and signal peptides, respectively. Transformation technology can take advantage of the complexity of gene regulation, by deliberately engineering these components of a given transgene's sequence.

Here we review the current state-of-the-art of *Triticeae* transgene expression systems. We cover both transient and stable gene transfer methods, as both are important for specific applications. A particular focus has been given to promoters and other regulatory sequences which can be exploited to control the localization in time and space and/or the abundance of transgene transcript and gene product in transgenic *Triticeae* species.

Methods of gene transfer

Transient expression systems

The value of transient transgene expression in plants has been only recently comprehensively reviewed (Jones et al., 2009; Shepherd, 2009). Briefly, "transient" implies expression over a period ranging from a few hours to several days. Transience, in part at least, reflects the expression of non-integrated recombinant DNA, which by definition is not replicated subsequent to its introduction. In some cases, genomic integration does occur, but the period of transgene expression is delimited by the life span of the recipient cell, which is unable to proliferate under the imposed experimental conditions. Many transient expression systems rely for their efficacy on supplying a large number of transgene copies per transformed cell, while in stable transgenics, a high transgene copy is frequently associated with silencing rather than amplification of transgene expression. Furthermore, transgene expression is not, in the case of transient systems, dependent on the genomic site of integration. The phenotypic consequences of transient transgenesis are often more consistently detectable, although post-transcriptional transgene silencing resulting from high transgene copy numbers can also affect transient expression systems.

A major feature of transient expression is that it avoids the time-consuming and laborious generation and maintenance of stable transgenic lines. This advantage is especially relevant in the context of the cereals which remain less readily amenable to stable transformation than are many dicotyledonous species. A range of physical, chemical and biological methods of DNA delivery into various cereal tissue types is available, making transient expression assays particularly attractive both for basic research and for some biotechnological applications.

The development of the micro-projectile (tungsten or gold) bombardment (or so called "biolistic" or "ballistic") technique of delivering DNA into a cereal cell represented an important breakthrough for the assessment of gene function via transient over-expression. As each transfer event necessarily affects only an individual cell, the expression of the transgene can be monitored at the single cell level. DNA vectors designed for transient over-expression need not include more than the target expression cassette. Since the biolistic approach allows for the inclusion of multiple vectors in a single bombardment, various transgenes and/or construct types such as scorable markers (*gus*, Jefferson, 1987 or *gfp*, Davis and Vierstra, 1998) can be simultaneously assayed in the same cell. Co-bombardment can also be used to genetically modify the target cell to provide the appropriate conditions for transgene expression; an example of this approach was the use of an *Mlo* over-expression construct to suppress the basal powdery mildew (*Blumeria graminis*) resistance of bombarded barley cells, and in so doing, produce a host–pathogen interaction environment which was more conductive for the expression of the transgenes under test (Shirasu et al., 1999; Elliott et al., 2002).

The biolistic method has been extensively applied in the *Triticeae* cereals to estimate the activity of promoter/*gus*-fusions. It has been repeatedly demonstrated that the number of detectable GUS-positive cells is correlated with promoter activity (Onate et al., 1999; Rubio-Somoza et al., 2006). More recently, a comparison of the strength of four different promoters in stably transgenic barley lines with their activities in transiently transformed leaves also revealed a strong correlation (Himmelbach et al., 2010). Consequently, transient tests can provide reliable information on the relative promoter activity in transgenic plants, yet absolute quantitative levels of expression unfortunately cannot be predicted in this way for stably transformed lines. Thus, stably transformed plants remain the gold standard for promoter studies, however, the biolistic approach can provide a viable alternative, especially where the aim is to pre-screen many candidate sequences or to rapidly acquire comparative gene expression information.

Although the biolistic method is not restricted to any particular plant cell type, the target cells clearly need to be physically accessible to bombardment, a requirement which does limit its applicability in the cereals. Most transient expression experiments in this group of plants have therefore been conducted on either the grain endosperm (Knudsen and Müller, 1991) or the leaf epidermis (Douchkov et al., 2005). Since only a small proportion of the cells is transformed, the accumulation of sufficient material for molecular biological and biochemical analysis can be problematical. This limitation has driven the development of enhanced tissue capture methods such as laser capture micro-dissection (Emmert-Buck et al., 1996) and microfluidic single-cell analysis (Stone et al., 2004; Marcus et al., 2006a,b). The ease and rapidity of the transient expression approach have been greatly improved by the recent combination of GATEWAY™ cloning technology with robotics-based microscopic evaluation. It is now technically possible to perform functional analyses of thousands of genes within only a fraction of the time needed to generate sufficient populations of stably transgenic plants (Douchkov et al., 2005; Ihlow et al., 2008). The benefit of this high-throughput approach system has been well illustrated by the exploration of both the host–pathogen interaction in the barley leaf epidermis (Panstruga, 2004; Zimmermann et al., 2006; Shen et al., 2007) and by a phenomics-based study of gene expression in dehydration-stressed barley (Marzin et al., 2008).

The expression of genes responsible for the accumulation of protein in the grain is of particular interest in the cereals, and has been led by the development of a polyethylene glycol (PEG)-mediated gene transfer method applied to barley and wheat